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# Morphological and Molecular Investigation of New Polypores Fungi *Picipes anjaliae* sp. nov. (*Basidiomycota*: *Polyporales*: *Polyporaceae*) from India

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## ABSTRACT

**In this manuscript, *Picipes anjaliae* sp.nov. is described and illustrated from western ghat of Kolhapur district of Maharashtra state, India. This new species is characterized by distinctly long central stipe with brownish black cuticle; annual, yellowish brown to reddish-brown deeply infundibuliform pileus (5-35 mm diam.) and irregular to angular pores (3-6 per mm). The poroposed species ismorphologically and phylogenetically quite close to *Polyporus tubaeformis* (P. Karst.) Ryvarden & Gilb. and *Picipes auriculatus* B.K. Cui, Xing Ji & J.L. Zhou from which is differs in having a long centrally attached stipe (up to 26 cm long and 2 mm diam.), small pileus (<3cm diam.) and longer spores measuring 6.2–9.5 µm. Molecular phylogenetic conclusion also support the noval status of *P. anjaliae* sp. nov.**

**Keywords**: *Picipes*, Taxonomy, Phylogeny, *Polyporaceae*, White rot, India.

## INTRODUCTION

The genus *Picipes* Zmitr. & Kovalenko was described by Ivan Zmitrovich and Aleksandr Kovalenko (Zmitrovich, Kovalenko, 2016).The genus is characterized by annua lstipitate basidiomata, polyporoid morphotype, infundibuliform pileus, covered with hard cuticle, without scales, smoke gray to castaneous or deeply brown; stipe covered with brownish to black cuticle; pores small (more than 5 per mm); hyphal system dimitic with uninflated both corioiloid and fibrous skeletals; clamps can either present or absent; basidiospores cylindrical, smooth, hyaline; on wood of frondose and coniferous trees causing a white rot. Molecular genetic studies made it possible to clarify the taxonomic status and transfer another 10 species to the genus *Picipes*, and another 18 species were described as new to science in the genus, two of which were transferred to the genus *Polyporus* s. str. (Bhunjun et al. 2022, Cui et al. 2019, Dai et al. 2014, Ji et al. 2022, Zhou et al. 2016). Thus, based on Species fungorum data base accessed on 30-09-2024, the genus *Picipes* currently includes 29 taxa.

## MATERIAL AND METHODS

During the present study, the mature fruiting bodies of new species *P. anjaliae* were collected from the fallen angiosperm branches (unidentified) at an elevation of 160 56' 32" N, 730 47' 42" E from Amba reserve forest, Shahuwadi, Kolhapur district (M.S.), India. Fresh specimens were photographed in the field. Cotton blue lacto-phenol mounts were observed under Lawrence and Mayo N-300M research microscope. Specimen was identified by using relevant literature (Cui *et al.* 2019; Dai 1996; Dai et al. 2014; Zhou *et al.* 2016). Macro-micro morphological and phylogenetic analysis has been described in this paper.

Line drawings of various microscopic characteristics were drawn under camera lucida. The holotype collections have been preserved following Pradhan *et al.* (2015) and deposited in the Ajrekar Mycological Herbarium-AMH, Agharkar Research Instittute, Pune with accession no.AMH-10775.

## TAXONOMIC DESCRIPTION

***Picipes anjaliae*** Patil Y. S. & Jadhav S. N.

**Figure 1 and 2**

MycoBank No.: 856280

Diagnosis; Basidiocarps annual, centrally stipitate, deeply infundibuliform, with yellowish brown to reddish-brown pileus, pore surface whitish to cream, later brown to pale ochre, pores irregular to round or angular to slightly subangular, 6-8 per mm when young, 3-6 per mm when mature; hyphal systemdimitic; basidiospores narrowly ellipsoid to cylindrical, 6.2-9.5 × 2.5-3.5µm.

Holotype: India, Maharashtra State, Kolhapur district, Shahuwadi tehsil, Amba Reserve forest, on fallen angiosperm branch (unidentified), 16.92694

N 73.80333 E, 22/09/2024, Patil Y.S. field no.Y23V1P1 (**Holotype,** Ajrekar Mycological Herbarium-AMH, Agharkar Research Instittute, Pune, Maharashtra, India with accession no.AMH-10775).

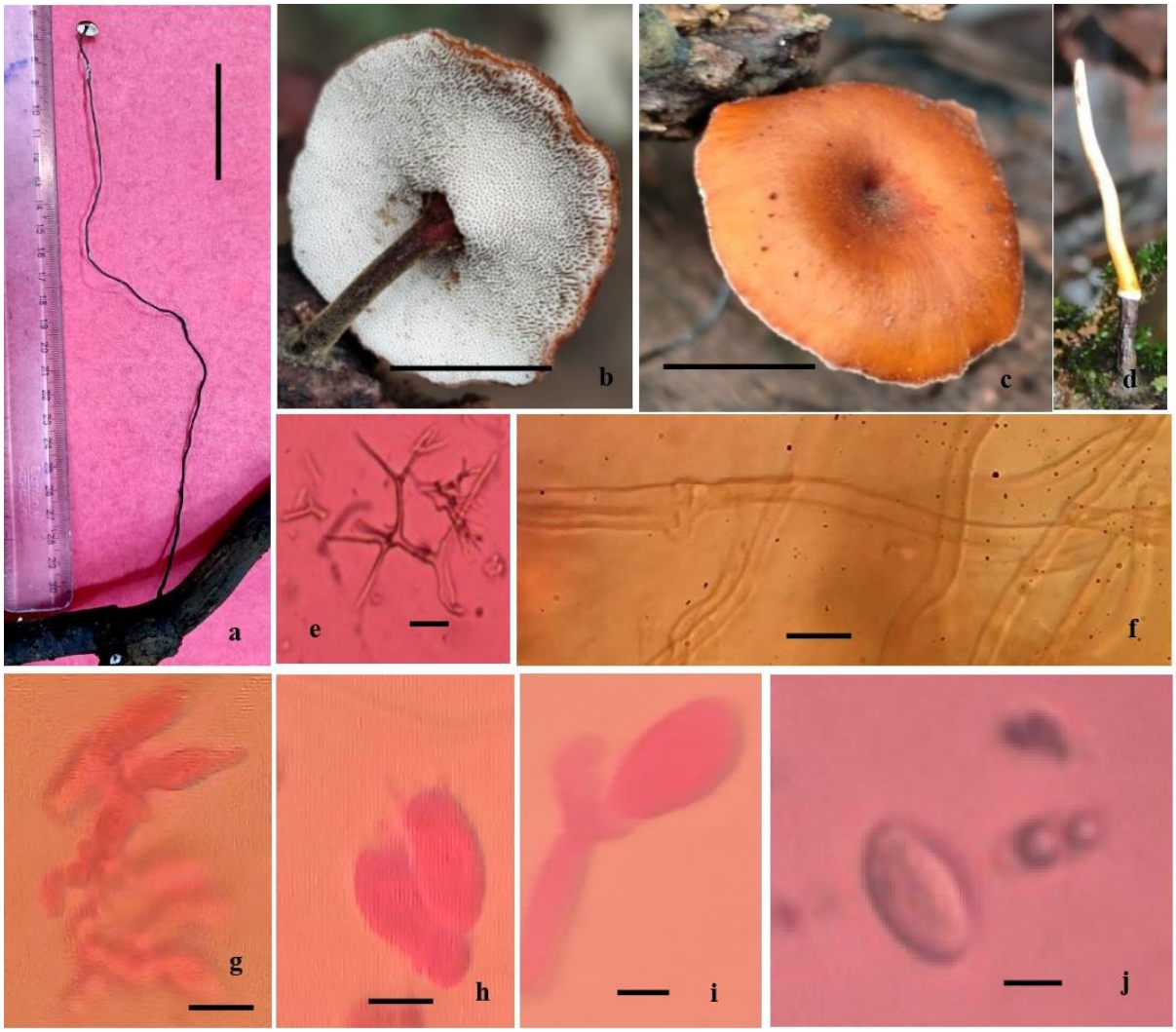



**Figure 1**: *Picipes anjaliae* (holotype). a-c, fruiting bodies; d, serial rhizomorph; e, skeletal-binding hyphae; f, generative hyphae; g, cystidioles; h, basidia with basal clamp connection and basidioles; I, basidiole with basal clamp connection; j, basidiospore. Scale bar: a=5 cm; b,c=1 cm; e,g=10 µm; h-j=5 µm.

***Etymology:*** Specific epithet *'anjaliae'* has been namedin honour of Dr. Anjali Rajendra Patil, Head, Department of Botany, Rajaram College, Kolhapur (MS), India for her significant contribution to the mycoflora of Maharashtra state.

**Macroscopic characters: Basidiocarp** annual**,** centrally stipitet with aerial rhizomorph. **Pileus** 5-35 mm, orbicular to semi-orbicular, with even or slightly lobate margin, planoconvex with depressed centre when young, slightly to deeply infundibuliform with inflexed margin when mature; yellowish brown to reddish-brown when young, with darker centre, then dark chestnut brown, surface velvety, then glabrous and finely radially wrinkled. **Context** white to buff, becoming woody hard upon drying and up to 1 mm thick. **Tubes** up to 2 mm long, sub-decurrent when juvenile, then deeply decurrent. **Pores** narrow, 6-8 per mm when young, 3-6 per mm when mature, irregular to round or angular to slightly subangular, whitish to cream when young, later brown to pale ochre when dry. **Stipe** very short to very long, bearing a terra-brown to black cuticle, up to 26 cm long and up to 2 mm wide, cylindrical to slightly broad towards the base or narrower in the middle and widening towards both ends, tomentose to finely hairy when young.

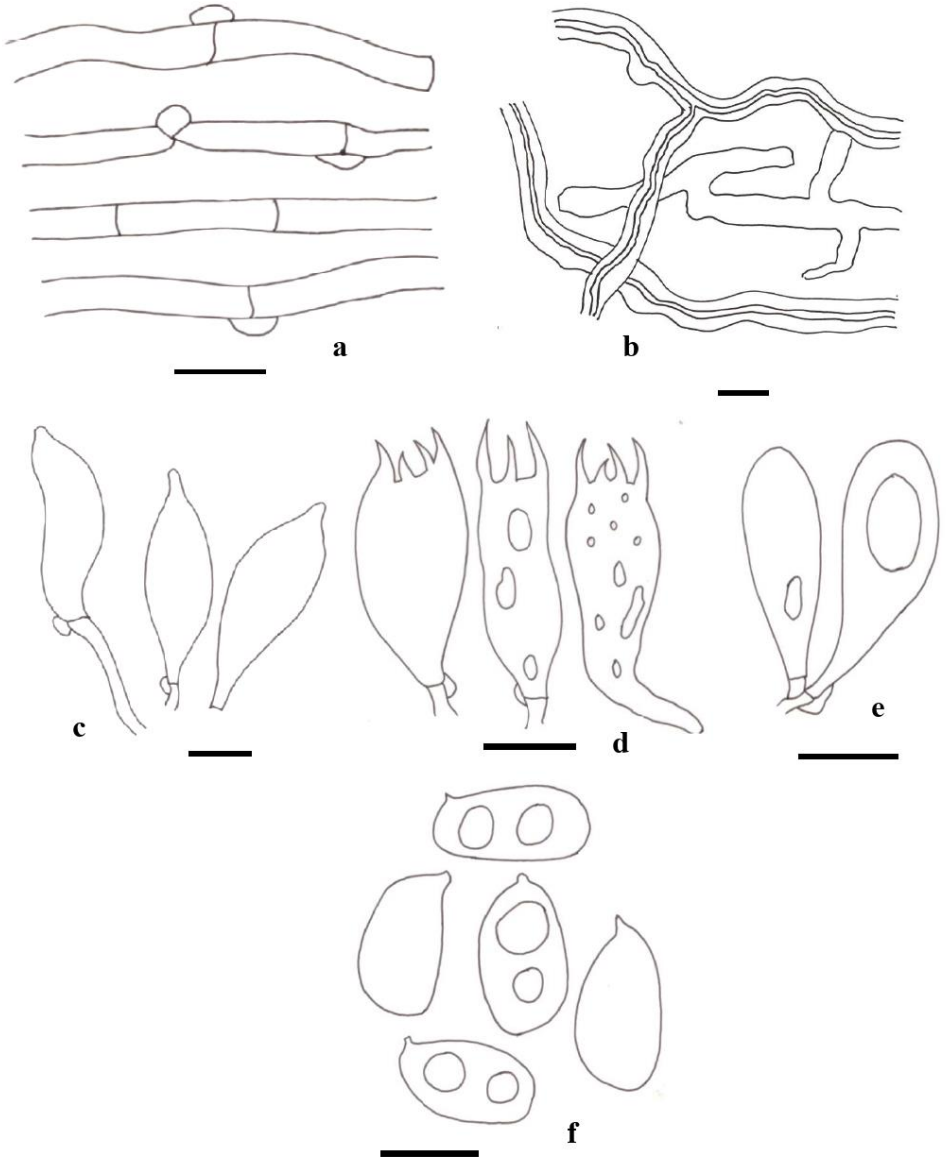



**Figure 2**: *Picipes anjaliae* (holotype). a, generative hyphae; b, skeletal-binding hyphae; c, cystidioles; d, basidia with basal clamp connection; e, basidiole with basal clamp connection; f, basidiospore. Svale bar: a-b=10 µm, c-f=5 µm

**Microscopic characters: Hyphal system** dimitic and interwoven. Generative hyphae hyaline, thin-walled, 1.5-7 µm wide, with clamp connections. Skeletal-binding hyphae hyaline, thick-walled, up to 8µm wide, with arboriform branches and tapering ends, CB+, tissue unchanged in KOH.**Context** made up of generative hyphae that are infrequent, colourless, thin-walled, frequently branched, 1.3-6.5µm in diameter, skeletal-binding hyphae dominant, colourless, thick-walled with a narrow lumen, to solid, arboriform branched, interwoven, 1.4-4.8µm in diameter. **Basidia** clavate, with basal clamp connection, 4-sterigmata, 12-20×5-7µm, **Basidioles** in shape similar to basidia, smaller than basidia, 8.5-15.5×5-7.5µm **cystidioles** less, subulate, 12.5-20×4-5.5µm. **Basidiospores** cylindrical, smooth, thin-walled, usually bearing one or two guttules, 6.2-9.5 × 2.5-3.5µm.

**Rot type** - white rot.

**Specimens examined:** India, Maharashtra State, Kolhapur district, Shahuwadi tehsil, Amba reserve forest, on fallen angiosperm branch (unidentified), 22/09/2024, Y.S. Patil (field no. $Y_{23}V_1P_1$)

**DNA extraction, Polymerase Chain Reaction, and sequencing**

Genomic DNA was extracted from dried (50°C) herbarium tissue (10–50 mg) using the 'Fungal

gDNA Mini Kit' (Xcelris Genomics, Ahmedabad, India). nLSU region was amplified with the primer pair LR0R and LR5. PCR procedure for nLSU was as followed. For purification of the amplified PCR product, a QIAquick® Gel Extraction Kit was used (QIAGEN, Hilden, Germany) (Saha *et al.* 2022)

DNA sequencing was outsourced to Paeon Laboratories Kolhapur, Maharashtra, India. BioEdit sequence alignment editor ver. 7.2.5 (Hall 1999) was used to check the quality of the freshly generated sequencing data.

**Phylogenetic analysis**

ThenLSU sequence so obtained was submitted to GenBank (**PQ456460**) (www.ncbi.nlm.nih.gov), and related *Picipes* sequences were identified by a BLASTn search (http://blast.ncbi. nlm.nih.gov/). Total of 36 sequences including*Trametes conchifer* voucher Dai 8359 as outgroup used in the phylogenetic analysis are indicated in **Figure 3**. Maximum Parsimony (MP) phylogenetic analyses was performed using the default settings and 1000 bootstrap replicates with the Subtree-Pruning-Regrafting (SPR) algorithm for datasets which was determined using MEGA XI (Kumar *et al.* 2021). The tree was rooted with the sequences that showed the highest similarity of *Picipes* and *Polyporus* in GeneBank (**Figure 3**).

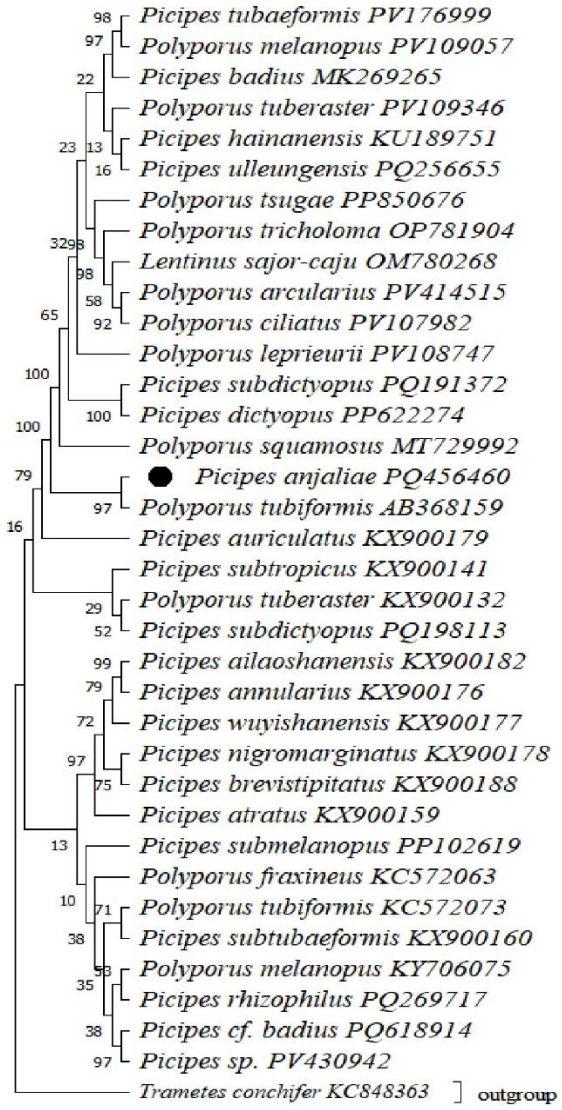



**Figure 3**: Phylogram showing relationship of *Picipes anjaliae* to closely related species of *Picipes* based on nLSU sequence of rDNA. *Picipes anjaliae* is placed with bullets (bott) to highlight its phylogenetic position in the tree.

BLAST analyses of *P. anjaliae* sequences showed highest similarity with*Polyporus tubaeformis* (BP ¼ 97) and *Picipes auriculatus* (BP ¼ 79). Phylogenetic analysis of the nLSU region inferred by Maximum Parsimony (MP) strongly supports *P. anjaliae* as a distinct species of genus *Picipes* (**Figure 3**). Although *P. anjaliae* forms a distinct branch sister to *Polyporus tubaeformis*with strong support (BP ¼ 97) but it is morphologically different in size of pileus (5-35 mm), long centrally stipitate basidiocarp (up to 26 cm) and larger pore (3-6 per mm). From phylogenetic point of view *P. anjaliae* is also quite close to*Picipes auriculatus* (BP ¼ 79) except that*P. auriculatus* has laterally stipitate basidiomata, short stipe (up to 3 mm long) and smaller spore 5.8–6.8 µm in comparison to *P. anjaliae* which centrally stipitate and has longer spores 6.2–9.5 µm.

## CONCLUSION

*Picipes anjaliae* share morphological characters with *Picipes subtropicus, Polyporus tubaeformis, Picipes auriculatus* but, *P. anjaliae* differes from *Picipes subtropicus, Polyporus tubaeformis* in the size of the pileus (5-35 mm), long centrally stipitate basidiocarp (up to 26 cm) and larger pore size ranging from (3-6 per mm). As compared to *Picipes anjaliae*, *P. auriculatus* has laterally stipitate basidiomata, small stipe (3 mm) and smaller spores (5.8–6.8 µm) instead of longer spore (6.2–9.5 µm) in *P. anjaliae.*

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