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Additions to the Indian *Phylloporus* (*Boletaceae*) based on morphology and molecular phylogeny

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ABSTRACT

***Phylloporus maculatus* and *P. yunnanensis* are reported as new records from Himalayan region of India. Detailed macro- and microscopic descriptions together with phylogenetic analyses of the nuclear ribosomal large subunit (nrLSU) are presented.**

Keywords: Basidiomycota, Himalaya, macrofungi, phylogeny, Sikkim, Uttarakhand

INTRODUCTION

Boletaceae is mostly featured by their poroid or tubulose nature of the hymenophore except *Phylloporus* Quél., a well-known genus of this family displaying lamellate hymenophore instead of poroid hymenophore (Corner, 1970; 1974; Watling 2008; Neves and Halling, 2010; Neves *et al.*, 2012; Hosen and Li, 2015; 2017). About 85 named species are reported so far in this genus throughout the globe (www.indexfungorum.org; Hosen and Li, 2017). In India, species of *Phylloporus* are poorly represented. So far only two species, namely *P. rhodoxanthus* (Schwein.) Bres. and *P. septocystidiatus* C.K. Pradeep & K.B. Vrinda are documented from India (Abraham, 1993; Pradeep *et al*., 2015).

In the present communication, *P. maculatus* N.K. Zeng, Zhu L. Yang & L.P. Tang and *P. yunnanensis* N.K. Zeng, Zhu L. Yang & L.P. Tang, originally described from China, are reported for the first time from India. *Phylloporus maculatus* was collected under temperate broadleaf forest dominated by *Castanopsis* from Kewzing in Sikkim (Eastern Himalaya), whereas *P. yunnanensis* was collected from subalpine mixed broadleaf and coniferous forest at Dhakuri in Uttarakhand (Western Himalaya) of India. Detailed descriptions, illustrations and comparisons with phenotypically similar and phylogenetically related species are presented in the ongoing account.

MATERIAL AND METHODS

Morphological studies: Macromorphological characters were recorded from the fresh basidiomata in the field and at the basecamp. After recording the macromorphological characters, basidiomata were dried with a field drier. Photographs of these fresh and dry basidiomata and microphotographs were taken with Nikon D300s, Cannon SX 220 HS and Nikon-DS-Ri1 (dedicated to Nikon Eclipse Ni compound microscope) cameras. Colour codes and terms are from Kornerup and Wanscher (1978). Micromorphological characters were observed with the help of a compound microscope (Nikon Eclipse Ni-U). Sections from dried specimens were mounted in a mixture of 3% KOH, 1% Phloxine and 1% Congo red or in distilled water. Micromorphological drawings were prepared with a drawing tube (attached to the Nikon Eclipse Ni) at 1000×. Basidium length excludes sterigmata. Basidiospore measurements were recorded in profile view from 20 basidiospores taken from a spore print. Spore measurements and length/width ratios (Q) are recorded here as: minimum-mean-maximum. Methods for scanning electron microscopy follow Das *et al.* (2015). Herbarium codes follow Thiers (continuously updated).

DNA extraction, PCR amplification and sequencing: Genomic DNA was extracted from 100 mg of dry basidioma using the InstaGeneTM Matrix Genomic DNA isolation kit (Biorad, USA) following the manufacturer's instructions. PCR amplification primers were LR0R and LR7 (nrLSU region) (Velgalys and Hester, 1990). PCR amplification on "ABI Veriti" thermal cycler protocols for the LSU gene were 5 min at 95°C, 30 cycles of 1 min at 95°C, 30 s at 52°C, 2 min at 72°C, and a final 7 min extension step at 72°C. The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Germany). Both strands of the PCR fragments were sequenced on the 3730xl DNA Analyzer (Applied Biosystems, USA) using the amplifying primers and assembled using sequencer (Gene Codes Corporation, USA). The newly generated nrLSU sequences (MF458302) and (MF458301) for *P. yunnanensis* and *P. maculatus*, respectively were deposited in GenBank.

Phylogenetic analysis: A total of 49 nrLSU sequences were retrieved from GenBank based on BLAST search (Altschul *et al.*, 1997) and recent publications (Neves *et al*., 2012; Zeng *et al*., 2013; Hosen and Li, 2015; 2017). The retrieved nrLSU sequences were aligned in MAFFT 7.305 (Katoh and Standley, 2013), and manually edited on Bioedit v.7.0.9 (Hall, 1999). Maximum Likelihood (ML) was performed using RAxML v.7.2.6 (Stamatakis, 2006). GTRGAMMA model was chosen as the best fit model for the dataset by using MrModeltest 2.3 (Nylander, 2004), and statistical support values were obtained using nonparametric bootstrapping (BS) with 1000 replicates. For this analysis *Boletellus ananas* (HQ161853) was selected as the outgroup taxon for rooting purpose following Wu *et al*. (2014).

RESULTS

Phylogenetic inferences: Phylogenetic analysis of nrLSU with 51 sequences including two newly isolated sequences show the monophyly of *Phylloporus* with moderate support

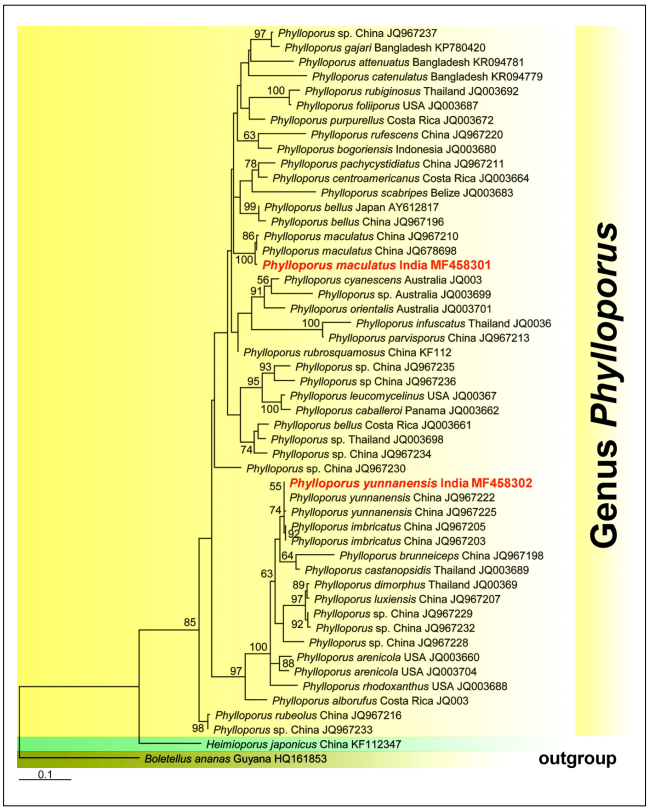

Fig. 1. Phylogeny of DC 16-34 (*Phylloporus maculatus*, in bold and red font) and MEH 16 P-03 (*P. yunnanensis*, in bold and red font) inferred from Maximum Likelihood analysis of LSU sequences using RAxML.

(85% ML BS, Fig. 1), which is consistent with the recent phylogenetic analyses of *Phylloporus* (Neves *et al.*, 2012; Zeng *et al.*, 2013; Hosen and Li, 2015; 2017). Sequences derived from the Indian collections (DC 16-34, MEH 16 P-03) are nested with the respective East Asian *Phylloporus* species (**Fig. 1**). One collection (DC 16-34) of *Phylloporus* is found to be closest to the Chinese *P. maculatus* with strong BS support value. Similarly, MEH 16 P-03 is clustered with *P. yunnanensis* and *P. imbricatus*.

Taxonomy

1. *Phylloporus maculatus* N.K. Zeng, Zhu L. Yang & L.P. Tang, *Fungal Diversity* **58**: 86 (2013).

Figs. 2 & 3

Pileus 30-66 mm diam., convex when young, becoming plano-convex when mature; surface subvelvety to densely tomentose with brown patches (7D7) over yellowish white (3A2) background, unchanging when bruised, turning reddish brown (9D4) with 3% KOH; margin regular, undulated and appendiculate with narrow sterile flap of tissue, concolorous with pileus surface. Hymenophore lamellate, decurrent, distant, 4-5/cm at pileal margin, mustard yellow (3B6), becoming bluish green (25D5) on bruising; lamellulae in 3 series. Stipe 55-80 × 4-8 mm, central, cylindric, solid; surface pastel yellow to pale yellow (3A34), faint striations near juncture of pileus, gradually fading towards base, surface with brown (6E7) pruina, basal

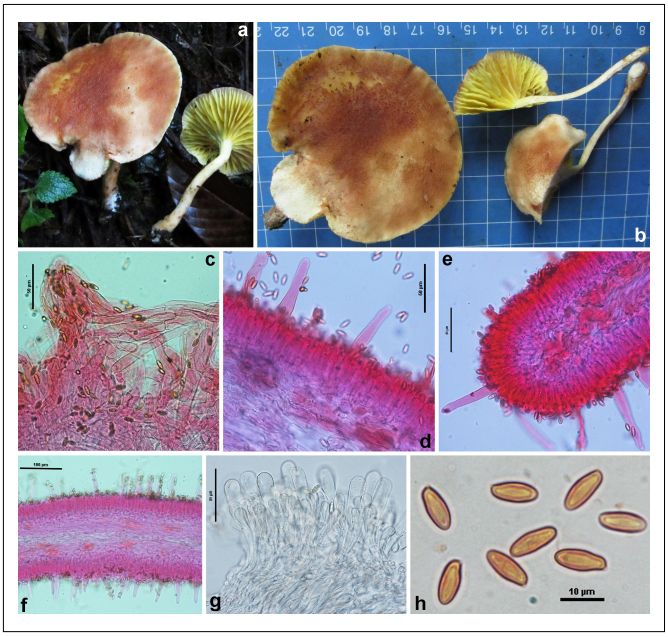

Fig. 2. (A-f) *Phylloporus maculatus.* a & b. Fresh basidioma in the field. c. Pileipellis d. Pleurocystidia, e. Cheilocystidia, f. Lamellar trama. g. Caulocystidia. h. Basidiospores; Bars: c-e & g = 50 mm; f = 100 µm; h = 10 µm.

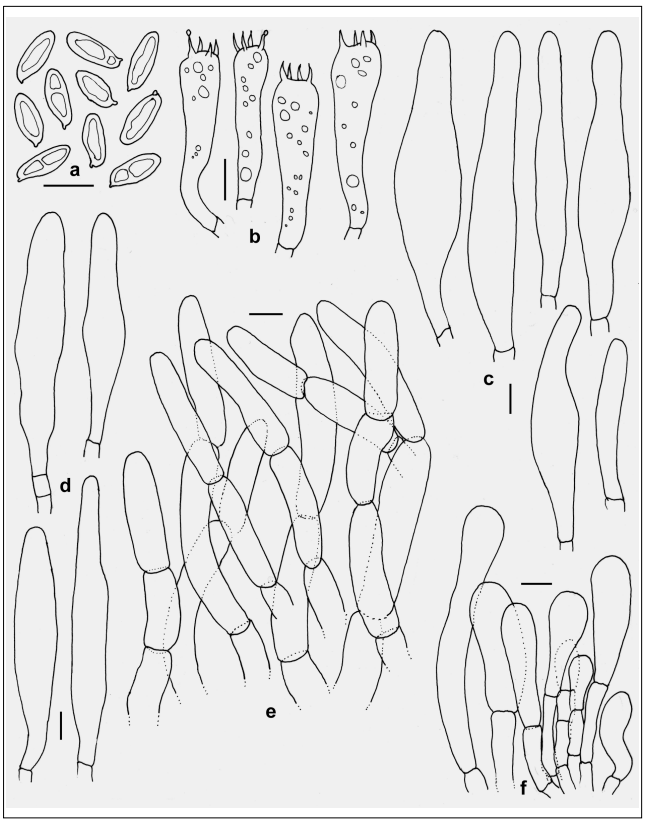

Fig. 3. (a-f) *Phylloporus maculatus* (DC 16-34). a. Basidiospores b. Basidia c. Pleurocystidia d. Cheilocystidia e. Pileipellis f. Caulocystidia. Bars: a-f = 10 µm.

mycelium white. Context solid in both pileus and stipe, 7-9 mm wide in pileus, pale yellow (1A3), unchanging on exposure. Spore print olive brown.

Basidiospores 10-11.6-12.5 × 3.6-4.0-4.5 µm (n = 20, Q = 2.38-2.6-3.1), ellipsoid to oblong, subfusoid, inequilateral, with bacillate ornamentation under SEM, oliveaceous. Basidia 36-43 × 9-16 µm, 4-spored, clavate. Lamellae edge fertile, composed of basidia and cystidia. Cheilocystidia 70-90 × 13-16 µm, uncommon, emergent 45-70 µm, same as pleurocystidia. Pleurocystidia 60-116 × 9-24 µm, emergent 20-82 µm, numerous, subfusiform to fusiform or ventricose. Pileipellis 80-120 µm thick, a trichoderm, composed of erect to suberect septate hyphae, sometimes slightly interwoven, few encrusted; terminal cells 30-55 × 7-15 µm, cylindrical with rounded to subfusoid apices. Stipitipellis 100-150 µm thick, trichoderm, composed of hyphae, basidia and cystidia in clusters; caulocystidia 35-70 × 5-15 µm, cylindro-clavate to clavate; caulobasidia similar to hymenial basidia but less in number. Clamp connections absent.

Habit and habitat: Under *Castanopsis* sp. in temperate broadleaf forest.

Distribution: Known from China and India (South district, Sikkim).

Specimen examined: India: Sikkim, South district, Kewzing, 2015 m asl., 27°17'42.7″ N 88°21'30.3″ E, 22 August, 2016, D. Chakraborty and K. Das, DC 16-34 (CAL).

2. *Phylloporus yunnanensis* N.K. Zeng, Zhu L. Yang & L.P. Tang, *Fungal Diversity* **58**: 95 (2013).

Figs. 4 & 5

Pileus 30-40 mm diam., uplifted when young, gradually becoming applanate to plano-convex at maturity; surface tomentose, when young becoming glabrous when mature and dry; surface maize yellow, to deep yellow (4A6-4A8) to topaz (5C5) when young, brown or yellowish brown or slightly deep when mature, unchanging when bruised, violet brown to maroon (11E5-11F6) with 3% KOH; margin entire, regular, undulated and appendiculate with narrow sterile flap of tissue, concolorous with pileal surface. Hymenophore lamellate, decurrent, more or less subdistant to rather close (11-13/cm at pileal margin), citric yellow to orange yellow, greyish green (27E7) on bruising; lamellulae in 3 series. Stipe 25-35 × 4-8 mm, central, cylindric, with distinct striations in the upper region, solid; surface light yellow to butter yellow (4A4-4A5) when young, light brown to brownish orange (5C-D6-7) at maturity, unchanging on bruising, basal mycelium yellowish. Context 5-6 mm wide in pileus, solid in pileus and stipe; yellowish white to pale yellow (4A2-4A3), unchanging when exposed, brownish with KOH, light olive green with $FeSO_4$, slightly ochraceous with guaiacol. Spore print olive brown. Taste indistinct to mild. Odor distinctly pleasant.

Basidiospores 10.2-11.3-12.9 × 3.6-4.2-4.8 µm (n = 20, Q = 2.39-2.66-3.11), ellipsoid to oblong, inequilateral, smooth under light microscope, olivaceous. Basidia 33-45 × 8-9 µm, 4-spored, clavate; sterigmata 3-5 × 1-1.5 µm. Lamellae edge fertile. Cheilocystidia common, similar to that of

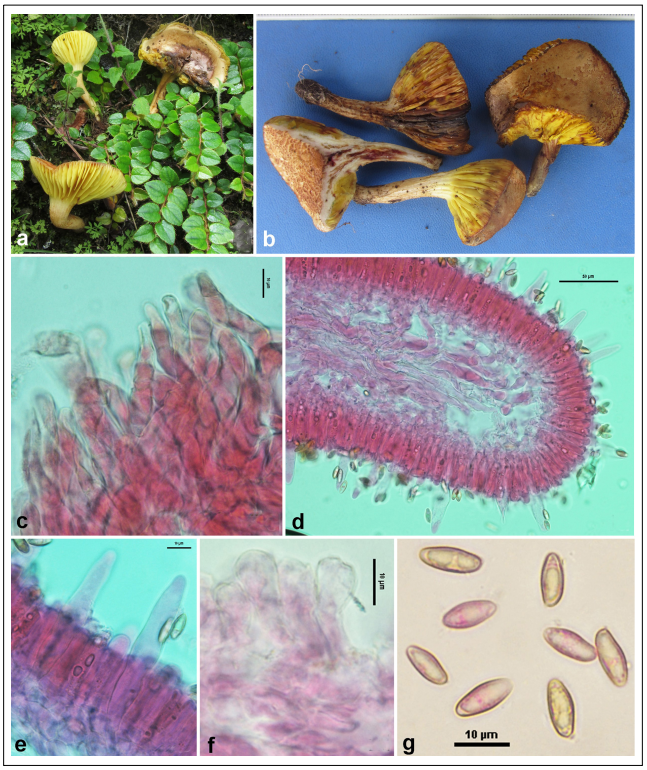

Fig. 4. (a-g) *Phylloporus yunnanensis*. a & b. Fresh basidioma in the field. c. Pileipellis. d. Lamellar edge. e. Pleurocystidia. f. Caulocystidia. g. Basidiospores. Bars: c, e-g = 10 µm; d = 50 µm.

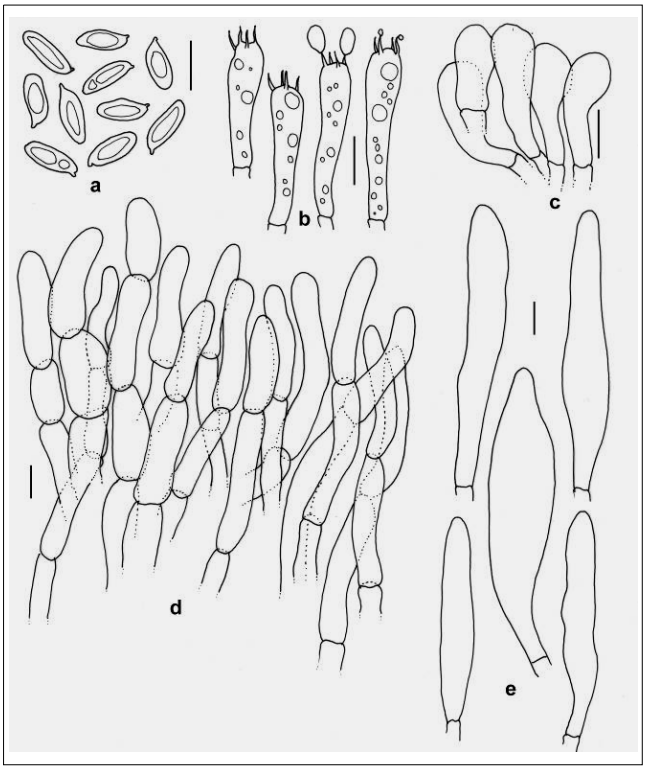

Fig. 5. (a-e) *Phylloporus yunnanensis*. a. Basidiospores. b. Basidia. c. Caulocystidia. d. Pileipellis. e. Pleurocystidia. Bars: a-e = 10 µm.

pleurocystidia. Pleurocystidia 60-100 × 10-20 µm, emergent 35-60 µm, numerous, fusoid to subventricose or ventricose, thin-walled. Pileipellis 160-200 µm thick, a trichoderm, composed of erect to suberect or sometimes interwoven hyphae; terminal cells 6.5-15 µm wide, cylindrical with rounded to subfusoid apex. Stipitipellis 40-55 µm thick, a trichoderm, upper part composed of hyphae, basidia and cystidia; caulocystidia 19-30 × 9-11 µm, subclavate to clavate.; Caulobasidia similar to hymenial basidia but less in number. Clamp connections absent.

Habit and habitat: Under *Quercus* sp. in temperate broadleaf forest.

Distribution: Known from China and India (Bageshwar district, Uttarakhand).

Specimen examined: India: Uttarakhand, Bageshwar district, Dhakuri forest area, along the Jhandidhar-Dhakuri pathway, 2857 m asl., 30º04'50.5″ N 79º55'00.5″ E, 3 Aug., 2016, M.E. Hembrom, MEH 16 P-03 (CAL).

DISCUSSION

In the molecular phylogenetic analysis based on nrLSU sequences we found that both Indian species are close to the Chinese species *P. maculatus* and *P. yunnanensis* with strong bootstrap support (**Fig. 1**). It is worth mentioning that distinction between *P. imbricatus* and *P. yunnanensis* is not possible based on morphology along with LSU sequence data. However, phylogenetic distinction is evident between *P. imbricatus* and *P. yunnanensis* when multiple sequences were analyzed (Zeng et al., 2013; Hosen and Li, 2017). It should be noted that, the Chinese *P. yunnanensis* and *P. imbricatus* have only a single base pair difference (genetic distance 0.08%) in nrLSU sequence out of 1298 nucleotide sites, while the Chinese *P. maculatus* has three base pairs difference (genetic distance 0.34%) out of 878 nucleotides with Indian *P. maculatus*. Another Indian collection (MEH 16 P-03) has a single base pair difference (genetic distance 0.12%) with the Chinese *P. imbricatus* out of 832 nucleotide sites but no difference with the Chinese *P. yunnanensis*, suggesting that Indian collection is *P. yunnanensis* rather than *P. imbricatus*. Moreover, morphological data and ecological preference also supports the Indian collection for *P. yunnanensis*. Therefore, despite having minor or negligible differences between the Chinese collection and Indian collection we treated our specimen as *P. yunnanensis*.

Moderately large basidiomes with brown densely tomentose pileus, bluing of lamellae, yellowish stipe with brownish pruina, trichoderm pattern of pileipellis and white basal mycelium characterize *P. maculatus*.. The Indian collection matches both morphologically and genetically with the description of the type from China (Zeng *et al*., 2013) except for the slightly larger (41-59 × 12-18 µm) cheilocystidia and narrow hyphae of pileipellis (8-25 µm). *Phylloporus bellus* (Massee) Corner, *P. rhodoxanthus* (Schwein.) Bres. and *P. rubrosquamosus* N.K. Zeng, Zhu L. Yang & L.P. Tang are some of the comparable species with the present collection. *Phylloporus bellus*, originally described from China (Zeng *et al.*, 2013), can be separated on account of its yellowish basal mycelium. *Phylloporus rhodoxanthus* differs by its non-staining lamellae, yellow stipe and presence of yellow basal mycelium. *Phylloporus rubrosquamosus* is distinct from *P. maculatus* by brownish red squamulose pileus and larger basidiospores (10-13 × 4.5-5 µm) (Zeng *et al*., 2013).

Phylloporus yunnanensis is distinct by its centrally depressed densely tomentose pileus, cyanescent lamellae, unchanging context brownish stipe and yellowish basal mycelium (Zeng *et al*., 2013). Earlier studies show that *P. imbricatus* and *P. yunnanensis* are quite confusing in the field as they share common morphological features except for the stature which is more robust in *P. imbricatus* (Zeng *et al*., 2013). However, ecological preferences of the two species are sufficient to warrant it as distinct (Zeng *et al*., 2013).

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