

Morpho and Molecular Studies on *Phellinotus badius* from Tamil Nadu, India

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ABSTRACT

***Phellinotus*, a pathogenic neotrophic fungi, inhabiting on living Fabaceae members. This study demonstrates the first report with molecular evidence of *Phellinotus badius* from Dindigul and Villupuram districts of Tamil Nadu, India. Morphological and phylogenetic analyses inferred from combined ITS and LSU nrDNA region was carried out. Maximum likelihood and Bayesian analyses placed the current isolates in *Phellinotus badius* clade. Taxonomic and phylogenetic relationships of *P. badius* is discussed.**

Keywords: Hymenochaetoid fungi, Phylogenetics, Styrylpyrone, Wood inhabiting fungi.

INTRODUCTION

Hymenochaetaceae Donk comprises xanthochoric fungi, having styrylpyrone based yellow pigment that gives black colour while reacting with 5% KOH. Earlier, the species concept of this family was based primarily on the type of hyphal system (monomitic or dimitic) and the annual or perennial nature of the basidiomata (Cunningham, 1947). Species with dimitic hyphal system and perennial basidiomes have placed under *Phellinus* (Fiasson and Niemelä, 1984; Corner, 1991; Wagner and Fischer, 2001). However, *Phellinus* was proposed to be polyphyletic in early 20th century and subsequent molecular phylogenetics studies confirmed many smaller natural species groups with distinct morphological features within this genus (Gilbertson and Ryvardeen, 1987; Larsen and Cobb-Pouille, 1990; Niemelä *et al.*, 2001; Wagner and Fischer, 2002; Ryvardeen, 2005; Zhou and Qin, 2013).

Based on the morphological and molecular approach, *Phellinotus* (Cooke) Salvador-Mont., Popoff & Drechsler-Santos was delineated from *Phellinus rimosus* species complex with *P. neoaridus* as the type genus (Drechsler-Santos *et al.*, 2016). *Phellinotus* is characterized by perennial basidiomes, duplex context with dark lines, monodimitic hyphal system, mycelial core, basidiospores turns chestnut brown in aqueous KOH (Drechsler-Santos *et al.*, 2016). Phylogenetic analysis revealed that '*phellinotus clade*' is monophyletic group that accommodates *Phellinotus*, six allied well-established genera and some unresolved taxa found

in *Fomitiporella s.l.* (Salvador-Montoya *et al.*, 2022). At present, *Phellinotus* comprises 8 species viz., *P. badius*, *P. magnoporatus*, *P. neoaridus*, *P. piptadeniae*, *P. resinaceus*, *P. scaber*, *P. teixeirae* and *P. xerophyticus* (www.indexfungorum.org). *Phellinotus badius* seems to be distributed in North/Central America and Caribbean region (Cunningham, 1965; Larsen and Cobb-Pouille, 1990). In the current study, we provide morpho and molecular data of *Phellinotus badius* from Villupuram and Dindigul districts, Tamil Nadu, India.

MATERIALS AND METHODS**Morpho-taxonomic studies and Phylogenetic analyses**

The specimens were collected from, Malaiyanur (Villupuram district) and Sirumalai (Dindigul district), Tamil Nadu, India. Morphological features of basidiomata viz., size, shape, annual or perennial, colour, texture, and margin (acute or obtuse), colour (Kornerup and Wanscher, 1978), xanthochoric reaction of the context tissue (context tube layer and pores were recorded from the fresh samples. Microscopic characters such as hyphal system, setae, cystidiole, basidiole, basidia and basidiospores were observed using sterile distilled water, 5% aqueous KOH (w/v), Cotton blue (CB), and Melzer's reagent (IKI). The basidiospores, cystidiole, basidiole and basidia were observed using phloxine dye. The mean length and width of the basidiospores, their Q values were also recorded (derived from an average of 30 basidiospores). The samples were deposited in the

herbarium of Madras University Botany Laboratory (MUBL), Centre for Advanced Studies in Botany, University of Madras, Chennai, Tamil Nadu, India.

Pure culture was raised from the piece of context tissue of the basidiome using potato dextrose agar medium and stored at 10°C in PDA slants. From the mycelial mat, 50 mg was used for genomic DNA extraction following the protocol of Doyle and Doyle (1987) and modified by Góes-Neto *et al.* (2005). Internal transcribed spacer region and large subunit of nuclear ribosomal DNA region was amplified using the primers ITS1/ITS4 and LR7/LROR with the recommended thermal conditions (White *et al.*, 1990; Vilgalys and Hester, 1990), respectively. The resultant products were outsourced for sequencing at Eurofins Genomics India (Karnataka, India). The newly generated sequences from the current study along with the sequences retrieved from GenBank (NCBI) based on Blastn search were used for phylogenetic analysis. *Fomitiporella caryophylli* (CBS 448.76) is used as outgroup. The nrITS and nLSU sequences were aligned separately in MEGA X v10.0.2 and edited manually to increase the similarity of the alignment (Kumar *et al.*, 2018) and the same was used for the maximum likelihood

(ML) analysis and Bayesian Inference (BI) analysis using RAxMLGUI 2.0 (Edler *et al.*, 2021) and MrBayes 3.2.7a (Ronquist *et al.*, 2012) respectively. Bootstrap support values for Maximum likelihood (ML, first value) equal to or greater than 60% and Bayesian Inference (BI, second value) equal to or greater than 0.8 are given above the nodes. The newly generated sequences are indicated in red and bold.

RESULTS AND DISCUSSION

A total of 1812 characters were in the concatenated nLSU and nrITS dataset (910 for nLSU and 902 for nrITS), of which 1008 were constant, 665 variable, and 418 parsimony informative. Maximum likelihood (ML) trees were constructed using RAxML GUI 2.0 by 1000 replicates (Edler *et al.*, 2021). Bayesian inference was performed using two independent runs of six chains of Metropolis-coupled Markov chain Monte Carlo reconstructions for 10,000,000 generations, with tree samples obtained every 100 generations. The phylogenetic analysis revealed that our two isolates (MUBL1124 and MUBL1125) closely clustered with the South Korean isolate (CBS 449.76) and other Indian isolates (LDCMY3, KRMICRO, and MVMB) with 100% ML and 1.00 BPP (**Figure 1**).

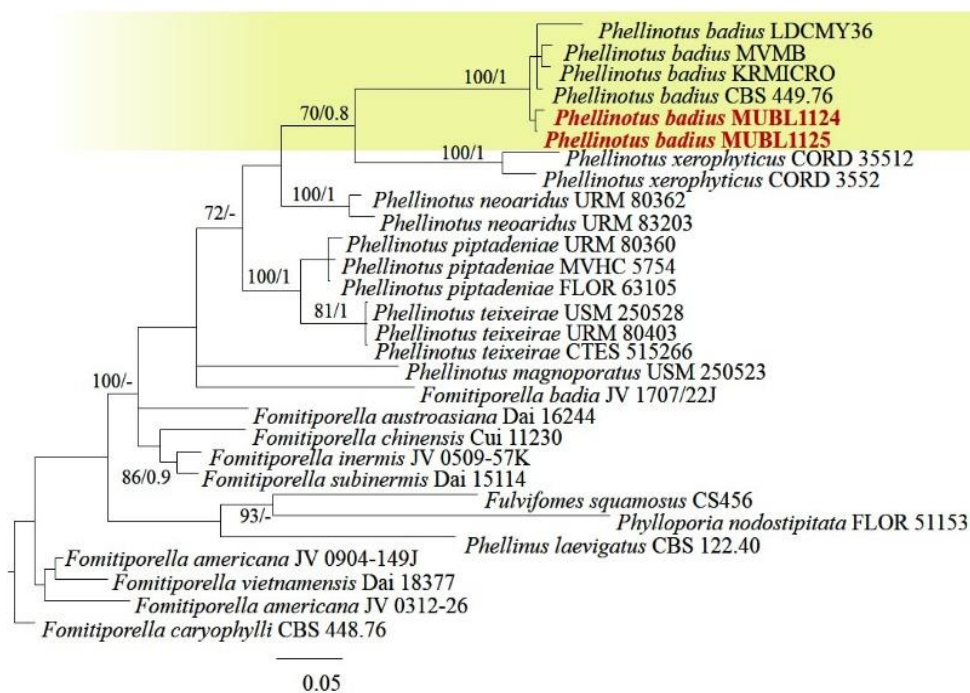


Figure 1: Maximum likelihood consensus tree inferred from the combined ITS and LSU. Bootstrap support values for Maximum likelihood equal to or greater than 70% and Bayesian Posterior probabilities from MCMC analyses equal to or greater than 0.8 are given above the nodes. The tree is rooted with *Fomitiporella caryophylli* (CBS 448.76). The newly generated sequences are indicated in Maroon.

Phellinotus badius (Cooke) Salvador-Mont., Popoff & Drechsler-Santos. Journal of Fungi 8 (3, no. 216): 8 (2022) (**Figure 2**).

≡ *Fomes badius* Cooke, Grevillea 14 (69): 18 (1885).

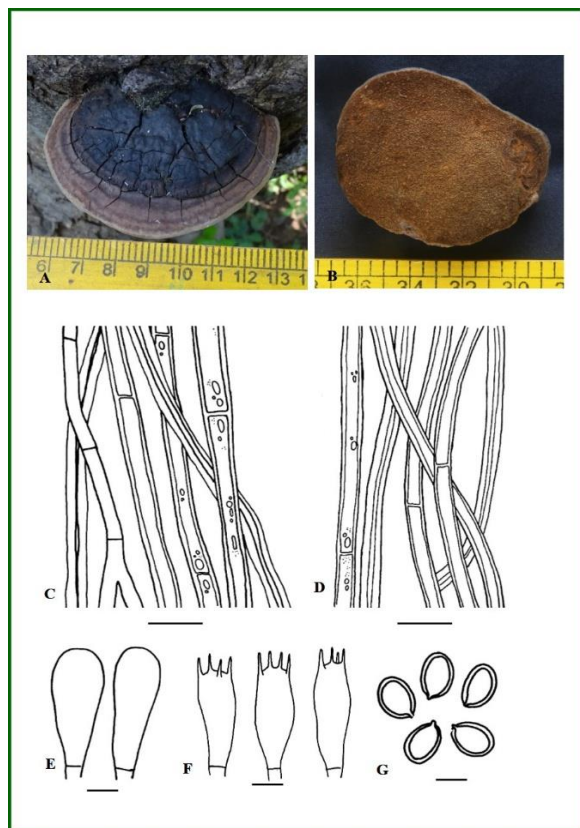


Figure 2: Morpho-taxonomic features of *Phellinotus badius* (MUBL1125); **A.** Basidioma; **B.** Pore surface; **C.** Context hyphae; **D.** Trama hyphae; **E.** Basidiolae; **F.** Basidia; **G.** Basidiospores. (Scale bar: **C-G** = 5 μ m).

Basidioma: perennial, sessile, hoof-shaped to unguulate up to 10 cm wide, 7 cm broad and 2 cm thick, hard, woody. **Pileal surface:** yellowish brown (5E8) when young becoming dark brown (6F7) with age, weakly zonate, rimose, crust up to 0.2 mm thick; **margin:** round, 2 cm thick. **Context:** duplex with black line, bright, yellowish brown (5E5), corky when fresh, hard on drying, up to 15 mm thick, faintly zonate, turns brownish grey (6F2) in KOH. **Pore surface:** light brown (6D5) to brown (6E8). **Pores:** round, 4-6 per mm. **Tube layer:** yellowish brown (5E5), indistinctly stratified, tubes up to 3 mm long. **Hyphal system:** strictly dimitic, generative hyphae dominant; both skeletal and generative hyphae acyanophilous; tissue darkening with KOH without swelling. **Context:** generative hyphae thin to thick-walled, hyaline to yellow,

simple septate, branched, 2–5 μ m diam.; skeletal hyphae, thick-walled with narrow lumen, unbranched, yellowish brown, aseptate, 2–5.2 μ m diam. **Trama:** generative hyphae thin to thick-walled, yellow to brown, septate, rarely branched, 2.3–5.5 μ m diam.; skeletal hyphae, thick walled with narrow to wide lumen, yellowish brown, aseptate, unbranched, 2.1–5 μ m diam. **Cystidioles** and **setae** absent. **Basidioles:** clavate, 8–16.5 \times 4–7.2 μ m. **Basidia:** clavate to broadly clavate, with four sterigmata, 9–18 \times 6–8.5 μ m. **Basidiospores:** broadly ellipsoid to subglobose, thick-walled, smooth, yellow in water, turning rust brown in KOH, (4.8–) 5.1–7.7 (–7.9) \times (5.1–) 5.4–5.9 (–6.2) μ m (n = 50/2), Q = 1.5 (Q range 1.3–1.7), acyanophilous and inamyloid.

Specimen examined: Tamil Nadu, Villupuram district, Malaiyanur, 12°20'34.7"N 79°20'06.0"E, on living angiosperm tree *Tamarindus indica*, 25 October 2016, Sugantha Gunaseelan (MUBL1124) (ITS - PQ432936; LSU - PQ432937); Sirumalai, 10°15'06.1"N 78°00'59.2"E, on living angiosperm tree dead wood, 31 December 2022, Ramesh Murugadoss (MUBL1125) (ITS - PQ423099; LSU - PQ423100).

Phellinotus badius was earlier reported from northern parts of India but lacks molecular evidence (Kumari *et al.*, 2021). *P. badius* can be easily recognized by its golden brown lustrous duplex context with granular pore, absence of setae, and large subglobose yellowish spores and is similar to earlier descriptions (Sharma, 2012; Parihar *et al.*, 2019). Sundari *et al.* (2018) reported *P. badius* from Pudhupatti, Dharmapuri district, Tamil Nadu however lacks molecular details. Our study represents the first report of *P. badius* with molecular details (ITS and LSU) from Dindigul and Villupuram districts of Tamil Nadu. This investigation further expands our knowledge on geographical distribution of *P. badius* in India.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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