



Molecular phylogeny revealed two new records of wood-rotting fungi from Velha Tehsil (Rajgad) of Pune District, Maharashtra, India

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Abstract

In the present study, two new records of wood rotting polypores viz. *Earliella scabrosa* and *Trametes flavida* (Family- Polyporaceae) for Pune District of Maharashtra state, India have reported. Polypores are essential to the health of forest ecosystems because they are decomposers. Despite their significance, meagre is known about the distribution and diversity of polypores in the Velha Region of the Pune district. The collection of specimens and field study was conducted of study area. Based on morphological characteristics, the specimens were identified and reconfirmed based on molecular characterization. We conducted a phylogenetic analysis using ITS sequencing data to ascertain the taxonomic placement of our taxon. DNA sequences for wood decaying polypores collected in Madheghat, Velha tehsil, India, were generated and utilised for phylogenetic studies to validate identification. Polypores *Earliella scabrosa* and *Trametes flavida* have been identified and recorded for the first time in the Pune district of Maharashtra state, India. Molecular-based methods are necessary to identify the various species of fungi accurately. Thus, monitoring both natural and man-induced disturbances and evaluating the necessity and effectiveness of conservation are made possible by an awareness of the biodiversity of macrofungi.

Keywords: Polypores, *Earliella scabrosa*, *Trametes flavida*, Phylogeny, Pune District.

Introduction

Polypores (wood rotting fungus) are an ecologically diversified group, ranging from saprotrophic, parasitic to mycorrhizal (Olou et al., 2023; Patil et al., 2025). They are crucial to food webs because they participate in the recycling of nutrients and carbon in the soil and break down difficult-to-digest organic compounds like cellulose and lignin into forms that other species can utilise (Stokland et al., 2012; Bajpai et al., 2019; Akhtar and Mannan, 2020; Cui et al., 2025). Apart from their ecological significance, polypores are also useful to humans because they can be used as food, medicine, and supplies for mycoremediation (Singh, 2006; Perez-Moreno, 2021; Olou et al., 2023; Kumar et al., 2026). Macrofungi play an important ecological role in their habitats and contribute to ecosystems' overall 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; Zhao et al., 2026). Polypores are important, yet their diversity and distribution are frequently disregarded in the tropics.

Consequently, many species may vanish without being found, identified, or documented (Blackwell, 2011). Because polypores are primarily tuned towards wood from certain plant species, and because the tropics are losing forests at an alarming rate (Krah et al., 2018; Song et al., 2026; Gavit et al.,

2026). Similarly, not much is known about the aphyllorphorales species in the Velha tehsil of Pune District, India (Ranadive et al., 2011). In the current investigation, characterization of two polypores have been conducted and recorded as new reports from the studied area of the Velha region of Pune District, India.

The Velha region is located in Maharashtra's Western Ghats and is known for its diverse sceneries and ecosystems. Overall, 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 Aphyllorphorales of the Pune District. A total of 256 species of Aphyllorphoraceous 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 Aphyllorphorales from a molecular standpoint.

DNA marker sequencing, often known as barcoding, has become a prominent method for a variety of research, including species identification and molecular phylogenetic extrapolation (Savolainen et al., 2005; Hibbett et al., 2013). The ITS region has been widely regarded by the mycological community as the best identifier, with a high possibility of accurate identification for species in a variety of fungal groups (Deng and Zhao, 2023). As a result, representatives of the Aphyllorphorales (family Polyporaceae) were gathered and investigated in this work to give molecular phylogenetic evidence based on internal transcribed spacer (ITS) region sequences that validate the documentation of two new records from the Velha tehsil (Rajgad) of the Pune District.

2. Material and Methods

2.1. Study area

The current investigation is on the Velha region is part of the Pune District (Latitude is 18.30628 and Longitude is 73.66054) in the Western Ghats of Maharashtra. The region is distinguished by a wide range of landscapes and ecosystems. Overall, the Velha region has typical damp deciduous vegetation with a few patches of semi-evergreens (Champion and Seth, 1968).

2.2. Collection, Examination, Isolation, and Preservation

The specimens were collected from Madhe Ghat in the Velha tehsil (Rajgad) of Pune District, India, by visiting the months of July to September. These basidiomycetes-focused wood-rotting polypores were photographed in their natural surroundings. The geographic coordinates of occurrence were recorded for each specimen. Small pieces of fresh fruiting bodies were placed in paper bags to be used in the extraction of DNA. Depending on their size, the remaining fruiting bodies were air-dried at room temperature for 1-2 days. The dried fruiting bodies were used for further morphological observations, and the remaining were preserved in brown paper bags.

2.3. Identification

The specimens were identified using morphological inspection by observing microstructural features based on the recorded morphological information like size, shape, colour, surface texture, colour, attachment, and photographs of the specimens. For the anatomical features, thin sections through the basidiocarps were taken for comment using a sharp blade under compound light microscope and mounted in 10 % aqueous solution of potassium hydroxide mixed with 1 % Phloxine in water (Olou et al., 2023). Melzer's reagent and cotton blue were used for the microscopic examination at 100× magnification (Olympus compound microscope). Species were recognised mostly using identification keys prepared by Ryvarden and Johansen (1980), Gilbertson and Ryvarden (1987), and Gorjón and Bericchia (2010).

2.4. DNA extraction

By homogenising tissue in liquid nitrogen for 2 minutes and floating it in lysis buffer, genomic DNA was recovered from desiccated samples using the Buzina et al. (2001) technique. The lysis buffer was composed of the following components: Tris (100 mM), EDTA (50 mM), and 3% Sodium Dodecyl Sulphate (SDS). To separate the component tube was centrifuged at 10,000 g for ten minutes. After that, the DNA-containing filtrate was cautiously shifted into a sterile, 1.5 ml Eppendorf tube that was

devoid of nuclease. The next action was to add Phenol in an equivalent volume of Chloroform and isoamyl alcohol (PCI) into the supernatant. Then Eppendorf tube is thoroughly inverted to mix all components. The mixture was then centrifuged (8000g) for 12 minutes in order to separate the DNA-containing aqueous layer into a new 1.5 ml Eppendorf tube. An equal volume of cold isopropyl alcohol was added to the pooled aqueous layer to precipitate the DNA. To form a DNA pellet at the tube's bottom, the tube was then centrifuged (8000g) for 10 minutes after being held at -20°C for 20 minutes. The DNA pellet was washed with 70% chilled ethanol (500µl) of by centrifuging at 8000g for five minutes. After removing the supernatant, the DNA pellet was dried at 55°C to ensure that all of the ethanol was disappeared. It was then resuspended in 40µl of 1X TE buffer.

2.5. Polymerase Chain Reaction (PCR) amplification and purification

The nuclear ribosomal ITS (Internal Transcribed Spacer) region was amplified using the ITS1 and ITS4 primers (White et al., 1990, Borde et al., 2021). The PCR was carried in a 25 µl reaction mixture with 1 µl of DNA template (10 ng), 0.25 U of Taq DNA polymerase (Sigma-Aldrich, India), 2.5 µl of Taq DNA polymerase buffer (10X), 1 µl of dNTPs (deoxynucleotide triphosphate) at a concentration of 200 µM (Sigma-Aldrich, India), 1 µl of 10 p mol primer and the remaining volume was made up with sterilized ultra-pure water. An Eppendorf thermal cycler (Eppendorf, Hamburg, Germany) was used for the amplification. The thermal cycling parameters were as follows: five minutes of initial denaturation at 95°C; 30 amplification cycles, each of which included denaturation at 95°C for one minute; thirty seconds of annealing at 55°C; one minute of extension at 72°C; and one final extension step at 72°C for seven minutes. After that, the PCR products were purified by removing impurities or unincorporated primers and nucleotides using a PCR cleanup kit (Axygen Scientific Inc., CA, USA). The purified product of PCR from the cycle, sequencing process were sequenced using an automated DNA sequencer, the ABI Avant 3100 (Applied Biosystems, USA). ChromasLite version 2.01 was used to modify and merge the raw DNA sequencing data into a logical sequence. The resulted sequence data were deposited in the in the NCBI nucleotide sequence database.

2.6. Phylogenetic Analysis

We used ITS sequencing data to conduct a phylogenetic analysis in order to establish our taxonomic and phylogenetic position. Reference sequences and the outgroup were chosen from GenBank and pertinent literature. Using Chromas Pro software, consensus sequences were produced. BLASTn examinations of the resulting ITS sequences were used to identify fungal cultures. By building a maximum likelihood (ML) approach tree with MEGA v.7.0 and the Kimura 2-parameter model, further identity validation was accomplished (Saitou and Nei, 1987; Tamura et al., 2011). Nodal support was evaluated by analysing 1000 bootstrap repetitions. The number of anticipated substitutions per location is shown by the scale bar. The phylogenetic tree was rooted with *Trametes flavida* CBS 158. An ITS region-based maximum likelihood (ML) phylogenetic tree was used to place all the *Earliella* sp. in their respective clades, which further confirmed the identification. The ITS region-based ML phylogenetic tree was constructed for *Trametes* sp. in their respective clades and was rooted with *Tyromyces chioneus* CL- 63 for further identification and confirmation.

3. Results

3.1. Taxonomy

3.1.1. *Earliella scabrosa* (Pers.) Gilb. & Ryvarden

Occurrence: *Artocarpus* decaying wood log.

Description: The fruit body of this fungus is tough and leathery, widely concentrically zonate, at first cream, then with a dark reddish brown, dark brick, to dark chestnut cuticle covering nearly the whole surface with a white margin (Fig 1). The pore surface is whitish when it is fresh and upon drying it becomes cream colour. The flesh is white. The resupinate basidiomata with their breaking hymenial surface, simple-septa-carrying generative hyphae in the dimorphic hyphal system and the smooth, colourless, somewhat thick-walled hyphae are the characteristics that define this species.

Fruiting body: Annual basidiomata, adnate, resupinate, about 8 cm in length, 4 cm broad, and 80–100µm thick, odourless, tasteless when young. The lower surface is creamy, smooth, and cracks with

age. Cream colour, sterile edge and thin.

Hyphal structure: Simple septa are carried by dimitic, generative hyphae in the hyphal system.

Basidiospores: Ellipsoid, amyloid, smooth, colourless, and somewhat thick-walled.

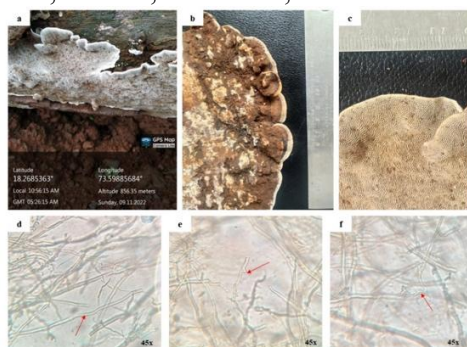


Fig. 1 - *Earliella scabrosa*: (a) Habit, (b) Sporocarp- abhymenial surface, (c) Sporocarp- hymenial surface, (d) Skeletal hyphae, (e) Generative hyphae, (f) Binding hyphae.

3.1.2. *Trametes flavida* (Lev.) Zmitr., Wasser & Ezhov

Occurrence: Decaying wood log of *Ficus* spp.

Description: The annual, sessile to sub-stipitate basidiomes (fruiting bodies) have a thick, petite central or adjacent stalk that is 3.6–5.7 cm in diameter and 1-7 cm elongated (Fig 2). Measurements: 2.4–20 × 3-29 × 0.4–1.4 cm; superior surface: white, somewhat zonate, smooth when fresh, glabrous after drying; margin: uneven, thin, occasionally thick, and round; context: coriaceous to corky, 0.12–1 cm thick. Shape: dimidiate, fan-shaped to round, usually solitary, but infrequently imbricated.

Hymenial Structure: White to wood brown and lamellate on the hymenial surface; round to angular, maze-like, buff colour in the context; trimitic hyphal system; skeletal and generative hyphae are hyaline with thick-walls, binding hyphae is hyaline.

Basidiospores: Basidiospores with thin walls that are non-amyloid.



Fig. 2 - *Trametes flavida*: (a) Habit, (b) Sporocarp- abhymenial surface, (c) Sporocarp- hymenial surface, (d) Skeletal hyphae, (e) Generative hyphae, (f) Binding hyphae.

3.2. Molecular identification and phylogenetic analysis

The phylogenetic position of our taxon was determined by a phylogenetic analysis based on ITS sequencing data. Reference sequences and an out-group were chosen from GenBank and pertinent literature. To validate the identification, the ITS sequences of every fungus were matched with those found in the GenBank database. The sequences from the ITS regions of the material were compared with the NCBI database (<https://www.ncbi.nlm.nih.gov/>) using BLASTn. The specimen has been submitted and deposited at Department of Botany, Savitribai Phule Pune University, Pune, India. ITS and accession number of the collected specimen *Earliella scabrosa*. OR781817 forms a clade with existing sequences with accession numbers OP651753, MK268897, MN892530 and OP390870 in the phylogeny with bootstrap support (86%) (Fig. 4). Therefore, the collected sample was confirmed to be *Earliella scabrosa* (Polyporaceae).

The ITS sequences of collected specimen *Earliella scabrosa* with accession no. OR781817 forms a clade with previously reported sequences with accession Nos. OP651753, MK268897, MN892530 and OP390870 in phylogeny with bootstrap provision (86%) (Fig. 3). Therefore, it was confirmed that the collected specimen is *Earliella scabrosa* (Family Polyporaceae). Similarly, one more species was identified and described from the region. The ITS sequences of the specimen *Trametes flavida* (Accession no. OR782107 & OR782108) form a clade in the phylogenetic tree (Fig. 4) with previously submitted sequences with accession Nos. MH855616, MK736968 was identified as *Trametes flavida* with a bootstrap value (97%).

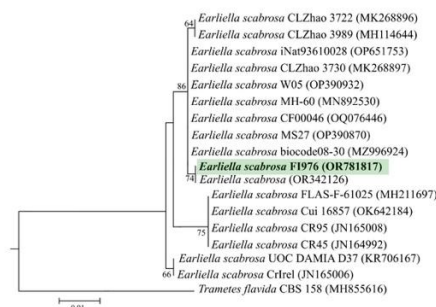


Fig. 3 - Phylogenetic tree and identification of fungus *Earliella scabrosa* construction from ITS sequence from Neighbor-Joining and Maximum likelihood with 100 bootstrap values using *Trametes flavida* as an outgroup.

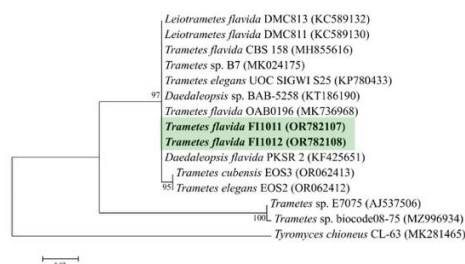


Fig. 4 - Phylogenetic tree and identification of fungus *Trametes flavida* construction from ITS sequence from Neighbor-Joining and Maximum likelihood with 100 bootstrap values using *Tyromyces chioneus* as an outgroup.

4. Discussion

In the present study, explored samples were recognized by morphoanatomical features. Additionally, we produced DNA sequences for wood-rotting polypores 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 a widespread and apt for accurate identification (Schoch et al., 2012).

In the present study, polypores *Earliella scabrosa* and *Trametes flavida* have been identified and recorded for the first time in the Pune district of Maharashtra, India. These are the two new records for the Velha tehsil (Rajgad tehsil) of Pune district. 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. The specimens were identified based on morpho-taxonomical studies, and these two species were not listed (Ranadive et al., 2013). Few more studies have been conducted on Aphyllophorales, in which 57 species are reported from 29 genera, and 7 families have been recorded from Karnataka, India. In which, *Earliella scabrosa* and *Trametes flavida* were not reported. In addition, molecular characterization was not carried out in previous study (Adarsh et al., 2019). It was observed that the molecular studies are missing in earlier reports, and no reports are recorded on the occurrence of *Earliella scabrosa* and *Trametes flavida* in the Pune district. Therefore, as per our acquaintance, these are two new records for Velha Tehsil of Pune district, Maharashtra, India.

5. Conclusion

Western Ghat is a biodiversity hotspot in India. The Velha region belongs to the Western Ghats of Maharashtra and is in the Pune District. There is a lack of molecular data and information on fungi. The present study has assessed the macrofungi at the molecular level, where the two new records of polypores viz. *Earliella scabrosa* and *Trametes flavida* (family Polyporaceae) have been reported for the first time from Pune District. By merging cutting-edge technology with time-tested techniques, PCR and other molecular-based techniques have given rise to novel instruments for identifying fungal species. Therefore, understanding macrofungal biodiversity at the community and species level allows relevant authorities to track both natural and anthropogenic disturbances and assess conservation needs and effectiveness.

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Declarations

Ethics approval and consent to participate

This work does not involve any human participation nor live animals performed by any of the listed authors.

Conflict of interest

The all authors declare no competing interests.

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