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First report of *Callistosporium luteoolivaceum* from Western Himalaya, Pakistan

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Abstract — *Callistosporium luteoolivaceum* was collected from pure pine forests of Western Himalaya, Pakistan, and identified based on morphological characters and nrDNA ITS sequences. It is characterized by dull yellow or olive yellow pilei, lamellae, and stipes that turn reddish brown with age and basidiospores that turn purplish in KOH. This species is new to the mycobiota of Pakistan.

Key words — Abbottabad, *Basidiomycota,* Khyber Pakhtoonkhaw, saprobic fungi, *Tricholomataceae*

Introduction

Geographically, the areas of Pakistan and Indian-held Kashmir fall within the Western Himalaya. The climate ranges from tropical at the base of the mountains to permanent ice and snow at the highest elevations. The Himalayan forests have been reliably identified as a 'biodiversity hotspot', a global priority for the conservation of biodiversity (Wikramanayake et al. 2002; Qamar et al. 2011).

During a 2012 mushroom collecting trip in pure pine forests in the Himalayan range, many species of saprobic macrofungi were collected. Amongst them, *Callistosporium luteoolivaceum* was identified morphologically and molecularly as a new record from Pakistan. This very variable species occurs worldwide in temperate or tropical forests mostly outside of boreal and subalpine regions (Redhead 1982). According to Kuo (2006) this little mushroom is a decomposer found primarily on dead conifer wood. No *Callistosporium* species has previously been reported from Pakistan.

Materials & methods

Basidiomata were collected, photographed, vouchered, dried under fan heater and characterized morphologically. Specimen sections were mounted in 5% KOH for

observation under a MX4300H biological microscope (Meiji Techno Co., Ltd., Japan). Phloxine was used to increase contrast of structures, and Melzer's reagent was used to test for dextrinoidity of basidiospores.

Measurements of morphological features (basidiospores, basidia, cystidia, and pileus and stipe hyphae) were taken from at least 20 measurements made with an ocular micrometer and 100× oil-immersion objective, where x = arithmetic mean of spore length and width for all spores measured, and Q = spore length divided by spore width. Line drawings were made with camera lucida. Color designations are from Munsell (1975). The collection was conserved in the Herbarium of the Department of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Genomic DNA was extracted from a small piece of pileus by a modified CTAB method (Bruns 1995). The internal transcribed spacers (ITS1 and ITS2) and the 5.8S nuclear ribosomal gene were amplified with primer pairs ITS1F/ITS4 (White et al. 1990; Gardes and Bruns 1993) using the Extract-N-Amp plant DNA extraction Kit (Sigma-Aldrich, St. Louis, MO, USA). PCR cycling parameters were as follows: initial denaturation (94 °C for 1 min), 35 cycles (94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min), and final extension 72 °C (8 min). Amplified PCR products were sent for purification and bidirectional sequencing to Macrogen (Republic of Korea). One *C. luteoolivaceum* sequence and other related sequences were retrieved from GenBank and aligned by Muscle using default setting in Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura et al. 2011). Sequences were manually edited and assembled using BioEdit (www.mbio.ncsu.edu/bioedit/bioedit.html). Following Dentinger et al. (2011) for complete ITS sequences, all sequences were trimmed with the conserved motifs 5′-(…GAT) CATTA– and –GACCT (CAAA…)-3′ and the alignment portion between them were included in analysis. A sequence generated for this study was submitted to GenBank. Percent Identities (PID) and DNA divergence were calculated by Megalign (DNA Star Inc.).

Taxonomy

Callistosporium luteoolivaceum (Berk. & M.A. Curtis) Singer, Lloydia 9: 117 (1946). Figs 1, 2

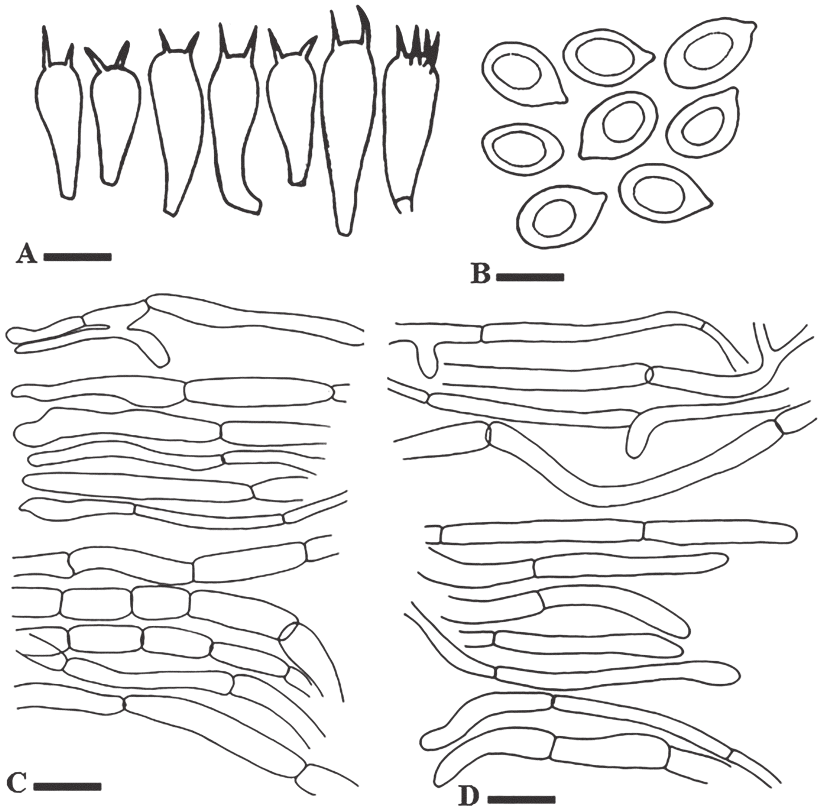
Pileus 27–31 mm diam., convex to plano-convex, flat, thin (collybioid); margin straight or flaring, smooth; surface dull, smooth, olive yellow near margin (5YR7/10); disc moderately indented, dark brown (5YR1/4) at the center. Lamellae regular, adnate, close to crowded, pale yellow or olive yellow (5YR7/8); margin wavy. Stipe 55–58 mm, central, equal, hollow, striate, pale yellow to olive yellow (5YR7/8); annulus absent; volva absent. Rhizomorphs white, few. Odor and taste not recorded. Basidiospores 4.6–6.6 × 3–4.5 µm [x = 5.2 × 3.8 µm, Q = 1.36], ellipsoid in profile, smooth, thin-walled, purplish in KOH, apiculus present; Basidia 16.9–24.5 × 5.4–7.8 µm, clavate, two to four spored, thin-walled, some turning deep purple in KOH; sterigmata 2.8–4.2 µm. Trama hyphae, parallel, thin-walled, hyaline in KOH. Cheilocystidia 12–26.6 × 4.7–8 µm, clavate or cylindrical, hyaline, thin-walled. Pleuro-

Callistosporium luteoolivaceum (MSM #004, LAH). Basidiomes. Scale bars = 7 mm.

cystidia absent. Pileipellis a cutis; hyphae cylindrical, 4–6 µm, thin-walled, hyaline in KOH, some with brown pigments; pileal cystidia clavate, 34–61 × 5.1–8.2 µm, thin walled, hyaline in KOH. Stipe hyphae cylindrical, 3.5–17 µm, hyaline in KOH. All structures inamyloid. Clamp connections absent.

Material examined: **PAKISTAN**, **Khyber Pakhtoonkhaw**, Abbottabad, Shimla, under *Pinus roxburghii* Sarg., 14 September 2012, MSM #004 (LAH; GenBank KJ101607).

Discussion: *Callistosporium luteoolivaceum* was first described from eastern North America. It is taxonomically complicated, and its variable characters and wide world distribution has resulted in its being described many times as a new taxon. Redhead (1982) critically examined *C. elaeodes* Bon, *C. favrei* Singer, *C. graminicolor* Lennox, *C. luteofuscum* Singer, *C. luteofuscum* var. *major* Singer, *C. majus* Singer, and *C. xanthophyllum* (Malençon & Bertault) Bon and reduced them to synonymy under *C. luteoolivaceum* because their distinguishing features intergrade. Despite the intergradations of critical characters, there remains a great diversity of forms within the broad species concept which when viewed individually appear quite distinct from one another (Redhead 1982).



Callistosporium luteoolivaceum (MSM #004, LAH).
(A) Basidia. (B) Basidiospores. (C) Stipe elements. (D) Pileipellis and pileal cystidia.
Scale bars: A = 10 µm; B = 4.5 µm; C, D = 17.5 µm.

Our collection is in remarkable agreement with the description provided by Redhead (1982). This is the first report of *C. luteoolivaceum* from Pakistan. It has been reported from North and South America, Europe, and Asia (Hongo 1981; Horak 1987; Manimohan & Leelavathy 1989; Redhead 1982; Singer 1970). This species is listed among the rare and notable macrofungi of British Columbia (Redhead 1997: 6–8).

Sequencing of the nrITS region of *C. luteoolivaceum* yielded fragments of 690 base pairs when its PCR product was sequenced. Initial blast analysis showed our Pakistani sequence (GenBank KJ101607) with 97% maximum identity with *C. xanthophyllum* (GenBank JF907781; AF325667) and *C. luteoolivaceum* (GenBank AF325666). Further analysis reinforced the blast results, showing

97.5% identity and 0.5% genetic divergence between our Pakistani sequence and the *C. luteoolivaceum* sequence (GenBank AF325666).

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