



## RESEARCH ARTICLE

**3** OPEN ACCESS



# Report of 10 Unrecorded Fungi from Freshwater Environment in Korea

Wonsu Cheon, Sangkyu Park, Yoosun Oh, Jaeduk Goh and Hye Yeon Mun

Freshwater Bioresources Research Department, Fungal Resources Research Division, Nakdonggang National Institute of Biological Resources, Gyeongsangbuk-do, Republic of Korea

## **ABSTRACT**

Research on fungi isolated from freshwater environments contribute to expanding the fungal diversity and species inventory of freshwater ecosystems, as well as aiding in understanding the roles they play within these ecosystems. This study presents detailed descriptions and phylogenetic tree of 10 fungal species isolated from various freshwater environments in South Korea. These species were isolated from freshwater environments, such as plantlitter, sediment, and filtered freshwater. Identification of based on morphological characteristics and phylogenetic analysis using the DNA sequences from the internal transcribed spacer (ITS), elongation factor (EF1), calmodulin (cmd),  $\beta$ -tubulin (btub), and RNA polymerase II (rpb2) coding genes, depending on the species. The following species were recorded for the first time in Korea: Plectosphaerella pauciseptata NNIBRFG186, Striatibotrys rhabdosporus NNIBRFG2094, Gibellulopsis serrae NNIBRFG1912, Trichoderma hunanense NNIBRFG33378, T. Albofulvopsis NNIBRFG34098, Curvularia americana NNIBRFG34293, Phacidium mollerianum NNIBRFG28409, Mucor brunneogriseus NNIBRFG50199, M. laxorrhizus NNIBRFG37671, and M. pseudolusitanicus NNIBRFG39976.

## ARTICLE HISTORY

Received 3 September 2024 Revised 2 December 2024 Accepted 3 December 2024

### **KEYWORDS**

Freshwater fungi: unrecorded fungi; Korea

## 1. Introduction

Freshwater fungi were first discovered by Saccardo in 1880 with the identification of Heliscus lugdunensis. Subsequent discoveries by de Wildeman (1893) and Ingold (1942) led to further studies, with an estimated 3069-4145 species reported by various researchers. According to a global survey of freshwater fungi by Calabon et al., the following species were recorded: 2968 species of Ascomycota, 333 species of Chytridiomycota, 221 species of Rozellomycota, species of Basidiomycota, 19 species Mucoromycota, 15 species of Aphelidiomycota, 6 species of Entomophthoromycota, 5 species of Mortierellomycota, 4 species of Olpidiomycota, 3 species of Zoopagomycota, and 2 species of Sanchytriomycota [1].

Fungi play essential roles in ecosystems and are found in various habitats. The genus Plectosphaerella inhabits substrates, such as plants, soil, and insects, with many species being significant pathogens causing substantial agricultural losses [2]. The genus Striatibotrys, segregated from Stachybotrys, has been established as a distinct taxonomic group [3,4]. It currently comprises six species, including both sexual and asexual forms, with longitudinally striate conidia as a defining morphological feature [3,5]. Gibellulopsis is recognized as a genus distinct from Verticillium, with Gliocladium cibotii identified as a close relative [6].

Meanwhile, Trichoderma is a genus of significant economic and ecological importance, widely distributed across various ecosystems and frequently utilized in agriculture and industry [7]. Curvularia americana waspathogenic fungus first described in northern Italy as the cause of leaf and crown necrosis in turfgrass species [8]. The genus Phacidium was introduced by Fries in 1815 and has undergone continuous taxonomic revision, with P. lacerum currently regarded as the type species. [9]. Finally, Mucor is a large genus with over 300 species reported. It is characterized by fast-growing colonies and unique sporangial structures [10,11].

This article aims to report, for the first time in Korea, 10 species belonging to Ascomycota that were isolated from freshwater environments. It explores the ecological and phylogenetic characteristics of these fungal genera, providing detailed insights into their diversity and highlighting their potential applications.



## 2. Materials and methods

## 2.1. Sampling and isolation of fungi

To isolate fungi inhabiting freshwater environments, samples were collected from various freshwater sources. Freshwater samples were immediately filtered on-site using a hand pump, passing 50 mL of field water through an MCE membrane filter (HAWP04700, MF-Millipore<sup>TM</sup>, Tullagreen, Ireland). The filter was then placed on water agar (WA: 20 g/L agar, 1L distilled water, and streptomycin 100 ppm) medium. The samples were brought to the laboratory and incubated at 15 °C, followed by single spore isolation. The pure isolated fungi were inoculated onto potato dextrose agar (PDA; BD, Franklin Lakes, NJ) medium and incubated at 20 °C.

For submerged plant material collected from freshwater, the samples were washed twice with sterile water, then placed in sterile water and incubated at 20°C for about a week. After incubation, 100 µL of the incubated water was spread on WA medium and incubated at 15°C for 2d. Soil samples were diluted to 102, 103, and 104, and 200 µL of each dilution was spread on PDA medium, then incubated at 15°C to isolate the fungi. The isolated fungi were preserved in 15% glycerol and stored at -80°C.

## 2.2. Identification of fungi

To identify the isolated strains, mycelia cultured on PDA were harvested and placed in a tube containing glass beads, followed by homogenization. DNA was then extracted using the Nucleospin Plant II DNA Extraction Kit (Macherey-Nagel, Düren, Germany). The extracted DNA was used for amplifying various

Table 1. Primers used in this study.

No.	Gene	Primer name	Primer sequencing	Reference
1	ITS	ITS4	TCC TCC GCT TAT	[13]
			TGA TAT GC	
		ITS5	GGA AGT AAA AGT	
			CGT AAC AAG G	
2	bTUB	Bt2a	GGT AAC CAA ATC	[14]
			GGT GCT GCT	
			TTC	
		Bt2b	ACC CTC AGT GTA	
			GTG ACC CTT	
			GGC	
3	CAM	CMD5	CCG AGT ACA AGG	[15]
			ARG CCT TC	
		CMD6	CCG ATR GAG GTC	
			ATR ACG TGG	
4	RPB2	5F2	gay gay mgw gat	[16]
			CAY TTY GG	
		7CR	CCC ATR GCT TGY	
			TTR CCC AT	
5	TEF	EF1-983F	GCY CCY GGH CAY	[17]
			CGT GAY TTY AT	
		EF1-1567R	ACH GTR CCR ATA	
			CCA CCR ATC TT	

gene, and PCR was performed using the primers listed in Table 1. DNA sequence alignment, editing, and phylogenetic tree construction were conducted using MEGA version 11.0.13 [12]. The strains were observed using a model Eclipse Ni-U microscope (Nikon, Tokyo, Japan).

## 3. Taxonomy

Plectosphaerella pauciseptata AJL Phillips, Carlucci & ML Raimondo (2012)

Mycobank No.: MB564524

Description: Colonies grew fast at 25 °C and reached 58 mm on MEA, 49 mm on PCA, 55 mm on PDA, and 52 mm on V8A, 9 d after inoculation. The color of the colony was hyaline with a smooth aerial mycelial surface on PCA, hyaline with a smooth aerial mycelial surface on MEA, hyaline to creamy white with short aerial mycelia on V8A, and white to light pink with fluffy aerial mycelia on PDA. Conidiophores were solitary, hyaline, and smooth, thin-walled. Conidiogenous cells were phialidic, sometimes polyphialidic, hyaline, smooth, and apex straight. Conidia were ellipsoid or ovoid, apex rounded, hyaline, mostly aseptate, sometimes 1-septate. Aseptate conidia were measured  $4.7-10.2 \,\mu m \times 1.7 3.7 \,\mu\text{m}$  ( $x=7.50\pm1.32 \,\mu\text{m} \times 2.98\pm0.39 \,\mu\text{m}$ , L/W ratio = 2.51, n=71). 1-septate conidia were measured 5.8-×  $2.0-3.7 \, \mu m$  $(x = 8.56 \pm 1.81 \,\mu\text{m})$  $2.87 \pm 0.43 \,\mu\text{m}$ , L/W ratio = 2.98, n = 24) (Figure 1).

Habitat: Plant litter in freshwater

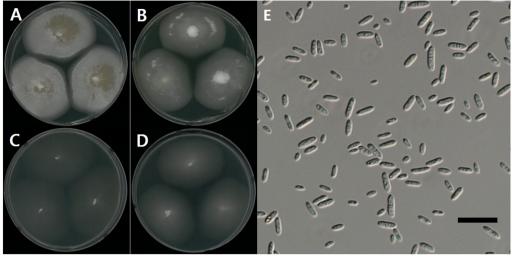
Specimen examined: Buk-cheon, Sinchon-ri, Naeseo-myeon, Sangju-si, Gyeongsangbuk-do, Republic of Korea; October 23 2015; NNIBRFG186 (ITS, KU751875; EF1, OQ737705).

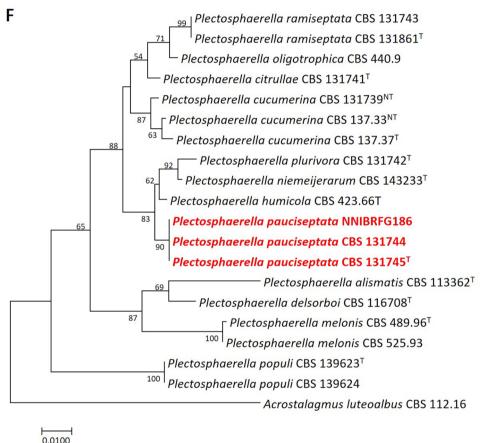
Note: The type material of P. pauciseptata, JAUCC1788, was isolated from tomato root in Italy [18]. In this study, NNIBRFG186 was isolated from plant litter in stream. Other specimens identified as P. pauciseptata have been isolated from plant tissues, such as root of tomato, watermelon, cucumber, or collar of tomato and melon [18].

Striatibotrys rhabdosporus Lombard & L Crous (2016)

Mycobank No.: 821468

Description: Colonies grew slowly at 25°C and reached 17 mm on CMDA, 17 mm on MEA, 28 mm on OA, 18 mm on PDA, 23 mm on V8A, and 23 mm on YPDA, 7 d after inoculation. The color of the colony was creamy white with fluffy aerial mycelial





**Figure 1.** Morphological characters and phylogenetic tree of *Plectosphaerella pauciseptata* NNIBRFG186. Mycelial growth at 25 °C for 9 d on PCA (a), PDA (B), MEA (C), and V8A (D). Morphology of conidia (E), scale bar, 10μm, (F) Molecular phylogenetic analysis by Maximum Likelihood method based on the Tamura-Nei model. Sequence combination with ITS and EF1 were used in phylogenetic analysis. T indicates type material. Outgroup was *Acrostalagmus luteoalbus*.

surface on CMDA and PDA, white with cottony aerial mycelial surface on MEA, light white-brown from center to lemonish yellow margin with short aerial mycelia on OA, light gray with cottony aerial mycelia surface on V8A, and light gray with short aerial mycelia and wet from center on YPDA. Conidiogenous cells were phialidic, hyaline to subhyaline, smooth,  $10-14\times4-7\,\mu\text{m}$ . Conidia were acrogenous, ellipsoid to subcylindrical, initially hyaline to dark brown, aseptate,  $7.0-13.4\,\mu\text{m}$  ×

 $3.8-5.9 \,\mu\text{m}$  ( $x = 11.26 \pm 1.26 \,\mu\text{m} \times 4.96 \pm 0.47 \,\mu\text{m}$ , L/W ratio =2.26, n = 71) (Figure 2).

Habitat: Sediment in freshwater

**Specimen examined:** Nakdonggang-river, Ha-ri, Nonggong-yeup, Dalsung-gun, Daegu, Republic of Korea; May 17 2016; NNIBRFG2094 (ITS, OQ727430; *RPB2*, OQ737702; *cmdA*, OQ737703; *btub*, OQ737704).

**Note:** The type material of *S. rhabdosporus*, CBS 528.80, was isolated from soil in Germany [3]. In this

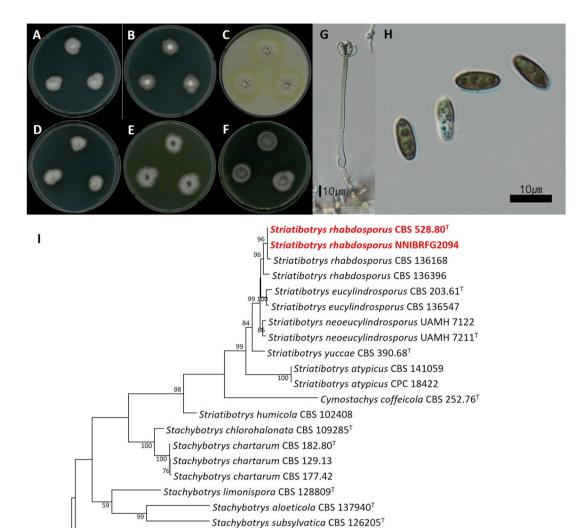


Figure 2. Morphological characters and phylogenetic tree of Striatibotrys rhadosporus NNIBRFG2094. Mycelial growth at 25 °C for 7d on CMDA (a), MEA (B), OA (C), PDA (D), V8A (E), and YPDA (F). Morphology of conidiophore (G) and conidia (H), scale bar, 10µm. (I) Molecular phylogenetic analysis by maximum likelihood method based on the Tamura-Nei model. Sequence combination with ITS, RPB2, cmdA, and btub were used in phylogenetic analysis. T indicates type material. Outgroup were memnoniella and brevistachys genus.

Memnoniella echinata CBS 216.32

Brevistachys globosa CBS 397.73

Brevistachys lateralis CBS 141058 Memnoniella brunneoconidiophora CBS 136191<sup>T</sup>

Stachybotrys dolichophialis DAOMC 227011<sup>T</sup>

study, NNIBRFG2094 was isolated from sediment in river. Other specimens identified as S. rhabdosporus have been isolated from diverse materials, such as plant debris, soil, asbestos cement tile, wall board, and so on [3]. In phylogenetic analysis, NNIBRFG2094 forms a clade with other strains of S. rhabdosporus, including type material. However, conidia of NNIBRFG2094 are slightly larger than those of CBS 528.80.

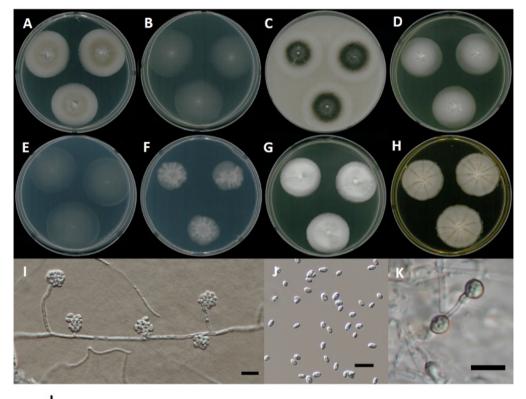
0.050

#### **Gibellulopsis** serrae (Maffei) Llópez Crous (2018)

Mycobank No.: MB828040

Description: Colonies grew 37 mm on PDA, 37 mm on MEA, 40 mm on OA, 32 mm on PCA, 39 mm on CMA, 24mm on CDA, 32mm on DG18A, and 34 mm on YPDA, 10 d after inoculation at 25 °C. The color of the colony was light gray to brown with fluffy aerial mycelia on PDA, hyaline with a smooth aerial mycelial surface on MEA, hyaline to dark brown with felty aerial mycelia at center on OA, apricot with a smooth aerial mycelial surface on PCA, hyaline with a smooth aerial mycelial surface on CMA, light gray with rough margin on CDA, creamy white with fluffy aerial mycelia on DG18A, and light khaki with floccose aerial mycelia on YPDA. Optimal temperature for growth is 25°C at most media condition. Conidiogenous cells were phialidic and hyaline. Conidia were ellipsoid to cylindrical with round ends, hyaline, 1-celled. Conidia were measured  $3.6-6.7\,\mu m \times 1.9-3.6\,\mu m$ 

 $(x=4.86\pm0.60\,\mu\text{m} \times 2.64\pm0.32\,\mu\text{m}, n=50)$ . Chlamydospores were mostly intercalary, singly or in chains, globose, hyaline to dark brown, thick-walled. Chlamydospores were measured 5.3–7.7  $\mu$ m ×



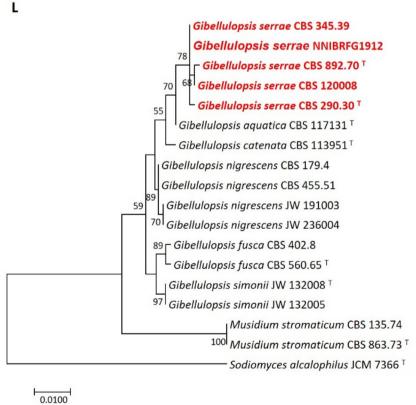


Figure 3. Morphological characters and phylogenetic tree of *Gibellulopsis serrae* NNIBRFG1912. Mycelial growth at 25°C for 10 d on PDA (a), MEA (B), OA (C), PCA (D), CMA (E), CDA (F), DG18 (G), and YPDA (H). Morphology of conidiophore (I), conidia (J), and chlamydospores (K) (scale bar, 10μm). (L) Graph of mycelial growth rate under different temperature. (M) Molecular phylogenetic analysis by maximum likelihood method based on the Tamura-Nei model. Sequence combination with ITS and LSU were used in phylogenetic analysis. T indicates type material. The outgroup was *Sodiomyces alcalophilus*.



 $4.2-6.9 \,\mu\text{m}$   $(x=6.40\pm0.58 \,\mu\text{m})$  $5.49 \pm 0.59 \,\mu m$ n=30) (Figure 3).

Habitat: Sediment in freshwater

**Specimen examined:** Gilan-cheon, Andeok-myeon, Cheongsong-gun, Gyeongsangbuk-do, Republic of Korea; April 20 2016; NNIBRFG1912 (ITS, OR425150; LSU, OR418416).

Note: The type material of G. serrae CBS 290.30 was isolated from human eve in Italy [1]. In this study, NNIBRFG1912 was isolated from sediment in stream. Other specimens identified as G. serrae have been isolated from various sources including seed, soil, human blood, wood pulp, other fungal mass, and plant tissues [19].

# Trichoderma hunanense K Chen & **WY Zhuang (2017)**

Mycobank No.: MB570395

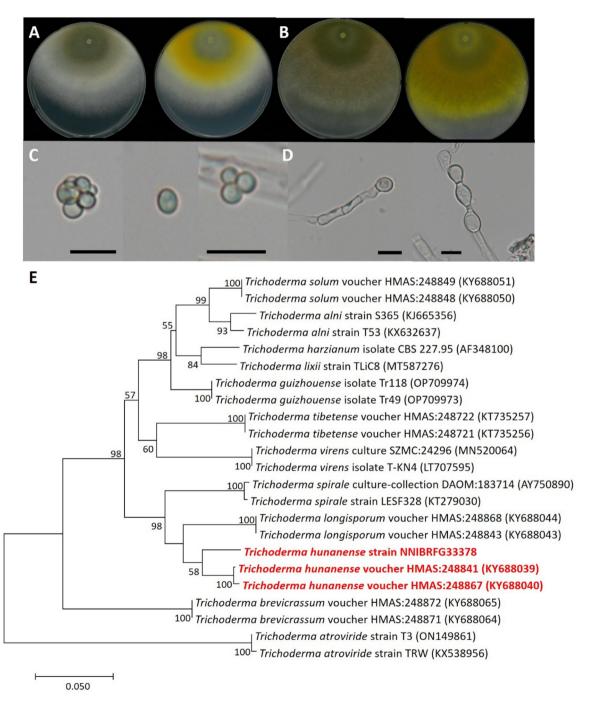


Figure 4. Morphological characters and phylogenetic tree of Trichoderma hunanense NNIBRFG33378 (A,B) Mycelial growth at 25°C for 7 d on PDA (A), MEA (B). Morphology of conidia (C) and conidiophore (D), scale bar, 10µm, (E) Molecular phylogenetic analysis by neighbor-joining method based on the Tamura-Nei model. Sequence of TEF gene was used in phylogenetic analysis. The outgroup was Trichoderma atroviride.

Description: On PDA after 7d at 20°C, colony radius 58-65 mm, radial, indistinct zonate, aerial hyphae spreading abundant throughout the colony. Concentric ring was observed at the radius 45-50 mm of colony, but not finely zonated. On CMD after 7d at 20°C, colony radius 68-77 mm, white, radial, aerial hyphae less abundant than PDA, and several concentric ring was observed. Colony was denser at the colony margin and absent in colony center. Light yellowish pigment noted at the periphery of colony. Conidiophore branching structures, septum, hyphae hyaline (transparent) and smooth. Conidia formed on the tips of branches, green, smooth, ellipsoid to subglobose, with smooth surfaces, single celled, and no septa,  $3.5-5.8 \mu m \times 2.9-3.5 \mu m$  (Figure 4).

**Habitat:** Freshwater sediment from mountainous stream

**Specimen examined:** Gwangyang-si, Jeollanam-do, Republic of Korea, March 25 2021, NNIBRFG33378, Nakdonggang National Institute of Biological Resources (Sangju-si, Gyeongsangbuk-do, Republic of Korea).

**Note:** Sequence analysis of NNIBRFG33378 using *TEF* gene showed high similarity to *Trichoderma hunanense* strain HMAS 284481 (96.77%). The phylogenetic tree clearly places the isolate (*T. hunanense*, NNIBRFG33378) within a clade containing reference strains of *T. hunanense* [20]. The isolate is shown in bold red font, confirming its close relation to strains HMAS:248841 (KY688039) and HMAS:248867 (KY688040) (Figure 4(E)).

# Trichoderma albofulvopsis WT Qin & WY Zhuang (2016)

Mycobank No.: MB570242

**Description:** Colony on PDA after 7 d at 20 °C, colony radius 55–62 mm, aerial hyphae frequent, forming radial strands, white and cottony. On CMD after 7 d at 20 °C, colony radius 59–70 mm, aerial hyphae sparse, forming radial strands, white, and downy. Phialides commonly divergent in whorls of 2–5, lageniform, subulate or slightly ampulliform, often subulate in the middle of the whorls, 5.5–12.0 μm × 2.0–3.3 μm. Conidia light green, smooth, and subglobose to ellipsoidal, 2.9–3.8 μm × 2.1–3.0 μm. Some autolytic excretion was observed (Figure 5).

**Habitat:** Freshwater sediment from mountainous stream

**Specimen examined:** Namwon-si, Jeollabuk-do, Republic of Korea, April 2 2021, NNIBRFG34099, Nakdonggang National Institute of Biological

Resources (Sangju-si, Gyeongsangbuk-do, Republic of Korea).

**Note:** Sequence analysis of NNIBRFG34099 using *TEF* gene showed high similarity to *Trichoderma albofulvopsis* strain 9930 (99.43%). Based on neighbor-joining (NJ) trees, this strain clustered in the same clade as *T. albofulvopsis* [21].

# Curvularia americana D Cunha, Madrid, Gené & Cano (2014)

Mycobank No.: MB806052

**Description:** Colonies on PDA, MEA, and V8 attaining 44 mm, 32 mm, and 58 mm in diameter after 7 d at 20 °C, funiculose and greenish grey to dark green at the center, effuse, and grayish white toward the periphery, with a fimbriate margin; reverse olive to dark green. Conidia 4(-5)-celled, straight to slightly curved, broadly ellipsoidal,  $13-28\times7-15\,\mu\text{m}$ , usually with the third cell unequally sided and larger than the others, second and third cells pale brown to brown, smooth-walled, apical and basal cell sub-hyaline, apical cell smooth-walled and basal cell often verruculose hilum non-protruding, flat, darkened, and thickened,  $1.5-3\,\mu\text{m}$  wide (Figure 6).

**Habitat:** Filtered freshwater of *Curvularia americana* from freshwater

**Specimen examined:** Yeongam-gun, Jeollanam-do, Republic of Korea, April 22 2021, NNIBRFG34293, Nakdonggang National Institute of Biological Resources (Sangju-si, Gyeongsangbuk-do, Republic of Korea).

**Note:** Sequence analysis of NNIBRFG34293 using ITS region showed high similarity to *C. americana* (100%, HG779020), morphology of NNIBRFG34293 was similar to *C. americana* [22]. Based on the maximum likelihood (ML) trees, this strain clustered in the same clade as *C. americana*.

## Phacidium mollerianum (Thüm) Crous (2014)

Mycobank No.: MB809680

**Description:** Colonies on PDA spreading with sparse aerial mycelium and feathery margin, white in the center, and slightly yellowish at the margin. Conidiomata were observed 2–3 weeks after culturing on PDA medium, multilocular, up to 420  $\mu$ m diameter. Conidiophores branched, up to 45  $\mu$ m long. Conidia hyaline, smooth granular, subcylindrical 15–16.7×4.1–4.5  $\mu$ m with a length/width. Appendage not observed (Figure 7).

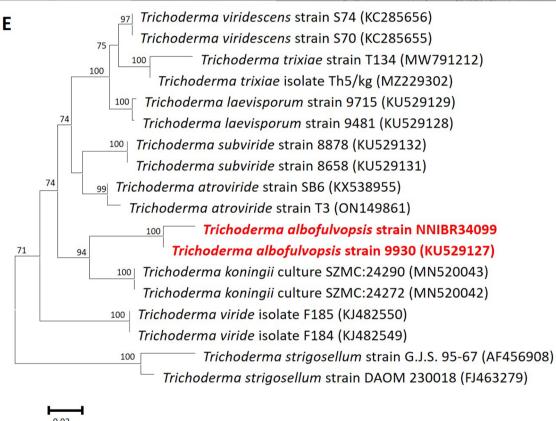


Figure 5. Morphological characters and phylogenetic tree of Trichoderma albofulvopsis NNIBRFG34099. Mycelial growth at 25°C for 7d on PDA (A) and MEA (B). Morphology of conidia (C) and conidiophore (D), scale bar, 10µm. (E) Molecular phylogenetic analysis by Neighbor-Joining method based on the Tamura-Nei model. Sequence of TEF gene was used in phylogenetic analysis. The outgroup was Trichoderma strigosellum.

**Habitat:** Filtered freshwater from valley

**Specimen examined:** Damyang-gun, Jeollanam-do, Republic of Korea, April 27 2020, NNIBRFG28409, Nakdonggang National Institute of Biological Resources (Sangju-si, Gyeongsangbuk-do, Republic of Korea).

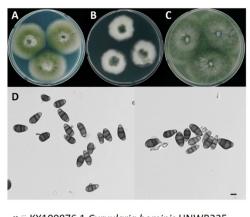
Note: Sequence analysis of NNIBRFG28409 using ITS region showed high similarity to Phacidium mollerianum (100%, KR873247). Based on NJ tree,

NNIBRFG28409 clustered in the same clade as P. mollerianum. Conidia with the apical mucoid appendage was observed only in water [9].

Mucor brunneogriseus AK Sarbhoy (1968)

Mycobank No.: MB334525

Description: Colonies on PDA attaining 90 mm in diameter after 7 d at 20 °C. The colony appears white



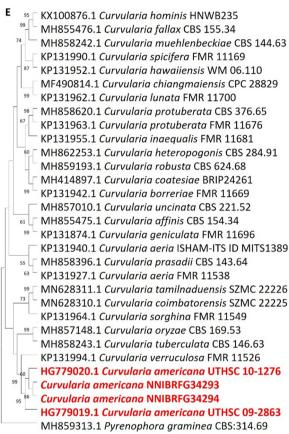


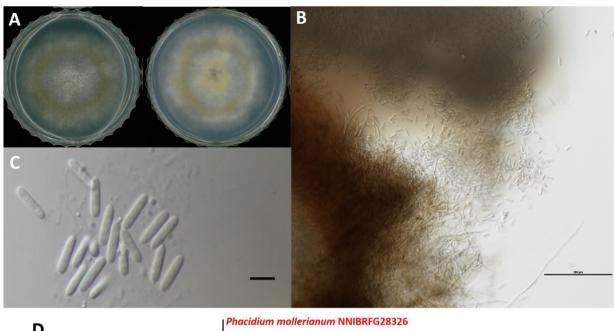
Figure 6. Morphological characters and phylogenetic tree of Curvularia americana NNIBRFG34293. Mycelial growth at 25 °C for 9d on PDA (a), MEA (B), and V8A (C). Morphology of conidia (D), scale bar, 10µm. (E) Molecular phylogenetic analysis by maximum likelihood method based on the Tamura-Nei model. Sequence combination with ITS was used in phylogenetic analysis. The outgroup was Pyrenophora graminea.

to pale gray, denser, slightly darker central area which fades to a lighter edge, giving it a fluffy, cotton-like appearance. The colony was circular in shape, with a uniform radial pattern spreading from the center to the edge. The edges are soft and diffused, fuzzy appearance. The structures appear transparent to slightly refractive and reflect the abundance and maturity of sporangia back white to light yellow. Sporangia light brown, globose to subglobose Columellae 25(-32)-32.3(-45.8)μm diameter. applanate, subglobose, hyaline, with or without collars. Sporangiospores hyaline, thick walled, and smooth, spherical to short oval 4.6-8.8 µm diameter. Chlamydospores hyaline, smooth-walled, oval 7.6-9.2 µm diameter. The presence of septa and small vesicle-like inclusions within the larger cells (Figures 8 and 9).

Habitat: Filtered freshwater from river

Specimen examined: Gimposi, Gyeonggi-do, Republic of Korea, April 6 2023, NNIBRFG50199, Nakdonggang National Institute of Biological Resources (Sangju-si, Gyeongsangbuk-do, Republic of Korea).

Note: Sequence analysis of NNIBRFG50199 using ITS region showed similarity to M. brunneogriseus (98.72%, NR\_145283). Based on NJ tree, NNIBRFG50199 clustered in the same clade as M. brunneogriseus [23].



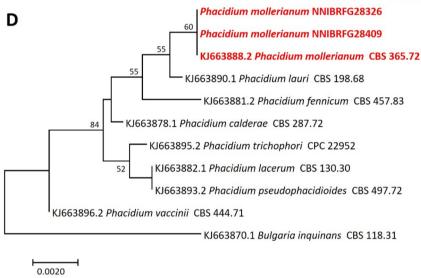


Figure 7. Morphological characters and phylogenetic tree of Phacidium mollerianum NNIBRFG28409. Mycelial growth at 25 °C for 9d on PDA (a). Morphology of breaked conidiomata (B), x100, scale bar, 100 μm, conidia (C), x400, scale bar, 10μm. (D) Molecular phylogenetic analysis by neighbor-joining method based on ITS seguence on the Kimura-2-parameter model. The outgroup was Bulgaria inquinans.

## Mucor laxorrhizus Y Ling (1930)

Mycobank No.: MB252978

Description: Colonies on PDA, MEA and V8 attaining 90 mm in diameter after 7 d at 20 °C. The colony color, white to dark grey, reflects the abundance and maturity of sporangia. Sometimes rather long, unbranched aerial hyphae first cover the surface of the medium. The sporangiophores hyaline to slightly brownish, up to 20(-25) µm diameter repeated branching of a mixed type, either straight branches at right angles or recurved and sympodial, rarely starts prior to sporangium formation. Sporangia at first white, then either blackish grey or brownish grey, with encrusted rather persistent walls which break at maturity or remain persistent in smaller sporangia, up to 100(-140) μm diameter. Columellae applanate, subglobose or conical, sessile, 9-50×11-60 μm, occasionally larger, smoke color to bluish with large collars. Sporangiospores subglobose, (-3)4-6(-7) µm diameter, containing a few granules, slightly roughened in outline, bluish grey (Figures 8 and 10).

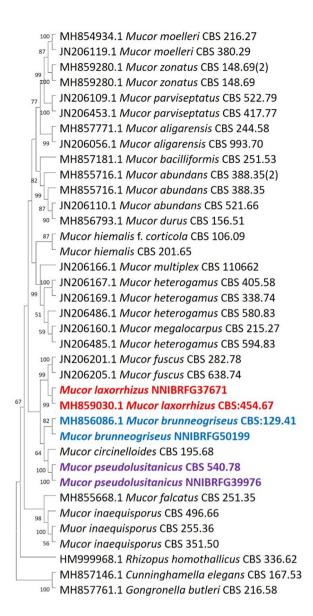


Figure 8. Molecular phylogenetic analysis of Mucor genus was inferred using the neighbor-joining method. The percentage of replicate trees in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method. This analysis included 37 nucleotide sequences. There were a total of 795 positions in the final dataset. The outgroup was Gongronella butleri and Cunninghamella elegans.

Habitat: Filtered freshwater of Mucor laxorrhizus from freshwater

Specimen examined: Hampyeong-gun, Jeollanam-do, Republic of Korea, May 7 2020, NNIBRFG37671, Nakdonggang National Institute of Biological Resources (Sangju-si, Gyeongsangbuk-do, Republic of Korea).

Note: Sequence analysis of NNIBRFG37671 using ITS region showed high similarity to M. laxorrhizus (100%, MZ325971), morphology of NNIBRFG34293 was similar to M. laxorrhizus. Based on the ML trees, this strain clustered in the same clade as M. laxorrhizus [24].

Mucor pseudolusitanicus L Wagner & G Walther (2020)

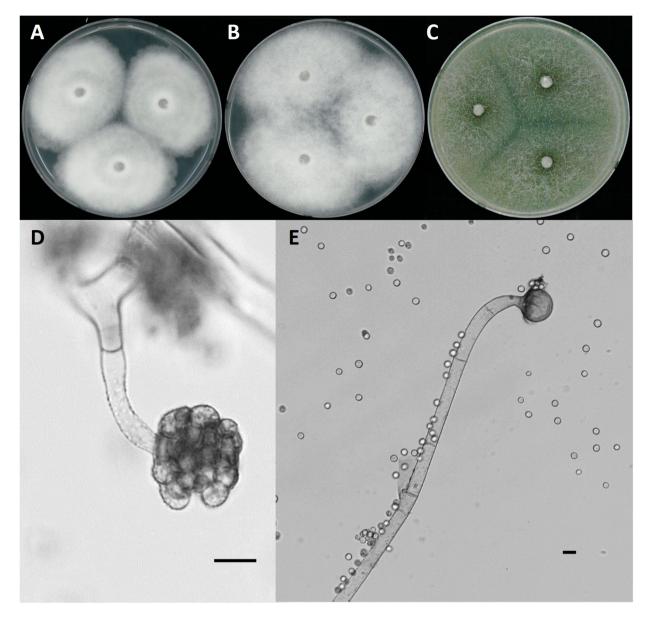
Mycobank No.: MB828293



Figure 9. Morphological characters of Mucor brunneogriseus NNIBRFG50199. (A) Mycelial growth at 25 °C for 9 d on PDA, (B) sporangia, sporangiospore, chlamydospore, x400, scale bar, 10µm.

Description: Colonies on PDA attaining 90 mm in diameter after 7d at 20°C. The colony has a white to light grayish coloration, the front a uniform gray tone, while the back paler with a slight yellowish

central area, the colony was circular and radially symmetric, texture appears fluffy or cottony, with a dense, fuzzy appearance extending toward the edges. The brownish Sporangia slightly spherical



**Figure 10.** Morphological characters of *Mucor laxorrhizus* NNIBRFG37671. Mycelial growth at 25 °C for 9 d on PDA (a), MEA (B) and V8A (C), (D,E) sporangiophore, collumellae, sporangiospore, x400, scale bar, 10µm.

22.7–24.3 µm. Columellae subglobose, hyaline with collars. Sporangiospores hyaline, oval to ellipsoidal, smooth surfaces, varying in size,  $5.1–5.7\times2.6–3.9$  µm. Chlamydospores and zygospores not observed (Figures 8 and 11).

Habitat: Filtered freshwater from stream

**Specimen examined:** Yangpyeongun, Gyeonggi-do, Republic of Korea, April 15 2022, NNIBRFG39976,

Nakdonggang National Institute of Biological Resources (Sangju-si, Gyeongsangbuk-do, Republic of Korea).

**Note:** The strain *M. pseudolusitanicus*, first reported in 2020, was known to grow at 35 °C but did not produce spores at this temperature [25]. NNIBRFG39976 showed high similarity with *M. pseudolusitanicus* (100%, OM212655.1) and was confirmed to belong to the same clade in the NJ tree.

Figure 11. Morphological characters of Mucor pseudolusitanicus NNIBRFG39976. (A) Mycelial growth at 25°C for 9d on PDA, (B) sporangiophore, collumellae, sporangiospore, x400, scale bar, 10µm.

# **Disclosure statement**

No potential conflict of interest was reported by the author(s).

# **Funding**

This work was supported by a grant from the Nakdonggang National Institute of Biological Resources (NNIBR),

funded by the Ministry of Environment (MOE) of the Republic of Korea (NNIBR20251105).

# References

[1] Calabon MS, Hyde KD, Gareth Jones EB, et al. Freshwater fungi numbers. Fungal Divers. 2022;114(1):3-235. doi: 10.1007/s13225-022-00503-2.

- [2] Yang XQ, Ma SY, Peng ZX, et al. Diversity of Plectosphaerella within aquatic plants from southwest China, with P. endophytica and P. sichuanensis spp. nov. MycoKeys. 2021;80:57-75. doi: 10.3897/mycokeys.80.64624.
- [3] Lombard L, Houbraken J, Decock C, et al. Generic hyper-diversity in Stachybotriaceae. Persoonia. 2016; 36:156-246. doi: 10.3767/003158516X691582.
- [4] Crous PW, Wingfield MJ, Guarro J, et al. Fungal planet description sheets: 154-213. Persoonia. 2013;31(1):188-296. doi: 10.3767/003158513X675925.
- [5] Lechat C, Hairaud M, Rubio E. A new species of Striatibotrys from Asturias (Spain), S. asturiensis. Ascomycete.org. 2017;9:153-155.
- [6] Zare RG. Gibellulopsis, a suitable genus for Verticillium nigrescens, and Musicillium, a new genus for V. theobromae. nova hedwigia. 2007;85(3-4):463-489. doi: 10.1127/0029-5035/2007/0085-0463.
- [7] Zhao R, Mao LJ, Zhang CL. Three new species of Trichoderma (Hypocreales, Hypocreaceae) from soils in China. MycoKeys. 2023;97:21-40. doi: 10.3897/ mycokeys.97.101635.
- [8] Gilardi G, Mocioni M, Gullino ML, et al. Curvularia americana and Cuvularia tropicalis cause leaf and crown necrosis on Bermuda grass in Italy. Phytopathol Mediterr. 2022;61(3):431-437. doi: 10.36253/phyto-13825.
- [9] Crous PW, Quaedvlieg W, Hansen K, et al. Phacidium and Ceuthospora (Phacidiaceae) are congeneric: taxonomic and nomenclaturla implications. IMA Fungus. 2014;5(2):173-193. doi: 10.5598/imafungus.2014.05.02.02.
- [10] Lima DX, Barreto RW, Lee HB, et al. Novel mucoralean fungus from a repugnant substrate: Mucor merdophylus sp. nov., isolated from dog excrement. Curr Microbiol. 2020;77(10):2642-2649. doi: 10.1007/ s00284-020-02038-8.
- [11] Hurdeal VG, Gentekaki E, Hyde KD, et al. Novel Mucor species (Mucoromycetes, Mucoraceae) from northern Thailand. MycoKeys. 2021;84:57-78. doi: 10.3897/mycokeys.84.71530.
- [12] Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022-3027. doi: 10.1093/molbev/ msab120.
- [13] White TJ, Bruns TD, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, editors. PCR protocols: a guide to methods and applications. London: Academic Press; 1990. p. 315-322.

- [14] Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol. 1995;61(4):1323-1330. 10.1128/aem.61.4.1323-1330.1995.
- [15] Hong S-B, Cho H-S, Shin H-D, et al. Novel Neosartorya species isolated from soil in Korea. Int J Syst Evol Microbiol. 2006;56(2):477-486. doi: 10.1099/ijs.0.63980-0.
- [16] O'Donnell K, Sarver BAJ, Brandt M, et al. Phylogenetic diversity and microsphere array-based genotyping of human pathogenic fusaria, including isolates from the multistate contact lens-associated U.S. keratitis outbreaks of 2005 and 2006. J Clin 2007;45(7):2235–2248. doi: Microbiol. 10.1128/ JCM.00533-07.
- [17] Rehner SA, Buckley E. A Beauveria phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. Mycologia. 2005;97:84-98.
- [18] Carlucci A, Raimondo ML, Santos J, et al. Plectosphaerella species associated with root and collar rots of horticultural crops in southern Italy. Persoonia. 2012;28(1):34-48. doi: 10.3767/ 003158512X638251.
- [19] Giraldo A, Crous PW. Inside Plectosphaerellaceae. Stud Mycol. 2019;92(1):227-286. doi: 10.1016/j.simyco.2018.10.005.
- [20] Chen K, Zhuang WY. Discovery from a large-scaled survey of Trichoderma in soil of China. Sci Rep. 2017;7(1):9090. doi: 10.1038/s41598-017-07807-3.
- [21] Qin WT, Zhuang WY. Seven wood-inhabiting new species of the genus Trichoderma (Fungi, Ascomycota) in Viride clade. Sci Rep. 2016;6:27074. doi: 10.1038/ srep27074.
- [22] Madrid H, Cunha KCd, Gené J, et al. Novel Curvularia species from clinical specimens. Mol Phylogen Evol Fungi. 2014;33(1):48-60. 10.3767/003158514X683538.
- [23] Sarbhoy AK. Revision of the sphaerosporus group of Mucor. Trans Br Mycol Soc. 1968;51(1):25-32. doi: 10.1016/S0007-1536(68)80118-4.
- [24] Schipper MAA. Mucor laxorrhizus. Stud Mycol. 1989;31:151-155.
- [25] Wagner L, Stielow JB, de Hoog GS, et al. A new species concept for the clinically relevant Mucor circinelloides complex. Persoonia. 2020;44(1):67-97. doi: 10.3767/persoonia.2020.44.03.