

The genus *Laetiporus* (Basidiomycota) in Brazil: a new species and a new record

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Abstract: *Laetiporus squalidus* is described as a new species; it is distinguished by effused-reflexed basidioma with numerous small and broadly attached pilei, cream to pale brown when fresh to light ochraceous after dry upper surface and ellipsoid to broadly ellipsoid basidiospores. Phylogenetic analysis with ITS and nLSU regions corroborate the position and identity of this species. Furthermore, *L. gilbertsonii*, a Pan-American species, is recorded for first time from Brazil, based on ITS region and a more accurate analysis of morphological characteristics.

Key words: mycodiversity, neotropical, polypores, xylophilous fungi

INTRODUCTION

Species of the genus *Laetiporus* Murrill are cosmopolitan poliporoid basidiomycetes that cause brown cubical rot in living hardwoods and conifers, as well are considered a destructive pathogen of trees (Corner 1984).

Laetiporus sulphureus has been treated as a species complex that varies on its morphological features as fruiting bodies and basidiospores, its hosts-specificity on dead or living wood of gymnosperms or angiosperms and recently, molecular studies have confirmed eight well-supported clusters within *L. sulphureus* species (Burdall & Banik 2001, Lindner & Banik 2008, Ota & Hattori 2003).

Vasaitis *et al.* (2009) characterized the genetic variation and molecular relationships of *L. sulphureus* s. l. from Europe, South America, Africa and Asia. The results indicate that each resolved cluster represents a distinct taxon within *L. sulphureus* complex. Specimens from Uruguay were used like representatives from South America. The analysis showed that these specimens from Uruguay clustered strongly with

the taxon currently known in North America as *L. sulphureus* (cluster E) and other ten individual, all collected exclusively from eucalypts, grouped into a well-supported cluster with *L. gilbertsonii* Burds. (cluster F). Surprisingly, a second taxon (cluster C) included 49 individual of *Laetiporus* with a host range and distribution exclusively from Europe did not group with any of the *Laetiporus* species defined from North America by Lindner & Banik (2008).

This discovery calls into doubt the propriety to use the European name *L. sulphureus* for the taxon occurring in North America. Further morphological studies of macro- and microscopic traits and a more details knowledge of host range and distribution of both taxon occurring in America (cluster F) and Europe (cluster C) is necessary before a definition of *L. sulphureus* s.s. can be established.

During recent surveys in Brazilian Atlantic rainforest, an interesting species of *Laetiporus* was found and herein is described as new. *Laetiporus gilbertsonii* is recorded for the first time from Brazil.

MATERIAL AND METHODS

Morphological analysis

Specimens were collected in areas of Atlantic rainforest in São Paulo state, between 2013 and 2014, dried and deposited at the herbarium SP (Instituto de Botânica, São Paulo, Brazil). Sections of the basidioma were studied macroscopically and described according to color, texture, size, hymenial surface, etc., and for microscopic analyses thin sections of the basidioma were mounted on microscope slides prepared with 5% KOH solution and for hyaline structures, was added 1% aqueous phloxine solution for the analysis of basidiospores, hyphal system and sterile elements (Teixeira 1995, Coelho 2005).

DNA sequencing and phylogenetic analysis

DNA was extracted from fruiting bodies using Sigma-Aldrich Gen Elute™ Plant Genomic DNA

Miniprep Kit. ITS region was amplified with primers ITS1 and ITS4 and nLSU region was amplified with primers LR0R and LR7 (White *et al.* 1990) with Sigma-Aldrich ReadMix™ Taq PCR P4600 kit. Forward and reverse sequencing were compared and merged in Sequencher (v. 4.1.1). Sequences were aligned with MAFFT (<http://mafft.cbrc.jp/alignment/server/>) v.7, and optimized manually with Geneious v. 8.1.

Four phylogenetic analyses were performed. Bayesian analysis (BA) was performed in MrBayes 3.2.2 (Ronquist *et al.* 2012). Best model of evolution used in BA were estimated by using JModeltest 2.c1.4 (Darriba *et al.* 2012). Maximum likelihood (ML), Maximum parsimony (MP) and Neighbor-Joining (NJ) analysis were performed using MEGA6 (Tamura *et al.* 2013). Trees from ML, MP and NJ analysis were compared with those resulting from the BA. Bootstrap values (above 50%) were used to support the respective nodes compatible between four trees.

RESULTS

Taxonomy

Laetiporus squalidus R.M.Pires, Motato-Vásquez & Gugliotta, **sp. nov.** MycoBank MB 811581

Basidioma annual, effused-reflexed forming numerous narrow pileus at the margins, thin, soft when fresh to hard when dry. Pileus broadly attached, up to 2.0–2.5 × 1.0–2.0 × 0.4–1.0 cm, upper surface glabrous, azonate, slightly rugose, cream to pale brown when fresh to light ochraceous after dry. Margin undefined. Context homogeneous, azonate, concolorous with the pileus. Pore surface cream when fresh to brown when dry, pores rounded, decurrent, 3–5 per mm, tubes concolorous with the pore surface, up to 0.2 cm deep, dissepiments entire, thin to slightly thick. Hyphal system dimitic, tubes trama composed of generative hyphae simple-septate, hyaline, smooth and thin to thick-walled, frequently branched, 4.0–7.0 µm wide, and skeletal hyphae hyaline, straight, thick-walled, 4–6 µm wide. The context composed of generative hyphae simple-septate, unbranched and inflated, (10–)11–21(–25) µm wide, binding hyphae richly branched, hyaline, thick-walled, (4.0–)5.5–7.0 µm wide. Basidia clavate, hyaline, 4-sterigmated, thin-walled, 16.0–20.5 × 8.0–10.0 µm. Basidiospores abundant, ellipsoid to broadly ellipsoid, hyaline, smooth, slightly thick-walled, usually with one or two oil drops, inamyloid, undextrinoid, (5.0–)6.5–7.0 × 4.0–5.0 µm ($X_m =$

5.8 × 4.4 µm), $n = 30/1$, $Q = (1.1–)1.2–1.5$, $Q_m = 1.3$.

Comments — *Laetiporus squalidus* is very similar to *L. sulphureus* complex, sharing the same hyphal system and basidiospores shape, but these can be easily distinguished by the presence of effused-reflexed basidioma that form narrow pileus at the margins in the former and the pileate to laterally stipitate basidioma in the latter; besides that, the spores are slightly narrower in *L. sulphureus* (Burdall & Banik 2001).

Laetiporus gilbertsonii Burds, Harvard Papers in Botany 6:48 (2001)

Basidioma annual, laterally stipitate, sessile or with broad stipe attachment, soft when fresh, hard when dry. Pileus dimidiate up to 5.4–14.5 × 5.8–11.1 × 0.6–2 cm, upper surface striated, azonate, yellow to pale brown when fresh and yellowish-brown to pale orange after dry. Margin involute. Context homogeneous, azonate, dense, up to 2.0 cm thick, cream to white. Pore surface cream to pale brown when fresh to yellowish-brown when dry, pores rounded, decurrent on stipe, 5–7 per mm, and tubes concolorous with the pore surface, up to 0.1 cm deep, dissepiments entire, thin to slightly thick. Hyphal system dimitic, pileus context with generative hyphae simple-septate, inflated, thin-walled, smooth, 10–18(–19) µm wide, and binding hyphae hyaline, densely branched, thick-walled, 5–10(–11) µm wide. Tubes trama composed of generative hyphae simple-septate, frequently branched, parallels in arrangement, hyaline, smooth and thin-walled, 5.0–7.0 µm wide, and skeletal hyphae, hyaline, sinuous and undulating, thick-walled, 5.0–6.5 µm wide. Basidia clavate, hyaline, 4-sterigmated, thin-walled, 16.0–20.0 × 8.0–9.5 µm. Basidiospores broadly ovoid, hyaline, smooth, thin-walled, inamyloid, undextrinoid, 5.0–5.5 × 4.0–5.0 µm ($X_m = 5.2 × 4.4 µm$), $n = 60/3$, $Q = 1.0–1.3$, $Q_m = 1.2$.

Comments — Microscopically, the broadly ovoid basidiospores differentiate it from other species of *Laetiporus* occurring in Brazil. Our materials differ from the description presented by Burdall & Banik (2001) in the pore size (5–7 per mm in the Brazilian material and 2–4 per mm in the type), however agree in the other macro and microscopic features and our identification is also supported by molecular data.

Molecular phylogeny

Analysis of nLSU region was performed in order to assess the relative position of the new taxon within the family Polyporaceae Corda. For nLSU, the final matrix consisted of nineteen sequences from

GenBank and a new nLSU sequence generated. The results showed the new species forming a distinct clade close related with the *Laetiporus* group (Figure 1). The best bootstrap supports were produced from NJ and ML analysis (83% and 53%, respectively). BA and MP analysis had similar topology but the bootstrap values weren't high enough.

Phylogenetic tree of ITS region were produced with fifty-six sequences from GenBank and five new ITS sequence generated from this study (Figure 2). This phylogenetic reconstruction resulted in a distinct clade of *Laetiporus squalidus* sp. nov. with high PP in BA (1.00) and high bootstrap in MP and NJ (98 and 93, respectively), and grouped the four Brazilian *Laetiporus* species sequenced for this work with the *Laetiporus gilbertsonii* group (Clade F).

DISCUSSION

Laetiporus squalidus sp. nov. is characterized by its pale whitish and irregular pilei and by its large connective hyphae in the context and basidiospores $6.5-7.0 \times 4.0-5.0 \mu\text{m}$. Microscopically, *L. squalidus* is quite similar to *L. sulphureus* complex, differing mainly in its macromorphology, as the shape and color of the pileus. What is a good way to characterize the species and differs from the other species within the group.

The phylogenetic analysis supported *L. squalidus* as a distinct species on both ITS and nLSU region. Lindner & Banik (2008) discuss the status of *L. persicinus* (Berk & M.A. Curtis) Gilb., a close related species to *L. sulphureus* complex but divergent in morphological traits, including lacking of bright orange coloration in the upper surface and tubes that stain blackish-brown. *Laetiporus squalidus* also exhibits different characteristics from other species of the genus as the pileus shape and the cream to pale brown upper surface. In this study, the sequence of *L. persicinus* obtained from GenBank databases revealed no significant similarity of this species to any known genus, although it appears related to the *L. sulphureus* complex clade in other different studies (Ortiz-Santana *et al.* 2013). *Laetiporus squalidus* present the same condition, it appear close to *L. sulphureus* complex and *L. persicinus* in the phylogenetic trees. More studies are required to determine the position of *L. persicinus* within the genus *Laetiporus*, or transfer this to a different or new genus.

CONCLUSIONS

Laetiporus gilbertsonii (clade F) is now referred to Brazil. The phylogenetic analysis supports the Brazilian specimens within the clade F with 0.98 in BA analysis, 57% and 60% in PA and NJ analyzes, respectively. The present work including Brazilian *Laetiporus* specimens had similar results to Vasaitis *et al.* (2009) and intensifies its argument about of the Pan-American distribution of *L. gilbertsonii*.

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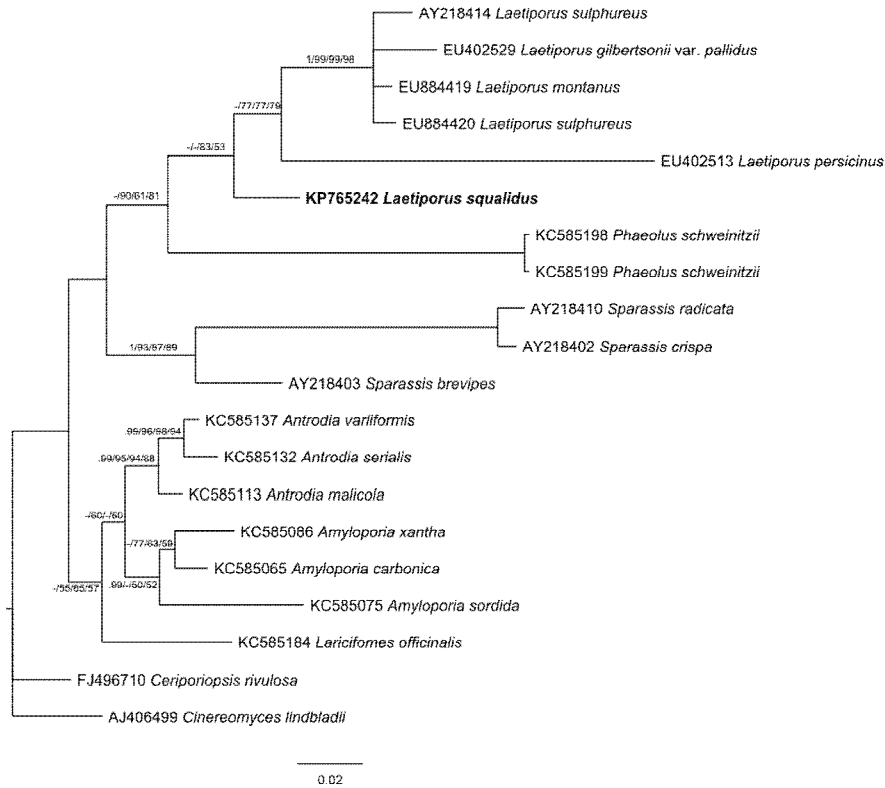


Figure 1: Phylogenetic placement of the *L. squalidus* inferred from BA, MP, NJ and ML from nLSU sequences. Name of taxon in bold font represent the new species. Support values consist of the Bayesian posterior probability (PP) (>0.95) followed by MP, NJ and ML bootstrap values (>50%).

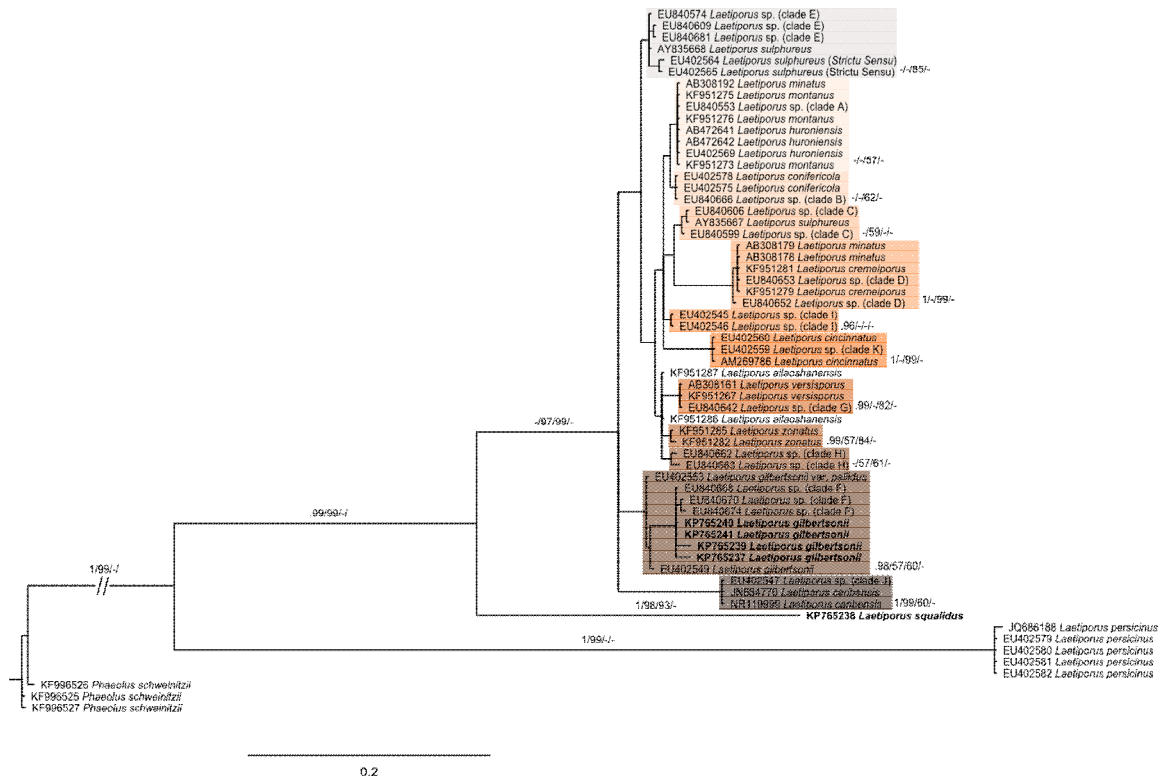


Figure 2: Phylogenetic analysis of *L. squalidus* sp. nov. and *L. gilbertsonii* based in BA, MP, NJ and ML from ITS sequences. Names of taxa in bold font represent the studied species. Support values consist of the Bayesian posterior probability (PP) (>0.95) followed by MP, NJ and ML bootstrap (BS) values (>50%). The different colored boxes represent clades within the *L. sulphureus* group.