RESEARCH ARTICLE

A New Record on *Umbelopsis vinacea* and *Mucor hiemalis* f. *corticola* Isolated from Korea

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ABSTRACT

In the screening of fungal diversity, two strains were collected from the soil of Yeongcheon and dissected guts from the bodies of Chinese rice grasshopper (*Oxya chinensis*), Chinese grasshopper (*Acrida cinerea*), and Far eastern devil grasshopper (*Oedaleus infernalis*) from Daejeon in Korea. They were identified as *Umbelopsis vinacea* (KNU-YC-1801B) and *Mucor hiemalis* f. *corticola* (KNU-20F7, KNU-20F8, KNU-20F9). Multigene phylogenetic analyses of the internal transcribed spacer (ITS) regions, and large subunit (LSU) sequence data confirmed two unreported taxa along with their morphology. The results of molecular phylogeny firmly supported the detailed description and illustration for each taxon. As far as we know, both *Umbelopsis vinacea* and *Mucor hiemalis* f. *corticola* are the first reported taxa in Korea.

Keywords: Grasshopper, *Mucor hiemalis* f. *corticola*, Soil, *Umbelopsis vinacea*

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INTRODUCTION

Amos and Barnett introduced the genus *Umbelopsis* with the type species namely *U. versiformis* [1]. The members of the subgenus *Micromucor* were reclassified from Mortierellaceae to the Mucoraceae as the species of the genera *Micromucor* and *Umbelopsis* [2]. Later the species of *Micromucor* and *Umbelopsis* were combined into the genus *Umbelopsis* [3]. The genus *Umbelopsis* comprised two major clades of species, which cannot be differentiated unequivocally by a particular set of morphological characters [4]. Based on molecular evidence these two genera were confirmed to be monophyletic and all these fungi were consequently treated as the members of *Umbelopsis* [4].

The genus *Mucor* is part of the family Mucoraceae under the order of Mucorales within the phylum of Mucoromycota [5]. The genus are characterized by fast-growing colonies and the production of simple and/or branched sporangiophores as well as globular sporangia [2,6]. In the genus *Mucor*, there are more than 50 species that were identified based on the molecular and morphological characteristics [7]. Recently, the numbers of *Mucor* species were increased with a lot of new taxa worldwide [8]. The various species

of *Mucor* are used in biotechnology applications including bioremediation (remove oil from water) [9], production of biofuel [10], bioproteins [11], pharmaceuticals, industrial enzymes and chemicals [12]. In some rare cases, *Mucor* species such as *M. circinelloides* can be involved in human infection [13].

The aim of the current study was to carry out the morphological and molecular analyses of opportunistic fungi from the ecosystems in Korea. Moreover, the accurate identification of the species is very important for further research on antifungal efficacy as well as biotechnology applications.

MATERIALS AND METHODS

Soil sampling and fungal isolation

In 2018 and 2019, the soil samples were collected from Yeongcheon (35°57'56.4"N, 128°59'27.5"E), and grasshoppers were obtained from Daejeon (36°22'33.7"N, 127°22'36.4"E), Korea, respectively. The soil was gathered from a depth of 15 to 30 cm. After air drying, the sample was transferred to polythene zipper bags and then stored at 4°C before using. For the examination, 1 g of the soil was suspended in 10 mL of sterile distilled water and gently vortexed. The suspension was serially diluted, and 100 μL of each sample was spread on potato dextrose agar plates (PDA; Difco, Detroit, MI, USA). The guts of Chinese rice grasshopper (*Oxya chinensis*), Chinese grasshopper (*Acrida cinerea*), and Far eastern devil grasshopper (*Oedaleus infernalis*) were dissected. The dissected guts were then grinded with double distilled water (DDW), spread on PDA media, and cultured at 25°C for 3-4 days. The pure cultures of fungal strains were transferred on fresh PDA plates and incubated at 25°C, and then molecular analyses were carried out.

Morphological characterization

To inspect the morphology, the strain KNU-YC-1801B was cultured on three different media, namely, PDA, malt extract agar (MEA; Difco, Detroit, MI, USA), and corn meal agar (CMA; Difco, Detroit, MI, USA), for 5 days and incubated at 25°C [14]. The strains KNU-20F7, KNU-20F8, and KNU-20F9 were cultured on PDA and MEA and incubated at 25°C for 3-5 days, after which the cultural and morphological characteristics were examined [15,16]. Then the colony characteristics including color, shape, and size were noted following incubation. A light microscope (BX-50; Olympus, Tokyo, Japan) was utilized to observe the morphological structures of the strain.

Genomic DNA extraction, PCR amplification, and sequencing

The genomic DNA of the strains KNU-YC-1801B, KNU-20F7, KNU-20F8, and KNU-20F9 was extracted with the use of the HiGene Genomic DNA prep kit (Biofact, Daejeon, Korea) following the manufacturer instructions. Polymerase chain reaction (PCR) was carried out to amplify using the primers ITS1F/ITS4 [17,18] for the internal transcribed spacer (ITS) regions, NL1/NL4 [19], and LROR/LR5 [20] for the large subunit (LSU). Then, the amplified PCR products were purified with ExoSAP-IT (Thermo Fisher Scientific, Waltham, USA) and sequenced by Macrogen Co. Ltd. (Daejeon, Korea).

Phylogenetic analyses

Additional sequences were retrieved from the National Center for Biotechnology Information (NCBI) complemented the sequences created in this study (Table 1). The sequences were carried out to show the BLAST search results of the closest members with the taxa. The evolutionary distance matrices were determined with the use of Kimura's two-parameter model for the neighbor-joining (NJ) algorithm [21]. The tree topology deduced the phylogenetic relationships by using the software program MEGA7.0 with the bootstrap analysis of 1000 replications [22].

Table 1. List of species used in this study and their GenBank accession numbers for phylogenetic analysis.

Species	Strain Numbers	GenBank Accession Numbers	
		ITS	LSU
Umbelopsis angularis	CBS 603.68^T	NR137072	KF727442
U. autotrophica	CBS 310.93^T	NR111558	NG042541
U. changbaiensis	Um 018	KC489481	KF727444
U. dimorpha	CBS 110039^T	NR111664	KF727471
U. fusiformis	CBS 385.85	JN206386	KF727463
U. isabellina	CBS 208.32	KC489497	KF727460
U. nana	CBS 858.68	JN206391	MH870964
U. ovata	CBS 499.82	JN206395	JN206572
U. ramanniana	NRRL 5844	KM017730	KM017710
U. roseonana	CBS 473.74	KF765510	KF727464
U. vinacea	CBS 212.32	MH855292	NG058032
U. vinacea	CBS 236.82	KC489499	KF727462
U. vinacea	**NIBRFGC000502246**	**LC535359**	**LC535363**
Mortierella verticillata	NRRL 6337	AF157145	AF157199
Mucor amphibiorum	CBS 763.74	MH860895	MH872631
M. circinelloides	CBS 195.68^T	NR126116	NG055735
M. circinelloides f. *griseocyanus*	CBS 116.08^T	NR126136	NG056283
M. circinelloides f. *janssenii*	CBS 205.68^T	NR126123	MH870832
M. circinelloides	UWFP 1079	AY213658	AY213710
M. ellipsoideus	UTHSC 02-2090	JF299211	FN650660
M. endophyticus	CBS 385.95^T	NR111661	MH874169
M. fragilis	CBS 236.35	JF299225	JN206422
M. fragilis	LMSA 1.09.161	JF723587	JF723713
M. fragilis	LMSA 1.09.196	JF723586	JF723714
M. fragilis	LMSA 1.09.199	JF723585	JF723715
M. fuscus	CBS 132.22	JF723619	MH866227
M. hiemalis f. *corticola*	CBS 366.68	JN206139	MH870871
M. hiemalis* f. *corticola	**KNU-20F7**	**LC535360**	**LC535364**
M. hiemalis* f. *corticola	**KNU-20F8**	**LC535361**	**LC535365**
M. hiemalis* f. *corticola	**KNU-20F9**	**LC535362**	**LC535366**
M. hiemalis f. *hiemalis*	CBS 337.71A	MH860154	MH871923
M. hiemalis f. *hiemalis*	CBS 337.71B	MH860155	MH871924
M. indicus	CBS 226.29^T	NR077173	NG057878
M. irregularis	CBS 103.93	JX976257	JX976212
M. luteus	CBS 243.35^T	NR120224	NG057969
M. nederlandicus	CBS 735.70	MH859923	MH871720
M. nidicola	H13	KX375786	KX375769
M. ramosissimus	CBS 135.65^T	NR103627	NG056280
M. variisporus	CBS 837.70	MH859970	NG057972
M. velutinosus	UTHSC 02-1981	JF299212	FN650672
M. zonatus	CBS 148.69^T	NR103638	NG057917
Cokeromyces recurvatus	CBS 158.50^T	NR077172	NG058813

The newly generated sequences were indicated in bold, ITS: Internal transcribed spacer; LSU: 28S rDNA gene.

RESULTS AND DISCUSSION

***Umbelopsis vinacea* (Dixon-Stew.) Arx, Sydowia 35:20 (1984) [MB#115505] (Fig. 1)**

Specimen examined: Yeongcheon (35°57'56.4"N, 128°59'27.5"E), isolated from soil. The stock culture (NIBRFGC000502246) was deposited in the National Institute of Biological Resources (NIBR) as a metabolically inactive culture.

Morphology of the strain KNU-YC-1801B

On PDA, the colonies 21.0-24.0 mm diam. after a 5-day incubation at 25℃; surface white, non-aerial, and slightly velvety; reverse white, then pale purplish after 2-3 weeks (Fig. 1A). On MEA, colonies 26.0-29.0 mm diam. and cottony felt, slightly reddish in color in the center; reverse brown to yellowish (Fig. 1B). On CMA, colonies 15.0-18.0 mm diam., slow growth, slightly pinkish in the center with age (Fig. 1C). Sporangiophores branched, 33.0-91.6 μm long, one septum, with 2.6-3.4 μm near the base and 2.4-2.8 μm near the tip (Fig. 1D-F). Chlamydospores elliptical, globose to subglobose, single or compact, oil droplets and with a diameter of 8.8-12.9 × 4.8-11.4 μm (Fig. 1G-I). Sporangia globose to subglobose, brown, multi-spored, and brown, with a diameter of 7.2-14.0 μm (Fig. 1J and 1K). Sporangiospores distinctly angular, five to seven edges, hyaline and one-celled containing oil droplet, with a diameter of 3.3-5.2×2.5-4.6 μm (n=100) (Fig. 1L). Zygospores not observed. The cultural and morphological characteristics were the same with previously identified *U. vinacea* (Table 2).

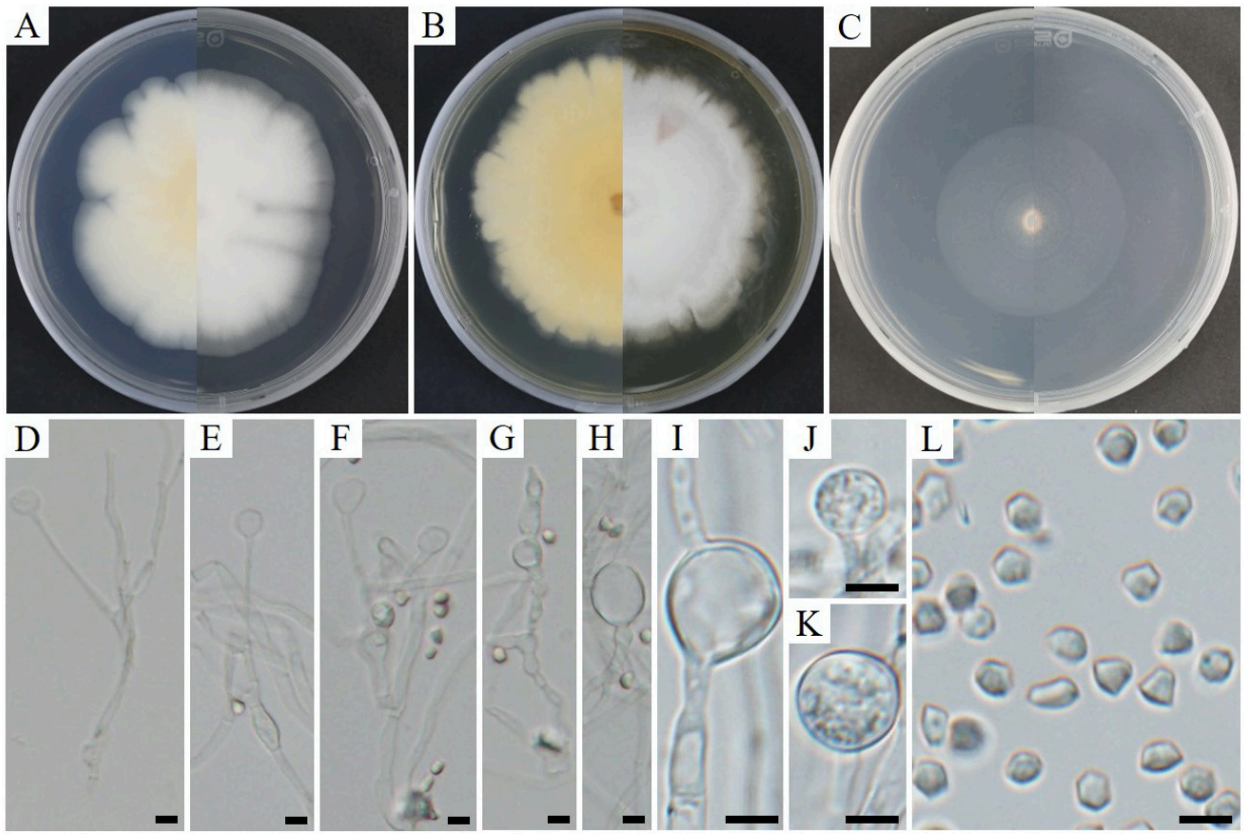

Fig. 1. Cultural and morphological characteristics of *Umbelopsis vinacea* KNU-YC-1801B. Colony on potato dextrose agar (A), malt extract agar (B), and corn meal agar (C), correspondingly following 14 days at 25℃; sporangiophores showing branching type (D-F); chlamydospores (G-I); sporangia (J, K); sporangiospores (L). Scale bars: D-L=5 μm.

Table 2. Morphological characteristics of the strain KNU-YC-1801B with the reference to *Umbelopsis vinacea*.

Characteristics	*Umbelopsis vinacea* KNU-YC-1801B[a]	*Umbelopsis vinacea*[b]
Colony		
Shape and color	Colonies on MEA were cottony felt, not zonate, slightly reddish color in the center, then toward the edge; colonies were slightly pinkish in the center when it became old on CMA	Colonies were cottony felt, not zonate, light russet-vinaceous in the center, lighter gradually toward the edge, sometimes forming small sectors on MEA; on CMA slightly pinkish in the center when old
Size (diam.)	MEA=26-29 mm; CMA=15-18 mm after 5 days at 25℃	MEA=35.0 mm; CMA=26.0 mm after 5 days at 20℃
Sporangiophores		
Shape and size (diam.)	Branched, 33.0-91.6 μm long, 2.6-3.4 μm wide near the base and 2.4-2.8 μm near the tip	Simply branched, 47.4-94.8 μm long, 2.4-2.8 μm wide near the base and 1.8-2.4 μm near the tip
Sporangia		
Shape and color	Globose to subglobose, brown, multi-spored	Globose to subglobose, reddish-brown, multi-spored
Size (diam.)	7.2-14.0 μm	9.9-12.3 μm
Chlamydospores		
Shape	Elliptical, globose to subglobose, single or compact	Elliptical or globose, single or bunching
Size (diam.)	8.8-12.9×4.8-11.4 μm	6.9-10.2×5.9-7.9 μm
Sporangiospores		
Shape and size (diam.)	Angular, 3.3-5.2×2.5-4.6 μm	Distinctly angular, 4.7-5.5×3.6-4.4 μm

MEA: Malt extract agar; CMA: Corn meal agar; diam.: Diameter.

[a]Fungal strain studied in this paper, [b]Sources of the descriptions [14].

Molecular phylogeny of the strain KNU-YC-1801B

Genetic sequences of the ITS regions and 28S rDNA were examined to determine the evolutionary relationships with the strains acquired from GenBank (Table 1). The nucleotide sequences, 561 and 868 bp, were obtained from the ITS regions and 28S rDNA, respectively. The BLAST results have shown maximum 99.37% and 99.21% similarities from ITS regions with the strains of *Umbelopsis vinacea* NEFU37 and *U. vinacea* CBS 236.82, respectively. The large subunit (LSU) has shown the highest (100% and 99.54%) identities with the strains of *U. vinacea* (CBS 236.82, CBS 212.32, CGMCC 3.16357), respectively. The combined sequences of ITS regions and 28S rDNA genes were utilized to carry out the phylogenetic analysis. The phylogenetic tree designated that the strain KNU-YC-1801B was grouped together with the previously identified strain *U. vinacea* (Fig. 2). Therefore, the phylogenetic results firmly support the fact that the strain KNU-YC-1801B is *Umbelopsis vinacea*. The strain KNU-YC-1801B was deposited in the National Institute of Biological Resources (NIBRFGC000502246).

Lately, *Umbelopsis changbaiensis* was isolated from amphibian feces and soil samples in Korea [23]. *U. dimorpha* was isolated from the roots of two terrestrial orchids (*Cypripedium japonicum* and *C. macranthum*) during the screening of orchid endophytic fungi in Korea [24]. Furthermore, *U. ramanniana* was found to be an endophytic fungus that is related to *Pinus thunbergii* in coastal shelterbelts of Korea [25]. Both *U. nana* and *U. vinacea* are isolated from forest soils in Japan [26]. Also, other strains of *U. vinacea* were isolated from sandy loam soil, forest soil, and soil under bushes in Australia and China, respectively [14]. The strain KNU-YC-1801B was isolated from the soil in Korea. For this reason, further studies are needed to provide an in-depth knowledge as regards this species. In this study, we report *Umbelopsis vinacea* for the first time in Korea.

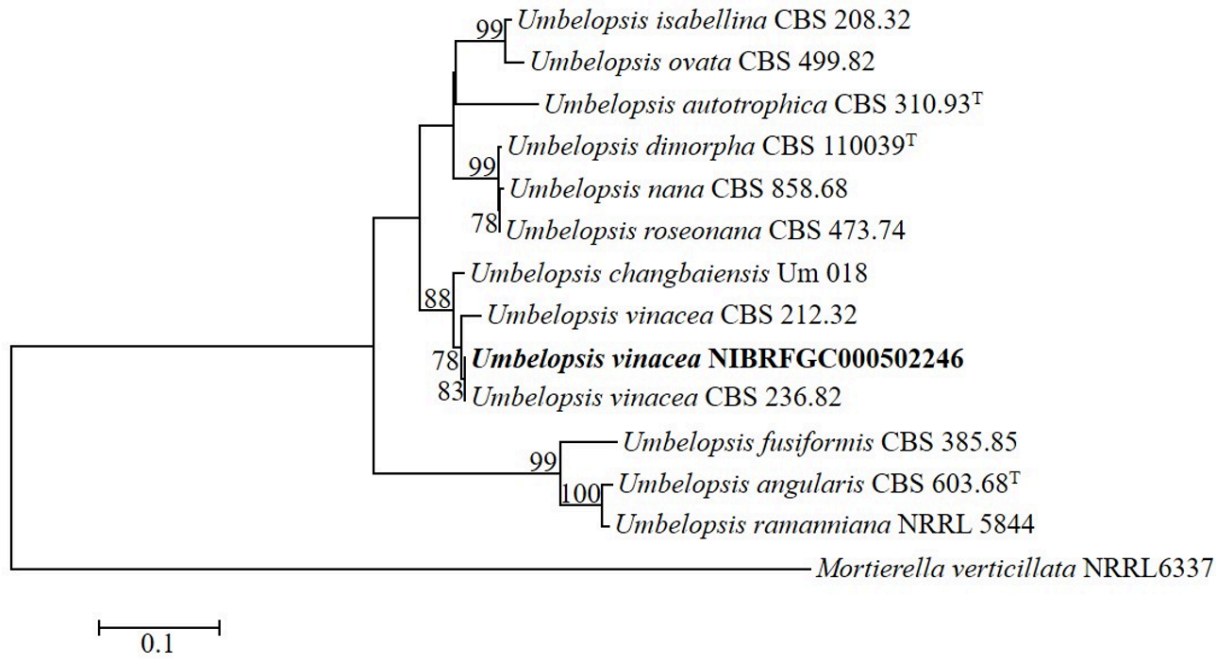

Fig. 2. Neighbor-joining phylogenetic tree based on the combined sequences of internal transcribed spacer (ITS) regions and large subunit (LSU), indicating the relationship between *Umbelopsis vinacea* and the closest *Umbelopsis* spp. The tree was rooted using *Mortierella verticillata* NRRL 6337 as an outgroup. The strain isolated in this study is in bold, and the bootstrap values are based on 1000 replications (values smaller than 60% were not shown). Bar, 0.1 substitutions per nucleotide position.

***Mucor hiemalis f. corticola* (Hagem) Schipper, Studies in Mycology 4:31 (1973) [MB#348494] (Fig. 3)**

The strains KNU-20F7, KNU-20F8, and KNU-20F9 were assessed and were found to be the same and clustered together with molecular phylogeny (Fig. 4). Therefore, KNU-20F7, KNU-20F8, and KNU-20F9 strains were recognized as *Mucor hiemalis* f. *corticola*. For this reason, one strain (KNU-20F7) was chosen to describe this taxon.

Specimen examined: Daejeon (36°22'33.7"N, 127°22'36.4"E), isolated from grasshoppers. The stock cultures (KNU-20F7, KNU-20F8, and KNU-20F9) were maintained at fungal plant pathology laboratory, Kyungpook National University, Korea as a metabolically inactive culture.

Morphology of the strain KNU-20F7

On PDA, the colonies 90 mm in diam. after a 5-day incubation at 25°C; grew rapidly, filled the petri dish, up to 15 mm in height, white to light gray at first, and then it turned pale yellow (Fig. 3A). On MEA, the colonies 90 mm in diam. also after a 5-day incubation at 25°C; grew quickly, light gray in color, and then turned pale yellow (Fig. 3B). Columellae hyaline to light brown, conical, subglobose to ellipsoidial, diameter of 17.5-35.4 × 10.5-26.3 μm, with the presence of a clear collar (Fig. 3C-F). Sporangiophores branched monopodially or sympodially, comprising yellowish contents, with a diameter of 4.4-12.6 μm. Sporangia hyaline to light brown at first and then dark brown when they matured, diameter of 14.6-31.0 × 11.2-17.9 μm, with an average diameter of 13.6-19.6 μm (Fig. 3G-I). Sporangiospores oval, ellipsoidal to broadly ellipsoidal, a diameter of 5.1-9.8 × 3.1-5.0 μm (n=30), with an average diameter of 3.8-6.1 μm (Fig. 3J). The cultural and morphological characteristics were similar to those previously reported *M. hiemalis* f. *corticola* (Table 3). This result suggests that the fungal strain KNU-20F7 was closely associated with *M. hiemalis* f. *corticola*.

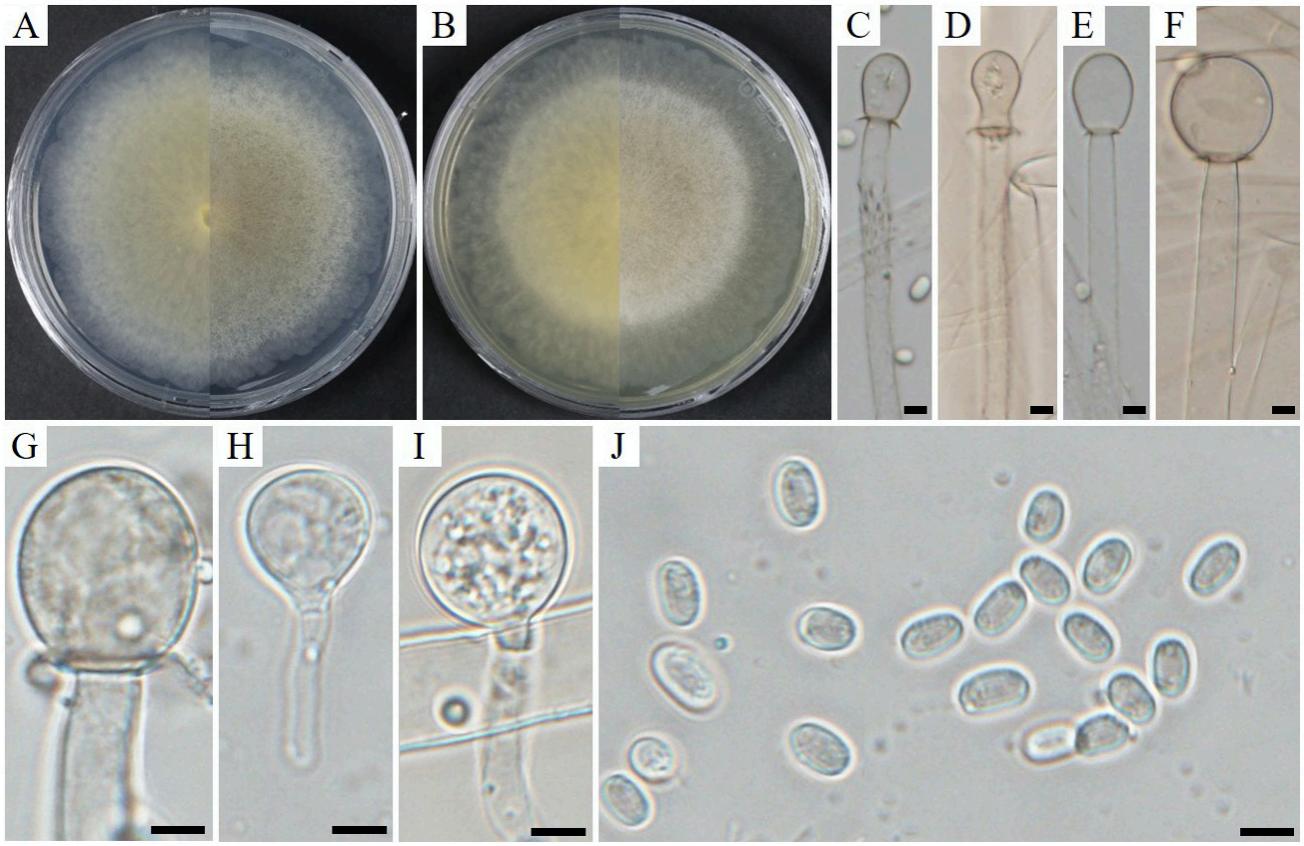

Fig. 3. Cultural and morphological characteristics of *Mucor hiemalis* f. *corticola* KNU-20F7. Colony on potato dextrose agar (A), malt extract agar (B), columella with the presence of a clear collar (C-F), sporangiophores and sporangia (G-I), sporangiospores (J). Scale bars: C-J=5 μm.

Table 3. Morphological characteristics of the strain KNU-20F7 with the reference to *Mucor hiemalis* f. *corticola*.

Characteristics	*Mucor hiemalis* f. *corticola* KNU-20F7[a]	*Mucor hiemalis* f. *corticola*[b]
Colony		
Shape and size (diam.)	Colonies light gray, up to 15 mm in height, filling petri dish at 25℃ in 5 days on PDA	Colonies light gray, up to 15 mm in height, filling petri dish at 20℃ in 5 days
Sporangiophores		
Shape	Branched monopodially or sympodially containing yellowish contents	Branched monopodially or sympodially, with yellowish contents
Shape and Size (diam.)	4.4-12.6 μm	5.0-12.5 μm
Sporangia		
Shape	Hyaline to light brown initially and then dark brown when became matured	White or hyaline at first and dark brown at maturity
Size (diam.)	15.0-31.0 μm	40.0-82.8 μm
Chlamydospores		
Shape	Conical, subglobose to ellipsoidal	Subglobose to ellipsoidal
Size (diam.)	17.5-35.4×10.5-26.3 μm	16.2-50.0×18.9-52.5 μm
Sporangiospores		
Shape	Oval, ellipsoidal to broadly ellipsoidal	Mainly ellipsoidal to broadly ellipsoidal
Shape and Size (diam.)	5.1-9.8×3.1-5.0 μm	3.9-9.0×2.9-5.9 μm

PDA: Potato dextrose agar, [a]Fungal strain studied in this paper, [b]Sources of the descriptions [15].

Molecular phylogeny of the strain KNU-20F7

The nucleotide sequences of the ITS (619 bp) and 28S rDNA (643 bp) were acquired and compared to the GenBank database with the use of BLAST to categorize the isolated fungal strain at the species level (Table 1). The BLAST search results of ITS sequences reflected 100% similarity with the strains of *Mucor hiemalis* f. *corticola* CBS 362.68 and 99.84% similarities with the different strains of *M. hiemalis* f. *corticola* (KCCM60281, CBS 366.68). The large subunit (LSU) gene has shown 98.59-100% similarities with the strains of *M. hiemalis* f. *corticola* (CBS 106.09, CBS 366.68, CBS 362.68). The strains KNU-20F8 and KNU-20F9 also manifested a maximum of 99.83-100% similarities from ITS regions (593, 608 bp) with the different strains of *M. hiemalis* f. *corticola* (CBS 366.68, CBS 362.68, F95, F96). The 28S rDNA (643, 641 bp) gene has shown 98.29-99.84% similarities with the different strains of *M. hiemalis* f. *corticola* (CBS 366.68, CBS 106.09). A combination of ITS regions and 28S rDNA genes sequences was utilized to carry out the phylogenetic analysis with the use of NJ method to know the exact taxonomic position of the strain. The phylogenetic tree has shown that the strains KNU-20F7, KNU-20F8, and KNU-20F9 were grouped together with the previously known *M. hiemalis* f. *corticola* (Fig. 4). Therefore, the phylogenetic results support the fact that the strain KNU-20F7 is *M. hiemalis* f. *corticola*.

However, the members of the genus *Mucor* are usually isolated from diversified sources including soil, fruit, vegetables, stored grains, insects, or dung [27]. Recently, there are some *Mucor* species, namely, *M. abundans*, *M. aligarensis*, *M. moelleri*, and *M. heterogamus*, that have been reported from freshwater and sediment samples in Korea [28]. *Mucor ardhlaengiktus* and *M. gigasporus* were identified from amphibian feces and soil samples in Korea [23]. Furthermore, the zygomycetous fungal strain named *M. ramosissimus* was also isolated from freshwater samples in Busan, Korea [29]. A fruit soft rot due to *M. piriformis* occurred on sweet persimmon storages in Gyeongnam Province, Korea [30].

So, further investigation is needed to explore the etiology of *M. hiemalis* f. *corticola* and the pathogenicity based on Korean ecological and environmental conditions. In this study, we also firstly report *M. hiemalis* f. *corticola* in Korea.

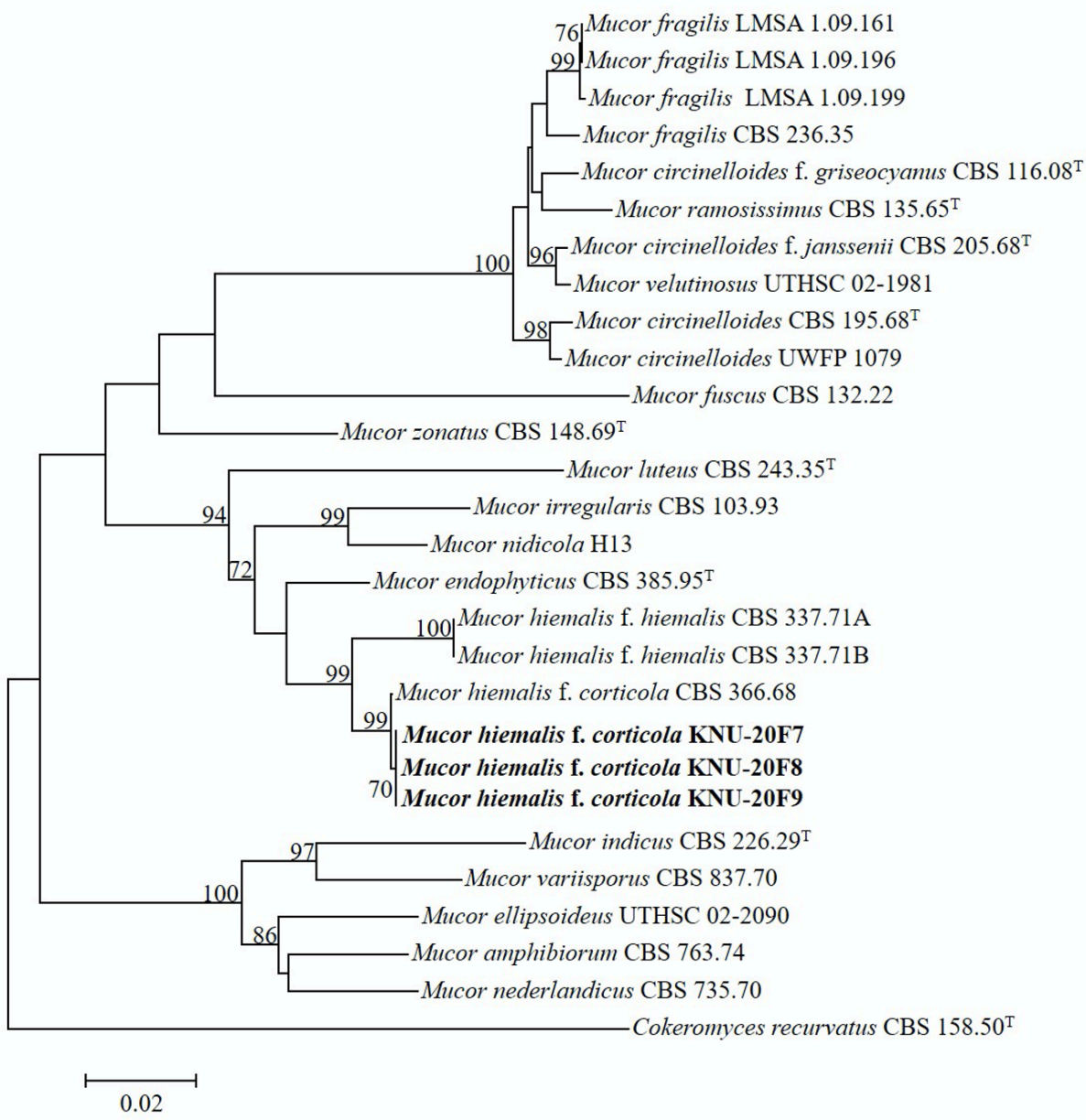

Fig. 4. Neighbor-joining phylogenetic tree based on the combined sequences of internal transcribed spacer (ITS) regions and large subunit (LSU), indicating the relationship between *Mucor hiemalis* f. *corticola* and the closest *Mucor* spp. The tree was rooted using *Cokeromyces recurvatus* CBS 158.50^{T} as an outgroup. The strain isolated in this study is in bold, and the bootstrap values are based on 1,000 replications (values smaller than 60% were not shown). Bar, 0.02 substitutions per nucleotide position.

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REFERENCES

1. Amos RE, Barnett HL. *Umbelopsis versiformis*, a new genus and species of the imperfects. Mycologia 1966;58:805-8.
2. von Arx JA. On Mucoraceae s. str. and other families of the Mucorales. Sydowia 1982;35:10-26.
3. Yip HY. *Umbelopsis fusiformis* sp. nov. from a wet sclerophyll forest and a new combination for *Mortierella ovata*. Trans Br Mycol Soc 1986;86:334-7.
4. Meyer W, Gams W. Delimitation of *Umbelopsis* (Mucorales, Umbelopsidaceae fam. nov.) based on ITS sequence and RFLP data. Mycol Res 2003;107:339-50.
5. Fresenius G. Beitrage zur Mykologie. Frankfurt: Heinrich Ludwig Brömmer Verlag; 1850.
6. Schipper MAA. A study on variability in *Mucor hiemalis* and related species. Stud Mycol 1973;4:1-40.
7. Gherbawy Y, Kesselboth C, Elhariry H, Hoffmann K. Molecular barcoding of microscopic fungi with emphasis on the mucoralean genera *Mucor* and *Rhizopus*. In: Gherbawy Y, Voight K, editors. Molecular identification of fungi. Berlin Heidelberg: Springer-Verlag; 2010. p. 225-65.
8. Phookamsak R, Hyde KD, Jeewon R, Bhat DJ, Jones EBG, Maharachchikumbura SSN, Raspe O, Karunarathna SC, Wanasinghe DN, Hongsanan S, et al. Fungal diversity notes 929-1035: Taxonomic and phylogenetic contributions on genera and species of fungi. Fungal Divers 2019;95:1-273.
9. Srinivasan A, Viraraghavan T. Oil removal from water using biomaterials. Bioresour Technol 2010;101:6594-600.
10. Alam MZ, Kabbashi NA, Hussin SN. Production of bioethanol by direct bioconversion of oil-palm industrial effluent in a stirred-tank bioreactor. J Ind Microbiol Biotechnol 2009;36:801-8.
11. Jamal P, Alam MZ, Umi N. Potential strain to produce bioprotein from cheaper carbon source: Hope for millions. Am J Biochem Biotech 2007;3:42-6.
12. Yazdi MT, Zarrini G, Mohit E, Faramarzi MA, Setayesh N, Sedighi N, Mohseni FA. *Mucor hiemalis*: A new source for uricase production. World J Microbiol Biotechnol 2006;22:325-30.
13. Skiada A, Pagano L, Groll A, Zimmerli S, Dupont B, Lagrou K, Lass-Florl C, Bouza E, Klimko N, Gaustad P, et al. Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European confederation of medical mycology (ECMM) working group on Zygomycosis between 2005 and 2007. Clin Microbiol Infect 2011;17:1859-67.
14. Wang YN, Liu XY, Zheng RY. *Umbelopsis changbaiensis* sp. nov. from China and the typification of *Mortierella vinacea*. Mycol Prog 2014;13:657-69.
15. Domsch KH, Gams W, Anderson HT. Compendium of soil fungi. London: Academic Press; 1980.
16. Madden AA, Stchigel AM, Guarro J, Sutton D, Starks PT. *Mucor nidicola* sp. nov., a fungal species isolated from an invasive paper wasp nest. Int J Syst Evol Micr 2012;62:1710-4.
17. Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Mol Ecol 1993;2:113-8.
18. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, editors. PCR protocols: A guide to methods and applications. New York: Academic Press; 1990. p. 315-22.
19. O'Donnell K. *Fusarium* and its near relatives. In: The fungal holomorph: Mitotic, meiotic and

pleomorphic speciation in fungal systematics. Reynolds DR, Taylor JW, editors. Wallingford: CAB International; 1993. p. 225-33.
20. Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 1990;172:4238-46.
21. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980;16:111-20.
22. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 2016;33:1870-4.
23. Nguyen TTT, Hyde KD, Lee HB. *Cunninghamella binariae*, *Mucor ardhlaengiktus*, *Mucor gigasporus* and *Umbelopsis changbaiensis*, newly recorded species from amphibian feces and soil in Korea. Phytotaxa 2019;425:19-34.
24. Lee BH, Han HK, Kwon HJ, Eom AH. Diversity of endophytic fungi isolated from roots of *Cypripedium japonicum* and *C. macranthum* in Korea. Kor J Mycol 2015;43:20-5.
25. Min YJ, Park MS, Fong JJ, Quan Y, Jung S, Lim YW. Diversity and saline resistance of endophytic fungi associated with *Pinus thunbergii* in coastal shelterbelts of Korea. J Microbiol Biotechnol 2014;24:324-33.
26. Watanabe T, Watanabe Y, Fukatsu T, Kurane R. Notes on two *Umbelopsis* species: *U. nana* and *U. vinacea* from Japan. Mycoscience 2001;42:235-7.
27. Walther G, Pawłowska J, Alastruey-Izquierdo A, Wrzosek M, Rodriguez-Tudela JL, Dolatabadi S, Chakrabarti A, de Hoog GS. DNA barcoding in Mucorales: an inventory of biodiversity. Persoonia 2013;30:11-47.
28. Nguyen TTT, Jeon YJ, Mun HY, Goh J, Chung N, Lee HB. Isolation and characterization of four unrecorded *Mucor* species in Korea. Mycobiology 2020;48:29-36.
29. Nguyen TTT, Lee HB. Isolation and characterization of three Zygomycetous fungi in Korea: *Backusella circina*, *Circinella muscae*, and *Mucor ramosissimus*. Mycobiology 2018;46:317-27.
30. Kwon JH, Ahn GH, Park CS. Fruit soft rot of sweet persimmon caused by *Mucor piriformis* in Korea. Mycobiology 2004;32:98-101.