



Diversity of endophytic fungi in the leaflets and branches of *Poincianella pyramidalis*, an endemic species of Brazilian tropical dry forest

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ABSTRACT

Plants harbour diverse communities of fungal species in their internal compartments. Endophytic fungi help their hosts to establish, survive, and adapt to different environments. Here, we examined the diversity of endophytic fungi in the leaflets and branches of *Poincianella pyramidalis*, a plant species endemic to the Brazilian tropical dry forest (Caatinga). A total of 360 fragments of leaflets and branches were analysed and 189 endophytic fungi were isolated and distributed among 21 ascomycetous genera based on their ITS and LSU rDNA sequences. *Diaporthe* was the most frequently identified genus, followed by *Didymella* and *Rhytidhysterion*. The colonisation rate of plant fragments was higher in the branches (74 %) than in leaflets (14 %). The richness of the genera of endophytic fungi was also higher in the branches than in leaflets, whereas no difference was observed in endophyte diversity between the plant parts, based on Shannon-Wiener and Fisher alpha diversity indices. Our results indicate that endemic plant species from Brazilian dry forest, such as *P. pyramidalis*, are predominantly colonised by ascomycetous fungi, especially members of the class Dothideomycetes.

Keywords: Ascomycetous fungi, Caatinga, *Diaporthe*, Dothideomycetes, taxonomy

Introduction

Endophytic fungi represent a large polyphyletic group of microorganisms that can reside in practically any healthy plant tissue without causing visible infections (Arnold & Herre 2003; Arnold & Lutzoni 2007; Banerjee 2011; Brader *et al.* 2017; Dastogeer *et al.* 2017). A single plant can harbour a large variety of endophytes, especially in mature tissues present in the aerial parts of the plant (Arnold 2008; Nisa *et al.* 2015). This symbiosis between

endophytes and their plant hosts can have profound impacts on plant communities and ecosystems, leading to favourable physiological and ecological relationships for environmental balance (Hardoim *et al.* 2015; Brader *et al.* 2017). In addition, fungal endophytes can synthesise several bioactive natural products, thereby making them an important biotechnological resource (Bezerra *et al.* 2012, 2015; Silva *et al.* 2018; Pádua *et al.* 2019).

Endophytic diversity can be influenced by plant morphology, chemical and physiological composition, tissue

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type, seasons, climate conditions, and biogeographical regions (Herrera *et al.* 2010; Hardoim *et al.* 2015; Massimo *et al.* 2015). Most studies have focused on the diversity of endophytic fungi from temperate and humid tropical forests (Arnold & Lutzoni 2007; Banerjee 2011), whereas few studies have estimated the fungal endophyte diversity in tropical dry regions (Bezerra *et al.* 2012, Bezerra *et al.* 2017a; b; Dastogeer *et al.* 2017; Pádua *et al.* 2019; Silva *et al.* 2019; Bezerra *et al.* 2019).

The Caatinga is the largest semiarid tropical ecoregion in South America, occupying an area of 912,529 km² in Brazil (Moro *et al.* 2016; Silva *et al.* 2017). Comprising nine ecoregions, this biogeographic domain is dominated by a seasonally dry tropical forest (SDTF) influenced by low rainfall regimes (Silva *et al.* 2017; Pedrosa *et al.* 2019). Although the Caatinga presents harsh abiotic conditions (e.g., high temperatures, soil with nutrient deficiency and high salinity), this domain has an adapted biota, endemic plant and animal species, and previously undiscovered microorganisms have been found here in recent years (JC Santos *et al.* 2011; Silva & Souza 2018).

Poincianella pyramidalis (Fabaceae, Caesalpinioideae) (synonym *Caesalpinia pyramidalis*) is a plant endemic to the Caatinga which has antimicrobial, antifungal, antioxidant, anti-inflammatory, and antinociceptive properties (Cruz *et al.* 2007; AC Santos *et al.* 2011; Silva *et al.* 2015; Chaves *et al.* 2019). This species is an economically important tree for the production of firewood, fuel, alcohol, and soap. *Poincianella pyramidalis* easily adapts to different soil types and its populations can grow rapidly, which confers an essential role in the restoration of Caatinga ecosystems (Cabral *et al.* 2013; Pagotto *et al.* 2015; Chaves *et al.* 2016).

Few studies have investigated the association of fungal endophytes with *P. pyramidalis*, reporting the presence of seven genera (e.g. *Colletotrichum*, *Cladosporium*, *Phyllosticta*, *Trichoderma*, and *Diaporthe*), but without the wide taxonomic and diversity analyses of different plant tissues (Gonçalves *et al.* 2013; Moura *et al.* 2016; Sena Filho *et al.* 2016; Souza *et al.* 2016). Despite the key role of endophytic fungi in the adaptation and evolution of plant species and the maintenance of ecosystem services, limited knowledge is available regarding the diversity of microorganisms associated with endemic plant species of the Caatinga dry forest in Brazil. Thus, we tested the following hypotheses: i) the leaflets and branches of *P. pyramidalis* harbour a great diversity of endophytic fungi, ii) the communities of fungal endophytes differ between plant tissues, and iii) the fungal richness of *P. pyramidalis* can contain taxonomic novelties. To verify these hypotheses, the aim of this study was to evaluate the diversity and community structure of endophytic fungi from the leaflets and branches of *P. pyramidalis*, a species endemic to the Brazilian tropical dry forest (Caatinga).

Materials and methods

Study site

Samples were collected from an area of tropical dry forest (Caatinga) at the Fazenda Tamanduá (07°02'20" S, 37°26'43" W), a property belonging to the Mocó Agropecuária Ltda (Cabral *et al.* 2013), in Paraíba state. This property has an area of about 3,073 ha, of which 900 ha is part of the Private Natural Heritage Reserve of the Caatinga. The site has an average altitude of 240 m, with an average annual rainfall of 600 mm (Silva *et al.* 2012; Silva *et al.* 2014). The climate is typical of semiarid tropical regions (Bsh) according to the classification by Köppen (1948). The dominant vegetation is composed of arboreal, xerophilous, woody, and often spiny formations.

Sampling

Healthy leaflets and branches from nine individual trees of *P. pyramidalis* (Tul.) L. P. Queiroz up to 3 m tall were randomly collected in May 2013 during the dry season in the Caatinga. After collection, the plant material was packed into paper and nylon bags and processed within 48 h. The collection was authorised by the Ministério do Meio Ambiente (MMA)/Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio); permission number: 40331-1/ authentication code 87451826 issued on 4 November 2013.

Isolation of fungal endophytes

The plant material was disinfected following the same methodology used by Bezerra *et al.* (2015). Briefly, the leaflets and branches were first washed in tap water and neutral liquid soap, followed by disinfection using 70 % alcohol for 60 s, 2-2.5 % sodium hypochlorite for 180 s, 70 % ethanol for 30 s, and then washed three times in sterilised distilled water. Thereafter, the leaflets and branches were cut into fragments of approximately 1 cm². After disinfection, a total of 180 leaflets and 180 branch fragments (20 fragments of each plant tissue from each tree) were prepared. The fragments were transferred into Petri dishes containing potato dextrose agar (PDA) supplemented with chloramphenicol (100 mg/L) and tetracycline (50 mg/L) to inhibit bacterial growth. The plates were incubated at 28 ± 2 °C for up to 30 days. Fungal growth was observed daily, and all colonies were isolated, purified, and preserved in a solution of water and 10 % glycerol for later identification. As a control of surface disinfestation, 1 mL of water from the last wash was transferred to Petri dishes containing PDA medium supplemented with antibiotics and incubated under the same conditions.

Identification of fungal endophytes

Endophytic fungi were identified based on morphology through the observation of macro- and micro-morphological



characteristics of the somatic and reproductive structures, and by DNA sequence analysis. Representative endophytic cultures are deposited in the culture collection Micoteca URM Prof. Maria Auxiliadora Cavalcanti (WCDM 604), and prepared microscopic slides in the Herbário URM Pe. Camille Torrend, both at the Universidade Federal de Pernambuco, Recife, Brazil.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from pure cultures using a Wizard® SV Genomic DNA Purification System Extraction Kit (Promega) following the manufacturer's instructions. Two loci were studied, and the primers ITS1/ITS4 (White *et al.* 1990) and LR0R/LR5 (Vilgalys & Hester 1990; Vilgalys & Sun 1994) were used to amplify part of the internal transcribed spacer (ITS) and nuclear ribosomal small subunit (LSU) regions of the rDNA, respectively. Amplification reactions were performed following the methodology described by Bezerra *et al.* (2017b). Amplicon purification and sequencing reactions were performed as described by Silva *et al.* (2019).

Phylogenetic analyses

The sequences obtained were initially compared with corresponding sequences deposited in GenBank using the BLASTn tool, later aligned with selected sequences using the MAFFT v. 6 online interface (Katoh & Toh 2010) and edited in MEGA v. 7 (Kumar *et al.* 2016). Maximum Likelihood (ML) and Bayesian Inference (BI), and the analyses were performed on the CIPRES Scientific Portal (Miller *et al.* 2010). For ML analyses, RAxML-HPC BlackBox (8.2.12) (Stamatakis 2008) was used within the GTR+I+G standard nucleotide substitution model. The BI analysis (1×10^6 generations) was performed on MrBayes at the XSEDE (CIPRES) using the nucleotide substitution model generated by the MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The models were estimated separately for each gene region (ITS = GTR+I+G and LSU = GTR+G). The obtained trees were visualised using FigTree v.1.4.0 (Rambaut 2012). The DNA sequences generated in this study were deposited in the GenBank database of the NCBI (ITS: MN912308-MN912350 and LSU: MN912266-MN912307, Tab. S1 in material supplementary), and the alignment was deposited in TreeBASE (study ID 25646).

Colonization rate, absolute and relative frequencies

The colonisation rate (TC %) was calculated as the ratio between the number of fungal growth fragments (Nf) and the total number of fragments (Nt) ($FI = Nf / Nt \times 100$) (Araújo *et al.* 2002). The relative frequency (RF) of isolation was calculated as the ratio between the number of isolates of a species to the total number of isolates (Photita *et al.* 2001).

Ecological data analyses

The abundance of fungal families among the leaflet and branch samples were compared using the package 'phyloseq'

(McMurdie & Holmes 2013) and the graph was generated with the 'ggplot2' package (Wickham 2016). The Shannon-Wiener diversity index was calculated by the equation $H' = -\sum (P_i \ln P_i)$, where $P_i = n_i / N$, n_i = number of endophytic isolates, and N = total number of endophytic isolates. At the end of the analysis, H' values were converted to Exp (H'). Richness was determined by the number of species in each sample (Shannon & Weaver 1949). Fisher's alpha index was determined by the equation $S = \alpha * \ln(1 + n / \alpha)$ (Fisher *et al.* 1943).

Based on the Bray-Curtis distance, permutation multivariate analysis of variance (PERMANOVA) was performed to test whether the endophytic fungal communities differed between plant tissues, and the variation in the composition of the endophytic fungal communities was visualised using non-metric multidimensional scaling (NMDS). Analyses were performed using relative abundance data. Species accumulation curves were determined, and the total richness was compared with the estimated richness using the Chao1 index and Jackknife to evaluate the sampling effort efficiency. For these analyses, we used the 'agricolae' (Mendiburu 2017), 'vegan' (Oksanen *et al.* 2018), and 'iNEXT' (Hsieh *et al.* 2016) packages. All statistical analyses were conducted in R v.3.5.0 (R Development Core Team 2018).

Results

A total of 189 endophytic fungi were isolated from 360 leaflet and branch fragments. However, 30 isolates (five from the leaflets and 25 from the branches) did not develop after preservation. The remaining 159 endophytes (137 from branches and 22 from leaflets) were found to be distributed in 16 families in Ascomycota (Figs. 1, 2). The colonisation rate of the plant tissue by the endophytic fungi was higher in the branches (74 %) than in the leaflets (14 %).

The phylogenetic analyses, based on the sequence combination of ITS and LSU rDNA, consisted of 125 sequences comprising 1779 characters (including gaps). The phylogram grouped the endophytic fungi into 21 genera belonging to 10 orders of Ascomycota (Amphisphaeriales, Botryosphaeriales, Capnodiales, Diaporthales, Eurotiales, Hypocreales, Hysteriales, Kirschsteinioteliales, Mucocarpales, and Pleosporales) (Fig. 1). Of the 21 genera, 13 (*Caatingomyces*, *Camarographium*, *Didymella*, *Epicoccum*, *Fusarium*, *Kirschsteiniotelia*, *Lasiodiplodia*, *Pseudopithomyces*, *Phoma*, *Preussia*, *Rhytidhysterion*, *Trichoderma*, and *Truncatella*) were present exclusively in the branches, five (*Byssoschlamys*, *Curvularia*, *Pyrenophora*, *Muyocopron*, and *Purpureocillium*) were present exclusively in the leaflets, and three (*Alternaria*, *Diaporthe*, and *Sarocladium*) were isolated from both the branch and leaflet tissues. *Diaporthe* sp. and *D. inconspicua* were the most frequently identified taxa, and other endophytic fungi were rarely found ($fr < 10\%$). Overall,



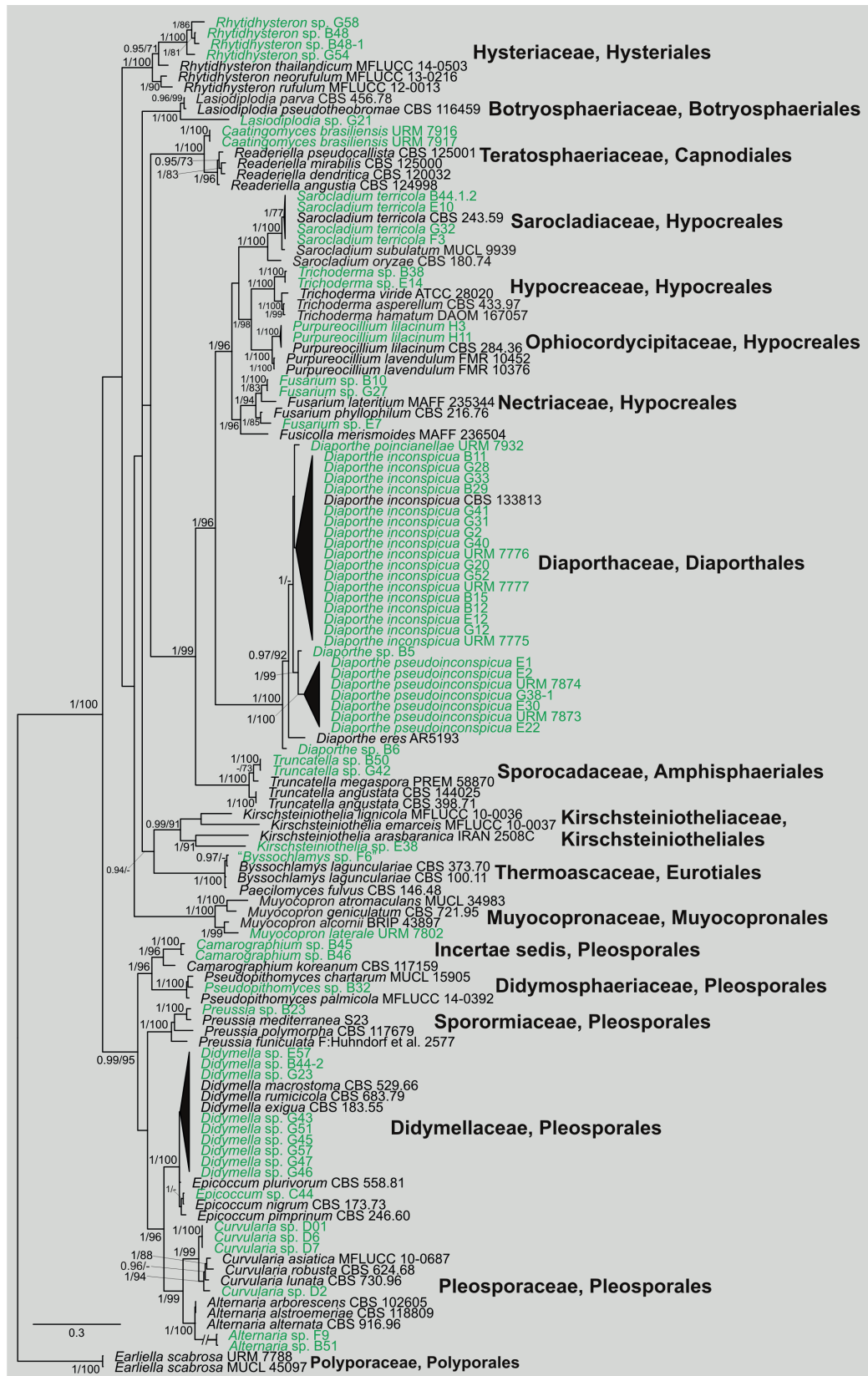


Figure 1. Phylogram generated from Bayesian inference (BI) analysis based on a combined LSU and ITS rDNA dataset from endophytic fungi isolated from *Poincianella pyramidalis* in the Caatinga forest (Brazil) and sequences obtained from GenBank. Posterior probabilities from BI above 0.95 and ML bootstrap support values above 70 % are shown near nodes. The tree was rooted to *Earliella scabrosa* (URM 7788 and MUCL 45097).

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10 taxa (*Byssosclamyces* sp., *Curvularia pallescens*, *Diaporthe miriciae*, *D. poincianellae*, *Pyrenophora* sp., *Kirschsteiniiothelia* sp., *Lasiodiplodia* sp., *Pseudopithomyces* sp., *Muyocopron laterale*, and *Preussia* sp.) were recovered only once (Tab 1).

The genera richness, Shannon-Wiener, and Fisher alpha diversity indices were 6.67, 1.04, and 4.88, respectively (Fig. 3). The accumulation curve of endophytic fungi did not reach stability; however, the Chao1 and Jackknife richness estimated the isolation of 31 and 30 genera, respectively (Fig. 4).

Discussion

So far, few studies have analysed the diversity of endophytic fungi from Caesalpinioideae species, including *P. pyramidalis* (Gonçalves *et al.* 2013; Moura *et al.* 2016; Sena Filho *et al.* 2016; Souza *et al.* 2016). This endophytic fungal association has been reported by Hilarino *et al.* (2011) in expanded and unexpanded mature leaves of *Bauhinia brevipes*; and by Bezerra *et al.* (2015) in the leaves, stems, sepals, and seeds of *Bauhinia forficata* in Brazil. Endophytic fungi were also isolated from the bark and stems of *Paubrasilia echinata* (= *Caesalpinia echinata*) (Campos *et al.* 2015). Studies on endophytic fungi associated with *P. pyramidalis* reported isolates of *Colletotrichum*, *Cladosporium*, *Phyllosticta*, *Nodulisporium* (Gonçalves *et al.* 2013), and *Paecilomyces* (Moura *et al.* 2016). *Trichoderma* species isolated as endophytes from *P. pyramidalis* were used for the treatment and biocontrol of diseases caused

by *Fusarium* in pineapple plantations (Souza *et al.* 2016), and an important terpenoid with potential anti-cancer effects was reported from the endophyte *Diaporthe* sp. of *P. pyramidalis* (Sena Filho *et al.* 2016).

The high colonisation rate of endophytic fungi in the branches of *P. pyramidalis* has also been reported in other hosts (Liu *et al.* 2010; Bezerra *et al.* 2015; Russo *et al.* 2016). For example, Liu *et al.* (2010), studied the branches and leaves of 23 species of evergreen plants in China and 92 % of the recovered endophytic fungi were from the branches. In India, Sunayana *et al.* (2014) isolated endophytic fungi from *Vitex negundo* and recovered 143 isolates from bark, twig, and leaf tissues, observing a colonisation rate of 22.22 %, 22.66 %, and 21.33 %, respectively. In northern Thailand, Suwannarach *et al.* (2012) isolated 2,774 endophytes from the leaves and stems of *Cinnamomum bejolghota* and reported a colonisation rate varying between 97.8 % and 99.3 % from samples collected during the dry season, and between 94.8 % and 99.7 % from plant material collected during the rainy season. The variation in colonisation rates may be influenced by the differences in plant tissues, endophyte interactions, ecosystems, and the environmental conditions (Suwannarach *et al.* 2012; Sunayana *et al.* 2014; Haridoim *et al.* 2015).

The variability of environmental characteristics observed in the Caatinga may influence the richness and abundance of endophytic fungi in the branches because they last longer than the leaves (see Sun *et al.* 2011; Sun *et al.* 2012a). Most of the Caatinga flora is constituted of deciduous vegetation, which is dominated by trees that lose about 70 % of their

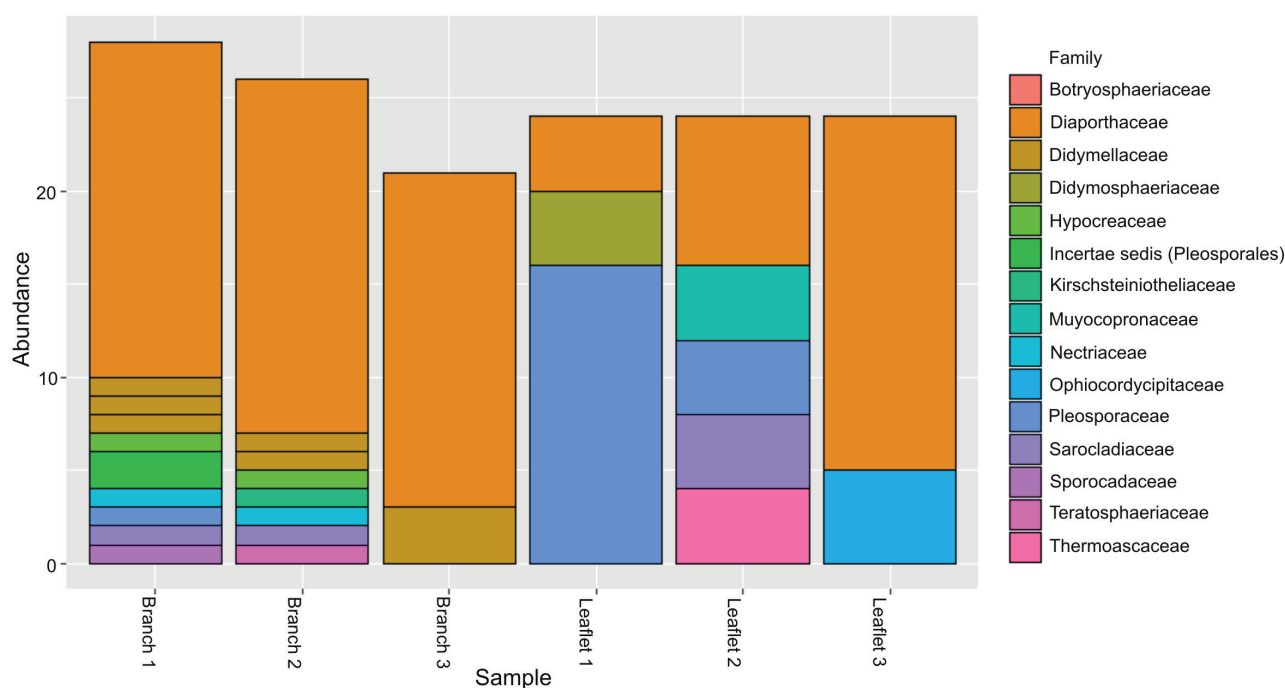


Figure 2. Taxonomic composition of endophytic fungi isolated from leaflet and branch samples of *Poincianella pyramidalis* in the Caatinga forest, Brazil.

small leaves during the dry season (Silva *et al.* 2017). The distribution pattern of the endophytes in the leaves is imbalanced (Cannon & Simmons 2002) and the abundance may vary according to their maturity (Arnold & Herre 2003). For example, plant tissues had a significant effect (15.1 % variation) on the composition of the fungal endophyte community in the twigs and leaves of *Betula platyphylla*, *Quercus liaotungensis*, and *Ulmus macrocarpa* in a mixed forest in China (Sun *et al.* 2012a), and evergreen plants had a higher incidence of endophytes when compared to deciduous plants (Lau *et al.* 2013).

Most endophytes found in *P. pyramidalis* belong to the genera described worldwide as endophytes (Khiralla *et al.* 2016; Rana *et al.* 2017; Verekar *et al.* 2017; Pádua *et al.* 2019), mainly belonging to Ascomycota in plants from different ecosystems (Arnold & Lutzoni 2007; Gazis & Chaverri 2010; Sunayana *et al.* 2014; Pádua *et al.* 2019). Fungal taxa, known as generalist fungi that grow rapidly in non-selective culture media, such as *Diaporthe*, *Colletotrichum*, *Curvularia*, and *Fusarium* are often found in different tropical plants (Arnold & Lutzoni 2007; Siqueira *et al.* 2011; Chowdhary & Kaushik 2015; Verekar *et al.* 2017). Also, some taxa found in this work had low frequency. Similar results showed that most tropical communities had a log-normal pattern distribution and few common rare taxa (Gazis & Chaverri 2010; Hilarino *et al.* 2011; Bezerra *et al.* 2013).

One interesting observation from our study was that the endophytes URM 7916 and URM 7917 were isolated from the branches of *P. pyramidalis*. These endophytic fungi were described as a new genus, *Caatingomyces* (type species *C. brasiliensis*), belonging to the family Teratosphaeriaceae (*Capnodiales*, *Dothideomycetes*) (Hyde *et al.* 2019). Teratosphaeriaceae comprises numerous cryptic species that can be defined phylogenetically based on the sequence analyses of ITS and LSU rDNA (Crous *et al.* 2009; Quaedvlieg *et al.* 2014). Species belonging to Teratosphaeriaceae are commonly found as phytopathogens; however, *Readeriella considerianae* has already been reported as endophytic fungi of *Eucalyptus grandis* × *E. camaldulensis* in South Africa (Marsberg *et al.* 2014). Another example is endophyte URM 7802, which was identified as *Muyocopron laterale* (Hernández-Restrepo *et al.* 2019) in a genus mainly found as saprobes and plant pathogens (Tibpromma *et al.* 2016; Hernández-Restrepo *et al.* 2019), but also as an endophyte (Bills & Polishook 1992, as *Mycoleptodiscus atromaculans*; Andrioli *et al.* 2012, as *Mycoleptodiscus indicus*; Bezerra *et al.* 2012, as isolate PF108).

The genus *Diaporthe* is commonly cited as an endophyte and has been found in different plant hosts that inhabit tropical as well as temperate areas. For example, *Diaporthe* was found as an endophyte in *Bauhinia brevipes* (Caesalpinioideae) (Hilarino *et al.* 2011), *Cinnamomum*

Table 1. Absolute (f) and relative (fr) frequency of endophytic fungi isolated from *Poincianella pyramidalis* leaflets and branches of Caatinga area.

Endophytic fungi	f	fr
<i>Alternaria</i> sp.	2	1.05
<i>Byssosclamyces</i> sp.	1	0.52
<i>Caatingomyces brasiliensis</i> T.G.L. Oliveira, Souza-Motta, O.M.C. Magalhães & J.D.P. Bezerra	2	1.05
<i>Camarographium</i> sp.	3	1.58
<i>Curvularia pallescens</i> Boedijn	1	0.52
<i>Curvularia</i> sp.	3	1.58
<i>Diaporthe inconspicua</i> R.R. Gomes, Glienke & Crous	17	8.99
<i>Diaporthe miriciae</i> R.G. Shivas, S.M. Thomps. & Y.P. Tan	1	0.52
<i>Diaporthe poincianellae</i> T.G.L. Oliveira, O.M.C. Magalhães & J.D.P. Bezerra	1	0.52
<i>Diaporthe pseudoinconspicua</i> T.G.L. Oliveira, J.D.P. Bezerra, A.R. Machado, Souza-Motta & O.M.C. Magalhães	7	3.70
<i>Diaporthe</i> sp.	85	44.97
<i>Didymella</i> sp.	8	4.23
<i>Pyrenophora</i> sp.	1	0.52
<i>Epicoccum nigrum</i> Link	2	1.05
<i>Fusarium</i> sp.	3	1.58
<i>Kirschsteiniotelia</i> sp.	1	0.52
<i>Lasiodiplodia</i> sp.	1	0.52
<i>Pseudopithomyces</i> sp.	1	0.52
<i>Muyocopron laterale</i> (Alcorn & B. Sutton) Hern.-Restr., J.D.P. Bezerra & Crous	1	0.52
<i>Phoma</i> sp.	2	1.05
<i>Preussia</i> sp.	1	0.52
<i>Purpureocillium lilacinum</i> (Thom) Luangsa-ard, Houbaken, Hywel-Jones & Samson	2	1.05
<i>Rhytidhysterion</i> sp.	5	2.64
<i>Sarocladium terricola</i> (J.H. Mill., Giddens & A.A. Foster) A. Giraldo, Gené & Guarro	4	2.11
<i>Trichoderma</i> sp.	2	1.05
<i>Truncatella</i> sp.	2	1.05
Total	159	



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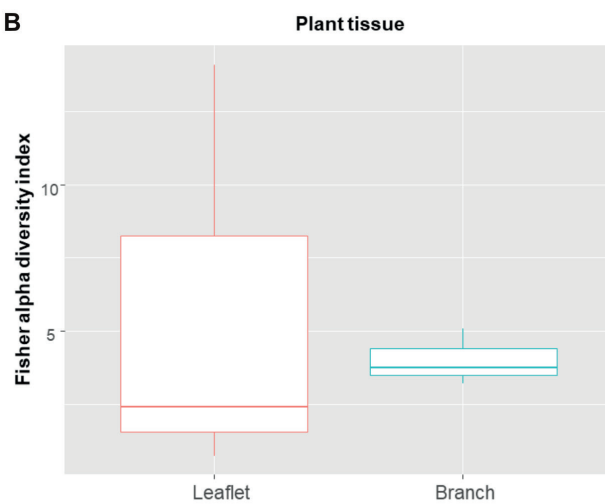
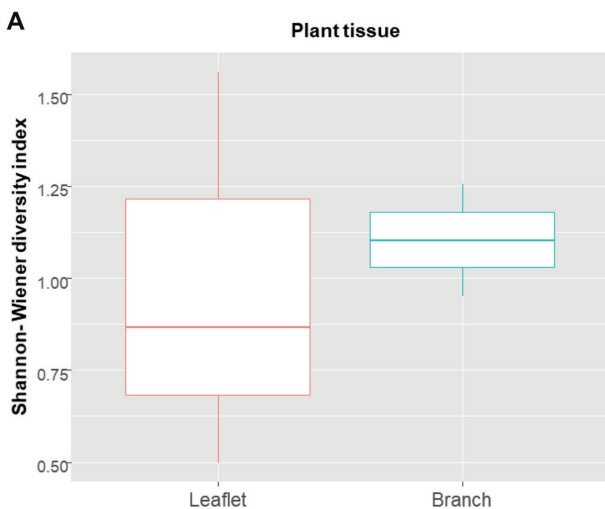
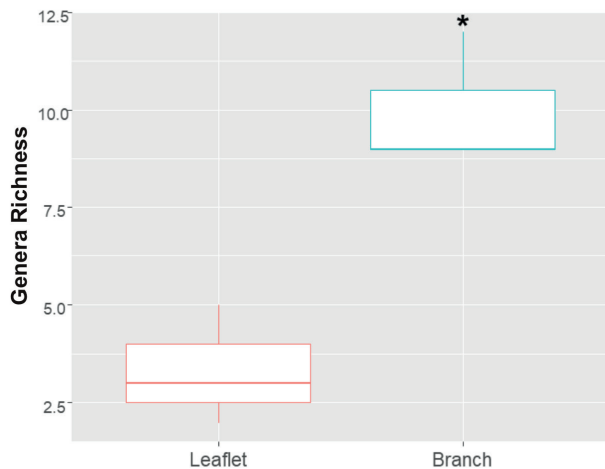


Figure 3. Boxplot indicating the richness (A) and diversity based on Shannon–Wiener (B) and Fisher indices (C) of endophytic fungi isolated from the leaflets and branches of *Poincianella pyramidalis* in the Caatinga forest, Brazil. Asterisks (*) indicate significantly higher values of the evaluated attribute based on one-way ANOVA. The median (central dot), quartile (box), maximum and minimum (whiskers) are shown.

bejolghota (Lauraceae) (Suwannarach *et al.* 2012), *Trichilia elegans* (Meliaceae) (Rhoden *et al.* 2012), *Delonix regia* (Fabaceae) (Zhou *et al.* 2014), *Costus spiralis* (Costaceae) (Marson-Ascêncio *et al.* 2014), and *Myracrodruon urundeuva* (Anacardiaceae) in Brazil (Pádua *et al.* 2019). Members of *Diaporthe* are cosmopolitan and are mainly found as saprobes, phytopathogens, and opportunistic pathogens (Udayanga *et al.* 2011; Gomes *et al.* 2013; Dissanayake *et al.* 2017). Several new species have been described in *Diaporthe*, including *D. pseudoinconspicua* and *D. poincianellae*, that we isolated from the branches of *P. pyramidalis* (Crous *et al.* 2018a; b). Additionally, *Diaporthe* presents species with the capacity to produce enzymes and other secondary metabolites (Maiquel *et al.* 2016; Yan *et al.* 2018; Pádua *et al.* 2019).

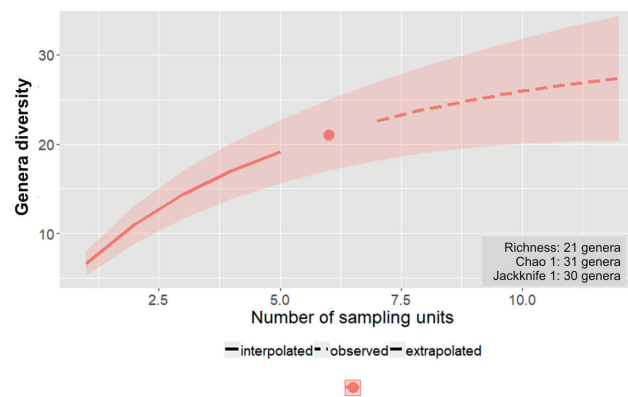


Figure 4. Genera accumulation curve for endophytic fungi recovered from leaflets and branches of *Poincianella pyramidalis* in the Caatinga forest, Brazil, showing the observed and estimated richness based on the Chao 1 and Jackknife 1.

Some genera of endophytes reported in this study are commonly found in the leaves and branches of other hosts: species of *Diaporthe*, *Fusarium*, *Lasiodiplodia*, and *Trichoderma* are found in branches of *Theobroma cacao* in Brazil (Rubini *et al.* 2005); *Alternaria* and *Epicoccum* have been reported from the branches of *Prunus cerasus* in the Czech Republic (Hortová & Novotný 2011); *Alternaria*, *Diaporthe*, and *Fusarium* spp. have been reported from branches of *Vitex rotundifolia* in Taiwan (Yeh & Kirschner 2019); and *Diaporthe* and *Phoma* species were registered from the branches of *Litsea cubeba* in China (Wu *et al.* 2019). Endophytes from leaflets were mainly found isolates of *Diaporthe* and *Alternaria* from *Hevea brasiliensis* in Brazil (Vaz *et al.* 2018) and Peru (Gazis & Chaverri 2010); *Alternaria*, *Diaporthe*, and *Curvularia* from leaflets of *Prosopis juliflora* in India (Srivastava & Anandrao 2015); *Byssoschlamys*, *Curvularia*, and *Alternaria* from leaves of *Euphorbia prostrata*, *Calotropis procera*, and *Catharanthus roseus* in Sudan (Khiralla *et al.* 2016); and *Sarocladium* from leaves of *Myracrodruon urundeuva* in Brazil (Pádua *et al.* 2019).

Overall, the genera richness (6.67) and the Shannon–Wiener diversity index (1.04) recovered from *P. pyramidalis*

were lower than those recorded in other studies. Analysing the endophytic mycobiota of *Bauhinia forficata*, Bezerra *et al.* (2015) recorded greater species richness (11) and a Shannon-Wiener diversity index of 2.206 in the stems, and Sunayana *et al.* (2014) recorded a higher Shannon-Wiener diversity index (2.48) in the twigs of *Vitex negundo*. Evaluating the leaves and stems of *Cinnamomum bejolghota*, Suwannarach *et al.* (2012) registered a diversity index varying between 1.598–1.924 in the dry season, and between 2.088–2.305 in the rainy season. Similar results were reported by Sun *et al.* (2012b), who studied 10 plant hosts from desert areas in China and recorded a low diversity of endophytic fungal communities in the stems and leaves (Shannon index = 0.59 to 1.92, Fisher- α index = 0.82 to 5.68). In the arid regions of northern Australia, Dastogeer *et al.* (2017) observed the diversity of endophytic fungi in *Nicotiana* and recorded a high alpha diversity (Shannon-Wiener diversity index (H') = 2.61 ± 0.17) and a high frequency (60.8%) of isolation in roots compared to the stem and leaf tissues.

This study of endophytic fungi from the leaflets and branches of *P. pyramidalis* in the Caatinga dry forest contributes significantly to the existing knowledge regarding fungal diversity. A difference in the endophytic community was observed between plant tissues, dominated by rare taxa. Moreover, the colonisation rate and species richness were higher in the branches than in the leaflets, demonstrating that the fungal endophyte community from *P. pyramidalis* forms an important and a specific mycobiome. Future studies focusing on the association of endophytes with other plants from dry tropical forests will be important for maintaining the preservation of vegetation cover, and for delineating conservation policies to protect plant hosts and fungal species in their natural environment.

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