

## RESEARCH ARTICLE

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

Sung-Eun Cho<sup>1</sup>, Young-Nam Kwag<sup>1</sup>, Sang-Kuk Han<sup>1</sup>, Dong-Hyeon Lee<sup>2</sup>, and Chang Sun Kim<sup>1\*</sup><sup>1</sup>Forest Biodiversity Division, Korea National Arboretum, Pocheon 11186, Korea<sup>2</sup>Division of Forest Diseases and Insect Pests, National Institute of Forest Science, Seoul 02455, Korea

\*Corresponding author: changsun84@korea.kr

## ABSTRACT

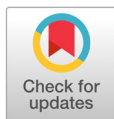
Two new records of *Scleroderma* species from South Korea are described here. Comprehensive taxonomic studies of *Scleroderma* specimens were conducted at the Korea National Arboretum. Based on morphological and molecular data (fungal barcode sequences), two new records (*S. laeve* and *S. nastii*) were 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

## 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.



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## 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 BigDye™ 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.

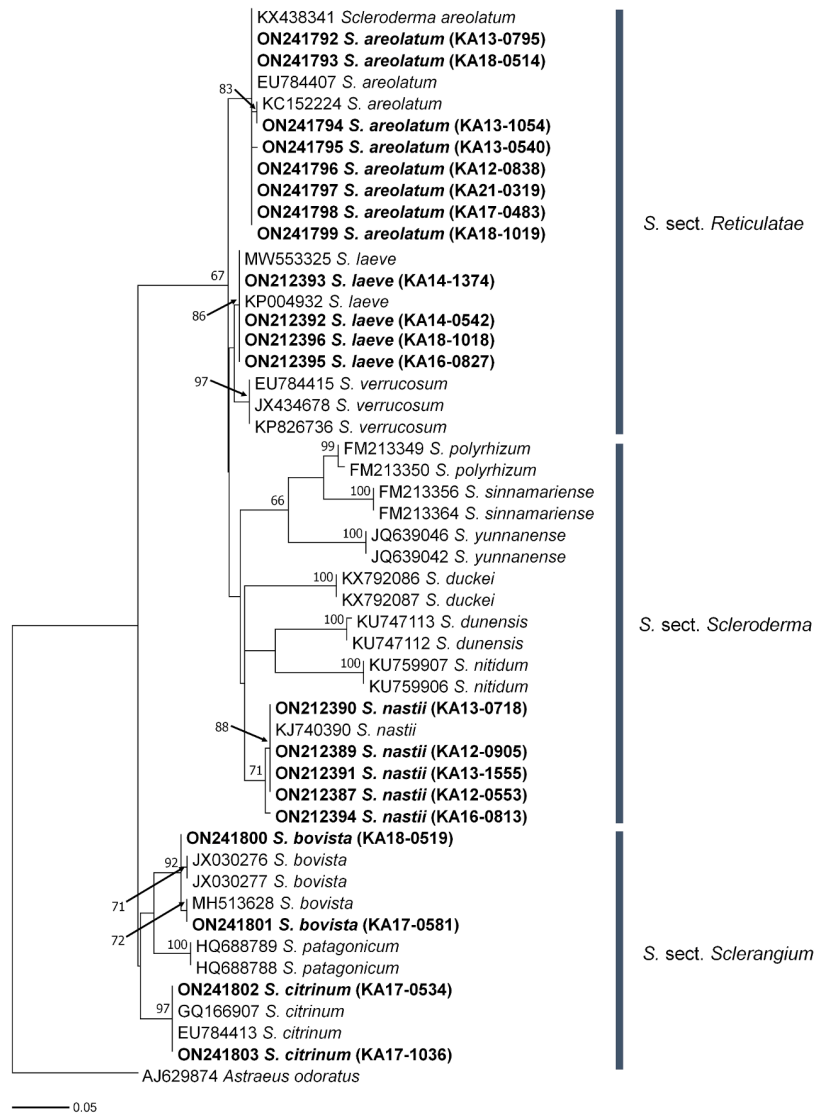
## Results

### 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.

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

Species	Specimen ID	Country	ITS	Reference
<i>S. areolatum</i>	KA12-0838	Korea	ON241796	This study
	KA13-0795	Korea	ON212390	This study
	KA13-0540	Korea	ON241795	This study
	KA13-1054	Korea	ON241794	This study
	KA17-0483	Korea	ON241798	This study
	KA18-0514	Korea	ON241793	This study
	KA18-1019	Korea	ON241799	This study
	KA21-0319	Korea	ON241797	This study
	RBG Kew K(M)125392	England	EU784407	Brock et al. [15]
	GO-2010-148	Mexico	KC152224	-
<i>S. areolatum</i>	M20	Poland	KX438341	Hilszczańska et al. [16]
<i>S. bovista</i>	KA17-0581	Korea	MN294906	Cho et al. [14]
	KA18-0519	Korea	ON241800	This study
	01A_1	USA	JX030276	-
	01Q_1	USA	JX030277	-
	160723-42	China	MH513628	Zhang et al. [8]
<i>S. citrinum</i>	KA17-0534	Korea	ON241802	This study
	KA17-1036	Korea	ON241803	This study
	RBG Kew K(M)17485	England	EU784413	Brock et al. [15]
	F:PRL5772	USA	GQ166907	-
<i>S. duckei</i>	INPA:272127	Brazil	KX792086	Baseia et al. [17]
	UFRN:Fungos 2795	Brazil	KX792087	Baseia et al. [17]
<i>S. dunensis</i>	UFRN:Fungi 1359	Brazil	KU747113	-
	UFRN:Fungi 2033	Brazil	KU747112	-
<i>S. laeve</i>	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	-
<i>S. nastii</i>	KA12-0553	Korea	ON212387	This study
	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]
<i>S. nitidum</i>	Trappe 26232	Argentina	HQ688789	Nouhra et al. [1]
<i>S. nitidum</i>	UFRN:Fungos 2550	Brazil	KU759906	-
	UFRN:Fungos 1759	Brazil	KU759907	-
<i>S. patagonicum</i>	Trappe 26236	Argentina	HQ688788	Nouhra et al. [1]
	Trappe 26232	Argentina	HQ688789	Nouhra et al. [1]
<i>S. polyrhizum</i>	POLSCL1	USA	FM213349	Phosri et al. [18]
	POLSCL2	USA	FM213350	Phosri et al. [18]
<i>S. sinnamariense</i>	SINSCL1 (SCLK4)	Thailand	FM213356	Phosri et al. [18]
	SINSCL9	Thailand	FM213364	Phosri et al. [18]
<i>S. verrucosum</i>	RBG Kew K(M)30670	England	EU784415	Brock et al. [15]
	A4	China	JX434678	-
	CM077	Algeria	KP826736	-
<i>S. yunnanense</i>	Ji003	China	JQ639046	Zhang et al. [19]
	Ji001C	China	JQ639042	Zhang et al. [19]
<i>Astraeus odoratus</i>	ASTRAE-58	Thailand	AJ629874	Phosri et al. [20]



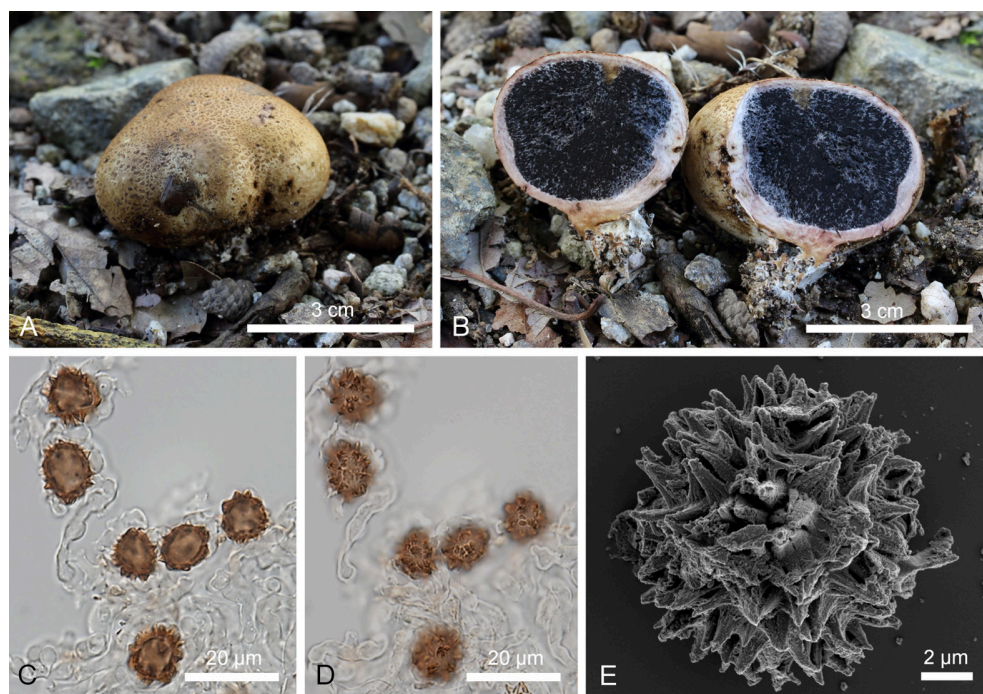
**Fig. 1.** RAxML tree based on internal transcribed spacer (ITS) sequences of *Scleroderma* species. Sequences generated in this study are indicated in bold text.

### 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.

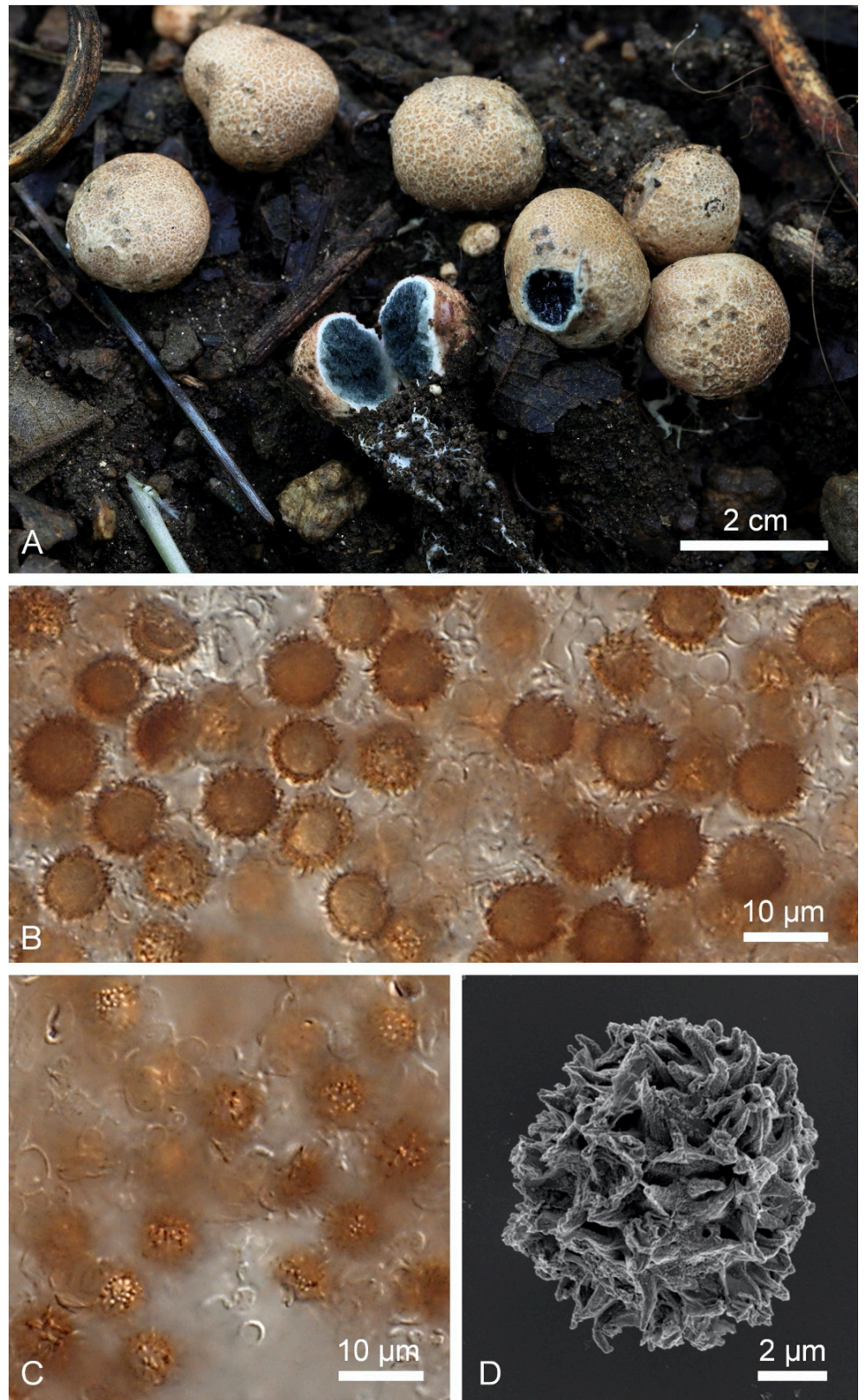
**Specimens examined.** Korea. Jeollanam-do, Suncheon, Jul. 15, 2014, KA14-0542; Gyeonggi-do, Pocheon-si, Sept. 4, 2014, KA14-1374; Gyeonggi-do, Pocheon-si (37°44'57.67"N 127°9'59.44"E, alt. 112 m), Jul. 28, 2016, KA16-0827; Gyeonggi-do, Goyang-si (37°39'32.98"N 126°45'38.32"E, alt. 16.8 m), Sept. 18, 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  $\mu\text{m}$  in diameter including ornaments or 7.5-10.0  $\mu\text{m}$  in diameter excluding ornaments; average=9  $\mu\text{m}$ . 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 echinulate .....3
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 µm .....*S. nastii*
  - Basidiospores larger, < 10 µm .....*S. laeve*

## CONFLICT OF INTERESTS

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

## ACKNOWLEDGEMENTS

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