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# *Lactarius olivaceoumbrinus*: a new addition to Indian mycobiota from Uttarakhand, India

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**ABSTRACT**

*Lactarius olivaceoumbrinus* (*Lactarius* subg. *Piperites* sect. *Atroviridi*) is presented with morphological and anatomical descriptions for the first time from India. Supported illustrations and phylogenetic conformity of identification is also provided.

**Key words:** Russulaceae, new record, taxonomy, phylogeny, Western Himalaya.

## INTRODUCTION

Playing one of the key roles in ecosystem functions, ectomycorrhizal fungi not only promote the growth, development and overall health of host-plants but also form vast metabolic networks (Leake et al. 2004; Courty et al. 2010).

*Lactarius* Pers. is a large ectomycorrhizal genus consisting of three subgenera i.e., *Lactarius* subg. *Piperites* (Fr. ex J. Kickx f.) Kauffman, *Lactarius* subg. *Russularia* (Fr. ex Burl.) Kauffman and *Lactarius* subg. *Plinthogali* (Burl.) Hesler & A.H. Sm. Members of the genus have been reported in ectomycorrhizal association with numerous trees and shrubs, and their important ecological role as late-stage root colonizers in a range of ectotrophic plant communities is largely appreciated (Das et al. 2015; Hutchinson 1999).

In an attempt of macrofungal exploration through Uttarakhand Himalaya, a large number of lactarioid species were collected. Thorough morphological examination along with the phylogeny, based on nrITS (Internal Transcribed Spacer of nuclear ribosomal RNA) region of one of these taxa revealed it as a new record for the Indian mycobiota namely, *Lactarius olivaceoumbrinus* Hesler & Smith (subg. *Piperites* sect. *Atroviridi*). In present communication, this taxon is presented with detailed description and supported illustrations.

## MATERIALS AND METHODS

### Morphological study

Macromorphological characters were duly noted in the forest or basecamp from the fresh and dissected young to mature basidiomata. Macrochemical colour reactions on pileus, stipe surface, context and latex were also recorded. Images of the fresh basidiomata were captured with the help of Canon Power Shot SX 50 HS and Sony Cyber Shot W730. Color codes and terms mostly follow Methuen

Handbook of Color (Kornerup & Wanscher 1978). Micromorphological characters were observed with the help of a compound microscope (Olympus CH20i) from dry material mounted in a mixture of 5% KOH, 1% Phloxin and 1% Congo red. Drawings of micromorphological elements were made with the Camera lucida at 2000× magnification. Microphotographs were captured with the digital cameras attached to the respective compound microscopes: Olympus CH20i and Olympus-CX21iLED. Basidiospores were mounted in Melzer reagent and measured in lateral view excluding the height of ornamentations.

Scanning Electron Microscope (SEM) images (of basidiospores) were obtained from dry basidiospores that were directly mounted on a double-sided adhesive tape pasted on a metallic specimen-stub and then scanned with silver coating at different magnifications in high vacuum mode (20 KV) to observe patterns of spore-ornamentation. SEM-studies were carried out with a ZEISS EVO 18 SPECIAL EDITION model installed at USIC Dept., HNBGU Srinagar (Garhwal), India. Herbarium codes follow Thiers (continuously updated).

**DNA extraction, PCR amplification and sequencing**

Genomic DNA was isolated by 100mg of basidiomata using XcelGen Plant Fungal gDNA kit (XG2416-01) following the manufacturer's instructions. The ITS gene region was amplified with primer pairs ITS1 and ITS4 (White et al. 1990). PCR amplification was performed using thermocycler (ABI, Veriti) programmed for 3 min at 95°C, followed by 30 cycles of 45 sec at 48°C, 45 sec at 72°C and a final stage of 10 min at 72°C. The PCR amplicon was purified with exosap enzymatic purification as per the manufacturer instruction (ABI). After the purification the products were subjected to Sanger sequencing using ABI, 3730XL DNA analyzer using BdT v.3.1 chemistry. Each forward and reverse reaction of PCR amplified products were sequenced separately. Forward and Reverse DNA sequencing reaction of PCR amplicons of respective samples was carried out with ITS1and ITS4 primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer.

**Phylogenetic analysis**

Phylogenetic analyses based on internal transcribed spacer (ITS) sequences data were carried out to establish the phylogenetic placement of our isolated taxon. Multiple sequence alignment was performed using MAFFT v.7 (Katoh & Standley 2013). Phylogenetic analyses were undertaken based on maximum likelihood (ML) criterion implemented in MEGA 6.0. (Tamura et al. 2013). One-thousand bootstrap replicates were analyzed to obtain nodal support values.

## RESULTS AND DISCUSSION

**Phylogeny**

The multiple ITS sequences of 27 species (including our isolate, with genbank acc. no. KX845556) of *Lactifluus* and *Lactarius* were analyzed. All the sequences belonging to *Lactarius* sect. *Atroviridi* were clustered in a clade. Our Indian isolate (KX845556) was found to form a well supported clade (83% bootstrap) with sequences of *L. olivaceoumbrinus* (genbank acc. no. KJ146720 and KC581315).

**Taxonomy**

***Lactarius olivaceoumbrinus*** Hesler & Smith, North American species of *Lactarius*: 219 (1979) **Figs. 2 & 3**

**Pileus** 46−62 mm in diam., convex to planoconvex with depressed centre, sometimes infundibuliform when mature, with or without umbo, surface dry, leathery, smooth to appressed fibrillose toward margin, azonate or zonate increasingly toward margin, olive yellow (2D6) to olive (2F4− 2F5), dull yellow (3B3−3B4) to greyish yellow (3B5) at margin, turning purplish red (13B7− 13B8) with KOH; margin entire, decurved to plane. **Lamellae** subdecurrent, close to crowded, forked near the juncture of the stipe, lamellulae present, pale yellow (1A3) at first, brownish yellow (5C7−5C8) with maturity, yellowish brown (5D8) on bruising. **Stipe** 28− 62 × 11− 17 mm, cylindric, equal to slightly tapering downward, surface dry, glabrous, dull yellow (3B3), greyish yellow (3B4) or darker (4B3) to olive (2F5), spotted with olive brown (4D8) scrobiculi. **Context** (3−6) mm thick, thin at pileus mid radius, hollow in stipe, white to dirty white, turning greyish yellow (4B3) on exposure and reddish lilac (14C5) with KOH. **Latex** abundant, white. **Taste** acrid, mild when isolated. **Odor** unpleasant.

**Basidiospores** 6.0−**6.9**−8.0 × 5.0−**6.0**−6.5 µm, Q = 1−**1.13**−1.27 (n = 45), globose to broadly ellipsoid, rarely ellipsoid, ornamentations up to 0.5 µm high, composed of large and small warts connected with broad to thin ridges, but never forming a complete reticulum, with small isolated warts; plage inamyloid. **Basidia** 40−61 × 8−12 µm, subclavate, 2−4 spored, sterigmata 4−6 µm long. **Subhymenium** up to 27 µm thick, pseudo-parenchymatous. **Pleuromacrocystidia** 31−75 × 4−10 µm, abundant, emergent up to 18 µm, fusiform to subfusiform; apex round, acute to subcapitate; contents granular. **Lamellar edges** sterile. **Cheilomacrocystidia** 29−65 × 4.5−10 µm, subfusiform; apex round to subcapitate. **Pseudocystidia** up to 4 µm wide, slight to non emergent, cylindric, never forked. **Lactifers in hymenophoral trama** up to 10.5 µm thick.

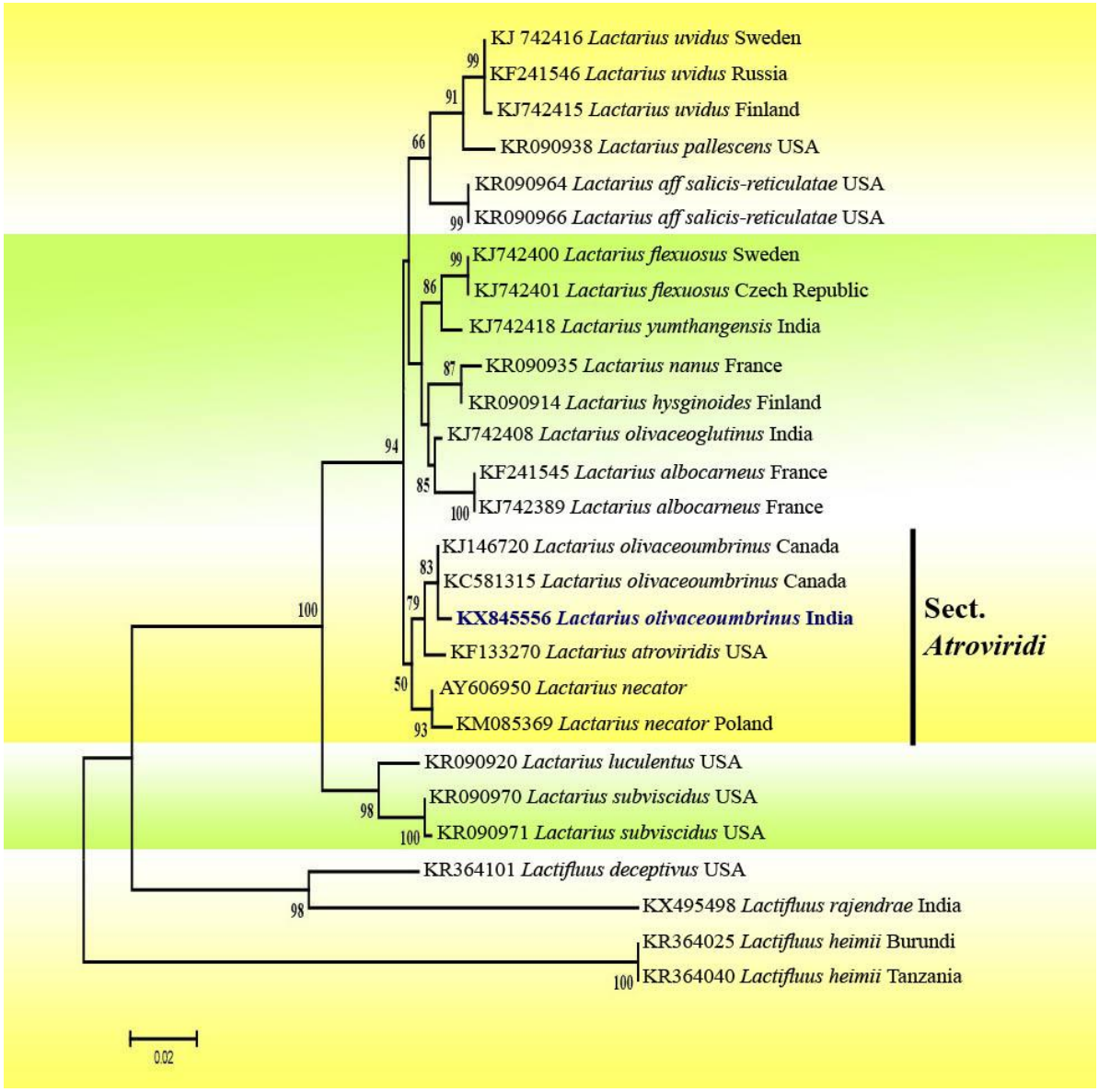


**Fig. 1.** Phylogeny of Indian specimen of *L. olivaceoumbrinus* (in blue fonts) inferred from Maximum Likelihood analysis of ITS sequences using Mega 6.0.

**Pileipellis** an ixocutis, 43−103 µm thick, composed of septate to aseptate hyphae; septate hyphae up to 5 µm wide, aseptate hyphae thinner, up to 3.8 µm wide. **Stipitipellis** an ixocutis, up to 100 µm thick, hyphae 2.5−5.5 µm wide.

**Habitat & distribution:** Under *Abies pindrow* in subalpine coniferous forests in India and also known from Canada.

**Materials examined:** INDIA, Uttarakhand, Rudraprayag district, Chopta, 2943 m, N30° 28.623′ E079° 11.492′, 30 August 2015, P. Uniyal, CAL-1404; Tungnath trek, 3236 m, N30° 29.359′ E079° 12.506′, 20 July 2015, P. Uniyal, PU 15-723.

**Notes:** In the field, *L. olivaceoumbrinus* (belonging to *L.* sect. *Atroviridi*) is quite distinct by its olivaceous pileus, pale yellow to brownish yellow lamellae which turn yellowish brown on bruising, lilac color reaction of context with KOH, scrobiculate stipe, white, acrid latex and occurrence under conifers. Micromorphologically, it is characterized by globose to broadly ellipsoid basidiospores with low ornamentations (up to 0.5 µm) which never form a complete reticulum, abundant macrocystidia and ixocutis pattern of pilei- and stipitipellis. Morphology of our Indian material is on complete conformity with North American counterpart except the wider (w = 6−9 µm) basidiospores with longer (up to 1.5 µm) prominences in latter (Hesler & Smith 1979).


**Fig. 2. (**a) Fresh basidiomata in field, (b) Latex on gills, (c) Context turning purple with KOH, (d) Transverse section through pileipellis, (e) Pseudocystidia, (f) Cheilomacrocystidia, (g) Transverse section of hymenium showing abundant pleuramacrocystidia, (h) SEM image of basidiospores. Scale bars: a = 100 mm; d & g = 25 µm; e =10 µm; f = 20 µm; h = 2 µm.

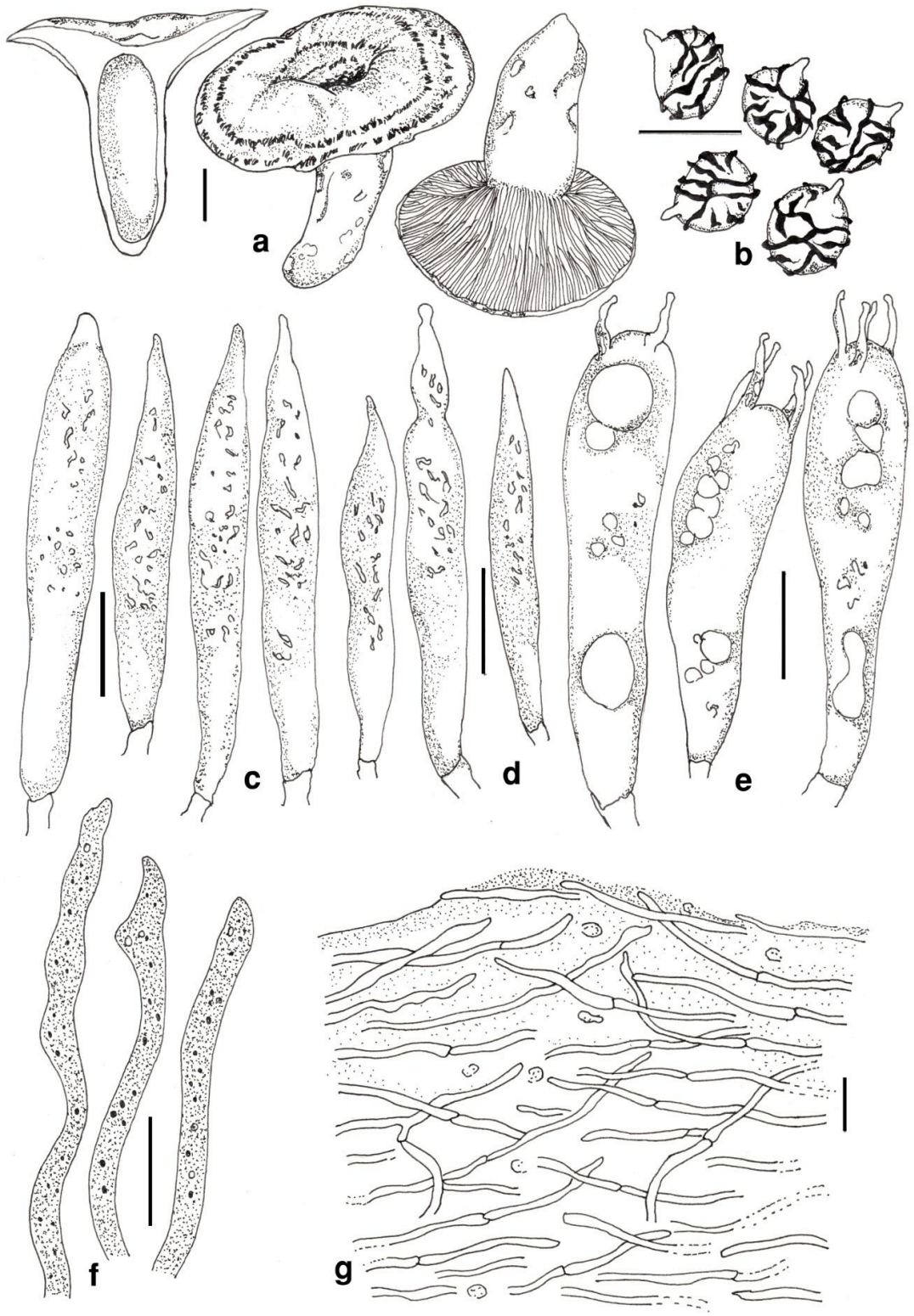


**Fig. 3.** **(**a) Fresh and dissected basidiomata, (b) Basidiospores, (c) Pleuromacrocystidia, (d) Cheilomacrocystidia, (e) Basidia, (f) Pseudocystidia (g) Pileipellis. Scale bars: a = 10 mm; b-g = 10 µm.

Morphologically, *L. atroviridis* Peck (labelled with genbank no. KF133270 in Fig. 1) is quite similar to *L. olivaceoumbrinus* because of olive-green pileus with dry, fibrillose surface and scrobiculate stipe but differs in presence of conspicuous, concentric spots on pileus margin, longer ellipsoid basidiospores (7−9 × 5.5−6.5 µm) and occurrence mostly under oaks (Hesler & Smith, 1979). Purple color reaction of context with KOH is also shown by *L. plumbeus* (Bull.: Fr.) Gray (= *L. turpis*), which is easily separable by dull olivaceous color of basidiome, higher spore ornamentations (up to 1 µm) and thicker (150−200 µm) pileipellis (Heilmann-Clausen et al. 1998).

*Lactarius* sect. *Atroviridi* is well represented in North America followed by European continent (Hesler & Smith 1979; Heilmann-Clausen et al. 1998). However, none of them is reported from the Asian continent so far. Therefore, the report of *L. olivaceoumbrinus* from India is quite significant in establishing the distributional and phylogenetic pattern among the genus *Lactarius*.

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