

New record of *Pluteus losulus* (*Pluteaceae*) from India

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ABSTRACT

A noteworthy *Pluteus* was collected on several occasions during our diversity study on the *Pluteaceae* of Kerala State, India. Morphological and molecular studies (nrITS) confirmed it as *Pluteus losulus* of sect. *Pluteus*. It is described in detail based on collections made in Kerala State, India with field photographs, microphotographs and phylogeny. This forms the first record of the species from India.

Keywords: *Celluloderma*, First record, *Hispidoderma*, Kerala State, nrITS

INTRODUCTION

The genus *Pluteus* Fr., typified by *Pluteus cervinus* (Schaeff.) P. Kumm., currently includes about 500 species and is recognized world over (He *et al.*, 2019). It is quite common in tropical habitats and is characterized by basidiomes with free pink lamellae, pinkish brown, inamyloid basidiospores and inversely bilateral convergent hymenophoral trama (Singer 1986). Primarily based on the characteristics of the hymenial cystidia and the pileipellis three sections were traditionally recognized in the genus *viz.*, *Pluteus*, *Celluloderma* Fayod and *Hispidoderma* Fayod (Singer, 1986; Justo *et al.*, 2011).

Reports on Indian *Pluteus* are scanty and sporadic and to date, only 32 taxa were reported from this vast country of which 22 taxa were reported from Kerala State (Farook *et al.*, 2013). It seems that the genus *Pluteus* is particularly diverse in the region. In the course of our studies on the *Pluteaceae* of Kerala, we came across an interesting species of *Pluteus* on several occasions on a dead angiosperm tree trunk. Detailed morphological and molecular studies confirmed it as *Pluteus losulus* Justo. Morphological, molecular and phylogenetic details of the species are discussed in detail in the present article.

MATERIALS AND METHODS

Morphological studies

Conventional morphology based taxonomic methods were employed for this study. Macroscopic characters were drawn from fresh specimens. Basidiomes were dried in a hot air oven (40-50°C) overnight for preservation. Pieces of tissue and sections of the dried basidiomes were mounted in 3% KOH solution and stained with 1% Congo red. Microscopic characters were examined and measured using an Olympus CX43 optical microscope. Microphotographs were made with a Magcam DC10 digital camera attached to the same microscope. Twenty basidiospores per collection were measured for length and width. Basidiospore measurements include both the mean and the standard deviation for both the length (avL) and the width (avW), together with the range of spore quotient (Q, length/width ratio) and its mean value (Qm). Basidiospore measurements exclude the hilar appendix. Colour notations refer to Kornerup and Wanscher (1978). All collections examined are deposited in the

Mycological Herbarium of Jawaharlal Nehru Tropical Botanic Garden and Research Institute [TBGT (M)].

DNA sequencing and phylogenetic analysis

Genomic DNA was extracted from fresh specimens of *Pluteus* following protocols in Izumitsu *et al.* (2012). The nuclear ribosomal Internal Transcribed Spacer region (nrITS) was amplified and sequenced from the newly collected specimens of *Pluteus* species. PCR reactions were performed with the primer pair ITS1 and ITS4 (White *et al.*, 1990). The protocols for PCR amplification and sequencing followed Kumar *et al.* (2018a). The newly generated sequences are deposited in GenBank [OM442928, OM442933 (nrITS)].

The molecular phylogenetic analysis was performed using nrITS sequences newly generated from the Kerala material and those retrieved from GenBank. The nrITS sequence of *Pluteus losulus* (627bp, 638bp) along with those retrieved from GenBank were aligned using MAFFT web tool (www.ebi.ac.uk/Tools/msa/mafft/) with default settings. The final aligned data matrix of nrITS sequences from 31 taxa, including *Pluteus chrysaegis* Berk. & Broome as outgroup, were then imported into BioEdit v7.2.6.1 (Hall 1999) for manual adjustment. Maximum likelihood (ML) analysis was performed in MEGA X with 1000 rapid bootstrap replicates. Hasegawa-Kishino-Yano model (HKY+G) was selected as the best substitution model as per BIC score, by running model test in the same software. The phylogram inferred from ML analysis is displayed with MEGA X (Kumar *et al.*, 2018b).

RESULTS

Molecular phylogeny

The phylogeny inferred from ML analysis of the data matrix of nrITS sequences is provided in Fig. 9. The phylogenetic analysis data shows that the Indian collections of *Pluteus* shows 100% ML bootstrap support with *Pluteus losulus* Justo from China (MH231231), São Tomé (MG968803) and DR Congo (HM562129).

Taxonomy

Pluteus losulus Justo, *Mycol. Progr* **10** (4): 473, 2011

Pluteus cervinus var. *ealaensis* Beeli, *Bull. Soc. R. Bot. Belg.* **61** (1): 81, 1928. **Figs. 1-8**

Basidiomata large, fleshy, pluteoid. Pileus 42-85 mm in diam., conico-convex, broadly convex, companulate to plano-convex with a conical or submammillate umbo; surface chocolate brown to dark brown (6E5/6F4/6F5/6F7/7E4/7F4) appressed fibrillose striate, often splitting especially towards margin to appear fibrillose rimose, exposing the pinkish (6B3) context below, shiny, nonhygrophanous, dry; margin straight, entire to variously incised, striate. Lamellae free, remote, orange white to reddish or pinkish white (6A2/6A3/7A2), up to 10 mm, close to crowded with lamellulae of different lengths; edge concolorous to the sides, entire. Stipe 35-140 × 4-10 mm, central, cylindrical to slightly twisted at base, equal or slightly tapering upwards from a broad base, solid, brittle; surface off white in the upper half, lower part brownish gray (6B2/7C2), fibrillose striate, pruinose at extreme base. Context white, 4 mm thick at the pileus disc, soft. Mycelial mat and rhizoids absent. Odor mild, not characteristic.



Figs. 1-8: *Pluteus losulus*. 1-2. Habit in situ; 3. Basidia; 4. Basidiospores; 5. Cheilocystidia; 6. Pleurocystidia; 7. Pileipellis; 8. Caulocystidia. Scale bars: 1-2= 20 mm; 3-6, 8= 10 µm; 7= 50 µm

Basidiospores $6.4-8.4 \times 6-8 \mu\text{m}$ ($avL=8.04$, $avW=6.41$); $Q=1.05-1.33$; $Q_m=1.25$, subglobose to broadly ellipsoid, hyaline, smooth, slightly thick-walled. Basidia $24-42 \times 6-12 \mu\text{m}$, clavate to broadly clavate, 4-spored, smooth, thin-walled, hyaline. Lamella edge sterile with crowded cheilocystidia. Cheilocystidia $26-60 \times 10.5-21 \mu\text{m}$, abundant, versiform, clavate to broadly clavate, cylindro-clavate, narrowly utriform, lageniform to sublageniform, hyaline, thin-walled. Pleurocystidia abundant, metuloidal, $45-69.6 \times 10-30 \mu\text{m}$, narrowly fusiform to fusiform, ventricose to narrowly utriform with 2, 3-4 apical/lateral hooks, thick-walled (up to $3 \mu\text{m}$), hyaline. Hymenophoral trama inversely bilateral with convergent hyphae, $2-32 \mu\text{m}$ wide, inflated.

Subhymenium pseudoparenchymatous. Pileal trama composed of interwoven hyphae, $4-29 \mu\text{m}$ wide, inflated, thin-walled, hyaline. Pileipellis a cutis passing to a trichoderm towards the disc, pileal hyphae parallel, $7-12.5 \mu\text{m}$ wide, thin-walled, with pale brown intracellular contents. Trichodermial terminal elements $44-156 \times 6-22 \mu\text{m}$, cylindrical to narrowly fusiform, some strongly tapering towards apex, thin-walled, with light brown contents. Stipitipellis a cutis composed of parallel hyphae $6-13.8 \mu\text{m}$ wide, thin-walled, hyaline. Caulocystidia present more at the basal region, scattered in middle region, absent or not observed at apex, $27-65 \times 6-18 \mu\text{m}$, versiform, narrowly fusiform, lageniform, narrowly to broadly lageniform with a long whip like, flexuous apex, rarely with excrescences at apex, with intracellular brown pigments, thin-walled. Clamp connections frequent, present in all tissues.

Habit, habitat and phenology: Solitary or in groups on dead decaying tree stumps, in tropical evergreen forest, Kerala State, India. May, June, January.

Specimens examined: India, Kerala State, Thiruvananthapuram district, Palode, JNTBGRI campus, 15 May 2017, TBGT(M)16721; Palode, Plavara, 1 June 2018, TBGT(M)17508; JNTBGRI campus 13 May 2020, TBGT(M)18055; *ibid.*, 30 July 2020, TBGT(M)18193; *ibid.*, 13 January 2021, TBGT(M)18472.

DISCUSSION

Pluteus losulus is characterized by medium to large fleshy basidiomata, conico-convex, umbonate, appressed fibrillose brownish pileus, subglobose to broadly ellipsoid basidiospores, versiform cheilocystidia, apically hooked metuloidal pleurocystidia, presence of abundant clamp connections and caulocystidia. *Pluteus losulus* was originally described as a variety of *P. cervinus* (Schaeff.) P. Kumm. But later molecular and morphological studies confirmed it as a distinct species not related to *P. cervinus* or to any clamped species in that group (Justo *et al.*, 2011).

The Kerala collections are similar both morphologically and phylogenetically with the descriptions of *P. losulus* by various workers (Justo *et al.*, 2011; Desjardin *et al.*, 2018; Hosen *et al.*, 2018). Additional features observed in the Indian collections are appressed fibrillose to rimose fibrillose pileus with a submammillate umbo; subglobose to broadly ellipsoid basidiospores, pileipellis a cutis passing to a trichoderm towards center and versiform caulocystidia with a long whip like flexuous apex.

The phylogeny was inferred from the ML analysis of data matrix of nrITS sequence (Fig. 9). The Kerala collections of *Pluteus losulus* forms a subclade with the specimens of *P. losulus* from China (MH231231), São (MG968803) and DR Congo (HM562129) confirming its conspecificity (Fig. 9). The similarity of the Indian collections with the African *P. losulus* species (HM562129) indicates its wide geographical distribution pattern and a similar pattern of distribution can also be observed in species like *P. cervinus*, *P. amphicystis*

Singer, and *P. Martinicensis* Singer & Fiard. However, the dispersal mechanism (natural/man made) for their wide distribution is yet to understand.

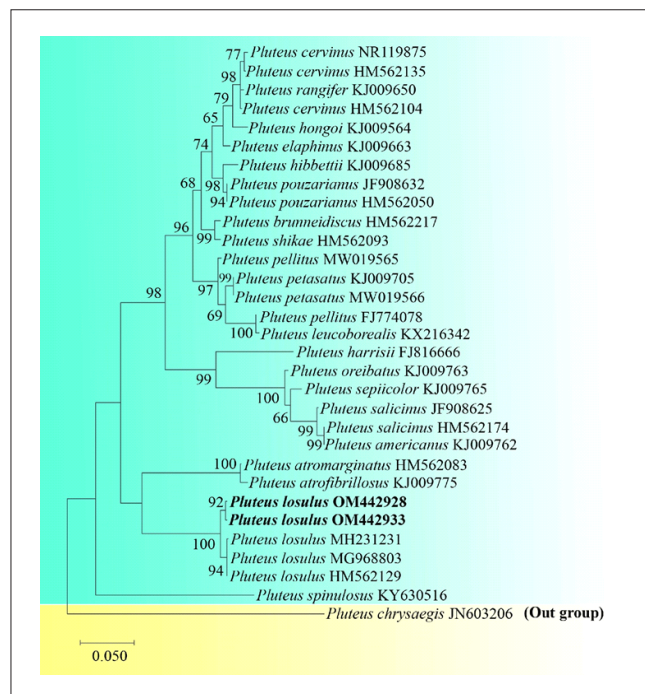


Fig. 9: Maximum Likelihood tree generated from ITS sequence data. BS values greater than 50% are shown above/below branches. Collections from Kerala, India are given in bold. GenBank accession numbers are given after the name of each taxon.

The report of *Pluteus losulus* from India is significant phytogeographically and points to a wide geographic distribution pattern, similar to those observed and reported by earlier workers (Justo *et al.*, 2011; Pradeep *et al.*, 2012; Menolli *et al.*, 2015).

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