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Mucor variicolumellatus L. Wagner & G. Walther (Mucorales, Mucoromycota): a first record for the Neotropics

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Abstract
Two specimens of *Mucor variicolumellatus* L. Wagner & G. Walther were isolated from soil samples collected in an upland rainforest area located in Pernambuco state, Brazil. Their identity were confirmed by morphophysiology and ITS rDNA sequence analysis. Both specimens are distinguished from other species within the *Mucor circinelloides* complex by producing obovoid, ovate and strawberry-shaped columellae. A detailed description and illustration of the specimens are presented. This is the first record of *M. variicolumellatus* in the Neotropics.

Keywords
ITS rDNA; Mucoromyceta; taxonomy.

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Introduction

Upland rainforest are disjunctions of Montane Seasonal Semideciduous Forest, which is one of the vegetation types belonging to the Brazilian Atlantic Forest domain (Freire et al. 2018). In northeastern Brazil, upland rainforest areas are commonly inserted in the semiarid region, surrounded by Caatinga vegetation. When compared to the ecosystems of Caatinga, the upland rainforest areas exhibit higher humidity and lower average temperatures (Medeiros and Cestaro 2019), thus favoring the high diversity of plants, animals and microorganisms, including fungi. However, the disordered using of the Brazilian upland rainforest areas has caused irreversible degradation processes, with the fragmentation and loss of habitats and ecosystems, impacting on biological diversity and putting at risk this still poorly studied forests (Tabarelli and Santos 2004).

Despite of the important ecological role played by fungi in edaphic environments as saprobes, parasites and symbionts (Stamford et al. 2005), the species richness of fungi in the Brazilian upland rainforest areas is still underestimated (Flora do Brasil 2020). The distribution records of the order Mucorales Dumort. from these ecosystems are restricted to the contributions of Santiago et al. (2011, 2013), Crous et al. (2018), de Souza et al. (2018), and Lima et al. (2018), including three new recently described species of *Mucor* Fresen.

The genus *Mucor* is characterized by rapid mycelial growth and formation of non-apophysate sporangia in simple or branched sporangiophores (Benny et al. 2014). Molecular studies have demonstrated that this genus is polyphyletic, and includes some species complexes, such as the *M. circinelloides* complex (MCC) (Pawłowska et al. 2013; Walther et al. 2013; Wagner et al. 2019). The MCC consists of 14 species, including the recently described *Mucor variicolumellatus* L. Wagner & G. Walther (Wagner et al. 2019).

Mucor variicolumellatus was previously reported by Zycha (1935) in a *Tremella* Dill. ex L. carpophore and it was mistakenly identified as *M. fragilis* Bainier. Wagner et al. (2019) described the new species based on morphophysiological and molecular data. Here we present a detailed description and illustration of *M. variicolumellatus* isolated from soil samples in an upland rainforest area of the semiarid region of Brazil. This is the first record of *M. variicolumellatus* in the Neotropics.

Methods

Isolation, purification, and identification. Soil samples were collected in an upland rainforest area, in the city of Taquaritinga do Norte (Fig. 1), state of Pernambuco, Brazil. The samples were placed in clean plastic bags and stored in styrofoam boxes for transportation to the laboratory. For isolation, five milligrams of soil were added to Petri dishes containing wheat germ agar medium (Benny 2008) supplemented with chloramphenicol (100 mg/L) to prevent bacterial growth. The dishes were stored in the laboratory at room temperature (28 ± 2 °C) for seven days under alternate light and dark periods. Fragments of mycelium were removed from the Petri dishes under a stereomicroscope (Leica EZ4) and transferred to Petri dishes with malt extract agar (MEA) (Benny 2008). The specimens were identified by comparing the macroscopic and microscopic characteristics as described by Schipper (1976), Álvarez et al. (2011), and Wagner et al. (2019). Lyophilized cultures of *M. variicolumellatus* are deposited in the culture collection Micoteca URM of the Federal University of Pernambuco.

Molecular analysis. Fungal biomass was obtained from MEA cultures in test tubes stored at (25 ± 2 °C) for up to six days. Genomic DNA was extracted as described by Oliveira et al. (2016). The primer pairs ITS1/ITS4 (White et al. 1990) were used to amplify the rDNA ITS1–5.8S–ITS2 region. The polymerase chain reaction was conducted as described by Oliveira et al. (2014). The final amplicons were purified with the Invitrogen PureLink PCR Purification Kit and sequenced. The newly obtained sequences were deposited in GenBank.

Phylogenetic reconstructions were obtained by analyzing the ITS1–5.8S–ITS2 sequence data. The sequences of *Mucor* spp. were obtained from GenBank and aligned with the sequences generated in the present study. The sequences were aligned with Clustal X (Larkin et al. 2007) and edited with BioEdit (Hall 1999). Prior to phylogenetic analysis, the optimal nucleotide substitution model was estimated using Topali 2.5 (Milne et al. 2004). Bayesian inference analysis (two runs over $1{\times}10^6$ generations with a burn-in of 2500) was performed using

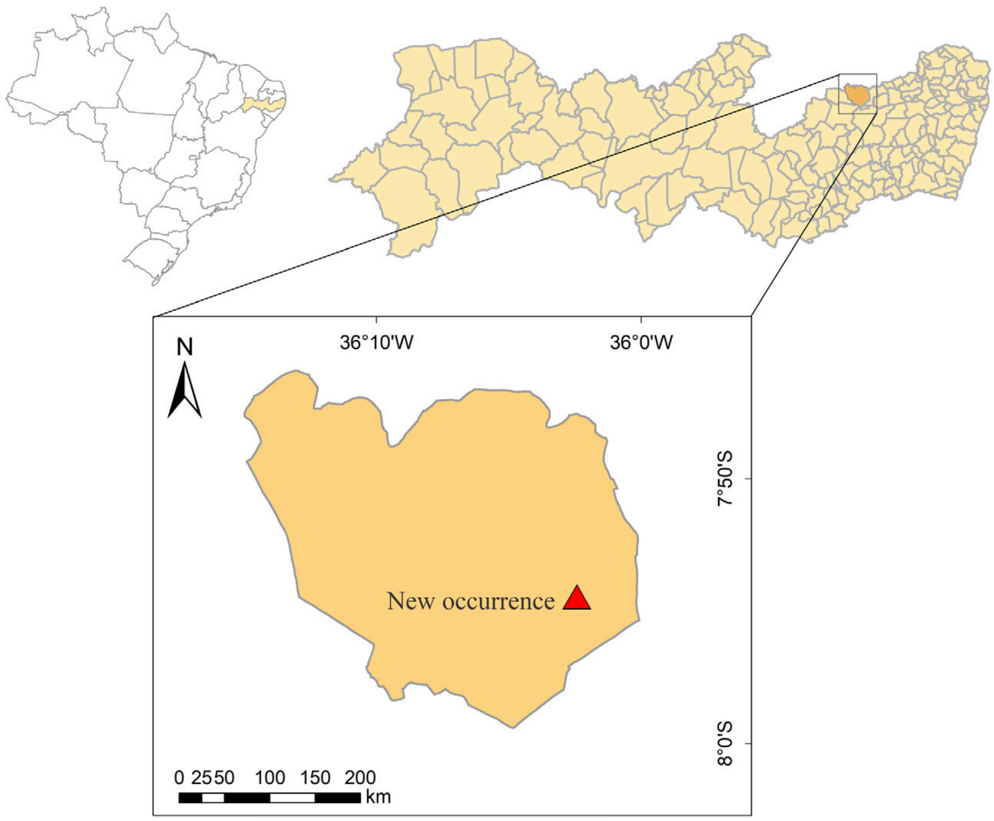

Figure 1. The location in Taquaritinga do Norte city, where *Mucor variicolumellatus* URM 7769 was found.

MrBayes 3.1.2, and maximum likelihood analysis (with support estimated by bootstrap analysis with 1000 replicates) was performed using PhyML (Guindon and Gascuel 2003), launched from Topali 2.5.

Results

Mucor variicolumellatus **L. Wagner & G. Walther**, Persoonia 44: 67–97. 2019.
Figure 2A–H

Material examined. BRAZIL• Pernambuco, Taquaritinga do Norte; 07°54′28″S, 036°02′25″W; 1.056 m a.s.l.; 26 Dec. 2017; Carlos Alberto Fragoso de Souza leg.; habitat: soil; URM 7769; GenBank: MT124621.

Additional material examined. BRAZIL• Pernambuco, Taquaritinga do Norte; 07°54′28″S, 036°02′25″W; 1.056 m a.s.l.; 03 Apr. 2018, Carlos Alberto Fragoso de Souza leg.; habitat: soil; URM 7867; GenBank: MT124622.

Distribution. Brazil, Germany, Mawali, and USA.

Identification. Colonies firstly white then turning into pale gray, cottony, colonizing the entire Petri dish (9 cm diam) and touching the plate lid in five days, at 25°C, in MEA, reverse uncolored. Rhizoids present, slightly branched, up to 32 μm length, hyaline. Sporangiophores, hyaline to slightly pallid gray, 10–20 μm in diameter, repeatedly sympodially branched, with long or short branches, erect, some slightly curved, smooth-walled. Sporangia initially hyaline to pale orange then becoming brown gray to dark gray, globose, 20–90 μm in diameter, wall deliquescent. Columellae hyaline, often

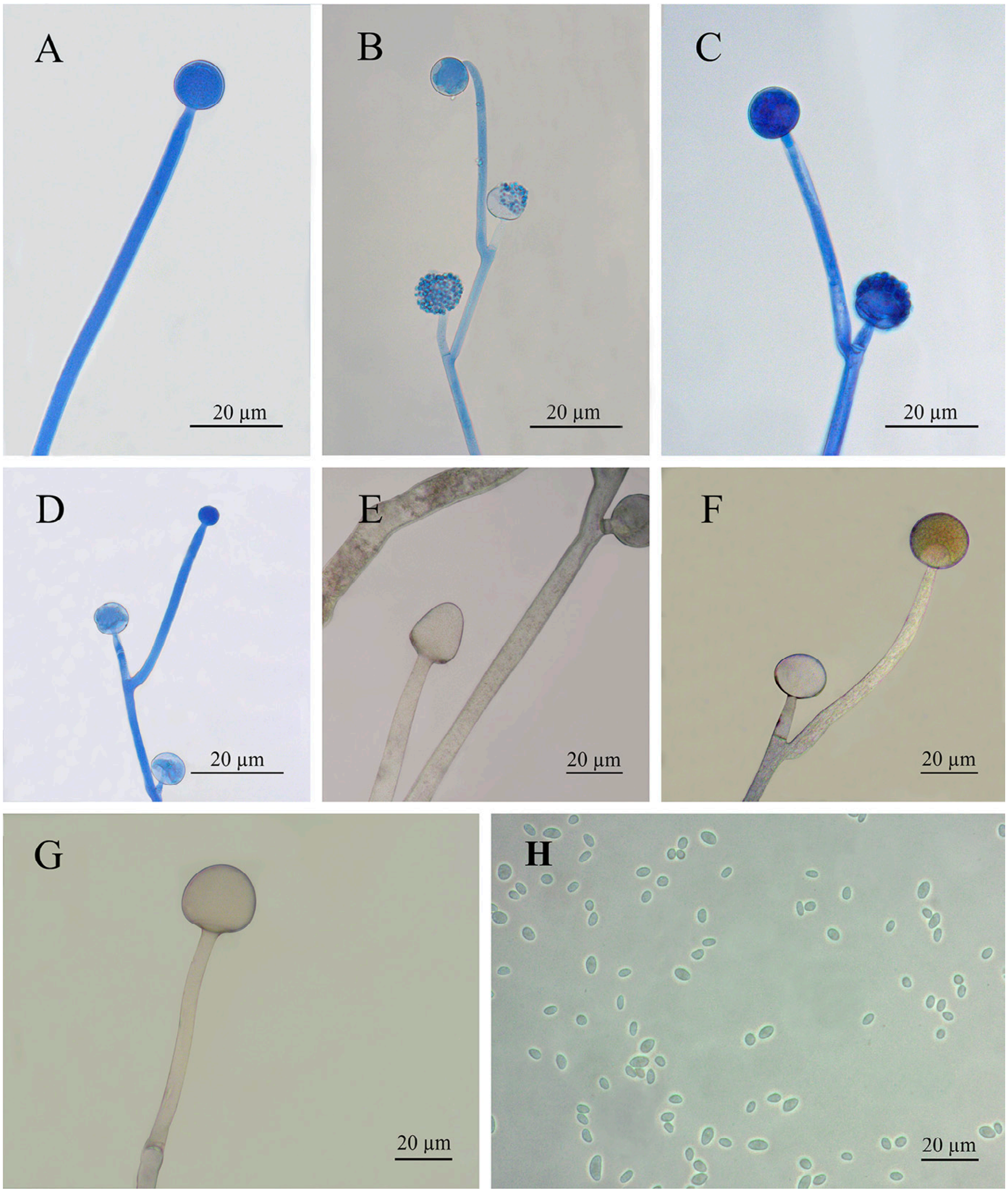

Figure 2. *Mucor variicolumellatus*. **A.** Unbranched sporangiophore with immature sporangium. **B, D.** Sympodially branched sporangiophore with globose sporangia and columella. **C.** Once branched sporangiophore with sporangia. **E.** Sporangiophore with conical columella. **F.** Once-branched sporangiophore with sporangium and columella. **G.** Unbranched sporangiophore with columella. **H.** Sporangiospores.

globose, 13–50 (–55) µm in diameter, obovoid, ovate and strawberry-shaped, 13.5–52 × 13–45 (–60) µm, rarely ellipsoidal to cylindrical 22–33.5 × 20–30, and conical 20–30 × 13–25 µm, smooth-walled, with collar commonly observed. Sporangiospores hyaline, variable in size, ellipsoidal, (3–) 4–8 × 2.5–4.5 µm, smooth-walled. Chlamydospores ellipsoidal to elongated ellipsoidal. Zygospores not observed.

Discussion

The *Mucor circinelloides* complex (MCC), as defined by Walther et al. (2013), currently comprises 14 recognized species, including *M. variicolumellatus*. According to Wagner et al. (2019), despite the morphological similarity between members of the MCC, all species can be distinguished by morphophysiological characteristics and phylogenetically, through molecular markers like ITS rDNA region. Our phylogenetic and morphological analyses support *M. variicolumellatus* as distinct from the other described MCC species.

Species of the MCC constitute a heterogeneous group of fungi, with oligotrophic or mesotrophic representatives adapted to a wide variety of substrates (Wagner et al. 2019). Although species of this complex are admittedly cosmopolitan, data on its geographic distribution are still quite fragmented, especially in Neotropical regions. *Mucor variicolumellatus* has been reported only in Germany, Malawi and the USA, isolated from *Tremella*, maize meal and humans (Álvarez et al. 2011; Wagner et al. 2019). Here we report the first record of *M. variicolumellatus* for the Neotropics, that was isolated from soil in an upland rainforest area of Pernambuco, Brazil.

Morphologically, *M. variicolumellatus* is distinguished from other MCC species by the production of obovoid, ovate and strawberry-shaped columellae. The morphological characteristics described here show close similarity to the original description given by Wagner et al. (2019) and Álvarez et al. (2011) (as *M. fragilis*), despite some features of our isolate, such as strawberry-shaped to conical and cylindrical columellae, were not reported by them. However, we do not consider these differences enough to characterize a new variety.

In the ITS rDNA phylogenetic tree (Fig. 3), *M. variicolumellatus* sequences grouped closely with those of *M. pseudolusitanicus* L. Wagner & G. Walther, that share morphological similarities. However, the sporangiophores of *M. variicolumellatus* do not show swellings at the base as frequently observed in *M. pseudolusitanicus*. Differences in sporangia diameters have also been observed, with *M. pseudolusitanicus* producing smaller sporangia (up to 75 µm). Additionnally, *M. pseudolusitanicus* produces globose, semiglobose or occasionally slightly elongated but never obovoid columellae, different from those of *M. variicolumellatus*, which are obovoid, ovate and strawberry-shaped (Wagner et al. 2019).

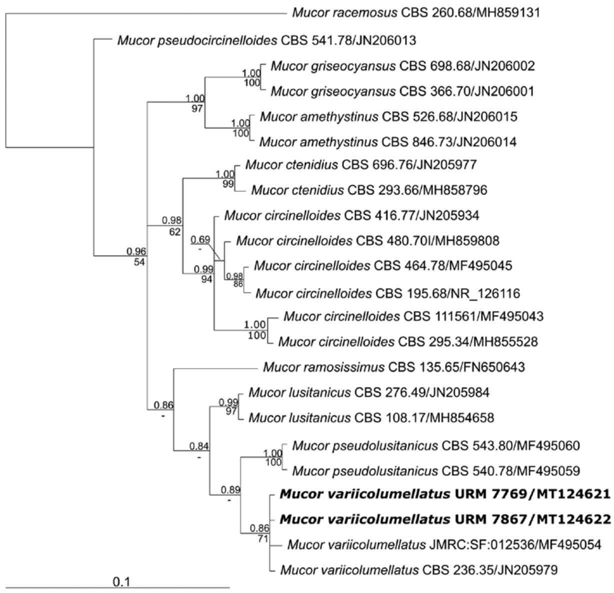

Figure 3. Phylogenetic tree of *Mucor variicolumellatus* and related species constructed using sequences of the ITS region. *Mucor racemosus* was used as outgroup. Sequences are labeled with their database accession numbers. Support values are from Bayesian inference and maximum likelihood analysis. The new Brazilian sequences are in boldface.

Our specimens, isolated as saprobe from soil, represent the first record in the Neotropics thereby expanding the knowledge of the geographical distribution of Mucoralean fungi.

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Authors' Contributions

DXL and CLFL collected the material; DPC performed the specified methodology; CAFS, ALCMAS and EVM wrote the text; ALCMAS and CAFS identified the species and built the plate.

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