

Chlorolepiota brunneotincta: a new species (Agaricaceae) from India

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Abstract: The genus *Chlorolepiota* Sathe & Deshpande is a monotypic genus represented by *C. mahabaleshwerences* Sathe & Deshpande. In this paper, a new species, *Chlorolepiota brunneotincta*, is described, based upon macroscopic, microscopic, and molecular studies undertaken on the collection from Solan Himachal Pradesh (India).

Key words: Macrofungi, basidiomycetes, taxonomy, ITS, PCR, India

1. Introduction

The genus *Chlorolepiota* Sathe & Deshpande is a basidiomycetes fungus of the family Agaricaceae, represented by a single species worldwide (Kirk et al., 2008). Unique characteristics of *Chlorolepiota brunneotincta* sp. nov. are its greenish lamellae, the presence of versiform cheilocystidia, nontruncate spores, and characteristic browning on bruising. On the basis of macroscopic and microscopic characteristics and ITS sequences, the presently proposed species was compared with *C. mahabaleshwerences* Sathe & Deshpande, which is the only species of *Chlorolepiota* worldwide (Kirk et al., 2008).

2. Materials and methods

2.1. Morphological studies

The field characteristics pertaining to gross morphology, shape, color and size of pileus, stipe etc. were noted down in the field key for mushroom collection (Atri et al., 2005). The color terminology used was that of Kornerup and Wanscher (1978). The specimens were hot air dried and preserved in cellophane paper bags containing 1,4-dichlorobenzene. The microscopic structures were observed by cutting freehand sections after reviving a part of the dried specimens in KOH solution, and staining the sections in 1% cotton blue and 2% congo red. The holotype specimen has been deposited in the Herbarium of the Botany Department (PUN), Punjabi University. The photographs and microscopic details are given in

Figures 1–3. These collections are morphologically and microscopically identified according to Pegler (1977, 1986), Sathe et al. (1980), Asef and Muradov (2012), Seyidova and Hüseyin (2012), and Kumari et al. (2012, 2013).

2.2. ITS sequencing and phylogenetic analysis

Genomic DNA from the fruit bodies of *Chlorolepiota brunneotincta* was isolated as described by Vankan et al. (1991). The ITS region of the rDNA from genomic DNA of the sample was amplified by using PCR employing ITS1 and ITS4 primers. The resultant PCR products were viewed after electrophoresis in agarose gel. The results showed that the ITS region of each studied taxon produced a single band of approximately 700 bp. The sequence was analyzed using the gapped BLASTn <http://www.ncbi.nlm.nih.gov> search algorithm and aligned to the nearest neighbors. The evolutionary distances among *Chlorolepiota brunneotincta* and its related taxa were calculated with MEGA5 software after aligning the sequences with ClustalW. The newly generated sequence was deposited at GenBank (accession no. JQ928940).

3. Results

3.1. Taxonomy

Chlorolepiota brunneotincta N.S.Atri, B.Kumari & R.C.Upadhyay sp. nov. (Figures 1–3)

Myco Bank no: MB800485

Type: India, Himachal Pradesh, Solan, Kather growing scattered on soil in *Pinus roxburghii* forest, 1650 m, 17.08.2008, B.Kumari s.n. (Holotype: PUN 4711).

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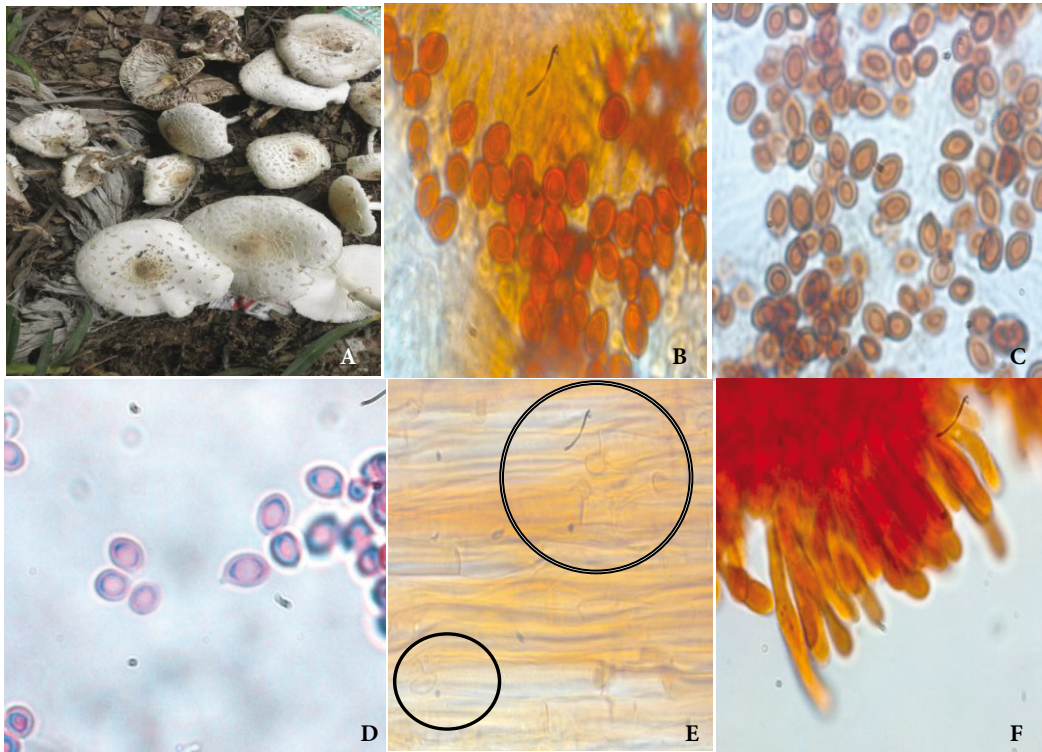


Figure 1. A- Field photograph of *Chlorolepiota brunneotincta*, B- basidiospores in Congo red, C- basidiospores showing dextrinoid in Melzer's reagent, D- basidiospores in cresyl blue showing metachromatic reaction, E- clamp connection in stipe surface hyphae, F- clusters of cheilocystidia.

Diagnosis: Pileus 2.5–5.5 cm broad, surface white covered with brown fibrillose scales. Lamellae free, crowded. Basidiospores ellipsoid, dextrinoid, aporus. Cheilocystidia with clavate to cylindrical, subcapitate or even slightly lageniform shape and flesh and stipe surface browning on bruising.

Description. Carpophores: 2.5–6.5 cm in height. Pileus 2.5–5.5-cm wide; surface moist, white (1A1), convex to campanulate with a prominent broad tawny or brown (7E5) umbo; covered with brown (7E5) fibrillose scales over yellowish white (4A2) background; margin striate, regular, splitting at maturity; cuticle fully peeling; flesh up to 3-mm thick, white, changing to brownish red (10 D7) on bruising; taste and odor mild; spore deposit yellowish white (4A2) with greenish shade. Lamellae free, collariate, unequal, of 3–4 lengths, crowded, 2–4-mm broad, white (1A1), to greenish white, becomes brownish green on drying; gill edges smooth. Stipe central, up to 6.3-cm long, 0.2–0.6-cm broad, white, color changes to brownish red (10 D7) on bruising, equal in diameter, hollow, scaly; annulate, annulus single, peronate (Figure 1). **Basidiospores** 7.0–9.0 × 5.5–7 μm (Q = 1.28) ellipsoid, thick-walled, non-truncate, appears aporous, with rounded apex studied with a poral plug, dextrinoid, cyanophilous, con-

gophilous, metachromatic in cresyl blue (Figures 1 and 2). Basidia 18.0–30.0 × 7.0–10.0 μm, clavate (Figure 2), tetrasporic; sterigmata up to 4-μm long; gill edges sterile. Cheilocystidia 25.5–44.5 × 6.5–9 μm, crowded, clavate to cylindrical, subcapitate or even slightly lageniform (Figures 1 and 2). Pleurocystidia absent. **Pileus** cuticle a disrupted trichoderm of cylindrical to narrowly clavate elements measuring up to 10.0–16.0 μm in width (Figure 2); context homoiomerous; gill trama regular; tramal hyphae measuring 8.0–20.0 μm; subhymenium pseudoparenchymatous, well-developed. Stipe cuticle hyphae parallel running throughout, measuring 3.2–14.5 μm in width. Clamp connections present in the basal mycelium, stipe surface hyphae (Figure 1) and absent at the base of basidia. Hyphal construction monomitic. Elements of annulus 2.5–8.5-μm wide, subcylindrical, inflated, septate, smooth, hyaline in KOH, thin-walled.

Habitat: Growing scattered under *Pinus roxburghii* on soil in *Pinus roxburghii* forest.

Additional collection examined (Paratypes): India, Himachal Pradesh, Mandi, Bakarata, 850 m, 25.06.2010, carpophores scattered on soil in *Pinus roxburghii* forest, B.Kumari s.n. (PUN 4420); Punjab, Patiala, Thaper

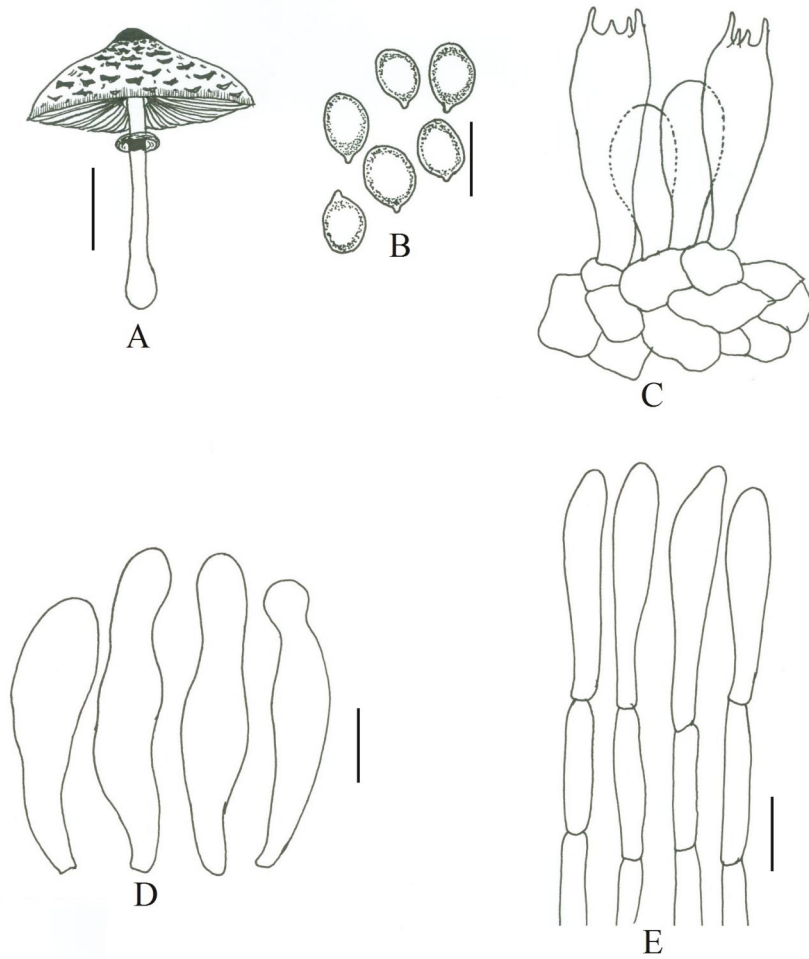


Figure 2. *Chlorolepiota brunneotincta*: A- carpophore, B- basidiospores, C- basidia, D- cheilocystidia, E- pileus trichodermial elements. Scale bars: A = 2 cm, B-E = 10 μ m.

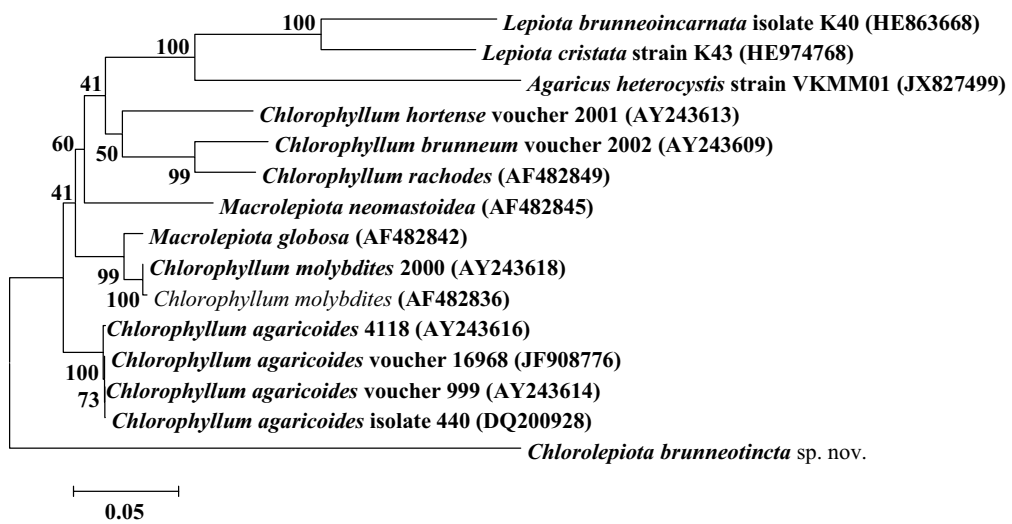


Figure 3. Phylogeny of *Chlorolepiota brunneotincta* generated from maximum likelihood analysis of ITS sequences. The numbers on the nodes indicate how often (no. of times, %) the species to the right are grouped together in 100 bootstrap samples. The scale gives the substitution rate.

University campus (250 m), growing gregarious on soil, 28.06.2008 *M.Kaur & B.Kumari s.n.* (PUN 4114).

Etymology: Browning of flesh and stipe surface on bruising.

3.2. Molecular sequencing: A single band of ~700 bp was obtained on amplification of the ITS region of the fungus. The sequence of ~691 bp ITS region of the fungus showed 84% identity with the *Chlorophyllum molybdites* strain. The phylogenetic tree was constructed with the MEGA5 software (Figure 3) (Tamura et al., 2011). All positions containing gaps and missing data were eliminated. In the tree, sequences of reference strain were obtained from the NCBI GenBank. ITS sequences of *Chlorolepiota brunneotincta* are a new addition to the NCBI GenBank.

4. Discussion and conclusion

The presently examined collections fall within the overall taxonomic limits of the genus *Chlorolepiota* in view of its close relatedness with *Chlorophyllum* Masee & *Macrolepiota* Singer. The presently examined collection closely resembles *Chlorophyllum molybdites* in outward morphology, scaly pileus, and unique green coloration of lamellae and spores, but not in the double complex annulus, the shape of cheilocystidia, nontruncate spores, and a palisade of hyphen with clavate to subfusiform terminal elements. In its gross morphology and presence of clamp connections it resembles *Macrolepiota*. However, the comparatively short stature of the carpophores, the green color of lamellae, the absence of apical truncation, and hyaline cap in the basidiospores make the present collection rather distinct from *Macrolepiota* (Vellinga 2003; Vellinga et al., 2003; Ge et al., 2010). *Chlorolepiota brunneotincta* also demonstrates a resemblance with *Chlorophyllum hortense* on account of basidiospores without germ pores, reddening of stipe when bruised, a simple annulus, and presence of clamp connection; it differs by lacking 2-spored basidia and hymeniderm made up of broadly clavate, terminal elements. It is also quite different from *Chlorophyllum sphaerosporum* in that it lacks pileipellis, a hymenidermal layer, and clavate to broadly clavate terminal elements (Ge and Yang, 2006).

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Most of its features come rather close to the taxonomic details of *Chlorolepiota*, a genus described by Sathe and Deshpande (1979) from Poona, which is characterized by a macrolepiotoid habit, a pruinose yellow greenish spore deposit, and absence of clamp connections. In comparison to *Chlorolepiota mahabaleshwariensis*, the presently studied collection has smaller spores ($7-9 \times 5.5-7 \mu\text{m}$ instead of $11.4-15.7 \times 5.7 (9.3) \mu\text{m}$), browning flesh and stipe surface on bruising, versiform cheilocystidia and clamp connections in the basal mycelium, and stipe surface hyphae. Since in the allied 2 genera (*Macrolepiota* and *Chlorophyllum*) the presence of clamp connection is a prominent characteristic, a feature that the present variant also possesses but it is reported to be absent in *Chlorolepiota* (Sathe & Deshpande, 1979), which seems to be an aberration as it is based on a limited observation because the genus is monotypic and the description is single-collection based, no importance has been given to this feature in the present case.

The distinguishing features of the presently examined collection provide sufficient grounds for establishment of a new species. Conclusions arrived at regarding the identity of *Chlorolepiota brunneotincta* through classical taxonomic techniques were confirmed through molecular sequencing of the ITS region of their genomic DNA. The sample exhibited 84% homology with the sequence of *Chlorophyllum molybdites* and Lepiotaceae in the parsimony analysis, which clearly takes it away from *Chlorophyllum*. Along with other features, taking browning of flesh and stipe surface on bruising into consideration as a unique quality of this variant of *Chlorolepiota*, a new species *Chlorolepiota brunneotincta* has been proposed to accommodate this collection.

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