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Scleroderma suthepense, a new ectomycorrhizal fungus from Thailand

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Abstract — A new species of earthball, described herein as *Scleroderma suthepense,* was found under *Prunus cerasoides* in Doi Suthep-Pui National Park, Thailand. Morphological characteristics indicate it belongs to section *Scleroderma*. Molecular analysis showed differences from previously described *Scleroderma* species. A description, illustration, and comparisons with related taxa are provided.

Key words — gasteromycete, phylogenetic analysis, taxonomy

Introduction

Ectomycorrhizal fungi have symbiotic associations with many tree species in forests around the world and over 7000 species have been described (Taylor & Alexander 2005). *Scleroderma* (*Boletales*; Binder & Hibbett 2006) has worldwide distribution in temperate and tropical regions and forms ectomycorrhizas with a broad range of woody plants including members of families *Caesalpiniaceae*, *Dipterocarpaceae*, *Fagaceae*, *Myrtaceae*, *Phyllanthaceae*, and *Pinaceae* (Munyanziza & Kuyper 1995; Sims et al. 1997; Sanon et al. 2009). There are 131 *Scleroderma* names in Index Fungorum (http://www.indexfungorum.org/Names/Names.asp). In Thailand, only ten species of *Scleroderma* have been reported, *S. areolatum*, *S. bovista*, *S. cepa*, *S. citrinum*, *S. dictyosporum*, *S. flavidum*, *S. lycoperdoides*, *S. polyrhizum*, *S. sinnamariense*, and *S. verrucosum* (Chandrasrikul et al. 2011). *Scleroderma* species have been used to increase the growth of tree seedling, both in nurseries and in the field (Chen et al. 2006). Several species of *Scleroderma* have been proposed based on morphological characteristics of their basidiomes and basidiospores (Guzmán 1970; Sims et al. 1995; Guzmán et al. 2004). Recent studies have also used molecular analysis to analyze inter- and intraspecific variation in *Scleroderma* species (Phosri et al. 2009; Nouhra et al. 2012).

During an investigation of ectomycorrhizal fungi associated with tree species in northern Thailand, we found an interesting species of *Scleroderma* under *Prunus cerasoides* Buch.-Ham. ex D. Don (*Rosaceae*), which we describe as a new species in the present paper. To confirm their taxonomic status the phylogenetic relationship among 25 isolates of *Scleroderma* were also determined by analysis of complete sequences of the internal transcribed spacer (ITS) regions of the RNA gene.

Materials & methods

Morphology studies

Basidiomes of *Scleroderma* were collected from Medicinal Plant Garden (18°48′20″N 98°54′52″E, elevation 1072 m), Doi Suthep-Pui National Park, Chiang Mai Province, Thailand in May 2012. Basidiomes were wrapped in aluminum foil or kept in plastic specimen boxes until transport back to the laboratory where notes on macromorphological features and photographs were taken within 24 h. Macromorphological data were derived from fresh specimens, whereas micromorphological data were derived from dried specimens mounted in 95% ethanol followed by distilled water, 3% KOH or Melzer's reagent. Size data of anatomical features are based on at least 50 measurements of each structure. The specimens were dried at 40–45 °C and deposited at the Research Laboratory for Excellence in Sustainable Development of Biological Resources, Faculty of Science, Chiang Mai University, Thailand (SDBR-CMU).

Molecular studies

Genomic DNA of a dried specimen was extracted according to a CTAB method (Kumla et al. 2012). The ITS regions of the RNA gene were amplified by polymerase chain reaction (PCR) with primers ITS4 and ITS5 under the following thermal conditions: 94 °C for 2 min; 35 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min, and 72 °C for 10 min. The PCR products were then checked on 1% agarose gels stained with ethidium bromide and visualized under UV light. PCR products were purified by using PCR clean up Gel extraction NucleoSpin® Extract II Purification Kit (Macherey-Nagel, Germany, Catalog no. 740 609.50) following the manufacturer's protocol. The purified PCR products were directly sequenced. Sequencing reactions were preformed and the sequences were automatically determined in the genetic analyzer (1ST Base, Malaysia) using the PCR primers mentioned above. Sequences were used to query GenBank via BLAST (http://blast.ddbj.nig.ac.jp/top-e.html). For the phylogenetic analysis, a multiple alignment subroutine in Clustal X (Thompson et al. 1997) and a maximum-parsimony analysis using the PAUP beta 10 software version 4.0 (Swofford 2002) were carried out.

Results

Taxonomic description

Scleroderma suthepense Kumla, Suwannarach & Lumyong, **sp. nov.** PLATE 1

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Differs from *Scleroderma meridionale* by its longer basidiospore spine length.

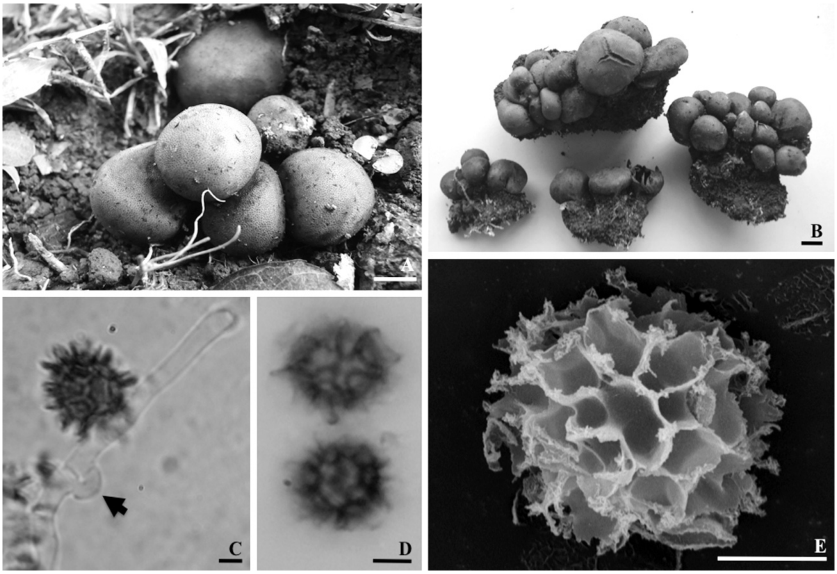

Plate 1. *Scleroderma suthepense.* A, B: Basidiomes. C: Hypha with a clamp connection (arrowed). D: Basidiospores as observed under a compound microscope. E: Basidiospore as observed with a scanning electron microscope. Scale bars: A, B = 1 cm; C–E = 5 µm.

Type—Thailand, Chiang Mai Province, Muang District, Doi Suthep-Pui National Park, Medicinal Plant Garden, 18°48′20″N 98°54′52″E, elevation 1072 m, rainforest dominated by *Prunus cerasoides*, *Cinchona pubescens*, and other trees, on sandy loam, 15 May 2012, Jaturong Kumla & Nakarin Suwannarach (**Holotype**, SDBR-CMU55-SC2; GenBank sequence, JX205215).

Etymology—*suthepense*, referring to Doi Suthep-Pui National Park, where the new species was found.

Basidiomes globose or subglobose 1.1–3.5 cm diameter, 1.0–3.9 cm height, rhizomorphs well developed, white to yellow, 0.5–1.2 cm height. Peridium 0.5–1.0 mm thick when fresh, leathery, surface partially smooth with scattered, small and thin scales, greyish yellow to greyish brown, consisting of two layers. The outer layer consists of cylindrical, thick-walled, yellowish brown hypha up to 8.0 µm diameter, with scattered clamp connections, turning to reddish brown with KOH. The inner layer consists of cylindrical, thick-walled, hyaline hyphae up to 6.0 µm diameter, with clamp connections. Gleba when mature dark greyish brown to back and pulverulent. Basidia not observed. Basidiospores globose to subglobose, strongly reticulate with spines, 8.0–13.0 µm diameter (n = 50) including ornamentation (Plate 1D,E), spine 1.0–2.5 µm in length, dark yellowish brown in water or KOH and not changing in Melzer's reagent.

Ecology & distribution — Terrestrial on sandy loam, under *Prunus cerasoides* in dipterocarp forest; known only from Thailand.

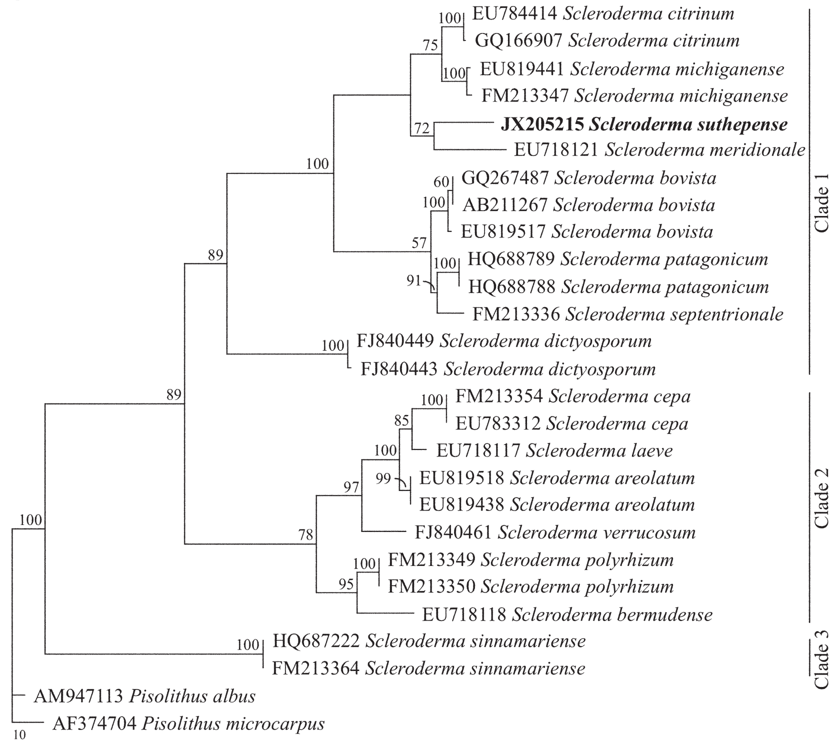

Plate 2. A maximum parsimonious tree inferred from a heuristic search of ITS 1, 5.8S ribosomal RNA gene, and ITS 2 alignments of 27 sequences. *Pisolithus albus* and *P. microcarpus* were used to root the tree. Branches with bootstrap values ≥50% are shown above each branch and the bar represents 10 substitutions per nucleotide position.

Molecular analysis

The 679-bp ITS sequence of *S. suthepense* (JX205215) was deposited in GenBank and analyzed phylogenetically with 24 other *Scleroderma* sequences and the outgroup (*Pisolithus albus* and *P. microcarpus*) obtained from the GenBank database. Heuristic searches produced a tree length of 803 steps, CI = 0.685, RI = 0.823, RC = 0.563 and HI = 0.315. One maximum-parsimony tree is shown in Plate 2. A phylogenetic dendrogram separates *Scleroderma* fungi into three clades. Clade 1 contains species with reticulate spores (sect. *Scleroderma*), while clades 2 and 3 include species with spiny or subreticulate spores. *Scleroderma suthepense* stands within clade 1 together with *S. bovista*, *S. citrinum*, *S. dictyosporum*, *S. meridionale*, *S. michiganense*, *S. patagonicum*, and *S. septentrionale* and forms a sister taxon with *S. meridionale* (72% bootstrap support). Clade 2 includes six species (*S. areolatum*, *S. bermudense, S. cepa*, *S. laeve*, *S. polyrhizum, S. verrucosum*). Clade 3 comprises a single species, *S. sinnamariense*.

Discussion

Based on basidiospore morphology, *Scleroderma* species are separated into three sections; sect. *Aculeatispora* (spiny spore), sect. *Sclerangium* (subreticulate spore), and sect. *Scleroderma* (reticulate spore) (Guzmán 1970; Sims et al. 1995, 1997). However, it is difficult to distinguish *Scleroderma* species based only on morphology because basidiome size and shape change depending on the soil and environment (Sanon et al. 2009). *Scleroderma suthepense*, described as a new species in the present study, was found beneath *Prunus cerasoides* and is placed in sect. *Scleroderma* based on its reticulate basidiospores and hyphal clamp connections. Its basidiospores are similar in size to those of *S. dictyosporum*, *S. meridionale,* and *S. septentrionale* (Table 1). Our ITS sequence analysis clearly separates *S. suthepense* from the other reticulate spored *Scleroderma* species in sect. *Scleroderma,* although *S. suthepense* appears closely related to *S. meridionale*, which has a shorter basidiospore spine length (0.5–1.5 μm) and is reported only in North America (Sims et al. 1995; Guzmán & Ovrebo 2000). Our analysis separates the reticulate spored clade (clade 1) and the spiny or subreticulate spored clades (clades 2 and 3) with 89% bootstrap support (Plate 2). This result was similar to previous molecular phylogenetic studies, which separated *Scleroderma* species into two moderately to strongly supported clades (Phosri et al. 2009; Nouhra et al. 2012). Further studies are required to confirm an ectomycorrhizal synthesis between *S. suthepense* and various host plants.

Table 1. Basidiospore sizes of *Scleroderma suthepense* and other species in *Scleroderma* sect. *Scleroderma*.

Species	Spore diameter (μm)	References
S. bovista	11–16	Sims et al. (1995), Cortez et al. (2011)
S. citrinum	11–16	Sims et al. (1995), Cortez et al. (2011)
S. dictyosporum	9–14	Guzmán (1970), Cortez et al. (2011)
S. hypogaeum	17–20	Sims et al. (1995)
S. meridionale	8–17	Sims et al. (1995), Guzmán & Ovrebo (2000)
S. michiganense	14–20	Sims et al. (1995)
S. minutisporum	4–7	Alfredo et al. (2012)
S. patagonicum	19–28	Nouhra et al. (2012)
S. septentrionale	8–15	Jeppson & Piatek (2005)
S. suthepense	8–13	Present study

Such knowledge is important for selection and management of ectomycorrhizal fungi for greenhouse and in field inoculation programs in Thailand.

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