RESEARCH ARTICLE

Six Unrecorded Species of Sordariomycetes Isolated from Soil in the Republic of Korea

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ABSTRACT

Sordariomycetes is the second largest class within the Ascomycota and an ecologically and economically important group in fungal taxonomy. As a result of investigating fungal diversity in the soil of Anseong, Cheonan, Wando, and Jeju Island, we identified six species belonging to Sordariomycetes that had not been previously recorded in Korea. To confirm the taxonomic position of these species, we performed morphological observations and phylogenetic analyses based on the nucleotide sequences of the internal transcribed spacer (ITS), 28S ribosomal RNA gene (LSU rDNA), TEF1-α gene (*TEF1-α*), β-tubulin gene (*β-TUB*), and RNA polymerase II gene (*RPB2*). As a result, six species (*Cladorrhinum samala, Fusarium miscanthi, Gymnascella dankaliensis, Microascus gracilis, Papulaspora equi*, and *Paecilomyces purpureus*) were listed as newly confirmed species in Korea.

Keywords: Soil, Sordariomycetes, Unrecorded fungi

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INTRODUCTION

The class Sordariomycetes of the Ascomycota phylum was recently recognized as comprising 45 orders, 167 families, and 1,499 genera [1,2]. This type of fungus exists in various environments, including soil, air, fresh water, and oceans, and in animals and plants [3–7]. The lifestyle of fungi in this class also includes endophytes, parasites, human pathogens, plant pathogens, mushroom pathogens, fish and animal pathogens, and antagonists of bacteria that could be used for biological control agents [7–12]. Additionally, certain groups of Sordariomycetes are useful in industrial application [13].

Geographically, Korea belongs to the mid-latitude temperate climate zone and has four distinct seasons. It is also surrounded by the sea on three sides and consists of inland and islands areas [14]. As Korea has various climates and environments, it can be a valuable place for exploring diverse fungal species. As the fungi of Sordariomycetes are highly diverse in morphology, growth form, and habitat, we are interested in exploring the biodiversity and species distribution of this ecologically and economically important taxon. To study fungal diversity in soil across different geographical regions in Korea, we collected soil samples from the inland regions of Anseong and Cheonan and the island regions of Wando and Jeju. As a result of investigating the fungal diversity of the collected soil, we identified six species of Sordario fungi that had not been recorded in Korea. These species are known as plant pathogens, industrial agents, biological control

agents, and metabolite producers. In this study, we report on the molecular and morphological identification and characterization of these species.

MATERIALS AND METHODS

Collecting soil samples and isolation of fungi. We collected soil samples in Anseong, Cheonan, Wando and Jeju Island. After removing the external soil to a radius of 10 cm and depth of 10 cm, we used a trowel to collect soil to a depth of 10 cm. Samples soils were sealed in clean sample bags and then used for analysis. We placed 3 g of each of the six soil samples and 30 mL of primary sterile distilled water in a 50-mL tube and mixed them in a vortex mixer for 10 minutes. The mixed solution was diluted up to 100 times using the serial dilution method; moreover, 100 μL of the diluted solution was inoculated onto DG18 (DiCholran glycerol 18% agar, MB cell, Seoul, Korea) and smeared using a glass smear. The plated medium was sealed with parafilm and cultured in an incubator at 25°C for 7 days. The grown fungi were inoculated onto PDA (Potato Dextrose Agar, Difco, Detroit, MI, USA) using a sterilized inoculation loop and cultured in an incubator at 25°C for 7 days. Single spore cultures were obtained from PDA-grown fungi and assigned Dankook University Culture Collection (DUCC) numbers. Among the strains assigned DUCC numbers, representative strains were deposited at the National Institute of Biological Resources (NIBR), Incheon, Republic of Korea, where they were given NIBRFGC numbers.

Fungal morphological examination and molecular maker gene analysis. For morphological analysis, the isolated fungi were cultured on PDA, MEA (Malt Extract Agar, MBcell, Seoul, Korea), CYA (Czapek Yeast extract Agar, MBcell, Seoul, Korea), and OA (Oatmeal Agar, Difco, Detroit, MI, USA) media in the dark at 25°C for 7 days. We observed the morphological characteristics of the pure isolates under a light microscope (BX53: Olympus, Tokyo, Japan) and measured the length and size of the ultrastructure. We extracted genomic DNA from the isolated fungus using a Direct DNA prep kit (NaviBiotech, Cheonan, Korea). The extracted DNA was subjected to PCR (Bio-Rad T100 Thermal Cycler) using the universal primers of internal transcribed spacer (ITS), 28S ribosomal RNA gene (LSU rDNA), TEF1-α gene (*TEF1-α*), β-tubulin gene (*β-TUB*), and RNA polymerase II gene (*RPB2*). PCR conditions and primer sequences are provided in Table 1. The amplified PCR products were subjected to 1% agarose gel electrophoresis to confirm DNA amplification and the size of amplified DNA. The PCR amplified product was purified using the High Pure Product Purification Kit (Roche, Indianapolis, IN, USA) and sequenced by Macrogen Corp. (Daejeon, Korea).

Table 1. PCR condition and primer sequences used in this study

Target region	Primer name	Primer sequence (5' – 3')	PCR condition	Reference
ITS	ITS1	GAAGTAAAAGTCGTAACAAGG	95℃ 5min; 35 cycles: 95℃ 30s, 56℃ 30s, 72℃ 1min; 72℃ 5min	[19]
	ITS4	TCCTCCGCTATTGATATGC		[20]
LSU rDNA	LR0R	ACCCGCTGAACTTAAGC	95℃ 5min; 35 cycles: 95℃ 30s, 56℃ 30s, 72℃ 1min; 72℃ 5min	[21]
	LR5	TCCTGAGGGAAACTTCG		
TEF1-α	TEF1	GCCATCCTTGGAGATACCAGC	95℃ 5min; 35 cycles: 95℃ 30s, 55℃ 30s, 72℃ 1min; 72℃ 5min	[22]
	TEF728	CATCGAGAAGTTCGAGAAGG		
β-TUB	Bt2a	GGTAACCAAATCGGTGCTGCTTTC	95℃ 10min; 35 cycles: 95℃ 50s, 52℃ 50s, 72℃ 1min; 72℃ 5min	[23]
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC		
RPB2	RPB2-5f	GAYGAYMGWGATCAYTTYGG	95℃ 10min; 35 cycles: 95℃ 30s; 60℃ 1min; 72℃ 1min; 72℃ 7min	[24]
	RPB2-7cR	CCCATRGCTTGYTTRCCCAT		

ITS: internal transcribed spacer; LSU rDNA: 28S ribosomal RNA gene; *TEF1-α*: translation elongation factor 1-α gene; *β-TUB*: β-tubulin gene; *RPB2*: RNA polymerase II gene.

Molecular phylogenetic analysis. The determined nucleotide sequences were compared for homology with the sequences of marker genes of fungi in the National Center for Biotechnology Information (NCBI, 2023) database using GenBank BLASTN (http://blast.ncbi.nlm.nih.gov/) tool and the species with the closest nucleotide sequence similarity were searched. To analyze phylogeny of the DUCC strains, multiple sequence alignment was performed using the CLUSTAL W alignment tool in the MEGA 11 program with reference sequences obtained from the GenBank [15]. The reference sequences used in this study are listed in Tables 2–7. We used the aligned sequences to generate a molecular phylogenetic tree of the strains using the Kimura two-parameter model and maximum likelihood (ML) in the MEGA 11 program [16,17]. The reliability of phylogenetic tree branches was analyzed using 1,000 bootstrap analyses [18].

Table 2. Reference sequences used for phylogenetic analysis of strain NIBRFGC000510203

Scientific name	Strain	Source	Country	ITS	LSU rDNA
Cladorrhinum brunnescens	CBS 643.75A	-	Netherlands	NR_137152	NG_073587
Cladorrhinum carnegieae	CBS 150814	*Carnegiea gigantea*	USA	NR_197920	PP791451
Cladorrhinum coprophilum	HGUP191016	*Rosa roxburghii*	China	MZ724892	MZ724986
Cladorrhinum foecundissimum	BCCM 6980	-	-	KT321080	KT312993
Cladorrhinum globisporum	LC5415	Water	China	KU746680	KU746726
Cladorrhinum hyalocarpum	CBS 322.70	-	Netherlands	NR_172536	MH871448
Cladorrhinum intermedium	CBS 433.96	Soil	India	NR_165594	MK926859
Cladorrhinum olerum	CBS 120012	Wombat dung	Australia	-	MT731522
Cladorrhinum queenslandicum	BRIP 63076a	*Poa annua*	Australia	NR_197571	PP766573
Cladorrhinum samala	CBS 302.90	-	-	KT321079	KT312992
Cladorrhinum samala	INTA-AR 112	Soybean crop	-	KT321075	KT312988
Cladorrhinum samala	**NIBRFGC000510203**	**Soil**	**Republic of Korea**	**OQ991278**	**OQ991295**
Cladorrhinum tomentosum	Francoise Candoussau (S)	*Brassica oleracea*	Spain	-	KF557691
Podospora bulbillosa	CBS 304.90	Desert soil	Egypt	NR_077199	NG_073583
Podospora dacryoidea	INTA-AR 70	Soybean crop	-	KT321062	KT312976
Podospora flexuosa	FMR 10415	Soil	Spain	NR_154757	NG_058773
Triangularia microsclerotigena	CBS 290.75	*Musa* sp.	Turkey	NR_154758	NG_073584
Triangularia phialophoroides	CBS 301.90	Desert sand	Egypt	NR_077198	NG_073586
Valsa nivea	MFLUCC 15-0860	*Salix acutifolia*	Russia	KY417737	KY417771

ITS: internal transcribed spacer; LSU rDNA: 28S ribosomal RNA gene.
Strain identified in this study and its accession numbers are indicated in bold.

Table 3. Reference sequences used for phylogenetic analysis of strain NIBRFGC000510209 strain

Scientific name	Strain	Source	Country	*TEF1-α*	*RPB2*	*β-TUB*
Fusarium denticulatum	CBS 407.97	*Ipomoea batatas*	USA	MN534000	MN534274	MN534068
Fusarium pseudocircinatum	LC13677	*Syzygium samarangense*	China	MW580543	MW474489	MW533822
Fusarium sacchari	CBS 223.76	*Saccharum officinarum*	India	MW402115	KU604309	MW402313
Fusarium andiyazi	CBS 134430	-	-	KC954401	KU604232	KU603867
Fusarium annulatum	CBS 792.91	Gladiolus	Netherlands	MW402153	MW402774	MW402354
Fusarium brevicatenulatum	CBS 404.97	*Striga asiatica*	Madagascar	MN533995	MN534295	MN534063
Fusarium concentricum	CBS 102157	*Macaranga pruinosa* stem	Malaysia	MW401963	MW402728	MW402164
Fusarium ficicrescens	CBS 125178	*Ficus carica*	Iran	KU604452	KT154002	KP662896
Fusarium foetens	CBS 110286	Begonia elatior hybrid	Netherlands	MT011001	MW928825	MT011049
Fusarium fujikuroi	LC7864	*Poaceae* sp.	China	MW580493	MW474439	MW533772
Fusarium globosum	CBS 431.97	*Zea mays* seed	South Africa	MW402131	MW402816	MW402330
Fusarium lactis	NRRL 25200	-	-	MN193862	MN193890	U61551
Fusarium mangiferae	CBS 119853	Mango	South Africa	MN534016	MN534270	MN534140
Fusarium metavorans	LC5933	Submerged wood	China	MW620178	MW474703	MW534057
Fusarium miscanthi	NRRL 26231	-	-	KU171725	JX171634	AF060384
Fusarium miscanthi	LC7503	Water	China	MW594318	MW474551	MW533922
Fusarium miscanthi	**NIBRFGC000510209**	**Soil**	**Republic of Korea**	**PQ611945**	**PQ611946**	**PQ611947**
Fusarium ngaiotongaense	LC13834	*Armeniaca mume*	Japan	MW620176	MW474701	MW534055
Fusarium nygamai	CBS 749.97	*Sorghum bicolor*	Australia	MW402151	KU604262	MW402352
Fusarium oxysporum	CBS 221.49	*Camellia sinensis*	Southeast Asia	MH484963	MH484872	MH485054
Fusarium paranisikadoi	LC2824	Unidentified grass	China	MW594317	MW474550	MW533921
Fusarium proliferatum	CBS 480.96	Soil	Papua New Guinea	MN534059	MN534272	MN534129
Fusarium udum	CBS 747.79	*Cajanus cajan*	India	MN534045	MN534258	MN534141
Fusarium verticillioides	CBS 122159	-	-	KR071707	KU604224	KU603856

TEF1-α: translation elongation factor 1-α gene; *β-TUB*: β-tubulin gene; *RPB2*: RNA polymerase II gene.
Strain identified in this study and its accession numbers are indicated in bold.

Table 4. Reference sequences used for phylogenetic analysis of strain NIBRFGC000510207

Scientific name	Strain	Source	Country	ITS	LSU rDNA
Arthropsis truncata	CBS 584.82	-	Peru	NR_159641	NG_056973
Gymnascella afilamentosa	CBS 658.71	-	USA	NR_160135	NG_057626
Gymnascella aurantiaca	CBS 405.84	Mouse dung	Netherlands	OM468610	OM515116
Gymnascella dankaliensis	**NIBRFGC000510207**	**Soil**	**Republic of Korea**	**OQ991280**	**OQ991297**
Gymnascella dankaliensis	CBS 352.68	-	Netherlands	MH859156	MH870867
Gymnascella dankaliensis	CBS 339.65	-	India	MH858597	MH870236
Gymnascella littoralis	CBS 454.73	-	Canada	NR_155105	MH872451
Gymnascella nodulosa	CBS 577.63	-	India	NR_160094	NG_064039
Gymnascella sp.	BiMM-F285 (SV3)	Dust	Austria	OL527727	OL527728
Gymnascella stercoraria	LC4076	Compost	China	NR_160561	NG 067788
Gymnascella thermotolerans	LC3877	Soil	China	NR_160560	NG 067787
Narasimhella hyalinospora	CBS 548.72	Guinea pig dung	India	NR_130659	NG_057618
Narasimhella poonensis	CBS 393.71	-	India	NR_178142	NG_088077

ITS: internal transcribed spacer; LSU rDNA: 28S ribosomal RNA gene.
Strain identified in this study and its accession numbers are indicated in bold.

Table 5. Reference sequences used for phylogenetic analysis of strain NIBRFGC000510198

Scientific name	Strain	Source	Country	ITS	LSU rDNA	*β-TUB*
Microascus alveolaris	CBS 139501	Human	USA	NR_155393	NG_069390	KX924263
Microascus alveolaris	CBS 494.70	Marine sediment	Norway	MH859814	MH871586	LN850855
Microascus atrogriseus	CBS 295.52	Culture contaminant	UK	NR_155415	KX924030	KX924265
Microascus campaniformis	CBS 138126	Human BAL	USA	NR_132937	HG380495	LM652606
Microascus cinereus	CBS 365.65	Soil	India	MH858612	MH870254	KX924270
Microascus cirrosus	CBS 217.31	*Prunus* sp.	Italy	MH855194	MH866642	KX924273
Microascus cirrosus	CBS 267.49	*Sciurus vulgaris*	Netherlands	KX923840	AF400865	KX924275
Microascus gracilis	CBS 369.70	Wheat flour	Japan	NR_165206	HG380467	KX924297
Microascus gracilis	**NIBRFGC000510198**	**Soil**	**Republic of Korea**	**OP604172**	**OP604180**	**OP973115**
Microascus macrosporus	CBS 662.71	Soil	USA	LM652423	LM652517	LM652636
Microascus pyramidus	CBS 668.71	Pocket mouse	USA	KX923926	MH872050	LN850876
Microascus senegalensis	CBS 594.78	Skin	Algeria	LN850781	LN850830	LN850878
Microascus terreus	CBS 601.67	Soil	Ukraine	LN850783	LN850832	LN850880
Microascus terreus	CBS 665.71	Soil	USA	KX923938	-	KX924372
Microascus trigonosporus var. *trigonosporus*	CBS 150.64	*Allium cepa* seed	USA	MH858399	MH870027	KX924262
Yunnania carbonaria	MUCL 9027	Soil	Panama	NR_154546	HG380462	LM652695

ITS: internal transcribed spacer; LSU rDNA: 28S ribosomal RNA gene; *β-TUB*: β-tubulin gene.
Strain identified in this study and its accession numbers are indicated in bold.

Table 6. Reference sequences used for phylogenetic analysis of strain NIBRFGC000510208

Scientific name	Strain	Source	Country	ITS	LSU rDNA
Apiosordaria hamata	CGMCC 3.15231	Wetland soil	China	KP878307	KP878305
Cephalotrichum hinnuleum	CBS 289.66	Deer dung	Australia	NR_159771	NG_069723
Microthecium sepedonioides	CBS 265.79	Soil	-	KP970638	KP970661
Papulaspora equi	**NIBRFGC000510208**	**Soil**	**Republic of Korea**	**OQ991279**	**OQ991296**
Papulaspora equi	CBS 128690	-	USA	MH865116	MH876556
Papulaspora equi	CBS 573.89	Ocular lesion	USA	NR_154293	NG_057961
Papulaspora sp.	Kw207/12	-	-	HF952115	HG003579
Paralulworthia halima	CMG 69	Submerged wood	Portugal	MT235737	MT235754

ITS: internal transcribed spacer; LSU rDNA: 28S ribosomal RNA gene.
Strain identified in this study and its accession numbers are indicated in bold.

Table 7. Reference sequences used for phylogenetic analysis of strain NIBRFGC000512619

Scientific name	Strain	Source	Country	ITS
Aphanocladium album	CBS 401.70	Myxomycete	Netherlands	AJ292401
Lophiostoma multiseptatum	HHUF 27309	Dead herbaceous twigs	Japan	NR_138018
Lophiostoma ravennicum	MFLUCC 140005	*Juncus* sp.	Italy	NR_148079
Metapochonia rubescens	CBS 352.70	-	USA	MH859706
Metapochonia rubescens	CBS 464.88	-	UK	NR_160177
Metarhizium acridum	ARSEF 7486	-	Niger	NR_132019
Metarhizium album	ARSEF 1942	-	-	NR_152950
Metarhizium anisopliae	ARSEF 7487	-	Eritrea	NR_132017
Metarhizium baoshanense	CCTCC M2016589	Soil	China	NR_166220
Metarhizium brunneum	ARSEF 2107	Coleoptera	USA	NR_132023
Metarhizium chaiyaphumense	BCC 78198	-	Thailand	NR_173299
Metarhizium cylindrosporum	ACCC 30114	-	China	NR_169887

Table 7. Reference sequences used for phylogenetic analysis of strain NIBRFGC000512619

Scientific name	Strain	Source	Country	ITS
Metarhizium flavoviride	ARSEF 2133	-	-	NR_131992
Metarhizium frigidum	ARSEF 4124	-	-	NR_132012
Metarhizium gaoligongense	CCTCC M2016588	Soil	China	NR_169915
Metarhizium globosum	ARSEF 2596	-	India	NR_132020
Metarhizium granulomatis	UAMH 11028	Chameleon liver	Denmark	NR_132013
Metarhizium gryllidicola	BCC 82988	-	Thailand	NR_175627
Metarhizium guizhouense	CBS 258.90	-	China	NR_163550
Metarhizium lepidiotae	ARSEF 7488	-	Australia	NR_132018
Metarhizium majus	ARSEF 1914	Coleoptera	Philippines	NR_152952
Metarhizium novozealandicum	FI-698 (F10)	Lepidoptera	New Zealand	AF139851
Metarhizium novozealandicum	FI-1125 (DAT-F368)	Soil	Australia	AF139853
Metarhizium phasmatodeae	BCC 49272	-	Thailand	NR_175628
Metarhizium pinghaense	CBS 257.90	-	China	NR_077205
Metarhizium robertsii	ARSEF 2575	-	USA	NR_132011
Metarhizium viride	CBS 348.65	*Chameleo lateralis*	Madagascar	NR_111173
Nigelia martialis	RLC707iNat84182994	Insect-larva	Ecuador	OQ871845
Paecilomyces brunneolus	CBS 370.70	*Cucumis sativus*	Japan	NR_149328
Paecilomyces clematidis	BRNU 677844	*Clematis* sp.	Czech Republic	NR_182939
Paecilomyces dactylethromorphus	CBS 251.55	Acetic acid	Brazil	NR_149330
Paecilomyces divaricatus	CBS 284.48	Mucilage bottle with library paste	USA	NR_145143
Paecilomyces formosus	CBS 990.73B	-	Japan	NR_149329
Paecilomyces fulvus	CBS 146.48	Bottled fruit	UK	NR_103603
Paecilomyces laguncularіae	CBS 373.70	Wood	Brazil	NR_145144
Paecilomyces niveus	CBS 100.11	-	-	NR_144910
Paecilomyces purpureus	GZDXIFR-GZSL-5	-	-	EF640809
Paecilomyces purpureus	**NIBRFGC000512619**	**Soil**	**Republic of Korea**	**OR742033**
Paecilomyces purpureus	**DUCC15611**	**Soil**	**Republic of Korea**	**OR742034**
Paecilomyces tenuis	GZUIFR-C43-1	-	-	NR_184868
Paecilomyces variotii	CBS 101075	Fruit beverage	Japan	NR_130679
Paecilomyces verticillatus	GZDXIFR-49-01	-	-	DQ836182
Paecilomyces zollerniae	CBS 374.70	Wood	Brazil	NR_103602
Papiliomyces shibinensis	GZUH SB13050311	Lepidopteran pupa	China	NR_154178
Pochonia cordycipiticonsociata	CGMCC:317366	Larvae	China	KM263567
Pochonia cordycipiticonsociata	P678	Soil	Poland	OK584612
Pochonia suchlasporia	PP14b	*Hevea brasiliensis*	Peru	FJ884150
Pochonia suchlasporia	C2S1-2009a	Soil	China	HQ660437
Pochonia suchlasporia	C3S3-2009b	Soil	China	HQ660438
Purpureocillium atypicola	NBRC 9205	Trapdoor spider	Japan	LC848307
Purpureocillium lavendulum	CBS 128677	-	Spain	NR_166039
Purpureocillium lilacinum	NRRL 895	-	-	NR_165946
Purpureocillium sodanum	IBRC-M 30175	Salt crystals	Iran	KX668542
Purpureocillium takamizusanense	NBRC:110232	Unidentified cicada	Japan	LC008205
Purpureomyces khaoyaiensis	BCC12687	Lepidoptera	Thailand	JN049868
Purpureomyces khaoyaiensis	BCC12587	*Homo sapiens*	Japan	KX983461
Purpureomyces pyriformis	BCC85348	*Homo sapiens*	Japan	MN781927
Purpureomyces pyriformis	BCC85349	*Homo sapiens*	Japan	MN781928
Purpureomyces pyriformis	BCC85074	*Homo sapiens*	Japan	MN781929

ITS: internal transcribed spacer.
Strains identified in this study and their accession numbers are indicated in bold.

RESULTS AND DISCUSSION

Cladorrhinum samala (Subram. & Lodha) W. Gams & Mouch., Mycotaxon 48: 429 (1993) [MB#360514]

Strains examined: Seoun-myeon, Anseong-si, Gyeonggi-do, Republic of Korea (36°55'35.8"N 127° 14'29.4"E), isolated from soil in which pear trees was buried for disease control, collected on January 6, 2023, strain DUCC24252(NIBRFGC000510203).

Macro morphological characteristics (Fig. 1A–D)

The morphology of the colony was observed after 7 d of incubation in the dark condition at 25°C. In all media, the colonies were fully grown to fill the plate. White mycelia initially grow radially in a chrysanthemum-like pattern, eventually turning into a dull light green, with the center remaining white and periphery shifting to a dull light green. Over time, dense cotton-like mycelia appear, and after one month, the color of this cottony mycelium turns buff.

Microscopic morphological characteristics (Fig. 1E–I)

Microsclerotia were attached to thick, dark brown mycelia. They were irregularly shaped, comprising several cells with rounded to polygonal shapes, measuring 79.4–89.4 × 89.8–108.7 μm. Phialides were formed laterally or at the terminal end of the branch, 4.66–8.34 × 2.09–2.85 μm, one conidiogenous opening, bearing conspicuous collarettes. Collarettes size was 1.01–4.3 × 0.6–2.69 μm. Conidia were mostly spherical, often resembling water drops that have a cylindrical with a clump of cut underparts, hyaline, 1.85–2.98 × 2.15–2.85 μm, observed after two months of incubation. Microsclerotia were observed, but teleomorph was not observed.

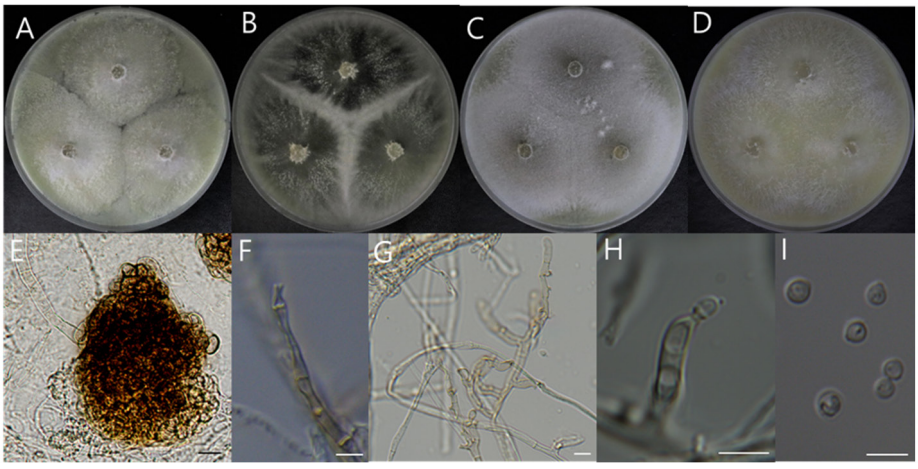

Fig. 1. Morphological characteristics of *Cladorrhinum samala* NIBRFGC000510203 incubated at 25°C for 7 days. (A) PDA; (B) MEA; (C) CYA; (D) OA; (E) Micro sclerotium; (F) Phialides; (G, H) Conidiophores and phialides; (I) Conidia. Scale bar = 10 μm. PDA: Potato Dextrose Agar; MEA: Malt Extract Agar; CYA: Czapek Yeast extract Agar; OA: Oatmeal Agar.

Molecular characteristics (Fig. 2)

The ITS and LSU rDNA sequences of strain NIBRFGC000510203 were used for phylogenetic analysis. The size of ITS and LSU rDNA sequences were 520 bp and 875 bp, respectively. These two sequences were concatenated and aligned with sequences of other *Cladorrhinum* species, resulting in a final sequence of 939 bp including gaps. As a result of constructing an ML phylogenetic tree based on the 939 bp concatenated sequence, strain NIBRFGC000510203 formed the same clade with *C. samala* INTA-AR 112 and *C. samala* CBS 302.90.

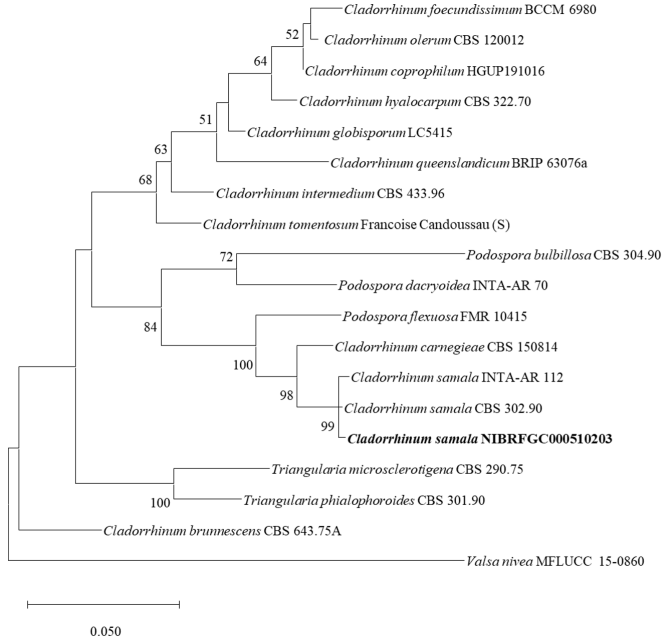

Fig. 2. The phylogenetic tree based on the concatenated nucleotide sequences of the ITS and LSU rDNA using the ML method with the Kimura 2-parameter model. The strain identified in this study is indicated in bold. The number of nodes represents the reliability value through 1,000 bootstrap replicates. Bootstrap values whose node reliability was less than 50 were removed. *Valsa nivea* MFLUCC 15-0860 was used as an outgroup. ITS: internal transcribed spacer; LSU rDNA: 28S ribosomal RNA gene; ML: maximum likelihood.

Notes

In 1964, *Cladorrhinum samala* was first reported as an isolate from cow dung in the Chakrata district and characterized by numerous funnel-shaped or cup-shaped collarettes on brown mycelia [25]. Some *Cladorrhinum* spp. have potential as biological control agents against plant pathogenic fungi and are promising candidates for developing biofertilizers for plant production. *Cladorrhinum* spp. are also known to produce phytase, an enzyme used in the processing of animal feed [26]. In this study, strain NIBRFGC000510203 was also observed with numerous funnel-shaped or cup-shaped collarlets. Phylogenetic analysis using the combined ITS and LSU rDNA sequences showed that it formed the same clade as *C. samala*. Based on the results of morphological and molecular analysis, we identified NIBRFGC000510203 as *Cladorrhinum samala*, a species not previously recorded in Korea.

Fusarium miscanthi W. Gams, Klamer & O'Donnell, Mycologia 91: 264 (1999) [MB#461045]

Strains examined: Seoun-myeon, Anseong-si, Gyeonggi-do, Republic of Korea (36°55'35.8"N 127° 14'29.4"E), isolated from soil in which pear trees was buried for disease control, collected on January 6, 2023, strain DUCC22601(NIBRFGC000510209).

Macro morphological characteristics (Fig. 2A–D)

We observed the morphology of the colony after 7 days of incubation in the dark condition at 25°C. On PDA, 46–47mm diameter radial, circular, slightly fluffy, mycelia tend to white, little purple hyphae in the middle, purple pigment was found. On MEA, 39–40-mm diameter, white mycelia, hyphae at the end were aerial hyphae. On CYA, 45–46-mm diameter, pinkish white, fluffy mycelia. On OA, 49–50mm diameter, white colored, flat, mycelia density lower than other media.

Microscopic morphological characteristics (Fig. 3E–H)

Hyphae were septate, thin walled, mostly cylindrical. Conidiophores were solitary, sometimes lacking a basal septum, 8.5–12.9 × 2.3–2.9 μm, producing one-celled microconidia of two kinds. Pyriform microconidia were one-celled, smooth surface, circular to elliptical with one pointed end like a droplet, 4.3–8.6 × 2.3–3.3 μm. Fusiform microconidia were one-celled, smooth surface, thin walled, cylindrical shape, 5.5–7.7 × 2.8–6.7 μm. Macroconidia were 2–3 septate, smooth surface, tapering apical cell, 24–26.5 × 3–4.7 μm. Chlamydospores were absent, and teleomorph was not observed.

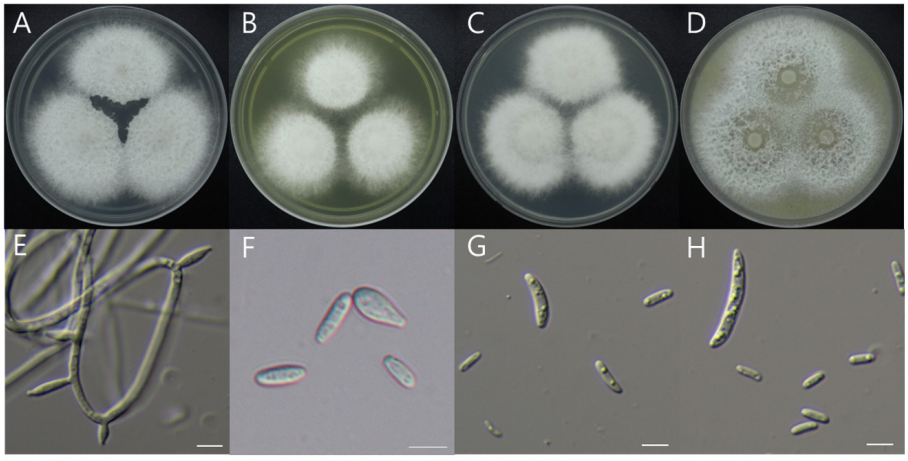

Fig. 3. Morphological characteristics of *Fusarium miscanthi* NIBRFGC000510209 incubated at 25°C for 7 days. (A) PDA; (B) MEA; (C) CYA; (D) OA; (E) Aerial conidiophores; (F) Pyriform and fusiform microconidia; (G) Fusiform microconidia; (H) Fusiform microconidia and macroconidia. Scale bar = 10 μm. PDA: Potato Dextrose Agar; MEA: Malt Extract Agar; CYA: Czapek Yeast extract Agar; OA: Oatmeal Agar.

Molecular characteristics (Fig. 4)

The sequences of *TEF1-α, β-TUB*, and *RPB2* of strain NIBRFGC000510209 were analyzed. The size of *TEF1-α, β-TUB*, and *RPB2* sequences was 522 bp, 315 bp, and 1,055 bp, respectively; these three sequences were concatenated. The concatenated sequence aligned with sequences of other *Fusarium* species, resulting in a final sequence of 1,497 bp including gaps. As a result of constructing an ML phylogenetic tree based on the 1,497 bp concatenated sequence, strain NIBRFGC000510209 formed the same clade with *F. miscanthi* LC7503 and *F. miscanthi* NRRL 26231.

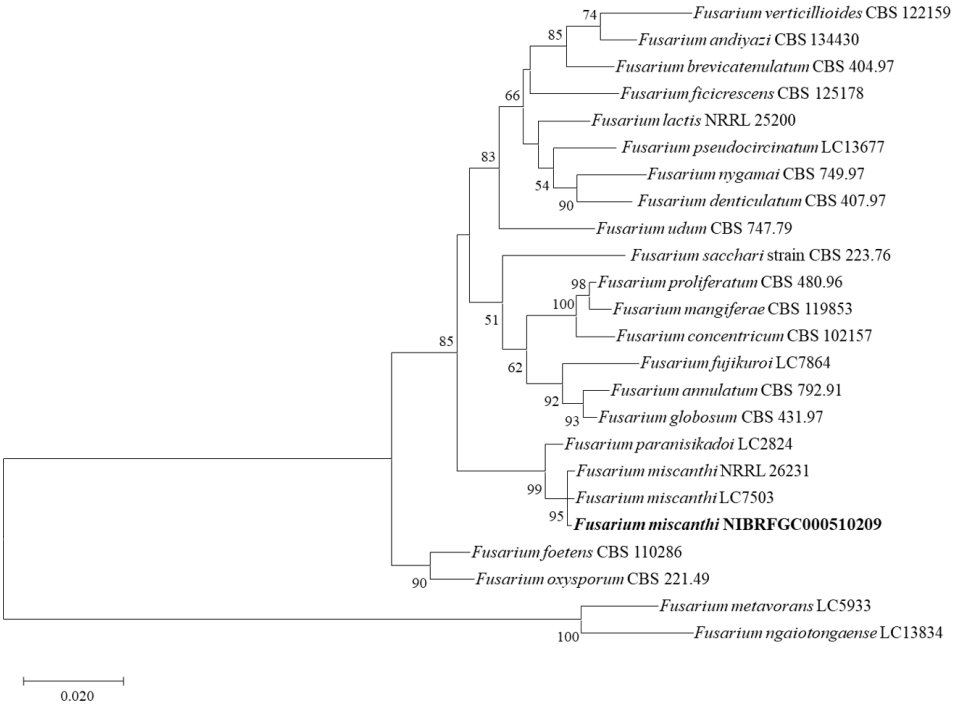

Fig. 4. The phylogenetic tree based on the concatenated nucleotide sequences of the *TEF1-α, RPB2,* and *β-TUB* using the ML method with the Kimura 2-parameter model. The strain identified in this study is indicated in bold. The number of nodes represents the reliability value through 1,000 bootstrap replicates. Bootstrap values whose node reliability was less than 50 were removed. *Fusarium metavorans* LC5933 and *Fusarium ngaiotongaense* LC13834 were used as outgroups. *TEF1-α*: translation elongation factor 1-α gene; *β-TUB*: β-tubulin gene; *RPB2*: RNA polymerase II gene; ML: maximum likelihood.

Notes

In 1998, *Fusarium miscanthi* was first reported from straw of *Miscanthus sinensis* in Denmark and characterized by two forms of microconidia, 3–5 septated macroconidia, and solitary or sporodochial conidiophores. [27]. *F. miscanthi* causes rhizome rot disease under greenhouse conditions and is also a known cause of ear rot in maize [28,29]. In this study, strain NIBRFGC000510209 also has solitary

conidiophore, two forms of microconidia and macroconidia. However, the size of the macroconidia was small, and the number of septa in the macroconidia was 2–3, which was less than in previous reports. Nevertheless, in phylogenetic analysis using the combined *TEF1-α*, *β-TUB* and *RPB2* sequences, strain NIBRFGC000510209 formed the same clade as *F. miscanthi*. Based on the results of morphological and molecular analysis, strain NIBRFGC000510209 was *Fusarium miscanthi*, a species not previously recorded in Korea.

Gymnascella dankaliensis (Castell.) Currah, Mycotaxon 24: 77 (1985) [MB#104292]

Strains examined: Seonggeo-eup, Seobuk-gu, Cheonan-si, Chungcheongnam-do, Republic of Korea (36°52'23.5"N 127°12'30.2"E), isolated from soil in which pear trees was buried for disease control, collected on July 7, 2022, strain DUCC24354(NIBRFGC000510207).

Macro morphological characteristics (Fig. 5A–D)

We observed the morphology of the colony after 7 d of incubation in the dark condition at 25°C. On PDA, strain NIBRFGC000510207 grew slowly, to 17.62–19.66mm. Conversely, on CYA, it showed the fastest growth at 32.17–32.69 mm. On MEA and OA, colonies were irregular, slightly fluffy, and white to buff colored.

Microscopic morphological characteristics (Fig. 5E–H)

Gymnothecia were yellow to brown colored, spherical, enclosed structure surrounded by a thick wall, 209.57–309.17 × 193.92–269.46 µm. Asci were yellow to orange colored, eight-spored, and 9.05–14.79 × 6.6–13.24 µm. Ascospores were circular or elliptical, one celled, ivory to yellow colored, and 3.16–5.77 × 2.9–5.45 µm. Anamorph was not observed.

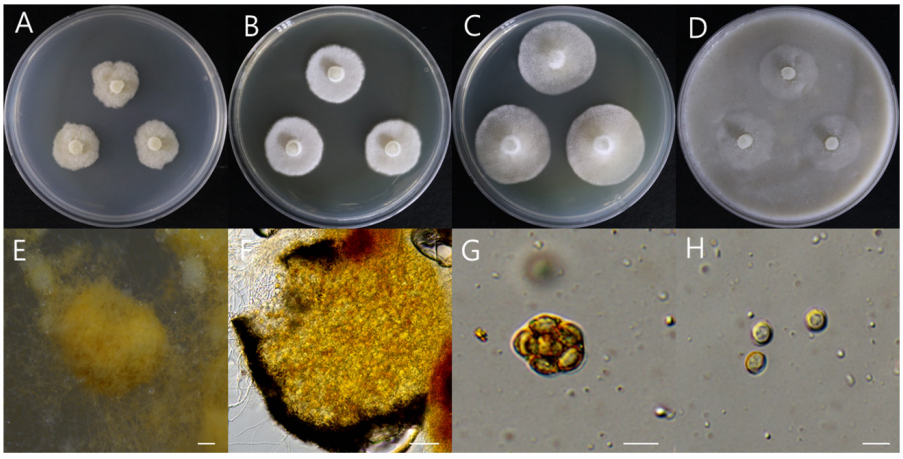

Fig. 5. Morphological characteristics of *Gymnascella dankaliensis* NIBRFGC000510207 incubated at 25°C for 7 days. (A) PDA; (B) MEA; (C) CYA; (D) OA; (E, F) Gymnothecium; (G) Ascus; (H) Ascospore. Scale bar: E, F = 100 µm, G, H = 10 µm. PDA: Potato Dextrose Agar; MEA: Malt Extract Agar; CYA: Czapek Yeast extract Agar; OA: Oatmeal Agar.

Molecular characteristics (Fig. 6)

The ITS sequences and LSU rDNA sequences of strain NIBRFGC000510207 were used for phylogenetic analysis. The size of ITS and LSU rDNA sequences was 567 bp and 911 bp, respectively. These two sequences were concatenated and aligned with sequences of other *Gymnascella* species, resulting in a final sequence of 1,138 bp including gaps. As a result of constructing an ML phylogenetic tree based on the 1,138-bp concatenated sequences, strain NIBRFGC000510207 formed the same clade with *G. dankaliensis* CBS 339.65 and *G. dankaliensis* CBS 352.68.

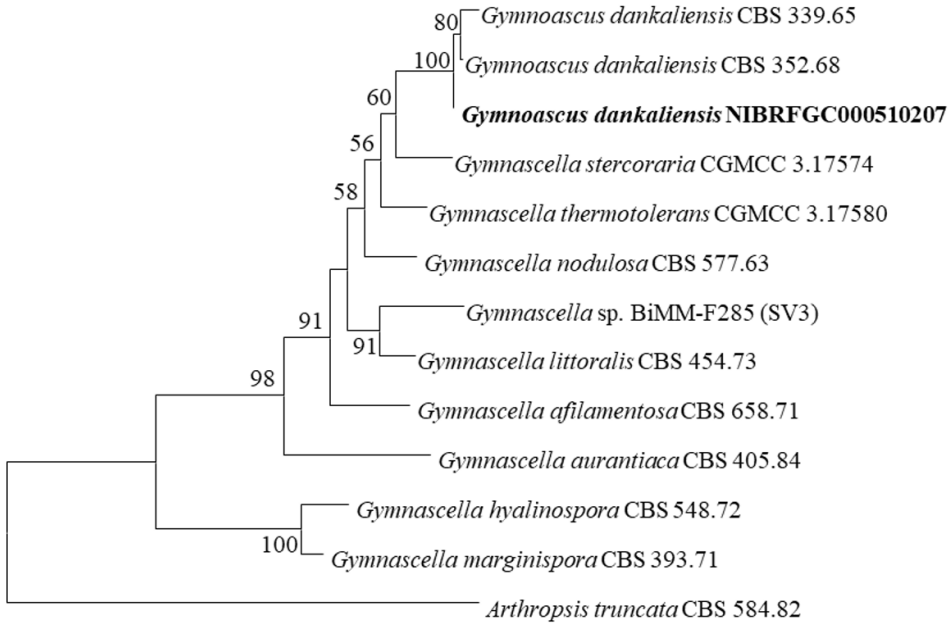

Fig. 6. The phylogenetic tree based on the concatenated nucleotide sequences of the ITS and LSU rDNA using the ML method with the Kimura 2-parameter model. The strain identified in this study is indicated in bold. The number of nodes represents the reliability value through 1,000 bootstrap replicates. Bootstrap values whose node reliability was less than 50 were removed. *Arthropsis truncata* CBS 584.82 was used as an outgroup. ITS: internal transcribed spacer; LSU rDNA: 28S ribosomal RNA gene; ML: maximum likelihood.

Notes

Gymnascella dankaliensis was first isolated from the hairy skin of a camel in 1937 under the scientific name *Trichophyton dankaliense* and characterized by round yellow, orange or red brown ascospores encircled by undifferentiated filaments [30]. *G. dankaliensis* is a keratinophilic fungus that causes superficial infections in humans [31]. Keratinophilic fungi can produce keratinases that degrade keratin materials in the soil [32]. Gymnastatins F and G extracted from the fungus were found to exhibit potent growth inhibition against P388 cancer cell lines [33]. Dankastatin B extracted from the species is involved in the antiproliferative effect on breast cancer cells by targeting C65 in the VDAC3 channel, a mitochondrial pore-forming protein [34]. In this study, strain NIBRFGC000510207 represents the morphological characteristics of *G. dankaliensis*, which exhibits undifferentiated hyphae surrounding yellow ascospores, and formed the same clade with *G. dankaliensis* CBS 339.65 and *G. dankaliensis* CBS 352.68 in phylogenetic analysis.

Based on the results of morphological and molecular analysis, strain NIBRFGC000510207 was identified as *G. dankaliensis*—a species not previously recorded in Korea.

Microascus gracilis (Samson) Sand. Den., Gené & Guarro, Persoonia 36: 17 (2015) [MB#809209]

Strains examined: Seonghwan-eup, Seobuk-gu, Cheonan-si, Chungcheongnam-do, Republic of Korea 36°55'35.8"N 127°14'29.4"E), isolated from rhizosphere soil of pear tree, collected on November 19, 2022, strain DUCC15381(NIBRFGC000510198).

Macro morphological characteristics (Fig. 6A–D)

The colony morphology was observed after 7 days of incubation in the dark condition at 25°C. Colonies grew slowly, flat, circular colony on all four media (PDA, MEA, CYA, and OA). The colony diameters varied among the four media (9–16 mm). The best media for mycelial growth was CYA. Colony colors were white on PDA, gray in the middle and white outside on MEA, gray in the middle and light brown outside on CYA, and dark gray in the middle and white outside on OA.

Microscopic morphological characteristics (Fig. 7E–S).

Hyphae were hyaline, branched, septate, thin-walled, and 2–3 μm in size. Ascomata are dark, subspherical to spherical, and 160–220 μm in diameter. The development of ascomata took 14 days on OA but more than 3 weeks on the other three media. Asci are oval-shaped, 10.5–16.4 × 6.5–9.1 μm in size, hyaline-filled and transparent, with eight ascospores. Ascospores are 4.3–6.6 × 2.7–3.9 μm in size, oval-shaped, slightly tapered at both ends, pale green, thin-walled. Conidiophores are 6.5–11.6 × 2–3.3 μm in size, cylindrical, internally transparent, pale green, and thin walled. Annellides are 6.4–10.4 × 2.1–3.1 μm in size, pale green, transparent, gourd-shaped, with a transparent interior, and 1–3 at the tip of a conidiophore. Conidia are oval, light green, hyaline and transparent, 3.3–4.6 × 2.6–3.5 μm in size. Microscopic morphological characteristics did not vary substantially among the four media.

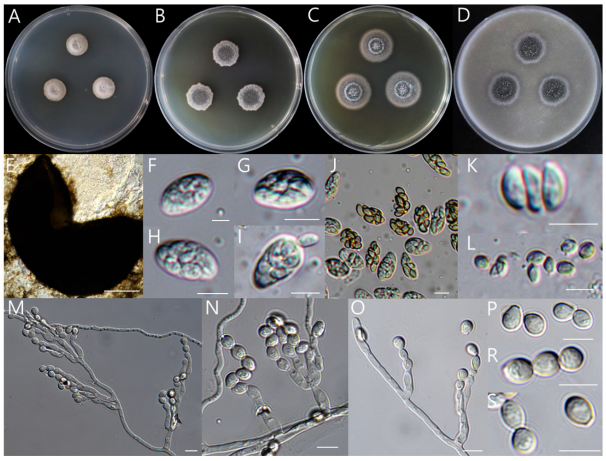

Fig. 7. Morphological characteristics of *Microascus gracilis* NIBRFGC000510198 incubated at 25°C for 7 days. (A) PDA; (B) MEA; (C) CYA; (D) OA; (E) Ascocarp; (F–J) Asci; (K, L) Ascospore; (M–O) Conidiophore and conidiogenous cells; (P–S) Conidia. Scale bar: E = 100 μm, F–S = 10 μm. PDA: Potato Dextrose Agar; MEA: Malt Extract Agar; CYA: Czapek Yeast extract Agar; OA: Oatmeal Agar.

Molecular characteristics (Fig. 8)

We analyzed the sequences of the ITS, LSU rDNA, and *β-TUB*. The size of the ITS, LSU rDNA, and *β-TUB* sequences of strain NIBRFGC000510198 were 485 bp, 909 bp, and 516 bp, respectively. These three sequences were concatenated. The concatenated sequence aligned with sequences of other *Microascus* species, resulting in a final sequence of 1,332 bp including gaps. As a result of constructing an ML phylogenetic tree based on the concatenated sequences, strain NIBRFGC000510198 formed the same clade with *M. gracilis* CBS 369.70.

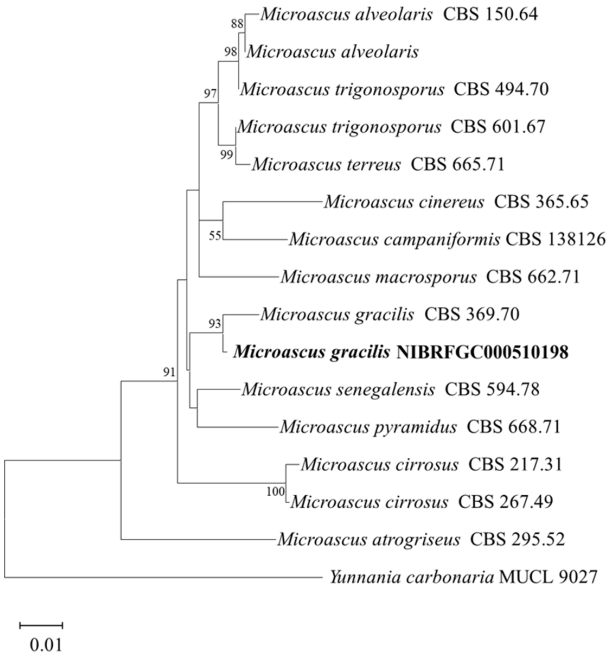

Fig. 8. The phylogenetic tree is based on the concatenated nucleotide sequences of the ITS, LSU rDNA, and *β-TUB* using the ML method with the Kimura 2-parameter model. The strain identified in this study is indicated in bold. The number of nodes represents the reliability value through 1,000 bootstrap replicates. Bootstrap values whose node reliability was less than 50 were removed. *Yunnania carbonaria* MUCL 9027 was used as an outgroup. ITS: internal transcribed spacer; LSU rDNA: 28S ribosomal RNA gene; *β-TUB*: β-tubulin gene; ML: maximum likelihood.

Notes

Microascus gracilis has been isolated mainly from food in Asia and North and South America, as well as from soil in Europe. Morphologically, *M. gracilis* is characterized by lunate ascospores, conidiophores irregularly branched, and subglobose to ellipsoidal conidia [35]. It caused mycetoma to humans and disseminated infection in a lung transplant patient [36,37]. Strain NIBRFGC000510198 was observed to have morphological characteristics similar to those of *M. gracilis,* such as lunate ascospores and oval shaped conidia. Based on the results of morphological and molecular analysis, strain NIBRFGC000510198 was identified as *Microascus gracilis*, a species not previously recorded in Korea.

Papulaspora equi Shadomy & D.M. Dixon, Mycopathologia 106 (1): 35 (1989) [MB#125460]

Strains examined: Yangseong-myeon, Anseong-si, Gyeonggi-do, Republic of Korea (37°01'47.3"N 127°11'15.4"E), isolated from soil in which pear trees was buried for disease control, collected on January 6, 2023, strain DUCC24276(NIBRFGC000510208).

Macro morphological characteristics (Fig. 9A–D)

The colony morphology was observed after 7 days of incubation in the dark condition at 25°C. In all media, strain NIBRFGC000510208 was fully grown to fill the plate. Colonies were fluffy, radially grown, gray colored, white clumped mycelia form at the edge. Subsequently, dark brown pigmentation resembling melanin developed, and a dense dot pattern was observed on the reverse side.

Microscopic morphological characteristics (Fig. 9E–H)

Hyphae were hyaline, thick, and 6.7–9.7 μm wide. Bulbils were observed after one month of incubation, were light-brown colored, consisted of densely thick hyphae, and were 100.01–105.53 × 76.64–86.44 μm. Initially, the hook of the bulbil was produced in a tangled form at the end of the hyphae. Afterward, the bulbil hook formed tightly coiled hyphae. Cells that composed the bulbils were swollen, spherical, thick-walled, and 9.2–11.67 × 9.65–12.75 μm. Teleomorph was not observed.

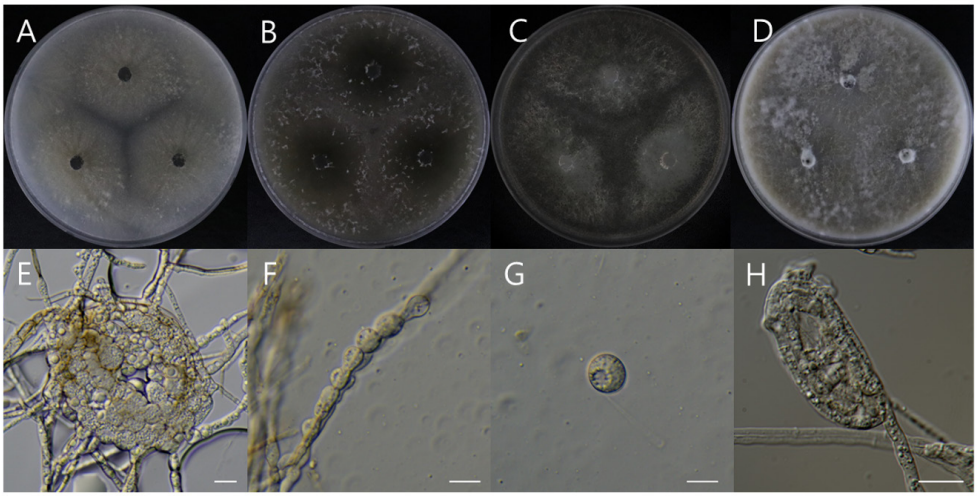

Fig. 9. Morphological characteristics of *Papulaspora equi* NIBRFGC000510208 incubated at 25°C for 7 days. (A) PDA; (B) MEA; (C) CZA; (D) OA; (E) Bulbils; (F, G) Cells that compose the bulbils on OA; (H) Hook of bulbil on PDA. Scale bar = 10 μm. PDA: Potato Dextrose Agar; MEA: Malt Extract Agar; CYA: Czapek Yeast extract Agar; OA: Oatmeal Agar.

Molecular characteristics (Fig. 10)

The ITS and LSU rDNA sequences of strain NIBRFGC000510208 were used for phylogenetic analysis. The size of ITS and LSU rDNA sequences was 523 bp and 897 bp, respectively. These two sequences were concatenated and aligned with sequences of other *Papulaspora* species, resulting in a final sequence of 1,071 bp including gaps. Sequence similarity between strain NIBRFGC000510208 and *P. equi* CBS 573.89

(type strain) and CBS 128690 was 99.9%. It is higher than the accepted similarity threshold 90.0% in the clustering of strains for this species in MycoBank. As a result of constructing an ML phylogenetic tree based on the 1,071 bp concatenated sequence, strain NIBRFGC000510208 formed the same clade with *P. equi* CBS 573.89 (type strain) and CBS 128690

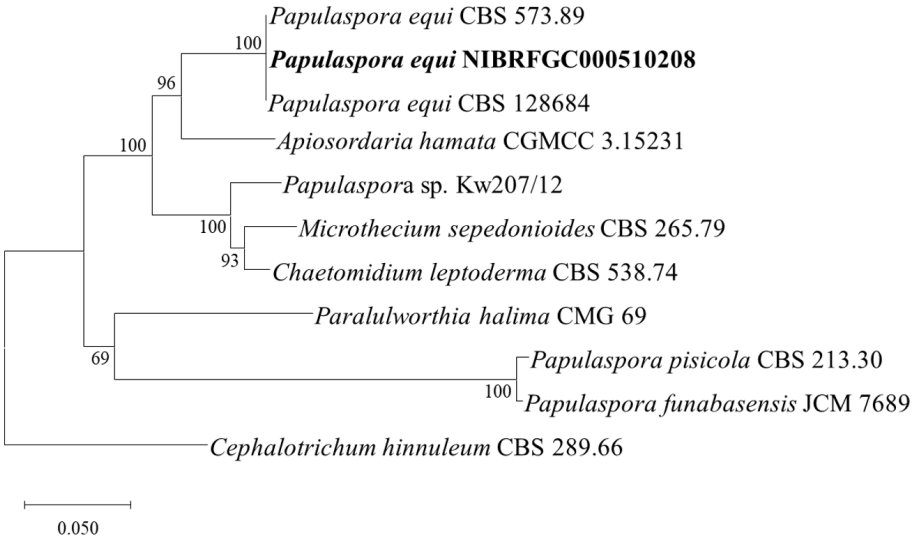

Fig. 10. The phylogenetic tree based on the concatenated nucleotide sequences of the ITS and LSU rDNA using the ML method with the Kimura 2-parameter model. The strain identified in this study is indicated in bold. The number of nodes represents the reliability value through 1,000 bootstrap replicates. Bootstrap values whose node reliability was less than 50 were removed. *Cephalotrichum hinnuleum* CBS 289.66 was used as an outgroup. ITS: internal transcribed spacer; LSU rDNA: 28S ribosomal RNA gene; ML: maximum likelihood.

Notes

The genus *Papulaspora* has been isolated from various objects, including soil, plants, and animals, but *Papulaspora equi* is a pathogenic fungus associated with the eyes. It is a potential zoonotic pathogen isolated from the infected eyes of horses and animals. Morphological characteristics of *P. equi* include the bulbils with an irregularly lobed surface and two sizes of hyphae; narrow hyphae 1–2.5 μm wide and broad hyphae 8 μm wide [38]. Additionally, *P. equi* is a rare causative agent of fungal keratomycosis in humans [39]. Strain NIBRFGC000510208 had morphologically similar characteristics to *P. equi*, such as thick hyphae and bulbils composed of thick cells. In phylogenetic analysis using the combined ITS and LSU rDNA sequences, strain NIBRFGC000510208 formed the same clade as that with the type strains *P. equi* CBS 573.89 and *P. equi* CBS 128690. Based on the results of morphological and molecular analysis, strain NIBRFGC000510208 was identified as *Papulaspora equi,* a species not previously recorded in Korea.

Paecilomyces purpureus Z.Q. Liang & Y.F. Han, Mycotaxon 101: 273 (2007) [MB#510901]

Strains examined: Seogwipo-si, Jeju Island, Republic of Korea (33°20'14.1"N 126°37'25.6"E), isolated from the hyposphere soil of Wrinkled mushroom, collected on June 19, 2023, strain DUCC15606(NIBRFGC000512619).

Macro morphological characteristics (Fig. 11A–D)

The colony morphology was observed after 7 days of incubation in the dark condition at 25°C. Colonies were circular, raised, and fluffy and had filiform margins. On CYA, growth reached approximately 30.53 mm, while on MEA, it grew slowly, reaching about 28.47 mm. On PDA and OA, the colony had a purple center, and on the reverse side, it was yellowish white-colored. On OA, synnemata formed within 7 days of culture, while in other media (PDA, MEA, CYA), it took 14 days.

Microscopic morphological characteristics (Fig. 11E–H)

Synnemata were formed by clusters of hyphae—gregarious, thick at the base and narrowing toward the terminal end, flat at the terminal end, 4.1–6.8 mm in length, 0.6–1 mm diam in base, and 0.4–0.6 mm diam in apex. Conidia are formed at the terminal end and margin. Vegetative hyphae were septate, hyaline, branched, smooth walled, 1.8–2.1 diam. Conidiophores were cylindrical, solitary, smooth walled, erect, or slightly curved, 6.5–7.2 × 2.1–2.4 µm. Phialides had occurred from vegetative hyphae or conidiophores and were ellipsoidal, cylindrical, and sometimes awl-shaped, and 11.28–18.03 × 1.55–2.3 µm. Conidia were single celled, hyaline, smooth walled, ovoid to ellipsoidal, continuously produced from phialides, and 3.24–3.52 × 2.62–2.84 µm. Teleomorph was not observed.

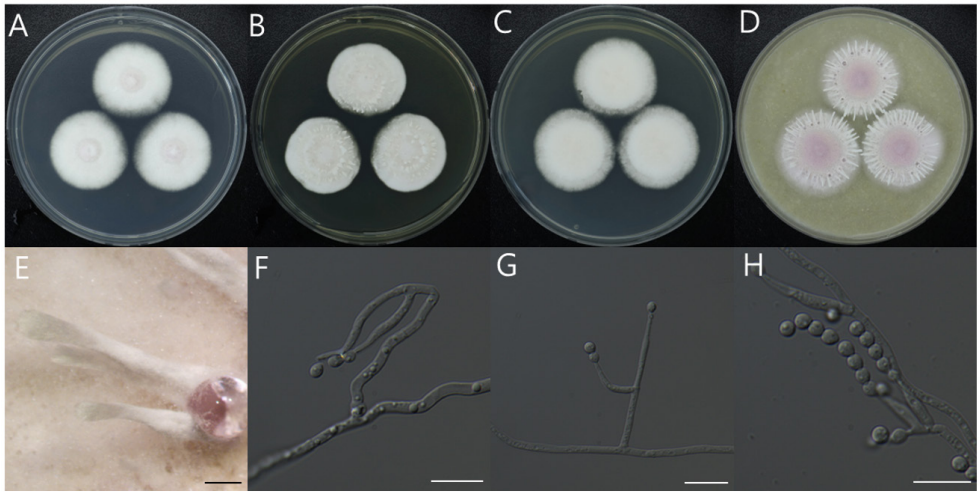

Fig. 11. Morphological characteristics of *Paecilomyces purpureus* NIBRFGC000512619 incubated at 25°C for 7 days. (A) PDA; (B) MEA; (C) CYA; (D) OA; (E) synnemata; (F–H) Conidiophore, Conidiogenous cell and conidia; Scale bar (E = 1mm; F–H =10 µm). PDA: Potato Dextrose Agar; MEA: Malt Extract Agar; CYA: Czapek Yeast extract Agar; OA: Oatmeal Agar.

Molecular characteristics (Fig. 12)

The size of the ITS sequence was 491 bp. As a result of aligning sequence with the reference sequences of other *Paecilomyces* species, a final sequence of 506 bp including gaps was obtained. The ITS sequence of strain NIBRFGC000512619 was used for phylogenetic analysis. As a result of constructing an ML phylogenetic tree based on the 506 bp ITS sequences, strain NIBRFGC000510208 formed the same clade as that with *P. purpureus* GZDXIFR-GZSL-5.

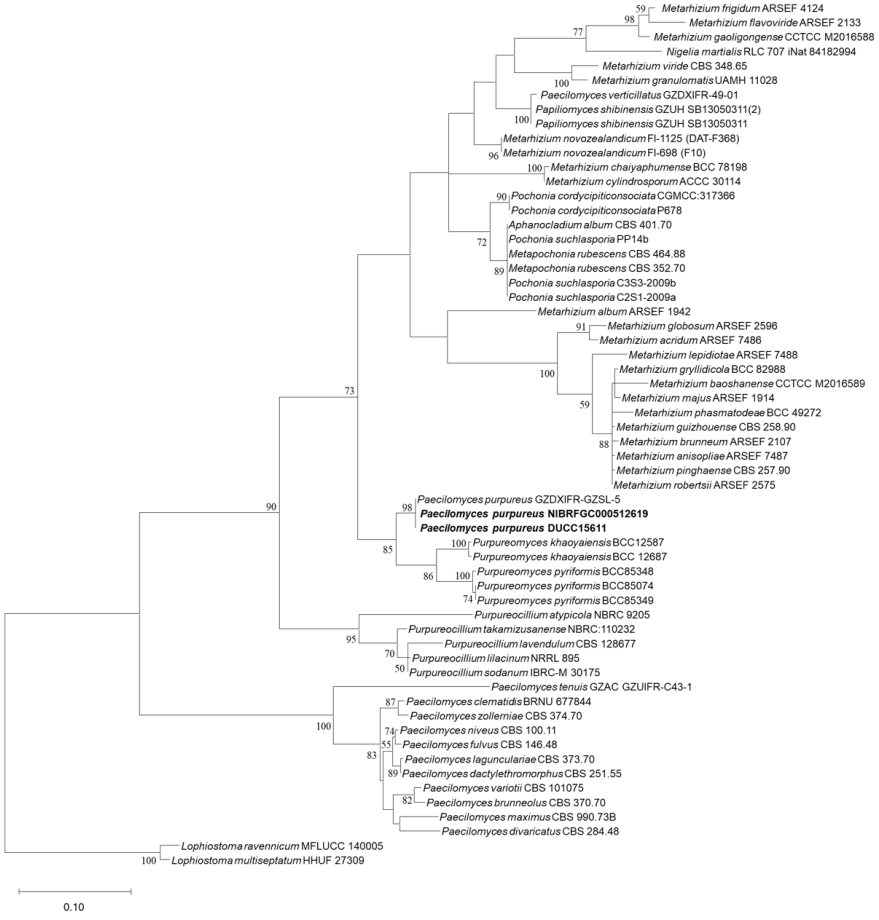

Fig. 12. The phylogenetic tree based on the nucleotide sequences of the ITS using the ML method with the Kimura 2-parameter model. Strains identified in this study are indicated in bold. The number of nodes represents the reliability value through 1,000 bootstrap replicates. Bootstrap values whose node reliability was less than 50 were removed. *Lophiostoma ravennicum* MFLUCC140005 and *Lophiostoma multiseptatum* HHUF 27309 were used as outgroups. ITS: internal transcribed spacer; ML: maximum likelihood.

Notes

Some members of the genus *Paecilomyces* are biostimulants of plant growth and crop yield [40]. They also produce secondary metabolites with herbicidal and cytotoxic activity [41,42]. The purple-red pigment produced by *P. lilacinus* TD16 is an important natural product for various applications. This species has antimicrobial activity against a wide range of bacteria and fungi, which suggests potential applications in industry [43]. In the genus, some species can be pathogenic to humans. *P. lilacinus* and *P. variotii* can mainly cause infections in immunocompromised individuals [44]. Morphologically, *P. purpureus* exhibited purple colonies; synnemata formed after long-term culture; phialides with a swollen basal portion or awl-shaped structure occurring directly or arising as 2–3 phialides from conidiophores; and single-celled, subglobose to fusiform conidia [45]. Strain NIBRFGC000512619 exhibited the morphological characteristics of *P. purpureus*, including purple synnemata, phialides with a swollen basal portion or awl-shaped structure occurring directly or arising as 2–3 phialides from conidiophores. In the phylogenetic analysis based on ITS rDNA sequences, strain NIBRFGC000512619 clustered with the same clade as *P. purpureus* GZDXIFR-GZSL-5. Based on morphological and molecular analyses, strain NIBRFGC000512619 was identified as *Paecilomyces purpureus*, which has not been previously recorded in Korea.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

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