
Description and affinities of *Agaricus martineziensis*, a rare species

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Agaricus martineziensis, previously reported from Argentina and Mexico, is described from Brazil, and its phylogenetic affinities evaluated by nLSU rDNA sequence data showed a close relation with *Agaricus aridicola* [= *Gyrophragmium dunalii*].

Key words: *Agaricaceae*, *Agaricales*, *Agaricus aridicola*, Brazil, *Gyrophragmium dunalii*

Introduction

Agaricus martineziensis Heinem. was described from Argentina with the name *Agaricus volvatus* (Martinez, 1957) probably due to the well developed volva at the base of stipe. Because of nomenclatural reasons it was renamed (Heinemann, 1990). Apparently it is a rare species since it was reported once only from the type locality, and afterwards from Mexico (Guzmán and García-Saucedo, 1973; Guzmán, 1980; Guzmán-Dávalos *et al.*, 1983; Bandala-Muñoz *et al.*, 1988). This paper reports the species occurrence for the first time from Brazil and also its affinities with other *Agaricus* species.

Materials and methods

The two specimens studied were collected at the "Parque Estadual das Fontes do Ipiranga" (PEFI). PEFI is a reserve to the south of São Paulo City (23°39'S e 46°37'W), with an altitude of 798 m and occupying a total area of 549.31 ha. More information can be found in De Vuono (1985) and Bicudo *et al.* (2002).

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Table 1. Overview of species, GenBank accession numbers and references for the sequences.

Fungus	GenBank number	Reference
<i>Agaricus abruptibulbus</i> Pk.	AF59228	Mitchell and Bresinsky, 1999
<i>Agaricus arvensis</i> Schaeff.	U11910	Chapela <i>et al.</i> , 1994
<i>Agaricus bisporus</i> (J. Lge) Imbach	AF059227	Mitchell and Bresinsky, 1999
<i>Agaricus campestris</i> L.: Fr.	AF059221	Mitchell and Bresinsky, 1999
<i>Agaricus lanipes</i> Moell. & J. Schaeff.) Sing.	AF059229	Mitchell and Bresinsky, 1999
<i>Agaricus maskae</i> Pil.	AF059230	Mitchell and Bresinsky, 1999
<i>Agaricus martineziensis</i> Heinem.	AY668957	this work (SP307818)
<i>Agaricus semotus</i> Fr.	AF059224	Mitchell and Bresinsky, 1999
<i>Agaricus silvicola</i> (Vitt.) Sacc.	AF059223	Mitchell and Bresinsky, 1999
<i>Agaricus xanthoderma</i> Gen.	AF059222	Mitchell and Bresinsky, 1999
<i>Chlorophyllum brunneum</i> (Farl. & Burt) Vellinga	AF482886	Vellinga <i>et al.</i> , 2003 (as <i>Macrolepiota</i> sp.)
<i>Gyrophragmium dunalii</i> (Fr.) Mont.	AF261478	Moncalvo <i>et al.</i> , 2002
<i>Lepiota humei</i> Murr.	U85284	Johnson and Vilgalys, 1998
<i>Longula texensis</i> (Berk. & Curt.) Zeller	AF261479	Moncalvo <i>et al.</i> , 2002

The identification was accomplished using macro-and microscopic characters of the basidiomes and the pertinent literature.

DNA was extracted with the QIAamp Mini Kit (Qiagen, Mississauga, Ontario, Canada). Dry cap tissue (ca. 50 mg) was homogenised in 360 μ L buffer ATL. Proteinase K (40 μ L, provided with the kit), 40 μ L RNase A (20 mg mL⁻¹), and 400 μ L buffer AL were added, and the homogenate was incubated at 56°C for 30 min. 440 μ L ethanol (95%) was added and the mixture was centrifuged briefly to sediment the solids. The supernatant was loaded on a Qiagen column and processed as recommended by the manufacturer. The DI/D2 large subunit rDNA was amplified by adding purified DNA (1 μ L, A260 = 2.5) to a 50 μ L PCR reaction. The primers were NL1 and NL4, and the conditions were as described by Kurtzman and Robnett (1998). Sequencing was performed with the same primers in an ABI sequencer at the John P. Robarts Research Institute, London, Ont. The sequence was compared to those available in GenBank. Similar sequences were selected for alignment and construction of a phylogenetic tree by neighbour-joining analysis using the program DNAMAN (Vaudreuil, Québec, Canada). Bootstrap values were determined from 1000 pseudoreplicates. The sequence was deposited in GenBank and was assigned the accession number AY668957. Table 1 shows the GenBank accession number and the references for all sequences utilised.

Results

Agaricus martineziensis Heinem., Bull. Jard. Bot. Nat. Belg. 60: 339. 1990.

(Figs. 1-3, 5-10)

≡ *Agaricus volvatus* Martínez, Revta. Invest. Agríc. 11: 299. 1957. [nom. inv.: non *Agaricus volvatus* Peck, Rep. N.Y. St. Mus. Nat. Hist. 24: 59. 1872; non *Agaricus volvatus* (Pearson) Heinem., Bull. Jard. Bot. Brux. 26: 24. 1956.]

Type: Argentina, Buenos Aires, Vicente López, Florida, leg. L.C. Goldner e A.F. de Goldner, 13-2-1955 (BAB 77.480).

Pileus 14-21 cm broad, applanate at maturity, slightly umbonate, centre smooth, dry, whitish, margin entire, squamulose. *Squamules* large, platelike to fasciculate, concentric, brown to ferruginous brown, fleshy. *Odour* unpleasant. *Lamellae* at first white becoming deep brown, free, crowded, margin entire. *Stipe* 12 × 3-5.5 cm, stout, bulbous, violaceous brown to white above the annulus, and white towards the base. *Annulus* superior, thick, fleshy, white, with white squamules. *Volva* saccate, white to greyish, well-developed, partially buried on the soil. *Basidiospores* 5.5-7 × 4-5.5 μm (Q = 1.25-1.33), dark brown in KOH, ovate, smooth, thick-walled, without germ pore, inamyloid. *Basidia* 26.5-29.5 × 8.5-17 μm, clavate, slightly thick-walled, bearing four long sterigmata, up to 4 μm. *Pleurocystidia* absent. Lamella edge heteromorphous with scattered cheilocystidia. *Cheilocystidia* 19.5-28 × 8.5-10 μm, clavate or piriform, hyaline, thin-walled. *Hymenophoral trama* irregular, inamyloid, hyaline, with septate, inflated, thin walled hyphae, 3-19.5 μm diameter. *Subhymenial layer* well-developed, up to 21 μm wide, parenchymatous. *Pileipellis* a stratified trichodermium, hyaline to brown, consisting of short chains of about 4-7 elements, subglobose to cylindrical, 14-22.5 × 10-15.5 μm, terminal elements with a brown vacuolar pigment. *Volva* formed by interwoven, cylindrical to inflated, septate hyphae, 4-14 μm diameter, thin-walled, hyaline to pale brown in KOH, inamyloid, with more or less globose elements, 4-46 μm diam. *Pileal squamules* composed of cylindrical to inflated, septate hyphae, 4-12.5 μm diam., thin-walled, hyaline to pale yellow, non amyloid, with more or less thick walled globose elements, 4-49 μm diam.

Habitat: On soil.

Known distribution: Argentina (Martínez, 1957; Heinemann, 1990), Mexico (Guzmán, 1980; Guzmán and García-Saucedo, 1973; Guzmán-Dávalos *et al.*, 1983; Bandala-Muñoz *et al.*, 1988).

Material examined: BRAZIL, SP, São Paulo, Parque Estadual das Fontes do Ipiranga, 23°29'S - 46°37'W, 700 m alt., on soil without vegetation, 13 June 2001, U.C. Peixoto (SP307635); 14 December 2001, U.C. Peixoto (SP307818).

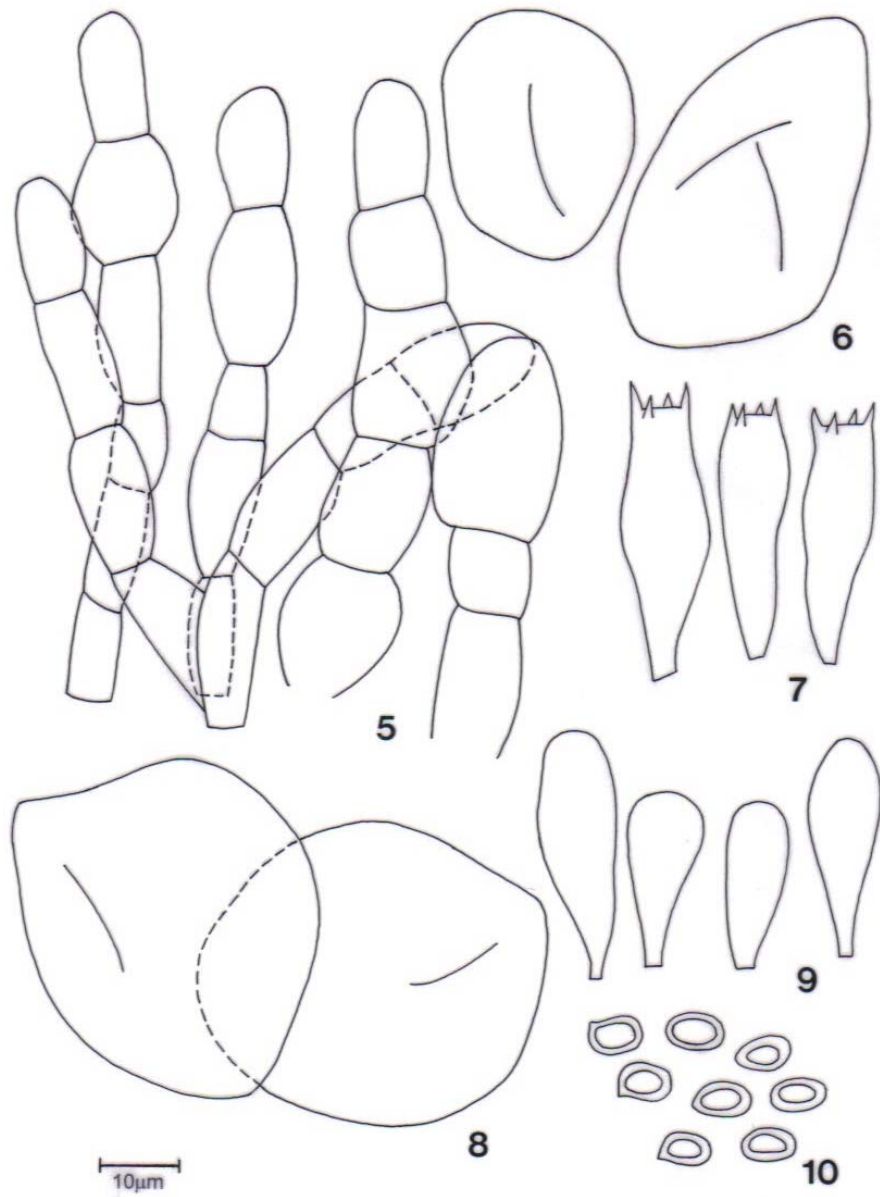


Figs. 1-4. *Agaricus* species. **1-3.** *Agaricus martineziensis* (SP307635). **4.** *Agaricus aridicola*. Bars = 1 cm.

Notes: The two specimens listed above were collected at the same place, but at different times. At each time just one basidiome was formed. Since then, no more have appeared during periodic examination.

The Brazilian material is the same in all aspects when compared with the descriptions published by Martínez (1957) and Heinemann (1990), although Heinemann (1990) did not mention the presence of a well-developed annulus that appears clearly in the illustrations provided by Martínez (1957). The papers reporting the Mexican material are not provided with descriptions and, there is only a schematic figure in Guzmán (1980).

Guzmán and García-Saucedo (1973) considered the species of Pearson (1950) described from South Africa as *Psalliota volvata* Pearson, as a synonym of *A. volvatus* Martínez. Heinemann (1956) made the new combination *Agaricus volvatus* (Pearson) Heinem. (nom. invalid, non *A. volvatus* Peck) and later gave a new name for the species of Martínez (Heinemann, 1990), considering both species as distinct. Looking at the description and illustration given by Pearson (1950), it does not appear to be the same fungi from Martínez. *Agaricus volvatus* (Pearson) Heinem. differs macroscopically from *A. martineziensis* by the smaller size, the cap colour that is white and with



Figs. 5-10. *Agaricus martineziensis* (SP307818). **5.** Pileipellis. **6.** Squamules cells. **7.** Basidia. **8.** Volva cells. **9.** Cheilocystidia. **10.** Spores. Bar = 10 µm.

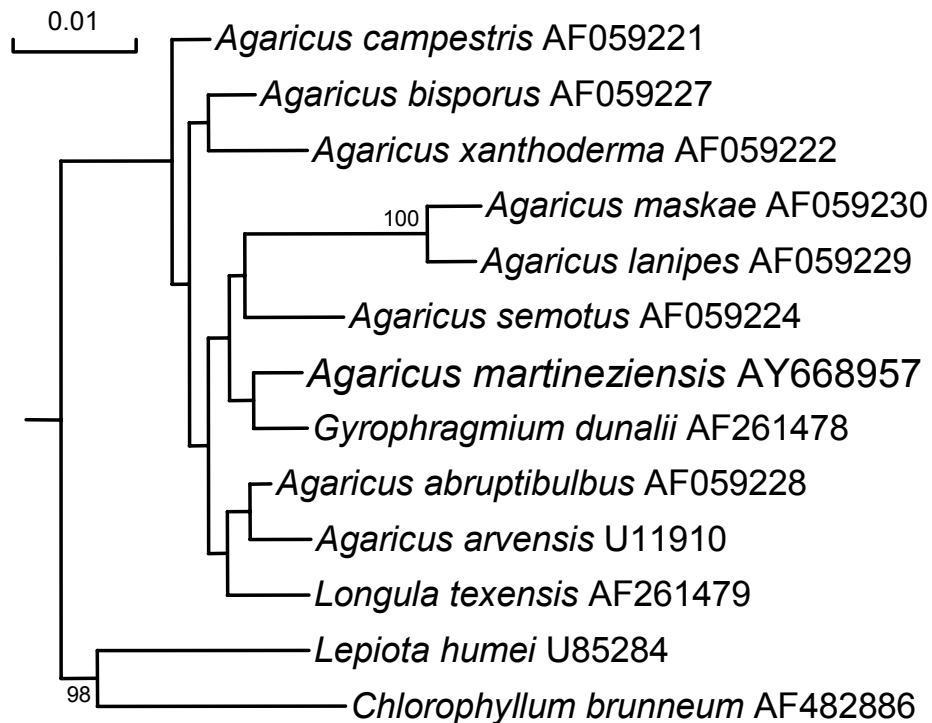


Fig. 11. Phylogenetic tree generated from nLSU rDNA by neighbour-joining analysis.

violaceous or pinkish tinge, and by the absence of an annulus. An annulus is well-developed in the material of *A. martineziensis* both from Argentina and Brazil.

The genus *Agaricus* is well-characterised in the *Agaricaceae* by the free gills that change colour from white to deep brown and the central stipe with an annulus. It is considered to represent a natural group, but identification at species level is quite difficult.

The phylogenetic tree generated from nLSU rDNA sequence data represented in Fig. 11 shows that *Gyrophragmium dunalii* (Fig. 4), now known as *Agaricus aridicola* Geml, Geiser & Royse (Geml *et al.*, 2004) is sister to *A. martineziensis* and suggests that the latter species is best placed in subgenus *Agaricus* section *Arvenses* of Heinemann (1977).

Kreisel (1973) used morphological data to conclude that *Gyrophragmium* is a secotiaceous genus in the family *Agaricaceae*, with a close relationship with *Agaricus* L.: Fr. s. str. Recent sequencing studies corroborated this affinity and that of other secotioid fungi such as *Longula texensis* (Moncalvo *et al.*, 2002), *Endoptychum depressum* (Vellinga *et al.*, 2003) and *Barcheria willisiana*, a recently described monotypic genus from Australia (Lebel *et al.*, 2004).

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