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Fuscoporia minutissima: A New Report of Family Hymenochaetaceae for Maharashtra State of India

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ABSTRACT

The present study reports a new record of *Fuscoporia minutissima* from the Maharashtra state of India. *F. minutissima* is a poroid global member of the Hymenochaetaceae family that degrades wood. During the study, an unknown specimen of wood-inhabiting fungus was collected from the Velha region of the Pune District of Maharashtra. The species was described based on morphologic features of the fruiting body and phylogenetic analysis of the ITS region. This report also provides comprehensive macro-morphology and micro-morphology of newly documented fungus. Morphological and molecular studies based on the ITS nucleotide dataset confirmed that the specimen represents a new record for Maharashtra state. The current study will improve the fungal diversity checklist of Maharashtra. The findings could also assess the necessity for fungal ecology conservation and monitor anthropogenic and natural interruptions.

Key words: *Hymenochaetaceae, Fuscoporia minutissima, ITS, Phylogenetic analyses, Maharashtra state.*

Introduction

The Family Hymenochaetaceae is a significant group within the fungal kingdom, and its taxonomy plays a crucial role in understanding its ecological importance and evolutionary relationships within ecosystems (Nickerson *et al.*, 2013; Adarsh *et al.*, 2019). Members of this family are primarily wood-rotting and play important ecological roles in the decomposition of wood. The production of tough, woody fruiting bodies characterizes many species within the Hymenochaetaceae family. Key features of Hymenochaetaceae include the presence of a hymenium, the spore-bearing surface often found on the underside of the fruiting body. The fruiting bodies can take various forms, such as brackets or crusts, and they are typically durable and long-lasting (Raymundo *et al.*, 2009; Chen *et al.*, 2023). *Fuscoporia* is broadly dispersed in America, Oceania, Europe and Asia. The genus is a synonym of *Poria* or *Phellinus* for an extended period (Overholts, 1953; Lowe, 1966; Ryvarden and Johansen, 1980; Larsen and Cobb-Poulle, 1990). Macrofungi play an important ecological role in their habitats and contribute to ecosystems' diversity and health. They act as natural dispersants and enrich nutrient cycling in the ecosystem by decomposing organic matter (e.g., fallen leaves, dead plants, woody debris), which

would be digested and reused by plants and other microorganisms (Olou *et al.*, 2023). Many species of fungi may vanish without being found, identified, or documented (Blackwell, 2011). Polypores are primarily tuned towards wood from certain plant species, and the tropics are losing forests at an alarming rate (Krah *et al.*, 2018). Similarly, little is known about the aphyllophorales species in the Velha tehsil of Pune District, India (Ranadive *et al.*, 2011). Therefore, in the current investigation, the characterization and studies have been conducted on two new records of the Velha region of Pune District, India.

Furthermore, the ecological importance of specific localities within ecosystems is crucial for understanding the key controlling roles in ecological processes, and the construction of ecological networks based on ecological resistance is an important method for protecting ecological environments and optimizing landscape patterns (Nickerson *et al.*, 2013). In conclusion, the taxonomy and phylogeny of the Family Hymenochaetaceae is essential for understanding its ecological importance, evolutionary relationships, and roles within ecosystems.

The Velha area is located in Maharashtra's Western Ghats and is known for its diverse sceneries and ecosystems. Overall, the study area has typical damp deciduous vegetation with a few patches of semi-evergreens (Champion and Seth, 1968). Few reports are available on the diversity of Aphyllophorales in Maharashtra state of India. A total of 256 Aphyllophoraceous fungi found in Maharashtra State are listed in a checklist, comprising 86 species from 20 non-poroid families and 170 species from 10 poroid families (Ranadive *et al.*, 2011). A few notable species from the Pune District were reported. The species include *Daedalea* sp. and *Ganoderma* sp. *Phellinus* sp. etc. (Ranadive *et al.*, 2011). However, due to a lack of molecular sequence data, certain specimens were not included in DNA-based polypore investigations. They were aware that fungal identification based on morphology might occasionally produce inaccurate findings due to deceptive morphological traits. (Olou *et al.*, 2023). There is potential to research the variety of Aphyllophorales from a molecular standpoint.

DNA marker sequencing, often known as barcoding, has become a prominent method for various research, including species identification and molecular phylogenetic extrapolation (Hibbett *et al.*, 2013; Savolainen *et al.*, 2005). The mycological community has widely regarded the ITS region as the best identifier, with a high possibility of accurate identification for species in various fungal groups (Deng and Zhao, 2023).

As a result, representatives of the Aphyllophorales (family Hymenochaetaceae) were collected and investigated in this work to give molecular phylogenetic evidence based on internal transcribed spacer (ITS) region sequences that validate the documentation of a new record from the Velha region of the Pune District, Maharashtra state of India.

Materials and Methods

Study area

The current investigation is on the Velha region, part of the Pune District (18° 17' 46.824" N, 73° 38' 16.08" E.) in the Western Ghats of Maharashtra. A wide

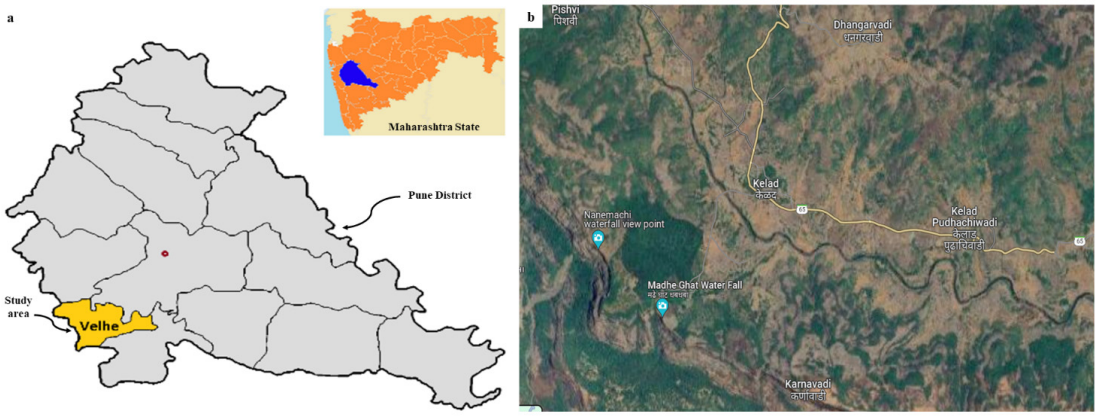

Fig. 1. Study area: (a) Map of Maharashtra State, showing the Pune District with highlighting study area Velha region (Source: https://images.app.goo.gl/pgvvhzjb4uukEzUR8) (b) Google satellite map of study area (Source: https://maps.app.goo.gl/JdBzkccNP7v3ouvW6).

range of landscapes and ecosystems distinguishes the region. (Fig. 1). The vegetation in the Velha area is damp and deciduous, with a few semi-evergreen patches (Champion and Seth, 1968).

Collection, Examination, Isolation, and Preservation

Between 2022 and 2023, samples were collected every year from July to September at Madhe Ghat (Velha tehsil) in the Pune district of India. These wood-rotting, basidiomycete-focused polypores are photographed in their natural environment. Small pieces of the fresh fruiting bodies are placed in paper bags for DNA extraction. Depending on the size of the fruiting bodies, they are air-dried for 1 to 2 days at room temperature. The dried fruiting bodies are used for morphological observations. The remaining fruiting bodies are stored in brown paper bags. The collected specimens are stored at Agharkar Research Institute's Ajrekar Mycological Herbarium, Pune, India.

Identification

The microstructural examination was based on recorded morphological information such as size, shape, colour, surface texture, colour, attachment, and photographs of the specimens. For anatomical features, thin sections of basidiocarp were taken for comment under a compound light microscope using a sharp blade and mounted in 10% potassium hydroxide + 1% Phloxine aqueous solution in water (Olou *et al.,* 2023) using Melzer's reagent + cotton blue. Microscopic examination was performed at 100× magnification under the Olympus compound microscope. Species were generally identified using identification keys developed in 1980 by Ryvarden and Johansen (1980), 1987 Gilbertson and Ryvarden (1990), and Gorjón and Bericchia (2010).

DNA extraction

Using the Buzina *et al.,* (2001) method, tissue was homogenized in liquid nitrogen for two minutes and then floated in a lysis buffer to recover genomic DNA from desiccated samples. The components of the lysis buffer were 3% Sodium Dodecyl Sulphate (SDS), 50 mM EDTA, and 100 mM Tris. The component tube was separated by centrifuging it for ten minutes at 10,000 g. Next, the DNA filtrate was cautiously shifted into a sterile 1.5 ml Eppendorf tube that was nuclease-free. The following step was adding phenol to the supernatant in an amount equal to that of chloroform and isoamyl alcohol (PCI). The Eppendorf tube is then entirely inverted to combine all of the ingredients. After that, the mixture was centrifuged (8000 g) for 12 minutes to extract the DNA-containing aqueous layer, which was then transferred into a fresh 1.5 ml Eppendorf tube. To precipitate the DNA, an equivalent volume of cold isopropyl alcohol was added to the pooled aqueous layer. After being kept at -20 °C for 20 minutes, the tube was centrifuged (8000 g) for 10 minutes to create a DNA pellet at the bottom of the tube. The DNA pellet was centrifuged at 8000g for five minutes after being cleaned with 70% cold ethanol (500 µl). The ethanol was evaporated, and the DNA pellet was dried at 55 °C after removing the supernatant. Subsequently, it was again suspended in 40ìl of 1X TE buffer.

Polymerase Chain Reaction (PCR) amplification and purification

The ITS1 and ITS4 primers were used to amplify the nuclear ribosomal ITS (Internal Transcribed Spacer) region (White *et al.,* 1990; Borde *et al.,* 2021). One milliliter of DNA template (10 ng), 0.25 units of Taq DNA polymerase (Sigma-Aldrich, India), 2.5 milliliters of Taq DNA polymerase buffer (10X), one milliliter of deoxynucleotide triphosphate (dNTPs) at a concentration of 200 micrograms (Sigma-Aldrich, India), one milliliter of 10 pmol primer, and the remaining volume was made up with ultra-pure sterilized water. The amplification was performed using an Eppendorf thermal cycler (Eppendorf, Hamburg, Germany). The following were the thermal cycling parameters: Initial denaturation occurred for five minutes at 95 °C. There were 30 amplification cycles, with one minute of denaturation at 95 °C, thirty seconds of annealing at 55 °C, one minute of extension at 72 °C, and one final extension step lasting seven minutes at 72 °C. Next, the PCR products were purified using a PCR cleanup kit (Axygen Scientific Inc., CA, USA) to eliminate contaminants or unincorporated primers and nucleotides. An automated DNA sequencer, the ABI Avant 3100 (Applied Biosystems, USA), was used to sequence the purified PCR product from the cycle. The raw DNA sequencing data was modified and combined into a logical sequence using Chromas Lite version 2.01. The resulting sequence data were deposited in the NCBI nucleotide sequence database.

Results and Discussion

Taxonomy

Fuscoporia minutissima

Occurrence: *Vitex negundo* stem

Fruiting body: It is perennial, having a pileus with imbricate basidiocarps. A basidiocarp is hard and corky when it is dried and it is odourless while fresh (Fig. 2). The pilei are primarily imbricate, with 6.0 × 4.0 × 1.2 cm in length, width and thickness respectively. The pileal exterior shows reddish-brown colour, concentrically sulcate with regions; the border width is 1.2 mm with honey-yellow, and obtuse to slightly acute. Glancing pore surface that ranges from greyish brown edge of 1 mm wide; round, 11–14 pores/mm; comparatively profuse, complete. Context: hard corky, yellowish in colour with ~6 mm in thickness and 5 mm in length.

Hyphae: It is dimitic, simple septate generative hyphae. Context: Skeletal hyphae are mostly brown in appearance with thick-walls. It has medium to narrow lumen, unbranched, occasionally septate and 2–4 µm in diameter. Uncommon generative hyphae which is hyaline, thin to thick–walled, unbranched and simple septate.

Tubes: The majority of skeletal hyphae are yellow-brown, with strong walls and a medium-to-narrow lumen. They are often septate, roughly straight, subparallel along the tubes, and have a diameter of 4-5 µm. The generative hyphae are an uncommon sight, mostly seen near the borders of dissepiment and subhymenium. They are hyaline, thin-walled, often branching, simple septate, and measure 1.2–3 µm in diameter. A portion of them is embedded in the hymenium and at the borders of the dissepiment. Measuring 15–35 × 5–10 µm, hymenial setae are subulate and mostly produced from tramal hyphae. Cystidioles have thin walls and a hyaline appearance. They measure 8–11 × 4.3–5.2 µm. Measuring 9–14 × 3–5.8 µm, basidia are small, clavate to barrel-shaped, with four sterigmata and a simple septum at the base. Lastly, basidioles, which have a considerably smaller form than basidia, predominate on the hymenium.

Spores: The basidiospores are smooth, thin-walled, hyaline, broadly ellipsoidal to subglobose, and have a guttle. They measure 3 mm in length and 2.2 mm in width.

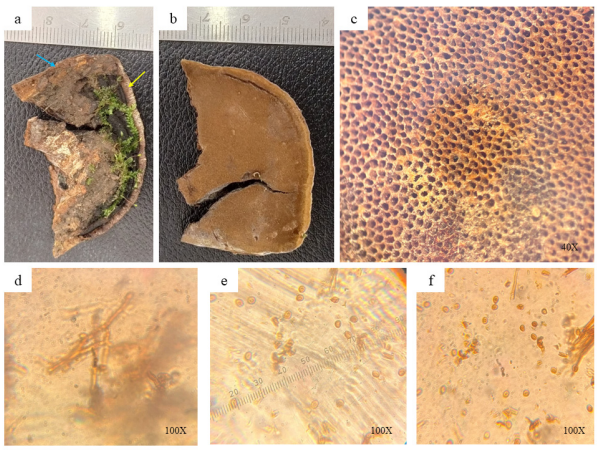

Fig. 2. *Fuscoporia minutissima*: (a) Sporocarp- abhymenial surface- yellow arrow indicates sporocarp & blue arrow indicates host plant, (b) Sporocarp- hymenial surface, (c) Hymenial pores, (d) Generative hyphae, (e & f) Spores.

Phylogenetic Analysis

The phylogenetic position of our taxon was determined by a molecular analysis based on ITS nucleotide sequencing data. Reference sequences and an outgroup were chosen from GenBank and pertinent literature. To validate and identify, the ITS sequences of collected fungus were correlated with those found in the GenBank database. The nucleotide sequences from the ITS regions of the specimen were compared with the NCBI database (https://www.ncbi.nlm.nih.gov/) using BLASTn. The specimen has been submitted and deposited at Ajrekar Mycological Herbarium (AMH), ARI, Pune, India. The ITS and accession number of the collected specimen Fuscoporia minutissima PP038005 forms a clade with existing nucleotide sequences with accession numbers OQ817711.1 and OQ817712.1 in the phylogeny with bootstrap support (1000) (Fig. 3). Therefore, the collected fungal specimen was identified and confirmed to be *Fuscoporia minutissima* (Family- Hymenochaetaceae).

The studied fungal specimen was described by morphology and identified by molecular features. The produced nucleotide sequences of specimen collected from the Madheghat, Velha tehsil, India and used them for phylogenetic investigation to validate morphological proof of identity. All recently-created sequences fall properly into the conforming clades. The present study displays that even though the ITS region is recognized as widespread and apt for accurate identification (Schoch *et al.*, 2012).

This is the first report where *Fuscoporia*

minutissima (Family- Hymenochaetaceae) has been identified and recorded for the first time in the Maharashtra state of India. The specimen studied was collected from the Velha region of the Pune District.

A checklist published on order Aphyllophorales by Randive *et al.* (2011) recorded 256 species, including 170 from 10 poroid families and 86 from 20 non-poroid families. However, *Fuscoporia* was not listed in the checklist. Previous reports on the order Aphyllophorales recorded 20 species belonging to 8 families and 14 genera from 15 different localities in the Western Ghats of Pune district, Maharashtra. However, the present reported species were not listed in the earlier study, where specimens were identified based on morpho-taxonomical characteristics (Ranadive *et al.*, 2013). In 2013, Randive overviewed an Aphyllophorales from India and *Fuscoporia minutissima* was not listed among a total 1175 species from 52 families and 190 genera of poroid and non-poroid Aphyllophorales. In recent studies, studied fungi have not been reported. (Ranadive, 2013; Hakimi *et al.*, 2013; Yemul *et al.*, 2019; Gore and Mali, 2023).

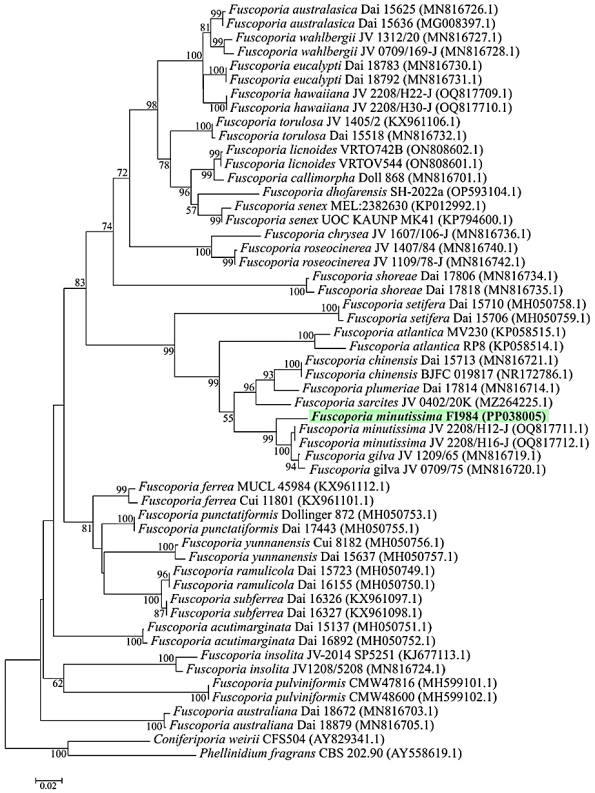

Fig. 3. Phylogenetic tree and identification of fungus *Fuscoporia minutissima* construction from the ITS sequence from Neighbor-Joining and Maximum likelihood with 1000 bootstrap supports using *Coniferiporia weirii* and *Phellinidium fragrans* as an outgroup.

Conclusion

India's Western Ghat is a hotspot for biodiversity. The Velha region belongs to the Western Ghats of Maharashtra and is in the Pune District. Information about fungus and molecular data is scarce. The current study evaluated macrofungi at the molecular level, reporting Maharashtra's first-ever discovery of a novel polypore, *Fuscoporia minutissima* (family Hymenochaetaceae). PCR and other molecular-based methods, which combine state-of-the-art technology and tried-and-true methods, have led to the development of new tools for identifying fungal species. Thus, knowledge of macrofungal biodiversity at the species and community level enables pertinent authorities to monitor changes caused by humans and natural processes and evaluate the necessity and efficacy of conservation efforts.

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Conflict of Interest

The authors declare no conflict of interest.

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