



## Mycorrhizal fungi associated with plantations of *Pinus taeda* L. from the National University of Asunción, Paraguay

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### Abstract

Four species of ectomycorrhizal fungi associated with *Pinus taeda* L. are cited. This species is estimated to have been introduced to Paraguay during the 20<sup>th</sup> century and the mycorrhizal mushrooms were inoculated in the roots of the trees, therefore they are considered non-native species. *Geastrum minumun* Schwein., *Pisolithus arrizhus* (Scop.) Rauschert, *Suillus granulatus* and *Scleroderma bovista* Fr. are cited for the first time for Paraguay. The macroscopic and microscopic characteristics are included as well as a brief description of *Pinus taeda* L.

**Key words** – ectomycorrhizal – exotic fungi – gymnosperms

### Introduction

Mycorrhizal fungi are those that form mutualistic associations with plants. The main benefit of the symbiotic association lies in the exchange of nutrients. The consequence of the ability of mycorrhizal fungi to intercede in the process of release of nitrogen (N) and phosphorus (P) in its ionic form can be considered not only in terms of preferential supply of nutrients to the host plant, but also on early intervention in the event of mineralization, reducing the supply of P and N in ionic form required for non-mycorrhizal competitors. Analytical studies reveal that the most abundant symbiotic relationship is with the group of Basidiomycota (Becerra & Zak 2011, Read & Perez-Moreno 2003).

Extensive plantations of exotic pine, mainly *Pinus radiata* have been (and continue to be) settled in the Southern Hemisphere, from the second half of the 9<sup>th</sup> century. For example, 1.5 million hectares have been introduced in Chile, more than 1 million hectares in New Zealand, more than 700,000 hectares in South Africa, and 800 000 ha in Australia (Dustamet *al.* 1998).

In Paraguay, the first plantations, usually in small areas, were established before and during World War II by private owners and companies like Fassardi Ltd. (Plantations of eucalyptus and cedar), the “Azucarera Paraguaya SA” (400-500 ha of eucalyptus plantations, mainly *E. saligna*), aimed at the production of industrial wood. Between 1955 and 1966, the National Agricultural Institute (IAN), emphasized the production of exotic plants and over 100 exotic tree species were planted for research purposes. Of the 18 species of tropical and subtropical pines introduced in 1959, only three stayed as better adapted: *Pinus elliottii* Engelm., *P. taeda* L., *P. caribaea* var. *caribaea* Morelet, also emerged trials with plantations of native species “Peterevy” (*Cordia trichotoma*) (Vell.) Steud.) and Paraná pine (*Araucaria angustifolia* (Bertol.) Kuntze). By the end of 1980, the total planted area in the country was approximately 3.800 ha. Between the month of

may and June of 1981, the Directorate of the Department of Forest Production from the Faculty of Agricultural Engineering (FIA), installed in their fields plantation trials of *P. elliottii* y *P. taeda*, in strips of 230 x 24 meters north-south and remnants of these pine plantations of over 30 years old are left today. The seeds were obtained from the US Forest Tree Seed Center, in 1980-1981. The origin of *P. taeda* covers the east of Texas to Georgia and west Florida (Enciso 2002, Noguera 2003, Larrobla & Berni 1982 López 1974).

With the introduction of these tree species, several ectomycorrhizal fungi were also introduced and established in exotic plantations (Nouhra *et al.* 2012).

To generate and enrich the information on the study of mycorrhizal fungi introduced to Paraguay with exotic pine species and expand the known facts of the exotic species introduced, *P. taeda*, *G. minimum*, *P. arrizhus*, *S. granulatus* and *S. bovista* are described, representing the first records for Paraguay, and the exotic species *P. taeda* is also taxonomically characterized.

## Materials & Methods

Samples collected from plantations of *Pinus taeda* located inside the campus of the National University of Asunción were analyzed at the Laboratory of Analysis of Plant Resources and deposited in the herbarium of the Faculty of Natural and Exact Sciences, National University of Asunción. The sample corresponding to the species *P. taeda* was deposited in the Herbarium of the Faculty of Chemistry (HFCQ) from the National University of Paraguay. Macroscopic data of size, color and consistency refer to fresh material. For microscopic study of structures, KOH preparations were analyzed at 5%. Samples were observed through an optical microscope with 40X and 100X objectives with immersion oil. Vegetative structures (capillitium hyphae) were stained with Congo red and Phloxine. The materials are preserved in the herbarium of the Faculty of Natural and Exact Sciences (FACEN) of the National University of Asunción, except for samples belonging to the *Scleroderma bovista* species, which were deposited in the herbarium of the University of Alcalá de Henares.

## Results

### *Pinus taeda* L., Species Plantarum 2: 1000. 1753.

Known distribution – *P. taeda* is native to the southeastern United States, crossing the Mississippi River and spanning across the states of Arkansas and Texas. It grows mostly on brittle land. In the north it develops especially over dry and sandy soils; further south, preferably in low and wet land, it is found in areas of low to medium altitude and temperate climate - warm and rainy summer (Figuerola 2007). In Paraguay it is common in the Departments of Cordillera, Central, Alto Paraná and Itapúa (Larrobla & Berni 1982, Morales *et al.* 2002, Noguera 2003).

Material examined – Paraguay, Central Department, City of San Lorenzo, University Campus, Nov 2014, *L. Britos* 35 (HFCQ).

### *Geastrum minimum* Schwein., Schriften Naturf.Ges. Leizig 1: 58 (1822) Figs. 7, 8, 9–11

Mature basidiomata expanded, epigeous, small, star-shaped, 3.5 cm diam. Exoperidium sac-shaped, whitish gray, opening from the apex in 5-10 rays. The rays are triangular and medium-sized at maturity, 1 cm length and different diameters, whitish to gray; non-hygroscopic, papyraceous when dry, beige. Mycelial layer with abundant detritus and incrustations strongly adhered to the outer layer by a whitish mycelium, mixed with organic matter and sand, this makes the mycelial layer inappreciable; hyphae from the mycelial layer 1–2 µm diam., hyaline, thin-walled, narrow lumen, with septa and clamps. Fibrous layer beige to yellowish white, hyphae 4-6 µm diam., parallel, smooth, hyaline, narrow lumen, strongly intertwined. Pseudoparenchymatous layer whitish to gray when fresh, beige and slightly brittle when dry, hyphae of different shapes and sizes: angular, rounded or isodiametric 30–50 × 20–30 µm, hyaline with thin walls. Endoperidium globose to sac-shaped, 1–1.5 high × 0.5–1 cm diam., gray, in mature samples gray to brownish gray. In the apex it presents a peristome delimited by a white ring of 4 mm diam., not very evident, of a clearer tone than the rest of the endoperidial surface, crowned by a fibrillose ostiole, 0.5–1 mm



**Figs 1–6** – Basidiomata of fungi associated to *Pinus taeda* plantations 1 *Pisolithus arhizus* 2 *P. arhizus* in transversal section 3 *Scleroderma bovista* 4 *S. bovista* in transversal section 5 *Suillus granulatus* 6 *S. granulatus* in transversal section 7 *Geastrum minimum* 8 *G. minimum* in colonies

diam.; on very mature samples the peristome does not seem delimited since it takes the same color as the endoperidium and the ostiole presents a darker brown colour. Endoperidium hyphae 3.5–5  $\mu\text{m}$ , thin-walled, smooth, narrow lumen. Capillicium hyphae 3–8  $\mu\text{m}$  diam., yellowish to brown on KOH, some with thin walls, others with thick walls up to 3  $\mu\text{m}$  diam. Mature basidiospores 3–5  $\mu\text{m}$  diam., globose, warty, brown to golden brown and a short pedicel in the apex. Immature spores hyaline, at first subovoid, then globose.

Habitat – Gregarious or solitary, growing on soil.

Known distribution – In *Costa Rica* cited for the first time by Calonge et al.(2005); in Brazil, for the States of Rio Grande do Norte (Fazolino 2009, Trierveiler-Pereira & Baseia 2009), Rio Grande do Sul (Rick 1961) and Paraná (De Meijer 2006). This represents the first record for Paraguay.

Material examined – Paraguay, Central Department, City of San Lorenzo, University Campus, Oct 2013, *M. Campi* 31 (HFACEN).

***Pisolithus arhizus*** (Scop.) Rauschert, *Zeitschrift für Pilzkunde* 25(2): 50 (1959). Figs. 1, 2, 12,13

Mature **basidiomata** 5.2-6 cm diam., epigeous, variable shapes: globose to subglobose, claviform to pyriform, constrict in the basal part to form a short and irregular stipe 2-2.6 cm length. In the lower part of it an olive green to yellowish **mycelial cord** grows with incrustations of the substrate strongly adhered. **Peridium** thin  $\pm$ 1mm thick, smooth and hard, solid and heavy appearance, yellowish brown, it tears leaving localized olive to black spots. **Peridium** hyphae 5.3-5.6  $\mu$ m diam., hyaline, thin and smooth walls, with septa and clamps. **Gleba** tough, compact and multilocular, divided in strati or mosaics composed by irregular and angular pseudoperidioles 4 x 2 mm. The color varies with the degree of maturation; in the basal part they are yellow of slimy texture, in the middle portion they are light brown with some yellow lines, and in the upper part they are dark brown of dusty texture; this delimitation of colors suggests that the maturation occurs from the apex to the base; at maturity it becomes a dry and dusty structure with hard consistency. **Gleba hyphae** septate with clamps  $pp\mu$ m diam. **Spores** spherical with spines, 8-10  $\mu$ m without including the spines, which are up to 2  $\mu$ m long. Septate hyphae, with clamps, 5.3-5.6  $\mu$ m in both peridium and gleba, amyloid, hyaline in KOH.

Habitat – Gregarious or solitary. Found on soil near Pinaceae plants.

Known distribution – In Brazil it is cited for the States of Espirito Santo (Vinha 1988), São Paulo (Bononi *et al.* 1981), and Rio Grande do Sul (Guerrero & Homrich 1999). This represents the first record for Paraguay.

Material examined – Paraguay, Central Department, City of San Lorenzo, University Campus, Aug 2013, *M. Campi* 35 (HFACEN).

Notes – This species is considered edible when immature (García et al. 2006). It is used in reforestation to restore degraded systems forming mycorrhiza (Roldan *et al.* 1996).

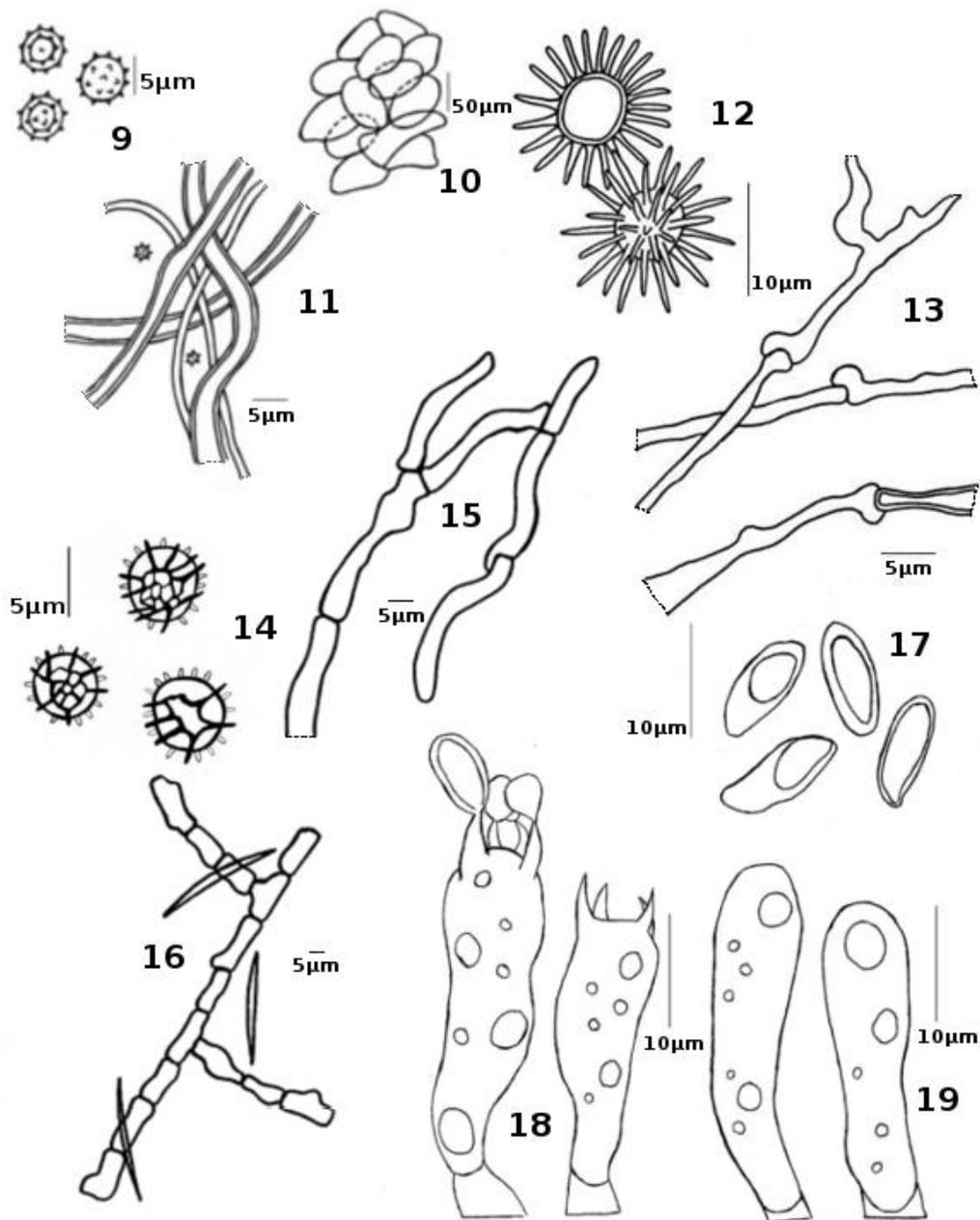
***Scleroderma bovista*** Fr., *Syst. mycol. (Lundae)* 3(1): 48 (1829). Figs. 3, 4, 14–16

**Basidiomata** 1,5-4,5 cm diam., globose to subglobose, sessile or with a rudimentary pseudostipe, base with groups of compact mycelial threads strongly adhered to the substrate. **Peridium** 0,9-1,4 mm thick, beige or pale yellow to dark brown, smooth to verrucose with tiny brown scales, rubbery when fresh, coriaceous when dry. The dehiscence occurs in the apical part where it breaks into vertical grooves. **Gleba** grayish at maturity, powdery, with yellowish to auburn veins. **Exoperidium** composed of cylindric hyphae, 5  $\mu$ m diam., thin-walled, hyaline to yellowish, congophilic. Chain of spheric hyphae, thin-walled, 10–12  $\times$  5–8  $\mu$ m diam. encrusted with crystals, some with clamps; inner layer composed of thin-walled cylindrical hyphae, 5-6  $\mu$ m diam. **Spores** 11–16  $\mu$ m including spines, globose, reticulated, reticulum 1–3  $\mu$ m in length, gold brown, dark in KOH.

Habitat – gregarious, growing on soil.

Known distribution – From Brazil (Guzmán 1970), for the states of: Santa Catarina (Giachini *et al.* 2000 - as *Scleroderma fuscum* [Corda] E. Fisch.), Pernambuco (Gurgel *et al.* 2008), for Argentina: Buenos Aires (Spegazzini 1880, Guzmán 1970, Wright & Albertó 2006), La Pampa (Domínguez De Toledo 1989) and Misiones (Guzmán 1970). This represents the first record for Paraguay.

Examined material – Paraguay, Central Department, City of San Lorenzo, University Campus, Aug 2013, *M. Campi* 44 (HFACEN).



**Figs 9–11** – *Geastrum minimum*: 9 Spores. 10 Pseudoparenchymatous layer. 11 Capillitium hyphae. **Figs 12–13** – *Pisolithus arhizus*: 12 Spores. 13 Exoperidium hyphae. **Figs 14–16** – *Scleroderma bovista*: 14 Spores. 15 Hyphae from the outer layer of the exoperidium. 16. Inner layer of the exoperidium hyphae **Figs 17–19** – *Suillus granulatus*: 17 Spores. 18. Basidia. 19 Cystidia.

Notes – Nouhra (2012) explains that this is a common species in plantacions, parks and forest of pines in Argentina. The combination of spines and reticulum in the wall of the spores and the color of the peridium separates *S. bovista* from the *Scleroderma* species of the region.

*Suillus granulatus* (L.) Roussel, Flore du Calvados et terrains adjacents, composée suivant la méthode de Jussieu: 34. (1806) Figs. 5, 6, 17–19

**Pileus** 20–60 mm diam., convex to flat, moist surface, slimy, smooth, yellow-brown to reddish-brown, margin whole and curved. **Cuticle** smooth and slimy, separates easily from the cap. **Hymenium** poroid, adnate short tubes, 3–5 mm in length, whitish when young, yellowing with age; toward the hymenial surface the tubes end in angular pores, yellowish, 1–2 per mm. **Context** fleshy yellowish. **Stipe** 15–45 × 5–13 mm, central to slightly eccentric, whitish when immature, yellowish

with age, solid, cylindrical, with apical characteristics granulations, reddish to chestnut, with white mycelium at the base. No ring. **Spores** 9-11 × 2-4 μm, ellipsoidal, fusiform, with small apex and center gutule, smooth, yellowish-brown in mass, inamyloid. **Basidia** 18-30 × 5,5-7 μm, clavate, 4 sporated, no clamps. **Cystidia** 16-30 × 5-6,5 μm, fusiform with brownish gutules. **Pileipellis** cutis type, formed by cylindrical entangled gelled hyphae.

Habitat – on soil. Winter to autumn season. Over three individuals.

Known distribution – In Brazil for the state of Santa Catarina (Giachini *et al.* 2000); in Argentina for the states of Entre Ríos, Tucumán, Corrientes, Misiones (Niveiro *et al.* 2009) and Buenos Aires (Niveiro *et al.* 2009, Deschamps 2002).

Material examined – PARAGUAY, CENTRAL DEPARTMENT, City of San Lorenzo, University Campus, May 2013, *M. Campi* 62 (HFACEN).

Notes – In young specimens is characteristic the exudation of whitish latex droplets on the hymenium, than later drop to the stipe and leave the granulations features. Blanco *et al.* (2012) explains that ecologically the *Suillus* species are ectomycorrhizal which establish symbiosis with conifers, especially pines (*Pinus* spp.), being typical of young pine forests and reforestation. Niveiro *et al.* (2009) mentions that are distributed almost worldwide, coinciding with the area of the host species which are spread across much of the world due to reforestation.

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