KALIANE SÍRIO ARAÚJO

DIVERSIDADE DE FUNGOS ENDOFÍTICOS DE *Hevea* spp. DA FLORESTA AMAZÔNICA BRASILEIRA: IDENTIFICAÇÃO E SELEÇÃO DE ISOLADOS DE *Penicillium* E DE *Talaromyces* COM POTENCIAL PARA O CONTROLE DE FITOPATÓGENOS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de *Doctor Scientiae*.

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APROVADA: 22 de fevereiro de 2018.

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Dedico

Aos meus pais Lauro Conceição Araújo (*in memoriam*) e Gisélia Síro Araújo.
Aos meus irmãos Kalisson Rodrigo S. Araújo e Katiane S. Araújo

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RESUMO

Araújo, Kaliane Sírio, D.Sc., Universidade Federal de Viçosa, fevereiro de 2018. **Diversidade de Fungos endofíticos de *Hevea* spp. da floresta amazônica brasileira: identificação e seleção de isolados de *Penicillium* e de *Talaromyces* com potencial para controle de fitopatógenos.** Orientadora: Marisa Vieira de Queiroz. Coorientadores: Olinto Liparini Pereira e Eduardo Seiti Gomide Mizubuti.

Hevea brasiliensis e *Hevea guianensis* são espécies nativas da floresta amazônica brasileira e de grande importância econômica por produzirem a borracha natural. Estas espécies vegetais são consideradas grandes reservatórios de fungos endofíticos. Dentro deste grupo de micro-organismos, as espécies endofíticas pertencentes ao gênero *Penicillium* e *Talaromyces* são conhecidas por atuarem no controle biológico de fitopatógenos e na promoção de crescimento vegetal. Entretanto, não existem estudos na literatura sobre a diversidade de fungos endofíticos que habitam os tecidos das seringueiras da floresta amazônica brasileira, bem como pesquisas sobre isolados de *Penicillium* spp. e *Talaromyces* spp. endofíticos dessas plantas. Neste estudo é descrita a diversidade de fungos endofíticos nas folhas, no caule e nas raízes de *H. brasiliensis* e *H. guianensis*. Além disso, espécies pertencentes aos gêneros *Penicillium* e *Talaromyces* foram identificadas e o potencial antagônico destas ao crescimento de diferentes fitopatógenos foi analisado. O total de 549 e 92 fungos foram isolados do interior dos tecidos de *H. brasiliensis* e *H. guianensis*, respectivamente. O filo Ascomycota foi dominante em ambos os hospedeiros. A diversidade dos fungos foi maior no caule em *H. brasiliensis* e no caule e nas raízes de *H. guianensis*, embora a frequência de colonização destes micro-organismos tenha sido maior nas folhas destas seringueiras. *Colletotrichum*, *Diaporthe*, *Fusarium*, *Trichoderma* e *Penicillium* foram os gêneros mais representativos entre os isolados nos dois hospedeiros. Em ambos os estudos foi verificada uma tendência ao agrupamento dos isolados das folhas e uma distribuição homogênea dos fungos do caule e das raízes, bem como entre os pontos de coletas. Em um estudo mais detalhado dos isolados pertencentes aos gêneros *Penicillium* e *Talaromyces*, foram identificadas espécies novas de *Penicillium*. Também, foi constatada variabilidade genética intra e interespecífica entre as espécies de *Penicillium* e *Talaromyces* pelo método de IRAP e REMAP.

Em adição, as espécies de *Penicillium* e *Talaromyces* foram capazes de reduzir o crescimento de diferentes fitopatógenos. Os fungos endofíticos isolados neste estudo podem ser uma alternativa promissora como produtores de compostos bioativos de grande interesse para a indústria e para o agronegócio.

ABSTRACT

Araújo, Kaliane Sírio, D.Sc., Universidade Federal de Viçosa, February, 2018. **Diversity of endophytic fungi of *Hevea* spp. of the Brazilian Amazon forest: identification and selection of *Penicillium* and *Talaromyces* isolates with potential for phytopathogen control**. Adviser: Marisa Vieira de Queiroz. Co-advisers: Olinto Liparini Pereira and Eduardo Seiti Gomide Mizubuti.

Hevea brasiliensis e *Hevea guianensis* are native species of the Brazilian Amazon forest and of great economic importance for the producing natural rubber. These plant species are large reservoirs of endophytic fungi. Inside this group of microorganisms, the endophytic species belonging to the genus *Penicillium* and *Talaromyces* are known to act in the biological control of phytopathogens and plant growth promotion. However, there are no studies in the literature on the diversity of endophytic fungi that inhabit the tissues of rubber trees in the Brazilian Amazon forest, nor any research on *Penicillium* spp. and *Talaromyces* spp. endophytic of these plants. In this study it is described the diversity of endophytic fungi in the leaves, stem and in the roots of *H. brasiliensis* and *H. guianensis*. In addition, species belonging to the genus *Penicillium* and *Talaromyces* were identified and their antagonistic potential to the growth of different phytopathogens was analyzed. The total of 549 and 92 fungi was isolated from the interior of the tissues of *H. brasiliensis* and *H. guianensis*, respectively. The Ascomycota phylum was dominant in both hosts. The diversity of fungi was greater in the stem of *H. brasiliensis* and in the stem and roots of *H. guianensis* and the frequency of colonization of these microorganisms was higher in the leaves of these rubber trees. *Colletotrichum*, *Diaporthe*, *Fusarium*, *Trichoderma* and *Penicillium* were the most representative genera between the isolates in the two hosts. In both studies, a tendency was observed for the grouping of the leaf isolates and a homogeneous distribution of stem and root fungi, as well as between collection points. In a more detailed study of the isolates belonging to the genus *Penicillium* and *Talaromyces*, new species of *Penicillium* were identified. Also, intra and interspecies genetic variability among *Penicilium* and *Talaromyces* species were verified by the IRAP and REMAP method. In addition, the *Penicillium* and *Talaromyces* species were able to reduce the growth of different phytopathogens. The endophytic fungi isolated in this study may be a

promising alternative as producers of bioactive compounds of great interest to industry and agribusiness.

INTRODUÇÃO

A floresta amazônica é um acervo significativo de diversidade de espécies vegetais, como também de micro-organismos endofíticos que vivem no interior dessas plantas (micro-organismos endofíticos). Dentre essas espécies vegetais, as seringueiras (*Hevea* spp.) se destacam por serem de grande interesse econômico para o Brasil, principalmente a *Hevea brasiliensis* por ser cultivada comercialmente para a produção de borracha natural.

Entre os micro-organismos associados às plantas, os fungos endofíticos têm sido estudados devido a sua alta diversidade taxonômica e funcional, podendo atuar como agentes de controle biológico de fitopatógenos e na promoção do crescimento de culturas de grande importância econômica para o país, na produção de compostos bioativos (metabólitos secundários) de interesse industrial e na biorremediação de diferentes compostos químicos.

Até o momento, já foram realizados estudos sobre a diversidade de fungos endofíticos em seringueiras na floresta amazônica no Peru, Camarões e no México, e sobre a seleção de fungos endofíticos de folhas de diferentes cultivares de *H. brasiliensis* para controlar e/ou inibir o crescimento do *Pseudocercospora ulei*, fitopatógeno causador da doença mal-das-folhas e principal fator limitante para a produção de borracha natural no Brasil.

Entretanto, não existia até a realização deste estudo qualquer relato sobre os fungos endofíticos que colonizam seringueiras da floresta amazônica brasileira. Por causa disso, nesta tese são apresentados estudos pioneiros e desafiadores que visaram a caracterização da diversidade de fungos endofíticos de seringueiras presentes em duas bacias hidrográficas da floresta amazônica brasileira situadas nos estados do Acre e do Amazonas, tendo sido realizada coletas em 51 árvores para a obtenção desses dados. No capítulo I é abordada a diversidade de fungos endofíticos em *Hevea brasiliensis* e no capítulo II a diversidade de fungos endofíticos filamentosos em *Hevea guianensis*.

Dentre os diferentes grupos de fungos endofíticos, as espécies dos gêneros *Penicillium* e *Talaromyces* têm sidos estudadas e relatadas na literatura como produtoras de novos compostos de interesse econômico, como importantes agentes de controle biológico de fitopatógenos e por atuarem no incremento da produção vegetal. Dessa forma, no capítulo III é descrita a

identificação de novas espécies, a variabilidade genética intra e interespecífica e o potencial para a produção de antimicrobianos de isolados de *Penicillium* spp. endofíticos de seringueiras da floresta amazônica brasileira.

CAPITULO 1

Diversity of endophytic fungi in *Hevea brasiliensis* native to the Brazilian Amazon forest

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Diversity of endophytic fungi in *Hevea brasiliensis* native to the Brazilian Amazon forest

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ABSTRACT

To characterize the fungal communities that inhabit the tissues of *Hevea brasiliensis* of the Brazilian Amazon forest, a total 549 endophytic fungi were isolated, of which 211 were from plants of drainage basins located in the state of Acre and 338 in the state of Amazonas. These fungi were grouped into 115 OTUs. The phylum Ascomycota was dominant (92.71%) and most abundant isolates belonged to families Glomerallaceae (genus *Colletotrichum*), Diaporthaceae (genus *Diaporthe*), Nectriaceae (genus *Fusarium*), Hypocreaceae (genus *Trichoderma*) and Trichocomaceae (genus *Penicillium*). Fungal diversity was higher in the stems and roots than in the leaves, whereas the fungal abundance was higher in the leaves. Comparisons between the fungal communities revealed different genera distributions and abundances in different tissues, with a tendency for leaf isolates to group together. Conversely, homogeneous distributions were found for fungi isolated from the stems and roots and from different sampling sites. Fungi genera isolated in this study are closely related to species that have potential to produce metabolites of interest for industry and sustainable development of agribusiness.

INTRODUCTION

The Amazon forest possesses the highest diversity of plant species worldwide, with potential for industrial, medicinal, food and biotechnological applications and *Hevea brasiliensis* (Willd. ex Adr. de Juss) Muell.-Arg (rubber tree) stands out among these plant species (Gasparotto et al., 2012; Rocha et al., 2011). This tree is grown for the commercial production of natural rubber. Natural rubber (latex) is a secondary metabolite produced by rubber trees, with higher quality and wider industrial applications than synthetic rubber (Gasparotto et al., 2012).

Natural rubber production in Brazil has been suffering large economic losses for decades, especially due to the high incidence of *Pseudocercospora ulei* (South American Leaf Blight – SALB) in northern Brazil (Hora Júnior et al., 2014) and *Colletotrichum gloeosporioides*, *Colletotrichum acutatum* (anthracnose), *Oidium hevea* (mildew) and *Phytophtora* spp. (striated cancer or panel cancer) in the central west and northeast regions of Brazil (Gasparotto et al., 2012). These pathogens have the potential for introduction to rubber tree plantations in Asia and Africa, which would result in economic losses and even bankruptcy of the industries that depend on their raw material worldwide.

Endophytes are microorganisms that live inside plant organs during at least part of their life cycle without causing apparent damage to their hosts (Petrini, 1991). Of these microorganisms, endophytic fungi form a highly diverse polyphyletic group. Their richness, lifestyles and activities within plants from different environments underscore their ecological importance to the maintenance of some plant species in the environment (Arnold, 2007; Brundrett, 2002; Saikkonen et al., 2011, 2004; Saunders et al., 2010).

The isolation of endophytic fungi depends on the rigor of the method used (Leite et al., 2013; Prior et al., 2014). Isolation by culture is adequate for fungal morphological characterization and physiological testing (Bernardi-Wenzel et al., 2010).

Endophytic fungi have high taxonomic and functional diversity, and the endophytic community profile is strongly determined by the host due to its physiological and biochemical conditions and its interaction with the abiotic medium (Porras-Alfaro and Bayman, 2011). These microorganisms function as

control agents for plant pathogens in crops with high economic importance (Ben Amira et al., 2017; Contina et al., 2017; Landero Valenzuela et al., 2015; Larran et al., 2016; Mbarga et al., 2014; Rocha et al., 2011), promote plant growth, produce lytic extracellular enzymes (Babu et al., 2015; Khan et al., 2008; Murali and Amruthesh, 2015) and act as bioremediation agents through the degradation of chemical compounds (Russell et al., 2011).

Studies have been performed with the aim of characterizing endophytic fungal communities associated with *Hevea* spp. growing in their native habitats (Peru) and in rubber tree plantations in Brazil, Peru, Cameroon (Africa) and Mexico (Chaverri et al., 2011; Gazis et al., 2012, 2011; Gazis and Chaverri, 2010; Gazis, 2012). These studies have revealed high diversity of endophytic fungi in tissues of *Hevea* spp., especially in the stems. In addition, a new endophytic fungal species named *Trichoderma amazonicum* (Chaverri et al., 2011), a new class Xylonomycetes (Gazis et al., 2012) and the diversity of endophytic Basidiomycetes in rubber trees have been described (Martin et al., 2015).

Until the moment, no studies have characterized the diversity of endophytic fungi in native rubber trees from the Brazilian Amazon forest or the differences between endophytic fungal communities from different tissues (leaf, stem and root) of *H. brasiliensis*. Thus, the aim of this study was to isolate, identify and analyze the diversity of endophytic fungi colonizing tissues of *H. brasiliensis* (leaf, stem and root) from two drainage basins in the Brazilian Amazon forest in the states of Acre and Amazonas.

MATERIALS AND METHODS

Collection and isolation of endophytic fungi

Leaf, stem and root cortex fragments were collected from healthy *Hevea brasiliensis* trees growing in two drainage basins of the Brazilian Amazon forest in the states of Acre and Amazonas. The samples were collected from 17 trees distributed in seven random sampling sites in the state of Acre and 28 trees distributed in 15 random sampling sites in the state of Amazonas (Fig. S1 / Table S1).

Endophytic fungi were isolated using modified methods and proposed by Wirsel et al. (2001), Evans et al. (2003) and Leite et al. (2013). Leaves were placed in paper bags that were placed inside plastic bags and stored at 4 °C (Stone et al. 2004). Root cortex fragments were placed in tubes containing silica gel, transported to the laboratory and immediately processed. Stem cortex fragments (3 to 5 cm) were obtained after bark removal using a sterile scalpel and immediately inoculated onto YMC culture medium (10 g of malt extract, 2 g of yeast extract and 15 g of agar dissolved in 1 L of distilled water and autoclaved) (Evans et al., 2003).

In the laboratory, the leaves and root fragments were washed in running water for 10 min to remove soil residues and dust and then sterilized. The leaves were cut into fragments of approximately 0.25 cm^2 and sterilized in 70% ethanol containing Tween 80 for 1 min. Then, the fragments were immersed in sodium hypochlorite (2.5% active chloride) for 8 min and washed twice with sterile distilled water for 2 min. To test the efficiency of the surface sterilization, the adaxial surface of some leaf fragments was pressed onto isolation medium (Schulz et al., 1998). The roots were washed with sterile water, cut into fragments of approximately 5 cm and sterilized in 70% ethanol and Tween 80 (0.02%) for 1 min. Then, the fragments were immersed in hydrogen peroxide (3%) for 3.5 min and washed twice with sterile distilled water for 2 minutes. All plant tissue fragments were placed in Petri dishes containing YMC medium supplemented with the antibiotics streptomycin and tetracycline and incubated for 10 days at 25 °C ± 2 °C in the dark.

The disinfestation process was optimized to eliminate epiphytic and saprophytic microorganisms and to allow only the isolation of endophytic fungi. The time of exposure of the leaf and root fragments to sodium hypochlorite and hydrogen peroxide, respectively, and their concentrations were previously tested and adjusted, and aliquots of the last washing solutions of the leaf and root fragments were inoculated into liquid YMC culture medium to verify that the sterilization was successful.

All isolates were subjected to single-spore purification and grown in YMC medium at 25 °C ± 2 °C under a 12 h photoperiod for seven days. Subsequently, all isolates were preserved in 10% glycerol (five disks of fungal mycelium in 2-mL

polypropylene microtubes) and sterile distilled water (Castellani, 1939) and stored at ambient temperature and 4 °C.

DNA extraction and amplification and sequencing of the rDNA internal transcribed spacer (ITS) region

Mycelia from viable isolates grown in YMC medium without the addition of antibiotics for seven days were transferred into Eppendorf tubes containing 0.2 mL of glass beads. Fungal DNA was extracted using an extraction kit Wizard® Genomic DNA Purification Kit (Promega) according Pinho et al. (2012), with modifications. The extracted DNA was analyzed by electrophoresis in a 0.8% agarose gel, and the DNA concentration and purity were determined by spectrophotometry (ratio A_{260}/A_{230}) (Nanodrop 2000, Thermo Scientific).

Molecular identification of the endophytic fungal isolates was based on the rDNA ITS region, which was amplified by PCR using the ITS1F (5' CTTGGTCATTTAGAGGAAGTAA 3') (Gardes and Bruns, 1993) and ITS4 (5' TCCTCCGCTTATTGATATGC 3') primers (White et al., 1990). PCR was performed in a 25-µL final volume containing 50 ng of sample DNA, 25 mM $MgCl_2$, 10 mM dNTPs, 5 µM of each primer (ITS1F and ITS4), one unit of the GoTaq® Green MasterMix 2X and MilliQ water using a thermal cycler Eppendorf Mastercycler (Eppendorf, Germany). The PCR program consisted of an initial denaturation at 95 ºC for 3 min, 36 cycles at 95 ºC for 1 min, 50 ºC for 1 min and 72 ºC for 1 min and final extension at 72 ºC for 7 min. The amplicons were analyzed by electrophoresis in a 1.2% agarose gel and sent to Macrogen, Korea, for DNA purification and sequencing.

The sequence electropherograms were analyzed using the *Geneious* 8.0.4 software. Sense and antisense DNA sequences were assembled in contiguous sequences, aligned, manually corrected and compared with sequences deposited in the NCBI (National Center for Biotechnological Information) and UNITE (Unified system for the DNA based fungal species) databases using the BLAST software with an algorithm for local alignment of nucleotide sequences (BLASTN). The fungal isolates were identified to the species or genus level by comparison of the obtained sequences with the sequences deposited in the databases based on the lowest e-value, highest query coverage and highest identity obtained. The ITS region sequences

obtained in the present study were deposited in GenBank with accession numbers between **MG751184** and **MG751298**.

Sequences presenting ≥ 98% similarity were considered to belong to the same OTU (Operational Taxon Unit) based on the ITS region, and sequences presenting < 98% similarity were considered to belong to different OTUs, even when they belonged to the same genus. These percentages were selected based on studies that showed intraspecific variation of 1.96% (standard deviation = 3.73) for Ascomycota, 3.33% (standard deviation = 5.62%) for Basidiomycota and 3.24% (standard deviation = 6.12%) for Zygomycota (Nilsson et al., 2009).

Phylogenetic analyses

The nucleotide sequences of the rDNA ITS region for each OTU found were aligned with reference sequences obtained from the databank using the MEGA6 software: Molecular Evolutionary Genetics Analysis Version 6.0 (Tamura et al., 2013).

The phylogenetic analysis was performed by Bayesian inference (BI) (Yang and Rannala, 1997) using the MrBayes 3.2.1 software (Huelsenbeck and Ronquist, 2001) . The best evolutionary models were previously selected based on the Akaike Information Criterion (AIC) using the MrModeltest v2.3 software (Nylander, 2004). The selected evolutionary models for the phylogenetic trees based on the ITS region were GTR+I+G for phylum Ascomycota, GTR+G for phylum Basidiomycota, and HKY+G for phylum Zygomycota.

Two independent runs with four Monte Carlo Markov Chains (MCMC) were run for 10 million generations, with trees sampled and retained every 1000 generations. The first one million trees were discarded as burn-in, and the remaining trees were summarized to generate a majority-rule consensus tree.

BI posterior probabilities were added to the respective branches of the trees inferred by BI. Posterior probabilities under 95% indicated low data reliability with low statistical support and were omitted (Harada et al., 1995).

Reference sequences of endophytic fungi representing each OTU analyzed in the present study were deposited in GenBank are represented in Table S2.

Diversity analysis

The diversity of the endophytic fungal species was measured using diversity indices using the species richness and relative abundance as parameters.

Prediction (extrapolation) and rarefaction (interpolation) models of the initial sample were used to compare the species richness and diversity of endophytic fungi isolated from samples with different sizes collected in the states of Acre (17 trees) and Amazonas (28 trees) and to compare the abundance of endophytic fungi isolated from different *H. brasiliensis* tissues (leaf, stem and root). Rarefaction and extrapolation with Hill numbers are empirical estimates that tend to be an increasing function of the sampling effort and are used to characterize taxonomic, phylogenetic or functional diversity (Chao et al., 2014).

Hill (1973) integrated species richness and species abundances into a class of measures currently called Hill numbers or effective number of species using the equation: ${}^{q}D = \left(\sum_{i=1}^{s} p_i^q\right)^{1/(1-q)}$, where S is the number of species (i) in the sample with relative abundance *p*i, i = 1, 2, ..., S, and *q* is the relative frequency. A *unified* sampling framework for extrapolation and rarefaction models of species richness (^{q}D, when *q* = 0) for *both individual*-based (*abundance*) and *sample*-based (*incidence*) *data was used* to extend measures of taxon diversity incorporating the relative abundance (for any ^{q}D, *q* > 0), with q = 1 for the exponential of Shannon's entropy index and q = 2 for the inverse of Simpson's concentration index.

The Shannon Wiener index evaluates species richness and abundance, with 0 indicating a community with only one species and a high value indicating communities with many species, each with few individuals (Hill, 1973). Simpson's diversity index estimates the probability that two individuals randomly selected from a community will belong to the same species (Simpson, 1949).

Analysis of the diversity indices and calculation of standard errors with 95% confidence intervals with 1000 bootstrap replicates were performed using the R 3.1.2 software.

Similarity analysis

A non-metric multidimensional analysis (nMDS) was performed to evaluate similarities between different tissues (root, stem and leaf) and between the states of Acre and Amazonas. Distances dissemilarity were measured using the Bray-Curtis index with the vegan package of the R software (Oksanen, 2015)

RESULTS

A total of 549 endophytic fungal were cultivated and purified from *H. brasiliensis*, of which 211 (133 from leaves, 42 from stems and 36 from roots) were from 17 trees from the state of Acre and 338 (207 from leaves, 69 from stems and 62 from roots) were from 28 trees from the state of Amazonas (Tables S3 and S4).

Ascomycota strains were predominant, accounting for 92.71% (509 endophytic fungi) of all isolates, in both states. Basidiomycota and Zygomyccota represented 1.82% (10 isolates) and 5.46% (30 isolates) of all of the endophytic fungal isolates, respectively.

The 549 endophytic filamentous fungal isolates were grouped into 115 OTUs (Tables S4, S5 and S6). Of the isolates belonging to Ascomycota, 62 OTUs belonged to class Sordariomycetes (405 isolates), 15 OTUs to class Dothideomycetes (38 isolates), 18 OTUs to class Eurotiomycetes (60 isolates) and 3 OTUs (six isolates) were not identified at the class level.

The DNA sequences of the isolates subjected to phylogenetic analysis by BI were grouped with sequences from type or reference isolates deposited in GenBank and UNITE belonging to Ascomycota (Fig. 1), Basidiomycota (Fig. S2) and Zygomycota (Fig. S3). A total of 107 (19.5%) isolates could not be classified at the genus level based on the UNITE database (Table S5). However, 88 of these isolates belonging to order Diaporthales and an isolate of OTU101 identified as Sordariomycetes were grouped in the phylogenetic tree with reference isolates of genus *Diaporthe* (Fig. 1).

The diversity of the endophytic fungi was higher in the stems and roots than in the leaves, although the richness was similar for all tissues (q0) (Table 1, Fig. 2, Fig. S4).

A richness of 115 OTUs was observed, but this value was extrapolated to 179.161 using an empirical estimate proposed by Chao et al. (2014) to compare species richness from samples with different sizes. Therefore, the estimated richness of the endophytic fungi was not significantly different, even though it was higher for the stems and roots than for the leaves. Moreover, the Shannon (q1) and Simpson (q2) diversity indices were higher for the stems and roots than for the leaves (Table 1, Fig. 2, Fig. S4).

For rubber trees from the state of Acre, the rarefaction and extrapolation empirical estimations revealed no significant differences in fungal richness (q0) or diversity (q1 and q2) between the leaves, stems and root cortexes for the 211 isolates (Table 1, Fig. 2, Fig. S4).

For rubber trees from the state of Amazonas, the estimated fungal richness (q0) and diversity (q1 and q2) were higher for the stems and roots than for the leaves (Table 1, Fig. 2, Fig. S4).

Despite the differences between the samples from Acre and Amazonas, no significant differences in the richness of endophytic fungi were observed between the two states. However, the diversity of the endophytic fungi was higher for the state of Acre than for the state of Amazonas (Table 1, Fig. 2, Fig. S4).

Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis distances between OTUs indicated a tendency for leaf isolates to group together and to present a distribution different from the stem and root cortex isolates ($r = 0.302$, $p < 0.001$). Many isolates from the two states shared the same OTU, and the NMDS plot of the OTUs showed a tendency for a homogenous distribution of OTUs between the two states ($r = 0.303$, $p < 0.001$) (Fig. 3).

Genus *Colletotrichum* (26%) was most abundant genus and predominated in both states. Fungi belonging genus *Diaporthe* (19%), *Fusarium* (9%), *Trichoderma* (6%), *Penicillium* (7%) and *Mucor* (4.7%) were also among the most represented in the different plant tissues. These genera also presented significant abundances of the total endophytic fungi for both states (Acre and Amazonas) (Fig. 4a and b).

Genus *Colletotrichum* constituted 28.57% of the total leaf isolates for the state of Acre and 50.72% for the state of Amazonas. Family Diaportaceae also constituted a significant percentage of the total leaf isolates from rubber trees from the states of Acre (39.09%) and Amazonas (23.67%). The genera *Fusarium* (8.69%), *Lasiodiplodia* (4.34%) and *Aspergillus* (3.38%) were also isolated from leaves in considerable amounts, albeit in lower proportions than *Colletotrichum* spp. and family Diaportaceae.

The genera *Trichoderma* and *Fusarium* presented high abundances in the stem cortex (Fig. 4b). *Trichoderma* constituted 14.28% and 17.39%, and *Fusarium* constituted 21.42% and 23.18% of the total endophytic fungi isolated from the stems of rubber trees from the states of Amazonas and Acre, respectively.

Penicillium was the third most abundant genus isolated and mainly colonized the stems and root cortexes of the rubber trees (Fig. 4a and b). Genus *Mucor* constituted 11.11% of the total root isolates for the state of Acre and 14.51% for the state of Amazonas.

However, different fungi from Ascomycota phylum were isolated with lower abundances, including *Purpureocillium, Hypoxylon*, *Aspergillus*, *Arthrinium*, *Curvularia*, *Nodulisporium*, *Lasiodiplodia*, *Alloconiothyrium*, *Talaromyces*, *Daldinia*, *Pilidiella*, *Pestalotiopsis*, *Chaetominum, Phoma*, *Nigrospora*, *Paradictyoarthrinium*, *Readeriella*, *Preussia*, *Simplicillium*, *Annulohypoxylon*, *Sarocladium*, *Spegazzinia*, *Wardomyces*, *Cladosporium*, *Neoscytalidium*, *Neosartorya* and *Torula*.

From Basidiomycota, the genera *Cylindrobasidium*, *Fomitopsis*, *Coprinellus*, *Trametes*, *Ganoderma*, *Bjerkandera*, *Phanerochaete* and *Phlebiopsis* were isolated, whereas only the genera *Umbelopsis*, *Syncephalastrum* and *Mucor* were isolated from Zygomycota phylum.

Differences in the endophytic fungal community profiles of *H. brasiliensis* were observed between the states of Acre and Amazonas, especially regarding the least abundant genera (Fig. 5). For example, *Spegazzinia*, *Fomitopsis*, *Phoma*, *Talaromyces* and *Cylindrobasidium* were some of the least abundant genera isolated from the leaves of rubber trees from the state of Acre, whereas *Lasiodiplodia*, *Pestalotiopsis*, *Phyllosticta* and *Wardomyces* were some of the least abundant genera from rubber trees from the state of Amazonas (Fig. 5).

The genera *Syncephalastrum*, *Umbelopsis*, *Neoscytalidium*, *Bjerkandera*, *Trametes*, *Talaromyces*, *Pestalatiopsis* and *Cladosporium* were isolated from the stem cortexes of rubber trees from the state of Amazonas, whereas the genera *Simplicillium*, *Purpureocillium*, *Pilidiella* and *Sarocladium* were isolated from the state of Acre (Fig. 5).

Phomopsis, *Ganoderma*, *Phanerochaete*, *Phoma*, *Talaromyces*, *Wardomyces*, *Syncephalastrum*, *Neosartorya* and *Cladosporium* were some of the least abundant genera in the roots from rubber trees from the state of Amazonas, whereas *Lasiodiplodia*, *Coprinellus*, *Aspergillus*, *Annulohypoxylon*, *Colletotrichum*, *Chaetominum* and *Torula* were some of the least abundant genera in the roots from rubber trees from the state of Acre. Notably, the genera *Arthrinium*, *Purpureocillium* and *Alloconiothyrium* were only isolated from the roots in both states (Fig. 5).

DISCUSSION

Fungal diversity of *H. brasiliensis* from the Brazilian Amazon forest was higher in the stems and roots than in the leaves, whereas the fungal abundance was higher in the leaves. Comparisons revealed different fungi genera distributions and abundances in different tissues, with a tendency for leaf isolates to group together. Conversely, homogeneous distributions were found for fungi isolated from the stems and roots and from different sampling sites.

The ITS region is considered a universal DNA barcode marker for fungi and is used for identification of endophytic fungal isolates at the genus level (Schoch et al., 2012). The use of ITS region sequences enabled the identification of a high number of isolates obtained at the genus level, and diversity was easily analyzed for a large variety of different fungal species.

The estimated diversity of endophytic fungi can be affected by the criteria and methods used for species identification. Species diversity in a community of endophytic fungi is estimated by counting the number of different species or by determining OTUs (Angelini et al., 2012; Chaverri et al., 2011; Gazis and Chaverri, 2010; Martins et al., 2016). A global value of intraspecific variation between 0 and 3% has been recommended, because the ITS region does not

vary equally for all fungal genera, and this variation is not easily correlated with the identity between ITS sequences within the same species (Nilsson et al., 2009). Therefore, sequences with similarities higher than or equal to 98% were considered to belong to the same OTU in the present study.

The endophytic fungal community was composed of 44 genera. However, 107 isolates (19.49%) were not identified at the genus level based on comparisons with fungal sequences deposited in the UNITE database (Tables S2, S5 and S6). These isolates were grouped close to the reference isolate sequences deposited in the UNITE and GenBank databases in the same classes, orders and families (Fig. 1). Thus, these isolates may represent new species, and further multigenic and taxonomic analyses are needed for their precise identification.

The observed dominance of Ascomycota (92.71%) relative to Basidiomycota (1.82%) and Zygomycota (5.46%) in the endophytic fungal community of *H*. *brasiliensis* from Brazil was also observed for *H*. *brasiliensis* from Peru (Ascomycota 96.6%, Basidiomycota 1.1% and Zygomycota 2.3%) (Gazis and Chaverri, 2010). A high proportion of Ascomycota was also observed in other studies of endophytic diversity (both culturable and non-culturable) from different microbiomes (Coleman-Derr et al., 2016; Leite et al., 2013; Fernandes et al., 2015; Hanada et al., 2010; Martins et al., 2016; Zhang and Yao, 2015).

Although the estimated richness (q0) of endophytic fungi was not significantly different between the leaves, stems and root cortexes, fungal diversity (Shannon and Simpson) was higher in the stems and root cortexes than in leaves. The high diversity of endophytic fungi in the stem and roots cortexes is due to the high equitability in the distribution of these microorganisms in these tissues.

For the state of Amazonas, the estimated richness and diversity of the endophytic fungi was higher for the stems and root cortexes than for the leaves. However, no significant differences in the estimated richness and diversity of endophytic fungi were observed for the state of Acre, which had a smaller sample size than the state of Amazonas (Table 1, Fig. 2, Fig. S4), and we suppose that interfered with the estimation of species richness and diversity.

In the present study, the interpolation (rarefaction) and extrapolation (prediction) models proposed by Chao et al. (2014), which allow the comparison

of samples with different sizes, were used in the Hill number analysis of the Shannon and Simpson richness and diversity indices. The diversity of endophytic fungi was higher in the stems and root cortexes, although the frequency of colonization was higher in the leaves. In addition, NMDS based on the Bray-Curtis distances between OTUs showed a tendency for leaf isolates to group together and separate from the stem and root cortex isolates (Fig. 3).

The geographical location, forest structure, climate conditions, tissue chemical composition, interspecific competition between fungi and occurrence of diseases can affect the microbial community distribution and abundance (Gazis and Chaverri, 2010; Martins et al., 2016; Suryanarayanan and Vijaykrishna, 2001). In the present study, the clustering analysis based on the Bray-Curtis similarity measure indicated differences in the distribution and abundance of endophytic fungal genera between different tissues (leaf, stem and root cortex) of rubber trees from Brazil. This finding supports the hypothesis that the medium in which the organs grow (soil or air) and the type of host tissue affect the fungal community (Martins et al., 2016; Suryanarayanan and Vijaykrishna, 2001; Wearn et al., 2012).

Martins et al. (2016) observed that the endophytic fungi colonizing leaves and branches of olive trees formed a narrow group that was clearly separated from the endophytic fungi isolated from the roots and stated that the virtual absence of these endophytes from olive tree organs aboveground should be due to their inability to travel to other plant tissues. However, this scenario was not observed in the present study.

Clustering analysis of the endophytic fungal community showed that the endophytic fungi isolated from leaves grouped together, whereas the distribution of the stem and root isolates was more homogeneous. This finding indicates systemic growth of endophytic fungi from the roots into the stem, with the soil serving as an important inoculum source. However, differences in the endophytic fungal community were not observed between different sampling sites in the states of Acre and Amazonas (Fig. 3), indicating that the geographical location of the host plant did not affect the endophytic fungal communities of the rubber trees. Although higher diversity of endophytic fungi was observed for the state of Acre than for Amazonas, both states presented similar species richness (Table 1, Fig. 2, Fig. S4).

In several studies of the diversity of endophytic fungi in tropical plants, including the present study of rubber trees from Brazil, *Colletotrichum* was identified as a dominant genus (Leite et al., 2013; Fernandes et al., 2015; Ferreira et al., 2017, 2015, Rojas-Jimenez et al., 2016). This result was in contrast to the findings of Gazis and Chaverri (2010), who characterized the endophytic fungi of rubber trees from the Peruvian Amazon forest and observed a low frequency of *Colletotrichum.* This discrepancy may be explained by the different locations of the sampling sites and the different isolation methods used, including different culture media, which may have resulted in different endophytic community profiles for rubber trees from Peru and Brazil.

Regarding the distribution and abundance of endophytic fungal isolates in the present study, the genera *Colletotrichum*, *Penicillium*, *Trichoderma*, *Aspergillus* and *Fusarium*, isolates of family Diaportaceae and *Mucor* from family Mucoraceae were the most abundant but presented different frequencies in different host tissues (Fig. 4, Table S4). In contrast, Gazis and Chaverri (2010) observed that the most frequent genera in rubber trees from Peru were *Penicillium*, *Pestalotiopsis* and *Trichoderma* and that genera *Alternaria*, *Annulohypoxylon*, *Cladosporium*, *Cochiobolus*, *Endomelanconiopsis*, *Entonaema*, *Epicoccum*, *Fusarium, Guignardia*, *Leptosphaerulina*, *Khuskia*, *Umbelopsis* and *Colletotrichum* were rare, with frequencies ranging between 1% and 3%. We again suggest that this discrepancy shows variation between the endophytic fungal communities of rubber trees from the Amazon forests of Brazil and Peru.

Rocha et al. (2011) isolated a total of 435 endophytic fungi from leaves of three *H. brasiliensis* cultivars so far from its center of origin. These cultivars present different levels of resistance to dieases. The isolates identified were *Fusarium* sp./*Giberella* sp., *Glomerella* sp./*Colletotrichum* sp., *Microspheropsis* sp./*Paraphaeosphaeria* sp., *Myrothecium* sp., *Pestalotiopsis* sp. and *Diaporthe* sp. Of these genera, *Microsphaeropsis* sp., *Pestalotiopsis* and *Myrothecium* sp. were isolated from pathogen-resistant cultivars.

In our study, the genera *Pestalotiopsis*, *Hypoxylon*, *Curvularia*, *Lasiodiploidia*, *Purpureocillium*, *Fomitopsis*, *Spegazzinia*, *Cylindrobasidium*, *Simplicillium*, *Pilidiella*, *Sarocladium*, *Coprinellus*, *Annulohypoxylon*, *Chaetominum*, *Ganoderma*, *Phanerochaete*, *Talaromyces*, *Wardomyces*,

Syncephalastrum, *Neosartorya*, *Cladosporium*, *Trametes*, *Umbelopsis*, *Phyllosticta* and *Bjerkandera* presented frequencies lower than 7% and were considered rare. These genera were also observed with lower frequencies in other studies of the diversity of tropical endophytic fungi (Leite et al., 2013; Fernandes et al., 2015; Ferreira et al., 2017, 2015).

Most of the endophytic fungi identified in the present study are potentially know as mutualistic species and may be tested in the future study as biological control agents of different pathogens and as promising sources of bioactive natural products.

Rubber tree plantations are attacked by different pathogens, including *P. ulei* (Hora Júnior et al., 2014), *C. gloeosporioides*, *C. acutatum*, *O. hevea* and *Phytophthora* spp. (Gasparotto et al., 2012), which are responsible for losses in natural rubber production. Rocha et al. (2011) observed that 30 isolates from rubber trees cultivars, far from its center of origin, belonging to *Fusarium* sp., *Gibberella* sp., *Glomerella cingulata*, *Microsphaeropsis* sp., *Myrothecium* sp., *Pestalotiopsis* sp. and *Phomopsis* sp. inhibited the germination of *P. ulei* conidia by 80%. In addition, several studies confirmed the biotechnological potential of the dominant endophytic fungal genera observed in the present study.

Trichoderma spp. is a biocontrol agent of *Drechslera tritici repentis* and an important component of integrated management of wheat tan spot disease (Larran et al., 2016) and the nematode *Globodera pallida* in potato plantations (Contina et al., 2017). Furthermore, isolates from genus *Trichoderma* act as control agents of root rot caused by *Fusarium solani* in olive trees (Ben Amira et al., 2017). Application of a *Trichoderma*-based formulation significantly decreased the incidence of *Phytophthora megakarya*, which is a pathogen of cocoa plants, in Cameroon, Africa (Mbarga et al., 2014), and of *Colletotrichum gloeosporioides*, which causes anthracnose in papayas (Landero Valenzuela et al., 2015).

Trichoderma and *Aspergillus* species inhibited the growth of wood-decomposing fungi (Tiwari et al., 2011), and *Penicillium* species promoted plant growth by acting on flora conservation, reforestation and growth of agriculturally important crops (Khan et al., 2008; Murali and Amruthesh, 2015). Isolates from these genera are great producers of antimicrobial compounds of pharmacological interest (Houbraken et al., 2012) and enzymes, such as pectinases, with high

applicability to industrial processes, especially in the food and textile industries (Banu et al., 2010; Rangarajan et al., 2010). *Penicillium* spp. isolates may act as biological control agents of plant pathogens, especially through the production of antimicrobial compounds and extracellular lytic enzymes (Babu et al., 2015; Guijarro et al., 2017; Khan et al., 2008; Murali and Amruthesh, 2015).

Many endophytic fungal genera presenting frequencies lower than 7% in the present study were previously described as important for agro-industry. For example, isolates from genus *Lasiodiplodia* were reported to have antifungal activity against *Blumeria graminis* f. sp. *Tritici* (Xiang et al., 2016), *Chaetomium* and *Aspergillus* species effectively suppressed leaf infection by *Sclerotinia sclerotium* in *Brassica napus* (rape), *Alternaria*, *Arthrinium* and *Fusarium* species produced volatile compounds that inhibited the growth of *S. sclerotiorum; Alternaria* and *Fusarium* species promoted the growth of *B. napus* (Zhang et al., 2014)and isolates of genus *Cladosporium* decreased the severity of tan spot disease caused by *D*. *tritici repentis* (Larran et al., 2016).

Purpureocillium lilacinum is entomopathogenic endophytic fungi that can control the cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae) (Castillo Lopez et al., 2014). In addition, strains of *P. lilacinum* and *Pochonia chlamydosporia* decreased gall formation and the egg masses of *Meloidogyne enterolobii* adhering to the roots of tomato and banana seedlings, thereby enabling the management of *M. enterolobii* populations under some conditions as long as the levels of soil infestation were low (Ilva et al., 2017). *Talaromyces flavus* can be used in potato management as a biological control agent of the disease caused by *Verticillium albo-atrum* (Naraghi et al., 2010).

In addition to endophytic fungal species of agro-industrial interest, isolates of *Pestalotiopsis microspora* are considered environmental bioremediators, because they can grow in medium with polyurethane polyester polymer by using this compound as a single carbon source under aerobic and anaerobic conditions, resulting in its degradation (Russell et al., 2011).

The diversity of this fungal community described in this study was higher in the stems and roots than in the leaves, whereas the fungal abundance was higher in the leaves. There is a difference in the abundance and in the distribution of fungal genera within the tissues of *H. brasiliensis*, with a tendency for leaf isolates to group together. Conversely, homogeneous distributions were found

for fungi isolated from the stems and roots and from different sampling sites. New studies are necessaries to the investigate the systemic growth of endophytic fungi from the roots into the stem, with the soil serving as an important inoculum source.

The microorganisms isolated and identified in the present study will enable new studies on the detection of producers of metabolites with desirable bioactive properties for biological control, plant growth promotion and use in different branches of industry, especially the increase of sustainable agribusiness. In addition, new species of endophytic fungi may be described through multilocus phylogenetic and taxonomic analyses.

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ATTACHMENTS

Fig. 1. Phylogenetic tree obtained by Bayesian Influence (BI) using sequences from the ITS region of the rDNA of the 95 Operational Taxonomic Units (OTUs) that grouped a total of 509 endophytic fungi pertaining to the phylum Ascomycota. The posteriori probability values below 95% were omitted.

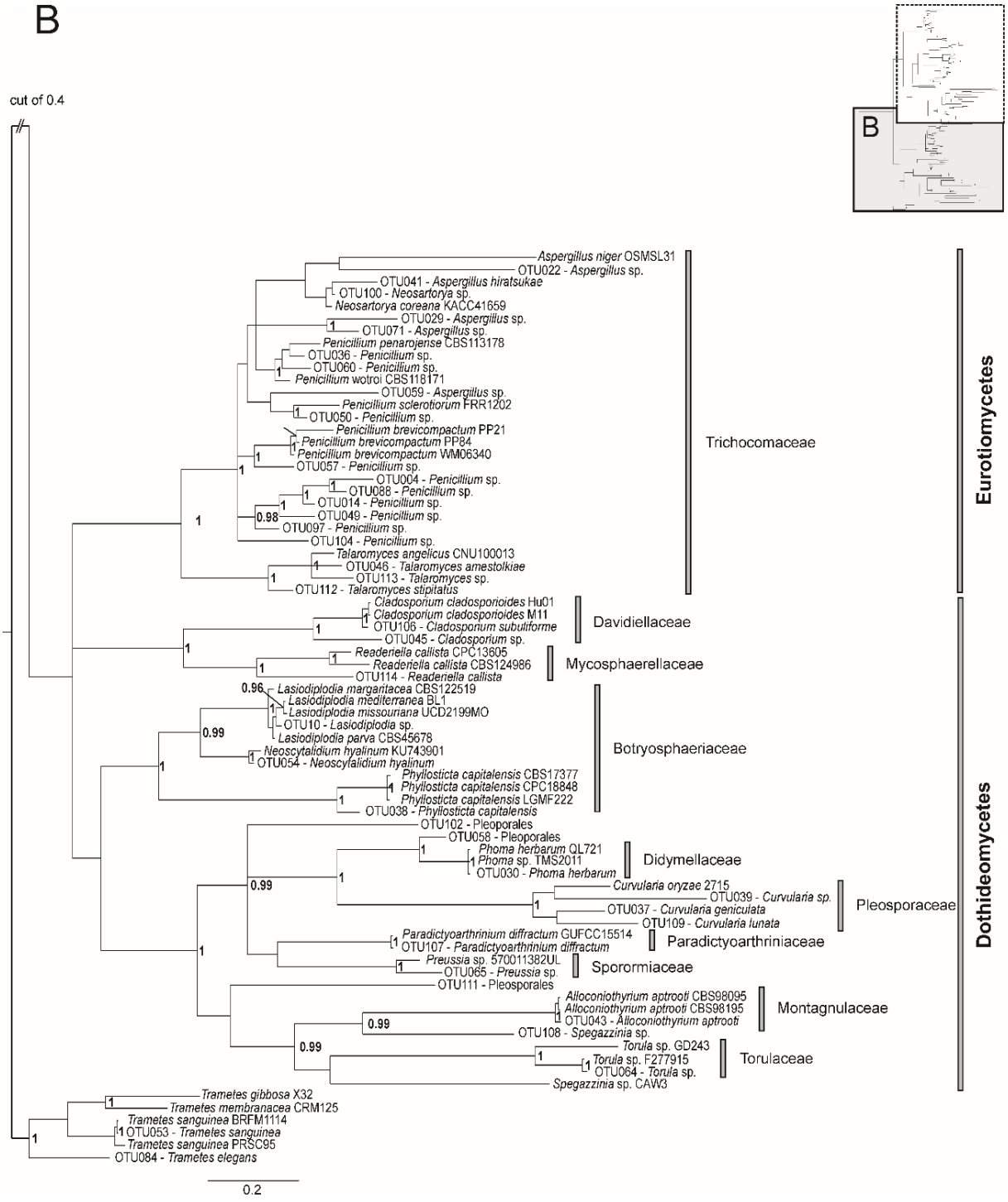

Fig. 1. Phylogenetic tree obtained by Bayesian Influence (BI) using sequences from the ITS region of the rDNA of the 95 Operational Taxonomic Units (OTUs) that grouped a total of 509 endophytic fungi pertaining to the phylum Ascomycota. The posteriori probability values below 95% were omitted.

Table 1. Comparison of the asymptotic estimation of richness (q0), exponential Shannon index (q1) and inverse of the Simpson concentration index (q2) of the endophytic fungi between the different niches of Brazilian rubber trees and between states Acre and Amazonas, uses an approach from diversity Hill numbers with 95% confidence interval [(*)].

Samples	Tissues	Richness (q0)	Shannon (q1)	Simpsom (q2)
Diversity analysis by tissue				
AC + AM	Leaf	46.778 ± 0.871 (A)	17.090 ± 0.912 (A)	6.129 ± 0.912 (A)
	Stem	57.789 ± 0.895 (A)	38.792 ± 0.966 (B)	19.074 ± 0.966 (B)
	Root	74.594 ± 0.777 (A)	53.240 ± 0.872 (B)	27.082 ± 0.872 (B)
AC	Leaf	30.118 ± 0.748 (A)	22.307 ± 0.820 (A)	11.993 ± 0.820 (A)
	Stem	29.737 ± 0.805 (A)	24.364 ± 0.932 (A)	13.657 ± 0.932 (A)
	Root	28.628 ± 0.834 (A)	26.054 ± 0.948 (A)	17.745 ± 0.948 (A)
AM	Leaf	27.175 ± 0.858 (A)	9.551 ± 0.928 (A)	3.958 ± 0.928 (A)
	Stem	41.285 ± 0.862 (AB)	30.963 ± 0.941 (B)	18.361 ± 0.941 (B)
	Root	61.252 ± 0.664 (B)	48.967 ± 0.772 (B)	21.071 ± 0.772 (A)
Diversity analysis by state				
Acre	-	92.633 ±0.922 (A)	45.140 ±0.922 (B)	23.022 ±0.922 (B)
Amazonas	-	86.509 ±0.909 (A)	27.407 ±0.909 (A)	9.123 ±0.909 (A)

[(*)] Means followed by equal letters in the column do not differ by program R version 3. 1. 2. with bootstrap of 1000 replicates, 5% probability.

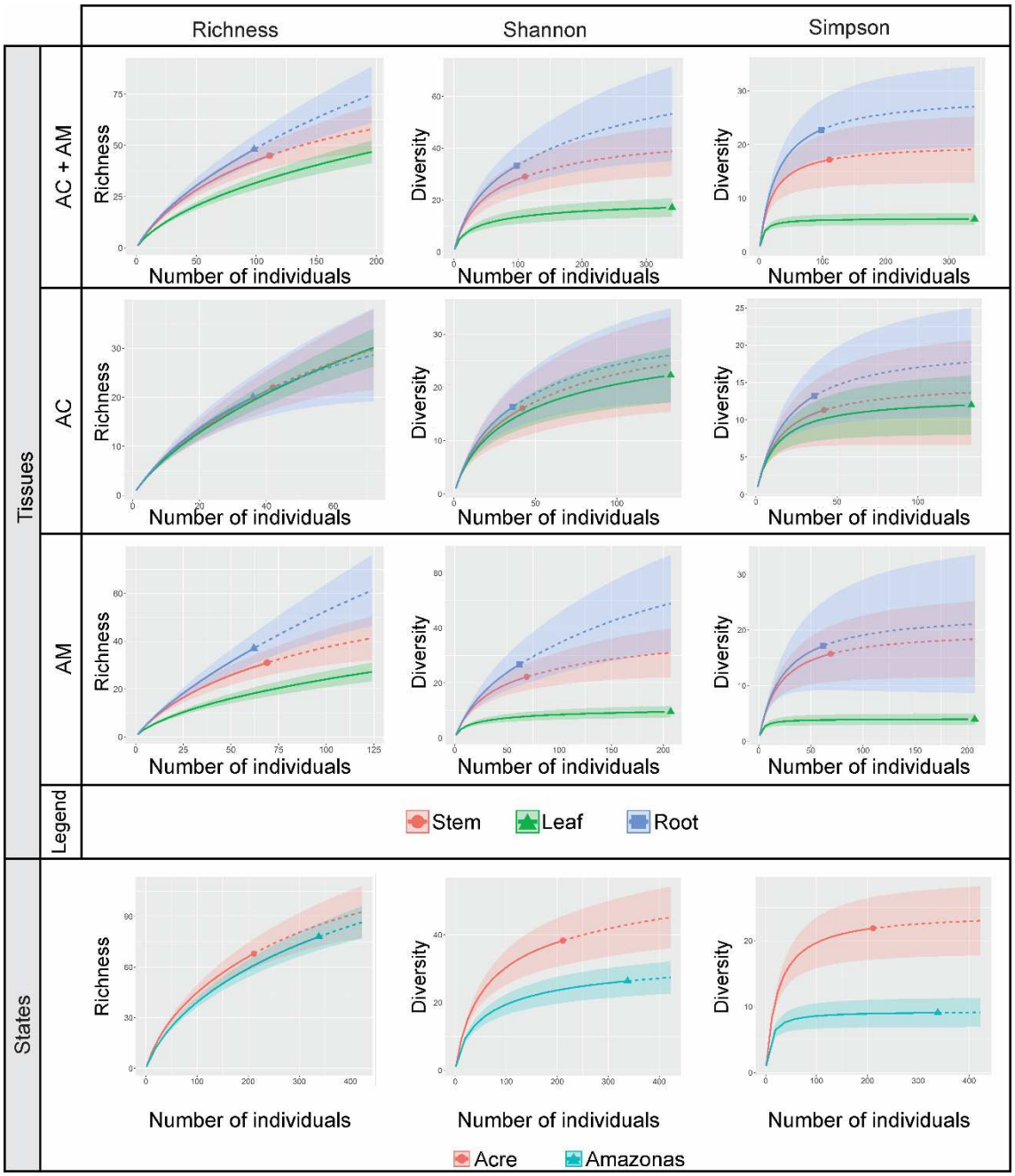

Fig. 2. Curve of rarefaction (solid line) and extrapolation (dashed line) to twice the size of the reference sample. Comparison of species richness (q=0), Shannon Wiener's exponential entropy (q = 1), the inverse concentration of Simpson (q = 2) according Hill numbers, as tripod of diversity of species, of endophytic fungi in the different niches: stem (red line), leaf (green line), root (blue line) of *Hevea brasiliensis* independent of state, and in states of Acre and Amazonas, and between the states of Acre (red line) and the state of Amazonas (blue line), with 95% confidence intervals obtained by the bootstrap method with 200 replications.

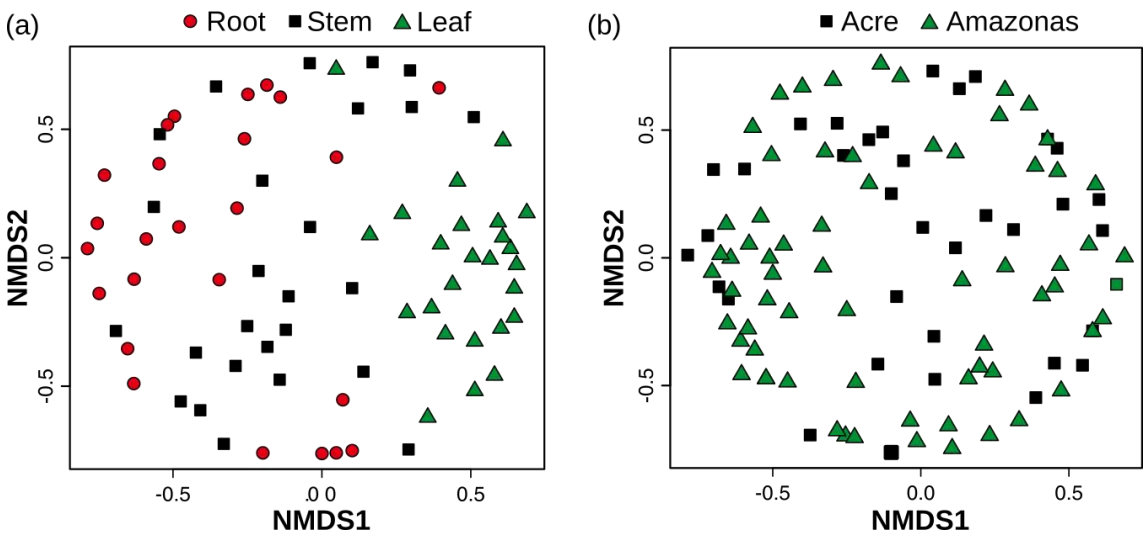

Fig. 3. Multidimensional non-metric (NMDS) scaling based on Bray-Curtis distance between fungal samples obtained from different (a) tissues and (b) regions (states) of *Hevea brasiliensis*.

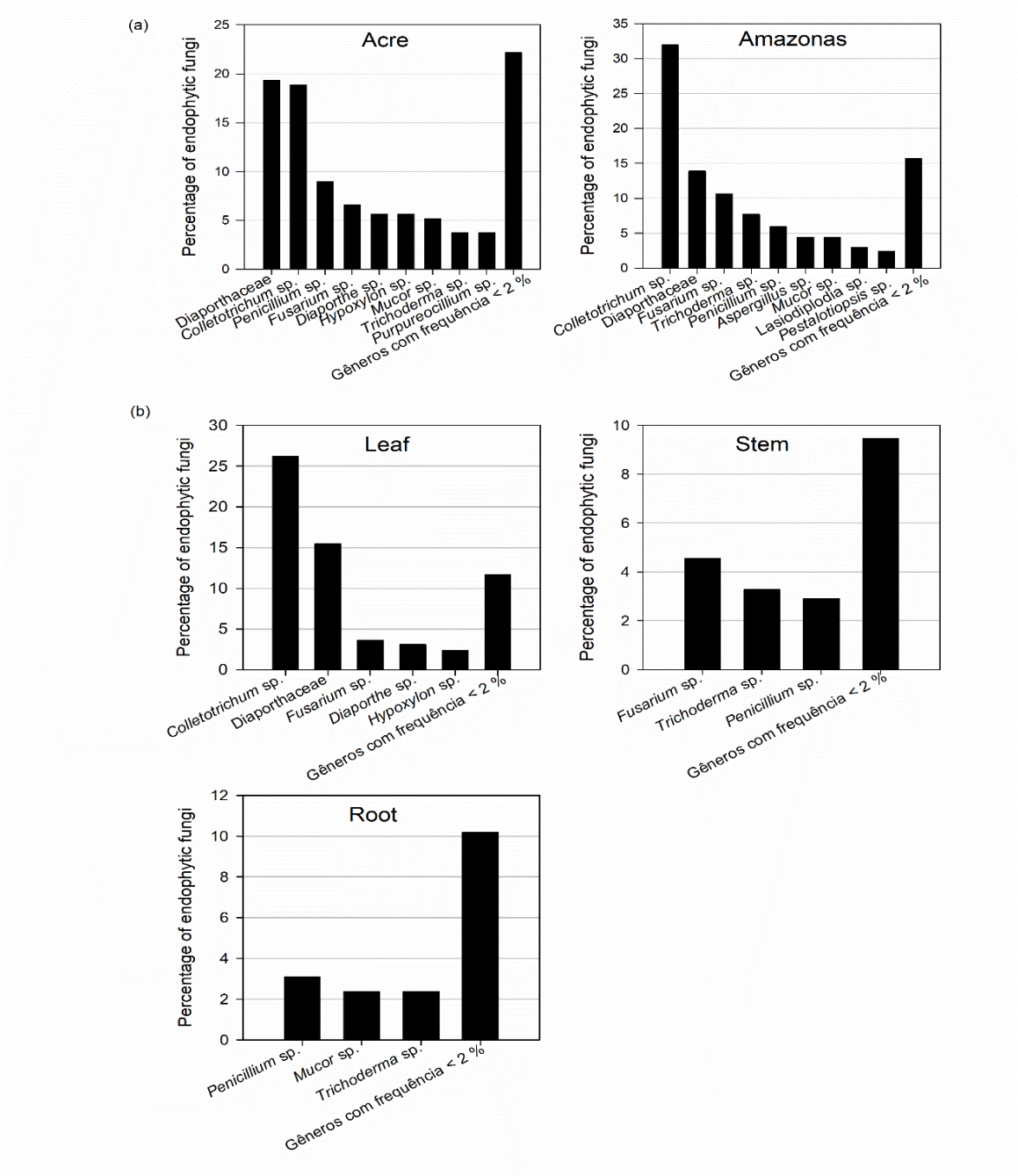

Fig. 4. Percentage of genera of endophytic fungi isolated from *Hevea brasiliensis* from different (a) host states and (b) tissues. Only genres with a percentage equal to or greater than 2% are shown.

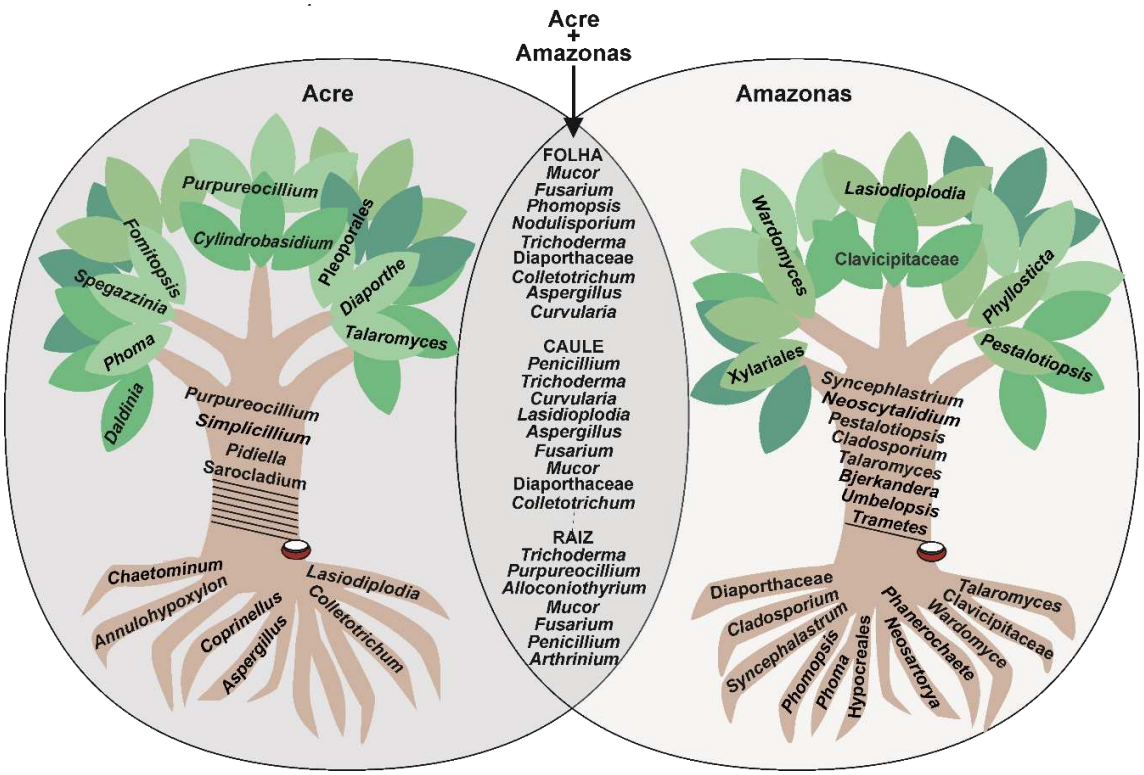

Fig. 5. Venn diagram in which the sets contain the genus of endophytic fungi of *Hevea brasiliensis* present in the different tissues found exclusively in the states of Acre (Acre) or in the state of Amazonas (Amazonas). In intersection are the genera of the fungi isolated from the rubber trees of both the states (Acre + Amazonas).

SUPPLEMENTARY MATERIAL

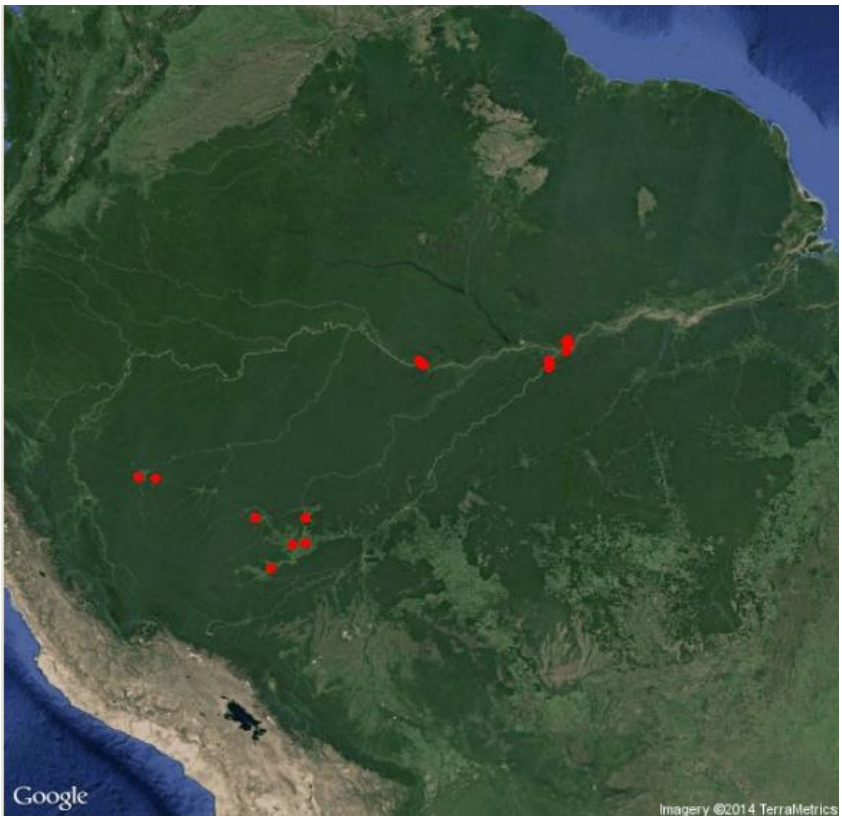

Fig. S1. Location of collection regions located in native forest in the states of Acre and Amazonas, Brazil. The collection sites are marked in red.

Table S1. Geographic coordinates of collection points in the states of Acre (AC) and Amazonas (AM).

Place	Latitude	Longitude
Bonal (AC)	09°57'03.5" S	67°13'21.4" W
Xapuri – Reserva Chico Mendes(AC)	10°49'59" S	68°23'11.2" W
Reserva embrapa (AC)	10°02'16.7" S	67°40'45.4" W
Boca do Acre (AC)	09°07'34.9" S	67°14'57.1" W
Cruzeiro do Sul – Iguarapé Campinas (AC)	07°45"50.9" S	72°15'07.5" W
Cruzeiro do Sul - (AC)	07°44'05,3" S	72°49'46,8" W
Cazumbá - Sena Madureira (AC)	09°08'01.7" S	68°57'04.7" W
Itacoatiara - Terra firme (AM)	03°02'37,6" S	58°30'11,8" W
Itacoatiara - Terra firme (AM)	03°00'09" S	58°27'06.1" W
Itacoatiara - Rio Arari (AM)	03°24'34.00" S	58°29'18.3" W
Itacoatiara - Rio Arari (AM)	03°08'58.9" S	58°22'48.3" W
Itacoatiara - Bosque (AM)	03°08'58.9" S	58°26'16.8" W

Nova Olinda do Norte - Santa Luzia (AM)	03°53'55.0" S	50°05'53.6" W
Nova Olinda do Norte - Rio Madeira (AM)	03°57'08.3" S	59°05'16.8" W
Nova Olinda do Norte - Rio Madeira (AM)	03°50'49.9" S	59°03'02.7" W
Nova Olinda do Norte - Rio Madeira (AM)	03°43'31.9" S	59°04'15.3" W
Coari - Rio Copeá (AM)	03°53'07.6" S	63°17'18.6" W
Coari - Rio Copeá (AM)	03°52'52.7" S	63°17'59.7" W
Coari - Rio Copeá (AM)	03°50'44.1" S	63°20'29.4" W
Coari - Rio Copeá (AM)	03°48'08.8" S	63°21'45.5" W
Coari - Rio Copeá (AM)	03°45'52.7" S	63°23'51.9" W
Coari - Rio Copeá (AM)	03°43'56.1" S	63°26'59.8" W

Table S2. Access number of the sequences of the reference isolates deposited in GenBank.

Closest species in GenBank	Isolates	Accession nº	Closest species in GenBank	Isolates	Accession nº
Diaphorte helianthi	A1	AJ312356.1	*Cladosporium cladosporioides*	Hu01	EF405864
Diaporthe phaseolorum	E99382	AY577815.1	*Cladosporium cladosporioides*	M11	HM595523
Phomopsis sp.	GJS83377	AF102999.1	*Readeriella callista*	CPC13605	KF901541
Phomopsis sp.	ZH5C1	FJ037753.1	*Readeriella callista*	CBS124986	KF442522
Coniella wangiensis	CBS1322539	NR111764.1	*Lasiodiplodia margaritacea*	CBS122519	KT852959
Purpureocillium lilacinum	CBS28436	MF975787.1	*Lasiodiplodia mediterranea*	BL1	KJ638312
Fusarium equiseti	Xsd08114	FJ481025	*Lasiodiplodia missouriana*	UCD2199MO	HQ288226
Fusarium polyphialidicum	CM7	AY745991.1	*Lasiodiplodia parva*	CBS45678	KF766192
Fusarium proliferatum	BLE1	FN868470.1	*Neoscytalidium hyalinum*	--------	KU743901
Fusarium proliferatum	CBS122158	DQ655730	*Phyllosticta capitalensis*	CBS17377	KF206179
Trichoderma koningiopsis	GJS04199	FJ442654	*Phyllosticta capitalensis*	CPC18848	KF206255
Trichoderma ovalisporum	DIS172i	DQ323438	*Phyllosticta capitalensis*	LGMF222	KF206204
Trichoderma sp.	GS2014a	FJ884177.1	*Phoma herbarum*	QL721	AB369456.1
Colletotrichum boninense	CBS123755	JQ005153	*Phoma sp.*	TMS2011	HQ630963.1
Colletotrichum gloesporioides	CBS112999	JQ005152	*Curvularia oryzae*	2715	EU27519
Colletotrichum gloesporioides	PP143	FJ884081.1	*Paradictyoarthrinium diffractum*	GUFCC15514	JN128869
Colletotrichum sp.	PP9	FJ884085.1	*Preussia sp.*	570011382UL	FJ210521

Colletotrichum cliviae	CBS125375	NR_137097	*Alloconiothyrium aptrooti*	CBS98095	JX496121
Simplicillium Cylindrosporum	JCM18171	AB603997	*Alloconiothyrium aptrooti*	CBS98195	JX496122
Chaetomium globosum	CBS33267	KX976570.1	*Torula sp.*	GD243	MF034135.1
Annulohypoxylon moriforme	CBS123579	KU683751	*Torula sp.*	F277915	KC427082.1
Hypoxylon gibriacense	MUCL52698	NR_137100	*Spegazzinia sp.*	CAW3	KY073418
Hypoxylon samuelsii	MUCL51843	KC968916	*Cylindrobasidium laeve*	AFTOLID453	DQ205682
Nodulisporium hinnuleum	CBS28662	NR_145212	*Coprinellus disseminatus*	B1b0855P302	JQ922135.1
Daldinia sp.	PP29b	FJ884087	*Coprinellus sp.*	INBio3713C	HM771019
Hypoxylon haematostroma	MUCL53301	NR_144918	*Fomitopsis sp.*	7R81	FJ372676
Arthrinium phaeospermum	XSD132	EU326200	*Trametes gibbosa*	X32	KC176329.1
Nigrospora sphaerica	KP10050	KX834821	*Trametes membranacea*	CRM125	JN164956.1
Nigrospora sphaerica	AWB1017G	KX833088	*Trametes sanguinea*	BRFM1114	JX082366.1
Pestalotiopsis palmarum	PP98	FJ884144	*Trametes sanguinea*	PRSC95	JN164982.1
Pestalotiopsis palmarum	PP99	FJ884145.1	*Phanerochaete sordida*	UASWS0827	JX139732.1
Pestalotiopsis microspora	29SES	EF451800	*Phanerochaete sp.*	AX95	KC507268.1
Wardomyces moseri	CBS16480	LN850995.1	*Phlebiopsis flavidoalba*	CFMR2762	KX065956
Aspergillus niger	OSMSL31	KR017026.1	*Phlebiopsis flavidoalba*	CFMR4167	KX065957
Neosartorya coreana	KACC41659	AB299414.1	*Bjerkandera adusta*	X04	KC176308.1
Penicillium penarojense	CBS113178	NR_138289	*Bjerkandera adusta*	X42	KC17633.1
Penicillium wotroi	CBS118171	NR_119813	*Bjerkandera adusta*	X53	KC176339.1
Penicillium sclerotorum	FRR1202	AY373931.1	*Bjerkandera adusta*	X75	KC176354.1

Penicillium brevicompactum	PP21	FJ884116	*Syncephalastrum monosporum*	CBS56791	NR_138374.1
Penicillium brevicompactum	PP84	FJ884117.1	*Ganoderma lucidum*	AAU_V	KM375927.1
Penicillium brevicompactum	WM06340	EF568063	*Ganoderma* sp.	BAB-4928	KR349640.1
Talaromyces angelicus	CNU100013	KF183638	*Ganoderma* sp.	BAB-4982	KR349638.1
Mucor luteus	CBS24335	NR_120224.1	*Ganoderma* sp.	BAB-4984	KR349639.1
Umbelopsis autotrophica	CBS31093	KC489480.1			

Table S3. Number total of endophytic fungi recovered from *Hevea brasiliensis* tissues of the Brazilian Amazon forest.

State	Number of endophytic fungi of *Hevea brasiliensis*		
	Leaf	Stem	Root
Acre	133	42	36
Amazonas	207	69	62
Total	**549**		

Table S4. OTUs and number of endophytic fungi isolated from leaf, stem and roots of rubber tree of the Amazon forest in the states of Acre and Amazonas.

OTUs	Number of isolated of *Hevea brasiliense* of Acre			Number of isolated of *Hevea brasiliense* of Amazonas		
	Leaf	**Stem**	**Roots**	**Leaf**	**Stem**	**Roots**
OTU001 - *Colletotrichum* sp.	29	0	1	100	3	0
OTU002 - *Fusarium oxysporum*	2	9	2	15	11	1
OTU003 – Diaporthaceae	18	1	0	9	1	0
OTU004 - *Penicillium* sp.	2	0	6	2	5	2
OTU005 – Diaporthaceae	7	0	0	14	0	1
OTU006 - *Trichoderma* sp.	1	2	0	1	8	1
OTU007 - *Purpureocillium lilacinum*	1	4	3	0	0	5
OTU008 – Diaporthaceae	3	0	0	12	0	0
OTU009 - *Mucor irregulares*	2	1	4	2	2	3
OTU010 - *Lasiodiplodia* sp.	0	1	1	9	1	0
OTU011 -*Trichoderma brevicompactum*	0	0	0	0	0	11
OTU012 - *Phomopsis* sp.	8	0	0	2	0	0
OTU013 - *Pestalotiopsis mangiferae*	0	0	0	2	3	1
OTU014 - *Penicillium* sp.	0	5	2	0	1	1
OTU015 - *Mucor* sp.	2	0	0	0	1	4
OTU016 - *Colletotrichum* sp.	5	0	0	2	0	0
OTU017 - *Colletotrichum* sp.	1	0	0	1	0	0
OTU018 – Diaporthaceae	3	0	0	3	0	0
OTU019 - *Hypoxylon anthochroum*	6	0	0	0	0	0
OTU020 – Diaporthaceae	5	0	0	1	0	0
OTU021 - *Daldinia eschscholtzii*	3	0	0	0	0	0
OTU022 - *Aspergillus* sp.	0	0	1	1	4	0
OTU023 – Ascomycota	2	0	2	0	1	1
OTU024 - *Mucor* sp.	1	1	0	1	0	2
OTU025 - *Trichoderma harzianum*	0	1	1	0	3	0
OTU026 – Diaporthaceae	4	0	0	0	0	0
OTU027 - *Fusarium solani*	0	0	1	0	3	0
OTU028 - *Colletotrichum* sp.	2	0	0	1	0	0
OTU029 - *Aspergillus* sp.	0	0	0	4	0	0
OTU030 - *Phoma herbarum*	1	0	0	0	0	3

OTU031 - *Wardomyces moseri*	0	0	0	2	1	1
OTU032 - *Fusarium proliferatum*	0	0	0	3	0	1
OTU033 - *Arthrinium malaysianum*	0	0	3	0	0	1
OTU034 - *Hypoxylon sublenormandii*	4	0	0	0	0	0
OTU035 – Diaporthaceae	0	0	0	3	0	0
OTU036 - *Penicillium* sp.	1	0	0	0	0	2
OTU037 - *Curvularia geniculata*	0	2	0	0	1	0
OTU038 - *Phyllosticta capitalensis*	0	0	0	1	0	0
OTU039 - *Curvularia* sp.	1	1	0	0	0	0
OTU040 - *Nodulisporium* sp.	1	0	0	2	0	0
OTU041 - *Aspergillus hiratsukae*	0	0	0	2	0	1
OTU042 - *Chaetomium globosum*	0	0	1	0	0	0
OTU043 - *Alloconiothyrium aptrootii*	0	0	1	0	0	1
OTU044 - *Pestalotiopsis* sp.	0	0	0	0	2	0
OTU045 - *Cladosporium* sp.	0	0	0	0	0	2
OTU046 - *Talaromyces amestolkiae*	1	0	0	0	0	1
OTU047 - Clavicipitaceae	0	0	0	1	0	1
OTU048 – Diaporthaceae	0	0	0	2	0	0
OTU049 - *Penicillium* sp.	1	0	0	0	0	0
OTU050 - *Penicillium* sp.	0	2	0	0	0	0
OTU051 - *Nigrospora sphaerica*	0	2	0	0	0	0
OTU052 - *Phlebiopsis flavidoalba*	0	0	0	0	1	0
OTU053 - *Trametes sanguínea*	0	0	0	0	2	0
OTU054 - *Neoscytalidium hyalinum*	0	0	0	0	2	0
OTU055 - *Hypoxylon* sp.	2	0	0	0	0	0
OTU056 – Hypocreales	0	0	1	0	0	1
OTU057 - *Penicillium* sp.	0	0	0	0	2	0
OTU058 – Pleosporales	1	1	0	0	0	0
OTU059 -*Aspergillus* sp.	0	0	0	0	2	0
OTU060 - *Penicillium* sp.	0	0	0	0	1	1
OTU061 - *Diaporthe* sp.	0	0	0	2	0	0
OTU062 - *Trichoderma* sp.	0	2	0	0	0	0
OTU063 - *Pilidiella wangiensis*	0	1	0	0	0	0
OTU064 – Chaetomiaceae	0	0	2	0	0	0
OTU065 - *Preussia* sp.	1	0	0	0	0	0
OTU066 - *Syncephalastrum* sp.	0	0	0	0	0	1
OTU067 - *Diaporthe eucalyptorum*	1	0	0	0	0	0
OTU068 - *Fusarium* sp.	0	0	0	0	1	0
OTU069 - *Daldinia bambusicola*	1	0	0	0	0	0
OTU070 - *Phomopsis* sp.	1	0	0	0	0	0
OTU071 - *Aspergillus* sp.	0	0	0	0	0	1
OTU072 - *Trichoderma* sp.	0	1	0	0	0	0
OTU073 – Sordariomycetes	1	0	0	0	0	0
OTU074 - *Fusarium* sp.	0	0	0	0	1	0
OTU075 - *Colletotrichum* sp.	1	0	0	0	0	0
OTU076 - *Hypoxylon* sp.	0	0	0	1	0	0

OTU077 – Xylariales	0	0	0	1	0	0
OTU078 - *Syncephalastrum* sp.	0	0	0	0	0	1
OTU079 - *Simplicillium obclavatum*	0	1	0	0	0	0
OTU080 - *Ganoderma* sp.	0	0	0	0	0	1
OTU081 - *Umbelopsis* sp.	0	0	0	0	1	0
OTU082 - *Cylindrobasidium* sp.	1	0	0	0	0	0
OTU083 - *Bjerkandera* sp.	0	0	0	0	1	0
OTU084 - *Trametes elegans*	0	0	0	0	0	1
OTU085 - *Phanerochaete australis*	0	0	0	0	0	1
OTU086 - *Fomitopsis palustres*	1	0	0	0	0	0
OTU087 - *Coprinellus radians*	0	0	1	0	0	0
OTU088 - *Penicillium* sp.	0	0	0	0	0	1
OTU089 - *Annulohypoxylon moriforme*	0	0	1	0	0	0
OTU090 – Diaporthaceae	0	0	0	1	0	0
OTU091 - *Colletotrichum* sp.	0	0	0	1	0	0
OTU092 - *Sarocladium* sp.	0	1	0	0	0	0
OTU093 - *Arthrinium* sp.	0	0	1	0	0	0
OTU094 - *Phomopsis* sp.	0	0	0	0	0	1
OTU095 - *Phomopsis* sp.	0	0	0	1	0	0
OTU096 - *Syncephalastrum* sp.	0	0	0	0	1	0
OTU097 - *Penicillium* sp.	0	0	0	0	0	1
OTU098 - *Nigrospora oryzae*	1	0	0	0	0	0
OTU099 - *Phomopsis* sp.	1	0	0	0	0	0
OTU100 - *Neosartorya* sp.	0	0	0	0	0	1
OTU101 – Sordariomycetes	1	0	0	0	0	0
OTU102 - Pleosporales	0	1	0	0	0	0
OTU103 - *Arthrinium* sp.	0	0	0	1	0	0
OTU104 - *Penicillium* sp.	0	0	0	0	0	1
OTU105 - *Trichoderma* sp.	0	0	0	0	1	0
OTU106 - *Cladosporium subuliforme*	0	0	0	0	1	0
OTU107 - *Paradictyoarthrinium diffractum*	0	1	0	0	0	0
OTU108 - *Spegazzinia* sp.	1	0	0	0	0	0
OTU109 - *Curvularia lunata*	0	0	0	1	0	0
OTU110 - *Trichoderma brevicompactum*	0	0	0	0	0	1
OTU111 – Pleosporales	0	0	1	0	0	0
OTU112 - *Talaromyces stipitatus*	0	0	0	0	1	0
OTU113 - *Talaromyces* sp.	0	0	0	0	0	1
OTU114 - *Readeriella callista*	0	1	0	0	0	0
OTU115 - *Diaporthe* sp.	1	0	0	0	0	0
Total of isolates	**133**	**42**	**36**	**207**	**69**	**62**

Table S5. Taxonomic positioning of endophytic fungi grouped in each OTU.

OTUs	Phylum	Class	Order	Family	Genus	Species
OTU001	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum*	*Colletotrichum* sp.
OTU002	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium oxysporum*
OTU003	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	unclassified	unclassified
OTU004	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp
OTU005	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	unclassified	unclassified
OTU006	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma sp*
OTU007	Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	*Purpureocillium*	*Purpureocillium lilacinum*
OTU008	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	unclassified	unclassified
OTU009	Zygomycota	Incertae sedis	Mucorales	Mucoraceae	*Mucor*	*Mucor irregularis*
OTU010	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Lasiodiplodia*	*Lasiodiplodia* sp
OTU011	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma brevicompactum*
OTU012	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Phomopsis*	*Phomopsis* sp.
OTU013	Ascomycota	Sordariomycetes	Xylariales	Amphisphaeriaceae	*Pestalotiopsis*	*Pestalotiopsis mangiferae*
OTU014	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp.
OTU015	Zygomycota	Incertae sedis	Mucorales	Mucoraceae	*Mucor*	*Mucor* sp.
OTU016	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum*	*Colletotrichum* sp.
OTU017	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum*	*Colletotrichum* sp.
OTU018	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	unclassified	unclassified
OTU019	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Hypoxylon*	*Hypoxylon anthochroum*
OTU020	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	unclassified	unclassified
OTU021	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Daldinia*	*Daldinia eschscholtzii*
OTU022	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus* sp.
OTU023	Ascomycota	unclassified	unclassified	unclassified	unclassified	unclassified
OTU024	Zygomycota	Mucoromycotina	Mucorales	Mucoraceae	*Mucor*	*Mucor* sp.
OTU025	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma harzianum*

OTU026	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	unclassified	unclassified
OTU027	Ascomycota	Sordariomycetes	TaHypocreales	Nectriaceae	*Fusarium*	*Fusarium solani*
OTU028	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum*	*Colletotrichum* sp.
OTU029	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus* sp.
OTU030	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	*Phoma*	*Phoma herbarum*
OTU031	Ascomycota	Sordariomycetes	Microascales	Microascaceae	*Wardomyces*	*Wardomyces moseri*
OTU032	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium proliferatum*
OTU033	Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	*Arthrinium*	*Arthrinium malaysianum*
OTU034	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Hypoxylon*	*Hypoxylon sublenormandii*
OTU035	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	unclassified	unclassified
OTU036	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp.
OTU037	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Curvularia*	*Curvularia geniculata*
OTU038	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Phyllosticta*	*Phyllosticta capitalensis*
OTU039	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Curvularia*	*Curvularia* sp.
OTU040	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Nodulisporium*	*Nodulisporium* sp.
OTU041	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus hiratsukae*
OTU042	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Chaetomium*	*Chaetomium globosum*
OTU043	Ascomycota	Dothideomycetes	Pleosporales	Montagnulaceae	*Alloconiothyrium*	*Alloconiothyrium aptrootii*
OTU044	Ascomycota	Sordariomycetes	Xylariales	Amphisphaeriaceae	*Pestalotiopsis*	*Pestalotiopsis* sp.
OTU045	Ascomycota	Dothideomycetes	Capnodiales	Davidiellaceae	*Cladosporium*	*Cladosporium* sp.
OTU046	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*Talaromyces amestolkiae*
OTU047	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	unclassified	unclassified
OTU048	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	unclassified	unclassified
OTU049	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp.
OTU050	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp.
OTU051	Ascomycota	Sordariomycetes	Trichosphaeriales	Trichosphaeriaceae	*Nigrospora*	*Nigrospora sphaerica*

OTU052	Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	*Phlebiopsis*	*Phlebiopsis flavidoalba*
OTU053	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Trametes*	*Trametes sanguinea*
OTU054	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Neoscytalidium*	*Neoscytalidium hyalinum*
OTU055	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Hypoxylon*	*Hypoxylon* sp.
OTU056	Ascomycota	Sordariomycetes	Hypocreales	unclassified	unclassified	unclassified
OTU057	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp.
OTU058	Ascomycota	Dothideomycetes	Pleosporales	Pleosporales	*unclassified*	*unclassified*
OTU059	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus* sp.
OTU060	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp.
OTU061	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Diaporthe*	*Diaporthe* sp.
OTU062	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma* sp.
OTU063	Ascomycota	Sordariomycetes	Diaporthales	Schizoparmaceae	*Pilidiella*	*Pilidiella wangiensis*
OTU064	Ascomycota	Dothideomycetes	Pleosporales	Torulaceae	*Torula*	*Torula* sp.
OTU065	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Preussia*	*Preussia*
OTU066	Zygomycota	Mucoromycotina	Mucorales	Syncephalastraceae	*Syncephalastrum*	*Syncephalastrum* sp.
OTU067	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Diaporthe*	*Diaporthe eucalyptorum*
OTU068	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium* sp.
OTU069	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Daldinia*	*Daldinia bambusicola*
OTU070	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Phomopsis*	*Phomopsis* sp.
OTU071	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus* sp.
OTU072	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma* sp.
OTU073	Ascomycota	Sordariomycetes	unclassified	unclassified	*unclassified*	*unclassified*
OTU074	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium* sp.
OTU075	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum*	*Colletotrichum* sp.
OTU076	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Hypoxylon*	*Hypoxylon* sp.
OTU077	Ascomycota	Sordariomycetes	Xylariales	unclassified	unclassified	unclassified
OTU078	Zygomycota	Mucoromycotina	Mucorales	Syncephalastraceae	*Syncephalastrum*	*Syncephalastrum* sp.
OTU079	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	*Simplicillium*	*Simplicillium obclavatum*

OTU080	Basidiomycota	Agaricomycetes	Polyporales	Ganodermataceae	*Ganoderma*	*Ganoderma* sp.
OTU081	Zygomycota	Mucoromycotina	Mucorales	Umbelopsidaceae	*Umbelopsis*	*Umbelopsis* sp.
OTU082	Basidiomycota	Agaricomycetes	Corticiales	Corticiaceae	*Cylindrobasidium*	*Cylindrobasidium sp*
OTU083	Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	*Bjerkandera*	*Bjerkandera*
OTU084	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Trametes*	*Trametes elegans*
OTU085	Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	*Phanerochaete*	*Phanerochaete australis*
OTU086	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	*Fomitopsis*	*Fomitopsis palustris*
OTU087	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Coprinellus*	*Coprinellus radians*
OTU088	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp.
OTU089	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Annulohypoxylon*	*Annulohypoxylon moriforme*
OTU090	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	unclassified	unclassified
OTU091	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum*	*Colletotrichum* sp.
OTU092	Ascomycota	Sordariomycetes	Hypocreales	Incertae sedis	*Sarocladium*	*Sarocladium* sp.
OTU093	Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	*Arthrinium*	*Arthrinium* sp.
OTU094	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Phomopsis*	*Phomopsis* sp.
OTU095	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Phomopsis*	*Phomopsis* sp.
OTU096	Zygomycota	Mucoromycotina	Mucorales	Syncephalastraceae	*Syncephalastrum*	*Syncephalastrum* sp.
OTU097	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp.
OTU098	Ascomycota	Sordariomycetes	Trichosphaeriales	Trichosphaeriaceae	*Nigrospora*	*Nigrospora oryzae*
OTU099	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Phomopsis*	*Phomopsis* sp.
OTU100	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Neosartorya*	*Neosartorya* sp.
OTU101	Ascomycota	Sordariomycetes	unclassified	unclassified	unclassified	unclassified
OTU102	Ascomycota	Dothideomycetes	Pleosporales	unclassified	unclassified	unclassified
OTU103	Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	*Arthrinium*	*Arthrinium* sp.
OTU104	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp.
OTU105	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma* sp.
OTU106	Ascomycota	Dothideomycetes	Capnodiales	Davidiellaceae	*Cladosporium*	*Cladosporium subuliforme*
OTU107	Ascomycota	Dothideomycetes	Pleosporales	Paradictyorthriniaceae	*Paradictyoarthrinium*	*Paradictyoarthrinium diffractum*

OTU108	Ascomycota	unidentified	unidentified	unidentified	*unidentified*	*Spegazzinia* sp.
OTU109	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Curvularia*	*Curvularia lunata*
OTU110	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma brevicompactum*
OTU111	Ascomycota	Dothideomycetes	Pleosporales	unclassified	*unclassified*	*unclassified*
OTU112	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*Talaromyces stipitatus*
OTU113	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*Talaromyces* sp.
OTU114	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Readeriella*	*Readeriella callista*
OTU115	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Diaporthe*	*Diaporthe* sp.

Table S6. Isolates identification codes of each OTU.

OTUs	Identification codes of endophytic fungi
OTU001	131F1F-AC; 384F4F-AC; 489F17F-AM; 545F27F-AM; 322F5F-AC; 605F5F-AM; 734F9F-AM; 618F4F-AC; 792F1F-AC; 285F7F-AM; 499F18F-AM; 8F3F-AC; 382F29F-AM; 660F22F-AM; 437F22F-AM; 475F17F-AM; 123F5F-AC; 349F1F-AC; 121F5F-AC; 44F9C-AM; 407F7F-AC; 405F5F-AC; 230F1F-AC; 226F1F-AC; 224F5F-AC; 125F1F-AC; 122F1F-AC; 120F7F-AC; 119F1F-AC; 65F5F-AC; 283F11F-AM; 124F1F-AC; 435F9C-AM; 636F1F-AC; 611F9F-AM; 466F10F-AM; 434F11F-AM; 39F13F-AM; 336F12R-AC; 300F9F-AM; 269F13F-AM; 726F13F-AM; 665F29F-AM; 260F13F-AM; 462F13F-AM; 256F22F-AM; 471F10F-AM; 34F4F-AM; 442F14F-AM; 481F4F-AM; 126F1F-AC; 578F15F-AM; 55F4F-AC; 500F4F-AC; 443F4F-AC; 383F4F-AC; 373F4F-AC; 315F7F-AC; 238F1F-AC; 186F16C-AM; 128F7F-AC; 783F21F-AM; 404F26F-AM; 210F22F-AM; 436F16F-AM; 560F17F-AM; 337F29F-AM; 440F14F-AM; 454F11F-AM; 707F17F-AM; 670F14F-AM; 503F14F-AM; 144F17F-AM; 676F14F-AM; 680F15F-AM; 43F29F-AM; 577F22F-AM; 306F22F-AM; 719F17F-AM; 583F22F-AM; 516F17F-AM; 483F24F-AM; 403F24F-AM; 292F27F-AM; 258F22F-AM; 250F22F-AM; 438F9F-AM; 304F25F-AM; 282F24F-AM; 788F27F-AM; 529F24F-AM; 463F9F-AM; 330F11F-AM; 279F23F-AM; 272F18F-AM; 348F29F-AM; 784F29F-AM; 725F25F-AM; 673F13F-AM; 654F24F-AM; 613F17F-AM; 608F29F-AM; 590F9F-AM; 579F22F-AM; 576F13F-AM; 527F13F-AM; 511F19F-AM; 478F5F-AM; 446F13F-AM; 441F23F-AM; 380F19F-AM; 351F29F-AM; 342F29F-AM; 338F29F-AM; 323F15F-AM; 310F10F-AM; 299F22F-AM; 297F19F-AM; 289F22F-AM; 288F24F-AM; 281F23F-AM; 278F22F-AM; 277F24F-AM; 273F18F-AM; 259F23F-AM; 254F19F-AM; 166F17F-AM; 10F25F-AM; 736F17F-AM; 457F19F-AM; 448F13F-AM; 286F22F-AM; 284F19F-AM; 9F25F-AM

OTU002	467F29C-AM; 570F29R-AM; 742F9C-AM ; 781F24F-AM; 695F16C-AM; 710F22C-AM; 447F29C-AM; 697F3C-AM; 416F8C-AM; 456F11C-AC; 354F16F-AC; 789F11F-AM; 733F11F-AM; 727F2C-AC; 720F18F-AM; 703F9C-AM; 677F22C-AM; 668F3C-AM; 642F4C-AC; 635F24F-AM; 631F17F-AM; 621F11F-AM; 614F2C-AC; 603F19F-AM; 554F22C-AC; 553F5C-AC; 543F15F-AM; 484F29F-AM; 459F4C-AC; 445F22F-AM; 423F18C-AC; 399F24F-AM; 387F16F-AC; 361F14R-AC; 334F2R-AC; 276F24F-AM; 271F23F-AM; 799F22F-AM; 319F19C-AC; 806F2C-AM
OTU003	632F18F-AC; 531F14F-AC; 535F14F-AC; 345F16F-AC; 469F14F-AC; 61F14F-AC; 802F14F-AC; 88F25C-AM; 96F17F-AM; 201F19F-AC; 390F19F-AC; 420F27F-AM; 195F11C-AC; 138F19F-AC; 101F19F-AC; 193F19F-AC; 497F19F-AC; 152F14F-AC; 566F17F-AM; 501F4F-AM; 546F14F-AC; 375F16F-AC; 32F9F-AM; 187F14F-AC; 145F13F-AM; 139F16F-AC; 108F9F-AM; 791F13F-AM; 99F9F-AM
OTU004	198F11R-AC; 287F18F-AM; 684F9C-AM; 211F7F-AC; 212F14F-AC; 652F22C-AM; 174F20C-AM; 477F10R-AM; 298F4R-AM; 564F2R-AC; 629F14F-AM; 767F20C-AM; 624F12R-AC; 634F20C-AM; 562F11R-AC; 360F5R-AC; 294F7F-AC; 524F11R-AC
OTU005	251F22F-AM; 255F23F-AM; 225F19F-AC; 50F19F-AC; 398F19F-AC; 393F1F-AC; 377F19F-AC; 355F16F-AC; 28F29R-AM; 627F19F-AC; 184F15F-AM; 536F15F-AM; 439F15F-AM; 568F15F-AM; 49F25F-AM; 487F3F-AM; 460F2F-AM; 33F2F-AM; 588F3F-AM; 729F13F-AM; 521F3F-AM; 80F3F-AM
OTU006	762F17F-AC; 432F8C-AM; 763F25F-AM; 764F2C-AM; 20F5C-AM; 610F5C-AM; 619F6C-AM; 21F13C-AC; 245F13C-AC; 265F6C-AM; 58F6C-AM; 24F6C-AM; 815F11R-AM
OTU007	690F4R-AM; 552F18C-AC; 313F15R-AM; 519F12R-AC; 6F4F-AC; 538F18R-AM; 42F18C-AC; 328F18C-AC; 248F15R-AM; 743F5R-AC; 173F12R-AC; 683F18C-AC; 786F21R-AM
OTU008	78F14F-AC; 565F19F-AM; 465F24F-AM; 421F21F-AM; 412F21F-AM; 688F19F-AC ; 409F27F-AM; 316F25F-AM; 580F22F-AM; 374F16F-AC; 303F25F-AM; 263F23F-AM; 169F24F-AM; 790F24F-AM; 82F22F-AM
OTU009	431F19F-AM; 592F14F-AC; 582F22R-AM ; 149F22R-AC; 429F18F-AC; 574F22R-AC; 509F18F-AM; 158F12C-AM; 151F22R-AC; 607F12C-AM; 505F26R-AM; 532F18C-AC; 550F14R-AC; 609F13R-AM
OTU010	231F25F-AM; 612F22R-AC; 244F16C-AC; 237F2F-AM; 714F25F-AM; 417F27F-AM; 424F27F-AM; 537F27F-AM; 415F27F-AM; 252F6C-AM; 633F14F-AM; 746F14F-AM
OTU011	17F5R-AM; 15F5R-AM; 19F5R-AM; 14F5R-AM; 246F5R-AM; 530F5R-AM; 515F5R-AM; 585F5R-AM; 18F5R-AM; 57F5R-AM; 60F5R-AM
OTU012	109F22F-AM; 548F4F-AC; 340F16F-AC; 666F14F-AC; 449F14F-AC; 191F19F-AC; 170F14F-AC; 723F14F-AC; 704F15F-AM; 397F7F-AC
OTU013	586F16R-AM; 559F1C-AM; 584F16C-AM; 492F13F-AM; 794F16F-AM; 803F16C-AM
OTU014	321F7C-AC; 46F7C-AC; 41F5R-AC; 646F5R-AC; 451F7R-AM; 40F4C-AC; 196F16C-AC; 51F5C-AM; 657F18C-AC
OTU015	221F28R-AM; 650F19F-AC; 106F16C-AM; 616F24R-AM; 517F29R-AM; 795F19F-AC; 796F27R-AM
OTU016	379F29F-AM; 341F29F-AM; 381F1F-AC; 370F1F-AC; 13F1F-AC; 132F1F-AC; 395F1F-AC

OTU017	591F21F-AM; 5F7F-AC
OTU018	485F14F-AC; 589F2F-AM; 647F2F-AM; 257F11F-AM; 498F14F-AC; 801F14F-AC
OTU019	502F23F-AC; 513F23F-AC; 185F23F-AC; 48F23F-AC; 692F1F-AC; 691F1F-AC
OTU020	133F16F-AC; 400F16F-AC; 214F14F-AC; 482F16F-AC; 541F4F-AM
OTU021	242F7F-AC; 391F7F-AC; 567F4F-AC
OTU022	207F3C-AM; 630F3C-AM; 713F5R-AC; 587F9C-AM; 30F10C-AM; 737F8F-AM
OTU023	274F22F-AC; 573F14R-AC; 311F11R-AC; 267F22F-AC; 686F21R-AM; 754F27C-AM
OTU024	160F3R-AM; 428F12R-AM; 491F14F-AC; 232F21C-AC; 526F9F-AM
OTU025	25F1C-AM; 549F18C-AC; 518F1C-AM; 247F12R-AC; 765F5C-AM
OTU026	182F1F-AC; 353F1F-AC; 385F16F-AC; 595F18F-AC
OTU027	522F22C-AM; 56F4R-AC; 708F22C-AM; 709F22C-AM
OTU028	369F1F-AC; 350F1F-AC; 816F29F-AM
OTU029	2F3F-AM; 38F3F-AM; 1F3F-AM; 534F24F-AM
OTU030	411F29R-AM; 464F29R-AM; 394F7F-AC; 490F29R-AM
OTU031	656F22F-AM; 661F18F-AM; 653F3C-AM; 662F22R-AM
OTU032	209F25F-AM; 410F22F-AM; 296F27R-AM; 425F25F-AM
OTU033	392F20R-AC; 356F20R-AC; 35F5R-AM; 504F22R-AC
OTU034	148F18F-AC; 127F18F-AC; 640F18F-AC; 777F18F-AC
OTU035	625F25F-AM; 137F25F-AM; 766F25F-AM
OTU036	641F18F-AC; 507F5R-AM; 658F5R-AM
OTU037	638F21C-AM; 227F11C-AC; 663F11C-AC
OTU038	266F23F-AM
OTU039	239F22F-AC; 506F22C-AC
OTU040	262F22F-AM; 320F22F-AM; 563F22F-AC
OTU041	678F8F-AM; 604F8R-AM; 682F8F-AM
OTU042	243F2R-AC
OTU043	681F4R-AM; 711F5R-AC
OTU044	426F25C-AM; 95F25C-AM
OTU045	318F17R-AM; 644F17R-AM
OTU046	705F18F-AC; 86F10R-AM
OTU047	474F13F-AM; 750F14R-AM
OTU048	419F22F-AM; 735F19F-AM

OTU049	655F16F-AC
OTU050	213F5C-AC; 728F5C-AC
OTU051	228F7C-AC; 236F7C-AC
OTU052	103F9C-AM
OTU053	105F16C-AM; 745F16C-AM
OTU054	606F17C-AM; 659F17C-AM
OTU055	444F23F-AC; 581F2F-AC
OTU056	309F16R-AM; 69F20R-AC
OTU057	314F2C-AM; 37F27C-AM
OTU058	468F14C-AC; 544F14F-AC
OTU059	203F2C-AM; 54F2C-AM
OTU060	473F8R-AM; 510F1C-AM
OTU061	305F14F-AM; 557F3F-AM
OTU062	22F20C-AC; 23F18C-AC
OTU063	493F18C-AC
OTU064	113F12R-AC; 114F12R-AC
OTU065	339F7F-AC
OTU066	452F29R-AM
OTU067	556F14F-AC
OTU068	495F28C-AM
OTU069	533F23F-AC
OTU070	528F14F-AC
OTU071	366F3R-AM
OTU072	597F2C-AC
OTU073	376F4F-AC
OTU074	317F8C-AM
OTU075	229F1F-AC
OTU076	331F17F-AM
OTU077	569F15F-AM
OTU078	648F28R-AM
OTU079	312F4C-AC
OTU080	107F13R-AM

OTU081	717F11C-AM
OTU082	671F1F-AC
OTU083	31F16C-AM
OTU084	479F2R-AM
OTU085	542F3R-AM
OTU086	679F11F-AC
OTU087	741F23R-AC
OTU088	667F7R-AM
OTU089	72F19R-AC
OTU090	414F27F-AM
OTU091	118F29F-AM
OTU092	293F4C-AC
OTU093	602F22R-AC
OTU094	27F22R-AM
OTU095	264F23F-AM
OTU096	261F2C-AM
OTU097	637F5R-AM
OTU098	712F16F-AC
OTU099	779F14F-AC
OTU100	770F4R-AM
OTU101	140F1F-AC
OTU102	71F5C-AC
OTU103	68F11F-AM
OTU104	7F5R-AM
OTU105	84F15C-AM
OTU106	761F16C-AM
OTU107	117F5C-AC
OTU108	130F7F-AC
OTU109	693F8F-AM
OTU110	16F5R-AM
OTU111	520F14R-AC
OTU112	206F20C-AM

OTU113	623F7R-AM
OTU114	651F18C-AC
OTU115	194F14F-AC

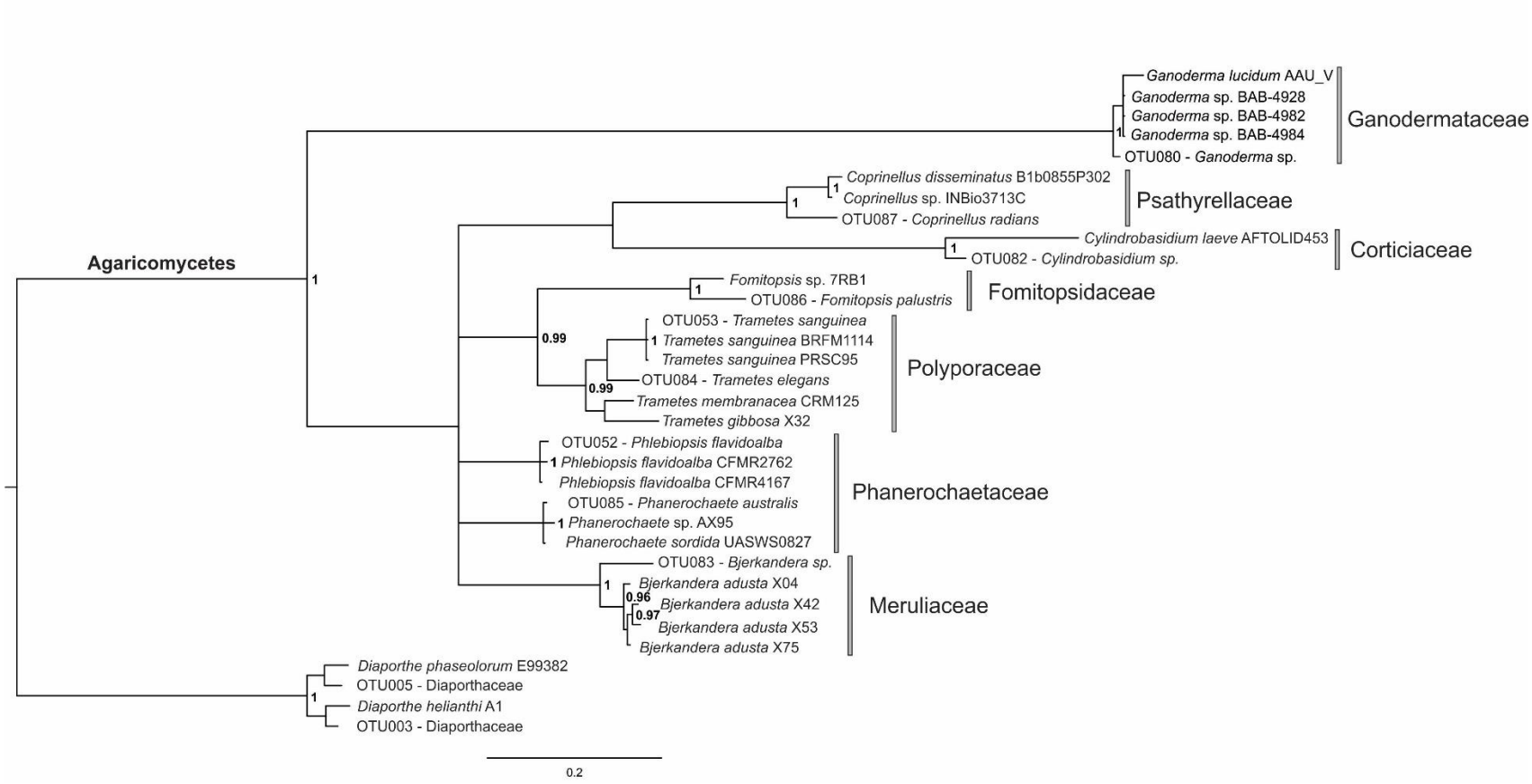

Fig. S2. Phylogenetic tree obtained by Bayesian Influence (BI) using sequences from the ITS region of the rDNA of the 08 Operational Taxonomic Units (OTUs) pertaining to the phylum Basidiomycota. A posteriori probability values below 95% were omitted.

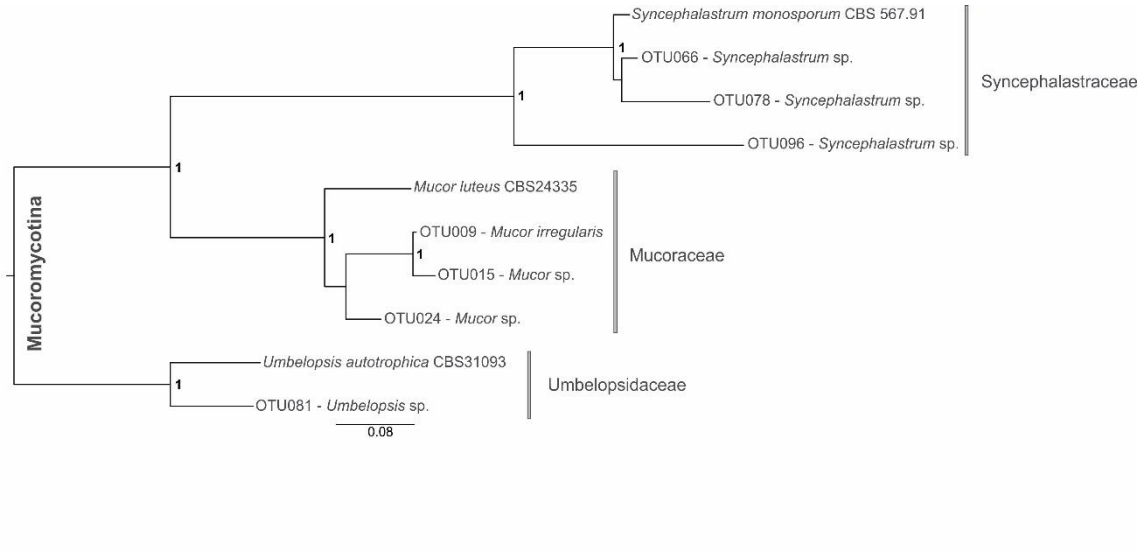

Fig. S3. Phylogenetic tree obtained by Bayesian Influence (BI) using sequences from the ITS region of the rDNA of the 08 Operational Taxonomic Units (OTUs) belonging to the Zygomycota phylum. A posteriori probability values below 95% were omitted.

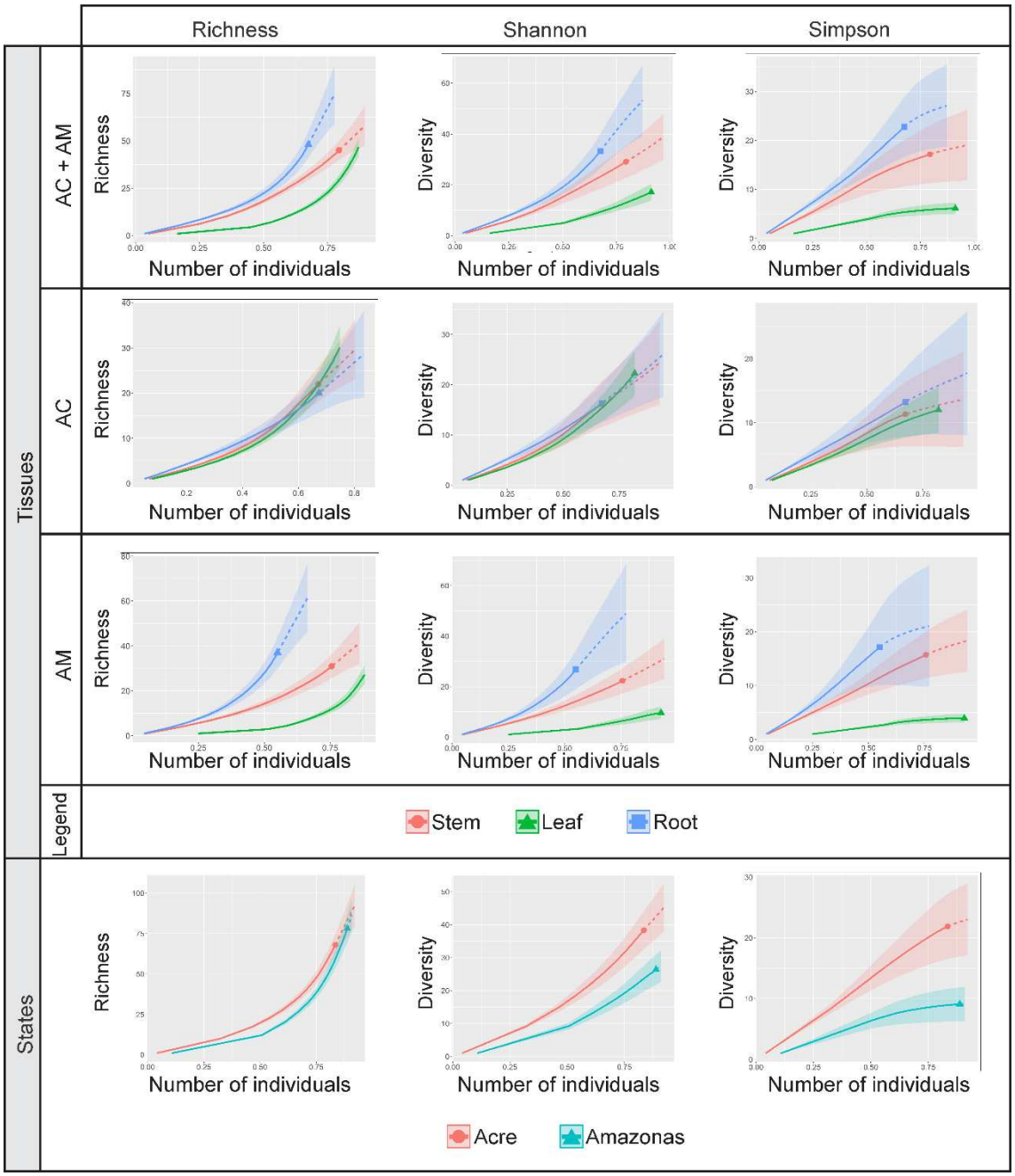

Fig. S4. Curve of rarefaction (solid line) and extrapolation (dashed line) to twice the coverage of the reference sample. Comparison of species richness (q=0), Shannon Wiener's exponential entropy (q = 1), the inverse concentration of Simpson (q = 2) according Hill numbers, as tripod of diversity of species, of endophytic fungi in the different niches: stem (red line), leaf (green line), root (blue line) of *Hevea brasiliensis* independent of state, and in states of Acre and Amazonas, and between the states of Acre (red line) and the state of Amazonas (blue line), with 95% confidence intervals obtained by the bootstrap method with 200 replications.

CAPITULO 2

Diversity of endophytic filamentous fungi of *Hevea guianensis*: a latex producer native tree from the Brazilian Amazon

Araújo KS, Brito VN, Veloso TGR, Leite TS, Pereira OL, Mizubuti ESG, Queiroz, MV. Diversity of endophytic filamentous fungi of *Hevea guianensis*: a latex producer native tree from the Brazilian Amazon. Microbiological Research. Em processo de submissão.

Diversity of endophytic filamentous fungi of *Hevea guianensis*: a latex producer native tree from the Brazilian Amazon

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ABSTRACT

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 isolate endophytic fungi and study their diversity 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 rubber tree. These isolates were grouped into 28 operational taxonomic units (OTUs), with isolates that showed 98% sequence similarity in the internal transcribed spacer (ITS) region considered to belong to the same OTU. The dominant phylum was Ascomycota, which represented 96.73% of the analysed fungi. The stem cortex showed the greatest fungal richness and diversity, although the frequency of isolates was highest in the leaves. In addition, when comparing the fungal communities among the tissues, a heterogeneous distribution of the leaf isolates was observed in relation to the stem and root isolates. Among the genera obtained, *Colletotrichum* was the most well-represented and the most abundant in the leaves, and *Diaporthe* was the second most abundant genus in the leaves. *Penicillium* was the second most well-represented genus among all recovered isolates and the main genus obtained from the roots. The genera *Lasiodiplodia*, *Purpureocillium*, *Phyllosticta*, *Daldinia* and *Pseudofusicoccum* were recovered only from the leaves. The genera *Mucor* and *Pestalotiopsis* were isolated from the leaves and roots, and the genera *Trichoderma* and *Fusarium* were isolated from the stems and roots of *H. guianensis*. Thus, we describe the endophytic communities in the different tissues of *H. guianensis* and isolates of representative genera of great biotechnological interest, such as *Trichoderma*.

INTRODUCTION

The Amazon rainforest is the largest biodiversity centre on the planet, and the first description of genus *Hevea* and its species *Hevea guianensis* by Fusee Aublet (1775) (Sethuraj and Mathew, 2012) occurred in this centre. Later, other species were described, such as *Hevea brasiliensis* (1824), *Hevea pauciflora*, *Hevea spruceana* and *Hevea rigidifolia* (1854), *Hevea nitida* var. *toxicodendroides*, *Hevea microphylla*, *Hevea camporum*, *Hevea benthamiana* (1962) and *Hevea camargocina* (1981) (Muller, 1865; 1874; Murca, 1981; Schulte, 1977; 1987; Sethuraj and Mathew, 2012).

Commercially acceptable latex and rubber have been obtained from *H. brasiliensis*, *H. benthamiana* and *H. guianensis*. However, the species *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 Pereira, 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* spp. (striated canker or panel canker) (Gasparroto and Perreira, 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 (Arnold, 2007; Koide et al., 2017; Saunders et al., 2010; Zheng et al., 2017), increase plant production (Babu et al., 2015; Khan et al., 2008; Murali and Amruthesh, 2015) and control phytopathogens (Ben Amira et al., 2017; Contina et al., 2017; Landero Valenzuela et al., 2015; Larran et al., 2016; 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 been conducted with the objective of describing 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 (Chaverri et al., 2011; Gazis et al., 2012, 2011; Gazis and Chaverri, 2010; Rocha et al., 2011; Gazis, 2012) and with *H. brasiliensis* in the Brazilian Amazon (Chapter 1). These studies demonstrated the occurrence of a high diversity of endophytic fungi mainly inside the stem despite the high colonization rate of endophytes on the leaves (Chaverri et al., 2011; Gazis et al., 2012, 2011; Gazis and Chaverri, 2010; Gazis, 2012; Chapter 1) 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).

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 *Hevea guianensis* trees located in the Amazon rainforest, Acre, and distributed at different sampling points (Suppl. Fig. 1 and Suppl. Table 1).

The methods proposed by Wirsel et al. (2001), Evans et al. (2003) and Leite et al. (2013) were used to isolate the endophytic fungi with modifications. The leaves were packed into paper bags, which in turn were placed into plastic bags and stored at 4 °C (Stone et al., 2004). The root cortex fragments were transported to the laboratory immediately after collection in silica gel tubes. 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 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 hours of white light and 12 hours in the dark for seven days. Then, the isolates were preserved in 10% glycerol and 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 the method of Pinho et al. (2012) with modifications. 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 GoTaq® Green MasterMix 2X unit (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 backward and forward 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 XXXXX to XXXXX.

Subsequently, the ITS region sequences were grouped into operational taxonomic units (OTUs), with sequences with ≥ 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. Nilsson et al. (2009) found an intraspecific variation of 1.96% (standard deviation = 3.73%) for Ascomycota, 3.33% (standard deviation = 5.62%) for Basidiomycota and 3.24% (standard deviation = 6.12%) for Zygomycota.

Phylogenetic analysis

The nucleotide sequences of the rDNA ITS region of each OTU and the reference or type sequences (Supplementary Material 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 1000th 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 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 Materials 2 and 3).

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)) (Fig. 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 (Fig. 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 ($R = 0.302$, $p < 0.001$) (Fig. 3).

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 (Fig. 4, Table 1, Supplementary Materials 2 and 3).

The *Penicillium* (family Trichocomaceae) genus had the second highest number of fungi isolated from *H. guianensis*, representing 11.95% of the total fungi recovered (Fig. 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 Materials 2 and 3). Also, *Diaporthe* (family Diaporthaceae) is the second genus most abundant within of the tissue of *H. guianensis*, mainly within of the leaf tissue, corresponding to 11.95% (11 isolates) of the isolates distributed into the four OTUs (Table 1, Supplementary Materials 2 and 3).

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 Materials 2 and 3).

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 Materials 2 and 3).

DISCUSSION

The fungi isolated from diferent tissues of *H. guianensis* were more diversity 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. *H*. *guianensis* is a latex-producing rubber tree that is widely exploited in its natural habitat but is not cultivated (Sethuraj and Mathew, 2012). In comparison to the species *H. brasiliensis*, which is extensively cultivated, few studies have investigated *H. guianensis* 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 (Gazis and Chaverri, 2010; Rocha et al., 2011).

The similarity between *H. guianensis* and *H. brasiliensis* makes identification of these species difficult in their natural environment. However, we distinguished 6 *H. guianensis* trees among the *H. brasiliensis* trees in the Amazon forest in the state of Acre. A total of 92 endophytic fungi were isolated from these *H. guianensis* trees, mainly from the leaves.

The 92 fungi belonged to 18 different genera and clustered into 28 OTUs (Table 1) were identified when the ITS region sequence was used as a barcode. The use of ITS region facilitated the identification of different genera and their clustering into OTUs with 98% similarity within phyla Ascomycota, Basidiomycota and Zygomycota (Fig. 1). Many studies have estimated the diversity and distributions of species in a microbial community by counting OTUs (Angelini et al., 2012; Gazis et al., 2011; Gazis and Chaverri, 2010; Koide et al., 2017; Martins et al., 2016; Chapter 1), 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 (Fernandes et al., 2015; Ferreira et al., 2017; Gazis and Chaverri, 2010; Hanada et al., 2010; Leite et al., 2013; Martins et al., 2016; Chapter 1), phylum Ascomycota was most abundant in the endophytic fungi community of *H. guianensis* (96.73%), particularly class Sordariomycetes (68.47%). In addition, the estimated richness (q = 0), Shannon diversity (q = 1) and Simpson diversity (q = 2) were significantly higher in the stems of the rubber trees (Fig. 2 and Table 2), 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), which found a high diversity of fungi recovered from the stem cortex despite obtaining a higher frequency of isolate colonization in the leaves of Peruvian rubber trees. In the chapter 1, it was found greater fungal richness and diversity in the stems and roots of *H. brasiliensis* from the Brazilian Amazon in the state of Amazonas than in the leaf tissues.

The nMDS analysis based on the Bray-Curtis distances between OTUs from the leaves, stems and roots of *H. guianensis* revealed a trend 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 (Fig. 3). This result corroborates the study conducted in the chapter 1, who analysed the diversity of endophytic fungi in *H. brasiliensis*.

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 (Gazis and Chaverri, 2010; Martins et al., 2016; Suryanarayanan and Vijaykrishna, 2001; Zheng et al., 2017). An important hypothesis suggested in the chapter 1 to explain the clustering of the isolates from the roots and stems and their separation from the isolates obtained from the leaves of the rubber trees was the systemic growth of endophytic fungi from the roots to the stem, with soil as an important inoculum source.

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*. *Colletotrichum*, *Penicillium* and *Diaporthe* were also predominant genera isolated from the tissues of *H. brasiliensis* from Amazon forest in the states of Acre and Amazonas (chapter 1). 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 frequente genera. Thus, there are diffferences among the endophytic fungal communities in rubber trees from diferente 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 (Leite et al., 2013; Fernandes et al., 2015, Ferreira et al., 2015, 2017, Rojas-Jimenez et al., 2016, Chapter 1). Although, Gazis and Chaverri (2010) observed a low frequency of *Colletotrichum* with of the tissue of *H. brasiliensis*, a closely related species from Peruvian Amazon forest.

Penicillium was the second most commonly isolated genus from *H. guianensis*. Fifty percent of the endophytic fungi isolated from the roots belonged to the genera *Penicillium*. *Trichoderma*, *Fusarium*, *Pestalotiopsis*, *Curvularia* and *Mucor*. A difference was found in the community profiles of the fungi recovered

from the roots of *H. guianensis* and the isolates obtained from the roots of *H. brasiliensis* in the Brazilian Amazon (chapter 1), which shared only the genera *Penicillium*, *Trichoderma*, *Mucor* and *Fusarium*.

The fungal genera recovered from the rubber tree stems in this study showed greater equitability and were identified as *Colletotrichum* sp., *Fusarium oxysporum*, *Trichoderma* sp., *Penicillium* sp., *Phlebiopsis flavidoalba*, *Letendraea helminthicola* and *Chaunopycnis* sp. When comparing the isolates recovered from the stems of *H. guianensis* with those obtained from the stems of *H. brasiliensis* from the Brazilian Amazon, the genera *Penicillium*, *Trichoderma*, *Fusarium* and *Colletotrichum* were found in both hosts; however, they exhibited differences in the fungal community profiles obtained from the different host species. This difference was also found in chapter 1 when analysing endophytic fungi isolated at lower frequencies from the stems of *H. brasiliensis* in the states of Acre and Amazonas.

Regarding the less frequently isolated genera, *Purpureocillium* and *Daldinia* were recovered from the leaves of *H. guianensis* and *H. brasiliensis* in the state of Acre, as reported in chapter 1. In addition, the genera *Lasiodioplodia*, *Phyllosticta* and *Pestalotiopsis* were recovered from the leaves of *H. guianensis* from the state of Acre and were also isolated from the leaves of *H. brasiliensis* in the state of Amazonas (chapter 1).

Among the factors that might affect 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 with regard to tolerance to extreme temperature and humidity, as could be the case with rubber trees in the Amazon.

Some species closely 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.

Others fungi genera with potential for biological control of diseases, inductors of plant resistance to abiotic stress and/or growth promoters in plants include *Penicillium* (Babu et al., 2015; Guijarro et al., 2017; Murali and Amruthesh, 2015), *Lasiodiplodia* (Xiang et al., 2016), *Fusarium* (Rocha et al., 2011; Zhang et al., 2014), *Purpureocillium* (Castillo 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 (Gazis and Chaverri, 2010; Gibertoni et al. 2016; chapter 1). 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 southeastern 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 stem cortex showed the greatest fungal richness, equitability and diversity, although the frequency of isolates was highest in the leaves. In addition, a heterogeneous distribution of the leaf fungal isolates was observed in relation to the stem and root isolates.

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.

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Table 1. Number and frequency of colonization of endophytic fungal isolates from the leaves, stems and roots of *Hevea guianensis* from the Amazon forest in the state of Acre per OTU.

OTUs	Total of isolates of *Hevea guianensis*			Frequency of colonization of isolates (%)		
	Leaf	Stem	Roots	Leaf	Stem	Roots
OTU001 - *Colletotrichum* sp.	27	1	0	40.90909	12.5	0
OTU002 - *Fusarium oxysporum*	0	1	2	0	12.5	11.11111
OTU003 - Diaporthaceae	6	0	0	9.090909	0	0
OTU004 - *Penicillium* sp.	0	0	9	0	0	50
OTU005 - Diaporthaceae	3	0	0	4.545455	0	0
OTU006 - *Trichoderma* sp.	0	1	3	0	12.5	16.66667
OTU007 - *Purpureocillium lilacinum*	2	0	0	3.030303	0	0
OTU008 - *Lasiodiplodia* sp.	2	0	0	3.030303	0	0
OTU009 - *Phomopsis* sp.	1	0	0	1.515152	0	0
OTU010 - *Pestalotiopsis mangiferae*	4	0	1	6.060606	0	5.555556
OTU011 - *Penicillium* sp.	0	1	0	0	12.5	0
OTU012 - *Mucor* sp.	1	0	1	1.515152	0	5.555556
OTU013 - *Colletotrichum* sp.	1	0	0	1.515152	0	0
OTU014 - *Colletotrichum* sp.	5	0	0	7.575758	0	0
OTU015 - *Daldinia eschscholtzii*	3	0	0	4.545455	0	0
OTU016 - Diaporthaceae	1	0	0	1.515152	0	0
OTU017 - *Colletotrichum* sp.	1	0	0	1.515152	0	0
OTU018 - *Phyllosticta capitalensis*	2	0	0	3.030303	0	0

OTU019 - *Curvularia* sp.	0	0	1	0	0	5.555556
OTU020 - *Pseudofusicoccum stromaticum*	3	0	0	4.545455	0	0
OTU021 - *Chaetomium globosum*	1	0	0	1.515152	0	0
OTU022 - *Penicillium* sp.	0	1	0	0	12.5	0
OTU023 - *Phlebiopsis flavidoalba*	0	1	0	0	12.5	0
OTU024 - *Phyllosticta citriasiana*	2	0	0	3.030303	0	0
OTU025 - *Pilidiella wangiensis*	1	0	0	1.515152	0	0
OTU026 - Hypocreales	0	0	1	0	0	5.555556
OTU027 - *Letendraea helminthicola*	0	1	0	0	12.5	0
OTU028 - *Chaunopycnis* sp.	0	1	0	0	12.5	0
Total	**66**	**8**	**18**	**----**	**----**	**----**

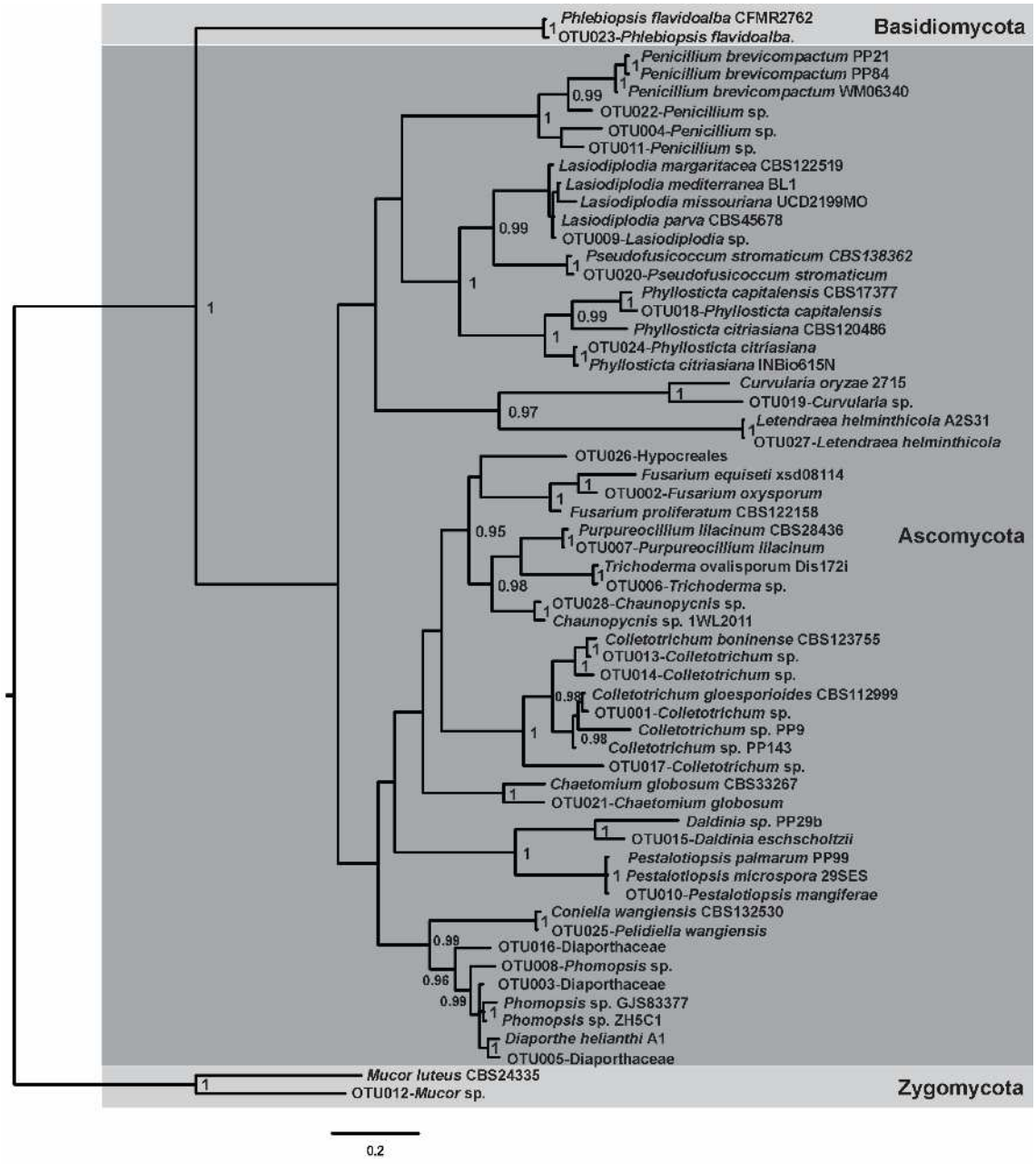

Fig. 1 Phylogenetic tree obtained by Bayesian inference (BI) using sequences from the rDNA ITS region of the 28 operational taxonomic units (OTUs) that clustered all 92 endophytic fungi belonging to the phyla Ascomycota, Basidiomycota and Zygomycota. Posterior probability values below 95% were omitted.

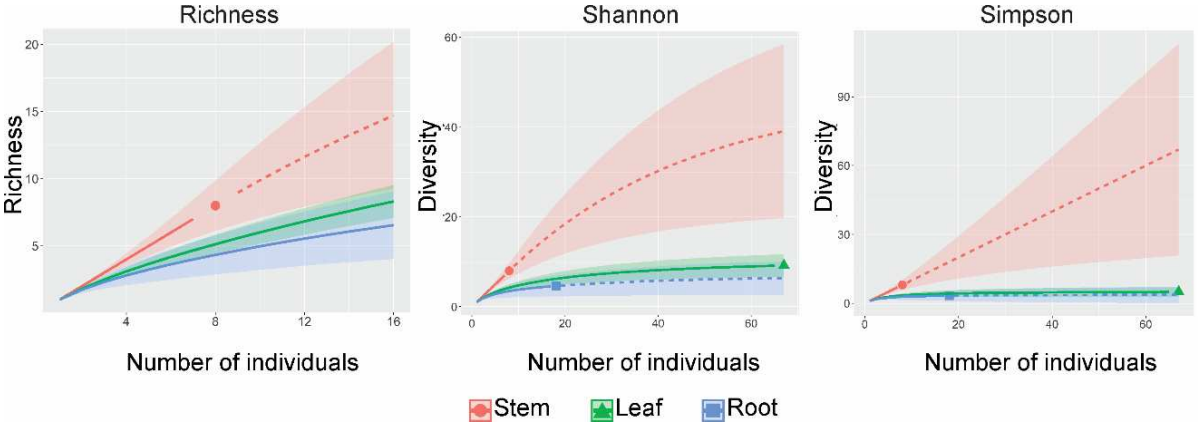

Fig. 2 Rarefaction (solid line) and extrapolation (dashed line) curves for twice the size of the reference sample. The rarefaction (solid line) and extrapolation (dashed line) curves compare the species richness (q = 0), exponential of Shannon's entropy index (q =1) and inverse of Simpson's concentration 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 concetration index (q = 2) of endophytic fungi among the different *Hevea guianensis* niches with their 95% confidence intervals (*).

Samples	Tissues	Richness (q0)	Shannon (q1)	Simpsom (q2)
AC	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 followed by the same letters in the column do not differ according to the R program version 3.1.2 with 1000 bootstrap replicates at 5% probability.

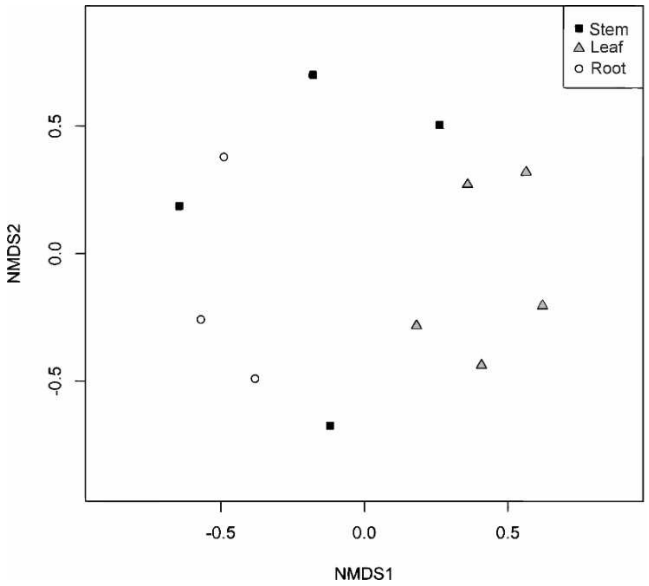

Fig. 3 Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis distance between fungal samples obtained from different *Hevea guianensis* tissues.

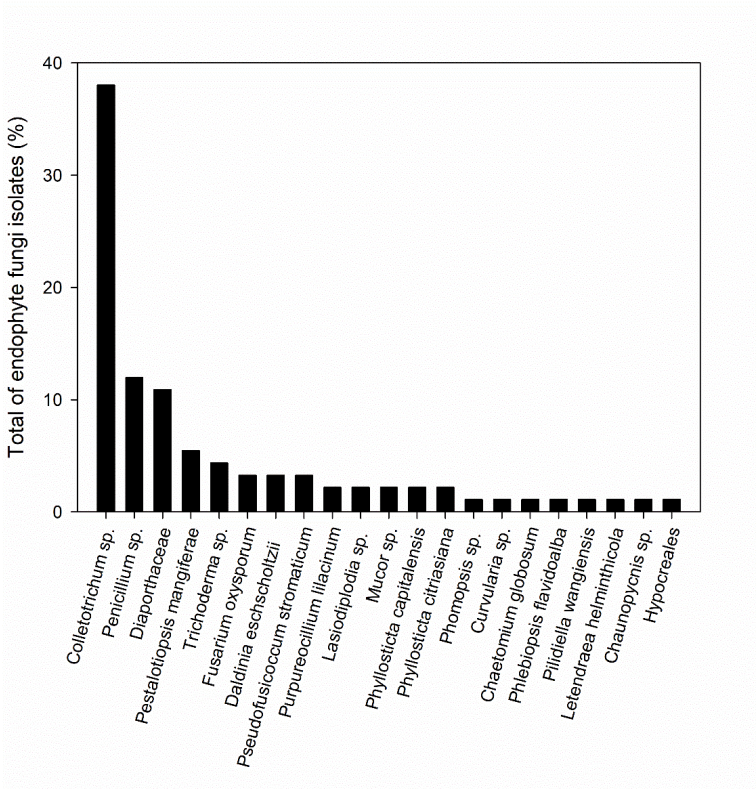

Fig. 4 Percentage of endophytic fungi isolated from *Hevea guianensis* in the Brazilian Amazon.

SUPPLEMENTARY MATERIAL

Table 1. Supplementary Material 1. Accession numbers of the isolate sequences used as references deposited in GenBank.

Closest species in GenBank	Isolates	Accession nº	Closest species in GenBank	Isolates	Accession nº
Diaphorte helianthi	A1	AJ312356.1	*Lasiodiplodia margaritacea*	CBS122519	KT852959
Phomopsis sp.	GJS83377	AF102999.1	*Lasiodiplodia mediterranea*	BL1	KJ638312
Phomopsis sp.	ZH5C1	FJ037753.1	*Lasiodiplodia missouriana*	UCD2199MO	HQ288226
Coniella wangiensis	CBS1322539	NR111764.1	*Lasiodiplodia parva*	CBS45678	KF766192
Purpureocillium lilacinum	CBS28436	MF975787.1	*Phyllosticta capitalensis*	CBS17377	KF206179
Fusarium equiseti	Xsd08114	FJ481025	*Phyllosticta citriasina*	CBS120486	NR_145217.1
Fusarium proliferatum	CBS122158	DQ655730	*Phyllosticta citriasina*	INBio615N	KU204611.1
Trichoderma ovalisporum	DIS172i	DQ323438	*Curvularia oryzae*	2715	EU27519
Colletotrichum boninense	CBS123755	JQ005153	*Chaunopycnis* sp.	1WL2011	JN198447.1
Colletotrichum gloesporioides	CBS112999	JQ005152	*Letendraea helminthicola*	A2S31	KJ774053.1
Colletotrichum gloesporioides	PP143	FJ884081.1	*Pseudofusicoccum stromaticum*	CBS138362	KP872348.1
Colletotrichum sp.	PP9	FJ884085.1	*Phlebiopsis flavidoalba*	CFMR2762	KX065956
Chaetomium globosum	CBS33267	KX976570.1	*Lasiodiplodia margaritacea*	CBS122519	KT852959
Daldinia sp.	PP29b	FJ884087	*Penicillium brevicompactum*	PP21	FJ884116
Pestalotiopsis palmarum	PP99	FJ884145.1	*Penicillium brevicompactum*	PP84	FJ884117.1
Pestalotiopsis microspora	29SES	EF451800	*Penicillium brevicompactum*	WM06340	EF568063

Table 2. Supplementary Material 2. 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	Eurotiomycetes	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.

Table 3. Supplementary Material 3. Identification codes of the isolates composing each OTU.

OTUs	Isolados
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

CAPITULO 3

Identificação, variabilidade gênica e potencial antimicrobiano de *Penicillium* spp. e *Talaromyces* spp. endofíticos de seringueiras da floresta amazônica brasileira

Araújo KS, Queiroz CB, Pereira OL, Queiroz MV. Identificação, variabilidade gênica e potencial antimicrobiano de Penicillium e Talaromyces endofíticos de seringueiras da floresta amazônica brasileira. Em andamento.

Identificação, variabilidade gênica e potencial antimicrobiano de *Penicillium* spp. e *Talaromyces* spp. endofíticos de seringueiras da floresta amazônica brasileira

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RESUMO

Espécies dos gêneros *Penicillium* e *Talaromyces* são comumente encontradas em diversos habitats, inclusive no interior das plantas. Elas produzem uma variedade de metabólitos para a planta e são capazes de defender o seu hospedeiro do ataque de fitopatógenos. Devido a importância do estudo dessas espécies, foram identificados fungos pertencentes aos gêneros *Penicillium* e *Talaromyces,* isolados das folhas, caules e raízes de seringueiras da floresta amazônica brasileira. A identificação foi realizada por meio da análise da região ITS, das sequências dos genes *benA*, *caM* e *RPB2* e das sequências das regiões gênicas concatenadas. Além disso, foi realizada a análise da variabilidade genética entre estes isolados pelas técnicas IRAP (*Inter-Retrotransposon Amplified Polymorphism*) e REMAP (*Retrotransposon-Microsatellite Amplified Polymorphism*) e foi avaliado o potencial antagônico destes fungos endofíticos aos fitopatógenos *Colletotrichum karstii* (seringueira), *Colletotrichum laticiphilium* (seringueira), *Colletotrichum lindemuthianum* (feijoeiro) e *Fusarium verticillioides* (milho) pelo método de cultura pareada. Treze espécies de *Penicillium* e três espécies de *Talaromyces* foram identificadas, sendo sugerida a ocorrência de 7 espécies novas de *Penicillium* pertencentes as seções *Lanata-divaricata*, *Sclerotiora* e *Citrina.* A espécie *Penicillium citrinum* compõe 54% dos isolados em estudo. As técnicas IRAP e REMAP foram capazes de mostrar polimorfismo em todas as espécies analisadas e foram eficientes para o estudo da variabilidade genética intraespecífica e interespécies dessas espécies. Todas as espécies identificadas foram capazes de reduzir o crescimento dos fitopatógenos *C. karstii*, *C. laticiphilum*, *C. lindemuthianum* e *F. verticillioides* em até 55%, 59%, 88,21% e 39,57%, respectivamente. Estes isolados podem ser uma alternativa promissora para o controle biológico de fitopatógenos.

INTRODUÇÃO

Fungos pertencentes ao gênero *Penicillium* são comumente encontrados em diversos habitats, como no solo, em plantas, no ar, em produtos alimentares, e causam um grande impacto na vida dos seres humanos (Frisvad et al. 2004; Khan et al. 2008; Houbraken et al. 2012; Babu et al. 2015; Murali & Amruthesh 2015). Isolados de várias espécies de *Penicillium* já foram descritos como fungos endofíticos de plantas (Khan et al. 2011; Khan et al. 2013).

Penicillium é um gênero anamorfo de fungos filamentosos, descrito primeiramente por Link (1809). Antigamente, as novas espécies pertencentes ao gênero eram descritas com base no conceito de espécies morfológicas (Thom, 1930; Raper & Thom, 1949; Ramírez, 1982). A classificação taxonômica do gênero *Penicillium* é desafiadora e está sendo revista continuamente, uma vez que a classificação baseada nas características fenotípicas é incongruente em relação a classificação filogenética (Houbraken & Samson, 2011; Samson et al. 2011; Visagie et al. 2014). Um exemplo disso é o isolado de Fleming produtor de penicilina, identificado como *Penicillium chrysogenum*, que foi re-identificado como *Penicillium rubens* pela análise filogenética multilocus e pela análise de extrólitos (Houbraken et al. 2011).

A análise filogenética multilocus revelou que as espécies do subgênero *Biverticillium* não pertencem ao gênero *Penicillium* (família Aspergillaceae) e foram agrupadas ao gênero *Talaromyces* (família Trichocomaceae) (Houbraken & Samson, 2011; Samson et al. 2011). Samson et al. (2011) demonstraram que os membros do subgênero *Biverticillium* e *Talaromyces* são acomodados em um clado monofilético e que espécies dos subgêneros *Aspergiloides*, *Furcatum* e *Penicillium* formam um clado independente.

O gênero atualmente contém 354 espécies aceitas, incluindo a nova seção taxonômica para acomodar as espécies *Aspergillus paradoxus*, *Aspergillus malodoratus*, *Aspergillus crystallinus*, as quais pertencem filogeneticamente ao gênero *Penicillium* (Visagie et al. 2014). Posteriormente, outras espécies novas foram descritas por meio de uma abordagem polifásica, tais como: *Penicillium salamii* (Perrone et al. 2015), *Penicillium chroogomphum* (Rong et al. 2016) e *Penicillium pedermalense* (Laich & Andrade 2018).

Na literatura são retratados alguns exemplos de espécies de *Penicillium* e *Talaromyces* endofíticas e do solo rizosférico de seus hospedeiros capazes de serem agentes de controle biológico (Ting et al. 2012; Dethoup et al. 2018), promover o crescimento vegetal, atuar na conservação da flora, no reflorestamento e no desenvolvimento de culturas de importância agrícola, como *Penicillium citrinum* (Khan et al. 2008), *Penicillium oxalicum* (Murali & Amruthesh 2015) e *Penicillium menonorum* (Babu et al. 2015). O fungo *Penicillium ochrochloron* produz quitinase que afeta o crescimento de larvas de *Helicoverpa armígera*, que é uma praga que infecta culturas como milho e soja, e esse fungo vem sendo estudado para formulações de pesticidas baseadas em enzimas (Patil & Jadhav, 2015).

Outras espécies, como *Penicillium frequentans* isolado dos galhos de pêssego é um agente de biocontrole cuja formulação está sendo testada (Guijarro et al. 2017). *Penicillium oxalicum* foi testado como inoculante biológico sendo aplicado em diferentes tipos de solos e obteve uma ótima taxa de sobrevivência a diferentes condições de temperatura, condições hídricas e concentrações de matéria orgânica no solo (Larena et al. 2014).

Além dos metabólitos secundários com atividade biológica de interesse agrícola, existem espécies de *Penicillium* endofíticas que produzem metabólitos com alto nível de diversidade química de interesse farmacológico, como *Penicilium chrysogenum* isolada de *Fargonia cretica*, capaz de controlar células tumorais humanas (Gao et al. 2017). *Penicillium* sp. isolado a partir de tubérculos de *Pinellia ternata* produz metabólitos secundários com propriedades antifúngicas contra micro-organismos patógenos de humanos (*Staphylococcus aureus* e *Pseudomonas aeruginosa*) (Yang et al. 2017).

A floresta amazônica é o maior centro de biodiversidade mundial e as espécies *Hevea brasiliensis* e *Hevea guianensis* são endêmicas da floresta amazônica brasileira e cultivadas comercialmente em outras regiões do Brasil e em países do continente asiático (Gasparroto et al. 2012). Essas espécies de plantas, conhecidas como seringueiras, apresentam grande importância econômica por serem produtoras de látex natural (Gasparroto et al. 2012; Rocha et al. 2011). Em estudos anteriores sobre a diversidade de fungos endofíticos em seringueiras da floresta amazônica brasileira foi constatada uma grande abundância de isolados do gênero *Penicillium*, principalmente em suas raízes

(Capítulos 1 e 2). Por causa disso, este estudo visa à identificação de espécies de *Penicillium* endofíticas de seringueiras da floresta amazônica brasileira utilizando a análise filogenética multilocus, a análise da variabilidade genética e a avaliação do potencial antimicrobiano destes isolados para o controle de diferentes fitopatógenos.

MATERIAL E MÉTODOS

Isolados e meios de cultura

Os fungos endofíticos pertencentes aos gêneros *Penicillium* e *Talaromyces* foram isolados das folhas, caules e raízes das seringueiras, *H. brasiliensis* e *H. guianensis*, sadias e nativas de duas Bacias hidrográficas da Floresta Amazônica Brasileiras localizadas no estado do Acre e no estado do Amazonas (Tabela 1) (Capítulos 1 e 2). Os fungos foram cultivados em BDA (17 g/L de ágar; 20 g/L de dextrose e 200g/L de batata) ou YMC (10 g de extrato de malte, 2 g de extrato de levedura, 15 g de ágar dissolvidos em 1L de água destilada e, posteriormente esterilizado em autoclave), de acordo com o experimento, a temperatura de 25°C, com fotoperíodo de 12h. Os isolados fazem parte da Micoteca do Laboratório de Genética Micoteca do Laboratório de Genética Molecular de Fungos – BIOAGRO – CaMpus UFV – Viçosa/MG (Brasil).

Extração de DNA, amplificação e análise filogenética

Os micélios dos isolados foram cultivados em meio YMC por sete dias e transferidos para tubos com 0,2 mL de *beads* de vidro (425 a 600 µM). Para a extração do DNA fúngico foi utilizado o kit de extração Wizard® Genomic DNA Purification Kit (Promega) conforme Pinho et al. (2012). A integridade do DNA extraído foi verificada por eletroforese em gel de agarose 0,8%, fotodocumentada (Locus) e estimada a concentração e a pureza do DNA por espectrofotometria (Nanodrop 2000, Thermo Scientific).

As sequências dos *primers* iniciadores usados para a amplificação de cada região gênica [ITS e genes *benA* (β-tubulina), *caM* (calmodulina) e *RPB2* (subunidade maior da RNA polimerase II)] estão descritas na tabela 2 e os

respectivos ciclos de amplificação estão inclusos na tabela 3. Para a reação de PCR (*Polymerase Chain Reaction*) foram utilizados 5 µM do primer Forward e 5 µM do primer Reverse, 50 ng do DNA da amostra, 25 mM de $MgCl_2$, 10 mM de dNTP, uma unidade de GoTaq®Green MasterMix 2X (Promega,WI,USA) e 9,25 µL de água ultra pura para constituir o total de 25 µL da reação. Os produtos da PCR foram purificados e sequenciados pela empresa Macrogen, Korea. Os eletroferogramas das sequências obtidas foram analisados pelo programa Geneious 8.0.4. As sequências consenso foram comparadas com o banco de dados GenBank, do National Center for Biotechnological Information, NCBI, utilizando o algoritmo local de sequências nucleotídicas (Blastn).

Para a análise filogenética, as sequências obtidas foram alinhadas no software MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0 (Tamura et al. 2013). A análise filogenética individual da região ITS do rDNA e de cada região gênica (*benA*, *caM* e *RPB2*) e a análise concatenada/multilocus dessas regiões foram realizadas por meio da Inferência Bayesiana (IB) utilizando o programa MrBayes 3.2.1 (Huelsenbeck & Ronquist 2001). O melhor modelo evolutivo utilizado em cada gene ou locus foi selecionado pelo programa MrModeltest v2.3 (Nylander, 2004) baseado no menor valor Akaike Information Criterion (AIC). O modelo evolutivo escolhido para as sequências das regiões ITS e do gene *benA* foi GTR+I+G, e para as sequências do gene caM foi SYM+G e do gene *RPB2* foi SYM+I+G. A análise filogenética foi realizada com duas corridas independentes com quatro cadeias Markovianas Monte Carlo (MCMC) rodadas por 10.000.000 gerações e as árvores foram amostradas e retidas a cada 1000 gerações. As primeiras amostras de árvores (1.000.000) foram descartadas na fase de *burnin* e as árvores remanescentes foram sumarizadas para gerar uma árvore consenso da maioria.

Os valores de probabilidade à *posteriori* da IB, foram adicionados aos seus respectivos ramos na árvore encontrada pela IB. Os valores de probabilidade *a posteriori* abaixo de 95 % foram omitidos. As sequências de referências e tipos dos *Penicillium* e *Talaromyces* analisados neste estudo depositadas no GenBank estão representados na tabela S1.

Variabilidade genética

A análise da variabilidade genética entre os isolados de *Penicillium* spp. e *Talaromyces* spp. foi realizada pelas técnicas de IRAP (*Inter-Retrotransposon Amplified Polymorphism*) e REMAP (*Retrotransposon-Microsatellite Amplified Polymorphism*) (Kalendar et al. 1999). Para as reações de PCR foram utilizados 2,5 mM de $MgCl_2$, 0,4 mM de dNTP, 0,2 µM cada primer, 50 ng de DNA, uma unidade de GoTaq®Green MasterMix 2X (Promega, WI,USA) para o volume final de 25 µL. Os amplicons foram separados por eletroforese em gel de agarose (1,5 %), com o uso do marcador 1kb DNA Lander (Promega). Os *primers* usados estão descritos na tabela 2 e os respectivos ciclos de amplificação estão inclusos na tabela 3. Foram consideradas para análise somente as bandas que apresentaram reprodutibilidade nas três repetições de amplificações. As bandas correspondentes para cada combinação de *primers* usadas na amplificação foram identificadas pela presença (1) e ausência (0). O dendrograma (bootstrap com 1,000 replicatas) foi construído pelo método de UPGMA (Unweighted Pair Group Method using Arithmetic averages), utilizando o pacote pvclust implementado no programa R versão 2.14 (R development Core Team, 2007).

Potencial antimicrobiano por cultura pareada

A capacidade dos isolados de *Penicillium* spp. e *Talaromyces* spp. endofíticos das seringueiras de inibir o crescimento dos fitopatógenos *Colletotrichum karstii* e *Colletotrichum laticiphilium* (causadores de antracnose na seringueira), *Colletotrichum lindemuthianum* (causador de antracnose no feijoeiro) e *Fusarium verticillioides* (causador da podridrão da espiga e infecta sementes e plântulas do milho) foi testada pelo método de cultura pareada descrito por Dennis & Webster (1971). Primeiramente, os isolados de *Penicillium*, *Talaromyces* e os fitopatógenos foram cultivados separadamente em meio Batata-Dextrose-Ágar – BDA por cinco e sete dias a 28 °C, respectivamente. Em seguida, os fungos endofíticos e os fitopatógenos foram inoculados em lados opostos da placa de Petri de 90 mm com 20 mL de meio de cultura BDA. Neste processo, foram inoculados um disco de micélio do fungo fitopatogênico (5 mm de diâmetro) no meio de cultura na extremidade da placa. Após 48 horas, foi inoculado um disco de micélio do fungo endofítico (5 mm de diâmetro) no meio

de cultura na extremidade oposta ao do fitopatógeno. As placas foram incubadas em BOD a 25°C, fotoperiodo de 12h. Placas inoculadas com o fungo fitopatogênico sem a presença do fungo endofítico foram usadas como controle negativo. A determinação da inibição foi realizada pela mensuração do diâmetro da colônia do fitopatógeno até que o controle atingisse o máximo de crescimento em placa. A porcentagem de inibição do crescimento micelial dos fungos fitopatogênicos na presença dos endofíticos antagonistas foi calculado pela seguinte fórmula: (DM – dm) / DM x 100, onde DM refere-se ao diâmetro médio das colônias de três replicatas do fungo fitopatógeno na ausência do endofítico (controle), e dm o diâmetro médio da colônia das três replicatas na presença do fungo endofítico (Whipps, 1987). Os valores de diâmetro foram mensurados em milímetros, submetidos à análise ANOVA e as médias comparadas pelo teste de Tukey a 5% de significância, utilizando o software R (R Core Team, 2017).

RESULTADOS

O total de 47 fungos endofíticos pertencentes ao gênero *Penicillium* e três isolados do gênero *Talaromyces* recuperados dos tecidos de *H. brasiliensis* e *H. guianensis* foram identificados, analisados quanto a variabilidade genética e a capacidade de inibição de diferentes fitopatógenos.

Análise filogenética

A identificação molecular dos isolados utilizando as sequências da região ITS e dos genes *benA*, *caM* e *RPB2* concatenadas revelou 13 espécies de *Penicillium* e três espécies de *Talaromyces*. Do total de espécies de *Penicillium* endofíticas isoladas, possivelmente sete são espécies novas (Figuras 1, 2, 3, 4 e 5).

As espécies obtidas neste estudo foram agrupadas em 11 clados na árvore da sequência ITS (Figura 1), em 14 clados nas árvores das sequências gênicas *benA* (Figura 2), *caM* (Figura 3) e em 15 clados nas árvores das sequências do gene *RPB2* (Figura 4) e na árvore concatenada (Figura 5), todos altamente suportados, com valores de probabilidade à *posteriori* da IB próximo ou igual a 1.00.

Em todas as árvores, foi verificado que o clado pertencente a espécie *Penicillium citrinum* agrupou o maior número de isolados, representando 52 % do total de isolados analisados (Figuras 1, 2, 3, 4 e 5). Entretanto, o gene *caM* dos isolados 629F14F-AM, 756F8R-AC e 767F20C-AM identificados como *P. citrinum* não foi amplificado, mesmo após várias tentativas e mudanças no programa usado na PCR. Os demais isolados foram identificados como *Penicillium crustosum* (37F27C-AM e 314F2C-AM), *Penicillium steckii* (667F7R-AM), *Penicillium sumatrense* (197F3C-AC), *Penicillium shearii* (637F5R-AM), *Penicillium paxilli* (7F5R-AM), *Talaromyces cnidii* (86F10R-AM), *Talaromyces thailandensis* (623F7R-AM) e *Talaromyces amestolkiae* (705F18F-AC) (Figura 5).

Ao analisar as árvores individuais foi observado que os isolados 54F2C-AM e 203F2C-AC formam um clado independente e com probabilidade à *posteriori* igual a 1,00. Dessa forma, é possível que estes isolados constituam uma espécie nova estritamente relacionada as espécies *Penicillium adametzioides* e *Penicillium angulare*, como é constatado em todas as árvores, e as espécies *Penicillium austrosinicum* e *Penicillium maximae*, quando é analisada as árvores da região ITS e dos genes *benA* e *caM* (Figuras 1, 2 e 3).

Os isolados 213F5C-AC e 728F5C-AC formaram um clado independente e relacionado as espécies *P. maximae* e *P. austrosinicum* na árvore do gene *caM* (Figura 3) e na árvore concatenada, e com as espécies *P. austrosinicum* e *Penicillium multicolor* na árvore do gene *RPB2* (Figura 4) com probabilidade à posteriori próxima a 1,00. Apesar destes isolados agruparem com o isolado da espécie *P. maximae* e *P. austrosinium* na árvore da região ITS e que o isolado 213F5C-AC pertença ao mesmo clado do *P. maximae* na árvore do gene *benA*, é sugerido que os isolados 213F5C-AC e 728F5C-AC constituem uma espécie nova.

Embora o isolado 510F1C-AM agrupe com o isolado da espécie *Penicillium rolfsii* na árvore do gene *RPB2* e este gene não tenha sido amplificado para a devida análise do isolado 473F8R-AM, é sugerido que estes isolados constituem uma espécie nova. Estes isolados formam clados independentes e altamente suportados nas árvores da região ITS e dos genes *benA* e *caM*, e na árvore concatenada.

Os resultados observados nas árvores da região ITS (Figura 1), do gene *RPB2* (Figura 4) e na árvore concatenada (Figura 5), sugerem que o isolado 507F5R-AM é uma espécie nova e que o clado independente composto pelos isolados 658F5R-AM e 641F18F-AC constituem uma outra espécie nova, ambas estritamente relacionadas com as espécies *Penicillium cataractum* e *Penicillium mariae-crucis*. Contudo, os isolados 507F5R-AM e 658F5R-AC formaram um clado independente e estritamente relacionado com a espécie *P. cataractum* na árvore do gene *benA* e o isolado 641F18F-AC agrupou com a espécie *P. cataractum*. Ao analisar a árvore do gene c*aM*, verifica-se que os isolados 507F5R-AM, 658F5R-AC e 641F18F-AC formaram clados e agruparam com o isolado da espécie *P. mariae-crucis*. Assim, é necessário a realização de uma abordagem mais detalhada para a determinação destes isolados como espécies novas.

Na árvore concatenada, os isolados 40F4C-AC, 196F16C-AC, 657F18C-AC formaram um clado independente com probabilidade à *posteriori* igual a 1.00 e estritamente relacionados com os isolados da espécie *Penicillium meleagrinum*, podendo estes isolados constituírem uma espécie nova. Assim como estes fungos endofíticos, os isolados 46F7C-AC e 646F5R-AC podem constituir uma espécie nova por formarem um clado independente com probabilidade à *posteriori* igual a 1.00, porém estritamente relacionados com isolados da espécie *P. sumatrense*. Esses resultados foram, também, verificados na análise das sequências do gene *RPB2* e do gene *caM* em relação aos isolados 46F7C-AC e 646F5R-AC, porém estes não foram devidamente separados dos clados das espécies *P. meleagrinum* e *P. sumatrense,* dentro das demais regiões gênicas analisadas.

Variabilidade gênica

O total de 44 loci foram amplificados, 23 loci para o IRAP e 21 loci para o REMAP. A combinação dos perfis eletroforéticos gerados produziu 21 loci e foi observado 100% de polimorfismo. Em relação à diversidade gênica de Nei (H_E), a média foi 0,2643.

Os agrupamentos observados no dendograma foram bem suportados, tendo apenas um clado com o valor igual à 65 e os demais clados apresentaram uma variação entre o valor mínimo de bootstrap igual à 74 e o valor máximo de

bootstrap igual à 100. O total de 50 fungos endofíticos analisados foi dividido em dois grupos principais com valores de bootstrap iguais à 95 e 94 (Figura 6). Dentro do primeiro grupo pode ser observada a formação de clados que agruparam os isolados 667F7R-AM e 7F5R-AM, identificados pela análise filogenética como *P. steckii* e *P. paxilli* com bootstrap igual à 1,0 (Figura 5). Embora, sejam de espécies diferentes estes isolados apresentaram algumas bandas correspondentes, principalmente no perfil eletroforético de IRAP (Figura 7). Ainda no primeiro grupo, pode-se observar a formação de clados que agruparam isolados das espécies *P. maximae* (728F5C-AC e 213F5C-AC), *P. sumatrense* (46F4C-AC, 646F5R-AC e 197F3C-AC) e *P. meleagrinum* (40F4C-AC, 657F18C-AC, 196F16C-AC) e espécies pertencentes ao gênero *Talaromyces* (623F7R-AM, 705F18F-AC e 86F10R-AM), cujos perfis eletroforéticos de IRAP e REMAP podem ser observados e comparados nas figuras 7 e 8.

O segundo grupo é composto por um grande clado bem suportado que agrupa 26 isolados identificados como *P. citrinum* (Figura 6). Dentro do grupo do *P. citrinum* pode-se verificar a formação de quatro grupos de isolados com perfis de bandas mais similares na amplificação do IRAP (Figura 7). Os isolados 174F9R-AC, 767F20C-AM e 643F20C-AM apresentam perfis bandas do IRAP mais similares e isso pode ser verificado na figura 7, ao analisar as suas respectivas posições no gel: 02, 09 e 10. Outro clado formado de espécies de *P. citrinum* é composto pelos isolados 634F20C-AM, 767F20C-AM, 174F9R-AC, 294F7F-AC, 171F9R-AC, 211F7F-AC, 287F18F-AM, 756F8R-AC, 202F8R-AC, 755F8R-AC, 694F8R-AC, 752F8R-AC, 198F11R-AC, 629F14F-AM, 753F8R-AC, 689F9R-AC, 798F8R-AC, 360F5R-AC, 524F11R-AC e 562F11R-AC.

Os isolados 634F20C-AM e 767F20C-AM podem ser o mesmo fungo, por serem da mesma espécie e terem sidos isolados do mesmo tecido da mesma planta. O mesmo é verificado para os isolados 294F7F-AC e 211F7F-AC, os isolados 756F8R-AC, 202F8R-AC, 755F8R-AC, 694F8R-AC, 752F8R-AC, 753F8R-AC e 798F8R-AC, bem como para os isolados 171F9R-AC, 689F9R-AC, e os isolados 198F11R-AC, 524F11R-AC e 562F11R-AC.

Os isolados que compõem o clado da espécie *P. maximae* (728F5C-AC e 213F5C-AC) podem ser o mesmo fungo, assim como os isolados da espécie *P. adametzoides* (54F2C-AM e 203F2C-AM) (Figura 6).

Atividade antimicrobiana

Os isolados de *Penicillium* foram capazes de inibir o crescimento dos fitopatógenos *C. karstii*, *C. laticiphilum*, *C. lindemuthianum* e *F. verticillioides* (Figuras 9, 10, 11, 12 e 13).

Conforme a comparação das médias de cada tratamento pelo teste tukey a 5% de significância, os isolados 473F8R-AM e 510F1C-AM (possível espécie nova estritamente relacionada com *P. rolfsii*) foram os isolados que promoveram maior porcentagem de inibição em *C. karstii*, 55% e 54,86%, respectivamente (Figura 9). Pode ser observado que um pequeno halo de inibição foi formado e que existe competição por espaço e nutrientes entre os isolados, com maior taxa de crescimento do fungo endofítico em comparação ao fitopatógeno (Figura 10).

Os isolados 198F11R-AC (*P. citrinum*), 211F7F-AC (*P. citrinum*) e 705F18F-AC (*T. amesttolkiae*) também inibiram o *C. karstii* em alta porcentagem, 47,34%, 46,91% e 49,37%, respectivamente (Figura 9). Os isolados 198F11R-AC e 705F18F-AC afetaram o crescimento de *C. karstii* por competição, produzindo um pequeno halo de inibição entre eles (Figura 10).

Os isolados 171F9R-AC (*P. citrinum*), 197F3C-AC (*P. sumatrense*), 202F8R-AC (*P. citrinum*), 212F14F-AC (*P. citrinum*), 294F7F-AC (*P. citrinum*), 314F2C-AM (*P. crustosum*), 360F5R-AC (*P. citrinum*), 37F27C-AM (*P. crustosum*), 46F4C-AC (*P. sumatrense*), 562F11R-AC (*P. citrinum*), 564F2R-AC (*P. citrinum*), 623F7R-AM (*T. thailandensis*), 641F18F-AC (*P. cataractum*), 646F5R-AC (*P. sumatrense*), 657F 18C-AC (*P. sumatrense*), 689F9R-AC (*P. citrinum*), 694F8R-AC (*P. citrinum*), 752F8R-AC (*P. citrinum*), 753F8R-AC (*P. citrinum*), 756F8R-AC (*P. citrinum*), 86F10R-AM (*T. siamensis*) apresentaram efeito antagônico ao *C. karstii* com porcentagens de inibição entre 37,98% a 45,29% (Figura 9). Sendo que os isolados 623F7R-AM e 689F9R-AC reduziram o crescimento do fitopatógeno por inibição e competição por espaço e nutrientes e os isolados 314F2C-AM e 37F27C-AM competiram por espaço e nutrientes (Figura 10).

Os isolados 174F9R-AC (*P. citrinum*), 196F16C-AC e 40F4C-AC (possíveis espécies novas estritamente relacionadas a *P. meleagrinum*), 213F5C-AC (possível espécie nova estreitamente relaciona a *P. maximae*), 287F18F-AM (*P. citrinum*), 507F5R-AM (*P. cataractum*), 524F11R-AC (*P.*

citrinum), 624F12R-AC (*P. citrinum*), 629F14F-AM (*P. citrinum*), 652F22C-AM (*P. citrinum*), 658F5R-AM (*P. cataractum*), 728F5C-AC (espécie nova estritamente relacionada a *P. maximae*), 753F8R-AC (*P. citrinum*), 755F8R-AC (*P. citrinum*), 798F8R-AC (*P. citrinum*), 7F5R-AM (*P. paxilli*) produziram halo de inibição (Figura 10) e conforme a análise estatística estes isolados inibiram em 32,40 % a 37,21% o crescimento do fitopatógeno (Figura 10).

Já os isolados 54F2C-AM e 203F2C-AM (possíveis espécies novas estreitamente relacionadas a *P. adametzoides*), 298F4R-AM (*P. citrinum*), 477F10R-AM (*P. citrinum*), 634F20C-AM (*P. citrinum*), 637F5R-AM (*P. shearii*), 667F7R-AM (*P. steckii*), 684F9C-AM (*P. citrinum*), 767F20C-AM (*P. citrinum*), embora tenham produzidos halos de inibição contra o fitopatógeno *C. karstii* (Figura 10), apresentaram um menor percentual de taxa de inibição que os demais isolados, variando entre 23,91% a 31,38%, que os demais isolados (Figura 9).

Todos os isolados do gênero *Penicillium* apresentaram efeito antagônico ao *C. laticiphilium* (Figuras 9 e 11). Os isolados que apresentaram resultados mais significativos em relação ao antagonismo foram 473F8R-AM e 510F1C-AM (possível espécie nova restritamente relacionada com *P. rolfsii*), 86F10R-AM (*T. siamensis*) e 37F27C-AM (*P. crustosum*), tendo o percentual de inibição do crescimento do fitopatógeno variado entre 63,68% a 51,61% (Figura 9). Entretanto, assim como em *C. karstii*, os isolados 473F8R-AM, 510F1C-AM e 37F27C-AM apresentaram uma taxa de crescimento superior em relação ao *C. laticiphilium* e produziram um pequeno halo de inibição (Figura 11).

Em relação ao *C. laticiphilium*, os isolados representados no gráfico pela letra “b” promoveram um percentual de inibição variando entre 34,26% e 46, 78% (Figura 9). Entretanto, deste grupo, é válido ressaltar que os isolados 171F9R-AC, 202F8R-AC, 212F14F-AC, 287F18F-AM, 298F4R-AM, 562F11R-AC, 624F12R-AC, 629F14F-AM, 634F20C-AM, 652F22C-AM, 694F8R-AC, 753F8R-AC da espécie *P. citrinum*, o isolado 641F18F-AC da espécie *P. cataractum* e 667F7R-AM da espécie *P. steckii* produziram halo de inibição (Figura 11). Além destes isolados, os fungos 211F7F-AC, 294F7F-AC, 756F8R-AC (*P. citrinum*) e os isolados 524F11R-AC e 798F8R-AC pertencentes ao grupo dos fungos representados pela letra “c” e “d”, respectivamente, embora tenham promovido uma menor redução do crescimento do fitopatógeno em comparação aos outros

isolados, produziram halo de inibição e perceptível a zona escura na extremidade das hifas do fitopatógeno.

Em relação ao fitopatógeno *C. lindemunthianum*, embora todos os isolados tenham conseguido reduzir o crescimento do fitopatógeno por inibição, os fungos endofíticos 198F11R-AC, 756F8R-AC e 684F9C-AM, pertencentes a espécie *P. citrinum*, e 473F8R-AM e 510F1C-AM (possível espécie nova estritamente relacionada com *P. rolfsii*) inibiram fortemente o crescimento do *C. lindemunthianum* (Figura 12), promovendo uma redução no seu crescimento de 85,42%, 84,34%, 84,34%, 85,75% e 88,21%, respectivamente (Figura 9). Entretanto, os isolados 212F14F-AC, 314F2C-AM, 641F18F-AC, 646F5R-AM, 755F8R-AC e 728F5C-AC foram retirados da análise por produzirem atividade inibitória ao *C. lindemunthianum* com variações bem diferentes entre as repetições, necessitando da realização de mais testes com estes isolados. O mesmo ocorreu com os isolados 212F14F-AC e 728F5C-AC nos ensaios com o fitopatógeno *F. verticillioides*.

Os isolados 46F4C-AC (*P. sumatrense*), 174F9R-AC (*P. citrinum*), 213F5C-AC (possível espécie nova estreitamente relaciona a *P. maximae*), 507F5R-AM (*P. cataractum*), 623F7R-AM (*T. thailandensis*), 634F20C-AM (*P. citrinum*), 641F18F-AC (*P. cataractum*), 646F5R-AC (*P. sumatrense*), 658F5R-AM (*P. cataractum*) e 798F8R-AC (*P. citrinum*) produziram halo de inibição reduziram em 31,76%, 33,08%, 29,14%, 33,61%, 32,08%, 31,04%, 39,57%, 29,23%, 38,29% e 28,44% o crescimento do *F. verticillioides* (Figuras 9 e 13).

DISCUSSÃO

Identificação molecular dos fungos endofíticos do gênero *Penicillium* e *Talaromyces*

Neste estudo foram identificados filogeneticamente 11 espécies de fungos endofíticos pertencentes ao gênero *Penicillium* e três espécies do gênero *Talaromyces* isolados das folhas, caule e raízes de *H. guianensis* e *H. brasiliensis*. De acordo com os dados apresentados, são sugeridas sete espécies novas de *Penicillium* (Figuras 1, 2, 3, 4 e 5) que devem ser caracterizadas de forma mais detalhada.

Embora os fungos do gênero *Penicillium* apresentem características morfológicas bem definidas e que dão significado ao seu nome por apresentarem aparência semelhante ao um “pequeno pincel” (Link, 1809), a identificação a nível de espécie pode ser confusa e trabalhosa. O advento dos estudos moleculares para a classificação de espécies do gênero *Penicillium* tem facilitado os pesquisadores no estudo dos subgêneros e das seções que compõem o gênero (Berbee, 1995; Houbraken et al. 2010; 2012; Houbraken & Samson, 2011; Visagie et al. 2013; 2014; 2016). Além disso, existe a perspectiva de agregar ao gênero as suas formas teleomórficas com o objetivo de alcançar a meta estabelecida no Código Internacional de Nomenclatura em Botânica (ICBN), em 2011, de “um fungo: um nome” (Norvel, 2011).

A reconstrução filogenética realizada foi bem suportada (Figuras 1, 2, 3, 4 e 5), e a identificação das espécies dos fungos endofíticos em estudo foi baseada na análise das sequências da região ITS (Figura 1), dos genes *benA* (Figura 2), *caM* (Figura 3) e *RPB2* (Figura 4) e na análise concatenada/multilocus dessas sequências (Figura 5). Primeiramente, pode ser observado em todas as árvores filogenéticas que as espécies do gênero *Talaromyces* foram acomodadas em um clado monofilético e independente do clado das espécies de *Penicillium*.

Os gêneros *Talaromyces* e *Eupenicillium* eram tidos como teleomorfos do gênero *Penicillium*, mas com os estudos moleculares, eles são agora considerados na nova nomenclatura como sinônimos de *Penicillium* sensu stricto (Houbraken & Samson, 2011). Samson et al. (2011) demonstraram que os membros do subgênero *Biverticillium* e *Talaromyces*, espécies com conidióforo biverticilado simétrico e fiálides lanceoladas, são acomodados em um clado monofilético e que espécies dos subgêneros *Aspergiloides*, *Furcatum* e *Penicillium* formam um clado independente.

As espécies do gênero *Talaromyces* identificadas foram *Talaromyces thailandensis* (623F7R-AM) e *Talaromyces cnidii* (86F10R-AM) isolados das raízes de *H. brasiliensis* da floresta amazônica do estado do Amazonas, e *Talaromyces amestolkiae* (705F18F-AC) isolado das folhas de *H. brasiliensis* da floresta amazônica no estado do Acre (Figuras 1, 2, 3, 4 e 5).

Em relação aos fungos endofíticos pertencentes ao gênero *Penicillium*, foram identificadas espécies das seções *Fasciculata* (*Penicillium crustosum*),

Lanata-divaricata (*Penicillium cataractum* e *Penicillium rolfsii*), *Sclerotiora* (*Penicillium angulare* e *Penicillium maximae*) e *Citrina* (*Penicillium citrinum*, *Penicillium steckii*, *Penicillium meleagrinum*, *Penicillium sumatrense*, *Penicillium shearii* e *Penicillium paxilli*). Isso foi verificado nas árvores de todas as regiões gênicas e na árvore concatenada. Sendo que a seção *Fasciculata* pertence ao subgênero *Penicillium* e as seções *Lanata-divaricata*, *Sclerotiora* e *Citrina* pertencem ao subgênero *Aspergilloides* (Houbraken & Samson, 2011).

Houbraken & Samson (2011) estudaram a relação filogenética entre *Penicillium* e outros membros da família Trichocomaceae, utilizando a análise multilocus e o princípio "um fungo: um nome", eles segregaram os gêneros pertencentes à Trichocomaceae dentro de três famílias, sendo que a primeira família foi nomeada de Aspergillaceae, a qual pertence o gênero *Penicillium*. Estes pesquisadores verificaram que a família Aspergillaceae foi dividida em sete clados, dentre estes o clado pertencente ao gênero *Penicillium*, denominando este grupo de *Penicillium* sensu stricto. O clado *Penicillium* sensu stricto foi dividido em dois subclados, cujo primeiro acomoda os subgêneros *Aspergilloides* e *Furcatum*, e o segundo é constituído pelo subgênero *Penicillium* (Houbraken & Samson, 2011). Esses achados corroboram com a nossa pesquisa, uma vez que ocorreu a formação de dois clados dentro do grupo do *Penicillium*, segregando os fungos do subgênero *Penicillium* e do subgênero *Aspergilloides*, nas árvores das regiões do gene *benA* (Figura 2) e *RPB2* (Figura 4), e na árvore concatenada (Figura 5).

A maioria dos fungos endofíticos do gênero *Penicillium* isolados das seringueiras pertencem ao subgênero *Aspergilloides*, abrangendo 95,74% (45 isolados) do total destes fungos. Dentro do subgênero *Aspergilloides*, a seção de maior destaque foi a *Citrina* por representar 76,60% (36 isolados) dos fungos do gênero *Penicillium* isolados, sendo que *P. citrinum* compõem 57,45% (27 isolados) das espécies de *Penicillium* obtidas nesta pesquisa. Embora *P. citrinum* seja relatado na literatura como uma espécie endofítica (Khan et al. 2008; Ting et al. 2012), ela não foi isolada das folhas e do caule de seringueiras peruanas (Gazis & Chaverri, 2010). Já Ferreira et al. (2015), obtiveram dois isolados da espécie *P. citrinum* ao estudar a diversidade de fungos endofíticos em *Vellozia gigante*, endêmica de campos rupestre no Brasil.

Neste estudo foram identificados isolados das espécies *P. sheari*, *P. citrinum*, *P. sumatrense*, *P. paxilli*, *P. crustosum*, *P. steckii*, *T. siamensis*, *T. thailandesis*, *T. amestolkiae* e sete possíveis espécies novas estritamente relacionadas as espécies *P. rolfsii*, *P. cataractum*, *P. adametzoides*, *P. maximae*, *P. meleagrinum*, *P. sumatrense*. Gazis & Chaverri (2010) isolaram 33 fungos pertencentes ao gênero *Penicillium* e não foram isoladas espécies do gênero *Talaromyces*. As espécies de *Penicillium* endofíticas obtidas das seringueiras peruanas foram *Penicillium paxilli*, *Penicillium sclerotiorum*, *Penicillium brevicompactum*, *Penicillium aculeatum*, *Penicillium* aff. *glabrum*, *Penicillium* aff. *spinulosum*, *Penicillium chrysogenum* e *Penicillium meleagrinum*.

No estudo de diversidade de fungos endofíticos isolados dos galhos de *Pinus sylvestris* L. no norte da Espanha foram obtidas as espécies *Penicillium glabrum*, *Penicillium melinii*, *Penicillium polonicum*, *Penicillium minioluteum* e não foram obtidas espécies do gênero *Talaromyces* (Sanz-ros et al. 2015). Ao comparar as espécies obtidas neste estudo com as espécies do gênero *Penicillium* encontradas em *Pinus sylvestris* L. no norte da Espanha foi verificada um perfil de espécies diferente, assim como foi verificado nas espécies encontradas nas seringueiras peruanas. Provavelmente, a localização geográfica e a competição interespecífica entre os fungos podem ter corroborado para a obtenção de perfis de espécies de *Penicillium* diferentes entre os hospedeiros, sobretudo entre as seringueiras brasileiras e peruanas.

Anteriormente, nós usamos as sequências ITS para a análise de todos os isolados endofíticos das seringueiras (capítulos 1 e 2), mas para uma análise mais detalhada dentro do gênero *Penicillium*, nós resolvemos empregar outras sequências indicadas para o gênero. A sequência do gene *benA* é frequentemente utilizado como um barcode secundário para identificar *Penicillium* a nível de espécie, por ser uma região de fácil amplificação e capaz de distinguir as espécies estreitamente relacionadas (Visagie et al. 2014; Laich & Andrade 2018). Outros possíveis marcadores secundários que podem auxiliar na identificação dos isolados de *Penicillium* a nível de espécie são os genes *caM* e *RPB2*, pois apresentam o mesmo poder discriminatório semelhante ao *benA*, porém o *RPB2* tem uma vantagem adicional da falta de íntrons no *amplicon*, permitindo um alinhamento robusto quando utilizado na filogenia. Entretanto, as vezes é difícil à amplificação dos genes c*aM* e *RPB2* (Visagie et al. 2014). Isso

foi verificado neste estudo, uma vez que os isolados 756F8R-AC, 629F14F-AM e 767F20C-AM não tiveram o seu gene *caM* amplificado e não houve a amplificação do gene *RPB2* do isolado 473F8R-AM. Assim, Visagie et al. (2014) sugerem a utilização do ITS, *benA*, *CaM* e *RPB2* numa análise multilocus para a identificação de espécies novas e conforme os resultados apresentados na árvore concatenada, os isolados mencionados anteriormente podem ser espécies novas, necessitando da caracterização morfológica destes para a afirmação dessa hipótese.

O conjunto de dados com sequências incompletas é um fator limitante para a determinação de uma espécie nova de *Penicillium* (Visagie et al. 2014). Assim, a maioria dos estudos descrevem e caracterizam as espécies de *Penicillium* utilizando a identificação filogenética multigênica, a caracterização morfológica e a descrição do perfil de extrólitos (metabolitos secundários) produzido por cada isolado (Houbraken et al. 2010; 2011; 2016; Visagie et al. 2013; 2014; 2016; Perrone et al. 2015; Rong et al. 2015).

As possíveis espécies novas de *Penicillium* deste estudo pertencem as seções *Citrina*, *Sclerotiora* e *Lanata – divaricata*. *Penicillium* da seção *Citrina* são identificados por meio da análise das sequências da região ITS e do gene *RPB2* e caracteres fenotípicas, em que compartilham a produção de conidióforos simetricamente biverticilados, fialídes em forma de frasco (7,0 – 9,0 µm de comprimento) e conídios relativamente pequenos (2,0-3,0 µm de diâmetro). Algumas espécies podem produzir cleistotécio de cor acastanhada contendo ascosporos flangeados. Os caracteres fenotípicos mais importantes para distinguir as espécies são as taxas de crescimento em temperaturas diferentes e as cores reversas da colônia no meio de ágar CYA, MEA e YES; forma, tamanho e ornamentação de conídios e a produção de esclerócios ou cleistotécio. Os padrões de extrólitos e as sequências parciais de *caM* e *benA* podem ser usadas para identificação baseada em sequências de DNA (Houbraken & Samson, 2011; Houbraken et al., 2011).

As espécies novas da seção *Sclerotiora* são determinadas pelas características macro e micromorfológicas em quatro meios de crescimento padrão (CYA, MEA, YES e CZ) e a filogenia é realizada com base nos dados de sequência das regiões ITS e dos genes *benA*, *caM* e *RPB2*, o que revela que todas as espécies com conidióforo biverticilados formam um subclado bem

suportado nesta seção (Houbraken & Samson, 2011; Wang et al. 2017). As espécies *P. maximae* e *P. austrosinicum* pertencem a seção *Sclerotiora* e ambas apresentam conidióforos monoverticilados (Houbraken & Samson, 2011; Visagie et al. 2013; Wang et al. 2017). As colônias da espécie *P. maximae* apresentam crescimento rápido e micélios laranja-rosados que mascaram a esporulação, principalmente em meio de cultivo MEA (Visagie et al. 2013).

Sequências da região ITS e do gene *benA* foram utilizados para identificar a espécie *P. adametzioides* (Deng et al. 2012). A espécie *P. adametzioides* pertencente a seção *Sclerotiora* e pode ser diferenciada diferencia da espécie *P. angulare*, a qual é intimamente relacionada, pela sua restrita taxa de crescimento, pela produção de pigmentos em meio CYA e, morfologicamente, pelo tamanho do seu conidióforo (Deng et al. 2012). *Penicillium angulare* apresenta conidióforos com tamanhos superiores a 60 mm de comprimento (Peterson et al. 2004).

Laich & Andrade (2016) descreveram uma nova espécie da seção *Lanata – divaricata*, *Penicillium pedernalense*, por meio da análise parcial do gene *benA* e da região ITS, por estes separarem claramente a espécie nova dentro do clado da seção, e da caracterização morfológica e fisiológica em meios MEA, CYA, CYAS, CZ, AO, CREA e DG18. A maioria das espécies dessa seção crescem rapidamente e formam colônias amplamente espalhas, são fortemente divaricatas e conidióforo monoverticilados (Houbraken & Samson, 2011). A espécie *P. caratactum* pertence a seção *Lanata – Divaricata*, como observado neste estudo, é intimamente relacionada com a espécie *P. mariae-crucis*. O que diferencia *P. mariae-crucis* da espécie *P. cataractum* é a sua produção de esclerócios e colônias marrom-avermelhadas, além disso a espécie *P. cataractum* apresenta crescimento mais restrito em meio MEA (Visagie et al. 2016).

Variabilidade genética dos isolados de *Penicillium* e *Talaromyces* por marcadores IRAP e REMAP

Esta é a primeira pesquisa que analisou a variabilidade genética de diferentes espécies de *Penicillium* por meio do uso dos marcadores IRAP e REMAP.

Na literatura são relatados estudos que analisam a variabilidade genética de espécies de *Penicillium* utilizando RAPD (*Randomly Amplified Polymorphic* DNA) e AFLP (*Amplified Fragment Length Polymorphism*) (Lund et al. 2003; Kure et al. 2003). Entretanto, as técnicas IRAP e REMAP foram escolhidas para estudar a variabilidade genética das espécies de *Penicillium* e *Talaromyces* endofíticas por apresentarem vantagens sobre outras técnicas pela simplicidade, pela versatilidade em permitir a combinação de vários *primers* que anelam regiões conservadas de retrotransposons (IRAP) ou retrotransposons e microssatélites (REMAP), pela alta reprodutibilidade por usar *primers* específicos, pela fácil manipulação por envolver a técnica de PCR e usar gel de agarose, além do baixo custo e elaboração quando comparadas as outras técnicas (Santana et al. 2012; 2012).

Neste estudo foram avaliados 47 isolados de *Penicillium* e 3 isolados de *Talaromyces* de diferentes espécies. A combinação dos *primers* foi capaz de gerar polimorfismo em todas as espécies observadas e foi eficiente para estudo da variabilidade genética intraespecífica e interespécies dessas espécies. Esses resultados corroboram com estudo realizado por Santana et al. (2012), que dentre as espécies avaliadas, observaram eficiência desses *primers* na produção de bandas em sequências de DNA do isolado *Penicillium brevicompactum* CMON28.

Em nossos estudos foram amplificados 44 loci com 100% de polimorfismo e alta variabilidade genética, resultados semelhantes foram obtidos em outros estudos que utilizaram a técnica de IRAP e REMAP com outras espécies de fungos, tais como: *Moniliophthora perniciosa* (Santana et al. 2012), *Pseudocercospora fijiensis* (Queiroz et al. 2014) e isolados endofíticos do gênero *Diaporthe* obtidos a partir de *Phaseolus vulgaris* (Santos et al. 2015).

O dendograma gerado revelou a formação de dois grandes grupos, sendo que o primeiro grupo foi composto pelas espécies *P. stecki*, *P. paxilli*, *P. autrosinicum*, *T. amestolkiae*, *T. thailandensis*, *T. cnidii*, *P. sumatrense*, *P. adametzioides*, enquanto o segundo grupo foi constituído pelas espécies *P. citrinum*, *P. rolfsii*, *P. crustosum*, *P. cataractum* e *P. shearii* (Figura 6).

É observado que os marcadores IRAP e REMAP agruparam os isolados analisados conforme as suas respectivas espécies e gêneros identificados na análise filogenética. Entretanto, no primeiro grupo há formação de um clado com

os isolados das espécies *P. steckii* e o *P. paxilli*, assim como os isolados das espécies *T. amestolkiae* e *T. thailandensis* (Figura 6).

No dendograma pode ser verificada a formação de clados com isolados obtidos do mesmo tecido, da mesma planta e do mesmo estado, como por exemplo os isolados 728F5C-AC e 213F5C-AC (*P. maximae*). Foi verificado também que o clado dos isolados de *P. citrinum* agruparam isolados das raízes da mesma planta de *H. guianensis* (202F8R-AC, 752F8R-AC, 753F8R-AC, 755F8R-AC, 756F8R-AC e 798F8R-AC) aos isolados dos diferentes tecidos de seringueiras de *H. brasiliensis* dos estados do Acre e do Amazonas. Dessa forma, é constatado que os marcadores IRAP e o REMAP agruparam os isolados da mesma espécie e mesmo gênero e não por hospedeiro (*H. brasiliensis* e *H. guianensis*), tecido do hospedeiro (folha, caule e raiz) ou local de coleta (Acre e Amazonas).

A combinação dos dados analisados de IRAP e REMAP revelaram variabilidade genética interespécies de *Penicillium* e variações intraespecíficas, conforme os clados das espécies de *Penicillium* e *Talaromyces* identificadas na árvore filogenética. Os marcadores IRAP e REMAP agruparam em um único clado os isolados das espécies *P. sumatrense* e *P. melagrinum*, cujas espécies são filogeneticamente relacionadas (Figuras 5 e 6). Sendo assim, os resultados obtidos pela análise IRAP e REMAP foram congruentes aos resultados apresentados pela a análise filogenética das espécies de *Penicillium* e *Talaromyces*.

As técnicas IRAP e REMAP mostraram ser ferramentas eficientes para o estudo da variabilidade genética interespecífica e intraespecífica em *Penicillium* e *Talaromyces*. Nossos resultados mostram que os marcadores IRAP e REMAP podem ser utilizados como ferramentas para a distinção de isolados duplicados e para o monitoramento dessas culturas a longo prazo.

Potencial antimicrobiano dos isolados de *Penicillium* e *Talaromyces* a diferentes fitopatógenos

O total de 50 fungos endofíticos pertencentes aos gêneros *Penicillium* e *Talaromyces* foram avaliados quanto ao seu potencial de inibir o crescimento dos fitopatógenos *C. karstii*, *C. laticiphilum*, *C. lindemunthianum F. verticilioides*. Deste total, 41, 39, 44 e 10 fungos endofíticos produziram halo de inibição e

reduziram o crescimento dos fitopatógenos *C. karstii*, *C. laticiphilum*, *C. lindemunthianum* e *F. verticilioides*, respectivamente. Esses fungos reduziram o crescimento dos fitopatógenos *C. karstii*, *C. laticiphilum*, *C. lindemunthianum* e *F. verticilioides* em 55% a 32,23%, 59% a 37,1%, 88,21% a 29,54%, 39,57% a 28,44%, respectivamente.

Não foi evidenciada uma correlação entre a origem dos isolados quanto ao hospedeiro (*H. guianensis* e *H. brasiliensis*), ao tecido em que o fungo foi isolado (folha, caule e raiz) e ao estado em que foi realiza as coletas das amostras (Acre e Amazonas) em relação a eficiência destes fungos na redução do crescimento dos fitopatógenos. Entretanto é válido ressaltar que a maioria dos fungos foram isolados do caule e das raízes das seringueiras e apenas os isolados 211F7F-AC (*P. citrinum*), 212F14F-AC (*P. citrinum*), 287F18F-AM (*P. citrinum*), 294F7F-AC (*P. citrinum*), 629F14F-AM (*P. citrinum*), 641F18F-AM (*P. cataractum*) e 705F18F-AC (*T. amestolkiae*) foram isolados das folhas de *H. brasiliensis*.

Como pode ser verificado nas figuras 10, 11, 12 e 13, a maioria dos isolados e todas as espécies dos fungos em estudo são potenciais agentes de controle biológico dos diferentes fitopatógenos analisados. Contudo, é importante destacar que os isolados 46F4C-AC, 174F9R-AC, 213F5C-AC, 507F5R-AM, 623F7R-AM, 634F20C-AM, 641F18F-AC, 658F5R-AM, 798F8R-AC reduziram o crescimento dos quatro patógenos em estudo e foi evidenciado a produção de halo de inibição, assim como o isolado 646F5R-AC na inibição dos fitopatógenos *C. karstii*, *C. laticiphilum*, *C. lindemunthianum* e *F. verticilioides*.

Outra importante observação a ser considerada num estudo futuro, é o fato que os isolados 198F11R-AC, 756F8R-AC e 684F9C-AM, pertencentes a espécie *P. citrinum*, e 473F8R-AM e 510F1C-AM (compõem uma possível espécie nova estritamente relacionada com *P. rolfsii*) inibiram totalmente o crescimento do *C. lindemunthianum* (Figura 12). É também verificado que os micélios dos isolados 198F11R-AC, 473F8R-AM e 510F1C-AM alcançaram o fitopatógeno, mas os isolados 756F8R-AC e 684F9C-AM inibiram o crescimento de *C. lindemuthianum* mesmo com pouco crescimento no meio de cultivo, indicando que a inibição pode ter ocorrido pela produção de compostos voláteis.

Além disso, os endofíticos que produziram halo de inibição do crescimento dos fitopatógenos em estudo podem ter produzido alguns compostos que foram difundidos no meio de cultivo podem ter gerado algum tipo de alteração na morfologia dos fitopatógenos.

A maioria dos *Penicillium* spp. e *Talaromyces* spp. testados no controle do *C. karstii*, do *C. laticiphilium* e do *C. lindemunthianum*, fitopatógenos que provocam danos a parte área dos seus hospedeiros, e do *F. verticilioides*, causador da doença podridão-da-espiga, foram isolados das raízes e do caule. Entretanto, também foram obtidos isolados de *Penicillium* e isolados de *Talaromyces* da parte aérea das seringueiras.

Todas as espécies em estudo apresentaram potencial para produção de antimicrobianos. Na literatura são relatados estudos da atuação de diferentes espécies de *Penicillium* e *Talaromyces* endofíticos e de solo rizosférico como agentes de controle biológico e promotores de crecimento vegetal. *Talaromyces tratensis* KUFA 0091 é indicado como um agente de controle biológico promissor e um bom candidato para controlar a doença mancha marrom e panícula suja no arroz. Esse fungo é capaz de controlar o crescimento dos fitopatógenos *Alternaria padwickii*, *Bipolaris oryzae*, *Curvularia lunata* (Wakk) e *Fusarium moniliforme*, em testes de cultura pareada, em testes com seu extrato bruto e em condições de casa de vegetação (Dethoup et al. 2018).

Penicillium menonorum é capaz de produzir ácido indol acético, sideróforos, solubilizadores de fosfato e aumentar a massa seca de folhas e raízes, os conteúdos da clorofila, amido e proteínas do pepino, além de provocar mudanças significativas de carbono orgânico, na disponibilização de P, e aumentar significativamente a atividade da desidrogenase, quando aplicado no solo, indicando a atividade da população microbiana no solo (Babu et al. 2015).

Murali & Amruthesh (2015) retrataram em sua pesquisa a capacidade de *Penicillium oxalicum* de produzir as enzimas quitinases e peroxidase, além de promoverem o crescimento no milheto e induzir a resistência sistêmica deste ao míldio. *Penicillium striatisporum* foi isolado da rizosfera de pimentões e apresentou efeitos antagônicos muito elevados no crescimento do micélio de *Phytophthora* spp., *Cladosporium cucumerium* e *Sclerotinia sclerotiorum* (Ma et al. 2007).

Entre as espécies de *Penicillium* endofíticas analisadas nesta pesquisa, *Penicillium citrinum* obteve maior destaque por compor 54% dos isolados em estudo. Alguns estudos retratam a sua importância no controle biológico, por atuar na supressão da mortalidade de plântulas de banana ao induzir a resistência do hospedeiro ao *Fusarium oxysporum* f. sp. *cubense* Raça 4 (FocR4) (Ting et al. 2012), na promoção do crescimento da planta, devido a produção de giberelinas, além de ser uma espécie conhecida por produzir a micotoxina citrina e enzimas que degradam celulose, como celulase, endoglucanase e xilanase (Khan et al. 2008).

As espécies de *Penicillium* e *Talaromyces* identificadas neste estudo podem ser uma alternativa promissora como agentes de controle biológico e promotores de crescimento de plantas, que associados ao desenvolvimento de tecnologias viáveis para a produção em larga escala e condições de armazenamento adaptáveis para formulações comerciais, podem contribuir para o incremento de uma agricultura sem danos ao meio ambiente e ao homem.

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ANEXO

Tabela 1. Códigos dos fungos endofíticos pertencentes aos gêneros *Penicillium* e *Talaromyces* e as suas especificações referentes ao órgão da planta, o estado e o hospedeiro que foram isolados.

Isolados	Órgão	Estado	Hospedeiro
7F5R-AM	raiz	Amazonas	*H. brasiliensis*
37F27C-AM	caule	Amazonas	*H. brasiliensis*
40F4C-AC	caule	Acre	*H. brasiliensis*
46F7C-AC	caule	Acre	*H. brasiliensis*
54F2C-AM	caule	Amazonas	*H. brasiliensis*
86F10R-AM	raiz	Amazonas	*H. brasiliensis*
171F9R-AC	raiz	Acre	*H. brasiliensis*
174F20C-AM	caule	Amazonas	*H. brasiliensis*
196F16C-AC	caule	Acre	*H. brasiliensis*
197F3C-AC	caule	Acre	*H. guianensis*
198F11R-AC	raiz	Acre	*H. brasiliensis*
202F8R-AC	raiz	Acre	*H. guianensis*

203F2C-AM	caule	Amazonas	*H. brasiliensis*
211F7F-AC	folha	Acre	*H. brasiliensis*
212F14F-AC	folha	Acre	*H. brasiliensis*
213F5C-AC	caule	Acre	*H. brasiliensis*
287F18F-AM	folha	Amazonas	*H. brasiliensis*
294F7F-AC	folha	Acre	*H. brasiliensis*
298F4R-AM	raiz	Amazonas	*H. brasiliensis*
314F2C-AM	caule	Amazonas	*H. brasiliensis*
360F5R-AC	raiz	Acre	*H. brasiliensis*
473F8R-AM	raiz	Amazonas	*H. brasiliensis*
477F10R-AM	raiz	Amazonas	*H. brasiliensis*
507F5R-AM	raiz	Amazonas	*H. brasiliensis*
510F1C-AM	caule	Amazonas	*H. brasiliensis*
524F11R-AC	raiz	Acre	*H. brasiliensis*
562F11R-AC	raiz	Acre	*H. brasiliensis*
564F2R-AC	raiz	Acre	*H. brasiliensis*
623F7R-AM	raiz	Amazonas	*H. brasiliensis*
624F12R-AC	raiz	Acre	*H. brasiliensis*
629F14F-AM	folha	Amazonas	*H. brasiliensis*
634F20C-AM	caule	Amazonas	*H. brasiliensis*
637F5R-AM	raiz	Amazonas	*H. brasiliensis*
641F18F-AC	folha	Acre	*H. brasiliensis*
646F5R-AC	raiz	Acre	*H. brasiliensis*
652F22C-AM	caule	Amazonas	*H. brasiliensis*
657F18C-AC	caule	Acre	*H. brasiliensis*
658F5R-AM	raiz	Amazonas	*H. brasiliensis*
667F7R-AM	raiz	Amazonas	*H. brasiliensis*
684F9C-AM	caule	Amazonas	*H. brasiliensis*
689F9R-AC	raiz	Acre	*H. brasiliensis*
694F8R-AC	raiz	Acre	*H. guianensis*
705F18F-AC	folha	Acre	*H. brasiliensis*
728F5C-AC	caule	Acre	*H. brasiliensis*
752F8R-AC	raiz	Acre	*H. guianensis*
753F8R-AC	raiz	Acre	*H. guianensis*
755F8R-AC	raiz	Acre	*H. guianensis*
756F8R-AC	raiz	Acre	*H. guianensis*
767F20C-AM	caule	Amazonas	*H. brasiliensis*
798F8R-AC	raiz	Acre	*H. guianensis*

Tabela 2. *Primers* utilizados neste trabalho

Locus	Primer	Direção	Sequência do Primer (5′-3′)	Referência
ITS	ITS1	Forward	TCC GTA GGT GAA CCT GCG G	White et al. 1990
	ITS4	Reverse	TCC TCC GCT TAT TGA TAT GC	White et al. 1990
BenA	Bt2a	Forward	GGT AAC CAA ATC GGT GCT GCT TTC	Glass & Donaldson 1995
	Bt2b	Reverse	ACC CTC AGT GTA GTG ACC CTT GGC	Glass & Donaldson 1995
CaM	CMD5	Forward	CCG AGT ACA AGG ARG CCT TC	Hong et al. 2006
	CMD6	Reverse	CCG ATR GAG GTC ATR ACG TGG	Hong et al. 2006
	CF1	Forward	GCC GAC TCT TTG ACY GAR GAR	Peterson et al. 2005
	CF4	Reverse	TTT YTG CAT CAT RAG YTG GAC	Peterson et al. 2005
RPB2	5F	Forward	GAY GAY MGW GAT CAY TTY GG	Liu et al. 1999
	7CR	Reverse	CCC ATR GCT TGY TTR CCC AT	Liu et al. 1999
	5Feur	Forward	GAY GAY CGK GAY CAY TTC GG	Houbraken et al. 2012
	7CReur	Reverse	CCC ATR GCY TGY TTR CCC AT	Houbraken et al. 2012
IRAP	CLIRAP1	Forward	5’ CGTACGGAACACGCTACAGA 3’	Santos et al. 2012
	CLIRAP4	Reverse	5’ CTTTTGACGAGGCCATGC 3’	
REMAP	SSR1	Reverse	5’ GAGAGAGAGAGAGAGAGAC 3’	Santos et al. 2012
	CLIRAP4	Forward	5’ CTTTTGACGAGGCCATGC 3’	

Tabela 3. Condições de amplificação (PCR) utilizadas neste trabalho

Locus	Condições de amplificação
ITS	95 ºC: 3 min; 36 ciclos (95 ºC 1 min, 51 ºC, 1 min, 72 ºC, 1 min); 72 ºC, 7 min, mantido no final a 4 °C
BenA	95 ºC: 3 min; 36 ciclos (95 ºC 1 min, 55 ºC, 1 min, 72 ºC, 1 min); 72 ºC, 7 min
CaM	95 ºC: 3 min; 36 ciclos (95 ºC 1 min, 57 ºC, 59°C ou 60°C, 1 min, 72 ºC, 1 min); 72 ºC, 7 min
RPB2	95 ºC: 3 min; 36 ciclos (95 ºC, 1 min, 55 ºC, 2 min, com aumento de 0,2 ºC por segundo para 72 ºC, 72 ºC, 1 min); 72 ºC, 7 min
IRAP / REMAP	94 °C: 2 min; 6 ciclos (94 °C, 30s, 50 °C, 2 min, 72 °C, 2 min); 24 ciclos (94 °C, 30s, 50 °C, 2 min, 72 °C, 2 min, mais 72 °C, 30 s a cada seis ciclos); 72 °C, 10 min

Figura 1. Árvore filogenética obtida por Influência Bayesiana (IB) utilizando sequências da região ITS do rDNA dos isolados pertencentes aos gêneros *Penicillium* e *Talaromyces*. Os valores de probabilidade à posteriori abaixo de 95% foram omitidos.

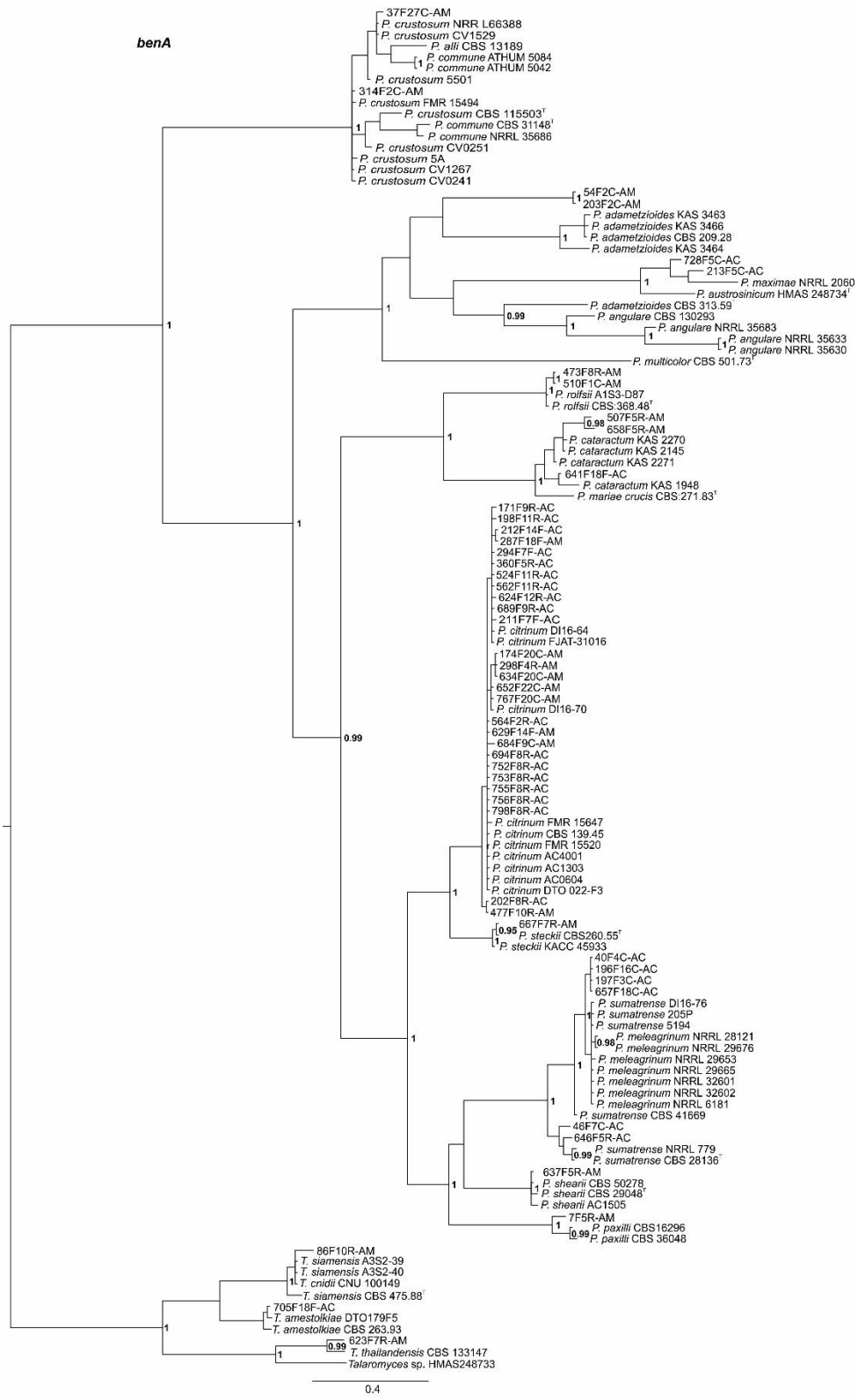

Figura 2. Árvore filogenética obtida por Influência Bayesiana (IB) utilizando sequências do gene *benA* (β-tubulina) dos isolados pertencentes aos gêneros *Penicillium* e *Talaromyces*. Os valores de probabilidade à posteriori abaixo de 95% foram omitidos.

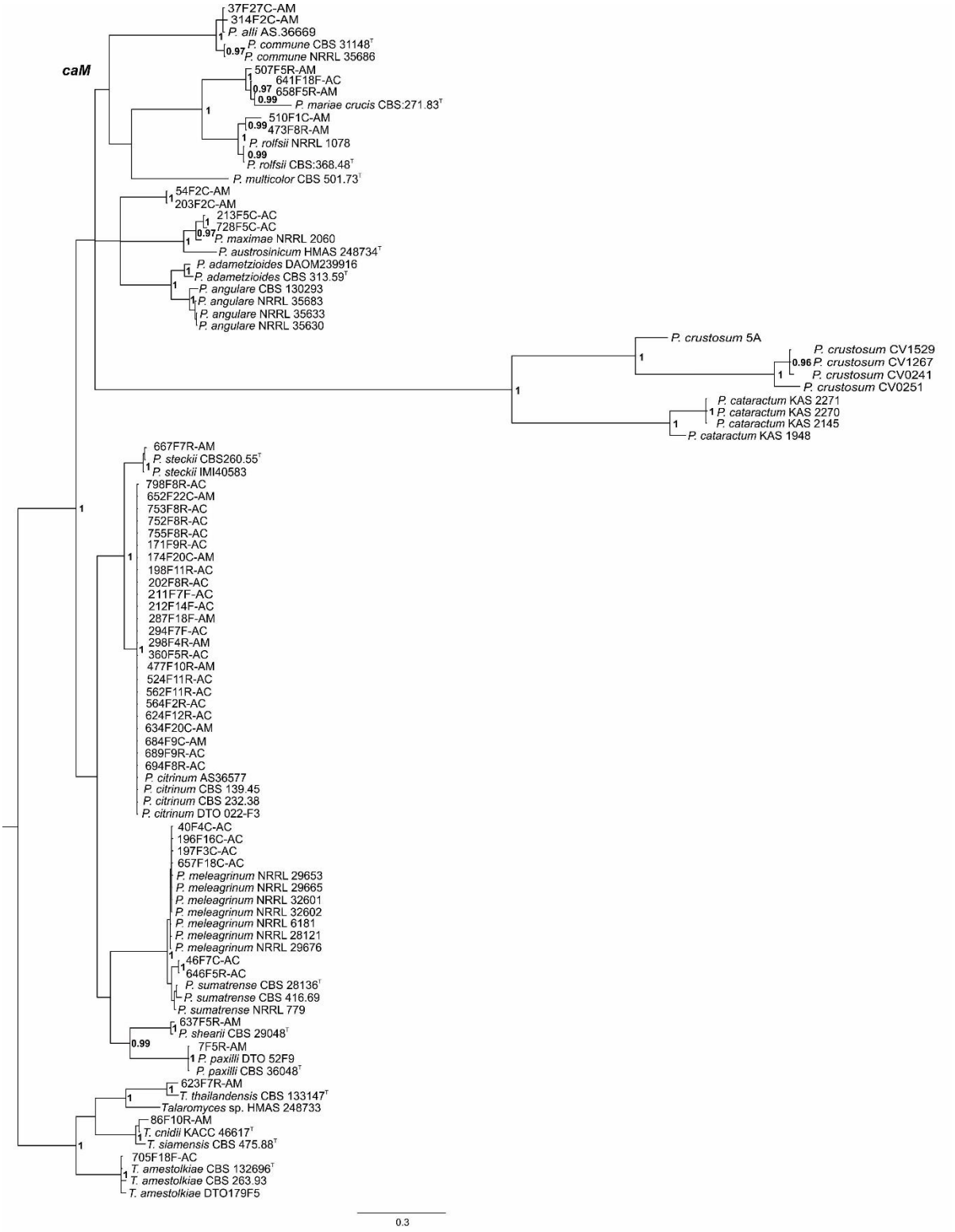

Figura 3. Árvore filogenética obtida por Influência Bayesiana (IB) utilizando sequências do gene *caM* (Calmodulina) dos isolados pertencentes aos gêneros *Penicillium* e *Talaromyces*. Os valores de probabilidade à posteriori abaixo de 95% foram omitidos.

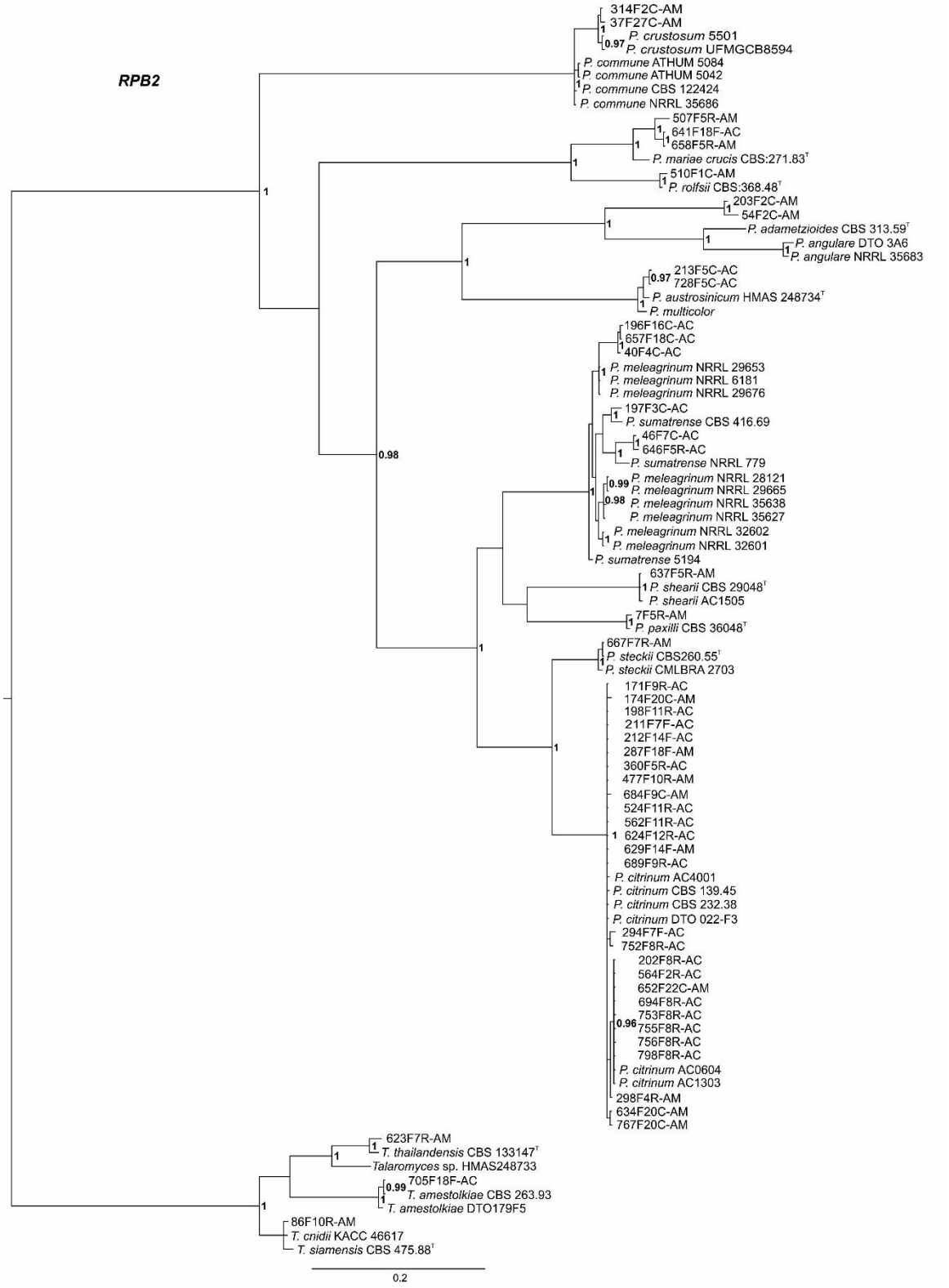

Figura 4. Árvore filogenética obtida por Influência Bayesiana (IB) utilizando sequências do gene *rpb2* (subunidade maior da RNA polymerase II) dos isolados pertencentes aos gêneros *Penicillium* e *Talaromyces*. Os valores de probabilidade à posteriori abaixo de 95% foram omitidos.

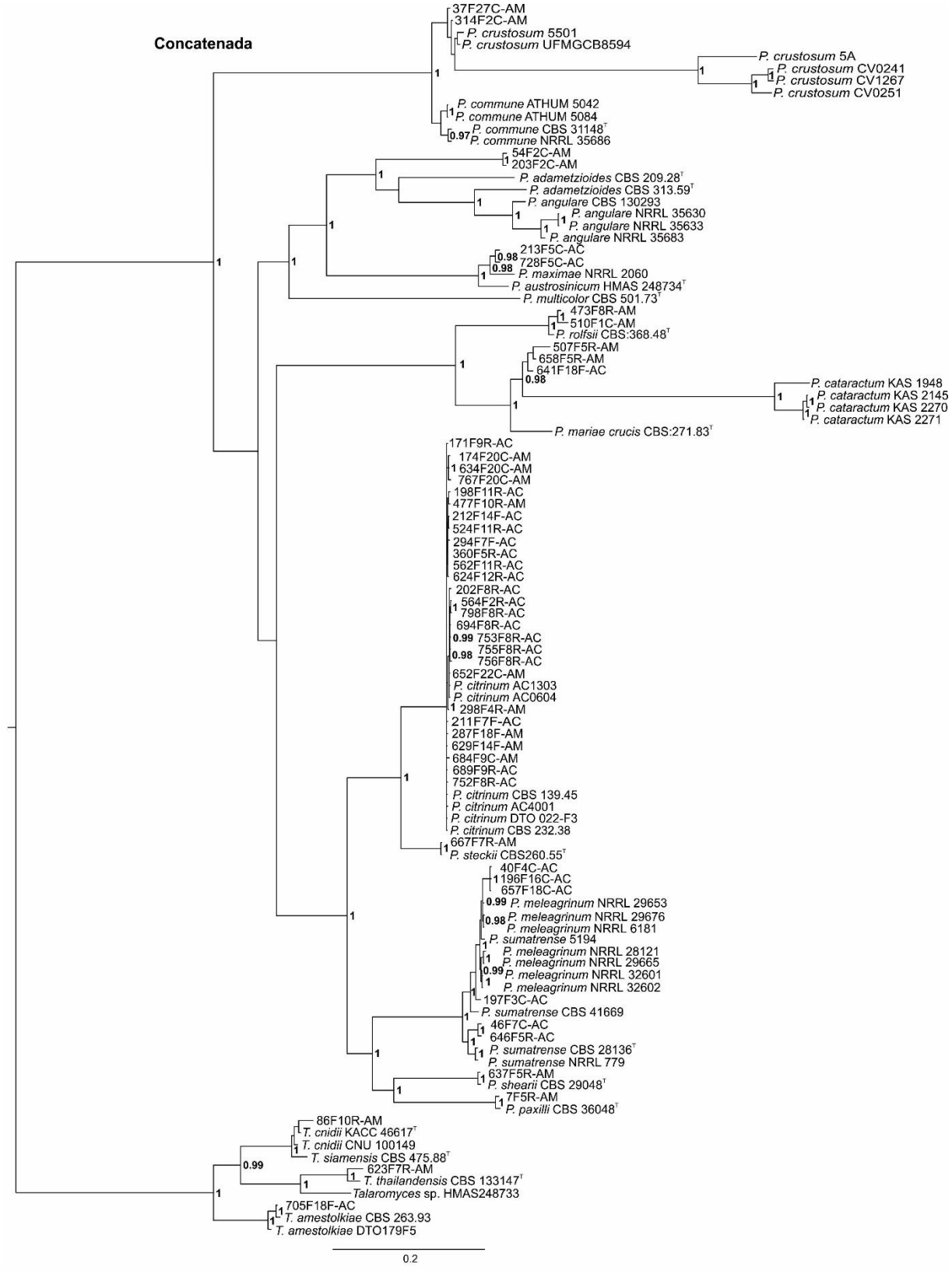

Figura 5. Árvore filogenética concatenada (ITS, *BenA*, *CaM* e *RPB2*) obtida por Influência Bayesiana (IB) utilizando sequências de DNA dos isolados pertencentes aos gêneros *Penicillium* e *Talaromyces*. Os valores de probabilidade à posteriori abaixo de 95% foram omitidos.

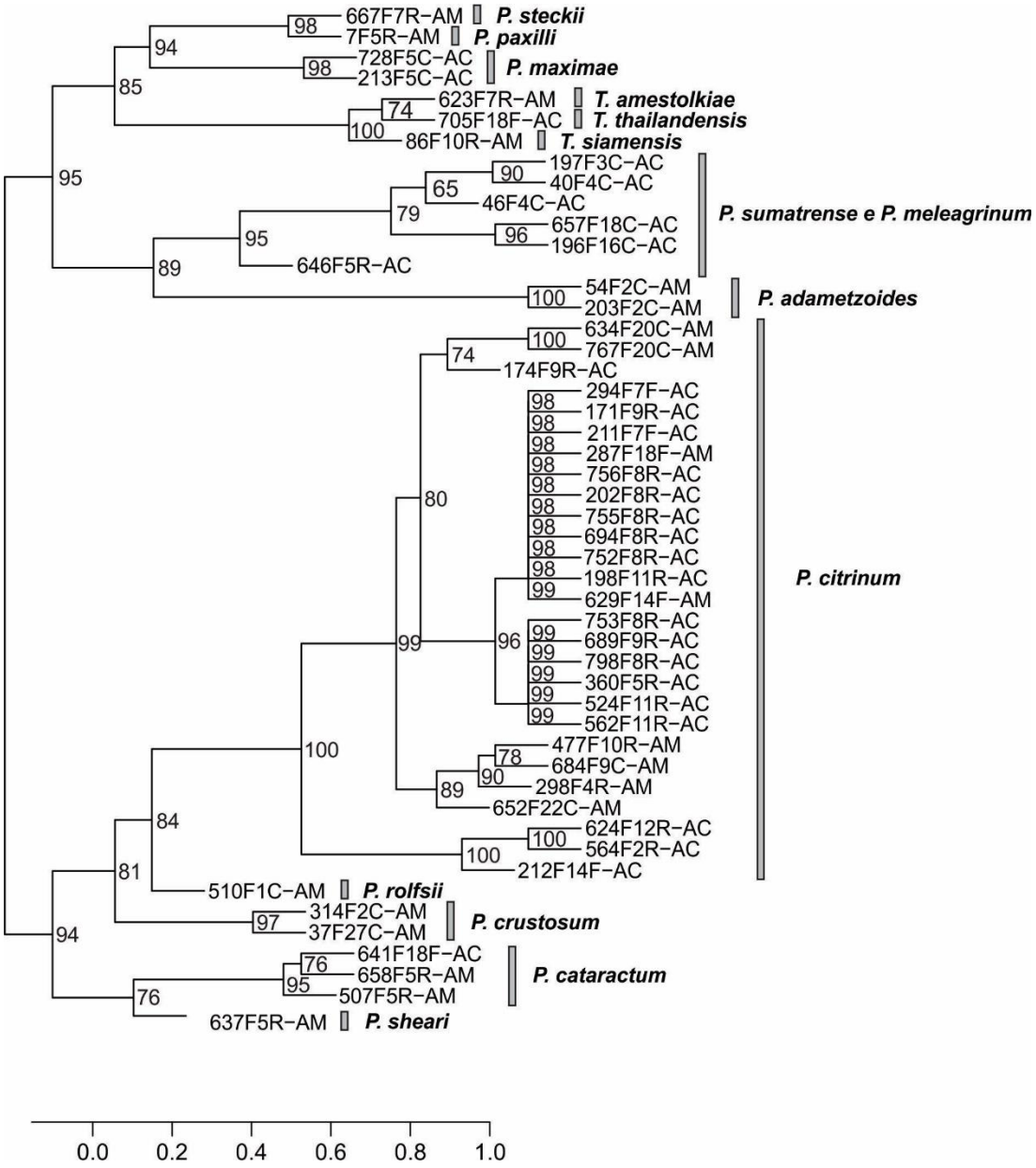

Figura 6. Dendograma de distância genética baseada na análise dos marcadores IRAP e REMAP. Os fungos endofíticos dos gêneros *Penicillium* e *Talaromyces* foram divididos por gênero e espécies. Os valores de Bootstrap foram obtidos a partir de 1.000 replicatas; o menor valor demonstrado foi igual a 65 e o restante dos valores foram maiores que 74. O dendograma foi construído pelo método UPGMA.

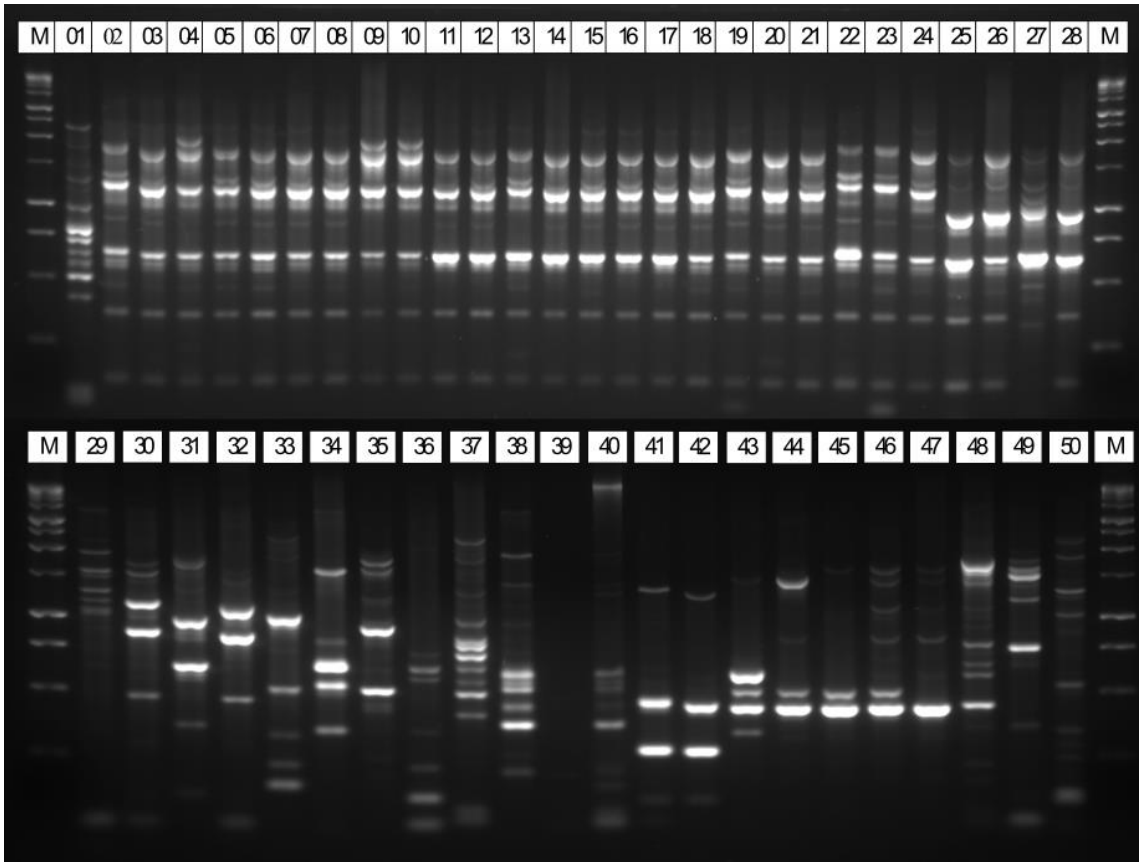

Figura 7. Perfil de amplicons produzidos por IRAP usando a combinação dos *primers* CLIIRAP1 e CLIIRAP4. Os fungos endofíticos enumerados de 01 a 50 são correspondes aos isolados 213F5C-AC, 212F14F-AC, 689F9R-AC, 174F20C-AM, 171F9R-AC, 294F7F-AC, 211F7F-AC, 287F18F-AM, 767F20C-AM, 634F20C-AM, 753F8R-AC, 756F8R-AC, 202F8R-AC, 798F8R-AC, 755F8R-AC, 694F8R-AC, 752F8R-AC, 360F5R-AC, 198F11R-AC, 524F11R-AC, 562F11R-AC, 564F2R-AC, 624F12R-AC, 629F14F-AM, 684F9C-AM, 477F10R-AM, 86F10R-AM, 298F4R-AM, 507F5R-AM, 658F5R-AM, 652F22C-AM, 641F18F-AC, 705F18F-AC, 646F5R-AC, 623F7R-AM, 667F7R-AM, 728F5C-AC, 37F27C-AM, 473F8R-AM, 314F2C-AM, 203F2C-AM, 54F2C-AM, 46F7C-AC, 40F4C-AC, 197F3C-AC, 196F16C-AC, 657F18C-AC, 7F5R-AM, 637F5R-AM e 510F1C-AM, respectivamente.

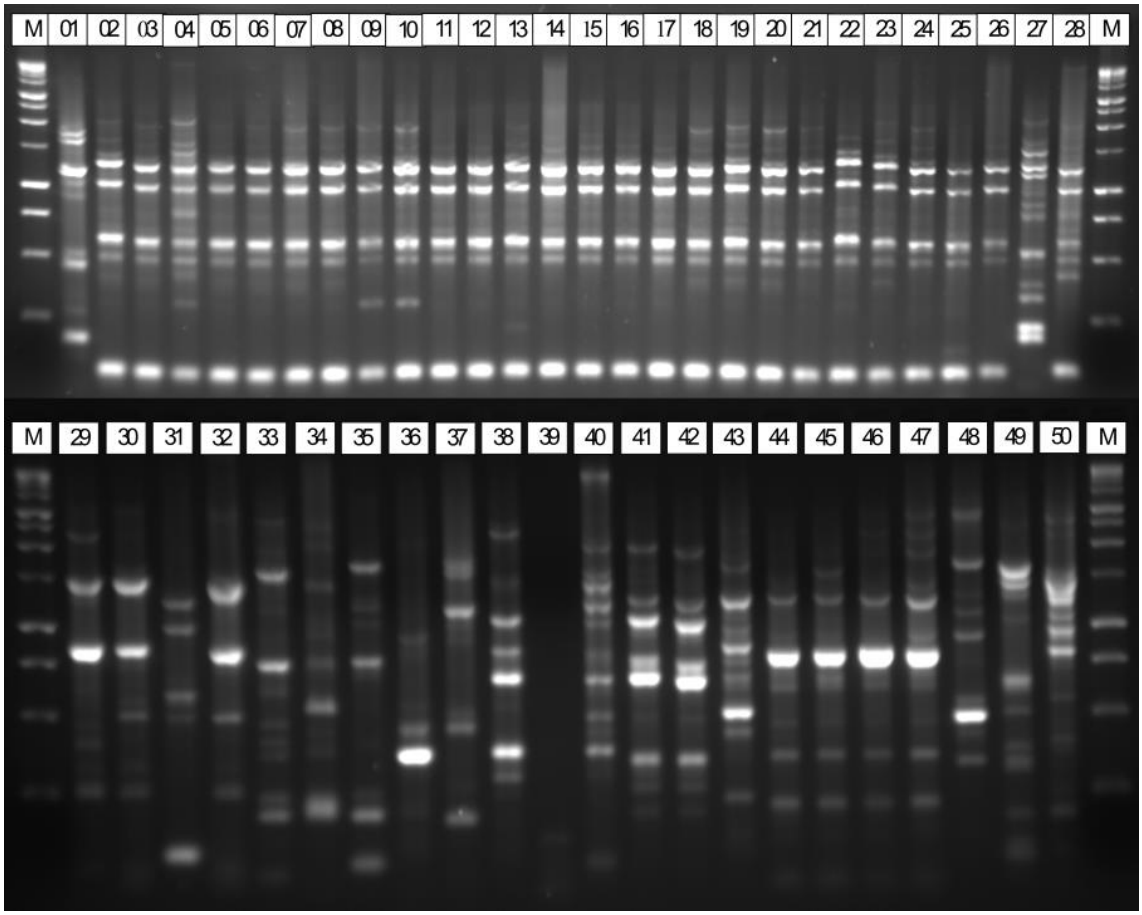

Figura 8. Perfil de amplicons produzidos por REMAP usando a combinação dos *primers* CLIIRAP1 e SSR1. Os fungos endofíticos enumerados de 01 a 50 são correspondes aos isolados 213F5C-AC, 212F14F-AC, 689F9R-AC, 174F20C-AM, 171F9R-AC, 294F7F-AC, 211F7F-AC, 287F18F-AM, 767F20C-AM, 634F20C-AM, 753F8R-AC, 756F8R-AC, 202F8R-AC, 798F8R-AC, 755F8R-AC, 694F8R-AC, 752F8R-AC, 360F5R-AC, 198F11R-AC, 524F11R-AC, 562F11R-AC, 564F2R-AC, 624F12R-AC, 629F14F-AM, 684F9C-AM, 477F10R-AM, 86F10R-AM, 298F4R-AM, 507F5R-AM, 658F5R-AM, 652F22C-AM, 641F18F-AC, 705F18F-AC, 646F5R-AC, 623F7R-AM, 667F7R-AM, 728F5C-AC, 37F27C-AM, 473F8R-AM, 314F2C-AM, 203F2C-AM, 54F2C-AM, 46F7C-AC, 40F4C-AC, 197F3C-AC, 196F16C-AC, 657F18C-AC, 7F5R-AM, 637F5R-AM e 510F1C-AM, respectivamente.

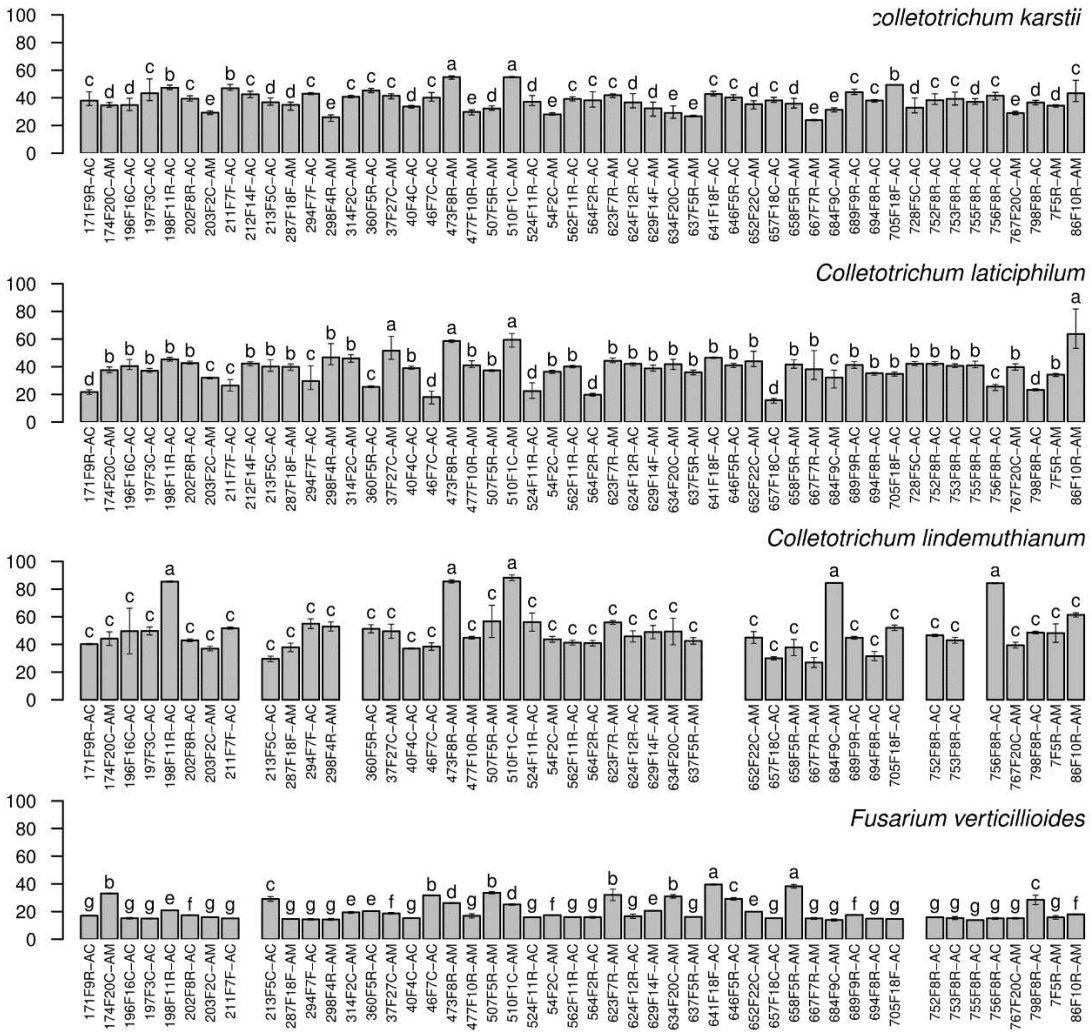

Figura 9. Porcentagem de inibição do crescimento dos fitopatógenos *Colletotrichum karstii*, *Colletotrichum laticiphilium*, *Colletotrichum lindemuthianum* e *Fusarium verticillioides* pelos fungos endofíticos. A porcentagem de inibição do crescimento micelial dos fungos fitopatogênicos na presença dos endofíticos antagonistas foi calculado pela fórmula: (DM – dm) / DM x 100, onde DM refere-se ao diâmetro médio das colônias do fungo fitopatógeno na ausência do endofítico (controle), e dm o diâmetro médio da colônia das três replicatas na presença do fungo endofítico. Os valores de diâmetro foram mensurados em milímetros, submetidos à análise ANOVA e as médias comparadas pelo teste de Tukey a 5% de significância.

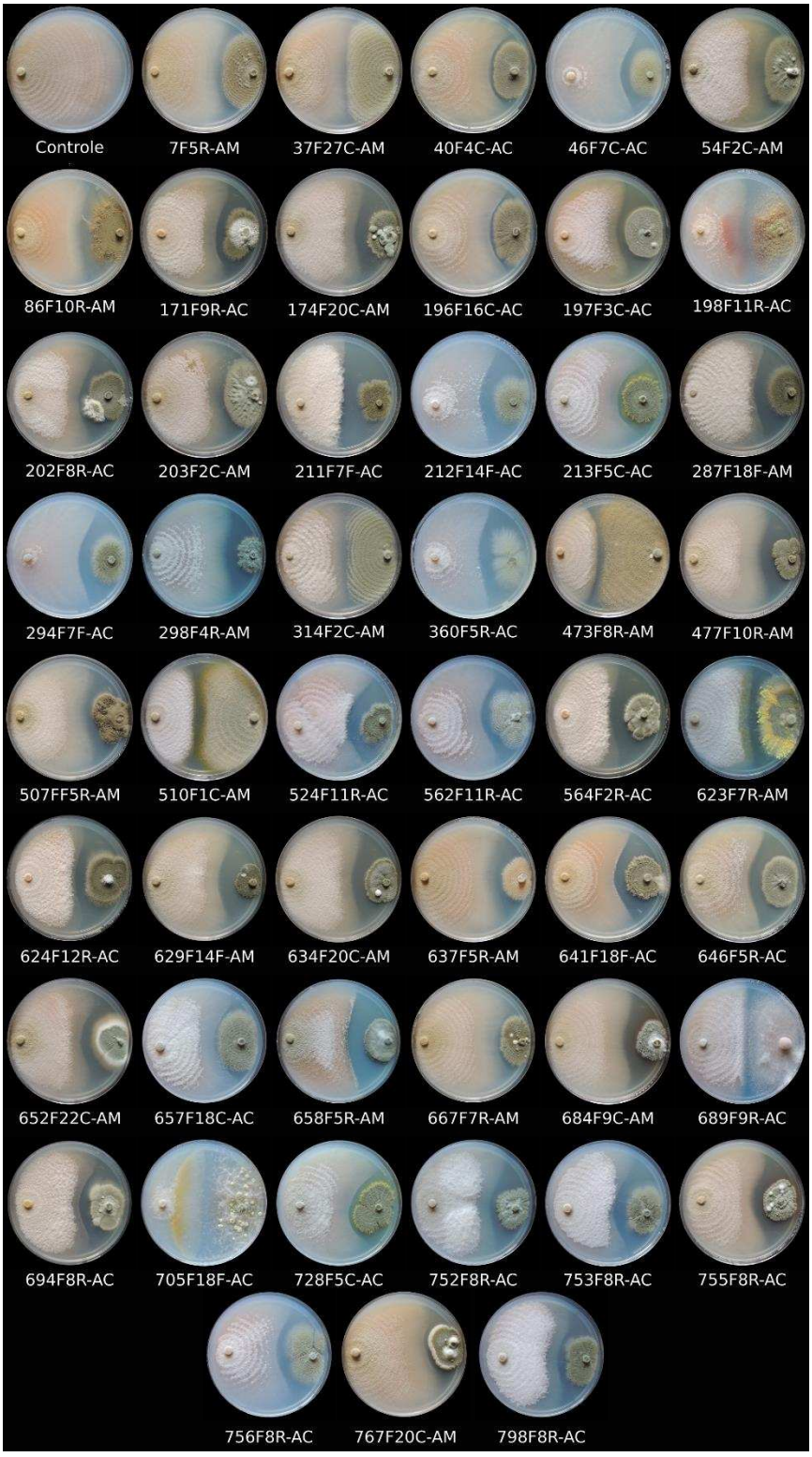

Figura 10. Atividade antimicrobiana dos isolados de *Penicillium* e *Talaromyces* endofíticos das seringueiras contra o *Colletotrichum karstii* pelo método de cultura dupla.

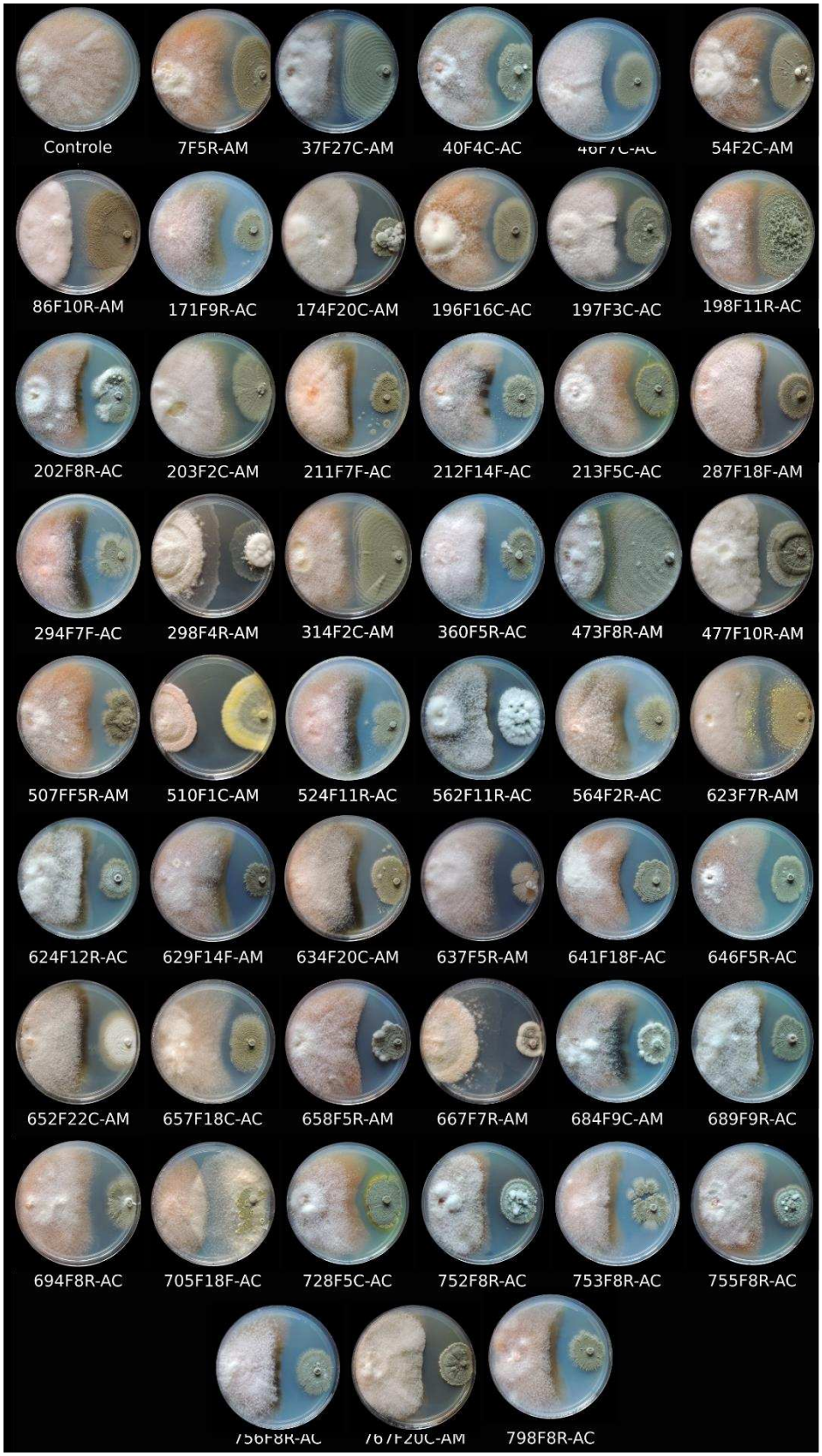

Figura 11. Atividade antimicrobiana dos isolados de *Penicillium* e *Talaromyces* endofíticos das seringueiras contra o *Colletotrichum laticiphilium* pelo método de cultura dupla.

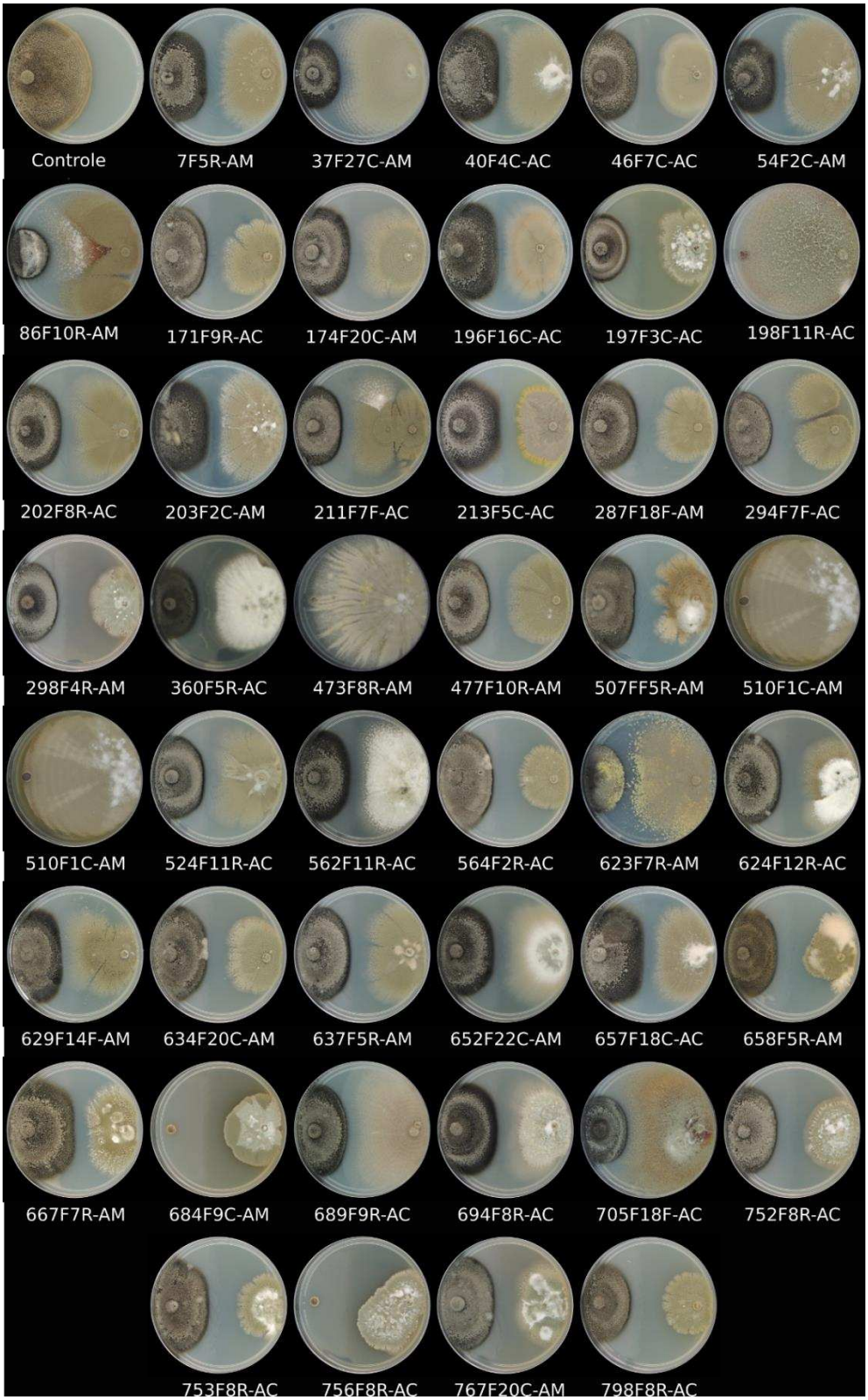

Figura 12. Atividade antimicrobiana dos isolados de *Penicillium* e *Talaromyces* endofíticos das seringueiras contra o *Colletotrichum lindemuthianum* pelo método de cultura dupla.

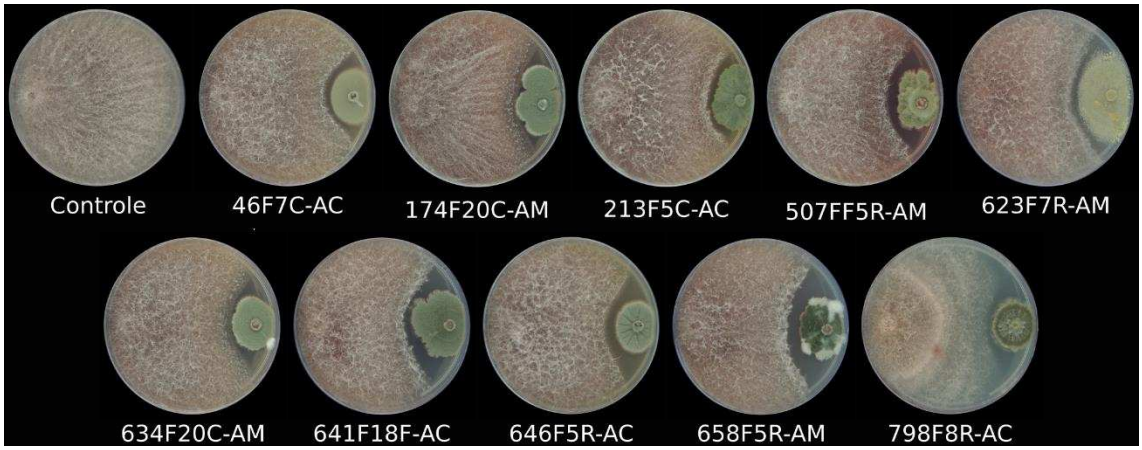

Figura 13. Atividade antimicrobiana dos isolados de *Penicillium* e *Talaromyces* endofíticos das seringueiras contra o *Fusarium verticillioides* pelo método de cultura dupla.

MATERIAL SUPLEMENTAR

Tabela S1. Número de acesso das sequências de DNA referentes a região ITS e dos genes *benA*, *CaM* e *RPB2* dos isolados tipos e de referência depositados no GenBank e utilizados nas análises filogenéticas.

ITS			*BenA*		
Closest species in GenBank	**Isolates**	**Accession nº**	**Closest species in GenBank**	**Isolates**	**Accession nº**
Penicillium crustosum	NRRL 968	AF033472	----------	----------	----------
Penicillium crustosum	UFMGCB8594	KP903322.1	----------	----------	----------
Penicillium crustosum	FRR 1669	NR_077153.1	----------	----------	----------
----------	----------	----------	*Penicillium crustosum*	NRRL 66388	KY172962
----------	----------	----------	*Penicillium crustosum*	FMR 15494	LT898266
----------	----------	----------	*Penicillium crustosum*	CBS 115503^{T}	AY674353
Penicillium crustosum	5501	KJ527442.1	*Penicillium crustosum*	5501	KJ527407.1
----------	----------	----------	*Penicillium crustosum*	5A	MF100874.1
Penicillium crustosum	CV0241	JX091403.1	*Penicillium crustosum*	CV0241	JX091536.1
Penicillium crustosum	CV0251	JX091404.1	*Penicillium crustosum*	CV0251	JX091530.1
Penicillium crustosum	CV1267	JX091401.1	*Penicillium crustosum*	CV1267	JX09153.7
Penicillium crustosum	CV1529	JX091402.1	*Penicillium crustosum*	CV1529	JX091538.1
Penicillium commune	CBS 311.48^{T}	NR_111143	*Penicillium commune*	CBS 31148^{T}	AY674366
Penicillium commune	ATHUM 5084	FJ004287	*Penicillium commune*	ATHUM 5084	FJ004398
Penicillium commune	ATHUM 5082	FJ004286.1	*Penicillium commune*	ATHUM 5082	FJ004397.1
Penicillium commune	NRRL 35686	EF200099.1	*Penicillium commune*	NRRL 35686	EF198566.1
Penicillium allii	CBS 13189	AJ005484	*Penicillium allii*	CBS 13189	AY674331
Penicillium brevicompactum	PP84	FJ884117	----------	----------	----------
Penicillium austrosinicum	HMAS 248734^{T}	NR_153272	*Penicillium austrosinicum*	HMAS248734^{T}	KX885041
----------	----------	----------	*Penicillium adametzii*	KAS 3463	JN625958
----------	----------	----------	*Penicillium adametzii*	KAS 3466	JN625961

----------	----------	----------	*Penicillium adametzii*	KAS 3464	JN625959
----------	----------	----------	*Penicillium adametzii*	KAS 3463	JN625958
Penicillium adametzii	CBS 209.28^{T}	NR_103661	*Penicillium adametzii*	CBS 209.28^{T}	JN625957
Penicillium adametzioides	CBS 313.59^{T}	NR_103660	*Penicillium adametzioides*	CBS 313.59^{T}	JN799642
Penicillium angularum	----------	AF125937.1	----------	----------	----------
Penicillium angulare	CBS 130293	KC773828.1	*Penicillium angulare*	CBS 130293	KC773779
Penicillium angulare	NRRL 35683	EF200096.1	*Penicillium angulare*	NRRL 35683	EF198563.1
Penicillium angulare	NRRL 35633	EF200088.1	*Penicillium angulare*	NRRL 35633	EF198555.1
Penicillium angulare	NRRL 35630	EF200087.1	*Penicillium angulare*	NRRL 35630	EF198554.1
----------	----------	----------	*Penicillium rolfsii*	A1S3-D87	KJ767045
Penicillium rolfsii	CBS 368.48^{T}	NR_111669	*Penicillium rolfsii*	CBS:368.48^{T}	GU981667
Penicillium wotroi	CBS 118171^{T}	NR_119813	----------	----------	----------
Penicillium mariae-crucis	CBS 271.83^{T}	NR_111506	*Penicillium mariae-crucis*	CBS:271.83^{T}	GU981630
Penicillium cataractum	KAS 1948	KT887843.1	*Penicillium cataractum*	KAS 1948	KT887804.1
Penicillium cataractum	KAS 2270	KT887864.1	*Penicillium cataractum*	KAS 2270	KT887825.1
Penicillium cataractum	KAS 2145	KT887847.1	*Penicillium cataractum*	KAS 2145	KT887808.1
Penicillium cataractum	KAS 2271	KT887865.1	*Penicillium cataractum*	KAS 2271	KT887826.1
Penicillium penarojense	CBS 113178^{T}	NR_138289	----------	----------	----------
----------	----------	----------	*Penicillium maximae*	NRRL 2060	KC773795.1
Penicillium multicolor	CBS 501.73^{T}	NR_111870.1	*Penicillium multicolor*	CBS 501.73^{T}	JN799645
Penicillium citrinum	CBS 139.45	GU944569.1	*Penicillium citrinum*	CBS 139.45	GU944545
Penicillium citrinum	NRRL 1841^{T}	NR_121224.1	----------	----------	----------
----------	----------	----------	*Penicillium citrinum*	FMR 15520	LT898241
----------	----------	----------	*Penicillium citrinum*	FMR 15647	LT898243
----------	----------	----------	*Penicillium citrinum*	DI6-64	LT559004.1
----------	----------	----------	*Penicillium citrinum*	DI16-70	LT559010.1
----------	----------	----------	*Penicillium citrinum*	FJAT-31016	KU737563

Penicillium citrinum	AC4001	KJ413367.1	*Penicillium citrinum*	AC4001	KJ413337.1
Penicillium citrinum	AC1303	KJ413362.1	*Penicillium citrinum*	AC1303	KJ413329.1
Penicillium citrinum	AC0604	KJ413361.1	*Penicillium citrinum*	AC0604	KJ413328.1
----------	----------	----------	*Penicillium citrinum*	DTO 022-F3	KM088687.1
Penicillium citrinum	NRRL 1841^{T}	NR_121224	----------	----------	----------
----------	----------	----------	*Penicillium steckii*	KACC 45933	JF521539
Penicillium steckii	CBS 260.55^{T}	NR_111488	*Penicillium steckii*	CBS 260.55^{T}	GU944522
----------	----------	----------	*Penicillium sumatrense*	205P	EU128573
Penicillium sumatrense	NRRL 779	AF033424.1	*Penicillium sumatrense*	NRRL 779	EF198503
----------	----------	----------	*Penicillium sumatrense*	DI16-76	LT559016
Penicillium sumatrense	CBS 281.36^{T}	NR_119812	*Penicillium sumatrense*	CBS 281.36^{T}	JN606639
Penicillium sumatrense	CBS 416.69	JN617707.1	*Penicillium sumatrense*	CBS 416.69	JN606641.1
Penicillium sumatrense	5194	KJ527465.1	*Penicillium sumatrense*	5194	KJ527430.1
Penicillium meleagrinum var. *viridiflavum*	NRRL 28121	EF198523	*Penicillium meleagrinum* var. *viridiflavum*	NRRL 28121	EF198504
Penicillium meleagrinum var. *viridiflavum*	NRRL 29653	EF198524	*Penicillium meleagrinum* var. *viridiflavum*	NRRL 29653	EF198505
Penicillium meleagrinum var. *viridiflavum*	NRRL 29665	EF198525.1	*Penicillium meleagrinum* var. *viridiflavum*	NRRL 29665	EF198506.1
Penicillium meleagrinum var. *viridiflavum*	NRRL 32601	EF198526.1	*Penicillium meleagrinum* var. *viridiflavum*	NRRL 32601	EF198508.1
Penicillium meleagrinum var. *viridiflavum*	NRRL 32602	EF198527.1	*Penicillium meleagrinum* var. *viridiflavum*	NRRL 32602	EF198509.1
Penicillium meleagrinum var. *viridiflavum*	NRRL 6181	EF198529.1	*Penicillium meleagrinum* var. *viridiflavum*	NRRL 6181	EF198512.1
Penicillium meleagrinum var. *viridiflavum*	NRRL 29676	EF198530.1	----------	----------	----------
----------	----------	----------	*Penicillium shearii*	CBS 50278	JN606850
Penicillium shearii	CBS 290.48^{T}	NR_111495	*Penicillium shearii*	CBS 29048^{T}	JN606840

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----------	----------	----------	*Penicillium paxilli*	CBS 16296	JN606804
Penicillium paxilli	CBS 360.48^{T}	NR_111483	*Penicillium paxilli*	CBS 36048^{T}	JN606844
----------	----------	----------	*Talaromyces siamensis*	A3S2-39	KJ767038
----------	----------	----------	*Talaromyces siamensis*	A3S2-40	KJ767039
Talaromyces siamensis	CBS 475.88^{T}	NR_103683	*Talaromyces siamensis*	CBS 475.88^{T}	JX091379
Talaromyces cnidii	CNU 100149	KF183639	*Talaromyces cnidii*	CNU 100149	KF183641
Talaromyces thailandensis	CBS 133147	JX898041	*Talaromyces thailandensis*	CBS 133147	JX494294
Talaromyces amestolkiae	DTO179F5	JX315660	*Talaromyces amestolkiae*	DTO179F5	JX315623
Talaromyces amestolkiae	CBS 263.93	JX315669.1	*Talaromyces amestolkiae*	CBS 263.93	JX315625.1
Talaromyces sp.	HMAS 248733	KX447531.1	*Talaromyces* sp.	HMAS 248733	KX885041.1
	CaM			***RPB2***	
Closest species in GenBank	**Isolates**	**Accession nº**	**Closest species in GenBank**	**Isolates**	**Accession nº**
Penicillium crustosum	5A	MF100894.1	----------	----------	----------
Penicillium crustosum	CV0241	JX141576.1	----------	----------	----------
Penicillium crustosum	CV0251	JX141577.1	----------	----------	----------
Penicillium crustosum	CV1267	JX141578.1	----------	----------	----------
Penicillium crustosum	CV1529	JX141579.1	----------	----------	----------
----------	----------	----------	*Penicillium crustosum*	5501	KJ527372
----------	----------	----------	*Penicillium crustosum*	UFMGCB8594	KT260175.1
Penicillium commune	CBS 311.48^{T}	KU896829	*Penicillium commune*	CBS 122424	KU904350.1
Penicillium commune	ATHUM 5084	FJ004287.1	*Penicillium commune*	ATHUM 5084	FJ004458
Penicillium commune	NRRL 35686	EF198594.1	*Penicillium commune*	NRRL 35686	EF198594.1
----------	----------	----------	*Penicillium commune*	ATHUM 5042	FJ004457.1
Penicillium allii	AS3.6669	AY678584	----------	----------	----------
Penicillium austrosinicum	HMAS248734^{T}	KX885051	*Penicillium austrosinicum*	HMAS248734^{T}	KX885032

Penicillium adametzioides	DAOM239916	JN686388	----------	----------	----------
Penicillium adametzioides	CBS 313.59^{T}	JN686387	*Penicillium adametzioides*	CBS 313.59^{T}	JN406578
----------	----------	----------	*Penicillium angulare*	DTO 3A6	JN406554
Penicillium angulare	CBS 130293	KC773804	----------	----------	----------
Penicillium angulare	NRRL 35630	EF198582.1	*Penicillium angulare*	NRRL 35630	EF198597
Penicillium angulare	NRRL 35633	EF198583.1	----------	----------	----------
Penicillium angulare	NRRL 35683	EF198591.1	----------	----------	----------
Penicillium rolfsii	NRRL 1078	AY443472	----------	----------	----------
Penicillium rolfsii	CBS:368.48^{T}	KF296375	*Penicillium rolfsii*	CBS:368.48^{T}	KF296455
Penicillium mariae-crucis	CBS:271.83^{T}	KF296374	*Penicillium mariae-crucis*	CBS:271.83^{T}	KF296439
Penicillium cataractum	KAS 1948	KT887765.1	----------	----------	----------
Penicillium cataractum	KAS 2145	KT887769	----------	----------	----------
Penicillium cataractum	KAS 2270	KT887786.1	----------	----------	----------
Penicillium cataractum	KAS 2271	KT887787.1	----------	----------	----------
----------	----------	----------	*Penicillium multicolor*	----------	EU427262.1
Penicillium multicolor	CBS 501.73^{T}	JN799646.1	----------	----------	----------
Penicillium maximae	NRRL 2060	KC773821.1	----------	----------	----------
Penicillium citrinum	CBS 139.45	GU944638	*Penicillium citrinum*	CBS 139.45	JF417416
			Penicillium citrinum	AC0604	KJ476421
Penicillium citrinum	AS36577	AY678555	*Penicillium citrinum*	AC1303	KJ476422
			Penicillium citrinum	AC4001	KJ476427
Penicillium citrinum	CBS 232.38	GU944633.1	*Penicillium citrinum*	CBS 232.38	JN606605.1
Penicillium citrinum	DTO 022-F3	KM089072.1	*Penicillium citrinum*	DTO 022-F3	KM089459.1
Penicillium steckii	IMI40583	EU644075	*Penicillium steckii*	CBS 260.55^{T}	JN606602
Penicillium steckii	CBS 260.55^{T}	GU944611	----------	----------	----------
Penicillium sumatrense	CBS 281.36^{T}	JN606368	----------	----------	----------

Penicillium sumatrense	CBS 416.69	JN606370.1	*Penicillium sumatrense*	CBS 416.69	JN606612
Penicillium sumatrense	NRRL 779	EF198522.1	*Penicillium sumatrense*	NRRL 779	EF198541
----------	----------	----------	*Penicillium sumatrense*	5194	KJ527395.1
Penicillium meleagrinum var. *viridiflavum*	NRRL 28121	EF198521	*Penicillium meleagrinum* var. *viridiflavum*	NRRL 28121	EF198532
Penicillium meleagrinum var. *viridiflavum*	NRRL 29653	EF198520	*Penicillium meleagrinum* var. *viridiflavum*	NRRL 29653	EF198533
Penicillium meleagrinum var. *viridiflavum*	NRRL 6181	EF198513	*Penicillium meleagrinum* var. *viridiflavum*	NRRL 6181	EF198540.1
Penicillium meleagrinum var. *viridiflavum*	NRRL 29665	EF198514.1	----------	----------	----------
Penicillium meleagrinum var. *viridiflavum*	NRRL 29676	EF198515.1	*Penicillium meleagrinum* var. *viridiflavum*	NRRL 29676	EF198535.1
Penicillium meleagrinum var. *viridiflavum*	NRRL 32601	EF198516.1	----------	----------	----------
Penicillium meleagrinum var. *viridiflavum*	NRRL 32602	EF198517.1	----------	----------	----------
Penicillium shearii	CBS 290.48^{T}	JN606560	*Penicillium shearii*	CBS 290.48^{T}	JN121482
Penicillium paxilli	DTO 52F9	JN606530	----------	----------	----------
Penicillium paxilli	CBS 36048^{T}	JN606566	*Penicillium paxilli*	CBS 36048^{T}	JN606610
Talaromyces siamensis	CBS 475.88^{T}	KF741960	*Talaromyces siamensis*	CBS 475.88^{T}	KM023279
Talaromyces cnidii	KACC 46617^{T}	KJ885266	*Talaromyces cnidii*	KACC 46617	KM023299
Talaromyces thailandensis	CBS 133147^{T}	KF741940	*Talaromyces thailandensis*	CBS 133147^{T}	KM023307
Talaromyces amestolkiae	CBS 132696^{T}	KF741937	----------	----------	----------
Talaromyces amestolkiae	CBS 263.93	JX315653.1	*Talaromyces amestolkiae*	CBS 263.93	JX315707
Talaromyces amestolkiae	DTO179F5	JX315650.1	*Talaromyces amestolkiae*	DTO179F5	JX315698
Talaromyces sp.	HMAS 248733	KX447528.1	*Talaromyces* sp.	HMAS 248733	KX447527.1