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Isomucor trufemiae J.I. de Souza, Pires-Zottarelli & Harakava (Mucorales, Mucoromycota): the second report worldwide and first from soil in northeastern Brazil

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Abstract
Isomucor trufemiae was isolated and described for the first time from soil samples collected in the state of São Paulo State, Brazil, in 2012. Eight years later, we isolated this species in the state of Pernambuco as the second record worldwide and the first record to northeastern Brazil. *Isomucor trufemiae* URM 8342 was isolated from a soil sample during a study on the diversity of Mucorales in a Montane Atlantic Forest area in the municipality of Bonito, Pernambuco, Brazil, and identified through morphological and molecular analyses (ITS and LSU sequences of rDNA). Aspects of the morphology and distribution of this species are commented in this manuscript.

Keywords
ITS rDNA, LSU rDNA, Mucoromycotina, taxonomy.

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Introduction

Montane Atlantic Forests are elevated areas within the semi-arid region of northeastern Brazil, which due to the altitude, that ranges from 500 to 1,500 m, present higher orographic rainfall (Rodrigues et al. 2008; De Queiroz et al. 2017), moderate temperatures and more humid soils when compared to the surrounding Caatinga vegetation (Tabarelli and Santos 2004). These characteristics reflect directly on the local vegetation, with semideciduous forests being predominant in this ecosystem (De Queiroz et al. 2017). In Brazil, 47 Montane Atlantic Forest areas are known in the states of Ceará, Paraíba, Pernambuco and Rio Grande do Norte (Tabarelli and Santos 2004), and studies have pointed out that Montane Atlantic Forest areas of Pernambuco are havens for mucoralean fungi including newly proposed taxa (Alves et al. 2017; Crous et al. 2018; Lima et al. 2018a; Crous et al. 2019; Lima et al. 2020). However, the knowledge of the genera *Isomucor* J.I Souza, Pires-Zottar. and Harakava is still scarce in Brazil, being restrict to the state of São Paulo (Flora do Brasil 2020).

Isomucor belongs to the order Mucorales Fr., subphylum Mucoromycotina Benny, and phylum Mucoromycota Doweld (Spatafora et al. 2016). It was proposed by de Souza et al. (2012) to accommodate *I. trufemiae* J.I. de Souza, Pires-Zottarelli & Harakava and *I. fuscus* (Berl. and De Toni) J.I. de Souza, Pires-Zottarelli & Harakava, the latter formally described as *Mucor fuscus* Bainier, and transferred to *Isomucor* by de Souza et al. (2012). However, according to the Species Fungorum (http://www.indexfungorum.org), *I. fuscus* is an invalid name (Art. 41.5; Turland et al. 2017), and therefore it must be treated as *M. fuscus*. Morphologically, *I. trufemiae* is characterized mainly by producing *Mucor*-like branched sporangiophores, globose sporangia and verrucose sporangiospores (de Souza et al. 2012). Sporangiola are also produced, which is a plesiomorphic character in the Mucoraceae Fries not present in all species of this family (Walther et al. 2013). Here we present a detailed description and illustration of *I. trufemie* isolated from soil in a Montane Atlantic Forest area located in the state of Pernambuco, northeastern Brazil.

Methods

The soil samples were collected in the city of Bonito (08°28′12″S, 035°43′44″W), located in the state of Pernambuco, Brazil (Fig. 1). The region has a rainy, tropical climate with a dry summer, with an average annual temperature ranging between 15 °C and 27 °C, and average annual rainfall around 1,100 mm (Rodal et al. 2005). The local vegetation is comprised of a mix of subperenifolia and hypoxophilic forests (IBGE 2019). For the isolation, 5 mg of soil were inoculated in a wheat germ agar medium (Benny 2008) containing chloramphenicol (100 mg/L). Colony growth was monitored for seven days in the dark at 25 °C. The morphological identification of the specimen was performed in potato dextrose agar and malt extract agar media (Benny 2008) by observing the microstructures (sporangiophores, sporangia, sporangiola, and sporangiospores characteristics), based on the description of de Souza et al. (2012). The genomic DNA extraction was performed according to the methodology described by Lima et al. (2018b). For amplification of the ITS region of rDNA, ITS1 and ITS4 primers were used (White et al. 1990), while for the LSU region, the LR1 and LSU2 primers were used (Schmitt et al. 2009). 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 using sanger sequencing at the Plataforma Multiusuária de Sequenciamento de DNA, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Brazil. The sequences obtained [ITS (MT237439) and LSU (MT237440)] were edited (edges trimmed) with BioEdit (Hall 1999), deposited in GenBank, and full alignments compared with sequences from the holotype [ITS (HQ592190) and LSU (NG060268)] available in that database using BLASTN.

Results

***Isomucor trufemiae* J.I. de Souza, Pires-Zottarelli & Harakava, 2012**; Mycologia 104 (1): 232–241.
Figure 2

New record. BRAZIL • 1 specimen; Pernambuco, Bonito; 08°28′12″S, 035°43′44″W; 10 March 2018; Catarina

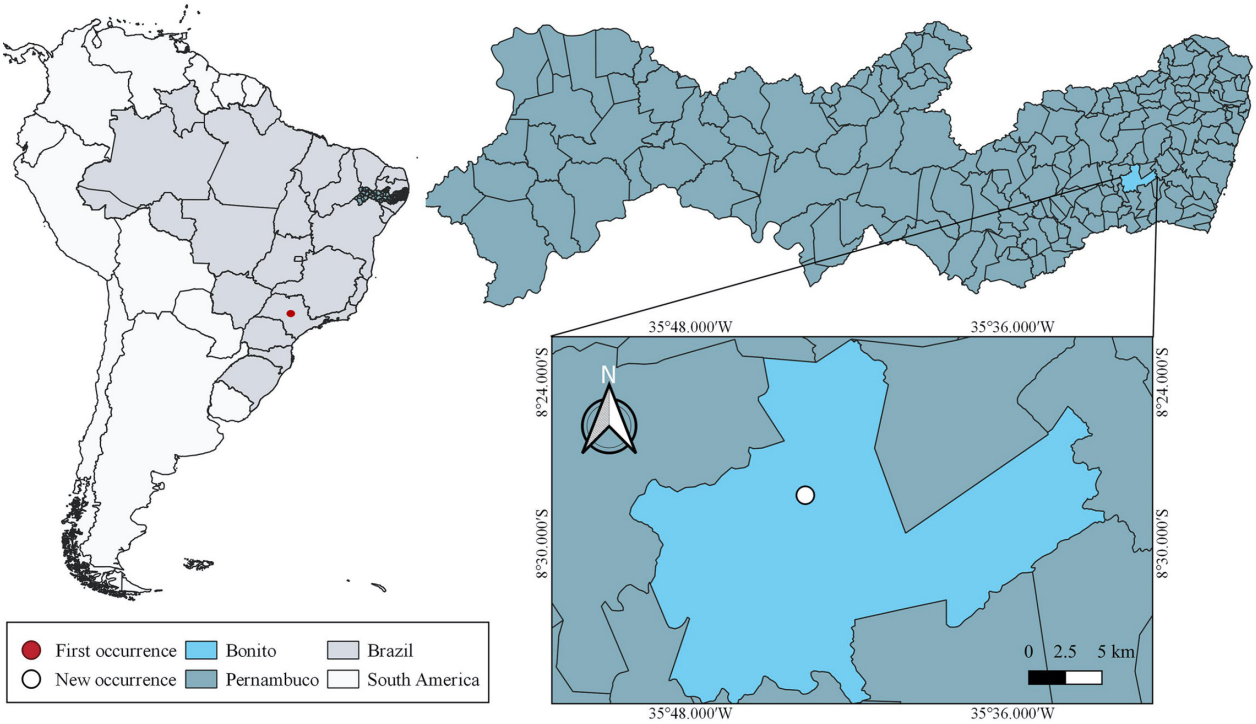

Figure 1. The location in Bonito city, where *Isomucor trufemiae* URM 8342 was found.

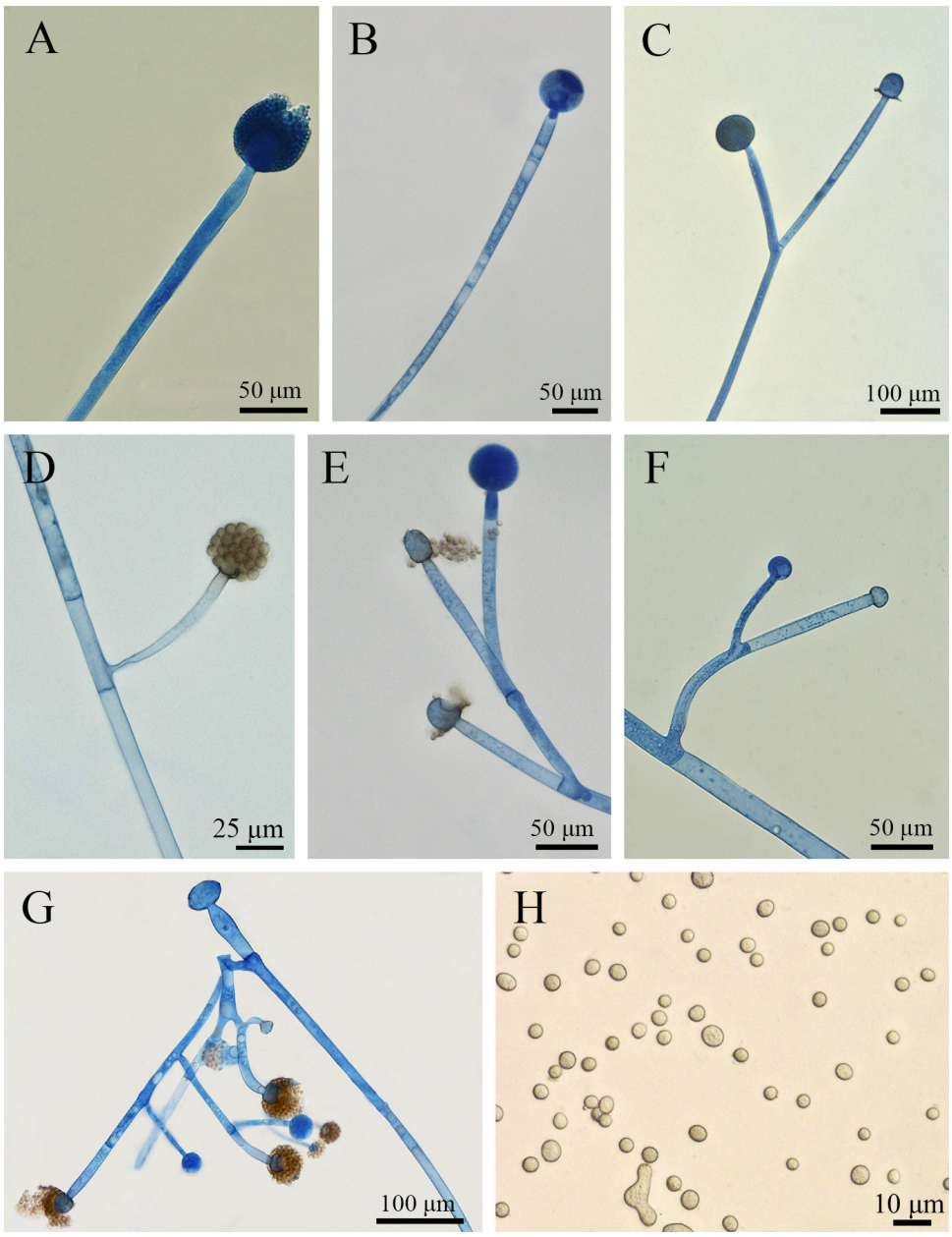

Figure 2. *Isomucor trufemiae*. **A, B.** Unbranched sporangiophore with sporangium. **C.** Branched sporangiophore with sporangium and columella. **D.** Unbranched sporangiophore with sporangiola. **E, F, G.** Branched sporangiophores with sporangia and columellae. **H.** Sporangiospores.

Letícia Ferreira de Lima leg.; in soil; URM 8342; GenBank: MT237439 (ITS), MT237440 (LSU).

Identification. Colony cotton-like, gray, dotted with black spots corresponding to sporangia, covering the entire Petri dish (9 cm diameter × 1.5 cm high) after seven days, on PDA at 25 °C; reverse yellowish-cream-colored. Odorless. Hyphae with irregularly spaced septa, with or without greenish-yellow, granular contents. Sporangiophores long, hyaline and smooth-walled, erect, mono- or sympodially branched with a short distance between the branches, 50–1700 (2000) × (7.5) 9.5–14.5 (19.5) µm. Lateral sporangiophores short, hyaline and smooth-walled, simple or monopodially branched, curved or erect, (36.5) 44–250 × 5–12 µm. Sporangia initially light brown becoming dark brown, non-apophysate, globose, 26.5–85 µm in diameter, smooth-walled and deliquescent. Columellae hyaline, cylindrical with truncate base, 24.5–48.5 × 14.5–36.5 µm, pyriform, 34–50 × 23–36.5 µm, and globose, 22–60 µm in diameter; collar evident. Sporangiola brown, globose, multispored, 15–30 µm in diameter. Columellae of sporangiola hyaline, flattened, 4.8–7.5 × 5–8.5 µm, globose, 9.5–22 µm in diameter, and conical, 12–17 × 11–14.5 µm. Sporangiospores, hyaline, globose, 4.8–7.5 µm in diameter, irregular, 7.3–19.5 × 4.8–7.3 µm, and ovoid, 7.3–9.7 × 4.8–7.3 µm, smooth-walled. Chlamydospores absent. Zygosporangia not observed. In the BLASTN analysis, the ITS (MT237439) and LSU (MT237440) sequences of URM 8342 showed 98.82% and 99.19% similarity with those obtained from the holotype: HQ592190 and NG060268, respectively.

Discussion

The molecular analysis (ITS and LSU rDNA regions) showed that URM 8342 is an *Isomucor trufemiae* and URM 8342 is morphologically quite similar to the holotype. However, some differences between both specimens have been verified. Although de Souza et al. (2012) observed vegetative mycelium uniformly septate, we observed hyphae with irregularly spaced septa. Our strain exhibits long sporangiophores sympodially branched with a short distance between the branches, while the holotype shows sympodial branches with a long distance between the branches. In addition, the lateral short sporangiophores of the holotype may show monopodial or sympodial branches, differing from URM 8342, which is often monopodially branched. Sporangia up to 85 µm in diameter are observed in URM 8342, smaller than those described by de Souza et al. (2012), which are up to 164 µm in diameter. The columellae of URM 8342 are up to 60 µm in diameter, smaller than the ones of the holotype, which are up to 110 µm in diameter. In addition, the sporangiospores of URM 8342 are bigger (up to 19.5 µm long) and are smooth-walled, different from those of the holotype that are up to 14.2 µm long and slightly rough-walled.

This work contributes to the knowledge of the distribution of *I. trufemiae*. So far, this species was reported only once in the Cerrado domain (holotype), in the state of São Paulo. This is the second record worldwide and the first report of *I. trufemiae* from a Montane Atlantic Forest area in northeastern Brazil.

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Authors' Contributions

JDAL and GCLC collected the material; DXL and LWSF performed the specified methodology; ALCMAS and CLFL identified the species; CLFL, ALCMAS and LMSG wrote the text.

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