The Korean Journal of Mycology www.kjmycology.or.kr

RESEARCH ARTICLE

Three Unrecorded Fungal Species from Fecal and Freshwater Samples in Korea

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Abstract

While evaluating fungal diversity in fecal and freshwater samples in Korea, three fungal strains, CNUFC-GHD83-1, CNUFC-RD8126, and CNUFC-YR2-1, were isolated from specific habitats including grasshopper and rat feces, and freshwater samples in Korea. On the basis of the morphological characteristics and phylogenetic analysis of the internal transcribed spacer (ITS) and 28S rDNA, the isolates CNUFC-GHD83-1, CNUFC-RD8126, and CNUFC-YR2-1 were identified as *Albifimbria terrestris*, *Cephaliophora tropica*, and *Mariannaea aquaticola*, respectively. These species have not been previously described in Korea.

Keywords: *Albifimbria terrestris*, *Cephaliophora tropica*, *Mariannaea aquaticola*, Specific habitat, Taxonomy

Introduction

Fungi are generally found in aerobic ecosystem, and colonize diverse substrates and play a wide diversity of roles. Many species are cosmopolitan but others are found only in restricted or specific niches or habitats [1]. While evaluating fungal diversity in unusual fungal niches, feces and freshwater have been considered for isolation of rare fungi with specific habitats in Korea [2, 3].

In fece ecosystems, fungi play an important role in the biodegradation of organic materials and the return of nutrients to the environment for reuse [4]. A number of studies on the diversity of fungi have been carried out using different animal dung substrates [2, 4, 5]. However, a few studies have been done using the feces from insect or rat. Stejskal et al. [6] reported that 35 species belonging to 11 genera such as *Alternaria*, *Arthrinium*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Eurotium*, *Geotrichum*, *Microascus*, *Mucor*, *Penicillium*, and *Thamnidium* were isolated from house mouse using dilution plate method. Nyberg and Persson [7] observed 24 species of 14 genera in mouse dung. One study of gut microflora of grasshopper (*Melanoplus sanguinipes*) has been reported, but there was no detailed information on the fungal communities [8]. In Korea, two new species (*Absidia*

OPEN ACCESS

Kor. J. Mycol. 2017 December, 45(4): 304-318
https://doi.org/10.4489/KJM.20170033

pISSN : 0253-651X
eISSN : 2383-5249

Received: 17 November, 2017
Revised: 27 November, 2017
Accepted: 27 November, 2017

stercoraria and *Mucor stercorarius*) and two new records (*Absidia glauca* and *Paecilomyces variotii*) have been currently reported from rat feces [9-12].

In freshwater ecosystems, fungi play a major role in the decomposition of complex organic compounds thus providing nutrients for other aquatic organisms. Based on a recent literature, there are approximately 622 species (170 genera) of Ascomycetes; more than 531 species of Hyphomycetes (55 genera); and species of Trichomycetes (3 orders, no longer regarded as fungi) among freshwater fungi; however, there is little information on freshwater fungi belonging to Basidiomycetes and Zygomycetes [3]. Intensive studies on the fungal diversity from various habitats including freshwater have been conducted in Korea. Despite intensive studies in freshwater habitats, our knowledge of the diversity of freshwater-derived fungi in general and freshwater Ascomycetes in particular is still lacking compared to that of terrestrial habitats [13].

Pezizomycotina is the largest subphylum of Ascomycetes. In the current classification, Pezizomycotina is divided into 11 classes based on rDNA phylogenies. Especially, the Pezizomycotina contains species which are important to both industry and agriculture. The most well-known order is Hypocreales.

The genus *Albifimbria* L. Lombard & Crous 2016 belongs to the subphylum Pezizomycotina, order Hypocreales, family Stachybotryaceae. It is characterized by the formation of verrucose setae surrounding the sporodochia and conidia with funnel-shaped, mucoid appendages. *Albifimbria* species are found in the soil, leaves, cotton, fruit, and air [14]. To date, *Albifimbria* includes only 4 species, i.e., *A. lateralis*, *A. terrestris*, *A. verrucaria*, and *A. viridis*.

The genus *Cephaliophora*, which belongs to the subphylum Pezizomycotina, order Pezizales, family Ascodesmidaceae, was established by Thaxter in 1903; it includes two coprophilous species, *Cephaliophora tropica* (type species) and *C. irregularis* [15]. According to available data from Index Fungorum, 7 species belonging to this genus are known. Members of this genus are characterized by the production of conidia synchronously from a swollen ampulla arising directly from vegetative hyphae and from a short lateral branch [16]. Species in this genus are regarded as nonpathogenic to humans, but can cause infectious keratitis [17, 18]. *C. tropica* is found as soil saprophytes and dominates subtropical and tropical areas [19]. Published literature has revealed the occurrence of *C. tropica* primarily in countries of the Asia continent, except South Korea [20].

The genus *Mariannaea* G. Arnaud ex Samson, which belongs to the subphylum Pezizomycotina, order Hypocreales, family Nectriaceae, was established by Samson, with *Mariannaea* (*M.*) *elegans* (Corda) Samson as type species [21]. The first and only teleomorph connection of *Mariannaea* to *Nectria* was introduced by Samuels and Seifert [22] in 1991. Species belonging to the genus *Mariannaea* have been frequently isolated from wood, bark, submerged wood in freshwater streams, insects, soil, and diseased roots [21-24]. To date, 15 species of *Mariannaea* have been described according to Index

Fungorum (www.indexfungorum.org). Numerous species have been reported from freshwater habitats, but there are very few reports from Korea [25]. A recent study reported *M. elegans* and *M. samuelsii* in beetles [26, 27].

During an inventory of fungal species from feces and freshwater samples, three interesting fungal strains belonging to the subphylum Pezizomycotina were assigned to the genera *Albifimbria*, *Cephaliophora*, and *Mariannaea*.

The objective of the present study was to morphologically and molecularly characterize three unrecorded species in Korea: *Albifimbria terrestris*, *Cephaliophora tropica*, and *Mariannaea aquaticola*.

Materials and Methods

Isolation of fungal strains from fecal samples

In September 2016, grasshoppers were collected at the CNU Arboretum located in Chonnam National University, Gwangju, Korea. Samples were transferred to the laboratory. After 6 hr, feces were collected. Rat fecal samples were collected from a garden at Chonnam National University, Gwangju, Korea using sterile forceps and transferred to the laboratory in plastic bags. The feces of grasshoppers and rats were placed on water agar (20 g of agar, 1 L of deionized water) and incubated at 25°C for 3~7 days. Hyphal tips were transferred to potato dextrose agar (PDA) plates using the tips of heat-stretched capillary tubes under a stereomicroscope.

Freshwater samples were collected from Yeongsangang River located in Gwangju Korea in Feb 2016. These samples were transferred in sterile 50 mL conical tubes, and stored at 4°C until examination. Fungi were isolated by a serial dilution plating method. In this technique, 1 mL water was mixed with 9 mL of sterile distilled water and the solution was shaken for 15 min at room temperature, and serial dilutions were made ranging from 10^{-1} to 10^{-4}. An aliquot of 0.1 mL from each dilution was transferred to PDA and incubated at 25°C for 3~7 days.

To isolate pure cultures, individual colonies with various morphologies were transferred to PDA plates. Pure isolates were maintained in PDA slant tubes and stored in 20% glycerol at -80°C at the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, Korea.

Morphological studies

For detailed morphological analyses, the CNUFC-GHD83-1, CNUFC-RD8126, and CNUFC-YR2-1 strains were cultured on PDA (Becton Dickinson, Sparks, MD, USA), corn meal agar (20 g of corn meal extract and 15 g of agar in 1 L of deionized water), oat meal agar (30 g of oat meal and 15 g of agar in 1 L of deionized water). The plates were incubated

at 25°C in the dark for 7 days. Samples were mounted in lactophenol solution (Junsei Chemical, Tokyo, Japan) and observed under an Olympus BX51 microscope with DIC optics (Olympus, Tokyo, Japan).

DNA extraction, PCR, and sequencing

Genomic DNA was extracted directly from the mycelia of fungal isolates using the Solg Genomic DNA Prep Kit (SolGent, Daejeon, Korea). The internal transcribed spacer (ITS) region and large subunit of 28S rDNA were amplified with the primer pairs ITS4 and ITS5 [28] and LROR and LR5F [29], respectively. The PCR amplification mixture (total volume, 20 μL) contained fungal DNA template, 5 pmol/μL each primer, and Accupower PCR Premix (Taq DNA polymerase, dNTPs, buffer, and a tracking dye; Bioneer, Daejeon, Korea). PCR products were purified using the AccuPrep PCR Purification Kit (Bioneer) according to the manufacturer's instructions. DNA sequencing was performed using an ABI 3700 Automated DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analysis

The fungal sequences obtained from the GenBank database were aligned using Clustal_X v.1.83 [30] and edited using BioEdit v.5.0.9.1 [31]. Phylogenetic analyses were performed using MEGA 6 [32] with maximum likelihood (ML) and Kimura's two-parameter correction method. *Myxospora* sp., *Pseudopithyella minuscula*, and *Stachybotrys chartarum* were used as outgroups. The reliability of internal branches was assessed using the p-distance substitution model with 1,000 bootstrap replications. The CNUFC-GHD83-1, CNUFC-GHD83-2, CNUFC-RD8125, CNUFC-RD8126, CNUFC-YR2-1, and CNUFC-YR2-2 sequences were deposited in the NCBI database under accession numbers as shown in Table 1.

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

Taxon name	Collection no. (Isolate no.)	GenBank accession no.	
		ITS	28S
Albifimbria lateralis	CBS117712^{T}	KU845881	KU845900
A. terrestris	CBS127838	KU845884	KU845903
A. terrestris	CBS126186^{T}	KU845883	KU845902
A. terrestris	CBS109378	KU845882	KU845901
A. terrestris	**CNUFC-GHD83-1**	**MG458216**	**MG458206**
A. terrestris	**CNUFC-GHD83-2**	**MG458217**	**MG458207**
A. verrucaria	CBS328.52^{T}	KU845893	KU845912

CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CNUFC, Chonnam National University Fungal Collection, Gwangju, South Korea; ITS, internal transcribed spacer; T, ex-type strain. Bold letters indicate isolates and accession numbers determined in our study.

Table 1. (Continued)

Taxon name	Collection no. (Isolate no.)	GenBank accession no.	
		ITS	28S
A. verrucaria	CBS962.95	KU845895	KU845914
A. viridis	CBS244.78	KU845897	KU845916
A. viridis	CBS449.71^{T}	KU845898	KU845917
A. viridis	CBS127346	KU845899	KU845918
Ascodesmis nigricans	CBS428.91		KC012665
Ascodesmis nigricans	CBS389.68		DQ168335
Cephaliophora tropica	CBS133.33		KC012669
Cephaliophora irregularis	CBS218.62		KC012668
Cephaliophora tropica	**CNUFC-RD8125**	**MG458219**	**MG458209**
Cephaliophora tropica	**CNUFC-RD8126**	**MG458218**	**MG458208**
Coprotus ochraceus	JHP-06.121		KC012673
Dimorphiseta terrestris	CBS127345^{T}	KU846314	KU846346
Eleutherascus lectardii	CBS626.71		DQ168334
Eleutherascus peruvianus	CBS101.75		DQ220330
Geopyxis vulcanalis	KH.04.37		KC012680
Glaziella aurantiaca	PR 6376		KC012681
Inaequalispora prestonii	CBS175.73^{T}	KU846316	KU846348
Ilyonectria capensis	CBS132815^{T}	JX231151	KM515908
Ilyonectria destructans	CBS264.65	AY677273	KM515927
Lasiobolidium orbiculoides	CBS344.73		DQ062995
Lasiobolus papillatus	KH.08.30		KC012687
Mariannaea aquaticola	MFU090225	GQ153838	GQ153837
M. aquaticola	MFU090223^{T}	GQ153834	GQ153833
M. aquaticola	MFU090224	GQ153836	GQ153835
M. aquaticola	**CNUFC-YR2-1**	**MG459018**	**MG459016**
M. aquaticola	**CNUFC-YR2-2**	**MG459019**	**MG459017**
M. camptospora	CBS209.73^{T}	AY624202	AY554241
M. catenulata	CBS491.62^{T}	KM231752	KM232009
M. chlamydospora	CGMCC3.17273^{T}	KX986134	KX986141
M. cinerea	CGMCC3.17274^{T}	KX986135	KX986142
M. clavispora	CBS149.87^{T}	KX986131	KX986138
M. dimorpha	HMAS266564^{T}	KF767353	KJ002443
M. elegans	CBS217.73A^{T}	KX986132	KX986139
M. elegans	DUCC400	JQ690354	
M. fusiformis	CGMCC3.17272^{T}	KX986133	KX986140
M. humicola	CBS740.95^{T}	KM231755	KM231619
M. lignicola	CGMCC3.17275^{T}	KX986136	KX986143

CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CNUFC, Chonnam National University Fungal Collection, Gwangju, South Korea; ITS, internal transcribed spacer; T, ex-type strain. Bold letters indicate isolates and accession numbers determined in our study.

Table 1. (Continued)

Taxon name	Collection no. (Isolate no.)	GenBank accession no.	
		ITS	28S
M. macrochlamydospora	FKI-4735^T	AB855777	AB855782
M. pinicola	CBS745.88^T	KM231754	AY554242
M. punicea	CBS239.56	AY624201	JF415981
M. samuelsii	CBS746.88^T	KM231757	KM231621
M. samuelsii	DUCC401	JX125048	
M. superimposita	CBS113472	AB855780	AB855785
Myxospora sp.	CBS100347	KU846465	KU846486
Nectria cinnabarina	AR4477^T	HM484548	HM484562
Parvothecium terrestre	CBS198.89^T	KU846468	KU846489
Peethambara sundara	CBS646.77^T	KU846471	KU846492
Peethambara sundara	CBS521.96	KU846470	KU846491
Pseudombrophila theioleuca	DHP3498		KC012696
Pseudopithyella minuscula	mh675		AY945849
Pulvinula constellatio	KH.03.64		DQ062987
Pulvinula convexella	KH.01.020		DQ062986
Pulvinula globifera	DHP DR-104		DQ220393
Smaragdiniseta bisetosa	CBS459.82	KU847229	KU847255
Stachybotrys chartarum	CBS129.13	KM231858	KM231738
Tarzetta catinus	KS.94.10A		DQ062984
Tarzetta pusilla	KH.03.66		DQ062983
Virgatospora echinofibrosa	CBS110115	KU847244	KU847270
Virgatospora echinofibrosa	MUCL39092	KU847245	KU847271

CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CNUFC, Chonnam National University Fungal Collection, Gwangju, South Korea; ITS, internal transcribed spacer; T, ex-type strain. Bold letters indicate isolates and accession numbers determined in our study.

Results

Phylogenetic analysis

A basic local alignment search tool (BLAST) search of ITS sequences against the NCBI database indicated that CNUFC-GHD83-1, CNUFC-RD8126, and CNUFC-YR2-1 sequences were most similar to sequences from *Albifimbria terrestris* (GenBank accession no. KU845884), *Cephaliophora tropica* (FJ792583), and *Mariannaea aquaticola* (KT224837) with 98.4% (570/579 bp), 99.3% (549/553 bp), and 99.2% (518/522 bp) homology, respectively. On the basis of the 28S rDNA sequence analysis, the three strains had homologies of 99.1% (819/826 bp), 99.7% (873/876 bp), and 99.9% (839/840 bp) with the sequences of *A. terrestris* (KU845902), *C. tropica* (KC012669), and *M. aquaticola* (GQ153837), respectively. On the basis of the ITS and 28S sequence analysis, the isolate

CNUFC-GHD83-1 was identical to *Albifimbria terrestris* (Fig. 1). CNUFC-RD8126 was identical to *Cephaliophora tropica* based on 28S sequences analysis (Fig. 2). CNUFC-YR2-1 was identical to *Mariannaea aquaticola* based on combined ITS and 28S sequences (Fig. 3).

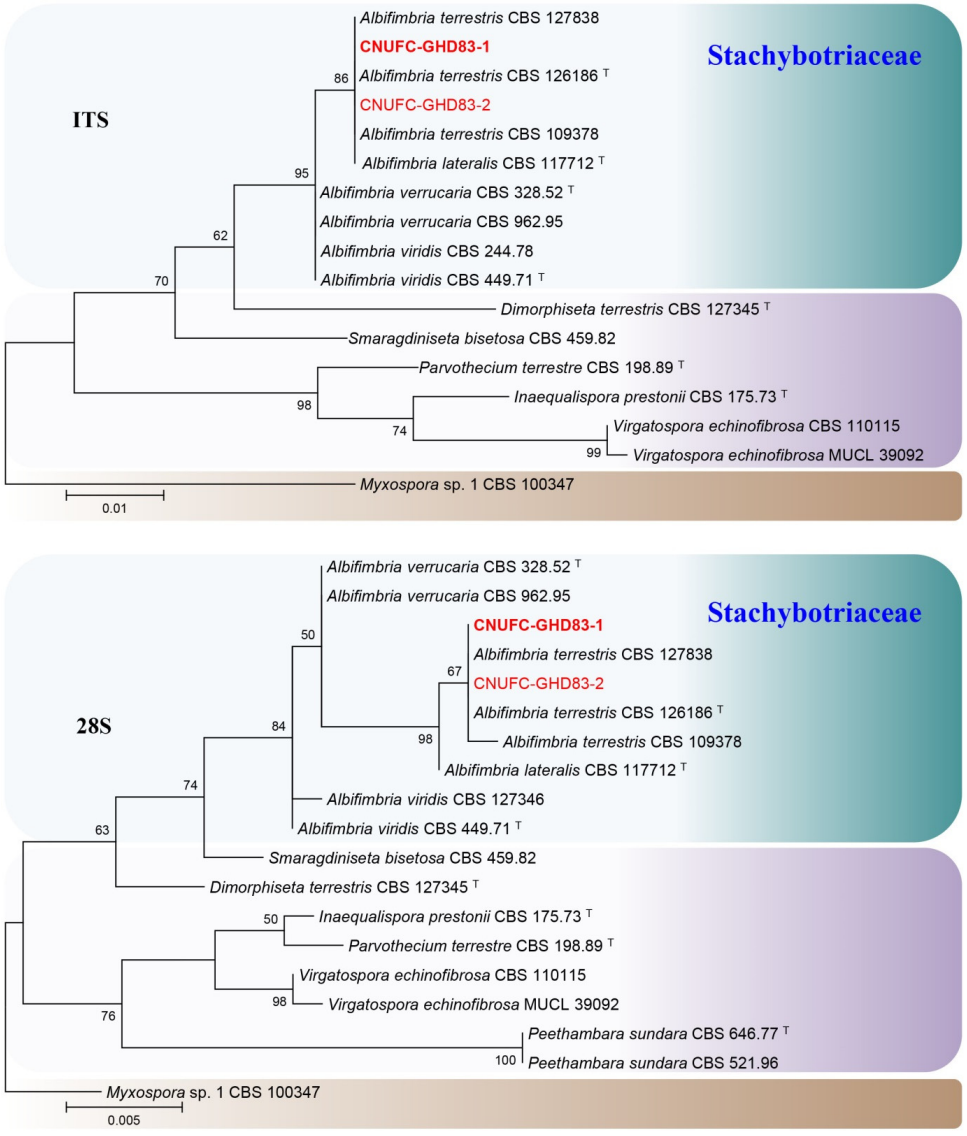

Fig. 1. Phylogenetic trees based on maximum likelihood analysis of the internal transcribed spacer (ITS) and 28S rDNA sequences for *Albifimbria terrestris* CNUFC-GHD83-1 and *A. terrestris* CNUFC-GHD83-2. *Myxospora* sp. was used as an outgroup. Bootstrap support values of ≥50% are indicated at the nodes. The bar indicates the number of substitutions per position.

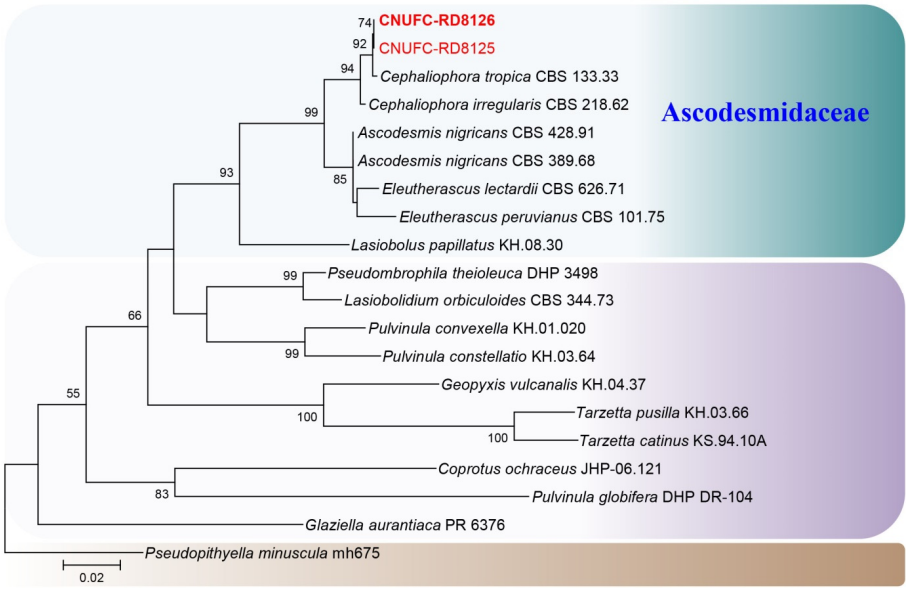

Fig. 2. Phylogenetic tree based on maximum likelihood analysis of 28S rDNA sequences for *Cephaliophora tropica* CNUFC-RD8125 and *C. tropica* CNUFC-RD8126. *Pseudopithyella minuscula* was used as an outgroup. Bootstrap support values of ≥50% are indicated at the nodes. The bar indicates the number of substitutions per position.

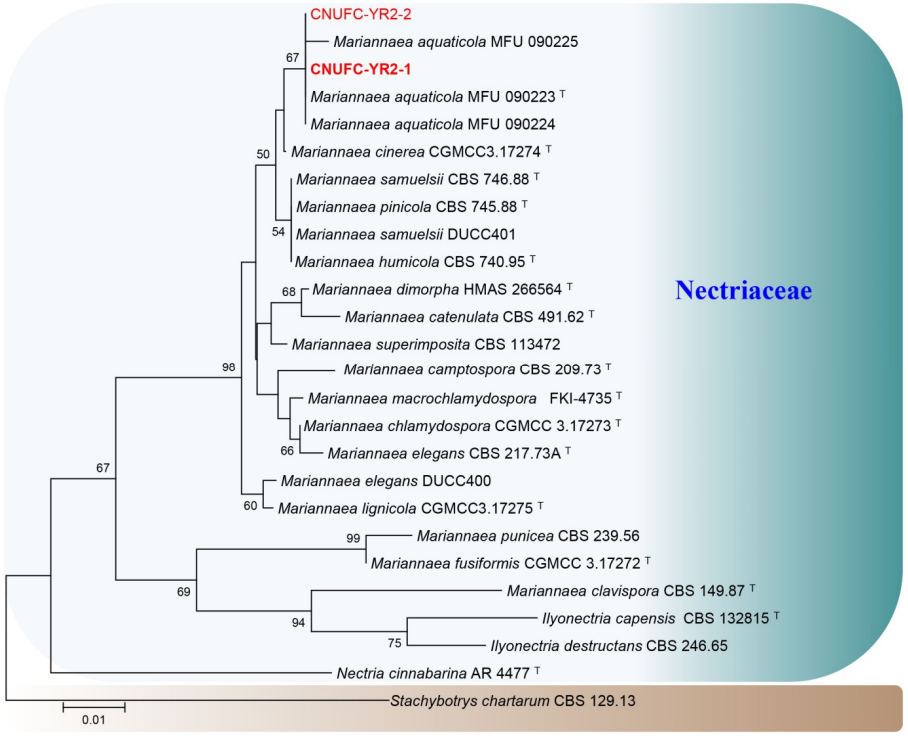

Fig. 3. Phylogenetic tree based on maximum likelihood analysis of the combined internal transcribed spacer and 28S rDNA sequences for *Mariannaea aquaticola* CNUFC-YR2-1 and *M. aquaticola* CNUFC-YR2-2. *Stachybotrys chartarum* was used as an outgroup. Bootstrap support values of ≥50% are indicated at the nodes. The bar indicates the number of substitutions per position.

Taxonomy of CNUFC-GHD83-1

Albifimbria terrestris L. Lombard & Crous, Persoonia 36: 177 (2016) (Table 2, Fig. 4).

Description: Colonies of the strain grew moderately on PDA, cotton in the center with a white margin, reaching 30~33 mm in diameter at 25°C after 7 days of incubation. Conidiogenous cells were phialidic, cylindrical, and measured 8.0~14.5 × 1.9~3.1 µm. Conidia were ellipsoidal and measured 5.6~7.2 µm × 2.2~3.5 µm.

Table 2. Morphological characteristics of CNUFC-GHD83-1 and *Albifimbria terrestris* on PDA medium at 25°C

Characteristics	Present isolate	*Albifimbria terrestris*[a]
Colony	moderate growth, cotton in the center, with a white margin	-
Conidiogenous cells	phialidic, cylindrical, and 8.0~14.5 × 1.9~3.1 µm	phialidic, cylindrical, and 5~11 × 1~3 µm
Conidia	ellipsoidal and 5.6~7.2 µm × 2.2~3.5 µm	ellipsoidal to limoniform, straight, and (4~)5~7 × 2~3 µm (av. 6 × 2 µm)

[a]From the description by Lombard et al. [14].
PDA, potato dextrose agar.

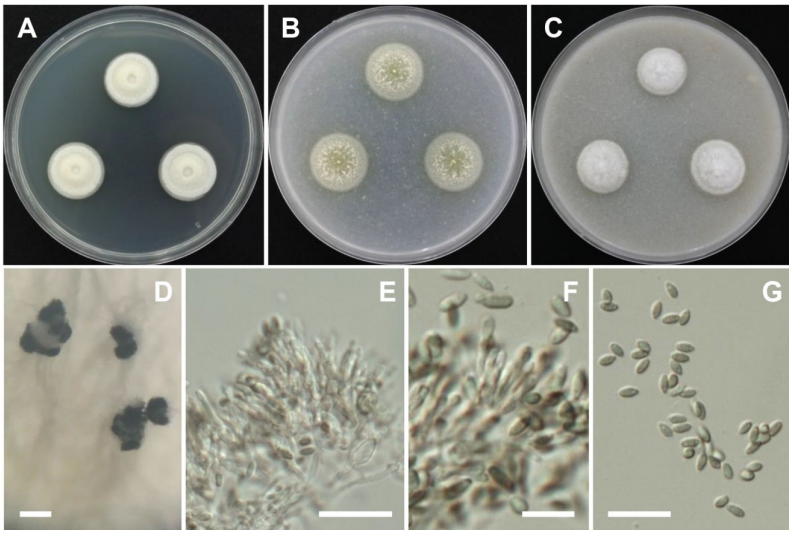

Fig. 4. Morphology of *Albifimbria terrestris* CNUFC-GHD83-1. A, Colonies on potato dextrose agar; B, Colonies on oat meal agar; C, Colonies on corn meal agar; D, Scattered aggregated conidiomata (observed under stereo microscope); E, Sporodochial conidiomata; F, Conidia on conidiogenous cells; G, Conidia (scale bars: D = 200 µm, E~G = 20 µm).

Taxonomy of CNUFC-RD8126

Cephaliophora tropica Thaxt., Botanical Gazette Crawfordsville 35: 157 (1903) (Table 3, Fig. 5).

Table 3. Morphological characteristics of CNUFC-RD8126 and *Cephaliophora tropica* on PDA medium at 25°C

Characteristics	Present isolate	*Cephaliophora tropica*[a]
Colony	rapid-growing, golden brown, reverse reddish brown	rapid-growing, brown
Conidiogenous cells	subspherical or clavate and 16.1~27.8 × 13.2~18.3 µm	clavate, 20~30 µm in diameter
Conidia	cylindrical, or slightly clavate, 1~4 septate, golden brown, and 27.7~43.8 × 15.5~20.8 µm	subcylindrical, 2~5 septate, pale chocolate-brown, and 50 × 19~20 µm

[a]From the description by Thaxter [15].
PDA, potato dextrose agar.

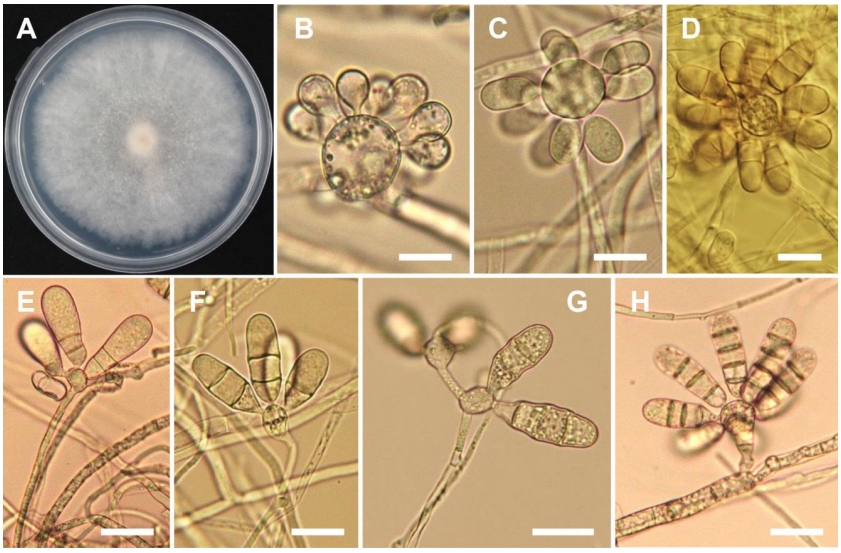

Fig. 5. Morphology of *Cephaliophora tropica* CNUFC-RD8126. A, Colony on potato dextrose agar; B~H, Subspherical conidiogenous cells and septate or aseptate conidia (scale bars: B~H = 20 µm).

Description: Colonies of the strain grew rapidly on PDA and were golden brown, filling a petri dish in 4 days at 25°C. The reverse colony was reddish-brown. Conidiogenous cells were subspherical to clavate, and measured 16.1~27.8 × 13.2~18.3 µm. Conidia were golden brown, 1~4 septate, cylindrical or slightly clavate, and measured 27.7~43.8 × 15.5~20.8 µm.

Taxonomy of CNUFC-YR2-1

Mariannaea aquaticola Kurniawati, L. Cai & K.D. Hyde, Mycological Progress 9 (3): 338 (2010) (Table 4, Fig. 6).

Table 4. Morphological characteristics of CNUFC-YR2-1 and *Mariannaea aquaticola* PDA medium at 25°C

Characteristics	Present isolate	*Mariannaea aquaticola*[a]
Colony	moderate growth, pale yellowish when young, browning with age; reverse dark brown	moderate growth, pale yellowish, with age brown to dark brown; reverse brown
Phialides	flask-shaped, and measured 11.9~21.7 × 2.2~3.5 µm	flask-shaped, and measured 14~25 × 2~3 µm
Conidia	ellipsoidal to fusiform, and measured 4.5~9.5 × 2.5~4.6 µm	ellipsoidal to fusiform, and measured 5~10 × 2~4.5 µm
Chlamydospore	Absent	Absent

[a]From the description by Cai et al. [23]
PDA, potato dextrose agar.

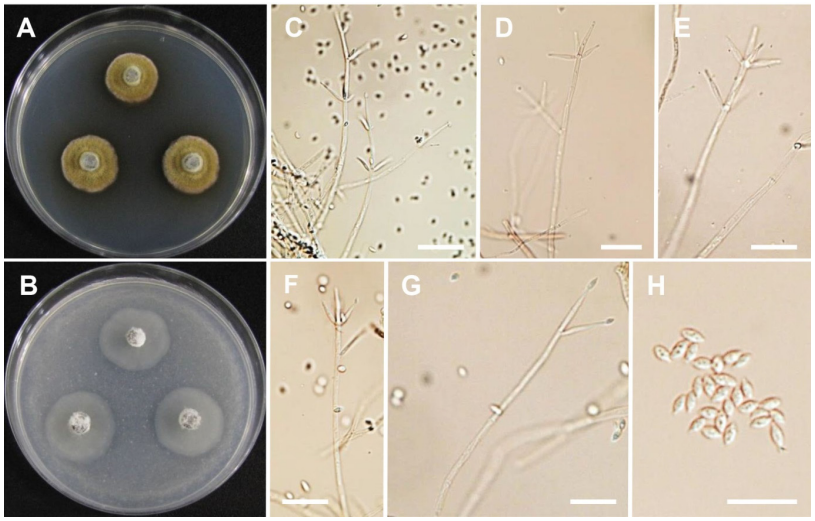

Fig. 6. Morphology of *Mariannaea aquaticola* CNUFC-YR2-1. A, Colonies on potato dextrose agar; B, Colonies on oat meal agar; C~G, Conidiophores and verticillate branches of phialides; H, Conidia (scale bars: C~H = 20 µm).

Description: Colonies of the strain grew moderately on PDA and were pale yellowish when young, while they became brown with age. Colonies reached 25~27 mm in diameter after 7 days of incubation at 25°C. Conidiophores were 2.75~5.2 µm wide, variable in length, erect, and bared short branches with whorls of 2~4 phialides. Phialides were flask-shaped, and measured 11.9~21.7 × 2.2~3.5 µm. Conidia were ellipsoidal to fusiform, and measured 4.5~9.5 × 2.5~4.6 µm. Chlamydospores were absent.

Discussion

Despite the wide intraspecific variation among some taxa, the rDNA ITS region is used as a critical barcode marker for the identification of fungi at the level of species. In the ITS and

LSU phylogenetic trees, CNUFC-GHD83-1 and CNUFC-GHD83-2 isolated from grasshopper fecal samples clustered in the clade containing *Albifimbria terrestris* CBS 126186 (type species) (Fig. 1). The morphological features of our isolates were in line with the description of *A. terrestris* by Lombard et al. [14]. The properties of *A. terrestris*, including the shape and size of the sporangiospores and conidiogenous cells, were similar to those of *A. verrucaria* and *A. viridis,* but differed slightly with respect to the size of conidia. Furthermore, in the phylogenetic tree, the strain formed a separate branch from *A. verrucaria* and *A. viridis*. Molecular data confirmed the morphological identification of CNUFC-GHD83-1 as *A. terrestris*. Lombard et al. [14] have reported the isolation of this species from soil. Accordingly, this is the first isolation of *A. terrestris* from a grasshopper fecal sample.

In the phylogenetic tree based on multiple genes, the strains CNUFC-YR2-1 and CNUFC-YR2-2 formed a group with *M. aquaticola* MUF090223 (type species). The results of our molecular data analysis were consistent with the phylogeny presented by Hu et al. [33]. The morphological characteristics of *M. aquaticola* isolate studied were generally similar to those previously described by Cai et al. [23]. Species of *Mariannaea* produce extracellular enzymes such as amylase, β-glucosidase, protease, and cellulase, and thus can degrade cellobiase, starch and xylan [26, 27]. This finding suggests that strain CNUFC-YR2-1 is a potentially useful source for biotechnological applications and deserves further investigation.

The majority of sequences retrieved from NCBI related to the isolated species were fungal 28S gene sequences, rather than ITS rDNA sequences. Accordingly, a phylogeny was also constructed using the 28S sequence region, which has been used previously to study phylogenetic relationships among fungi [28]. Interestingly, extensive information was available for the three species isolated in our study based on morphological characteristics. Additional studies are needed to improve sequence-based identification.

Our analyses of 28S rDNA sequences showed that the strains CNUFC-RD8126 and CNUFC- RD8125 clustered with *Cephaliophora tropica* with a well-supported branch, in full agreement with previous studies of Hansen et al. [34]. The CNUFC-RD8126 isolate was morphologically most similar to *Cephaliophora tropica,* as described by Thaxter [15]. However, conidia were 1~4 septate, which was fewer than the 2~5 septate conidia for *Cephaliophora tropica* described by Thaxter (2~5). Michalska et al. [20] reported 2~3 septate conidia.

Dung constitutes protein, nitrogen, vitamins, growth factors, minerals, carbohydrates, and water (pH, ~6.5), with varying moisture contents, which makes it a suitable environment for fungal growth and diversity [35, 36]. Although grasshoppers and rats are common animals worldwide, no fungal isolates have been reported from dung samples of grasshoppers in the world. This is the first study using grasshopper feces as isolation source of fungi.

Freshwater fungi form a ubiquitous and diverse group of organisms that grow on substrates that are predominantly aquatic or semi-aquatic. In addition to providing nutrients for the freshwater ecosystem, they are involved in the degradation of animal wastes and products, such as fish scales and hair and insect exoskeletons, and certain species can be pathogenic to plants and animals [37]. A greater number of freshwater fungi are waiting to be discovered in Korea, which will require broader studies in wider ranges of microhabitats and substrates, and involving larger sample sizes.

Studies of fungal diversity in dung and freshwater are needed because of limited records worldwide, despite the wide distributions of these taxa. Our findings will serve as a useful reference for mycologists interested in fungal diversity in dung and freshwater ecosystems. The current search was limited to Korea, and we expect greater mycobiota diversity to be uncovered in future.

Acknowledgements

This work was supported by the Project on Survey and Discovery of Indigenous Fungal Species of Korea funded by NIBR, and by the Project on Discovery of Fungi from Freshwater and Collection of Fungarium funded by NNIBR of the Ministry of Environment (MOE), and in part by the research fund (PJ01091404) from the National Institute of Animal Science under Rural Development Administration, Republic of Korea.

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