MYCOBIOLOGY
2019, VOL. 47, NO. 1, 20–30
https://doi.org/10.1080/12298093.2019.1574372

RESEARCH ARTICLE

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Characterization of Three Species of Sordariomycetes Isolated from Freshwater and Soil Samples in Korea

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ABSTRACT

During a survey of fungal diversity in the class Sordariomycetes, 3 fungal strains, CNUFC-KMHY6-1, CNUFC-MSW24-2-11, and CNUFC-GW2S-4 were isolated from soil and freshwater samples, respectively in Korea. The strains were analyzed both morphologically and phylogenetically on the basis of internal transcribed spacer and RNA polymerase II second largest subunit gene sequences. On the basis of their morphology and phylogeny, CNUFC-KMHY6-1, CNUFC-MSW24-2-11, and CNUFC-GW2S-4 isolates were identified as *Arcopilus aureus*, *Memnoniella echinata*, and *Stachybotrys sansevieriae*, respectively. To the best of our knowledge, *Ar. aureus* and *M. echinata* have not been previously recorded in Korea, and this is the first report of *S. sansevieriae* from freshwater niche.

ARTICLE HISTORY
Received 7 August 2018
Revised 1 December 2018
Accepted 18 January 2019

KEYWORDS
Arcopilus aureus; *Memnoniella echinata*; *Stachybotrys sansevieriae*; Sordariomycetes

1. Introduction

Sordariomycetes is the second largest class of the division Ascomycota, and it is typically characterized by non-lichenized, perithecial (flask-shaped) ascomata and inoperculate unitunicate asci [1–4]. Members of Sordariomycetes are found in different niches including terrestrial and freshwater habitats [5–7]. Some species are pathogens and endophytes of various plants, whereas others cause diseases in arthropods and mammals [3,8,9]. Many species are saprobes involved in decomposition and nutrient cycling [10], and some species are fungicolous [11].

The classification of Sordariomycetes has changed significantly in the past few decades [12–16]. Recently, Maharachchikumbura et al. [3] introduced 3 new subclasses on the basis of the morphology and combined analysis of 28S large subunit rDNA (LSU), 18S small subunit rDNA (SSU), translation elongation factor 1-alpha gene (TEF), and RNA polymerase II second largest subunit (RPB2) sequence data. Another study by Maharachchikumbura et al. [4] reported that Sordariomycetes has 6 subclasses, Sordariomycetidae, Hypocreomycetidae, Xylariomycetidae, Meliolomycetidae, Diaporthomycetidae, and Lulworthiomycetidae, 32 orders; 105 families; and 1331 genera. However, Hongsanan et al. [17] provided an updated backbone tree for Sordariomycetes on the basis of LSU, SSU, TEF, and RPB2 sequence data. On the basis of the results, a new subclass was introduced, and the class Sordariomycetes was reported to comprise 6 subclasses, Sordariomycetidae, Hypocreomycetidae, Xylariomycetidae, Savoryellomycetidae, Diaporthomycetidae, and Lulworthiomycetidae; 28 orders; and 105 families.

The genus *Arcopilus* belongs to the class Sordariomycetes, subclass Sordariomycetidae, order Sordariales, and family Chaetomiaceae. The genus *Arcopilus* was established by Wang et al. [18] with the type species *Arcopilus aureus*. In 2016, Wang et al. [18] revised the family Chaetomiaceae on the basis of phylogenetic analyses of RPB2, *β*-tubulin, internal transcribed spacer (ITS), and LSU nrDNA sequences and morphological comparisons. They proposed 5 new genera, namely, *Amesia*, *Arcopilus*, *Collariella*, *Dichotomopilus*, and *Ovatospora*, and transferred some species of *Chaetomium* to the new genera. Species of the genus *Arcopilus* usually have arcuate ascomatal hairs and often exhibit a colorful colony because of the ascomata and exudates [18]. Currently, the genus *Arcopilus* is composed of 5 species – *Ar. aureus* (≡*Chaetomium aureum*) (Chivers) X. Wei Wang & Samson, *Ar. cupreus* (≡*C. cupreum*) (Ames) X. Wei Wang & Samson, *Ar. fusiformis* (≡*C. fusiforme*) (Chivers) X. Wei Wang & Samson, *Ar. flavigenus* (≡*C. flavigenum*) (van Warmelo) X. Wei Wang & Samson, and *Ar. turgidopilosus* (≡*C. turgidopilosum*) (Ames) X. Wei Wang & Samson.

The genera *Memnoniella* and *Stachybotrys* belong to the class Sordariomycetes, subclass Hypocreomycetidae, order Hypocreales, and family

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Stachybotryaceae. These 2 genera have similar morphological characteristics. The only difference between these 2 genera is that *Memnoniella* species have long and dry chains of conidia, whereas *Stachybotrys* species have single-celled conidia aggregated in slimy heads [19,20]. Some authors have considered this is insufficient to distinguish between these genera and have suggested that the 2 genera should be combined under the older name of *Stachybotrys* [21,22]. This is supported by the molecular results based on the ITS phylogenetic analysis of Haugland et al. [23]. However, Lombard et al. [24] performed multi-locus sequence analysis of the family Stachybotriaceae and showed that the *Memnoniella* species grouped in a well-supported clade distinct from the *Stachybotrys* clade. Currently, the genus *Memnoniella* is composed of 11 species, and more than 50 species of *Stachybotrys* are accepted.

To date, *Arcopilus* and *Memnoniella* species have not been described in Korea. The aim of the present study was to perform morphological and molecular analyses to characterize 3 ascomycetes species in Korea: *Ar. aureus*, *M. echinata*, and *S. sansevieriae*.

2. Materials and methods

2.1 Isolation of fungal strains from freshwater and soil samples

In 2017, freshwater samples were collected from the Wonhyo Valley located at Mudeung Mt., Gwangju, and Geum River located in Gongju, Korea. The samples were transferred to sterile 50-mL conical tubes (SPL Life Sciences Co., Pocheon, Korea) and stored at 4 °C until examination. In 2017, soil samples were collected from Geumgol Mountain located in Jindo Island, Korea. These samples were transported in sterile 50-mL Falcon tubes and stored at 4 °C, until examination. Fungi were isolated using the serial dilution plating technique. Briefly, 1 mL of water or 1 g of soil was mixed with 9 mL of sterile distilled water, shaken for 7–10 min, and serially diluted from 10^{-1} to 10^{-4}. An aliquot of 0.1 mL from each dilution was transferred to potato dextrose agar (PDA; 39 g of potato dextrose agar in 1 L of deionized water) and incubated at 25 °C for 3–7 days. Individual colonies of fungi that showed varying morphologies were selected and transferred to PDA plates.

All pure isolates, including *Ar. aureus*, *M. echinata*, and *S. sansevieriae* 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. *Ar. aureus*, *M. echinata*, and *S. sansevieriae* strains isolated in our study were designated as CNUFC-KMHY6-1 and CNUFC-KMHY6-2, CNUFC-MSW24-2-11 and CNUFC-MSW24-2-12, CNUFC-GW2S-4 and CNUFC-GW2S-5, respectively. CNUFC-KMHY6-1 was also deposited at the Collection of National Institute of Biological Resources (NIBR, Incheon, Korea); CNUFC-MSW24-2-11 and CNUFC-GW2S-4 were deposited at the Culture Collection of the Nakdonggang National Institute of Biological Resources (NNIBR, Sangju, Korea).

2.2. Morphological studies

For detailed morphological studies, CNUFC-MSW24-2-11 and CNUFC-GW2S-4 strains were cultured on PDA, corn meal agar (CMA; 2 g of cornmeal and 2 g of agar in 1 L of deionized water), and oatmeal agar (OA; 30 g of oatmeal and 20 g of agar in 1 L of deionized water). CNUFC-KMHY6-1 strain was cultured on PDA, OA, and potato carrot agar (PCA; 20 g of potato, 20 g of carrot, and 20 g of agar in 1 L of deionized water). The plates were incubated at 15, 25, and 35 °C in the dark for 14 days. The samples were mounted in distilled water or lactophenol solution (Junsei Chemical Co. Ltd., Tokyo, Japan) and observed using an Olympus BX51 microscope with differential interference contrast optics (Olympus, Tokyo, Japan).

2.3. DNA extraction, PCR, and sequencing

Genomic DNA was extracted directly from the mycelia of the fungal isolates by using the Solgent Genomic DNA prep Kit (Solgent Co. Ltd., Daejeon, Korea). The ITS region and RPB2 were amplified with the primer pairs ITS4 and ITS5 [25], and fRPB2-5F and fRPB2-7cR [26], respectively. The PCR amplification mixture (total volume, 20 µL) contained fungal DNA template, 5 pmol/µL of each primer, and Accupower PCR Premix (Bioneer Corp., Daejeon, Korea). The PCR products were purified using the Accuprep PCR Purification Kit (Bioneer Corp.), according to the manufacturer's instructions. DNA sequencing was performed with an ABI 3700 Automated DNA sequencer (Applied Biosystems Inc., Foster City, CA).

2.4. Phylogenetic analysis

The fungal sequences obtained from the GenBank database (Table 1) were aligned using Clustal_X v. 2.0 [27] and edited with Bioedit v. 7.2.5 software [28]. Phylogenetic analyses were performed using MEGA 6 software [29], and maximum likelihood was constructed by Kimura's two-parameter correction method. The reliability of internal branches was assessed using the p-distance substitution model with 1000 bootstrap replications.

Table 1. Taxa, collection numbers, sequences, and GenBank accession numbers used in this study.

Taxon name	Collection No. (Isolate No.)	GenBank accession No.	
		ITS	RPB2
Achaetomium globosum	CBS 332.67	KX976570	KM655441
A. luteum	CBS 618.68	KX976571	KX976794
A. macrosporum	CBS 152.97	KX976573	KX976796
Arcopilus aureus	CBS 153.52	KX976582	KX976806
Ar. aureus	CBS 538.73	KX976583	KX976807
Ar. aureus	**CNUFC-KMHY6-1**	**MH685565**	**MH699880**
Ar. aureus	**CNUFC-KMHY6-2**	**MH685566**	**MH699881**
Ar. cupreus	CBS 560.80	KX976584	KX976808
Ar. flavigenus	CBS 337.67	KX976587	KX976811
Ar. fusiformis	CBS 484.85	KX976585	KX976809
Ar. turgidopilosus	CBS 169.52	KX976588	KX976812
Cymostachys coffeicola	CBS 252.76	KU846052	KU846081
C. fabispora	CBS 136180	KU846054	KU846082
Memnoniella brunneoconidiophora	CBS 109477	KU846138	KU846192
M. dichroa	CBS 526.50	KU846140	KU846194
M. echinata	CBS 216.32	KU846142	KU846196
M. echinata	CBS 343.50	KU846144	KU846198
M. echinata	CBS 627.66	KU846147	KU846201
M. echinata	DAOM 235365	KU846149	KU846203
M. echinata	**CNUFC-MSW24-2-11**	**MH685569**	**MH699882**
M. echinata	**CNUFC-MSW24-2-12**	**MH685570**	**MH699883**
M. humicola	CBS 463.74	KU846154	KU846208
M. oenanthes	CBS 388.73	KU846156	KU846210
M. putrefolia	CBS 101177	KU846158	KU846212
Ovatospora unipora	CBS 109.83	KX976689	KX976902
O. medusarum	CBS 148.67	KX976684	KX976897
Stachybotrys chartarum	CBS 136161	KU846702	KU846927
S. chlorohalonata	CBS 109285	AY180261	KU846954
S. limonispora	CBS 128809	KU846735	KU846959
S. sansevieriae	HGUP 0103	JX998165	JX987249
S. sansevieriae	KNU16-141	KY587783	
S. sansevieriae	**CNUFC-GW2S-4**	**MH685567**	**MH699878**
S. sansevieriae	**CNUFC-GW2S-5**	**MH685568**	**MH699879**
S. subcylindrospora	HGUP 0201	JX998163	JX998168
Striatibotrys rhabdospora	CBS 528.80	KU846760	KU846981
St. atypica	CBS 141059	KU846753	KU846973
Lasiosphaeria ovina	SMH4605	AY587923	AY600284
Peethambara sundara	CBS 521.96	KU846470	KU846508

Bold letters indicate isolates and accession numbers determined in our study.
ITS: internal transcribed spacer; RPB2: RNA polymerase II second largest subunit; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CNUFC: Chonnam National University Fungal Collection, Gwangju, South Korea; DAOM: Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada; HGUP: Herbarium of Guizhou University, Plant Pathology, China.

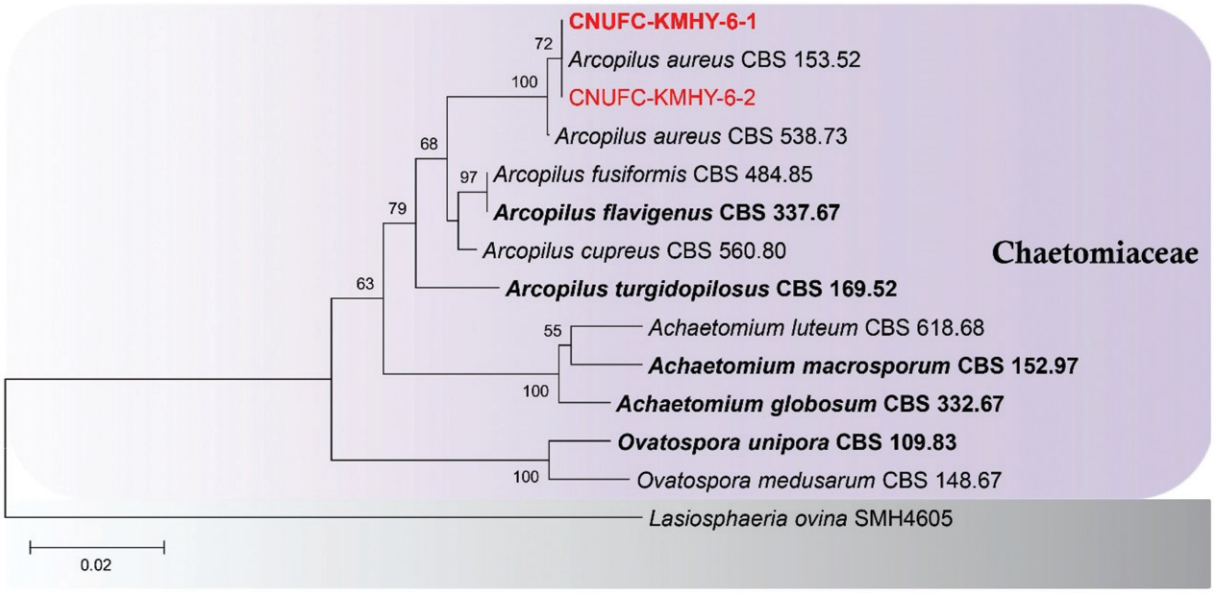

Figure 1. Phylogenetic tree based on neighbor-joining analysis of internal transcribed rDNA sequences for *Arcopilus aureus* CNUFC-KMHY6-1 and *Arcopilus aureus* CNUFC-KMHY6-2. *Lasiosphaeria ovina* was used as the outgroup. Bootstrap support values of >50% are indicated at the nodes. The bar indicates the number of substitutions per position. Ex-type strains are in bold.

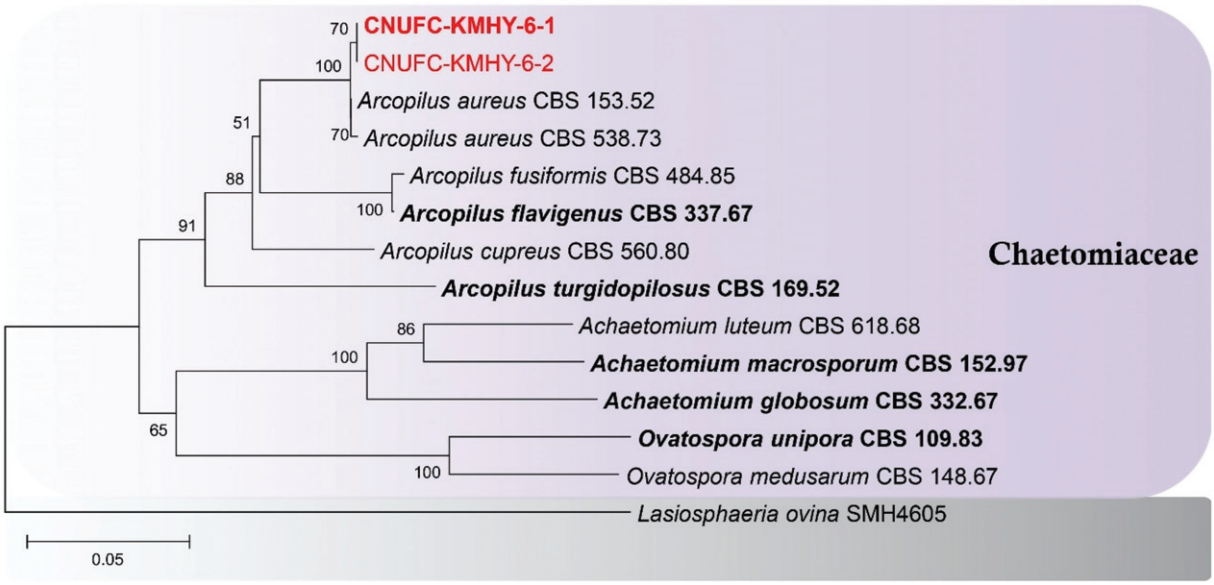

Figure 2. Phylogenetic tree based on neighbor-joining analysis of RNA polymerase II second largest subunit (RPB2) sequences for *Arcopilus aureus* CNUFC-KMHY6-1 and *Arcopilus aureus* CNUFC-KMHY6-2. *Lasiosphaeria ovina* was used as the outgroup. Bootstrap support values of >50% are indicated at the nodes. The bar indicates the number of substitutions per position. Ex-type strains are in bold.

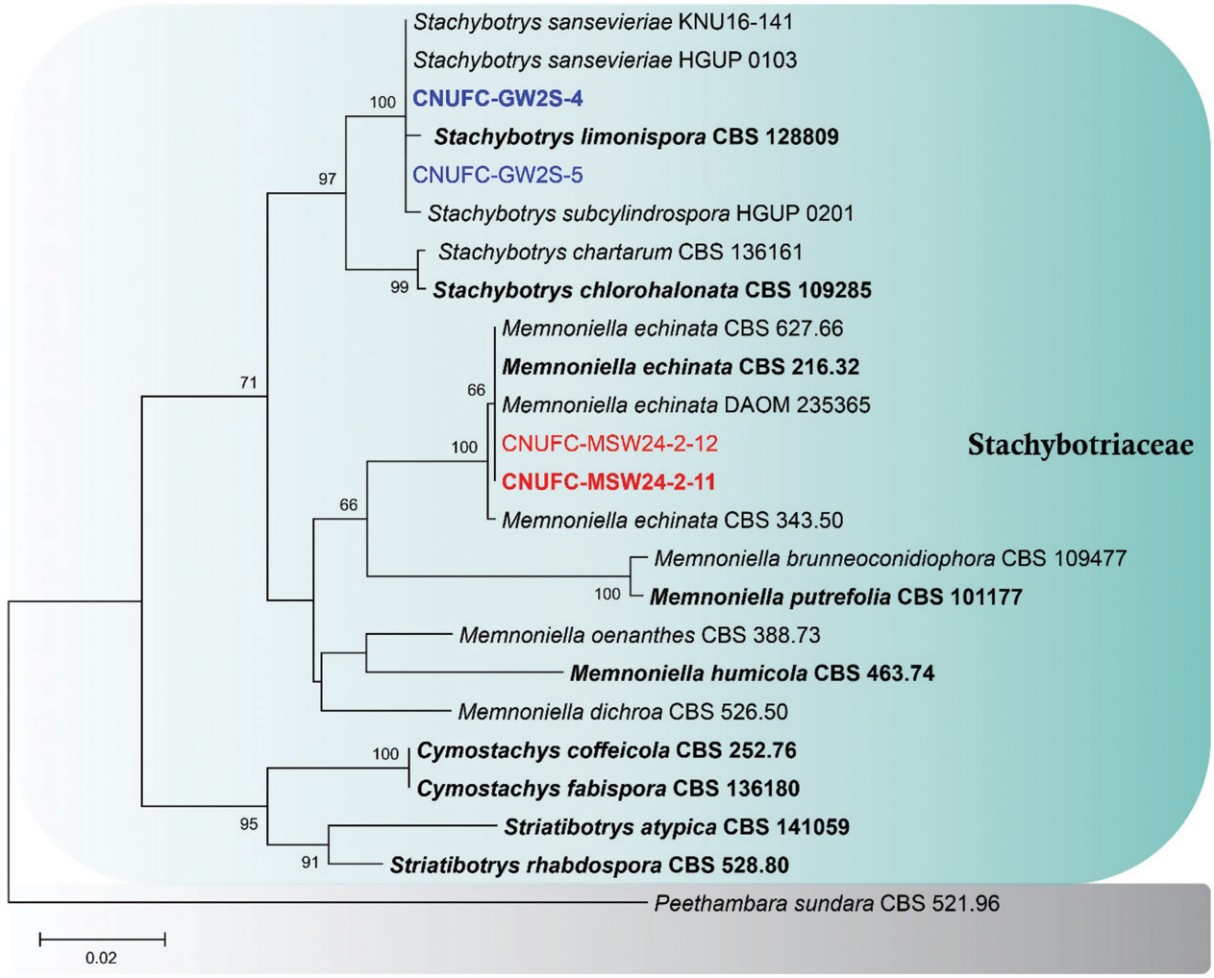

Figure 3. Phylogenetic tree based on neighbor-joining analysis of internal transcribed rDNA sequences for *Memnoniella echinata* CNUFC-MSW24-2-11, *Memnoniella echinata* CNUFC-MSW24-2-12, *Stachybotrys sansevieriae* CNUFC-GW2S-4, and *Stachybotrys sansevieriae* CNUFC-GW2S-5. *Peethambara sundara* was used as the outgroup. Bootstrap support values of >50% are indicated at the nodes. The bar indicates the number of substitutions per position. Ex-type and epi-type strains are in bold.

3. Results

3.1. Phylogenetic analysis

The phylogenetic analyses of the 2 sequence datasets (ITS and RPB2) showed that the strains CNUFC-KMHY6-1, CNUFC-KMHY6-2, CNUFC-MSW24-2-11, CNUFC-MSW24-2-12, CNUFC-GW2S-4, and CNUFC-GW2S-5 were placed within the same clade with species of *Arcopilus*, *Memnoniella*, and *Stachybotrys* (Figures 1–4).

In BLASTn for the ITS sequences, CNUFC-KMHY6-1, CNUFC-MSW24-2-11, and CNUFC-GW2S-4 showed 100% (500/500 bp), 100% (663/663 bp), and 100% (513/513 bp) sequence identity values with *Ar. aureus* (GenBank accession No. KX976582), *M. echinata* (GenBank accession No. KU846149), and *S. sansevieriae* (GenBank accession No. JX998165), respectively.

In BLASTn for the RPB2 sequences, CNUFC-KMHY6-1, CNUFC-MSW24-2-11, and CNUFC-GW2S-4 strains showed 99.8% (495/496 bp), 100% (526/526 bp), and 100% (743/743 bp) identity values with *Ar. aureus* (GenBank accession No. KX976806), *M. echinata* (GenBank accession No. KU846196), and *S. sansevieriae* (GenBank accession No. JX987249), respectively.

3.2. Taxonomy

3.2.1. Taxonomy of CNUFC-KMHY6-1

Arcopilus aureus (Chivers) X. Wei Wang & Samson, Stud. Mycol. 84: 217 (2016) (Table 2, Figure 5).

≡*Chaetomium aureum* Chivers, Proc. Amer. Acad. Arts & Sci. 48: 86 (1912).

Description: Colonies grew moderately at 25 °C on PDA, reaching 66 mm in diameter after 15 days at 25 °C. The colony color was initially white-to-orange, later greenish. The colony reverse was reddish purple. Ascomata were superficial, globose or oval, 76.3–157.8 × 96.7–165.8 µm. Lateral hairs were apically incurved. Terminal hairs were brown, mostly hook shape, verrucose, arcuate, slightly circinate to coiled.

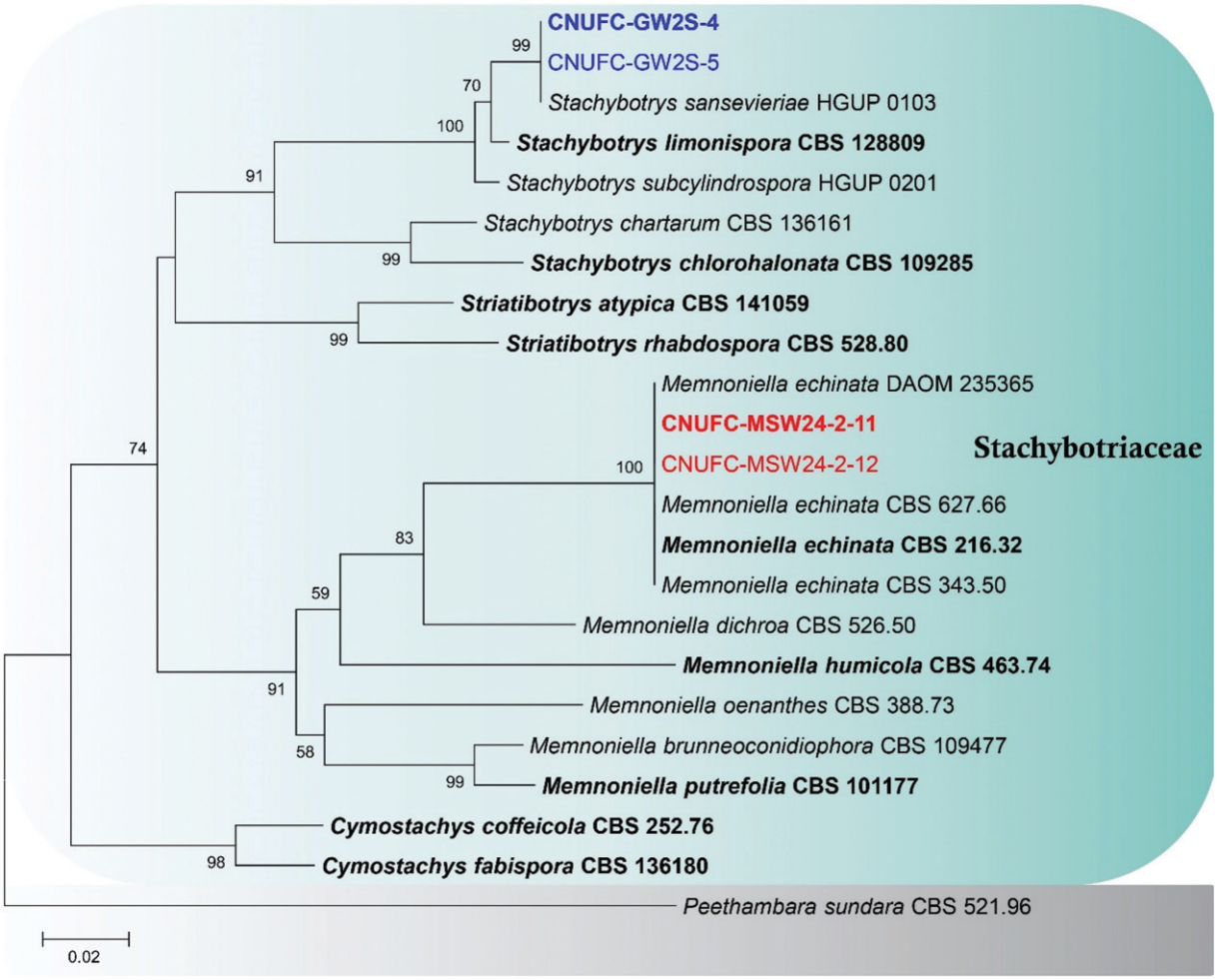

Figure 4. Phylogenetic tree based on neighbor-joining analysis of RNA polymerase II second largest subunit (RPB2) sequences for *Memnoniella echinata* CNUFC-MSW24-2-11, *Memnoniella echinata* CNUFC-MSW24-2-12, *Stachybotrys sansevieriae* CNUFC-GW2S-4, and *Stachybotrys sansevieriae* CNUFC-GW2S-5. *Peethambara sundara* was used as the outgroup. Bootstrap support values of >50% are indicated at the nodes. The bar indicates the number of substitutions per position. Ex-type and epi-type strains are in bold.

Table 2. Morphological characteristics of CNUFC-KMHY6-1 and the reference species *Arcopilus aureus* (≡*C. aureum*).

Characteristics	CNUFC-KMHY6-1	*Arcopilus aureus* (≡*C. aureum*)[a,b]
Colony color	Initially white-to-orange, later greenish, reverse reddish purple	First gray, pale olive then yellow
Ascomata	Superficial, globose or oval, 76.3–157.8 × 96.7–165.8 μm	Minute, globose or subglobose, 127 × 115 μm (110–140 × 105–123 μm)
Lateral hairs	Apically incurved	Numerous, slender, straight or flexed
Terminal hairs	Mostly hook shape, verrucose, arcuate, slightly circinate to coiled	Minutely roughened, straight or slightly recurved
Asci	Fasciculate, clavate, with 8 biseriate or irregularly arranged ascospores, 24.9–41.5 × 7.3–12.8 μm	Club-shaped, 8-spored, 42 × 10 μm
Ascospore	Brown when mature, 9.2–11.5 × 4.5–6.5 μm, sometimes irregular, fusiform, limoniform, lunate, with one or two apical germ pores	Irregularly ovate, apiculate at both ends, 9.8 × 5.4 μm (9.4–11 × 4.7–5.6 μm)

[a,b]From the description by Wang et al. [18] and Chivers [30].

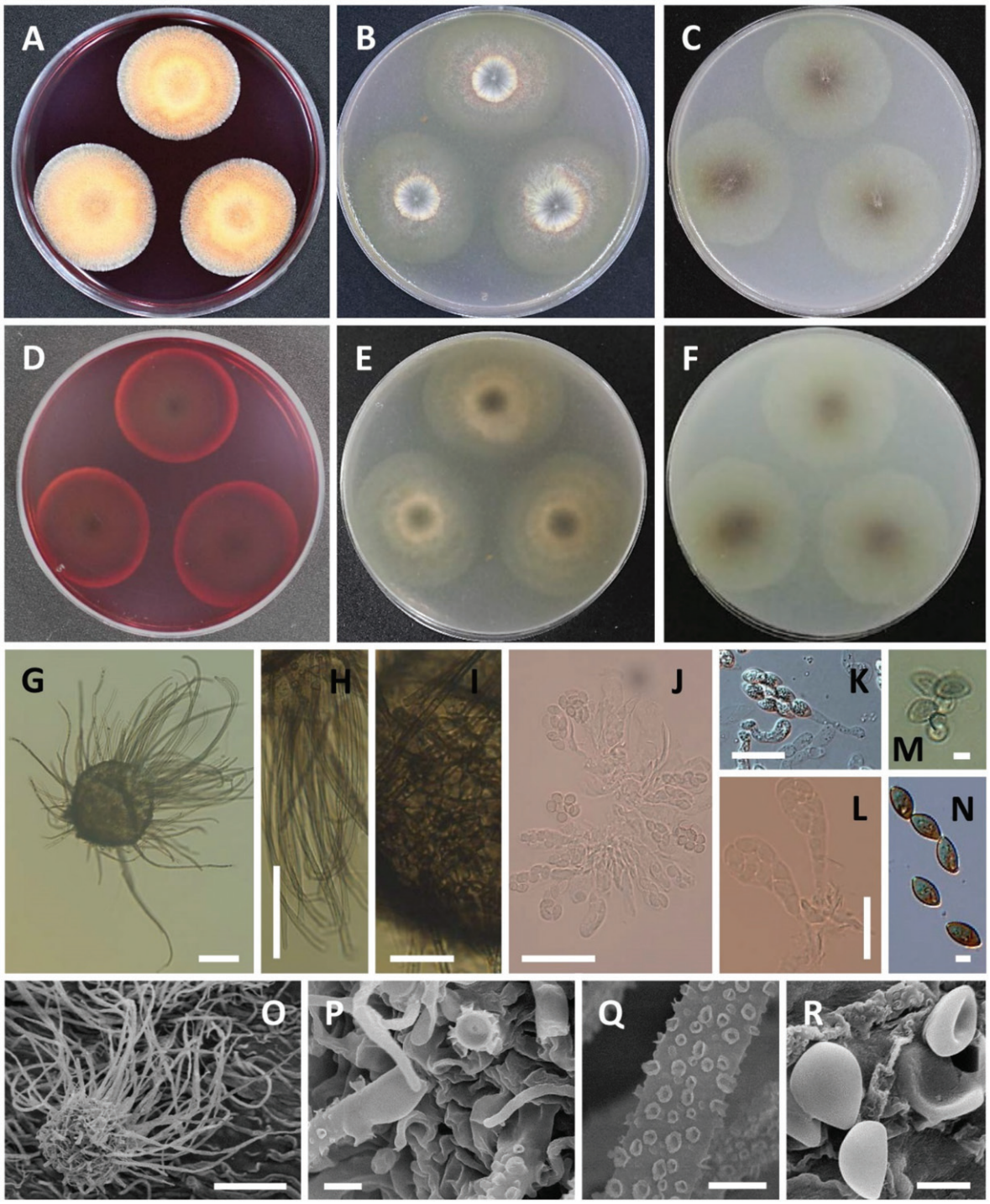

Figure 5. Morphology of *Arcopilus aureus* CNUFC-KMHY6-1. A) and D) Colony on potato dextrose agar (PDA); B) and E) Colony on potato carrot agar (PCA); C) and F) Colony on oatmeal agar (OA) [A)-C) top view, D)–F) reverse view)]; G) and O) Ascomata; H) and Q) Ascomatal hair; I) and P) Structure and surface of ascomatal wall; J)–L) Asci and ascospores; M), N), and R) Ascospores [Scale bars: G), H), and O) = 50 μm; I), K), and L) = 20 μm; J) = 40 μm; M) and N) = 5 μm; P) and Q) = 2.5 μm; R) = 5 μm).

Table 3. Morphological characteristics of CNUFC-MSW24-2-11 and the reference species *Memnoniella echinata*.

Characteristics	CNUFC-MSW24-2-11	*Memnoniella echinata*[a]
Colony color	Amber	Amber to Sienna
Conidiophore	Straight to slightly flexuous, smooth to slightly verrucose, unbranched, septate, 2.7–3.6 µm in width, variable in length	Simple, macronematous, mononematous, single, thick-walled, unbranched, erect, straight to slightly flexuous, septate, smooth to slightly verrucose, 40–100 × 4–6 µm, bearing 6–10 conidiogenous cells
Conidiogenous cell	Phialidic, clavate, smooth, 7.3–11.0 × 3.3–4.2 µm	Phialidic, clavate to subcylindrical, smooth, 7–10 × 2–5 µm, with conspicuous collarettes
Conidia	Globose to subglobose, verrucose, aseptate, 5.3–6.3 × 4.5–6.2 µm, formed in long dry chains	Acrogenous, aseptate, globose, verrucose, thick-walled, 3–6 × 3–5 µm (average, 5 × 4 µm), formed in long dry chains

[a]From the description by Lombard et al. [24].

Asci were fasciculate, clavate, with 8 biseriate or irregularly arranged ascospores, 24.9–41.5 × 7.3–12.8 µm. Ascospores were brown when mature, 9.2–11.5 × 4.5–6.5 µm, sometimes irregular, fusiform, limoniform, lunate, with one or two apical germ pores. The optimal growth temperature was 35 °C.

3.2.2. Taxonomy of CNUFC-MSW24-2-11

Memnoniella echinata (Riv.) Galloway, Trans. Brit. Mycol. Soc. 18: 165 (1933) (Table 3, Figure 6).

≡*Penicillium echinatum* Riv., Dei Parassiti Vegetali: 451 (1873).
≡*Haplographium echinatum* (Riv.) Sacc., Syll. Fung. 4: 307 (1886).
≡*Stachybotrys echinata* (Riv.) G. Sm., Trans. Brit. Mycol. Soc. 45: 392 (1962).
=*Periconia papyrogena* Sacc., Michelia 1: 273 (1878).
≡*Stachybotrys papyrogena* (Sacc.) Sacc., Fungi Ital.: tab. 900 (1881).
≡*Sterigmatobotrys papyrogena* (Sacc.) Oud., Ned. Kruidk. Arch., ser. 2, 4: 548 (1886).
=*Memnoniella aterrima* Höhn., Zentralbl. Bakt. ParasitKde, Abt. 2 60: 16 (1923).
=*Spinomyces japonica* Saito, J. Ferment. Technol. 17: 2 (1939).

Description: Colonies grew slowly on OA, reaching 28 mm diameter after 10 days at 25 °C. The colony color was amber. The colony reverse was also amber. Conidiophores were straight to slightly flexuous, smooth to slightly verrucose, unbranched, septate, 2.7–3.6 µm in width, and variable in length. Conidiogenous cells were phialidic, clavate, smooth, 7.3–11.0 × 3.3–4.2 µm. Conidia were globose to subglobose, verrucose, aseptate, 5.3–6.3 × 4.5–6.2 µm, formed in long dry chains. On OA, growth was faster than on CMA and PDA, and abundant sporulation was observed when grown on OA.

3.2.3. Taxonomy of CNUFC-GW2S-4

Stachybotrys sansevieriae G.P. Agarwal & N.D. Sharma, J. Indian Bot. Soc. 53: 78 (1974) (Table 4, Figure 7).

=*Stachybotrys indica* P.C. Misra, Mycotaxon 2: 107 (1975).

Description: Colonies grew slowly on PDA, reaching 23.5 mm diameter after 15 days at 25 °C. The colony color was brown to dark sepia. The colony reverse was dark sepia. Conidiophores were straight, unbranched, with septate, cylindrical, 28.1–73.8 µm in length, and 2.4–8.5 µm in width. Conidiogenous cells were phialidic, obovate, smooth, 8.3–12.5 × 3.5–6.1 µm. Conidia were ellipsoidal, unicellular, 5.4–10.1 × 3.5–6.9 µm. On PDA, growth was slower than on OA and CMA, and abundant sporulation was observed when grown on OA and CMA.

4. Discussion

In this study, two species of *Ar. aureus* and *M. echinata* were isolated from soil sample and one species of *S. sansevieriae* from freshwater sample in Korea. *Ar. aureus* and *M. echinata* have been recorded for the first time in Korea, and *S. sansevieriae* is the first report from freshwater niche in Korea.

In previous studies, SSU, LSU, TEF, and RPB2 sequence data have been used for phylogeny of the class Sordariomycetes [2,17]. In the present study, the phylogenetic trees for selected genera within the family Chaetomiaceae and Stachybotryaceae were inferred from ITS and RPB2 sequence data and provided to infer the phylogenetic position of the 3 species.

Our analyses of ITS and RPB2 sequences showed that the strains CNUFC-KMHY6-1 and CNUFC-KMHY6-2 were clustered with other *Ar. aureus* species in a well-supported clade with high bootstrap values (Figures 1 and 2). The morphological features of our isolate were generally similar to the description of *C. aureum* (≡*Ar. aureus*) by Chivers et al. [30]. Many *Chaetomium*-like fungi produce secondary metabolites with different biological activities. Interestingly, Dwibedi and Saxena [31] have reported that *Ar. aureus* produces resveratrol. Resveratrol is a polyphenolic flavonoid and widely used as a therapeutic moiety as well as a pharmacophore for the development of novel drugs because of its various beneficial effects [31]. It has been

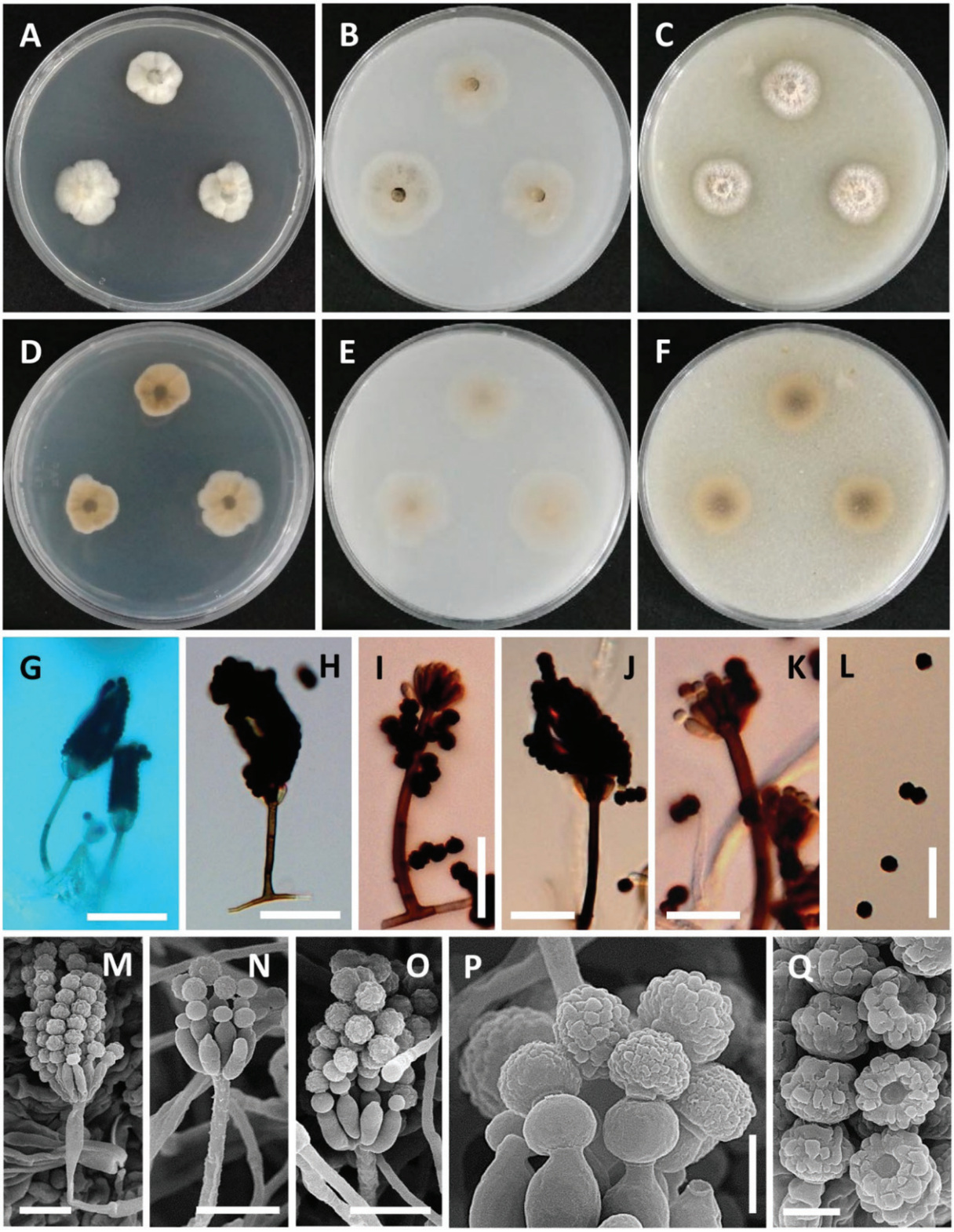

Figure 6. Morphology of *Memnoniella echinata* CNUFC-MSW24-2-11. A) and D) Colony on potato dextrose agar (PDA); B) and E) Colony on oatmeal agar (OA); C) and F) Colony on corn meal agar (CMA) [A)–C) top view, D)–F) reverse view]; G)–K) and M)–P) Conidiophores, phialides and conidia; L) and Q) Conidia [Scale bars: G) = 40 μm; H)–L) = 20 μm; M)–O) = 20 μm; P) = 2 μm; Q) = 5 μm).

reported to have beneficial effects in the treatment of neurological diseases like Alzheimer's, dementia, and Parkinson's diseases [32,33]. This finding suggests that the strain CNUFC-KMHY6-1 is a potentially useful source for medical and biotechnological applications and needs to be investigated further.

In the phylogenetic trees based on ITS and RPB2 sequences, the 2 investigated strains CNUFC-MSW24-2-11 and CNUFC-MSW24-2-12 were clustered with other *M. echinata* species in a well-supported clade with high bootstrap values (Figures 3 and 4). The morphological features of our isolate

Table 4. Morphological characteristics of CNUFC-GW2S-4 and the reference species *Stachybotrys sansevieriae*.

Character	CNUFC-GW2S-4	*Stachybotrys sansevieriae*[a]
Colony color	Brown to dark sepia	NA
Conidiophore	Macronematous, mononematous, erected, straight, unbranched, septae, cylindrical, 28.1–73.8 × 2.4–5.5 μm	Subhyaline to pale brown, up to 60 μm long
Conidiogenous cell	Phialidic, obovate, smooth, 8.3–12.5 × 3.5–6.1 μm	8–13 × 3–4 μm
Conidia	Ellipsoidal, unicellular, 5.4–10.1 × 3.5–6.9 μm	Navicular, dark brown, 6–9 × 3–4 μm

[a]From the description by Pinruan et al. [19].
NA: not available.

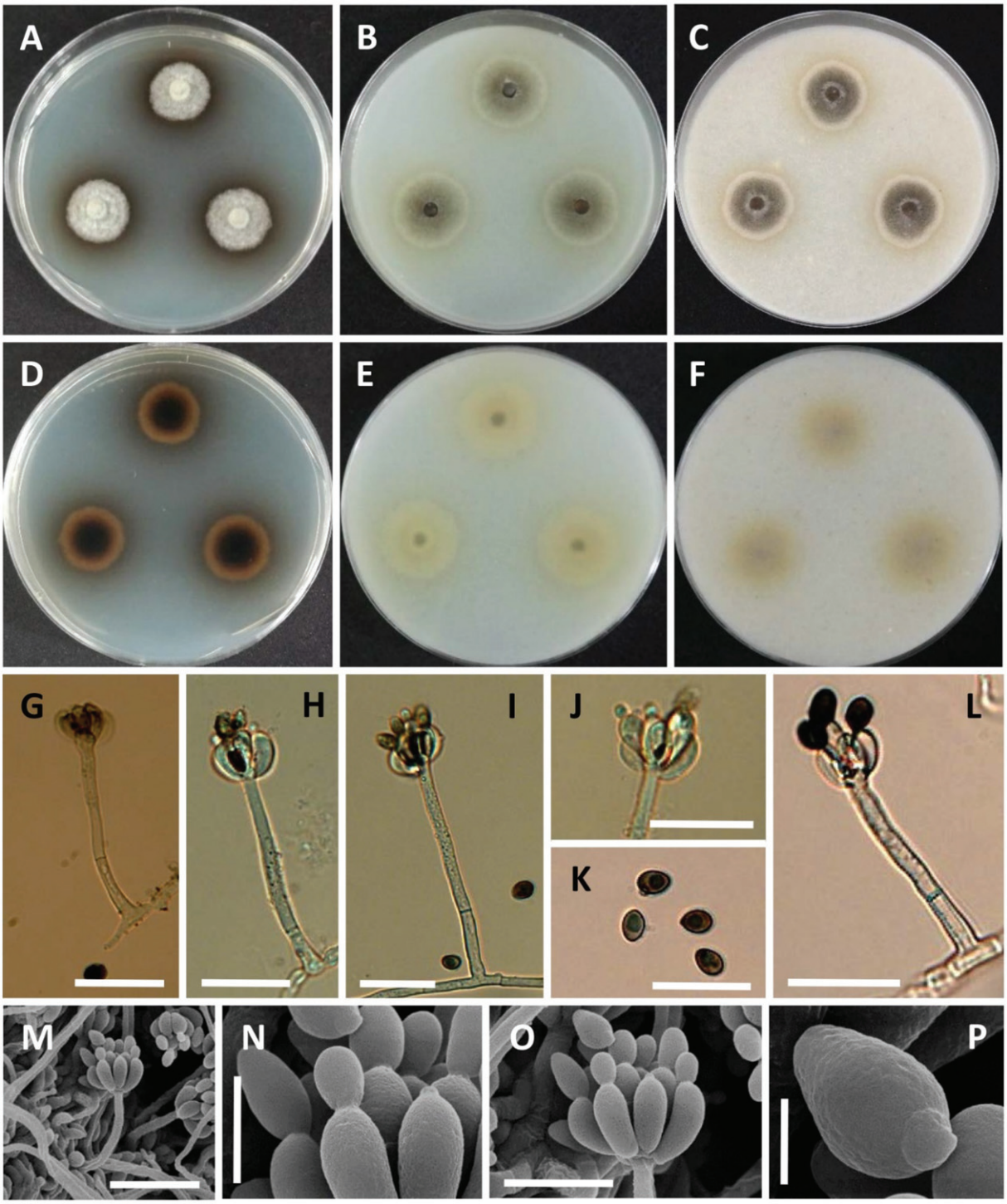

Figure 7. Morphology of *Stachybotrys sansevieriae* CNUFC-GW2S-4. A) and D) Colony on potato dextrose agar (PDA); B) and E) Colony on oatmeal agar (OA); C) and F) Colony on corn meal agar (CMA) [A)–C) top view, D)–F) reverse view]; G)–J), L), and M)–O) Conidiophores and phialides; K) and P) Conidia (Scale bars: G)–M) = 20 μm, N) = 5 μm, O) = 10 μm, P) = 2 μm).

were consistent with the description of *M. echinata* by Lombard et al. [24]. However, the size of the conidia was 5.3–6.3 × 4.5–6.2 µm, which was slightly larger than the conidia (3–6 × 3–5 µm) for *M. echinata* described by Lombard et al. [24].

In the ITS phylogenetic tree, the strains CNUFC-GW2S-4 and CNUFC-GW2S-5 were clustered in the same clade with *S. limonispora* CBS 128809, *S. sansevieriae* HGUP 0103, *S. sansevieriae* KNU16-141, and *S. subcylindrospora* HGUP 0201 (Figure 3). However, the RPB2 sequences of CNUFC-GW2S-4 and CNUFC-GW2S-5 were easily distinguishable and were well separated in the phylogeny (Figure 4). In 2017, the species of *S. sansevieriae* was isolated from field soils in Korea by Adhikari et al. [34] without detailed description such as the size of conidiophores, conidiogenous cells, and conidia. There was no any detailed phylogenetic analysis of the species in the family Stachybotriaceae.

Morphologically, *S. sansevieriae* reported here had a close similarity with the description by Pinruan et al. [19], although slight differences in the size of the conidia were noted. In comparison with other related species, our *S. sansevieriae* isolate presented ellipsoidal conidia (5.4–10.1 × 3.5–6.9 µm) that were larger than the ellipsoidal to limoniform conidia of *S. limonispora* ((6–)6.5–7.5(–9)×3–4 µm) [24] and smaller than the cylindrical to subcylindrical conidia of *S. subcylindrospora* ((9.7–)11.6–13.8(–14.7)×(2.9–)3.8–4.6(–5) µm) [35]. Furthermore, in the phylogenetic tree based on RPB2 sequence data, our strains formed a separate branch from *S. limonispora* CBS 128809 and *S. subcylindrospora* HGUP 0201.

Among species of the genus *Stachybotrys*, *S. chartarum* is known to produce mycotoxins, including the macrocyclic trichothecenes [36], as well as diverse immunosuppressant agents [37,38]. Our newly recorded isolate *M. echinata* is closely related to *S. chartarum*. According to Jarvis et al. [39,40], *M. echinata* produces mycotoxins, including cytotoxic trichothecenes, as well as several griseofulvins. Our findings contribute to the understanding of three Sordariomycetes genera *Arcopilus, Memnoniella*, and *Stachybotrys* and increase the number of undiscovered species from freshwater and soil in Korea. Further studies on these 3 newly reported fungal isolates from Korea need to be performed.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was in part supported by the Graduate Program for the Undiscovered Taxa of Korea, and in part by the Project on Survey and Discovery of Indigenous Fungal Species of Korea funded by NIBR and Project on Discovery of Fungi from Freshwater and Collection of Fungarium funded by NNIBR of the Ministry of Environment (MOE), and in part carried out with the support of Cooperative Research Program for Agriculture Science and Technology Development [PJ013744], Rural Development Administration, Republic of Korea. This work was in part supported by the BK21 plus program through the National Research Foundation (NRF) funded by the Ministry of Education of Korea.

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