


RESEARCH ARTICLE

First Report of *Albifimbria verrucaria* and *Deconica coprophila* (Syn: *Psilocybe coprophila*) from Field Soil in Korea

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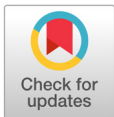
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ABSTRACT

During a survey of fungal diversity in Korea, two fungal strains, KNU17-1 and KNU17-199, were isolated from paddy field soil in Yangpyeong and Sancheong, respectively, in Korea. These fungal isolates were analyzed based on their morphological characteristics and the molecular phylogenetic analysis of the internal transcribed spacer (ITS) rDNA sequences. On the basis of their morphology and phylogeny, KNU17-1 and KNU17-199 isolates were identified as *Albifimbria verrucaria* and *Deconica coprophila*, respectively. To the best of our knowledge, *A. verrucaria* and *D. coprophila* have not yet been reported in Korea. Thus, this is the first report of these species in Korea.

Keywords: *Albifimbria verrucaria*, *Deconica coprophila*, Morphology



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INTRODUCTION

The genus *Albifimbria* L. Lombard & Crous 2016 belongs to the family Stachybotryaceae of Ascomycotic fungi. These fungi are characterized by verrucose setae and conidia bearing a funnel-shaped mucoidal appendage [1]. These fungi are distributed in the soil, leaves, fibers, fruit, and air [2]. To date, *Albifimbria* consists of four species, i.e. *A. lateralis*, *A. terrestris*, *A. verrucaria*, and *A. viridis*, of which *A. terrestris* has been previously reported in Korea [3]. *Albifimbria verrucaria* was previously classified under the genus *Myrothecium* [2]. *M. verrucaria* is associated with mycotoxicoses of livestock and humans [4-7]. This fungus is also associated with dieback and leafspot of various plant hosts [1, 8] resulting in exploitation of bioherbicides [9-11]. *Deconica coprophila* (Strophariaceae, Agaricales, Basidiomycota), commonly known as coprophilous and small mushroom, is distributed worldwide [12] and most commonly in tropical regions [13]. It is also known as *Psilocybe coprophila* [12]. A fungal diversity survey was carried out in 2017 in Sancheong and Yangpyeong, South Korea, through which two fungal species were assigned to the genera *Albifimbria* and *Deconica*. The objective of the present study is to morphologically and molecularly characterize these two unrecorded fungal species (*Albifimbria verrucaria* and *Deconica coprophila*) in Korea.

MATERIALS AND METHODS

Soil sampling and isolation of fungi

Soil sample collection was carried out in 2017 at different crop field locations at Gangwon-do province, Korea. In the present study, morphologically distinct fungal isolates, KNU17-1 and KNU17-199, were isolated from different crop field sites, 35.161771°N, 127.561884°E and 37.320693°N, 127.412046°E, respectively. Soil samples were collected from 15 cm below the surface, avoiding crop debris. Each soil sample was air dried and stored in a sterile polythene bag at 4°C. Collected soil was sieved through an autoclaved brass sieve of 2 mm aperture size. A conventional soil dilution technique [14] was followed to isolate the fungi from the soil. One g of fine soil was mixed with 9 mL of sterile distilled water and serial dilutions were made ranging from 10^{-1} to 10^{-5} . A 0.1 mL aliquot from each dilution was transferred into potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA) supplemented with $100 \mu\text{gL}^{-1}$ chloramphenicol (a bacteriostatic agent) in a Petri dish of size 90 mm and incubated at 25°C for 7-10 days. Thereafter, individual colonies of varied morphologies were transferred onto PDA plates. Pure isolates were maintained in PDA slant and stored in 20% glycerol at -80°C. KNU17-1 and KNU17-199 were deposited at the culture collection of the National Institute of Biological Resource (NIBR, Incheon, Korea) and received NIBR numbers of NIBRFGC000499998 and NIBRFG0000501887, respectively.

Morphological Characterization

For detailed morphological studies, the fungal isolates KNU17-1 and KNU17-199 were cultured on five different agar media, namely potato dextrose agar (PDA), oatmeal agar (OMA), malt extract agar (MEA), Czapek yeast extract agar (CYEA), and yeast extract sucrose agar (YESA). The isolates were inoculated at three points on Petri plates containing each medium and incubated at 26°C for 7 days under dark conditions. The fungal structures were examined using a light microscope (Olympus BX50F-3; Olympus Co., Tokyo, Japan). Photomicrographs were taken using an HK 3.1 CMOS digital camera (KOPTIC Korea Optics, Seoul, Korea) attached to an Olympus BX50F microscope. The micromorphology of the fungal isolates was examined using a scanning electron microscope (LEO Model 1450VP Variable Pressure Scanning Electron Microscope; Carl Zeiss, Cambridge, MA, USA).

Genomic DNA extraction, PCR, sequencing

For molecular identification, genomic DNA of the study isolates was extracted using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The internal transcribed spacer (ITS) region was amplified with the primer pairs ITS1 and ITS4 [15]. Polymerase chain reaction (PCR) amplification mixtures (total volume, 20 μL) for each gene were prepared in a 50 μL reaction comprising fungal DNA template, primers for each gene, Tag DNA polymerase, dNTPs, buffer, and a tracking dye. The amplification program included the following conditions: an initial denaturation step of 2 min at 95°C followed by 35 cycles of denaturation for 30 s at 95°C, annealing for 1 min at 72°C, and a final extension at 72°C for 10 min [16]. PCR amplification

products were purified with Microcon™ filters (Millipore, Burlington, MA, USA). The amplified PCR products were sequenced using an ABI Prism 3730 DNA analyzer (Applied Biosystem, Foster City, CA, USA).

Phylogenetic analysis

The fungal sequences obtained from the GenBank database (Table 1) were aligned using Clustal W [17]. All sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) program (NCBI, Bethesda, MD, USA). Phylogenetic trees were constructed by neighbor-joining method using Kimura's two parameter model [18] implemented in MEGA7 software [19]. The statistical confidence of tree topology was evaluated based on bootstrap analysis of 1,000 replicates. The ITS nrDNA sequences of the isolates KNU17-1 and KNU17-99 have been deposited in GenBank and have received accession numbers MH231755 and MH231771, respectively.

RESULTS AND DISCUSSION

Morphological characteristics of isolate KNU17-1

Colony of KNU17-1 in PDA attained a diameter of 60-65 mm in 7 days at 26°C. The front of the colony was white, whereas the back was light yellow (Fig. 1A, 1F). The shape of the colony was irregular, with moderate to dense sporulation. Elevation was flat. Margin was undulate. In OMA, the colony grew fast attaining a diameter of 70-75 mm. The front of the colony was cottony white and the back was rosy buff (Fig. 1B, 1G). The shape of the colony was irregular with moderate to dense sporulation. Margin was entire. Elevation was flat. In CYEA, the colony attained a diameter of 55-60 mm in 7 days at 26°C. The front of the colony was white and the back was light yellow (Fig. 1C, 1G). Sporulation was moderate. Margin was entire. Surface was smooth. Elevation was flat. In YESA, the colony attained a diameter of 65-70 mm in 7 days at 26°C. The front of the colony was white and the back was light yellow (Fig. 1D, 1I). Sporulation was moderate to dense. Margin was entire. Surface was smooth. Elevation was flat. In MEA, the colony attained a diameter of 60-65 mm in 7 days at 26°C. The front of the colony was white and the back was light yellow (Fig. 1F, 1J). Sporulation was moderate to dense. Margin was entire. Surface was smooth. Elevation was flat.

Micromorphology of the isolate KNU17-1

Aerial mycelium: hyphae (1.5-5 µm) were branched septate with clamp connection, with short branches, presence of anastomose. Hyphae abundant; chlamydospores present, Figure 1 (K-N). The morphological characteristics of the colonies were compared with previous descriptions of *Deconica coprophila* (Table 2) [20].

Table 1. Sequences used in this study and GenBank accession numbers

Taxon Name	Collection No.	Accession No.
<i>Albifimbria lateralis</i>	CBS 117712	NR_153548
<i>Albifimbria terrestris</i>	CBS 126186	NR_153549
<i>Albifimbria verrucaria</i>	CBS 962.95	KNU845895
<i>Albifimbria verrucaria</i>	CBS 328.52	NR_153550
<i>Albifimbria verrucaria</i>	CBS 121142	KU845896
<i>Alfaria caricola</i>	CBS 113567	KU845983
<i>Alnicola pallidifolia</i>	LIP PAM05082809	NR_153517
<i>Alnicola spectabilis</i>	LIP PAM05082811	NR_153516
<i>Anamika indica</i>	IB 19971305	NR_119451
<i>Anamika lactariolens</i>	LAU 88/95	NR_119524
<i>Cortinarius balteatoalbus</i>	PC R. Henry 82.98	NR_157924
<i>Cortinarius chromatophilus</i>	PC R. Henry 86.90	NR_157924
<i>Descolea infema</i>	CORD MES1315	NR_154032
<i>Dimorphiseta terrestris</i>	CBS 127345	NR_154009
<i>Gregatothecium humicola</i>	CBS 205.96	NR_154085
<i>Hebeloma aurantioumbrinum</i>	BR BR-MYCO 173985-64	NR_152902
<i>Hebeloma angustisporium</i>	TENN 023364	NR_119890
<i>Hebeloma brunneifolium</i>	TENN 029408	NR_119829
<i>Hebeloma griseopruinatum</i>	BR 5020169128570	NR_120099
<i>Hebeloma matritense</i>	BR MYCO 174910-19	NR_152905
<i>Hebeloma nothofagetorum</i>	HO 553022	NR_152886
<i>Hebeloma perexiguum</i>	BR BR-MYCO 173979-58	NR_137917
<i>Hebeloma pseudofragilipes</i>	BR MYCO 174911-20	NR_152906
<i>Hebeloma vesterholtii</i>	BR 5020166528762	NR_119724
<i>Hymenogaster raphanodorus</i>	SFSU F-000540	NR_119531
<i>Hebeloma subvictoriens</i>	MEL 2331640	NR_152885
<i>Macrolepiota deters</i>	HKAS 55306	NR_119832
<i>Myxospora aptrootii</i>	CBS 101263	NR_145080
<i>Myrospora crassiseta</i>	CBS 731.83	NR_155386
<i>Myrothecium Chiangmaiense</i>	MFLUCC 11-0506	NR_155389
<i>Myrothecium simplex</i>	CBS 582.93	NR_145079
<i>Pachhylepyrium carbonicola</i>	MICH 5270	NR_119906
<i>Parvothecium amazonense</i>	MUCL 54664	NR_155693
<i>Parvothecium terrestre</i>	CB 198.89	NR_145081
<i>Paramyrothecium terrestris</i>	CBS 564.86	NR_145078
<i>Paramyrothecium tellicola</i>	CBS 478.91	NR_155672
<i>Paramyrothecium foliicola</i>	CBS 113121	NR_145074
<i>Paramyrothecium breviseta</i>	CBS 544.75	NR_155670
<i>Paramyrothecium cupuliforme</i>	CBS 127789	NR_145073
<i>Paramyrothecium parvum</i>	CBS 257.35	NR_145076
<i>Paramyrothecium nigrum</i>	CBS 116537	NR_155671
<i>Paramyrothecium humicola</i>	CBS 127295	NR_145075
<i>Paramyrothecium viridisporum</i>	CBS 873.85	NR_155673
<i>Pholiota abieticola</i>	TENN 014000	NR_119907
<i>Pholiota caespitosa</i>	TENN 015908	NR_119908
<i>Pholiota virescentifolia</i>	TENN 020591	NR_119911
<i>Psathyroma cartervatim</i>	PDD 107742	NR_154327
<i>Psathyroma leucocarpum</i>	PDD 105593	NR_154328
<i>Psilocybe allenii</i>	PRM 899876	NR_119821
<i>Psilocybe coprophila</i>	PRM 899778	JX 235960
<i>Psilocybe cyanescens</i>	PRM 901481	NR_119478
<i>Smaragdiniseta bisetosa</i>	CBS 459.82	NR_145085
<i>Xepicula leucotricha</i>	CBS 256.57	NR_145088
<i>Xepicula crassiseta</i>	CBS 392.71	NR_145086

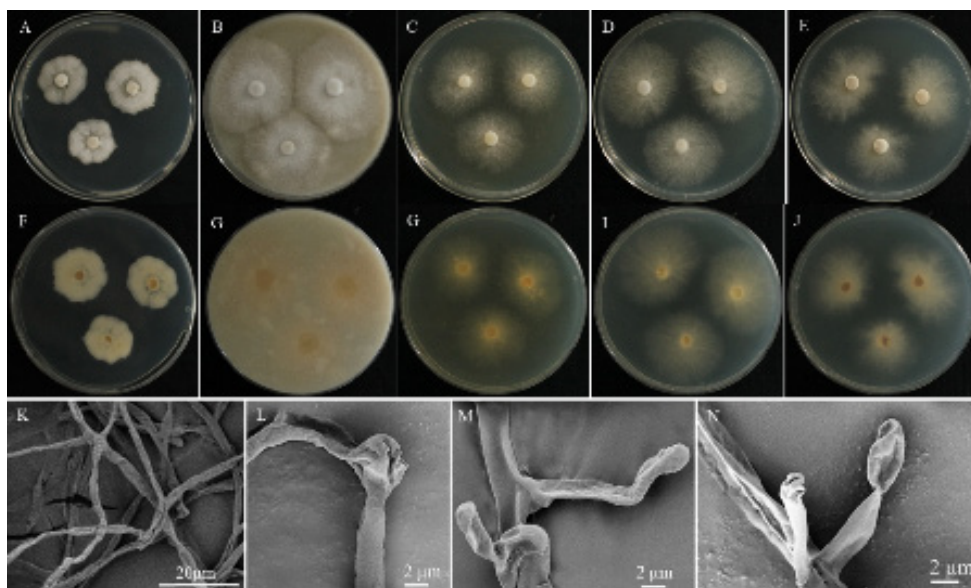


Fig. 1. Morphological characteristics of *Deconica coprophila* (KNU17-1) grown for 7 days at 26°C on potato dextrose agar (PDA), oatmeal agar (OMA), Czapek yeast extract agar (CYEA), yeast extract sucrose agar (YESA), and malt extract agar (MEA). (A-E) Observe colony from left to right. (F-J) Reverse colony from left to right grown on PDA, OMA, CYEA, YESA, and MEA. (K) Hyphae with anastomose (L) Intercalary chlamydospore (M, N) Hyphae with short branches.

Table 2. Morphological comparison of the present study isolate KNU17-1 with previously reported *Deconica coprophila* isolate

Characteristics	Study isolate KNU17-1	Previously reported <i>Deconica coprophila</i> ^a
Colony diameter (mm)	PDA: 60-65 OMA: 75-80 CYEA: 55-60 YESA: 65-70 MEA: 60-65	PDA: 70-75 OMA: N/A CYEA: N/A YESA: N/A MEA: N/A
Mycelium	Aerial mycelium	Aerial mycelium
Hyphae (structure)	Abundant, Branched, septate	Abundant, Branched and septate hyphae with granulose content
Hyphae (width)	1-4 μm	1.5-5 μm
Anastomoses	Present	Present
Chlamydospores	Present	Present (rare observed in one isolate)

^aSilva et al. 2016 [20].

N/A: Not available

Morphological characteristics of isolate KNU17-199

Colony of KNU17-199 in PDA attained a diameter of 40-45 mm in 14 days at 26°C. The front of the colony was white, whereas the back was reverse rosy buff (Fig. 2A, 2F). Shape was circular. Moderate to dense sporulation. Elevation was flat. Margin was entire. In OMA, the colony attained a diameter of 50-55 mm in 14 days at 26°C. The front of the colony was rosy buff and the back was light brown. Shape was irregular. Elevation was raised. Margin was irregular (Fig. 2B, 2G). In CYEA, the colony attained a diameter of 42-47 mm in 14 days at 26°C. The front side of the colony was white, whereas the back was dark brown in

the center and light yellow on the edges. The elevation was flat and margin was filiform (Fig. 2C, 2H). In YESA, the colony grew 35-40 mm in diameter in 7 days at 26°C. Front side of the colony was white while back was rosy buff in color. Shape was circular, margin was entire and flat elevation (Fig 2D, 2I). In MEA, colony attained diameter of 45-50 mm. Front side of the colony was white in color while back side was creamy light brown in color. Shape was irregular, elevation was flat and margin was entire (Fig 2E, 2J).

Micromorphology of the isolate KNU17-199

Hyphae were rarely branched, phialides were 3.6 in number and were cylindrical in shape and pointed towards the ends (Fig. 2K, 2L). Conidia were fusiform and pointed towards one end, 7-8 x 2.5-3.1 μm (Fig. 2M, 2N). The morphological characteristics observed for strain KNU17-199 were very similar to the previously described characteristics of *Albifimbria verrucaria* (Table 3) [2].

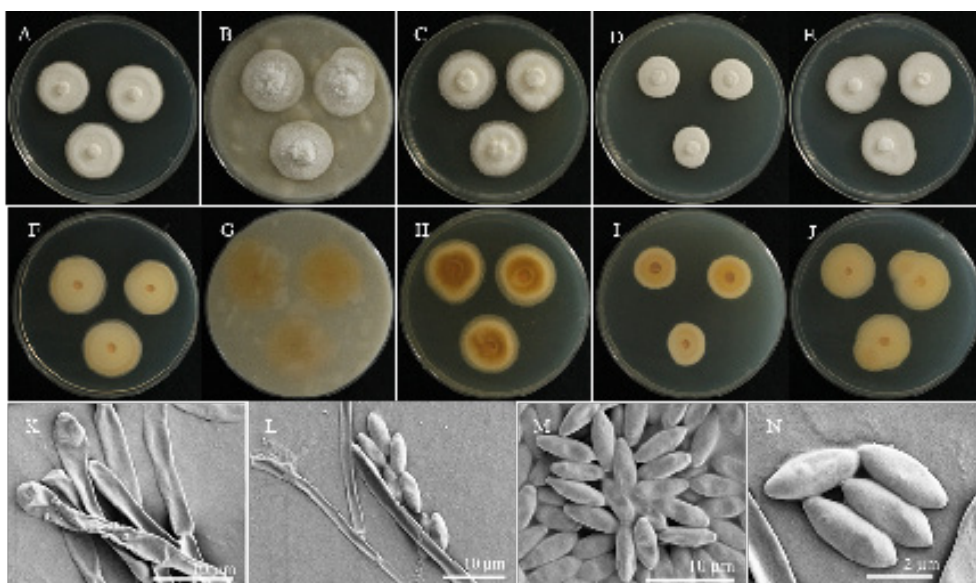


Fig 2. Morphological characteristics of *Albifimbria verrucaria* (KNU17-199) grown for 7 days at 25°C on potato dextrose agar (PDA), oatmeal agar (OMA), Czapek yeast extract agar (CYEA), yeast extract sucrose agar (YESA), and malt extract agar (MEA). (A-E) Observe colony from left to right. (F-J) Reverse colony from left to right grown on PDA, OMA, CYEA, YESA, and MEA. (K) Phialides. (L) Conidia on hyphae. (M, N) Spores.

Phylogenetic analysis of study isolates

Molecular phylogenetic analysis was carried out by comparing the ITS sequences of the study isolates with those of the reference strains in GenBank using the BLAST program. The phylogenetic tree evaluation based on a bootstrap analysis of 1,000 replicates revealed that the ITS region of strain KNU17-1 shared 100% identity with that of the strain JX235960 of *Psilocybe coprophila*. A concatenated dataset of ITS gene sequences was used to determine the molecular relationship between the present Korean isolate and *P. coprophila* retrieved from GenBank. A neighbor joining tree showed that KNU17-1 strain clustered in the

Table 3. Morphological comparison of the present study isolate KNU17-199 with the previously reported *Albifimbria verrucaria* isolate.

Characteristics	Study isolate KNU17-199	Previously reported <i>Albifimbria verrucaria</i> ^a
Colony diameter (mm)	PDA: 40-45 OMA: 50-55 CYEA: 42-47 YESA: 35-40 MEA: 45-50	PDA: 40-50 OMA: N/A CYEA: N/A YESA: N/A MEA: N/A
Mycelium	Floccose	Floccose, White to rosy buff, Reverse Roxy buff
Hyphae (structure)	Hyaline, rarely branched,	Hyaline, Smooth thin walled, rarely branched, septate, cells
Hyphae (size)	15-30× 1.4-2µm	15-30× 1.5-2µm
Phialides	3-6 in a whorl, Cylindrical	3-6 in a whorl, cylindrical, sometimes tapered toward apex
Spores (structures)	Fusiform, one end pointed the other protruding	Broadly fusiform, one end pointed the other protruding and truncate
Spores (size)	7-8×2.5-3.1µm	6.5-8×2-3µm

^aLombard et al. 2016 [2].
N/A: Not available

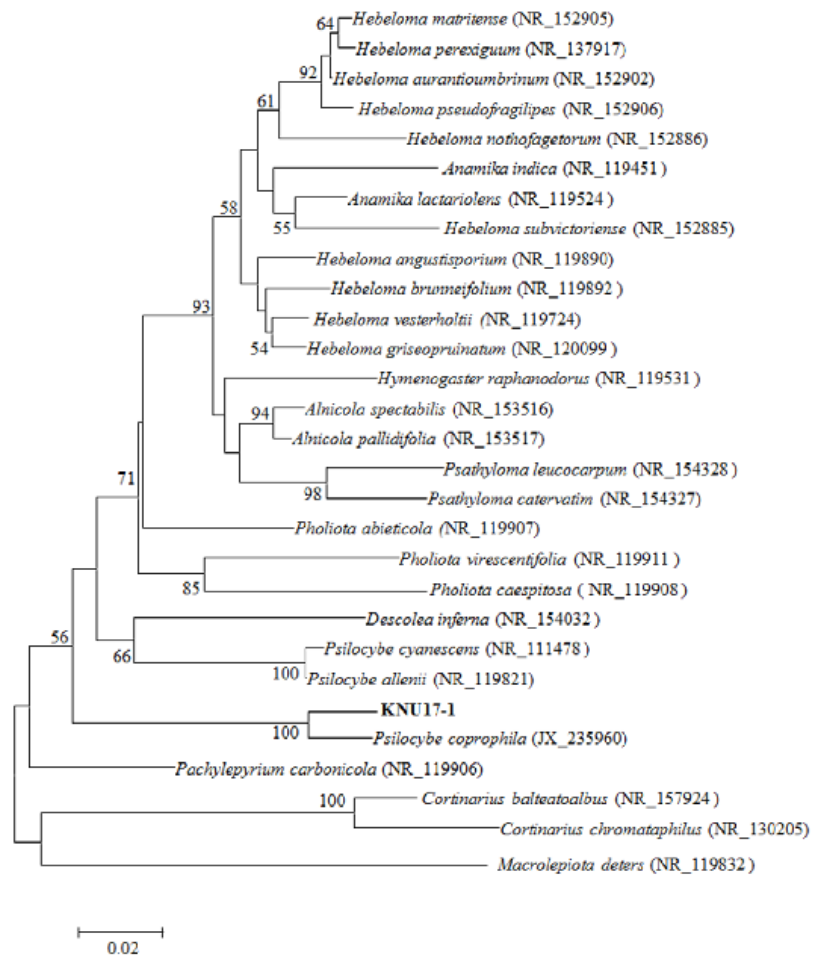


Fig 3. Neighbor-joining phylogenetic analysis of the partial 18S-ITS1-5.8S-ITS2-partial 28S rDNA sequences for *Psilocybe coprophila* KNU17-1, obtained from field soil in Korea. Species examined in this study are in bold. Bootstrap scores of >60 are presented at the nodes. The scale bar represents the number of substitutions per sites.

same clade as a *P. coprophila* strain, indicating that KNU17-1 is most closely related to *P. coprophila* (Fig. 3). *P. coprophila* is a synonym of *Deconica coprophila*, which was transferred to the genus *Psilocybe* by Paul Kummer in 1871 [21]. Similarly, the phylogenetic relationship from ITS sequence analysis indicated that KNU17-199 was grouped with the type strain *A. verrucaria* NR153550, KU845895, and KU845896 in a highly supported clade (98%) in our phylogenetic tree (Fig. 4). *A. verrucaria* was previously well known as *Myrothecium verrucaria* [2]. To our knowledge, this is the first report of *A. verrucaria* and *D. coprophila* in Korea and its identity is supported by the morphological and molecular characterization presented here.

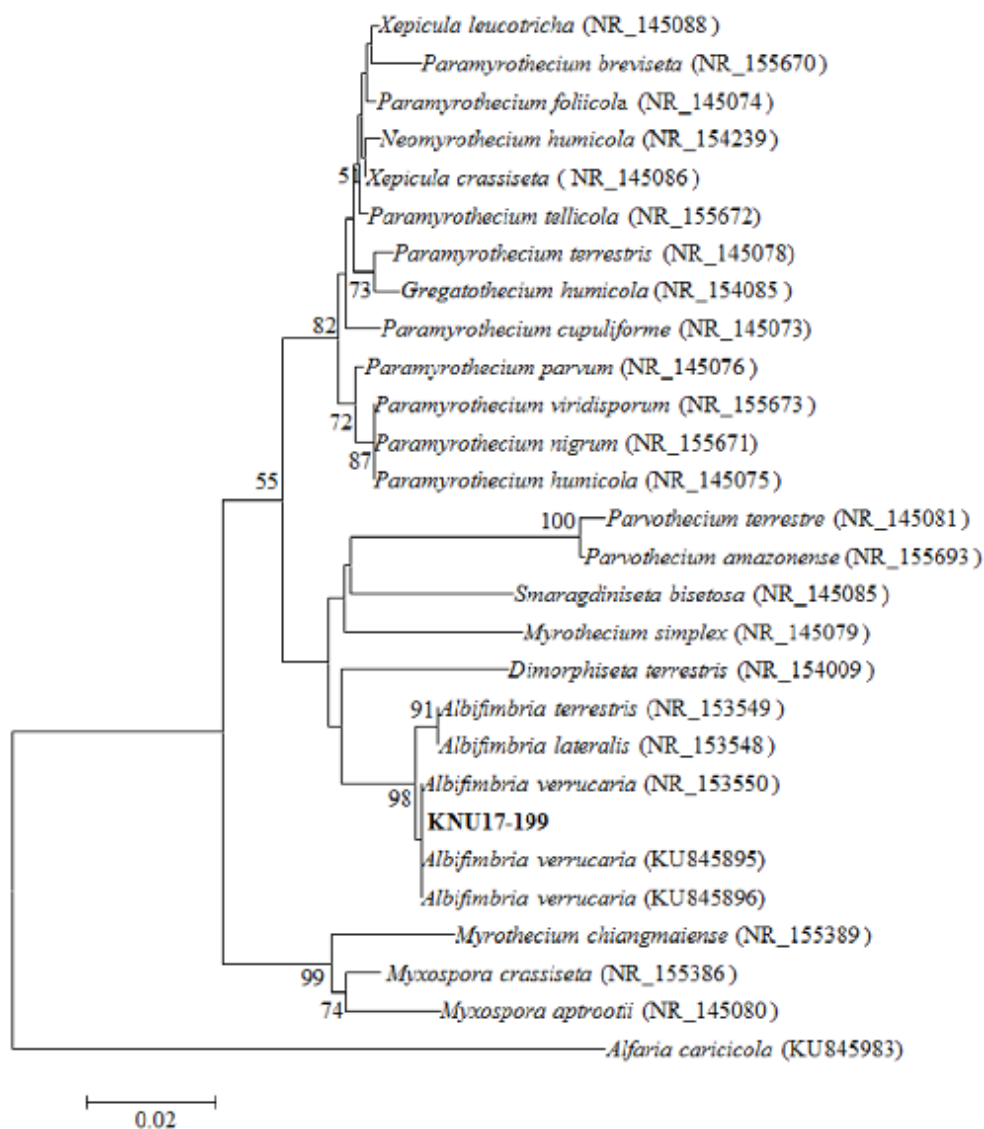


Fig 4. Neighbor-joining phylogenetic analysis of the partial 18S-ITS1-5.8S-ITS2-partial 28S rDNA sequences for *Albifimbria verrucaria* KNU17-199, obtained from field soil in Korea. Species examined in this study are in bold. Bootstrap scores of >60 are presented at the nodes. The scale bar represents the number of substitutions per sites.

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REFERENCES

1. Tulloch M. The genus *Myrothecium* Tode ex Fr. Mycol Papers 1972;130:1-42.
2. Lombard L, Houbraken J, Decock C, Samson RA, Meijer M, Reblova M, Groenewald JZ, Crous PW. Generic diversity in Stachybotriaceae. Personia 2016;36:156-246.
3. Lee SH, Park HS, Nguyen TTT, Lee HB. Characterization of three species of Sordariomycetes isolated from freshwater and soil samples in Korea. Mycobiology 2019;47:20-30.
4. Mortimer PH, Campbell J, Di Menna ME. Experimental myrotheciotoxicosis and poisoning in ruminants by verrucarins A and roridin A. Res Vet Sci 1971;12:508-15.
5. Martinovich D, Mortimer PH, Di Menna ME. Similarities between so called kikuyu poisoning of cattle and two experimental mycotoxicoses. NZ Vet J 1972;20:57-8.
6. Trapp SC, Hohn TM, McCormick S, Jarvis BB. Characterization of the gene cluster for biosynthesis of macrocyclic trichothecens in *Myrothecium roridum*. Mol Gen Genet 1998;257:421-32.
7. Abbas HK, Johnson BB, Shier WT. Phytotoxicity and mammalian cytotoxicity of macrocyclic trichothecene mycotoxins from *Myrothecium verrucaria*. Phytochemistry 2002;59:309-13.
8. Yang SM, Jong SC. Factors influencing pathogenicity of *Myrothecium verrucaria* isolated from *Eurphorbia esula* and species of Euphorbia. Plant Dis 1995;79:1036-8.
9. Boyette CD, Hoagland RE, Stelina KC. Biological control of the weed hemp sesbania (*Sesbania exaltata*) in rice (*Oryza sativa*) by the fungus *Myrothecium verrucaria*. Agronomy 2014;4:74-89.
10. Boyette CD, Hoagland RE, Weaver MA. Interaction of the bioherbicide *Myrothecium verrucaria* and glyphosphate for Kudzu control. Am J Plant Sci 2014;5:3943-56.
11. Weaver MA, Boyette CD, Hoagland RE. Management of kudzu by the bio herbicide, *Myrothecium verrucaria*, herbicides and integrated control programs. Biocontrol Sci Techn 2016;26:136-40.
12. Silva PS, Cortez VG, Silveira RMB. Synopsis of the Strophariaceae (Basidiomycota, Agaricales) from Floresta Nacional de Sao Francisco de Paula, Rio Grande do State, Brazil. Hoehnea 2012;39:479-87.
13. Guzman G. The genus *Psilocybe*. Beih Nova Hedw 1983;74:1-439.
14. Davet P, Rouxel F. Detection and Isolation of soil fungi. Enfield: Science Publishers; 2000.
15. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Snisky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 315-22.
16. Gilgado F, Cano J, Gene J, Guarro. Molecular phylogeny of the *Pseudallesheria boyddii* species complex: proposal of two new species. J Clin Microbiol 2005;43:4930-42.
17. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through weighing, position-specific gap penalties and weight

- matrix choice. Nucleic Acids Res 1994;22:4673-80.
18. Kimura M. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J Mol Evol 1980;16:111-20.
 19. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 2016;33:1870-4.
 20. Silva PS, Guzman DL, Silveira RMB. Cultural studies of *Psilocybe sensu lato* species (Agaricales, *Strophariaceae*). Mycosphere 2016;7:531-44.
 21. Kummer P. Der Führer in die Pilzkunde. Zerbst; 1871. p. 71.