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New records of *Hysterangium* (Basidiomycota) from a *Eucalyptus* plantation in southern Brazil

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ABSTRACT: (New records of *Hysterangium* (Basidiomycota) from a *Eucalyptus* plantation in southern Brazil). *Hysterangium affine* and *H. inflatum* are recorded for the first time from Brazil, based on specimens collected in a plantation of exotic *Eucalyptus*. Detailed descriptions and illustrations of the studied collections are presented, including an identification key to the species known from southern Brazil.

Key words: ectomycorrhizae, false-truffles, *Hysterangiales*, taxonomy.

RESUMO: (Novos registros de *Hysterangium* (Basidiomycota) em plantação de *Eucalyptus* no sul do Brasil). *Hysterangium affine* e *H. inflatum* têm sua ocorrência registrada pela primeira vez no Brasil a partir de espécimes coletados em plantação de *Eucalyptus*. São apresentadas descrições e ilustrações dos espécimes estudados, assim como uma chave de identificação das espécies de *Hysterangium* conhecidas no sul do Brasil.

Palavras-chave: ectomicorrizas, falsas-trufas, *Hysterangiales*, taxonomia.

INTRODUCTION

Hysterangium Vittad. is a genus of sequestrate (false-truffle) fungi, diagnosed by its hypogean to subhypogean habit and enclosed basidiomata that have a columella with a slimy gleba (due to the presence of a gelatinized trama) and smooth to rugose basidiospores (which are usually covered by a membranous utricle), and form ectomycorrhiza with species of trees in the *Fagaceae*, *Myrtaceae* and *Pinaceae* (Beaton *et al.* 1985, Castellano 1999). The genus comprises approximately 40 species (Castellano 1999) and belongs to the *Hysterangiaceae* E. Fisch., in the order *Hysterangiales* and subclass *Phallomycetidae* (Hosaka *et al.* 2006).

The genus is poorly known in Brazil, with only two recorded species, which is possibly due to its hypogean or subhypogean habit (Castellano 1999). *Hysterangium gardneri* E. Fisch. has been collected in the state of Santa Catarina (Giachini *et al.* 2000) and *H. australe* Speg. (Rick 1961) was recorded from the state of Rio Grande do Sul, comprising the only known records for the genus from this country. *Hysterangium thaxteri* Zeller & Dodge has also been reported for Brazil by Zeller & Dodge (1929), but this species is currently considered a member of *Gelopellis* Zeller (*Claustulaceae* G. Cunn.). In a revision of the South American *Hysterangium*, Castellano & Muchovej (1996) did not report any species of this genus from Brazil.

The present paper comprises some of the results of a survey of the gasteroid fungi from the state of Rio Grande do Sul (Cortez *et al.* 2008a, 2008b, 2009, 2010, 2011a, 2011b, Sulzbacher *et al.* 2010), and discusses *Hysterangium* specimens collected and identified by the authors.

MATERIALS AND METHODS

The field trips were carried out in a *Eucalyptus* plantation at the Estação Experimental de Silvicultura (FEPAGRO - 29°45' S, 53°43' W), in the municipality of Santa Maria, in the central region of the state of Rio Grande do Sul, in southern Brazil. FEPAGRO comprises an area of 280 ha and cultivates trees that are native (*Apuleia leiocarpa* J.F. Macbr., *Senna multijuga* (Rich.) H.S. Irwin & Barneby, and *Tabebuia* spp.) and exotic (*Hovenia dulcis* Thunb., *Platanus acerifolia* Willd., *Pinus* spp. and *Eucalyptus* spp.). The soil is of the Hapludult type, which is deep and poorly drained, and has low natural fertility (Abrão *et al.* 1988, Streck *et al.* 2008). The climate is subtropical humid (Cfa) according to Köppen's system, with mean temperature values higher than 22°C during the warmest month, an annual rainfall of about 1769 mm, and precipitation well distributed throughout the year (Schumacher *et al.* 2008).

The collected specimens were photographed and analyzed macro- and microscopically following Brunrett *et al.* (1996). Color names and codes were based

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on Kornerup & Wanscher (1978). The microscopic analysis of the basidiomata comprised the measurements of at least 30 microstructures (basidiospores, basidia and hyphae). Illustrations were made with the aid of a

drawing tube. Specimens were deposited in the SMDB (Department of Biology of Universidade Federal de Santa Maria) and ICN (Institute of Biosciences of Universidade Federal do Rio Grande do Sul) herbaria.

RESULTS AND DISCUSSION

Key to the *Hysterangium* species associated with *Eucalyptus* in southern Brazil

1. Basidiospores without utricle **1. *H. affine***
 1'. Basidiospores with a distinct hyaline utricle **2**
 2. Rhizomorphs present only at the base of the basidiomata, basidiospores with a winged utricle **2. *H. inflatum***
 2'. Rhizomorphs present at the base and sides of the basidiomata, basidiospores with an irregular, appressed utricle ***H. gardneri***

1. *Hysterangium affine* Masee & Rodway in Masee, *Bull. Misc. Inf. R. Bot. Gdn. Kew* 1898: 127, 1898.

Fig. 1A, 2A-C.

Basidiomata 7-12 mm diam., depressed globose to subglobose, with a distinct rhizomorphic base. Peridium <1 mm thick, brownish orange (6C3) to light brown (6D6), smooth and glabrous. Gleba loculate, gelatinized, greenish grey (27C2) to dull green (27D4), with irregular locules radially arranged on a dendroid columella. Rhizomorphs white (6A1) thin, short and very numerous, forming a fasciculate base in the basidiomata. Basidiospores 10-12.6 × 4.5-5.5 µm, ellipsoid to suboblong, apex and base tapered, hyaline in KOH, smooth and little thickened wall, with a sterigmatal attachment base, utricle absent. Basidia 22.5-26 × 4.5-6.7 µm, clavate, tetrasporic, hyaline. Peridium about 100-180 µm thick, composed of a cutis of interwoven hyphae 2.5-5 µm diam., with brownish walls, slightly thickened and encrusted by numerous, small crystals, not readily separable from gleba in the examined material. Trama formed by subparallel, smooth and thin-walled, hyaline hyphae, 2.5-7.5 µm diam.

Examined specimens: BRAZIL. RIO GRANDE DO SUL STATE. **Santa Maria**, Estação Experimental de Silvicultura, FEPAGRO, 15 May 2007, leg. *V.G. Cortez 091/07* (ICN). *Additional specimens examined:* ARGENTINA. BUENOS AIRES PROVINCE. **Mar del Plata**, Laguna de los Padres, 02 Nov. 1962, leg. *I. Gamundi & R.T. Guerrero*, det. *R. Singer* (ICN 6030).

Habitat: subhypogeous, under soil and fallen leaves of *Eucalyptus* sp., ectomycorrhizal.

Known distribution: Australia (Beaton *et al.* 1985), North (Zeller & Dodge 1929) and South America. Widespread in areas where species of *Eucalyptus* are cultivated.

Remarks: *Hysterangium affine* is diagnosed by the greenish color of the mature gleba, basidiospore size and shape, and the absence of a utricle (Beaton *et al.* 1985). Although more frequently reported as having an ectomycorrhizal association with species of *Eucalyptus* (Beaton *et al.* 1985), an association with *Quercus* has also been reported from North America (Zeller & Dodge 1929). *Hysterangium eucalyptorum* Lloyd, from Ecuador, is considered a synonym of this species (Zeller & Dodge

1929, Cunningham 1942, Beaton *et al.* 1985). *Hysterangium gardneri* E. Fisch., known from *Eucalyptus* plantations in Argentina and southern Brazil (Giachini *et al.* 2000), differs by its smaller basidiospores (8-10.5 × 3.3-4 µm) with wrinkled exosporium (Nouhra *et al.* 2008). *Hysterangium affine* is a new record for Brazil.

2. *Hysterangium inflatum* Rodway, *Pap. Proc. R. Soc. Tasm.* 1917: 108, 1918.

Fig. 1B, 2D-F.

Basidiomata 5-12 mm diam., subglobose to irregular, with a distinct rhizomorphic base. Peridium 0.2 mm thick, greyish yellow (1B3), to dull red (8B4) when fresh, drying olive brown (4D8), covered by soil and litter particles, smooth. Gleba loculate, olive (1F3), with elongate locules and a cartilaginous, dendroid, translucent columella. Rhizomorphs white (6A1), well developed at the base and sides of basidiomata, abundant within the substrate. Basidiospores 7.5-12.5 × 3-4 µm (ornamentation excluded), ellipsoid with a truncate base, surface smooth but enclosed in an inflated wing-like utricle (absent in immature spores) 0.8-2.5 µm, hyaline in KOH. Basidia 21-31 × 3-9 µm, clavate to cylindrical, 4-6 spored, hyaline, basal clamp connections present. Peridium 2-layered; external layer formed by narrow yellowish to brownish hyphae 1.6-3.3 µm in diam., slightly thickened walls, with clamp connections, and crystals adhered to the surface (1.5-) 2.5-4 µm diam.; internal layer composed by hyaline, smooth and thin-walled hyphae, interwoven to subregular, broader than the external layer, 5-20 µm diam. Trama gelatinized in young and mature basidiomata, formed by hyaline, smooth and thin-walled, 0.8-6 µm diam. hyphae, with clamp connections.

Examined specimens: BRAZIL. RIO GRANDE DO SUL. **Santa Maria**, Distrito de Boca do Monte, Estação Experimental de Silvicultura - FEPAGRO, 26 May 2009, leg. *M.A. Sulzbacher 201* (SMDB), 31 July 2009, leg. *M.A. Sulzbacher 200* (SMDB).

Habitat: subhypogeous, under soil and dead leaves of *Eucalyptus*, ectomycorrhizal.

Known distribution: Australia (Beaton *et al.* 1985), New Zealand (Castellano & Beever 1994), United States

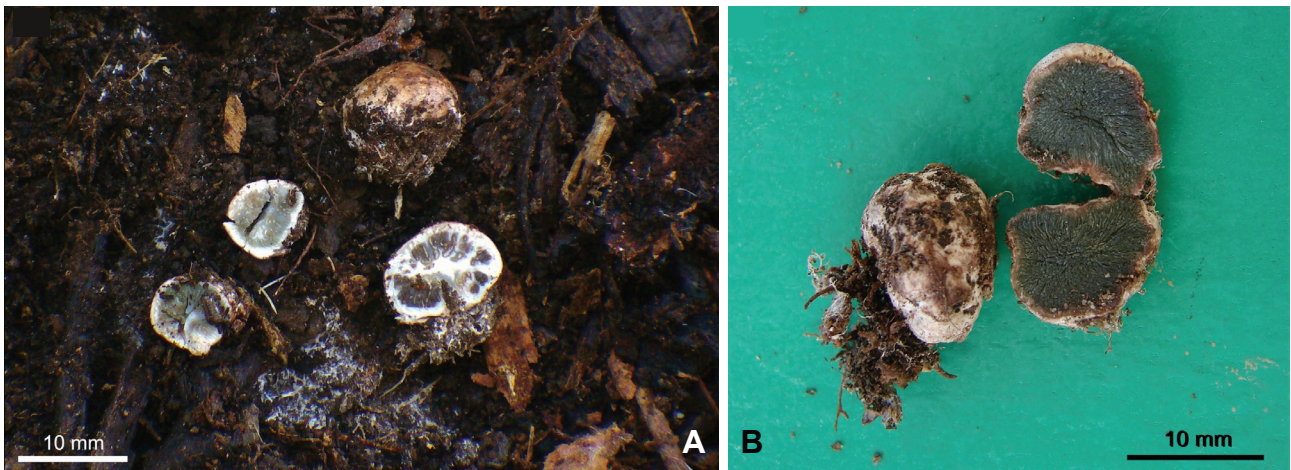


Figure 1. Basidiomata of *Hysterangium affine* (A) and *H. inflatum* (B). Photographs by V. G. Cortez (a) and M. A. Sulzbacher (b).

(Zeller & Dodge 1929), Spain (Lago & Castro 2004), France, Ecuador (Castellano & Muchovej 1996), and Brazil. Possibly widespread in plantations of species of *Eucalyptus* worldwide.

Remarks: *Hysterangium inflatum* is morphologically characterized by its ellipsoid and truncate basidiospores, each with an inflated wing-like utricle (Castellano & Muchovej 1996). The presence of numerous and large calcium oxalate crystals adhered to the surface of the

external layer of the peridium is also a diagnostic feature that has been observed on the rhizomorph hyphae (Malajczuk *et al.* 1987, Castellano 1999). *Hysterangium inflatum* forms within the upper humus layers or occasionally in the litter layer (Castellano 1999), and is reported to have ectomycorrhizal relationships with species of *Eucalyptus*, especially *E. globulus* (Castellano & Muchovej 1996). In Australia the mycelium of *H. inflatum* occupied ca. 10% of the soil surface area in some native

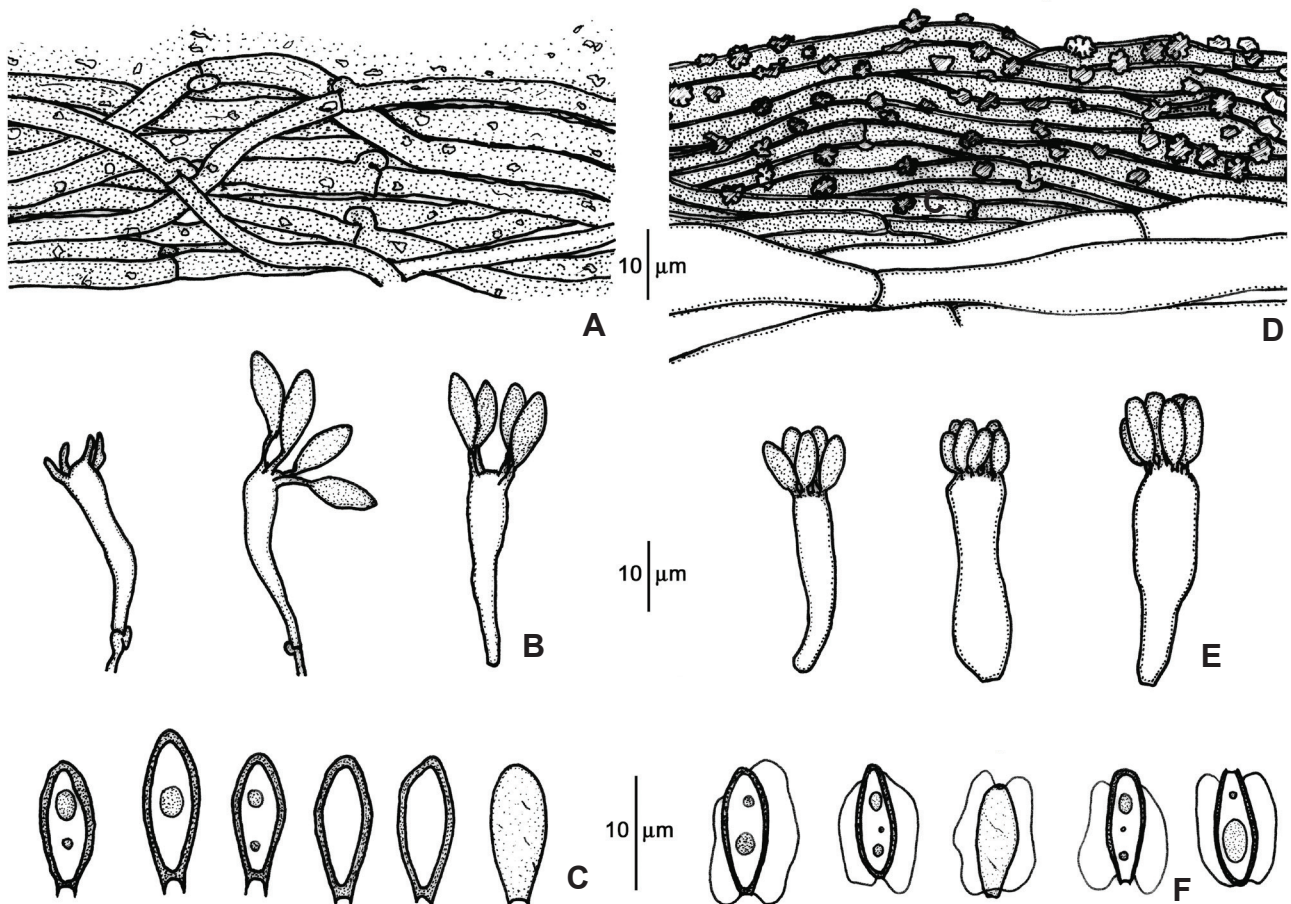


Figure 2. A-C. *Hysterangium affine*. A. Exoperidium. B. Basidia. C. Basidiospores. D-F. *Hysterangium inflatum*. D. Exoperidium. E. Basidia. F. Basidiospores. Line drawings by V. G. Cortez.

E. diversicolor F. Muell. forests, indicating its important role as a dominant ectomycorrhiza of those trees (Malajczuk *et al.* 1987).

The record of two previously unreported species of *Hysterangium* in Brazil is not surprising because these fungi are usually introduced via *Eucalyptus* roots, which are cultivated worldwide. However, these records emphasize the need of further studies in order to identify other mycorrhizal symbionts of these trees in southern Brazil. This is ultimately the first step towards a potential use of these symbionts in agroforestry programs. Members of the genus *Hysterangium* are considered of relevant interest to forestry programs because they form ectomycorrhizal relationships with several *Eucalyptus* species (Malajczuk *et al.* 1987, Castellano 1999). Unfortunately, little is known about the ectomycorrhizal fungi in southern Brazil where cultivation of *Eucalyptus* is economically important, and their possible use as an ectomycorrhizal inoculum remains unexplored. Isolating these fungi in culture, however, has been reported to be difficult (Castellano 1999), and our attempts to isolate the species of fungi in the present study also failed. Species of *Hysterangium* play decisive roles in the ecology of forests, contributing directly to the structure of soil communities (Castellano 1999), and, for this reason, will be the subject of future investigations.

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