

## RESEARCH ARTICLE

# A new record of *Scleroconidioma sphagnicola* isolated from soil in Korea

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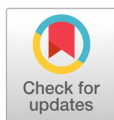
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## ABSTRACT

A fungal isolate, designated KNU-JJ-1824, was isolated from a soil sample collected from a field on Jeju, Korea. Colonies of the isolates cultured on PDA, MEA and CMAD for 14 days grew to diameters of 49~51 mm, 60~63 mm, and 47~50 mm, respectively. The colonies of this fungal isolate were dark green in color on both sides, and had irregular margins on the PDA media. Mycelia submerged and exhibited clear lines from the center to the edge on MEA media. On CMAD media, they were dark brown to greenish-brown, with narrow white margins. The shape of the conidia was guttulate, fusiform or clavate, and  $1.9\sim 3.4 \times 6.5\sim 10.2 \mu\text{m}$  in size. A molecular phylogenetic tree was constructed using dataset from small subunit (SSU) rDNA and the internal transcribed spacer (ITS) regions, and the isolate was found to cluster with *Scleroconidioma sphagnicola* UAMH 9731. Based on the results of the phylogenetic tree analysis and the cultural and morphological characteristics, the isolate was identified as *Scleroconidioma sphagnicola*. We report *S. sphagnicola* for the first time in Korea.

**Keywords:** Morphological characteristics, Phylogenetic analysis, *Scleroconidioma sphagnicola*



## OPEN ACCESS

pISSN : 0253-651X

eISSN : 2383-5249

Kor. J. Mycol. 2019 June, 47(2): 125-30

<https://doi.org/10.4489/KJM.20190016>

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**Received:** May 17, 2019

**Revised:** June 18, 2019

**Accepted:** June 19, 2019

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## INTRODUCTION

The genus *Scleroconidioma* Tsuneda, Currah & Thormann was identified for the first time in diseased leaves of *Sphagnum fuscum* in Canada, and later reported from Sweden and the Czech Republic [1-3]. It is similar to members of the genera *Phaeotheca* and *Phaeosclera*, but shows differences in conidiogenesis and cultural characteristics [4,5]. *Scleroconidioma sphagnicola*, the type and sole species of the genus *Scleroconidioma*, has distinctive characteristics. It is a strongly melanized fungus, allied to the Dothidiales, and represents a group of fungi found on plant litter [6,7]. It has the ability to adapt to stressful environmental conditions [8]. *S. sphagnicola* is an anamorphic ascomycete, and a pathogen of peat bogs (*Sphagnum fuscum*) as has been reported from Canada [6,9]. It was isolated in Sweden and the Czech Republic, where it was observed that the fungus has saprotrophically colonized wood and coniferous litter [2,3]. It has also been reported that *S. fuscum* cell walls are highly resistant to biological degradation due to a high content of phenolic compounds [10]. During surveying unreported fungal species in Korea, a fungal isolate KNU-JJ-1824 was isolated from soil collected on Jeju in 2018. It was identified as *S. sphagnicola*

based on morphological characteristics and phylogenetic analysis result. In this study, we firstly report and describe the characteristics of *S. sphagnicola* KNU-JJ-1824 which has not been reported in Korea.

## MATERIALS AND METHODS

### Soil sample collection and fungal isolation

Fungal isolate KNU-JJ-1824 was isolated from a soil sample collected on Jeju, Korea (N 33°25'20.3", E 126°33'04.0") in 2018. The sample was collected from the ground, air-dried, and stored in a plastic bag at 4°C until analysis. One gram of the soil sample was suspended in 10 mL of sterile distilled water. The suspension was vortexed, diluted, and a defined volume was spread on potato dextrose agar (PDA; Difco, Detroit, MI, USA). Individual colonies that developed on the agar were purified by subculture on fresh PDA and incubated at 25°C until mycelium development. The pure cultures were preserved on PDA slants at 4°C.

### Morphological characterization of cultures

For morphological analysis, isolate KNU-JJ-1824 was grown on potato dextrose agar (PDA), malt extract agar (MEA), and corn meal agar with 1% dextrose (CMAD) for 14 days [1]. After incubation, colony characteristics such as color, size, and shape were recorded. Samples were observed using a model BX-50 light microscope (Olympus, Tokyo, Japan).

### Genomic DNA extraction, PCR amplification and data analysis

Genomic DNA was extracted from strain KNU-JJ-1824 using HiGene Genomic DNA Prep Kits (BIOFACT, Daejeon, Korea), following the manufacturer's protocol. Small subunit ribosomal rDNA (SSU) and the internal transcribed spacer region (ITS) rDNA were amplified using the primers NS1/ITS4, while NS4, NS6, NS8 and ITS1F primers were used for inner sequence analysis [11,12]. The amplified PCR products were purified using ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced by Macrogen (Daejeon, Korea). Similarities between the sequences were analyzed using NCBI BLAST and GENETYX-WIN (ver. 3.2). A partial sequence of the SSU and ITS regions of allied species of *S. sphagnicola* were retrieved from NCBI to generate a phylogenetic analysis (Table 1). The sequences from KNU-JJ-1824 were deposited in NCBI GenBank under the accession number MK880096.

### Phylogenetic analysis

The isolates used to construct the phylogenetic tree are summarized in Table 1, with their strain and GenBank accession numbers. Phylogenetic trees were constructed based on concatenated SSU and ITS region sequences using the maximum likelihood method in the MEGA6 software program with a bootstrap analysis of 1,000 replications [13].

## RESULTS AND DISCUSSION

### Morphology of KNU-JJ-1824

The isolated KNU-JJ-1824 was cultured on three different media; PDA, MEA, and CMAD for 14 days, and incubated at 25°C. The diameters of the resulting colonies were 49–51 mm, 60–63 mm, and 47–50 mm, respectively. Both sides of the colonies were black and carbonaceous on PDA and had irregular margins and a solid texture. On MEA, mycelia submerged into the medium and made clear lines from the center to the edge. Colonies were dark brown to greenish brown with narrow white margins on CMAD (Fig. 1). Morphological characteristics were observed for colonies on the CMAD. Stromata with conidia, masses of sclerotic cells and clusters of shiny white tufts were observed (Fig. 2A, B and E). Conidia were pigmented, guttulate, variable in shape, but mostly fusiform or clavate,  $1.9\text{--}3.4 \times 6.5\text{--}10.2 \mu\text{m}$  in size (Fig. 2C). Bacilliform conidia were aseptate, hyaline, and  $0.9\text{--}1.2 \times 2.2\text{--}3.3 \mu\text{m}$  in size (Fig. 2D). Sympodial conidia arising directly from hyphae were observed (Fig. 2F). The morphological characteristics of KNU-JJ-1824 were similar to those previously reported for *S. sphagnicola* [1] (Table 2). Based on the morphological characteristics, the fungal isolate KNU-JJ-1824 was identified as *S. sphagnicola*.

**Table 1.** List of the sequences used in this study.

Species	Strain Number	GenBank Accession Number		
		SSU	ITS	SSU + ITS
<i>Columnosphaeria fagi</i>	CBS 171.93	AY016342	KT693737	-
<i>Delphinella strobiligena</i>	CBS 735.71	AY016341	MH860318	-
<i>Dothidea berberidis</i>	CBS 186.58	-	-	EU167601
<i>D. hippophaeos</i>	CBS 186.58	U42475	AF027763	-
<i>D. insculpta</i>	CBS 189.58	U42474	AF027764	-
<i>Scleroconidioma sphagnicola</i>	UAMH 9731	-	-	AY220610
<i>S. sphagnicola</i> <sup>a</sup>	KNU-JJ-1824	-	-	MK880096
<i>Stylodothis puccinioides</i>	CBS 193.58	AY016353	MH857753	-

<sup>a</sup>The fungal isolated used in this study.

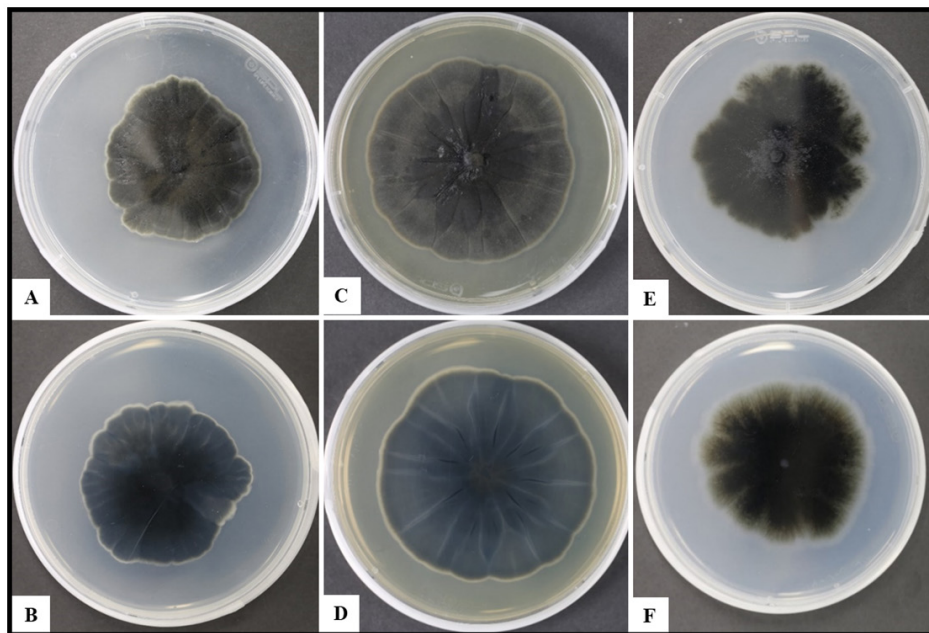
**Table 2.** Comparison of the morphological characteristics of an isolate KNU-JJ-1824 and the reference strain *Scleroconidioma sphagnicola*

Characteristics		<i>Scleroconidiom a sphagnicola</i> KNU-JJ-1824 <sup>a</sup>	<i>Scleroconidiom a sphagnicola</i> UAMH 9731 <sup>b</sup>
Colony	Shape and color	On CMAD, dark green to brown, irregular with white margin, smooth and becoming floccose with age	On CMAD, dark brown to greenish brown with a narrow white margin, smooth when young, becoming somewhat flocculose with age
	Size (mm)	CMAD: 47–50, PDA: 49–51, MEA: 60–63	CMAD: 49–51, PDA: N/A, MEA: N/A
Conidia	Shape and position	produced on conidiomata: guttulate, mostly fusiform or clavate, $1.9\text{--}3.4 \times 6.5\text{--}10.2 \mu\text{m}$ Conidia (bacilliform): aseptate, hyaline, $0.9\text{--}1.2 \times 2.2\text{--}3.3 \mu\text{m}$	produced on conidiomata: degree of pigmentation, commonly fusiform, clavate or spathulate, $1\text{--}3 \times 6.5\text{--}9 \mu\text{m}$ Conidia (bacilliform): hyaline, one-celled, $0.9\text{--}1 \times 3\text{--}5.5(7.5) \mu\text{m}$
Sclerotic cell		Abundant	Abundant

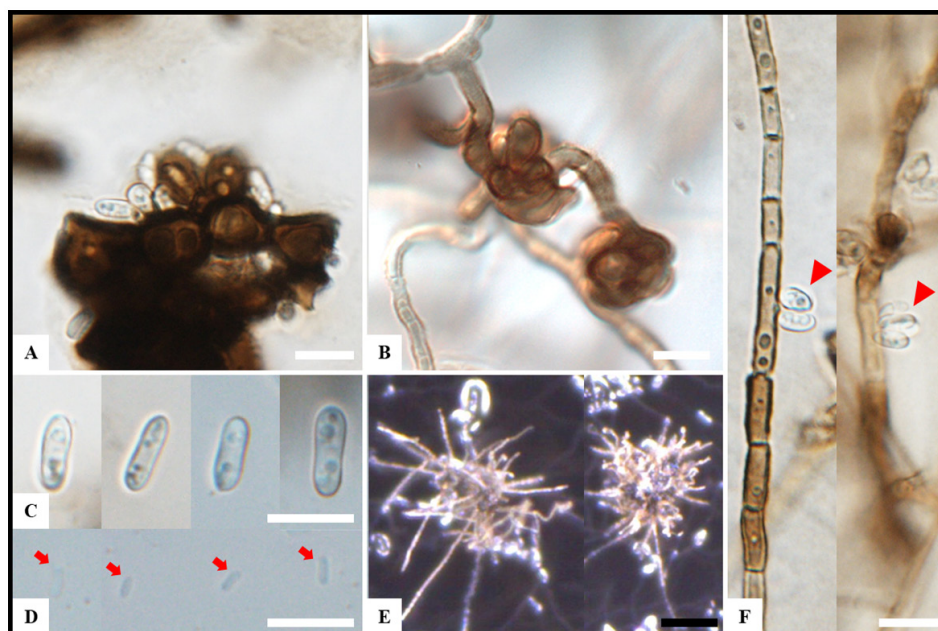
<sup>a</sup> The fungal isolate used in this study

<sup>b</sup> Tsuneda et al., 2000 [1]

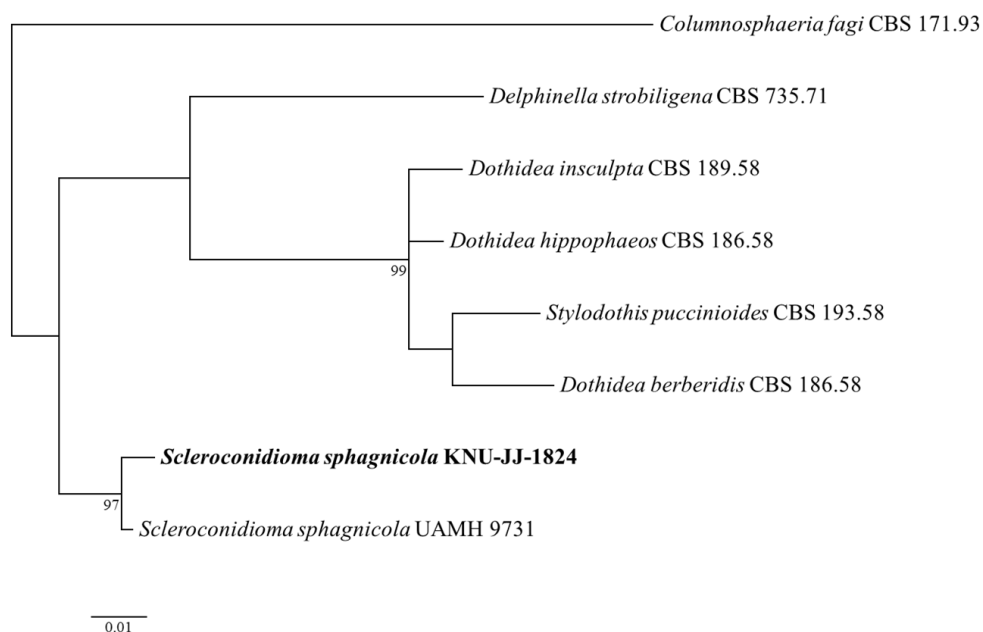
N/A: Not available



**Fig. 1.** Cultural characteristics *Scleroconidioma sphagnicola* KNU-JJ-1824 after 14 days in 25°C. A, B, Colonies on potato dextrose agar; C and D, Colonies on malt extract agar; E and F, Colonies on CMAD. Front (A, C, E) and reverse (B, D, F) sides.



**Fig. 2.** Morphological characteristics of *Scleroconidioma sphagnicola* KNU-JJ-1824. A, Stromata with conidia; B, Mass of sclerotic cells; C, Conidia; D, Conidia (Bacilliform); E, Shiny white tuft clusters; F, Sympodial conidia directly arising from hyphae (arrowhead). White scale bar = 10  $\mu$ m, Black scale bar = 200  $\mu$ m).



**Fig. 3.** Maximum likelihood phylogenetic tree based on a concatenated dataset of SSU and ITS gene sequences, showing the phylogenetic position of *Scleroconidioma sphagnicola*. The strain isolated in this study is shown in boldface. Bootstrap values based on 1,000 replications are shown at the branch points. *Columnosphaeria fagi* CBS 171.93 was used as an outgroup. Bar is 0.01 substitutions per nucleotide position.

### Molecular phylogeny of the isolate KNU-JJ-1824

A phylogenetic tree for isolate KNU-JJ-1824 was constructed to examine its phylogenetic relationship with allied species. Sequencing produced 2,240 bp, covering the small subunit (SSU) and the internal transcribed spacer (ITS) regions. BLAST search results revealed that strain KNU-JJ-1824 has 99% similarity with *S. sphagnicola* UAMH 9731 (AY220610), previously reported from Canada. A phylogenetic tree was constructed from partial sequences of the SSU and ITS regions using the neighbor-joining method. This tree indicated that strain KNU-JJ-1824 clustered with *S. sphagnicola* UAMH 9731 with 97% bootstrap value (Fig. 3). Furthermore, it has been reported that only *S. sphagnicola* UAMH 9731 is the sole species of the genus *Scleroconidioma*, it was confirmed that KNU-JJ-1824 was closely related with *S. sphagnicola*. Thus, *S. sphagnicola* KNU-JJ-1824 was confirmed using morphological and molecular phylogenetic characteristics and was deposited in the National Institute of Biological Resources (accession no. NIBRFGC000502240).

*S. sphagnicola* was described as an anamorphic necrotrophic parasite on *Sphagnum fuscum* in Canada [1]. In Korea, it has been reported that *Sphagnum* spp. grow naturally in Jeju island [14]. In this reason, it is expected that *S. sphagnicola* inhabiting *Sphagnum* spp. can be found on Jeju Island. According to our result, this is the first report of the presence of genus *Scleroconidioma* and *S. sphagnicola* in Korea.



## ACKNOWLEDGEMENTS

This research was supported by the project on the survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR 201801105) under the Ministry of Environment, Republic of Korea.

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