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Pleurotus shentelii (Pleurotaceae): a new edible mushroom from Trans-Himalayan region of Ladakh, India

Tahir MEHMOOD[1], Komal VERMA[1], Upendra SINGH[2], Rupam KAPOOR[3], Yash Pal SHARMA[1,*]

1. *Department of Botany, University of Jammu, Jammu, 180006, J&K, India.* ***2.*** *Department of Botany & Microbiology, H. N. B Garhwal University, Srinagar, Garhwal, 246174, Uttarakhand, India.* ***3.*** *Department of Botany, University of Delhi, Delhi, 110007, India. *Corresponding author's mobile: +9419157412; email: yashdbm3@gmail.com*

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ABSTRACT: *Pleurotus shentelii*, a new edible mushroom is described based on collections from the cold arid Trans-Himalayan region of Drass (Kargil) of Ladakh, India. It is characterized by white to whitish basidiomata turning brownish with age, elongated to cylindrical basidiospores, fusoid cheilocystidia with acuminate tip, and occurrence on roots and lower stem residues of members of Apiaceae (*Prongos pabularia* and *Ferula jaeschkeana*). The species is described, compared and discussed based on morphology and nrITS-based phylogeny.

KEY WORDS: Apiaceae, ethnomycology, ITS gene region, oyster mushroom, *Pleurotus tuoliensis*, western Himalaya.

INTRODUCTION

Pleurotus (Fr.) P. Kumm. (Pleurotaceae) is one of the most important edible mushroom genera distributed throughout the world. The members of this genus are characterized by decurrent lamellae, white spore prints, ellipsoid to cylindrical basidiospores, and hyphae with clamp connections (Corner, 1981; Singer, 1986; Vilgalys and Sun, 1994). Due to its economic potential, the genus is well-studied worldwide (Corner, 1981; Singer, 1986; Vilgalys *et al.*, 1993; Li and Yao, 2005; Kirk *et al.*, 2008; Huang *et al.*, 2010; Zervakis *et al.*, 2014). Approximately, 70 species of this genus are known worldwide (Chang, 1984; Julian *et al.*, 2019). In India, most studies have been related only to the cultivation and breeding of *Pleurotus* species while data on the taxonomy and phylogeny have been incomplete (Chavan and Barge, 1977; Sivanesan *et al.*, 1986; Shevale and Deshmukh, 2016; Sharma and Sharma, 2018). Up to 26 different species of *Pleurotus* have been identified so far from different regions of India (Upadhyay *et al*., 2017).

During a macrofungal exploration of edible mushroom resources in the Trans-Himalayan regions of the Union Territory of Ladakh, an interesting species of *Pleurotus* was collected several times. The thorough morphological and phylogenetic analyses of the internal transcribed spacer region (nrITS) revealed that the specimens represented an undescribed taxon of *Pleurotus*. Therefore, it is described here as a new species.

MATERIALS AND METHODS

Macro- and micromorphology

Macromorphological features were recorded from fresh specimens in the field along with the habitat and associated host plants. Macro-chemical test with 10% KOH was tested with pileus, stipe, and context of basidiomata. Colour codes follow Kornerup and Wanscher (1978). Photographs of the fresh basidiomata were captured with a Nikon D5300 camera. Microscopic characters were observed on dried materials mounted in 5% KOH, and stained with 1% phloxine, and 1% Congo red when necessary. Melzer's reagent was used to test the amyloidity of the basidiospores. Line drawings were made with the help of a camera lucida at 2000× magnification. Microphotographs of the various elements were taken using a digital camera attached to an Olympus CX33 compound microscope. Basidium length excludes the length of sterigmata. A total of 40 basidiospores from each of the three specimens were observed. Basidiospore measurements are represented as minimum-mean-maximum length × minimum-mean-maximum width and Q = length/width.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from 100 mg of dried fruitbodies using a fungal genomic DNA Mini Kit (RGCB, Thiruvananthapuram). The ITS gene region of the nuclear ribosomal DNA was amplified with primer pairs ITS1 and ITS4 (White *et al.*, 1990). PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), 1 µl DNA, 0.2 µl Phire Hotstart II DNA polymerase enzyme, 0.1 mg/ml BSA and 3% DMSO, 0.5 M Betaine, 5pM of forward and reverse primers. PCR amplification was carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems) programmed for 2 min at 96°C, followed by 30 cycles of 30 sec at 96°C, 40 sec at 50°C, and a final stage of 4 min at 60°C. The PCR products were purified with QIAquick Gel Extraction Kit (QIAGEN, Germany)

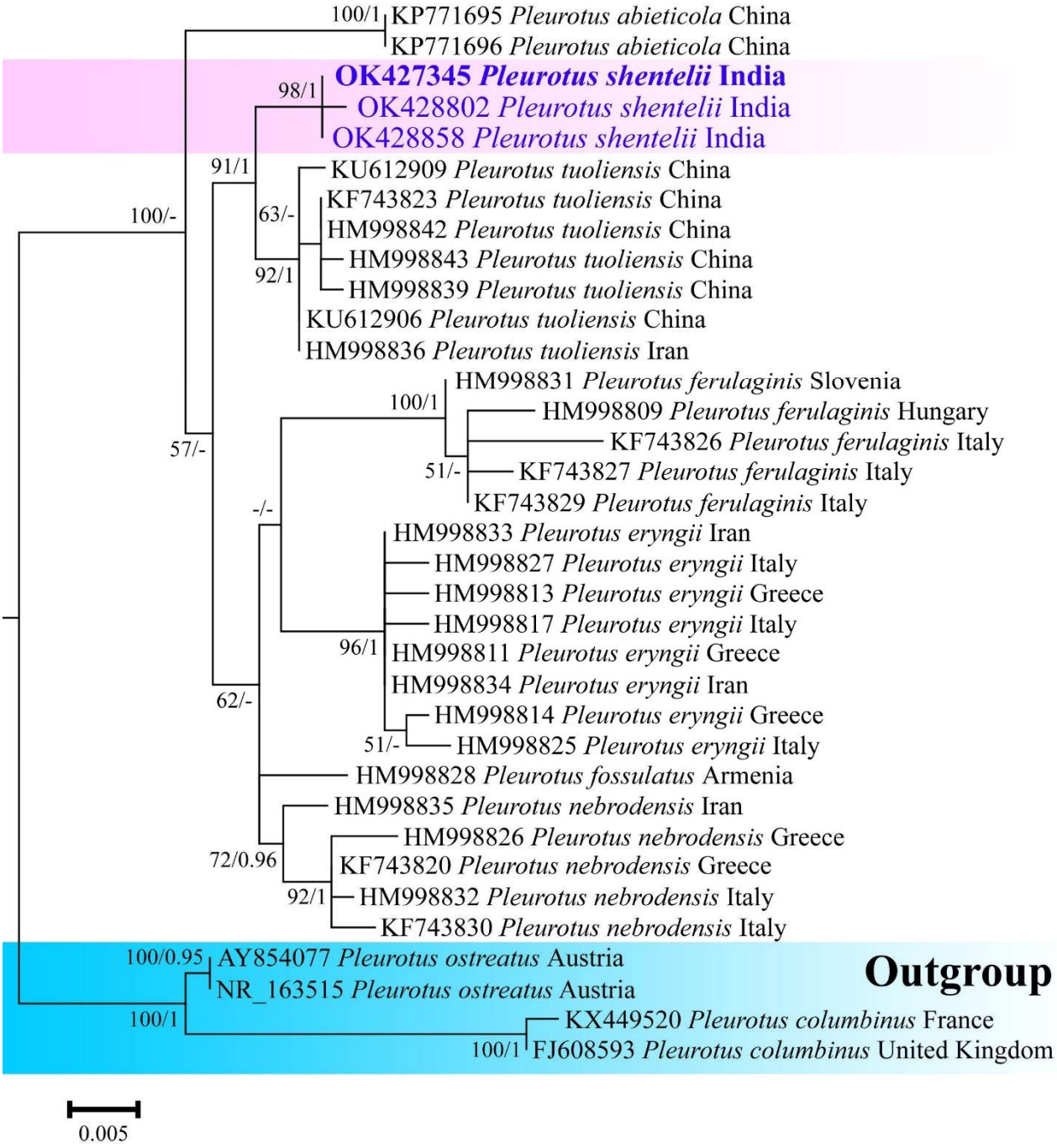

Fig. 1. Phylogeny of ***Pleurotus shentelii*** (holotype) generated in maximum likelihood analysis based on nrITS sequence data. Branches are labeled with bootstrap proportions (before the slash markers) higher than 50% and Bayesian posterior probabilities (after the slash markers) more than 0.90. Sequence derived from the holotype of the novel species ***Pleurotus shentelii*** is shown in bold and blue font.

and then subjected to Sanger sequencing in an automated DNA sequencer (ABI3730xl DNA Analyzer, Applied Biosystems, USA) using the same primers.

Phylogenetic analysis

Phylogenetic analyses were performed using nrITS sequences newly generated along with the sequences retrieved from previous published phylogenies (Zervakis *et al.*, 2014; Zhao *et al.*, 2016; Suwannarach *et al.*, 2020), BLAST searches (Altschul *et al.*, 1997), and data retrieved from GenBank (Clark *et al.*, 2016). *Pleurotus ostreatus* (Jacq.) P. Kumm. and *P. columbinus* Quél. were selected as outgroup taxa (Suwannarach *et al.*, 2020) (Fig. 1). Sequence alignment was performed using MAFFT v.7

(Katoh and Standley, 2013) and manually edited in Bioedit v 7.2.5 (Hall, 1999). To change the multiple alignment format, Alignment Transformation Environment (ALTER) was used (Glez-Peña *et al.*, 2010). Maximum Likelihood analysis was performed with the programme RAxMLGUI 1.5 (Silvestro and Michalak, 2012). One thousand bootstrap replicates were analyzed to obtain nodal support values. Bayesian inference was computed independently twice in MrBayes v.3.2.2 (Ronquist *et al.*, 2012), under different models. The best-fit substitution model of nucleotide evolution was carried out in MrModeltest 3.7 (Posada and Crandall, 1998). Bayesian posterior probabilities (BPP) were calculated in two simultaneous runs with the Markov chain Monte Carlo (MCMC) algorithm (Larget and Simon, 1999). Markov chains were run for 10 million generations, saving a tree every 100th generation. Default settings in MrBayes were used for the incremental heating scheme for the chains (3 heated and 1 cold chain), unconstrained branch length [unconstrained: exponential (10.0)], and uninformative topology (uniform) priors. The first 25% of trees were discarded as burn-in material (Hall, 2004).

RESULTS

Phylogeny

The nrITS dataset included 35 sequences of nrITS regions (including three of our specimens: *TMYPS– 021*, *TMKV–024* and *TMYPSKV–031*) from *Pleurotus*. This analysis revealed the similar fashion of lineages (clades) that were presented by Zervakis *et al.* (2014) and Suwannarach *et al.* (2020). Our proposed new species with three specimens (*TMYPS–021*, *TMKV–024* and *TMYPSKV–031*) appeared as sister to *Pleurotus tuoliensis* C.J. Mou, M.R. Zhao & Jin X. Zhang. However, our novel species of *Pleurotus* is separated in a distinct clade with very strong support (MLB: 91%, BPP: 1) showing its distinctiveness (Fig. 1)

Ethnomycology

The new species below described as *Pleurotus shentelii* is recognised as a very precious mushroom in the Kargil district of Ladakh because of its nutritional and medicinal potential. Locals frequently referred to it as "shentelii" in "Sheena" language. They pick it essentially every day from early April to early June. In the winter, when supplies are few, locals supplement their diets with dried basidiomata of the mushroom. Villagers, particularly women, make garlands (Fig. 2A–C) out of freshly harvested mountain mushrooms after drying the fruiting bodies for use in the winter. The natives describe this mushroom as one of the tastiest and most nourishing foods the cold desert region of Kargil has to offer, comparing it to "chicken" because of its flavour. It is superior in quality and has a prolonged shelf life, which is attributed to the freshness and lower water content of the fruiting body. In addition to combining it with soup and other regional specialties, locals also consume it raw after drying. The native residents have accumulated the required knowledge throughout time to identify and gather this mushroom from the mountains with ease. Furthermore, they now have an understanding of the phenology and ecology of this significant species.

Fig. 2. ***Pleurotus shentelii*** is a very precious mushroom in the Kargil district of Ladakh **A**. Local woman processing ***Pleurotus shentelii*** for consumption; **B**. Inhabitants making garlands to dry fruiting bodies to use during winter; **C–D**. Collecting dry fruiting bodies from Locals.

TAXONOMIC TREATMENT

Pleurotus shentelii Mehmood, Sharma Y.P, Verma K. & U. Singh, ***sp. nov.*** **Figs. 3–5**

MycoBank: MB841460

Type**:** India, Ladakh, Kargil, Pandrass, altitude 3550 m 34°0′24.54″N, 75°379.47″E., associated with the roots and stems of *Prongos pabularia*; June 16, 2021, *T. Mehmood & Y.P. Sharma, TMYPS–021* (CAL 1855, holotype!) GenBank Acc. No. OK427345 (nrITS).

Diagnosis: *Pleurotus shentelii* sp. nov. is characterized by its white to yellowish white, convex to plane basidiomata turning brownish with age, pileus surface initially lobulate then cracking at maturity elongated to cylindrical basidiospores, fusoid cheilocystidia with acuminate tip and occurrence on roots, and lower stem residues of *Prongos pabularia* Lindl. and

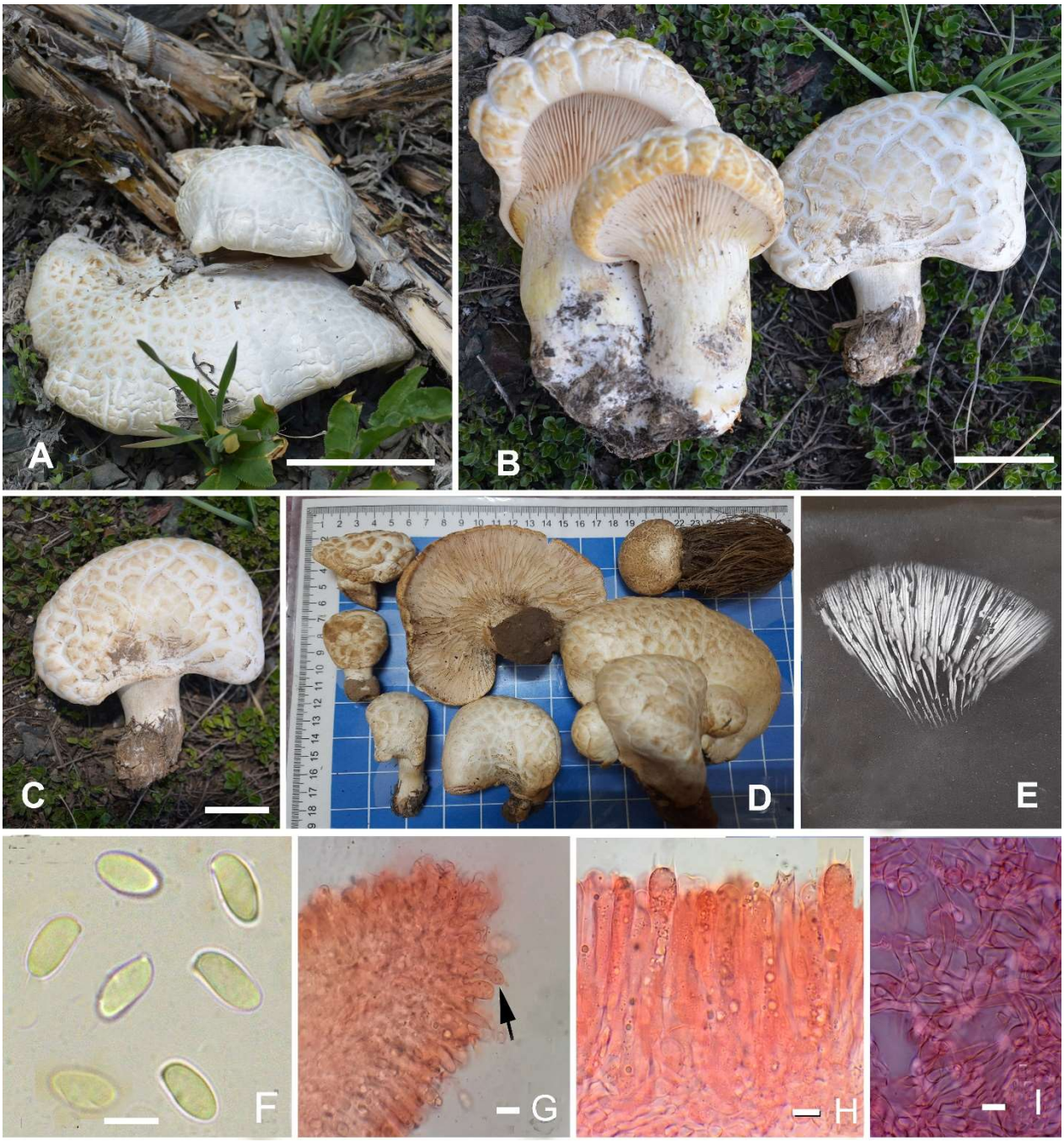

Fig. 3. ***Pleurotus shentelii*** (TMYPS-021, holotype). **A–D**. Fresh basidiomata in the field and base camp. **E**. Spore print. **F**. Basidiospores. **G**. Cheilocystidia. **H**. Basidia. **I**. Pileus context hyphae. Scale bars: (A–C) = 2 cm; (F–H) = 10 µm, (I) = 20 µm.

Ferula jaeschkeana Vatke (both belonging to the plant family Apiaceae) and nrITS sequence data.

Description: *Basidiomata* medium to large sized, fleshy, *Pileus* 50–200 × 5–15 mm; surface snow white, light yellowish, brownish (4A4–5C5) with age, convex to plane, pileus surface initially lobulate then cracking at maturity, dry, shiny; margin inrolled when young, incurved at maturity; pileus context 5–15 mm thick, white, unchanging, yellowish brown with 5% KOH. *Lamellae* 5–10 mm broad, strongly decurrent, whitish at first then turning yellowish white to light brown (5C5) with age, subdistant to crowded (6–10 lamellae/cm), lamellar edges entire; lamellulae 4–5 tiers, attenuated. Stipe 50–90 × 30–60 mm, lateral to strongly eccentric, subcylindric to cylindrical, initially white turning yellowish white to light yellowish (4A4) on maturity. Odour pleasant; taste sweet.

Basidiospores [60/3/3] (11–)14.3–**16.04**–17.8(–19) × (5.5–)6.2–**6.76**–7.3(–8.0) µm, Q = (1.69–)2.1–**2.4**–2.6(–3.0);

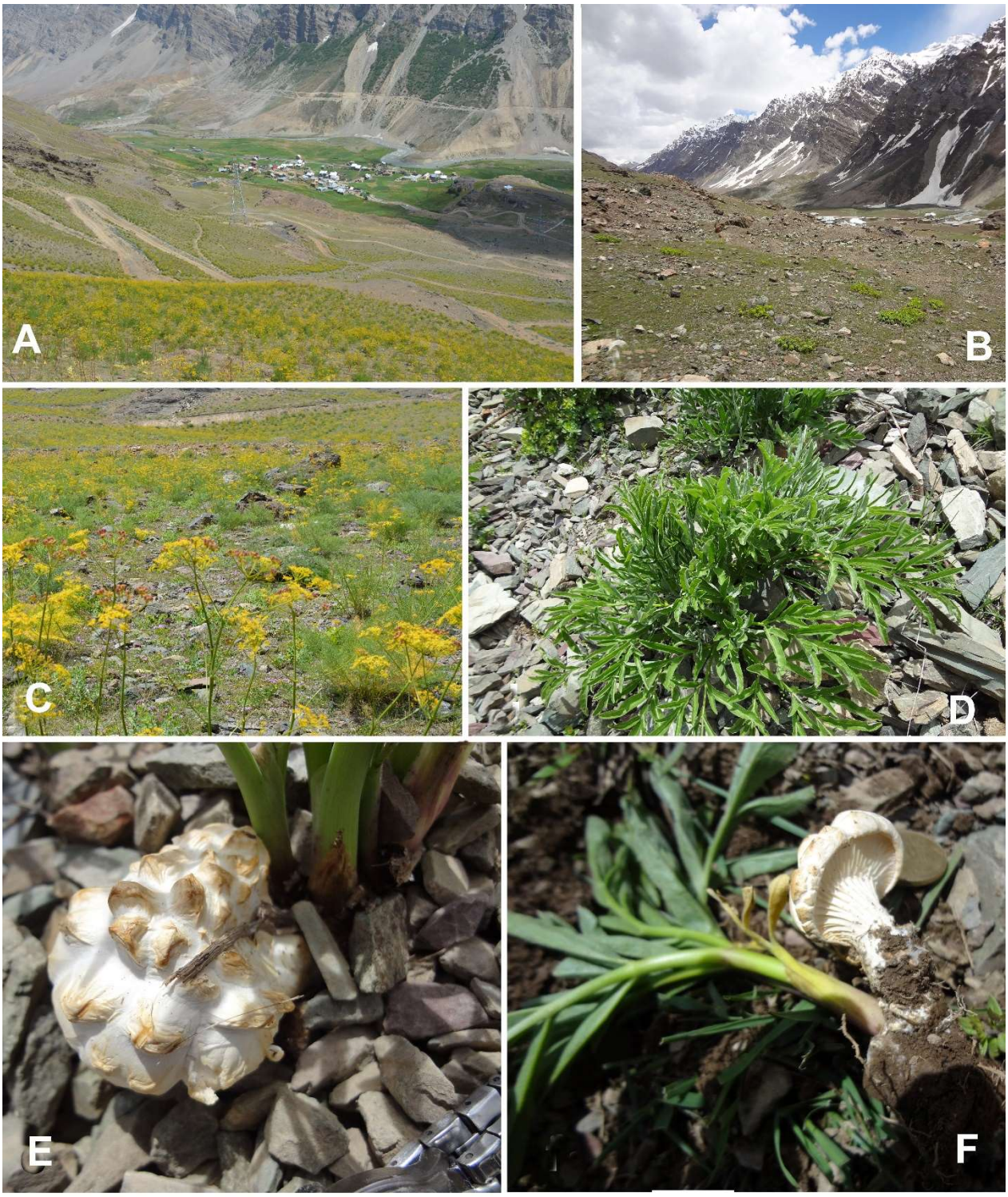

Fig. 4. ***Pleurotus shentelii*** **A–D**. Natural habitat. **E** & **F**. Association with the roots of ***Prongos pabularia***.

elongated to cylindrical, colourless and hyaline, thin-walled, smooth, inamyloid, white in prints. Basidia narrowly clavate, hyaline, thin-walled, 4-spored, 43–68 × 7.0–11 µm; sterigmata 3–7 µm long. Cheilocystidia abundant, fusoid cheilocystidia with acuminate tip, colourless and hyaline, thin-walled, 35–42 × 6–8 µm. Pleurocystidia absent. Lamellar trama monomitic, composed of irregularly arranged, colourless and hyaline, filamentous hyphae being 5–8 µm wide. Pileipellis 80–180 µm thick, a cutis composed of repent, parallelly arranged, yellowish to brownish filamentous hyphae being 4–10 µm wide; pileus context monomitic, composed

Table 1 The distinctive discriminating morphological characters for the ***Pleurotus eryngii*** species complex with our ***Pleurotus*** species**.**

Species	Pileus color	Lamellae	Basidiospores	Ecology	References
Pleurotus shentelii	White to yellowish white; brownish with age	whitish at first then turn yellowish white to light brown with age, strongly decurrent	14.3–17.8 × 6.2–7.3 µm; **Q** = 2.4	*Prongos pabularia, Ferula jaeschkeana*	Present study.
Pleurotus tuoliensis	Whitish to cream	White, crowded, decurrent	10–14 × 5.0–6.0 µm; **Q** = 2.2	*Ferula krylovii, F. ferulaeoides*	Zhao *et al.* (2016).
Pleurotus nebrodensis	Light ivory to cream	Deeply decurrent, whitish to pale yellow reticulum at stipe	12.2–17.4 × 5.5–8.2 µm; **Q** =2.27	*Prangos ferulacea*	Zervakis *et al.* (2001).
Pleurotus fossulatus	Whitish to cream to yellow ochraceous		9.0–14.0 × 4.5–6.0 µm; **Q** =2.24	*Eryngium, Smyrniopis, Kellusia*	Zervakis *et al.* (2014).
Pleurotus ferulaginis	Whitish to ochraceous to beige to brown	White to cream to ivory	11.3–13.4 × 4.6–5.1µm; **Q** = 2.54	*Ferulago campestris*	Zervakis *et al.* (2014).
Pleurotus eryngii	light brown to tan brown to grey brown	Decurrent, cream to light beige, anastomoses	9.1–13.5 × 4.6–6.7 µm; **Q** = 2.04.	*Eryngium* sp., *Opopanax chironium, Peucedanum* sp.	Zervakis *et al.* (2001).

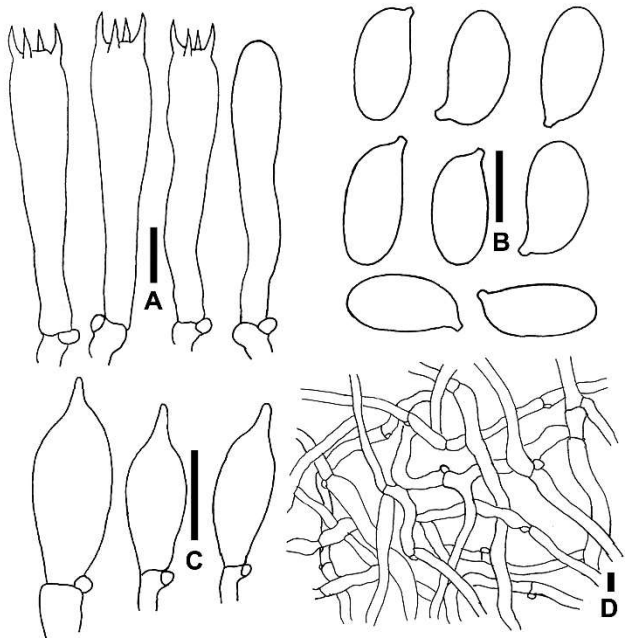

Fig. 5. Line drawings of ***Pleurotus shentelii*** (TMYPS-021, holotype). **A**. Basidia. **B**. Basidiospores. **C**. Hymenial cystidia near the lamellae sides **D**. Pileus context hyphae. Scale bars: (A–D) = 10 µm.

of radially to irregularly arranged, colourless and hyaline, filamentous 5–12 µm wide hyphae. Stipe context monomitic, composed of vertically to irregularly arranged filamentous 5–7 µm wide hyphae. Clamp connections abundant in all tissues.

Distribution: Known only from India.

Specimens examined**:** India, Ladakh, Kargil, Pandrass, altitude 3550 m 34°0′24.5″N, 75°379.40″E, (*TMKV-024*) 10 July 2021 (HBJU/TMYPS/03, paratype); Drass, Mushkoh, altitude 3400, 34°35'0.99"N, 75°32'12.99"E, 16 July 2021, *T. Mehmood, Y.P. Sharma & K.Verma, TMYPSKV–031*

Etymology: The specific epithet '*shentelii'* refers to the name of the mushroom in the local language of the type locality.

Habitat**:** Cold rocky meadows; altitude > 3400 m.

Phenology**:** April to June (fruiting period may be confirmed in future with more collections from different localities).

DISCUSSION

In the present study we identified the new species *Pleurotus shentelii* from India. Based on basidioma morphology and its association with umbellifers, this taxon shows close resemblance to some other species *viz*., *Pleurotus tuoliensis*, *P. nebrodensis* (Inzenga) Queli., *P. fossulatus* (Cooke) Sacc., *P. ferulaginis* Zervakis, Venturella & Cattarossi, and *P. eryngii* (DC.) Quél. However, *P. tuoliensis,* which is probably the closest known species both morphologically and phylogenetically to *P. shentelii*, can be distinguished by its cochleariform to flabelliform pileus with white to creamy white surface, slightly smaller basidiospores (10–14 × 5–6 µm; Q = 2.2) (Zhao *et al.*, 2016), while *P. nebrodensis* differs from *P. shentelii* by its light ivory to cream colour pileus, deeply decurrent, whitish to pale yellow reticulum at the stipe, subglobose to ellipsoid larger basidiospores 12.2–17.4 × 5.5–8.2 µm (Q = 2.27) (Zervakis *et al.* 2001). Likewise, *Pleurotus fossulatus*, in contrast to our species, shows a whitish to creamish yellow ochraceous pileus and comparatively smaller basidiospores (9.0–14.0 × 4.5–6.0 µm) (Zervakis *et al.*, 2014).

Pleurotus ferulaginis can be segregated from *P. shentelii* by its whitish to ochraceous to beige to brown pileus and comparatively slightly smaller basidiospores (11.3–13.4 × 4.6–5.1 µm) (Zervakis *et al.*, 2014). Another species of *Pleurotus*, i.e. *P. eryngii*, can be easily differentiated from the present taxon as the pileus colour of *P. eryngii* ranges from light brown to beige-brown (Zervakis *et al.*, 2001), whereas the pileus colour of *P. shentelii* is white to yellowish-brown. Thus, the morphology combined with phylogenetic analyses unequivocally resolves *P. shentelii* as a distinct and independent species of the genus *Pleurotus* which is associated with the roots and stem residues of apiaceous members (*Prongos pabularia* and *Ferula jaeschkeana*). Records of the prominent and distinctive macro-and

micromorphological characters are also provided for comparison (Table 1) to delineate the present taxon from its allied species.

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