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Basidiomycetes associated with decay of living ornamental trees. Basidiomicetos associados ao decaimento de árvores ornamentais vivas

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Abstract

Despite the high diversity of basidiomycetes in Brazil studies involving species attacking living trees are still scarce. Basidiomyceteous species associated with trunk rot of living trees were identified through morphological and phylogenetic analysis - β-tubulin, Internal Transcribed Spacer (ITS) and Large Subunit (28S). According to analyses, the isolates PD71 and PD72 have been identified as *Perenniporia martius* and both PD75 and PD76 as *Rigidoporus ulmarius*. The identification of species associated with the rot of *Delonix regia* and *Cinnamomum zeylanicum* bases and trunks is essential in evaluating the risk of falling and to control decay fungi in the living trees that are mainly used in the ornamentation of streets and squares.
Keywords: *Rigidoporus ulmarius*, *Perenniporia martius*, wood decay.

Resumo

Apesar da alta diversidade de basidiomicetos no Brasil, estudos envolvendo espécies que atacam árvores vivas ainda são escassos. Espécies de basidiomicetos associadas à podridão de tronco de árvores vivas foram identificadas através de análises morfológicas e filogenéticas - β-tubulina, ITS (Internal Transcribed Spacer) e 28S (Large Subunit). De acordo com as análises, os isolados PD71 e PD72 foram identificados como *Perenniporia martius* e PD75 e PD76 como *Rigidoporus ulmarius*. A identificação de espécies associadas à podridão de bases e troncos de *Delonix regia* e *Cinnamomum zeylanicum* é essencial na avaliação do risco de queda e no controle de fungos apodrecedores nas árvores vivas utilizadas principalmente na ornamentação de ruas e praças.
Palavras-chave: *Rigidoporus ulmarius*, *Perenniporia martius*, apodrecimento da madeira.

Introduction

Several basidiomycetes are able of colonizing living trees, causing disease such as trunk or branch rot, culminating in the decline of trees throughout their lives (Roccotelli et al., 2014, p. 855). This becomes more significant when trees are located in public areas such as parks and streets, due to the potential of injury to people (Paclt, 2009, p. 2).

There is a lack of studies about the occurrence of wood-decaying fungi on living trees in Brazil. Most of the time, trees are attacked by wood-rotting fungi, generally due to improper pruning or injuries caused by maintenance equipment. Such problems can cause trees to collapse and damage to pedestrians, vehicles and property (Silva et al., 2000, p. 123).

Most of the fungi that cause trunk / brunch rot of living trees in Brazil urban areas are not yet identified. Moreover, identification studies of fungi that have were conducted until now were based mainly on morphological characteristics. However in recent years, analyses of DNA sequence have been widely used to support morphology in the identification of species (Zhao et al., 2016, p. 92; Bhatt et al., 2018, p. e0197306; Shen et al., 2018, p. 67). Hence, the aim of this study was to characterize these isolates through analysis of morphological and phylogenetic data, using three regions β-tubulin, Internal Transcribed Spacer (ITS) and Large Subunit (LSU) of rDNA.

Material and Methods

Collection and isolation of fungi

Seventy-six isolates obtained from basidiomata associated with wood rot were isolated. Among these isolates, four were included in this study due to their association with living trees which are particularly used as ornamental trees of squares and streets. The fungal isolates PD71, PD72, PD75, and PD76 were deposited in the Collection of the Laboratory of Forest Pathology of Federal University of the Lavras. For this purpose, basidiocarps associated with two tree species *Delonix regia* (PD71 and PD72) and *Cinnamomum zeylanicum* (PD75 and PD76) were cut into small fragments and sterilized superficially, in 70% alcohol and 2% hypochlorite, prior to isolation. For their subsequent utilization, the isolates were stored in 0.85% saline (NaCl) solution at room temperature.

Morphological and physiological characterization

Morphological characterization was conducted by identifying basidiocarp characteristics, such as coloration, texture and shape. For this, small sections were made from the basidiocarp to measure 30 basidiospores, with the aid of an optical microscope with coupled camera (Labomed®). Meltzer reaction test were performed to detect dextrinoidity / amyloidity of hyphal structure and spores (Singer, 1986). Colonies were grown on Potato-Dextrose-Agar (PDA) at 25ºC with a 12h photoperiod. Observations were made over a seven-day period. In order to confirm the presence of chlamydospores, the center and edges of fungal colonies were observed in each Petri dish, at 7, 10 and 13 days.

Evaluation of the mycelial growth was conducted using disks of 5mm diameter transferred to 9 cm Petri dishes containing PDA. The Petri dishes were incubated in the dark at 15, 20, 25, 30 and 35ºC. The colony diameter was measured on a daily basis for six days. The Mycelial Growth Rate Index (IVCM) was utilized in accordance with the equation proposed by Oliveira (1991, p. 99): $IVCM = \frac{\Sigma(D-Da)}{N}$. Where: D = colony average diameter; Da= average diameter of previous evaluation; N= number of days after inoculation.

A completely randomized experimental design was used (CRD) and four replicates were employed at each temperature. The analysis of variance (ANOVA) was conducted for each species at the end of six days ($p<0.05$). The experiment was carried out using two factors – temperature and speed of mycelial growth per fungal isolate. Curves of growth-speed per temperature were adjusted with bases of residue average square and R2.

Phylogenetic analyses

In order to extract DNA, five mycelium disks of 7 mm diameter were transferred to flasks containing 200 ml of liquid medium (20g of malt extract / 1L of water) and incubated at 28˚C for 15 days. After this period, the mycelial mass was filtered, submitted to drying and kept at – 80 ºC until extraction. The DNA extraction was conducted in the Forest Pathology Laboratory of the Federal University of Lavras, using the "Wizard Genomic DNA Purification Kit®" (Promega, Madison, USA). The amplification and purification of genomic DNA were conducted according to the protocol of utilized kits. The amplification required the Thermo Scientific Dream Taq PCR Master Mix (2X) kit. The utilized initiators were the oligonucleotides ITS1 (5' TTCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') (White et al., 1990, p. 317) for the study of ITS region (Internal Transcribed Spacer), β-tubulin_f, (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and β-tubulin_r (5′ACCCTCAGTGTAGTGACCCTTGGC-3′) (Glass & Donaldson, 1995, p. 1325) for the partial β-tubulin gene sequence, and LROR (5´-ACCCGCTGAACTTAAGC-3´) and LR5 (5´-TCCTGAGGGAAACTTCG-3´) (White et al., 1990, p. 317) to amplify a section of the LSU region. Thermal cycling conditions for ITS and LSU were 94 ºC for 2 min; 35 cycles of 94 °C for 50 s, 55 °C for 1 min, 72 °C for 1 min, and 72 °C for 5 min. For β-tubulin gene; 94 ºC for 4 min; 37 cycles of 94 °C for 25 s, 54 °C for 30 s, 72 °C for 35 s, and 72 °C for 10 min, and for β-tubulin were 94 ºC for 4 min; 37 cycles of 94 ºC for 25 s, 54 °C for 30 s, 72 °C for 35 s, and 72 °C for 10 min. The product from PCR was purified using the Kit GFX PCR DNA® and Gel Band Purification®, in accordance with the protocol of the manufacturer and stored at -20°C until the time of sequencing.

The DNA amplicons were sequenced in both directions by Macrogen Company through capillary electrophoresis in the MEGA BACE® automatic sequencer. The edition of generated electropherograms was edited using SeqAssem program version 2007/2008 (Hepperle 2004). The edited sequences were compared to the GenBank database of the National Center for Biotechnological Information - NCBI, using the BLAST search option.

Multiple alignments of sequences of nucleotides were generated using the CLUSTALW tool, implemented by the program MEGA version 6.06 (Tamura et al., 2013, p. 2726). Phylogenetic trees were constructed using PAUP * software (Swofford, 2002) using Maximum parsimony (MP). A bootstrap analysis (1000 replicates) was also done on the dataset to determine the confidence levels of the branches. Bayesian inference was used to generate posterior probabilities (PP) for consensus nodes using MRBAYES v. 3.2.1 (Ronquist et al., 2012). The Monte Carlo Markov Chain (MCMC) (Larget & Simon, 1999) was run with 1,000,000 generations using the appropriate substitution evolution model determined by ModelTest. Trees were viewed and edited in Fig.Tree 1.3.1. (http://tree.bio.ac.uk/software). The combination of the three ITS, LSU and β-tubulin genes was determined with a partition homogeneity test PHT (Farris et al., 1994) (Table 1).

Table 1. Species used in the phylogenetic tree.

Original Identification	Collection number	Accession Number GenBank			Geographic Origin
		ITS	LSU	Beta-tubulin	
Ganoderma applanatum	ATCC44053	JQ520161.1	AY515339.1	JQ675614.1	Korea
Hornodermoporus latissima	Cui9368	KX081080.1	KX081143.1	-	China
Hornodermoporus latissima	Cui6630	KX081078.1	KX081140.1	-	China
Hornodermoporus latissima	Cui6625	-	JF706340.1	KF482834.1	China
Hornodermoporus martius	Cui7992	HQ876603.1	HQ654114.1	KF482835.1	China
Perenniporia africana	Cui8676	KF018120.1	KF018129.1	KF482769.1	-
Perenniporia corticola	Cui1465	JN048759.1	JN048779.1	-	-
Perenniporia lacerata	Cui7220	JX141448.1	JX141458.1	KF482787.1	China
Perenniporia lacerata	Dai11268	JX141449.1	JX141459.1	KF482788.1	China
Perenniporia lacerata	Wei2208	JX141450.1	JX141460.1	KF494965.1	China
Perenniporia martius	MUCL41678	FJ393860.1	FJ393860.1	-	Argentina
Perenniporia martius	MUCL41677	FJ393859.1	FJ393859.1	-	Argentina
Perenniporia minor	Cui5782	HQ883475.1	HQ654115.1	-	China
Perenniporia minor	Cui9198	KF495005.1	KF495016.1	KF494969.1	-
Perenniporia pyricola	Cui9149	JN048762.1	JN048782.1	KF482804.1	-
Perenniporia pyricola	Dai10265	-	JN048781.1	KF482805.1	China
Perenniporia rhizomorpha	Dai7248	JF706330.1	JF706348.1	-	China
Perenniporia rhizomorpha	Cui7507	HQ654107.1	HQ654117.1	KF482806.1	China
Perenniporia straminea	Cui8718	HQ876600.1	HQ654117.1	KF482810.1	China
Perenniporia subacida	Dai8224	HQ876605.1	JF713024.1	KF482814.1	China
Perenniporia subacida	Cui10053	KF495006.1	KF495017.1	KF482813.1	-
Perenniporia subtephropora	Dai10964	JQ861753.1	JQ861769.1	KF482816.1	China
Perenniporia tenuis	Wei2969	JQ001859.1	JQ001849.1	KF482818.1	China
Perenniporia tenuis	Cui5523	HQ848474.1	HQ848480.1	-	China
Perenniporia tibetica	Cui9459	JF706327.1	JF706333.1	KF482783.1	China
Perenniporia tibetica	Cui9457	JF706326.1	JF706332.1	KF482782.1	China
Perenniporia truncatospora	Cui6987	JN048778.1	HQ654112.1	KF482774.1	-
Rigidoporus microporus	M15	KJ559476.1	-	-	Malaysia
Rigidoporus microporus	FRIM646	HQ400709.1	-	-	Malaysia
Rigidoporus microporus	X1865	KJ559473.1	-	-	Indonesia
Rigidoporus microporus	M13	KJ559474.1	-	-	Malaysia
Rigidoporus microporus	M14	KJ559475.1	-	-	Malaysia
Rigidoporus microporus	X1864	KJ559472.1	-	-	Indonesia
Rigidoporus microporus	ED336	KJ559466.1	-	-	Iyanomo, Nigeria
Rigidoporus microporus	ED303	KJ559455.1	-	-	Iyanomo, Nigeria
Rigidoporus microporus	ED310	-	KJ559523.1	-	Iyanomo, Nigeria
Rigidoporus microporus	X1864e	-	KJ559526.1	-	Indonesia
Rigidoporus microporus	AB101	KJ559447.1	-	-	Akwete, Nigeria

Rigidoporus microporus	N405	KJ559471.1	-	-	Cameroon
Rigidoporus microporus	N402	KJ559468.1	KJ559525.1	-	Cameroon
Rigidoporus microporus	ED334	KJ559464.1	KJ559524.1	-	Iyanomo, Nigeria
Rigidoporus microporus	ED333	KJ559463.1	-	-	Iyanomo, Nigeria
Rigidoporus microporus	ED331	KJ559461.1	-	-	Iyanomo, Nigeria
Rigidoporus microporus	ED330	KJ559460.1	-	-	Iyanomo, Nigeria
Rigidoporus microporus	ED332	KJ559462.1	-	-	Iyanomo, Nigeria
Rigidoporus microporus	AB103	KJ559449.1	-	-	Akwete, Nigeria
Rigidoporus microporus	N401	KJ559467.1	-	-	Cameroon
Rigidoporus microporus	DT202	KJ559451.1	-	-	Oghara, Nigeria
Rigidoporus microporus	MUCL45064	KJ559482.1	-	-	Cuba
Rigidoporus microporus	889	KJ559479.1	-	-	Brazil
Rigidoporus microporus	MS564b	KJ559480.1	-	-	Peru
Rigidoporus microporus	MS318	KJ559481.1	-	-	Peru
Rigidoporus microporus	311	KJ559478.1	KJ559527.1	-	Brazil
Rigidoporus ulmarius	KM178999	KJ559446.1	-	-	United Kingdom
Rigidoporus ulmarius	N407	KJ559445.1	-	-	Cameroon
Oxyporus corticola	JV1206	KC176667.1	-	-	-
Oxyporus corticola	JV1109/8	-	KC176681.1	-	-
Oxyporus corticola	HMAS:254729	-	LC164936.1	-	-
Oxyporus corticola	JV0108/125	-	KC176679.1	-	-
Oxyporus corticola	JV0709/177	-	KC176680.1	-	-
Oxyporus corticola	JV1106/6	-	KC176677.1	-	-
Oxyporus corticola	DAI12632	-	KF111020.1	-	-
Oxyporus corticola	JV0908/1	-	KC176678.1	-	-
Oxyporus populinus	Dai12793	-	KF111021.1	-	-
Oxyporus populinus var. *schizoporoides*	LE2082622	-	KF856509.1	-	-
Oxyporus sp.	DSH-93-188	-	AF287877.1	-	-
Hyphodontia alienata	-	-	AY586727.1	-	-
phellinus ferrugineovelutius	CUI10042	-	KC782529.1	-	-
Hyphodontia hastata	1383b	-	DQ873620.1	-	-
Resincium furfuraceum	FP-101917	-	DQ863696.1	-	-
Phanerochaete viticola	FCUG3001	-	GQ845003.1	-	-
Rickenella fibula	-	-	AY586710.1	-	-
Alloclavaria purpurea	AFTOL-ID-1736	-	DQ457657.1	-	-
Alloclavaria purpurea	BD299	-	DQ284900.1	-	-
Antrodia carbonica	FDP105585R	-	EU232285.1	-	-

The same procedure previously described was done for the *Rigidoporus* samples. For the ITS analyses, sequences of *R. ulmarius* and *R. microporus* were added and *Oxyporus corticola* was used as outgroup. The LSU dataset was rooted with *Antrodia carbonica*, to show the relationship between *R. microporus*, our isolates and other members of the *Polyporales* and *Hymenochaetales*. Sequences were primarily analyzed using the Blast algorithm from NCBI (www.nih.ncbi.gov). Related sequences

with highest identities, deposited in GenBank were obtained by nucleotide blast searches at www.ncbi.nlm.nih.gov/blast/ Blast.cgi.

Results and Discussion

Morphological and molecular characterization of Perenniporia isolates

According to the observed morphological characteristics and phylogenetic analysis of regions ITS rDNA, LSU rDNA and β-tubulin, the isolates PD71 and PD72 were identified as *Perenniporia martius*. For *Perenniporia*, the sequences deposited in the GenBank (Zhao & Cui, 2012, p. 48) were used for comparison. *Ganoderma applanatum* was selected as outgroup.

The main morphological characteristics are: Basidiocarps pileate, solitary, semicircular, of hard consistency, with black to dark brown crust covering the pileus, in the upper sections. Pileus solitary with upper surfaces of coloration varying from dark brown to black and obtuse edges ranging between beige and white. Lower surfaces of light-brown to cream coloration. Pores 4 per mm2, (33.2) – 48.2 – 222 78.2 μm diam; tubes (47.6) – 73.5- 121.0 μm deep. Hyphae dextrinoid in all parts; Basidiospores of hyaline, sub-truncate, dextrinoid, with thick walls 7.0 – 9.5 x 3.3 – 5.6 μm; chlamydospores absent.

Regarding mycelial growth, after seven days, moderate growth was observed in the culture medium (PDA) with the colony diameter at 38.6mm and 29.7mm for isolates PD71 and PD72 respectively white mycelium. The temperature curves were adjusted in accordance with the dispositions of isolates in phylogenetic trees. Significant interaction was observed ($p<0.05$) between the isolates and the mycelial growth index (IVCM). The optimum temperature for the greatest IVCM of different isolates was 30°C. Mycelial growth followed the same pattern in all tested isolates. The isolates of *P. martius* (PD71 and PD72) partially differed in terms of growth speed, with the greatest IVCM of 3% at 25°C (PD71) and 5% at 30°C (PD72) (fig. 1).

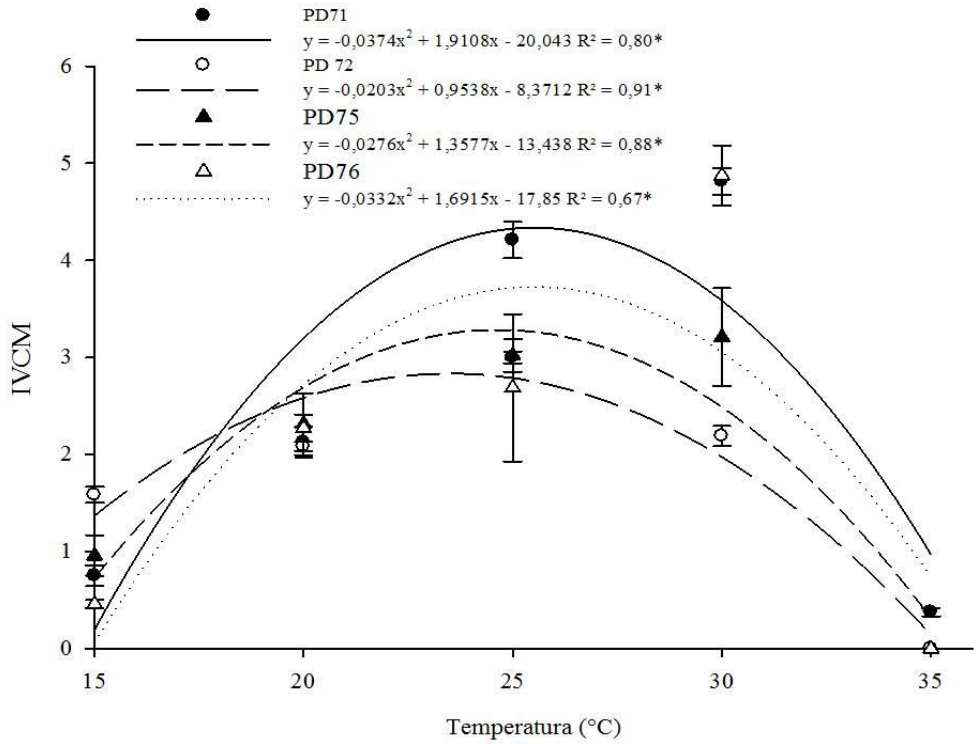

Figure 1 - Index of velocity of micelial growth IVCM (%) during 6 days at different temperatures of isolates PD71 e PD72 obtained from basidiocarps produced on *Delonix regia* and PD75 e PD76 obtained from basidiocarps produced on *Cinnamomum zeylanicum*

The morphological characteristics for both studied genera were similar to the ones found in literature. Ryvarden (1972, p. 140) and Decock & Figueroa (2000, p. 761) reported *P. martius* with porous surfaces, with cream to ochre coloration and presence of round pores with 4-5 per mm^2. Cream context, wood coloration, dark ochre to light gray and black in older basidiomata and hyphae were highly dextrinoid. The basidiospores were described as variable in size by Ryvarden (1972, p. 140) in all studied collections of 5-9 x 3-6µm. Similar sizes, 8.5-9.5 x 4-5µm and 6.1-10.1 x 3.9-5.4µm, respectively, were found in other studies (Teixeira, 1993, p. 48; Wright & Deschamps,1976, p. 129; Decock & Herrera Figueroa, 2000, p. 761). The presence of chlamydospores was not observed by Ryvarden (1972, p. 140) and Decock & Figueroa (2000, p. 761), as in this study.

For the concatenated tree of ITS, LSU and beta-tubulin regions, an alignment of 2071 characters of the 27 isolates resulted in 1070 constant characters and 882 parsimony non-informative characters and 382 parsimony informative.

Sequences from 27 isolates representing the sequence diversity of the genus *Perenniporia* were selected for three genes phylogenetic analyses combining ITS, LSU and BT sequences. The partition homogeneity test performed for the three regions of interest resulted in $P = 0.01$. An analysis of the combined dataset containing 29 sequences produced 24 trees of 1473 steps (Consistency index= 0.7563, Retention index= 0.7882, and Composite index=0.2437), and the trees were similar in topology, and thus one tree was selected for illustration (fig. 2). The two *Perenniporia* isolates, PD71 and PD72 from *Delonix regia*, grouped together *P. martius* with high bootstrap support (100%) and high values of posterior probability (1.00) (fig. 2)

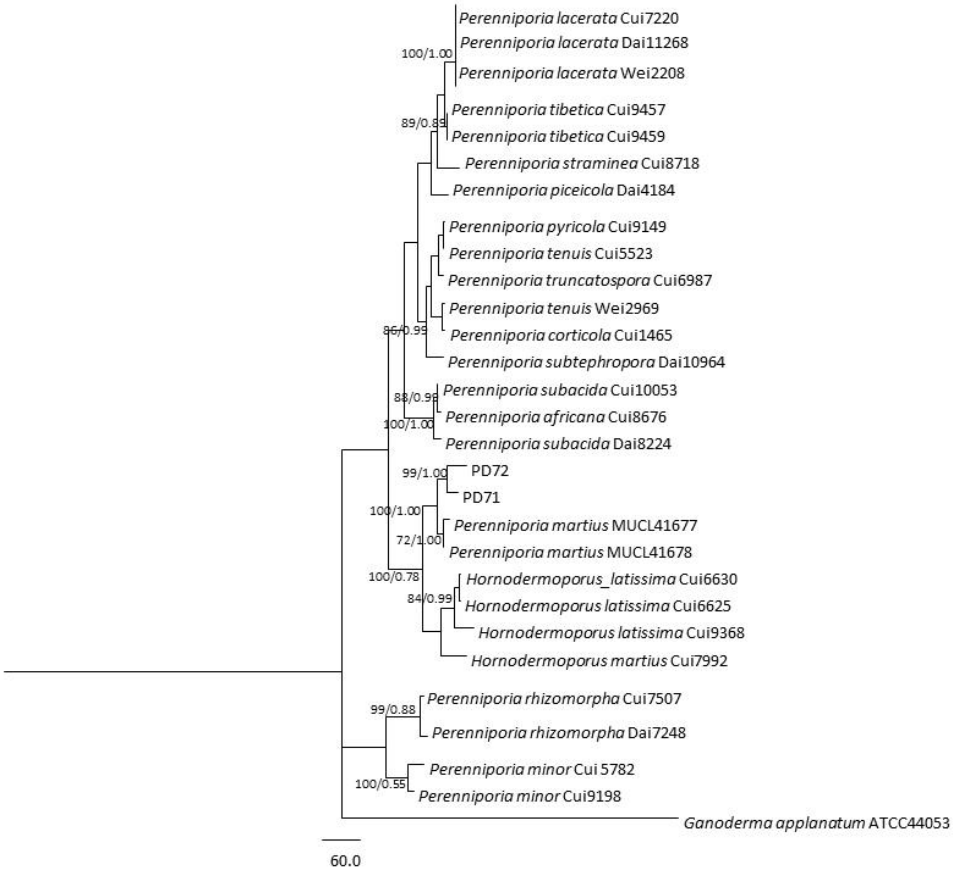

Figure 2 - Phylogenetic tree of *Perenniporia sp.* isolates inferred from a heuristic search of combined regions β-tubulin, Internal Transcribed Spacer (ITS) and Large Subunit (LSU) of rDNA sequences data. The length of the branches is indicated by the scale of the tree base and bootstrap values (1000 repetitions) are indicated in percentages above the internodes. The species used as *outgroup* was *Ganoderma applanatum*

Phylogeny of *Perenniporia* (PD71 and PD72), it has been verified that the genus is inserted in clade *Perenniporia s.l.*, where the type-specie of *P. medula-panis* genus is found in clade I. The species of *P. martius* and *P. latissima* are inserted into the complex *P. martius*, and are established as alternatives to *Hornodermoporus* in clade VII, as a monophyletic group inside *Perennipora s.l.* (Zhao et al., 2013, p. 56). The definition of clade in another genus was established using morphological characters previously defined by Teixeira (1993, p. 48), where *Hornodermoporus* was recognized for presenting pileate basidiocarps that were not branched and thin hyphae that were highly dextrinoid, oblong-ellipsoid basidiospores that were trunked and highly dextrinoid, with a presence of cystidia.

Perenniporia martius and *P. latissima* are closely related, and morphological similar. Previously, both species were considered as a single taxonomic entity (Ryvarden, 1972, p. 140). In the key established by Zhao et al. (2013, p. 56), the only distinctions in morphological characters were the hyphal system and basidiocarp growth. However, with the aid of molecular analysis, the two species were clearly divided within the clade.

Morphological and Molecular characterization of Rigidoporus isolates

According to the morphological characteristics and phylogenetic analysis of regions ITS rDNA, PD75 and PD76 were identified as *R. ulmarius*.

The morphological characteristics show: *Basidiocarps* sessile, effused-255 reflexed to resupinate, s*urface of pileus* glabrous, rough, white to cream and hymenophore yellow to cream. Edges were defined, intact, smooth, slightly bent downwards, with the same coloration as the porous surface. *Pores* round, 5 pores per mm^2 (132.8) – 166.9 – 214.8 µm diam.; tubes (125.5) – 145.5 – 209.9 µm deep, *hyphae* varied from varied from hyaline to yellow, dextrinoid. *Basidiospores* hyaline, slightly thick walled sub globular to globular 4.4 – 7.9 x 3.3 – 6.8 µm.

In the culture medium (PDA), isolates PD75 and PD76 presented moderate growth, with diameters of 27.37mm and 27.50mm respectively. White mycelium and formations of chlamydospores were observed for both isolates after 7 days in the center of colonies and after 10 days on the edges. The chlamydospores varied regarding shape from globular to sub globular and were hyaline with sizes of 10.0 – 21.2 x 7.6 – 9.0 for both isolates. Vesicles were also detected in colonies with length of (10.0) – 13.4 – 21.2µm and width of (9.6) – 11.6 – 14.1µm with shapes ranging from globular, sub globular to pear-shaped. The isolates PD75 and PD76 were observed with similar growth speed, with ≅ 4% of IVCM at 30°C (fig. 1).

The isolates identified as *R. ulmarius* showed similar characteristics to the ones reported by Ryvarden & Gilbertson (1994), whom observed basidiocarps with similar characters, corticolous of white to cream coloration and presence of 5-6 angular pores per mm. The reported basidiospores were negative in Meltzer (Ryvarden & Gilbertson, 1994, p. 602), hyaline to light yellow, with diameters of 6-7.5µm (Ryvarden, 1980, p. 545), 5-7µm and 6-8 x 5-6.5µm (Budington & Gilbertson, 1973, p. 412).

Regarding the cultural characteristics (PDA), similar observations were reported by Lombard et al (1960, p. 602), under the name *Fomes ulmarius*. The fungus shows an optimum growth at 28°C and develops a white mycelial blanket of 22-55 mm in diameter after seven days and hyaline chlamydospores in older cultures, with a globular to ovoid shape, 4.5-7µm (4.5-5.5 µm) diam. on grown on malt extract agar. Lombard et al. (1960, p. 602) also observed a possible misconception in previous publications, when thick-walled oval chlamydospores reaching 10 µm in diameter were reported, when in reality they were vesicles that differed from chlamydospores (Lombard et al. 1960, p. 602).

For isolates PD75 and PD76 were generated trees separately for ITS rDNA and LSU sequences, since there are not sequences of same isolates in the Genbank to compare. The ITS alignment comprised 29 sequences and 629 characters. Maximum parsimony analyses indicate that 445 characters were constant, 175 variable characters were parsimony- uninformative, and 111 were parsimony-informative. Bootstrap values of the phylogenetic analyses showed strong support for all clades. The two *Rigidoporus* isolates, PD75 and PD76 from *C. zeylanicum*, grouped together *R. ulmarius* with high bootstrap support (100%) and high values of posterior probability (1.00) (fig. 3).

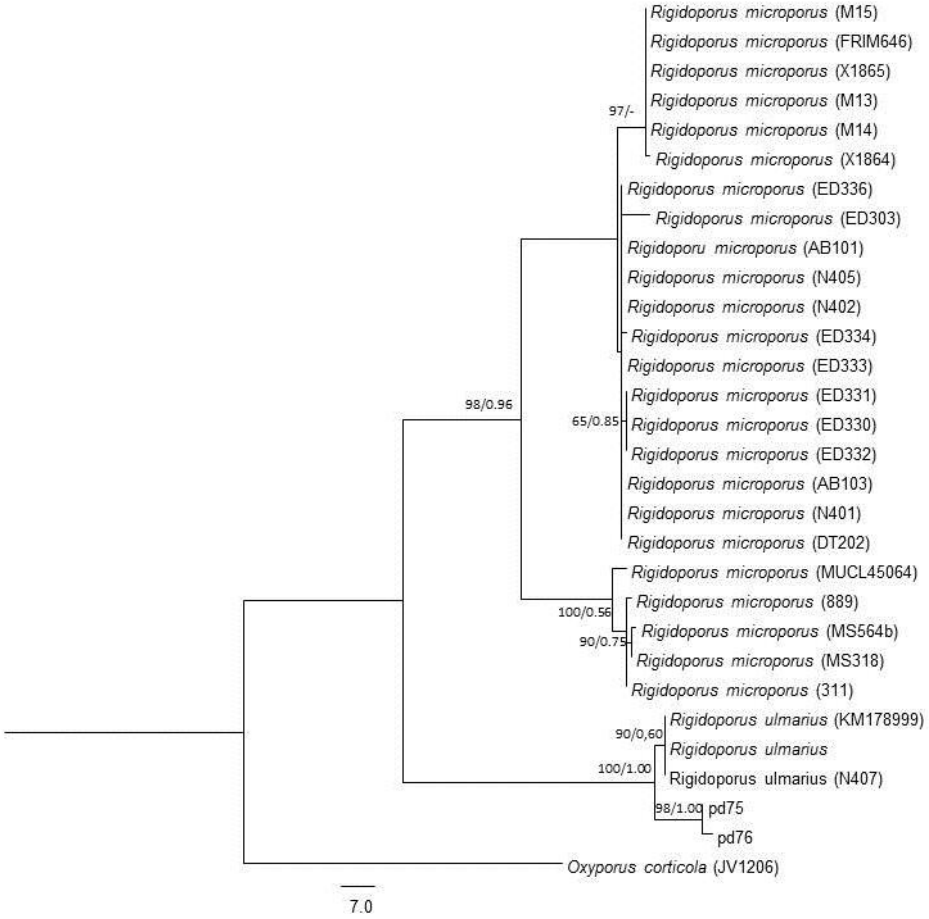

Figure 3 - Phylogenetic tree based Maximum Parsimony the Internal Transcribed Spacer (ITS) region of rDNA of species *Rigidoporus* spp. and isolates of *Rigidoporus* this study. The length of the branches is indicated by the scale of the tree base and bootstrap values (1000 repetitions) are indicated in percentages above the internodes. The specie used as *outgroup* was *Oxyporus corticola*

The LSU alignment was composed sequences from 26 taxa and 1097 characters. Maximum parsimony analyses indicate that 833 characters were constant, 199 variable characters were parsimony- uninformative, and 137 were parsimony-informative. *Rigidoporus microporus* isolates and isolates of this study clustered together, while the one from Brazil, representing clade III, formed a distinctive group. High bootstrap support of 100/99 showed the close relationship between *Oxyporus sp*. and isolates in this study (fig. 4).

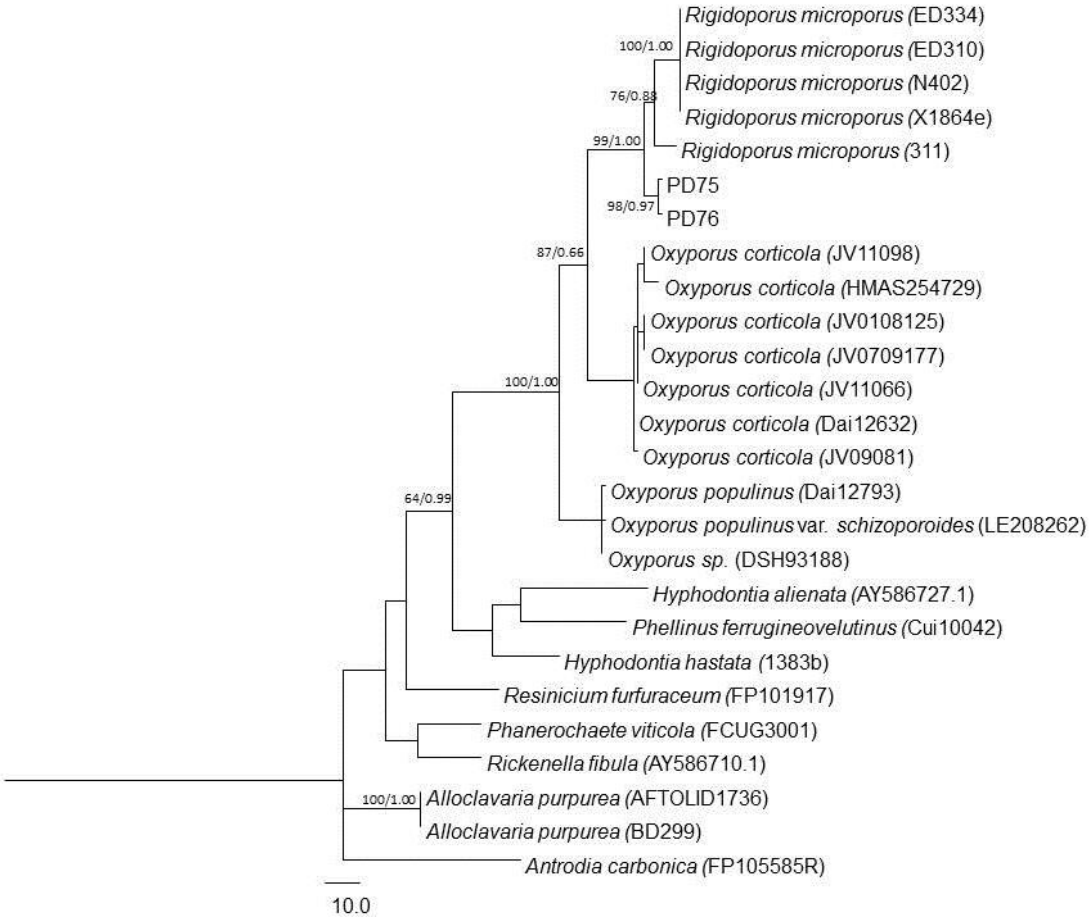

Figure 4 - Phylogenetic tree based Maximum Parsimony the Large Subunit (LSU) region of rDNA of species *Rigidoporus* spp. and isolates of *Rigidoporus* this study. The length of the branches is indicated by the scale of the tree base and bootstrap values (1000 repetitions) are indicated in percentages above the internodes. The specie used as *outgroup* was *Antrodia carbonica*

Molecular analysis resulted in the arrangement of *R. ulmarius* in different subclades. This result might reflect the genetic variability in the specie; however the phylogenetic analysis of other regions of genes is required to confirm this result. *Rigidoporus ulmarius* is correlated with *R. microporus*, but both species are separated within the clade (Kaewchai et al. 2010, p. 290; Oghenekaro et al., 2014).

Final considerations

The species *P. martius* and *R. ulmarius* are associated with base and trunk rot in living trees of *Delonix regia* and *Cinnamomum zeylanicum*. Such fungi can be potentially pathogenic and can cause internal decomposition of wood and high risk of falling over time. This knowledge about fungal species associated with living trees can cause diseases and deterioration of wood, is a fundamental step towards the monitoring of rot process in living trees and the control of diseases in trees.

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