

A new species of *Fuscoporia* (Hymenochaetaceae, Basidiomycota) from the Brazilian Atlantic Rainforest

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Abstract: *Fuscoporia atlantica* sp. nov. is described and illustrated based on specimens collected in Atlantic Rainforest areas of São Paulo state, Brazil, and the inferred phylogenetic relationships are based on sequence data from the ribosomal ITS and LSU regions. The new species is characterized by an effused-reflexed to pileate basidioma, golden-yellow-ferruginous pore surface, tiny pores, dimitic hyphal system with thin-walled hyphae at dissepiments edges encrusted, mostly uncinata or hooked hymenial setae and broadly ellipsoid to ellipsoid basidiospores.

Key words: basidiomycetes, neotropics, phylogeny, taxonomy

INTRODUCTION

Traditionally the species of the genus *Fuscoporia* Murrill have been included in *Phellinus* s.l., a morphologically heterogeneous and phylogenetically polyphyletic group (Fiasson & Niemelä 1984, Dai 1999, Wagner & Fischer 2001). However, molecular phylogenetic analyses have shown that *Fuscoporia* species form a clade independent from *Phellinus* s.s. (Wagner & Fischer 2002, Larsson *et al.* 2006).

The genus *Fuscoporia* was described to accommodate species occurring principally on coniferous and deciduous trees, with resupinate to pileate basidiomes; monomitic to dimitic hyphal system; encrusted generative hyphae at the dissepiments (which is described as a distinctive feature within *Phellinus* s.l.), hymenial setae and cylindrical to ellipsoid, hyaline, inamyloid, indextrinoid, thin-walled and smooth basidiospores (Murrill 1907, Fiasson & Niemelä 1984, Dai 1999, 2010; Wagner & Fischer 2001).

During the study of wood-inhabiting fungi in Atlantic Rainforest areas of the São Paulo state,

Brazil, an unknown species of *Fuscoporia* was found and it is described as new. The phylogenetic inference of its relationship based on ribosomal ITS and LSU sequence data demonstrated that it forms a monophyletic terminal clade, distinct from all the other *Fuscoporia* species for which DNA sequence data are available. The species is described here as *F. atlantica*.

MATERIALS AND METHODS

Morphological study

Specimens were collected in Atlantic Rainforest areas in the São Paulo state, Brazil, between 2012 and 2013. For microscopic analysis, free hand sections of the basidiomata were mounted on microscope slides with a drop of 3% KOH solution and 1% aqueous phloxine solution. Amyloid and dextrinoid reactions were observed in Melzer's reagent. The specimens were deposited in Herbário, Instituto de Botânica, São Paulo, Brazil (SP).

Sequencing

Total DNA was extracted from dried basidiomata. Extractions were carried out using the Sigma-Aldrich Gen Elute™ Plant Genomic DNA Miniprep Kit and later amplified with Sigma-Aldrich ReadMix™ Taq PCR P4600 (Sigma-Aldrich Corporation, St. Louis, MO, USA), following the manufacturer's recommendations. PCR reactions were performed in a thermal cycler (C1000 Touch™ Thermal Cycler Bio-Rad). Primers pairs used were ITS1 and ITS4 for the ITS region (including ITS1, 5.8S and ITS2) and LROR and LR7 for nLSU (White *et al.* 1990). PCR products were sequenced in both directions using the same primers as the amplification. Nucleotide sequences were determined using an Applied Biosystems 3730xl DNA Analyser (Macrogen, Korea). Edited sequences have been deposited in GenBank.

Phylogenetic analyses

All sequences were matched against the entire

GenBank database using BLAST (<http://blast.ncbi.nlm.nih.gov/>), and their pairwise identity was recorded. Sequences retrieved from GenBank (NCBI) included in the study were selected based on the quality, identity, length and alignment test to build the in-group. The selected out-group for ITS analyses was *Phellinidium fragrans* (M.J. Larsen & Lombard) M. Fisch., *P. weirii* (Murrill) Y.C. Dai, and *P. sulphurascens* (Pilát) Y.C. Dai, based in the results of the LSU region, that places *Phellinidium* as a sister group of *Fuscoporia*; and for LSU analyses was *Bondarzewia montana* (Quél.) Singer, *Russula violacea* Quél., and *Stereum hirsutum* (Willd.) Pers., based in previous work (Wagner & Fischer 2002). Nucleotide sequences were aligned automatically with MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/>), and then optimized manually with BioEdit 7.2.0 (Hall 1999). Best models of evolution were estimated by using JModeltest 2.c1.4 (Darriba *et al.* 2012). The substitution model used in the LSU dataset was TrN+I+G and for ITS dataset was TVM+G. Bayesian analysis was performed based on independent Markov chain Monte Carlo (MCMC). The run consisted of five million generations, two independent runs, and four independent chains. The sampling frequency was every 500 generations. The first ten percent of the analysis (burn-in phase) was discarded. Posterior probabilities (PP) were determined in MrBayes 3.2.2 (Ronquist *et al.* 2012) to test the support of the branches' nodes. PP values ≥ 0.95 were considered statistically significant, hence, all branches supported below this value were disregarded. Maximum likelihood (ML) analysis was performed using RaxML 7.5.4-standard (Stamatakis 2006) with 1000 rapid bootstrap replicates. Trees from ML analysis were compared with those resulting from the Bayesian analysis and bootstrap values $>50\%$ were used to support the respective nodes compatible between two trees.

RESULTS AND DISCUSSION

Taxonomy

Fuscoporia atlântica Motato-Vásquez, R.M. Pires & Gugliotta, **sp. nov.**

Types: Brazil. São Paulo state: Parque Estadual da Cantareira, 23°32'36"S 46°37'59"W, 27 June 2012, V. Motato-Vásquez, M.C. Westphalen & A.C. Bolaños 230 (**Holotype**, SP445618; GenBank KP058515, KP058517).

Etymology: *atlantica* – refers to the Brazilian Atlantic Rainforest biome, the only known habitat of this species.

Basidioma annual, pileate, sessile to effuse-reflexed, flexible when fresh to leathery when dry, solitary to imbricate, without odor or taste. Pileus broadly attached, semicircular, applanate, up to (5.0–)8.0–9.0 × 4.0–6.0 × 0.1–0.4 cm; upper surface concentrically zonate, radially wrinkled, glabrous to tomentose, brown to yellowish-brown, with a matte dark brown crust at the base. MARGIN thin, obtuse, entire, not involute when dry, sterile, up to 0.2 cm, yellowish-brown to black brown. Context homogeneous, dense, azonate, golden-yellow-ferruginous. Pore surface golden-yellow-ferruginous ($N_{30}A_{99}M_{30}$), pores rounded, invisible to the naked eye, 7–9 per mm, tubes concolor with the pore surface, up to 0.2 cm deep, dissepiments entire to slightly lacerated, thin to slightly thick. Hyphal system dimitic, generative hypha simple-septate, hyaline, golden yellow to rusty brown, thin to thick-walled, straight, sparsely branched, 3.0–6.5 μm diam., rarely branched, thin-walled hyphae at dissepiments edges encrusted; skeletal hyphae thick-walled, straight, unbranched, dark-brown, 3.0–6.0 μm . Hymenial setae abundant, subulate to ventricose, mostly uncinata or hooked, dark brown and thick-walled, 20–45 × 7–10 μm . Cystidioles abundant. Basidia clavate, hyaline, 4-sterigmate, thin-walled, 9–11 × 5–7 μm . Basidiospores abundant, broadly ellipsoid to ellipsoid, hyaline to pale yellow, smooth, thin-walled, inamyloid, 4–4.5 × (2–)3–3.5 μm ($X_m = 4.2 \times 2.8 \mu\text{m}$), $n = 60/2$, $Q = (1.1–)1.3–2$, $Q_m = 1.5$.

Additional specimens examined — *Fuscoporia atlantica*: **BRAZIL. SÃO PAULO STATE:** Parque Estadual da Serra do Mar, Núcleo Santa Virginia, 11 May 2013, R.M. Pires 8 (SP465829; GenBank KP058514, KP058516). *Fuscoporia gilva*: **BRAZIL. SÃO PAULO STATE:** Parque Estadual da Cantareira, 21 October 2011, V. Motato-Vásquez & A.M. Gugliotta 22 (SP417971); 16 February 2012, M. Capelari & J.J.S. Oliveira 4683 (SP445350). *Fuscoporia rhabarbarina*: **BRAZIL. SANTA CATARINA STATE:** 26 December 1988, F. Furlani & C.L. Leite 186 (FLOR 10929). **CANADA.** on wood, Dr. Richardson 4 (K187843, lectotype).

Comments — *Fuscoporia atlantica* is a distinct species that produces effused-reflexed to pileate basidiomata, radially wrinkled and tomentose pileus, with a matte dark brown crust at the base, a yellowish-brown when fresh to black brown after dry margin, tiny pores, hooked hymenial setae and broadly ellipsoid to ellipsoid basidiospores.

Fuscoporia atlantica is very similar to *F. gilva* (Schwein.) T. Wagner & M. Fisch., which differs microscopically by its straight setae and smaller basidiospores, and macroscopically by its crustless pileus base and its dark purplish brown pore surface (Loguercio-Leite & Wright 1995, Dai 1999). *Fuscoporia atlantica* is also morphologically similar to *F. rhabarbarina* (Berk.) Groposo *et al.*, which can be easily distinguished macroscopically by a glabrous pileus, with sulcate zones and a distinctive black crust covering the entire pileus surface, yellowish brown context; and microscopically by the smaller basidiospores (3–4 × 2–3 µm) and straight, ventricose hymenial setae (Groposo *et al.* 2007).

Phylogenetic analyses

For LSU analyses, the final matrix consisted of 51 sequences and 838 characters, from which 534 were parsimony-informative (63.72%). Bayesian analysis resulted in average standard deviation of split frequencies = 0.003721. Results shown in the tree based on LSU sequences (Figure 1) of different taxa of *Hymenochaetaceae* from GenBank, indicate that the new species presented in this study clustered within *Fuscoporia* genus, with full support. The analysis of the ITS was included 37 sequences and a total of 585 characters, from which 302 were parsimony-informative (51.62%). Bayesian analysis resulted in average standard deviation of split frequencies = 0.005049. The tree based on ITS sequences (Figure 2) recovered the newly described taxon among a limited number of the most related taxa within the genus *Fuscoporia*. This tree confirms the uniqueness of the new species, *F. atlantica*, supported by values of PP = 1 and bootstrap = 100%.

CONCLUSIONS

Our results based both in morphological and phylogenetic approaches showed a close relationship between *F. atlantica* and *F. gilva*.

This study support the introduction of a new taxon from the Brazilian Atlantic Rainforest, São Paulo, Brazil, another example of how poorly know is the fungal diversity in the area.

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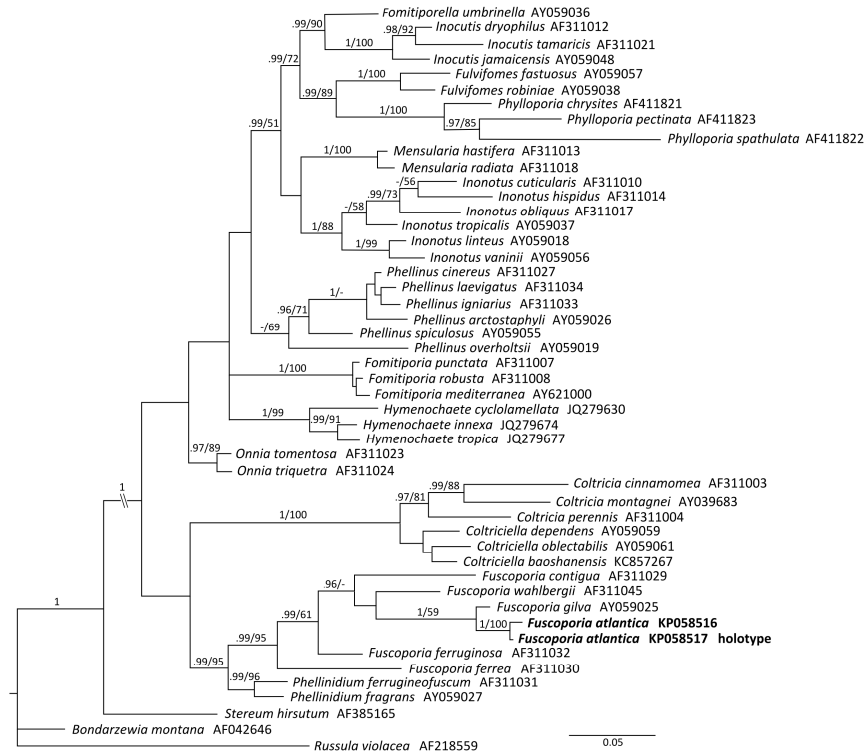


Figure 1: Phylogenetic placement of nLSU sequences from *Fuscoporia atlantica* and related species and genera, inferred from Bayesian analysis and ML. The new species is presented in bold font. Support values consist of the Bayesian posterior probability (PP) >0.95, followed by ML bootstrap (BS) values >50%.

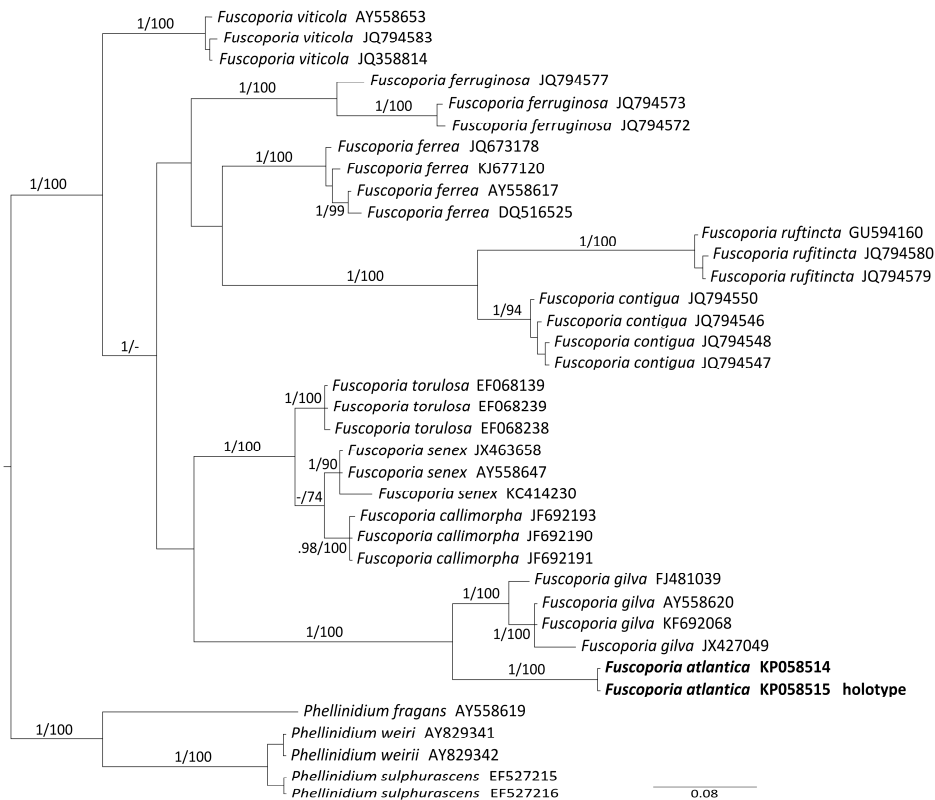


Figure 2: Phylogenetic analysis of ITS sequences from *Fuscoporia atlantica* and related species, based on Bayesian analysis and ML. The new species is presented in bold font. Support values consist of Bayesian posterior probability (PP) values >0.95, followed by ML bootstrap (BS) values >50%.