RESEARCH ARTICLE

Two New Records of Scleroderma Species (Sclerodermataceae, Boletales) in South **Korea**

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ABSTRACT

Two new records of Scleroderma species from South Korea are described here. Comprehensive taxonomic studies of Scleroderma specimens were conducted at the Korea National Arboretum. Based on morphological and molecular data (fungal barcode sequences), two new records (S. laeve and S. nastii) were confirmed. Herein, morphological descriptions, including Scanning Electron Microscope (SEM) images of basidiospore ornamentation, and a taxonomic key of Korean Scleroderma species are provided.

Keywords: Ectomycorrhizal fungi, Phylogeny, Sclerodermataceae, Taxonomy



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INTRODUCTION

Scleroderma Pers. (Sclerodermataceae, Boletales) is one of the most adaptable and widespread genera of ectomycorrhizal fungi [1]. This genus is associated with several trees or shrubs, such as species belonging to Abies, Betula, Coccoloba, Eucalyptus, Nothofagus, Pinus, Populus, and Quercus [2]. Morphologically, this fungus is characterized by a gasteroid basidiome with globose to subglobose basidiospores showing reticulate to echinulate ornamentation [3]. Several species of Scleroderma are employed as medicinal mushrooms or known to be poisonous [4,5]. The Index Fungorum lists a total of 131 Scleroderma species (http://www.indexfungorum.org, accessed on 18 Mar. 2022), and approximately 25 species are recognized morphologically.

Since 2012, we have conducted field trips to investigate the diversity of *Scleroderma* species from South Korea. As a result, we have discovered and described two new records within Sclerodermataceae (S. laeve and S. nastii). In this study, descriptions of Scleroderma species from Korea, including their morphological features and a phylogenetic analysis, are provided. In addition, this study is the first to review and identify specimens of Scleroderma stored in herbaria and collected in the field in Korea.

MATERIALS AND METHODS

Morphological observations

A total of 21 samples were collected during a mycological survey to investigate the diversity of mushrooms conducted from 2012-2021. The samples were examined for identification based on their macroscopic and microscopic characteristics in the present study. Dried materials were mounted in distilled water and 5% KOH using an Olympus BX53 microscope (Olympus Corporation, Tokyo, Japan) and Jenoptik ProgRes C14 Plus Camera (Jenoptik Corporation, Jena, Germany). Microscopic parameters were measured using ProgRes Capture Pro v.2.8.8. software (Jenoptik Corporation). A scanning electron microscopy (SEM) analysis of basidiospores was also performed. The systematics of the taxa included in this study were in accordance with the Index Fungorum (http://www.indexfungorum.org). Dried specimens were deposited in the herbarium of the Korea National Arboretum (Table 1).

DNA extraction, sequencing and phylogenetic analysis

For phylogenetic analysis, genomic DNA was extracted from the specimens using a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA). The ITS (internal transcribed spacer) regions of the rDNA were amplified with the ITS1 and ITS4 primers as previously described [6]. The polymerase chain reaction amplicons were purified using a QIAquick Purification Kit (Qiagen Inc.) and directly sequenced using an ABI Prism 377 Automatic DNA Sequencer (Applied Biosystems, Foster City, CA, USA) with a BigDyeTM Cycle Sequencing Kit (version 3.1; Applied Biosystems). Sequences belonging to Sclerodermataceae were downloaded from the GenBank database (National Center for Biotechnology Information; https://www.ncbi.nlm.nih.gov/). These sequences and sequences of an outgroup taxon, *Astraeus odoratus* (GenBank Accession No. AJ629874), were used for analysis. The dataset was aligned using MAFFT v.7 [7]. All other parameters were set to the default values. A phylogenetic tree was constructed using RAxML in the CIPRES Science Gateway (https://www.phylo.org). The relative robustness of the individual branches was estimated by bootstrapping with 1,000 replicates.

Results

Phylogenetic analysis

A total of 21 ITS sequences were obtained from *Scleroderma* specimens in this study. As a result, the phylogenetic positions of five species (*S. areolatum*, *S. bovista*, *S. citrinum*, *S. laeve*, and *S. nastii*) were resolved by conducting an RaxML analysis of the ITS sequences (Fig. 1). They together formed well-supported clades. The genus *Scleroderma* is morphologically divided into three sections (*Reticulatae*, *Scleroderma*, and *Sclerangium*) based on the surface structure of basidiospores and the presence of clamp connections [2,8]. According to the present phylogenetic analysis, *S. laeve* belongs to *S.* sect. *Reticulatae* and *S. nastii* belongs to *S.* sect. *Scleroderma*. Our phylogenetic analysis showed that the taxonomic study was well supported by ITS sequences.

Species Specimen ID Country ITS Reference S. arcolatum KA12-0838 Korea ON241796 This study KA13-0795 Korea ON212390 This study KA13-0540 Korea ON241795 This study KA13-054 Korea ON241794 This study KA13-1054 Korea ON241798 This study KA18-0514 Korea ON241793 This study KA18-0514 Korea ON241799 This study KA18-1019 Korea ON241797 This study KA21-0319 Korea ON241797 This study RBG Kew K(M)125392 England EU784407 Brock et al. [15] GO-2010-148 Mexico KC152224 - M20 Poland KX483341 Hilszczańska et al. [16] S. bovista KA18-0519 Korea ON241800 This study 01A_1 USA JX030276 - - 01Q_1 USA JX030276 - </th <th></th>	
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S. duckei INPA:272127 Brazil KX792086 Baseia et al [17]	
UFRN:Fungos 2795 Brazil KX792087 Baseia et al. [17]	
S. dunensis UFRN:Fungi 1359 Brazil KU747113 -	
UFRN:Fungi 2033 Brazil KU747112 -	
S. laeve KA14-0542 Korea ON212392 This study	
KA14-1374 Korea ON212393 This study	
KA16-0827 Korea ON212395 This study	
KA18-1018 Korea ON212396 This study	
ASIS24596 Korea KP004932 -	
ZLR46 China MW553325 -	
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KA12-0905 Korea ON212387 This study	
KA13-0718 Korea ON212390 This study	
KA13-1555 Korea ON212391 This study	
KA16-0813 Korea ON212394 This study	
NAST-FB11 Nepal KJ740390 Raut et al. [12]	
Trappe 26232 Argentina HQ688789 Nouhra et al. [1]	
S. nitidum UFRN:Fungos 2550 Brazil KU759906 -	
UFRN:Fungos 1759 Brazil KU759907 -	
S. patagonicum Trappe 26236 Argentina HQ688788 Nouhra et al. [1]	
Trappe 26232 Argentina HO688789 Nouhra et al. [1]	
S. polyrhizum POLSCL1 USA FM213349 Phosri et al. [18]	
POLSCL2 USA FM213350 Phosri et al. [18]	
S. sinnamariense SINSCL1 (SCLK4) Thailand FM213356 Phosri et al. [18]	
SINSCL9 Thailand FM213364 Phosri et al. [18]	
S. verrucosum RBG Kew K(M)30670 England EU784415 Brock et al. [15]	
A4 China IX434678 -	
CM077 Algeria KP826736 -	
S. vunnanense Ji003 China IO639046 Zhang et al [19]	
Ji001C China JO639042 Zhang et al [19]	
Astraeus odoratus ASTRAE-58 Thailand AJ629874 Phosri et al. [20]	

Table 1. Scleroderma specimens used in this study, including specimen numbers, localities, internal transcribed spacer (ITS), and GenBank accession numbers.



Fig. 1. RAxML tree based on internal transcribed spacer (ITS) sequences of *Scleroderma* species. Sequences generated in this study are indicated in **bold** text.

Taxonomy

Scleroderma laeve Lloyd, Mycol. Writ. (Cincinnati) 5 (Letter 63):11. 1916. (Figs. 1 and 2)

Korean name: Keun-Eorialbeoseot (큰어리알버섯); derived from the 'big basidiome' in Korean.

Description. Basidiome globose to subglobose, 15-45 mm diameter. Peridium thin, 1.0-1.5 mm thick, pale yellowish-brown, finally reddish-brown smooth to warty due to irregular cracking. Gleba compact, dark grayish-brown or reddish-brown. Basidiospores n=25, globose to subglobose, 10.0-13.0 µm in diameter including ornaments or 9.0-12.5 µm in diameter excluding ornaments; average=11 µm. Basidia not observed. Clamp connections absent.



Fig. 2. Morphological characters of *Scleroderma laeve*. A, B: Basidiomes of *S. laeve*. C, D: Basidiospores under light microscope (LM), E: Basidiospore under scanning electron microscope (SEM).

Habitat. Growing on soil or sand, July to September.

Specimens examined. Korea. Jeollanam-do, Suncheon, Jul. 15, 2014, KA14-0542; Gyeonggi-do, Pocheon-si, Sept. 4, 2014, KA14-1374; Gyeonggi-do, Pocheon-si (37°44′57.67″N 127°9′59.44″E, alt. 112 m), Jul. 28, 2016, KA16-0827; Gyeonggi-do, Goyang-si (37°39′32.98″N 126°45′38.32″E, alt. 16.8 m), Sept. 18, 2018, KA18-1018.

Remarks. Based on the similarity of its morphological characteristics (shapes and size of the basidiome) to reported descriptions, this species was considered to be *S. areolatum* in Korea. However, the rhizomorph of *S. laeve* is well developed, and *S. areolatum* has a sessile or short pseudostipitate rhizomorph. (Fig. 2) [9]. The description of *S. laeve* collected from Korea was similar to Guzmán's description [10]. According to a previous report by Kasuya et al. [11], this species occurs together with *Lithocarpus edulis* (Fagaceae) and sandy soil. The Korean samples were usually collected on soil, mainly in parks and gardens to date.

Scleroderma nastii Raut, in Raut, Basukala, Shrestha & Poudel, Stud. Fung. 5(1):53. 2020. (Figs. 1 and 3) Korean name: Jageun-Eorialbeoseot (작은어리알버섯); derived from the 'small basidiome' in Korean. Description. Basidiome epigeous, globose to subglobose, 10-15 to 10-20 mm, smooth, somewhat areolated, blackish-brown. Peridium 1 mm or less than 1 mm thick, pseudostipitate. Gleba compact, fleshy to dusty, blackish-brown. Basidiospores n=25, globose to subglobose, thick-walled, subreticulated to irregularly reticulated, 8.0-11.0 µm in diameter including ornaments or 7.5-10.0 µm in diameter excluding ornaments; average=9 µm. Basidia not observed. Clamp connections absent.



Fig. 3. Morphological characters of *Scleroderma nastii*. A: Basidiomes of *S. nastii*. B, C: Basidiospores under light microscope (LM), D: Basidiospore under scanning electron microscope (SEM).

Habitat. Solitary to caespitose on soil under leaves and litter of Pinus species.

Specimens examined. Korea. Gyeonggi-do, Pocheon-si, under *Pinus koraiensis*, Jul. 12, 2012, KA12-0553; Gyeonggi-do, Pocheon-si, under *P. koraiensis*, Jul. 27, 2012, KA12-0905; Gyeongsangnam-do, Sancheong-gun, 20 Aug. 2013, KA13-0718; Gyeonggi-do, Pocheon-si, under mixed forest, Oct. 30, 2013, KA13-1555; Gyeonggi-do, Pocheon-si (37°45′12.59″N 127°9′55.52″E, alt. 109 m), Jul. 28, 2016, KA16-0813.

Remarks. According to the original description of *S. nastii* [12], the basidiospores were described as showing an irregular reticulum under SEM. However, SEM photos of the specimens in Korean collections showed that this species had somewhat echinulate basidiospores (Fig. 3). In addition, an original description [12] showed that the habitat of the species was the leaves and litter of *Quercus* trees. However, the Korean samples were often collected from the litter of *Pinus* trees or mixed forests. These findings indicate that this species occurs not only on *Quercus* but also on *Pinus*. This is the second record of the species since the original description.

DISCUSSION

In Korea, seven species of *Scleroderma* (*S. areolatum*, *S. bovista*, *S. cepa*, *S. citrinum*, *S. flavidum*, *S. lycoperdoides*, *S. verrucosum*) have been recorded (Korea National Arboretum; http://nature.go.kr/kfni/index. do) [13]. In the phylogenetic analysis, the phylogenetic positions of five species (*S. areolatum*, *S. bovista*, *S. citrinum*, *S. laeve*, and *S. nastii*) were confirmed based on ITS regions (Fig. 1) [14]. The species of genus *Scleroderma* show similar shapes and sizes of their basidiomes, but phylogenetic analysis based on ITS sequences allowed the *Scleroderma* species that we analyzed to be easily identified. Furthermore, *S. areolatum* can be recognized as the most common species in Korea. Although four *Scleroderma* species (*S. cepa*, *S. flavidum*, *S. lycoperdoides*, and *S. verrucosum*) have been officially recorded in Korea, these species have not been collected in our field forays since 2012. Among these species, *S. verrucosum* has been recorded as a type species in the genus *Scleroderma* (index Fungorum; http://www.indexfungorum.org/). Therefore, additional collection efforts are needed to confirm their existence in Korea. Based on the specimens collected in this study, a morphological taxonomic key for five species from South Korea is provided below.

Key to Scleroderma species from South Korea

1. Basidiospores subreticulated or reticulated	2
- Basidiospores echinulated	
2. Basidia not observed ·····	·····S. bovista
- Basidia present ·····	······S. citrinum
3. Rhizomorphs well developed ·····	4
- Rhizomorphs sessile to shortly substipitate	······S. areolatum
4. Basidiospores small, $> 10 \ \mu m$	·····S. nastii
- Basidiospores larger, < 10 μm ·····	·····S. laeve

CONFLICT OF INTERESTS

No conflict of interest was reported by the author(s).

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