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A new species of *Lactarius* subg. *Lactarius* from Indian Himalaya with vinaceous brown latex

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ABSTRACT

Forays to Himachal Pradesh followed by morpho-taxonomy and molecular phylogeny of a wild mushroom led to the discovery of a novel species in the genus *Lactarius*. This species is characterized by faintly zonate, viscid, brownish red to vinaceous brown (with a silvery aspect) pileus, greyish red stipe with a darker ring-like zone at the juncture of stipe and lamellae, vinaceous brown or slightly darker latex and occurrence under *Abies* in temperate to subalpine forests. In view of its unique features, the milk cap is being described as a novel species, named as *L. vinaceopurpureus* sp. nov. In this manuscript, detailed morphology, illustrations, nrITS-based molecular phylogeny and a comparison with allied species has been provided.

Keywords: *Morphology, novelty, taxonomy, macrofungi, nrITS, molecular phylogeny*

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INTRODUCTION

Milkcaps are represented by two genera: *Lactarius* Pers. and *Lactifluus* (Pers.) Roussel. The genus *Lactarius* (*Russulaceae, Basidiomycota*) is readily recognized among ectomycorrhizal mushrooms by the presence of latex and amyloid ornamentation of basidiospores (Persoon, 1797). Unlike the genus *Lactifluus*, the genus *Lactarius* shows slimy to viscid, zonate to azonate pileus and absence of thick-walled elements in pileipellis or hymenium (Das, 2023). More than 640 species have been described worldwide (Kalichman *et al.*, 2020; Bera and Das, 2023). The genus *Lactarius* (henceforth abbreviated as "*L.*") is currently divided into three subgenera: *L.* subg. *Lactarius* (Fr. ex J. Kickx f.) Kauffman, *L.* subg. *Russularia* (Fr.) Kauffman, and *L.* subg. *Plinthogalus* (Berk.) Hesler & A.H. Sm. (Hesler & Smith, 1979; Heilmann-Clausen *et al.*, 1998; Buyck *et al.*, 2010; Verbeken and Nuytinck, 2013). The species of *L.* subg. *Lactarius* are characterized by a viscid to glutinous or often shiny pileus, which may be zonate or azonate and sometimes with a hairy margin, a dry or viscid, often scrobiculate stipe, variable latex colour, and a pileipellis of the ixocutis to ixotrichoderm type (Hesler and Smith, 1979; Heilmann-Clausen *et al.*, 1998; Le *et al.*, 2007; Verbeken and Nuytinck, 2013). The *L.* sect. *Deliciosi*, one of the prominent groups in this subgenus, is distinguished by orange, red, brown or bluish basidiocarps exuding latex of similar colour that mainly stain the context greenish on bruising. However, delimiting species within the section remains challenging due to morphological similarity and close phylogenetic relationships (Nuytinck *et al.*, 2007). Furthermore, several taxa are locally recognized but remain undescribed or taxonomically overlooked (Wang *et al.*, 2015; Wisitrassameewong *et al.*, 2015). In Asia, many species previously identified with European and North American taxa have been shown to represent distinct endemic lineages (Nuytinck *et al.*, 2006, 2007; Nuytinck and Ammirati, 2014). Thus, integrative approaches combining molecular phylogenetic and morphological analyses are essential to clarify species limits and intercontinental relationships within *Lactarius* (Nuytinck *et al.*, 2007).

In the rainy season of 2024 and 2025, during the routine macrofungal surveys in the Hatu Peak and adjoining areas of Shimla district, Himachal Pradesh (India), some collections of *L.* sect. *Deliciosi* were made from the temperate to subalpine mixed (broadleaf and coniferous) forests. After a thorough

morphological characterization and molecular phylogenetic estimation, it is revealed that this species of *Lactarius* has never been reported and is proposed herein as a novel species with a detailed morphological description and nrITS-based phylogenetic estimation.

MATERIAL & METHODS

Morphological study

During macrofungal surveys conducted in the Shimla District of Himachal Pradesh in 2024 and 2025 fresh basidiomata (ranging from young to mature) belonging to the genus *Lactarius*. were collected by the authors. Macromorphological features were recorded from fresh specimens, and photographs of both fresh and processed samples were captured using a Canon Power Shot SX 50 HS camera in the field and at the basecamp, respectively. The colours of fresh specimens were determined using the Methuen Handbook of Colour (Kornerup and Wanscher, 1978). Collected samples were properly dried with an aluminium field dryer and later deposited at the Central National Herbarium, Botanical Survey of India (CAL). Micromorphological studies were carried out using free-hand sections prepared from dried specimens. The sections were primarily mounted in 3% KOH and 1% Phloxine; in some cases, a combination of 1% Phloxine and 1% ammoniacal Congo red was used for counterstaining. Thirty basidiospores were examined in Melzer's reagent and measured in profile view, excluding ornamentation. Basidiospore measurements and length/width ratios (Q) are presented as minimum–mean–maximum values. Basidium length does not include sterigmata. Illustrations of micromorphological structures were prepared using a drawing tube attached to an Olympus CX41 light microscope at 1000× magnification. Measurements were taken using CellSens Standard software, which operates with the OLYMPUS BX-53 microscope. Photomicrographs were captured with an OLYMPUS DP-22 camera mounted on the OLYMPUS BX-53 compound microscope. Basidiospores were further examined under a Field Emission Scanning Electron Microscope (FESEM). Dry spores (taken from lamellae) were directly mounted on double-sided adhesive tape affixed to a metallic stub, coated with gold, and scanned in high vacuum mode (30 kV) to study spore ornamentation patterns. SEM observations were conducted using a FEI Quanta FEG 250 model, which is installed at Centre for Research in Nanoscience and Nanotechnology (CRNN), at the University of Calcutta, India. Dried specimens were deposited at Central National Herbarium (CAL), Howrah, India. Herbarium codes used in this study follow Thiers (continuously updated).

DNA extraction, polymerase chain reaction (PCR) and sequencing: Genomic DNA was extracted from 100 mg of dried basidiome using the InstaGene™ Matrix Genomic DNA Isolation Kit, following the manufacturer's protocol. The nrITS gene region was amplified using the primer pairs ITS1-F and ITS4 (White et al., 1990; Gardes and Bruns, 1993). PCR protocol of Das *et al.* (2017) was followed for the amplification of ITS1, 5.8S and ITS2 regions (nrITS). The amplified PCR products were purified using the QIAquick PCR Purification Kit. Both strands of the purified fragments were sequenced on a 3730 xl DNA Analyzer using the same amplification primers. Sequence quality was evaluated with Sequence Scanner Software version 1. Sequence alignment and necessary editing of the obtained sequences were performed in Geneious version 5.1 (Drummond *et al.*, 2010). In total, two sequences were generated from two separate collections of the taxon and subsequently deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/).

Phylogenetic Analysis

The nrITS sequences of the newly described *Lactarius* species and their close relatives were retrieved from nBLAST searches against GenBank (https://www.ncbi.nlm.nih.gov/genbank), and from relevant published phylogenies (Uniyal *et al.*, 2018; Bera *et al.*, 2020; Verma *et al.*, 2021). The nrITS dataset was aligned using the online version of MAFFT v.7 (https://mafft.cbrc.jp/alignment /software/) with the L-INS-i algorithm under the default alignment parameters. The alignment was inspected and manually adjusted using MEGA v.7 (Kumar *et al.*, 2016) to ensure positional homology and to trim ambiguous regions based on conserved motifs.

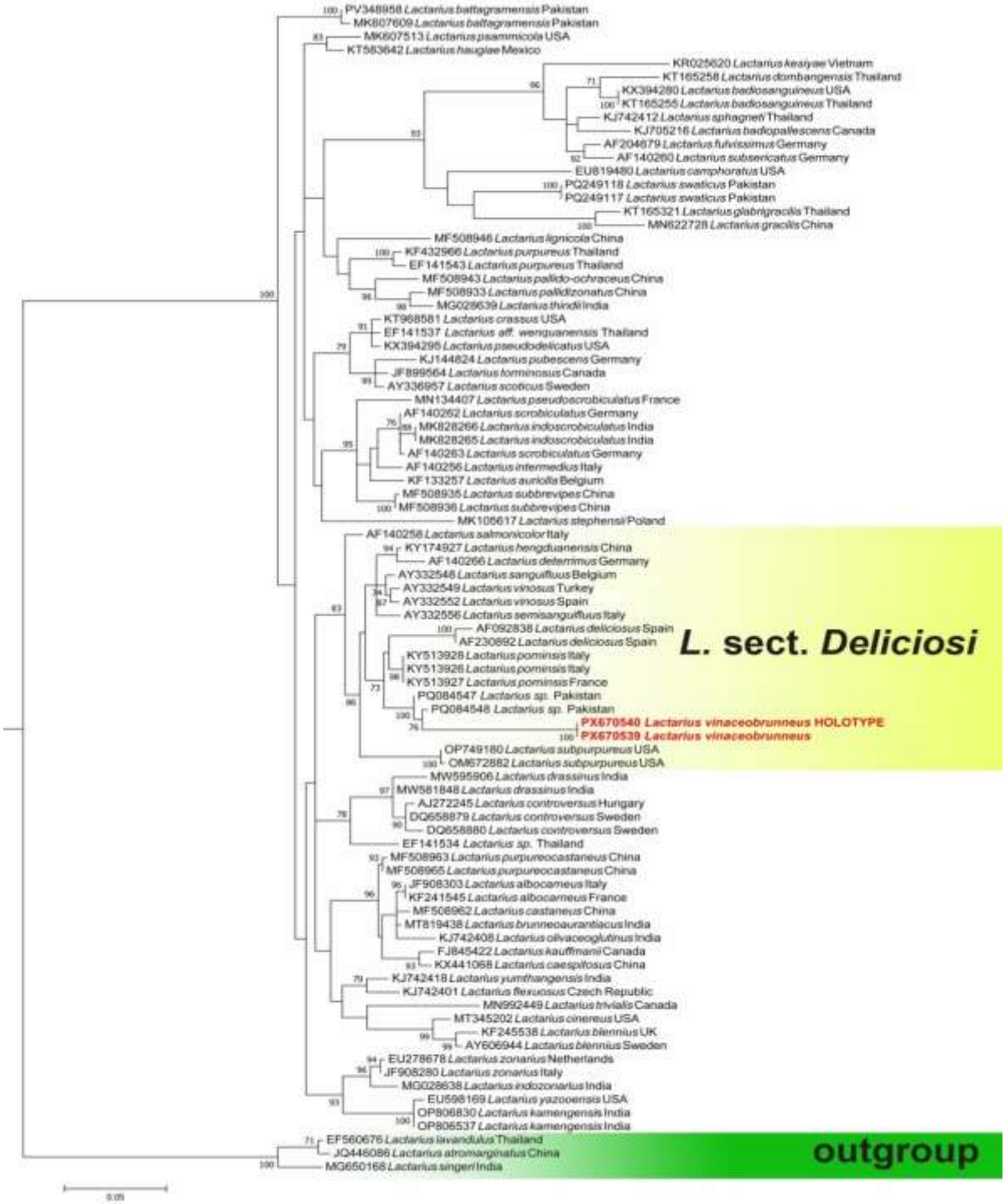


Figure 1. Phylogram generated from nrITS-DNA sequences. The evolutionary history was inferred by using the Maximum Likelihood method in RAxMLGUI version 2.0. Bootstrap support values (>70%) obtained from the ML analysis are shown above or below the branches at nodes. Two sequences (PX670540 & PX670539) derived from two Indian collections of *Lactarius vinaceobrunneus* are shown in bold and red font.

The final nrITS dataset comprised 84 taxa and 735 nucleotide positions, including gaps, with *L. singeri* Uniyal, K. Das & R. P. Bhatt, *L. atromarginatus* Verbeken & E. Horak and *L. lavandulus* H.T. Le and D. Stubbe designated as outgroup taxa (Verma *et al.,* 2021). Among these sites, 225 were distinct patterns**,** 238 were parsimony-informative**,** 72 were singleton sites**,** and 392 were conserved. Phylogenetic inference was performed using the maximum likelihood (ML) method with RAxMLGUI version 2.0 (Edler *et al.*, 2020), employing the GTRGAMMA model. To evaluate nodal support, an ultrafast bootstrap with 1,000 replicates was conducted. Maximum likelihood bootstrap (MLbs)

values ≥70% are displayed in the phylogenetic tree (Figure 1).

RESULTS

Phylogenetic estimation

In our maximum likelihood analysis, the sequences (GenBank accession numbers: PX670540 and PX670539) belonging to *L. vinaceobrunneus* appeared as monophyletic with full support (MLB = 100%) (Fig. 1) and was found to be sister to an unidentified *Lactarius* sp. from Pakistan. These two Asian species, in turn, cluster with another clade bearing two genetically close species: *L. porninsis* and *L. deliciosus.* However, the present species (*L. vinaceobrunneus*) is clearly recovered as a distinct species with moderate to strong support (MLB = 76%).

Taxonomy

Lactarius vinaceobrunneus Su. Datta and K. Das sp. nov. (Figure 2 and Figure 3)

MycoBank No. MB 861551

Genbank No. PX670540 (holotype: ITS) and PX670539 (ITS)



Figure 2. Photo-micrographs of *Lactarius vinaceobrunneus* (CAL 2377, holotype). A–D. Basidiomata; E. Basidia and marginal cells; F. Pleuropseudocystidia; G. Pleuromacrocystidia; H. Cheilomacrocystidia; I. Pileipellis; J. Photo-micrograph showing the basidiospores under compound microscope; K. Basidiospores under SEM. Bars: E–J = 10 µm; K = 2 µm

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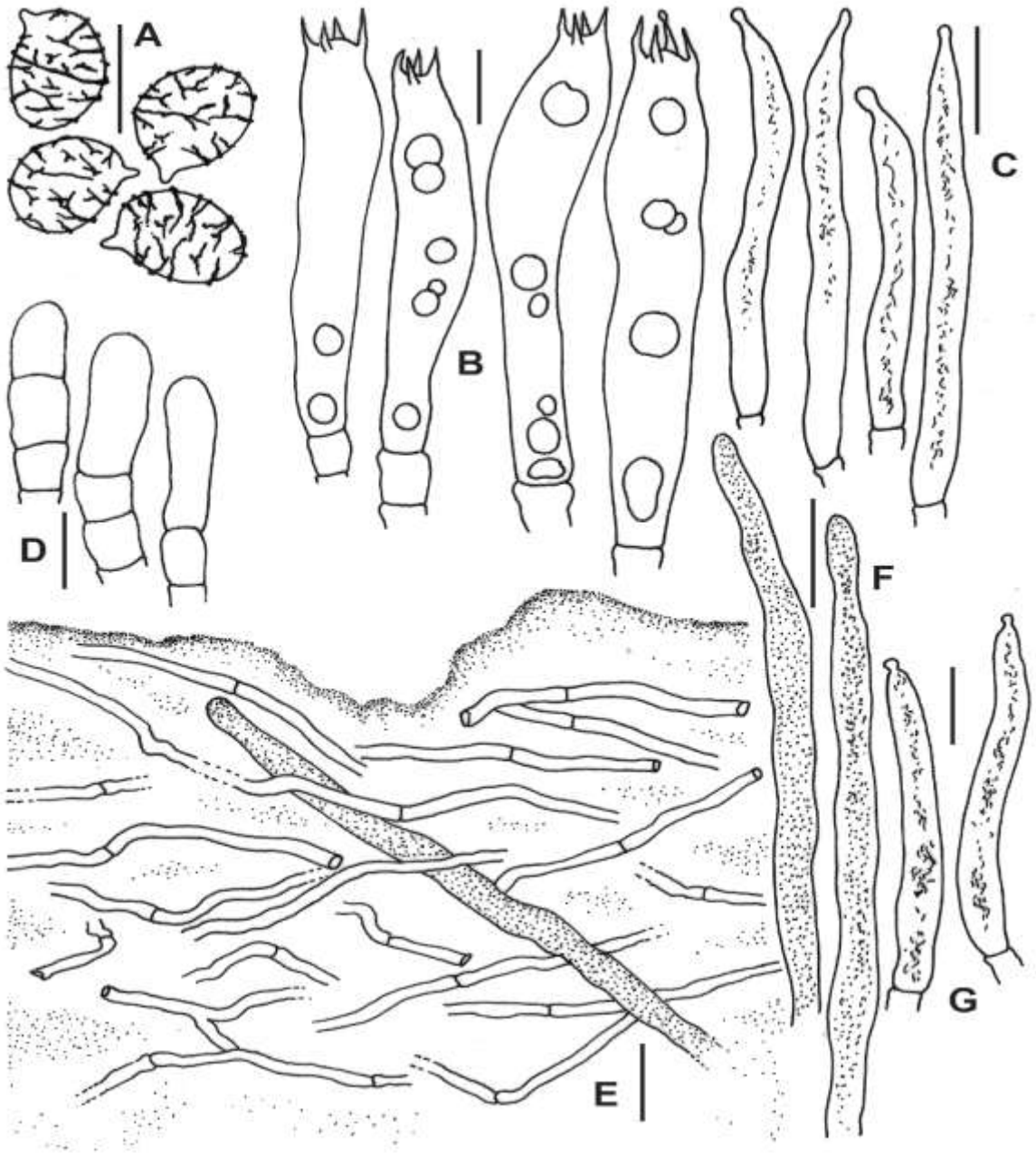


Figure 3: Micromorphological drawings of *Lactarius vinaceobrunneus* (CAL 2377, holotype). A. Basidiospores; B. Basidia; C. Pleuromacrocystidia; D. Marginal cells; E. Cross-section through pileus showing pileipellis interspersed with vesicles; F. Pleuropseudocystidia; G. Cheilomacrocystidia. Bars: A–G = 10 µm.

Diagnosis: *Lactarius vinaceobrunneus* can be separated from closely allied North American species *L. subpurpureus* by vinaceous brown or violet-brown non-scrobiculate pileus with a silvery aspect, distinctively shorter (23–40 mm long) non-scrobiculate stipe, occurrence under *Abies* sp. and nrITS-based sequence data.

Type: India, Himachal Pradesh, Shimla district, between Hatu Peak and Narkanda, 31°15.014′N, 77°29.642′E, 2974 m a.s.l., under *Abies* sp., in subalpine mixed forest, 29 Aug 2024, *leg. Sudeshna Datta & Kanad Das*, SD-KD 24-L002 (holotype! CAL 2377).

Etymology: Refers to the vinaceous brown latex of basidiomata.

Pileus 45–130 mm diam., convex with slightly depressed centre when young, gradually becoming planoconvex with shallow depressed centre; surface moist, smooth, viscid; pilear surface vinaceous brown, reddish brown to violet brown (10–11 E 6–

8), matted fibrillose with a silvery aspect, faintly zonate to azonate, more prominent with maturity, becoming opal to greyish-green (25C–D6) when bruised; surface turning (10C6) with 5% KOH but unchanging with 10% $FeSO_4$; not peeling easily; brittle in consistency; margin inrolled when young, gradually becoming decurved, wavy, interrupted. Lamellae subdecurrent, greyish red to brownish violet (11D5–6), distant to subdistant (3–7/ cm at the pilear margin), rarely forked near stipe; lamellulae in 4 series, edge entire, becoming greyish-green (25E6) when bruised. Stipe 23–40 × 7–15 mm, central to slightly eccentric; cylindrical, slightly narrowing towards the base, smooth to faintly longitudinally fibrillose, greyish red (11D4–5) with a silvery aspect, with a darker or vinaceous brown ring-like zone at the juncture of stipe and lamellae, strigose at the base; becoming dark green (25F4) on handling, turning violet brown (10E6) with KOH but unchanging with $FeSO_4$; basal mycelium yellowish white. Context in pileus thin, white, immediately becoming (11E5–6) on exposure, unchanging with 5% KOH and 5% $FeSO_4$. Context hollow in the stipe, white, immediately changing to (11E 5–6), and unchanging with 5% KOH and $FeSO_4$. Latex moderate, vinaceous brown to brownish red (10D 7–8) or darker. Taste bitter. Odor mild and pleasant. Spore deposit could not be obtained.

Basidiospores 6.3–8.42–10.4 × 4.5–6.41–8.1 µm, (n = 30, Q = 1.11–1.33–1.70), usually globose to broadly ellipsoid; ornamentation amyloid, up to 0.5 µm high, composed of short and long ridges and warts aligned or partly connected with connectors forming an incomplete reticulum; suprahilar spot inamyloid. Basidia 41.8–59.4 × 8.1–10.4 µm, clavate to subclavate, 4-spored; sterigmata 2.7–5.8 × 0.5–1.5 µm. Pleuromacrocystidia 29.7–50.4 × 2.3–6.3 µm, emergent up to 15.8 µm, cylindric to subcylindric with subfusoid, mucronate to subcapitate apices, thin-walled; content fibrous. Pleuropseudocystidia abundant, 3.2–4.5 µm wide, emergent up to 10 µm, cylindrical, unbranched, yellow in KOH, content granular to crystalline, tortuous at base. Lamellae edges fertile with basidia, basidioles, marginal cells and cystidia. Cheilomacrocystidia similar to pleuromacrocystidia. Marginal cells 25.7–28.8 × 4.5–6.7 µm, 1–2 septate, subcylindric to subclavate, hyaline. Subhymenium up to 13.2 µm thick, cellular. Hymenophoral trama composed of lactifers and few nests of sphaerocytes connected with connecting hyphae. Pileipellis up to 120 µm thick, forming an ixocutis, composed of loose thin-walled, repent, septate, branched, sometimes shrivelled hyphae (1.5–2.5 µm wide) and few lactifers. Stipitipellis an ixocutis, stipe-trama composed of loose septate hyphae of 1.0–2 µm wide, with rosettes of sphaerocytes connected with connecting hyphae. Clamp connections absent in all tissues.

Additional specimen examined: INDIA: Himachal Pradesh: Shimla District, between Hatu Peak and Narkanda, 31°14.628’ N, 077°30.345’ E, Alt. 3547 m a.s.l., 10 Aug. 2025, *leg. Sudeshna Datta & Kanad Das*, SD-KD 25-016 (CAL 2378).

Remarks: *Lactarius vinaceobrunneus* is characterized by a vinaceous brown or violet-brown, faintly zonate to azonate, viscid pileus with a silvery aspect, vinaceous brown to brownish red latex, greyish red stipe with a darker ring-like zone, and the occurrence under *Abies* in temperate to subalpine forests. The combination of these morphological features undoubtedly place it within *L.* section *Deliciosi*. It is referred to as “Khunio Chiaun” by the local communities or forest dwellers and frequently consumed as a delicacy.

The present species bears close resemblance to the European *Lactarius vinosus* (Quél.) Bataille, which exudes deep wine-red (11E/F5) latex that dries to brownish (8E8). However, *L. vinosus* differs in having a vinaceous-red (7D6), distinctly zonate pileus and a brownish red to wine-red, scrobiculate stipe (Nuytinck and Verbeken, 2005). Another closely related North American taxon, *L. subpurpureus* Peck, is similar in both macromorphology and latex coloration but differs in its pinkish to vinaceous buff, zonate to scrobiculate pileus, and moderately longer (30–80 mm), often scrobiculate stipe, sterile lamellae edge and much longer marginal cells (cheiloleptocystidia) (12.5–18 × 4–6 µm) and being ectomycorrhizal with *Tsuga* (Hesler and Smith, 1979; Nuytinck *et al.*, 2006). This species (*L. subpurpureus*) has also been reported from Kumaon Himalayas in India (Das and Sharma 2005) which closely resembles the present novel species in morphology as well as the ectomycorrhizal association with *Abeis,* however, the identity of this species was not confirmed at that time with molecular data. Hence the intercontinental conspecificity could not be confirmed. Phylogenetically, the present species (*L. vinaceobrunneus*) is closely allied to *L. porninsis* Rolland (originally reported from Europe), yet the latter can be distinguished morphologically by its distinctly zonate, bright orange to brown-orange pileus and a creamy buff to ochraceous buff,

indistinctly scrobiculate stipe, white unchanging latex and association with *Larix* (Nuytinck and Verbeken, 2005). This Indian species is also genetically closer to European *L. deliciosus* (L.) Gray but the latter can easily be separated from the present novel species by the orange to salmon orange pileus, orange with deeper orange scrobicules and bright orange (5/6A8) latex that slowly changes to red (Nuytinck and Verbeken 2005).

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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