

RESEARCH ARTICLE

First Reports of Unrecorded Mortierellomycetes and Umbelopsidomycetes Fungi from Freshwater Ecosystems in Korea

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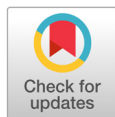
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ABSTRACT

In this study, we isolated several fungal strains from filtered water and sediment collected from rivers and streams. The strains were identified by molecular phylogenetic analyses of rDNA sequences (internal transcribed spacer [ITS], large subunit of ribosomal DNA [LSU]). The morphological characteristics of the fungi were investigated using microscopy, and the culture characteristics of fungi grown on several media were examined. We identified four species previously unknown in South Korea, namely, *Dissophora globulifera*, *Linnemannia exigua*, *Mortierella rishikesha* and *Umbelopsis autotrophica*.

Keywords: *Dissophora*, *Linnemannia*, *Mortierella*, *Umbelopsis*



OPEN ACCESS

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2023 September, 51(3): 155-165
<https://doi.org/10.4489/kjm.20230018>

Received: June 23, 2023

Revised: September 12, 2023

Accepted: September 18, 2023

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INTRODUCTION

Zygomycetous fungi, such as Mortierellomycota and Mucoromycota, are classified based on their reproductive characteristics. They produce zygospores for sexual reproduction, and conduct asexual reproduction via sporangia [1]. In yesteryears, the two phyla were grouped in a same phylum, Zygomycota; however, recently, they have been classified as two separate phyla [2]. Several species belonging to Mortierellomycota and Mucoromycota have been isolated from the soil, plant roots, decaying plant materials, endophytic plant litter, insect guts, mosses, animal dung, and freshwater environments [3,4].

Fungal species belonging to Mortierellomycota generally possess anastomosing hyphae and thalli with dichotomously branching multispored spherical sporangia [2]. They are found worldwide in various habitats including aquatic and terrestrial environments [2]. As stated in Mycobank, 21 genera have been reported as of 2022 [5].

The genus *Dissophora* of Mortierellaceae contained three species as of 2021 [6]. Using low-coverage genome sequences, this genus forms a clade distinct from *Mortierella* species [3]. *Dissophora* fungi are known to be found in forest litter and soil, unlike common *Mortierella* species [3]. Fungal species of this genus have fertile, septate aerial stolons that are differentiated from vegetative hyphae [3].

In the genus *Linnemannia* of family Mortierellaceae, 26 species have been reported as of 2022 [7]. Numerous fungal species of this genus have been isolated from soils, and are associated with the plant rhizosphere or litter [3]. This genus is classified according to the sporangia characteristics and sporangiospore morphology [3].

In the genus *Mortierella* of family Mortierellaceae, 173 species were reported in 2023 [5]. Many *Mortierella* species grow well at cooler temperatures and few species have been reported to be mycoparasites [3]. Various *Mortierella* species have been found in a wide range of habitats, including soil, water, living plant roots, and plant debris [3]. In South Korea, four *Mortierella* species have been isolated from freshwater sources [8,9].

Several fungal species belonging to Mucoromycota have been reported to be plant-associated fungi (e.g., plant symbionts, plant debris decomposers, or pathogens) [10]. They generally produce globose, smooth, or ornamented zygospores and exhibit rapid mycelial growth [10].

The genus *Umbelopsis* belongs to the family Umbelopsidaceae, order Umbelosidales, class Umbelopsidomycetes, and phylum Mucoromycota [2]. To date, 22 species of this genus have been reported [11]. Several species have been isolated from the soil, plant debris, roots, and human skin [11,12]. This genus is classified according to characteristics such as the morphology of the sporangia, sporangiospores, and colonies [12].

This is the first report of four fungal species (namely *Dissophora globulifera*, *Linnemannia exigua*, *Mortierella rishiksha*, and *Umbelopsis autotrophica*) found in South Korea from environmental samples such as freshwater, plant litter, and sediment in streams. The molecular, phylogenetic, and morphological characteristics of these species were also investigated.

MATERIALS AND METHODS

Isolation of fungal strains

Fungal strains were isolated from the freshwater environmental samples (filtered water and stream and river sediments) collected in South Korea. The collection information for all the strains identified in this study is listed in Table 1. A dilution method was used to isolate fungal strains from the sediment. The diluted suspensions (200 μ L) of sediment or soil with distilled water (1:100 and 1:1,000) were spread on potato dextrose agar (PDA; 3.9% PDA powder [w/v]; Difco, Sparks, MD, USA) containing 100 ppm of streptomycin, and fungal strains were isolated in pure form after incubation for 4-5 days at 25°C by repeating this step. To isolate fungal strains from freshwater, 50 mL of freshwater was filtered through a membrane filter. Membrane filter was attached on water agar (WA; 1% agar powder; Duksan Pure Chemicals Co., Ltd., Ansan, Korea) with 100 ppm of streptomycin and incubated at 20°C overnight. After removing membrane, hyphal tips and germinated conidia were observed under a microscope, transferred onto a 24-well plate containing V8 agar (V8A; 8% V8 juice [v/v] and 1.5% agar [w/v], adjusted to pH 6.0

using 10 N NaOH), and incubated for 7 days at 25°C in the dark. All strains identified in this study were grown on malt extract agar (MEA; 2% malt extract [w/v] and 2% agar [w/v]), oatmeal agar (OA; 7.25% OA powder [w/v]; Difco), corn meal dextrose agar (CMDA; 2% cornmeal [w/v], 2% glucose [w/v], and 2% agar), and yeast extract peptone dextrose agar (YPDA; Duchefa Biochemie, Haarlem, Netherlands).

Table 1. Information of strains used in this study.

Species	Strain No.	Source	Collection date	Location (GPS)
<i>Dissophora globulifera</i>	NNIBRFG26655	Freshwater	February 13, 2020	Cheonnae-ri, Jewon-myeon, Geumsan, Chungcheongnam-do (36°7'57.9"N, 127°35'31.2"E)
<i>Linnemannia exigua</i>	NNIBRFG5579	Freshwater	March 16, 2018	Yangji-ri, Goro-myeon, Gunwi-gun, Gyeongsangbuk-do (36°9'45"N, 128°49'31"E)
<i>Mortierella rishiksha</i>	NNIBRFG6579	Sediment in freshwater	July 27, 2018	Neunggang-ri, Susan-myeon, Jecheon-si, Chungcheongbuk-do (36°59'27"N, 128°12'30"E)
<i>Umbelopsis autotrophica</i>	NNIBRFG4148	Sediment in freshwater	May 25, 2017	Ocheon-ri, Yeonil-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do (35°59'54.6"N, 129°21'44.3"E)

DNA extraction, polymerase chain reaction (PCR), and DNA sequencing

Fungal genomic DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Düren, Germany). For the molecular identification of fungi, PCR amplification was performed for the internal transcribed spacer (ITS) rDNA region using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [13], and the large subunit of rDNA (LSU) was identified using primers LR0R (5'-ACCCGCTGAACCTTAAGC-3') and LR7 (5'-TACTACCACCAAGATCT-3') [14]. Amplicons were sequenced by a DNA sequencing service (Macrogen Inc., Seoul, Korea) using the same primers as those used for amplification. A homology search of the DNA sequences was conducted using BLAST algorithms from the National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov>).

Phylogenetic analysis

Previously published reference sequences [3-4,7,9-12,15] were obtained from the NCBI database (Table 2). The sequences were edited using DNASTar (version 5.05, DNASTar Inc., Madison, WI, USA). The accession numbers of the sequences used in this study are listed in Table 2. Phylogenetic trees were constructed using maximum likelihood (ML) analysis, which was performed using the default settings of MEGA 7.1 [16], except for the replacement with Tamura-Nei model. Bootstrapping analysis of 1,000 replicates was performed to test the robustness of each grouping.

Table 2. Taxa, collection numbers, and GenBank accession numbers used in the present study.

Species	Collection no. (Isolate no.)	GenBank accession no.	
		ITS	LSU
<i>Dissophora globulifera</i>	CBS 108.68	JX975847	KC018332
<i>Dissophora globulifera</i>	CBS 746.68	JX976026	KC018371
<i>Dissophora globulifera</i>	CBS 858.70 (NT)	JX975915	HQ667368
<i>Dissophora globulifera</i>	NNIBRFG26655	ON715845	ON715854
<i>Dissophora ornata</i>	CBS 347.77 (T)	MH861073	HQ667356
<i>Dissophora decumbens</i>	CBS 592.88	JX975954	HQ667355
<i>Linnemannia exigua</i>	CBS 655.68	JX976047	HQ667406
<i>Linnemannia exigua</i>	CBS 865.68 (T)	MH859237	MH870965
<i>Linnemannia exigua</i>	NNIBRFG5521	ON715840	ON715849
<i>Linnemannia exigua</i>	NNIBRFG5579	ON715841	ON715850
<i>Linnemannia gamsii</i>	CBS 110630	JX976106	KC018410
<i>Linnemannia gamsii</i>	CBS 253.36	JX975968	HQ667415
<i>Linnemannia hyalina</i>	CBS 115655 (T)	HQ630355	HQ667432
<i>Linnemannia hyalina</i>	FSU10532	JX975992	KC018289
<i>Linnemannia hyalina</i>	FSU509	JX975981	KC018291
<i>Linnemannia schmuckeri</i>	CBS 156.78	JX975854	KC018372
<i>Mortierella alpina</i>	CBS 210.32	JX975853	HQ667421
<i>Mortierella alpina</i>	FSU2698	JX976004	KC018272
<i>Mortierella antarctica</i>	CBS 194.89	JX976087	KC018345
<i>Mortierella antarctica</i>	CBS 609.70 (T)	JX975907	HQ667423
<i>Mortierella bainieri</i>	CBS 220.35	JX975901	KC018324
<i>Mortierella elongatula</i>	CBS 488.70 (T)	JX975967	HQ667425
<i>Mortierella epicladia</i>	CBS 355.76 (T)	JX976130	HQ667396
<i>Mortierella histoplasmatoides</i>	CBS 321.78	HQ630309	HQ667386
<i>Mortierella humilis</i>	CBS 443.68	JX976002	HQ667402
<i>Mortierella jenkinsii</i>	CBS 188.73	JX975999	KC018389
<i>Mortierella jenkinsii</i>	CBS 667.70	JX976088	KC018422
<i>Mortierella jenkinsii</i>	CBS 850.70	JX975849	KC018352
<i>Mortierella parvispora</i>	CBS 315.61	JX976104	HQ667374
<i>Mortierella rishikesha</i>	CBS 652.68 (T)	JX976110	HQ667385
<i>Mortierella rishikesha</i>	NNIBRFG6579	ON715844	ON715853
<i>Mortierella rishikesha</i>	NNIBRFG5173	ON715843	ON715852
<i>Mortierella rishikesha</i>	NNIBRFG5021	ON715840	ON715851
<i>Mortierella verticillata</i>	CBS 131.66	JX975886	KC018446
<i>Mortierella verticillata</i>	CBS 220.58 (T)	JX975905	JN940873
<i>Mortierella verticillata</i>	CBS 225.35	JX975940	JN940871
<i>Mortierella zonata</i>	CBS 228.35 (T)	JX975983	HQ667433
<i>Mucor anbudans</i>	CBS 521.66	JN206110	JN206457
<i>Umbelopsis autotrophica</i>	CBS 310.93 (T)	HQ630285	JN940878
<i>Umbelopsis autotrophica</i>	NNIBRFG4148	ON715839	ON715848
<i>Umbelopsis nana</i>	CBS 858.68	JN206391	KF727467
<i>Umbelopsis nana</i>	CBS 730.70	KC489506	KF727470
<i>Umbelopsis roseonana</i>	CBS 473.74	KF765510	AB090302
<i>Umbelopsis versiformis</i>	CBS 150.81	KC489496	KF727459
<i>Umbelopsis vinacea</i>	CBS 222.29	HQ630286	AB090304
<i>Umbelopsis vinacea</i>	CBS 236.82	KC489499	AB090305
<i>Umbelopsis vinacea</i>	CBS 212.32	KC489498	KF727461
<i>Umbelopsis vinacea</i>	CBS 913.85	JN206376	JN206562
<i>Umbelopsis westae</i>	CBS 870.85	JN206379	AB090306
<i>Umbelopsis angularis</i>	CBS 603.68	JN206380	HM849710

Table 2. Taxa, collection numbers, and GenBank accession numbers used in the present study(continued).

Species	Collection no. (Isolate no.)	GenBank accession no.	
		ITS	LSU
<i>Umbelopsis dimorpha</i>	CBS 110039 (HT)	JN206387	HM849709
<i>Umbelopsis fusiformis</i>	CBS 385.85 (T)	JN206386	JN206560
<i>Umbelopsis gibberispora</i>	CBS 109328 (T)	JN206384	JN206564
<i>Umbelopsis isabellina</i>	CBS 100559	JN206396	JN206571
<i>Umbelopsis isabellina</i>	CBS 250.95	JN206399	JN206574
<i>Umbelopsis ovata</i>	CBS 499.82 (IT)	JN206395	JN206572
<i>Umbelopsis ramanniana</i>	CBS 101226	JN206383	JN206566
<i>Umbelopsis ramanniana</i>	CBS 112976	JN206382	JN206568
<i>Umbelopsis swartii</i>	CBS 868.85 (T)	JN206378	JN206567

Morphological analysis

The microstructures of the fungal species were observed under an Eclipse Ni light microscope (Nikon, Tokyo, Japan) equipped with a Ds-Ri2 digital camera (Nikon). At least 50 individuals were examined to observe and measure each structure. For scanning electron microscopy, we followed the protocol described by Alves et al. [17]. Photographs were captured using a scanning electron microscope (SEM; SU8220, Hitachi, Tokyo, Japan).

RESULTS AND DISCUSSION

Phylogenetic analysis

Phylogenetic analysis, using a combination of ITS and an LSU, was performed to identify fungal strains and infer their phylogenetic relationships with other similar species. As shown in Fig. 1, the strain NNIBRFG6579 formed a clade with *M. rishiksha* strain CBS 652.68. The sequences of NNIBRFG6579 showed high similarity—100% (ITS), 99.69% (LSU), and 100% (EF1)—to those of *M. rishiksha*. NNIBRFG5579 formed a clade with *L. exigua* strain CBS 965.68. The sequences of NNIBRFG5579 showed high similarity—99.69% (ITS) and 99.42% (LSU)—to those of *L. exigua*. NNIBRFG26655 formed a clade with CBS 858.70 of *D. globulifera*. The sequences of NNIBRFG26655 showed high similarity—99.52% (ITS) and 99.90% (LSU)—to those of *D. globulifera*. NNIBRFG4148 formed a clade with the CBS 310.63 of *U. autotrophica*. The sequences of NNIBRFG4148 showed high similarity—100% (ITS) and 100% (LSU)—to those of *U. autotrophica*.

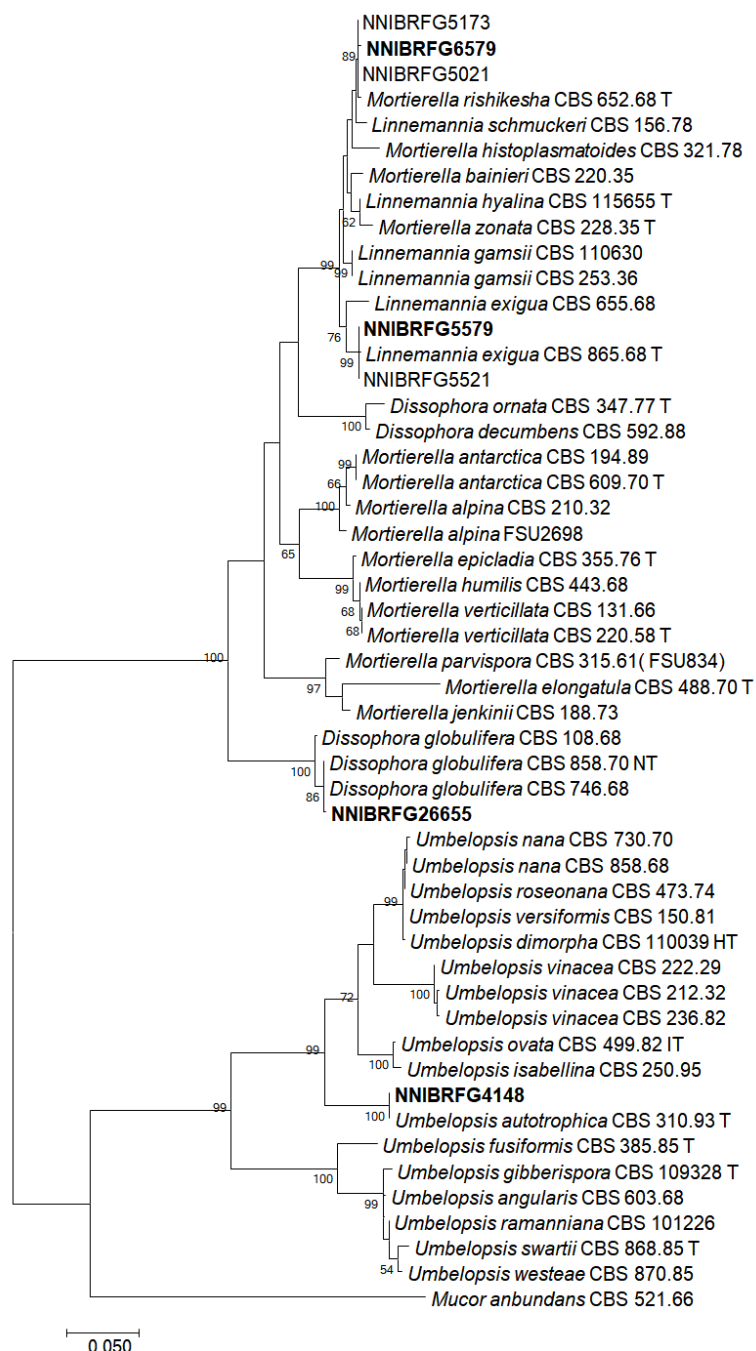


Fig. 1. Phylogenetic tree of *Dissophora globulifera*, *Linnemannia exigua*, *Mortierella rishiksha*, *Umbelopsis autotrophica* and their related species, based on maximum-likelihood analysis of the combination of internal transcribed region sequences (ITS) and large subunit of ribosomal DNA (LSU) sequences. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications. *Mucor anabundans* is outgroup. Bold type font indicated the strains used in this study. T, type material.

Three fungal species of Mortierellomycetes and one species of Umbelopsidomycetes were discovered from freshwater environment and identified through molecular identification.

TAXONOMY

Dissophora globulifera (O. Rostrup) Vandepol & Bonito, Fungal Diversity 104: 279 (2020) [MB#833727] (Fig. 2A, 3A-D)

Description: Colonies grew fast at 25 °C and reached 55, 58, 42, 56, 64, and 60 mm on CMDA, MEA, OA, PDA, V8A, and YPDA, respectively, 7 d after inoculation. The colony color was hyaline with a smooth aerial mycelial surface on CMDA and MEA, hyaline-to-creamy white with short aerial mycelia on OA, translucent white with fluffy aerial mycelia showing a concentric pattern on PDA, translucent white with cottony aerial mycelia showing a concentric pattern on V8A, and translucent white with fluffy aerial mycelia on YPDA. Sporangia were hyaline and 19.0-35.3 µm diameter ($\bar{x}=26.8\pm5.34$ µm, $n=10$). Sporangiospore were hyaline, globose, echinulate, and 6.8-9.0 µm in diameter ($\bar{x}=7.8\pm0.55$ µm, $n=50$). Chlamydospores were round-to-oval, thick-walled, and 9.3-12.9 µm in diameter ($\bar{x}=11.1\pm1.45$ µm, $n=10$).

Habitat: Filtered freshwater from a stream

Specimen examined: Cheonnae-ri, Jewon-myeon, Geumsan, Chungcheongnam-do, Republic of Korea; 13 Feb, 2020, NNIBRFG26655, Nakdonggang National Institute of Biological Resources (collected by Jaeduk Goh).

Note: *D. globulifera* was first described as isolates from soil or organic debris in soil [18]. The type specimen MBT#8101(neotype) was isolated from the decaying root of *Dactylis glomerata* [3]. Other strains reported as *D. globulifera* have been isolated from agricultural soil in Netherlands, Schweden, England, and Austria [3]. In this study, NNIBRFG26655 was isolated from filtered freshwater streams.

Linnemannia exigua (Linnem.) Vandepol & Bonito, Fungal Diversity 104: 283 (2020) [MB#835752] (Fig. 2B, 3E-H)

Description: Colonies grew slowly at 25°C and reached 47, 46, 45, 30, 49, and 45 mm on CMD, MEA, OA, PDA, V8A, and YPDA, respectively, 7 d after inoculation. The colony was colored hyaline with a smooth aerial mycelial surface on CMDA and MEA, creamy white with a fluffy aerial mycelial surface on OA, light yellow with dense aerial mycelia on PDA, translucent white with cottony aerial mycelia on V8A, and creamy white with short aerial mycelia on YPDA. Sporangia were hyaline, many-spored, and 21.3-41.2 µm in diameter ($\bar{x}=30.8\pm5.72$ µm, $n=15$). Sporangiospores were hyaline in color, ellipsoid-to-cylindrical, and 3.5-10.9 µm × 2.6-8.1 µm ($\bar{x}=8.1\pm1.57$ µm × 5.6 ± 0.99 µm, length/width (L/W) ratio=1.44, $n=50$). Chlamydospores are globose, thick-walled with irregular appendage, and 13.2-32.4 µm in diameter ($\bar{x}=21.9\pm6.74$ µm, $n=15$).

Habitat: Filtered freshwater from a stream

Specimen examined: Yangji-ri, Goro-myeon, Gunwi-gun, Gyeongsangbuk-do, Republic of Korea, 16 Mar 2018, NNIBRFG5579, Nakdonggang National Institute of Biological Resources (collected by Namil Chung).

Note: Other strains, such as *L. exigua*, were mainly isolated from agricultural soil in Spain, India, and Germany [15]. In this study, NNIBRFG5579 was isolated from filtered freshwater streams.

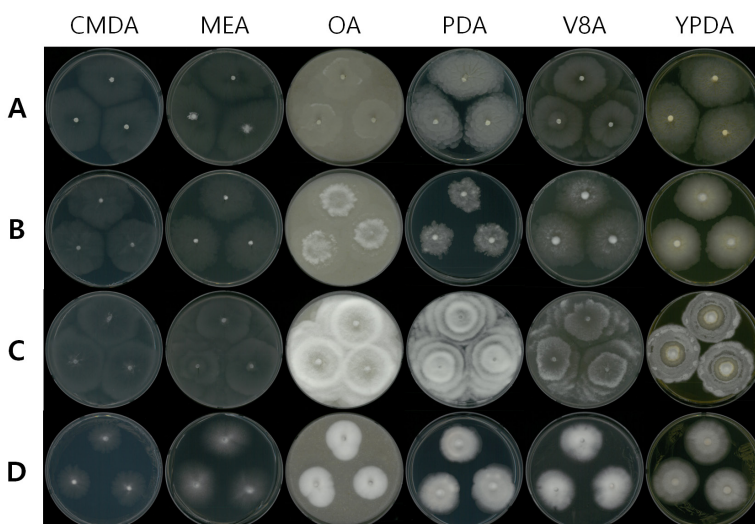


Fig. 2. Mycelial growth on CMDA, MEA, PDA, OA, V8A, and YPDA for 7 days at 25°C. (A) *Dissophora globulifera* strain NNIBRFG26655. (B) *Linnemannia exigua* strain NNIBRFG5579. (C) *Mortierella rishikesha* strain NNIBRFG6579. (D) *Umbelopsis autotrophica* strain NNIBRFG4148. CMDA, corn meal dextrose agar; MEA, malt extract agar; PDA, potato dextrose agar; OA, oatmeal agar; V8A, V8-juice agar; YPDA, yeast extract peptone dextrose agar.

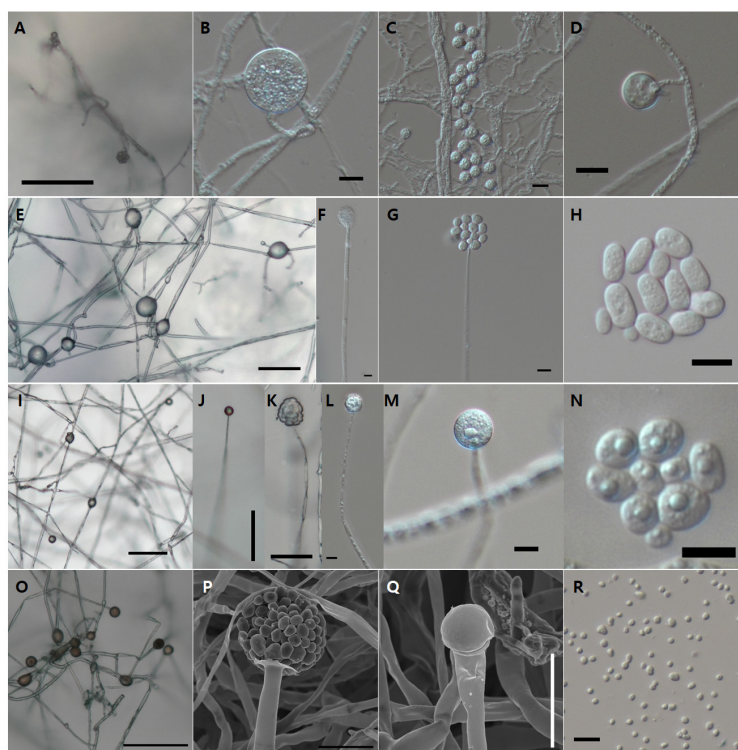


Fig. 3. Microscopic observation. A-D, sporangiophore (A-B), sporangiospore (C) and chlamydospore (D) morphology of *Dissophora globulifera* strain NNIBRFG26655; E-H, sporangiophore (E-G) and sporangiospore (H) morphology of *Linnemannia exigua* strain NNIBRFG5579; I-N, sporangiophore (I-M) and sporangiospore (N) morphology of *Mortierella rishikesha* strain NNIBRFG6579; O-R, sporangiophore (O-Q) and sporangiospore (R) morphology of *Umbelopsis autotrophica* strain NNIBRFG4148. Scale bars are 10 µm (B-D, F-H, L-N, P-R), 50 µm (K), and 100 µm (A, E, I, J, O).

***Mortierella rishiksha* B.S. Mehrotra & B.R. Mehrotra, ZentBl. Bakt. ParasitKde, Abt. 2: 184 (1964) [MB#334512] (Fig. 2C, 3I-N)**

Description: Colonies grew fast at 25°C and reached 52, 65, 71, 72, 69, and 48 mm on CMD, MEA, OA, PDA, V8A, and YPDA, respectively, 7 d after inoculation. The colony was hyaline with a smooth aerial mycelial surface on CMDA and MEA, creamy white with a fluffy aerial mycelial surface showing a concentric pattern on OA and PDA, translucent white with cottony aerial mycelia showing a concentric pattern on V8A, and creamy white at the margin with aerial mycelia on YPDA. Sporangia were hyaline, many-spored, and 21.8–37.7 µm in diameter ($\bar{x}=31.3 \pm 5.51$ µm, $n=20$). Sporangiospores were hyaline, globose-to-oval, and 5.1–13.6 µm × 3.6–8.8 µm ($\bar{x}=9.6 \pm 1.91$ µm × 6.1 ± 1.17 µm, L/W ratio=1.57, $n=50$). No chlamydospores were observed in this study.

Habitat: Sediment of a stream

Specimen examined: Neunggang-ri, Susan-myeon, Jecheon-si, Chungcheongbuk-do, 27 July, 2018, NNIBRFG6579, Nakdonggang National Institute of Biological Resources (collected by Jaeduk Goh).

Note: *M. rishiksha* was first isolated from the forest soil in India [15]. The lack of literal information, morphological characteristics, and additional sequences (EF1) of NNIBRFG6579 were directly compared with *M. rishiksha* CBS 652.68.

***Umbelopsis autotrophica* (E.H. Evans) W. Gams, Mycological Research 107 (3): 349 (2003) [MB#373417] (Fig. 2D, 3E-F)**

Description: Colonies grew slightly slow at 25°C and reached 28, 37, 31, 37, 35, and 38 mm on CMDA, MEA, OA, PDA, V8A, and YPDA, respectively, after 7 d of inoculation. The colonies were hyaline with a smooth aerial mycelial surface on CMDA, hyaline-to-white with a cottony aerial mycelial surface on MEA, creamy white with dense aerial mycelia on OA, white-to-light pink with fluffy aerial mycelia on PDA, white with cottony aerial mycelia on V8A, and white with fluffy aerial mycelia on YPDA. Sporangia were light pink-to-vinaceous, many-spored, globose, with columellae, and 15.2–25.8 µm in diameter ($\bar{x}=19.7 \pm 2.53$ µm, $n=50$). Sporangiospores were hyaline-to-light pink, globose, and 5.1–13.6 µm × 3.6–8.8 µm ($\bar{x}=9.6 \pm 1.91$ µm × 6.1 ± 1.17 µm, L/W ratio=1.57, $n=50$).

Habitat: Sediment of a freshwater source

Specimen examined: Ocheon-ri, Yeonil-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do, Republic of Korea, 25 May 2017, NNIBRFG4148, Nakdonggang National Institute of Biological Resources (collected by Hye Yeon Mun).

Note: *U. autotrophica* was first isolated from forest soil crumbs, root segments, and other plant material [19]. In this study, one strain was isolated from freshwater sediment.

CONFLICT OF INTERESTS

The authors declared no conflicts of interest.

ACKNOWLEDGMENTS

This research was supported by the research program named “Investigation of Fungal Resources in freshwater environment” (NNIBR202301106) of the Nakdonggang National Institute of Biological Resources.

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