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# Diversity of culturable endophytic fungi of *Hevea guianensis*: A latex producer native tree from the Brazilian Amazon

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***Hevea guianensis*** **is a species of rubber tree native to the Amazon rainforest. This tree is highly exploited for latex extraction but is not cultivated. Therefore, few studies have investigated its microbiota. The aim of this study was to analyze the diversity of endophytic fungi in the leaves, stems and roots of *H. guianensis* trees from the Brazilian Amazon. A total of 92 fungi were isolated from different tissues of this plant species. These isolates were grouped into 28 operational taxonomic units (OTUs). The dominant phylum was Ascomycota (96.73%). The stem cortex showed the greatest fungal richness and diversity, although the frequency of isolates was highest in the leaves. The fungal isolates of leaves were highly heterogeneous than those of stem and roots. *Colletotrichum* was the most well-represented and abundant genera in the leaves; *Diaporthe* was the second most abundant genus in the leaves; *Penicillium* was the main genus obtained from the roots; the genera *Lasiodiplodia*, *Purpureocillium*, *Phyllosticta*, *Daldinia* and *Pseudofusicoccum* were recovered only from the leaves; whereas the genera *Trichoderma* and *Fusarium* were isolated from the stems and roots of *H. guianensis*. Thus, we describe the endophytic fungi of *H. guianensis* of great biotechnological interest, such as *Trichoderma*.**

**Key words:** Rubber tree, biodiversity, endophytic fungi.

## INTRODUCTION

The Amazon rainforest is the largest biodiversity region on the planet, and the first description of genus *Hevea* and its species *Hevea guianensis* in 1775 by Fusee Aublet (Sethuraj and Mathew, 2012) was made in this

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forest. Later, other species were described, such as *Hevea brasiliensis* [(Willd. ex Adr. De Jess) Muell.-Arg] in 1824, *Hevea pauciflora* (Spruce ex Benth), *Hevea spruceana* (Benth) and *Hevea rigidifolia* [(Spruce ex Benth) Muell.-Arg] in 1854, *Hevea nitida* var. *toxicodendroides* (Mart. ex Muell.-Arg), *Hevea microphylla* (Ule), *Hevea camporum* (Ducke), *Hevea benthamiana* (Muell.-Arg) in 1962 and *Hevea camargocina* (Pires) in 1981 (Muller, 1865, 1874; Murca, 1981; Schultes, 1977, 1987; Sethuraj and Mathew, 2012).

Commercially acceptable latex and rubber is obtained from *H. brasiliensis*, *H. benthamiana* and *H. guianensis*. However, *H. guianensis* is exploited for latex extractivism but is not cultivated, unlike *H. brasiliensis*, a closely related species, which is exploited, and extensively cultivated (Sethuraj and Mathew, 2012). Thus, few studies have attempted to obtain *H. guianensis* cultivars resistant to diseases for the production of better quality latex (Cardoso et al., 2014) or to describe its endophytic community, which is capable of producing metabolites of biotechnological interest and with potential applications for the biological control of phytopathogens (Gazis and Chaverri, 2010; Rocha et al., 2011).

*Hevea* species seem to have evolved in the Amazon rainforest under high and constant humidity, which favours the colonization of pathogens; thus, the development of some degree of resistance is considered essential for plant survival (Gasparotto and Rezende, 2012). Natural rubber production in Brazil has been affected for decades by the high incidence of pathogens, including *Pseudocercospora ulei* (South American leaf blight - SALB) (Hora-Júnior et al., 2014), *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* (anthracnose), *Oidium hevea* (powdery mildew), and *Phytophthora* species (striated canker or panel canker) (Gasparoto and Rezende, 2012). Therefore, countries in Southeast Asia, such as Thailand, Indonesia, Vietnam, India and Malaysia, are the largest producers of rubber worldwide (FAO, 2017).

The symbiotic interaction between microorganisms and plants is an alternative to ensure the preservation of native species because it can increase plant resistance to biotic and abiotic stresses (Zheng et al., 2017; Koide et al., 2017; Saunders et al., 2010; Arnold, 2007), increase plant production (Babu et al., 2015; Murali and Amruthesh, 2015; Khan et al., 2008) and control phytopathogens (Ben Amira et al., 2017; Contina et al., 2017; Larran et al., 2016; Valenzuela et al., 2015; Mbarga et al., 2014; Rocha et al., 2011). However, little is known about the interaction between endophytic fungi and plants from the Amazon. Some studies have examined the communities of microorganisms associated with *H. brasiliensis* and *H. guianensis* distributed in native habitats and rubber trees plantations in Peru, Cameroon (Africa) and Mexico (Gazis, 2012; Gazis et al., 2012, 2011; Chaverri et al., 2011; Rocha et al., 2011; Gazis and Chaverri, 2010). These studies demonstrated the occurrence of a high diversity of endophytic fungi mainly inside the stem despite the high colonization rate of endophytes inside the leaves (Gazis, 2012; Gazis et al., 2012, 2011; Chaverri et al., 2011; Gazis and Chaverri, 2010) and enabled the discovery of a new species of endophytic fungus identified as *Trichoderma amazonicum* (Chaverri et al., 2011), a new class of Xylonomycetes (Gazis et al., 2012) and a wide diversity of basidiomycetes in Peruvian rubber trees (Martin et al., 2015). In addition, it is evidenced in these studies that molecular techniques are efficient tools for the identification of cultivable fungi and for the analysis of their diversity in their habitat.

However, no study has described the diversity of endophytic fungi in *H. guianensis* in the Brazilian Amazon or the differences in the profiles of these microorganisms in the communities present in the different niches of these rubber trees. In addition, few studies have promoted knowledge about the diversity, conservation and biotechnological exploitation of endophytic microorganisms in different Brazilian biomes, although several state and federal programmes have encouraged research on natural resources and biodiversity (Sette et al., 2013; Valencia and Chambergo, 2013).

Thus, this study describes the diversity of endophytic fungi in *H. guianensis* trees in the Brazilian Amazon and the community profiles of these microorganisms in the leaves, stems and roots of this latex producer.

## MATERIALS AND METHODS

### Isolation of endophytic fungi from different tissues of *H. guianensis*

Leaf, stem and root samples were obtained from six healthy *H. guianensis* trees of similar size, located in the Amazon rainforest, Acre, and distributed at different sampling points between the coordinates 07°44'05.3" S / 72°49'46.8" W and 10°02'16.7" S / 67°40'45.4" W. The collections were carried out in native fragments of forest located near the cities of Xapuri, Boca do Acre and Rio Branco. The samples were collected in the month of July and the trees were randomly selected.

The methods proposed by Wirsel et al. (2001), Evans et al. (2003) and Leite et al. (2013) with modifications were used to isolate the endophytic fungi. The leaves were collected at the interface of the center to the periphery of the tree. The leaves that showed good sanity were packed into paper bags, which in turn were placed into plastic bags and stored at 4°C (Stone et al., 2004). Fragments of the cortex of the lateral roots near the primary root of the tree were collected and the root cortex fragments transported to the laboratory immediately after collection in silica gel tubes. Also, fragments of the stem cortex were collected at breast height and on the same side of the tree. The 3-to-5-cm fragments of the stem cortex were obtained after removal of the outer bark with the aid of a properly sterilized scalpel and immediately inoculated into YMC culture medium (10 g of malt extract, 2 g of yeast extract and 15 g of agar dissolved in 1 L of heated distilled water and then autoclaved) (Evans et al., 2003).

The leaves were washed in running water for 10 min, cut into fragments of approximately 0.25 $cm^2$ and subsequently subjected to disinfestation treatments. During the disinfestation process, the leaf

fragments were immersed in 70% ethanol solution containing Tween 80 (0.02%) for 1 min, transferred to sodium hypochlorite solution (2.5% active chlorine) for 8 min and then washed twice in sterile distilled water for 2 min. To test the efficiency of the surface disinfestation method, the adaxial portion of some leaf fragments was pressed onto the culture medium used for the isolation (Schulz et al., 1998).

For disinfestation, the roots were washed in sterilized water, cut into fragments of approximately 5 cm, immersed in 70% ethanol and Tween 80 (0.02%) for 1 min, immersed in hydrogen peroxide (3%) for 3.5 min and washed twice in sterilized distilled water for 2 min per wash. Five fragments of leaves and roots were transferred into each Petri dish containing YMC medium plus the antibiotics streptomycin (50 µg/ml) and tetracycline (50 µg/ml). The plates were incubated for 10 days at 25°C ± 2°C in the dark.

The concentrations and exposure times of the leaf and root fragments in sodium hypochlorite and hydrogen peroxide, respectively, were previously tested to obtain and adjust the optimal conditions for endophyte isolation and the proper elimination of epiphytic and saprophytic microorganisms. The effectiveness of the disinfestation process was verified by the inoculation of aliquots of the last washing solution from the leaf and root fragments into liquid YMC medium.

After growth of colonies, the fungi were subjected to monosporic purification and cultured in YMC medium at 25°C ± 2°C for a photoperiod with 12 h of white light and 12 h in the dark for seven days. Then, the isolates were preserved in 10% glycerol, distilled and sterilized water (Castellani, 1939) and stored at 4°C in the Mycology Collection of the Laboratory of Molecular Genetics of Fungi (BIOAGRO - UFV Campus - Viçosa/MG, Brazil).

### DNA extraction, amplification and sequencing of the rDNA ITS region

The fungi were grown in YMC medium for seven days, and their mycelia were transferred to Eppendorf tubes with 0.2 ml of glass beads (425 to 600 µm). The DNA from these mycelia was extracted using the Wizard® Genomic DNA Purification Kit (Promega) according to manufacturer instructions with modifcations proposed by Pinho et al. (2012). The extracted DNA was quantified and evaluated for purity by spectrophotometry ($A_{260}/A_{280}$ ratio) (Nanodrop 2000, Thermo Scientific).

The internal transcribed spacer (ITS) region (ITS1-5.8s-ITS2) of the rDNA was amplified by PCR using the primers ITS 1F (5' CTTGGTCATTTAGAGGAAGTAA 3') (Gardes and Bruns, 1993) and ITS 4 (5' TCCTCCGCTTATTGATATGC 3') (White et al., 1990). Each amplification reaction used 50 ng of DNA, 25 mM $MgCl_2$, 10 mM dNTPs, 5 µM ITS 1F, 5 µM ITS 4, 1 unit of GoTaq® Green MasterMix 2X (Promega, WI, USA) and ultrapure water to 25 µl. The Eppendorf Mastercycler thermocycler (Eppendorf, Germany) was programmed to perform an initial denaturation step at 95°C for 3 min, followed by 36 cycles at 95°C for 1 min, 50°C for 1 min and 72°C for 1 min. After the cycles, there was a final extension at 72°C for 7 min. Next, the PCR products were separated by 1.2% agarose gel electrophoresis and sent to the commercial company Macrogen (Korea) for DNA purification and sequencing.

The forward and reverse sequences of each DNA strand were analysed using the Geneious 8.0.4 program and grouped into contigs. Next, using the BLAST program, the sequences were compared to the sequences deposited in the GenBank database of the National Center for Biotechnological Information (NCBI) and UNITE (Unified system for the DNA-based fungal species) using a nucleotide sequence alignment algorithm (BLASTN). In this process, the sequences from this study with lower e-values, greater query coverage and greater identity in correspondence to the sequences present in the database were considered to belong to the species or genus referring to the isolate with greater sequence identity. Sequences from the ITS regions of the isolates from this study were deposited in GenBank under accession numbers MK026979 to MK027005 and MK027293. Subsequently, the ITS region sequences were grouped into operational taxonomic units (OTUs), with sequences having ≥ 98% similarity considered to belong to the same OTU. Sequences with < 98% similarity were considered to belong to different OTUs even though they were of the same genus, based on Nilsson et al. (2009) results.

### Phylogenetic analysis

The nucleotide sequences of the rDNA ITS region of each OTU and the reference or type sequences (Table 1) obtained from the database were aligned with the program MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0 (Tamura et al., 2013). The cluster was performed using Bayesian inference (BI) (Yang and Rannala, 1997) in the program MrBayes 3.2.1 (Huelsenbeck and Ronquist, 2001) with the GTR+I+G evolutionary model according to the Akaike Information Criterion (AIC) parameter chosen in the program MrModeltest v2.3 (Nylander, 2004). The phylogenetic trees were inferred in the program MrBayes, in which two independent runs with four Monte-Carlo Markovian chains (MCMC) were run for 10,000,000 generations, with the trees sampled and retained every $1000^{th}$ generation. During the burn-in phase, the first 1,000,000 tree samples were discarded, and the remaining trees were summarized to generate a consensus tree. *A posteriori* probability BI values above 95% were added in the tree branches and indicated high data reliability with strong statistical support (Harada et al., 1995).

### Endophytic fungal diversity

The diversity of endophytic fungal species was measured using the prediction (extrapolation) and rarefaction (interpolation) models of the initial sample to compare species richness and biodiversity among the different fungi isolated from the tissues (leaf, stem and root) of *H. guianensis*.

Extrapolation and rarefaction models based on Hill numbers are empirical estimates that tend to be an increasing function of sampling effort. *q* determines the measure of relative frequency, and the models determine a unified approach for individual-based (abundance) and sample-based (incidence) data for species richness ($^{q}$D, where $q = 0$). To measure taxon diversity by incorporating the relative abundance, we assume $^{q}$D, where $q > 0$ and q = 1 for the exponential of Shannon's index and q = 2 for the inverse of Simpson's concentration index (Chao and Colwell, 2014).

The diversity index analysis and calculation of the standard error within a 95% confidence interval with 1000 bootstrapping replicates were performed in R version 3.1.2.

### Analysis of similarity among fungi in different plant tissues

Non-metric multidimensional scaling (nMDS) analysis was used to evaluate the similarity among the fungal communities isolated from the different tissues (leaf, stem and root) of the rubber trees. In this analysis, the distances were measured using the Bray-Curtis index within the R vegan package (Oksanen, 2015).

## RESULTS

A total of 92 endophytic fungi were isolated from the tissues of *H. guianensis* trees (leaf: 66 isolates, stem: 8 isolates and root: 18 isolates) located at different

**Table 1.** Number and frequency of endophytic fungi isolated from the leaves, stems and roots of *Hevea guianensis* from the Amazon forest in the state of Acre per operational taxonomic unit (OTU).

| Operational taxonomic units (OTUs) | Total of isolates of *H. guianensis* | | | Frequency of colonization of isolates (%) | | |
|---|---|---|---|---|---|---|
| | Leaf | Stem | Roots | Leaf | Stem | Roots |
| OTU001 - *Colletotrichum* spp. | 27 | 1 | 0 | 40.9 | 12.5 | 0 |
| OTU002 - *Fusarium oxysporum* | 0 | 1 | 2 | 0 | 12.5 | 11 |
| OTU003 - Diaporthaceae | 6 | 0 | 0 | 9.0 | 0 | 0 |
| OTU004 - *Penicillium* spp. | 0 | 0 | 9 | 0 | 0 | 50 |
| OTU005 - Diaporthaceae | 3 | 0 | 0 | 4.5 | 0 | 0 |
| OTU006 - *Trichoderma* spp. | 0 | 1 | 3 | 0 | 12.5 | 16.6 |
| OTU007 - *Purpureocillium lilacinum* | 2 | 0 | 0 | 3.0 | 0 | 0 |
| OTU008 - *Lasiodiplodia* spp. | 2 | 0 | 0 | 3.0 | 0 | 0 |
| OTU009 - *Phomopsis* spp. | 1 | 0 | 0 | 1.5 | 0 | 0 |
| OTU010 - *Pestalotiopsis mangiferae* | 4 | 0 | 1 | 6.0 | 0 | 5.5 |
| OTU011 - *Penicillium* spp. | 0 | 1 | 0 | 0 | 12.5 | 0 |
| OTU012 - *Mucor* spp. | 1 | 0 | 1 | 1.5 | 0 | 5.5 |
| OTU013 - *Colletotrichum* spp. | 1 | 0 | 0 | 1.5 | 0 | 0 |
| OTU014 - *Colletotrichum* spp. | 5 | 0 | 0 | 7.5 | 0 | 0 |
| OTU015 - *Daldinia eschscholtzii* | 3 | 0 | 0 | 4.5 | 0 | 0 |
| OTU016 - Diaporthaceae | 1 | 0 | 0 | 1.5 | 0 | 0 |
| OTU017 - *Colletotrichum* spp. | 1 | 0 | 0 | 1.5 | 0 | 0 |
| OTU018 - *Phyllosticta capitalensis* | 2 | 0 | 0 | 3.0 | 0 | 0 |
| OTU019 - *Curvularia* spp. | 0 | 0 | 1 | 0 | 0 | 5.5 |
| OTU020 - *Pseudofusicoccum stromaticum* | 3 | 0 | 0 | 4.5 | 0 | 0 |
| OTU021 - *Chaetomium globosum* | 1 | 0 | 0 | 1.5 | 0 | 0 |
| OTU022 - *Penicillium* spp. | 0 | 1 | 0 | 0 | 12.5 | 0 |
| OTU023 - *Phlebiopsis flavidoalba* | 0 | 1 | 0 | 0 | 12.5 | 0 |
| OTU024 - *Phyllosticta citriasiana* | 2 | 0 | 0 | 3.0 | 0 | 0 |
| OTU025 - *Pilidiella wangiensis* | 1 | 0 | 0 | 1.5 | 0 | 0 |
| OTU026 - Hypocreales | 0 | 0 | 1 | 0 | 0 | 5.5 |
| OTU027 - *Letendraea helminthicola* | 0 | 1 | 0 | 0 | 12.5 | 0 |
| OTU028 - *Chaunopycnis* spp. | 0 | 1 | 0 | 0 | 12.5 | 0 |
| Total | 66 | 8 | 18 | - | - | - |

collection points throughout the state of Acre (Table 1). Of the total, 96.73% (89 isolates), 1.08% (one isolate) and 2.17% (two isolates) belonged to the phyla Ascomycota, Basidiomycota and Zygomycota, respectively.

Within the Ascomycota phylum group, 16 OTUs (63 isolates) belonged to class Sordariomycetes, 6 OTUs (11 isolates) to class Dothideomycetes and 4 OTUs (15 isolates) to class Eurotiomycetes. Only 1 OTU (two isolates) belonged to class Mucoromycotina of phylum Zygomycota, and 1 OTU (one isolate) belonged to class Agaricomycetes of phylum Basidiomycota (Supplementary Tables 1 and 2).

The sequences of the isolates used as representatives of each OTU and subjected to phylogenetic analysis via Bayesian inference were grouped with the sequences of the type and reference isolates deposited in GenBank and Unite. Phyla Zygomycota, Ascomycota and Basidiomycota formed clusters, and the genera within these phyla formed clades within their respective families with well-supported branches (greater than 95% bootstrap support (BS) and 0.95 posterior probabilities (PP) (Figure 1).

When comparing the richness and diversity of the fungi recovered from the different plant tissues, greater richness ($q = 0$) and Shannon ($q = 1$) and Simpson ($q = 2$) diversity were observed in isolates obtained from the stem cortex of *H. guianensis.* The richness and diversity values of the fungi isolated from the leaves and roots of these rubber trees did not differ significantly (Figure 2 and Table 2).

The nMDS analysis based on the Bray-Curtis distances between OTUs showed a trend towards cluster formation and a heterogeneous distribution of fungi recovered from leaf tissue compared to isolates from the stem and root cortex ($R = 0.302$, $p < 0.001$) (Figure 3).

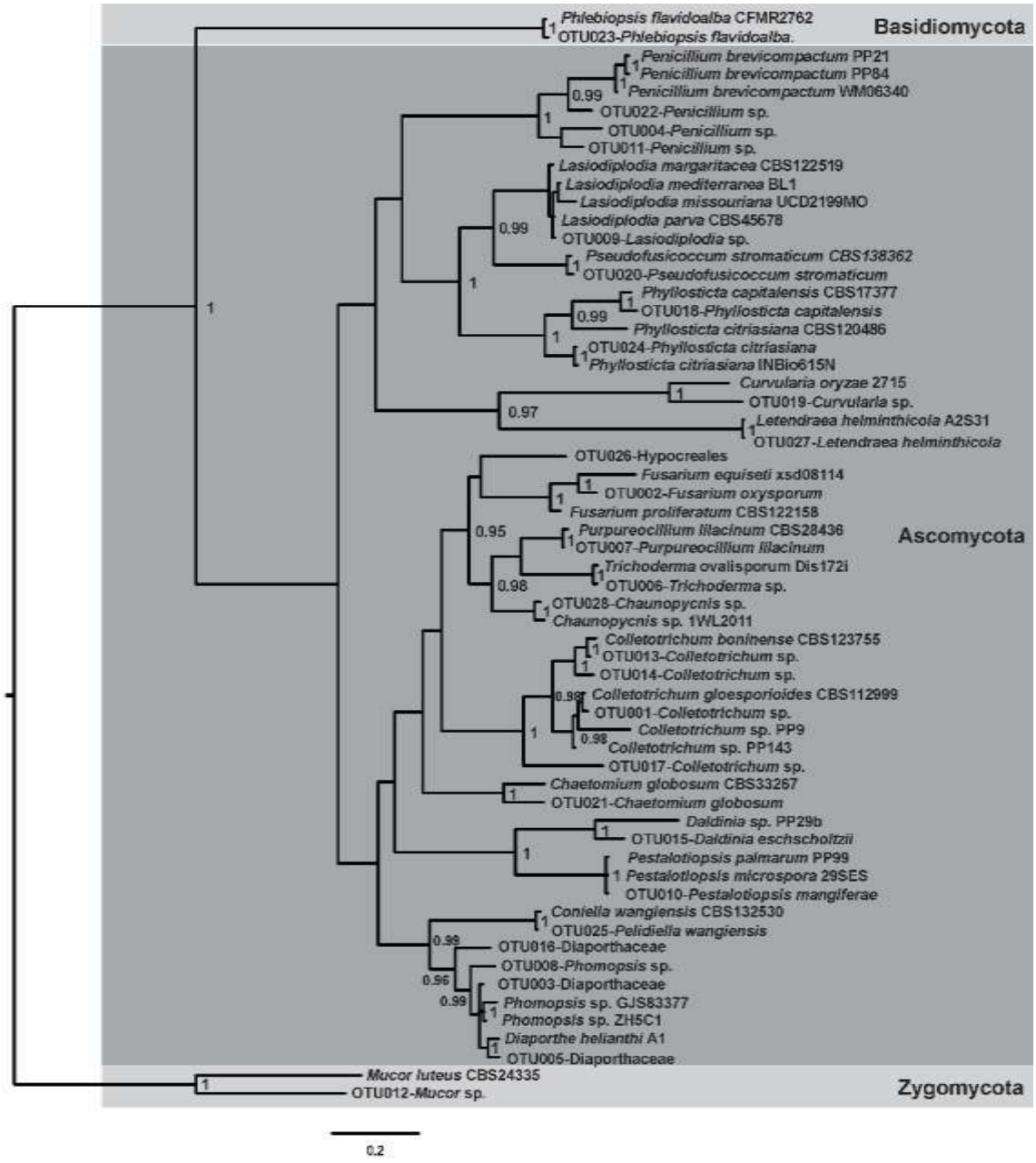


**Figure 1.** Phylogenetic tree obtained by Bayesian inference (BI) using sequences from the rDNA ITS region of the 28 operational taxonomic units (OTUs) that clustered all 92 endophytic fungi belonging to the phyla Ascomycota, Basidiomycota and Zygomycota. Posterior probability values below 95% were omitted.

Of the 92 isolates cultured from *H*. *guianensis* tissues, 38% of the fungi (35 isolates) belonged to the genus *Colletotrichum* (family Glomerellaceae), of which 97.14% (34 isolates) were obtained from the leaf fragments of the rubber tree; only 2.85% (one isolate) of the isolates from this genus were isolated directly from the stem cortex of the plant (Figure 4, Table 1, Supplementary Tables 1 and 2).

The *Penicillium* (family Trichocomaceae) genus had the second highest number of fungi isolated from *H. guianensis*, representing 11.95% of the total fungi recovered (Figure 4). Representatives of this genus were isolated mainly from the roots, totalling 50% (nine isolates) of the fungi obtained from the roots of *H. guianensis* (Table 1, Supplementary Tables 1 and 2 Material). Also, *Diaporthe* (family Diaporthaceae) is the second genus most abundant within of the tissue of *H. guianensis*, mainly within of the leaf tissue, corresponding

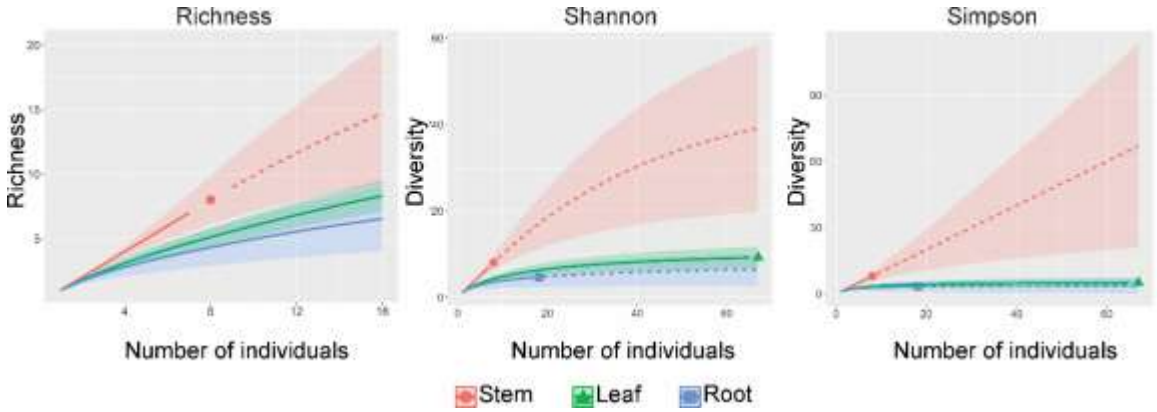


**Figure 2.** Rarefaction (solid line) and extrapolation (dashed line) curves for twice the size of the reference sample. The rarefaction (solid line) and extrapolation (dashed line) curves compare the species richness (q = 0), exponential of Shannon's entropy index (q =1) and inverse of Simpson's concentration index (q = 2) according to the Hill numbers of endophytic fungi in the different *Hevea guianensis* niches: stem (red line), leaf (green line) and root (blue line), with 95% confidence intervals obtained by the bootstrap method with 200 replications.

**Table 2.** Comparison of the asymptotic richness estimator (q = 0), exponential of Shannon's entropy index (q = 1) and inverse of Simpson's concentration index (q = 2) of endophytic fungi among the different *Hevea guianensis* niches with their 95% confidence intervals (*).

| Sample | Tissues | Richness (q0) | Shannon (q1) | Simpsom (q2) |
|---|---|---|---|---|
| Acre | Leaf | 8.311 ± 0.658[a] | 9.193 ± 0.897[a] | 4.928 ± 0.897[a] |
| | Stem | 14.710 ± 0.302[b] | 39.122 ± 0.909[b] | 20.852 ± 0.909[b] |
| | Root | 6.549 ± 0.771[a] | 2.659 ± 0.948[a] | 3.670 ± 0.948[a] |

Means in a column followed by the same letter do not differ at 5% probability according to Tukey procedure using R software version 3.1.2 with 1000 bootstrap replicates.

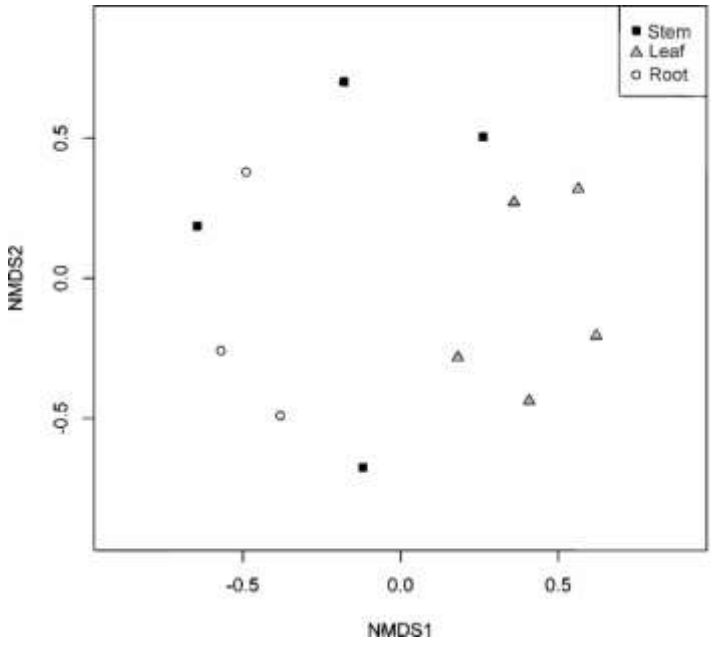


**Figure 3.** Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis distance between fungal samples obtained from different *Hevea guianensis* tissues.

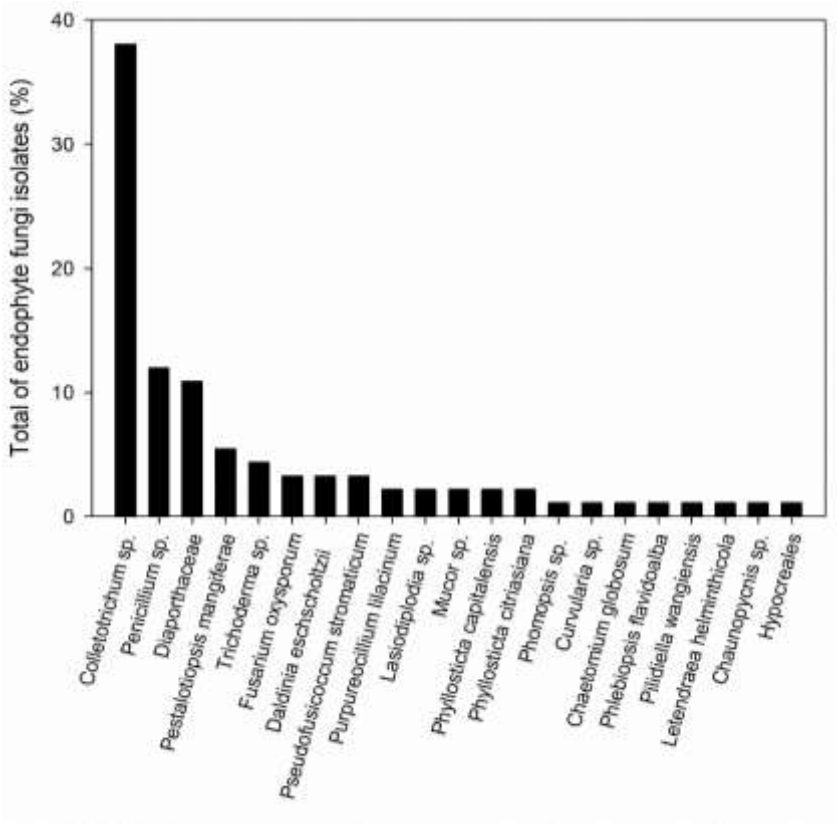


**Figure 4.** Percentage of endophytic fungi isolated from *Hevea guianensis* in the Brazilian Amazon (State Acre).

to 11.95% (11 isolates) of the isolates distributed into the four OTUs (Table 1, Supplementary Tables 1 and 2).

Fungi of the genus *Pestalotiopsis* were isolated from the leaves and roots, whereas isolates of the genera *Trichoderma* and *Fusarium* were obtained from the stems and roots and the genera *Phyllosticta*, *Daldinia* and *Pseudofusicoccum* were recovered only from the leaves of the rubber trees, with representation of 5.43, 4.34, 3.26, 4.34, 3.26 and 3.26%, respectively (Table 1, Supplementary Tables 1 and 2).

Among the genera that presented lower abundances, representatives of the genus *Mucor* were isolated from the leaves and roots of *H. guianensis*. Fungi belonging to the genera *Lasiodiplodia* and *Purpureocillium* were only isolated from the leaves of the rubber trees, corresponding to 2.17% of the total fungi recovered (Table 1, Supplementary Tables 1 and 2).

## DISCUSSION

The fungi isolated from different tissues of *H. guianensis* were more diverse and showed greatest richness in stem cortex than in the roots or leaves, although the frequency of isolates was highest in the leaves. In addition, the endophytic fungi of the leaves showed heterogeneous distribution in relation to the stem and root isolates.

This study is the first to describe the diversity of endophytic fungi in leaves, stems and roots of *H. guianensis* in the Brazilian Amazon. In comparison to the species *H. brasiliensis*, which is extensively cultivated, few studies have investigated *H. guianensis*, exploited in its natural habitat but is not cultivated in terms of the production of varieties that have been genetically improved for disease resistance or production of better quality latex (Cardoso et al., 2014) and descriptions of microorganisms in the tissues with potential biotechnological applications (Rocha et al., 2011; Gazis and Chaverri, 2010).

The morphological similarity between *H. guianensis* and *H. brasiliensis* makes identification of these species difficult in their natural environment. However, we located and identified six *H. guianensis* trees among the *H. brasiliensis* trees in the Amazon forest in the state of Acre.

The 92 fungi isolated were identified when the ITS region sequence was used as a barcode. However, most of the microorganisms that inhabit the interior of plants and other niches are unculturable. Although there a limitation in determining the true richness of fungi that colonize plants, the use of ITS region facilitated the identification of different genera and their clustering into OTUs. Many studies have estimated the diversity and

distributions of species in a microbial community by counting OTUs (Koide et al., 2017; Martins et al., 2016; Angelini et al., 2012; Gazis et al., 2011; Gazis and Chaverri, 2010), and ITS region sequences have been used as an efficient universal barcode to discriminate fungal genera (Schoch et al., 2012) and to cluster these sequences into OTUs with intraspecific variations of 0 to 3% (Nilsson et al., 2009).

As observed in several other studies (Ferreira et al., 2017; Martins et al., 2016; Fernandes et al., 2015; Leite et al., 2013; Gazis and Chaverri, 2010; Hanada et al., 2010), phylum Ascomycota was most abundant in the endophytic fungal community of *H. guianensis* (96.73%), particularly class Sordariomycetes (68.47%). In addition, the estimated richness, Shannon diversity and Simpson diversity were significantly higher in the stems, despite the high proportion of fungi isolated from the leaves (71.73%). Fungi isolated from leaves were distributed into 18 OTUs when compared to the fungi recovered from the stems (8.69% of the total isolates clustered into 8 different OTUs) and from the roots (19.56% of the recovered fungi were present in 7 OTUs) of these rubber trees. The high diversity of endophytic fungi in the stem is due to the high equitability in the distribution of fungi identified and isolated in this plant tissue.

Regarding the estimation of richness and diversity of the endophytic fungi, the results obtained in this study corroborate those from the study of Gazis and Chaverri (2010), who found a high diversity of fungi in the stem cortex despite obtaining a higher frequency of isolate colonization in the leaves of Peruvian rubber trees.

In this study, a trend was revealed towards clustering of the isolates present in the leaves and the separation of these isolates from the fungi recovered from the stem cortex and roots. Several factors may affect the distribution and abundance of the microbial community, such as the environment, chemical composition of tissues and interspecific competition among microorganisms (Zheng et al., 2017; Martins et al., 2016; Gazis and Chaverri, 2010; Suryanarayanan and Vijaykrishna, 2001).

*Colletotrichum*, *Penicillium* and *Diaporthe* were the predominant genera, while *Trichoderma*, *Pestalotiopsis*, *Fusarium*, *Phyllosticta*, *Daldinia*, *Pseudofusicoccum*, *Mucor*, *Lasiodiplodia* and *Purpureocillium* were obtained in lower frequencies from the tissues of *H. guianensis*.

However, Gazis and Chaverri (2010) studied the diversity of endophytic fungi in Peruvian rubber trees and found that *Penicillium*, *Pestalotiopsis* and *Trichoderma* were the most frequent genera. Thus, there are diffferences among the endophytic fungal communities in rubber trees from different study areas.

In this study, *Colletotrichum* was isolated from the leaf fragments (38%) and from the stem cortex (2,85%) of the *H. guianensis*. *Diaporthe* is the second most important genus isolate inside the leaves. This result was also observed in several studies of the diversity of endophytic fungi in tropical plants (Ferreira et al., 2017; Fernandes et al., 2015; Leite et al., 2013). Nevertheless, Gazis and Chaverri (2010) observed a low frequency of *Colletotrichum* with the tissue of *H. brasiliensis*, a closely related species from Peruvian Amazon forest.

Fifty percent of the endophytic fungi isolated from the roots belonged to the genera *Penicillium*. *Trichoderma*, *Fusarium*, *Pestalotiopsis*, *Curvularia* and *Mucor* were also isolated from the roots of *H. guianensis.* Also, the fungal genera recovered from the rubber tree stems in this study showed greater equitability and were identified as *Colletotrichum* species, *Fusarium oxysporum*, *Trichoderma* species, *Penicillium* species, *Phlebiopsis flavidoalba*, *Letendraea helminthicola* and *Chaunopycnis* species.

Among the factors affecting the microbial community, climate and dispersion are processes that have been reported to significantly influence the endophytic fungal communities in plants (Koide et al., 2017; Zheng et al., 2017). This knowledge has great relevance because climate change can affect the natural environment and plantations of crops of commercial interest. Additionally, the environment can modify the dispersion of endophytic fungi and their effects on plants regarding tolerance to extreme temperature and humidity, as could be the case with rubber trees in the Amazon.

Some species close to fungi genera obtained in the present study are reported in the literature as potentially mutualistic species, which may be tested in the future as biological control agents of plant diseases, may confer resistance to abiotic stresses and/or promote plant growth. For example, in relation to studies on rubber trees, Rocha et al. (2011) isolated a total of 435 endophytic fungi from the leaves of three cultivars of *H. brasiliensis* that were resistant to diseases and found a higher abundance of fungi belonging to the genera *Colletotrichum*, *Diaporthe*, *Fusarium, Pestalotiopsis*, *Microspheropsis* and *Myrothecium*. These latter isolates were able to inhibit the germination of *Pseudocercospora ulei* conidia by 80%. The genera *Colletotrichum*, *Diaporthe*, *Pestalotiopsis*, and *Fusarium* were also obtained in the present study and could be tested as biological control agents in future studies.

Other fungi genera with potential for biological control of diseases, inductors of plant resistance to abiotic stress and/or growth promoters in plants include *Penicillium* (Guijarro et al., 2017; Babu et al., 2015; Murali and Amruthesh, 2015), *Lasiodiplodia* (Xiang et al., 2016), *Fusarium* (Zhang et al., 2014; Rocha et al., 2011), *Purpureocillium* (Lopez et al., 2014) and *Trichoderma* (Ben Amira et al., 2017; Contina et al., 2017; Larran et al., 2016; Mbarga et al., 2014).

In addition, the Amazon region has the greatest biodiversity on the planet as well as different endemism centres, and little is known about the communities of endophytic fungi present (Gibertoni et al., 2016; Gazis and Chaverri, 2010). Analysis of microbial culture collections showed the existence of 46 Brazilian culture

collections registered in the Genetic Heritage Management Council (CGEN) database belonging to the World Federation for Culture Collections, the majority of which were located in south eastern Brazil (Sette et al., 2013). The authors believe that there is still a lack of up-to-date information and studies aimed at obtaining and analysing microbial culture collections (Sette et al., 2013). This information and analysis can promote knowledge about the diversity, conservation and biotechnological exploitation of fungi.

The endophytic fungi isolated and identified from *H. guianensis* in the present study will be used in future studies focusing on the identification of new species, using different locus to phylogenetic analysis, and also their potential use in the promotion of plant growth, the biological control of diseases and in the production of bioactive metabolites of interest.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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**Supplementary Table 1.** Identification of endophytic fungi grouped in each OTU.

| OTUs | Phylum | Class | Order | Family | Genus | Species |
|---|---|---|---|---|---|---|
| OTU001 | Ascomycota | Sordariomycetes | Sordariomycetidae | Glomerellaceae | *Colletotrichum* | *Colletotrichum* sp. |
| OTU002 | Ascomycota | Sordariomycetes | Hypocreales | Nectriaceae | *Fusarium* | *Fusarium oxysporum* |
| OTU003 | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | - | ---------- |
| OTU004 | Ascomycota | EurotiomyTTcetes | Eurotiales | Trichocomaceae | *Penicillium* | *Penicillium* sp |
| OTU005 | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | - | ---------- |
| OTU006 | Ascomycota | Sordariomycetes | Hypocreales | Hypocreaceae | *Trichoderma* | *Trichoderma sp* |
| OTU007 | Ascomycota | Sordariomycetes | Hypocreales | Ophiocordycipitaceae | *Purpureocillium* | *Purpureocillium lilacinum* |
| OTU008 | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | *Phomopsis* | *Phomopsis* sp. |
| OTU009 | Ascomycota | Dothideomycetes | Botryosphaeriales | Botryosphaeriaceae | *Lasiodiplodia* | *Lasiodiplodia* sp |
| OTU010 | Ascomycota | Sordariomycetes | Xylariales | Amphisphaeriaceae | *Pestalotiopsis* | *Pestalotiopsis mangiferae* |
| OTU011 | Ascomycota | Eurotiomycetes | Eurotiales | Trichocomaceae | *Penicillium* | *Penicillium* sp. |
| OTU012 | Zygomycota | Mucoromycotina | Mucorales | Mucoraceae | *Mucor* | *Mucor* sp. |
| OTU013 | Ascomycota | Sordariomycetes | Sordariomycetidae | Glomerellaceae | *Colletotrichum* | *Colletotrichum* sp. |
| OTU014 | Ascomycota | Sordariomycetes | Sordariomycetidae | Glomerellaceae | *Colletotrichum* | *Colletotrichum* sp. |
| OTU015 | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | *Daldinia* | *Daldinia eschscholtzii* |
| OTU016 | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | - | ---------- |
| OTU017 | Ascomycota | Sordariomycetes | Sordariomycetidae | Glomerellaceae | *Colletotrichum* | *Colletotrichum* sp. |
| OTU018 | Ascomycota | Dothideomycetes | Botryosphaeriales | Botryosphaeriaceae | *Phyllosticta* | *Phyllosticta capitalensis* |
| OTU019 | Ascomycota | Dothideomycetes | Pleosporales | Pleosporaceae | *Curvularia* | *Curvularia* sp. |
| OTU020 | Ascomycota | Dothideomycetes | Botryosphaeriales | Botryosphaeriaceae | *Pseudofusicoccum* | *Pseudofusicoccum stromaticum* |
| OTU021 | Ascomycota | Sordariomycetes | Sordariales | Chaetomiaceae | *Chaetomium* | *Chaetomium globosum* |
| OTU022 | Ascomycota | *Eurotiomycetes* | Eurotiales | Trichocomaceae | *Penicillium* | *Penicillium* sp. |
| OTU023 | Basidiomycota | Agaricomycetes | Polyporales | Phanerochaetaceae | *Phlebiopsis* | *Phlebiopsis flavidoalba* |
| OTU024 | Ascomycota | Dothideomycetes | Botryosphaeriales | Botryosphaeriaceae | *Phyllosticta* | *Phyllosticta citriasiana* |
| OTU025 | Ascomycota | Sordariomycetes | Diaporthales | Schizoparmaceae | *Pilidiella* | *Pilidiella wangiensis* |
| OTU026 | Ascomycota | Sordariomycets | Hypocreales | - | - | ---------- |
| OTU027 | Ascomycota | Dothideomycetes | Pleosporales | Tubeufiaceae | *Letendraea* | *Letendraea helminthicola* |
| OTU028 | Ascomycota | Sordariomycetes | Hypocreales | Ophiocordycipitaceae | *Chaunopycnis* | *Chaunopycnis*sp. |

**OTU,** operational taxonomic unit.

**Supplementary Table 2.** Identification codes of the isolates composing each OTU.

| OTUs | Isolates |
|---|---|
| OTU001 | 800F8F-AC; 368F3F-AC; 598F9F-AC; 362F6F-AC; 12F6F-AC; 219F3F-AC; 222F3F-AC; 347F3F-AC; 61F3F-AC; 408F3F-AC; 402F3F-AC; 372F3F-AC; 358F3F-AC; 344F3F-AC; 235F6F-AC; 233F3F-AC; 223F10F-AC; 218F3C-AC; 168F3F-AC; 143F3F-AC; 11F10F-AC; 67F10F-AC; 62F3F-AC; 571F10F-AC; 241F10F-AC; 220F10F-AC; 805F3F-AC; 66F10F-AC |
| OTU002 | 664F10C-AC; 329F6R-AC; 215F6R-AC |
| OTU003 | 327F3F-AC; 716F3F-AC; 141F6F-AC; 326F6F-AC; 178F6F-AC; 514F6F-AC |
| OTU004 | 202F8R-AC; 171F9R-AC; 689F9R-AC; 752F8R-AC; 753F8R-AC; 756F8R-AC; 694F8R-AC; 755F8R-AC; 798F8R-AC |
| OTU005 | 512F9F-AC; 617F9F-AC; 333F8F-AC |
| OTU006 | 508F9R-AC; 26F9R-AC; 3F13C-AC; 4F9R-AC |
| OTU007 | 64F3F-AC; 364F10F-AC |
| OTU008 | 470F9F-AC; 626F9F-AC |
| OTU009 | 77F3F-AC |
| OTU010 | 172F10F-AC; 594F10F-AC; 422F10F-AC; 359F3F-AC; 165F8R-AC |
| OTU011 | 197F3C-AC |
| OTU012 | 555F10F-AC; 649F9R-AC |
| OTU013 | 129F6F-AC |
| OTU014 | 343F3F-AC; 406F6F-AC; 572F6F-AC; 401F6F-AC; 367F3F-AC |
| OTU015 | 240F3F-AC; 324F8F-AC; 365F3F-AC |
| OTU016 | 558F6F-AC |
| OTU017 | 234F6F-AC |
| OTU018 | 302F10F-AC; 307F10F-AC |
| OTU019 | 325F8R-AC |
| OTU020 | 396F3F-AC; 551F10F-AC; 601F10F-AC |
| OTU021 | 413F10F-AC |
| OTU022 | 620F10C-AC |
| OTU023 | 179F10C-AC |
| OTU024 | 135F3F-AC; 388F3F-AC |
| OTU025 | 363F6F-AC |
| OTU026 | 216F6R-AC |
| OTU027 | 674F10C-AC |
| OTU028 | 176F9C-AC |

**OTU,** operational taxonomic unit.