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Clitocella (Entolomataceae) - a new genus record for India

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Abstract

During an exploration in the virgin coniferous forest of Jammu and Kashmir, a little known taxon, *Clitocella popinalis* (Basidiomycota, Entolomataceae) was gathered along with other macrofungi. Based on morphological and molecular (nrITS sequence) data, detailed taxonomic information related to this species is described and illustrated for the first time from India. Morphologically and genetically allied taxa are also compared with this species.

Key words – Agaricales – internal transcribed spacer – Jammu and Kashmir

Introduction

The family Entolomataceae is one of the species rich families in Agaricales. Recent multi-gene analyses by Co-David et al. (2009) revealed that the family is monophyletic with two clades, the Entoloma clade and Rhodocybe-Clitopilus clade. More recently, Kluting et al. (2014) studied the Rhodocybe-Clitopilus clade in detail and recognized five genera with *Clitocella* K.L. Kluting, T.J. Baroni & S.E. Bergemann as new.

Species of the genus *Clitocella* are characterized by clitocyboid basidiocarps, long decurrent, narrow to very narrow lamellae, hyphae devoid of clamp connections and basidiospores with thin, evenly cyanophilic walls that are angular in polar view with 7–12 facets and are ornamented with indistinctly undulating pustules or minute bumps that are visible in profile and face views, spore print light pinkish in deposit.

Currently, only three species are known throughout the world including the type *Clitocella popinalis* Kluting, T.J. Baroni & S.E. Bergemann (Kluting et al. 2014) while Index Fungorum documents seven records of this genus so far. During our studies on the macrofungal diversity from district Poonch of Jammu and Kashmir, India, an interesting species of *Clitocella* was encountered. A detailed morphological and molecular study revealed that the species closely matches with *C. popinalis* (Bas et al. 1988, Kluting et al. 2014). Here we describe the species based on the morphological and phylogenetic analyses with comprehensive description along with photographs and relevant discussion. This forms the first report of the species after the type and also the first record of this genus from India.

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Materials & Methods

Morphological studies

The specimen under consideration was collected during September 2014 from Mughal Road, Poonch region of Jammu and Kashmir, India. The material was photographed in the field using a digital camera and extensive notes on the basidiocarps were accomplished before drying.

Microscopic features were obtained from dried material in 95% ethanol and then distilled water and then by mounting free-hand sections of basidiocarp tissues in 5% KOH, Melzer's reagent and Congo Red. Q value of the spore denotes length/width ratio of the basidiospores excluding ornamentation. Statistics for measurements of basidiospores are based on 30 measurements from each of the collected five basidiocarps. Scanning electron microscope (SEM) illustrations of basidiospores were obtained from dry basidiospores (spore print) with platinum coating at different magnifications in high vacuum mode to observe patterns of spore ornamentation. The examined specimen was deposited in the Calcutta University Herbarium (CUH), Kolkata, India

DNA extraction, PCR and sequencing

Genomic DNA was extracted from the dried basidiocarps following the procedure as described by Dutta et al. (2015). PCR reactions were performed with primer pairs ITS1 and ITS4 (White et al. 1990). The DNA fragments were amplified on an Applied Biosystems 2720 automated thermal cycler, following the PCR program used by Dutta et al. (2015). PCR products were purified using QIAquick® Gel Extraction Kit (QIAGEN, Germany) and was subjected to automated DNA sequencing on ABI3730xl DNA Analyzer (Applied Biosystems, USA) using primers identical with amplification for ITS rDNA region. The generated sequences were edited manually using BioEdit sequence alignment editor version 7.0.9.0 (Tom Hall, Ibis Biosciences, Carlsbad, USA). The edited sequences were then used for BLAST search in the GenBank database (www.ncbi.nlm.nih.gov).

Dataset representation and phylogenetic analysis

Total 21 sequences (nrITS) representing 16 species were used for phylogenetic analysis, of which 20 sequences retrieved from GenBank and one sequence was generated for this study. The sequences represent the genus viz. *Clitocella*, *Clitopilus*, *Entoloma* and *Rhodocybe*. Two sequence of *Tricholoma aurantium* were selected for rooting purpose (Kluting at al. 2014). All the sequences were aligned with the help of ClustalX (Thompson et al. 1997) using default setting.

The best fit model of sequence for phylogenetic study was determined with the help of MEGA v.7. The T92+G model was best fit model with lowest BIC scores of 4392.43 and AICc scores 4096.1. ML analysis was done based on T92+G (Tamura 1992) model where the initial tree (s) for the heuristic search was obtained by applying the neighbour-joining method and Bio NJ to a matrix of pairwise distances estimated using the maximum composite likelihood approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites {5 categories (+G, parameter = 0.3957)}.

Results & Discussion

Taxonomy

Clitocella popinalis (Fr.) K.L Kluting, T.J. Baroni & S.E. Bergemann Fig. 1a-e

Basidiocarp small to medium, clitocyboid. Pileus 10–32 mm diam., convex with a shallow depression at the centre; surface white, becoming buff yellow on drying, smooth, shiny, fleshy, non-striate. Lamellae buff yellow, decurrent, crowded, with lamellulae of 3 lengths; edges concolorous, smooth, entire. Stipe 42–45 × 4–5 mm, central, cylindrical, solid, equal with a broad base, tomentose; surface concolorous with the pileus, that turns brown on bruising; Odour strongly farinaceous. Taste bitter. Spore print light pink.

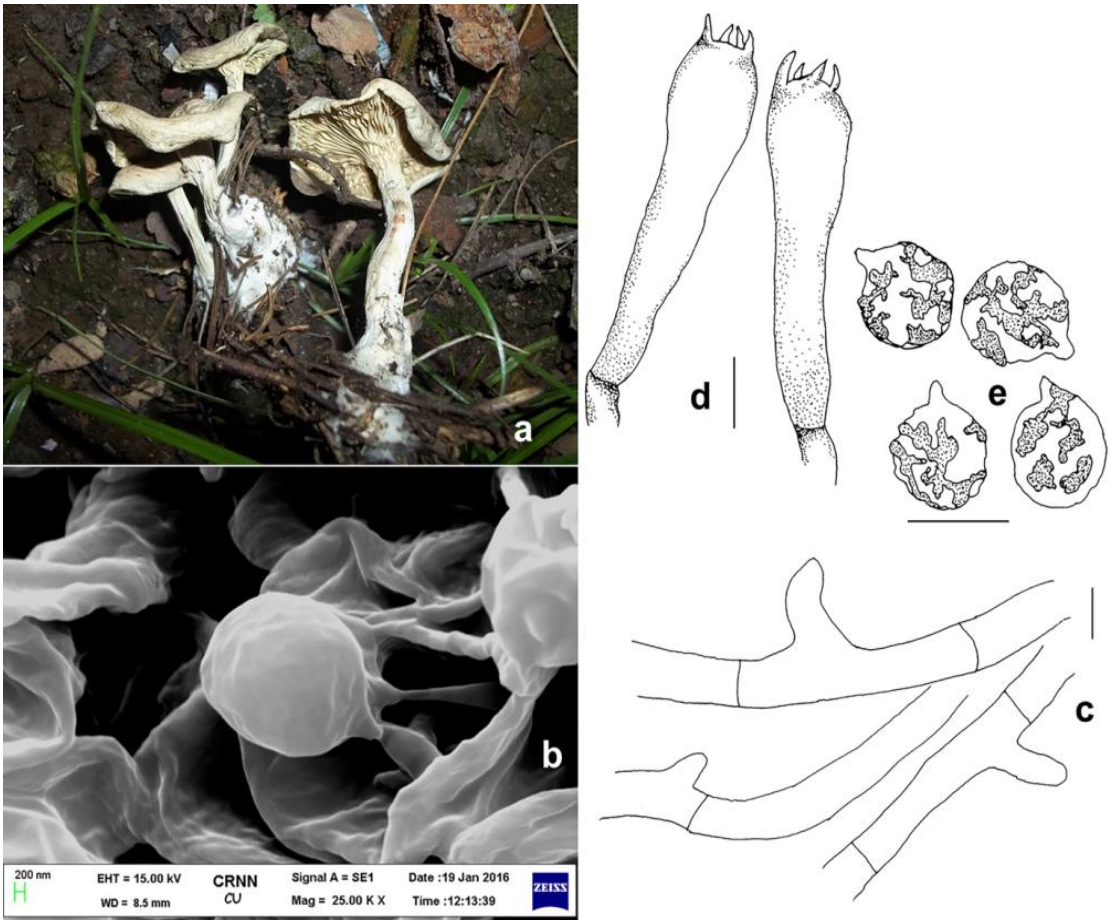

Fig. 1 – ***Clitocella popinalis***. **a.** Sporophores in natural habitat. **b.** Scanning electron micrograph of basidiospores. **c.** Pileus hyphae. **d.** Basidia. **e.** Basidiospores. Bars: c–e = 4 µm

Basidiospores 3.93–6.44 × 3.58–5.72 µm, a_vL= 5.1 , a_vW= 4.65, Q = 1.09–1.12, ovoid or subglobose to broadly ellipsoid, with a broadly rounded apex and base, suprahilar region never depressed, ornamentation consists of an open, indefinite reticulum as seen in SEM microphotograph, IKI –ve. Basidia 18–29 × 3.5–7.5 µm, clavate, 4-spored; sterigmata up to 3.2 µm long. Lamella edge fertile. Cheilocystidia and pleurocystidia absent. Hymenophoral trama more or less regular, made up of narrow to inflated, hyaline, thin-walled hyphae. Sub-hymenial layer weakly developed, composed of interwoven hyphae. Pileipellis a cutis, composed of 3.5–7.5 µm wide, hyaline, branched hyphae. Stiptipellis composed of 3.5–7.2 µm diam., thin-walled, hyaline, branched hyphae. Stipe trama parallel. Clamp-connections absent in all tissues.

Habit and Habitat – single to gregarious, humicolous, in coniferous forest dominated by *Cedrus deodara* (Roxb.) G. Don.

Specimen examined – INDIA: Jammu and Kashmir, Poonch District, Mughal road, 33º 37´ 59.26 " N and 74º 27´56.59" E, 21st September 2014, Harpreet Kour and Y.P. Sharma, HK550 (CUH AM033).

Remarks: *Clitocella popinalis* is characterised by the combination of characters like a small to medium sized clitocyboid basidiocarps coloured whitish to grayish, long-decurrent lamellae that are close to crowded or very crowded with a smooth lamellar edge, flesh-pinkish spore-print, basidiospores in the range of 3.93–6.44 × 3.58–5.72 µm and are angular in polar view with thin and evenly cyanophilic walls, ornamentation with obscurely defined undulating pustules or minute bumps that are visible in profile and face views and absence of hymenial cystidia and clamp-connections.

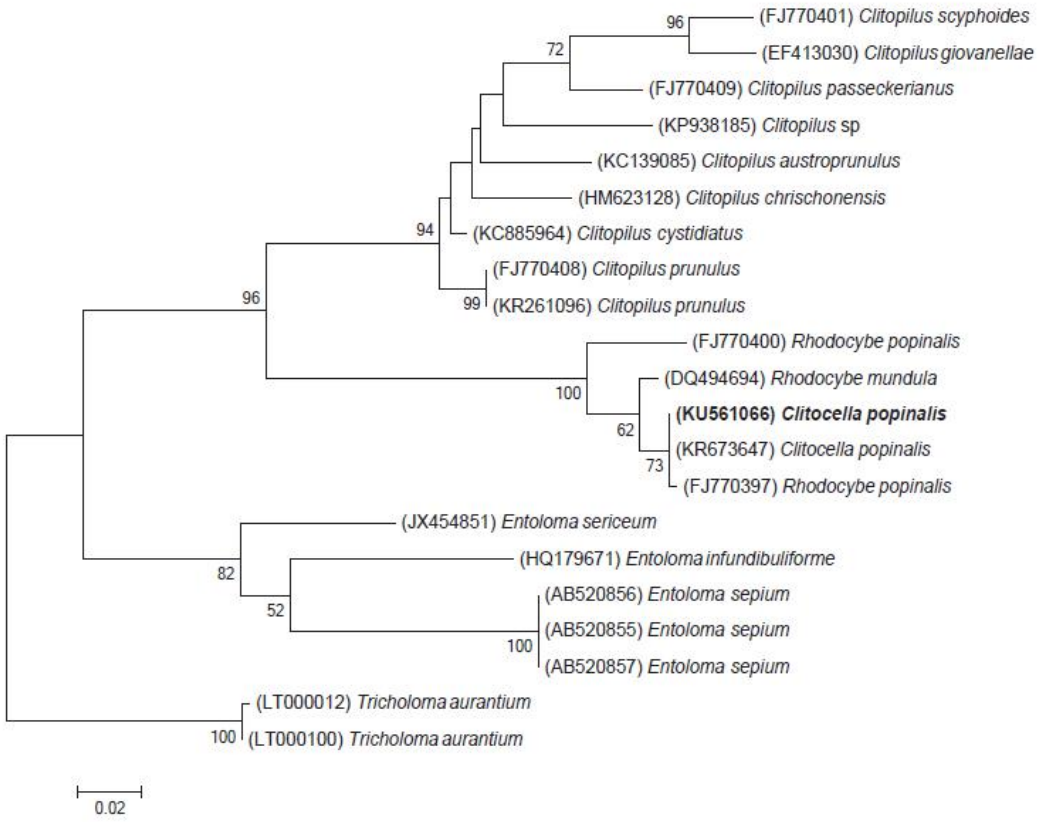

Fig. 2 – Maximum likelihood tree (-*InL* = 2022.9545) generated using T92+G model of nucleotide evolution. Maximum likelihood bootstrap value >50% are shown. *Clitocella popinalis* (KU561066) is placed in bold front.

Molecular analysis

The newly generated sequence was deposited in GenBank (www.ncbi.nlm.nih.gov) with the accession number KU561066. Based on megablast in NCBI GenBank database using the newly generated nrITS sequence, the closest hits was *Clitocella popinalis* [GenBank KR673647, Identities = 585/592(99%), Gaps = 3/592(0%); and GenBank FJ770397; Identities = 635/646 (98%), Gaps = 3/646(0%)], previously deposited from South Korea and UK respectively.

All the sequences were end trimmed to create a dataset of 602 nuclotides that included 412 parsimony informative characters. The ML analysis iteration recovered a single tree. We have selected the topology resulting from the first iteration to present this present study (Fig 2; -*InL* = 2022.9545) (Kumar et al. 2016). ML bootstrap values (BS) support many of the terminal nodes in the phylogeny (Fig 2).

Morphologically as well as phylogenetically, the genus *Clitocella* is closely related to *Clitopilus* (Fr. ex Rabenh.) P. Kumm. and *Clitopilopsis* Maire. However, *Clitopilus* can be differentiated on account of its longitudinally ridged basidiospores ornamentation while *Clitopilopsis* is discernible by its smooth thickened wall of basidiospores. *Rhodocybe* Maire and *Rhodophana* Kuhner, the other two genera of the clade can be differentiated by their well developed isolated pustules on the surface and are distinctly angular in polar view. Further, the presence of hyphal clamp connections is another important characteristic feature of genus *Rhodophana* Maire. Besides this, *C. mundala* (Fr.) Kluting, T.J. Baroni & Bergemann is other comparable species on the basis of morphology which, however, microscopically differs in having narrower basidiospores.

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References

Bas C, Kuyper TW, Noordeloos ME, Vellinga EC, Crevel R Van, Arnolds EJM. 1988 – Flora Agaricina Neerlandica - Critical monographs on the families of agarics and boleti occuring in the Netherlands. 1: Entolomataceae. Balkema, Rotterdam-Brookfield, pp 182.

Co-David D, Langeveld D, Noordeloos ME. 2009 – Molecular phylogeny and spore evolution of Entolomataceae. Persoonia 23, 147–176.

Dutta AK, Paloi S, Pradhan P, Acharya K. 2015 – A new species of *Russula* (Russulaceae) from India based on morphological and molecular (ITS sequence) data. Turkish Journal of Botany 39, 850–856.

http://www.ncbi.nlm.nih.gov.

Kluting KL, Baroni TJ, Bergemann SE. 2014 – Toward a stable classification of genera within the Entolomataceae: a phylogenetic re-evaluation of the Rhodocybe-Clitopilus clade. Mycologia 106, 1127–1142.

Kumar S, Stecher G, Tamura K. 2016 – MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger data sets. Molecular Biology and Evolution 33, 1870–1874.

Tamura K. 1992 – Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Molecular Biology and Evolution 9, 678–687.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997 – The CLUSTALX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25, 4876–4882.

White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: PCR Protocols: A Guide to Methods and Applications, (eds MA Innis, DH Gelfand, JJ Sninsky, TJ White). London Academic Press, London, pp 315–322.