Mycologia, 104(2), 2012, pp. 488–495. DOI: 10.3852/11-082

The species of *Scleroderma* from Argentina, including a new species from the *Nothofagus* forest

Eduardo R. Nouhra
Maria L. Hernández Caffot[1]
Nicolás Pastor

Instituto Multidisciplinario de Biología Vegetal, CONICET, Universidad Nacional de Córdoba, CC 495, 5000, Córdoba, Argentina

Esteban M. Crespo

Cátedra de Diversidad Vegetal I, Facultad de Química Bioquímica y Farmacia, Universidad Nacional de San Luis, 5700 San Luis, Argentina

Abstract: Five ectomycorrhizal species of *Scleroderma* were identified from herbarium and field-collected specimens from Argentina. A new hypogeous species, *Scleroderma patagonicum*, was recorded in association with native *Nothofagus* spp. in Patagonia. The epigeous species *S. albidum*, *S. areolatum*, *S. bovista and S. citrinum* were associated with various exotic tree species. A phylogenetic analysis based on the ITS region of *Scleroderma* species, including *S. patagonicum*, illustrates its distinct status within *Scleroderma*, including its placement among species with reticulate spores. Descriptions with SEM images of the spores and a key to the species are provided.

Key words: Argentina, exotic fungi, hypogeous *Scleroderma*, *Nothofagus* spp.

Introduction

From the early 20th century to the present *Eucalyptus*, Pinaceae and various northern hemisphere deciduous tree species have been planted extensively in Argentina (Dunstan et al. 1998). Most plantations comprise *Pinus* species, such as *P. elliottii* Engelm., *P. halepensis* Mill., *P. patula* Schiede ex Schltdl. & Cham., *P. ponderosa* Douglas ex Lawson & C. Lawson, *P. radiata* D. Don, and *P. taeda* L. (Izurieta et al. 1998, Mangieri 1977), as well as *Eucalyptus* species such as *E. camaldulensis* Dehnh., *E. sideroxylon* A. Cunn. ex Woolls, *E. tereticornis* Sm. and *E. viminalis* Labill. (Golfari 1985).

With the introduction of these tree species various ectomycorrhizal fungi also were introduced and became established in the exotic plantations. *Scleroderma* is one of the most adaptable and widespread genera of ectomycorrhizal fungi associated with forest tree plantations. Several *Scleroderma* isolates are used currently as ectomycorrhizal inoculum in tropical ecosystems (Marx et al. 1991, Sanon et al. 2009, Watling 2006).

Ectomycorrhizal fungi associated with exotic forest have been surveyed extensively elsewhere in the southern hemisphere (e.g. Birch 1937, Chu-Chou 1979, Chu-Chou and Grace 1983, Dunstan et al. 1998, Garrido 1986, Giachini et al. 2000). However in Argentina only a few mycologists have paid attention to ectomycorrhizal fungi (Barroetaveña et al. 2005, 2006, 2007; Becerra et al. 2002, 2005; Nouhra et al. 2008; Nuñez et al. 2009) and studies on sclerodermatoid fungi are non-existent.

The genus *Scleroderma* comprises gasteroid basidiomycetes with reticulate to echinulate globose spores (Sims et al. 1995). Several morphological and molecular studies have confirmed the systematic postition of the genus, placing it in the suborder Sclerodermatineae within the Boletales (Binder and Bresinsky 2002, Hughey et al. 2000, Louzan et al. 2007, Wilson et al. 2011). Most lineages within this suborder are recognized ectomycorrhizal taxa (Binder and Hibbett 2006, Watling 2006).

The aim of this study is to review and identify collections of *Scleroderma* from herbaria and field collections in Argentina. This includes collections from both native forests and plantations of exotic tree species. From these collections we discovered and herein describe the new taxon *Scleroderma patagonicum*. To provide a phylogenetic context for this species the ITS region was sequenced and compared to ITS data from related taxa. Descriptions of *Scleroderma* species from Argentina, including diagnostically informative macro and micro characters, SEM photography of the spores and a key to the species, are provided to aid identification.

Materials and methods

Basidiomata sampling.—Field-collected basidiomata were obtained in *Pinus* and *Eucalyptus* plantations in central Argentina, mostly during summer and autumn (Dec–Apr) 2004–2007, and in *Nothofagus pumilio* (Poepp. & Endl.) Krasser, *N. alpina* (Poepp. & Endl.) Oerst., *N. obliqua* (Mirb.) Oerst. and *N. dombeyi* (Mirb.) Oerst. forests in northern Patagonia in 2001. In all cases specimens were obtained by hand from the soil surface or by uncovering the basidiomata by raking forest litter. Field notes included

Submitted 10 Mar 2011; accepted for publication 19 Aug 2011.
[1] Corresponding author. E-mail: lhernandezcaffot@hotmail.com

TABLE I. GenBank accession numbers and collections of the taxa used in the phylogenetic analysis

Collection	Herbarium number	Origin	ITS accession No.
S. areolatum	JMP00080	USA	EU819438
S. areolatum	E00278290	USA	FM213352
S. bovista	RBG Kew K(M) 105588	England	EU784409
S. bovista	RT00034	USA	EU819517
S. cepa	RBG Kew K(M) 54373	England	EU784412
S. cepa	BCN-MPM 2525	Spain	FM213354
S. citrinum	RBG Kew K(M) 53906	England	EU784414
S. citrinum	F:PRL5772	USA	GQ166907
S. michiganense	E00278311	USA	FM213347
S. michiganense	JMP0083	USA	EU819441
S. patagonicum	J Trappe 26232 (CORD)	Argentina	HQ688788
S. patagonicum	J Trappe 26236 (CORD)	Argentina	HQ688789
S. polyrhizum	E00278315	USA	FM213349
S. polyrhizum	E00278313	USA	FM213350
S. septentrionale	A.D.Parker (2/10/97)	USA	FM213338
S. sinnamariense	SCLK4	Thailand	FM213356
S. sinnamariense	SINSCL_9	Thailand	FM213364
Pisolithus sp.	PISOLI_12	Thailand	AJ629887
Suillus sp.	K9158	New Zealand	GQ267488

information on location and associated hosts for each species along with a description of fresh macroscopic characteristics. Hypogeous specimens were cut in half and dried on a forced-air dehydrator at ± 40 C.

Herbarium material.—In addition to field sampled specimens several collections from different Argentinean herbaria also were studied and their characters are included in the species revision and key of characters. These herbaria were Museo Botánico de Córdoba (CORD); Herbario Micológico, Facultad de Ciencias Exactas y Naturales of the Universidad de Buenos Aires (BAFC); Instituto Spegazzini of the Universidad Nacional de la Plata and Instituto Miguel Lillo (LIL), Universidad Nacional de Tucumán.

Basidiomata identification and description.—For fresh collections, color, size, shape and other macro characters were recorded. Most collections were photographed in surface and cross-sectional views. Color names of fresh and dry specimens are in general terms described in the literature for different species. Otherwise color is used in accordance with Ridgway (1912).

Tissues for describing microscopic characters from both dry and recently collected specimens were prepared with a Zeiss stereo microscope at 10–40× magnification, rehydrated with water and 5% KOH and tested for chemical composition with Melzer's reagent, cotton blue and phloxine. Spore dimensions, including ornamentation, are based on 15 spores for each basidioma, including exceptional dimensions in parentheses. Microscopic characters were observed and photographed with a Zeiss Axiophot light microscope at 200–1000× magnification. Scanning electron microscopy (SEM) of spores was made with a Zeiss LEO 1450VP. Identification of taxa was aided by keys and specific references (Cunningham 1979; Domínguez de Toledo 1989; Guzmán 1967, 1970; Sims et al. 1995).

Molecular and phylogenetic analysis.—DNA was extracted from gleba tissue of dry basidiomata by CTAB chloroform method (Rogers and Bendich 1994). The ITS region, including the 5.8S rDNA locus, was amplified via PCR with ITS1-F and ITS4 primer pair (Gardes and Bruns 1993). PCR reactions were performed in 50 µL reaction tubes with 1.1 × Reddy Mix™ PCR Master Mix (2.5 mM $MgCl_2$) (ABgene®, Thermo Fisher Scientific Inc., UK) according to manufacturer instructions. PCR products were checked for positive amplification on 1% agarose gels. Amplified products were sent to Macrogen Inc. (Seoul, South Korea) for purification and sequencing with the BigDye™ terminator kit and run on ABI 3730XL.

ITS sequences of *Scleroderma patagonicum* collections generated for this study have been deposited into GenBank (TABLE I). These were combined into a dataset with additional GenBank sequences chosen based on completeness, provenance and use in recently published studies. A total of 19 sequences, including the outgroup taxa *Pisolithus* sp. (AJ629887) and *Suillus* sp. (GQ267488), were used for analyses. Alignment of the ITS dataset was performed with BioEdit 7.0.5.3 (Hall 1999). Further manual alignment was performed with MacClade 4.4.08 (Maddison and Maddison 2005).

Maximum parsimony (MP) analysis was done with PAUP 4.0b10 (Swofford 2002). The analysis was conducted under an equally weighted parsimony criterion with a heuristic search with TBR branch swapping, MULTREES option on and 1000 replicates of random addition sequences. Support for the individual nodes was tested with a parsimony bootstrap (BS) analysis using 1000 BS replicates, TBR and MULTREES option on, with simple random addition sequence.

RESULTS

Morphology.—Five species were clearly defined with morphological analysis. Only *Scleroderma patagonicum*

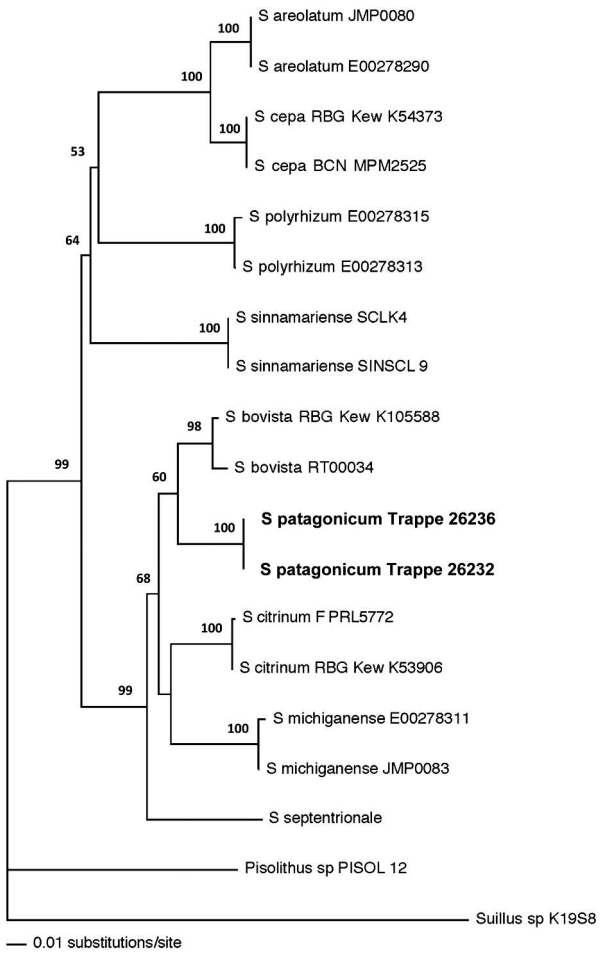

FIG. 1. One of two most parsimonious trees obtained from the analysis of nuclear ITS1, ITS2 and 5.8S rDNA markers. Bootstrap values are indicated at the respective internodes.

was registered in association with native vegetation. In addition four species were identified from herbarium and field-collected specimens as follows: *S. albidum*, *S. areolatum*, *S. bovista* and *S. citrinum*. Species misidentification and outdated synonymy were observed in herbarium collections (see SUPPLEMENTARY TABLE I).

Molecular analysis.—The final alignment had 19 sequences; characters were of type ''unord'' and have equal weight. Of the total 779 characters, 530 were uninformative and 249 were parsimony informative. Under a heuristic search two most parsimonious trees were obtained with a length of 715 steps, CI = 0.7510, RI = 0.7933 and RC = 0.5958. The phylogenetic analysis based on the ITS1, ITS2 and 5.8S rDNA loci provided strong support for considering *Scleroderma patagonicum* as a distinct species with 100% bootstrap support (FIG. 1). This topology clearly shows the placement of *S. patagonicum* clustered within the clade constituted by species with reticulate spores (*S. bovista*, *S. citrinum*, *S. michiganense* and *S. septentrionale*). In our analysis this cluster is well supported (99% BS).

TAXONOMY

Scleroderma patagonicum Nouhra & Hernández Caffot sp. nov. FIG. 2

MycoBank MB561772

Basidioma hypogaea, subglobosa vel globosa, 5–18 mm × 7–28 mm. Peridium ± 1 mm crassum, in juventute luteum brunneum, in maturitate atrum brunneum. Rhizomorphis sparsis vel abundis, concoloribus, appressis. Gleba brunnea atra vel atra, loculata, 300–900 µm, pulveracea. Sporis brunneis, globosis, 19–28 µm cum ornamentis reticulatis-cristatis. In *Nothofagus obliqua*, *N. dombeyi* et *N. alpina* silva.

Basidiomata hypogeous, up to 2.8 cm diam × 1.8 cm high, soft, subglobose to irregularly lobed, sessile, with well developed rhizomorphs aggregated at the base from a point of attachment, with a few rhizomorphs appressed to the sides, concolorous at first, then dark (FIG. 2a). Odor earthy to slightly musty. Mature specimens quite soft and powdery. Surface glabrous, off white when young and staining slightly pink where cut, then yellowish brown to dark brown at maturity (cinnamon buff to clay), with many aggregated soil particles, off white in cross section.

Peridium 450–600 µm thick, whitish in cross section, consisting of two layers. The outer constitutes cylindrical, brownish, clamped hyphae, up to 9 µm diam, walls up to 1.6 µm thick. Hyphae are cylindrical in cross section and are displayed in a tightly intermingled structure, with patches of dark, vertical hyphae with rounded ends in the surface (FIG. 2b). The inner section of this layer consists of similar clamped hyphae, although they are thin-walled and hyaline, up to 9 µm diam. A second layer consists of a pseudoparenchymatic structure of irregular to prismatic hyaline hyphae, 5–15 µm diam, intermingled with bundles of cylindrical, clamped, brownish hyphae; scattered oxalate crystals present. Conductive hyphae up to 5 µm diam were present in both layers. Exoperidium turning reddish brown with KOH.

Gleba when fresh white in youth, later becoming dark brown to black (dark grayish olive to olivaceous black), with whitish trama veins and constituted of globose chambers of 300–900 µm diam, black and powdery at maturity. Gleba containing spores, nurse cells and clamped, thin-walled, hyaline to yellowish brown hyphae (FIG. 2c). Tramal hyphae branched with some inflated septa, 5–16 µm diam with infrequent to abundant, prominent clamp connections.

Spores (13–)19–24(–28) µm diam including ornamentation, globose to subglobose, yellowish brown in KOH, reticulate (FIG. 2d, e); reticulum alveolate to

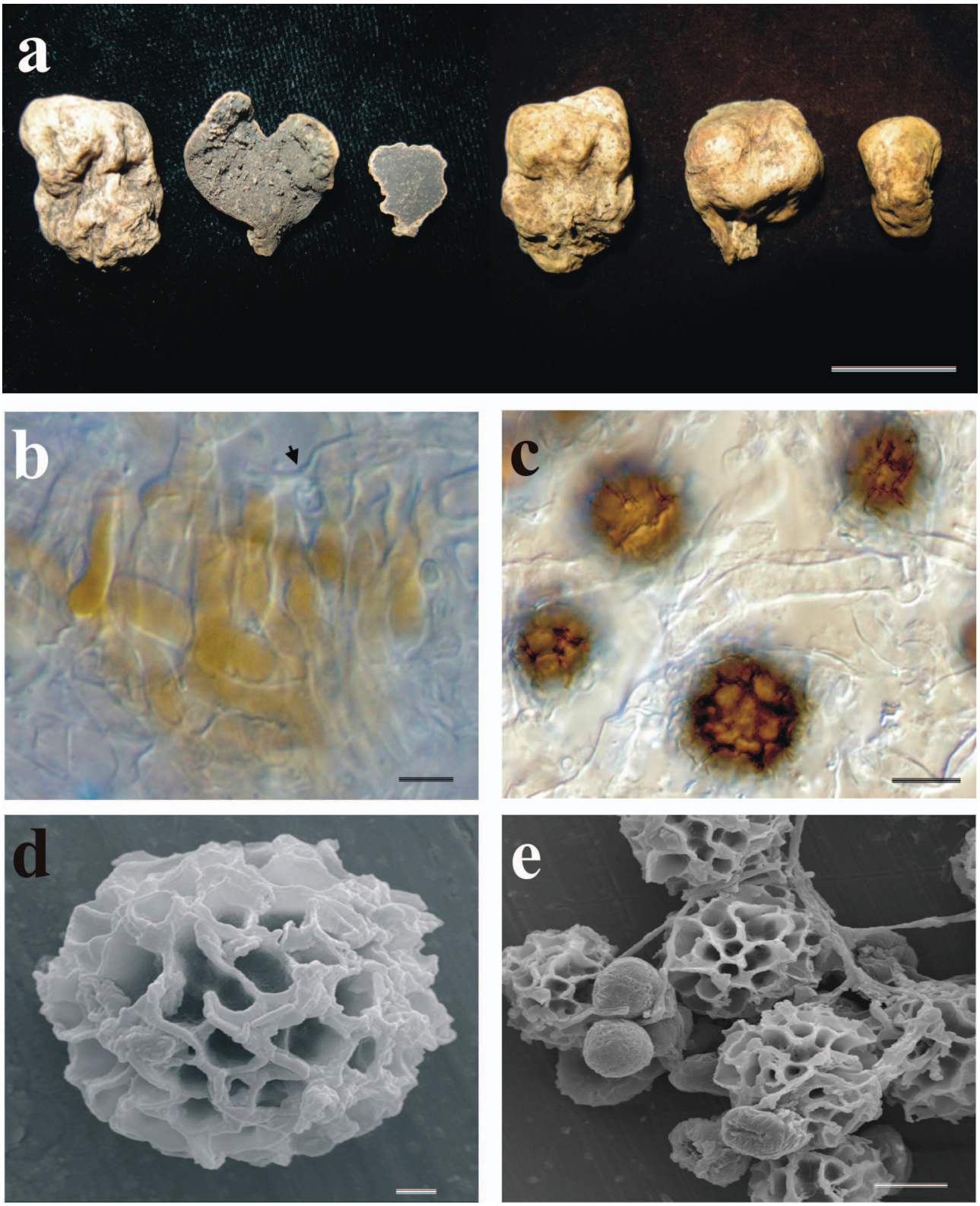

FIG. 2. Macro- and microscopic features of *Scleroderma patagonicum*. a. Basidioma. Bar = 1 cm. b. Outer peridium with cylindrical, brownish and clamped hyphae. Bar = 10 µm. c. Spores with clamped hyphae. Bar = 10 µm. d. Spore. Bar = 2 µm. e. Spores with nurse cells. Bar = 10 µm.

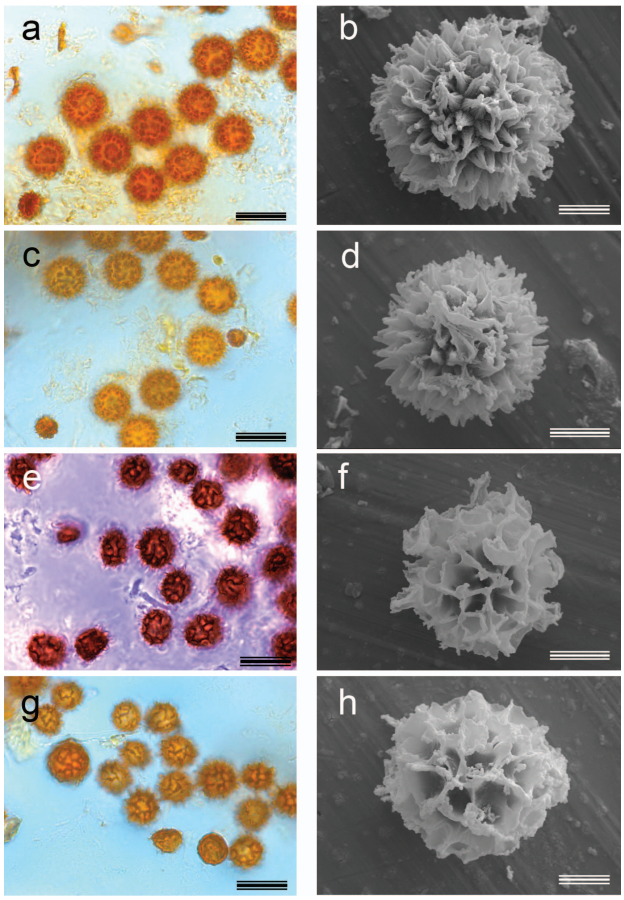

FIG. 3. Microscopic features of *Scleroderma* species from Argentina. a–b. *S. albidum* spores. c–d. *S. areolatum* spores. e–f. *S. bovista* spores. g–h. *S. citrinum* spores. Bars: a, c, e, g = 20 µm; b, d, f, h = 2 µm.

sinuous, 1–3.5(–4) µm high; immature spores almost smooth; apiculus visible.

Holotype: ARGENTINA. NEUQUEN: Hua Hum Road (48), Lanin National Park, 3.5 km from Chilean Border, 40°7.920′S, 71°38.575′W, 25-IV-2001, *J Trappe, 26236* (CORD, ISOTYPE OSC).

Habitat, host and season.—Basidiomata occurring single or in groups up to 20 between the organic debris and mineral soil in mixed forest of *Nothofagus obliqua*, *N. dombeyi* and *N. alpina* in autumn (Apr).

Etymology: Latin epithet *patagonicum* refers to the species distribution.

Specimens examined. ARGENTINA. NEUQUEN: 1.5 km from the Chilean Border, 40°7.219′S, 71°39.302′W, 24-IV-2001, *J. Trappe, 26232* (PARATYPES, CORD, OSC); ibidem, 1 km from Chilean Border, 40°7.174′S; 71°39.432′W, 25-IV-2001, *J. Trappe, 26219* (CORD, OSC).

Comments.—This is the first species recorded occurring in native forests and the first record of a *Scleroderma* species growing with *Nothofagus*.

Scleroderma albidum Pat. et Trab., Bull. Soc. mycol. Fr. 15(1):57 (1899).

Basidiomata 2–6 cm diam, globose to subglobose, sessile or possessing rhizomorphs aggregated at the base, sometimes forming a well developed pseudostipe. Surface smooth, scaly or cracked near the top, scales irregular up to 6 mm diam. Peridium somewhat rubbery when fresh, pale yellowish brown, bruising reddish brown, drying pale brown. Dehiscence stellate observed, but not frequent, occurring by rupture of the apical portion.

Peridium 1200–1400 µm thick, consisting of two layers; the outer peridium consists of cylindrical, yellowish brown hyphae from 3–6 µm diam, turning reddish brown with KOH; the inner layer consists of pseudoparenchymatic, thin-walled, hyaline hyphae 4–8 µm diam. Conductive hyphae present, irregular, yellowish brown with amorphous content, up to 5 µm diam. Clamp connections not observed. Gleba powdery at maturity, grayish green with yellowish tramal veins; constituted by spores, nurse cells and clamped hyphae.

Spores globose, echinulate, dark brown in KOH, (10.5–)12–14.5(–16.5) µm diam including ornamentation of crowded blunt spines (FIG. 3a, b). Basidia not observed.

Habitat, host and season.—Epigeous, exceptionally sub-hypogeous in soil, under leaves and litter in mixed plantation of *Pinus* spp., Mar–Jul.

Specimens examined: (see SUPPLEMENTARY TABLE I).

Comments.—Macroscopically similar to *S. bovista*, however *S. albidum* has a lighter peridium, echinulate spores and stellate dehiscence in old specimens.

Scleroderma areolatum Ehrenb., Sylv. Mycol. Berol. (Berlin) 15:27 (1818).

Basidiomata 2–4 cm diam, subglobose to pyriform, flattened on top, sessile or with small pseudostipe, with rhizomorphs aggregated at the base. Surface smooth in young specimens, developing polygonal scales on the apical portion; scales brown, distinctly dark. Peridium rubbery when fresh, yellowish white, paler when mature, some specimens distinctly yellow. Dehiscence occurs through an irregular apical pore or by rupture of the top portion.

Peridium 800–1000 µm thick when fresh, consisting of two layers; outer layer thin and not continuous. The outer layer consists of thin-walled interwoven brownish to hyaline hyphae, turning yellowish to reddish brown with KOH. The inner layer is a pseudoparenchymatic structure of hyaline hyphae, thick-walled up to 8 µm diam. Clamp connections not observed; conductive hyphae common. Gleba pow-

dery at maturity, brownish violet to dark olivaceous with abundant yellowish trama veins.

Spores globose, yellowish brown in KOH, echinulate, spines crowded, tapering to sharp points in mature spores, (10–)11–16(–17) µm diam including ornamentation, (FIG. 3c, d). Basidia not observed.

Habitat, host and season.—Epigeous to sub-hypogeous in soil, under leaves and litter of *Pinus* spp., *Populus* sp. and *Quercus* sp., Oct–May.

Specimens examined. (see SUPPLEMENTARY TABLE I).

Comments.—*S. areolatum* is easily distinguished by its thin yellowish peridium and small basidiomata.

Scleroderma bovista Fr., Syst. mycol. (Lundae) 3(1):48 (1829).

Basidiomata 2–7 cm diam, globose to subglobose, sometimes flattened on top; rhizomorphs aggregated at the base, sometimes forming a short basal cluster that retains soil particles. Surface smooth, scaly or finely cracked near the top; scales irregular up to 3 µm diam. Peridium somewhat rubbery when fresh, whitish to light brown or pale yellowish brown, bruising reddish brown to dark brown, drying pale brown. Dehiscence produced by rupture or cracking of the upper surface.

Peridium 900–1400 µm thick, consisting of two layers; the outer layer consist of cylindrical, thin-walled, yellowish to hyaline hyphae up to 6 µm diam, with scattered clamp connections. The inner layer consists of cylindrical, thick-walled (± 1 µm), hyaline hyphae up to 6 µm diam, conductive hyphae yellowish brown, 3–5 µm diam. Gleba grayish green with yellowish trama veins becoming powdery at maturity constituted by spores, nurse cells and clamped hyphae.

Spores globose, dark yellowish brown in KOH, reticulate with spines, (11–)12–14(–16) µm diam including ornamentation (FIG. 3e, f). Basidia not observed.

Habitat, host and season.—Epigeous or sub-hypogeous in forest litter and organic soil, growing under *Pinus radiata*, *P. elliottii*, *Cedrus* sp., *Quercus* sp. and *Betula* sp., Oct–Jun.

Specimens examined. (see SUPPLEMENTARY TABLE I)

Comments.—This exotic species is one of the most common in pine plantations, parks and forested gardens in Argentina. The combination of spines and reticulum on the spore wall, in addition to the peridium coloration aids the separation of *S. bovista* from other *Scleroderma* species in the region.

Scleroderma citrinum Pers., Syn. meth. fung. (Göttingen) 1:153 (1801).

Basidiomata globose to subglobose, sometimes flattened on top, 2–8 cm diam; sessile or with rhizomorphs aggregated at the base, rarely developing a pseudostipe. Surface smooth at the base, scaly and cracked at the sides and top, scales irregular or triangular, single or aggregated, concolorous or darker. Peridium somewhat rubbery when fresh, pale yellowish, to pale brown, some specimens notoriously yellow. Dehiscence occurs through an irregular apical pore or by rupture of the top portion.

Peridium 2000–3000 µm thick when fresh, consisting of three layers. The outer layer is thin and not continuous, forming a hymeniform structure of thin-walled, brownish hyphae, and it turns reddish brown with KOH. The middle layer is formed by interwoven hyphae forming a pseudoparenchimatic structure of hyaline hyphae up to 8 µm diam. The inner layer is less differentiated and hyphae usually thinner, up to 5 µm. Clamp connections present. Gleba brownish violet to olivaceous with yellowish trama veins, becoming powdery at maturity, constituted by spores, nurse cells and clamped hyphae.

Spores globose, yellowish brown in KOH, reticulate with spines, (9.5–)11–14(–16) µm diam including ornamentation (FIG. 3g, h). With SEM the reticulum is well developed but less intricate than in *S. bovista*. Basidia not observed.

Habitat, host and season.—Epigeous exceptionally sub-hypogeous, in soil, under leaves and litter in mixed plantation of *Pinus* spp. and *Betula* sp., Dec–May.

Specimens examined. (see SUPPLEMENTARY TABLE I).

Comments.—The most obvious features in *S. citrinum* are the combination of its yellowish coloration and abundant scales on the sides and top portion of the basidiomata.

KEY TO *SCLERODERMA* SPECIES FROM ARGENTINA

1. Basidiomata hypogeous, subglobose, irregular, associated with native *Nothofagus* forests (Patagonia). Spores globose to subglobose, reticulate, (13–)19–24(–28) µm diam including ornamentation . . . . . *S. patagonicum*
1′. Basidiomata epigeous, sessile or having a short sterile base of aggregated rhizomorphs. Species associated with exotic conifers and angiosperm trees 2
2. Basidiospores with echinulate ornamentation . . . 3
2′. Basidiospores with reticulate ornamentation . . . 4
3. Basidiomata subglobose to pyriform, 2–4 cm diam without a pseudostipe. Peridium thin, membranaceous and scaly, yellowish white, paler when mature, in some specimens notoriously yellow. Scales polygonal, brown, distinctly darker than the surface. Spores (10–)11–16(–17) µm diam; spines crowded, tapering to a sharp point in mature spores . . . . . *S. areolatum*

3′. Basidiomata globose to subglobose, 2–6 cm diam; sometimes forming a well developed pseudostipe. Peridium thick and rubbery when fresh, smooth, scaly or cracked near the top, pale yellowish brown, bruising reddish brown. Scales irregular up to 6 mm diam. Spores (10.5–)12–14.5(–16.5) µm diam with crowded blunt spines . . . . . *S. albidum*

4. Peridium smooth, scaly or finely cracked near the top, whitish to light brown or pale yellowish brown, bruising reddish brown to dark brown. Spores (11–)12–14(–16) µm diam, with a well developed and intricate reticulum . . . . . *S. bovista*

4′. Peridium smooth at the base, scaly and cracked; scales irregular or triangular, single or aggregated, pale yellowish to pale brown, in some specimens notoriously yellow. Spores (9.5–)11–14(–16) µm diam with a less intricate reticulum and with isolated spines . . *S. citrinum*

DISCUSSION

From the morphological analysis of more than 120 collections five species of *Scleroderma* were clearly differentiated and identified. From these, the new species *S. patagonicum* was the sole *Scleroderma* species associated with native forests in Argentina and the first record of *Scleroderma* in association with *Nothofagus*. The four remaining species, *S. albidum*, *S. areolatum*, *S. bovista* and *S. citrinum*, were always associated with exotic trees either from plantations or parks and gardens. Further sampling within native habitats, in particular the extensive areas dominated by *Nothofagus* forests in Patagonia, might reveal new species of *Scleroderma*. Several herbarium collections (23) were misidentified or currently labeled under outdated synonyms. Observation of key characters, such as spore ornamentation, peridium structure and associated hosts, aid their identification. In the case of echinulate spores as in *S. albidum* and *S. areolatum* SEM photography also was helpful to differentiate similar ornamentation types. However due to the intra-specific variability and inter-specific similarity observed in the spore ornamentation, this character by itself can be misleading when determining taxa at species level.

Phylogenetic analysis in addition to the unique morphological features, the hypogeous habit and the endemic character of *Scleroderma patagonicum* support its description as a distinct species. The phylogenetic analysis shows the placement of *S. patagonicum* among other species with reticulate spores comprising *S. bovista*, *S. citrinum*, *S. michiganense* and *S. septentrionale*. The value of spore ornamentation in determining the relationships between *Scleroderma* species had been highlighted by Guzmán and Ovrebo (2000). This group of reticulate spored *Scleroderma* was given high statistical support in our analysis (99% BS). This result was observed in another phylogenetic study of *Scleroderma* by Phosri et al. (2009). They presented a phylogenetic analysis that included two moderately to well supported clades that consisted of the spiny and subreticulate spored species in one clade and the reticulate spored species in the second.

Descriptions and a key of the species are expected to aid identification of *Scleroderma* species from Argentina. As suggested by Phosri et al. (2009) and Sanon et al. (2009), additional phylogenetic studies with the inclusion of new taxa is necessary to clarify various aspects of the *Scleroderma* taxonomy at a global scale.

ACKNOWLEDGMENTS

We thank Dr James Trappe for his valuable comments and for providing additional data on undescribed Australian specimens and Drs Andrew Wilson and Gabriel Garcia Moreno Horcajada for valuable reviews and comments. We also thank the Laboratorio de Microscopía Electrónica y Microanálisis (LABMEM) of the Universidad Nacional de San Luis, Argentina, for the SEM photomicrographs. This study was supported by CONICET (PIP No. 6193), which also provided a research position and fellowship for ERN and MLHC respectively. We acknowledge Arq. L.I. Cofré for plate designs.

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