

Morphological description and phylogenetic estimation of *Lactarius abieticola* (Russulaceae), a new record for Indian mycobiota

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भारतीय कवकजात के लिये नवीन अभिलेख *लेक्टैरियस अबेटिकोला* (रुसुलेसी) की बाह्याकारिकी एवं फायलोजेनेटिक विवेचना

इशिका बेरा, कनद दास एवं अनिकेत घोष

सारांश

प्रस्तुत शोध पत्र भारतीय हिमालय के सिक्किम एवं अरुणाचल प्रदेश से संग्रहित *लेक्टैरियस अबेटिकोला* (लि. सेक्ट. *डेलिकियोसी*) को प्रदर्शित किया गया है। इसके प्रमुख लक्षण एबिस वृक्ष की तली में उगने वाले मध्यम आकार के नारंगी रंग के बेसिडियोमाटा हल्के हरे रंग और गेंहूये रंग के छत्रक हैं। इसका विस्तृत आकारिकी वर्णन, चित्रण एवं एनआरआईटीएस-आधारित फायलोजेनेटिक विवेचना की गई है। यह जाति भारतीय कवकजात में एक नवीन अभिलेख है।

ABSTRACT

The present paper describes *Lactarius abieticola* (L. sect. *Deliciosi*) from Sikkim and Arunachal Pradesh of Indian Himalayas. It is characterized as a medium sized orange basidiomata growing under *Abies* spp. with faint greenish coloration and whitish aspect on the pileus. Detailed morphological descriptions, illustrations and nrITS-based phylogenetic estimation of the species are presented. This species is a new record to Indian mycobiota.

Keywords: Arunachal Pradesh, Morphology, nrITS, Phylogenetic inference, Russulales, Sikkim

INTRODUCTION

The major identifying character of the members of *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh is: latex color ranging from orange to yellow to vinaceous red, brown, indigo blue but changing to greenish on exposure either immediately or after few minutes due to some enzymatic reactions (Hesler & Smith, 1979; Heilmann-Clausen & al., 1998; Nyutinck & al., 2017) although there are two exceptions which are featured with unchanging white

latex: *L. porinsis* Rolland (Nyutinck & Verbeken, 2007) and *L. splendens* Hesler & A. H. Sm. (Nyutinck & al., 2017). Others characters in this group like zonate pileus and scrobiculate stipe make these species easily identifiable in the field. But lesser macromorphological variation makes their identification very difficult. Microscopically, the size of the basidiospores, the ornamentation and the abundance of the pleuro- and cheilomacrocystidia can be helpful in some cases but their vast variability is not reliable in a broader sense. This popular group has been

reported across the world from countries of North and Central America, Europe and Asia. Previously, 74 names have been published from around the world. But the mycologists are using the European and North American names to identify the Central American and Asian species only on basis of the morphological resemblance. *L. deliciosus* (L.: Fr.) Gray, the type species of this section, *L. salmonicolor* R. Heim & Leclair and *L. deterrimus* Gröger are the European names used commonly for all the look-alike Asian species (Nyutinck & al., 2007). But, now it is evident that combination of morphology and phylogeny clearly demarcates the species within this group. After phylogenetic analyses based on ITS and partial glyceraldehyde-3-phosphate dehydrogenase gene sequences along with the morphological study, nineteen names have been published so far; thirteen species (*L. abieticola* X.H. Wang, *L. akahatsu* Tanaka, *L. deliciosus* (L.: Fr.) Gray, *L. hatsudake* Tanaka, *L. hengduanensis* X.H. Wang, *L. horakii* Nyutinck & Verbeken, *L. laticolor* (S. Imai) Imazeki ex Hongo, *L. pseudohatsudake* X.H. Wang, *L. subindigo* Verbeken & E. Horak, *L. thakalorum* Bills & Cotter, *L. vividus* X.H. Wang, Nyutinck & Verbeken and two unidentified species), three varieties, one form and two invalid names from Asia (Nyutinck & al., 2006; Wang & al., 2015; Wang 2016). From India, six species i.e. *L. deliciosus* (L.) Gray, *L. deterrimus* Gröger, *L. fennoscandicus* Verbeken & Vesterh., *L. indigo* (Schwein.) Fr., *L. sanguifluus* (Paulet) Fr. and *L. subindigo* Verbeken & E. Horak are reported in this group but some are identified without the support of molecular data and thus questioning its conspecificity and identity.

During the routine macrofungal surveys in the subalpine-alpine forests of Sikkim and Arunachal Pradesh, India, some collections of *L. sect. Deliciosi* were made by two of us (KD and IB). After a thorough morphological characterization (macro- and micromorphology) coupled with nrITS-based phylogenetic estimation revealed these taxa as *L. abieticola* which has never been reported from India. In this communication, *L. abieticola* is reported for the first time from India with a detailed morphological description and ITS-based phylogenetic estimation.

MATERIAL AND METHODS

Morphological study

Macromorphological characters were recorded in the forest and in base-camp from the collected and dissected young to mature basidiomata. Images of the fresh basidiomata were captured with Canon PowerShot SX 220 HS and Nikon SLR D3400. Color codes and terms used here are mostly after Methuen Handbook of Color (Kornerup & Wanscher, 1978). Micromorphological characters were observed with a compound microscope (OLYMPUS CX-41). Free hand sections from dried

specimens were mounted in a mixture of 5% potassium hydroxide (KOH), 1% Phloxine and 1% Congo red or in distilled water. Micromorphological drawings were prepared with a drawing tube (attached to the OLYMPUS CX-41) at 1000× magnification. All measurements were taken with the help of CellSens Standard software, dedicated to OLYMPUS BX-53. The basidium length excludes sterigmata. Basidiospore measurements were recorded in profile view from 40 basidiospores. Spore measurements and length/width ratios (Q) are recorded here as: minimum–mean–maximum. Herbarium codes follow Thiers 2020 (continuously updated). Microphotography is made with the help of a dedicated camera OLYMPUS DP-22 attached with compound microscope OLYMPUS BX-53.

DNA extraction, PCR amplification and sequencing

Genomic DNA was isolated from dry herbarium specimens (10–50 mg) using the Fungal gDNA Mini Kit (RGCB, RFDE, Thiruvananthapuram). PCR protocol for the amplification of ITS1, 5.8S and ITS2 regions (nrITS) followed those in Das & al., (2017). PCR products were duly purified using a QIAquick Gel Extraction Kit (QIAGEN, Germany) and subjected to automated DNA sequencing on an ABI3730xl DNA Analyzer (Applied Biosystems, USA) adding the same primers used earlier for amplification. The final consensus sequences were deposited at GenBank to procure the accession numbers: MT986024 and MT986032 for the ITS sequence of *Lactarius abieticola*.

Phylogenetic analysis

Phylogenetic analysis based on nrITS sequences data were carried out to establish the phylogenetic placement of our isolated taxa. The nrITS sequences of Indian *Lactarius abieticola* plus similar ones acquired from Blast search (Altschul & al., 1997) against GenBank (Clark & al., 2016) and relevant published phylogenies (Wang 2016) were aligned with the help of ClustalX 2.1 (Larkin & al., 2007) using default settings. Aligned sequences were trimmed manually with MEGA7 (Kumar & al., 2016). To eliminate ambiguously aligned positions in the alignment as objectively as possible, the on-line program Gblocks ver. 0.91b (Talavera & Castresana, 2007) was used. The program was run with settings allowing for smaller blocks, gaps within these blocks and less strict flanking positions. Final aligned sequences were edited manually with the help of BioEdit 7.2.5 (Hall 1999). Sequences of ITS were phylogenetically analyzed using Maximum Likelihood (ML). Maximum Likelihood (ML) was computed in raxmlGUI 2.0 (Edler & al., 2019). One-thousand (1000) bootstrap replicates were analyzed to obtain nodal support values in case of ML analysis. We considered that clades with the bootstrap values exceeding 50% as well-supported.

RESULTS

Phylogenetic inferences

The final dataset consists of 39 nrITS sequences including our consensus sequences derived from Indian isolates. The final alignment comprised 649 characters including gaps. Phylogenetic tree (showing Indian collections in red and bold font) is presented in Fig. 1. Sequences derived from Indian collections (MT986024 and MT986032) are nested within the *L. abieticola* clade (indicated with a blue arrow) consisting of all the Chinese collections (representing KY174947, KY174935, KY174941, NR 152988, KY174949, KY174948, KY174938, KY174939 GenBank numbers) suggesting its strong similarity or conspecificity with the Asian species: *L. abieticola*.

TAXONOMY

Lactarius abieticola X.H. Wang, Cryptog. Mycol. 37(4): 497 (2016) (Fig. 1–3)

Pileus 30–65 mm diam., hemispheric to convex with slightly depressed centre when young, gradually becoming planoconvex to plano-applanate with wider and shallow depressed centre, faintly zonate; surface moist, smooth, greasy; pilear surface light orange to orange (5A5–7) with a whitish aspect, with orange (6B6) spots forming broad, concentric zones, becoming greyish green (26E5) when bruised; not peeling easily; brittle in consistency; margin inrolled when young, gradually becomes decurved, entire. *Lamellae* decurrent to subdecurrent, close (10 L+1/cm at pilear margin); rarely forked at the juncture of the stipe, lamellulae in 5 series, pale orange to light orange (5A4–5), edge entire. *Stipe* 55–60 × 10–15 mm, central; cylindrical with broader base, smooth to faintly pitted, concolorous to pileus. *Context* moderately thick at pileus, hollow in stipe, pale orange (5A4), unchanged with 3% KOH and FeSO₄, becoming brownish with guaiac. *Latex* scarce, slightly bitter, bright orange turning exposed gills greyish green (26E5) after few minutes. *Taste* bitter. *Odor* mild pleasant. *Spore print* chalky white (1A1–2).

Basidiospores 7.5–8.6–9.5 × 6.3–7.1–8.0 µm, (n = 40, Q = 1.03–1.16–1.30), usually globose to broadly ellipsoid; ornamentation amyloid, up to 0.6–1.1 µm high, composed of short and long ridges and warts partly connected with connectors in a more or less zebroid pattern; suprahilar spot inamyloid. *Basidia* 47.7–63.2 × 9.8–12 µm, clavate to subclavate, 4-spored; sterigmata 2.3–5.4 × 1.1–2.5 µm. *Pleuromacrocytidia* scarce to abundant, 44.3–85.8 × 5.1–8.5 µm, emergent up to 47 µm, cylindric to subcylindric with fusoid, subfusoid, mucronate, subcapitate to appendiculated apices, thin-walled; content dense, granular to crystalline. *Pleuropseudocystidia* abundant, 2.8–4.5 wide, emergent up to 10 µm, cylindrical, sometimes branched, tortuous

at base. *Lamellae* edge fertile. *Cheilomacrocytidia* absent to common, 50–74.3 × 4.7–6 µm, emergent up to 35 µm, cylindric to subcylindric with fusoid, subfusoid, mucronate, subcapitate to appendiculated apices, thin-walled; content dense, granular to crystalline. *Subhymenium* up to 20 µm thick, cellular. *Hymenophoral trama* composed of lactifers and few nests of sphaerocytes connected with connecting hyphae. *Pileipellis* up to 150 µm thick, an ixocutis, composed of loose thin-walled, repent, septate, branched, sometimes shrivelled hyphae (1.6–3.1 µm wide) and few lactifers. *Stipitipellis* up to 47 µm thick, an ixocutis, composed of loose septate hyphae of 1.5–3 µm wide. *Clamp connections* absent in all tissues.

Habitat/distribution: Growing in scattered on soil in association with *Abies* sp. in subalpine Himalaya.

Specimens examined: India, Sikkim, Yumthang, 27°47.723' N, 88°42.338' E, 3619 m a.s.l., 28 July 2019, K. Das, KD 19-005 (CAL 1823), Arunachal Pradesh, Ani Gompa, Tawang district, 27°37.964' N, 091°50.267' E, 3533 m a.s.l., 18 Aug 2018, I. Bera, IB 18-010 (CAL 1824).

DISCUSSION

The combination of macro- and micromorphological characters such as medium sized orange colored basidiomata (pileus 30–65 mm diam, stipe 55–60 × 10–15 mm) with faint greenish coloration on bruising, the white aspect on pileus, a faintly zonate cap, orange unchanging latex, ixocutis nature of pileipellis and stipitipellis and its occurrence under *Abies* sp. undoubtedly, identifies the present species as *L. abieticola*. Morphologically, the Indian specimens are mostly in conformity with that of the holotype described from China (Wang 2016). However, Indian materials show rather smooth to faintly pitted stipe, close lamellae, globose to broadly ellipsoid basidiospores, longer cheilomacrocytidia (50–74.3 × 4.7–6 µm). Also, little variabilities in the micromorphological characters in two different collections from Sikkim and Arunachal Pradesh have been observed. Cheilomacrocytidia is totally absent in IB 18-010 (collection of Arunachal Pradesh) but found in KD 19-005 (Collection of Sikkim). Likewise, the pleuropseudocystidia is frequently branched in KD 19-005 but not in IB 18-010.

The European *L. salmonicolor* R. Heim & Leclair, North American *L. thyinos* A.H. Sm. and Asian *L. laeticolor* (S. Imai) Imazeki ex Hongo are quite similar in morphologically with *L. abieticola*. The orange viscid basidiomata with rare greenish coloration, the almost smooth stipe surface and the association with *Abies* sp. can confuse *L. abieticola* with the former three species in the field but the latter differs by the whitish aspect on the pileus and a much smaller basidiomata (Hesler & Smith, 1979; Heilmann-Clausen & al., 1998; Wang 2016).

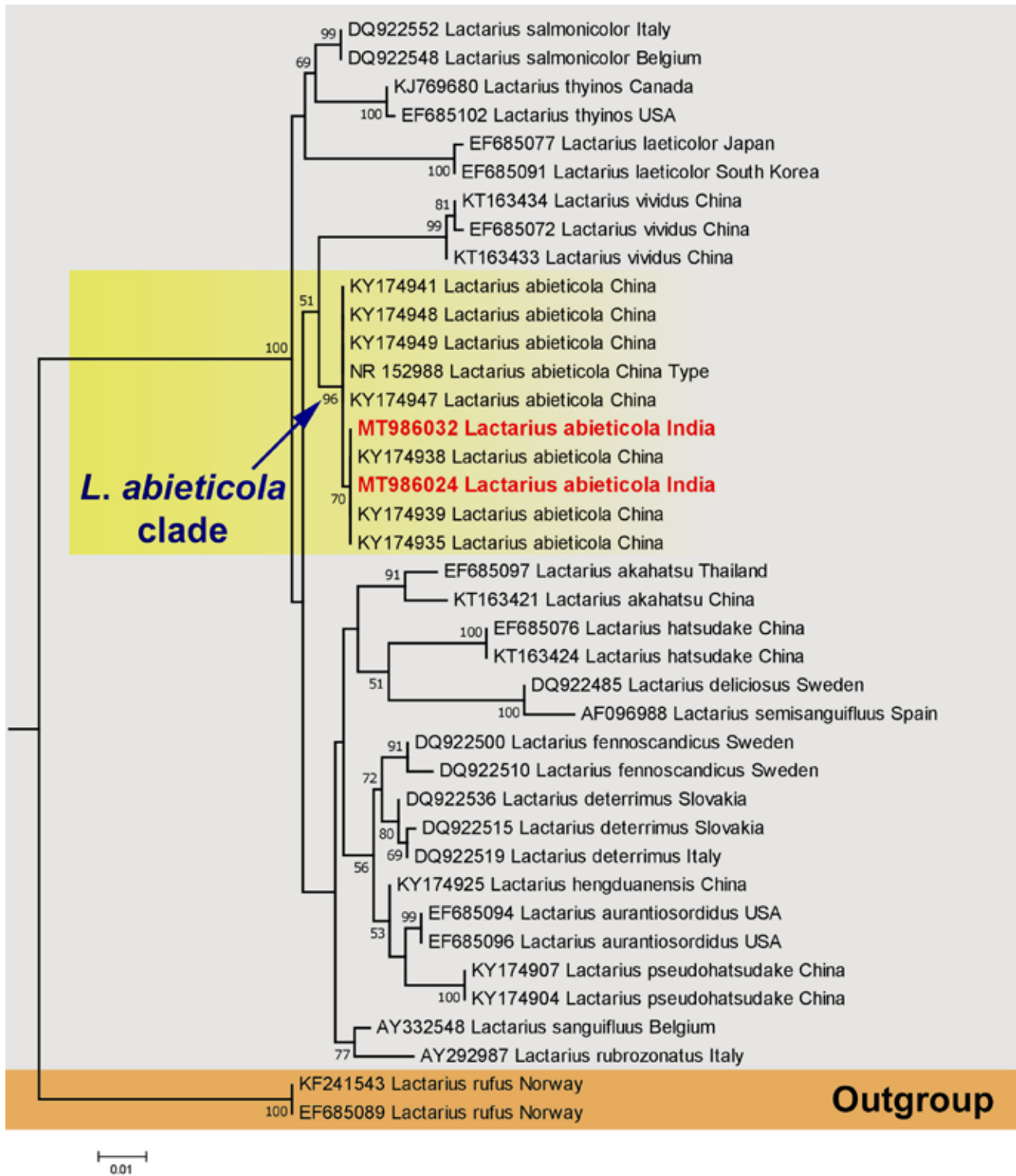


Fig.1. Phylogram generated from ITS-rDNA sequences: The evolutionary history was inferred by using the Maximum Likelihood method in raxmlGUI 2.0 beta. Bootstrap support values (>50%) obtained from the ML analysis are shown above or below the branches at nodes.

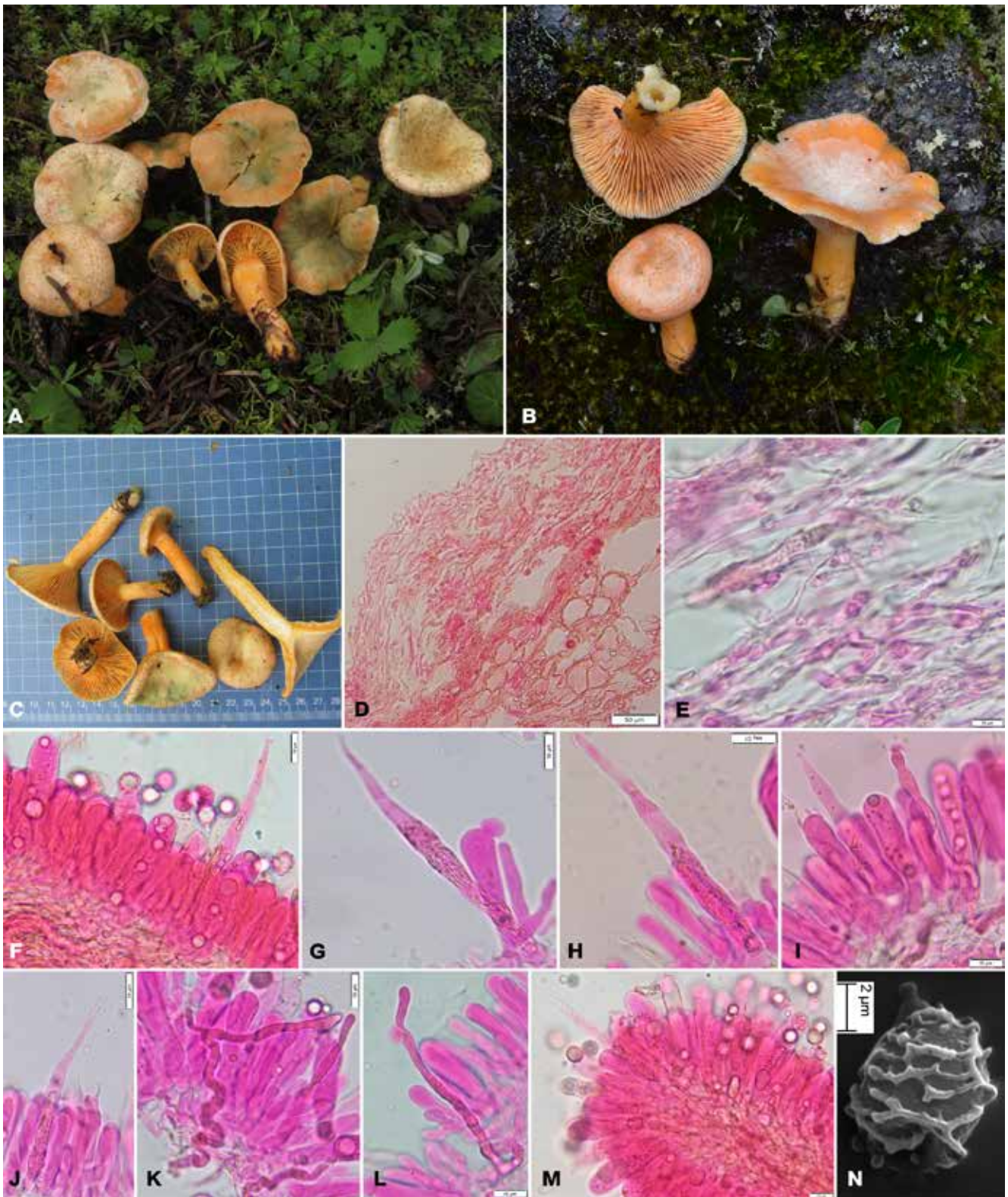


Fig.2. *Lactarius abieticola* (KD 19-005 and IB 18-010). A-C. Fresh basidiomata in the field and basecamp. D & E. Transverse section through pileipellis. F-J. Pleuromacrocystidia. K & L. Pleuropseudocystidia. M. Cheilomacrocystidia. N Basidiospore. Scale bars: d=50 µm, e-m= 10 µm, n=2 µm.

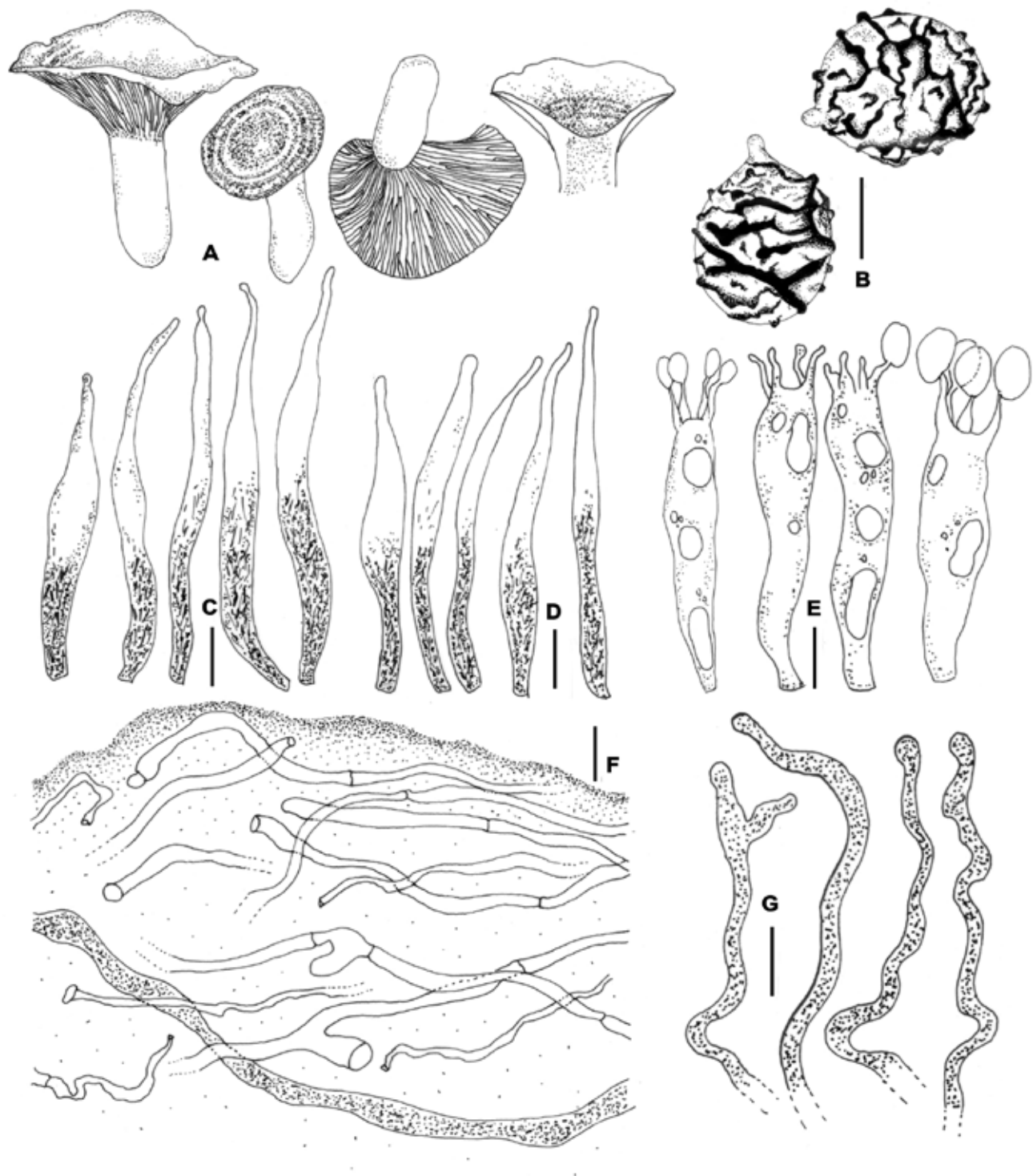


Fig.3. Line drawings of *Lactarius abieticola* (KD 19-005 and IB 18-010). A. Fresh basidiomata in the field and basecamp. B. Basidiospores. C. Pleuromacrocystidia. D. Cheilomacrocystidia. E. Basidia. F. Pileipellis. G. Pleuropseudocystidia. Scale bars: B = 5 μ m; C–G = 10 μ m.

Another Asian species, *L. vividus* X.H. Wang, Nuytinck & Verbeken almost resemble *L. abieticola* but presence of often greenish patches on pileus, subdistant-distant lamellae [close (10 L+l/cm in *L. abieticola*)] and exclusive association with *Pinus taiwanensi* of the former clearly distinguishes it from *L. abieticola* (Wang & al., 2015).

Finally, based on authors' vast field experiences in Indian Himalaya, herbarium consultation and literature studies, it can be concluded that all the Himalayan collections of *L. sect. Deliciosi* that are found under *Abies* spp. and inadvertently identified earlier (Saini & Atri, 1982; 1984; Das & Sharma, 2005) as *L. deliciosus* are none other than *L. abieticola*, the species in discussion.

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