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Open Access

Isolation and characterization of three unrecorded yeast species in the order *Tremellales* from soil, wildflowers, and strawberries in Korea

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The purpose of this study was to isolate and identify wild yeasts from soil in Busan, wildflowers in Okcheon-gun, and strawberries in Jeju, Republic of Korea. Among the isolated strains, three were unrecorded in the Republic of Korea. To identify wild yeast strains, pairwise sequence comparisons of D1/D2 region of the 26S rRNA gene sequence were done using Basic Local Alignment Search Tool (BLAST). The cell morphologies were observed by phase contrast microscope and assimilation test are done using API 20C AUX kit. All strains were assigned to the phylum *Basidiomycota*. The two unrecorded yeast strains, BS-6 and PFL-3, belong to the genus *Hannaella* (family *Bulleribasidiaceae*, order *Tremellales*, class *Tremellomycetes*). Strain PST-43 belongs to the genus *Papiliotrema* (family *Rhynchogastremataceae*, order *Tremellales*, class *Tremellomycetes*). All three strains had oval shaped and polar budding cells. This research showed the morphological and biochemical properties of the three unreported yeast species in Korea.

Keywords: 26s rRNA, *Tremellales*, unrecorded yeasts

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Introduction

The genus *Hannaella* is a basidiomycetous yeast in the family *Bulleribasidiaceae* (order *Tremellales*, class *Tremellomycetes*, phylum *Basidiomycota*). *Hannaella* species were isolated from Leaf of *Parthenocissus* sp., air and sediments from the deep-sea floor of Suruga Bay, Japan (Nagahama *et al*., 2003; Wang and Bai, 2008; Li *et al*., 2020). The genus *Hannaella* comprises twelve distinct species and has *Hannaella sinensis* as a type species (https://www.mycobank.org). The genus *Hannaella* belongs to the family *Bulleribasidiaceae*, and the typical characteristics associated with the family *Bulleribasidiaceae* include pseudohyphae and true hyphae maybe present, ballistoconidia present or not. If formed, they are rotationally symmetric, turbinate, flabelliform, globose or subglobese. Budding cells present and cells are oval, clamp connections are absent. Colonies are cream to yellowish, mucoid, smooth and shiny. Additionally, fermentation usually absent and diazonium blue B and urease reaction are positive. The major CoQ system is CoQ-10 (Wang and Bai, 2008).

The genus *Papiliotrema* is a basidiomycetous yeast in the family *Rhynchogastremataceae* (order *Tremellales*, class *Tremellomycetes*, phylum *Basidiomycota*). *Papiliotrema* species were isolated from flower of *Miconia* sp., water sample in Brazil, and bromeliad leaves (Machado Pagani *et al*., 2016). The genus *Papiliotrema* comprises

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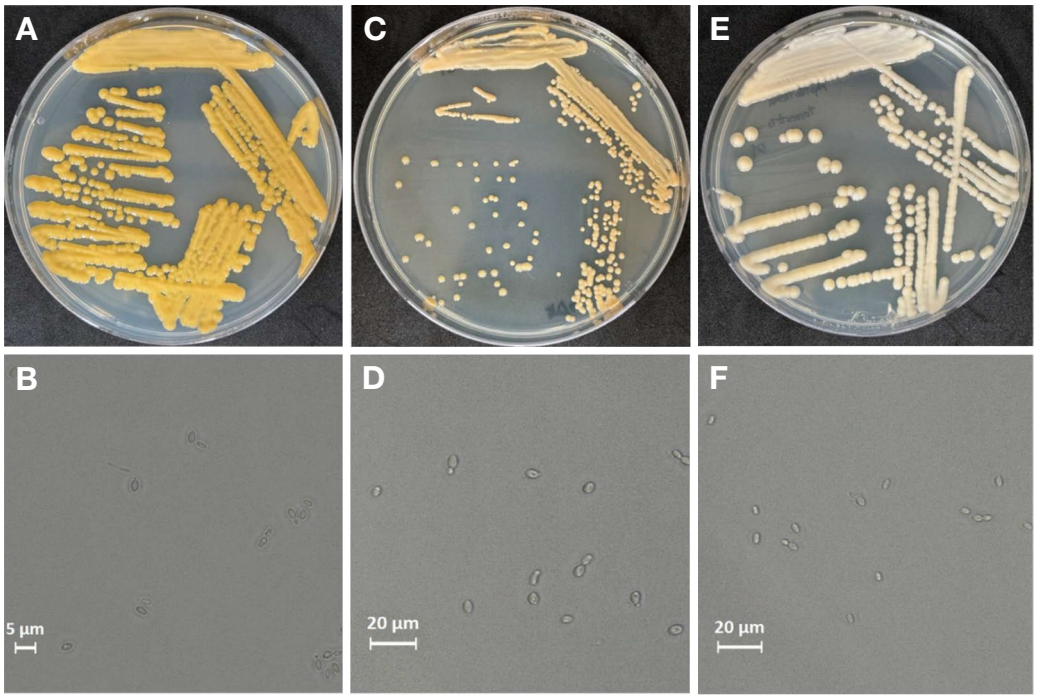


Fig. 1. Morphology from cells from the unrecorded strain *H. pagnoccae* BS-6 incubated at 25°C, and strains *H. sinensis* PFL-3 and P. *baii* PST-43 incubated at 10°C. All strains were grown after 3 days on YPD agar. The colonies of *H. pagnoccae* BS-6 (A), *H. sinensis* PFL-3 (B), and *P. baii* PST-43 (C). The budding cells of *H. pagnoccae* BS-6 (C), *H. sinensis* PFL-3 (D), and *P. baii* PST-43 (F). Bars: 5 μm (B) and 20 μm (D, F).

twelve distinct species and has *Papiliotrema bandonii* as a type species (https://www.mycobank.org). The genus *Papiliotrema* belongs to the family *Rhynchogastremataceae*, and the typical characteristics associated with the family *Rhynchogastremataceae* include pseudohyphae and true hyphae maybe present, basidiocarps maybe present. If present, minute, ca. 0.5 mm in diameter. Colonies are pale to brownish yellow. Budding cells present, clamp connections and haustorial branches on dikaryotic hyphae present. Ballistoconidia can be formed. Sexual reproduction observed in some species. Additionally, fermentation absent and nitrate usually not utilized. The major CoQ system is CoQ-10 (Liu *et al*., 2015).

In this study, wild yeasts were isolated from soil in Busan, wildflowers in Okcheon-gun, and strawberries in Jeju, Republic of Korea. As a result of isolation and identification, a number of strains were found to be previously reported species, and three strains were identified as unrecorded in Korea. These unrecorded strains were assigned to the genera *Hannaella* and *Papiliotrema*. In this study, their phenotypic characteristics were also examined to support species identification.

Materials and Methods

Samples were collected from three different regions in the Republic of Korea. Strain BS-6 was isolated from a wildflower in Haeundae-gu, Busan (35°10′43.4″N, 129°8′13.6″E) on January 12, 2024. Strain PFL-3 was obtained from a wildflower in Okcheon-gun, Chungcheongbuk-do (36°13′40.2″N, 127°37′14.8″E) on January 19, 2024. Strain PST-43 was isolated from a strawberry in Jeju-si, Jeju-do (33°27′54.8″N, 126°29′09.7″E) on January 10, 2024. The samples were serially diluted in distilled water and the suspension was spread in a Yeast Extract Peptone Dextrose (YPD) agar (Difco, USA) and incubated at 25°C for 3 days. The strains are preserved in a metabolically inactive state at the Korean Agricultural Culture Collection, Korea.

The cell morphologies of strains were done by the LEICA (DM500), using yeast strains incubated in YPD agar for 3 days. Phase contrast microscope images and the colonies of the strains BS-6, PFL-3 and PST-43 are shown in Fig. 1. To characterize the biochemical features, API 20C AUX (bioMérieux) were tested according to the manufacturer's instructions.

The genomic DNA were extracted after incubated on YPD agar for 3 days. The D1/D2 region of the 26S rRNA gene sequence was amplified by PCR using NL1 (5′-GCA TATCAATAAGCGGAGGA AAAG-3′) and NL4 (5′-GGT CCGTGTTTCAAGACGG-3′) primers (Kurtzman and Robnett, 1998).

Pairwise sequence comparisons were made using the Basic Local Alignment Search Tool (BLAST) (Altschul *et*

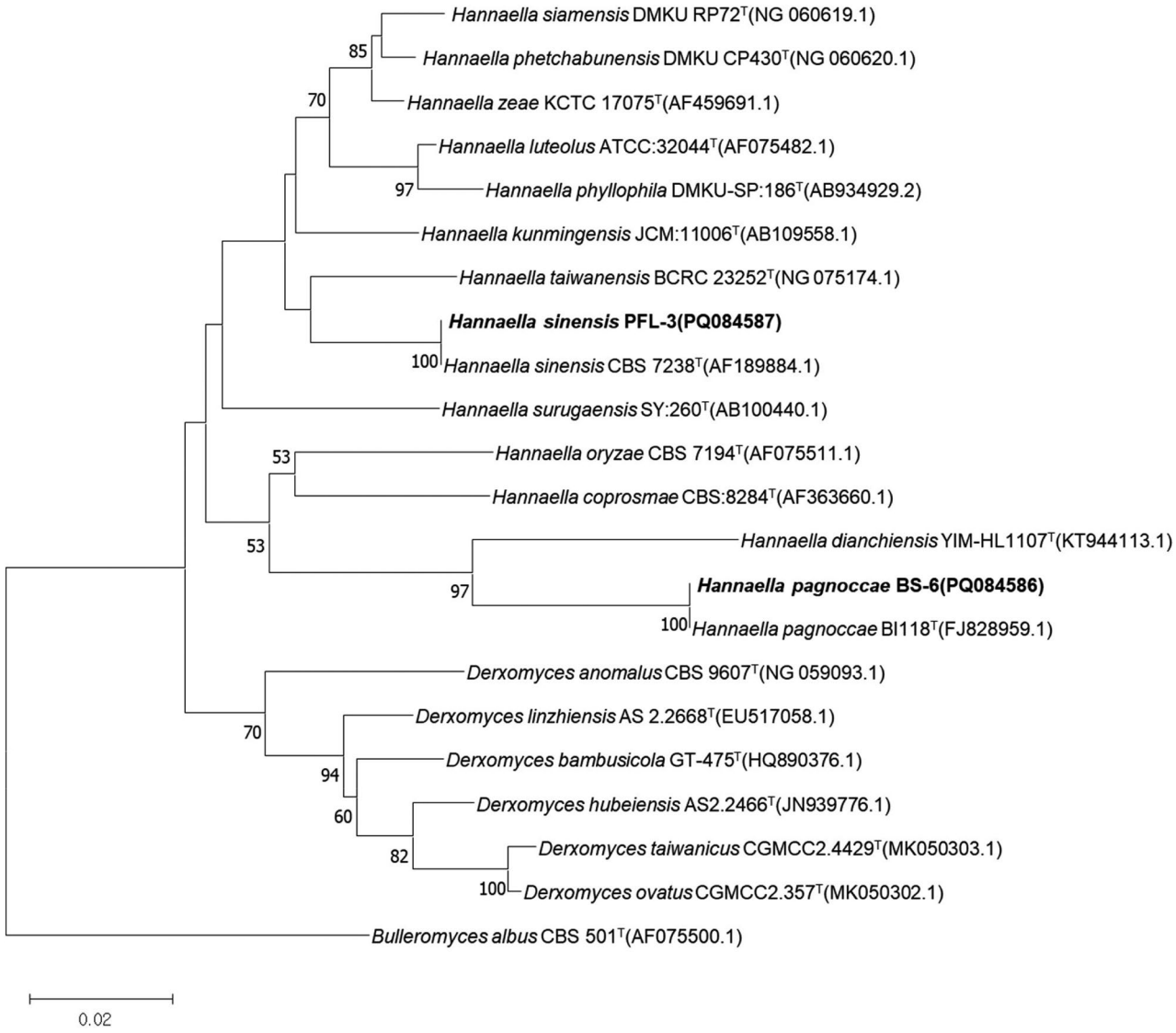


Fig. 2. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strains BS-6 and PFL-3 with closely related species. Bootstrap values (>50%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.02 substitutions per nucleotide position.

al., 1997) and to align with the sequences of related species retrieved from GenBank. The MYCOBANK (https://www.mycobank.org/) database identified strain types for each species and close strains gene sequence were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/) for 26s rDNA in a row (Fell *et al*., 2000).

The phylogenetic trees based on the D1/D2 domain of LSU rRNA gene sequence were reconstructed by the neighboring joining algorithm of MEGA 11 program (Tamura *et al*., 2021) The evolutionary distance was calculated using the two-parameter model of Kimura (Kimura, 1983) for neighborhood bond analysis and the confidence level of the class was estimated through bootstrap analysis (1,000 replicates) (Felsenstein, 1985).

Results and Discussion

Ten wild yeasts strains were collected from soil in Busan, wildflowers in Okcheon-gun, and strawberries in Jeju, Republic of Korea. The yeast strains were identified by analyzing D1/D2 domain of 26s rRNA gene sequence similarities that were calculated by the NCBI BLAST. Result of identification based on the D1/D2 domain of 26s rRNA gene sequence, three yeast strains were identified as domestic unrecorded yeast species. The taxonomic composition and identification results are listed in Table 1.

All strains were assigned to the families *Cystofilobasidiaceae* (1 strain), *Rhynchogastremataceae* (4 strains), *Bulleribasidiaceae* (2 strains), *Bulleraceae* (2 strains) and

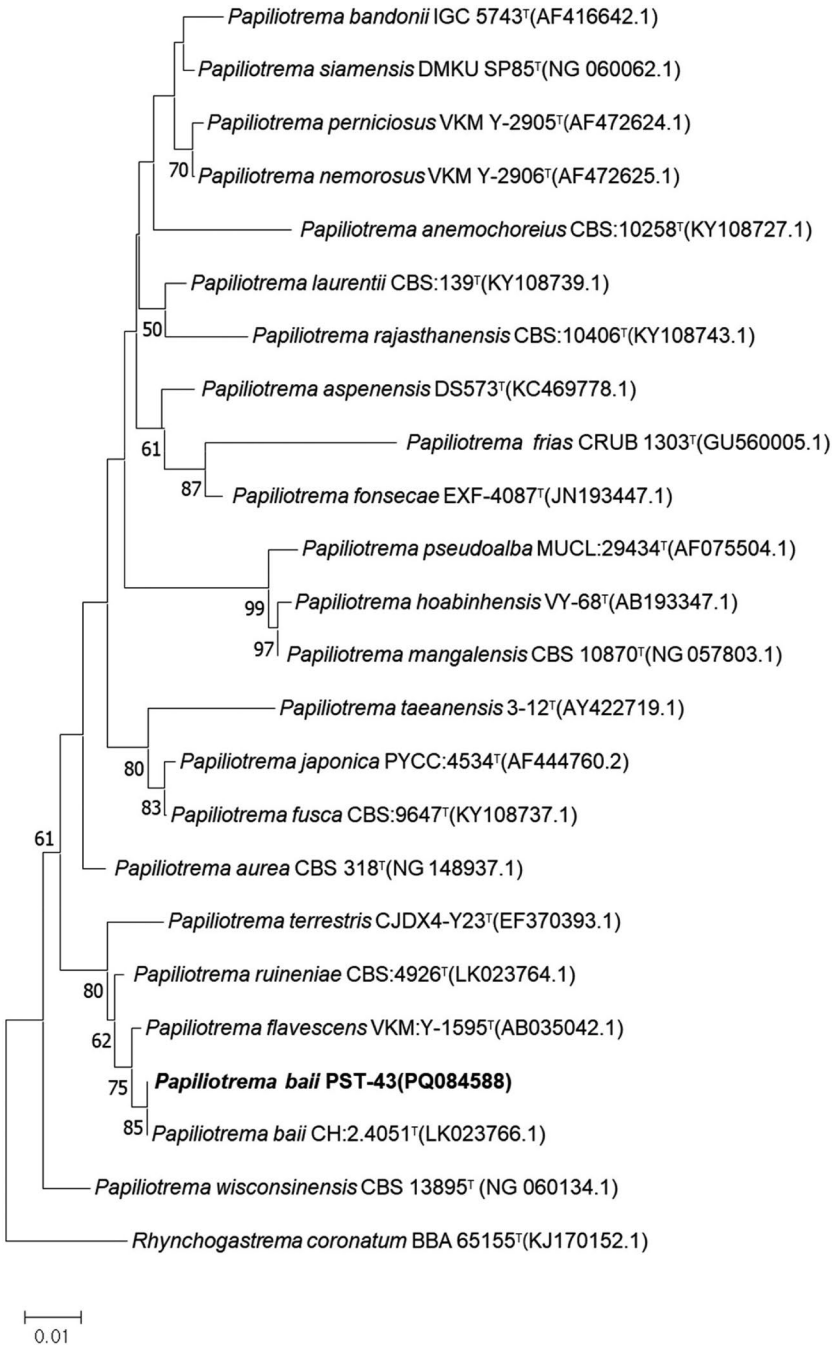


Fig. 3. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain PST-43 with closely related species. Bootstrap values (>50%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

Filobasidiaceae (1 strain) of the phylum *Basidiomycota*. The unrecorded yeast strains BS-6 and PFL-3 belong to the phylum *Basidiomycota* and the genus *Hannaella*, strain PST-43 belong to the phylum *Basidiomycota* and the genus *Papiliotrema*. The phylogenetic tree shows that the isolated strains are closely related to the *Hannaella* and *Papiliotrema* species with the highest 26S rRNA gene sequence similarity (Figs. 2 and 3) and thus supports close relationships, respectively.

Description of *Hannaella pagnoccae* BS-6

Colonies are convex, smooth, and yellow colored after 3 days of incubation on YPD agar at 25°C. In the API

Table 1. Yeasts isolated strains from soil in Republic of Korea.

Phylum	Class	Order	Family	Strain ID	Most closely related species	26S rRNA similarity	Source	Record in Korea
		Cystofilobasidiales	*Cystofilobasidiaceae*	MST-12	*Cystofilobasidium infirmominiatum*	617/618 (99.84%)	Strawberry	Reported
				PST-15	*Papiliotrema flavescens*	564/566 (99.65%)	Strawberry	Reported
				PST-43	*Papiliotrema baii*	554/554 (100.00%)	Strawberry	Unreported
			Rhynchogastremataceae	BS-3	*Papiliotrema horticola*	522/524 (99.61%)	Soil	Reported
		Tremellales		BS-5	*Papiliotrema horticola*	522/524 (99.61%)	Soil	Reported
Basidiomycota	*Tremellomycetes*			BS-6	*Hannaella pagnoccae*	511/511 (100.00%)	Soil	Unreported
			Bulleribasidiaceae	PFL-3	*Hannaella sinensis*	616/616 (100.00%)	Wildflowers	Unreported
				PST-25	*Bullera alba*	607/609 (99.67%)	Strawberry	Reported
			Bulleraceae	PST-35	*Bullera alba*	609/612 (99.67%)	Strawberry	Reported
		Filobasidiales						
			Filobasidiaceae	MFL-3	*Naganishia liquefaciens*	604/604 (100.00%)	Wildflowers	Reported

All strains were cultured under 25°C for 3 days.

Table 2. Phenotypic characteristics that differentiate *H. pagnoccae* BS-6, *H. sinensis* PFL-3 and related species and strain.

	1	2	3	4
Growth on				
Temp (°C)	10−30	10−30	20−25	19−25
Assimilation of				
Sucrose	+	+	+	+
Raffinose	+	+	+	+
Galactose	+	+	+	+
Trehalose	+	+	+	+
Maltose	+	+	+	+
Melezitose	+	+	+	+
Methyl-α-D-glucoside	−	−	+	+
Cellobiose	w	+	+	+
D-Xylose	+	+	+	+
L-Arabinose	+	+	+	+
Glycerol	−	−	v	+
N-Acetyl-D-Glucosamine	−	w	−	ND
2-Keto-D-Gluconate	+	+	ND	+
Adonitol	+	w	+	v
Xylitol	w	w	+	+
Inositol	−	−	+	+
D-Sorbitol	+	+	−	ND
D-Lactose (bovine origin)	−	−	v	−

Strains: 1, *H. pagnoccae* BS-6; 2, *H. sinensis* PFL-3; 3, *H. pagnoccae* BI118^{T}; 4, *H. sinensis* CBS 7238^{T}.
+, positive; w, weakly positive; −, negative; v, variable; ND, No Data.

20C AUX test, strain BS-6 is positive for glucose, calcium 2-keto-gluconate, L-arabinose, D-xylose, adonitol, D-galactose, D-sorbitol, D-maltose, D-saccharose, D-trehalose, D-melezitose, D-raffinose; weak positive for xylitol, D-cellobiose, but negative glycerol, inositiol, methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine, D-lactose (Table 2).

Strain BS-6 (KCTC 37293) was isolated from soil collected in Busan, Republic of Korea.

Description of *Hannaella sinensis* PFL-3

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

Strain PFL-3 (KCTC 37302) was isolated from wildflowers collected in Okcheon-gun, Chungcheongbuk-do, Republic of Korea.

Table 3. Phenotypic characteristics that differentiate *P. baii* PST-43 and related species and strain.

	1	2	3
Growth on			
Temp (°C)	10−30	25−30	19−25
Assimilation of			
Sucrose	+	+	+
Raffinose	+	+	+
Galactose	+	+	+
Trehalose	+	+	+
Maltose	+	+	+
Melezitose	+	+	+
Methyl-α-D-glucoside	−	+	v
Cellobiose	+	+	+
D-Xylose	+	+	+
L-Arabinose	+	+	+
Glycerol	+	+	v
N-Acetyl-D-Glucosamine	−	ND	−
2-Keto-D-Gluconate	+	+	+
Adonitol	+	+	+
Xylitol	+	+	+
Inositol	+	+	+
D-Sorbitol	+	v	v
D-Lactose (bovine origin)	+	+	+

Strains: 1, *P. baii* PST-43; 2, *P. baii* PYCC 6532^T; 3, *P. flavescens* CBS 942^T.
+, positive; −, negative; v, variable; ND, No Data.

Description of *Papiliotrema baii* PST-43

Colonies are convex, smooth, and cream colored after 3 days of incubation on YPD agar at 25°C. In the API 20C AUX test, strain PST-43 is positive for glucose, glycerol, calcium 2-keto-gluconate, L-arabinose, D-xylose, adonitol, xylitol, D-galactose, inositol, D-sorbitol, D-cellobiose, D-lactose, D-maltose, D-saccharose, D-trehalose, D-melezitose, D-raffinose. But, negative for methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine (Table 3).

Strain PST-43 (KCTC 37303) was isolated from strawberry collected in Jeju, Republic of Korea.

Conflicts of Interest

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

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New records of Mycetophilidae (Diptera: Bibionomorpha) from Ulleung Island in South Korea

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Ulleung Island, characterized by its distinctive geological traits, has a unique array of fauna and flora. Although numerous studies have been conducted on insect fauna of this island. Mycetophilidae has never been investigated. Therefore, we conducted field investigations at three sampling locations on Ulleung Island from 2022 to 2024, utilizing sweeping and Malaise traps. As a result, we document seven species across six genera, including two previously recorded species (*Boletina nitida* and *Monoclona laosilvatica*) and five new country records (*Allodia fuliginosa*, *Allodia laccariae*, *Clastobasis serrulate*, *Mycomya tridens*, and *Mycetophila assimilis*) from Ulleung Island. Diagnosis, photographs of morphological characters, and habitat information are provided.

Keywords: checklist, fungus gnats, Korean fauna, taxonomy, volcanic island

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Introduction

Ulleung Island is a volcanic island of South Korea, situated in the East Sea (N 37°28′53.48″, E 130°54′34.91″). Spanning an area of 72.857 km^2, it is located 130.3 km from the Korean Peninsula at its closest point through Uljin, Gyeongsangbuk-do (Lim and Lee, 2012). The island hosts a unique ecosystem influenced by an oceanic climate, a result of its geological detachment from the mainland. Numerous studies have focused on the faunistic diversity of insects in Ulleung Island. The family Mycetophilidae represents one of the largest groups within the infraorder Bibionomorpha in Diptera, encompassing eight subfamilies, 230 genera, and nearly 4,500 species that have been described globally (Kaspřák *et al*., 2019). They have a mycophagous larval stage, and adults act as pollinators in nature. The adults are typically found in moist environments, such as under tree roots and on rocks covered in mosses near aquatic systems. Despite their great diversity, Mycetophilidae had not previously been documented in Ulleung Island, even though its insect fauna includes 18 orders, 179 families, and a total of 1,177 species (Cho, 1929; Kim *et al*., 1971; Lee and Kwon, 1981; Kwon *et al*., 1996; Lee and Jung, 2001; Lim *et al*., 2013). Thus, this study aims to document the fauna of Mycetophilidae species in Ulleung Island for the first time. In this research, six genera and seven species of Mycetophilidae were identified, including five newly recorded species. Further investigations on this island are necessary to fully understand the fauna of Mycetophilidae in this unique volcanic island.

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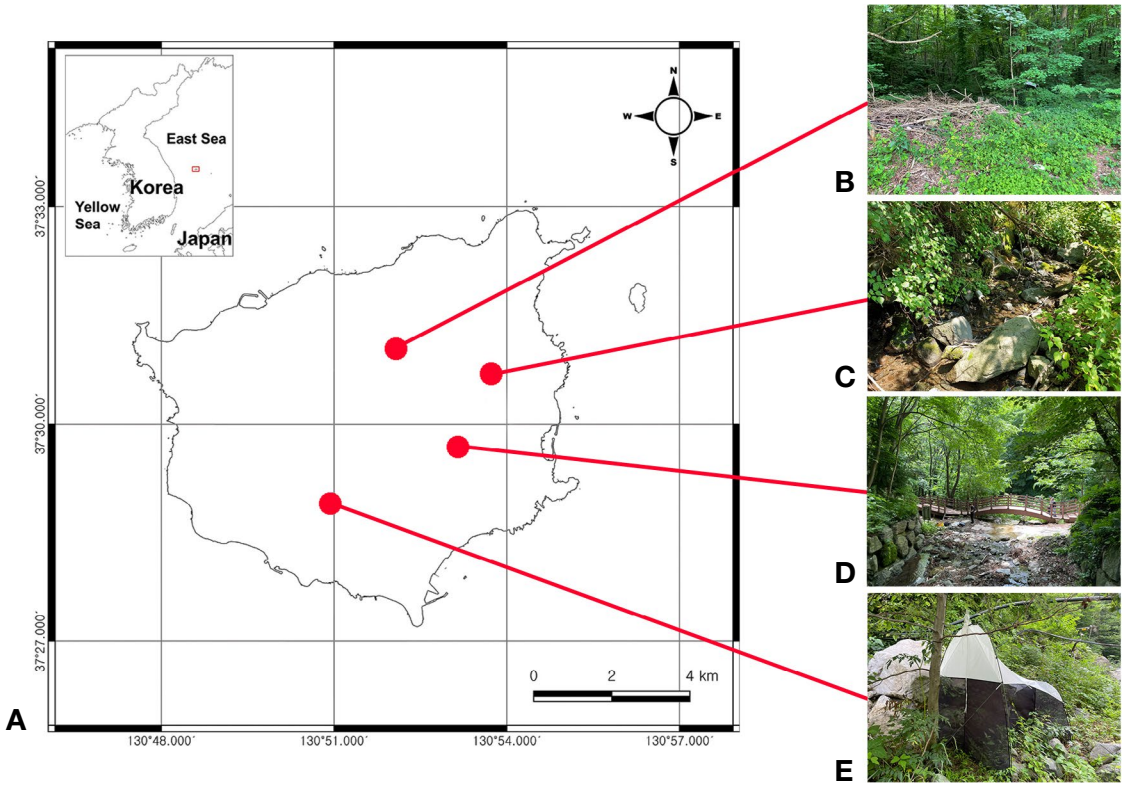


Fig. 1. The map of sampling sites and its habitat in Ulleung Island. A: Map, B: Nari Basin, C: Naesujeoncheon Stream, D: Bongrae waterfall, E: Namyangcheon stream.

Materials and Methods

Adult fungus gnats were collected from four sampling locations, primarily well-preserved forests, using sweeping nets and Malaise traps from May to September (spring to autumn) 2024 (Fig. 1). Additional specimens collected in 2022, which are deposited in the Korea University Entomological Museum (KUEM), were also used. All specimens were preserved in 80% ethanol. Male genitalia were dissected and placed overnight in 10% KOH to clear and remove tissues. Subsequently, genitalia were mounted on slide glass with Hoyer's solution and examined under a dissecting microscope (Olympus SZX7, Japan). Photographs of the specimens were taken with a digital camera (Nikon Z7 with NIKKOR 105 mm macrolens, Japan). All specimens examined in this study were deposited in KUEM.

The terminology of adult morphological features generally follows Kirk-Spriggs and Sinclair (2017a; 2017b), and Zaitzev (1994; 2003). The distribution of each species is provided according to Bechev (2000).

Taxonomic Accounts

Order Diptera Linnaeus, 1758 파리목
Infraorder Bibionomorpha Hennig, 1954 털파리하목
Superfamily Sciaroidea Billberg, 1820 버섯파리상과
Family Mycetophilidae Newman, 1834 버섯파리과
Genus *Allodia* Winnertz, 1863 등줄버섯파리속 (신칭)

1. *Allodia fuliginosa* Wu, Zheng & Xu, 2003 (Fig. 2A)
노란등줄버섯파리 (신칭)
Allodia fuliginosa Wu, Zheng & Xu, 2003: 349.

Material examined. 2♂ (No. NIBRIN0001073532, No. KUEM DIP 200012 preserved in 80% ethanol), Bongrae waterfall, Dodong-ri, Ulleung-eup, Ulleung-gun, Gyeongsang-buk-do, South Korea, 37°29′51.11″N 130°53′12.53″E, 27−31.V.2024, malaise trap, Coll. C Lim, SH Park, W Kim.
Diagnosis. Mesoscutum yellowish brown without discal bristles; Sc ends on R, apical part of R_5 almost straight, r-m 0.8−1.0 times as long as m-stalk. Tergites 2−4 lighter markings at anterior angles. Gonostylus complex; dorsal branch flat; medial branch with small black collar-like setae; ventral branch curved and sharp.
Distribution. South Korea (new record), China.

2. *Allodia laccariae* Sasakawa & Ishizaki, 2003 (Fig. 2B)
등줄버섯파리 (신칭)
Allodia laccariae Sasakawa & Ishizaki, 2003: 104.

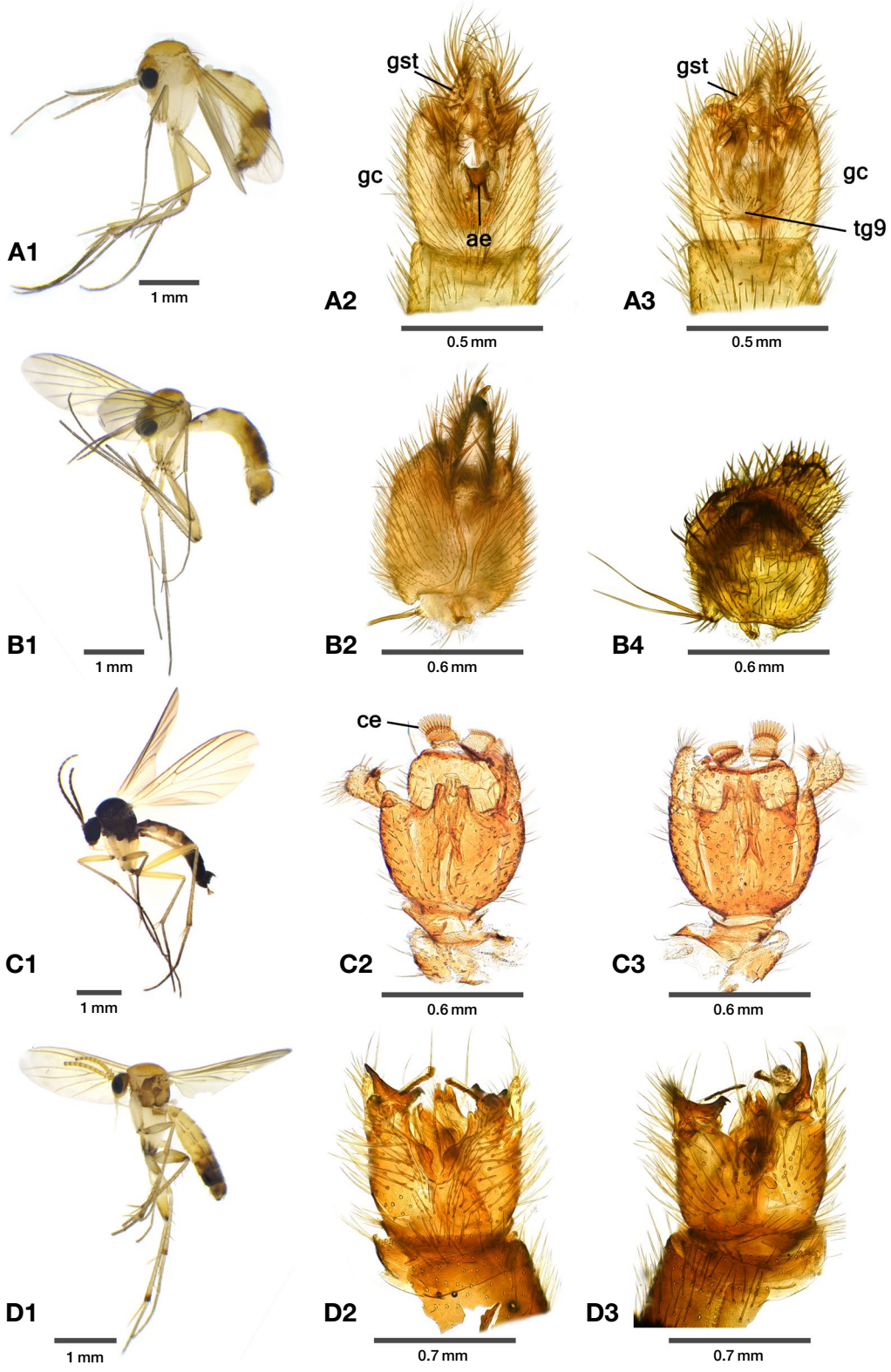


Fig. 2. Male habitus and terminalia. 1: habitus, 2: terminalia ventral view, 3: terminalia dorsal view. 4: terminalia lateral view. A: *Allodia fuliginosa*, Wu, Zheng & Xu, 2003, B: *Allodia laccariae* Sasakawa & Ishizaki, 2003, C: *Boletina nitida* Grzegorzek, 1885, D: *Clastobasis serrulata* Sasakawa, 2005. Abbreviations: ae, aedeagus; ce, cercus; gc, gonocoxite; gst, gonostylus; tg9, tergites9.

Material examined. 1♂ (No. NIBRIN0001073531, preserved in 80% ethanol), Naesujeoncheon Stream, Jeodong-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, South Korea, 37°30′41.6″N 130°54′00.9″E, 05.VII.2022, sweeping, Coll. J Kim, W Lee. 1♂ (No. KUEM DIP 200013, preserved in 80% ethanol), Bongrae waterfall, Dodong-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, South Korea, 37°29′51.11″N 130°53′12.53″E, 24.V−10.IX.2024, Malaise trap, Coll. C Lim, SH Park, W Kim.
Diagnosis. Mesoscutum supra-alar and post-alar bristles long; Sc very short, R_1 as long as R, r-m as long as M fork, with two to four microtrichia on distal part, base of cubital fork usually before that of M fork. Tergite 9 very small, with two long apical bristle. Gonocoxites slightly united with each other on ventral side; gonostylus with dorsal and ventral stylomeres.
Distribution. South Korea (new record), Japan.

Genus *Boletina* Staeger, 1840 꼬리버섯파리속

3. *Boletina nitida* Grzegorzek, 1885 (Fig. 2C)
밝은꼬리버섯파리
Boletina nitida Grzegorzek, 1885: 204.

Material examined. 3♂ (No. KUEM DIP 200014−200016, preserved in 80% ethanol), Namyangcheon stream, Namyang-gil, Seo-myeon, Ulleung-gun, Gyeongsangbuk-do, South Korea, 37°28′50.1″N 130°51′11.6″E, 24.VI−10.IX.2024, Sweeping, Coll. J Lee, S Mok, SH Park.
Diagnosis. Mediotergite black and bare; laterotergite with three long setae; C extending beyond R_5, Sc complete and reaching C, R_1 longer than r-m at less four times, M_1 and M_2 forked at Rs; legs entirely yellow with black setae. Tergites 2−4 brown and yellow, 5−8 black, 9 with two cerci; cerci with long pectinate seta. Gonocoxites wider than tergite 9; gonostylus club shape with setose; inner side and three strong setae.
Distribution. Czech Republic, Georgia, Hungary, South Korea, United Kingdom.
Remarks. This species was first reported by Lee and Bae in 2022.

Genus *Clastobasis* Skuse, 1890 숲버섯파리속(신칭)

4. *Clastobasis serrulata* Sasakawa, 2005 (Fig. 2D)
노란숲버섯파리(신칭)
Clastobasis serrulata Sasakawa, 2005: 279.

Material examined. 1♂ (No. NIBRIN0001073530, preserved in 80% ethanol), Naesujeoncheon Stream, Jeodong-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, South Korea, 37°30′41.6″N 130°54′00.9″E, 04.VII.2022, Sweeping, Coll. J Kim, W Lee.
Diagnosis. Mesoscutum shiny except for whitish pollinose lateral margin and yellowish humeral area, and posterolateral corner; Sc ending on C, R_1 almost as long as r-m, M-petiole as long as or slightly shorter than r-m, CuA_1 not connected to CuA_2 at base. Tergites 2 brown posterior section on 1/3 poin. Gonocoxite well-projected posteriorly ventral lobe.
Distribution. South Korea (new record), Japan.

Genus *Monoclona* Mik, 1886 민날개버섯파리속

5. *Monoclona laosilvatica* Ševčík, 2001 (Fig. 3A)
굵은엄지버섯파리
Monoclona laosilvatica Ševčík, 2001: 158.

Material examined. 2♂ (No. KUEM DIP 200017−200018, preserved in 80% ethanol), Naesujeoncheon Stream, Jeodong-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, South Korea, 37°30′41.6″N 130°54′00.9″E, 04.VII.2022, Sweeping, Coll. J Kim, W Lee. 1♂ (No. KUEM DIP 200019, preserved in 80% ethanol), Namyangcheon Stream, Namyang-gil, Seo-myeon, Ulleung-gun, Gyeongsangbuk-do, South Korea, 37°28′50.1″N 130°51′11.6″E, 24.VI−10.IX.2024, Sweeping, Coll. J Lee, S Mok, SH Park.
Diagnosis. Mesonotum yellow, densely covered with short darker setae and with three indistinct brownish longitudinal stripes; M_1+M_2 half length of r-m. Abdomen dark brown and yellow; tergites 3−5 with broad dark brown bands, not reaching to anterior, posterior and lateral margins of tergites; all tergites covered with dark setae. Gonostylus narrow, shorter than gonocoxites, internal processus of a gonostylus simple, almost straight, bearing two setae, subapical one being longer and more distinct, and a dark apical tooth.
Distribution. Laos, South Korea.
Remarks. This species was first reported by Lee and Bae in 2023.

Genus *Mycomya* Rondani, 1856 가시버섯파리속

6. *Mycomya tridens* Lundstrom, 1911 (Fig. 3B)
낫발꼬리버섯파리(신칭)
Mycomya tridens Lundstrom, 1911: 394.

Material examined. 1♂ (No. NIBRIN0001073529, preserved in 80% ethanol), Nari Basin, Buk-myeon, Ulleung-gun, Gyeongsangbuk-do, South Korea, 37°30′59.2″N 130°52′09.4″E, 26.V.2024, Sweeping, Coll. C Lim, SH Park, W Kim. 1♂ (No. KUEM DIP 200020, preserved in 80% ethanol), Bongrae waterfall, Dodong-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, South Korea, 37°29′51.11″N 130°53′12.53″E, 24.V−10.IX.2024, Malaise trap, Coll. C Lim, SH Park, W Kim.
Diagnosis. Antenna with yellow pale scape and pedicel;

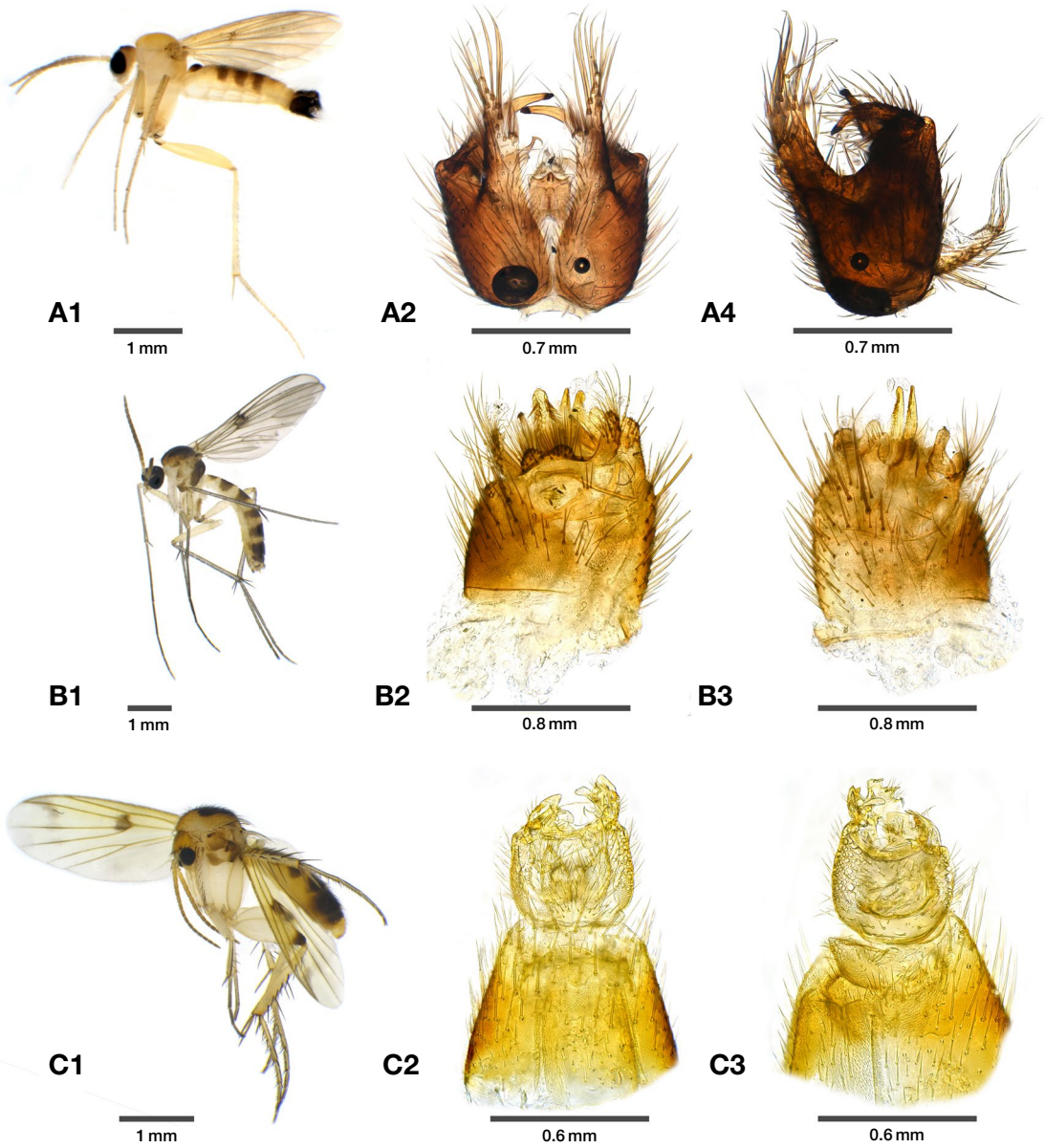


Fig. 3. Male habitus and terminalia. 1: habitus, 2: terminalia ventral view, 3: terminalia dorsal view. 4: terminalia lateral view. A: *Monoclona laosilvatica* Sevcik, 2001, B: *Mycomya tridens* Lundstrom, 1911, C: *Mycetophila assimilis* Matile, 1967.

flagellomeres brown. Sc ending on C, Sc_2 ending on R_1 distally to between Rs and R_4, Cu fork below M fork; legs uniformly yellow. Abdomen yellow with brown patterns.
Distribution. South Korea (new record), Austria, France, Georgia, Germany, Hungary, Poland.

Genus *Mycetophila* Meigen, 1803
물가버섯파리속(신칭)

7. *Mycetophila assimilis* Matile, 1967 (Fig. 3C)
여섯점버섯파리(신칭)
Mycetophila assimilis Matile, 1967: 212.

Material examined. 1♂ (No. NIBRIN0001073533, preserved in 80% ethanol), Naesujeoncheon Stream, Jeodong-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, South Korea, 37°30′41.6″N 130°54′00.9″E, 04.VII.2022, Sweeping, Coll. J Kim, W Lee. 2♂ (No. KUEM DIP 200021–200022, preserved in 80% ethanol), Bongrae waterfall, Dodong-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, South Korea, 37°29′51.11″N 130°53′12.53″E, 27.V.2024, Sweeping, Coll. C Lim, SH Park, W Kim. 1♂ (No. KUEM DIP 200023, preserved in 80% ethanol), Nari Basin, Bukmyeon, Ulleung-gun, Gyeongsangbuk-do, South Korea, 37°30′59.2″N 130°52′09.4″E, 28.V.2024, Sweeping, Coll.

C Lim, SH Park, W Kim. 1♂ (No. KUEM DIP 200024, preserved in 80% ethanol), Bongrae waterfall, Dodong-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, South Korea, 37°29′51.11″N 130°53′12.53″E, 25.VI.2024, Sweeping, Coll. C Lim, SH Park.

Diagnosis. Mesonotum with three dark longitudinal stripes, middle stripe more distinct than lateral stripes; R located before Rs with 11 setulae below. Tergites 1 yellow, tergites 2–4 yellow with dark brown bands.

Distribution. South Korea (new record), France, Germany, Russia.

Conflicts of Interest

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

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A report of 31 unrecorded bacterial species belonging to the phylum *Actinomycetota* in Korea

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During a comprehensive investigation of indigenous prokaryotic species in Korea, a total of 31 bacterial strains assigned to the phylum *Actinomycetota* were isolated from diverse environmental habitats, including soil, mud, wetland, tidal field, and seawater. Based on their high 16S rRNA gene sequence similarities (>98.7%) and the formation of robust phylogenetic clades with the closest type species, each strain was assigned to an independent and predefined bacterial species. Because there have been no publications or official reports regarding 31 isolates in Korea, these 31 species of 12 families within seven orders of the phylum *Actinomycetota* are reported as unrecorded species in Korea. In addition, 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, *Actinomycetota*, bacterial diversity, indigenous prokaryotic species in Korea, taxonomy, unrecorded species

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Introduction

The *Actinomycetota* is the largest bacterial phylum that was renamed from "*Actinobacteria*" in 2021 (Goodfellow, 2012; Oren and Garrity, 2021). Most species of the phylum *Actinomycetota* are found in diverse environments, and they are Gram-positive bacteria. At the time of writing, the taxonomic classification based on 16S rRNA gene sequences showed the phylum *Actinomycetota* includes six classes: *Acidimicrobiia*, *Actinomycetes*, *Coriobacteriia*, *Nitriliruptoria*, *Rubrobacteria*, *Thermoleophilia* (Oren and Garrity, 2021). In 2023, we collected the number of environmental samples from diverse habitats in Korea and isolated various novel and unrecorded bacterial species. In this study, we describe 31 unrecorded bacterial species belonging to the phylum *Actinomycetota* that were not previously reported in Korea.

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Table 1. Summary of strains isolated belonging to the phyla *Actinomycetota* and their taxonomic affiliations.

Phylum	Class	Order	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
Actinomycetota	*Actinomycetia*	*Microbacteriales*	*Microbacteriaceae*	*Microbacterium*	19H1F11	NIBRBAC000510546	*Microbacterium oryzae*	99.79	Soil	R2A	30°C, 2d
					19H7F20	NIBRBAC000510545	*Microbacterium petrolearium*	99.14	Soil	R2A	30°C, 2d
					19H7G11	NIBRBAC000510544	*Microbacterium sediminis*	99.52	Soil	R2A	30°C, 2d
					AN1007	NIBRBAC000510618	*Microbacterium kunmingense*	99.10	Wetland	R2A	28°C, 3d
					WC15	NIBRBAC000510792	*Microbacterium lindanitolerans*	100.00	Soil	R2A	28°C, 4d
				Agreia	B5	NIBRBAC000510738	*Agreia bicolorata*	99.02	Soil	R2A	25°C, 3d
				Leucobacter	17H1P12	NIBRBAC000510547	*Leucobacter iarius*	99.79	Soil	R2A	30°C, 2d
				Microcella	MMS23-DY36	NIBRBAC000510835	*Microcella alkalica*	100.00	Soil	R2A	30°C, 3d
			Nocardiaceae	*Rhodococcus*	17H2F15	NIBRBAC000510554	*Rhodococcus corynebacterioides*	99.65	Soil	R2A	30°C, 2d
					CAU 1749	NIBRBAC000510786	*Rhodococcus gannanensis*	98.81	Mud	GYE	30°C, 3d
				Prescottella	BU40	NIBRBAC000510755	*Prescottella soli*	99.86	Soil	R2A	25°C, 3d
			Mycobacteriaceae	*Mycobacterium*	AN126	NIBRBAC000510600	*Mycobacterium peregrinum*	100.00	Wetland	R2A	28°C, 3d
				Mycolicibacterium	KR3-105	NIBRBAC000510748	*Mycolicibacterium mucogenicum*	99.16	Soil	R2A	25°C, 3d
			Gordoniaceae	*Gordonia*	MBLB3299	NIBRBAC000510675	*Gordonia amicalis*	99.93	Soil	R2A	30°C, 3d
		Propionibacteriales	*Nocardioidaceae*	*Nocardioides*	KR1UV-15	NIBRBAC000510730	*Nocardioides baculatus*	99.30	Soil	R2A	25°C, 3d
					MBLB3548	NIBRBAC000510676	*Nocardioides luteus*	99.24	Soil	R2A	30°C, 3d
					WU27	NIBRBAC000510798	*Nocardioides soli*	98.96	Soil	R2A	28°C, 3d
					YJW22	NIBRBAC000510804	*Nocardioides cavernaquae*	99.72	Soil	R2A	28°C, 3d
				Aeromicrobium	KR3UV-104	NIBRBAC000510741	*Aeromicrobium lacus*	98.80	Soil	R2A	25°C, 3d
				Mumia	MBLB3055	NIBRBAC000510672	*Mumia xiangluensis*	98.83	Soil	MA	30°C, 3d
		Micrococcales	*Micrococcaceae*	*Arthrobacter*	ASW17	NIBRBAC000510800	*Arthrobacter ramosus*	99.63	Soil	R2A	28°C, 3d
					EW49	NIBRBAC000510788	*Arthrobacter woluwensis*	100.00	Soil	PTYG	28°C, 4d
					WP28	NIBRBAC000510796	*Arthrobacter mangrovi*	99.79	Soil	R2A	28°C, 3d
				Citricoccus	MBLB3007	NIBRBAC000510671	*Citricoccus zhacaiensis*	98.99	Soil	MA	30°C, 3d
				Paenarthrobacter	MMS23-GA13	NIBRBAC000510821	*Paenarthrobacter aurescens*	98.75	Soil	AIA	30°C, 3d
		Dermatophilales	*Dermacoccaceae*	*Flexivirga*	DG3-2	NIBRBAC000510733	*Flexivirga alba*	99.31	Soil	R2A	25°C, 3d
			Intrasporangiaceae	*Knoellia*	AN362	NIBRBAC000510605	*Knoellia subterranea*	98.76	Wetland	R2A	28°C, 3d
			Ornithinimicrobiaceae	*Ornithinimicrobium*	WE9	NIBRBAC000510846	*Ornithinimicrobium algicola*	99.22	Mud	MA	28°C, 3d
		Cellulomonadales	*Promicromonosporaceae*	*Isoptericola*	SBCC-2277	NIBRBAC000510510	*Isoptericola cucumis*	99.58	Tidal field	MA	25°C, 3−5d
		Brevibacteriales	*Brevibacteriaceae*	*Brevibacterium*	CAU 1727	NIBRBAC000510784	*Brevibacterium sanguinis*	99.59	Seawater	MA	30°C, 3d
	Thermoleophilia	*Solirubrobacterales*	*Solirubrobacteraceae*	*Solirubrobacter*	AN992	NIBRBAC000510617	*Solirubrobacter phytolaccae*	98.81	Wetland	R2A	28°C, 3d

Abbreviations of the agar medium: R2A, Reasoner's 2A; MA, Marine; AIA, Actinomycetota Isolation; PTYG, Peptone Tryptone Yeast extract Glucose; GYE, Glucose Yeast Extract.

Materials and Methods

A total of 31 bacterial strains assigned to the phylum *Actinomycetota* were isolated from various environmental habitats, including soil, mud, wetland, tidal field, and seawater (Table 1). All environmental samples were processed independently, serially diluted, and spread onto diverse culture agar media, including Reasoner's 2A agar (R2A, BD, USA), marine agar 2216 (MA; BD), *Actinomycetota* isolation agar (AIA; BD), peptone tryptone yeast extract glucose agar (PTYG; 0.25 g peptone, 0.25 g tryptone, 0.5 g yeast extract, 0.5 g glucose, and 0.03 g $MgSO_4$ in 1 liter) and glucose yeast extract agar (GYE; HIMEDIA, India) and incubated at 25−30°C for 2−5 days (Table 1). The designated strain identifications (IDs), most closely related species, isolation sources, culture media, and incubation conditions of the isolates are described in Table 1. All strains were isolated as pure cultures and stored as 10−20% glycerol suspension at −80°C and as lyophilized ampoules.

The colony morphology of the strains was observed by eye or a magnifying glass after the cells were cultivated to their exponential phases on their culture agar media. Cellular morphologies and cell sizes were examined by using either transmission electron or scanning electron microscopy. Gram staining tests were performed using a Gram-staining kit according to the standard procedures. Biochemical characteristics were evaluated using API 20NE kit (bioMérieux, France), according to the manufacturer's instructions.

Bacterial DNA extraction, PCR amplification, and 16S rRNA gene sequencing were performed using standard procedures, as described previously (Baek *et al*., 2023). The 16S rRNA gene sequences of the strains assigned to the phylum *Actinomycetota* were compared with those of the type strains of validated bacterial species by using the nucleotide similarity search program in the EzBioCloud server (http://www.ezbiocloud.net/identify) (Yoon *et al*., 2017). For phylogenetic analyses, multiple alignments of the 16S rRNA gene sequences between the isolates and established bacterial species were carried out using the MUSCLE program (Edgar, 2004). Evolutionary distances were calculated using the Kimura two-parameter model and the phylogenetic trees were constructed using the MEGA11 program (Tamura *et al*., 2021), based on the neighbor-joining algorithm with 1,000 replications.

Results and Discussion

Thirty strains of *Actinomycetota* were distributed into two classes: 30 strains in the *Actinomycetia* and one strain in the *Thermoleophilia* (Table 1). One strain of the class *Thermoleophilia* was assigned to the genus *Solirubrobacter* of the family *Solirubrobacteraceae* in the order *Solirubrobacter*ales (Table 1). Thirty strains in the class of *Actinomycetia* belonged to six different orders: *Microbacteriales* (14 strains), *Propionibacteriales* (6 strains), *Micrococcales* (5 strains), *Dermatophilales* (3 strains), *Cellulomonadales* (1 strain), and *Brevibacteriales* (1 strain). Fourteen strains in the order *Microbacteriales* belonged to four different families: *Microbacteriaceae* (8 strains), *Nocardiaceae* (3 strains), *Mycobacteriaceae* (2 strains), and *Gordoniaceae* (1 strain). Eight strains in the family *Microbacteriaceae* belonged to four different genera: *Microbacterium* (5 strains), *Agreia* (1 strain), *Leucobacter* (1 strain), and *Microcella* (1 strain). Three strains in the family *Nocardiaceae* belonged to two different genera: *Rhodococcus* (2 strains) and *Prescottella* (1 strain). Two strains in the family *Mycobacteriaceae* belonged to two different genera: *Mycobacterium* (1 strain) and *Mycolicibacterium* (1 strain). One strain in the family *Gordoniaceae* was assigned to the genus *Gordonia*. Six strains in the order *Propionibacteriales* belonged to the family *Nocardioidaceae* (6 strains), and these 6 strains in the family *Nocardioidaceae* belonged to three different genera: *Nocardioides* (4 strains), *Aeromicrobium* (1 strain), and *Mumia* (1 strain). Five strains in the order *Micrococcales* belonged to the family *Micrococcaceae* (5 strains), and these five strains in the family *Micrococcaceae* belonged to three different genera: *Arthrobacter* (3 strains), *Citricoccus* (1 strain), and *Paenarthrobacter* (1 strain). Two strains of the order *Dermatophilales* belonged to three different families: *Dermacoccaceae* (1 strain), *Intrasporangiaceae* (1 strain), and *Ornithinimicrobiaceae* (1 strain). One strain in the family *Dermacoccaceae* was assigned to the genus *Flexivirga*, one strain in the family *Intrasporangiaceae* was assigned to the genus *Knoellia*, and the other strain in the family *Ornithinimicrobiaceae* was assigned to the genus *Ornithinimicrobium*. One strain in the order *Cellulomonadales* was assigned to the genus *Isoptericola* of the family *Promicromonosporaceae*. One strain in the order *Brevibacteriales* was assigned to the genus *Brevibacterium* of the family *Brevibacteriaceae* (Fig. 2). In conclusion, we report 31 unrecorded bacterial species belonging to the phylum *Actinomycetota* which were isolated in Korea.

Description of *Microbacterium oryzae* 19H1F11

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are round, smooth, and yellow-colored after two days of incubation at 30°C on R2A agar. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, and trisodium citrate. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urase activities. Does not utilize *N*-acetyl-glucosamine, capric acid, adipic acid, malic acid, and phenylacetic acid. Strain 19H1F11

(=NIBRBAC000510546) was isolated from soil in Hanam, Gyeonggi-do, Korea.

Description of *Microbacterium petrolearium* 19H7F20

Cells are Gram-stain-positive, motile, and rod-shaped with flagellum. Colonies are circular and white-colored after two days of incubation at 30°C on R2A agar. Positive for esculin hydrolysis and β-galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, and potassium gluconate. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urase, and gelatinase activities. Does not utilize *N*-acetyl-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 19H7F20 (=NIBRBAC000510545) was isolated from soil in Goyang, Gyeonggi-do, Korea.

Description of *Microbacterium sediminis* 19H7G11

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, convex, and white-colored after two days of incubation at 30°C on R2A agar. Positive for esculin hydrolysis and β-galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, and malic acid. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urase, and gelatinase activities. Does not utilize *N*-acetyl-glucosamine, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain 19H1F11 (=NIBRBAC000510544) was isolated from soil in Goyang, Gyeonggi-do, Korea.

Description of *Microbacterium kunmingense* AN1007

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are round, entire, raised, and pale yellow-colored after three days of incubation at 28°C on R2A agar. Positive for esculin hydrolysis and utilize D-mannose. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain AN1007 (=NIBRBAC000510618) was isolated from wetland in Anseong, Gyeonggi-do, Korea.

Description of *Microbacterium lindanitolerans* WC15

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, entire, convex, entire, smooth, and yellow-colored after four days of incubation at 28°C on R2A agar. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase activities. Negative for indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain WC15 (=NIBRBAC000510792) was isolated from soil in Chuncheon, Gangwon-do, Korea.

Description of *Flexivirga alba* DG3-2

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, slightly convex, glistening, and cream yellow-colored after three days of incubation at 25°C on R2A agar. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain DG3-2 (=NIBRBAC000510733) was isolated from soil in Daegu, Korea.

Description of *Agreia bicolorata* B5

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, slightly convex, glistening, and cream yellow-colored after three days of incubation at 25°C on R2A agar. Positive for esculin hydrolysis and β-galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannitol, and D-maltose. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-mannose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain B5 (=NIBRBAC000510738) was isolated from soil in Seoul, Korea.

Description of *Leucobacter iarius* 17H1P12

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are smooth, convex, opaque, and white-colored after two days of incubation at 30°C on R2A agar. Positive for esculin hydrolysis and β-galactosidase activity. Utilizes *N*-acetyl-glucosamine, D-maltose, and trisodium citrate. Negative for nitrate reduction, indole production, glucose fermentation arginine dihydrolase, urease, and gelatinase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, and phenylacetic acid. Strain 17H1P12 (=NIBRBAC000510547) was isolated from soil in Hanam, Gyeonggi-do, Korea.

Description of *Microcella alkalica* MMS23-DY36

Cells are Gram-stain-negative, rod-shaped without flagellum. Colonies are circular, smooth, glistening, and yellow-

colored after three days of incubation at 30°C on R2A agar. Positive for nitrate reduction, glucose fermentation, and esculin hydrolysis. Negative for indole production, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain MMS23-DY36 (=NIBRBAC 000510835) was isolated from soil in Incheon, Korea.

Description of *Rhodococcus corynebacterioides* 17H2F15

Cells are Gram-stain-negative, rod-shaped without flagellum. Colonies are circular, convex, translucent, and light cream-colored after two days of incubation at 30°C on R2A agar. Positive for esculin hydrolysis and β-galactosidase activitiy. Utilizes D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, malic acid, and trisodium citrate. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-mannose, D-maltose, capric acid, and phenylacetic acid. Strain 17H2F15 (=NIBRBAC000510554) was isolated from soil in Guri, Gyeonggi-do, Korea.

Description of *Rhodococcus gannanensis* CAU 1749

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, rought, entire, pulvinate, opaques, and cream-colored after three days of incubation at 30°C on GYE. Positive for nitrate reduction. Negative for indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CAU 1749 (=NIBRBAC000510786) was isolated from mud in Jeongeup, Jeollabuk-do, Korea.

Description of *Prescottella soli* BU40

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, slightly convex, mucoid, and pale orange-colored after three days of incubation at 25°C on R2A agar. Positive esculin hydrolysis and utilizes D-glucose. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize 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 BU40 (=NIBRBAC000510755) was isolated from soil in Seoul, Korea.

Description of *Mycobacterium peregrinum* AN126

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are round, entire, flat, and white-colored after three days of incubation at 28°C on R2A agar. Positive for nitrate reduction and utilizes D-glucose, D-mannose, D-mannitol, potassium gluconate, and malic acid. Negative for indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize L-arabinose, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain AN126 (=NIBR BAC000510600) was isolated from wetland in Anseong, Gyeonggi-do, Korea.

Description of *Mycolicibacterium mucogenicum* KR3-105

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, slightly convex, and pale orange-colored after three days of incubation at 25°C on R2A agar. Positive for nitrate reduction, esculin hydrolysis, urease, gelatinase, and β-galactosidase activities. Negative for indole production, glucose fermentation, and arginine dihydrolase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain KR3-105 (=NIBRBAC000510748) was isolated from soil in Gangneung, Gangwon-do, Korea.

Description of *Gordonia amicalis* MBLB3299

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are irregular, convex, translucent, and orange-colored after three days of incubation at 30°C on R2A agar. Positive for nitrate reduction, esculin hydrolysis, urease, and β-galactosidase activities. Utilizes D-glucose, L-arabinose, D-mannitol, and D-maltose. Negative for indole production, glucose fermentation, arginine dihydrolase, and gelatinase activity. Does not utilize D-mannose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain MBLB3299 (=NIBRBAC000510675) was isolated from soil in Incheon, Korea.

Description of *Nocardioides baculatus* KR1UV-15

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, slightly convex, glistening, and cream-colored after three days of incubation at 25°C on R2A agar. Positive for nitrate reduction, esculin hydrolysis, gelatinase, and β-galactosidase activities. Negative for indole production, glucose fermentation, arginine dihydrolase, and urease activity. Does not utilize D-glucose,

L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain KR1UV-15 (=NIBRBAC000510730) was isolated from soil in Gangneung, Gangwon-do, Korea.

Description of *Nocardioides luteus* MBLB3548

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, flat, opaque, and cream-colored after three days of incubation at 30°C on R2A agar. Positive for esculin hydrolysis, urease, and β-galactosidase activities. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain MBLB 3548 (=NIBRBAC000510676) was isolated from soil in Incheon, Korea.

Description of *Nocardioides soli* WU27

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, entire, convex, smooth, and cream-colored after three days of incubation at 28°C on R2A agar. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes D-mannitol and D-maltose. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activities. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain WU27 (=NIBRBAC000510798) was isolated from soil in Sunchang, Jeollabuk-do, Korea.

Description of *Nocardioides cavernaquae* YJW22

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, entire, convex, smooth, and white-colored after three days of incubation at 28°C on R2A agar. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain YJW22 (=NIBRBAC000510804) was isolated from soil in Yeongwol, Gangwon-do, Korea.

Description of *Aeromicrobium lacus* KR3UV-104

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, convex, glistening, and cream-colored after three days of incubation at 25°C on R2A agar. Positive for indole production, esculin hydrolysis, and gelatinase activity. Utilizes D-glucose, D-mannitol, and malic acid. Negative for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, and β-galactosidase activities. Does not utilize L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain KR3UV-104 (=NIBRBAC000510741) was isolated from soil in Gangneung, Gangwon-do, Korea.

Description of *Mumia xiangluensis* MBLB3055

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, entire margine, raised, and cream-colored after three days of incubation at 30°C on MA. Positive for esculin hydrolysis and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain MBLB 3055 (=NIBRBAC000510672) was isolated from soil in Ulleung, Gyeongsangbuk-do, Korea.

Description of *Arthrobacter ramosus* ASW17

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, entire, convex, smooth, and white-colored after three days of incubation at 28°C on R2A agar. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activities. Does not utilize capric acid and adipic acid. Strain ASW17 (=NIBRBAC000510800) was isolated from soil in Suncheon, Jeollanam-do, Korea.

Description of *Arthrobacter woluwensis* EW49

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are irregular, undulate, umbonate, smooth, and white-colored after four days of incubation at 28°C on PTYG. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activities. Does not utilize L-arabinose, capric acid, and adipic acid. Strain EW49 (=NIBRBAC000510788) was isolated from soil in Chuncheon, Gangwon-do, Korea.

Description of *Arthrobacter mangrovi* WP28

Cells are Gram-stain-positive, coccus-shaped without flagellum. Colonies are circular, entire, convex, smooth, and white-colored after three days of incubation at 28°C on R2A agar. Positive for nitrate reduction and esculin hydrolysis. Utilizes D-glucose, D-mannitol, malic acid, and phenylacetic acid. Negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, and trisodium citrate. Strain WP28 (=NIBRBAC000510796) was isolated from soil in Pyeongtaek, Gyeonggi-do, Korea.

Description of *Citricoccus zhacaiensis* MBLB3007

Cells are Gram-stain-positive, coccus-shaped without flagellum. Colonies are irregular, glistening, translucent, and yellow-colored after three days of incubation at 30°C on MA. Positive for nitrate reduction and esculin hydrolysis. Utilizes malic acid, trisodium citrate, and phenylacetic acid. Negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, and adipic acid. Strain MBLB3007 (=NIBRBAC000510671) was isolated from soil in Ulleung, Gyeongsangbuk-do, Korea.

Description of *Paenarthrobacter aurescens* MMS23-GA13

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, convex, entire, and pale yellow-colored after three days of incubation at 30°C on AIA. Positive for indole production, glucose fermentation, esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid. Negative for nitrate reduction, arginine dihydrolase, and urease activities. Does not utilize capric acid and adipic acid. Strain MMS23-GA13 (=NIBRBAC000510821) was isolated from soil in Muju, Jeollabuk-do, Korea.

Description of *Knoellia subterranea* AN362

Cells are Gram-stain-positive, oval-shaped without flagellum. Colonies are round, entire, raised, and yellow-colored after three days of incubation at 28°C on R2A agar. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase activities. Utilizes D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, and potassium gluconate. Negative for indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize L-arabinose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain AN362 (=NIBRBAC000510605) was isolated from wetland in Anseong, Gyeonggi-do, Korea.

Description of *Ornithinimicrobium algicola* WE9

Cells are Gram-stain-negative, rod-shaped without flagellum. Colonies are circular, entire, convex, smooth, and yellow-colored after three days of incubation at 28°C on MA. Positive for nitrate reduction, esculin hydrolysis, and gelatinase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease, and β-galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain WE9 (=NIBRBAC000510846) was isolated from mud in Incheon, Korea.

Description of *Isoptericola cucumis* SBCC-2277

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, slightly convex, smooth, glistening, and cream-colored after 3−5 days of incubation at 25°C on MA. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, malic acid, and trisodium citrate. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activities. Does not utilize D-maltose, capric acid, adipic acid, and phenylacetic acid. Strain SBCC-2277 (=NIBRBAC000510510) was isolated from tidal field in Taean, Chungcheongnam-do, Korea.

Description of *Brevibacterium sanguinis* CAU 1727

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, smooth, entire, convex, opaques, and cream-colored after three days of incubation at 30°C on MA. Positive for esculin hydrolysis, arginine dihydrolase, and gelatinase activity. Utilizes D-glucose, *N*-acetylglucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid. Negative for nitrate reduction, indole production, glucose fermentation, urease, and β-galactosidase activities. Does not utilize L-arabinose, D-mannose, D-mannitol, capric acid, and adipic acid. Strain CAU 1727 (=NIBRBAC000510784) was isolated from seawater in Busan, Korea.

Description of *Solirubrobacter phytolaccae* AN992

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are round, entire, raised, and white-colored after three days of incubation at 28°C on R2A agar. Posi-

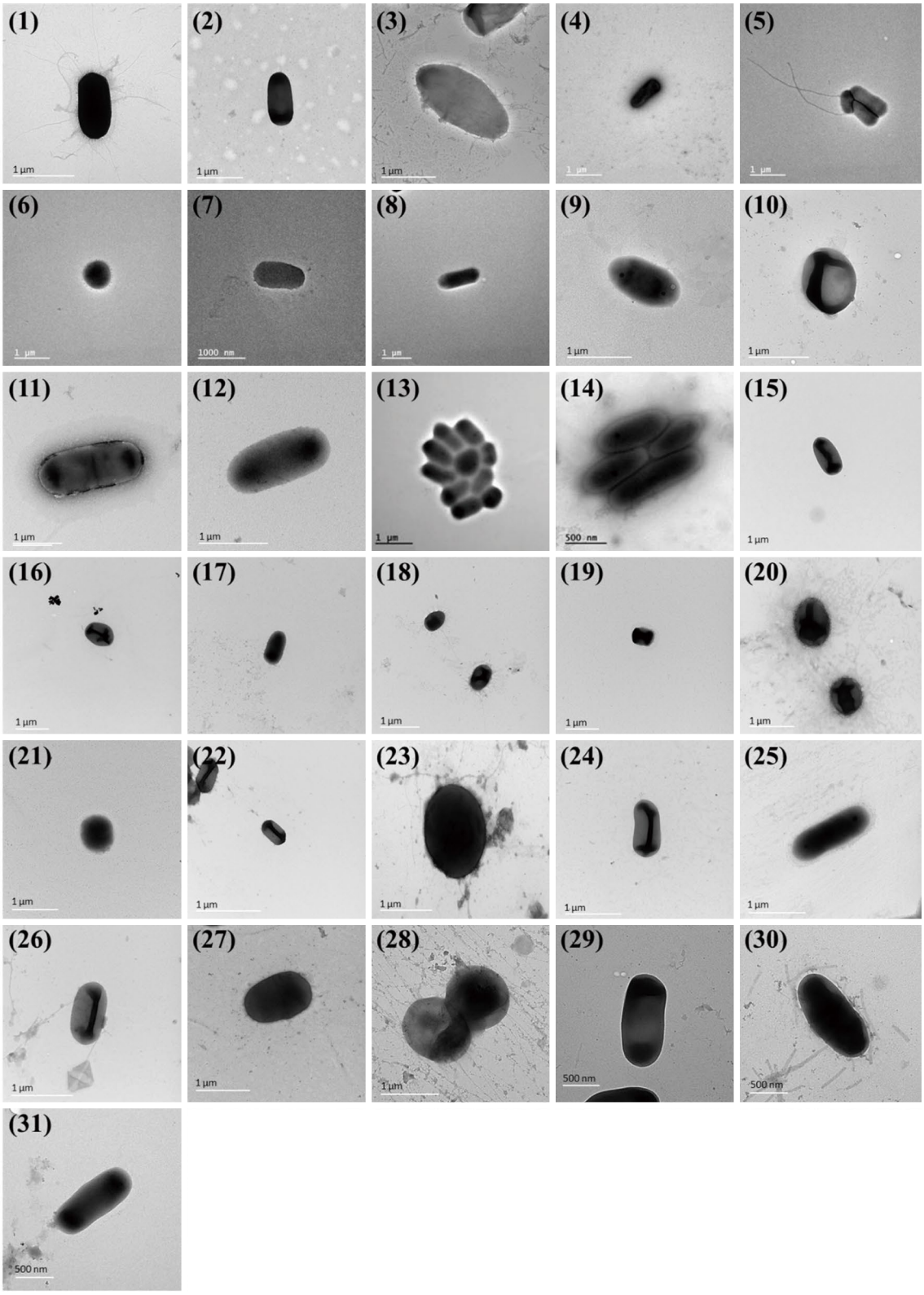


Fig. 1. Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. The cells were cultured at their optimal growth conditions. Strain: 1, SBCC-2277; 2, CAU 1727; 3, CAU 1749; 4, 19H7G11; 5, 19H7F20; 6, 19H1F11; 7, 17H1P12; 8, 17H2F15; 9, AN126; 10, AN362; 11, AN992; 12, AN1007; 13, MMS23-GA13; 14, MMS23-DY36; 15, KR1UV-15; 16, DG3-2; 17, B5; 18, KR3UV-104; 19, KR3-105; 20, BU40; 21, EW49; 22, WC15; 23, WP28; 24, WU27; 25, ASW17; 26, YJW22; 27, WE9; 28, MBLB3007; 29, MBLB3055; 30, MBLB3299; 31, MBLB3548.

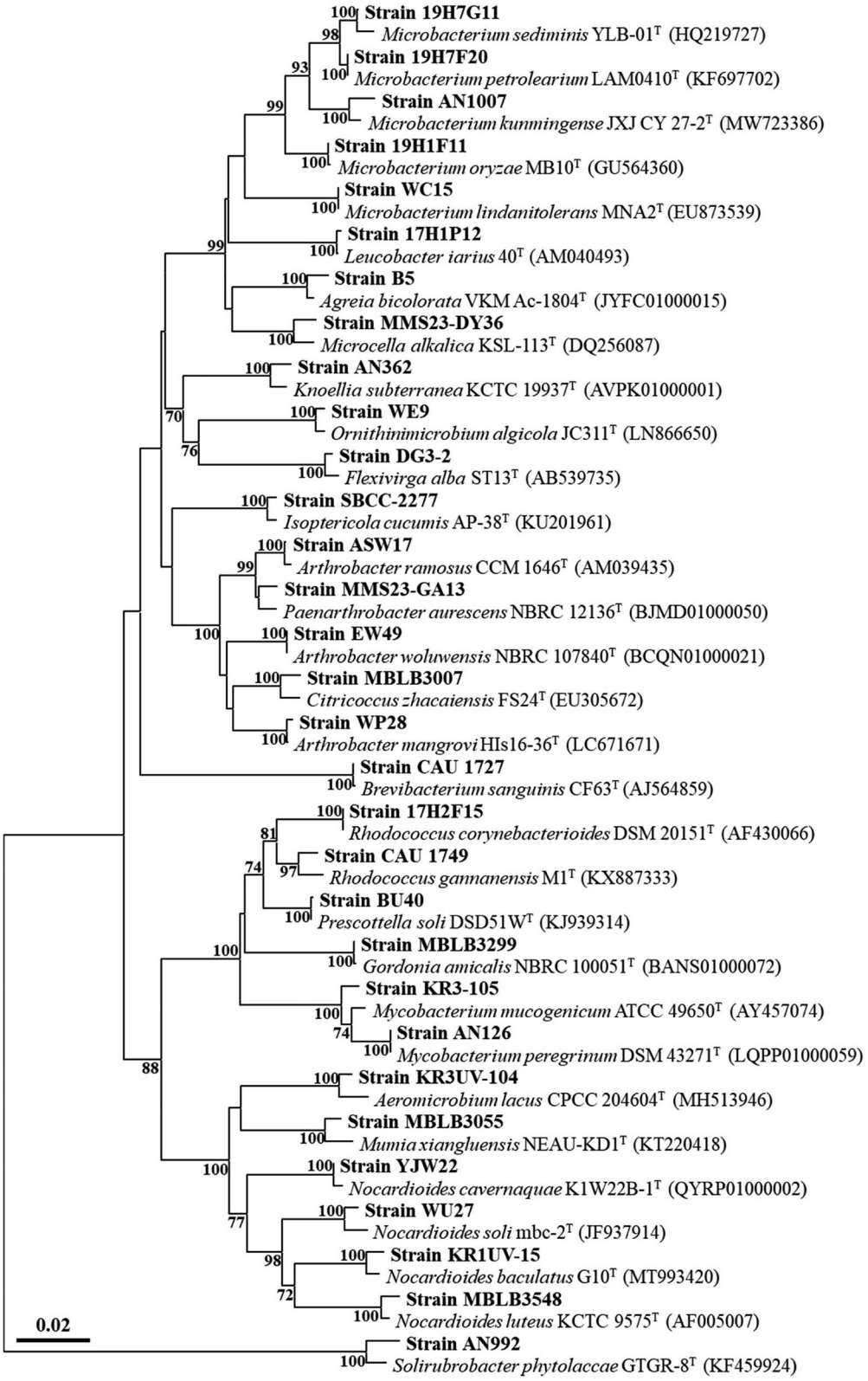


Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic relationship between the strains isolated in this study and their relatives in the phyla *Actinomycetota*. Bootstrap values (>70%) are shown above nodes. Scale bar: 0.02 changes per nucleotide.

tive for esculin hydrolysis. Utilizes D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, and malic acid. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize D-mannose, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain AN992 (=NIBR BAC000510617) was isolated from wetland in Anseong, Gyeonggi-do, Korea.

Conflicts of Interest

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

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Distributional spread and DNA barcoding of invasive species, *Thalamoporella californica* (Levinsen, 1909) (Bryozoa, Cheilostomatida, Thalamoporellidae) in Korean waters

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Thalamoporella californica (Levinsen, 1909) is an invasive bryozoan species first found on ropes at the Tongyeong Yacht Marina in 2020. It was subsequently found in Geojedo Island (Beopdong-ri and Gujora Harbor), Tongyeong Yacht Marina, Hansando Island in the South Sea, and Yangpo Yacht Marina in the East Sea. This suggests that the distribution of *T. californica* has gradually expanded from its initial detection in Korean waters. We also analyzed the DNA barcode region of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) of *T. californica*. The intra-specific *COI* sequence variation of *T. californica* collected from three different localities in this study ranged from 0.002 to 0.014. *T. californica* and *T. lioticha* were separated by a genetic distance of 0.328−0.338. The present study provides *COI* sequences that can be utilized in molecular research on *T. californica* and the genus *Thalamoporella* and basic information to eDNA-based methods for quick identification of marine invasive species.

Keywords: Bryozoa, *COI*, distribution, DNA barcode, fouling, Korean waters, *Thalamoporella californica*

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Introduction

The invasion of exotic marine species is an escalating global concern as it poses a significant threat to biodiversity and the economy (Bax *et al*., 2001). This issue is further compounded by the unpredictability of the damage caused by these non-native marine species (Bax *et al*., 2001). Marine exotic species that are highly tolerant to environmental changes and capable of rapid growth by attaching to artificial structures, particularly in ports and harbors, have a high potential to become invasive (Gordon and Mawatari, 1992; Almeida *et al*., 2015). For example, a bryozoan, *Membraniporopsis tubigera* (Osburn, 1940), was reported to block fishing nets in New Zealand in 2001 (Gordon *et al*., 2006). Subsequently, mass bloom events were observed at Cassino Beach, Brazil, and La Coronilla Beach, Uruguay, in 2003, where the species covered up to 2 km of the beach (Gappa *et al*., 2010). In Korean waters, *Bantariella bocki* (Silén, 1942), first invaded the black corals on Munseom in Jejudo Island in 2005 (Moon and Song, 2007; Seo *et al*., 2009) since this species was described from Bonin Islands in Japan (Silén, 1942). In response to this invasion, Moon and Song (2007) proposed the removal method of *B. bocki* to conserve black corals in Jeju waters. Five species of *Bugula neritina* (Linnaeus, 1758), *Bugulina californica* (Robertson, 1905), *Celleporaria brunnea* (Hincks, 1884), *Schizoporella unicornis* (Johnston, 1847) and *Tricellaria occidentalis* (Trask, 1857) have been known as newly introduced bryozoans of Korea (Shin *et al*., 2013). Three of *Jellyella tuberculata* (Bosc, 1802), *T. occidentalis* and *Watersipora subtorquata* (d'Orbigny, 1852) were legally designated as harmful bryozoans in Korea in 2019 (Park *et al*., 2020). The technique to rapidly identify these marine invasive species and monitor

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their introduction to Korean waters and distributional dispersion was suggested through molecular methods. *Bugulina californica* and *Bugula neritina* were detected by environmental DNA assay with simple sampling seawater (Kim *et al*., 2018; 2021). Following the early detection of marine invasive species through DNA monitoring, we expect to see the development of technologies to eliminate disturbing and harmful species from the marine ecosystem shortly. For this reason, DNA barcoding is essential for quick identification of marine invasive species.

The cheilostome genus *Thalamoporella* Hincks, 1887 contains 50 species, with the largest diversity among the five genera in the family Thalamoporellidae Levinsen, 1902 worldwide (Bock, 2025). Three *Thalamoporella* species have been reported to inhabit Korea: *T. californica*, *T. lioticha*, and *T. sibogae* (Chae *et al*., 2022). Among them, *T. californica* was first found on rope hanging at the Tongyeong Yacht Marina in 2020 (Chae *et al*., 2022). Afterward, the authors monitored this species to investigate its distributional spread to different localities and whether it attached to algae instead of fouling the artificial substrata. In 2021, this species was found only in the same locality where it was first reported and exclusively on artificial substrata, such as ropes (Chae *et al*., 2022).

The accurate morphological identification of species is central to biodiversity research, but several technical challenges exist (Padial *et al*., 2010; Twyforwd *et al*., 2024). Significant efforts are being made to obtain genetic data from various species for identification, and DNA barcoding is a method used to distinguish species by sequencing standardized DNA regions (Hebert *et al*., 2003; Hebert and Gregory, 2005). Currently, integrative taxonomy has been proposed to catalogue the biodiversity of the planet with an integrative and evolutionary framework by morphologists and molecular biologists (Padial *et al*., 2010). In Britain and Ireland, DNA barcoding for the Darwin Tree of Life Project (DToL), aiming to sequence and assemble high-quality reference genomes for all eukaryotic species, was developed and implemented (Twyforwd *et al*., 2024). Unfortunately, there is a lack of DNA barcoding studies on bryozoans (Taylor and Waeschenbach, 2015). In this study, we present the cytochrome *c* oxidase subunit I (*COI*) sequence as a barcode marker for the molecular identification of *T. californica* for the first time.

Materials and Methods

The colonies of *Thalamoporella californica* were collected from five localities in Korea (Table 1). The colonies from Hansando Island were attached to algae [*Codium fragile* (Suringar) Hariot, 1889] and 20–30 mm sized stone, while the others were found hanging on ropes in harbors and yacht marinas and fouling on nets at the fish farm. All were collected using hand and forceps. After separating them to prevent contamination with other organisms, the samples were preserved in 99.99% ethyl alcohol. For species identification, the external features of the zooids were observed under a stereomicroscope (Stemi SV6, Carl Zeiss, Germany). Parts of the specimens were bleached with hot aqueous sodium hypochlorite (10–25%), washed, and gold coated (MCM-100, SEC, Korea) before being examined with a scanning electron microscope (SNE-3200M Mini-SEM, SEC, Korea) set to an accelerating voltage of 15 kV. Measurements were made using Image J on SEM images of the zooids. Voucher specimens were deposited at Woosuk University (MBRBK-9828, MBRBK-9827, MBRBK-9826, MBRBK-9825, MBRBK-9824, MBRBK-9823).

Table 1. Information on sampling localities of *T. californica* in Korea in this study.

Locality	Coordinates	Date	Substratum
Tongyeong Yacht Marina	34°49′42″N, 128°26′05″E	14 Oct 2020 20 Oct 2021 4 Jul 2022 13 Oct 2022 26 Jul 2023 25 Oct 2023 3 Jul 2024 5, 16 Oct 2024	Rope
Hansando Island	34°47′04″N, 128°29′32″E	3 Sep 2024	*Codium fragile* (Suringar), Stone
Beopdong-ri at Geojedo Island	34°48′46″N, 128°31′20″E	22 Aug 2022	Fishing net
Gujora Harbor	34°49′56″N, 128°42′10″E	26 Jul 2023 25 Oct 2023 3 Jul 2024 5, 16 Oct 2024	Rope
Yangpo Yacht Marina	35°52′40″N, 129°31′10″E	27 Jul 2023 26 Oct 2023 4 Jul 2024 17 Oct 2024	Rope

The genomic DNA of *T. californica* was extracted following the manufacturer's protocol using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The *COI* sequences of *T. californica* were amplified using LCO 1490 and HCO 2198 primers (Folmer *et al*., 1994), and the sequence length of all specimens was 658 bp throughout this study. A total of six sequences were deposited in GenBank under the accession numbers PQ454604 and PQ454605 (Tongyeong Yacht Marina), PQ454606 and PQ454607 (Hansando Island) and PQ454608 and PQ454609 (Yangpo Yacht Marina). Geneious Prime v2021.2.2 (Biomatters, Auckland, New Zealand) was used to compare the sequen-

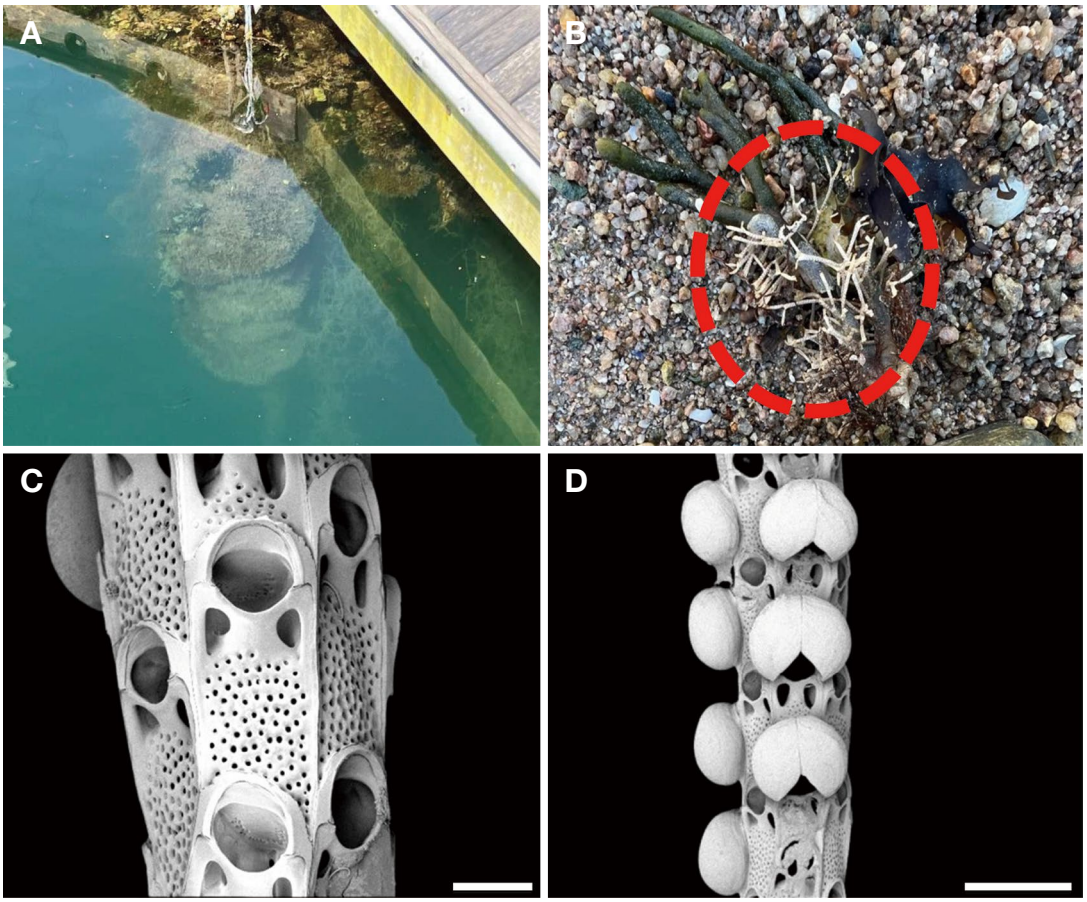


Fig. 1. *Thalamoporella californica*. A. Colony fouled the ropes at Tongyeong Yacht Marina; B. Colony attached to algae at Hansando Island; C. Detailed autozooid showing orifice, a pair of opesiules and porous frontal wall; D. Ovicell typical in thalamoporellids. Scale bars: C, D = 300 μm.

ces with *T. lioticha* (Ortmann, 1890) and *Steginoporella magnifica* Harmer, 1900, belonging to the same superfamily. *T. lioticha* is the only *Thalamoporella* species with *COI* sequences registered in NCBI (473 bp), and *S. magnifica* is known to be the closest to the genus *Thalamoporella*. The sequence of *Heteropora alaskensis* Borg, 1933, which belongs to a different class, was used as the outgroup. The sequences for *T. lioticha* from Korea, *S. magnifica* from New Zealand (612 bp), and *H. alaskensis* from San Juan Island (U.S.A.) (658 bp) were obtained from NCBI with the accession numbers HQ896191.1, JF950435.1, and MH242798.1, respectively, and they were referenced for comparison with *T. californica*. The intra- and intergenetic distance of *T. californica* were calculated using the Kimura-2-parameter (K2P) model with the software MEGA X (Kumar *et al*., 2018).

Results and Discussion

Class Gymnolaemata Allman, 1856
Order Cheilostomatida Busk, 1852
Suborder Thalamoporellina Ostrovsky, 2013
Superfamily Thalamoporelloidea Levinsen, 1902
Family Thalamoporellidae Levinsen, 1902
Genus *Thalamoporella* Hincks, 1887

***Thalamoporella californica* (Levinsen, 1909)**
New Korean name: 캘리포니아안방이끼벌레
(cal-li-po-ni-a-an-bang-i-kki-beol-le)

Materials examined. See Table 1 for Information on sampling localities, dates and substrata in which *T. californica* was collected in Korea. All materials were collected from waters shallower than 5 m deep.

Diagnosis. Colony light beige (Fig. 1A, B), erect, dichotomously branching. Zooids elongated, rectangular or oval, 0.38–0.56 mm long (0.45 ± 0.07 mm), 0.19–0.30 mm wide (0.24 ± 0.05 mm). Orifice subcircular, 0.12–0.18 mm long (0.14 ± 0.01 mm), 0.12–0.16 mm wide (0.14 ± 0.01 mm). Pair of opesiules with different sizes. Cryptocyst flat with densely distributed small perforations (Fig. 1C). Ovicells very large, ectoecium, symmetrical along central groove, 0.39–0.45 mm wide (0.43 ± 0.04 mm) (Fig. 1D).

Distribution. Korea (South Sea, East Sea), East Pacific (Monterey Bay to southern California, Colombia, Galapagos Islands).

Remarks on the distribution expansion of *T. californica* in Korea. *Thalamoporella californica* is regarded as an invasive species in Korea, with its primary distribution in the eastern Pacific (Chae *et al*., 2022). Since this species was first found in Korea on artificial substrata, such as ropes hanging at the Tongyeong Yacht Marina in 2020, the volume of colonies was found to be doubled only in the same locality the following year (Chae *et al*., 2022). *T. californica* has been consistently monitored in the eight harbors and ports of Incheon, Dangjin, Bieung, Nokdong, Gujora, Ulsan, Donghae and Sokcho, and five yacht marinas of Mokpo, Soho, Tongyeong, Busan and Yangpo from 2020 to 2024 (Table 2). The monitoring of *T. californica* resulted in Table 3. As shown in Table 3, *T. californica* has occurred continuously from Tongyeong Yacht Marina since 2020, and its presence was confirmed in Gujora Harbor and Yangpo Yacht Marina in 2023 and 2024. In addition to the monitored localities, *T. californica* was found fouling the fishing net of the fish farm in Beopdong-ri at Geojedo Island, in 2022, causing damage to the fish farming industry (Table 1). It is noteworthy that this species attached to the algae [*Codiun fragile* (Suringar) Hariot, 1889)] and stone in Hansando Is. was additionally found in 2024 (Table 1). This situation confirmed the occurrence of *T. californica* in additional localities beyond its first found site, suggesting that its distribution within Korean waters may be expanding. Regarding the substratum, *T. californica*, is originally known to be encrusted to *Macrocystis* holdfasts from the intertidal to 15 m deep in the eastern Pacific (Soule *et al*., 1999). However, this species was reported on only ropes in Korean waters (Chae *et al*., 2022) and additionally found on natural substrata, such as algae and stone, in 2024 in Korean waters, too. It is necessary to consistently monitor to which substratum this species is attached to consistently.

Remarks on the DNA barcoding of *T. californica* and other species. We obtained six new partial *COI* sequences from *T. californica* collected from three localities (Hansando Is., Tongyeong and Yangpo Yacht Marinas) in Korean waters and calculated genetic distances within and between species for evaluating DNA barcoding resolution of *COI*. Because *Thalamoporella lioticha* was GenBank's only available congeneric species, *Steginoporella magnifica*, belonging to the same superfamily, was included in this study to enhance the accuracy of species identification using its *COI*. Additionally, we included the outgroup *Heteropora alaskensis* according to the preceding analysis (Knight *et al*., 2011; Lee *et al*., 2011; Leray and Paulay, 2018). The *COI* gene of each species for the genetic comparison turned out to be 658 bp in length and then was

Table 2. Monitored localities of Korean harbors, ports and yacht marinas.

No.	Locality	Coordinates
1	Incheon Port	37°25′41″N, 126°37′01″E
2	Dangjin Port	36°59′48″N, 126°47′40″E
3	Bieung Harbor	35°56′14″N, 126°31′45″E
4	Mokpo Yacht Marina	34°47′01″N, 126°23′18″E
5	Nokdong Harbor	34°42′20″N, 127°11′50″E
6	Soho Yacht Marina	34°44′14″N, 127°39′02″E
7	Tongyeong Yacht Marina	34°49′42″N, 128°26′05″E
8	Gujoro Harbor	34°49′56″N, 128°42′10″E
9	Busan Yacht Marina	35°09′29″N, 129°08′43″E
10	Ulsan Harbor	35°29′02″N, 129°25′47″E
11	Yangpo Yacht Marina	35°52′40″N, 129°31′10″E
12	Donghae Port	37°29′28″N, 129°07′29″E
13	Sokcho Port	38°12′39″N, 128°35′47″E

Table 3. Occurrence of *T. californica* in monitored localities from 2020 to 2024.

Date	Locality													Data source
	1	2	3	4	5	6	7	8	9	10	11	12	13	
27–31 Jul 2020							○							Chae *et al*., 2022
12–16 Oct 2020							○							This study
7, 19–23 Jul 2021							○							Chae *et al*., 2022
18–22 Oct 2021							○							This study
2–6 Jul 2022							○							This study
11–15 Oct 2022							○							This study
24–28 Jul 2023							○	○			○			This study
23–28 Oct 2023							○	○			○			This study
1–5 Jul 2024							○	○			○			This study
5, 14–18 Oct 2024							○	○			○			This study

※ Information on locality numbers is indicated in Table 2.

Table 4. Genetic distance (K2P) based on 417 bp of *COI* sequences in *Thalamoporella californica* and three bryozoans.

No.	Species	Accession No.	1	2	3	4	5	6	Data source
1	*Thalamoporella californica*	PQ454604							This study
2	*Thalamoporella californica*	PQ454605	0.003						This study
3	*Thalamoporella californica*	PQ454606	0.005	0.008					This study
4	*Thalamoporella californica*	PQ454607	0.006	0.009	0.002				This study
5	*Thalamoporella californica*	PQ454608	0.006	0.003	0.011	0.012			This study
6	*Thalamoporella californica*	PQ454609	0.008	0.005	0.012	0.014	0.002		This study
7	*Thalamoporella lioticha*	HQ896191.1	0.328	0.331	0.328	0.328	0.335	0.338	Lee *et al*., 2011
8	*Steginoporella magnifica*	JF950435.1	0.331	0.336	0.329	0.331	0.339	0.342	Knight *et al*., 2011
9	*Heteropora alaskensis*	MH242798.1	0.457	0.451	0.454	0.457	0.451	0.454	Leray and Paulay, 2018

aligned into 417 bp. The intra-specific variations in *T. californica* were 0.2−1.4%, and the genetic differences of *T. californica* compared to three other bryozoans are as follows: 32.8−33.8% with *T. lioticha* within the same genus, 32.9−34.2% with *S. magnifica* within the same superfamily, and 45.1−45.7% with *H. alaskensis* from a different class (Table 4). In this study, the K2P model was used for genetic sequence analysis, focusing on simple species identification rather than phylogenetic relationship analysis. The sequence differences in the *COI* gene region indicate that *T. californica* from three different localities belong to the same species. This study does not provide a clear understanding of where *T. californica* was first introduced or how it spread. For more accurate information on how *T. californica* invaded Korean waters from a single location and spread in distribution, it is necessary to verify using microsatellite analysis (Mackie *et al*., 2012).

As a result of this study, *T. californica*, a member of the genus *Thalamoporella*, has been added to GenBank, where previously only *T. lioticha* was registered. The longer 658 bp *COI* sequence of *T. californica* compared to the 473 bp sequence of *T. lioticha*, which was already deposited in GenBank, is likely to provide better species discrimination. By providing DNA barcoding data of *T. californica* along with its ecological photographs and information in this study, these features can be utilized to facilitate the distinction of *T. californica* from other congeners.

Conflicts of Interest

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

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Unrecorded bacterial species from halophilic and contaminated environments in Korea: Descriptions of 33 species isolated in 2021 and 2022

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This study describes 33 previously unrecorded bacterial species strains isolated from specialized halophilic and contaminated environments in Korea. The bacterial strains were isolated from unique ecological niches, including solar saltern, mudflats, and contaminated soils from highly polluted sites such as landfills and parking lots. These habitats harbor diverse microbial communities, offering insights into bacterial adaptation and distribution. The findings contribute to the understanding of microbial diversity in extreme and anthropogenically impacted environments. Based on high 16S rRNA gene sequence similarity (>98.7%) and the formation of a robust phylogenetic clade with the closest species, each strain was determined to belong to a predefined bacterial species. There is no official report that these 33 species belonged to 4 phyla, 7 classes, 17 orders, 19 families, and 27 genera, which were assigned to *Muricauda*, *Kaistella*, *Hymenobacter* and *Flavobacterium* of the phylum *Bacteroidota*; *Streptomyces*, *Rhodococcus*, *Rathayibacter*, *Nocardioides*, *Cryobacterium*, *Arthrobacter* and *Aeromicrobium* of the phylum *Actinomycetota*; *Thioclava*, *Sulfitobacter*, *Spiribacter*, *Simplicispira*, *Shewanella*, *Roseovarius*, *Qipengyuania*, *Pseudomonas*, *Maritalea*, *Marinobacter*, *Halomonas*, *Devosia*, *Chromohalobacter* and *Brevundimonas* of the phylum *Pseudomonadota*; *Virgibacillus* and *Alkalihalobacillus* of the phylum *Bacillota*. Gram-staining results, colony and cell morphologies, basic biochemical characteristics, isolation sources, and strain IDs are also described in the species description section.

Keywords: 16S rRNA gene, culture-dependent isolation, environmental microbiota, phylogenetic profiling, taxonomic characterization

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Introduction

Environmental microorganisms play crucial roles in maintaining ecosystem stability, particularly in extreme habitats where selective pressures shape unique microbial communities. Contaminated soils, subjected to prolonged exposure to pollutants such as heavy metals and hydrocarbons, develop complex microbial ecosystems. These environments drive microbial adaptation and diversification, often leading to the emergence of specialized taxa capable of surviving under nutrient-limited and chemically fluctuating conditions. Notably, landfill and parking lot soils, frequently exposed to petroleum derivatives, heavy metal leachates, and industrial runoff, serve as reservoirs for metabolically versatile bacterial communities. Studies have identified members of the genera *Sphingomonas* and *Flavobacterium* in these environments, with certain species exhibiting hydrocarbon degradation capabilities

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and heavy metal resistance, thereby contributing to biogeochemical cycling and pollutant detoxification (Raghad, 2019). Similarly, halophilic environments, characterized by high salinity levels, impose distinct selective pressures that shape microbial diversity. Halophilic microorganisms, including extreme halophiles, have evolved specialized metabolic pathways and enzymatic systems that allow them to survive and function in high-salinity conditions. For instance, species of the genus *Halobacterium* have been isolated from such environments, demonstrating unique metabolic adaptations that not only enable survival in hypersaline conditions but also hold biotechnological potential. These halophilic bacteria are of particular interest for applications such as the bioremediation of saline industrial wastewater and the enzymatic treatment of salt-rich pollutants (Martínez-Espinosa, 2024). As part of a broader effort to explore microbial diversity in specialized environments, we conducted a large-scale investigation of bacterial communities in both contaminated and halophilic habitats across Korea during 2021 and 2022. Supported by the National Institute of Biological Resources (NIBR), this study identified bacterial species spanning seven classes—*Actinomycetes*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Bacilli*, *Cytophagia*, and *Flavobacteriia*—distributed across 17 different orders. Here, we present an overview of 33 previously unreported bacterial species discovered during this research program, contributing to a deeper understanding of microbial adaptations in specialized and extreme environments.

Materials and Methods

A total of 33 bacterial strains affiliated to the *Actinomycetes*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Bacilli*, *Cytophagia* and *Flavobacteriia* were isolated from unique ecological niches, including salt evaporation ponds, mudflats, and contaminated soil samples. Mud, seawater and soil samples were processed separately, spread onto diverse culture media including R2A and marine agar 2216 (+5% NaCl), and incubated at 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 using 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 followed the standard procedures outlined by Lane (1991). The 16S rRNA gene sequences from the 47 bacterial strains were compared to sequences in GenBank using BLASTN and also analyzed through the EzBioCloud database (Yoon *et al*., 2017). For phylogenetic analysis, sequence alignments were performed with the Clustal_X program (Thompson *et al*., 1997), and any gaps were manually edited using BioEdit (Hall, 1999). Evolutionary distances were computed using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed via the neighbor-joining method (Saitou and Nei, 1987) in the MEGA X software (Kumar *et al*., 2018), with bootstrap values calculated from 1,000 replications (Felsenstein, 1985).

Results and Discussion

The 33 strains were distributed in 7 classes of the *Actinomycetes*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Bacilli*, *Cytophagia* and *Flavobacteriia*; 2 strains for the class *Bacilli*, 7 strains for the *Actinomycetes*, 9 strains for the *Alphaproteobacteria*, 1 strain for the *Betaproteobacteria*, 9 strains for the *Gammaproteobacteria*, 1 strain for the *Cytophagia* and 4 strains for the *Flavobacteriia* (Table 1). These strains were Gram-staining-negative or positive, chemoheterotrophic, rod, short-rod, or coccoid shaped showing in Fig. 1.

The strains in the phylum *Actinomycetota* (Fig. 2) were found to belong to 1 classes 7 separate genera: *Streptomyces* (1 species), *Rhodococcus* (1 species), *Rathayibacter* (1 species), *Nocardioides* (1 species), *Cryobacterium* (1 species), *Arthrobacter* (1 species), and *Aeromicrobium* (1 species).

Fig. 3 shows phylogenetic assignment of 19 strains of the phylum *Pseudomonadota* belong to 3 classes 14 separate genera: *Thioclave* (1 species), *Sulfitobacter* (1 species), *Roseovarius* (2 species), *Qipengyuania* (1 species), *Maritalea* (1 species), *Devosia* (2 species), *Brevundimonas* (1 species), *Simplicispira* (1 species), *Spiribacter* (2 species), *Shewanella* (1 species), *Pseudomonas* (1 species), *Marinobacter* (2 species), *Halomonas* (2 species), and *Chromohalobacter* (1 species).

Fig. 4 shows phylogenetic assignment of 7 strains of the phyla *Bacillota* and *Bacteroidota* belong to 3 classes 6 separate genera: *Virgibacillus* (1 species), *Alkalihalobacillus* (1 species), *Hymenobacter* (1 species), *Muricauda* (2 species), *Kaistella* (1 species), and *Flavobacterium* (1 species).

Here we report 33 unrecorded bacterial species in Korea belonging to 7 classes of 4 phyla.

Table 1. The taxonomic affiliations of isolated strains belonging to the classes *Actinomycetes*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Bacilli*, *Cytophagia* and *Flavobacteriia*.

Class	Order	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Isolation site	Medium	Incubation conditions (°C, days)
Actinomycetes	*Microbacteriales*	*Microbacteriaceae*	*Rathayibacter*	Du119	NIBRBAC000509522	*Rathayibacter rubneri*	100.00	Contaminated soil	Anseong-si	R2A	25°C
Actinomycetes	*Micrococcales*	*Micrococcaceae*	*Arthrobacter*	Du194	NIBRBAC000509525	*Arthrobacter glacialis*	99.18	Contaminated soil	Anseong-si	R2A	25°C
Actinomycetes	*Streptomycetales*	*Streptomycetaceae*	*Streptomyces*	P23	NIBRBAC000509526	*Streptomyces lateritius*	100.00	Contaminated soil	Anseong-si	R2A	25°C
Actinomycetes	*Mycobacteriales*	*Nocardiaceae*	*Rhodococcus*	P101	NIBRBAC000509528	*Prescottella defluvii*	100.00	Contaminated soil	Anseong-si	R2A	25°C
Actinomycetes	*Microbacteriales*	*Microbacteriaceae*	*Cryobacterium*	P108	NIBRBAC000509529	*Cryobacterium soli*	99.78	Contaminated soil	Anseong-si	R2A	25°C
Actinomycetes	*Propionibacteriales*	*Nocardioidaceae*	*Nocardioides*	Du230	NIBRBAC000509530	*Nocardioides pacificus*	99.44	Contaminated soil	Anseong-si	R2A	25°C
Actinomycetes	*Propionibacteriales*	*Nocardioidaceae*	*Aeromicrobium*	Du262	NIBRBAC000509532	*Aeromicrobium phoceense*	100.00	Contaminated soil	Anseong-si	R2A	25°C
Alphaproteobacteria	*Rhodobacterales*	*Rhodobacteraceae*	*Roseovarius*	T3	NIBRBAC000508810	*Roseovarius tibetensis*	99.93	Salt evaporation pond	Taean-gun	MA + 5% NaCl	30°C
Alphaproteobacteria	*Rhodobacterales*	*Rhodobacteraceae*	*Roseovarius*	T12	NIBRBAC000508811	*Roseovarius tolerans*	100	Salt evaporation pond	Taean-gun	MA + 5% NaCl	30°C
Alphaproteobacteria	*Sphingomonadales*	*Erythrobacteraceae*	*Qipengyuania*	SC77	NIBRBAC000508816	*Qipengyuania vesicularis*	99.36	Mudflats	Hwaseong-si	MA + 5% NaCl	25°C
Alphaproteobacteria	*Rhizobiales*	*Hyphomicrobiaceae*	*Maritalea*	SC80	NIBRBAC000508817	*Maritalea mobilis*	99.93	Mudflats	Hwaseong-si	MA + 5% NaCl	25°C
Alphaproteobacteria	*Rhodobacterales*	*Rhodobacteraceae*	*Thioclava*	SC162	NIBRBAC000508820	*Thioclava nitratireducens*	100	Mudflats	Hwaseong-si	MA + 5% NaCl	28°C
Alphaproteobacteria	*Rhodobacterales*	*Rhodobacteraceae*	*Sulfitobacter*	SC164	NIBRBAC000508821	*Sulfitobacter faviae*	99.86	Mudflats	Hwaseong-si	MA + 5% NaCl	25°C
Alphaproteobacteria	*Caulobacterales*	*Caulobacteraceae*	*Brevundimonas*	Du49	NIBRBAC000509520	*Brevundimonas mediterranea*	99.71	Contaminated soil	Anseong-si	R2A	25°C
Alphaproteobacteria	*Rhizobiales*	*Devosiaceae*	*Devosia*	Du137	NIBRBAC000509523	*Devosia epidermidihirudinis*	98.72	Contaminated soil	Anseong-si	R2A	25°C
Alphaproteobacteria	*Rhizobiales*	*Devosiaceae*	*Devosia*	Du184	NIBRBAC000509524	*Devosia limi*	99.43	Contaminated soil	Anseong-si	R2A	25°C
Bacilli	*Bacillales*	*Bacillaceae*	*Virgibacillus*	GP109	NIBRBAC000508814	*Virgibacillus flavescens*	99.33	Mudflats	Hwaseong-si	MA + 5% NaCl	25°C
Bacilli	*Bacillales*	*Bacillaceae*	*Alkalihalobacillus*	SC171	NIBRBAC000508822	*Alkalihalobacillus pseudofirmus*	99.93	Mudflats	Hwaseong-si	MA + 5% NaCl	30°C
Betaproteobacteria	*Burkholderiales*	*Comamonadaceae*	*Simplicispira*	Du248	NIBRBAC000509531	*Simplicispira psychrophila*	99.31	Contaminated soil	Anseong-si	R2A	25°C
Cytophagia	*Cytophagales*	*Hymenobacteraceae*	*Hymenobacter*	Du96	NIBRBAC000509521	*Hymenobacter actinosclerus*	99.44	Contaminated soil	Anseong-si	R2A	25°C
Flavobacteriia	*Flavobacteriales*	*Flavobacteriaceae*	*Muricauda*	SC11	NIBRBAC000508815	*Muricauda ruestringensis*	99.52	Mudflats	Hwaseong-si	MA + 5% NaCl	25°C
Flavobacteriia	*Flavobacteriales*	*Flavobacteriaceae*	*Muricauda*	SC140	NIBRBAC000508818	*Muricauda chongwuensis*	99.24	Mudflats	Hwaseong-si	MA + 5% NaCl	25°C
Flavobacteriia	*Flavobacteriales*	*Flavobacteriaceae*	*Flavobacterium*	Du4	NIBRBAC000509519	*Flavobacterium chryseum*	99.34	Contaminated soil	Anseong-si	R2A	25°C
Flavobacteriia	*Flavobacteriales*	*Flavobacteriaceae*	*Kaistella*	P84	NIBRBAC000509527	*Kaistella daneshvariae*	100.00	Contaminated soil	Anseong-si	R2A	25°C
Gammaproteobacteria	*Oceanospirillales*	*Halomonadaceae*	*Halomonas*	A8	NIBRBAC000508805	*Halomonas azerica*	99.11	Salt evaporation pond	Taean-gun	MA + 5% NaCl	30°C
Gammaproteobacteria	*Alteromonadales*	*Alteromonadaceae*	*Marinobacter*	B9	NIBRBAC000508806	*Marinobacter aquaticus*	99.37	Salt evaporation pond	Taean-gun	MA + 5% NaCl	37°C
Gammaproteobacteria	*Alteromonadales*	*Alteromonadaceae*	*Marinobacter*	B20	NIBRBAC000508807	*Marinobacter vulgaris*	99.73	Salt evaporation pond	Taean-gun	MA + 5% NaCl	37°C
Gammaproteobacteria	*Oceanospirillales*	*Halomonadaceae*	*Halomonas*	D12	NIBRBAC000508808	*Halomonas saccharevitans*	99.72	Salt evaporation pond	Taean-gun	MA + 5% NaCl	30°C
Gammaproteobacteria	*Oceanospirillales*	*Halomonadaceae*	*Chromohalobacter*	D37	NIBRBAC000508809	*Chromohalobacter salexigens*	99.93	Salt evaporation pond	Taean-gun	MA + 5% NaCl	37°C
Gammaproteobacteria	*Chromatiales*	*Ectothiorhodospiraceae*	*Spiribacter*	T18	NIBRBAC000508812	*Spiribacter roseus*	99.79	Salt evaporation pond	Taean-gun	MA + 5% NaCl	37°C
Gammaproteobacteria	*Chromatiales*	*Ectothiorhodospiraceae*	*Spiribacter*	T26	NIBRBAC000508813	*Spiribacter salinus*	99.11	Salt evaporation pond	Taean-gun	MA + 5% NaCl	37°C
Gammaproteobacteria	*Alteromonadales*	*Shewanellaceae*	*Shewanella*	SC153	NIBRBAC000508819	*Shewanella carassii*	99.79	Mudflats	Hwaseong-si	MA + 5% NaCl	35°C
Gammaproteobacteria	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*	Du18	NIBRBAC000509533	*Pseudomonas sivasensis*	100	Contaminated soil	Anseong-si	R2A	25°C

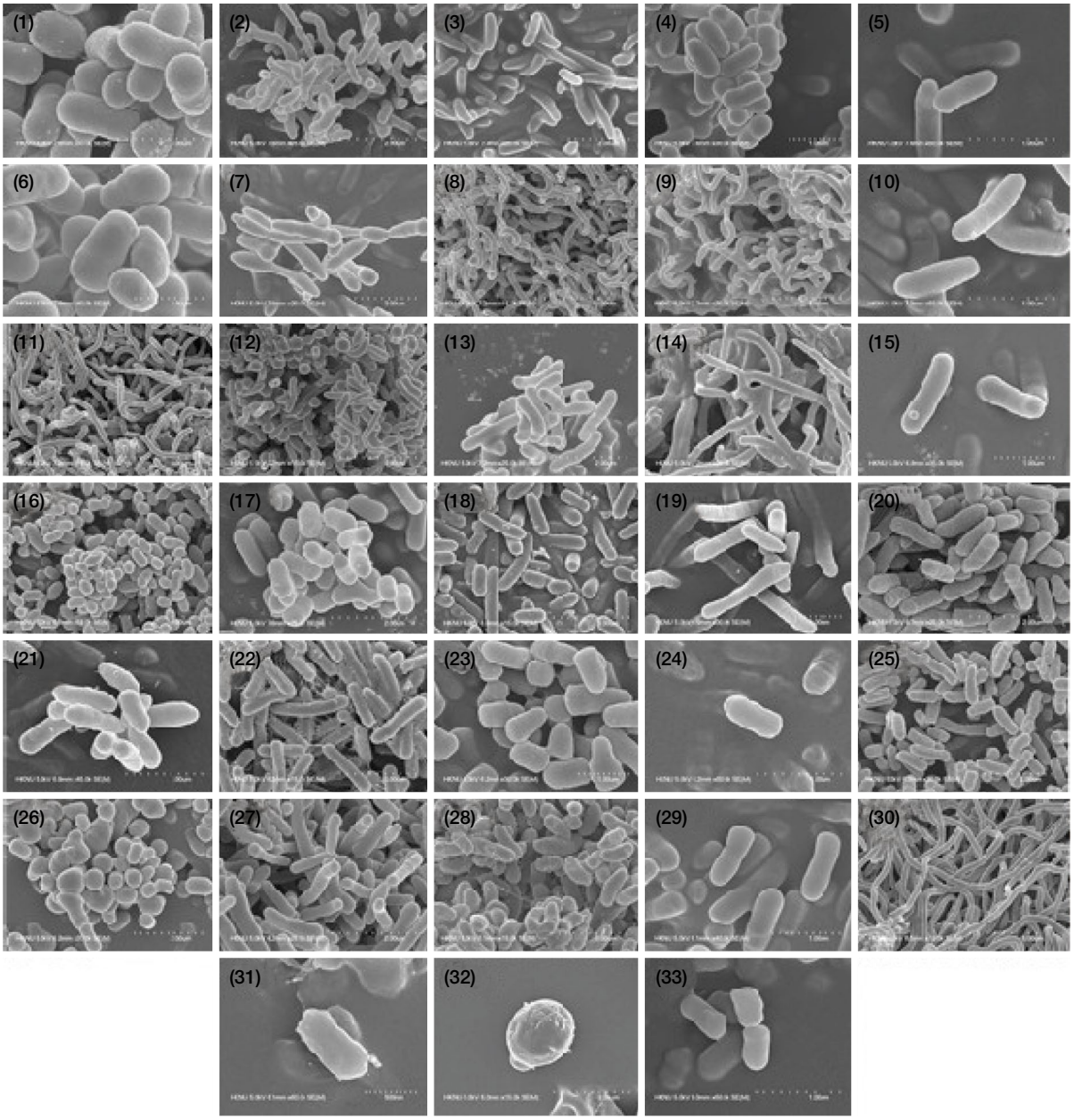


Fig. 1. Scanning electron micrograph of cells of the strains isolated in this study. Strains: 1, A8; 2, B9; 3, B20; 4, D12; 5, D37; 6, T3; 7, T12; 8, T18; 9, T26; 10, GP109; 11, SC11; 12, SC77; 13, SC80; 14, SC140; 15, SC153; 16, SC162; 17, SC164; 18, SC171; 19, Du4; 20, Du18; 21, Du49; 22, Du96; 23, Du119; 24, Du137; 25, Du184; 26, Du194; 27, Du230; 28, Du248; 29, Du262; 30, P23; 31, P84; 32, P101; 33, P108.

Description of *Halomonas azerica* A8

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, entire, convex and pale brown-colored after 3 days incubation on marine agar with 5% sodium chloride at 30°C. In the API 20NE system, positive for glucose acidification, utilization of D-glucose, D-mannitol, D-maltose, potassium gluconate, malic acid and trisodium citrate, but negative for nitrate reduction, indole production, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, β-galactosidase activity, oxidase activity, utilization of L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, capric acid, adipic acid and phenylacetic acid. Strain A8 (=NIBRBAC000508805) was isolated from salt evaporation pond in Nam-myeon, Taean-gun, Chungcheongnam-do, Republic of Korea (36° 36′27.0″N 126°18′17.1″E).

Description of *Marinobacter aquaticus* B9

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, convex and ivory-

colored after 3 days incubation on marine agar with 5% sodium chloride at 37°C. In the API 20NE system, positive for oxidase activity, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, β-galactosidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain B9 (=NIBRBAC000508806) was isolated from salt evaporation pond in Nam-myeon, Taean-gun, Chungcheongnam-do, Republic of Korea (36°36′27.0″N 126°18′17.1″E).

Description of *Marinobacter vulgaris* B20

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, convex and cream-colored after 3 days incubation on marine agar with 5% sodium chloride at 37°C. In the API 20NE system, positive for nitrate reduction, urease activity, utilization of D-glucose, L-arabinose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipic acid and malic acid, but negative for indole production, glucose acidification, arginine dihydrolase, esculin hydrolysis, gelatin hydrolysis, β-galactosidase activity, oxidase activity, utilization of D-mannose, D-mannitol, capric acid, trisodium citrate and phenylacetic acid. Strain B20 (=NIBRBAC000508807) was isolated from salt evaporation pond in Nam-myeon, Taean-gun, Chungcheongnam-do, Republic of Korea (36°36′27.0″N 126°18′17.1″E).

Description of *Halomonas saccharevitans* D12

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, entire, convex and ivory-colored after 3 days incubation on marine agar with 5% sodium chloride at 30°C. In the API 20NE system, positive for nitrate reduction and oxidase activity, but negative for indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, β-galactosidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain D12 (=NIBRBAC000508808) was isolated from salt evaporation pond in Nam-myeon, Taean-gun, Chungcheongnam-do, Republic of Korea (36°36′27.0″N 126°18′17.1″E).

Description of *Chromohalobacter salexigens* D37

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, convex, smooth and ivory-colored after 3 days incubation on marine agar with 5% sodium chloride at 37°C. In the API 20NE system, positive for nitrate reduction, β-galactosidase activity, oxidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, malic acid and trisodium citrate, but negative for indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, utilization of *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain D37 (=NIBRBAC000508809) was isolated from salt evaporation pond in Nam-myeon, Taean-gun, Chungcheongnam-do, Republic of Korea (36°36′27.0″N 126°18′17.1″E).

Description of *Roseovarius tibetensis* T3

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, convex, smooth and ivory-colored after 3 days incubation on marine agar with 5% sodium chloride at 30°C. In the API 20NE system, positive for nitrate reduction, gelatin hydrolysis, β-galactosidase activity, oxidase activity, utilization of D-glucose, D-mannitol and *N*-acetyl-D-glucosamine, but negative for indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, utilization of L-arabinose, D-mannose, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain T3 (=NIBRBAC000508810) was isolated from salt evaporation pond in Nam-myeon, Taean-gun, Chungcheongnam-do, Republic of Korea (36°36′27.0″N 126°18′17.1″E).

Description of *Roseovarius tolerans* T12

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, convex, smooth and ivory-colored after 3 days incubation on marine agar with 5% sodium chloride at 30°C. In the API 20NE system, positive for nitrate reduction, oxidase activity and utilization of *N*-acetyl-D-glucosamine, but negative for indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, β-galactosidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain T12 (=NIBRBAC 000508811) was isolated from salt evaporation pond in Nam-myeon, Taean-gun, Chungcheongnam-do, Republic of Korea (36°36′27.0″N 126°18′17.1″E).

Description of *Spiribacter roseus* T18

Cells are Gram-staining-negative, non-flagellated and Curved-rod-shaped. Colonies are circular, convex and pink-colored after 7 days incubation on marine agar with 5% sodium chloride at 37°C. In the API 20NE system, positive for urease activity and oxidase activity, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, esculin hydrolysis, gelatin hydrolysis and β-galactosidase activity, utilization of D-

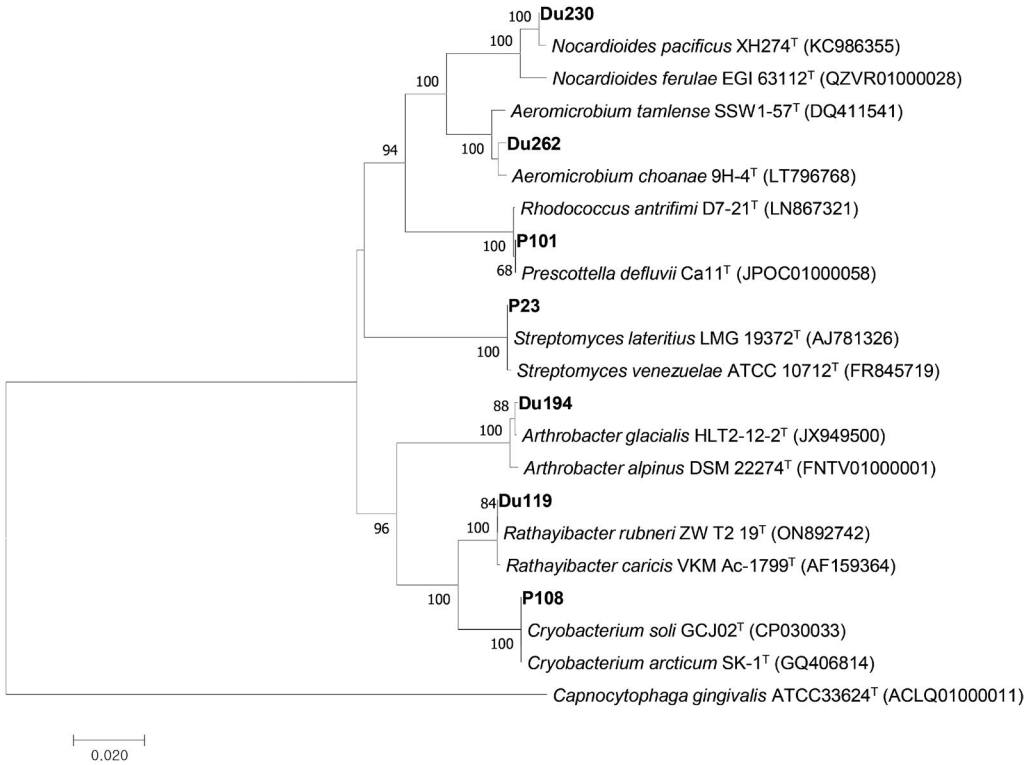


Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between the strains in the phylum *Actinomycetota*. Bootstrap values (>60%) based on 1,000 replications are shown on the branches. The tree was constructed using the neighbor-joining method. Bar, 0.02 substitutions per nucleotide position.

glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain T18 (=NIBRBAC000508812) was isolated from salt evaporation pond in Nam-myeon, Taean-gun, Chungcheongnam-do, Republic of Korea (36°36′27.0″N 126°18′17.1″E).

Description of *Spiribacter salinus* T26

Cells are Gram-staining-negative, non-flagellated and Curved-rod-shaped. Colonies are circular, convex and pink-colored after 7 days incubation on marine agar with 5% sodium chloride at 37°C. In the API 20NE system, positive for oxidase activity, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, β-galactosidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain T26 (=NIBRBAC000508813) was isolated from salt evaporation pond in Nam-myeon, Taean-gun, Chungcheongnam-do, Republic of Korea (36°36′27.0″N 126°18′17.1″E).

Description of *Virgibacillus flavescens* GP109

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are circular, convex and pale orange-colored after 3 days incubation on marine agar with 5% sodium chloride at 25°C. In the API 20NE system, positive for gelatin hydrolysis and oxidase activity, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, β-galactosidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain GP109 (=NIBRBAC000508814) was isolated from mudflats in Seosin-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°07′15.2″N 126°41′06.0″E).

Description of *Muricauda ruestringensis* SC11

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, convex and yellow-colored after 3 days incubation on marine agar with 5% sodium chloride at 25°C. In the API 20NE system, positive for esculin hydrolysis, gelatin hydrolysis, oxidase activity, utilization of D-mannose and *N*-acetyl-D-glucosamine, but negative for nitrate reduction, indole production, glucose

acidification, arginine dihydrolase, urease activity, β-galactosidase activity, utilization of D-glucose, L-arabinose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain SC11 (=NIBRBAC000508815) was isolated from mudflats in Ujeong-eup, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′49.2″N 126°45′60.0″E).

Description of *Qipengyuania vesicularis* SC77

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, convex and red-colored after 3 days incubation on marine agar with 5% sodium chloride at 25°C. In the API 20NE system, positive for oxidase activity, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, β-galactosidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain SC77 (=NIBRBAC000508816) was isolated from mudflats in Ujeong-eup, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′49.2″N 126°45′60.0″E).

Description of *Maritalea mobilis* SC80

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, convex and ivory-colored after 3 days incubation on marine agar with 5% sodium chloride at 25°C. In the API 20NE system, positive for nitrate reduction, glucose acidification, β-galactosidase activity, oxidase activity, utilization of D-glucose, D-mannose, *N*-acetyl-D-glucosamine and D-maltose, but negative for indole production, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, utilization of L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain SC80 (=NIBRBAC000508817) was isolated from mudflats in Ujeong-eup, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′49.2″N 126°45′60.0″E).

Description of *Muricauda chongwuensis* SC140

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, convex and yellow-colored after 3 days incubation on marine agar with 5% sodium chloride at 25°C. In the API 20NE system, positive for esculin hydrolysis, oxidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid and trisodium citrate, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease activity, gelatin hydrolysis, β-galactosidase activity, utilization of D-maltose and phenylacetic acid. Strain SC140 (=NIBRBAC000508818) was isolated from mudflats in Ujeong-eup, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′49.2″N 126°45′60.0″E).

Description of *Shewanella carassii* SC153

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, convex and pale orange-colored after 3 days incubation on marine agar with 5% sodium chloride at 35°C. In the API 20NE system, positive for gelatin hydrolysis, oxidase activity, utilization of D-glucose, L-arabinose, *N*-acetyl-D-glucosamine, capric acid and malic acid, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, β-galactosidase activity, utilization of D-mannose, D-mannitol, D-maltose, potassium gluconate, adipic acid, trisodium citrate and phenylacetic acid. Strain SC153 (=NIBRBAC000508819) was isolated from mudflats in Ujeong-eup, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′49.2″N 126°45′60.0″E).

Description of *Thioclava nitratireducens* SC162

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, convex and ivory-colored after 3 days incubation on marine agar with 5% sodium chloride at 28°C. In the API 20NE system, positive for nitrate reduction, esculin hydrolysis, β-galactosidase activity, oxidase activity, utilization of D-glucose, L-arabinose, D-mannitol and D-maltose, but negative for indole production, glucose acidification, arginine dihydrolase, urease activity, gelatin hydrolysis, utilization of D-mannose, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain SC162 (=NIBRBAC000508820) was isolated from mudflats in Ujeong-eup, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′49.2″N 126°45′60.0″E).

Description of *Sulfitobacter faviae* SC164

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, convex and white-colored after 3 days incubation on marine agar with 5% sodium chloride at 25°C. In the API 20NE system, positive for oxidase activity and utilization of D-glucose, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, β-galactosidase activity, utilization of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain SC164 (=NIBRBAC000508821) was isolated from mudflats in Ujeong-eup, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′49.2″N 126°45′60.0″E).

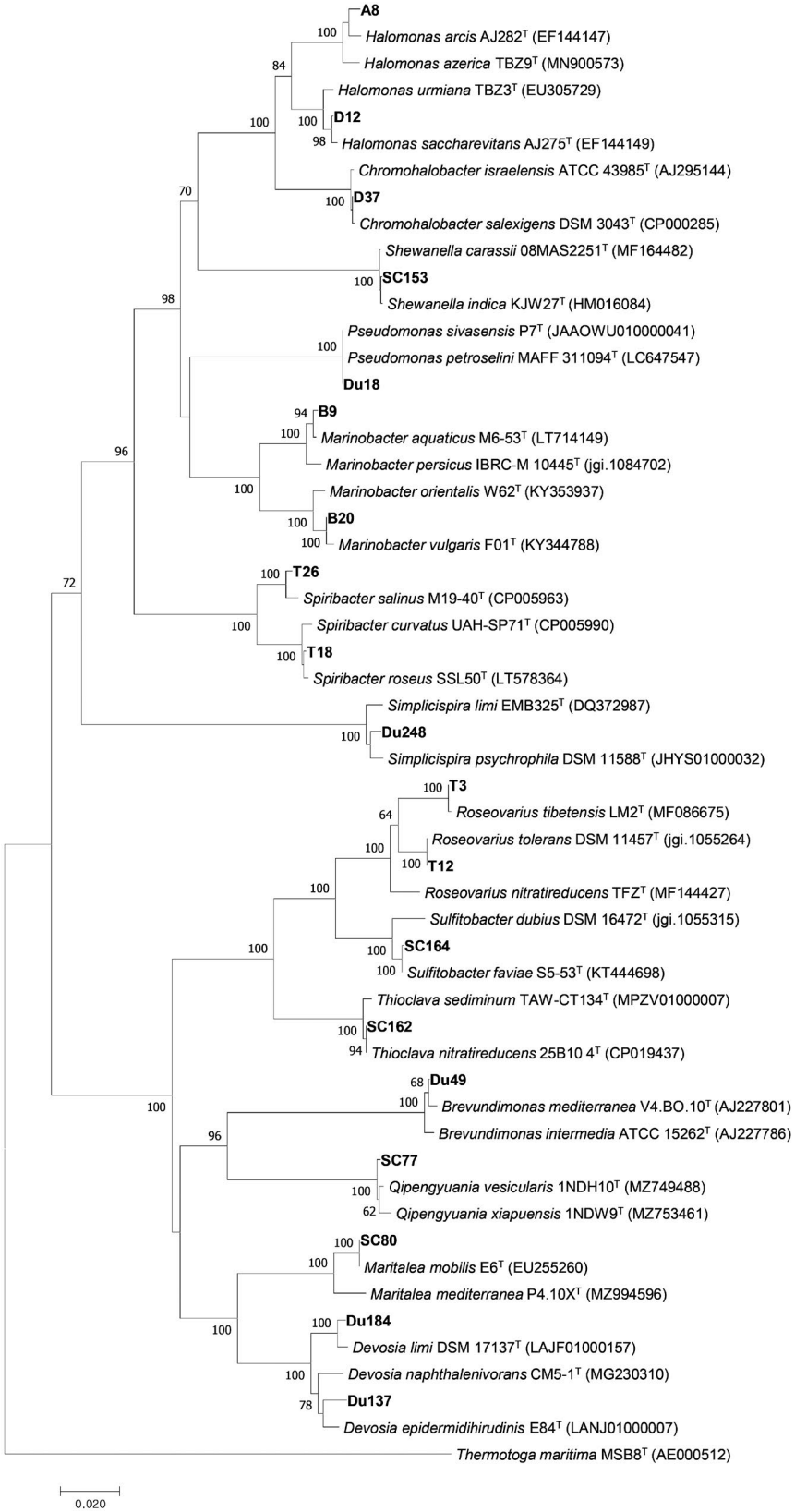


Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between the strains in the phylum *Pseudomonadota*. Bootstrap values (>60%) based on 1,000 replications are shown on the branches. The tree was constructed using the neighbor-joining method. Bar, 0.02 substitutions per nucleotide position.

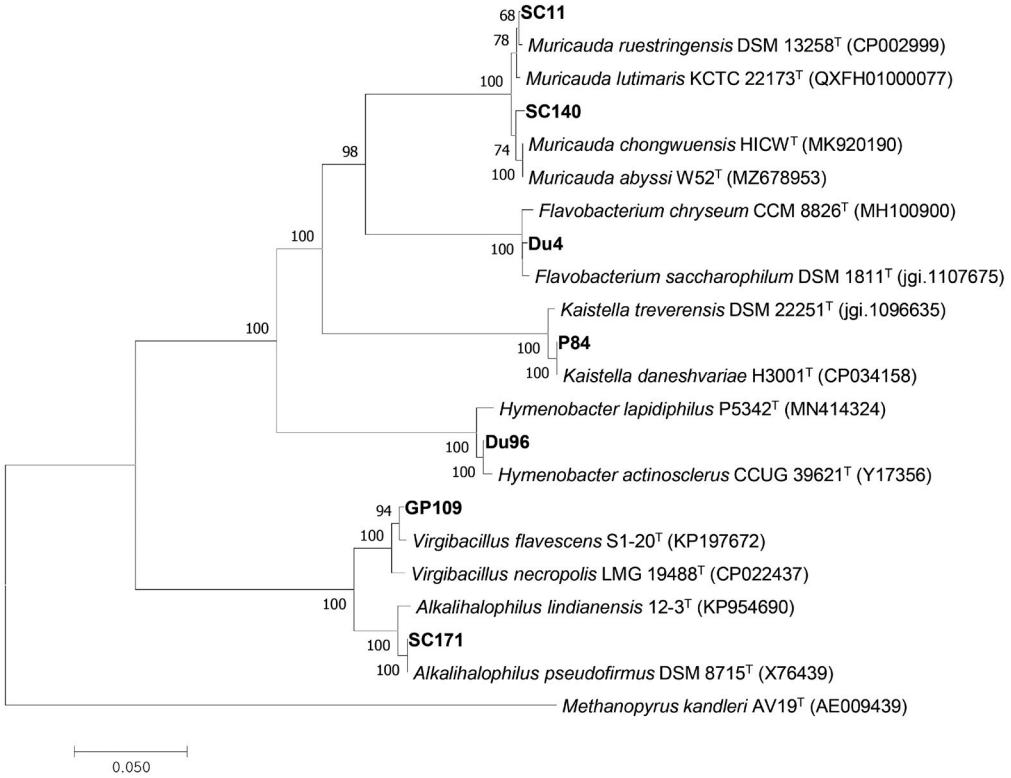


Fig. 4. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between the strains in the phylum *Bacillota* and *Bacteroidota*. Bootstrap values (>60%) based on 1,000 replications are shown on the branches. The tree was constructed using the neighbor-joining method. Bar, 0.05 substitutions per nucleotide position.

Description of *Alkalihalobacillus pseudofirmus* SC171

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are circular, convex and pale yellow-colored after 3 days incubation on marine agar with 5% sodium chloride at 30°C. In the API 20NE system, positive for nitrate reduction, oxidase activity, utilization of *N*-acetyl-D-glucosamine, malic acid and trisodium citrate, but negative for indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, β-galactosidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain SC171 (=NIBRBAC000508822) was isolated from mudflats in Ujeong-eup, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′49.2″N 126°45′60.0″E).

Description of *Flavobacterium chryseum* Du4

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, entire, convex and yellow-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for glucose acidification, arginine dihydrolase, esculin hydrolysis, β-galactosidase activity, oxidase activity, utilization of D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine and D-maltose, but negative for nitrate reduction, indole production, urease activity, gelatin hydrolysis, utilization of D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain Du4 (=NIBRBAC000509519) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Pseudomonas sivasensis* Du18

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, entire, convex and cream-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for arginine dihydrolase, gelatin hydrolysis, oxidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, malic acid and trisodium citrate, but negative for nitrate reduction, indole production, glucose acidification, urease activity, esculin hydrolysis, β-galactosidase activity, utilization of D-maltose, adipic acid and phenylacetic acid. Strain Du18 (=NIBR BAC000509533) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Brevundimonas mediterranea* Du49

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, entire, convex and pale orange-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for esculin hydrolysis, oxidase activity, utilization of D-glucose, D-maltose and malic acid, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease activity, gelatin hydrolysis, β-galactosidase activity, utilization of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain Du49 (=NIBRBAC000509520) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Hymenobacter actinosclerus* Du96

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, entire, convex and red-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for oxidase activity, utilization of D-glucose, *N*-acetyl-D-glucosamine, D-maltose and malic acid, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, β-galactosidase activity, utilization of L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain Du96 (=NIBR BAC000509521) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Rathayibacter rubneri* Du119

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are circular, entire, convex and cream-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for urease activity, esculin hydrolysis, β-galactosidase activity, oxidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate and phenylacetic acid, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, gelatin hydrolysis and utilization of capric acid. Strain Du119 (=NIBR BAC000509522) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Devosia epidermidihirudinis* Du137

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, entire, raised and orange-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for urease activity, esculin hydrolysis, β-galactosidase activity, oxidase activity, utilization of D-glucose, D-maltose, potassium gluconate and phenylacetic acid, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, gelatin hydrolysis, utilization of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, capric acid, adipic acid, malic acid and trisodium citrate. Strain Du137 (=NIBR BAC000509523) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Devosia limi* Du184

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, entire, convex and white-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for urease activity, esculin hydrolysis, β-galactosidase activity, oxidase activity, utilization of D-mannose and phenylacetic acid, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, gelatin hydrolysis, utilization of D-glucose, L-arabinose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid and trisodium citrate. Strain Du184 (=NIBR BAC000509524) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Arthrobacter glacialis* Du194

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are circular, entire, convex and pale yellow-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for nitrate reduction, urease activity, esculin hydrolysis, β-galactosidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate and malic acid, but negative for indole production, glucose acidification, arginine dihydrolase, gelatin hydrolysis, oxidase activity, utilization of capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain Du194 (=NIBRBAC000509525) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Streptomyces lateritius* P23

Cells are Gram-staining-positive, non-flagellated and filamentous shaped. Colonies are filamentous, umborate and white-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for nitrate reduction, esculin hydrolysis, β-galactosidase activity, utilization of D-glucose, D-mannose, *N*-acetyl-D-glucosamine, potassium gluconate and malic acid, but negative for indole pro-

duction, glucose acidification, arginine dihydrolase, urease activity, gelatin hydrolysis, oxidase activity, utilization of L-arabinose, D-mannitol, D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain P23 (=NIBRBAC000509526) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Kaistella daneshvariae* P84

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, entire, convex and cream-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for arginine dihydrolase, urease activity, esculin hydrolysis, β-galactosidase activity, oxidase activity, utilization of D-glucose, D-maltose and potassium gluconate, but negative for nitrate reduction, indole production, glucose acidification, gelatin hydrolysis, utilization of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain P84 (=NIBRBAC000509527) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Prescottella defluvii* P101

Cells are Gram-staining-positive, non-flagellated and coccoid shaped. Colonies are circular, entire, raised and white-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for glucose acidification, esculin hydrolysis, oxidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine and potassium gluconate, but negative for nitrate reduction, indole production, arginine dihydrolase, urease activity, gelatin hydrolysis, β-galactosidase activity, utilization of D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain P101 (=NIBRBAC000509528) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Cryobacterium soli* P108

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are circular, entire, convex and yellow-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for esculin hydrolysis, β-galactosidase activity, oxidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose and potassium gluconate, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease activity, gelatin hydrolysis, utilization of capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain P108 (=NIBRBAC000509529) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Nocardioides pacificus* Du230

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are circular, entire, raised and pale yellow-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for nitrate reduction, esculin hydrolysis, gelatin hydrolysis, β-galactosidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipic acid and malic acid, but negative for indole production, glucose acidification, arginine dihydrolase, urease activity, oxidase activity, utilization of capric acid, trisodium citrate and phenylacetic acid. Strain Du230 (=NIBRBAC000509530) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Simplicispira psychrophila* Du248

Cells are Gram-staining-negative, non-flagellated and rod shaped. Colonies are circular, entire, raised and cream-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for oxidase activity and utilization of malic acid, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, β-galactosidase activity, utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain Du248 (=NIBRBAC 000509531) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Description of *Aeromicrobium phoceense* Du262

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are circular, entire, raised and yellow-colored after 3 days incubation on R2A at 25°C. In the API 20NE system, positive for nitrate reduction, esculin hydrolysis, β-galactosidase activity, utilization of D-glucose, D-maltose, potassium gluconate and adipic acid, but negative for indole production, glucose acidification, arginine dihydrolase, urease activity, gelatin hydrolysis, oxidase activity, utilization of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain Du262 (=NIBR BAC000509532) was isolated from contaminated soil in Seokjeong-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°00′25.3″N 127°15′39.4″E).

Conflicts of Interest

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

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Characterization of three unrecorded yeast species, *Nakaseomyces glabratus*, *Crinitomyces flavificans* and *Kazachstania africana*, isolated from *Batillaria cumingii*, *Batillaria multiformis* and soil in Republic of Korea

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The purpose of this study is to isolate and identify wild yeasts from *Batillaria cumingii*, *Batillaria multiformis* and soil samples collected in Mokpo-si and Shinan-gun, Republic of Korea. Out of the fourteen strains identified, ten had been previously reported, whereas four 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. Of the four unreported yeast strains, D-7 and 11-2 belong to the genus *Nakaseomyces* (family: *Saccharomycetaceae*, order: *Saccharomycetales*, class: *Saccharomycetes*) and the genus *Kazachstania* (family: *Saccharomycetaceae*, order: *Saccharomycetales*, class: *Saccharomycetes*), respectively. Meanwhile, K-9 and K-22 belong to the genus *Crinitomyces* (family: *Trichomonascaceae*, order: *Dipodascales*, class: *Dipodascomycetes*). The 26S rRNA gene sequences of strains D-7, K-9, K-22, and 11-2 have been deposited in GenBank under the accession numbers PV244027, PV244028, PV244029, and PV243995, respectively. All strains had oval-shaped and multilateral budding cells. This research elucidated the morphological and biochemical properties of these three previously unreported yeast species in Korea.

Keywords: 26s rRNA, *Crinitomyces*, *Kazachstania*, *Nakaseomyces*, unreported yeast, yeast isolation

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Introduction

With the enforcement of the Nagoya Protocol in 2014, access to and utilization of microbial resources among nations have been significantly restricted. Consequently, securing the diversity of domestic microbial resources, studying their characteristics for resource development, and utilizing them as fundamental materials for industrial applications have become essential. Yeasts, as one of the microbial resources, play a crucial role in various industries. However, despite their potential, only 1−5% of yeast species have been identified, and most of their diversity remains unexplored (Choudhary and Johri, 2009). To date, yeasts have primarily been isolated from fermented foods

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and raw materials, with some being applied in the food fermentation industry (Kim *et al*., 1999). However, research on the classification of yeasts from natural habitats and their industrial application potential remains limited. Therefore, isolating and classifying yeasts from various natural environments, along with conducting further research, is essential to gain a deeper understanding of their ecological roles, develop them as resources, and maximize their industrial applications.

In this study, wild yeast strains were isolated from *Batillaria cumingii* and *Batillaria multiformis* samples collected in Mokpo-si, Republic of Korea, as well as from soil samples collected in Shinan-gun, Republic of Korea, to investigate the yeast diversity present in these environments. As a result of isolation and identification, many yeast strains were found to be previously reported species, while some were identified as unreported species. These unreported species were classified into the genera *Kazachstania*, *Nakaseomyces*, and *Crinitomyces*.

As a result, four previously unrecorded yeast strains were discovered in domestic ecosystems. Their phenotypic characteristics were thoroughly investigated to evaluate their taxonomic identities and potential applications.

Materials and Methods

Samples were collected from Mokpo-si and Shinan-gun, 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 for 3 days. The strains are preserved in a metabolically inactive state at Honam National Institute of Biological Resources, 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 D-7, K-9, K-22 and 11-2 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′-GCAT ATCAATAAGCGGAGGAAA AG-3′) and NL4 (5′-GGTC CGTGTTTCAAGACGG-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 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 method (Saitou and Nei, 1987) with

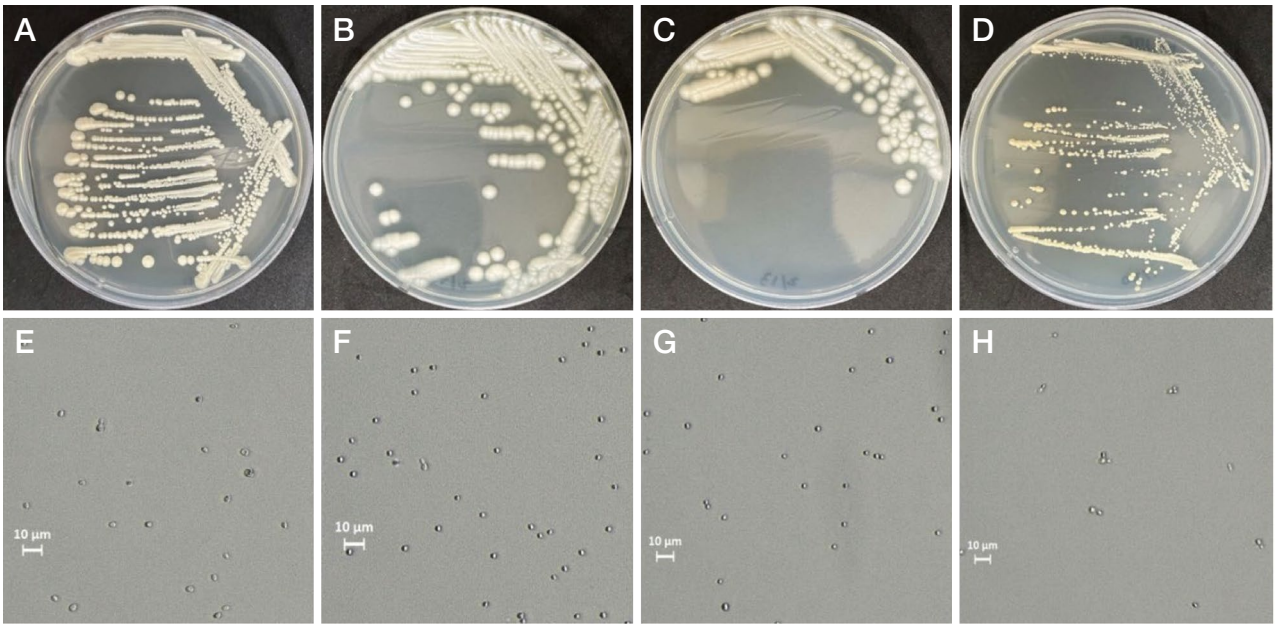


Fig. 1. Morphology of cells from the unrecorded strains D-7, K-9, K-22 and 11-2 incubated at 25ºC. All strains were grown after 3 days on YPD agar. The colonies of *Nakaseomyces glabratus* D-7 (A), *Crinitomyces flavificans* K-9 (B), *Trichosporiella flavificans* K-22 (C) and *Kazachstania africana* 11- 2 (D). The budding cells of of *Nakaseomyces glabratus* D-7 (E), *Crinitomyces flavificans* K-9 (F), *Trichosporiella flavificans* K-22 (G), and *Kazachstania africana* 11-2 (H). Bars, 10 μm.

Table 1. Yeasts isolated strains from *Batillaria cumingii*, *Batillaria multiformis* and soil in Republic of Korea.

Phylum	Class	Order	Family	Strain ID	Sample	Most closely related species	26S rRNA similarity	Record in Korea
Ascomycota	*Saccharomycetes*	*Saccharomycetales*	*Saccharomycetaceae*	11-2	Soil	*Kazachstania africana*	496/496 (100%)	Unreported
				D-7	*Batillaria cumingii*	*Nakaseomyces glabratus*	580/580 (100%)	Unreported
	Dipodascomycetes	*Dipodascales*	*Trichomonascaceae*	K-9	*Batillaria multiformis*	*Crinitomyces flavificans*	362/362 (100%)	Unreported
				K-22	*Batillaria multiformis*	*Crinitomyces flavificans*	511/511 (100%)	Unreported
	Dothideomycetes	*Dothideales*	*Saccotheciaceae*	15-5	Soil	*Aureobasidium leucospermi*	499/500 (99%)	Reported
				19-2	Soil	*Aureobasidium xishuangbannaense*	500/500 (100%)	Reported
Basidiomycota	*Microbotryomycetes*	*Sporidiobolales*	*Sporidiobolaceae*	15-1	Soil	*Rhodotorula graminis*	540/540 (100%)	Reported
	Tremellomycetes	*Tremellales*	*Rhynchogastremaceae*	15-3	Soil	*Papiliotrema flavescens*	560/560 (100%)	Reported
				Y-1	Soil	*Papiliotrema aurea*	516/517 (99%)	Reported
				Y-2	Soil	*Papiliotrema aurea*	514/514 (100%)	Reported
			Trimorphomycetaceae	17-1	Soil	*Saitozyma podzolica*	561/561 (100%)	Reported
		Cystofilobasidiales	*Cystofilobasidiaceae*	16-2	Soil	*Cystofilobasidium macerans*	560/560 (100%)	Reported
		Filobasidiales	*Filobasidiaceae*	15-7	Soil	*Filobasidium magnum*	562/562 (100%)	Reported
				19-3	Soil	*Filobasidium magnum*	562/562 (100%)	Reported

All strains were cultured under 25°C for 3 days.

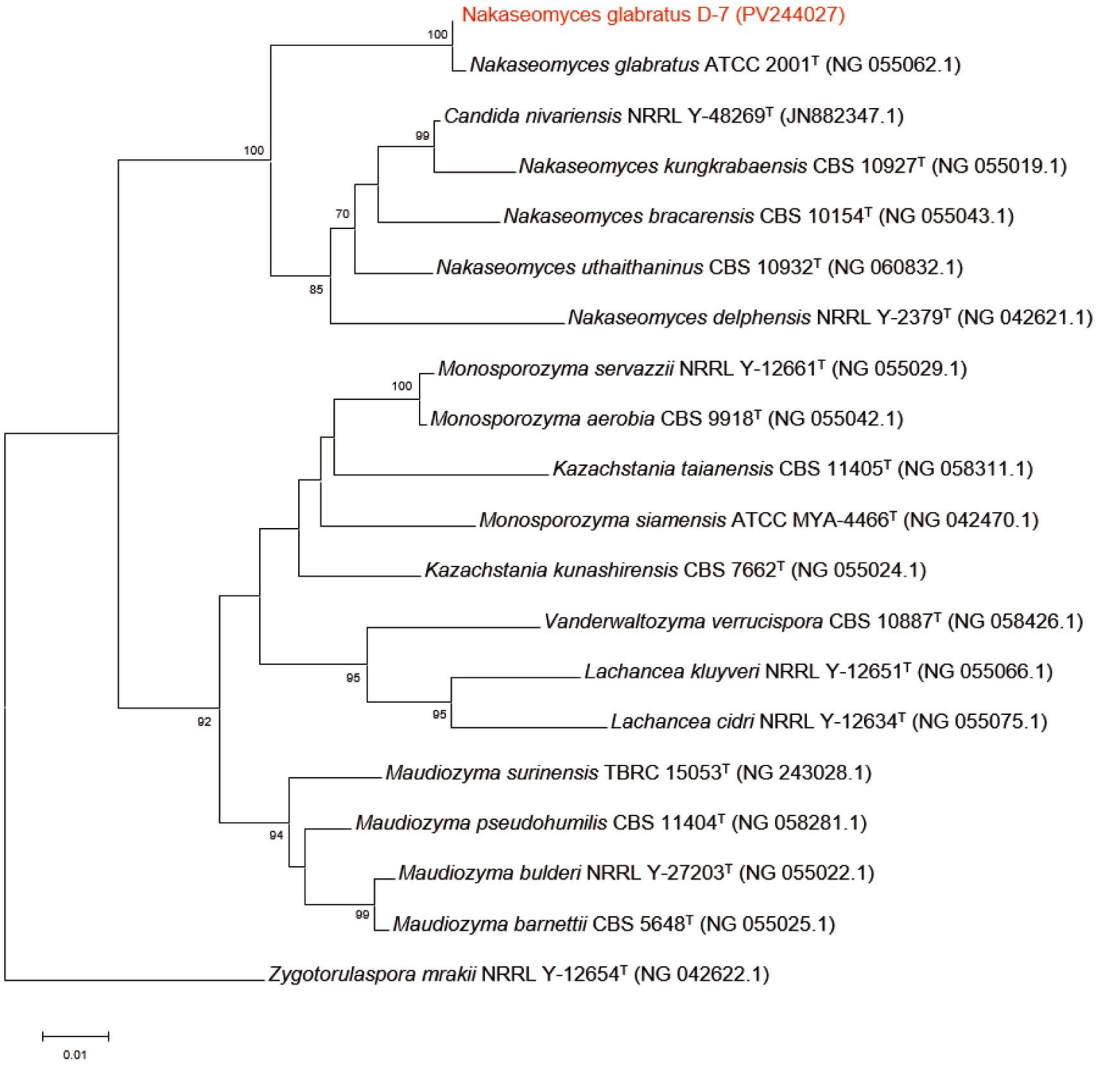


Fig. 2. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strains D-7 with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

the MEGA 11 program (Tamura *et al*., 2021). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1980) for neighborhood bond analysis, and the confidence levels of the clades were estimated 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 and Discussion

Among the fourteen wild yeast strains analyzed, eleven were isolated from soil samples collected in Shinan-gun, while the remaining three were derived from *Batillaria cumingii* and *Batillaria multiformis* samples obtained in Mokpo-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. Six strains were classified into the families *Saccharomycetaceae* (2 strains), *Trichomonascaceae* (2 strains), and *Saccotheciaceae* (2 strains) within the phylum *Ascomycota*, while eight strains were classified into the families *Sporidiobolaceae* (1 strain), *Rhynchogastremaceae* (3 strains), *Trimorphomycetaceae* (1 strain), *Cystofilobasidiaceae* (1 strain), and *Filobasidiaceae* (2 strains) within the phylum *Basidiomycota*.

The species isolated from soil samples collected in Shin-

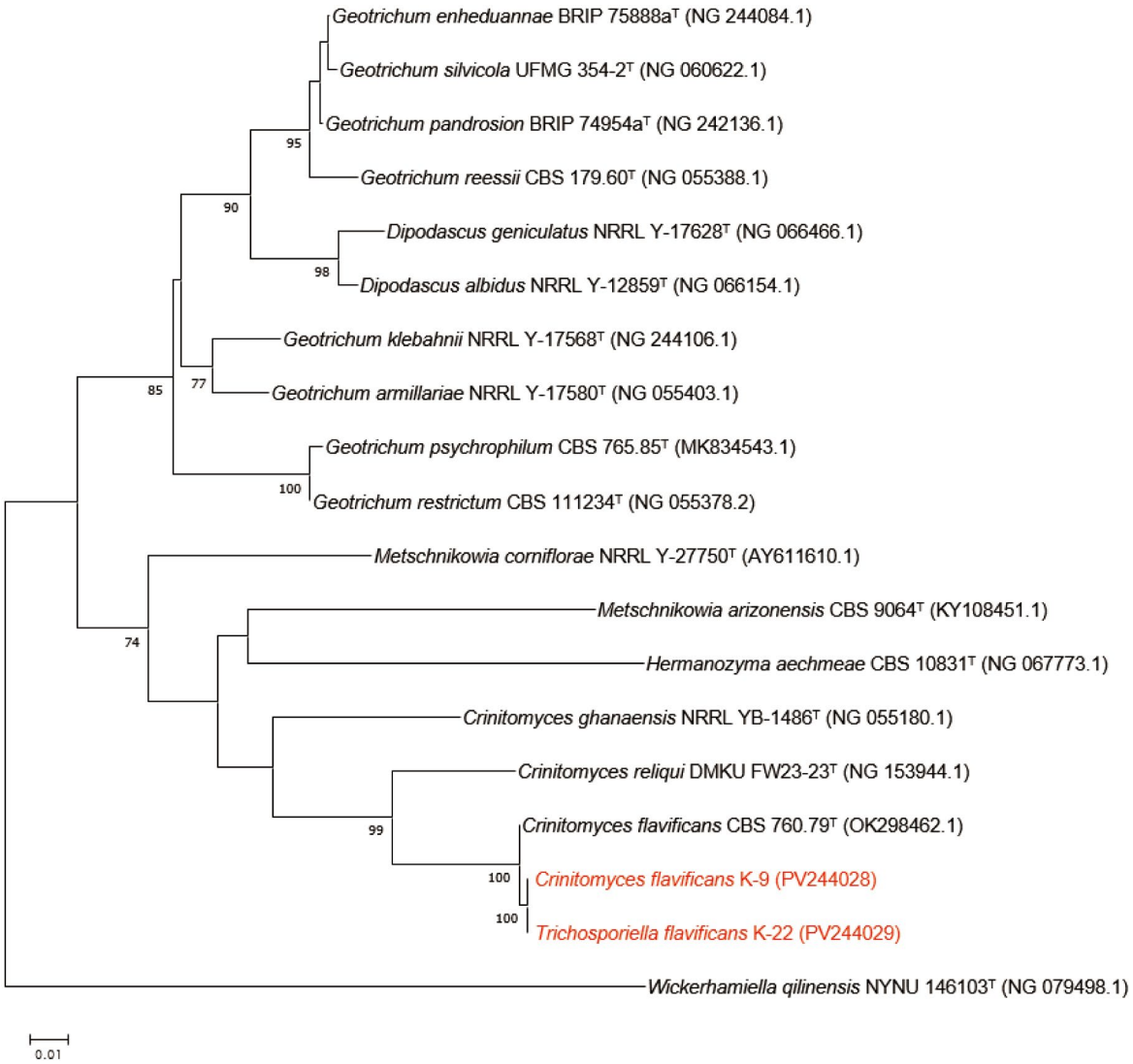


Fig. 3. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strains K-9 and K-22 with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

an-gun included 11-2, 15-5, 19-2, 15-1, 15-3, 15-7, Y-1, Y-2, 17-1, 16-2, and 19-3. Among these, 11-2 was identified as a previously unreported species in Korea. Additionally, the yeast strains D-7, isolated from *Batillaria cumingii* in Mokpo-si, and K-9 and K-22, isolated from *Batillaria multiformis* in Mokpo-si, were also identified as other previously unreported species in Korea. The taxonomic composition and identification results are detailed in Table 1.

The unreported yeast strains D-7, K-9, K-22 and 11-2 all belong to the phylum *Ascomycota*. Specifically, D-7 is classified in the genus *Nakaseomyces*, K-9 and K-22 are classified in the genus *Crinitomyces*, and 11-2 is classified in the genus *Kazachstania*. The phylogenetic tree shows that the isolated strains are closely related to the *Nakaseomyces* species, *Crinitomyces* species and *Kazachstania* species with the highest 26S rRNA gene sequence similarity (Figs. 2, 3 and 4), thereby supporting close relationships. Additional taxonomic and morphological characteristics of these three genera are outlined below. The genus *Kazachstania* is a member of the class *Saccharomycetes* within the phylum *Ascomycota*. This genus comprises 56 distinct species, with *Kazachstania viticola* designated as the type species (https://www.mycobank.org). The genus *Nakaseomyces* is a member of the class *Saccharomycetes* within the phylum *Ascomycota*. This genus includes 8 distinct species, with *Nakaseomyces delphensis* as the type species (https://www.mycobank.org). The genus *Crinitomyces* is a member of the class *Dipodascomycetes* within the phylum *Ascomycota*. This genus includes 3 distinct

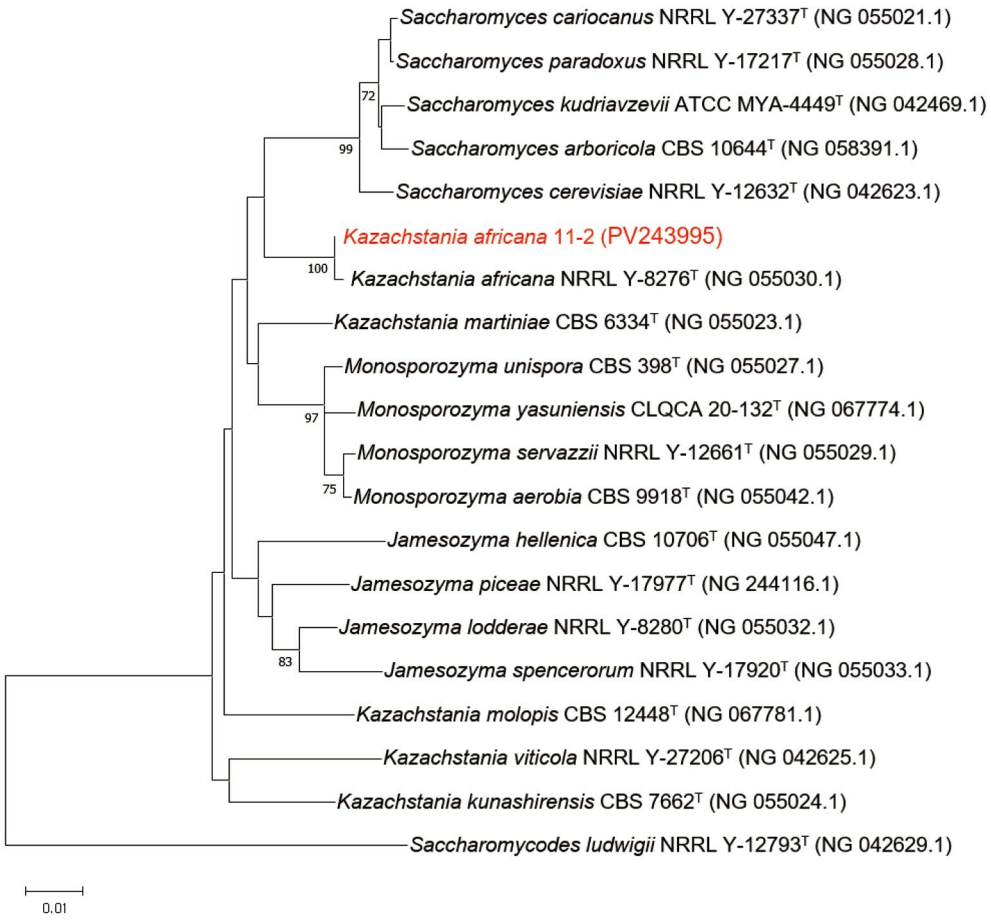


Fig. 4. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain 11-2 with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

species, with *Crinitomyces flavificans* as the type species (https://www.mycobank.org).

The genus *Kazachstania* belongs to the order *Saccharomycetales* and is considered a sister group to the genus *Naumovozyma* (Lachance, 2018). Asexual reproduction occurs via multilateral budding from a narrow base, with cells that can be spherical, ovoid, or elongate. Pseudohyphae may be produced, but true hyphae are not formed, and colonies are generally smooth, displaying colors ranging from white to tan. Sexual reproduction involves the formation of asci through conjugation between independent cells or between a mother cell and its progeny; these asci may persist or dissolve after maturation, and each ascus contains 1−16 ascospores that are spherical, ovoid, or reniform, with spore surfaces that can be either smooth or rough. Standard test reactions for yeast classification are generally limited in *Kazachstania*. Most species ferment glucose and are capable of assimilating galactose; however, nitrogen sources such as cadaverine, L-lysine, and ethylamine are scarcely utilized, and nitrate is not used. In addition, the primary respiratory quinone produced by this genus is Coenzyme Q-6, and the Diazonium Blue B reaction is negative. The type species of the genus is *Kazachstania viticola* (Vaughan-Martini *et al*., 2011).

The genus *Nakaseomyces* was proposed by Cletus P. Kurtzman in 2003 and established as an independent lineage by reclassifying several species formerly included in the genus *Candida* based on molecular phylogenetic analysis (Kurtzman, 2003). Asexual reproduction occurs via multilateral budding from a narrow base, with cells exhibiting spherical, ovoid, or elongate shapes, and neither pseudohyphae nor true hyphae are formed. Sexual reproduction involves the formation of deliquescent asci—produced either by conjugation between independent cells or via an unconjugated process—that contain 1−8 ascospores, which are reniform or bacilliform. *Nakaseomyces* species are capable of fermenting glucose, although galactose is neither fermented nor assimilated; nitrogen sources such as cadaverine, L-lysine, and ethylamine are scarcely utilized, and nitrate is not used. In addition, the primary

respiratory quinone produced by this genus is Coenzyme Q-6, and the Diazonium Blue B reaction is negative. The type species of the genus is *Nakaseomyces delphensis* (Takashima and Sugita, 2022).

The genus *Crinitomyces* belongs to the subphylum *Saccharomycotina* within the order *Saccharomycetales* and was reclassified as an independent genus based on molecular phylogenetic analyses, despite its close relationship with *Trichosporiella flavificans* and *Candida ghanaensis* (Sakpuntoon *et al*., 2022). Asexual reproduction in this genus occurs via multilateral budding from a narrow base, with cells that are either spherical or ellipsoidal in shape. In addition, true hyphae are formed and septate hyphae are observed. In contrast, sexual reproduction has not been observed, as no formation of asci has been detected. *Crinitomyces* species are capable of assimilating a variety of carbon sources and can ferment sugars such as glucose, galactose, and maltose, although nitrate is not utilized as a nitrogen source. Moreover, the primary respiratory quinone produced by this genus is Coenzyme Q-6; the Diazonium Blue B reaction is negative, and neither urea hydrolysis nor the production of starch-like compounds is observed. Members of *Crinitomyces* have been isolated from diverse environments, including rivers, sewage sludge, and wastewater treatment facilities. The type species of the genus is *Crinitomyces flavificans* (Sakpuntoon *et al*., 2022).

Description of *Nakaseomyces glabratus* D-7

Cells are oval-shaped, and budding is multilateral (Fig. 1). Colonies are convex, smooth, and white to light yellow colored after 3 days of incubation on YPD agar at 25°C. In the API 20C AUX test, strain D-7 is positive for glucose and D-trehalose; and negative for glycerol, calcium 2-keto-gluconate, L-arabinose, D-xylose, adonitol, xylitol, D-galactose, inositol, D-sorbitol, methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine, D-cellobiose, D-lactose, D-maltose, D-saccharose, D-melezitose and D-raffinose (Table 2).

Strain D-7 (HNIBR-FC132) was isolated from *Batillaria cumingii* collected in Mokpo-si, Jeollanam-do, Republic of Korea. The 26S rRNA gene sequence of strain D-7 has been deposited in GenBank under the accession number PV244027.

Table 2. Phenotypic characteristics of strain D-7 and related species.

	1	2	3	4
Growth on				
Temp (°C)	15−30	19−45	19−42	19−42
Assimilation of:				
Glucose	+	+	+	+
Glycerol	−	w	−	w
Calcium 2-Keto-Gluconate	−	v	ND	−
L-Arabinose	−	−	−	−
D-Xylose	−	−	−	−
Adonitol	−	−	−	−
Xylitol	−	−	−	−
D-Galactose	−	−	w	−
Inositol	−	−	w	−
D-Sorbitol	−	−	−	−
Methyl-α-D-Glucopyranoside	−	−	−	−
N-Acetyl-Glucosamine	−	−	−	ND
D-Cellobiose	−	−	−	−
D-Lactose	−	−	−	−
D-Maltose	−	w	w	−
D-Saccharose	−	−	+	−
D-Trehalose	+	v	w	+
D-Melezitose	−	−	−	−
D-Raffinose	−	−	−	−

Taxa: 1, *Nakaseomyces glabratus* D-7; 2, *Nakaseomyces glabratus* ATCC 2001^T; 3, *Nakaseomyces nivariensis* NRRL Y-48269^T; 4, *Nakaseomyces bracarensis* CBS 10154^T.
+, positive; w, weakly positive; −, negative; v, variable; ND, no data.

Table 3. Phenotypic characteristics of strains K-9 and K-22 and related species.

	1	2	3	4
Growth on				
Temp (°C)	15−30	15−30	15−40	15−37
Assimilation of:				
Glucose	+	+	+	v
Glycerol	−	−	+	+
Calcium 2-Keto-Gluconate	−	−	v	w
L-Arabinose	−	−	+	+
D-Xylose	−	−	+	+
Adonitol	−	−	v	+
Xylitol	−	−	ND	ND
D-Galactose	w	w	+	+
Inositol	−	−	−	w
D-Sorbitol	−	−	v	w
Methyl-α-D-Glucopyranoside	−	−	−	+
N-Acetyl-Glucosamine	+	+	+	+
D-Cellobiose	w	w	+	+
D-Lactose	−	−	−	−
D-Maltose	−	−	−	v
D-Saccharose	−	−	v	w
D-Trehalose	−	−	+	+
D-Melezitose	−	−	v	w
D-Raffinose	−	−	v	w

Taxa: 1, *Crinitomyces flavificans* K-9; 2, *Crinitomyces flavificans* K-22; 3, *Crinitomyces flavificans* CBS 760.79^T; 4, *Crinitomyces reliqui* DMKU FW23-23^T.
+, positive; w, weakly positive; −, negative; v, variable; ND, no data.

Table 4. Phenotypic characteristics of strain 11-2 and related species.

	1	2	3	4
Growth on				
Temp (°C)	15−30	19−37	19−35	19−35
Assimilation of:				
Glucose	−	+	+	+
Glycerol	−	−	−	−
Calcium 2-Keto-Gluconate	−	−	+	w
L-Arabinose	−	−	−	−
D-Xylose	−	−	−	−
Adonitol	−	−	−	−
Xylitol	−	−	−	−
D-Galactose	−	+	+	w
Inositol	−	−	−	−
D-Sorbitol	−	−	−	−
Methyl-α-D-Glucopyranoside	−	−	−	−
N-Acetyl-Glucosamine	−	−	−	ND
D-Cellobiose	−	−	−	−
D-Lactose	−	−	−	−
D-Maltose	−	−	−	−
D-Saccharose	−	−	−	+
D-Trehalose	−	w	+	w
D-Melezitose	−	−	−	w
D-Raffinose	−	−	−	+

Taxa: 1, *Kazachstania africana* 11-2; 2, *Kazachstania* africana NRRL Y-8276^T; 3, *Kazachstania martiniae* CBS 6334^T; 4, *Saccharomyces cariocanus* NRRL Y-27337^T.
+, positive; w, weakly positive; −, negative; v, variable; ND, no data.

Descriptions of *Crinitomyces flavificans* K-9, K-22

Cells are oval-shaped, and budding is multilateral from a narrow base (Fig. 1). Colonies are convex, filamentous, and white colored after 3 days of incubation on YPD agar at 25°C. In the API 20C AUX test, strains K-9 and K-22 are positive for glucose and *N*-acetyl-glucosamine; weakly positive for D-galactose and D-cellobiose; and negative for glycerol, calcium 2-keto-gluconate, L-arabinose, D-xylose, adonitol, xylitol, inositol, D-sorbitol, methyl-α-D-glucopyranoside, D-lactose, D-maltose, D-saccharose, D-trehalose, D-melezitose and D-raffinose (Table 3).

Strain K-9 (HNIBR-FC137) and Strain K-22 (HNIBR-FC144) were isolated from *Batillaria multiformis* collected in Mokpo-si, Jeollanam-do, Republic of Korea. The 26S rRNA gene sequences of strains K-9 and K-22 have been deposited in GenBank under the accession numbers PV244028 and PV244029, respectively.

Description of *Kazachstania africana* 11-2

Cells are oval-shaped, and budding is multilateral (Fig. 1). Colonies are convex, butyrous, and white to tan colored after 3 days of incubation on YPD agar at 25°C. In the API 20C AUX test, strain 11-2 is negative for all substrates; specifically, it does not utilize glucose, glycerol, calcium 2-keto-gluconate, L-arabinose, D-xylose, adonitol, xylitol, D-galactose, inositol, D-sorbitol, methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine, D-cellobiose, D-lactose, D-maltose, D-saccharose, D-trehalose, D-melezitose and D-raffinose (Table 4).

Strain 11-2 (HNIBR-FC176) was isolated from soil collected in Shinan-gun, Jeollanam-do, Republic of Korea. The 26S rRNA gene sequence of strain 11-2 has been deposited in GenBank under the accession number PV243 995.

Conflicts of Interest

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

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New records of the marine diatom parasites *Lagenisma coscinodisci* (Lagenismatales, Oomycetes) and *Paulsenella kornmannii* (Thoracosphaerales, Dinophyceae) in Korea

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This study presented the first record of two marine diatom parasites, *Lagenisma coscinodisci* (Oomycetes) and *Paulsenella kornmannii* (Dinophyceae), in Korea. Plankton samples were collected from Yongho Harbor, Busan, in March 2022, and diatoms infected by these parasites, *Coscinodiscus wailesii* by *L. coscinodisci* and *Eucampia zodiacus* by *P. kornmannii*, were isolated for morphological and molecular analyses. Both parasites exhibited developmental characteristics consistent with previous descriptions. The 18S rRNA gene sequences of *L. coscinodisci* (1,430 bp) and *P. kornmannii* (1,331 bp) were successfully obtained, with the latter being reported for the first time. Phylogenetic analysis placed *L. coscinodisci* within Oomycetes, clustering with *Thraustotheca clavata*, while *P. kornmannii* was positioned within Dinophyceae, closely related to *P. vonstoschii*. This study provides new insights into the biodiversity of parasitic protists in Korean waters and contributes the first molecular data for *P. kornmannii*.

Keywords: 18S rRNA, *Coscinodiscus wailesii*, *Eucampia zodiacus*, molecular phylogeny, morphology, parasitism

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Introduction

Diatoms are susceptible to infection by broad taxonomic groups of parasitic protists, including Aphelidiomycetes, Bigyromonadea, Chytridiomycetes, Dinophyceae, Ellobiopsea, Labyrinthulomycetes, Oomycetes, Phytomyxea, and Thecofilosea (Drebes and Schnepf, 1988; Schnepf *et al*., 1990; Cavalier-Smith, 1993; 2018; Kühn, 1997; Schweikert and Schnepf, 1996; Scholz *et al*., 2016; Dumack *et al*., 2017; Danz and Quandt, 2023; Cho and Kim, 2024). Among these, Oomycetes represent a diverse lineage of fungus-like protists that infect animals, plants, and algae in marine and freshwater ecosystems (Lee *et al*., 2015; Larousse and Galiana, 2017; Buaya and Thines, 2020; Buaya *et al*., 2021). *Lagenisma coscinodisci* Drebes, 1968 (Lagenismatales, Oomycetes) is a parasite of centric diatoms of the genus *Coscinodiscus* and is one of the few studies marine holocarpic pathogenic Oomycetes (Beakes *et al*., 2014). The species was initially reported as a fungal parasite, *Lagenidium* sp. infecting the marine diatom *Coscinodiscus centralis* from Hecate Strait, British Columbia (Parsons, 1962). Although Parsons illustrated the organism, he failed to describe sexual apparatus and sporangia using formalin-preserved plankton samples and only tentatively assigned it to *Lagenidium* sp. Later, in 1966, Drebes (1966) collected a very similar parasite to Parson's fungus from Helgoland (Germany). He clearly documented its morphology, asexual reproduction, and infection cycle through photomicroscopy, maintained the infection in laboratory-grown *Coscinodiscus* cultures. In 1968, Drebes formally

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named the fungus *Lagenisma coscinodisci*. Since then, the parasite has been observed in the North Sea (Drebes, 1966; 1968; 1974), along the west coast of the USA (Gotelli, 1971), and near Jamaica (Grahame, 1976).

The genus *Paulsenella* Chatton, 1920 (Dinophyceae) is an ectoparasitic dinoflagellate parasitizing marine diatoms. In 1911, Paulsen described an ectoparasite from the coastal waters of eastern Greenland, feeding on the diatoms *Chaetoceros borealis* and *Chaetoceros decipiens*, and named it tentatively *Apodinium chaetoceratis* Paulsen, 1911. Chatton (1920) later transferred it to a new genus, *Paulsenella* in his literature on parasitic dinoflagellates. Later, Drebes and Schnepf (1988) isolated three *Paulsenella* strains infecting *C. decipiens*, *Eucampia zodiacus*, and *Streptotheca thamesis*, respectively, from the Wadden Sea near List/Sylt (North Sea, Germany). They then compared their morphology, development, host range, and feeding mode using host-parasite cultures and described two new species, *Paulsenella kornmannii* Drebes & Schnepf, 1988 on *Eucampia zodiacus* and *Paulsenella vonstoschii* Drebes & Schnepf, 1988 on *Streptotheca thamesis*, based on their developmental processes and biological traits, including the timing of peduncle formation, the number of food vacuoles, and variation in cyst production (Drebes and Schnepf, 1988). The two *Paulsenella* species, *P. kornmannii* and *P. vonstoschii*, showed similar morphological characteristics, but they differed in host range and specificity. *P. kornmannii* exhibited high host specificity, infecting only the diatom *Eucampia zodiacus*, while *P. vonstoschii* showed a wide host range, infecting several diatom genera including *Streptotheca*, *Neostreptotheca*, *Lithodesmium*, and *Bellerochea* (Paulsen, 1911; Drebes and Schnepf, 1988; Konovalova, 2008; Purushothaman *et al*., 2023). To date, the molecular phylogenetic position has been reported only for *P. vonstoschii* (Kühn and Medlin, 2005). Therefore, molecular sequence data for other *Paulsenella* species are needed to elucidate the phylogenetic relationships within the genus.

In this study, the marine diatoms *Coscinodiscus wailesii* and *Eucampia zodiacus* were observed to be infected by the parasites *L. coscinodisci* and *P. kornmannii*, respectively, in Yongho Harbor, Busan, Korea in March 2022. We established parasite and host culture systems to examine their morphological and developmental characteristics as well as their 18S rRNA gene sequences. This study is the first to report the molecular phylogenetic position of *P. kornmannii* based on 18S rRNA gene sequences. Additionally, it presented the first record of these two parasitic species infecting marine diatoms in Korea.

Materials and Methods

Sample collection and cultivation

Plankton samples were collected using a 20 μm mesh net from the surface seawater at the Yongho Harbor of Busan in Korea (35°08′00″N, 129°06′55″E) in March 2022. Net samples were immediately transported to the laboratory within 15 min. The samples were stored at room temperature until further microscopic examination. Surface seawater temperature and salinity were measured *in situ* using a YSI Pro30 (YSI Inc. OH, USA).

Infected diatom cells were isolated using a capillary pipette, washed in serial drops of 0.2 μm syringe-filtered sterile seawater and put into a 24-well plate containing sterile seawater with naive host cells under an inverted microscope (Axio Imager A1, Carl Zeiss Inc., Oberkochen, Germany) (Yoo and Kim, 2020). Additionally, uninfected host diatom cells were isolated as describe above and placed into 24 well plate. Host cultures were maintained by weekly transfers with f/2 + Si medium (Guillard and Ryther, 1962). All cultures were incubated with a 14 : 10 light-dark cycle at 130 μmol photons m^{-2} s^{-1} at 20°C. The parasite cultures were transferred to naive host cultures twice a week.

For the specimen slide preparation, the culture of the diatom *C. wailesii* cells infected by the parasite *L. coscinodisci* strain MPL-CwLc01 and the net-tow sample of the diatom *Eucampia zodiacus* cells infected by the parasite *P. kornmannii* collected from the Yongho Harbor on March 14, 2022, were fixed with glutaraldehyde at a final concentration of 2%. Infected cells were individually isolated from the fixed samples and rinsed repeatedly with serial drops of distilled water. Glycerol gelatine was melted on a water bath at 60°C and dropped to a slide. The washed cells were mounted on the slide by mixing with glycerol gelatin and covered with a coverslip. The slides were deposited to the Honam National Institute of Biological Resources and the Nakdonggang National Institute of Biological Resources.

Light microscopy

Specimens were observed using an Axio Imager A2 (Carl Zeiss Inc., Oberkochen, Germany) and recorded at ×400 and ×630 magnifications using a digital cinema camera (Canon EOS R5C, Canon Inc., Tokyo, Japan) mounted on the microscope. Cell size was measured using Zen software (Carl Zeiss Inc.) based on still images extracted from video recordings. To examine the thecal plates of parasites, cells were fixed with glutaraldehyde (1% final concentration), stained with Calcofluor White (40 μg mL^{-1} final concentration), and observed under ultraviolet light (excitation at 360 nm and emission at 460 nm) using an Axio Imager A2 (Fritz and Triemer, 1985).

DNA extraction, PCR, and sequencing

Newly generated parasite zoospores were isolated, washed in serial drops of sterile filtered seawater, and put into a polymerase chain reaction (PCR) tube. DNA was extracted using the Chelex extraction method (Kim and

Park, 2014). The 18S rRNA gene regions were amplified using primer sets EUKA (5′-AAC CTG GTT GAT CCT GCC AGT-3′)/1209R (5′-GGG CAT CAC AGA CCT G-3′) or 328F (5′-ACC TGG TTG ATC CTG CCA G-3′)/329R (5′-TGA TCC TTC YGC AGG TTC AC-3′) (Giovannoni *et al*., 1988; Moon-van der Staay *et al*., 2000). PCR was performed by adding 2 μL of template DNA, 2 μL of each forward and reverse primer, and 14 μL of deionized sterile water to an AccuPower PCR premix (Bioneer, Daejeon, Korea). The reactions were performed using a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA) under the following conditions: an initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 45 s, and extension at 72°C for 45 s, with final extension at 72°C for 7 min (Cho and Kim, 2024). PCR products were visualized on 1% TAE agarose gels stained with Eco Dye (SolGent Co., Daejeon, Korea) and run at 110 V for 15 min. Purification was performed using Exo SAP-IT (Thermofisher Scientific). The purified products were then sequenced using the EUKA/1209R or 328F/329R primer sets with a Big-Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystem) and an ABI model 3730xl DNA Analyzer (Applied Biosystems: Foster City, CA, USA). Low-quality regions were trimmed, and sequence reads were assembled using ContigExpress (Vector NTI v. 10.1: Invitrogen, Grand Island, NY, USA). The resulting sequences were verified through a BLASTN search in NCBI.

Alignment and phylogenetic analyses

A total of 57 18S rRNA gene sequences, including three sequences of parasites generated from this study, along with 28 taxa of stramenopiles and 26 taxa of alveolata downloaded from GenBank (http://www. ncbi.nlm.nih.gov), were aligned using MAFFT v7.10 (Katoh and Standley, 2013; Katoh *et al*., 2019). The aligned dataset was trimmed the ambiguous site using MEGA11 (Tamura *et al*., 2021), and the resulting 1968 informative sites were applied for further phylogenetic analyses. Maximum likelihood analysis was performed using RAxML 8.0.0 (Stamatakis, 2014) based on the GTRGAMMA evolution model with 2,000 bootstrap replicates.

Results and Discussion

Taxonomic summary

Phylum Gyrista Cavalier-Smith, 1998 stat. n. 2018
Class Oomycetes Winter, 1880
Order Lagenismatales Dick, 2001
Family Lagenismataceae Dick, 2001
Genus *Lagenisma* Drebes, 1968

***Lagenisma coscinodisci* Drebes, 1968 (Fig. 1)**
Reference: Buaya *et al*. (2019).

Material examined. Marine water (salinity 32.2, temperature 12.1°C) collected from Yongho Harbor of Busan, Republic of Korea (35°08′00″N, 129°06′55″E) on March 02, 2022.
Accession number. PV110864.
Description. Zoospores are kidney-shaped and laterally biflagellate, measuring approximately 10.0 μm in length (range: 8.8–11.1 μm) and about 6.5 μm in width (range: 5.0–7.9 μm) *in vivo*. They attach to the exterior of the host frustule and subsequently penetrate into the host cell. Inside the host, the thallus initially develops as a small, undifferentiated structure that gradually expands. The thallus becomes visible within 12 hours following zoospore attachment. It spreads throughout the host cytoplasm, eventually occupying the entire host cavity. Approximately 18 hours after attachment, the thallus matures and differentiates into a zoosporangium, in which new zoospores are formed. The zoosporangium becomes densely packed with developing zoospores that gradually acquire motility. Around 22 hours after attachment, a discharge tube extends outside the host, within an hour, zoospores are released through the discharge tube. These motile zoospores actively exit the host, resume their free-swimming stage, and disperse to locate new hosts.
Host. *Coscinodiscus wailesii* Gran & Angst, 1931.
Distribution. Puget Sound, USA (Gotelli, 1971), Kingston Harbour, Jamaica (Grahame, 1976), Wadden Sea near List/Sylt, Germany (Schnepf and Drebes, 1977), Oosterschelde, Netherlands (Wetsteyn and Peperzak, 1991), and Helgoland Roads, Germany (Buaya *et al*., 2019)
Remark. The Korean strain of *Lagenisma coscinodisci* exhibited developmental characteristics identical to those reported by Gotelli in 1971 (Fig. 1). While the previously reported strain has a zoospore size of 7 × 8 μm, the Korean strain measured 6.5 × 10 μm, showing a similar width but a slightly greater length. The surface water temperature range for *L. coscinodisci* has been reported as 8.2–20.2°C (Wetsteyn and Peperzak, 1991); however, the Korean strain was observed within a narrower range of 12.1–13.5 °C. Similarly, while the documented salinity for *L. coscinodisci* is 29.8–32.4, the Korean strain was found within a range of 31.2–32.2. Previous studies have reported *L. coscinodisci* infecting various diatom species, including *Coscinodiscus concinnus*, *C. granii*, *C. wailesii*, and other *Coscinodiscus* species (Drebes, 1966; Chakravarty, 1974). However, in Korean field samples, despite the presence of various *Coscinodiscus* species, parasite infections were observed only in *C. wailesii*.
Voucher slide. Slide with glutaraldehyde-fixed specimens was deposited at the Honam National Institute of Biological Resources (HNIBRPR41).

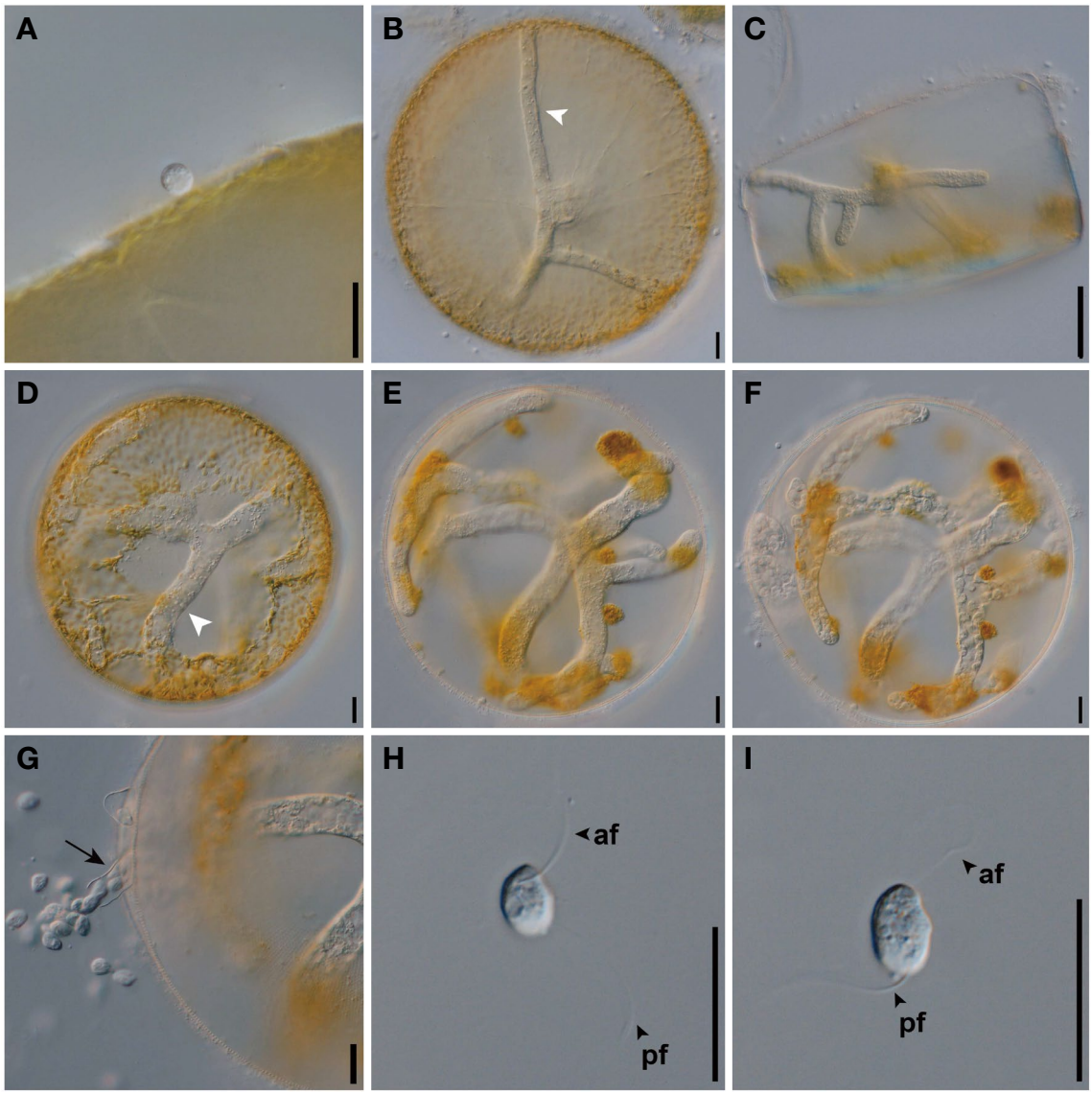


Fig. 1. Light micrographs showing *Lagenisma coscinodisci* infecting the marine diatom *Coscinodiscus wailesii*. (A) A zoospore attached to the host frustule. (B) Valve view of the diatom host showing the holocarpic formation of the parasite zoosporangium (white arrowhead) inside the host. (C) Lateral view of the diatom possessing a zoosporangium. (D) The growth stage of the thallus which splits into branches. (E) The thallus becomes more voluminous. (F) The thallus transforms into a zoosporangium inside *C. wailesii*. (G) Formation of zoospores and protrusion of a discharge tube (black arrow) from the host cell. (H) Flagella (black arrowhead) are laterally inserted. (I) Kidney-shaped zoospore. af; anterior flagellum, pf; posterior flagellum. Scale bars represent 20 μm.

Phylum Miozoa Cavalier-Smith, 1987 stat. n. 1999
Class Dinophyceae Pascher, 1914
Order Thoracosphaerales Tangen, 1982
Family Thoracosphaeraceae Schiller, 1930 emend. Tangen, 1982
Genus *Paulsenella* Chatton, 1920

***Paulsenella kornmannii* Drebes & Schnepf, 1988 (Fig. 2)**
Reference: Drebes and Schnepf (1988).

Material examined. Marine water (salinity 31.2, temperature 13.5°C) collected from Yongho Harbor of Busan, Republic of Korea (35°08′00″N, 129°06′55″E) on March 14, 2022.
Accession number. PV110865.
Description. The dinospore has a hemispherical or conical epitheca and a hemispherical hypotheca, measuring approximately 16.2 μm in length (range: 14.5–17.6 μm) and 12.8 μm in width (range: 11.8–14.5 μm) *in vivo*. It penetrates the host through an intercingular slit located in the host's girdle and forms a peduncle inside the host approximately 30 minutes after attachment. Using the peduncle,

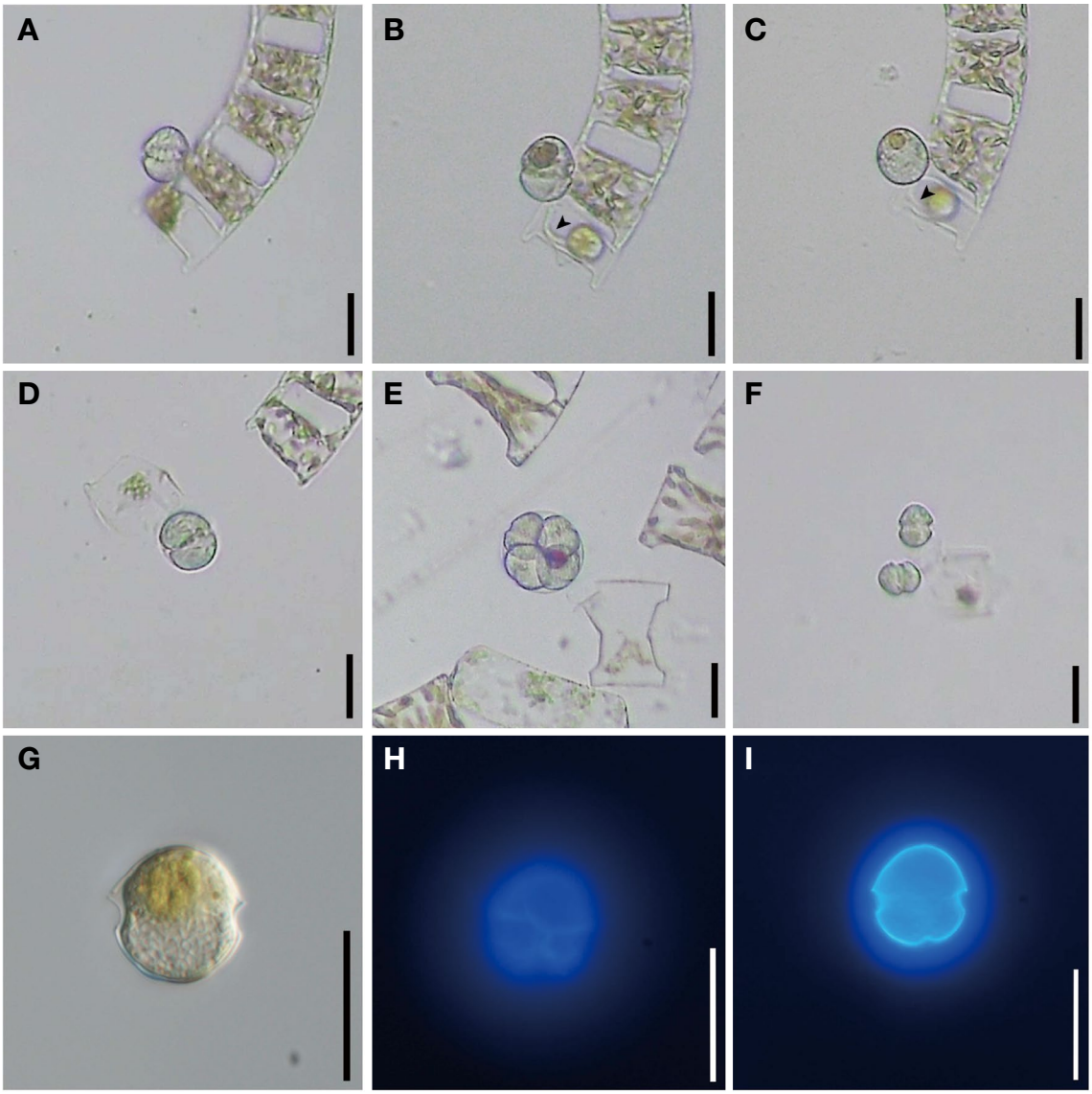


Fig. 2. Light micrographs showing *Paulsenella kornmannii* infecting the marine diatom *Eucampia zodiacus*. (A) A dinospore attached to the host frustule. (B) The parasite feeding on the host material using a peduncle (arrowhead). (C) The parasite transforming into an encysted trophont. (D) The first division of the encysted trophont. (E) The second division of the encysted trophont. (F) Newly produced dinospores being released from the cyst. (G–I) Dinospore with thecal plates. (H, I) Calcofluor-stained thecal plates of the dinospore. Scale bars represent 20 μm.

the dinospore absorbs the host materials and develops a single large vacuole. Within four hours after the peduncle is formed, the entire host cytoplasm is consumed. After feeding, it changes into a round shaped and transforms into an encysted trophont. During the trophont stage, it lacks both cingulum and sulcus. Approximately 19 hours after attachment, the first cell division occurs. The encysted trophont subsequently undergoes a series of divisions, ultimately producing two to four dinospores. Division is complete approximately 28 hours after attachment, after which the newly formed dinospores are released to locate new hosts.

Host. *Eucampia zodiacus* Ehrenberg, 1839.
Distribution. Wadden Sea near List/Sylt, Germany (Drebes and Schnepf, 1988).
Remark. The Korean strain of *Paulsenella kornmannii* exhibits morphological and developmental characteristics consistent with those described by Drebes and Schnepf (1988). Similar to the strain from the German Bight, the Korean strain has a hemispherical or conical epitheca and a hemispherical hypotheca. The Korean strain measures 14.5–17.6 μm in length and 11.8–14.5 μm in width, closely aligning with the size range reported for the German strain (15–19 μm in length, 12–15 μm in width). Both strains

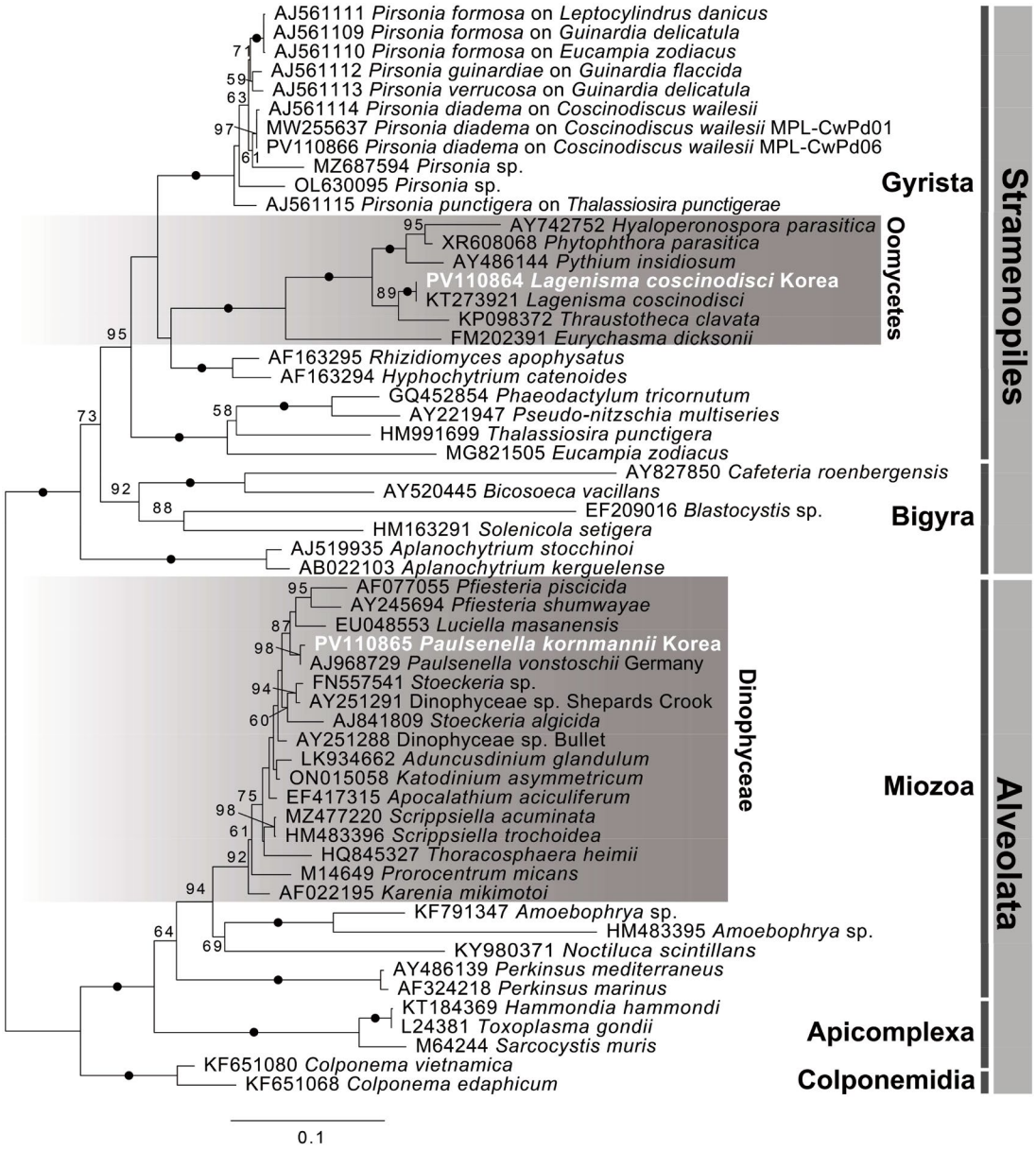


Fig. 3. Maximum likelihood phylogeny inferred from 18S rRNA gene sequences. Sequences generated from this study are presented in white. Bootstrap values (over 50%) are shown above nodes. Closed circles indicate robust support with 100% bootstrap values.

share key developmental traits, including penetration through the intercingular slit in the host's girdle region and formation of a peduncle upon attachment. Additionally, both develop a single large food vacuole and exhibit similar division patterns, producing two to four dinospores. Several developmental differences have been reported between the morphologically similar species *P. kornmannii* and *P. vonstoschii*. Among these developmental differences, *P. kornmannii* lacks the refractive granules and loses its flagella more rapidly than *P. vonstoschii*. In addition, *P. kornmannii* often extrudes the residual body of the food vacuole during the final cell division, whereas in *P. vonstoschii*, the residual body is typically retained within the dinospore (Drebes and Schnepf, 1988). The Korean strain of *P. kornmannii* was observed at temperatures ranging from 13.5 to 14.5°C and at a salinity of 31.2–31.8. However, environmental conditions for the German strain were not specified in the original description. Host specificity also remains consistent, as both strains infect *Eucampia zodiacus*.

Voucher slide. Slide with glutaraldehyde-fixed specimens was deposited at the Nakdonggang National Institute of Biological Resources (NNIBRDN2210).

rRNA gene sequences and molecular phylogeny

The 18S rRNA gene sequences of the two parasites, *L. coscinodisci* and *P. kornmannii*, were successfully obtained, with sequence lengths of 1,430 bp and 1,331 bp, respectively. Notably, this study represents the first publication of the molecular sequence of *P. kornmannii*. A BLASTN search revealed that the sequence of *P. kornmannii*, which infects the diatom *E. zodiacus*, exhibited 99.47% similarity to *P. vonstoschii* (AJ968729), a parasite infecting the diatom *Streptotheca thamesis*, isolated from the Wadden Sea near List/Sylt, Germany (Kühn and Medlin, 2005). The 18S rRNA gene sequence of the Korean strain of *L. coscinodisci* partially matched the Helgoland strain (KT273921) with 100% identity, but with relatively low query coverage (66%, 943 bp). Additionally, it showed 98.9% identity and 100% query coverage with *Crypticola clavulifera* (MF066365), an Oomycetes pathogen of mosquito larvae (Frances *et al*., 1989; Mendoza *et al*., 2018).

A maximum likelihood (ML) phylogenetic tree inferred from the 18S rRNA sequences demonstrated that the two parasitic species infecting marine diatoms belong to two distinct phyla, Miozoa and Gyrista (Fig. 3). The Korean and Helgoland (KT273921) strains of *L. coscinodisci* clustered together with *Thraustotheca clavata* (KP098372) with high bootstrap support (89%) and formed a sister lineage to a clade containing *Hyaloperonospora parasitica*, *Phytophthora parasitica*, and *Pythium insidiosum* within the class Oomycetes (Fig. 3). The Korean strain of *P. kornmannii* grouped closely with *P. vonstoschii* with strong bootstrap support (98%) and formed a sister lineage to a clade containing *Pfiesteria* and *Luciella* species, which received moderate bootstrap support (87%), within the class Dinophyceae (Fig. 3).

Conflicts of Interest

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

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A preliminary study of the butterfly diversity in Arusha region, northern Tanzania: Part 1

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Tanzania is one of the most butterfly-diverse countries in Africa, hosting numerous endemic species due to its diverse habitats and climatic conditions. Despite the presence of high biomass within key national parks, such as Kilimanjaro, Ngorongoro, and Manyara, studies on butterfly diversity in northern Tanzania remain fragmented and insufficient. To address this gap, we conducted a butterfly diversity survey in the Arusha region of northern Tanzania in 2024, collecting various butterfly species through field sampling. Additionally, specimens from northern Tanzania, housed at the Tanzania Wildlife Research Institute (TAWIRI), were cataloged and photographed. As a result, we identified and recorded 82 species, belonging to 49 genera, 14 subfamilies, and five families in Arusha region. This study is the first in a series of publications specifically summarizing butterfly diversity in northern Tanzania, providing structured baseline data on the previously unorganized butterfly diversity in the Arusha region. Furthermore, it includes ecological information and photographic data for all examined species, along with detailed collection site information from the surveyed regions.

Keywords: afrotropical region, eastern Africa, fauna, Lepidoptera, Papilionoidea

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Introduction

Tanzania, located between 1–11.75°S and 29.7–40.4°E, covers approximately 947,303 km^2 and encompasses a diverse range of climates and habitats, including rainforests, alpine regions, savannas, and coastal ecosystems. This environmental heterogeneity supports the country's high species diversity of flora and fauna (Kielland, 1990; Noort and Stone, 2000).

Currently, more than 1,400 butterfly species have been recorded in Tanzania, making it one of the biodiverse regions in East Africa, surpassing neighboring countries such as Kenya, Uganda, and Malawi (Kielland, 1990; Congdon and Collins, 1998; Noort and Stone, 2000). The country contains over 120 endemic butterfly species, which are associated with diverse topography, climates, and species differentiation hotspots, such as the Eastern Arc Mountains, known for their high endemism (Noort and Stone, 2000). However, ongoing threats such as deforestation, habitat loss, inadequate protected areas, and climate change are contributing to a rapid decline in butterfly diversity, placing several endemic species at risk of extinction (Noort and Stone, 2000).

Kielland (1990) conducted an extensive and comprehensive study on Tanzanian butterflies, later published as a book systematically detailing their distribution and ecol-

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ogy. Since then, various researchers have continued to investigate butterfly diversity and abundance in Tanzania; however, due to the country's vast size and diverse habitat characteristics, most studies have remained localized and fragmented (Cordeiro, 1990). In particular, northern Tanzania is an ecologically significant region that hosts several national parks, including Ngorongoro, Kilimanjaro, Arusha, Tarangire, and Manyara National Parks. Despite its exceptionally high wildlife biomass, this region remains largely understudied in terms of butterfly diversity (Coe *et al*., 1976; Loth and Prins, 1986; Cordeiro, 1990; 1995). To date, researchers such as Cordeiro (1990; 1995), and Liseki and Vanc-Wright (2011; 2013; 2014; 2016; 2018) have primarily focused their studies on Manyara and Kilimanjaro National Park. However, intensive research on the butterfly diversity of the Arusha region has yet to be conducted.

Therefore, this study examined butterfly diversity in the Arusha region of Tanzania, where research remains insufficient, through two field surveys conducted in 2024, during which specimens deposited at the Tanzania Wildlife Research Institute (TAWIRI) were analyzed, identifying a total of 82 butterfly species across five genera and 49 families. Furthermore, collection data and photographs of all identified species are provided as foundational resources for future butterfly research and conservation strategies in northern Tanzania.

Materials and Methods

Specimen collection

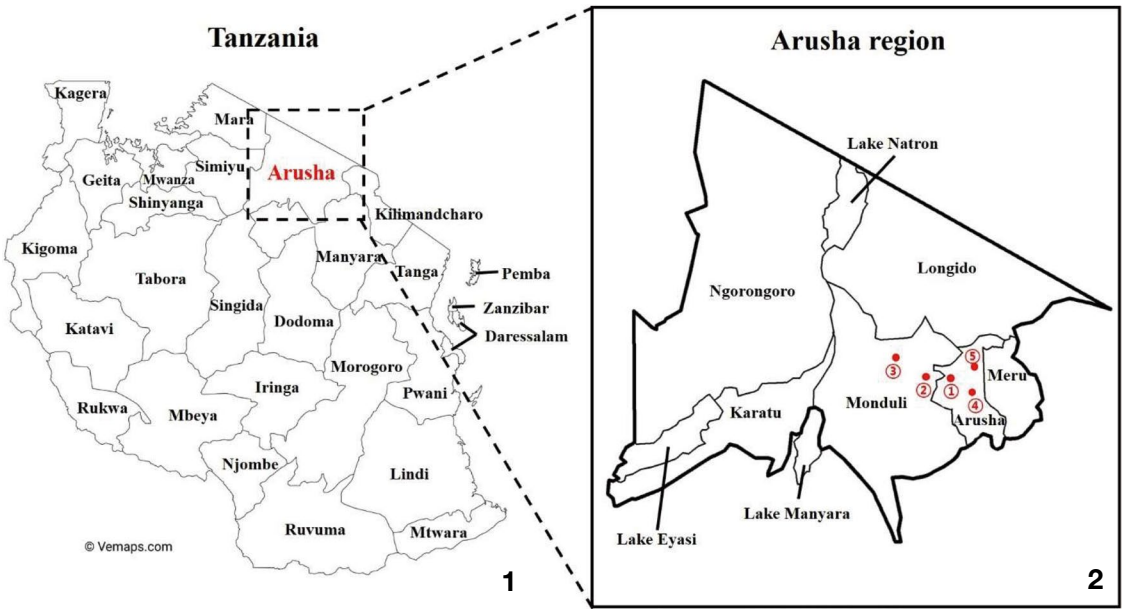
The specimens used in this study were collected during expeditions to Arusha region, Tanzania, in 2024, under the Memorandum of Understanding (MOU) between the National Institute of Biological Resources (NIBR), Ministry of Climate, Energy and Environment, Korea, and the Tanzania Wildlife Research Institute (TAWIRI), Tanzania, for cooperation concerning biological resources and information.

Collecting methods and photography

Butterflies were collected during the daylight hours using butterfly nylon nets (Bugdorm, Inc., Taiwan) from various habitats (Figs. 84–87). The collected specimens are deposited at the National Institute of Biological Resources (NIBR), Ministry of Climate, Energy and Environment, Incheon, Korea. Examined specimens were photographed using a Canon EOS 5Ds digital camera equipped with a Canon MP-E 65 mm F2.8 1–5× Macro photo lens (Canon, Inc., Japan) and illuminated with a Leica 5000HDI lamp. Specimens deposited at the Tanzania Wildlife Research Institute (TAWIRI) were photographed using a Nikon D810 digital camera paired with a Nikon AF-S 105 mm macro lens (Nikon, Inc., Japan).

Identification and classification

Butterfly identification in this study was based on data from Kielland (1990), Larsen (1991), Williams (2022), and articles published in the Journal of the Lepidoptera Society of Africa (Metamorphosis, metamorphosis.org.za). The classification of families follows Nieukerken *et al*. (2011), while subfamilies, genus and species are arranged in alphabetical order.



Figs. 1, 2. 1) Administrative map of Tanzania; 2) Administrative districts of the Arusha Region (Red dots indicate collecting sites listed in Table 1).

Collecting sites

The collecting sites in the Arusha region of Tanzania are listed in Table 1 and Figs. 1, 2.

Results and Discussion

In this study, a total of 82 butterfly species, representing 49 genera, 14 subfamilies, and five families, were identified. Specimens were collected during fieldwork conducted in 2024 and were further supplemented by the examination of reference specimens housed at the Tanzanian Wildlife Research Institute (TAWIRI). Although some specimens appeared to represent subspecies or exhibited notable morphological variation, all individuals were identified and classified at the species level to maintain consistency in this comprehensive and ongoing study. The complete list of examined species is provided in Table 2.

While the 82 butterfly species recorded represent only a small fraction of the approximately 1,400 species known

Table 1. Collecting sites in Arusha region, Tanzania.

Number	Sites	GPS information	Date	Altitude
1	Burka, Olasiti	S3°23′35.52″ E36°38′02.39″	29. Aug. 2024	1,346 m
2	Engorora village, Kisongo	S3°22′50.75″ E36°33′30.90″	03. Sep. 2024	1,346 m
3	Engutoto village, Monduli	S3°18′19.0″ E36°28′09.8″	14. May. 2024; 28. Aug. 2024	1,586 m
4	KARAMA Lodge, Arusha Urban	S3°22′47.0″ E36°42′53.0″	08. May. 2024	1,414 m
5	TAWIRI forest, Arusha Urban	S3°24′45.0″ E36°42′22.0″	08. May. 2024; 11. May. 2024; 27. Aug. 2024	1,308 m

Table 2. List of butterfly examined species in the Arusha region of Tanzania.

Number	Family	Subfamily	Species	Common name
1	Papilionidae	Papilioninae	*Graphium leonidas*	Veined swordtail
2	Papilionidae	Papilioninae	*Papilio dardanus*	Mocker swallowtail
3	Papilionidae	Papilioninae	*Papilio demodocus*	Citrus swallowtail
4	Papilionidae	Papilioninae	*Papilio echerioides*	White-banded swallowtail
5	Papilionidae	Papilioninae	*Papilio hornimani*	Horniman's swallowtail
6	Papilionidae	Papilioninae	*Papilio ophidicephalus*	Emperor swallowtail
7	Papilionidae	Papilioninae	*Papilio thuraui*	Black malachite
8	Hesperiidae	Hesperiinae	*Acleros mackenii*	Macken's dart
9	Hesperiidae	Hesperiinae	*Artitropa erinnys*	Bos-skemervegter
10	Hesperiidae	Hesperiinae	*Borbo fatuellus*	Long horned swift
11	Hesperiidae	Hesperiinae	*Borbo lugens*	Lesser-horned swift
12	Hesperiidae	Hesperiinae	*Celaenorrhinus ovalis*	Evans'orange sprite
13	Hesperiidae	Hesperiinae	*Metisella quadrisignatus*	Four spot sylph
14	Hesperiidae	Hesperiinae	*Zenonia zeno*	Orange-spotted skipper
15	Hesperiidae	Pyrginae	*Eagris sabadius*	Spotted sylph
16	Hesperiidae	Pyrginae	*Netrobalane canopus*	Buff-tipped skipper
17	Hesperiidae	Pyrginae	*Sarangesa haplopa*	–
18	Pieridae	Coliadinae	*Catopsilia florella*	African migrant
19	Pieridae	Coliadinae	*Eurema brigitta*	Broad-bordered grass yellow
20	Pieridae	Coliadinae	*Eurema desjardinsii*	Angled grass yellow
21	Pieridae	Coliadinae	*Eurema hapale*	Marsh grass yellow
22	Pieridae	Coliadinae	*Eurema senegalensis*	Forest grass yellow
23	Pieridae	Coliadinae	*Nepheronia thalassina*	Cambridge vagrant
24	Pieridae	Pierinae	*Belenois creona*	African common white
25	Pieridae	Pierinae	*Belenois gidica*	African veined white

Table 2. Continued.

Number	Family	Subfamily	Species	Common name
26	Pieridae	Pierinae	*Belenois raffrayi*	Raffray's white
27	Pieridae	Pierinae	*Belenois zochalia*	Forest white
28	Pieridae	Pierinae	*Colotis aurigineus*	African golden arab
29	Pieridae	Pierinae	*Colotis aurora*	Sulphur orange tip
30	Pieridae	Pierinae	*Colotis auxo*	Yellow orange tip
31	Pieridae	Pierinae	*Colotis euippe*	Smoky orange tip
32	Pieridae	Pierinae	*Colotis eunoma*	Dune crimson tip
33	Pieridae	Pierinae	*Leptosia alcesta*	African wood white
34	Pieridae	Pierinae	*Mylothris agathina*	Common dotted border
35	Pieridae	Pierinae	*Mylothris rueppellii*	Twin dotted border
36	Lycaenidae	Poritiinae	*Baliochila lipara*	Lipara buff
37	Lycaenidae	Poritiinae	*Pentila pauli*	Paul's pentila
38	Lycaenidae	Miletinae	*Lachnocnema bibulus*	Common woolly legs
39	Lycaenidae	Polyommatinae	*Anthene larydas*	Forest hairtail
40	Lycaenidae	Polyommatinae	*Azanus jesous*	African babul blue
41	Lycaenidae	Polyommatinae	*Azanus natalensis*	Natal babul blue
42	Lycaenidae	Polyommatinae	*Cacyreus lingeus*	Common bush blue
43	Lycaenidae	Polyommatinae	*Lampides boeticus*	Pea blue butterfly
44	Lycaenidae	Polyommatinae	*Leptotes pirithous*	Common zebra blue
45	Lycaenidae	Polyommatinae	*Zizeeria knysna*	African grass blue
46	Lycaenidae	Polyommatinae	*Zizina otis*	Dark grass blue
47	Lycaenidae	Polyommatinae	*Zizula hylax*	Tiny grass blue
48	Lycaenidae	Theclinae	*Chloroselas pseudozeritis*	Brilliant gem
49	Nymphalidae	Charaxinae	*Charaxes brutus*	White-barred emperor
50	Nymphalidae	Charaxinae	*Charaxes candiope*	Green-veined emperor
51	Nymphalidae	Charaxinae	*Charaxes varanes*	Southern african pearl emperor
52	Nymphalidae	Charaxinae	*Euxanthe wakefieldi*	Southern empress
53	Nymphalidae	Danainae	*Amauris albimaculata*	Layman
54	Nymphalidae	Danainae	*Amauris niavius*	Friar
55	Nymphalidae	Danainae	*Danaus chrysippus*	Plain tiger
56	Nymphalidae	Danainae	*Tirumala formosa*	Beautiful tiger
57	Nymphalidae	Heliconiinae	*Acraea acerata*	Sweet potato butterfly
58	Nymphalidae	Heliconiinae	*Acraea aganice*	Wanderer
59	Nymphalidae	Heliconiinae	*Acraea cabira*	Yellow-banded Acraea
60	Nymphalidae	Heliconiinae	*Acraea encedon*	White-barred Acraea
61	Nymphalidae	Heliconiinae	*Acraea jodutta*	Jodutta Acraea
62	Nymphalidae	Heliconiinae	*Acraea quadricolor*	Four-colour wanderer
63	Nymphalidae	Nymphalinae	*Catacroptera cloanthe*	Pirate
64	Nymphalidae	Nymphalinae	*Eurytela dryope*	Golden piper
65	Nymphalidae	Nymphalinae	*Hypolimnas misippus*	Danaid eggfly
66	Nymphalidae	Nymphalinae	*Junonia oenone*	Blue pansy
67	Nymphalidae	Nymphalinae	*Junonia orithya*	Eyed pansy
68	Nymphalidae	Nymphalinae	*Junonia sophia*	Little pansy
69	Nymphalidae	Nymphalinae	*Junonia terea*	Soldier pansy
70	Nymphalidae	Nymphalinae	*Junonia hierta*	Yellow pansy
71	Nymphalidae	Nymphalinae	*Junonia natalica*	Brown pansy
72	Nymphalidae	Nymphalinae	*Neptis penningtoni*	Speckled banded sailer
73	Nymphalidae	Nymphalinae	*Neptis saclava*	Malagasy spotted sailer
74	Nymphalidae	Nymphalinae	*Precis octavia*	Gaudy commodore

Table 2. Continued.

Number	Family	Subfamily	Species	Common name
75	Nymphalidae	Nymphalinae	*Precis tugela*	Dry-leaf commodore
76	Nymphalidae	Nymphalinae	*Protogoniomorpha anacardii*	Clouded mother-of-pearl
77	Nymphalidae	Nymphalinae	*Protogoniomorpha parhassus*	Mother-of-pearl
78	Nymphalidae	Nymphalinae	*Vanessa abyssinica*	Abyssinian admiral
79	Nymphalidae	Satyrinae	*Bicyclus safitza*	Common savanna bush brown
80	Nymphalidae	Satyrinae	*Bicyclus campus*	Hill bush brown
81	Nymphalidae	Satyrinae	*Heteropsis perspicua*	Marsh patroller
82	Nymphalidae	Satyrinae	*Melanitis leda*	Common evening brown
Total	5 families	14 subfamilies	82 species	

from Tanzania, this study provides valuable baseline data for future biodiversity research. It also helps address the current gap in documented butterfly diversity in northern Tanzania. Future research should aim to broaden both the geographic and temporal scope of surveys and employ a wider range of collection methods. Building on the findings of this study, we plan to carry out continuous butterfly surveys and maintain systematic records to advance and expand lepidopteran research in the region. This paper marks the first in a series of ongoing publications dedicated to the Northern Tanzania Butterfly Study.

Order Lepidoptera Linnaeus, 1758
Superfamily Papilionoidea Latreille, 1802
Family Papilionidae Latreille, 1802
Subfamily Papilioninae Latreille, 1802

***Graphium leonidas* (Fabricius, 1793) (Fig. 8)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Forest, 25.III.2022, (W. Sengo, A. Pauly), (S3°24′41.0″, E36°42′15.0″ Alt: 1,300 m), deposited in TAWIRI.
Distribution. Sub-Saharan Africa.
Remarks. This species inhabits savannahs and forest edges, occurring at elevations of up to 2,200 m above sea level altitude. Its flight is fast, erratic, and highly mobile, enabling long-distance dispersal. It frequently visits flowers and often gathers at mud pools (Williams, 2022).

***Papilio dardanus* Brown, 1776 (Fig. 7)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Ngurdoto campsite, 06.IV.2023, (J. Mbulu), (S03°17′14.2″, E36°54′03.7″ Alt: 1,460 m), deposited in TAWIRI.
Distributions. Sub-Saharan Africa.
Remarks. This species is widely distributed and commonly found in evergreen and riverside forests, as well as in dry forests in West Africa. In Tanzania, it occurs at elevations between 780 and 2,100 m above sea level. It is frequently attracted to flowers, as well as to puddles and decaying organic matter (Kielland, 1990; Williams, 2022).

***Papilio demodocus* Esper, [1798] (Fig. 4)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 08.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Sub-Saharan Africa.
Remarks. This species inhabits a wide range of environments, except for deserts and alpine regions, and is commonly found in parks and gardens. In Tanzania, it occurs at elevations of up to 2,600 m above sea level, while in Madagascar, it can survive in urban environments. It frequently visits flowers and occasionally congregates near rivers or puddles. Additionally, it is capable of long-distance travel alongside other species, participating in mixed butterfly mass migrations. At night, individuals gather in groups on tree trunks or grass stalks, exhibiting communal roosting behavior (Williams, 2022).

***Papilio echerioides* Trimen, 1868 (Fig. 9)**

Material examined. (3♂, 2♀), TANZANIA, ARUSHA: 3♂, 2♀, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Cameroon, Equatorial Guinea, Angola, Democratic Republic of Congo, Sudan, Ethiopia, Uganda, Rwanda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland.
Remarks. This species inhabits temperate and montane evergreen forests and occurs in Tanzania at elevations ranging from 250 to 2,400 m. It has relatively weak flight capability and typically flies close to the ground. It is often attracted to mud pools and animal waste (Williams, 2022).

***Papilio hornimani* Distant, 1879 (Fig. 5)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂,



Figs. 3, 4. Papilionidae. 3) *Papilio ophidicephalus*, male; 4) *Papilio demodocus*, male.

Maji ya chai river, 06.IV.2023, (J. Mbulu), (S03°18′10.0″, E36°52′57.0″ Alt: 1,399 m), deposited in TAWIRI.
Distribution. Kenya, Tanzania.
Remarks. This species primarily inhabits forests and occurs at elevations between 300 and 2,400 m above sea level in Tanzania. It has strong flight capabilities and is highly mobile. It frequently visits flowers and often gathers at mud pools (Kielland, 1990; Williams, 2022).

***Papilio ophidicephalus* Oberthür, 1878 (Fig. 3)**

Material examined. (2♂), TANZANIA, ARUSHA: 2♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Democratic Republic of Congo, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland.
Remarks. This species primarily inhabits evergreen forests and occurs in Tanzania at elevations ranging from 0 to 2,000 m. Its flight is slow and undulating, often observed near forested canyons and streams. It frequently visits flowers and often gathers at mud pools (Kielland, 1990; Williams, 2022).

***Papilio thuraui* Karsch, 1900 (Fig. 6)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Iringa, Ilamba, 12−18.V.2023, (W. Sengo), (S08°03′59.0″, E35°52′49.7″ Alt: 1,977 m), deposited in TAWIRI.
Distribution. Democratic Republic of Congo, Tanzania, Malawi, Zambia.
Remarks. This species was collected from southern Tanzania but is specifically recorded here to document and organize the specimen collection at TAWIRI. It primarily inhabits montane forests and occurs at elevations between 1,300 and 2,600 m above sea level in Tanzania. It frequently visits flowers and often gathers at mud pools (Kielland, 1990; Williams, 2022).

Family Hesperiidae Latreille, 1809
Subfamily Hesperiinae Latreille, 1809

***Acleros mackenii* (Trimen, 1868) (Fig. 19)**

Material examined. (3♂, 1♀), TANZANIA, ARUSHA: 2♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24’45.0″, E36°42’22.0″ Alt: 1,308 m); 1♀, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♂, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Sub-Saharan Africa.
Remarks. This species primarily inhabits humid regions and occurs at elevations of up to 2,000 m above sea level in Tanzania. It usually flies low along forest edges or in open spaces, often resting on leaves or grass. It frequently visits flowers for nectar and is also known to obtain moisture from bird droppings (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Artitropa erinnys* (Trimen, 1862) (Fig. 10)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Forest, 11.VII.2022, (A. Pauly), (S3°24′42.0″, E36°42′16.0″ Alt: 1,300 m), deposited in TAWIRI.
Distribution. Ethiopia, Kenya, Tanzania, Malawi, Mozambique, Zimbabwe, South Africa, Swaziland, Comoro Islands.
Remarks. This species primarily inhabits forests or shade near streams and occurring at altitude of 900 to 1,500 m above sea level in Tanzania. It is primarily active at dusk and frequently visits flowers for nectar (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Borbo fatuellus* (Hopffer, 1855) (Fig. 12)**

Material examined. (2♂), TANZANIA, ARUSHA: 2♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).



Figs. 5–9. Papilionidae. 5) *Papilio hornimani*, male; 6) *Papilio thuraui*, male; 7) *Papilio dardanus*, male; 8) *Graphium leonidas*, male; 9) *Papilio echerioides* (a. male, dorsal view; b. male, ventral view; c. female, dorsal view; d. female, ventral view).

Distribution. Sub-Saharan Africa.

Remarks. This species primarily inhabits wet forests, moist woodlands, and coastal bush, occurring at altitudes of up to 2,100 m above sea level in Tanzania. It is a common species, often resting on shrubs or grass stalks. It frequently visits flowers for nectar and often gathers at mud pools (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Borbo lugens* (Hopffer, 1855) (Fig. 13)**

Material examined. (2♂), TANZANIA, ARUSHA: 2♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).

Distribution. Democratic Republic of Congo, Uganda, Kenya, Tanzania, Malawi, Mozambique, Zimbabwe, South Africa.

Remarks. This species inhabits grassy clearings within dense woodlands and forests, occurring at altitudes of up to 2,000 m above sea level in Tanzania. It is a relatively common species, primarily active in the morning It frequently visits flowers for nectar and is often observed near water courses (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Celaenorrhinus ovalis* Evans, 1937 (Fig. 17)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Monduli, 14.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03° 18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Sierra Leone, Liberia, Ivory Coast, Ghana, Nigeria, Cameroon, Gabon, Congo, Central African Republic, Democratic Republic of Congo, Uganda, Kenya.
Remarks. This species inhabits forests, and considered relatively rare (Larsen, 1991; 2005; Williams, 2022).

***Metisella quadrisignatus* (Butler, 1894) (Fig. 16)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Monduli, 14.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03° 18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Uganda, Kenya, Tanzania, Malawi.
Remarks. This species inhabits wet grasslands, montane forest clearings, and open areas, occurring at altitudes of 1,700 to 2,600 m in Tanzania. It has a relatively slow flight and frequently visits flowers for nectar (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Zenonia zeno* (Trimen, 1864) (Fig. 18)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Monduli, 14.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03° 18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Nigeria, Cameroon, Angola, Democratic Republic of Congo, Ethiopia, Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, South Africa.
Remarks. This species inhabits forests, dense savannas, and coastal bush. In Nigeria, it is found on grassy slopes of the plateau, while in Tanzania, it occurs at altitudes of 400 to 2,600 m. It is a common species and is frequently observed along forest edges and near streams. It has a fast flight and primarily perches on shrubs or the ground (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

Subfamily Pyrginae Burmeister, 1878

***Eagris sabadius* (Gray, 1832) (Fig. 15)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Monduli, 14.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03° 18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Madagascar, Mauritius, Reunion, Seychelles, Comoro Islands.
Remarks. This species inhabits montane forests and occurs at altitudes of 400 to 2,000 m above sea level in Tanzania. It has a fast and irregular flight, and when resting on leaves or the ground, its wings are fully spread out (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Netrobalane canopus* (Trimen, 1864) (Fig. 11)**

Material examined. (1♀), TANZANIA, ARUSHA: 1♀, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Guinea, Sierra Leone, Ivory Coast, Ghana, Nigeria, Cameroon, Democratic Republic of Congo, Sudan, Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland.
Remarks. This species inhabits moist savannas and forests and occurs at altitudes of 0 to 1,700 m above sea level in Tanzania. It is common in the eastern and southern regions but rare in West Africa. It has a relatively small population, and its flight is not particularly fast. Additionally, it has been suggested that the coloration of adults resembles bird droppings, providing camouflage (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

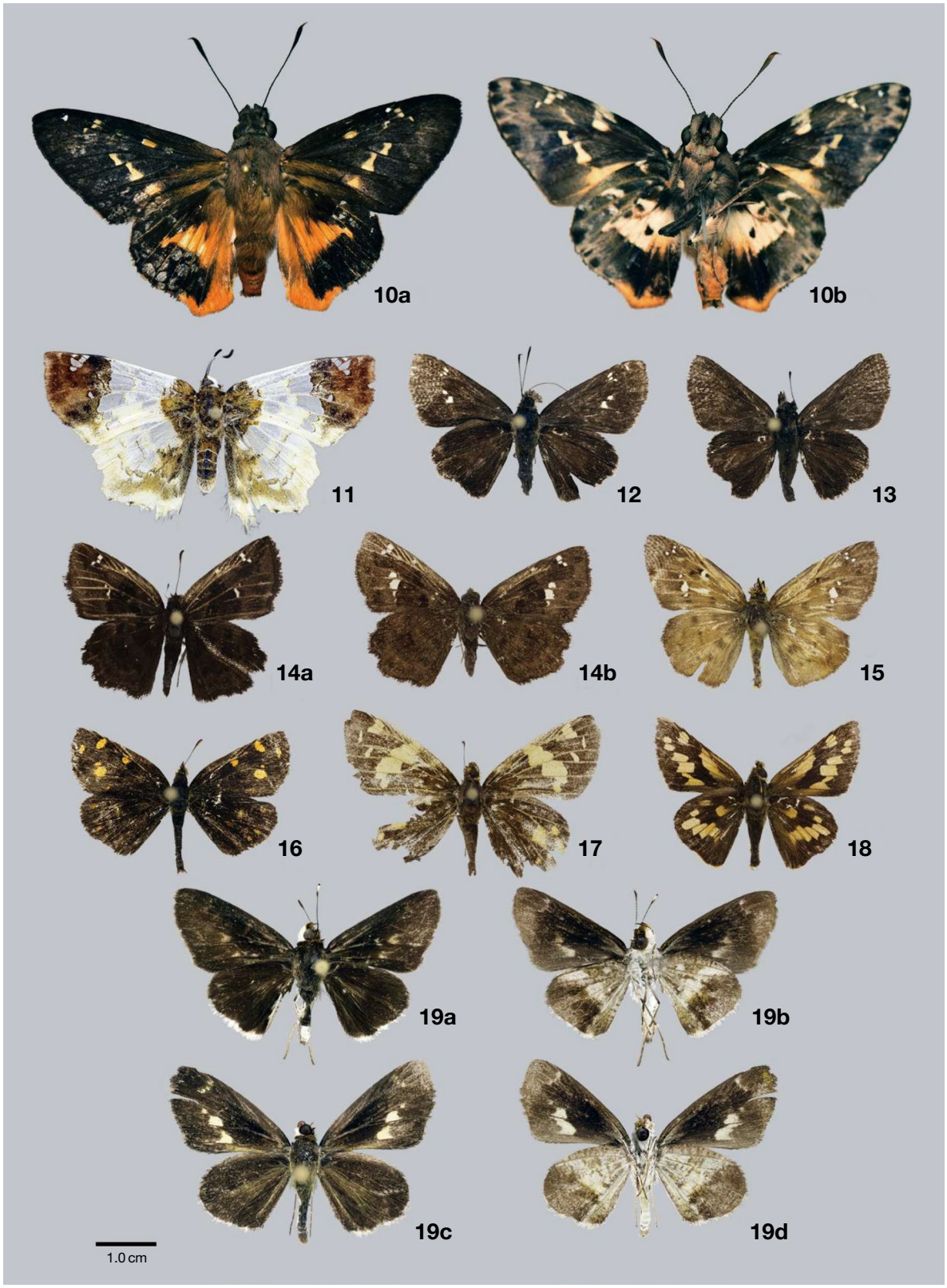
***Sarangesa haplopa* Swinhoe, 1907 (Fig. 14)**

Material examined. (1♂, 2♀), TANZANIA, ARUSHA: 1♀, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♀, Monduli, 14.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m); 1♂, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Democratic Republic of Congo, Sudan, Uganda, Kenya, Tanzania, Malawi.
Remarks. This species inhabits forests, and is considered relatively common (Larsen, 1991; Williams, 2022).

Family Pieridae Duponchel, [1835]
Subfamily Coliadinae Swainson, 1827

***Catopsilia florella* (Fabricius, 1775) (Fig. 33)**

Material examined. (1♂, 1♀), TANZANIA, ARUSHA: 1♀, TAWIRI Forest, 23.XII.2023, (W. Sengo, A. Pauly), (S3°24′41.0″, E36°42′15.0″ Alt: 1,300 m), deposited in TAWIRI; 1♂, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Sub-Saharan Africa, Madagascar, Reunion, Mauritius, Comoro Islands, Seychelles, Arabia, Saudi



Figs. 10–19. Hesperiidae. 10) *Artitropa erinnys*, male (a. dorsal view; b. ventral view); 11) *Netrobalane canopus*, female; 12) *Borbo fatuellus*, male; 13) *Borbo lugens*, male; 14) *Sarangesa haplopa* (a. male; b. female); 15) *Eagris sabadius*, male; 16) *Metisella quadrisignatus*, male; 17) *Celaenorrhinus ovalis*, male; 18) *Zenonia zeno*, male; 19) *Acleros mackenii* (a. male, dorsal view; b. ventral view; c. female dorsal view; d. ventral view).

Arabia, Canary Islands, Egypt, Israel, Lebanon.
Remarks. This species inhabits a wide range of habitats, excluding dense forests, true deserts, and frigid alpine zones. It is highly migratory and adaptable, with excellent flight capabilities that allow it to fly in straight lines or at high altitudes. Additionally, it is strongly attracted to flowers and often gathers at mud pools to absorb water (Williams, 2022).

***Eurema brigitta* (Stoll, [1780]) (Fig. 35)**

Material examined. (1♂, 1♀), TANZANIA, ARUSHA: 1♀, TAWIRI Forest, 23.XII.2023, (W. Sengo, A. Pauly), (S3°24′41.0″, E36°42′15.0″ Alt: 1,300 m), deposited in TAWIRI; 1♂, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Sub-Saharan Africa, Madagascar, Saudi Arabia, Yemen, Mauritius, Comoro Islands, Reunion, Cape Verde Islands, the Oriental Region, Japan.
Remarks. The species is also found in savannas, grasslands, forest clearings, and roadsides, and in Tanzania, it inhabits altitudes ranging from 0 to 2,400 m above sea level. It is a very common species with weak flight ability, typically flying close to the ground. Preferring humid environments, it often gathers in large groups at mud pools, wet sand, and animal feces to absorb water (Kielland, 1990; Williams, 2022).

***Eurema desjardinsii* (Boisduval, 1833) (Fig. 34)**

Material examined. (14♂, 14♀), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♀, Monduli, 14.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m); 3♂, 2♀, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 3♂, 2♀, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m); 8♂, 9♀, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Sub-Saharan Africa, Madagascar, Comoro Islands.
Remarks. This species inhabits forest edges, coastal bush, and dense savannas, occurring at altitudes ranging from 400 to 2,500 m in Tanzania, including montane habitats. It has weak flight capability, flies close to the ground, and exists in small, localized populations within its habitat. It feeds on nectar from flowers and also gathers in mud puddles to absorb moisture (Kielland, 1990; Larsen, 2005; Williams, 2022).

***Eurema hapale* (Mabille, 1882) (Fig. 37)**

Material examined. (3♂), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♂, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♂, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Sub-Saharan Africa, Madagascar.
Remarks. This species inhabits marshy areas in forests and moist savannas, often found along grassy stream banks within or near forests, and occurs at altitudes ranging from 0 to 2,000 m in Tanzania. Among the species in the genus *Eurema*, it has a very weak flight ability and occasionally gathers in mud puddles to absorb water (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

***Eurema senegalensis* (Boisduval, 1836) (Fig. 36)**

Material examined. (3♀), TANZANIA, ARUSHA: 1♀, Monduli, 14.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m); 1♀, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m); 1♀, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Guinea-Bissau, Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Equatorial Guinea, Gabon, Congo, Central African Republic, Democratic Republic of Congo, Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique.
Remarks. This species inhabits forests, heavy woodland, and occurs at altitudes ranging from 0 to 2,000 m in Tanzania. It is a common species and is frequently found with *Eurema hecabe*. It is attracted to flowers and often gathers at mud pools to absorb water (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

***Nepheronia thalassina* (Boisduval, 1836) (Fig. 26)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Monduli, 14.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Senegal, Gambia, Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Gabon, Congo, Angola, Central African Republic, Democratic Republic of Congo, Sudan, Uganda, Ethiopia, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Botswana, Namibia, Swaziland, South Africa.
Remarks. This species inhabits forests and riparian vegetation, especially secondary and dry forests, and is also found in woodlands. In Tanzania, it occurs at altitudes ranging from 0 to 1,700 m. It has a fast, straight flight and

can fly at high altitudes. It is strongly attracted to flowers but tends not to gather in mud pools (Kielland, 1990; Larsen, 1991; Williams, 2022).

Subfamily Pierinae Swainson, 1820

***Belenois creona* (Cramer, [1776]) (Fig. 21)**

Material examined. (7♂, 8♀), TANZANIA, ARUSHA: 1♂, 2♀, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 4♂, 8♀, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 2♂, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Sub-Saharan Africa, Madagascar, Comoro Islands.
Remarks. This species inhabits moist, frost-free savannas and frequently occurs along forest margins. In Tanzania, it occurs at altitudes ranging from 0 to 2,600 m. It is a very common species, strongly attracted to flowers, and frequently gathers in mud puddles. Additionally, this species participates in mixed migrations with up to a dozen or more butterfly species and also move in large-scale migrations alone (Larsen, 1991; Williams, 2022).

***Belenois gidica* (Godart, [1819]) (Fig. 22)**

Material examined. (2♂), TANZANIA, ARUSHA: 1♂, Monduli, 14.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m); 1♂, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Sub-Saharan Africa.
Remarks. This species inhabits frost-free savannas, and occurring in Tanzania at altitudes ranging from 0 to 2,100 m. It can be a common species at times, is strongly attracted to flowers, and often gathers in mud pools (Kielland, 1990; Williams, 2022).

***Belenois raffrayi* (Oberthür, 1878) (Fig. 23)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Maji ya chai river, 06.IV.2023, (J. Mbulu), (S03°18′10.0″, E36°52′57.0″ Alt: 1,399 m), deposited in TAWIRI.
Distribution. Ethiopia, Sudan, Uganda, Kenya, Rwanda, Burundi, Democratic Republic of Congo, Tanzania.
Remarks. This species inhabits montane forests and open grasslands, and occurring in Tanzania at altitudes ranging from 1,500 to 2,000 m. It has a fast flight and is strongly attracted to the flowers of herbaceous and shrubby plants. Additionally, it gathers readily in mud puddles (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Belenois zochalia* (Boisduval, 1836) (Fig. 20)**

Material examined. (2♂, 1♀), TANZANIA, ARUSHA: 1♀, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 2♂, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Nigeria, Cameroon, Democratic Republic of Congo, Uganda, Ethiopia, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Namibia, South Africa, Swaziland.
Remarks. This species inhabits forests and dense, moist woodlands, and occurring in Tanzania at altitudes ranging from 300 to 2,700 m. It flies quickly along the edge of the forest or in sunny open clearings while predominantly remaining active in the undergrowth. It is attracted to flowers and frequently gathers in mud puddles. Additionally, this species sometimes move in large-scale migrations (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

***Colotis aurigineus* (Butler, 1883) (Fig. 32)**

Material examined. (10♂), TANZANIA, ARUSHA: 10♂, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Sudan, Democratic Republic of Congo, Uganda, Kenya, Tanzania, Malawi, Zambia.
Remarks. This species inhabits mesic to dry savannas and can extend into sub-desert areas as well as forest clearings. In Kenya, it has been recorded in forest clearings at altitudes of 3,000 m, while in Tanzania, it occurs at altitudes ranging from 500 to 2,200 m (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Colotis aurora* (Cramer, [1780]) (Fig. 29)**

Material examined. (2♂, 1♀), TANZANIA, ARUSHA: 2♂, 1♀, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Mauritania, Ghana, Chad, Sudan, Democratic Republic of the Congo, Ethiopia, Somalia, Tanzania, East Africa, Malawi, Zambia, Zimbabwe, Botswana, Mozambique.
Remarks. This species inhabits woodlands and savannas, particularly common in dry bushland from 200 to 1,700 m. In Tanzania, it is found in most areas except higher mountains and very wet coastal regions (Kielland, 1990).

***Colotis auxo* (Lucas, 1852) (Fig. 31)**

Material examined. (9♂, 4♀), TANZANIA, ARUSHA: 3♂, 3♀, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 5♂, 1♀, Monduli, 28.VIII.2024, (S.W. Jung,



Figs. 20–26. Pieridae. 20) *Belenois zochalia* (a. male, dorsal view; b. male, ventral view; c. female); 21) *Belenois creona* (a. male, dorsal view; b. male, ventral view; c. female); 22) *Belenois gidica* (a. male, dorsal view; b. male, ventral view); 23) *Belenois raffrayi*, male; 24) *Mylothris agathina* (a. male, dorsal view; b. male, ventral view; c. female); 25) *Mylothris rueppellii*, female (a. dorsal view; b. ventral view); 26) *Nepheronia thalassina*, male.

Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m); 1♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Democratic Republic of Congo, Uganda, Rwanda, Burundi, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Botswana, South Africa, Swaziland.
Remarks. This species inhabits dry, frost-free savannas. It has a medium-fast flight, flies close to the ground, and is strongly attracted to flowers (Williams, 2022).

***Colotis euippe* (Linnaeus, 1758) (Fig. 28)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♂, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Senegal, Gambia, Guinea-Bissau, Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Burkina Faso, Togo, Benin, Nigeria, Cameroon, Chad, Central African Republic, Democratic Republic of Congo, Angola, Sudan, Ethiopia, Somalia, Uganda, Kenya, Tanzania, Malawi, Comoro Islands, Saudi Arabia, Yemen.
Remarks. This species inhabits savannas, coastal bush, forest margins, and disturbed forest areas. In Tanzania, it occurs at altitudes from 0 to 1,900 m. It is a common species, favoring dense shrubland or semi-shaded environments, and is strongly attracted to flowers (Kielland, 1990; Larsen, 2005; Williams, 2022).

***Colotis eunoma* (Hopffer, 1855) (Fig. 30)**

Material examined. (1♀), TANZANIA, ARUSHA: 1♀, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Kenya, Tanzania, Mozambique.
Remarks. This species inhabits coastal dune scrub. It has a very fast flight and is strongly attracted to flowers, with a particular preference for wild periwinkle and flame lilies (Williams, 2022). The individual collected appears to be a variation of the female of this species.

***Leptosia alcesta* (Stoll, [1782]) (Fig. 27)**

Material examined. (6♂, 12♀), TANZANIA, ARUSHA: 10♀, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 6♂, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 2♀, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Senegal, Gambia, Guinea-Bissau, Guinea, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Equatorial Guinea, Gabon, Congo, Central African, Republic, Angola, Democratic Republic of Congo, Sudan, Uganda, Ethiopia, Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland, Madagascar.
Remarks. This species inhabits forests and heavy woodland savannas, particularly along river courses, and is also found in disturbed areas within forest zones. In Tanzania, it occurs at altitudes from 0 to 1,850 m. It is a very common species found in various forest habitats, flying very weakly in the shaded lower layers of the forest. They are usually active around dawn and feed on nectar from small flowers in the forest. They do not gather in mud pools (Larsen, 2005; Williams, 2022).

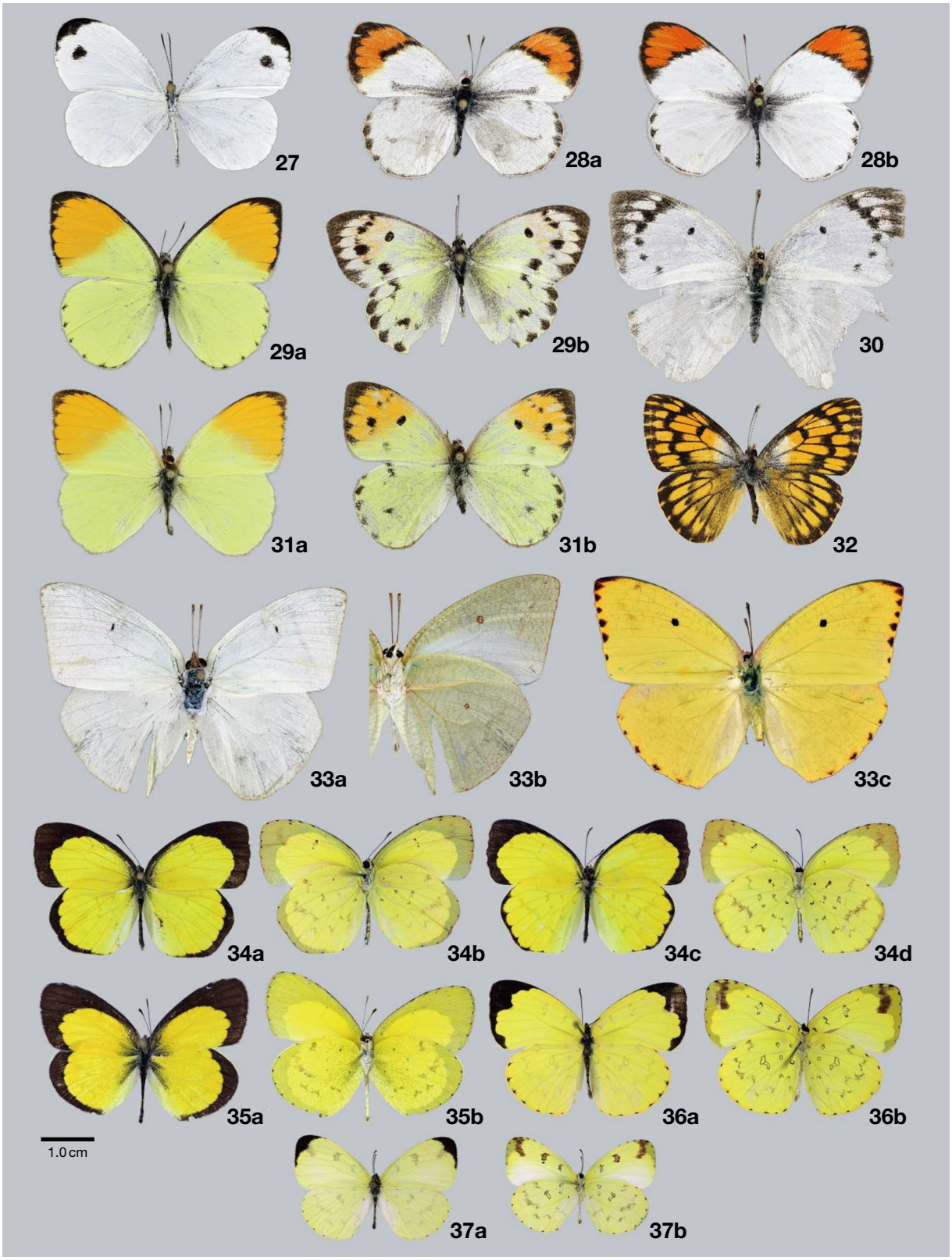
***Mylothris agathina* (Cramer, [1779]) (Fig. 24)**

Material examined. (1♂, 1♀), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24’45.0″, E36°42’22.0″ Alt: 1,308 m); 1♀, TAWIRI Forest, 19.VI.2023, (A. Pauly), (S3°24′41.0″, E36°42′15.0″ Alt: 1,300 m), deposited in TAWIRI.
Distribution. Cameroon, Gabon, Central African Republic, Democratic Republic of Congo, Uganda, Sudan, Ethiopia, Somalia, Kenya, Tanzania, Malawi, Zambia, Angola, Mozambique, Zimbabwe, Botswana, Namibia, South Africa, Swaziland.
Remarks. This species inhabits savannas and forests and is also commonly found in urban gardens and parks. In Tanzania, it occurs at altitudes from 0 to 2,200 m. It has a typical slow, floating flight, usually high in the trees. It is attracted to flowers and often gathers in mud puddles (Kielland, 1990; Williams, 2022).

***Mylothris rueppellii* (Koch, 1865) (Fig. 25)**

Material examined. (1♀), TANZANIA, ARUSHA: 1♀, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Nigeria, Sudan, Ethiopia, Democratic Republic of Congo, Uganda, Kenya, Tanzania, Malawi, Zambia, Angola, Mozambique, Zimbabwe, Botswana, South Africa, Swaziland.
Remarks. This species inhabits forests and heavily wooded savannas, occurring in Tanzania at altitudes ranging from 600 to 2,000 m. It is a common species with a slow, floating flight, primarily flying above trees. Additionally, it is strongly attracted to flowers (Kielland, 1990; Larsen, 1991; Williams, 2022).

Family Lycaenidae Leach, 1815
Subfamily Poritiinae Doherty, 1886



Figs. 27–37. Pieridae. 27) *Leptosia alcesta*, male; 28) *Colotis euippe*, male (a, b. dorsal view); 29) *Colotis aurora* (a. male; b. female); 30) *Colotis eunoma*, female; 31) *Colotis auxo* (a. male; b. female); 32) *Colotis aurigineus*, male; 33) *Catopsilia florella* (a. male, dorsal view; b. male, ventral view; c. female); 34) *Eurema desjardinsii*, male (a, c. dorsal view; b, d. ventral view); 35) *Eurema brigitta*, male (a. dorsal view; b. ventral view); 36) *Eurema senegalensis*, female (a. dorsal view; b. ventral view); 37) *Eurema hapale*, male (a. dorsal view; b. ventral view).

***Baliochila lipara* Stempffer & Bennett, 1953 (Fig. 39)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Kenya, Tanzania, Malawi, Mozambique, Zimbabwe, South Africa, Zambia.
Remarks. This species inhabits deciduous woodlands (savanna) and occurs at altitudes of 500 to 1,000 m in Tanzania. It has a habit of sitting on the bark of tree trunks or dry branches. Small individuals generally fly low, staying under the shade of trees (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Pentila pauli* Staudinger, 1888 (Fig. 38)**

Material examined. (10♂), TANZANIA, ARUSHA: 10♂, KARAMA Lodge, 08.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°22′47.0″, E36°42′53.0″ Alt: 1,414 m).
Distribution. Senegal, Guinea-Bissau, Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Gabon, Congo, Central African Republic, Angola, Democratic Republic of Congo, Sudan, Ethiopia, Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe.
Remarks. This species inhabits deciduous woodlands and forest margins, occurring at altitudes of 250 to 1,500 m in Tanzania. It has weak flying capabilities, flies low in the shade, and often sitting on grass (Kielland, 1990; Williams, 2022).

Subfamily Miletinae Reuter, 1896

***Lachnocnema bibulus* (Fabricius, 1793) (Fig. 40)**

Material examined. (8♀), TANZANIA, ARUSHA: 2♀, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m); 6♀, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Uganda, Burundi, Democratic Republic of Congo, Kenya, Tanzania, Malawi, Zambia, Angola, Mozambique, Zimbabwe, Botswana, Namibia, South Africa, Swaziland, Madagascar.
Remarks. This species inhabits savannas and open forests, occurring at altitudes of 0 to 2,000 m in Tanzania. It flies around trees or shrubs and rests on low vegetation. Adults are often observed sucking honeydew secreted by small sapsucking hemiptera (Kielland, 1990; Williams, 2022).

Subfamily Polyommatinae Swainson, 1827

***Anthene larydas* (Cramer, 1780) (Fig. 44)**

Material examined. (1♀), TANZANIA, ARUSHA: 1♀, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Senegal, Gambia, Guinea-Bissau, Guinea, Burkina Faso, Liberia, Sierra Leone, Ivory Coast, Ghana, Benin, Togo, Nigeria, Cameroon, Niger, Equatorial Guinea, Gabon, Angola, Congo, Central African Republic, Democratic Republic of Congo, Sudan, Uganda, Rwanda, Burundi, Ethiopia, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland.
Remarks. This species inhabits forests and heavy woodlands, occurring at altitudes of 0 to 1,800 m in Tanzania. It is a common species in West Africa but rarely found in western Kenya. It is strongly attracted to flowers, actively gathers in mud puddles, and prefers humid areas. Additionally, it has a characteristic of being strongly attracted to human sweat, urine, and wet sand (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

***Azanus jesous* (Guérin-Méneville, 1849) (Fig. 43)**

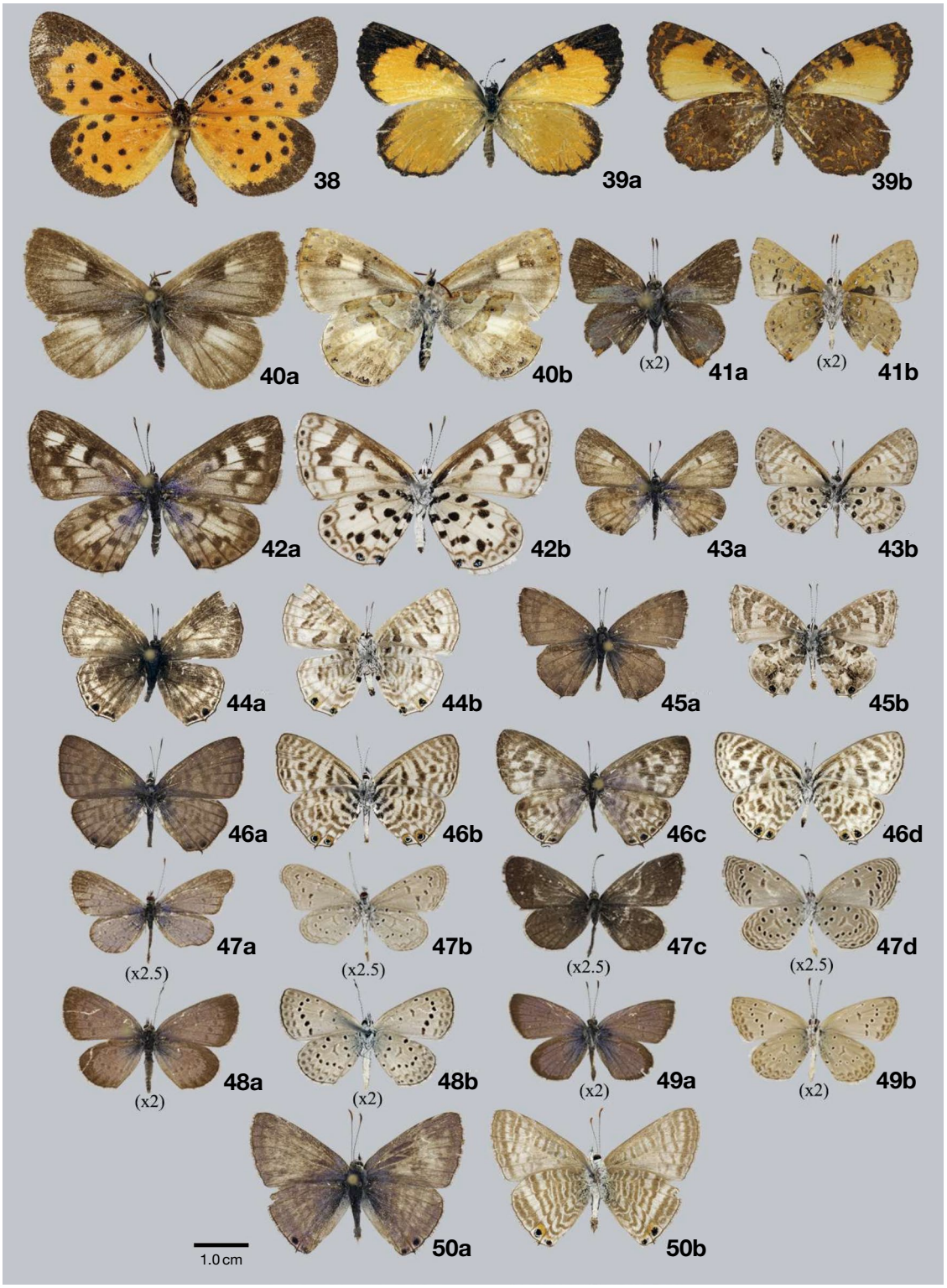
Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Senegal, Gambia, Guinea-Bissau, Guinea, Burkina Faso, Ghana, Benin, Nigeria, Ethiopia, Somalia, Uganda, Kenya, Tanzania, Zambia, Angola, Mozambique, Zimbabwe, Botswana, Namibia, South Africa, Swaziland, Lesotho, Arabia.
Remarks. This species inhabits savannas and Karoo regions, and occurring at altitudes of 300 to 2,600 m in Tanzania. It is a common species, characterized by its somewhat migratory nature. It is often observed flying around flowering acacia trees and is strongly attracted to flowers. Additionally, it tends to gather well in mud puddles and is often seen sitting on rotten animal carcasses (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

***Azanus natalensis* (Trimen, 1887) (Fig. 42)**

Material examined. (1♀), TANZANIA, ARUSHA: 1♀, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Senegal, Ghana, Benin, Nigeria, Ethiopia, Uganda, Kenya, Tanzania, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland.
Remarks. This species inhabits moist savannas, and occurring at altitudes of 300 to 2,000 m in Tanzania. It is the largest species in its genus but has the weakest flight capability. It is attracted to flowers, especially favoring acacia flowers, and also gathers in mud puddles (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Cacyreus lingeus* (Stoll, [1782]) (Fig. 45)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂,



Figs. 38–50. Lycanidae. 38) *Pentila pauli*, male; 39) *Baliochila lipara*, male (a. dorsal view; b. ventral view); 40) *Lachnocnema bibulous*, female (a. dorsal view; b. ventral view); 41) *Chloroselas pseudozeritis*, male (a. dorsal view; b. ventral view); 42) *Azanus natalensis*, female (a. dorsal view; b. ventral view); 43) *Azanus jesous*, female (a. dorsal view; b. ventral view); 44) *Anthene larydas*, female (a. dorsal view; b. ventral view); 45) *Cacyreus lingeus*, male (a. dorsal view; b. ventral view); 46) *Leptotes pirithous* (a. male, dorsal view; b. male, ventral view; c. female, dorsal view; d. female, ventral view); 47) *Zizula hylax* (a. male, dorsal view; b. male, ventral view; c. female, dorsal view; d. female, ventral view); 48) *Zizeeria knysna*, female (a. dorsal view; b. ventral view); 49) *Zizina otis*, male (a. dorsal view; b. ventral view); 50) *Lampides boeticus*, male (a. dorsal view; b. ventral view).

TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Gambia, Guinea-Bissau, Guinea, Sierra Leone, Burkina Faso, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Equatorial Guinea, Gabon, Uganda, Kenya, Tanzania, Zambia, Mozambique, Zimbabwe, Botswana, Namibia, South Africa, Swaziland, Lesotho.
Remarks. This species inhabits moist savannas, typically along stream banks in wooded habitats and is often found in gardens. It also occurs in open areas within forest zones and dry forests. In Tanzania, it is found at altitudes ranging from 0 to 2,300 m. It is a common species that usually flies low and slowly above the ground. Often sitting on flowers, grass stalks, or low vegetation, it is attracted to flowers and sometimes gathers in mud puddles (Kielland, 1990; Larsen, 2005; Williams, 2022).

***Lampides boeticus* (Linnaeus, 1767) (Fig. 50)**

Material examined. (3♂, 22♀), TANZANIA, ARUSHA: 2♂, 20♀, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m); 1♂, 2♀, Kisongo, 03.IX.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°22′50.7″, E36°33′30.9″Alt: 1,366 m).
Distribution. Afrotropical region, Saharo-Arabian region, Europe, Oriental region, Oceanian region, Sino-Japanese region.
Remarks. This species has strong migration abilities and is widely distributed from Africa to Australia, East Asia. In Africa, it inhabits a variety of habitats, ranging from subdeserts to open forests, and is commonly found in gardens. It has a high flight speed and typically flies low. It often visits flowers and readily gathers in mud puddles (Williams, 2022).

***Leptotes pirithous* (Linnaeus, 1767) (Fig. 46)**

Material examined. (4♂, 2♀), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♀, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 3♂, 1♀, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Sub-Saharan Africa, Madagascar, Mauritius, Reunion, Arabia, North Africa, Europe.
Remarks. This species inhabits most biomes except primary forests and deserts and is found from sea level to high altitudes. It is a very common species with a weak flight ability, often attracted to flowers and found around shrubs or small trees. Additionally, it drinks water in moist areas and gathers at animal carcasses (Larsen, 1991; Larsen, 2005; Williams, 2022).

***Zizeeria knysna* (Trimen, 1862) (Fig. 48)**

Material examined. (2♂, 4♀), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♀, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m); 3♀, Kisongo, 03.IX.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°22′50.7″, E36°33′30.9″ Alt: 1,366 m).
Distribution. Africa, Madagascar, Middle East, Spain, Southeast Asia, Australia.
Remarks. This species has strong migration abilities and is widely distributed from Africa to Southeast Asia and Australia. It inhabits a variety of habitats, including open areas in forest zones, suburban gardens, and parks, but is absent from dense forests and deserts. As one of the most common butterflies in Africa, it occurs extensively from sea level to high mountainous areas. Its flight capability is weak, and it flies low near the ground. It is frequently attracted to small flowers close to the ground and absorbs water in humid places (Kielland, 1990; Larsen, 2005; Williams, 2022).

***Zizina otis* (Fabricius, 1787) (Fig. 49)**

Material examined. (2♂), TANZANIA, ARUSHA: 1♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m); 1♂, Kisongo, 03.IX.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°22′50.7″, E36°33′30.9″ Alt: 1,366 m).
Distribution. Afrotropical region, Saharo-Arabian region, Oriental region, Oceanian region, Sino-Japanese region.
Remarks. This species appears to be a subspecies (*Zizina otis antanossa*), but it is treated as a distinct species in this study. It is widely distributed from Africa to Southeast Asia, East Asia, and Australia. In Africa, it inhabits grassy areas in savannas, occurring from sea level to high mountainous regions. It sometimes forms large groups and is frequently attracted to flowers or riverbeds (Larsen, 1991; Larsen, 2005; Williams, 2022).

***Zizula hylax* (Fabricius, 1775) (Fig. 47)**

Material examined. (2♂), TANZANIA, ARUSHA: 1♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″Alt: 1,346 m); 1♂, Kisongo, 03.IX.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°22′50.7″, E36°33′30.9″ Alt: 1,366 m).
Distribution. Afrotropical region, Saharo-Arabian region, Oriental region, Australian region, Oceanian region, Sino-

Japanese region.
Remarks. This species is widely distributed from Africa to Southeast Asia, East Asia, and Australia. In Africa, it inhabits a wide variety of habitats, particularly grasslands and grassy savannas, but is absent from primary forests. In Tanzania, it occurs from 0 to 2,600 m. It has a very weak flying ability, flying low, mainly within lower vegetation. Additionally, it is attracted to flowers and frequently gathers in mud pools (Kielland, 1990; Larsen, 2005; Williams, 2022).

Subfamily Miletinae Swainson, 1831

***Chloroselas pseudozeritis* (Trimen, 1873) (Fig. 41)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Central African Republic, Ethiopia, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Botswana, South Africa, Swaziland.
Remarks. This species inhabits deciduous woodlands and thornbush savannas, and occurring in Tanzania at altitudes between 1,000 and 1,500 m. It is a rare species that flies quickly around acacia trees, with a habit of perching on the tips of small branches. Additionally, it is strongly attracted to flowers (Kielland, 1990; Larsen, 1991; Williams, 2022).

Family Nymphalidae Rafinesque, 1815
Subfamily Charaxinae Guenée, 1865

***Charaxes brutus* (Cramer, [1779]) (Fig. 59)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Forest, 19.XII.2023, (W. Sengo, A. Pauly), (S3°24′41.0″, E36°42′15.0″ Alt: 1,300 m), deposited in TAWIRI.
Distribution. Senegal, Guinea-Bissau, Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Equatorial Guinea, Gabon, Congo, Central African Republic, Angola, Democratic Republic of Congo, Sudan, Uganda, Rwanda, Burundi, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Botswana, Namibia, South Africa, Swaziland.
Remarks. This species inhabits forests, degraded forests, dense savannas, and occurring in Tanzania at altitudes between 500 and 1,900 m. It is a relatively common species with a very fast flight, often sailing over the tops of trees. It is strongly attracted to fermented fruit and tree sap and also has the habit of gathering on rotten animal carcasses and animal feces (Kielland, 1990; Williams, 2022).

***Charaxes candiope* (Godart, [1824]) (Fig. 60)**

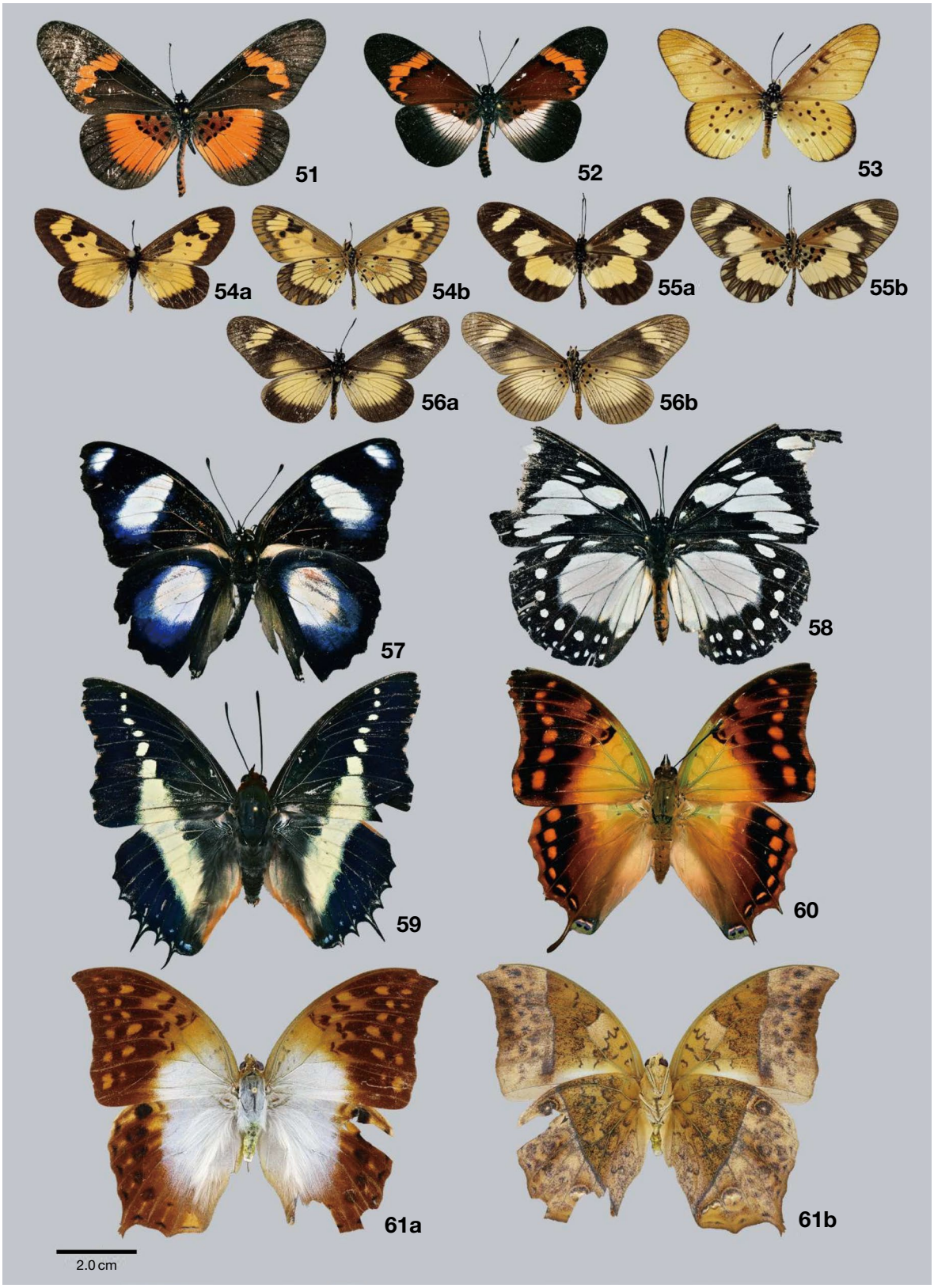
Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Forest, 24.XII.2023, (W. Sengo, A. Pauly), (S3°24′41.0″, E36°42′15.0″ Alt: 1,300 m), deposited in TAWIRI.
Distribution. Senegal, Gambia, Guinea, Sierra Leone, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Sao Tome and Principe Islands, Gabon, Angola, Central African Republic, Democratic Republic of Congo, Ethiopia, Uganda, Rwanda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Botswana, Namibia, South Africa, Swaziland.
Remarks. This species inhabits forests and savannas, excluding arid savannas, and occurs in Tanzania at altitudes from 0 to 2,600 m. It is a relatively common species with a very fast flight, often sailing over the tops of trees. It is strongly attracted to fermented fruits and fluids and is frequently seen gathering in mud puddles (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

***Charaxes varanes* (Cramer, 1777) (Fig. 61)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Senegal, Gambia, Guinea-Bissau, Guinea, Burkina Faso, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Equatorial Guinea, Gabon, Central African Republic, Democratic Republic of Congo, Sudan, Ethiopia, Somalia, Yemen, Oman, Uganda, Kenya, Tanzania, Malawi, Zambia, Angola, Mozambique, Zimbabwe, Botswana, Namibia, South Africa, Swaziland.
Remarks. This species primarily inhabits dry forests, open forests, dense savannas, and occurring in Tanzania at altitudes from 0 to 2,300 m. It is a common species with a moderate flight, often sailing over the tops of trees. It is strongly attracted to fermented fruits but not to animal feces or rotting animal carcasses (Kielland, 1990; Larsen, 2005; Williams, 2022).

***Euxanthe wakefieldi* (Ward, 1873) (Fig. 58)**

Material examined. (1♀), TANZANIA, ARUSHA: 1♀, TAWIRI Forest, 23.XII.2023, (W. Sengo, A. Pauly), (S3°24′41.0″, E36°42′15.0″ Alt: 1,300 m), deposited in TAWIRI.
Distribution. Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland.
Remarks. This species inhabits lowland and coastal forests, and occurring in Tanzania at altitudes from 0 to over 2,000 m. It is mainly active in semi-shaded areas of the forest, with a slow flight characterized by gliding and occasional wing flaps. It primarily flies along the edges of shrublands, resting on the edge of forests or on trees in open areas. Additionally, it is strongly attracted to fermented fruits (Kielland, 1990; Larsen, 1991; Williams, 2022).



Figs. 51–61. Nymphalidae. 51) *Acraea aganice*, male; 52) *Acraea quadricolor*, male; 53) *Acraea encedon*, male; 54) *Acraea acerata*, male (a. dorsal view; b. ventral view); 55) *Acraea cabira*, male (a. dorsal view; b. ventral view); 56) *Acraea jodutta*, male (a. dorsal view, b. ventral view); 57) *Hypolimnas misippus*, male; 58) *Euxanthe wakefieldi*, female; 59) *Charaxes brutus*, male; 60) *Charaxes candiope*, male; 61) *Charaxes varanes*, male (a. dorsal view; b. ventral view).

Subfamily Danainae Boisduval, 1833

***Amauris albimaculata* Butler, 1875 (Fig. 65)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Maji ya chai river, 06.IV.2023, (J. Mbulu), (S03°18′10.0″, E36°52′57.0″ Alt: 1,399 m), deposited in TAWIRI.
Distribution. Cameroon, Democratic Republic of Congo, Uganda, Rwanda, Burundi, Sudan, Ethiopia, Somalia, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland.
Remarks. This species inhabits forests, occurring in Tanzania at altitudes from 800 to over 2,200 m. It has a slow and lazy flight, usually flying above the trees. It is attracted to flowers and sometimes gathers in mud puddles or around animal waste (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Amauris niavius* (Linnaeus, 1758) (Fig. 66)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Maji ya chai river, 06.IV.2023, (J. Mbulu), (S03°18′10.0″, E36°52°57.0″ Alt: 1,399 m), deposited in TAWIRI.
Distribution. Guinea-Bissau, Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Equatorial Guinea, Gabon, Congo, Central African, Republic, Angola, Democratic Republic of Congo, Sudan, Ethiopia, Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Namibia, South Africa, Swaziland.
Remarks. This species appears to be a subspecies (*Amauris niavius dominicanus*), but it is treated as a distinct species in this study. This species inhabits drier forests and disturbed areas in the rainforest zone, and occurring in Tanzania at altitudes from 800 to over 2,300 m. It flies in a slow, gliding manner, is strongly attracted to flowers, and is also observed gathering at water, excrement, and rotting animal carcasses (Kielland, 1990; Larsen, 2005; Williams, 2022).

***Danaus chrysippus* (Linnaeus, 1758) (Fig. 64)**

Material examined. (5♂), TANZANIA, ARUSHA: 2♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 2♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″Alt: 1,346 m); 1♂, Kisongo, 03.IX.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°22′50.7″, E36°33′30.9″ Alt: 1,366 m).
Distribution. Afrotropical region, Saharo-Arabian region, Europe, Oriental region, Oceanian region, Sino-Japanese region.
Remarks. This species appears to be a subspecies (*Danaus chrysippus dorippus*), but it is treated as a distinct species in this study. It has strong migration abilities and is widely distributed from Africa to Australia, East Asia. In Africa, this species inhabits a wide range of habitats, excluding dense forests, and occurs from sea level to high mountains. It has a slow flight and contains toxic compounds in its body, which help protect it from vertebrate predators. It is strongly attracted to flowers and is occasionally observed gathering at mud puddles or the feces of carnivores (Larsen, 2005; Williams, 2022).

***Tirumala formosa* (Godman, 1880) (Fig. 67)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, KARAMA Lodge, 08.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°22′47.0″, E36°42′53.0″ Alt: 1,414 m).
Distribution. Nigeria, Cameroon, Equatorial Guinea, Gabon, Democratic Republic of Congo, Sudan, Ethiopia, Somalia, Uganda, Rwanda, Burundi, Kenya, Tanzania, Zambia.
Remarks. This species inhabits semi-montane forests, and occurring in Tanzania at altitudes from 300 to over 2,300 m. It usually flies above the forest canopy and has a slow, gliding flight pattern. It is easily attracted to flowers and occasionally gathers in mud pools. Additionally, this species is believed to mimic *Papilio rex* (Papilionidae) (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

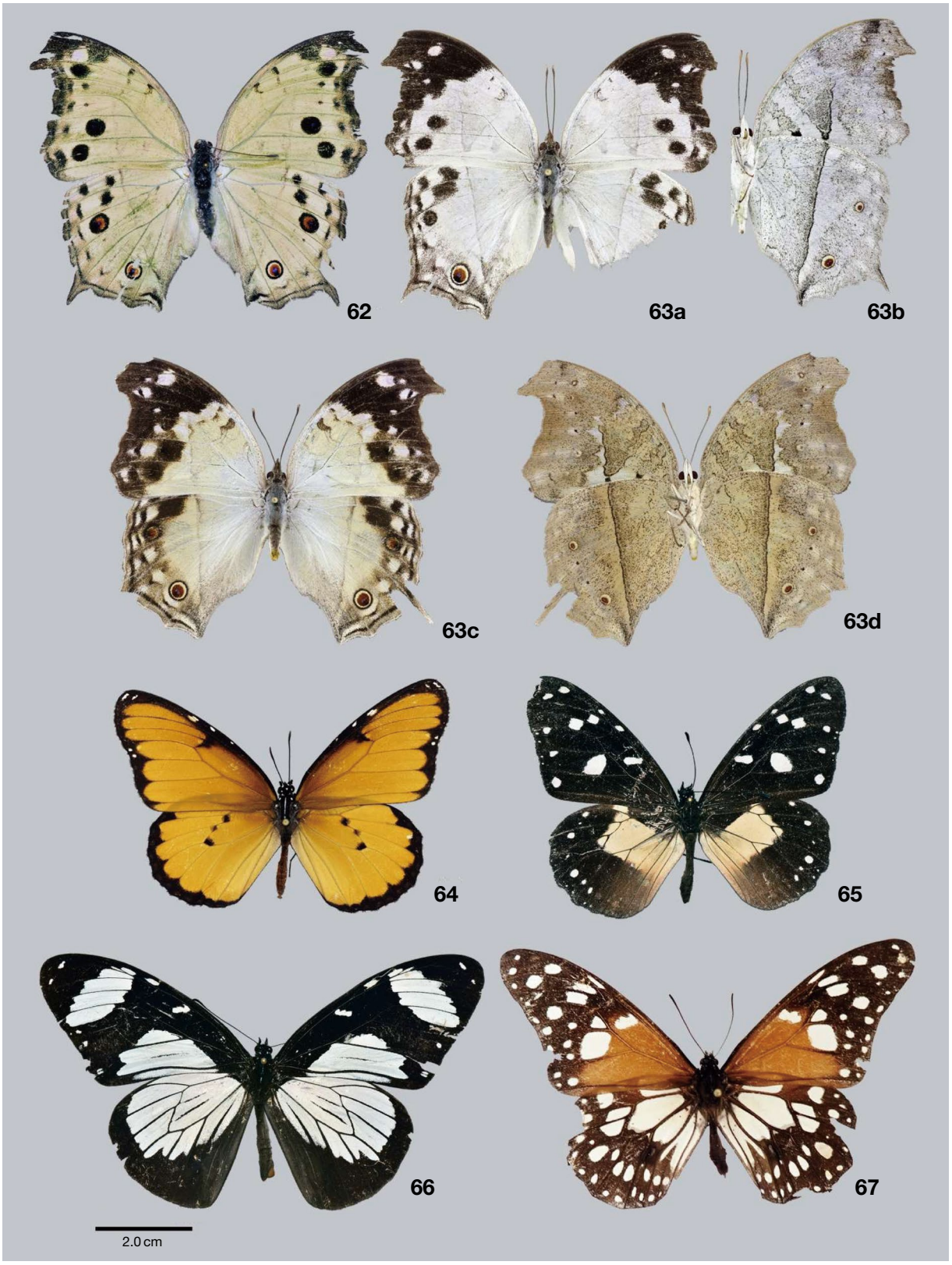
Subfamily Heliconiinae Swainson, 1822

***Acraea acerata* (Hewitson, 1874) (Fig. 54)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Guinea-Bissau, Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Niger, Cameroon, Equatorial Guinea, Gabon, Congo, Democratic Republic of Congo, Central African Republic, Sudan, Rwanda, Uganda, Kenya, Tanzania, Malawi, Zambia, Angola, Mozambique, Zimbabwe, Botswana, Namibia.
Remarks. This species primarily inhabits disturbed areas in forest zones, typically found near water or in riverine bush, and occurs in Tanzania at altitudes from 800 to 2,000 m. It has a weak flight capacity, flies close to the ground, and is known as a major pest of sweet potatoes (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Acraea aganice* Hewitson, [1852] (Fig. 51)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Forest, 20.XII.2023, (W. Sengo, A. Pauly) (S3°24′41.0″, E36°42′15.0″ Alt: 1,300 m), deposited in TAWIRI.
Distribution. Sudan, Ethiopia, Uganda, Kenya, Tanzania, Democratic Republic of Congo, Malawi, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland.



Figs. 62–67. Nymphalidae. 62) *Protogoniomorpha parhassus*, male; 63) *Protogoniomorpha anacardii*, male (a, c. dorsal view; b, d. ventral view); 64) *Danaus chrysippus*, male; 65) *Amauris albimaculata*, male; 66) *Amauris niavius*, male; 67) *Tirumala formosa*, male.

Remarks. This species appears to be a subspecies (*Acraea aganice nicega*), but it is treated as a distinct species in this study. It inhabits forests, coastal bush, heavy woodlands, and occurring in Tanzania at altitudes from 0 to 2,100 m. It is a common species with a slow flight, usually flying close to the ground. It perch high on twigs with closed wings, and is attracted to flowers or gathers in mud puddles (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Acraea cabira* (Hopffer, 1855) (Fig. 55)**

Material examined. (2♂), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Ethiopia, Uganda, Kenya, Tanzania, Democratic Republic of Congo, Malawi, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland.
Remarks. This species inhabits riverine forests, escarpment forests, heavy woodlands, and occurring in Tanzania at altitudes from 0 to 2,100 m. It has weak flying capabilities, primarily flies low, and is strongly attracted to flowers (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Acraea encedon* (Linnaeus, 1758) (Fig. 53)**

Material examined. (2♂), TANZANIA, ARUSHA: 1♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″Alt: 1,346 m); 1♂, Kisongo, 03.IX.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°22′50.7″, E36°33′30.9″Alt: 1,366 m).
Distribution. Senegal, Gambia, Guinea-Bissau, Guinea, Burkina Faso, Liberia, Sierra, Leone, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Gabon, Angola, Democratic Republic of Congo, Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Botswana, Namibia, South Africa, Swaziland, Saudi Arabia, Yemen, Comoro Islands, Madagascar.
Remarks. This species inhabits savannas, forest clearings, anthropogenic environments, and occurring in Tanzania at altitudes from 0 to 2,100 m. It is a common species with a slow flight, flying close to the ground and settling on low vegetation. It is strongly attracted to flowers, and communal resting behavior is regularly observed. Additionally, this species exhibits significant individual variation and is one of the species that forms a Müllerian mimicry relationship, including with *Danaus chrysippus* (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

***Acraea jodutta* (Fabricius, 1793) (Fig. 56)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Senegal, Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Equatorial Guinea, Gabon, Congo, Central African Republic, Angola, Democratic Republic of Congo, Sudan, Ethiopia, Uganda, Kenya, Tanzania, Zambia.
Remarks. This species inhabits forests, and occurring in Tanzania at altitudes between 800 and 1,600 m. It is a relatively common species with a weak flight, flying close to the ground. It is mainly active in the shade of the forest but also moves into sunlight to feed on flower nectar (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

***Acraea quadricolor* (Rogenhofer, 1891) (Fig. 52)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Forest, 20.XII.2023, (W. Sengo, A. Pauly), (S3°24′41.0″, E36°42′15.0″ Alt: 1,300 m), deposited in TAWIRI.
Distribution. Uganda, Burundi, Democratic Republic of Congo, Kenya, Tanzania.
Remarks. This species appears to be a subspecies (*Acraea quadricolor latifasciata*), but it is treated as a distinct species in this study. This species inhabits semi-montane, montane forests, and occurring in Tanzania at altitudes ranging from 900 to 2,300 m. It is a relatively common species that tends to fly high, and exhibiting a fluttering flight pattern (Kielland, 1990; Larsen, 1991; Williams, 2022).

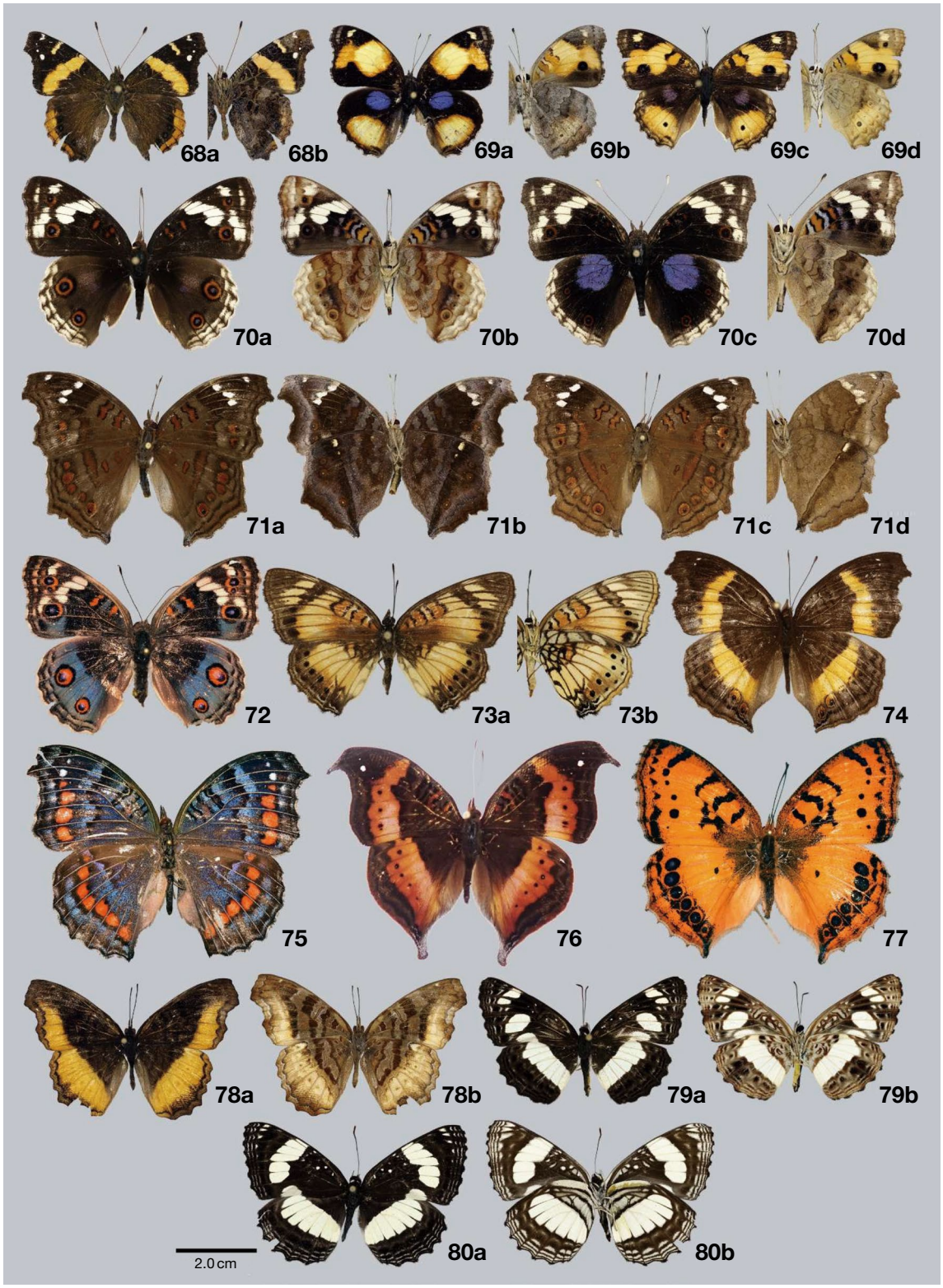
Subfamily Nymphalinae Swainson, 1827

***Catacroptera cloanthe* (Stoll, [1781]) (Fig. 77)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Forest, 20.XII.2023, (W. Sengo, A. Pauly), (S3°24′41.0″, E36°42′15.0″ Alt: 1,300 m), deposited in TAWIRI.
Distribution. Sub-Saharan Africa.
Remarks. This species inhabits grasslands, savannas, and areas near streams, marshes, and seepages on hillsides, occurring in Tanzania at altitudes from 300 to 2,200 m. It is sensitive and vigilant, quickly gliding away when threatened. It is strongly attracted to fermented fruits and often rests on the ground or on rocks (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Eurytela dryope* (Cramer, [1775]) (Fig. 78)**

Material examined. (5♂, 1♀), TANZANIA, ARUSHA: 1♂, KARAMA Lodge, 08.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°22′47.0″, E36°42′53.0″ Alt: 1,414 m); 3♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♂, 1♀,



Figs. 68–80. Nymphalidae. 68) *Vanessa abyssinica*, male (a. dorsal view; b. ventral view); 69) *Junonia hierta* (a. male, dorsal view; b. male, ventral view; c. female, dorsal view; d. female, ventral view); 70) *Junonia oenone* (a. male, dorsal view; b. male, ventral view; c. female, dorsal view; d. female, ventral view); 71) *Junonia natalica* (a. male, dorsal view; b. male, ventral view; c. female, dorsal view; d. female, ventral view); 72) *Junonia orithya*, male; 73) *Junonia sophia*, male (a. dorsal view; b. ventral view); 74) *Junonia terea*, male; 75) *Precis octavia*, male; 76) *Precis tugela*, male; 77) *Catacroptera cloanthe*, male; 78) *Eurytela dryope*, male (a. dorsal view; b. ventral view); 79) *Neptis saclava*, male (a. dorsal view; b. ventral view); 80) *Neptis penningtoni*, male (a. dorsal view; b. ventral view).

Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″Alt: 1,346 m).
Distribution. Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Gabon, Angola, Democratic Republic of Congo, Ethiopia, Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Botswana, South Africa, Swaziland, Saudi Arabia, Yemen, Madagascar, Comoro Islands.
Remarks. This species inhabits disturbed habitats in drier forests, forest-savanna ecotones, moist savannas, and occurring in Tanzania at altitudes from 0 to 2,200 m, though it is uncommon at higher elevations. It hovers around shrubs or low vegetation and is strongly attracted to tree sap and overripe fruit (Kielland, 1990; Larsen, 2005; Williams, 2022).

***Hypolimnas misippus* (Linnaeus, 1764) (Fig. 57)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Forest, 05.IV.2022, (A. Pauly), (S3°24′42.0″, E36°42′16.0″ Alt: 1,300 m), deposited in TAWIRI.
Distribution. Afrotropical region, Saharo-Arabian region, Oriental region, Australian region, Sino-Japanese region, Neotropical region, USA.
Remarks. This species has strong migration abilities and is distributed worldwide, except for the Palearctic region and some countries. In Africa, it inhabits savannas, grasslands, degraded habitats such as agricultural lands, and occurring in Tanzania at altitudes from 0 to over 2,300 m. It flies very fast and is strongly attracted to flowers. Additionally, females mimic several taxa, including the *Danaus chrysippus* (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

***Junonia oenone* (Linnaeus, 1758) (Fig. 70)**

Material examined. (5♂, 1♀), TANZANIA, ARUSHA: 1♂, 1♀, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♂, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m); 3♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Sub-Saharan Africa, Madagascar, Seychelles.
Remarks. This species inhabits savannas and grasslands and is a very common species. It often settles on the ground, roads, and trails and frequently gathers to feed on flower nectar (Kielland, 1990; Larsen, 2005; Williams, 2022).

***Junonia orithya* (Linnaeus, 1758) (Fig. 72)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Forest, 19.VII.2023, (A. Pauly *et al*.), (S3°24′42.0″, E36°42′16.0″ Alt: 1,300 m), deposited in TAWIRI.
Distribution. Afrotropical region, Saharo-Arabian region, Oriental region, Australian region.
Remarks. This species has strong migration abilities and is widely distributed from Africa to Southeast Asia and Australia. In Africa, it inhabits savannas and grasslands and is a common species. It flies close to the ground, frequently settling on the ground with its wings spread horizontally. Additionally, individuals feeding on flower nectar are often observed (Larsen, 2005; Williams, 2022).

***Junonia sophia* (Fabricius, 1793) (Fig. 73)**

Material examined. (4♂), TANZANIA, ARUSHA: 4♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m).
Distribution. Senegal, Gambia, Guinea-Bissau, Guinea, Sierra Leone, Liberia, Ivory, Coast, Burkina Faso, Ghana, Togo, Benin, Nigeria, Cameroon, Equatorial Guinea, Gabon, Central African Republic, Angola, Democratic Republic of Congo, Ethiopia, Uganda, Rwanda, Burundi, Kenya, Tanzania, Malawi, Zambia, Mozambique.
Remarks. This species inhabits forests and savannas, preferring open forests and disturbed habitats. In Tanzania, it occurs at altitudes from 400 to 2,000 m. It is a common species with weak flight capabilities, flying low among vegetation in search of flowers (Kielland, 1990; Larsen, 2005; Williams, 2022).

***Junonia terea* (Drury, 1773) (Fig. 74)**

Material examined. (7♂), TANZANIA, ARUSHA: 6♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♂, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Senegal, Gambia, Guinea-Bissau, Guinea, Sierra Leone, Liberia, Ivory Coast, Burkina Faso, Ghana, Togo, Benin, Nigeria, Cameroon, Equatorial Guinea, Gabon, Congo, Central African Republic, Angola, Democratic Republic of Congo, Sudan, Ethiopia, Somalia, Uganda, Rwanda, Burundi, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland.
Remarks. This species inhabits forests, woodlands, disturbed areas in forests, and occurring in Tanzania at altitudes from 0 to 2,200 m. It is a very common species, flying slowly along forest edges and in open areas around dense shrubland. In general, it does not fly more than 1–2 m above the ground and rests with its wings spread out on low vegetation. It is easily attracted to flowers and is often observed settling on animal feces (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

***Junonia hierta* (Fabricius, 1798) (Fig. 69)**

Material examined. (1♂, 2♀), TANZANIA, ARUSHA: 2♀, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Sub-Saharan Africa, Madagascar, Seychelles, Arabia, Egypt, Lebanon.
Remarks. This species inhabits grasslands, grassy savannas, and occurring in Tanzania at altitudes from 0 to 2,300 m. It is a very common species, often resting on the ground or low plants with its wings spread open. Additionally, it is easily attracted to flowers and gathers on animal waste (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Junonia natalica* (Felder & Felder, 1860) (Fig. 71)**

Material examined. (10♂), TANZANIA, ARUSHA: 6♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 4♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Angola, Democratic Republic of Congo, Uganda, Burundi, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Botswana, Namibia, South Africa, Swaziland, Madagascar.
Remarks. This species inhabits well-wooded savannas, riverine bush, coastal bush, and occurring in Tanzania at altitudes from 0 to 2,200 m. It is highly sensitive, flying quickly when it senses danger and frequently settling on the ground or low shrubs. It is strongly attracted to flowers and absorbs nutrients from damp places and animal feces (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Neptis penningtoni* van Son, 1977 (Fig. 80)**

Material examined. (2♂, 4♀), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 3♀, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m); 1♂, 1♀, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Kenya, Tanzania, Zambia, Mozambique, Zimbabwe.
Remarks. This species inhabits woodland, wooded hills, and occurring in Tanzania at altitudes from 900 to 1,700 m (Kielland, 1990; Williams, 2022).

***Neptis saclava* Boisduval, 1833 (Fig. 79)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Nigeria, Cameroon, Ethiopia, Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland, Madagascar.
Remarks. This species inhabits woodlands and forests, particularly along water courses, and occurring in Tanzania at altitudes from 0 to 2,200 m. It is one of the most common species in this genus, flying with a floating motion and intermittent flapping between trees. It is easily attracted to flowers and absorbs nutrients from fermented fruits and tree sap (Kielland, 1990; Larsen, 1991; Williams, 2022).

***Precis octavia* (Cramer, 1777) (Fig. 75)**

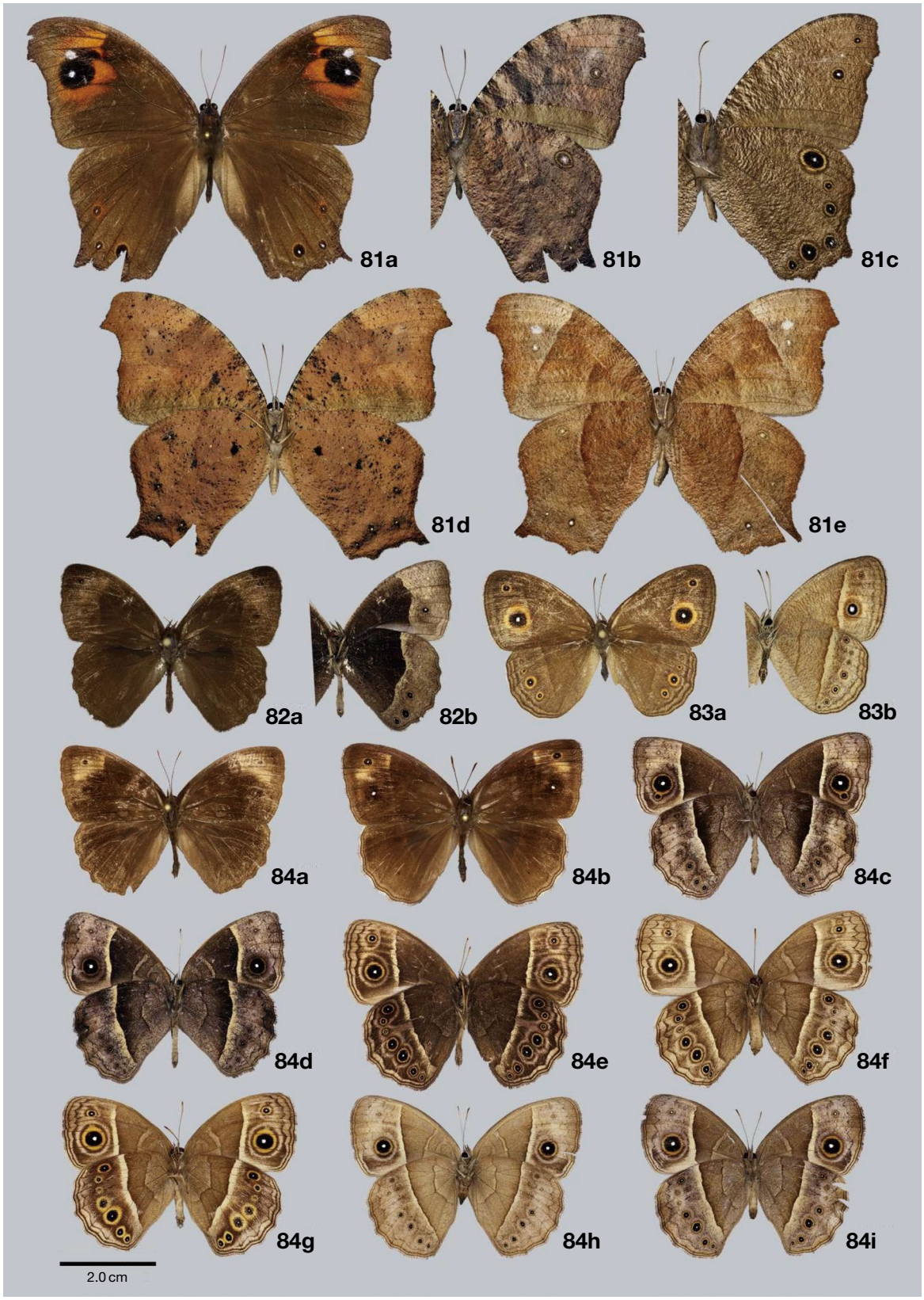
Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Iringa, Ilamba, 12−18.V.2023, (W. Sengo), (S08°03′59.0″, E35°52′49.7″ Alt: 1,977 m), deposited in TAWIRI.
Distribution. Senegal, Guinea-Bissau, Guinea, Burkina Faso, Sierra Leone, Liberia, Ghana, Togo, Benin, Nigeria, Cameroon, Gabon, Congo, Central African Republic, Democratic Republic of Congo, Sudan, Ethiopia, Somalia, Angola, Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Botswana, Namibia, South Africa, Swaziland.
Remarks. This species inhabits savannas and grasslands, particularly rocky areas, and occurring in Tanzania at altitudes from 800 to 2,600 m. It exhibits extreme seasonal dimorphism, which is more pronounced in certain subspecies. During cold periods, dry-season individuals rest in dark places, such as holes in levees or inside buildings. In warmer periods, they are easily attracted to flowers (Kielland, 1990; Larsen, 2005; Williams, 2022).

***Precis tugela* Trimen, 1879 (Fig. 76)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, Monduli, 14.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03° 18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Democratic Republic of Congo, Sudan, Ethiopia, Uganda, Rwanda, Burundi, Kenya, Tanzania, Malawi, Zambia, Angola, Mozambique, Zimbabwe, South Africa, Swaziland.
Remarks. This species inhabits forests and woodlands, and occurring in Tanzania at altitudes from 350 to 2,500 m. It has a fast, low flight and is highly sensitive, quickly escaping to the treetops when disturbed. Dry-season individuals tend to gather in groups in holes or protruding areas of embankments (Kielland, 1990; Williams, 2022).

***Protogoniomorpha anacardii* (Linnaeus, 1758) (Fig. 63)**

Material examined. (2♂), TANZANIA, ARUSHA: 2♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H.



Figs. 81–84. Nymphalidae. 81) *Melanitis leda*, male (a. dorsal view; b–e; ventral view); 82) *Bicyclus campus*, male (a. dorsal view; b. ventral view); 83) *Henotesia perspicua*, male (a. dorsal view; b. ventral view); 84) *Bicyclus safitza* (a. male, dorsal view; b. female, dorsal view; c–i. ventral view).

Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Sierra Leone, Ivory Coast, Ghana, Togo, Nigeria, Angola, Central African Republic, Democratic Republic of Congo, Ethiopia, Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Botswana, South Africa, Swaziland, Madagascar, Arabia.
Remarks. This species inhabits open forests, riverine bush, and frost-free savannas, and occurring in Tanzania at altitudes from 0 to 2,200 m. It is often seen in open spaces or along roadsides, and when disturbed, it flies up, perches on leaves, then slowly descends and returns to its original position. It is frequently observed feeding on nectar from tree flowers and absorbing nutrients from mud puddles and animal feces (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

***Protogoniomorpha parhassus* (Drury, 1782) (Fig. 62)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, KARAMA Lodge, 08.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°22′47.0″, E36°42′53.0″ Alt: 1,414 m).
Distribution. Sub-Saharan Africa.
Remarks. This species inhabits forests, heavy woodlands, and degraded forests, particularly near streams and rivers, occurring in Tanzania at altitudes from 0 to 2,400 m. It is a common species, flying along the edges of forests and perching on leaves. Males defend their territories by perching on leaves with their wings three-quarters spread, driving away passing butterflies. At night, they roost in small groups under large leaves. This species is strongly attracted to flowers, and it also absorbs nutrients from mud puddles and animal feces (Kielland, 1990; Larsen, 1991; 2005; Williams, 2022).

***Vanessa abyssinica* (Felder & Felder, 1867) (Fig. 68)**

Material examined. (2♂), TANZANIA, ARUSHA: 1♂, Monduli, 14.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03° 18′19.0″, E36°28′09.8″ Alt: 1,586 m); 1♂, Monduli, 28. VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03° 18′19.0″, E36°28′09.8″ Alt: 1,586 m).
Distribution. Ethiopia, Democratic Republic of Congo, Uganda, Rwanda, Kenya, Tanzania.
Remarks. This species appears to be a subspecies (*Vanessa abyssinica jacksoni*), but it is treated as a distinct species in this study. It inhabits montane forests, and occurring in Tanzania at altitudes from 1,500 to 2,700 m. It is readily attracted to banana-baited traps (Kielland, 1990; Williams, 2022).

Subfamily Satyrinae Boisduval, 1833

***Bicyclus safitza* (Westwood, [1850]) (Fig. 84)**

Material examined. (13♂, 2♀), TANZANIA, ARUSHA: 1♂, KARAMA Lodge, 08.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°22′47.0″, E36°42′53.0″ Alt: 1,414 m); 2♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♂, TAWIRI Office, 27.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 9♂, 2♀, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Senegal, Guinea-Bissau, Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Gabon, Central African Republic, Ethiopia, Uganda, Kenya, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, South Africa, Swaziland.
Remarks. This species inhabits savannas and forests, primarily Guinea savannas in West Africa, and occurs in Tanzania at altitudes from 0 to 2,200 m. It has a slow and low flight, mainly moving in the semi-shade of forests and dense bushes. Additionally, it is strongly attracted to fermenting fruits (Kielland, 1990; Larsen, 2005; Williams, 2022).

***Bicyclus campus* (Karsch, 1893) (Fig. 82)**

Material examined. (1♂), TANZANIA, ARUSHA: 1♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24'45.0″, E36°42'22.0″ Alt: 1,308 m).
Distribution. Guinea, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Angola, Democratic Republic of Congo, Sudan, Uganda, Kenya, Tanzania.
Remarks. This species inhabits hilly terrain in forest and savanna mosaics, as well as forest edges in mountainous regions and woodlands. In Tanzania, it occurs at altitudes from 800 to 2,000 m. Due to its preference for hilly landscapes, its distribution is extensive but patchy (Kielland, 1990; Williams, 2022).

***Heteropsis perspicua* (Trimen, 1873) (Fig. 83)**

Material examined. (3♂), TANZANIA, ARUSHA: 3♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).
Distribution. Cameroon, Sudan, Ethiopia, Uganda, Kenya, Democratic Republic of Congo, Rwanda, Burundi, Tanzania, Malawi, Zambia, Mozambique, Zimbabwe, Botswana, Namibia, South Africa, Swaziland.
Remarks. This species inhabits savannas, particularly along the banks of streams and rivers, and occurring in Tanzania at altitudes from 0 to 2,100 m. It flies low with a bobbing flight among tall grass, mainly in shady areas near water sources. Additionally, it is strongly attracted to fermenting fruit (Kielland, 1990; Williams, 2022).

***Melanitis leda* (Linnaeus, 1758) (Fig. 81)**

Material examined. (10♂), TANZANIA, ARUSHA:



Figs. 85–89. Collecting sites. 85) TAWIRI forest, Arusha Urban; 86) KARAMA Lodge, Arusha Urban; 87) Engutoto village, Monduli; 88) Burka, Olasiti; 89) Engorora village, Kisongo.

1♂, KARAMA Lodge, 08.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°22′47.0″, E36°42′53.0″ Alt: 1,414 m); 3♂, TAWIRI Office, 11.V.2024, (Y.H. Kim, J.H. Ko, S.B. Son), (S03°24′45.0″, E36°42′22.0″ Alt: 1,308 m); 1♂, Monduli, 28.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°18′19.0″, E36°28′09.8″ Alt: 1,586 m); 5♂, Burka, Olasiti, 29.VIII.2024, (S.W. Jung, Y.H. Kim, J.H. Ko, S.B. Son), (S03°23′35.5″, E36°38′02.3″ Alt: 1,346 m).

Distribution. Afrotropical region, Saharo-Arabian region, Oriental region, Australian region, Oceanian region, Sino-Japanese region.

Remarks. This species has strong migration abilities and is widely distributed from Africa to Southeast Asia, East Asia, Australia. In Africa, it inhabits forests and savannas, including arid savannas, and occurring in Tanzania at altitudes from 0 to 2,000 m. It is a very common species, hiding in the shade of trees or among fallen leaves during the day and becoming active at dusk. It possesses excellent camouflage abilities, and when threatened, it slowly flaps its wings before disappearing into the leaf litter. Additionally, it is strongly attracted to fermenting fruits (Kielland, 1990; Larsen, 2005; Williams, 2022).

Conflicts of Interest

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

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First record of *Dolichospermum mucosum* (Nostocales, Cyanobacteria) in Korea: A study from the Han River

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The heterocytous cyanobacterium *Dolichospermum mucosum* was observed in Cheongpyeong Lake, located in the mid-to-downstream region of the North Han River. This species is morphologically characterized by irregularly and circularly coiled trichomes, which are consistently surrounded by a transparent mucilaginous envelope at a distance of 19.8 ± 11.3 μm. Akinetes were solitary, nearly spherical, and positioned at a distance from the heterocysts, with a width-to-length ratio of 1.1 ± 0.2. In phylogenetic analyses based on the 16S rRNA and *rbcLX* genes, this strain clustered within the same clade as previously reported *D. mucosum*. However, in the phylogenetic analysis, it grouped mostly closely with *D. smithii*, which exhibited the highest nucleotide sequence similarity. Structural variations in Box-B and V3 helices enabled clear differentiation between *D. mucosum* and *D. smithii*. Based on these morphological and phylogenetic characteristics, the *Dolichospermum* strain was identified as *D. mucosum*, marking its first recorded occurrence in the Republic of Korea.

Keywords: *Dolichospermum*, heterocytous cyanobacteria, morphological and phylogenetic analysis, secondary structure, unrecorded species

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Introduction

Cyanobacteria are primary producers in freshwater ecosystems and play vital roles in ecosystem dynamics (Tomitani *et al*., 2006; Bae *et al*., 2020). Heterocytous cyanobacteria such as the genus *Dolichospermum* contribute to the nitrogen cycle by fixing atmospheric nitrogen within specialized cells called heterocysts (Paerl *et al*., 2001; Deng *et al*., 2024). Species of the genus *Dolichospermum* inhabit lakes and rivers and are among the key cyanobacterial taxa responsible for harmful algal blooms (HABs) (Chia *et al*., 2018; Song *et al*., 2022). Depending on their ability to produce cyanotoxins, *Dolichospermum* species can be either toxic or non-toxic (Li *et al*., 2016; Zhang *et al*., 2023). Therefore, investigating species diversity within this genus is essential for monitoring algal blooms and managing water quality in freshwater ecosystems.

This genus was historically known as *Anabaena* (Bornet and Flahault, 1888). However, a molecular phylogenetic study in 2009 revealed that benthic and planktonic species formed distinct clades in the 16S rRNA phylogenetic tree owing to genetic divergence. Consequently, planktonic species possessing gas vesicles were reclassified into the newly designated genus *Dolichospermum* (Wacklin *et al*., 2009).

Traditionally, *Dolichospermum* species have been identi-

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fied based on their morphological characteristics and phylogenetic analyses. However, relying solely on morphology can lead to misidentification owing to several factors. First, morphological variations may arise owing to long-term cultivation or environmental changes (Rippka *et al*., 1979; Watanabe *et al*., 2004; Wang *et al*., 2024). Second, the formation rate of the akinetes was low when the samples were collected from the field. Therefore, isolating and culturing a single strain for a certain period is essential for accurate species identification. However, if the akinetes remain immature after prolonged incubation, the morphological characteristics necessary for species identification may not be fully understood (Choi *et al*., 2018).

Therefore, to overcome the limitations of species identification based solely on morphological characteristics, molecular markers, such as the housekeeping gene 16S rRNA (Zapomelova *et al*., 2008), the *rbcLX* gene associated with photosynthetic function (Tuji and Niiyama, 2010; Singh *et al*., 2015), and the Internal Transcribed Spacer (ITS) region, which exhibits high intraspecific variation, are widely utilized for cyanobacterial identification s (Li and Brand, 2007).

Currently, 61 *Dolichospermum* species are registered in the international algal database AlgaeBase (https://www.algaebase.org). The Korean algal database maintained by the National Institute of Biological Resources (https://species.nibr.go.kr) records 12 species (*Dolichospermum affine*, *D. circinale*, *D. crassum*, *D. flos-aquae*, *D. hangangense*, *D. helicoideum*, *D. mendotae*, *D. planctonicum*, *D. sigmoideum*, *D. smithii*, *D. spiroides*, and *D. viguieri*), representing 19.7% of the globally documented species.

In this study, we aimed to identify and characterize a *Dolichospermum* strain newly discovered in the Han River system using morphological observations and molecular phylogenetic analyses.

Materials and Methods

Sampling site and sampling method

Water samples were collected from Cheongpyeong Lake (37°42′31.42″N, 127°27′07.30″E) in the downstream of the North Han River in July 2013. Plankton nets with mesh diameters of 20 μm were used to collect phytoplankton (Wehr and Descy, 1998). The collected samples were sealed in the field and transported immediately to the laboratory.

Cultivation

Single trichomes were isolated from the samples using a Pasteur pipette under an IX73 inverted microscope (Olympus, Tokyo, Japan), and the isolated cells were grown in 1 mL of CB medium (pH 9.0) (Ichimura and Itoh, 1977) and incubated in 48-well plates. After culturing for 2–3 weeks, the cell density increased, and it was confirmed under a microscope that there was no contamination and only a single type of cell existed. The culture was then transferred to a filter-cap type 25 cm^2 cell culture flask (SPL, cat. no. 70025). The culture was maintained under constant culture conditions (CB medium, 20°C, 45 μmol photons m^{-2} s^{-1} light intensity, 12 : 12h light : dark cycle). The strain has been maintained in culture for 12 years through monthly subculturing. The single culture obtained was deposited in the Culture Collection of the Korea Research Institute of Bioscience and Biotechnology (KRIBB). The culture was assigned the resource number (KCTC-AG60755).

Morphological characteristics

Morphological characteristics of *D. mucosum* were evaluated using a fresh strain. For morphological observation of this species, a BX51 upright microscope (Olympus, Tokyo, Japan) was used. For species identification based on morphological characteristics, *The Subwasserflora Von Mitteleuropa, Bd. 19/3, Cyanoprokaryota - 3. Teil /3rd Part: Heterocytous Genera* (J. Komárek, Springer) was used as a reference. Cell dimensions were measured using the Cell sens program v.1.8 (Olympus, Tokyo, Japan). The following morphological characteristics were determined for at least 30 cells: long and wide vegetative cells, heterocysts, akinetes, and distance between trichomes and the mucilaginous sheath.

DNA extraction

The biomass of the strain was harvested during the exponential growth stage. Genomic DNA was extracted from the samples using the Chargeswitch™ Forensic DNA Purification Kit (Invitrogen, Thermo Fisher Scientific).

PCR and sequencing

The polymerase chain reaction (PCR) mixture (total volume 20 μL) consisted of 11.9 μL sterile water, 2 μL 10× Taq buffer, 2 μL dNTP, 0.1 μL Ex Taq of the Takara Ex Taq PCR kit (Takara Shuzo, Osaka, Japan), 1 μL of each primer (0.01 mM), and 2 μL template DNA. PCR was performed on an i-cycler (Bio-Rad, Hercules, CA, USA) using 16S rRNA primers CYA108F (5′-ACG GGT GAG TAA CRC GTR A-3′) (Nübel *et al*., 1997) and 16S CYR (5′-CTT CAY GYA GGC GAG TTG CAG C-3′) (Urbach *et al*., 1992) and amplified using the following steps: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min, and a final extension step of 72°C for 5 min. PCR amplification of *rbcLX* was performed with primers CW (5′-CGT AHC TTC CGG TGG TAT CCA CG T-3′) and CX (5′-GGG GCA GGT AAG AAA GGG TTT CGT A-3′) (Rudi *et al*.,

1998). In addition, amplification of *rbcLX* was performed in two steps as follows: initial denaturation of the first step at 94°C for 4 min, two cycles of 94°C for 30 s, 40°C for 30 s, 72°C for 2 min, followed by initial denaturation of the second step at 94°C for 30 s, followed by 38 cycles of 55°C for 30 s, 72°C for 2 min, and a final extension reaction at 72°C for 7 min. Additionally, PCR amplification of 16S-23S ITS was performed with primers 322 (5′-TGT ACA CAC CGC CCG TC-3′) and 340 (5′-CTC TGT GTG CCT AGG TAT CC-3′) (Laloui *et al*., 2002). Amplification of 16S-23S ITS was performed in two steps as follows: initial denaturation of the first step at 94°C for 10 min, 38 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min and a final extension reaction at 72°C for 7 min.

Phylogenetic and secondary structures analysis

Phylogenetic analyses were performed using the sequences of our strain and other sequences of the same genus retrieved from a public database. They were aligned using the BioEdit program v.7.1.3 (Hall, 1999). The alignment was edited manually. We determined the partial sequences of the 16S rRNA (1,250 bp) and *rbcLX* (747 bp). The sequences were deposited in GenBank (http://www. ncbi.nlm.nih.gov) with the accession numbers (16S rRNA: MN207474, *rbcLX*: MN205557). The topology of the phylogenetic tree was derived using the maximum-likelihood (ML) method. The GTR + G + I (the General Time Reversible model incorporating variant sites and a gamma distribution) model was selected for ML analyses as determined by ModelTest in MEGA 12.0 (Kumar *et al*., 2024). MEGA software (version 12.0) was used for ML and neighbor-joining phylogenetic analyses using *p*-distance models. All analyses were performed using bootstrapping with 1,000 replicates. Box-B and V3 helices of the ITS were edited using the BioEdit program v.7.1.3 (Hall, 1999). The secondary structures were constructed using Mfold 3.1 (Zuker, 2003).

Results and Discussion

Morphological characterization of *D. mucosum* KCTC-AG60755

Family: Aphanizomenonaceae
Genus: *Dolichospermum*
Species: *Dolichospermum mucosum*

Basionym: *Anabaena mucosa* Komárková-Legnerová & Eloranta.

Synonyms: *Anabaena spiroides* f. *ukrainica* (Schkorbatow) Elenkin 1938; *Anabaena ucrainica* (Schkorbatow) M.Watanabe 1996; *Dolichospermum ucrainicum* (Schkorbatow) Tuji & Niiyama 2012.

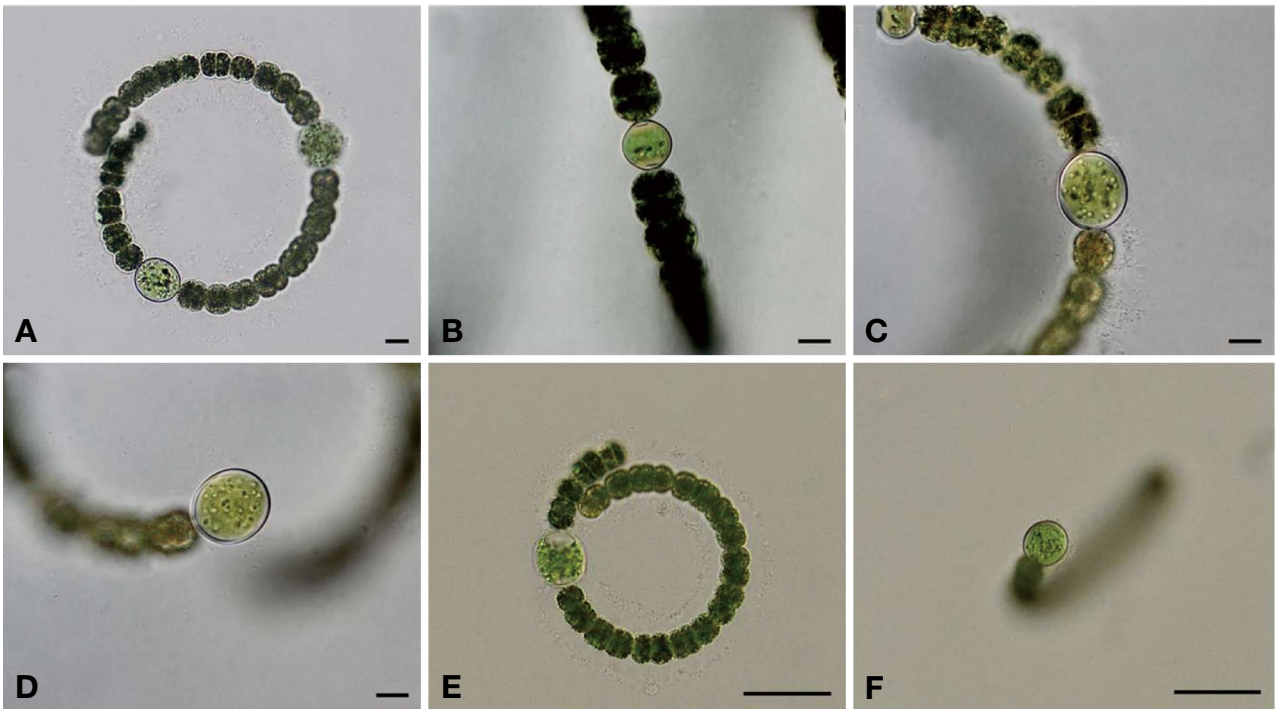


Fig. 1. Morphological characteristics of *Dolichospermum mucosum* (KCTCAG60755) isolated from the Han River. (A) Trichome. Transparent mucilaginous sheath was formed surrounding trichome. (B) Heterocyst. (C) Ripen akinete. (D) Akinete formed at the end of the trichome. (E) Trichome morphology of *D. mucosum* photographed in 2013. (F) Akinete formed at the end of the trichome, photographed in 2013. Scale bars = 10 μm.

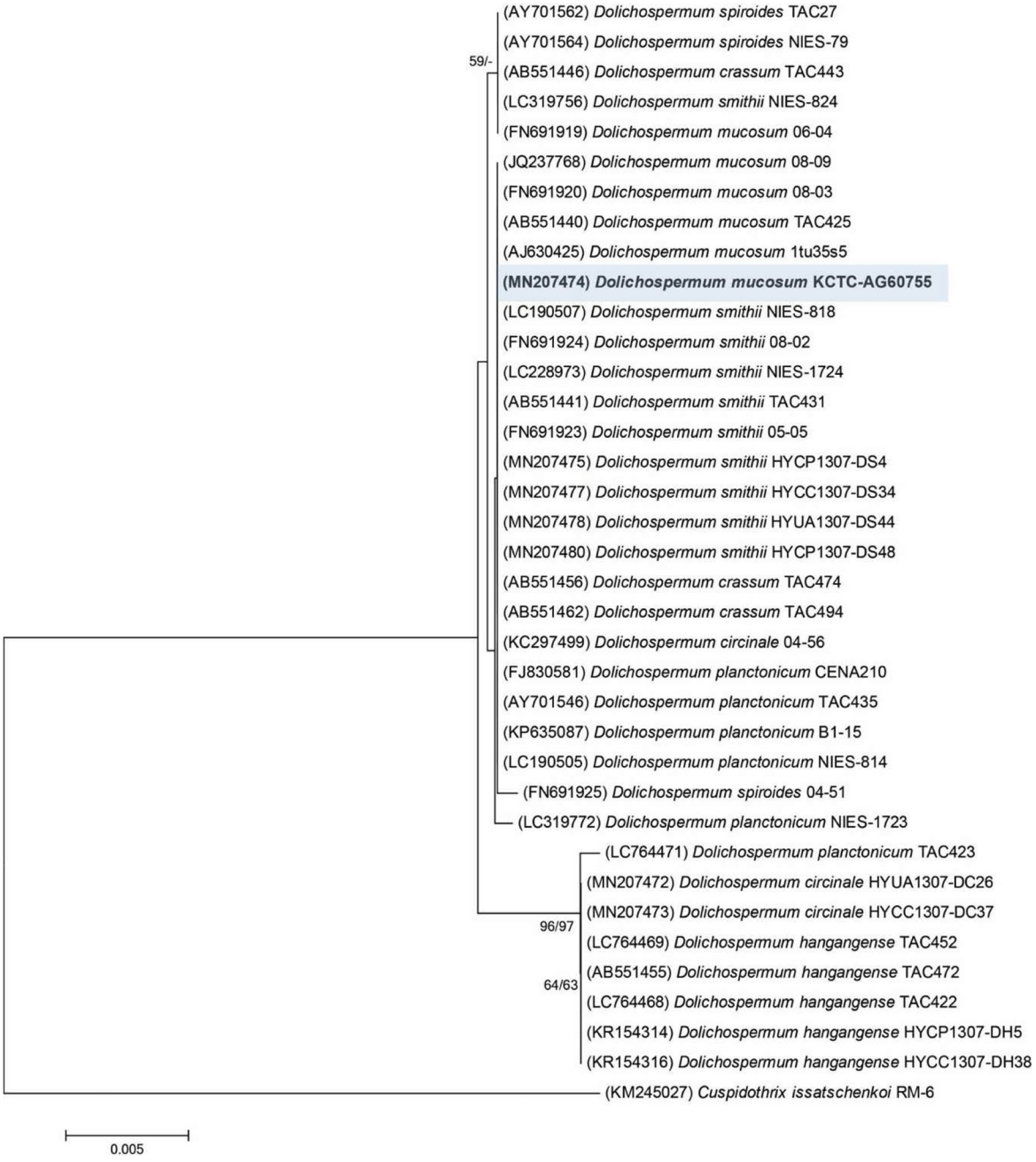


Fig. 2. Neighbor joining based on 16S rRNA (1,250 bp) sequences showing the clustering of cultured *Dolichospermum* spp. strains. Numbers above branches indicate bootstrap support (>50%) from neighbor joining/maximum likelihood. *D. mucosum* has skyblue background. *Cuspidothrix issatchenkoi* RM-6 was used as the out group.

Trichomes were free-floating and solitary, exhibiting an irregular, circularly coiled form that occasionally appears loosely coiled. A transparent mucilaginous envelope consistently surrounded and maintained a uniform distance from the trichome. The distance between the trichome and the mucilaginous sheath ranges from 9.9 to 25.9 μm (mean ± SD: 19.8 ± 11.3 μm). The trichomes were not tapered at their ends. Vegetative cells were spherical to barrel-shaped, dark blue-green, and contained numerous aerotopes, measuring 9.5–17.3 μm in length (12.5 ± 5.5 μm) and 4.3–11.1 μm in width (7.2 ± 4.8 μm) (Fig. 1A). Heterocysts were solitary, spherical, and exhibited a greenish hue, resembling hollow beads. They measure 9.6–13.7 μm in length (11.7 ± 2.9 μm) and 9.4–14.2 μm in width (11.4 ± 3.4 μm) (Fig. 1B). Akinetes were solitary, intercalary, and positioned at a distance from heterocysts. They

Table 1. Sequence similarity and *p*-distance between 5 closely related species and *D. mucosum* of the partial 16S rRNA gene sequences.

Species names of relatives	Strain names	Accession no.	Sequence similarity (%)	*p*-distance (%)
Dolichospermum smithii	HYCC1307-DS34	MN207477	100	0.0000
	HYUA1307-DS44	MN207478	100	0.0000
	HYCP1307-DS48	MN207480	100	0.0000
	HYCP1307-DS4	MN207475	99.92	0.0008
	05-05	FN691923	99.04	0.0096
	08-02	FN691924	98.96	0.0104
	TAC431/NIES-820	AB551441	98.96	0.0104
	NIES-818	LC190507	98.96	0.0104
	NIES-1724	LC228973	98.96	0.0104
Dolichospermum crassum	TAC474/NIES-1657	AB551456	99.04	0.0096
	TAC494/NIES-1665	AB551462	99.04	0.0096
Dolichospermum mucosum	08-03	FN691920	98.96	0.0104
	08-09	JQ237768	98.96	0.0104
	TAC425/NIES-1677	AB551440	98.96	0.0104
	1tu35s5	AJ630425	98.96	0.0104
Dolichospermum circinale	04-56	KC297499	98.96	0.0104
Dolichospermum planctonicum	NIES-814	LC190505	99.04	0.0096
	CENA210	FJ830581	98.96	0.0104
	B1-15	KP635087	98.88	0.0112

were nearly spherical, measuring 18.2−26.3 μm in length (21.5 ± 5.7 μm) and 19.5−30.4 μm in width (23.2 ± 7.7 μm). The width-to-length ratio of akinetes ranged from 1.0 to 1.2 (1.1 ± 0.2) (Fig. 1C). Akinetes were frequently formed at the ends of trichomes (Fig. 1D).

A distinguishing morphological characteristic of *D. mucosum* is the presence of spherical akinetes and a transparent mucilaginous sheath surrounding the trichome. However, this study identified a previously unreported feature: the mucilaginous sheath maintains a consistent distance from the trichomes. To quantify this trait, the distance between the trichome and the mucilaginous sheath was measured in more than 30 trichomes, and the range, mean value, and standard deviation were recorded. This characteristic is also depicted in an illustration of *D. mucosum* on page 712 of *Süßwasserflora von Mitteleuropa, Bd. 19/3: Cyanoprokaryota* (2013). Therefore, the distance between the trichome and the mucilaginous sheath can be considered a species-specific morphological feature of *D. mucosum*.

Long-term cultivation had no effect on the morphological characteristics of *D. mucosum*, including the coiled and circular trichome structure, wide gap between the trichome and mucilaginous sheath, spherical akinetes, and occasional formation of akinetes at the ends of the trichome (Fig. 1E, F). This finding indicated that the key morphological traits of *D. mucosum* were stable over time.

Molecular phylogeny of *D. mucosum*

The cultured strain of the cyanobacterium *D. mucosum* isolated in this study was registered in the NCBI database with the nucleotide sequences of the 16S rRNA and *rbcLX* regions (accession numbers: 16S rRNA, MN207474; *rbcLX*, MN205557). Phylogenetic analysis of the 16S rRNA gene showed that *D. mucosum* (KCTC-AG60755) formed a clade with four *D. mucosum*, nine *D. smithii*, two *D. crassum*, one *D. circinale*, and four *D. planctonicum* strains from the Korean and international culture collections (Fig. 2).

The nucleotide sequence similarity between *D. mucosum* and its closely related species ranged from 98.88% to 100%, with a *p*-distance of 0−0.0112%. These five species shared a minimum sequence similarity of 98.88%, making species-level identification challenging (Table 1). Thus, the 16S rRNA region is known to have low resolution for distinguishing species, particularly in heterocytous cyanobacteria (Wacklin *et al*., 2009; Zapomelova *et al*., 2009; Ki, 2010; Lee *et al*., 2020).

To address this limitation, a phylogenetic analysis of the *rbcLX* gene was conducted. *Dolichospermum mucosum* (KCTC-AG60755) formed a clade with one *D. mucosum* strain and four Korean *D. smithii* strains. Although phylogenetic analysis of the *rbcLX* region provided a higher resolution for species identification than that of the 16S

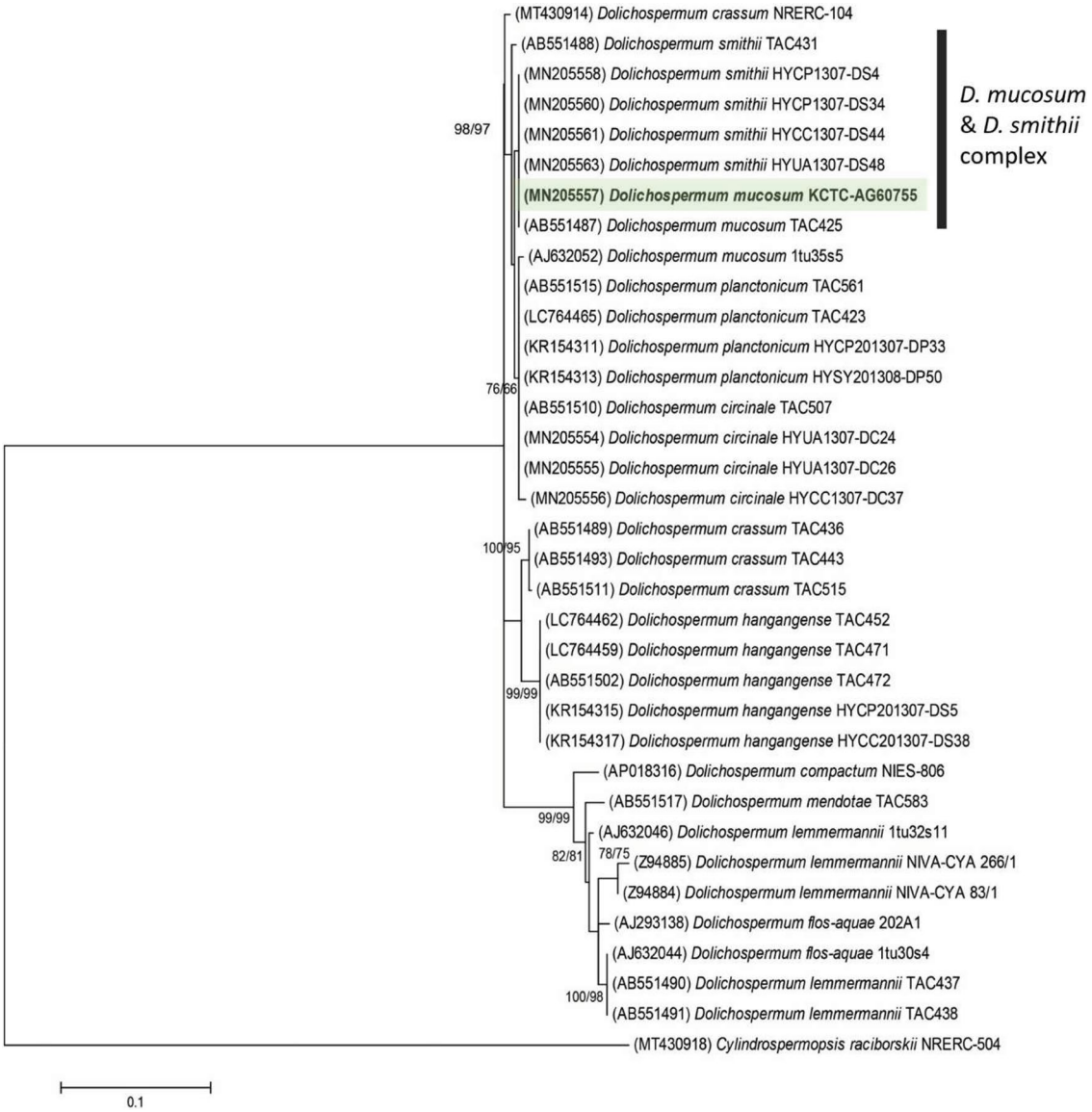


Fig. 3. Neighbor joining based on *rbcLX* (747 bp) sequence showing the clustering of cultured *Dolichospermum* spp. strains. Numbers above branches indicate bootstrap support (>50%) from neighbor joining/maximum likelihood. *D. mucosum* has green background. *Cylindrospermopsis raciborskii* NRERC-504 was used as the out group.

rRNA region, it still exhibited limitations in differentiating *D. mucosum* from *D. smithii* (Fig. 3).

The *rbcLX* gene encodes ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), a key enzyme involved in carbon fixation that plays a crucial role not only in cyanobacteria but also in various photosynthetic organisms. This gene exhibits high resolution for detecting subtle differences at the species level and provides reliable phylogenetic insights, particularly for heterocytous cyanobacteria (Gugger *et al*., 2002; Tuji *et al*., 2023).

Secondary structure

To differentiate *D. mucosum* from the closely related *D. smithii*, which could not be resolved through phylogenetic analysis, secondary structure analysis was performed on the Box-B and V3 helix regions within the 16S-23S ITS gene.

Both *D. smithii* and *D. mucosum* possessed three loops and a nucleotide sequence consisting of 48 bases in the Box-B helix. However, differences in loop size and nucleotide composition distinguish these species. The nucleotide sequence of the first loop differed between *D. mucosum* and *D. smithii* (AUAAAAU vs. UAUGC), whereas that of the second loop varied (CGACUAC vs. GUUAACGCA CCU) (Fig. 4A).

In the V3 region, the secondary structure of *D. smithii*

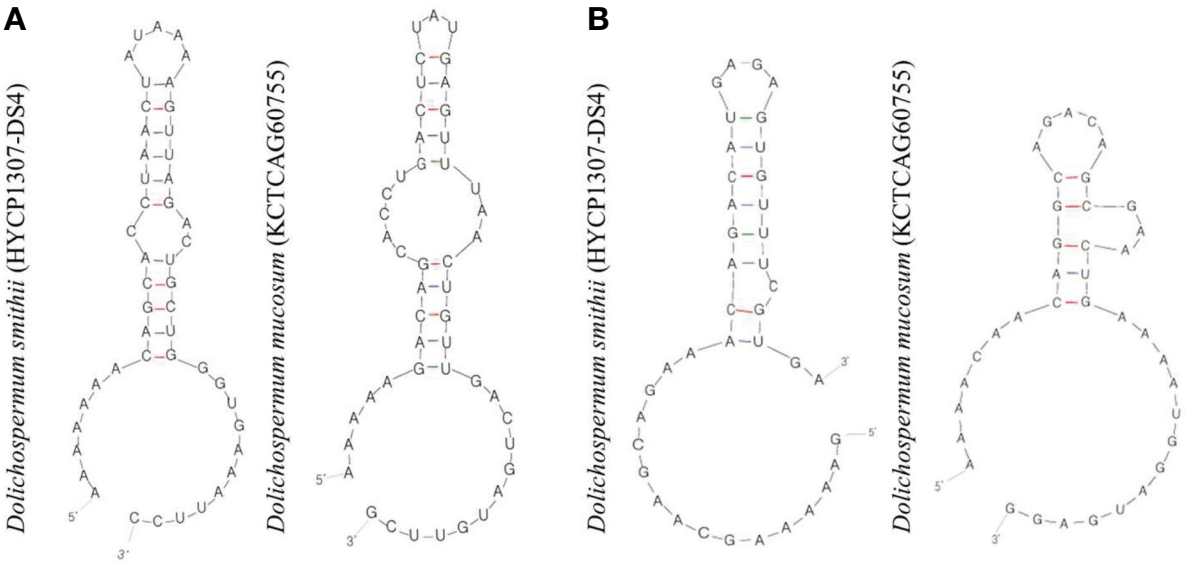


Fig. 4. Comparison of *Dolichospermum mucosum* and its closely related species *D. smithii* based on the secondary structures of (A) the Box-B helix and (B) the V3 helix within the 16S-23S ITS gene.

consisted of three loops with 39 bases. Similarly, *D. mucosum* possessed three loops; however, its total nucleotide count was 38 bases. Additionally, the size and shape of the loops differed significantly, allowing for a clear differentiation between the two species (Fig. 4B). The 16S-23S ITS gene is a non-coding region with high genetic variability among species, making it a valuable marker for species-level classification and for distinguishing closely related taxa (Johansen *et al*., 2011; Vaccarino and Johansen, 2012). Its secondary structure, which is composed of helices, loops, and stems, is species-specific and evolutionarily conserved, enhancing the reliability of species identification (Iteman *et al*., 2000; Kim *et al*., 2021). In addition, the Box-B and V3 helices are the most variable regions within heterocytous cyanobacteria and are widely used as molecular markers for species- and intraspecies-level identification (Li and Brand, 2007; Ni *et al*., 2008; Stanojkovic *et al*., 2022).

This is the first report of a previously unrecorded species, *D. mucosum* in Korea, with species identification based on an integrative approach combining morphological and molecular phylogenetic analyses. The findings of this study are expected to serve as valuable reference data for future studies on the identification and classification of heterocytous cyanobacteria.

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Conflicts of Interest

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

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NOTE

Two newly recorded species of Geometridae (Lepidoptera) from Korea

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The Lepidopteran family Geometridae is one of the most diverse and widely studied taxa in Korea, encompassing over 726 species. In this study, we report two species for the first time in Korea: *Problepsis albidior* Warren, 1899, belonging to the subfamily Sterrhinae, shows a whitish wing with a large medial eye spot encircled by ochreous and blackish scales. From its close relative, the male genitalia can be distinguished by the relatively rounded ceras on the eighth sternite, the strongly dentate inner margin of the tegumen, and two minute cornuti on the vesica. *Proomphe lobata* Warren, 1896, from the subfamily Larentiinae, features a light brown forewing with dark ochreous costal area, a pointed apex, a toothed distal margin, and a yellowish hindwing. The male genitalia are characterized by a strongly tapering uncus, a well-developed transtilla and anellus lobe, and a slender valva with a long, club-shaped harpe. A simple antrum, a long ductus bursae, and an ovate corpus bursae without a signum identify the female genitalia. Diagnoses, redescription, along with photographs of adults and their genitalia, are provided.

Keywords: biodiversity, Korea, moth fauna, *Problepsis*, *Proomphe*

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INTRODUCTION

The genus *Problepsis* Lederer (1853) was established with *Caloptera ocellata* Frivaldszky, 1845 as its type species and comprises over 51 species worldwide with high diversity in the Old World and Australia (Hausmann, 2004; Xue *et al*., 2018). Members of this genus are easily diagnosed by the presence of eye spots encircled by silvery scales on whitish or grayish white wings, and by the fused socii in the male genitalia (Hausmann, 2004; Xue *et al*., 2018). In Korea, five species of *Problepsis* have been recorded (NIBR, 2024).

The genus *Proomphe* established by Warren (1896) with *Proomphe lobata* Warren as the type species, includes only a single species occurring in the northern India, southwestern China, and Russian Far East. This monotypic genus exhibits remarkable wing shape that resembles *Dysathia* Warren (a synonym of *Heterophleps* Herrich-Schäffer, 1854) (Warren, 1896). The purpose of the present paper is to report two geometrid moths, *Problepsis albidior* Warren (1899) and *Proomphe lobata* Warren, 1896 for the first time in Korea.

MATERIALS AND METHODS

The examined materials in this study were collected using a 22-watt UV-light bucket trap with a 12 V battery (BioQuip, USA) and are deposited in the Department of Environmental Education, Mokpo National University and National Institute of Biological Resources, Incheon.

Species identification was based on the external mor-

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phology of adults including the genitalia. For genitalia slide preparation, the specimen was prepared by boiling the abdomen in 10% potassium hydroxide (KOH) for approximately 15−20 min. The scales and tissues were removed using a stereomicroscope (Leica S9i), stained with Chlorazol black, and mounted on slides in Euparal mounting medium. Photographs of the adults were taken using a Nikon D300 DSLR camera equipped with an AF-S Micro-Nikkor 105 mm f/2.8G IF-ED objective, and genitalia were photographed with built-in digital camera of a Leica S9i stereomicroscope.

Terminology refers to Hausmann (2001; 2004). Abbreviations used in this study are as follows: TS - type species; TL - type locality; GW - Gangwon-do; GB - Gyungsangbuk-do; JB - Jeollabuk-do; JN - Jeollanam-do.

Taxonomic Accounts

Order Lepidoptera Linnaeus, 1758
Family Geometridae Leach, 1815
Subfamily Sterrhinae Meyrick, 1892

***Problepsis* Lederer, 1853**

Problepsis Lederer, 1853: 74. TS: *Caloptera ocellata* Frivaldszky, 1845. TL: [Greece] Crete, [Turkey] Smyrna (= Izmir).
= *Argyris* Guenée, 1858.
= *Problepsiodes* Warren, 1899.
= *Caloptera* Frivaldsky, 1845 (preocc. Gistl, 1834).

***Problepsis albidior* Waren, 1899 (Figs. 1A, 2A−C)**

두눈큰애기자나방

Problepsis albidior Warren, 1899: 33. India: Kulu.

Material examined. 1 male, JN: Wando, Chungsan-myeon, Is. Chungsan-do, E126°53′06″ N34°12′23″, 97 m, 1.VI.2010, Sei-Woong Choi (NIBR RORYIN0000004888).

Diagnosis. The species is externally indistinguishable from the sister species, *Problepsis eucircota* Prout, but the species can be distinguished by the male eighth sternite and genitalia: *P. albidior* has a strongly dentate inner margin of the tegumen, while it is smooth in *P. eucircota*; *P. albidior* presents two minute cornuti on the vesica, whereas *P. eucircota* shows several large cornuti; and *P. albidior* features relatively rounded ceras, compared to the less rounded ceras in *P. eucircota*.

Redescription. Wingspan 33 mm. Antenna in male partially bipectinate with long rami, distal 1/4 filiform; labial palpi short, almost the same length as the eye diameter, barely projected beyond frons, dorsally blackish, ventrally whitish; frons blackish, lower middle yellowish white; vertex blackish. Thorax whitish, legs covered with long dark brownish hairs. Forewing ground color whitish; costa blackish; medially with a large eye spot encircled by ochreous and blackish scales and a small ochreous spot; subtermen with ochreous rounded line, followed by a series of faint, parallel dark dots; termen blackish dotted. Hindwing ground color whitish; medially with a dark grayish, flattened sphere-shaped marking and dark grayish tinges; subtermen grayish relatively thick, rounded line, followed by a parallel line with grayish dots.

Male abdomen and genitalia. 8th sternite anterior broad, medially narrowed, posteriorly three-branched form, wide ceras and relatively slim mappa in the middle. Uncus absent; socii fused in the middle; tegumen long, slender, upper inner margin strongly dentate; juxta sclerotized, tripod-shape; saccus broad. Valva sclerotized, strongly bifurcated; costa with a long, weakly hooked valvula; sacculus with a long, sclerotized and sharply pointed fibula. Aedeagus hammer-shaped with a large dilated caecum, apically deeply invaginated U-shaped with a short, sharply pointed process; cornuti two separate minute processes.

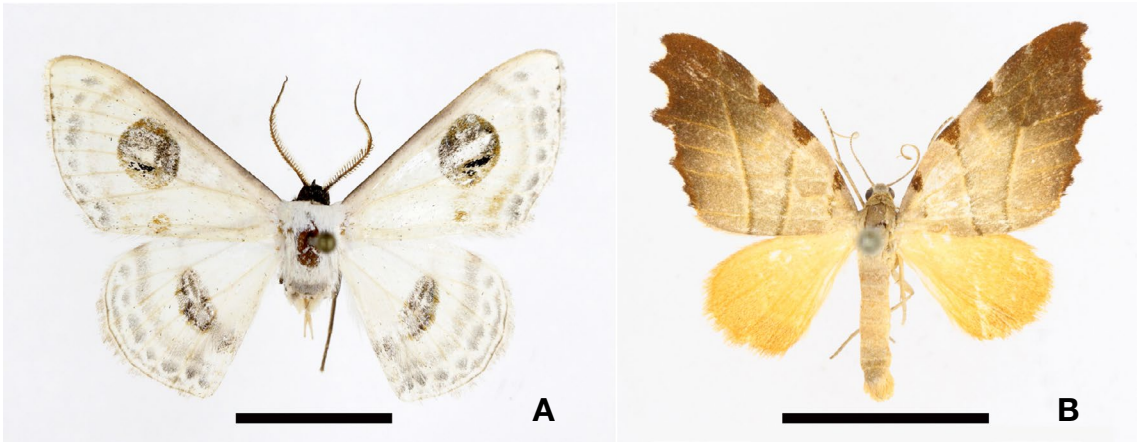


Fig. 1. Adults from Korea. Scale bar = 10 mm. A. *Problepsis albidior* Warren, B. *Proomphe lobata* Warren.

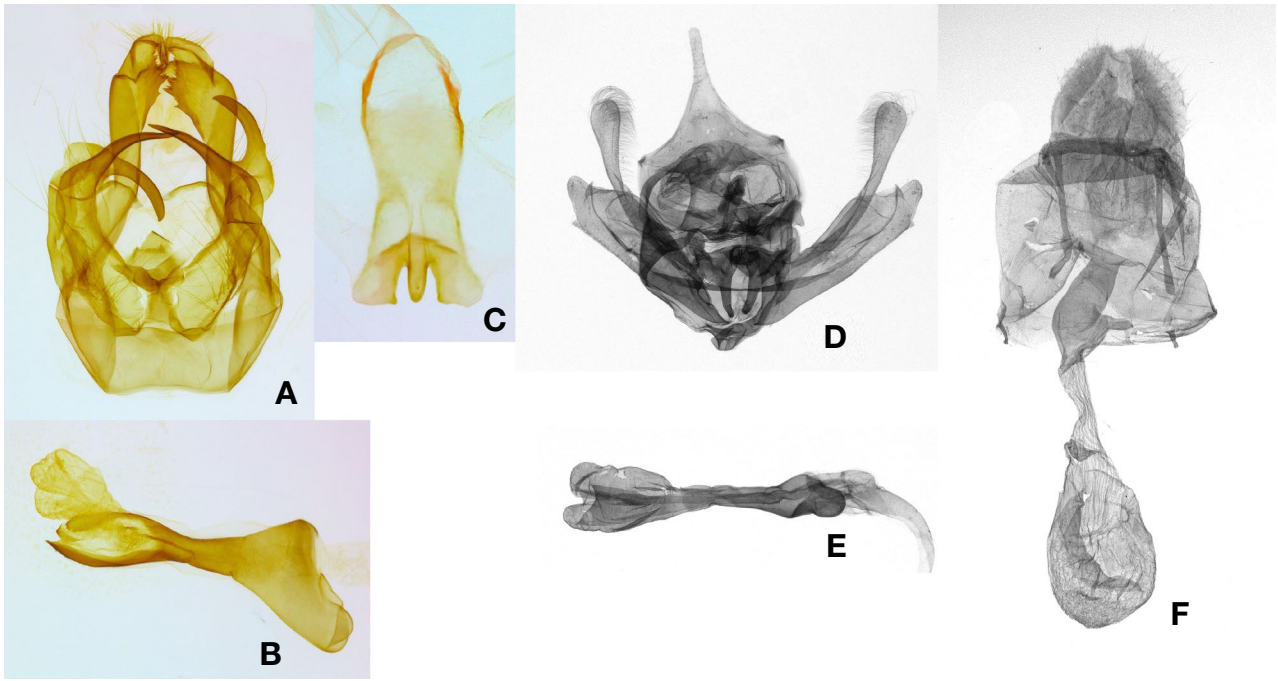


Fig. 2. Genitalia of two species of Geometridae from Korea. A, D. Male genital capsule, B, E. Aedeagus, C. 8th Sternite, F. Female genitalia. A–C. *Problepsis albidior* Warren, D–F. *Proomphe lobata* Warren.

Distribution. Korea, Japan, China (Gansu, Anhui, Zhejiang, Hubei, Hunan, Fujian, Guangdong, Hainan, Guangxi, Sichuan, Yunnan, Tibet), Taiwan, Indonesia, India.

Remarks. Including *Problepsis albidior*, a total of six species of the genus have now been recorded in Korea (NIBR, 2024). While the host plants of *Problepsis albidior* remain unidentified, many species within this genus are known to feed on plants from the Oleaceae (Singh, 1957; Holloway, 1997; Xue *et al*., 2018).

Subfamily Larentiinae Duponchel, 1845

***Proomphe* Warren, 1896**

Proomphe Warren, 1896: 316. TS: *Proomphe lobata* Warren, 1896. TL: [India, Meghalaja] Sikkim.

***Proomphe lobata* Warren, 1896 (Figs. 1B, 2D–F)**

고구려물결자나방 (신칭)

Proomphe lobata Warren, 1896: 316. TL: [India, Meghalaja] Sikkim.

Material examined. 2 males 1 female, GW: Samchuk, Nogok-ri, 24.VI.2023; 1 male, GW: Chuncheon, 15.VI. 1992; 1 male, GW: Taeback, Tong-ri, 8.VII.2008; 1 male 1 female, GW: Pyungchang-gun, Mt. Nambyungsan, 9.VI. 2016; 1 male, GW: Pyungchang, Mt. Jungangsan, 1.VII. 2011; 1 male, GW: Pyungchang, Mt. Jungangsan, 22.VI. 2017; 1 male, GB: Bongwha, Seungbu-ri, 11.VI.2023; 1 male, JB: Namwon, Banseon, E127°34′57.9″ N35°22′ 33.1″, 4.VI.2022. Sei-Woong Choi (NIBR PCQAIN0000 010654).

Diagnosis. *Proomphe lobata* is distinguishable by its forewings, which show a light brown ground color and dark ochreous costal area, a pointed apex, a toothed distal margin, three transverse medial lines, and yellowish hindwings without a medial line. This species exhibits a resemblance to *Heterophleps fusca* (Butler, 1878) by the presence of two dark-colored costal markings on the forewings. However, it can be readily distinguished by the toothed distal margin of the forewing and the yellowish hue of the hindwing. The male genitalia are characterized by a strongly tapering uncus, a well-developed transtilla and anellus lobe, and a slender valva with a long, club-shaped harpe. In females, the genitalia are identified by a simple antrum, a long ductus bursae that shows striated anteriorly and simple, sclerotized posteriorly, and an ovate corpus bursae lacking a signum.

Redescription. Wingspan 21 mm. Male antennae filiform; labial palpi short, almost not extending beyond the frons; frons square in shape, almost flat; vertex light grayish. Forewing ground color light brown with dark brown or dark ochreous, apex pointed, termen with toothed distal margin, the largest teeth on veins M_1 and M_3; costal margin with three triangular dark brown spots in the basal and medial areas, one light-brown fleck subapically, which is placed almost equidistantly to each other; three brownish

transverse lines from base to postmedian, underlined by yellow on one side; central fascia costally broad, tapered to dorsum. Hindwing ground color yellow, tinted with light brown suffusion along the outer margin.

Male genitalia. Uncus long, strongly tapering, almost triangular; tegumen broad, upper edge sclerotized with minute projected; transtilla with a pair of long apically projected processes; anellus lobe long, digitate; saccus weakly projected. Valva slender; costa membranous, medially expanded; harpe a long rod-shaped process with distally hairy and dilated, strongly projected beyond valva; sacculus long, slender with a short distal sharply pointed process. Aedeagus rod-shaped; cornutus absent.

Female genitalia. Papillae anales large, rounded; anterior apophyses very short, about 1/6 of posterior apophyses. Antrum simple, flat; ductus bursae long, anterior half with dense stripes, posterior half simple, sclerotized, weakly dilated; corpus bursae ovate, without a signum.

Distribution. Korea, China (Southwest), Russian Far East, India.

Remarks. Dr. Jaan Viidalepp (Tartu, Estonia), and Dr. Evgeny Beljaev (Vladivostok, Russia) prepared a description of a potential new taxon of *Proomphe* based on material from the Russian Far East. In addition, a specimen from southwestern China in Zoologische Staatssammlung München exhibited morphological differences compared to the type specimen in the British Museum, indicating the possibility of more than one species of *Proomphe* in the World.

Conflicts of Interest

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

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Erickson, L.E. and G. Gredrickson. 1990. Phylogeny and ontogeny of the thyroid gland. In: M.A. Greer (ed.), The Thyroid Gland (2nd ed.), Raven Press, New York. pp. 1-36.

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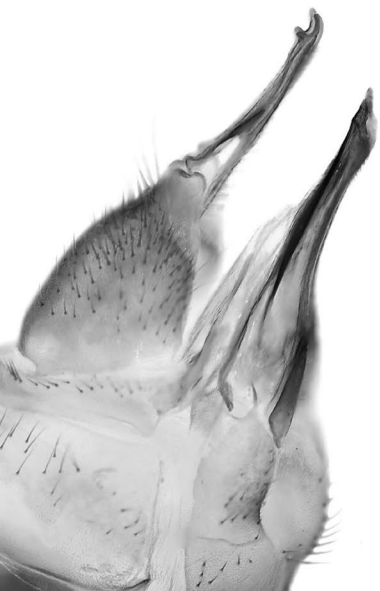
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Front Cover

This cover shows photographs of butterflies collected in the Arusha Region of northern Tanzania. The related article and detailed information can be found on pages 404–433. Photos by Jae-Ho Ko.