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# Additional notes on *Conchomyces bursiformis* (Agaricales), a rare monotypic agaric from India

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**Abstract**

Ample quantities of a crepidotoid agaric were collected from an evergreen forest in Kerala State, India and subsequent morphological and molecular analysis based on nLSU sequence confirmed it as *Conchomyces bursiformis*. A reappraisal along with comprehensive description, photographs, and discussion of this rare monotypic agaric is provided.

**Key words** – *Agaricus* – *Crepidotus* – Kerala – nLSU – taxonomy

## Introduction

During our ongoing study on the crepidotoid agarics of Kerala State, India an interesting crepidotoid species was collected in plenty from one of the evergreen forests. Initially we considered it as a species of the genus *Crepidotus* mainly due to its crepidotoid, lignicolous habit and colour. However, further microscopic and nLSU molecular analyses revealed that the species is *Conchomyces bursiformis* (Berk.) E. Horak, a rare monotypic agaric.

Berkeley (1860) collected an agaric with a puzzling mix of characters from Tasmania and described it as *Agaricus (Pleurotus) bursaeformis* Berk. This was later described afresh by different workers under different genera such as *Crepidotus, Pleurotus, Resupinatus, Hohenbuehelia* (Pegler 1965, Reid 1963, Singer 1947, 1951, 1969, 1986) until Horak (1981) confirmed it as *Conchomyces bursiformis* (as *C. bursaeformis*). More interestingly, none of these workers were aware that it had already been described as *C. verrucisporus* by Overeem from Java (Horak 1981). However, this genus was soon forgotten until Reid's (1963) transfer of *Agaricus bursaeformis* to *Hohenbuehelia*. Singer (1969) while accepting Reid's generic concept even erected a new subgenus *Reidia* to accommodate this and other related taxa.

Horak (1968) however hesitated to consider *Conchomyces verrucisporus* as a species of *Crepidotus* after assessing Overeem's description and kept it as doubtful, since neither it shows any obvious relationship to *Crepidotus* nor to any of the known agaric taxa. Later Horak collected this species at several occasions from Australasia (Horak 1981) and the topotype from Java. On further analysis and reference of these collections and the original description and paintings of *C.verrucisporus*, Horak confirmed that it was the same as Berkeley's Australasian species and made the appropriate combination *C. bursiformis* (as *C. bursaeformis*). Recently we collected this

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rare fungus in one of the evergreen forests in Kerala, India and we describe and discuss this agaric with some additional features.

## Materials & Methods

### *Morphological studies*

Conventional morphology based taxonomic methods as well as molecular methods were employed for this study. Colour notations refer to Kornerup & Wanscher (1978). Descriptive terms used in the descriptions follow Vellinga (1988). The materials examined are deposited at the Mycological Herbarium of Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Trivandrum (TBGT).

### *DNA sequencing and phylogenetic analysis*

Genomic DNA was extracted from dried basidiomes following protocols in Izumitsu et al. (2012). The nLSU region was amplified using primer pair LROR/LR7 (Vilgalys & Hester 1990). Amplification reactions were performed in a Veriti™ Thermal cycler (Applied Biosystems, USA) as per manufacturer's guidelines. It was then subjected for automated DNA sequencing on AB13730xl DNA Analyzer (Applied Biosystems, USA) using primers LROR/LR7. The generated sequence was edited manually using BioEdit sequence alignment editor version 7.2.6.1 (Hall 1999). The edited sequence (892 bp) was then used for BLASTn search in the GenBank database (www.ncbi.nlm.nih.gov). The newly generated sequence was deposited in GenBank (MF784865).The newly generated nLSU sequence (892bp) along with those retrieved from GenBank (11 nLSU sequences) was aligned using PRANK web tool (www.ebi.ac.uk/Tools/msa/prank/) with default settings. The final alignment was then imported into Aliview 1.18.1 (Larsson 2014) for manual adjustment. *Tubaria minima* (EF051055) was selected as outgroup for rooting purpose. The manually adjusted alignment was curated using the online tool Gblocks 0.91b (http://www.phylogeny.fr/one_task.cgi?task_type=gblocks) allowing all options for a less stringent selection. Maximum Likelihood (ML) analysis was performed with the curated alignment in MEGA 7.0.26 (Kumar et al. 2016) employing K2+G molecular evolution model with 1000 bootstrap replicates, which was selected by employing 'Automatic NJ tree' option and 'use all sites' option for missing/gap data information. Bootstrap values ≥70% were considered significant. The bootstrap consensus tree inferred from the ML analysis (Fig. 3) was displayed with MEGA 7.0.26 (Kumar et al. 2016).

## Taxonomy

***Conchomyces bursiformis*** (Berk.) E. Horak, Sydowia 34: 110 (1981) Figs 1–2

Basidiomata thick, fleshy, crepidotoid, rather tough. Pileus 16–53 mm diam., laterally attached, plano-convex, reniform to flabelliform; surface cream/light yellow (4A3/4A4) near the attachment, yellowish white (4A2) elsewhere, with pale orange/orange white (5A3/5A2) tinges, slightly downy, non-striate at the disc, hygrophanous becoming off white to ivory, sticky and viscid when fresh; margin straight, pellucid striate at the extreme margin, entire. Lamellae adnate, white, up to 2 mm wide, crowded with lamellulae of different lengths; edge concolorous to sides, entire. Stipe visible only in very young basidiomes, absent or reduced in mature specimens and attached directly to the substratum; surface cream (4A3), smooth, dry. Context thin, off white. Odor mild, not characteristic.

Basidiospores 5.5–7 × (5–) 5.5–6.5 µm, Q=1–1.14 µm, avL=6.4 µm, avW=5.6 µm, globose to subglobose, mostly subglobose (Q=1.1), moderately thick-walled, distinctly spinulose, inamyloid. Basidia 20–25.5 × 7–8µm, short clavate to clavate, 2, 4–spored, thin-walled, hyaline. Lamella edge sterile with abundant cheilocystidia. Cheilocystidia 19–36 × 8.5–16 µm, versiform, vesiculose clavate, clavate, narrowly ventricose, thin–walled, hyaline, tramal in origin. Pleurocystidia present more towards the gill edge, sparse and scattered elsewhere, 24–36 × 13.5–19

µm, ovoid, broadly lageniform, rostrate, thick-walled (metuloidal), without crystals, hyaline. Hymenophoral trama regular, hyphae 6.5 µm wide, thin-walled. Subhymenium pseudoparenchymatous. Pileal trama composed of interwoven, thin-walled, hyaline hyphae, 5–12 µm wide. Pileipellis a cutis, composed of loosely arranged hyphae embedded in a gelatinous matrix of about 130 µm thick. Pileal hyphae, 5.5–8 µm wide, light brown, incrusted with spiral bands of thickening. Pileocystidia present in groups, 30–56 × 12–20 µm, mostly clavate to vesiculose clavate, rarely narrowly lageniform, or cylindrical with subcapitate head, or rarely with branched or forked excrescences, incrusted with brown contents. Clamp connections present in all tissues.

Habit & habitat – Saprotrophic, scattered or in pairs on a dead liana in tropical evergreen forest, September.

Material examined – India, Kerala State, Thiruvananthapuram District, Kallar, 16 Sept. 2015, Bijeesh, TBGT15830.


**Fig. 1** – *Conchomyces bursiformis*. a–c Habit *in situ*. – Bars = 1 cm.

## Discussion

Medium, fleshy, tough crepidotoid basidiomata, globose to subglobose echinulate spores, presence of cheilocystidia and thick-walled metuloidal pleurocystidia, pileipellis a cutis embedded in a thick gelatinous matrix with distinct pileocystidia, and presence of clamp connections characterize *Conchomyces bursiformis*. The present collection agrees well with Horak's (1981) description of the topotype from Java in most of the macro and microscopic features. However, the present collection shows some additional features such as sticky/viscid pileus, presence of distinct pileocystidia, presence of a gelatinous layer in the pileipellis and spiral bands of thickening of the pileipellis hyphae which were not mentioned by earlier workers. We could not observe any crystals on the pleurocystidia though Horak (1981) mentioned it.

A BLASTn search in GenBank using nLSU sequence of the present material showed 100% identity and 100% query cover with zero e–value with *Conchomyces bursiformis* (GenBank AF261376). The bootstrap consensus tree obtained by ML analysis shows 99% bootstrap support with *Conchomyces bursiformis* (AF261376, AF042603). Therefore we prefer to consider the deviations observed in the macro and micro morphological features of the Indian material as additional features.

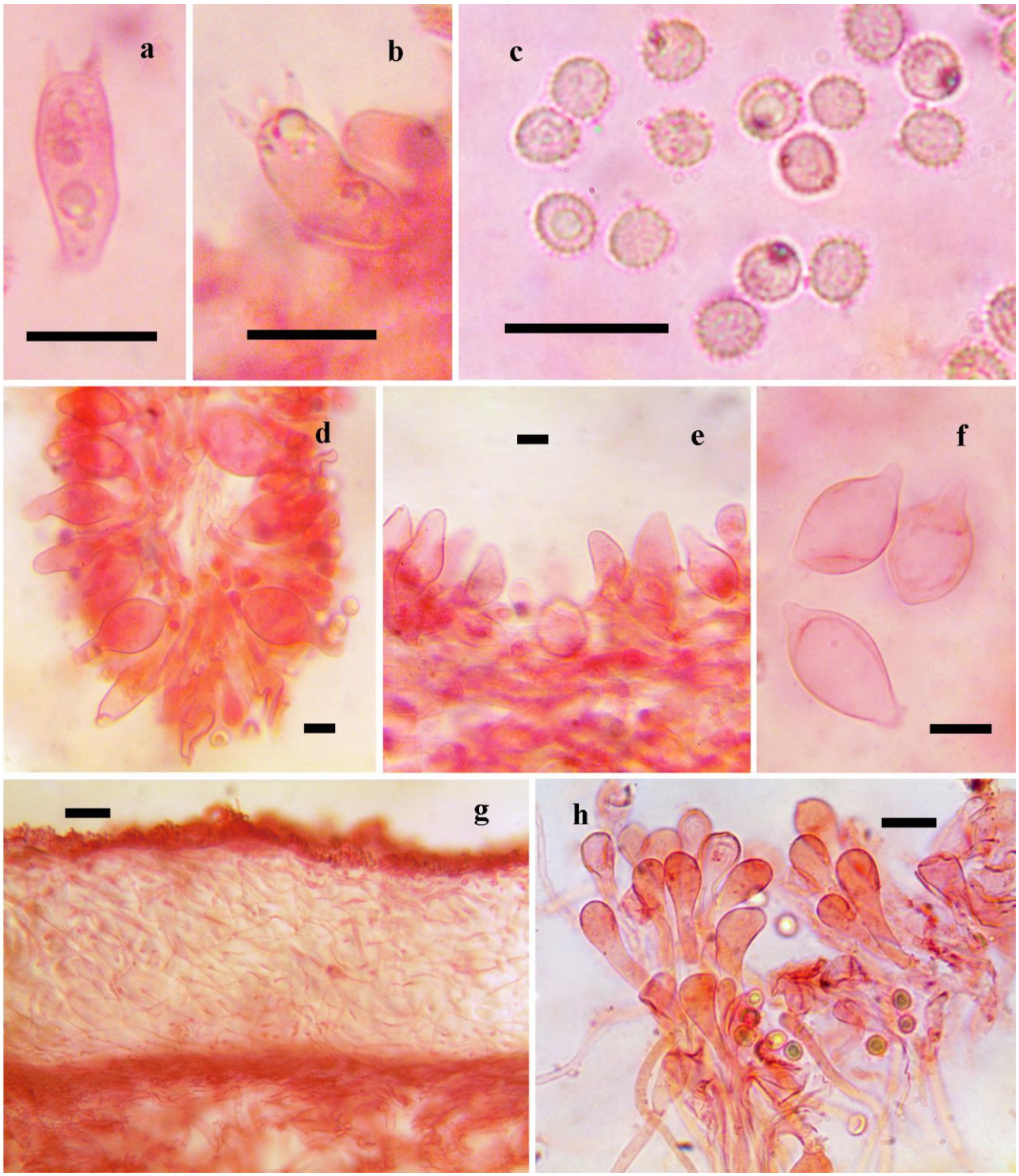


**Fig. 2** – *Conchomyces bursiformis*. a, b Basidia. c Spores. d Hymenophoral trama with cheilocystidia and pleurocystidia. e Cheilocystidia. f Pleurocystidia. g Pileipellis. h Pileocystidia. – Bars, a-f, h = 10 µm, g = 50 µm.

In a broad systematic treatment based on nLSU sequences on euagarics (Moncalvo et al. 2002), *Conchomyces* is clustered with *Gamundia, Caulorhiza, Myxomphalia* and *Fayodia* in a distinct clade – 'fayodia' (Clade – 28). Thus it supports Horak's view (1981) that *Conchomyces* is distinct and remote from other genera in euagarics.

According to Horak (1981) this monotypic genus is widespread in subantartic forests of southern South America and Australasia and also occurs in temperate montane forests in tropical and subtropical Australasia and Indomalaya. This species was earlier reported from Kashmir, India (Abraham & Kaul 1989).

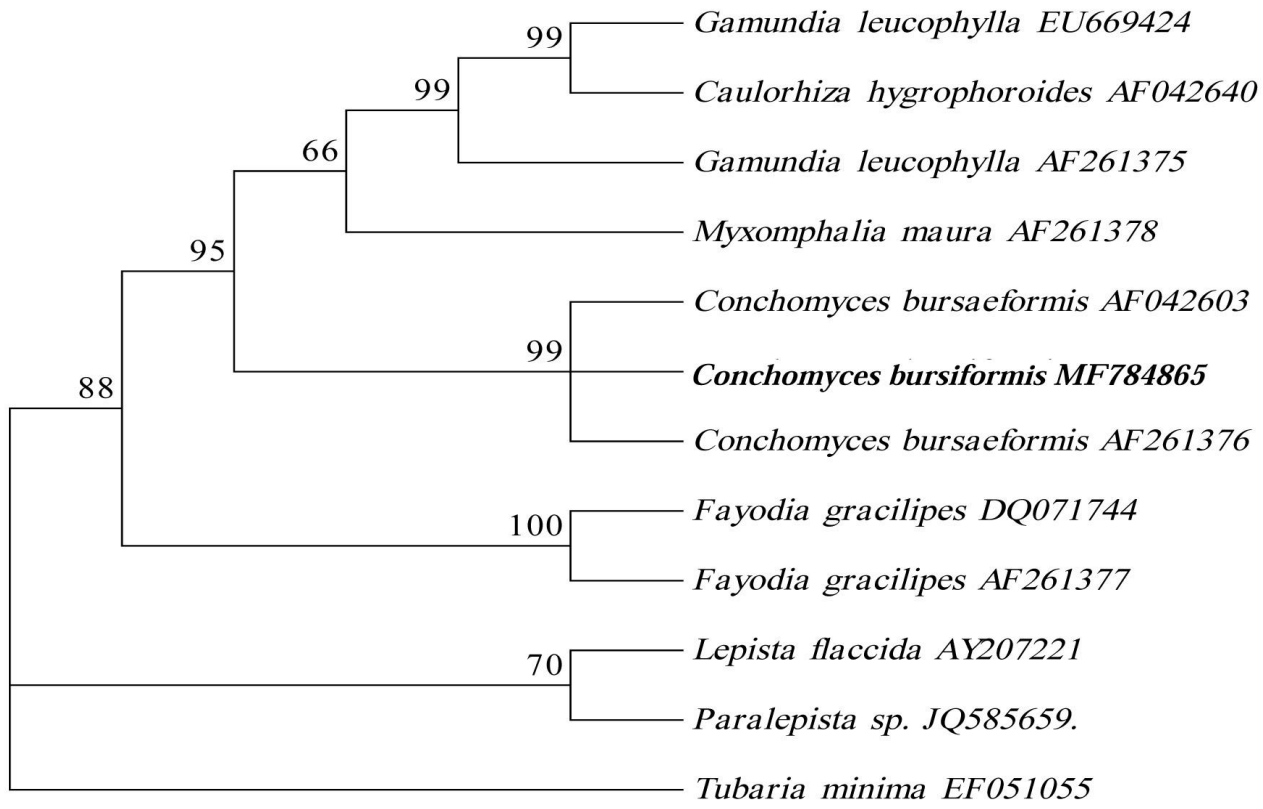


**Fig. 3** – Bootstrap consensus tree obtained from ML analysis using nLSU sequence data. Values above branches indicate Bootstrap support. BS values ≥ 70% are considered significant. GenBank accession numbers are given after the name of each taxon. *Conchomyces bursiformis* (MF784865) of the present study is shown in bold.

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