RESEARCH ARTICLE

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

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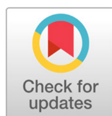
ABSTRACT

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, Basidomycota), 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 μgL^{-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, 20µL) 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 MicroconTM filters (Millipore, Burlingtong, 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.
Albifimbria lateralis	CBS 117712	NR_153548
Albifimbria terrestris	CBS 126186	NR_153549
Albifimbria verrucaria	CBS 962.95	KNU845895
Albifimbria verrucaria	CBS 328.52	NR_153550
Albifimbria verrucaria	CBS 121142	KU845896
Alfaria caricola	CBS 113567	KU845983
Alnicola pallidifolia	LIP PAM05082809	NR_153517
Alnicola spectabilis	LIP PAM05082811	NR_153516
Anamika indica	IB 19971305	NR_119451
Anamika lactariolens	LAU 88/95	NR_119524
Cortinarius balteatoalbus	PC R. Henry 82.98	NR_157924
Cortinarius chromataphilus	PC R. Henry 86.90	NR_157924
Descolea inferna	CORD MES1315	NR_154032
Dimorphiseta terrestris	CBS 127345	NR_154009
Gregatothecium humicola	CBS 205.96	NR_154085
Hebeloma aurantioumbrinum	BR BR-MYCO 173985-64	NR_152902
Hebeloma angustisporium	TENN 023364	NR_119890
Hebeloma brunneifolium	TENN 029408	NR_119829
Hebeloma griseopruinatum	BR 5020169128570	NR_120099
Hebeloma matritense	BR MYCO 174910-19	NR_152905
Hebeloma nothofagetorum	HO 553022	NR_152886
Hebeloma perexiguum	BR BR-MYCO 173979-58	NR_137917
Hebeloma pseudofragilipes	BR MYCO 174911-20	NR_152906
Hebeloma vesterholtii	BR 5020166528762	NR_119724
Hymenogaster raphanodorus	SFSU F-000540	NR_119531
Hebeloma subvictoriens	MEL 2331640	NR_152885
Macrolepiota deters	HKAS 55306	NR_119832
Myxospora aptrootii	CBS 101263	NR_145080
Myrospora crassiseta	CBS 731.83	NR_155386
Myrothecium chiangmaiense	MFLUCC 11-0506	NR_155389
Myrothecium simplex	CBS 582.93	NR_145079
Pachhylepyrium carbonicola	MICH 5270	NR_119906
Parvothecium amazonense	MUCL 54664	NR_155693
Parvothecium terrestre	CB 198.89	NR_145081
Paramyrothecium terrestris	CBS 564.86	NR_145078
Paramyrothecium tellicola	CBS 478.91	NR_155672
Paramyrothecium foliicola	CBS 113121	NR_145074
Paramyrothecium breviseta	CBS 544.75	NR_155670
Paramyrothecium cupuliforme	CBS 127789	NR_145073
Paramyrothecium parvum	CBS 257.35	NR_145076
Paramyrothecium nigrum	CBS 116537	NR_155671
Paramyrothecium humicola	CBS 127295	NR_145075
Paramyrothecium viridisporum	CBS 873.85	NR_155673
Pholiota abieticola	TENN 014000	NR_119907
Pholiota caespitosa	TENN 015908	NR_119908
Pholiata virescentifolia	TENN 020591	NR_119911
Psathyloma cartervatim	PDD 107742	NR_154327
Psathyloma leucocarpum	PDD 105593	NR_154328
Psilocybe allenii	PRM 899876	NR_119821
Psilocybe coprophila	PRM 899778	JX 235960
Psilocybe cyanescens	PRM 901481	NR_119478
Smaragdiniseta bisetosa	CBS 459.82	NR_145085
Xepicula leucotricha	CBS 256.57	NR_145088
Xepicula crassiseta	CBS 392.71	NR_145086

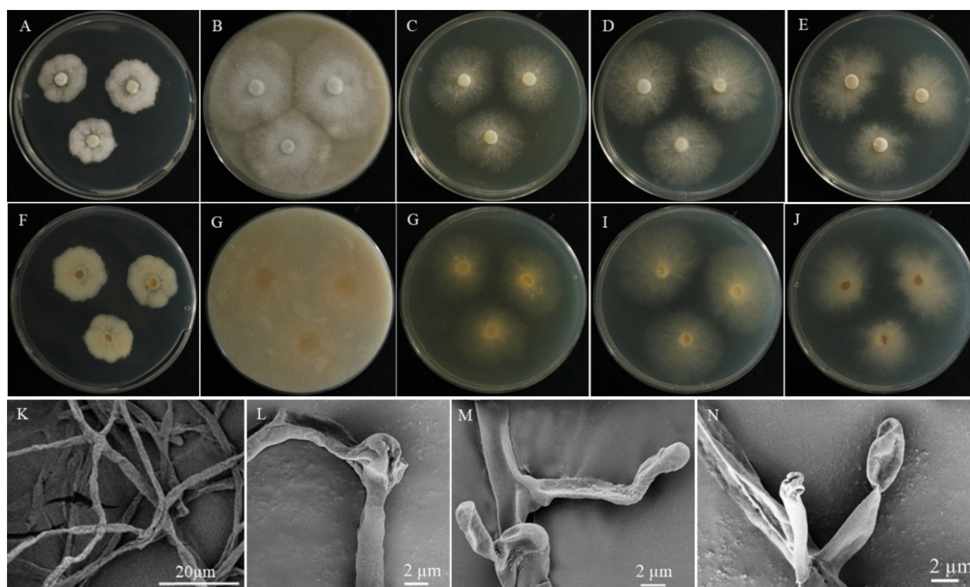

Fig. 1. Morphological characteristics of *Deconica coprophila* (KNU17-1) grown for 7 days at 26°C on potato dextrose agar (PDA), oatmeal agar (OMA), Czapek yeast extract agar (CYEA), yeast extract sucrose agar (YESA), and malt extract agar (MEA). (A-E) Observe colony from left to right. (F-J) Reverse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

[a]Silva 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].

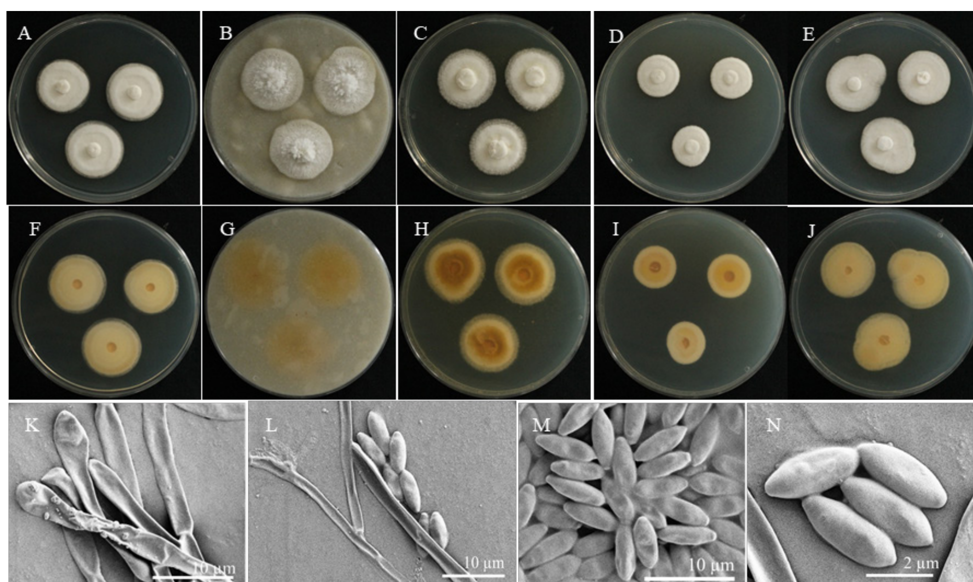

Fig 2. Morphological characteristics of *Albifimbria verrucaria* (KNU17-199) grown for 7 days at 25°C on potato dextrose agar (PDA), oatmeal agar (OMA), Czapek yeast extract agar (CYEA), yeast extract sucrose agar (YESA), and malt extract agar (MEA). (A-E) Observe colony from left to right. (F-J) Reverse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

[a]Lombard et al. 2016 [2].
N/A: Not available

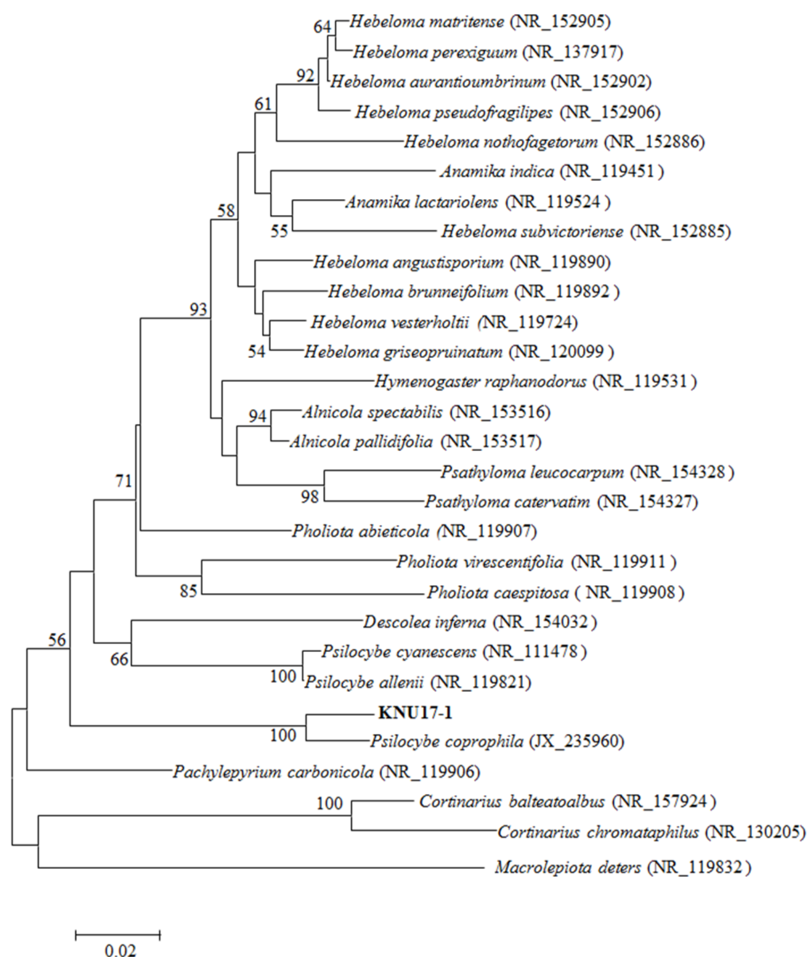

Fig 3. Neighbor-joining phylogenetic analysis of the partial 18S-ITS1-5.8S-ITS2-partial 28S rDNA sequences for *Psilocybe coprophila* KNU17-1, obtained from field soil in Korea. Species examined in this study are in bold. Bootstrap scores of >60 are presented at the nodes. The scale bar represents the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

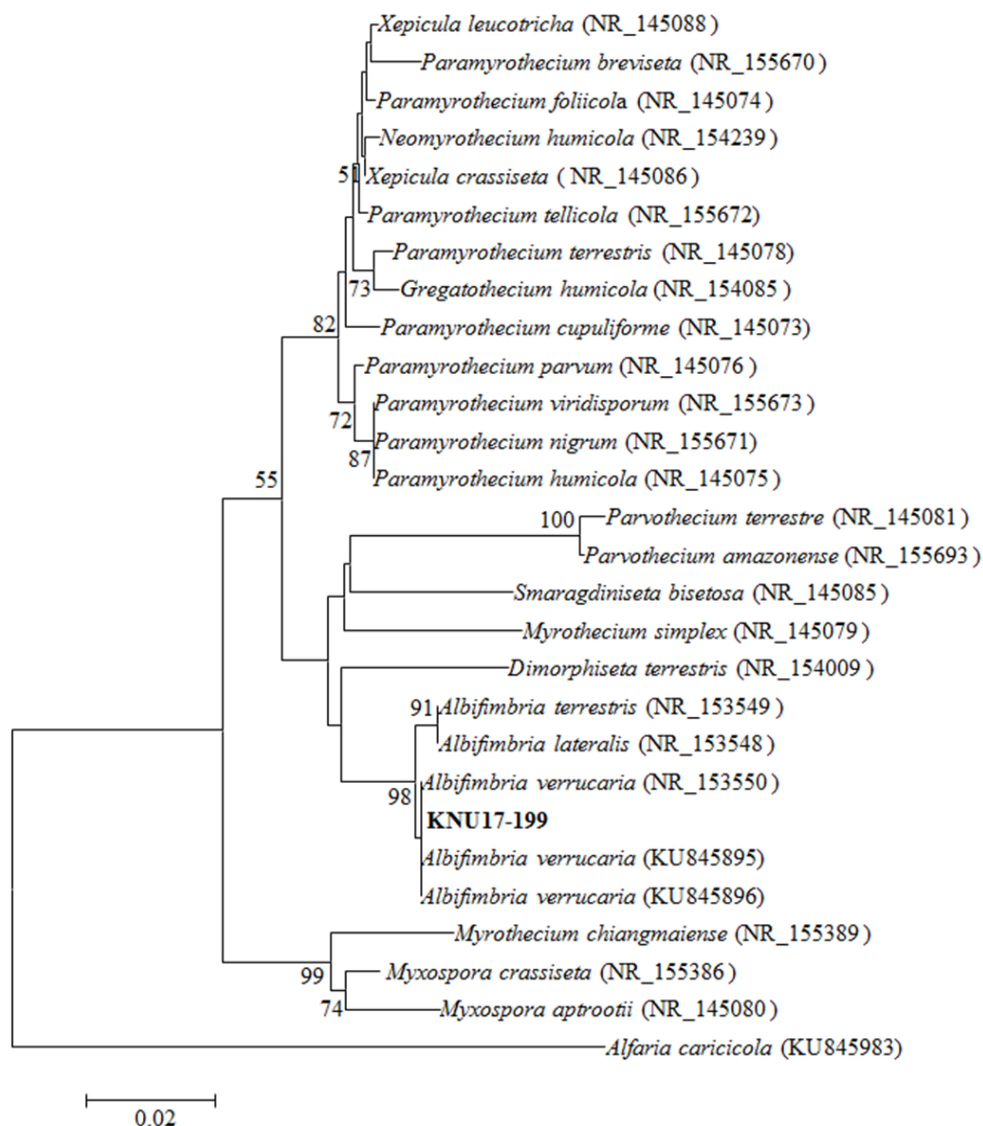

Fig 4. Neighbor-joining phylogenetic analysis of the partial 18S-ITS1-5.8S-ITS2-partial 28S rDNA sequences for *Albifimbria verrucaria* KNU17-199, obtained from field soil in Korea. Species examined in this study are in bold. Bootstrap scores of >60 are presented at the nodes. The scale bar represents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

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