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Original article

Filling a gap: endophytic fungal diversity in the leaves of *Miconia mirabilis* in the Atlantic Forest (Brazil) and the description of a new *Muyocopron* species

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ABSTRACT

Brazilian biomes, such as the Atlantic Forest, are hotspots for microbial diversity. Fungi at some stages of their life cycle can inhabit the interior of plants without apparent damage and are called endophytes. This study aimed to verify the culturable diversity of endophytic fungi present in the leaves of *Miconia mirabilis* in a conservation unit of the Atlantic Forest, in Northeast Brazil. A total of 273 endophytes were isolated, and 88 % of the estimated richness was recovered from 40 taxa with a Shannon diversity index of H' = 2.79. The most abundant order was Xylariales (37.73 %), with *Xylaria* cf. *cubensis* being the most abundant species, followed by Glomerellales (14.65 %), Eurotiales (3.66 %), and Diaporthales (3.33 %). Additionally, a novel endophytic fungal species of *Muyocopron* was identified. Our results indicate the potential of endophytic diversity in the plants of tropical forests, reinforcing the need for further studies to better understand their ecological relationships.

Keywords: Endophytic fungi; Phylogeny; Rainforest; Taxonomy; Xylariales.

Introduction

Among Brazilian biomes, the Atlantic Forest is one of the 34 biodiversity hotspots in the world (Leitman *et al*. 2015). Approximately, 90 % of its original area is degraded and just 28 % of its original vegetation is protected in conservation units, generating implications at different ecological levels (Rezende *et al*. 2018; Lembi *et al*. 2020). Presently, the lack of conservation is a major problem, leading to a considerable loss of biodiversity and its services (Foden & Young 2016). Therefore, it is necessary to preserve *in situ* this diversity in protected areas such as conservation units (Heywood 2019).

Conservation units (UCs) are established to conserve and protect biodiversity (Mattar *et al*. 2018); as a result, species that are threatened or vulnerable can be found in

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these areas. The UCs cover 18% of Brazilian territory, with 334 federal UCs managed by the Chico Mendes Institute for Biodiversity Conservation. Of these, 131 are located in the Atlantic Forest (ICMBio 2022). In Brazil, UCs are divided into two categories (Strict Protected Areas and Protected Areas with sustainable use); however, they are mainly located in areas with large population concentrations (Lembi *et al*. 2020) and, consequently, under pressure from anthropogenic activities (Bernard *et al*. 2014). Among the UCs of the Atlantic Forest, the Reserva Biológica de Pedra Talhada (REBIO de Pedra Talhada) was established on 13 December 1989 and is located in a transitional area between the Seasonal Tropical Forest and the Caatinga, with humid forests surrounded by dry forests (Nusbaumer *et al*. 2015; ICMBio 2022). Several species of animals, plants, and fungi have been reported in this reserve, including approximately 823 plant species (Studer *et al*. 2015). *Miconia mirabilis* (Melastomataceae, Myrtales) is a plant found in this UC. It grows rapidly, restores degraded areas, and provides food resources for the fauna (Pessoa *et al*. 2012). Although there are few studies on the ecological aspects of this species, to the best of our knowledge, no study has reported the association of endophytic fungi with *M. mirabilis*. Complex microbial communities colonise plants at different stages of interaction and perform various functions, both beneficial and harmful to the plant, depending on environmental factors (biotic and abiotic) and the genetic compositions of the host and microorganism (Rey & Schornack 2013; Brader *et al*. 2014). While studies have elucidated this interaction, it is known that when dealing with plant microbiome, fungi are an important part of plant development, growth, and adaptation. During all or part of their life cycle, they can exist within all plant tissues, without apparent damage, and are functionally defined as endophytic fungi (Khidir *et al*. 2010; Hardoim *et al*. 2015).

Although there is a growing interest in the understanding of endophytic biology and diversity, these fungi still correspond to a neglected wealth; therefore, further studies are needed to explore its diversity and distribution (Arnold & Lutzoni 2007; Baucom *et al*. 2012; Vaz *et al*. 2014; Baldrian 2017). The increase in conservation and accessibility to different biomes contribute to the increase in knowledge about the biology and life cycle of endophytic fungi. In particular, Brazilian biomes harbour substantial biodiversity and stand out due to specific characteristics (Noriler *et al*. 2018; Temperton *et al*. 2019; Rosa 2021).

This study aimed to verify the culturable diversity of endophytic fungi in the leaves of *M. mirabilis* growing in a UC of the Atlantic Forest in Brazil to contribute to the national and global fungal estimations and to understand the endophytic fungal diversity in a plant species of the Atlantic Forest in Brazil. We also introduced a new species of *Muyocopron* (Muyocopronaceae, Muyocopronales), demonstrating the capacity of *M. mirabilis* to harbour unknown fungal species.

Materials and methods

Study site

The leaves of *M. mirabilis* (Aubl.) L.O. Williams were collected during the rainy season (July 2018) from REBIO Pedra Talhada (36°22' to 36°28'W, 9°11' to 9°16'S) in the Atlantic Forest. The REBIO Pedra Talhada has an area of approximately 4469 ha and is located in the municipalities of Lagoa do Ouro and Correntes (Pernambuco) and Quebrangulo and Chã Preta (Alagoas) (Fig. 1). It is in the Borborema Plateau, a particular mountain formation in Northeast Brazil. The UC, a humid forest surrounded by dry forest, is in a transition area between the Seasonal Tropical Forest and Caatinga (Nusbaumer *et al*. 2015). This UC has a defined rainy season (May, June, and July) with an average annual accumulation of 1628 mm (more than 250 mm per month), and the dry season extends from October to

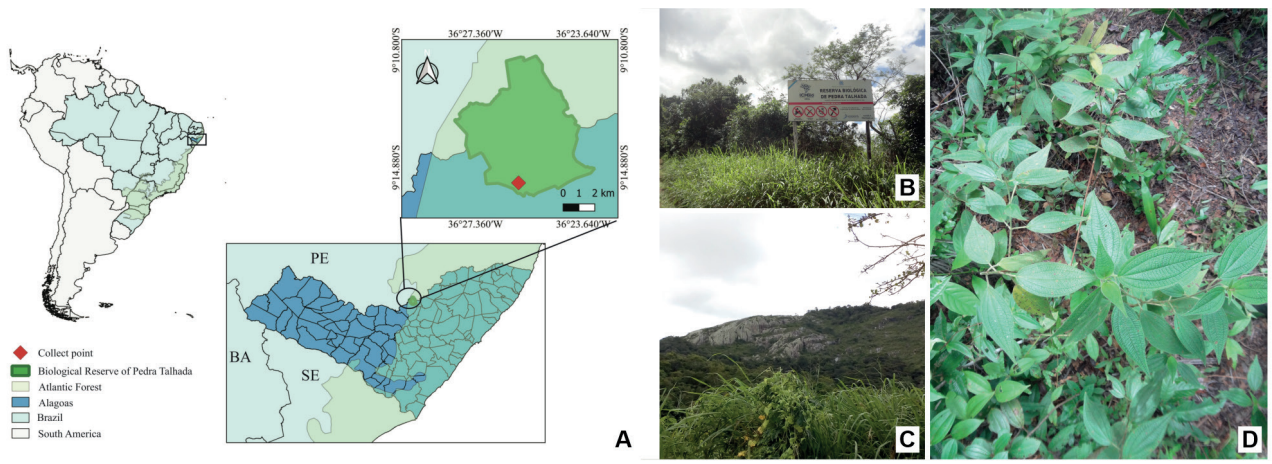

Figure 1. The geographical location of the Biological Reserve of Pedra Talhada, Atlantic Forest, Brazil (**A**), View of the main site of the studied area (**B-C**), and details of *Miconia mirabilis* (**D**).

February, with less than 50 mm rainfall per month (Studer *et al*. 2015; ICMBio 2022).

Plant collection was authorised by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) under the Ministério do Meio Ambiente (MMA); the permission number 63010 and authentication code 55412112 were issued on 26 April 2018.

Sampling and processing of plant materials

Healthy leaves from four individuals (labelled M1, M2, M3, and M4) of *M. mirabilis* were randomly collected. Plant materials (three to four leaves per individual) were individually packed in paper bags and processed within 48 h. To isolate endophytic fungi, the leaves were disinfected using the methods described by Bezerra *et al*. (2015). Briefly, asepsis of the leaves was performed by initial washing with water and detergent to remove impurities, followed by rinsing with distilled water. Subsequently, the leaves were washed in 70 % alcohol (1 min), 2–2.5 % sodium hypochlorite (3 min), and 70 % alcohol (1 min). Finally, the leaves were washed thrice (1 min each) in distilled and sterilized water.

The leaves were fragmented using a sterile scalpel blade, and 180 fragments of approximately 0.5 cm^2 were selected (45 fragments from each individual). The fragments were transferred to Petri dishes containing potato dextrose agar (PDA) and chloramphenicol (50 mgL^{-1}). The Petri dishes were incubated at 28 ± 2 °C for 10 days in a natural light-dark cycle. To verify the effectiveness of disinfection, 1 mL of the last washing water was transferred to the surface of the PDA, and the Petri dishes were incubated under the same conditions. Fungal growth was observed daily, and all fungal colonies were isolated and purified. After isolation, endophytes were preserved in water containing 10 % glycerol for later morphological and molecular analyses.

Identification of fungal endophytes

Endophytic fungi were identified based on their morphology by observing the macro- and micro-morphological characteristics of somatic and reproductive structures (Barnett & Hunter 1987; Hanlin 1990; Samson & Frisvad 2004; Hsieh *et al*. 2010; Gomes *et al*. 2013; Phillips *et al*. 2013; Hernández-Restrepo *et al*. 2019; Jayawardena *et al*. 2020). Later, the endophytic isolates were subcultured for DNA extraction and sequence analyses.

We deposited representative cultures of the endophytic isolates in the culture collection Micoteca URM Profa. Maria Auxiliadora Cavalcanti at the Universidade Federal de Pernambuco (UFPE), Recife, Brazil, and the permanent microscopic slides (holotype) of the new species in the herbarium at the Universidade Federal de Goiás (UFG), Goiânia, Brazil. In addition, we deposited all isolates in the working collection of the Laboratório de Micologia Ambiental at the UFPE and the ex-type strain of the new species in the working collection FCCUFG housed at the Laboratório de Micologia of the Instituto de Patologia Tropical e Saúde Pública of the UFG.

Morphological analysis of the new species

Endophytic isolates were cultured on malt extract agar (MEA) and PDA and incubated for two weeks at 28 °C in the dark. Colony colours were analysed using the Rayner colour chart (Rayner 1970). Lactic acid (60–80 %) was used as the mounting fluid to observe the micromorphological features. Macromorphological characteristics of the colonies were observed and described in two weeks. Micromorphological observations (such as conidiogenous cells and conidia) were performed after sporulation of the isolates on PDA for approximately 45 days, with the inclusion of sterile plant tissue in the agar plates (Hernández-Restrepo *et al*. 2019).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from seven-day cultures of endophytic fungi grown on PDA using the Wizard® SV Genomic DNA Purification System (Promega) Extraction Kit, following the manufacturer's instructions. Sequences of internal transcribed spacer (ITS) and intervening 5.8S rRNA, large subunit (LSU) rDNA, RNA polymerase II second largest subunit (*rpb2*), β-tubulin (*tub2*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were amplified using the primers ITS1/ITS4 or ITS5/ITS4 (White *et al*. 1990), LR0R/LR5 (Vilgalys & Hester 1990; Vilgalys & Sun 1994), fRPB2-5F2/fRPB2-7cR (Liu *et al*. 1999; Sung *et al*. 2007), Bt2a/Bt2b (Glass & Donaldson 1995) and GDF1/GDR1 (Weir *et al*. 2012), respectively.

Amplification, sequencing, and sequence editing were performed according to the methodology described by Bezerra *et al*. (2017) and Oliveira *et al*. (2020). The sequences obtained in this study were deposited in the NCBI GenBank database (Table S1).

Phylogenetic analyses

Phylogenetic analyses were performed using our sequences and reference sequences retrieved from the GenBank database (Hernández-Restrepo *et al*. 2019; Hyde *et al*. 2020). The sequences were aligned using the MAFFT v.7 online interface (Katoh & Standley 2013) and manually edited with the MEGA v.7 software (Kumar *et al*. 2016).

For fungal sequence analyses, we built alignments for each fungal genus and performed a maximum likelihood (ML) analysis (data not shown) following the instructions of Bezerra *et al*. (2017). For the phylogenetic inference of the new species, Bayesian inference (BI) and ML analyses were conducted using MrBayes v. 3.2.7a on XSEDE and RAxML-HPC BlackBox v. 8.2.12, respectively, in the CIPRES Science Portal (Miller *et al*. 2010; Ronquist *et al*. 2012; Stamatakis 2014). BI analysis was conducted with 1×10^6 generations and a burning value of 25 %, with chains sampled every

1000 generations, and ML analysis was conducted with 1000 bootstrap replicates. The best nucleotide model for BI analysis was estimated using MrModelTest v.2.3 software (Nylander 2004). The resulting phylogenetic trees were visualised using FigTree Software (Rambaut 2012). Values equal to or greater than 0.95 BI posterior probability (PP) and 70 % ML bootstrap support (ML-BS) were shown near the nodes. The alignment of *Muyocopron* was deposited in TreeBASE (Study ID S30510).

Data analyses

The endophytic colonisation rate (CR) of the fragments was calculated as the ratio of the number of fungal growth fragments (Nf) to the total number of fragments (Nt), that is CR = (Nf/Nt × 100) (Araújo *et al*. 2002). The total number of endophytes isolated was considered as absolute frequency, and the ratio of the number of isolates of each species to the total number of isolates gave the relative frequency (Photita *et al*. 2001).

Only endophytic isolates identified at the genus level were included in the ecological analyses. Richness (S) and Shannon–Wiener (H') indices were estimated for each sample (individuals M1, M2, M3, and M4). Richness was defined as the number of species in each sample. Shannon–Wiener index was calculated based on the equation H' = −∑ Ni ln(Ni) (Shannon & Weaver 1949). All the analyses were performed using the software R v. 4.1.0 (R Core Team 2021) under '*vegan*', '*agricolae*', and '*lsmeans*' R packages (Lenth 2016; Oksanen *et al*. 2020; Mendiburu 2021). To determine the species richness, abundance, and diversity indices, we used the functions '*biodiversity*', '*specpool*', and '*colSums*', respectively. Additionally, for Jaccard cluster analysis, the function '*vegdist*' under the R package '*vegan*' was used. Venn diagrams were created using the online software InteractiVenn (Heberle *et al*. 2015). To verify the sufficiency of the sampling effort based on the observed richness, curves of the accumulation and extrapolation of endophytic species were determined using iNEXT online (Chao *et al*. 2016).

Results

Fungal endophytes

In total, 168 leaf fragments of *M. mirabilis* were colonised (CR = 93.33 %), and 285 endophytic fungi were isolated. Twelve of the isolates did not grow after subculturing, and 273 endophytes were identified based on their morphology and DNA sequence analyses (Table S1). We were not successful in the morphological analysis and DNA amplification of 104 isolates, most of which were tentatively grouped as xylariaceous fungi (83 isolates), therefore, these isolates were not included in our ecological analyses. The isolates identified at in some specific level were considered and organized within the four samples (M1-M4) that represented each individual plant collected. Twenty-five isolates were only identified at the order level (Xylariales) and one at the family rank (Sporormiaceae) because they were phylogenetically distinct from the other isolates and DNA sequences included in our analyses (data not shown).

Endophytic isolates belonged to eight orders of Ascomycota named: Botryosphaeriales, Diaporthales, Eurotiales, Glomerellales, Muyocopronales, Pleosporales, Trichosphaeriales, and Xylariales. The order Xylariales showed the highest number of isolates (103), followed by Glomerellales (40), Eurotiales (10), and Diaporthales (9). Endophytes of other orders (such as Botryosphaeriales and Muyocopronales) were also obtained but with a low isolation frequency (Table 1). Two isolates of *Muyocopron* (Muyocopronaceae, Muyocopronales) were described as new species.

Diversity and richness of endophytes

Forty taxa comprised the total richness of endophytic fungi associated with the leaves of *M. mirabilis*. They were distributed in eight orders and ten families of Ascomycota. The species richness observed for each individual of *M. mirabilis* did not differ , with M1 (*S* = 15), M2 (*S* = 17), M3 (S = 17), and M4 (S = 18); however, the diversity of endophytic fungi based on the Shannon index (H' = 2.79) showed substantial differences, with M3 (H' = 2.55) being the most diverse among the individuals studied (Table 2).

Species accumulation curve and sampling sufficiency

Based on Simpson's diversity, two species accumulation and extrapolation curves were constructed: one for the four individuals of *M. mirabilis* and the other for the endophytic fungi isolated from *M. mirabilis* leaves. The sampling sufficiency curve in each case did not reach a plateau; however, by observing each sampled individual, the sampling effort was sufficient to recover 86 % of the expected richness in M1, 81 % in M4, 71 % in M2, and 72 % in M3 (Fig. 2A). In total, the sampling effort was sufficient to recover 88 % of the estimated richness of endophytic fungi in *M. mirabilis* leaves (Fig. 2A-B).

Total abundance of species

Based on the number of species, the total abundance of the species index was determined as the sum of all sampled areas. The results showed that *Xylaria* cf. *cubensis* was the most abundant species, followed by *Colletotrichum karstii* of the *C. boninense* species complex, *Colletotrichum* sp. 4 of the *C. gloeosporioides* species complex, and Xylariales sp. 1. In contrast, *Diaporthe ueckerae* showed the lowest abundance index; however, it is important to highlight that *Diaporthe* was one of the most specious genus. In addition,

an abundance chart was created for each individual to better understand the distribution of abundance among the samples. There were small differences in the total abundance of species, with M1 being the most abundant, followed by M4, M2, and M3 (Fig. 3).

Composition of the sampled individuals

A comparison of the taxa distribution revealed that only *C. karstii* of the *C. boninense* species complex, *Colletotrichum* sp. 4 of the *C. gloeosporioides* species complex, *Xylaria* cf. *cubensis*, and Xylariales sp. 4 were shared among the sampled

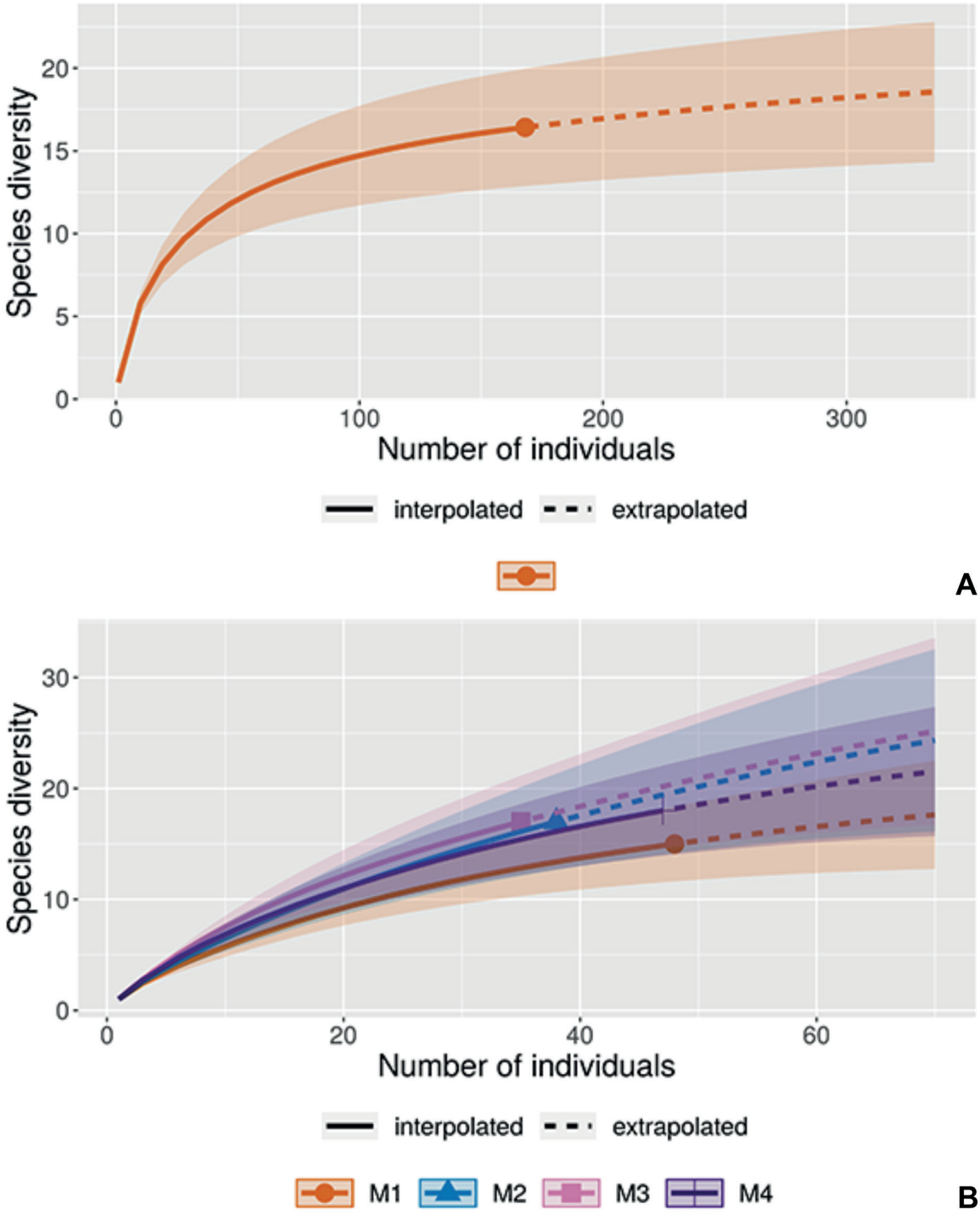

Figure 2. Taxa accumulation and extrapolation curve, based on Simpsons Diversity, for endophytic fungi recovered from leaves of *Miconia mirabilis* in an area of the Atlantic Forest, Brazil (**A**) and taxa accumulation and extrapolation curve, based on Simpsons Diversity, for the four individuals of *Miconia mirabilis* in an area of the Atlantic Forest, Brazil (**B**).

Table 1. Absolute (f) and relative (fr) frequency of endophytic fungi isolated from *Miconia mirabilis* leaves in an area of the Atlantic Forest, Brazil.

Endophytic fungi	M1	M2	M3	M4	f	fr
Botryosphaeriales						
Phyllostictaceae						
Phyllosticta capitalensis		1			1	0.59
Diaporthales						
Diaporthaceae						
Diaporthe paranensis			1		1	0.59
Diaporthe sp. 1			1	2	3	1.78
Diaporthe sp. 2	1				1	0.59
Diaporthe sp. 3	1				1	0.59
Diaporthe sp. 4			1		1	0.59
Diaporthe sp. 5				1	1	0.59
Diaporthe ueckerae				1	1	0.59
Eurotiales						
Aspergilaceae						
Aspergillus sydowii		1			1	0.59
Penicillium alagoense		1		3	4	2.38
Penicillium citrinum	1				1	0.59
Penicillium sp. 1		1	1	1	3	1.78
Talaromyces allahabadensis				1	1	0.59
Glomerellales						
Glomerellaceae						
Colletotrichum gigasporum (Gigasporum species complex)	1				1	0.59
Colletotrichum sp. 1 (Gigasporum species complex)	1				1	0.59
Colletotrichum karstii (Boninense species complex)	4	3	8	8	23	13.69
Colletotrichum sp. 1 (Gloeosporioides species complex)				1	1	0.59
Colletotrichum sp. 2 (Gloeosporioides species complex)		2			2	1.19
Colletotrichum sp. 3 (Gloeosporioides species complex)			1		1	0.59
Colletotrichum sp. 4 (Gloeosporioides species complex)	2	2	3	2	9	0.59
Colletotrichum sp. 5 (Gloeosporioides species complex)				1	1	0.59
Colletotrichum sp. 6 (Gloeosporioides species complex)			1		1	0.59
Muyocopronales						
Muyocopronaceae						
Muyocopron lulasilvae sp. nov.		2			2	1,19
Pleosporales						
Sporormiaceae						
Sporormiaceae sp. 1		1	1		2	1.19
Trichosphaeriales						
Trichosphaeriaceae						
Nigrospora hainanensis	2				2	1.19
Xylariales						
Xylariales sp. 1	3		3	2	8	4.76
Xylariales sp. 2		1			1	0.59
Xylariales sp. 3			1	1	2	1.19

Table 1. Cont.

Endophytic fungi	M1	M2	M3	M4	f	fr
Xylariales sp. 4	2	1	3	3	9	5.37
Xylariales sp. 5			2	1	3	1.78
Xylariales sp. 6			1	1	2	1.19
Hypoxylaceae						
Hypoxylon cf. *investiens*		1			1	0.59
Induratiaceae						
Induratia cf. *coffeanum*	3				3	1.78
Induratia cf. *thailandica*				2	2	1.19
Xylariaceae						
Nemania cf. *abortiva*	1	3			4	2.38
Xylaria cf. *arbuscula*			1	3	4	2.38
Xylaria cf. *cubensis*	22	15	2	13	52	30.95
Xylaria cf. *curta*	3	2			5	2.97
Xylaria cf. *laevis*		1			1	0.59
Xylaria cf. *multiplex*	1	1	4		6	3.57
Total					**169**	

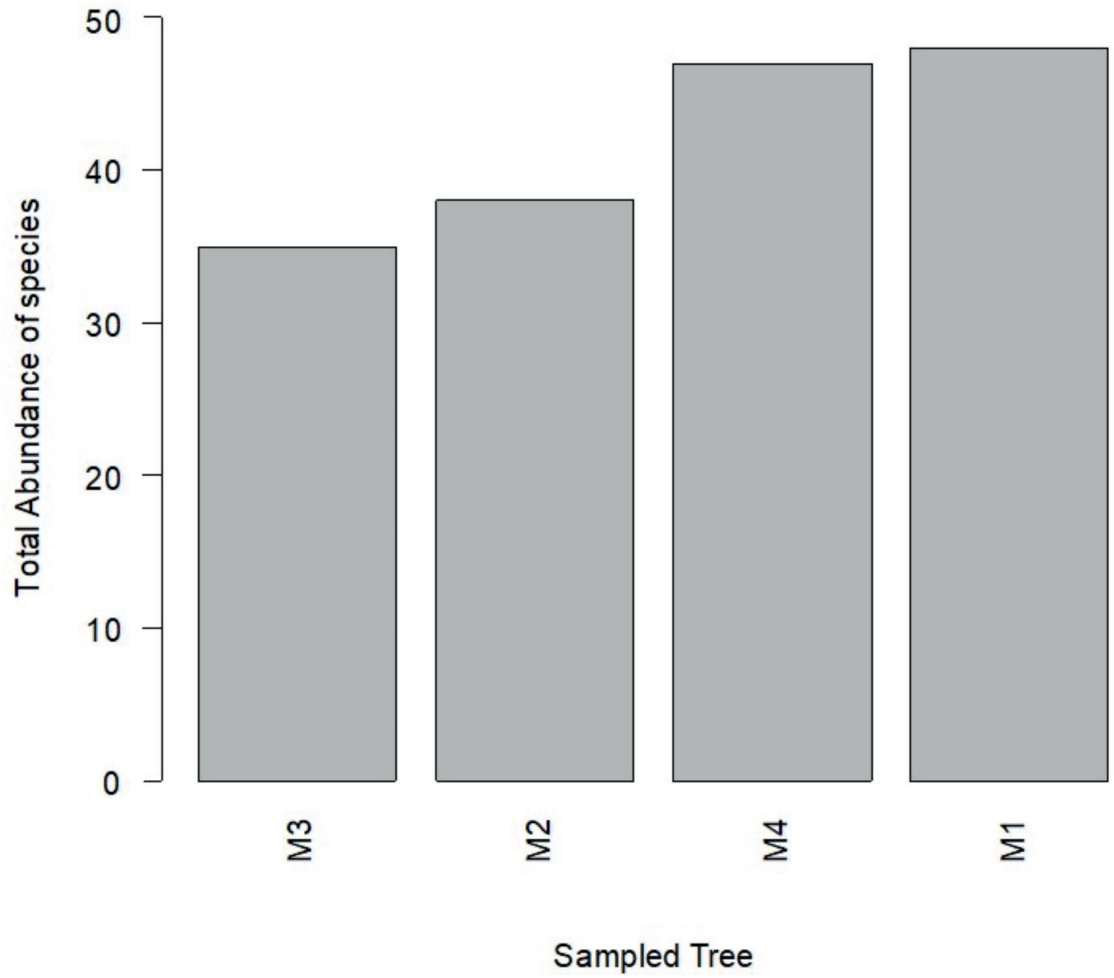

Figure 3. Abundance of endophytic fungi species, according to ascending order, per individual studied in *Miconia mirabilis* leaves of in an area of the Atlantic Forest, Brazil.

Miconia individuals. Notably, M3 and M4 shared five taxa (Xylariales sp. 5, Xylariales sp. 6, Xylariales sp. 3, *Xylaria* cf. *arbuscula*, and *Diaporthe* sp. 1), and M1 and M2 shared two species (*Xylaria* cf. *curta* and *Nemania* cf. *abortiva*). M1-M2-M3 and M1-M3-M4 shared only one species, *Xylaria* cf. *multiplex* and Xylariales sp. 1, respectively (Fig. 4A). Moreover, some species occurred exclusively in a single sampled individual: M1 (seven species), M2 (seven species), M3 (four species), and M4 (six species). The new species of *Muyocopron* occurred in only one individual (M2), and *Penicillium alagoense*, a species recently described from *M. mirabilis* as an endophyte (Crous *et al*. 2019), was isolated from two individuals (M2 and M4). Because the studied individuals shared a small number of species, the Jaccard cluster analysis method was used to investigate the similarity of the sampled individuals of *M. mirabilis*. Considering the species distribution, two main clades (Fig. 4B) were observed. The individuals M1-M2 similarity of 0.720 and M3-M4 a similarity of 0.781.

New species

The sequences of the endophytic fungi URM 8306 = FCCUFG 22 and URM 8724 = FCCUFG 23 were compared with corresponding sequences using the BLASTn tool searches, demonstrated their relationship with *Muyocopron* species (Muyocopronaceae, Muyocopronales). The ITS sequences had low similarity with those of *M. ficinum* MFLU 18-2515 (92.81 %), the LSU sequence had high similarity with that of *M. geniculatum* CBS 721.95 (98.12 %), and the *rpb2* sequence had low identity to *M. atromaculans* MUCL 34983 (87.64 %). The matrix of *Muyocopron* sequences consisted of 18 species and two outgroup sequences (Table S2), and the combined alignment contained 1516 characters (ITS = 686 and LSU = 830), including gaps. The GTR + I + G model was used for all ML analyses. For the BI analysis GTR + G and GTR + I + G models were used for of ITS and LSU, respectively.

Phylogenetic analyses using an individual dataset for each gene (data not shown) and a combined ITS–LSU matrix showed that the sequences of the new species belong to an independent lineage related to *M. atromaculans* and *M. ficinum* (ML–BS = 100 and PP = 1). (Fig. 5).

Taxonomy

Classification: Muyocopronaceae, Muyocopronales, Dothideomycetes

Muyocopron lulasilvae L.O. Ferro, Souza-Motta & J.D.P. Bezerra, *sp. nov.* Fig. 6

Type: Brazil, Alagoas state, Quebrangulo municipality, Reserva Biológica de Pedra Talhada, 09°15'26.8"S and

Table 2. Richness and Diversity based on Shannon-Wiener indices of endophytic fungi from leaves of *Miconia mirabilis* in an area of the Atlantic Forest, Brazil.

Individuals	Richness of species	Diversity (Shannon index)
M1	15	2.04
M2	17	2.28
M3	17	2.55
M4	18	2.45

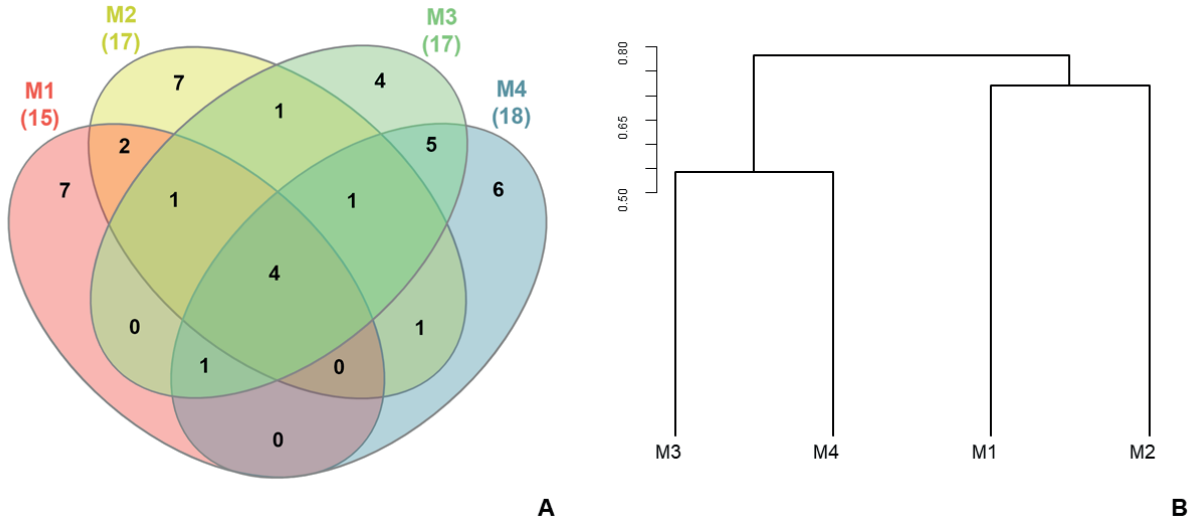

Figure 4. Venn diagram showing the richness of taxa of endophytic fungi from leaves of four individuals of *Miconia mirabilis* (exclusive and shared) in an area of the Atlantic Forest, Brazil (**A**) and Jaccard similarity index of the four individuals collected from *Miconia mirabilis* leaves in an area of the Atlantic Forest, Brazil. P = 0.96 (**B**).

36°25'53.7"W, as an endophyte from the leaves of *Miconia mirabilis* (Melastomataceae), July 2018, L.O. Ferro (Holotype Herbarium UFG 34288), ex-type culture in Micoteca URM 8306 = FCCUFG 22. MycoBank number: MB 849193.

Description: Asexual morph: *Hyphae* septate, branched, smooth, hyaline, pale brown and dark brown, 3–5.5(–8) µm wide. *Conidiomata* sporodochium-like, superficial, varying from a few combined cells to large aggregations, variable shape and size, pale to dark brown. *Conidiogenous cells* globose to ampulliform, smooth, with a collarette, pale to dark brown, (13.5–)16–19 × (8–)13(–15) µm. *Conidia* aseptate, lunate, curved, hyaline, smooth, guttulate, 16(–20) × 8(–11) µm. *Appressoria* not observed. Sexual morph not observed.

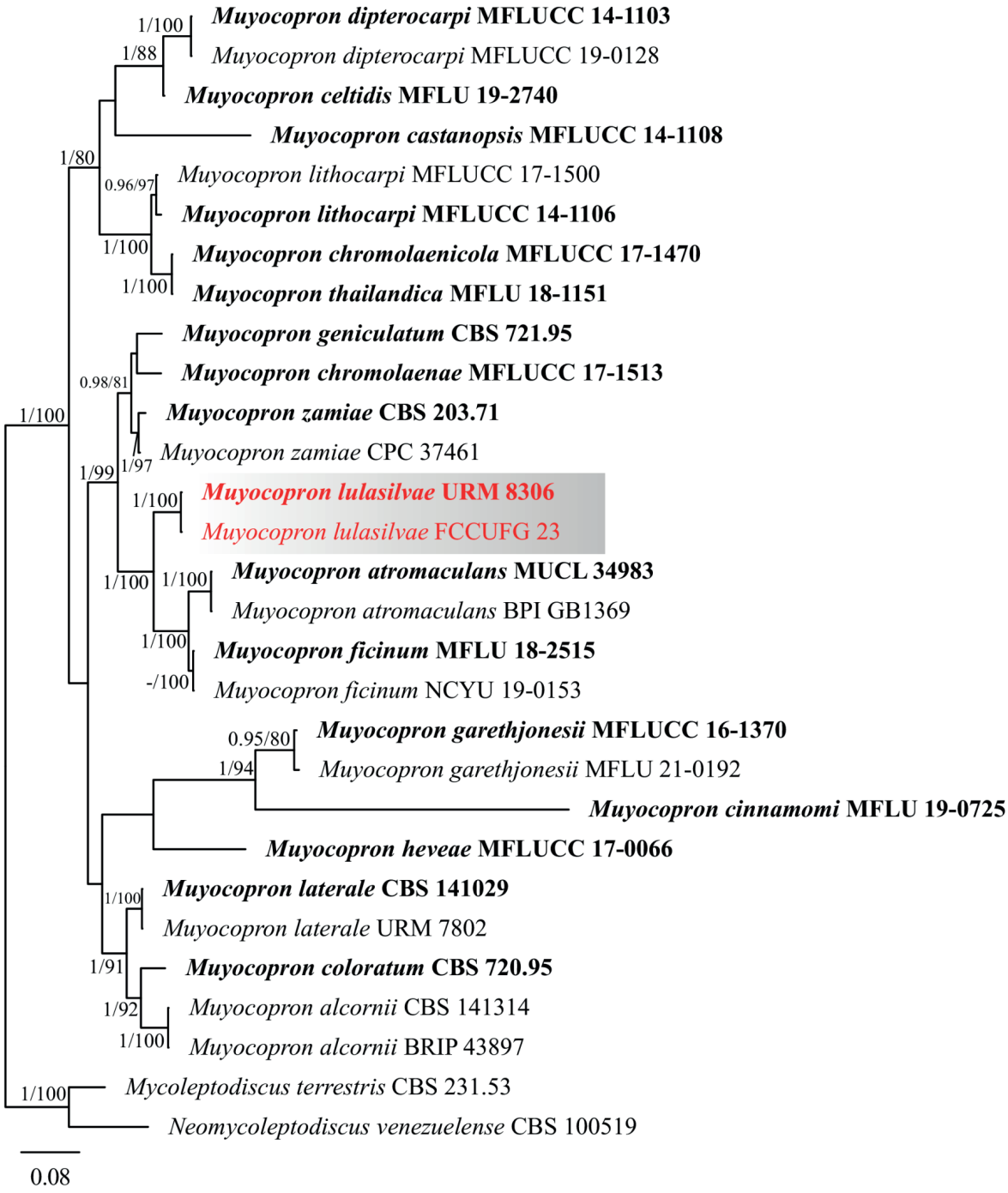

Figure 5. Bayesian phylogenetic tree based on a combined of LSU and ITS rDNA dataset of species included in *Muyocopron*. The new species described in this study (*Muyocopron lulasilvae*) is highlighted in red. Ex-type strains are in bold. Posterior probabilities from BI above 0.95 and ML bootstrap support values above 70 % are shown near nodes. The tree was rooted to *Mycoleptodiscus terrestris* CBS 231.53 and *Neomycoleptodiscus venezuelense* CBS 100519.

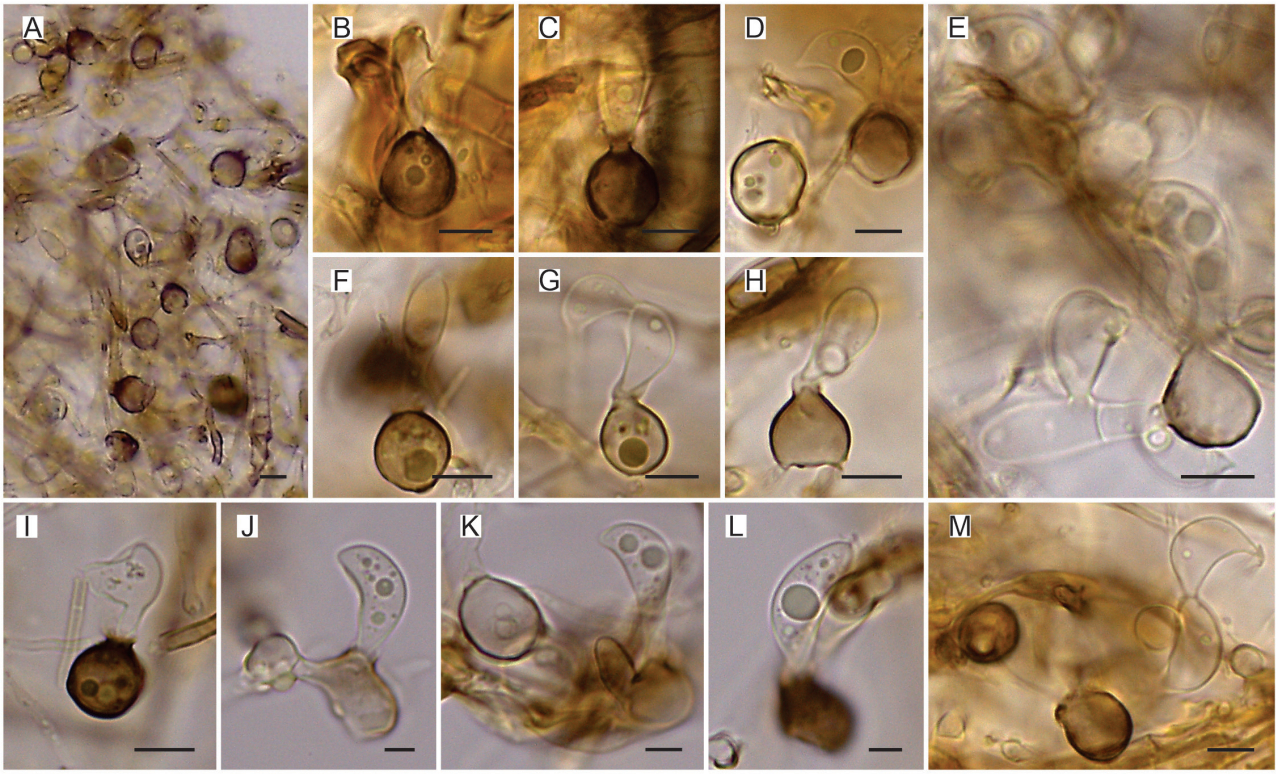

Figure 6. *Muyocopron lulasilvae* UFG 34288 (holotype). **A.** Conidiomata sporodochium-like. **B-M.** Conidiogenous cells and conidia. Scale bars: 10 µm.

Culture characters: The colonies on MEA reached 85 mm diameter after 2 weeks at 28 °C, aerial mycelium was scarce, brown, reverse without pigmentation. On PDA, colonies reached 67 mm diameter after 2 weeks at 28 °C, and had concentric circles, greyish, brown at the centre, and entire margin of purple colour; reverse orange to brown, without pigment. After approximately 45 days, on PDA, few reproductive structures were observed on the plant tissue surface.

Distribution and habitat: Endophytic fungus in the leaves of *Miconia mirabilis* found in the Atlantic Forest, Brazil.

Etymology: The name refers to Luiz Inácio Lula da Silva, the 39th President of Brazil, elected when the new species was described.

GenBank numbers: URM 8306 = FCCUFG 22, ITS = OR147103, LSU = OR157961, and RPB2 = OR413339. URM 8724 = FCCUFG 23, ITS = OR147104, LSU = OR157962, and RPB2 = OR413340.

Other materials examined: Brazil, Alagoas state, Quebrangulo municipality, Reserva Biológica de Pedra Talhada, 09°15'26.8"S and 36°25'53.7"W, as an endophyte from the leaves of *Miconia mirabilis* (Melastomataceae), July 2018, L.O. Ferro (URM 8724 = FCCUFG 23).

Notes: *Muyocopron lulasilvae* showed phylogenetic similarities with *M. atromaculans* and *M. ficinum*. Our new species differs from *M. atromaculans*, which was isolated as an endophyte in the USA, by having larger conidiogenous cells (13.5–19 × 8–15 µm in *M. lulasilvae* and 9.5–14.5 µm diameter in *M. atromaculans*) and conidia with no prominent appendages in *M. lulasilvae* and with terminal appendages in *M. atromaculans* (Hernández-Restrepo *et al.* 2019). *Muyocopron lulasilvae* also differs from *M. ficinum*, reported as a decomposer of dead leaves in China, which is known only based on the sexual morph (Tennakoon *et al.* 2021).

Discussion

Tropical forests are important hotspots of microbial diversity, which is estimated to account more than half of the terrestrial biodiversity (Lewis *et al.* 2015). In the early 20th century, researchers began to investigate whether these tropical areas are great reservoirs of endophytic fungal diversity (Arnold *et al.* 2000; Arnold & Lutzoni 2007). However, the ecological role of endophytic fungi and plant microbiome in these regions remains poorly understood. Several plants and biomes with high endophytic diversity have been investigated in an attempt not only to explore and increase fungal estimates, but also to elucidate the ecological patterns behind this diversity (Oita *et al.* 2021). To the best of our knowledge, ours is the first study to report the endophytic fungal diversity in the leaves of *M. mirabilis* growing in the Atlantic Forest, Brazil.

In our study, 285 endophytic fungi were isolated from 93.33 % of the colonised fragments. The high rate of endophytic colonisation may be associated with the properties of rainforests (Arnold *et al*. 2000). We obtained a similar number of fungal isolates compared with that of other studies on endophytes from rainforests (Arnold & Lutzoni 2007; Lima *et al*. 2012; Nascimento *et al*. 2015; Bonfim *et al*. 2016; Correia *et al*. 2017). However, the number of individuals and leaf fragments used to isolate the endophytes was lower than those of other studies. Generally, the richness and colonisation rate of endophytic fungi may not be ideal for full comparison, when we take into consideration the complexity of the endophytic microbiota, types of isolation methods, as well as the type of plant tissue and the effort of sampling (Cannon & Simmons 2002; Vieira *et al*. 2011).

The endophytic fungi were identified as belonging to 10 families, eight orders, and three classes (Dothideomycetes, Eurotiomycetes, and Sordariomycetes) of Ascomycota. The number of classes was similar to that presented by Arnold and Lutzoni (2007), who showed that endophytes from high latitudes belonged to few species and several classes of Ascomycota, whereas endophytic communities from tropical areas had several species within few classes of this phylum. Xylariales was the most abundant order among the endophytic fungi identified in this study, similar results can be seen in other studies and this order seems to prefer humid environments (Arnold & Lutzoni 2007; Daranagama *et al*. 2018; Zhou *et al*. 2019). Also, Xylariales is one of the most studied orders of filamentous fungi owing to its worldwide distribution and potential to produce bioactive compounds (Ma *et al*. 2022). *Xylaria* cf. *cubensis* was the most frequently isolated taxon, similar to that in other studies (Davis *et al*. 2003; Costa *et al*. 2012; Chen *et al*. 2013; Correia *et al*. 2017). Several studies have attempted to explain the emergence of xylariaceous fungi as endophytes. For example, Petrini (1995) observed that the role of these fungi could be linked to the decomposition of cellulose and lignin when plants begin to senesce, and since the past *X.* cf. *cubensis* is broadly known and studied as saprobe (Rogers 1984). In our study, the high prevalence of these fungi could be associated with the plant and environment from which they were collected. *Miconia mirabilis* is a common plant species in the degraded areas of humid tropical forests and has been used in the reforestation process (Pessoa *et al*. 2012).

Some fungal genera reported as pathogenic fungi can also be isolated as endophytes (Suryanarayanan *et al*. 2005; Hyde & Soytong 2008; Hardoim *et al*. 2015) and were isolated from *M. mirabilis*, belonging mainly to Glomerellales and Diaporthales. As a representative of a latent pathogens, *Colletotrichum* was the second most abundant genus in our study and has already been reported associated with several plant species (Siqueira *et al*. 2011; Mussi-Dias *et al*. 2012; Chow & Ting 2015; Singh *et al*. 2017).

Other abundantly isolated endophytes include species of *Diaporthe*, a cosmopolitan genus usually found as saprobes, phytopathogens, and endophytically in plants of different environments (Hilarino *et al*. 2011; Suwannarach *et al*. 2012; Dissanayake *et al*. 2017; Pádua *et al*. 2018). Although *Diaporthe* proved to be one of the most specious genera of the study, *D. ueckerae* showed the lowest abundance, with only one isolate. Silva *et al*. (2022) testing the production of L-asparaginase enzyme from different genus of endophytic fungi, presented as the best producer the *Diaporthe* isolate URM 8321 obtained originally in this study.

Isolates with lower abundance had only one or two representatives of each taxon, considered as rare taxa. These rare taxa are commonly found in endophytic studies, and this reduced number may be associated with the predominance of other genera in the endophytic community (Siqueira *et al*. 2011; Dos Santos *et al*. 2015). Another common fact in endophytic community studies is the discovery of new taxa (Bezerra *et al*. 2017; 2019; Cavalcanti *et al*. 2020). *Penicillium alagoense*, isolated in the present study was previously described as a new species (Crous *et al*. 2019) and *Muyocopron lulasilvae* is described here as a new endophytic species. Furthermore, the remaining possible novelties might be investigated in the future.

In this study we only analysed four individuals, however the ecological indices were relevant and showed good distribution and diversity of the endophytic fungi of *M. mirabilis* in the studied area. The richness observed in this study was similar to that reported by Correia *et al*. (2017), who analysed three species of *Begonia* in the Atlantic Forest (São Paulo state, Brazil). They found 71 morphospecies, and after phylogenetic analyses, they obtained a richness of 46 taxa with a Shannon index below that of our study (H' = 1.41). In another study in the Atlantic Forest with plants of the same order (Myrtales) as that of *M. mirabilis*, similar Shannon indices ranging from 2.25 to 2.39 were found (Vaz *et al*. 2014). In contrast to endophytic diversity in the Atlantic Forest, studies in the Caatinga forest showed lower diversity indices (Bezerra *et al*. 2015; Oliveira *et al*. 2020; Araújo-Magalhães *et al*. 2021); however, Pádua *et al*. (2018) recorded a higher diversity estimation (H' = 3.41) than our study when analysing the 'Brejo de altitude', an isolated fragment of humid forest (Atlantic Forest) found in the Caatinga forest. Although 88 % richness was recovered, more samples are needed to estimate the actual diversity of endophytic fungi. However, this pattern has been frequently observed in other studies of endophytic fungal communities (Gazis & Chaverri 2010; Correia *et al*. 2017; Pádua *et al*. 2018).

Our data provide the first insight into the endophytic community of *M. mirabilis* and may complement previous works on factors that determine endophytic assemblage in tropical forests, such as the Atlantic Forest, Brazil. The studied microbiome, which is part of an entire ecosystem, will help in understanding the complexity of the ecological

interactions. Our results reinforce the urge for conducting studies on mycobiome in environments with high biodiversity in order to highlight the need of conservation of protected areas.

Supplementary material

The following online material is available for this article:
Table S1. GenBank accession numbers of sequences obtained in this study.
Table S2. GenBank accession numbers of sequences of Muyocopronaceae species used in the phylogenetic analyses.

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