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# Two new species of genus *Lactarius* Pers. (Russulaceae) from Arunachal Pradesh, India

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**ABSTRACT**
*Lactarius* Pers. is one of the most frequently encountered ectomycorrhizal genera of the family Russulaceae. Because of its cosmopolitan nature, several taxa belonging to its three subgenera, *Lactarius* subg. *Lactarius* (Fr. ex J.Kickx f.) Kauffman, *L.* subg. *Russularia* (Fr.) Kauffman, and *L.* subg. *Plinthogalus* (Berk.) Hesler & A.H.Sm., have been documented worldwide portraying its diversity. Routine macrofungal explorations concentrating on *Lactarius* in two districts of Arunachal Pradesh (West Kameng and Tawang) have accounted for many species in temperate to subalpine forests. Recent observations have revealed two interesting species belonging to subgenera *Lactarius* and *Russularia*. Morphological features along with nrITS-LSU based phylogenetic analysis inferred these two species as new to science. *Lactarius kamengensis* sp. nov. (subg. *Lactarius*) is characterized as a medium-sized *Lactarius* with bitter to acrid taste, pale yellowish, vaguely zonate pileus, scrobiculate stipe, white latex turning pale yellowish and occurrence under *Castanopsis* (D.Don) Spach. Whereas, *Lactarius madhuriensis* sp. nov. is a unique high-altitude species belonging to subg. *Russularia* with brownish orange coloured, umbonate pileus, and watery white latex that turns yellowish.

**KEY WORDS**
India,
Basidiomycota,
Russulales,
Russulaceae,
new species.

**RÉSUMÉ**

*Deux nouvelles espèces du genre* Lactarius *Pers. (Russulaceae) de l'Arunachal Pradesh, Inde.*

*Lactarius* Pers. est l'un des genres ectomycorhiziens les plus fréquemment rencontrés dans la famille des Russulaceae. En raison de sa nature cosmopolite, plusieurs taxons appartenant à ses trois sous-genres, *Lactarius* subg. *Lactarius* (Fr. ex J.Kickx f.) Kauffman, *L.* subg. *Russularia* (Fr.) Kauffman, et *L.* subg. *Plinthogalus* (Berk.) Hesler & A.H.Sm., ont été documentés dans le monde entier, ce qui illustre sa diversité. Des explorations macrofongiques de routine se concentrant sur *Lactarius* dans deux districts de l'Arunachal Pradesh (West Kameng et Tawang) ont permis de recenser de nombreuses espèces dans les forêts tempérées à subalpines. Des observations récentes ont révélé deux espèces intéressantes appartenant aux sous-genres *Lactarius* et *Russularia*. Les caractéristiques morphologiques ainsi que l'analyse phylogénétique basée sur les nrITS-LSU ont permis de conclure que ces deux espèces sont nouvelles pour la science. *Lactarius kamengensis* sp. nov. (subg. *Lactarius*) est caractérisé comme un *Lactarius* de taille moyenne avec un goût amer à âcre, un pileus jaunâtre pâle, vaguement zoné, un stipe scrobiculé, un latex blanc tournant jaune pâle et une occurrence sous *Castanopsis* (D.Don) Spach. Alors que *Lactarius madhuriensis* sp. nov. est une espèce unique de haute altitude appartenant au sous-groupe *Russularia* avec un pileus omboné de couleur orange brunâtre et un latex blanc aqueux qui tourne jaunâtre.

**MOTS CLÉS**
Inde,
basidiomycètes,
Russulales,
Russulaceae,
espèces nouvelles.

## INTRODUCTION

The presence of latex and amyloid ornamentation of the basidiospores make the genus *Lactarius* Pers. quite identifiable amongst the other ectomycorrhizal mushrooms (Persoon 1797). The knowledge of this genus has found its prominence after its extensive study worldwide accounting for approximately 640 species recorded to date (Kalichman *et al.* 2020). Based on the various pileus textures and the consequent reflection in the microscopic pileipellis nature, genus *Lactarius* is presently classified 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 & Nuytinck 2013). Over the years, macrofungal forays have been quite fragmentary in the subtropical forests, however, expeditions in the subalpine habitats dominated by the ectomycorrhizal host tree species of *Abies* Mill., *Pinus* L., *Betula* L., *Salix* L. and *Alnus* Mill. have revealed high diversity in *Lactarius* globally (Hesler & Smith 1979; Heilmann-Clausen *et al.* 1998; Basso 1999; Basso *et al.* 2001; Wang 2007; Das *et al.* 2015; Uniyal *et al.* 2018; Verma *et al.* 2021, 2022).

The state of Arunachal Pradesh, often known as the "Land of the Dawn-lit-Mountains" is located at the extreme tip of the north-eastern part of India at the foothills of the Himalayas. The dominant climate of the state is a humid monsoon-based subtropical climate, but the higher elevated lands experience much cold temperatures with frequent snowfalls. However, the average temperature ranges from 15° to 21°C with an average rainfall of 300 cm (Mizuno & Tenpa 2015). The months of July to September witness heavy rainfall in Arunachal Pradesh (Sharma & Shukla 1992). With such suitable climatic conditions, Arunachal Pradesh has flourished its vegetation with many ectomycorrhizal host trees at altitudes between 1800 and 4000 m. The luxuriant and endemic flora makes Arunachal one of the 18 "Biodiversity Hot Spots" in the world. The Tawang district situated in the westernmost part of the state has subalpine to alpine vegetation with forests dominated by species of *Abies*, *Betula*, and *Rhododendron* L. On routine macrofungal forays, the commonly encountered *Lactarius* species mostly belong to *L.* subg. *Lactarius* from the subalpine forests of Tawang, reflecting its diversity. However, one unique species of *Lactarius* from subg. *Russularia* has been recently discovered alongside from another district in Arunachal Pradesh, West Kameng. In this current study, we propose two novel species, *Lactarius kamengensis* sp. nov. (*L.* subg. *Lactarius*) and *L. madhuriensis* sp. nov. (*L.* subg. *Russularia*).

## MATERIAL AND METHODS

### Morphological study

Macromorphological characters from immature to mature basidiomata were recorded in the forest and at basecamp. Images of the fresh basidiomata were captured with a Nikon SLR D3400. Colour codes and terms are mostly after the *Methuen Handbook of Colour* (Kornerup & Wanscher 1978). Chemical reaction tests with 5% Ferrous sulphate ($FeSO_4$), Guaiac, and 5% Potassium hydroxide (KOH) were done on the context of the specimens to observe any colour changes. All micromorphological characters (basidia, hymenial cystidia, elements of pileipellis, and basidiospores) were observed with an OLYMPUS CX-41 compound microscope. Free-hand sections from dried specimens were mounted in 1% ammoniacal Congo Red after a short treatment with KOH. To visualize the contents of the cystidia more clearly, one drop of 1% Phloxine was also added to it. Micromorphological drawings were prepared with a drawing tube attached to the microscope at 1000× magnification. All measurements were taken with the help of CellSens Standard software dedicated to an OLYMPUS BX-53. The basidium length excludes sterigmata. Basidiospores were examined in Melzer's reagent and measured in side view, excluding ornamentations.

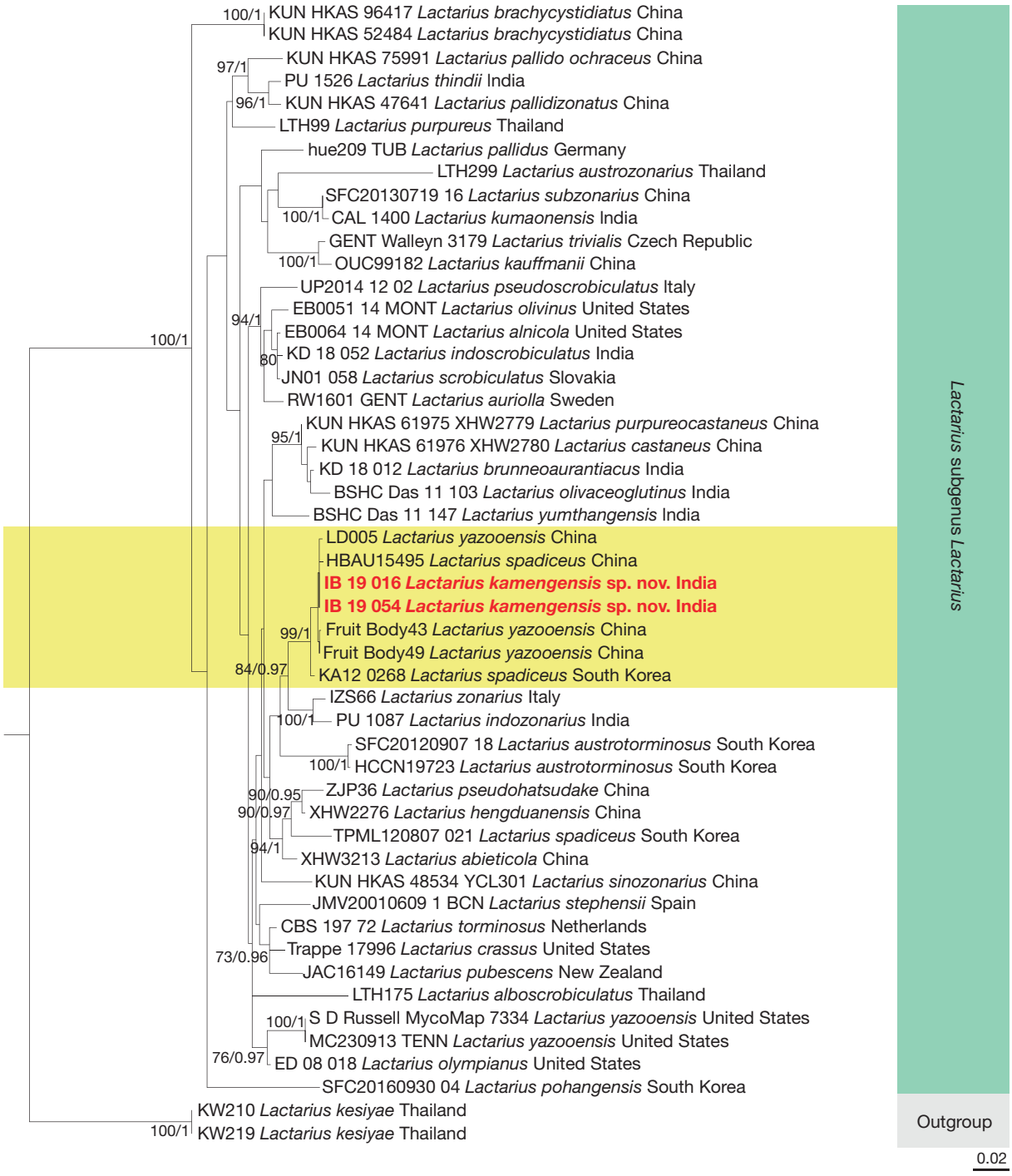


FIG. 1. — Phylogram inferred from Maximum Likelihood (ML) analysis by raxmlGUI 2.0 and Bayesian Inference by Mr.Bayes v.3.2.6 based on nrITS and nrLSU sequence data. Sequences derived from the novel Indian species are presented in **red** and **bold** and the respective clade is highlighted in **yellow**. Maximum Likelihood bootstrap support values (MLbs) ≥ 70% are shown on the left of "/" and Bayesian Posterior Probabilities (BPP) ≥ 0.95 are shown on the right or below of "/" at nodes.

Thirty basidiospore measurements were recorded. Basidiospore measurements and length/width ratios (Q) are recorded here as minimum-mean-maximum. Thirty measurements for each of the other micromorphological characters (basidia, hymenial cystidia, and elements of pileipellis) were also recorded to have a range. Photomicrographs were taken with a camera attached to the compound microscope.

### DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING

Genomic DNA was isolated from dry herbarium specimens (10-50 mg) using the Fungal gDNA Mini Kit (RGCB, RFDF, Thiruvananthapuram). The primers ITS1-F and ITS4, LR0R, and LR5 were used for the amplification (White *et al.* 1990; Matheny 2005). Polymerase Chain Reaction (PCR) was carried out in a PCR thermal cycler (Gene Amp PCR System 9700,

Applied Biosystems) programmed for two minutes at 96°C, followed by 30 cycles of 30 seconds at 96°C, 40 seconds at 50°C, two minutes at 72°C, and a final seven minutes extension step at 72°C. The PCR products were purified using a QIAquick Gel Extraction Kit and subjected to automated DNA sequencing on an ABI3730xl DNA Analyzer adding the same primers used earlier for amplification. The final consensus sequences were deposited at GenBank (https://www.ncbi.nlm.nih.gov/genbank/) to procure the accession numbers nrITS (OP806537, OP806830), nrLSU (OP811192, OP811193) for *Lactarius kamengensis* sp. nov. and nrITS (OP808232, OP808233), nrLSU (OP811194, OP811195) for *Lactarius madhuriensis* sp. nov.

### Phylogenetic analysis

Phylogenetic analyses based on nrITS and nrLSU sequences data were carried out to determine the phylogenetic placement of these two new taxa. The close relatives of the new taxa, and the outgroup species (*L. kesiyae* Verbeken. & K.Hyde in Figure 1, and *L. hatsudake* Nobuj.Tanaka and *L. torminosus* (Schaeff.) Gray in Figure 2) were acquired from the Blast search (Altschul *et al.* 1997) in GenBank (Clark *et al.* 2016) and relevant published phylogenies (Wisitrassameewong *et al.* 2016; Uniyal *et al.* 2018; Lee *et al.* 2019). Two datasets (nrITS and nrLSU) were created separately and were aligned using the online version of the multiple sequence alignment program MAFFT v7 (Katoh *et al.* 2019) with settings L-INS-i. The alignment was manually edited with MEGA7 (Kumar *et al.* 2016). Gblocks ver.0.91b (Talavera & Castresana 2007) was used to eliminate poorly aligned positions in the alignment. The program settings allowed results with smaller blocks, gaps within these blocks, and less strict flanking positions. Species delimitation was first examined using single locus phylogenies. When any significant conflict was not observed among the single locus phylogenies, we concatenated the two single-locus datasets (nrITS and nrLSU) into one bi-locus dataset using BioEdit v.7.0.9 (Hall 1999). Then the concatenated nrITS-nrLSU sequences were phylogenetically analyzed using Maximum Likelihood (ML) in raxmlGUI 2.0 (Edler *et al.* 2021) with the GTRGAMMA substitution model. ML analysis was executed by applying the rapid bootstrap algorithm with 10 000 replicates to obtain nodal support values. Maximum Likelihood bootstrap percentages (MLB) of 70% and above are considered significant support for clades. For the Bayesian Inference (BI), the nrITS alignments were separated into three distinct partitions: ITS1, 5.8S, and ITS2. The selection of substitution models was accomplished using jModelTest 2.0 (Guindon & Gascuel 2003; Darriba *et al.* 2012). The BI analyses were executed within MrBayes v.3.2.6 (Ronquist *et al.* 2012), employing four Markov chain Monte Carlo (MCMC) chains for a total of 1 000 000 iterations, with termination criteria set at a standard deviation of split frequencies falling below the 0.01 threshold. Trees were sampled at every 100th generation, with the initial 25% of trees being discarded as burn-in. The convergence of chains was assessed using Tracer 1.5 (Rambaut *et al.* 2018), ensuring that effective sample size (ESS) values exceeded 200, thus confirming the reliability of the results. In our phylogenetic analyses, gaps within the alignment were treated as missing data. Bayesian Posterior Probabilities (BPP) values exceeding 0.95 are considered as strong support.

## RESULTS AND DISCUSSION

### Phylogenetic inferences

The final dataset of *L. kamengensis* sp. nov. and *L. madhuriensis* sp. nov. consisted of 50 and 46 sequences including our novel species and two outgroup species with 1500- and 1744-characters including gaps, respectively. In the ML and BI analyses, our two novel taxa are presented in bold red font (Figs 1; 2).

In the nrITS-LSU-based phylogenetic analysis of *L. kamengensis* sp. nov. (Fig. 1), the sequences procured from the described species are found to be clustered in a clade with *L. yazooensis* Hesler & A.H.Sm. described from China and *L. spadiceus* H.Lee, Wisitr. & Y.W.Lim from China and South Korea with a strong bootstrap support (98%) and posterior probability (1). The Chinese specimens are actually misnamed *L. yazooensis*. The holotype of *L. spadiceus* (TPML120807_021) is classified within the section *Deliciosi* (Fr.) Redeuilh, Verbeken & Walleyn by Lee *et al.* (2019). This specimen is also morphologically very distinct from *L. kamengensis* sp. nov. However, in our phylogenetic estimation, it has been revealed that the specimen of *L. spadiceus* (KA120268) denoted as "Additional studied material" in the same study (Lee *et al.* 2019) got clustered within a clade that represents section *Zonarii* Quél. and is quite distantly related to the clade bearing its holotype. This indicates that the "Additional studied material" *L. spadiceus* and the holotype of *L. spadiceus* are two distinct species. Likewise, for a similar reason, the specimen named *L. spadiceus* (HBAU15495) from China is also a different species. In conclusion, the whole clade (highlighted by the yellow box), containing the described species, *L. yazooensis* from China and *L. spadiceus* from China and South Korea, represents *L. kamengensis* sp. nov.

In the resulting phylogenetic tree of *L. madhuriensis* sp. nov. (Fig. 2), sequences derived from the studied species have completely recovered as a separate clade with 100% bootstrap support.

Order RUSSULALES Kreisel
ex P.M.Kirk, P.F.Cannon & J.C.David
Family Russulaceae Lotsy
Genus *Lactarius* Pers.

***Lactarius kamengensis*** sp. nov.
(Figs 3; 4)

A medium-sized bitter to acrid tasting *Lactarius* with pale yellowish, vaguely zonate pileus, scrobiculate stipe, white latex turning pale yellowish and occurring under *Castanopsis* (D.Don) Spach.

Holotype. — **India**. Arunachal Pradesh, West Kameng district, Shergaon, 27°09.216'N, 92°16.174'E, alt. 2369 m a.s.l., scattered

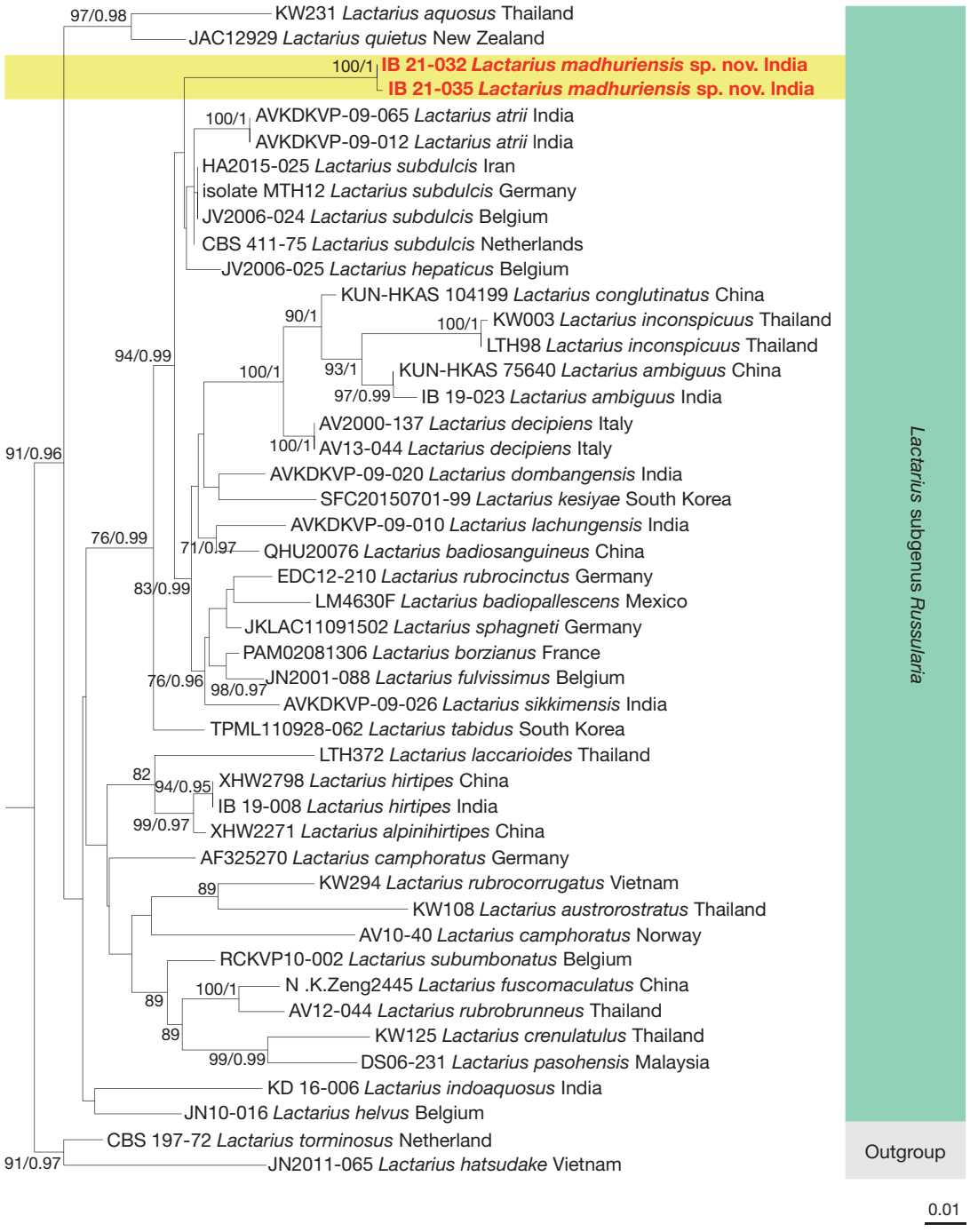


FIG. 2. — Phylogram inferred from Maximum Likelihood (ML) analysis by raxmlGUI 2.0 and Bayesian Inference by Mr.Bayes v. 3.2.6 based on nrITS and nrLSU sequence data. Sequences derived from the novel Indian species are presented in **red** and **bold** and the respective clade is highlighted in **yellow**. Maximum Likelihood bootstrap support values (MLbs) ≥ 70% are shown on the left of "/" and Bayesian Posterior Probabilities (BPP) ≥ 0.95 are shown on the right or below of "/" at nodes.

on soil under *Castanopsis* in temperate broadleaf forest, 28.VII.2019, I. Bera, *IB 19-016* (holo-, CAL[CAL 1878]!).

ADDITIONAL SPECIMEN EXAMINED. — **India**. Arunachal Pradesh, West Kameng district, Shergaon, 27°07.810'N, 92°15.116'E, alt. 2243 m a.s.l., scattered on soil under *Castanopsis* in temperate broadleaf forest, 30.VIII.2019, I. Bera, *IB 19-054* (CAL[CAL 1879]).

ETYMOLOGY. — Referring to the type locality, West "Kameng" district of Arunachal Pradesh.

GENBANK. — OP806537 (nrITS, holotype) and OP806830 (nrITS, *IB 19-054*), OP811192 (nrLSU, holotype) and OP811193 (nrLSU, *IB 19-054*).

MYCOBANK. — MB 848906.

## DESCRIPTION

Pileus 40-60 mm diam., convex when young, gradually becoming planoconvex on maturity; surface moist, smooth,

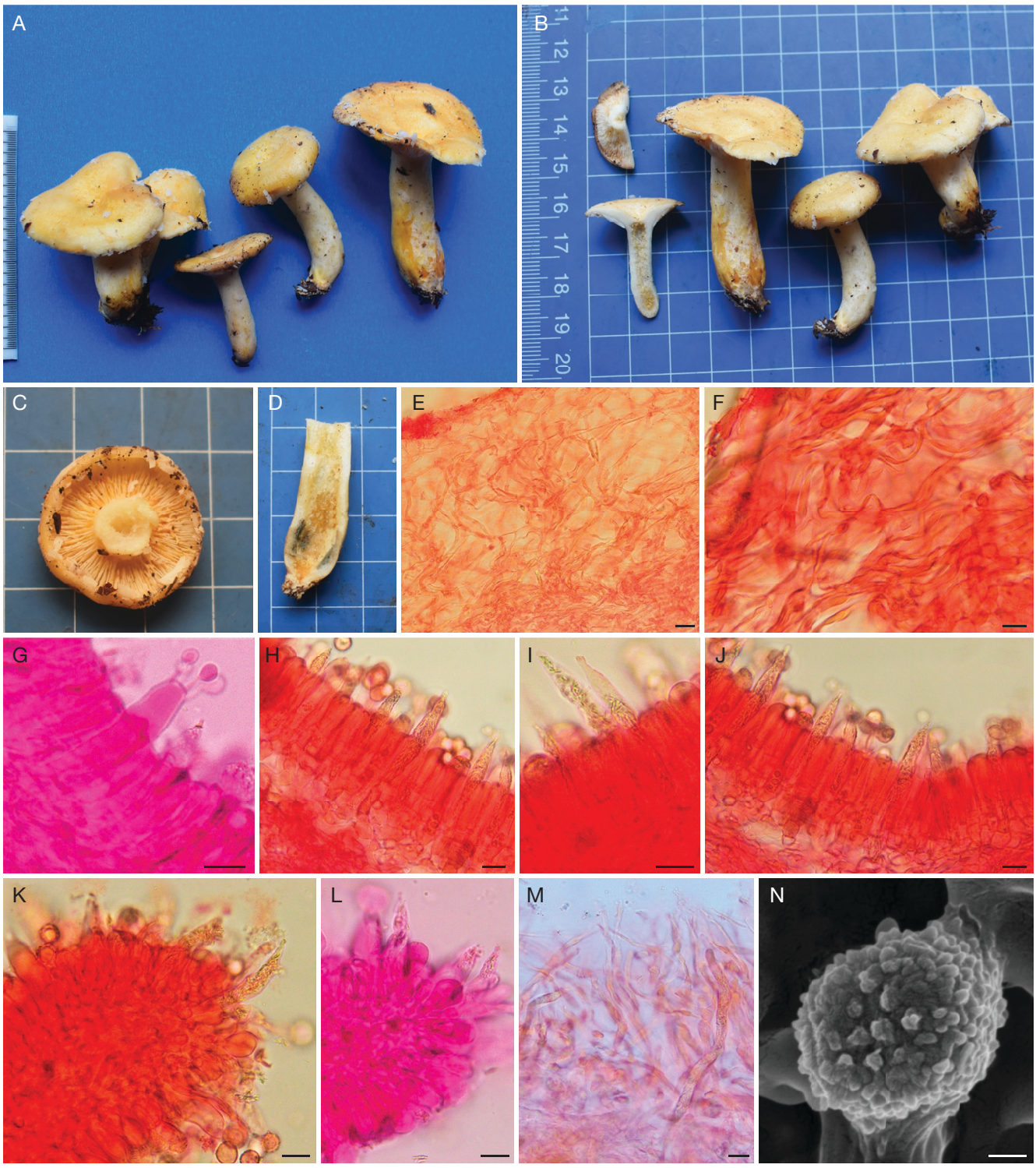


FIG. 3. — Photoplate of *Lactarius kamengensis* sp. nov. (holo-, CAL[CAL 1878]): **A**, **B**, fresh basidiomata in the field and basecamp; **C**, latex on cut lamellae; **D**, macrochemical reaction with guaiac; **E**, **F**, transverse section through pileipellis; **G**, basidia; **H-J**, pleuromacrocystidia; **K**, **L**, cheilomacrocystidia; **M**, transverse section through stipitipellis; **N**, basidiospore. Scale bars: E, 20 µm; F-M, 10 µm; N, 300 nm.

slightly viscid; surface zonate, with combination of pale yellow, greyish yellow to greyish dull yellow (2-4B3), a little darker on maturity but fading towards margin, mostly with whitish concentric, rather vague zones; margin entire, rarely lobed, incurved. Lamellae subdecurrent, yellowish white, crowded (20 L+l/cm at pilear margin); lamellulae present in 4-5 series; concolorous; edge entire. Stipe 40-70 × 18-20 mm, central, cylindrical; surface viscid, yellowish white (1A2) with distinct greyish yellow (4B4) scrobicules at base. Context in pileus thick, pithy in stipe, yellowish white (1-2A2), unchanging

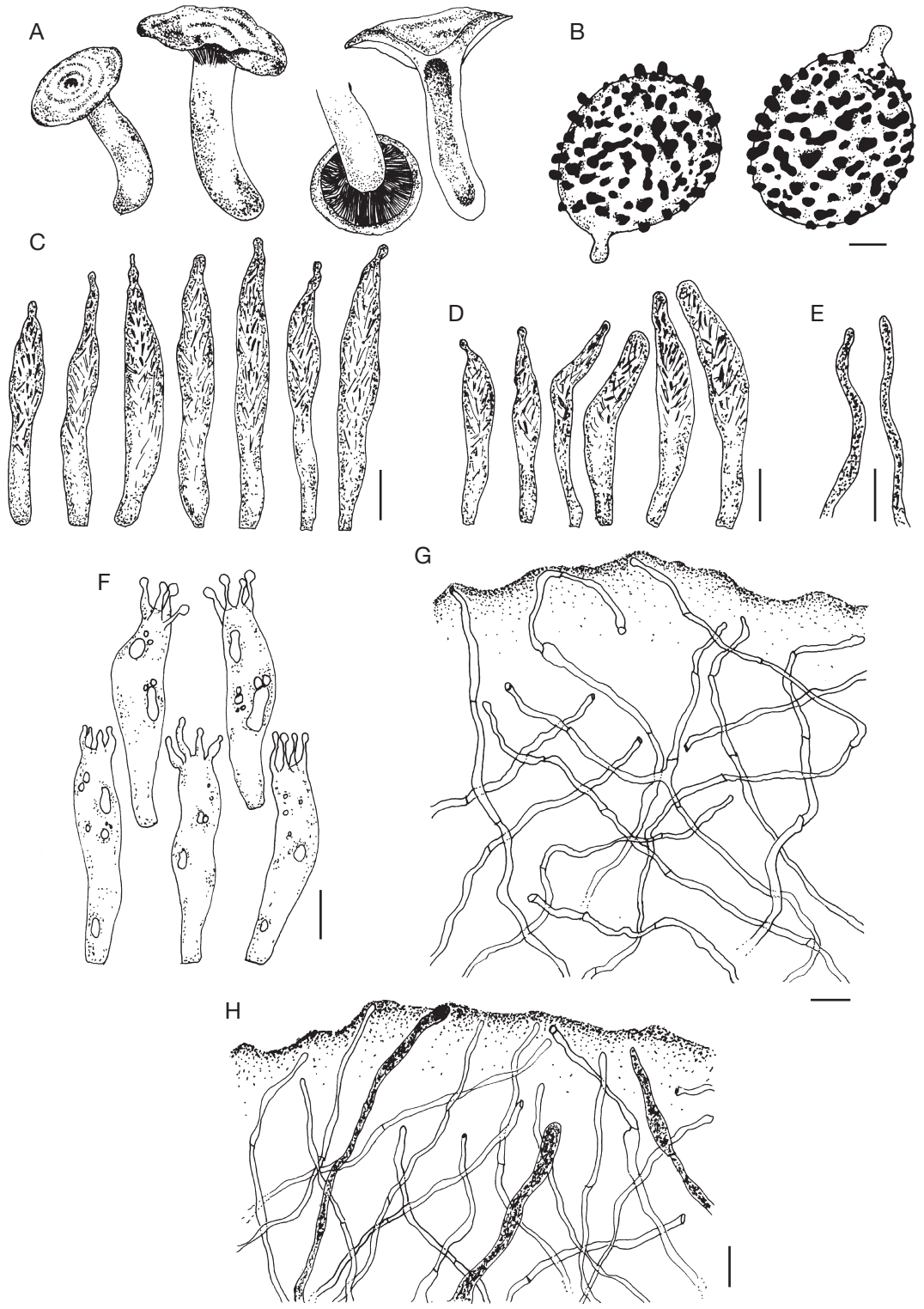


FIG. 4. — Line drawing of *Lactarius kamengensis* sp. nov. (holo-, CAL[CAL 1878]): **A**, fresh basidiomata in the field and basecamp; **B**, basidiospore; **C**, pleuromacrocystidia; **D**, cheilomacrocystidia; **E**, pleuropesudocystidia; **F**, basidia; **G**, transverse section through pileipellis; **H**, transverse section through stipitipellis. Scale bars: B, 2 µm; C-G, 10 µm.

in 3% KOH and FeSO4 but immediately turning greenish blue in guaiac. Latex white, turning pale yellow to light yellow within 10 seconds, very bitter. Taste quite bitter at first, then acrid. Odor mild. Spore print could not be obtained.

Basidiospores 7.4-8.5-9.8(10) × 6.2-7-7.89 µm, (n = 30, Q = 1.08-1.2-1.39), usually subglobose to ellipsoid; ornamentation amyloid, up to 1.1-1.7 µm high, composed of isolated warts, sometimes fused into short ridges but never forming any

reticulum; suprahilar spot inamyloid. Basidia 35.4-46.9 × 7.8-10.4 µm, subclavate, 4-spored; sterigmata 3-6 × 1-2.5 µm. Pleuromacrocystidia abundant, 47-70 × 4.5-8 µm, emergent up to 26 µm, subcylindric with fusoid, subfusoid to mucronate apices, thin-walled; content dense, granular to fibrous. Pleuropseudocystidia abundant, 1.2-1.4 µm wide, mostly non-emergent, cylindrical to slightly tortuous, with rounded apex. Lamellae edge heteromorphous with basidia, basidioles and cystidia. Cheilomacrocystidia rare, 37-55 × 5-7.5 µm, emergent up to 20 µm, subcylindric with subfusoid to appendiculated apices, thin-walled; content dense, granular to fibrous. Subhymenium up to 29 µm thick, cellular. Lamellar trama composed of lactifers, sphaerocytes, and connecting hyphae. Pileipellis up to 215 µm thick, an ixotrichoderm; suprapellis composed of interwoven, septate, mostly ascending hyphae. Stipitipellis up to 114 µm thick, an ixotrichoderm; suprapellis composed of interwoven, septate, mostly ascending hyphae intermixed with lactifers.

### Notes

The presence of viscid, zonate, pale yellowish pileus with scrobiculate stipe clearly indicates that the described species is a member of section *Zonarii* (Heilmann-Clausen *et al.* 1998). Moreover, bitter to acrid tasting medium-sized basidiomata with slightly viscid, zonate, greyish yellow to pale yellow pileus, scrobiculate (towards the base) stipe, white latex turning the cut lamellae light yellowish and the positive macrochemical reaction of the context with guaiac make the studied *Lactarius* sp. quite distinct in the field. Further, the micromorphological characters such as the basidiospore ornamentation of isolated warts sometimes connected with ridges and ixotrichoderm nature of the pileipellis and stipitipellis make it a recognizable species amongst its other relatives. The similar stature of the basidiocarp, its zonate yellowish pileus and the occurrence with broad-leaved trees remind of the European *L. zonarius* (Bull.) Fr. Yet striking dissimilarities in characters such as the pale pinkish buff pileus with ochraceous zones, tomentose margin, smaller spore ornamentations (only up to 0.75-1.0 µm high) and ixocutis pileipellis of *L. zonarius* segregate it from the currently studied specimens of *Lactarius* (Heilmann-Clausen *et al.* 1998). The zonate pileus character has been shared with some of the closest Asian species belonging to the subsect. *Zonarii* and associated with broad-leaved forest trees, *L. austrotorminosus* H.T.Le & A.Verbeken (Thailand), *L. austrozonarius* H.T.Le & A.Verbeken (Thailand), *L. sinozonarius* X.H.Wang (China), and *L. indozonarius* Uniyal, K.Das & Nuytinck (India). Despite that, pale orange to pinkish basidiomata with infundibuliform, papillate, hairy pileus with hairy margin and absence of cheilomacrocystidia in *L. austrotorminosus* and robust basidiomata (pileus 50-135 mm diam., stipe 25-115 × 15-35 mm) with reddish brownish scaly pileus, subdistant (6 L+l/cm) lamellae, larger basidiospores (7.2-*8.9*-12.2 × 7-*8.4*-10.2 µm) with higher ornamentations (2.5-3.5 µm high), the complete absence of cheilomacrocystidia and ixocutis nature of stipitipellis in *L. austrozonarius* evidently distinguish these Thai species from the present species (Le *et al.* 2007). On the other hand, the ochraceus brown hygrophanous pileus with forked lamellae, low ornamentations (0.5-1.0 µm high) of basidiospores, absence of cheilomacrocystidia and ixocutis nature of pileipellis in *L. sinozonarius* separate it out (Wang 2017). The Indian *L. indozonarius* has a larger pileus (60-122 mm diam.) with hairy margin and ixocutis pileipellis which are absent in the studied specimens (Uniyal *et al.* 2018).

*Lactarius yazooensis* (originally described from North America) might be confused with the described *Lactarius* due to its viscid, smooth, zonate pileus, whitish latex, similar-sized and ornamented basidiospores, and occurrences under the broad-leaved trees (Hesler & Smith 1979). But with the striking dissimilarities like the orange ochraceous to rusty orange basidiomata with larger pileus (50-150 mm broad), absence of cheilomacrocystidia, ixocutis nature of pileipellis and presence of caulocystidia, *L. yazooensis* is quite morphologically distinguishable from the current studied species of *Lactarius* (Hesler & Smith 1979). Yet, many look-alikes have been misnamed as *L. yazooensis* after the North American representatives clearly doubted their conspecificity. The phylogenetic analysis (depicted in Figure 1) states that the North American collections of *L. yazooensis* are distantly related to the Chinese misnamed *L. yazooensis* specimens that have formed the clade with *L. kamengensis* sp. nov.

## ***Lactarius madhuriensis*** sp. nov.
(Figs 5; 6)

A medium-sized *Lactarius* with the umbonate, brownish orange pileus, light brown stipe, watery white latex turning yellowish and occurring in association with *Abies*.

Holotype. — **India**. Arunachal Pradesh, Sangestar Tso (Madhuri Lake), Tawang district, 27°43.215'N, 91°49.473'E, alt. 3715 m a.s.l., scattered on moss bed in association with *Abies* in sub-alpine forest, 26.VII.2021, I. Bera, *IB 21-032* (holo-, CAL[CAL 1899]!).

Additional specimens examined. — **India**. Arunachal Pradesh, Sangestar Tso (Madhuri Lake), Tawang district, 27°43.276'N, 91°49.581'E, alt. 3705 m a.s.l., scattered on moss bed in association with *Abies* in sub-alpine forest, 26.VII.2021, I. Bera, *IB 21-035* (CAL[CAL 1900]).

Etymology. — Referring to the type locality Sangestar Tso which is famously known as "Madhuri" Lake.

GenBank. — OP808232 (nrITS, holotype) and OP808233 (nrITS, *IB 21-035*), OP811194 (nrLSU, holotype) and OP811195 (nrLSU, *IB 21-035*).

Mycobank. — MB 848907.

Habitat and distribution. — Growing scattered on moss bed in association with *Abies* in sub-alpine forest of Tawang district of Arunachal Pradesh.

### Description

Pileus 15-45 mm in diam., convex with a central umbo when young, gradually becoming planoconvex to infundibuliform, center with a broad shallow depression and an umbo; surface moist, viscid, smooth; brownish orange (7C6) with darker


FIG. 5. — Photoplate of *Lactarius madhuriensis* sp. nov. (holo-, CAL[CAL 1899]): **A**, **B**, **D**, fresh basidiomata in the field and basecamp; **C**, latex on lamellae; **E**, macrochemical reaction with guaiac; **F**, **G**, transverse section through pileipellis; **H**-**M**, pleuromacrocystidia; **N**, **O**, cheilomacrocystidia; **P**, transverse section through stipitipellis; **Q**, **R**, basidiospore. Scale bars: F-O, 10 µm; P, 20 µm; Q, R, 1 µm.

at the central region gradually becoming greyish orange to orange (6B4-6) and paler towards the margin; brittle in consistency; margin incurved, wavy, regular. Lamellae decurrent, rather crowded (17-18 L+l/cm at pilear margin), sometimes forked; lamellulae in five series; orange white to pale orange (5A2-3) turning brownish on maturity; edge entire. Stipe 30-55 × 2-5 mm, central, cylindrical, longitudinally striate, striation translucent; combination of light brown and brown

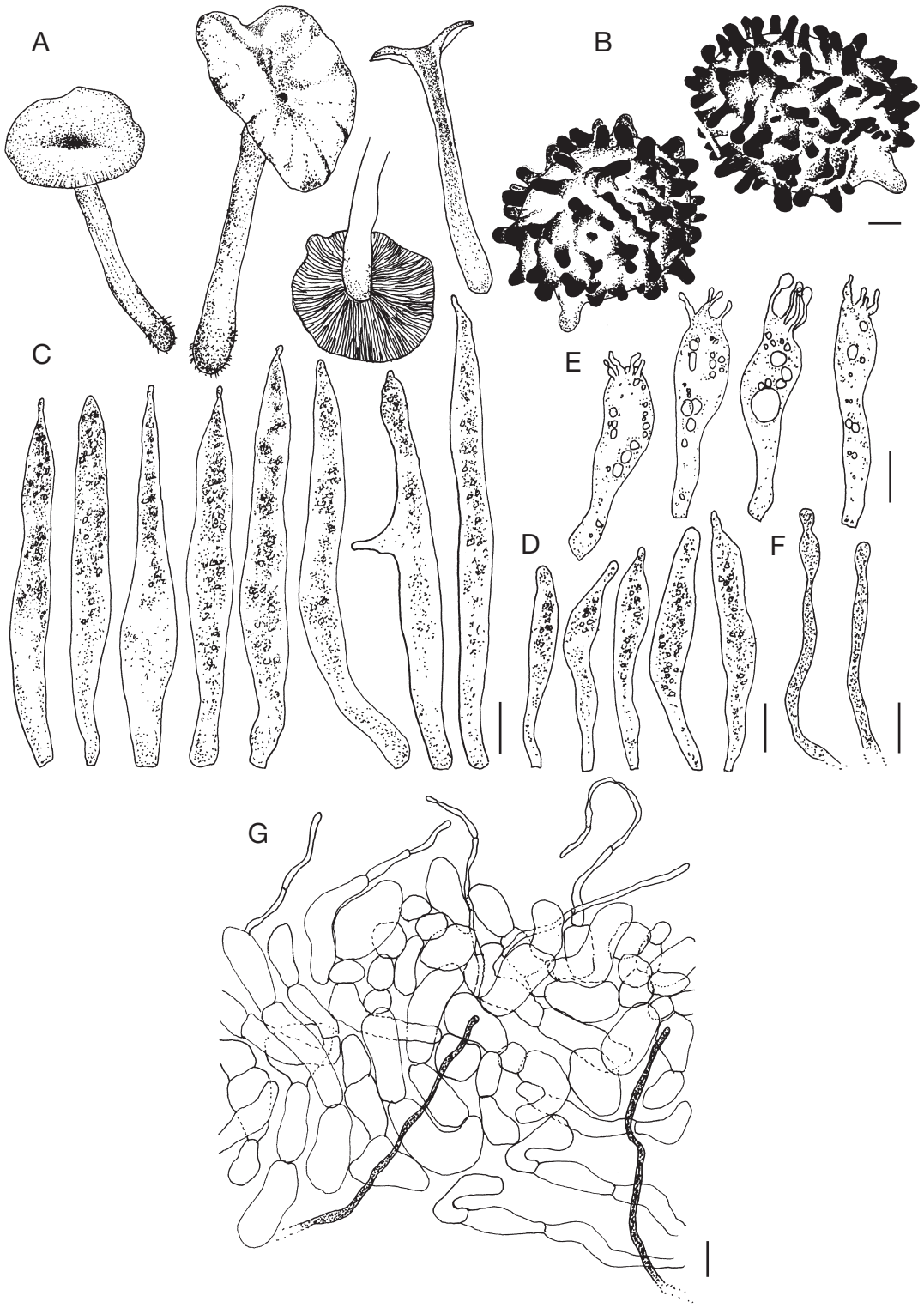


Fig. 6. — Line drawing of *Lactarius madhuriensis* sp. nov. (holo-, CAL[CAL 1899]): **A**, fresh basidiomata in the field and basecamp; **B**, basidiospore; **C**, pleuromacrocystidia; **D**, cheilomacrocystidia; **E**, basidia; **F**, pleuropesudocystidia; **G**, transverse section through pileipellis. Scale bars: B, 2 µm; C-G, 10 µm.

(6D5, 7E7). Context thin at pileus, hollow in stipe, concolorous to stipe, turning faint yellow with 3% KOH after one minute, no change with FeSO4 and immediately becoming greyish turquoise (24E5) with guaiac. Latex moderate, watery white changing to yellowish after few minutes, chalky when dry, turning exposed lamellae brownish after few minutes, bitter. Taste astringent. Odor mild pleasant. Spore print chalky white (1A1-2).

Basidiospores 7.2-8.1-8.9(9.6) × 5.2-6.3-6.9(7.6) µm, (n = 40, Q = 1.11-1.30-1.50[1.54]), usually subglobose to ellipsoid;

ornamentation amyloid; up to 0.9 µm high, irregular to linear warts sometimes connected with low ridges but never forming complete reticulum, presence of isolated warts; suprahilar spot inamyloid. Basidia 35-46 × 9-11 µm, subclavate, 4-spored; sterigmata up to 8.7 × 1.6 µm. Pleuromacrocystidia abundant, 73.4-112.9 × 6.3-9.7 µm, emergent up to 44.7 µm, cylindric to subcylindric with fusoid, subfusoid, mucronate to appendiculated apices, rarely branched, originating from subhymenium region. Pleuropseudocystidia up to 2.3-2.8 µm wide, mostly non-emergent, cylindrical to sometimes tortuous at apex, rarely branched. Lamellae edge heteromorphous with basidia, basidioles and cystidia. Cheilomacrocystidia 39-51.1 × 3.8-6 µm, emergent up to 21.8 µm, cylindric to subcylindric with rounded, fusoid, subfusoid to appendiculate apices. Subhymenium up to 20.13 µm thick, pseudoparenchymatous. Lamellar trama composed of lactifers, sphaerocytes, and connecting hyphae. Pileipellis up to 126 µm thick, hyphoepithelium; suprapellis composed of multiseptated, mostly repent hyphae (12.2-55 × 2-3.1 µm); subpellis pseudoparenchymatous composed of rounded to elongated or somewhat irregularly shaped cells (12.9-31.4 × 8-13 µm). Stipitipellis up to 65 µm thick, hyphoepithelium; suprapellis composed of hyphal elements; subpellis is of rounded to elongated to somewhat irregularly shaped cells.

### Notes

The somewhat sticky, brownish orange colored pileus with watery white latex turning yellowish on drying and pileipellis of hyphoepithelium nature are typical features of subg. *Russularia* (Heilmann-Clausen *et al.* 1998) which also correspond to the studied species. The unique characters of the novel species are planoconvex to infundibuliform shaped, umbonate, brownish orange, moist pileus with longitudinally striate light brownish stipe; latex changing to yellowish and turning exposed area brownish; positive reaction with guaiac and hyphoepithelial pileipellis.

The described species is quite identical to European *L. subdulcis* (Pers.) Gray, due to its small to medium-sized fruitbodies with similar colored convex to funnel shaped pileus, white bitter tasting latex, alike sized and ornamented basidiospores, similar pleuro- and cheilomacrocystidia. However, occassionally forked lamellae, wider stipe (25-65 × 4-18 mm), white unchanging latex, shorter cystidia of both lamellar face (40-90[-105] × 6-12 µm) and edge ([15-]20-35 × 4-8 µm), pileipellis oedotrichoderm and occurrence with *Fagus* L. are the striking differences possessed by *L. subdulcis* that make it distinct from the current species (Heilmann-Clausen *et al.* 1998). Another European *Lactarius*, *L. tabidus* Fr. can be confused with the studied species for similar characters such as orange brownish pileus with persistent umbo, latex turning pale yellow, astringent taste and hyphoepithelial pileipellis. But the radially wrinkled cap in matured basidiomata, basidiospores with mostly isolated acute warty ornamentation, and its association with *Betula*, clearly distinguish the former from the latter species (Heilmann-Clausen *et al.* 1998).

The Indian species, *L. lachungensis* Verbeken & Van de Putte also can be confused with the studied species for its unicolored warm orange-brownish basidiomata and similar habitat. Yet the distant (12 L+l/cm), never forking lamellae, unchanging latex, and the ixohyphoepithelium to ixotrichoepithelium pileipellis clearly separate *L. lachungensis* from the discussed species (Wisitrassameewong *et al.* 2016). The yellowing of the latex is shared by another Indian species, *L. flavigalactus* Verbeken & K.Das, but other specific characters like reddish brown basidiomata, ornamentation of the basidiospores with irregular ridges forming incomplete reticulum and the ixocutis nature of pileipellis clearly differentiate *L. flavigalactus* from the described species (Wisitrassameewong *et al.* 2016).

Although *L. atrii* Van de Putte & K.Das (described from India) (Fig. 2) is phylogenetically close to the studied species, morphological dissimilarities such as light brown basidiocarps with unchanging mild-tasting latex and ixocutis nature of pileipellis visibly distinguish the two species (Wisitrassameewong *et al.* 2016).

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## REFERENCES

Altschul S. F., Madden T. L., Schäffer A. A., Zhang J., Zhang Z., Miller W. & Lipman D. J. 1997. — Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25 (17): 3389-3402. https://doi.org/10.1093/nar/25.17.3389

Basso M. T. 1999. — *Fungi Europaei*. Volume 7: Lactarius *Pers.* Mykoflora, Alassio, 845 p.

Basso M. T., Neville P. & Poumarat S. 2001. — A new *Lactarius* species of the subsection *Scrobiculati* Hesler & AH Smith: *Lactarius pseudoscrobiculatus* Basso, Neville & Poumarat sp. nov. *Bulletin Famm*, n.s. 19: 13-20.

Buyck B., Hofstetter V., Verbeken A. & Walleyn R. 2010. — Proposals to conserve or reject names. 1919 Proposal to conserve *Lactarius* nom. cons. Basidiomycota with a conserved type. *Taxon* 59 (1): 295-296. https://doi.org/10.1002/tax.591031

Clark K., Karsch-Mizrachi I., Lipman D. J., Ostell J. & Sayers E.W. 2016. — GenBank. *Nucleic Acids Research* 44: D67-D72. https://doi.org/10.1093/nar/gkv1276

Darriba D., Taboada G. L., Doallo R. & Posada D. 2012. — jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9 (8): 772-772.

Das K., Verbeken A. & Nuytinck J. 2015. — Morphology and phylogeny of four new *Lactarius* species from Himalayan India. *Mycotaxon* 130 (1): 105-130. https://doi.org/10.5248/130.105

Edler D., Klein J., Antonelli A. & Silvestro D. 2021. — raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12 (2): 373-377. https://doi.org/10.1111/2041-210X.13512

Guindon S. & Gascuel O. 2003. — A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52 (5): 696-704. https://doi.org/10.1080/10635150390235520

HALL T. A. 1999. — BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.
HEILMANN-CLAUSEN J., VERBEKEN A. & VESTERHOLT J. 1998. — *The genus* Lactarius. The Danish Mycological Society, Odense, 287 p.
HESLER L. R. & SMITH A. H. 1979 — *North American species of* Lactarius. University of Michigan Press, United States, 841 p.
KALICHMAN J., KIRK P. M. & MATHENY P. B. 2020. — A compendium of generic names of agarics and Agaricales. *Taxon* 69 (3): 425-447. https://doi.org/10.1002/tax.12240
KATOH K., ROZEWICKI J. & YAMADA K. D. 2019. — MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20 (4): 1160-1166. https://doi.org/10.1093/bib/bbx108
KORNERUP A. & WANSCHER J. H. 1978. — *Methuen Handbook of Colour* (3rd edition). Eyre Methuen Ltd., London, 252 p.
KUMAR S., STECHER G. & TAMURA K. 2016. — MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33 (7): 1870-1874. https://doi.org/10.1093/molbev/msw054
LE H. T., NUYTINCK J., VERBEKEN A., LUMYONG S. & DESJARDIN D. E. 2007. — *Lactarius* in Northern Thailand: 1. *Lactarius* subgenus *Piperites*. *Fungal Diversity* 24 (1): 173-224.
LEE H., WISSITRASSAMEEWONG K., PARK M. S., VERBEKEN A., EIMES J. & LIM Y. W. 2019. — Taxonomic revision of the genus *Lactarius* (Russulales, Basidiomycota) in Korea. *Fungal Diversity* 95 (1): 275-335. https://doi.org/10.1007/s13225-019-00425-6
MATHENY P. B. 2005. — Improving phylogenetic inference of mushrooms with rpb1 and rpb2 nucleotide sequences (Inocybe; Agaricales). *Molecular Phylogenetics and Evolution* 35 (1): 1-20. https://doi.org/10.1016/j.ympev.2004.11.014
MIZUNO K. & TENPA L. 2015. — *Himalayan Nature and Tibetan Buddhist Culture in Arunachal Pradesh, India: A Study of Monpa*. Vol. 6. Springer, Tokyo, 196 p.
PERSOON C. H. 1797. — *Tentamen dispositionis methodicae fungorum in classes, ordines, genera et familias, cum supplement adjecto*. Petrum Philippum Wolf, Leipzig, 96 p.
RAMBAUT A., DRUMMOND A. J., XIE D., BAELE G. & SUCHARD M. A. 2018. — Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67 (5): 901-904. https://doi.org/10.1093/sysbio/syy032
RONQUIST F., TESLENKO M., VAN DER MARK P., AYRES D. L., DARLING A., HÖHNA S., LARGET B., LIU L., SUCHARD M. A. & HUELSENBECK J. P. 2012. — MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61 (3): 539-542. https://doi.org/10.1093/sysbio/sys029
SHARMA N. & SHUKLA S. P. 1992. — *Geography and development of hill areas: A case study of Arunachal Pradesh*. Mittal Publications, Delhi, 154 p.
TALAVERA G. & CASTRESANA J. 2007. — Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* 56 (4): 564-577.
UNIYAL P., NUYTINCK J. & DAS K. 2018. — *Lactarius* subg. *Lactarius* (Russulaceae) in Indian Himalaya: two new species with morphology and phylogenetic inferences. *Cryptogamie, Mycologie* 39 (4): 467-482. https://doi.org/10.7872/crym/v39.iss4.2018.467
VERBEKEN A. & NUYTINCK J. 2013. — Not every milkcap is a *Lactarius*. *Scripta Botanica Belgica* 51: 162-168.
VERMA K., MEHMOOD T., UNIYAL P. & SHARMA Y. P. 2022. — *Lactarius indoevosmus* and *L. kanadii* (Russulaceae), two new species from the northwestern Himalayas, India, inferred from morphology and molecular data. *Phytotaxa* 541 (2): 165-177. https://doi.org/10.11646/phytotaxa.541.2.6
VERMA K., MEHMOOD T., UNIYAL P., KAPOOR R. & SHARMA Y. P. 2021. — Two new species of genus *Lactarius* (Russulaceae) from North-western Himalaya, India. *Phytotaxa* 500 (4): 253-265. https://doi.org/10.11646/phytotaxa.500.4.1
WANG X. H. 2007. — Type studies of *Lactarius* species published from China. *Mycologia* 99 (2): 253-268. https://doi.org/10.1080/15572536.2007.11832585
WANG X. H. 2017. — Seven new species of *Lactarius* subg. *Lactarius* (Russulaceae) from southwestern China. *Mycosystema* 36 (11): 1463-1482.
WHITE T. J., BRUNS T., LEE S. J. W. T. & TAYLOR J. 1990. — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: a guide to methods and applications* 18 (1): 315-322.
WISITRASSAMEEWONG K., LOONEY B. P., LE H. T., DE CROP E., DAS K., VAN DE PUTTE K., EBERHARDT U., JIAYU G., STUBBE D., HYDE K. D., VERBEKEN A. & NUYTINCK J. 2016. — *Lactarius* subgenus *Russularia* (Basidiomycota, Russulales): novel Asian species, worldwide phylogeny and evolutionary relationships. *Fungal Biology* 120 (12): 1554-1581. https://doi.org/10.1016/j.funbio.2016.08.004

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