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Scleroderma yunnanense, a new species from South China

Chunxia Zhang [1], Xiao-E Xu [1], Jing Liu [1], Mingxia He [1], Wenbing Wang [1], Yun Wang [1, 2*] & Kaiping Ji [1]

[1] *Yunnan Institute for Tropical Crop Research, 99 Xuanwei Road, Jinghong 666100, Yunnan, China.*
[2] *The New Zealand Institute for Plant & Food Research Ltd., Private Bag 4704 Christchurch 8140, New Zealand*
* Correspondence to: *Yun.Wang@plantandfood.co.nz*

Abstract — *Scleroderma yunnanense* sp. nov. is described from Yunnan, China. Previously misidentified as *Scleroderma citrinum,* the new species is diagnosed by its echinulate-spiny spores and thick peridium. Molecular analysis supports erection of this new species. Perhaps unique for the genus, *S. yunnanense* is edible and considered a delicacy in Yunnan.

Key words — earth ball, gasteromycete, taxonomy

Introduction

Scleroderma (*Sclerodermataceae*, *Boletales*) has about 25 recognized species (Guzman 1970; Sims et al. 1995; Kirk et al. 2008), of which 10 are found in China (Liu 2005). The genus is widespread from the tropical to boreal regions and common in forests, parks, and farmland. Most mushroom books and scientific papers state that *Scleroderma* species are suspected, undesirable, inedible, or poisonous (Arora 1986; Bresinsky & Besl 1990; Mao 1998; Pacioni 1981; Stevenson & Benjamin 1962). Bresinsky & Besl (1990) state that consumption of raw *Scleroderma cepa* Pers. leads to severe abdominal pain, stomach cramp, and paralysis, while in New Zealand ingestion of an unknown *Scleroderma* species cooked in a casserole resulted in anaphylactic shock (I.R. Hall, pers. comm.). In stark contrast, the authors have found an edible *Scleroderma* species that is extensively collected and sold at local mushroom markets in Yunnan, China, without any reports of adverse affects on humans. The authors, who have eaten this mushroom on a number of occasions, have found it to be not just edible but choice and delicious. This species is commonly found growing in the tropical and warm-subtropical parts of Yunnan Province. Previously this mushroom was recorded from China as *S. citrinum* Pers. (Wang et al. 2004).

However, after re-examining recent collections both morphologically and molecularly we determined that this species has been incorrectly identified and warrants separation as a new species.

Materials & methods

The macroscopic and microscopic characters were described based on fresh and dried specimens collected from several locations in Yunnan, China. Sections were made with a freezing microtome (Jinhua Yidi Medical Equipment Factory, Zhejiang, China) and mounted in water and then lactophenol solution. Dry specimens were mounted in 5% KOH solution and examined under a Nikon E400 microscope. Spore samples were vacuum-dried, sputter-coated with gold, and examined with a FEI QUANTA200 Environmental Scanning Electron Microscope at Yunnan University.

The holotype and other examined specimens are deposited in KUN-HKAS, Kunming, Yunnan, China, except for one specimen held in the Herbarium of the Research Institute

Table 1. *Phlebopus* and *Scleroderma* collections included in the molecular analysis.

Species	Voucher	Origin	Genbank *
P. portentosus	H-01-3-1	China	GQ253574
S. areolatum	JMP0080	USA	EU819438
	RT00036	USA	EU819518
	F: PGK193	USA	GQ166910
S. aurantium	Strain 8-5	China	HM237174
S. bovista	K80S09	New Zealand	GQ267487
	K(M)105588	England	EU784409
	RT00034	USA	EU819517
S. cepa	JMP0081	USA	EU819439
S. citrinum	F:PRL5772	USA	GQ166907
	K(M)17485	England	EU784413
	CITSCL1	USA	FM213344
	K(M)53906	England	EU784414
	—	USA	FJ824090
S. polyrhizum	Strain 11-3	China	HM237173
S. sinnamariense	SINSCL9	Thailand	FM213364
	SINSCL8	Thailand	FM213363
	SINSCL6 (SCLD1)	Thailand	FM213361
	SINSCL7	Thailand	FM213362
	SINSCL2 (SCLP3)	Thailand	FM213357
S. verrucosum	K(M)30670	England	EU784415
S. yunnanense	KUN-HKAS 79633A	China	**JQ639040**
	KUN-HKAS 79633B	China	**JQ639041**
	KUN-HKAS 79633C	China	**JQ639042**
	KUN-HKAS 79633D	China	**JQ639043**
	KUN-HKAS 79664A	China	**JQ639044**
	KUN-HKAS 79664B	China	**JQ639045**
	KUN-HKAS 79665	China	**JQ639046**
	IFRD 414-012	China	FJ687275

*New sequences are presented in bold font.

of Resource Insects of Chinese Academy of Forestry (IFRD), Kunming, Yunnan, China.

Tissue samples were excised from the inner part of fresh basidiomata to avoid contamination. DNA was extracted from each sample with the E.Z.N.A. fungi DNA miniprep kit (Omega Biotech, GA, and USA). The ITS region was amplified with TransTaq™ DNA Polymerase High Fidelity (TransGen Biotech, China). Primer pairs ITS4/ITS5 were used to amplify the ITS region of the ribosomal RNA gene (White et al. 1990). PCR parameters were as follows: initial denaturation for 5 min at 95 °C followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and final extension at 72 °C for 10 min. PCR products were electrophoresed on 1.2% agarose gels with 1× TBE buffer. After purification with a Gel Extraction & PCR Purification Combo Kit (BioTeke, China), the PCR products were ligated into a pMD-18T vector (TakaRa, Japan) for DNA sequencing. Both strands were sequenced using the vector specific primers T7 and M13F (BGI Company). Sequences of seven samples were submitted to GenBank (Table 1). Closely related sequences revealed by BLASTN were obtained from the GenBank DNA database (http://www.ncbi.nlm.nih.gov/entrez/). A total of 29 sequences were used for phylogenetic analyses (Table 1).

A multiple sequence alignment was performed using the ClustalX programme (Thompson et al. 1998). Phylogenies were constructed using the MEGA 4.0 software package (Kumar et al. 2008). Neighbor-Joining (NJ) trees were constructed using the Kimura-2-parameter distance model and Gamma distribution parameter 0.55. Tree robustness was assessed with a 1000-pseudoreplicate Bootstrap analysis (Felsenstein 1985). Branches supported by >50% of bootstrap replicates are indicated on the tree. *Phlebopus portentosus* (GQ253574) was used as the outgroup.

Taxonomy

Scleroderma yunnanense Y. Wang, **sp. nov.** Figs. 1–12

MycoBank MB 805073

Differs from *Scleroderma cepa* by having clamp-connections and from *S. sinnamariense* in its thicker peridium with a hyaline inner surface and its non-reticulate basidiospore ornamentation of dense narrow pyramidal warts.

Type: China, Yunnan Province, Pure, 22°20'N 110°59'E, under *Pinus kesiya* var. *langbianensis*, 15 Sept. 2011: K.P. Ji S1101 (**Holotype**, KUN-HKAS 79633; GenBank JQ639040–JQ639043).

Etymology: *yunnanense* refers to the type locality.

Basidiomata subglobose to globose, 2.5–5.0(–7.0) cm in diameter, with scales, dirty whitish to yellowish, the base attached to the substrate via a tuft of mycelium and rhizomorphs, sometimes aggregated into a pseudostipe, ≤2 cm long, conspicuously covered with well-developed rhizomorphs composed of hyaline hyphae, usually 2.0–3.0(–5) μm in diam., with emanating right branching hyphae and numerous clamp-connections and with some hyphae with adherent crystalline structure. Peridium 2–7 mm thick, two-layered, the outer layer ≤500 μm thick, composed of thin and thick yellowish hyphae

towards the peridial surface, 1.5–2.0(–5) µm in diam.; the inner layer ≤6500 µm thick, composed of hyaline interwoven hyphae, 5.0–7.5 µm diam., clamp-connections rare. KOH on the surface of peridium light reddish and no reaction on peridial cross-section. GLEBA when young firm, white to whitish, and with numerous white capillitia, becoming grayish to purplish then purplish brown and finally powdery, composed of hyaline hyphae, 1.5–2.0 µm in diam., with a few dark, thick hyphae ≤3 µm in diam. BASIDIOSPORES globose to subglobose, (7.0–) 8.4 × 8.0(–9.5) µm including ornamentation, covered with dense narrow pyramidal warts, 1–1.2 µm long and 0.4–0.6(–8) µm thick at the base. ODOR mushroomy; TASTE sweet.

PHYLOGENETIC ANALYSIS — ITS-rDNA sequence analysis groups all *S. yunnanense* collections together with high (99%) bootstrap value and separate from the *S. citrinum* collections, which also group together with high (97%) bootstrap value (FIG. 13).

ECOLOGY & DISTRIBUTION — Sub-hypogeous, solitary or in small groups under *Pinus kesiya* var. *langbianensis* and *Betula alnoides* and forming ectomycorrhizae with one or both of these hosts, alt. 880–1300 m, fruiting from June to October in Yunnan, China. Known only from sub-tropical and tropical regions of Yunnan, China.

ADDITIONAL SPECIMENS EXAMINED: **CHINA, YUNNAN PROVINCE, Pure** — 22°20'N 110°59'E, under *Pinus kesiya* var. *langbianensis,* 15 Sept. 2011: K.P. Ji S1102 (KUN-HKAS 79664; GenBank JQ639044, JQ639045); under *Betula alnoides,* 15 Sept. 2011: K.P. Ji S1103 (KUN-HKAS 79665; GenBank JQ639046); **Puwen** — 22°30'N 101°03'E, under *Pinus kesiya* var. *langbianensis,* 24.Sep.2011: Ji S1104 (KUN-HKAS 79666), Ji S1105 (KUN-HKAS 79667), Ji S1106 (KUN-HKAS 79668); **Yiliang** — 24°92'N 103°14'E, 12.Sep.2007: T. Ma, YL007 (IFRD 414-012, as "*Scleroderma citrinum,*" GenBank FJ687275); **Wuiding** — 25°55'N 102°36'E, 12.Aug.1998: X.H. Wang 742, KUN-HKAS 35824, as "*Scleroderma citrinum*").

COMMENTS —The discovery of an edible *Scleroderma* species in a genus long considered toxic (or at least highly suspect) underlines the difficulties in generalizing on the edibility or toxicity of mushrooms. In the Yunnan area where *S. yunnanense* is widely consumed, there is presumably sufficient general knowledge to prevent the eating of inedible or toxic lookalikes. However, the

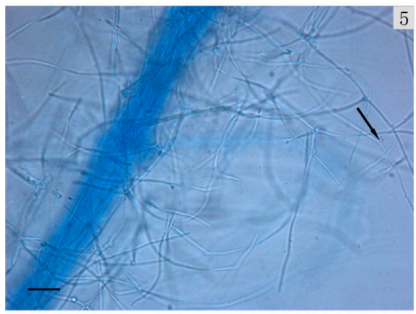
FIGS 1–8. *Scleroderma yunnanense.* 1: Sectioned fruiting body, showing scales and gleba. 2: Fruiting body, showing dense rhizomorphs in the soil. 3: Fruiting body with rhizomorphs, sectioned to show the pseudostipe. 4: Rhizomorphs on the fruiting body surface. 5: Rhizomorphs and emanating hyphae, showing right-angled hyphal branching (arrow) and clamp-connections. 6: Rhizomorphs and emanating hyphae, showing crystalline structure on the hyphae (arrow). 7: Outer peridial layer with vertically arranged hyphae. 8: Inner peridial layer of interwoven hyphae. Scale bars: 1, 3 = 1.5 cm; 2, 4 = 1 cm; 5 = 40 µm; 6 = 25 µm; 7, 8 = 50 µm.

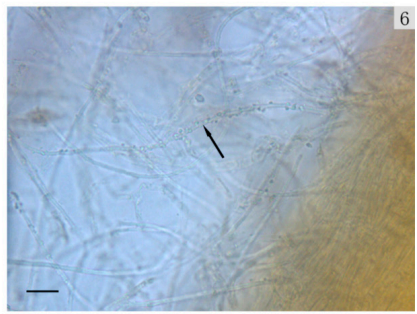

1

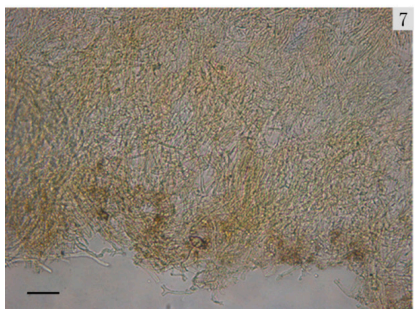

2


3


4


5


6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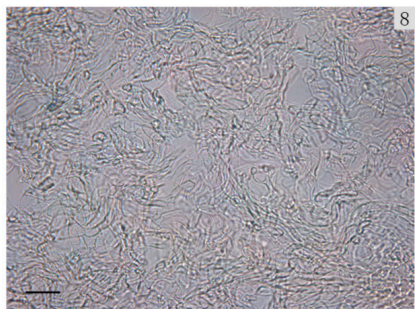
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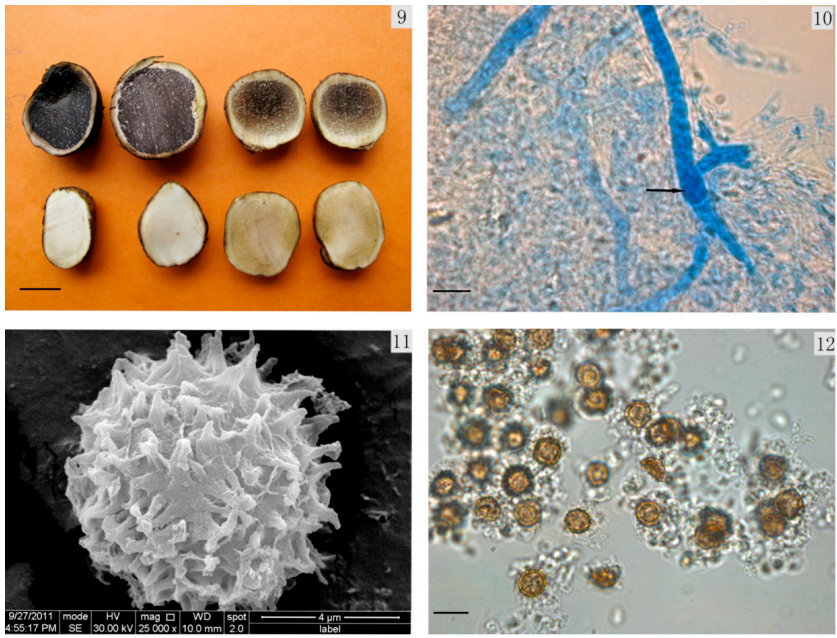

Figs 9–12. *Scleroderma yunnanense*. 9: Basidiomata showing various stages of maturity. 10: Gleba sporogenous tissue, showing thick hyphae (arrow). 11: Basidiospore (SEM). 12: Basidiospores. Scale bars: 9 = 2 cm; 10 = 2.5 µm; 11 = 4 µm; 12 = 10 µm.

likely publicity surrounding the edibility of *S. yunnanense* might now persuade some people to try eating potential lookalikes outside of the natural range of this species. Fortunately, the misidentification of *S. yunnanense* as *S. citrinum* Pers. (a toxic species) should be lessened based on the accurate morphological information that we present.

Morphologically *Scleroderma yunnanense* is similar to *S. cepa* Pers. in having spiny spores and thick peridium. However, it differs in having clamp-connections (Fig. 8). The ITS-rDNA sequence analysis shows that *S. yunnanense* is closely related to *S. sinnamariense* Mont. (Fig. 13). However, *S. sinnamariense* has a thinner (<1 mm) peridium, a bright sulfur yellow inner peridial surface, and partially reticulate spores, all of which clearly differ in *S. yunnanense*.

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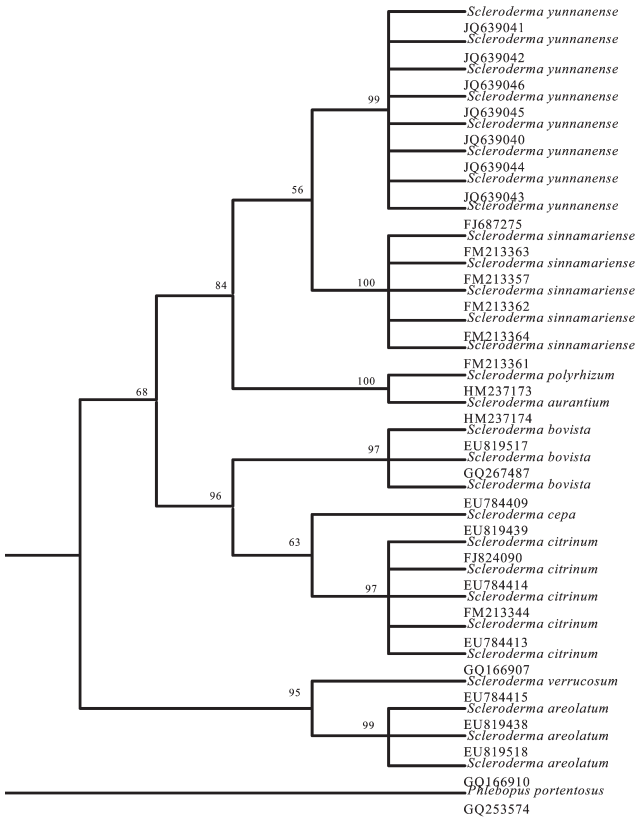

Fig. 13. ITS-based neighbor-joining phylogenetic tree of *Scleroderma* species. Sequences are labeled with their GenBank accession numbers (see Table 1). Bootstrap support values (1000 replications) >50% are shown on the nodes.

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