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SHORT COMMUNICATION

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Epulorhiza epiphytica sp. nov. isolated from mycorrhizal roots of epiphytic orchids in Brazil

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Abstract *Epulorhiza epiphytica* sp. nov. isolated from the roots of two Brazilian native epiphytic orchid species is described. In culture, it differs from the known species of *Epulorhiza* in the minute size of monilioid cells with foveate surfaces. This is the first report of an orchid mycorrhizal fungus from Brazil.

Key words Biodiversity · Orchid mycorrhiza · *Rhizoctonia*-like · Tropical fungi

The anamorphic genus *Epulorhiza*, one of the form genera of Basidiomycota, was proposed by Moore based on the combination of heterobasidiomycetous teleomorph and dolipore septa with imperforate parenthesomes (Moore 1987). The corresponding teleomorph is *Tulasnella* J. Schröt. of Tulasnellaceae, and the type species is *T. deliquescens* (Juel) Juel (Moore 1996). *Epulorhiza* is one of the most common genera reported to be associated with terrestrial orchid roots (Andersen and Rasmussen 1996; Currah et al. 1987, 1989, 1997; Currah and Zelmer 1992; Zelmer et al. 1996; Zelmer and Currah 1995; Zettler 1997; Zettler and Hofer 1998; Zettler et al. 2000). During a survey of mycorrhizal fungi of neotropical epiphytic orchids in southeastern Brazil, a distinctive type of isolate belonging to *Epulorhiza* was obtained from roots of *Epidendrum rigidum* Jacq. and *Polystachia concreta* (Jacq.) Garay & H.R. Sweet collected in the Atlantic Rain Forest. The minute size of monilioid cells produced in culture, their homogeneity in a single chain, and the foveate cell wall surfaces distinguished them from the known species of *Epulorhiza* (Currah et al. 1989, 1997; Currah and Zelmer 1992; Zelmer and Currah 1995). Thus, these isolates are described and illustrated as a new taxon based on their morphological characteristics.

Healthy root fragments of the epiphytic orchid species *E. rigidum* and *P. concreta* were collected in their native habitat in the Atlantic Forest at São Miguel do Anta City, Minas Gerais State, Brazil. The root fragments were transported within 1 h to the laboratory and washed under running tap water. To confirm the presence of pelotons in the cortical cells, root samples were sliced with a Leica CM 1850 freezing-stage microtome. The sections were mounted on glass slides with lactophenol and examined by light microscopy. Roots were surface-sterilized with 70% ethanol for 1 min, followed by 2% sodium hypochloride solution for 5 min, and rinsed five times with sterile distilled water. Roots were then decorticated with a sterile scalpel, macerated in a mortar, spread onto 25 ml of modified Melin–Norkrans agar (Marx 1969), and incubated at 28°C for 1 week. Some pelotons could be observed by light microscopy on the isolation medium being monitored for the presence of active hyphal growth. For the isolation of peloton-forming fungi, hyphal tips were selected, cut off with a sterile scalpel, transferred to Petri dishes containing potato dextrose agar (PDA, Difco), and incubated at 28°C for 1 week. To observe monilioid cells, 9-mm agar plugs, cut from the edge of the resulting colonies, were transferred to Petri dishes containing cornmeal agar (CMA, Difco), tap water agar, coconut milk agar, malt extract agar (Zelmer and Currah 1995), oat meal agar (30 g oat meal, 15 g agar, 1000 ml water), and sawdust agar medium (100 g *Eucalyptus grandis* sawdust, 15 g agar, 1000 ml water).

For taxonomy, the cultural characteristics were recorded on PDA medium incubated at 28°C in the dark. The number of nuclei per cell was observed in young hyphal cells stained with HCl–Giemsa (Sakena 1961). Enzymatic assays for the presence of cellulase and polyphenol oxidase followed the methods of Teather and Wood (1982) and Davidson et al. (1938), respectively. For transmission electron microscopy, 1-mm^3 agar blocks from cultures grown on PDA at 28°C for 1 week were prepared as described by

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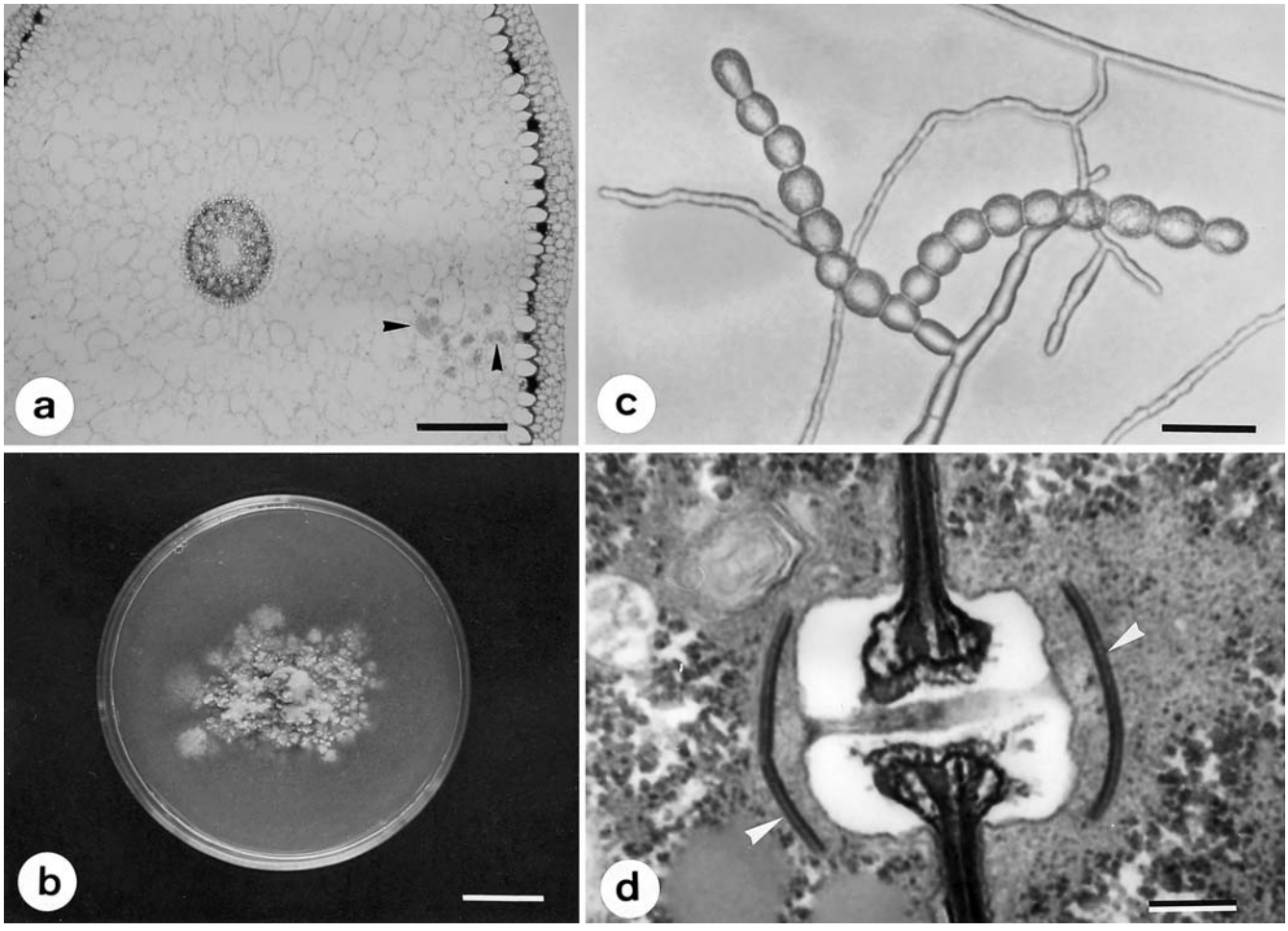

Fig. 1. *Epulorhiza epiphytica*. **a** Root section of *Polystachia concreta*, showing the pelotons formed by *E. epiphytica* (*arrowheads*). **b** Colony grown on PDA after 60 days. **c** Chains of monilioid cells. **d** Dolipore septum with imperforate parenthesomes, showing the three-layered lumen (*arrowheads*). *Bars* **a** 30 μm; **b** 2 cm; **c** 20 μm; **d** 0.2 μm

Matsuoka and Carvalho (1987) and examined with a Zeiss EM 109 electron microscope at 80 kV.

Pure cultures of the isolates were deposited in the culture collection of the Laboratório de Associações Micorrízicas, Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO/UFV).

Taxonomy

Epulorhiza epiphytica O.L. Pereira, Rollemberg & Kasuya, sp. nov. Fig. 1.

Coloniae in agaro dextroso solani tuberosi lentissime crescentes, albae vel brunneo lae, sebosae, mycelii aerii densii, margine submerso. Hyphae currentes, septatae, 2.0–2.5 μm diameter; cellulae hypharum binucleatae. Parenthesomata imperforata, ad marginem non recurvata. Lumen parenthesomatis tristratosum, ex strato centrali translucenti et stratis distalibus densis compositum. In agaro farinae zeae sclerotia absentia; cellulae moniloides 7.3–10 × 8.3–9.5 μm, globosae, catenas ex 8–10 cellulis aequabilibus constantea formantes, pariete superficie foveolato.

Holotypus: Colonia exsiccata ex VIC 22190 ex *Polystachia concreta* isolata ea in São Miguel do Anta, Minas Gerais, Brazil, Nov. 15, 1999 collectiva.

On PDA, white to brownish colonies; sebaceous aerial mycelium with submerged margins; hyaline, regularly septate runner hyphae, 2–2.5 μm in diameter, with dolipore-parenthesome septa; imperforate parenthesomes not recurved at the margin, composed of a central electron-transparent layer and two electron-dense layers surrounding the central layer; binucleate cells. On CMA, sclerotia absent; globose monilioid cells, 7.3–10 × 8.3–9.5 μm, with foveate surface in a single chain of 8–10 cells.

Enzymatic assays: polyphenol oxidase negative, weakly cellulolytic.

Holotype: dried colony on CMA, isolated from *P. concreta* collected at São Miguel do Anta, Minas Gerais, Brazil, Nov. 15, 1999; deposited at VIC Herbarium as VIC 22190.

Additional isolate: dried colony on CMA, isolated from *E. rigidum* collected at São Miguel do Anta, Minas Gerais,

Brazil, Nov. 15, 1999; deposited at VIC Herbarium as VIC 22191.

Etymology: epiphytica (Latin), referring to the epiphytical habitat of its hosts.

Currah et al. (1997) described a new species of *Epulorhiza*, *E. inquilina* Currah, Zettler & McInnis as a mycorrhizal symbiont of mature plants of *Platanthera* spp. and provided a key to distinguish the five known species of *Epulorhiza*. *Epulorhiza inquilina* differs from *E. epiphytica* by a superficial resemblance of the mycelium of the former to that of the genus *Ceratorhiza* R.T. Moore (Currah et al. 1997). *Epulorhiza epiphytica* is similar to *E. albertaensis* Currah & Zelmer, *E. calendulina* Zelmer & Currah, *E. anaticula* (Currah) Currah, and *E. repens* (N. Bernard) R.T. Moore in having narrow vegetative hyphae, scant aerial mycelium, colony with submerged margins, slow growth rates, and imperforate parenthesomes. All of these species also lack polyphenol oxidase activity on tannic acid medium. However, it differs in the minute size of its globose monilioid cells (7.5–10 × 8.0–9.5 μm), which do not vary in size in a single chain and show foveate cell surfaces. These characteristics differ sufficiently from those of the five known *Epulorhiza* species originally isolated from temperate terrestrial orchids. The morphological features of *E. epiphytica* were stable for more than 2 years when this fungus was grown on all the tested media. Thus, these features are distinctive and stable to serve as diagnostic characteristics for this new species.

Morphological features of the vegetative mycelium of *Rhizoctonia*-like fungi, including *Epulorhiza,* are considered to be of limited taxonomic value (Andersen 1990). However, the species concept of the group can be erected and validated on the basis of some cultural characteristics on specific media (Currah et al. 1989, 1997; Currah and Zelmer 1992; Zelmer and Currah 1995).

In our study, no hymenium production has been observed in culture, but *E. epiphytica* is expected to be assignable to *Tulasnella* based on parenthesomal ultrastructure (Andersen 1996; Moore 1996).

Most taxonomic works on orchid mycorrhizal fungi have dealt with those that associate with temperate terrestrial plants (Currah et al. 1987, 1989, 1997; Currah and Zelmer 1992; Zelmer et al. 1996; Zelmer and Currah 1995). *Epulorhiza* is considered to be a less common mycorrhizal partner of tropical epiphytic orchids (Richardson et al. 1993; Richardson and Currah 1995). In our work, however, this genus was exclusively isolated from two orchid species, but the actual geographic distribution of *Epulorhiza* remains largely unknown. Therefore, the survey of mycorrhizal fungi of epiphytic orchids needs to be expanded in the tropics to evaluate the fungal diversity associated with this large plant family.

This is the first report of an orchid mycorrhizal fungus from Brazil.

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