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Morphological and phylogenetic evidence for two new *Lactarius* species (Russulales, Basidiomycota) from India

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Abstract – *Lactarius ferruginascens* sp. nov. and *L. indoaquosus* sp. nov. are described from the East and South districts of Sikkim, a small Himalayan state in India. Macro- and micromorphological descriptions coupled with the illustrations and nrITS-based molecular analyses are given for both species. *Lactarius ferruginascens* is a species in *L.* subg. *Plinthogalus* with striking features such as a sticky pileus surface caused by the ixohymeniderm structure of the pileipellis, watery white latex which is turning rusty brown on the lamellae and the occurrence under *Abies* sp. in the subalpine mixed forest. *Lactarius indoaquosus* is a new representative of *L.* subg. *Russularia* with completely transparent and unchanging latex and is furthermore characterized by basidiospores with a zebroid ornamentation, the absence of hymenial macrocystidia and the occurrence under *Castanopsis* sp. in the temperate broadleaf forest. Both species are illustrated and compared with related taxa.

Eastern Himalaya / *Lactarius* subg. *Plinthogalus* / *Lactarius* subg. *Russularia* / nrITS / phylogeny / Russulaceae / taxonomy

INTRODUCTION

Milkcaps are forming an important group of macrofungi, striking in the field because of the exudation of latex and ecologically indispensable in all ecosystems worldwide as they belong to the largest genera of ectomycorrhizal fungi (EM). The traditional genus *Lactarius* was recently (Buyck *et al.* 2008) split in two milkcap genera: *Lactarius* (about 450 described species worldwide, 83 reported from India), *Lactifluus* (about 150 described species worldwide, 27 reported from

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India) and a mixed genus *Multifurca*. The latter one is a very small genus (7 described species worldwide and only 1 from India), with a remarkable distribution: North and Central America, Asia and Australia, but absent from Europe and Africa (Buyck *et al.* 2008; Atri *et al.* 2016).

Metagenomic studies support the view that the EM genus *Lactarius* is one of the most diverse and abundant genera at high altitudes and latitudes (Geml *et al.* 2009, 2011) forming EM with important plant families such as Fagaceae, Betulaceae or Pinaceae. Since 2008, we carry out an intensive exploration and inventory of macrofungi in Sikkim, a small Indian state bordering Nepal, Bhutan, Tibet and China. Sikkim belongs to the Eastern Himalaya Global Diversity Hotspot (Mittermeier *et al.* 2005) and its location on the border between the Oriental and Palearctic eco-regions, and the variation in elevation (300-8598 m) and climatic regime (tropical to cold desert) provide a wide range of vegetation types, from subtropical to temperate coniferous, broadleaf and mixed forests, alpine forests, shrubs and meadows (Bhupathy *et al.* 2009; Das *et al.* 2010). Before 2008 very little was known about the EM fungi, but since we started our fungal biodiversity study in the area 17 *Lactarius* species (14 new to science) and 10 *Lactifluus* species (5 new to science) were reported from this state (Das & Verbeken 2011, 2012; Van de Putte *et al.* 2012; Das & Chakraborty 2014; Das *et al.* 2015, 2017; Wisitrassameewong *et al.* 2016). Sixteen of these species are occurring in the subalpine zone (2700-5000 m), whereas eleven occur in the temperate zone (1500-2700 m). In this paper we introduce two more new *Lactarius* species: *L. ferruginascens* growing under *Abies* in the subalpine zone and *L. indoaquosus* growing under *Castanopsis* in the temperate zone (Fig. 1) of East and South districts of Sikkim respectively. Both species are presented with morphological details and molecular data of the nrITS region.

MATERIAL AND METHODS

Collecting and morphology

Field and macromorphological characters, ecological notes, macrochemical color tests (with the application of 10% KOH, guaiac and $FeSO_4$) were recorded from young to mature fresh basidiomata in the field and/or in the base camp. Specimens were dried with hot air using a portable aluminium field dryer. Images of fresh basidiomata and microphotographs were captured with Canon SX 120 and Nikon-DS-Ri1 (dedicated to "Nikon Eclipse N*i*" compound microscope) cameras respectively. Color codes and terms mostly follow Methuen Handbook of Color (Kornerup & Wanscher 1978). Micromorphological features were recorded with the help of the aforementioned compound microscope from free-hand sections of dry basidiomata mounted in 5% KOH, or stained in a mixture of 5% KOH and phloxin and mounted in 30% glycerol or distilled water. Micromorphological drawings were made with the help of a drawing tube (attached to Nikon-DS-Ni1) at 400× and 1000× magnifications. Basidium length excludes length of sterigmata. Basidiospores were mounted in Melzer's reagent and spore measurements were recorded in profile view from twenty spores. Spore-size measurements and length/width ratios (Q) are given as: minimum-**mean-**maximum. Herbarium codes follow Thiers (continuously updated).

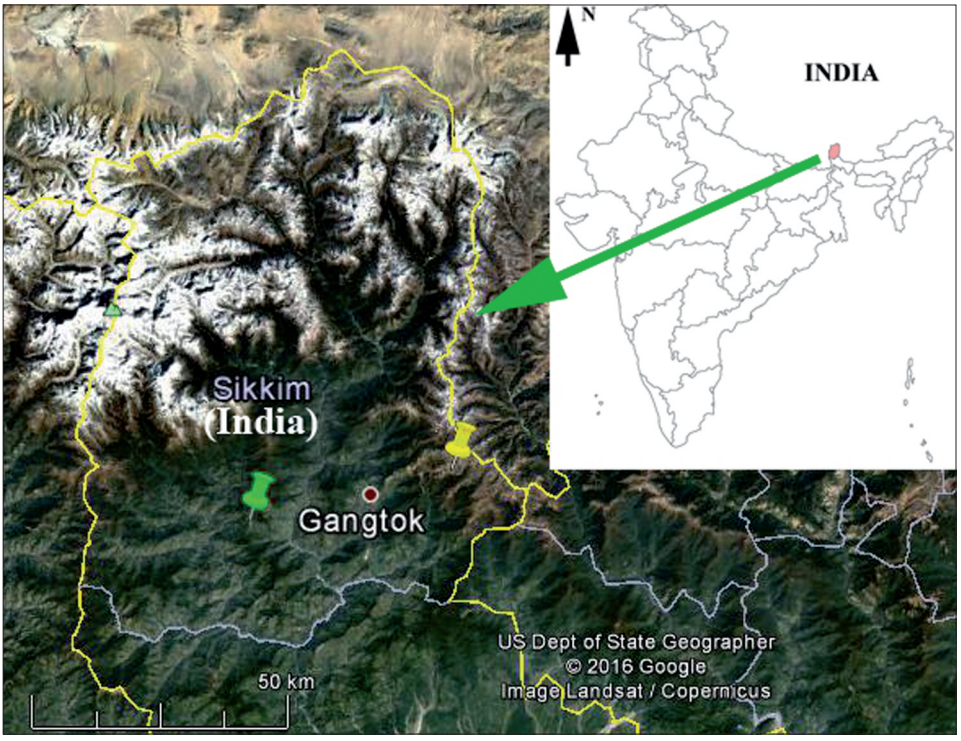


Fig. 1. Distributional map. Yellow and green pointers indicate the type localities for *Lactarius ferruginascens* sp. nov. and *L. indoaquosus* sp. nov. respectively in the state of Sikkim in India. Map is created with the help of Google Earth (www. google.com/earth).

DNA isolation, amplification and sequencing

Genomic DNA was extracted from dried herbarium specimens (10-50 mg) using the Fungal gDNA Mini Kit (Xcelris Genomics, Ahmedabad, India). The nuclear ribosomal ITS region was amplified using the primers ITS4 and ITS5 (White *et al.* 1990). PCR was performed in a 50 µl reaction using 2 µl template DNA (10-20 ng), 0.5 U Taq DNA polymerase (Sigma-Aldrich, India), 5 µl 10× Taq DNA polymerase buffer, 1 µl 200 µM of each dNTP (Sigma-Aldrich, India), 1 µL of each primer ITS4 and ITS5 (10 pmole μL^{-1}), and the remaining volume was made up by H_2O (Sterile Ultra Pure Water, Sigma-Aldrich). Amplification was done using an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) with the following parameters: 5 min step at 95°C, followed by 30 cycles of 1 min at 95°C, 30 s at 55°C, and 1 min at 72°C and a final 7 min extension step at 72°C. The PCR products were purified with an Axygen PCR cleanup kit (Axygen Scientific Inc, CA, USA). Sequencing of the PCR products was accomplished with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA), using the amplification primers. The cycle sequencing products were run on an ABI Avant 3100 automated DNA sequencer (Applied Biosystems, USA). The raw DNA sequencing files were edited and combined using ChromasLite v. 2.01. The final sequence data were deposited in the NCBI nucleotide sequence database (accession numbers: ITS-rDNA for KD 16-051 and KD 16-006 are KY867683 and KY867682 respectively).

Phylogenetic analysis

Phylogenetic analyses based on ITS-rDNA sequence data were carried out to establish the phylogenetic placement of the two new species. Two separate ITS datasets (respective reference sequences and out-groups) were assembled from previous studies on this genus, one on *L.* subg. *Plinthogalus* and another one on *L.* subg. *Russularia* (Le *et al.* 2007; Stubbe & Verbeken 2012; Lee *et al.* 2015; Wisitrassameewong *et al.* 2015; Wisitrassameewong *et al.* 2016; Hyde *et al.* 2016) and from BLAST (Altschul *et al.* 1997) as suggested by BLAST searches in GenBank (Clark *et al.* 2016). Alignments were performed using CLUSTAL W (http://www.ebi.ac.uk/clustalw/) and the relationships were inferred by the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura 1980). One-thousand bootstrap replicates were analyzed to obtain nodal support values. *L. deliciosus* and *L. austrotorminosus* were chosen as outgroup taxa for the phylogeny of *Lactarius ferruginascens* sp. nov.*,* similarly *L. friabilis, L. fuliginosus*, and *L. pterosporus* were considered as the outgroup taxa for the phylogeny of *Lactarius indoaquosus* sp. nov. The bootstrap values below 50% were not shown in the phylogenetic trees. The phylogenetic analyses were conducted in MEGA 6.0 (Tamura *et al.* 2013).

RESULTS

Phylogenetic inference

Our ITS-based phylogenetic analysis (Fig. 2) conducted with 36 nucleotide sequences supports (bootstrap 100%) the monophyly of *Lactarius* subg. *Plinthogalus* with *L. deliciosus* and *L. austrotorminosus* of *L.* subg. *Lactarius* as outgroup taxa. It shows that the sequence derived from KD 16-051 (i.e. *Lactarius ferruginascens,* GenBank KY867683) is clearly clustered amongst the other species of *L.* subg. *Plinthogalus*, but is recovered as a distinctly different taxon on a separate branch in a significantly supported (71% bootstrap) clade being sister to the branch bearing two species, *L. lignyotus* and *L. fallax*.

Similarly, our second ITS-based phylogenetic analysis (Fig. 20) undertaken with 56 nucleotide sequences places the taxa of *L.* subg. *Russularia* in a single clade (with *L. friabilis*, *L. fuliginosus* and *L. pterosporus* from *L.* subg. *Plinthogalus* as outgroup taxa). It shows that KD 16-006 (GenBank KY867682) i.e. *L. indoaquosus* is clearly nested in *L.* subg. *Russularia* but turns out to be an individual species on a separate and well supported (76% bootstrap) branch being sister to the clade containing *L. aquosus* and *L. quietus*.

Taxonomy

Lactarius ferruginascens K. Das &Verbeken, **sp. nov.** **Figs 2-19**

MycoBank: MB820868.

Diagnosis: Differing from the related species *L. lignyotus* and *L. crenulatus* by the presence of a glutinous to sticky pileus surface and an ixohymeniderm structure of the pileipellis.

Holotype: INDIA: **Sikkim**, East district, Kyangnosla Alpine Sanctuary, 27°22’21.0’’N, 88°46’31.1’’E, 3352 m asl., 24th Aug. 2016, *K. Das*, KD 16-051(CAL).

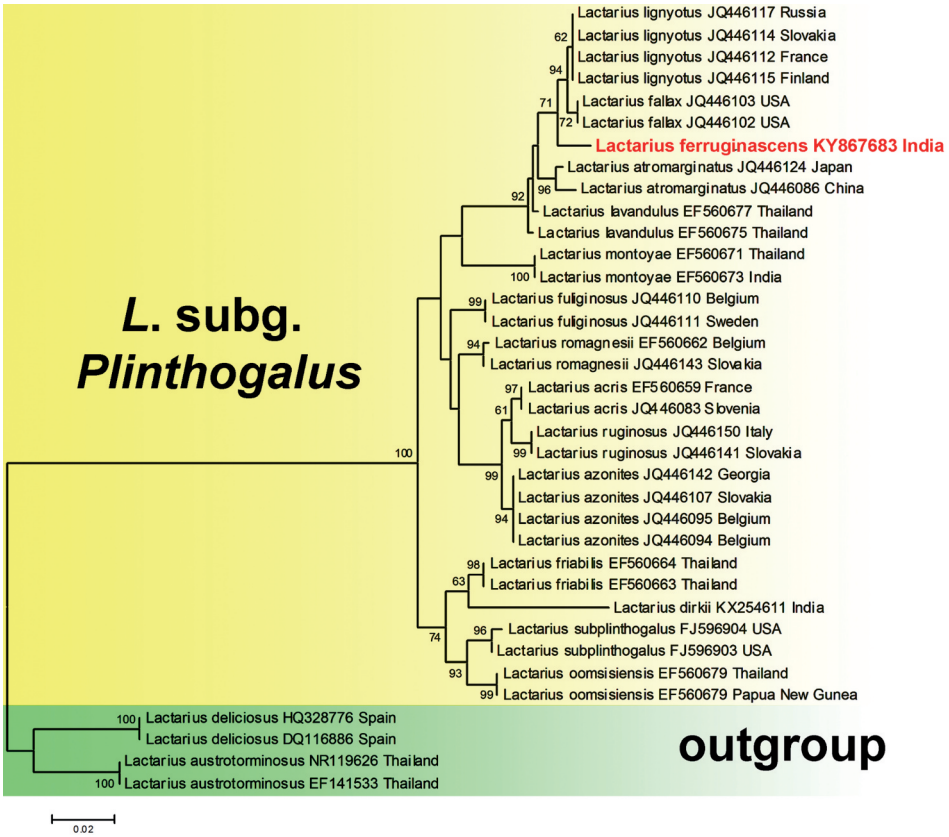
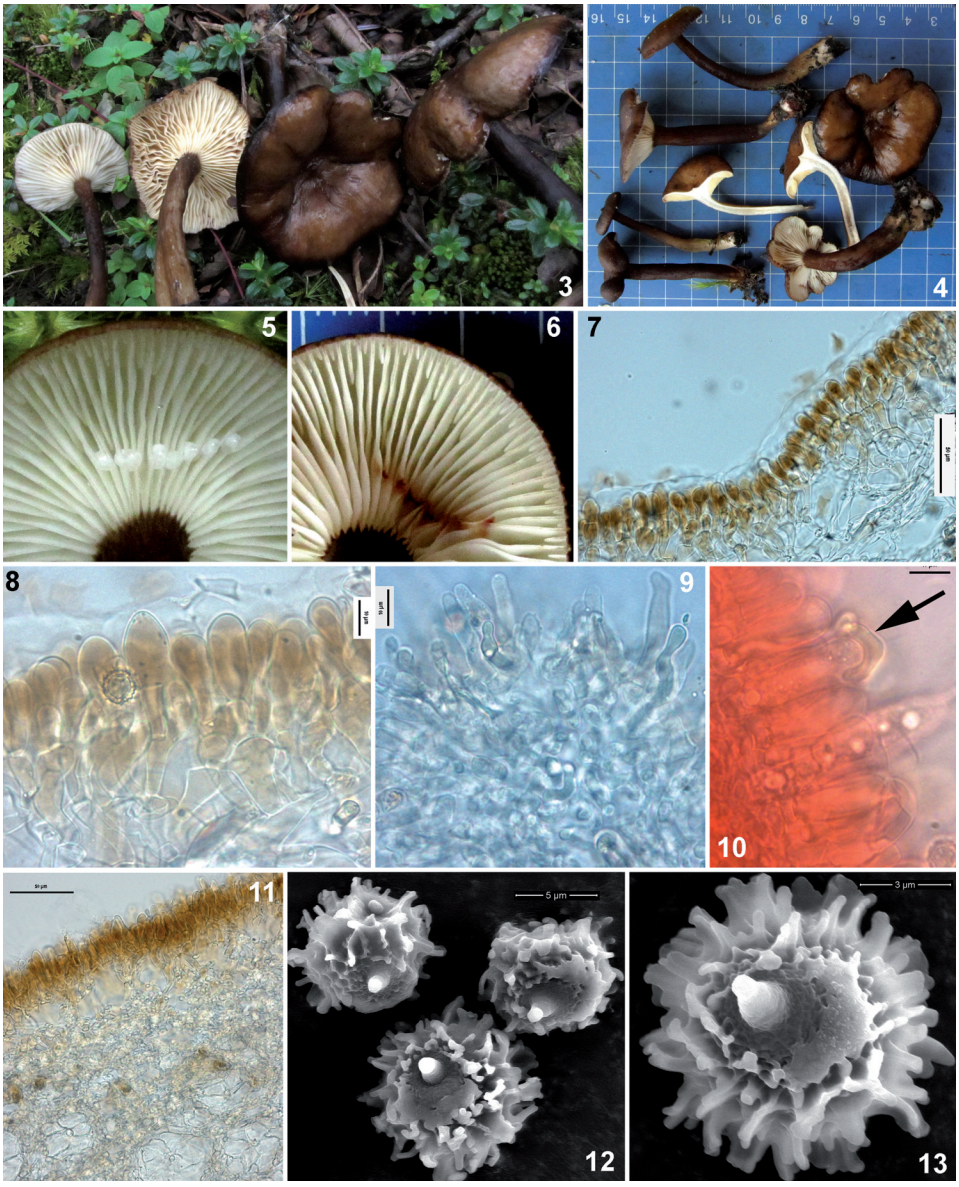


Fig. 2. Phylogram generated from ITS-rDNA sequences: The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [Kimura M. 1980]. The tree with the highest log likelihood (-2179.6198) is shown. *Lactarius ferruginascens* sp. nov. (KD 16-051) having GenBank accession number KY867683 is shown in red color. The *Lactarius deliciosus*, and *Lactarius austrotorminosus* were considered as the out group. Evolutionary analysis was conducted in MEGA6 (Tamura *et al.* 2013).

Etymology: Referring to the latex staining reddish brown to rust color on the lamellae.

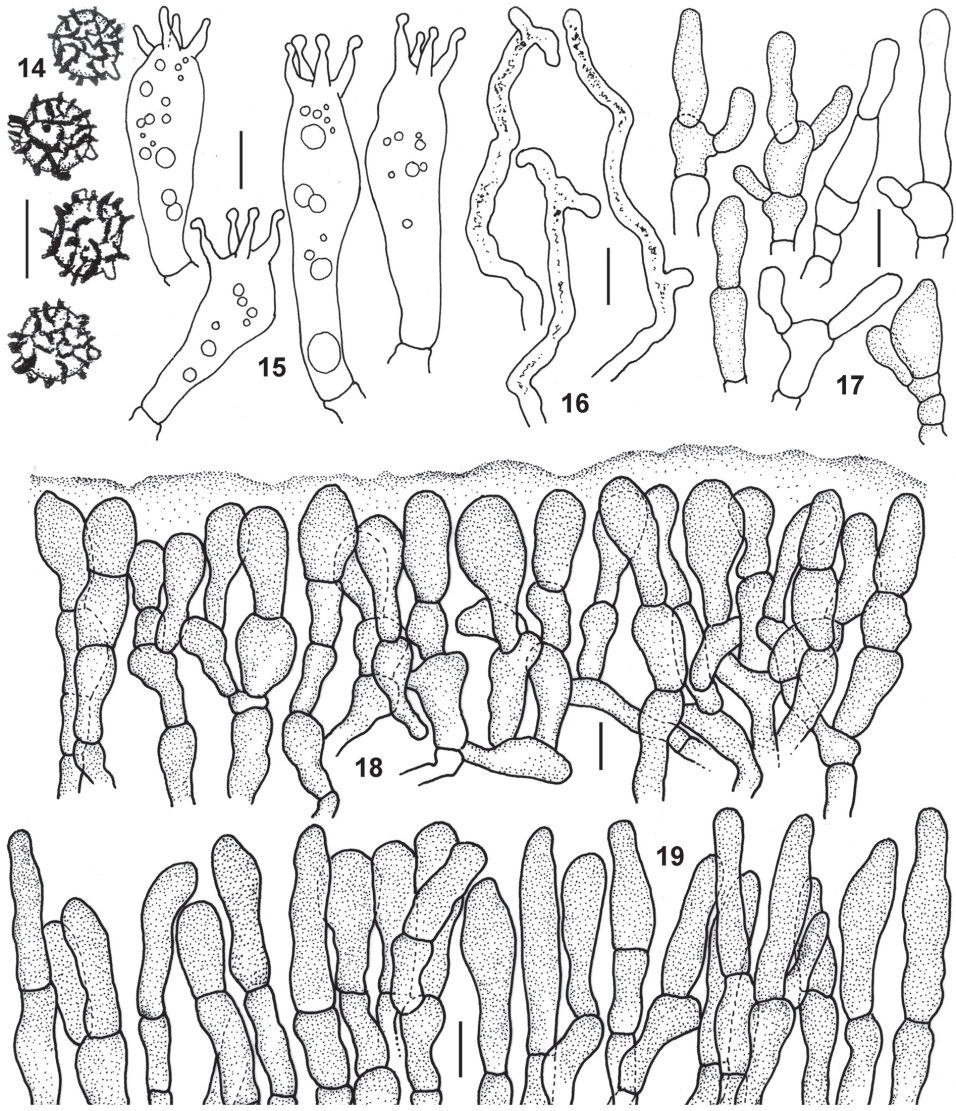
Pileus 18-47 mm diam., at first planoconvex with a central papilla, becoming infundibuliform or uplifted with depressed center and with a small papilla with maturity; surface glutinous, sticky, brown (6E5-8) to rusty brown or chocolate brown to grey; margin slightly incurved when young, decurved at maturity, non-striate, irregularly wavy or undulated to interrupted. **Lamellae** subdecurrent to decurrent, crowded (22-25/cm at pileus margin), never forked, initially white (2A2), gradually pale yellow (4A3) with maturity, with lamellulae in five series; edge entire, greyish brown (6D3) in part. **Stipe** 45-75 × 4-8 mm, central, cylindrical or slightly wider towards base, mostly curved, minutely pruinose, brown (6E4-5), grayish brown (8F3) to dark brown (9F4) or concolorous with the pileus but yellowish white to pale yellow (3A2-3) towards base and white at the extreme base. **Context** white (chalky), hollow or pithy in stipe, changing to orange white (6A2)

with $FeSO_4$, pale yellow (3A3) with KOH and brownish orange (6C4) with guaiac. **Latex** watery white, unchanging when isolated but, becoming brownish red to reddish brown (9C7-9D8) or rusty on cut lamellae after a long exposure, abundant, mild. **Odor** mild, spicy. **Taste** mild. **Spore print** not known.



Figs 3-13. *Lactarius ferruginascens* sp. nov. (KD 16-051, holotype). **3** & **4.** Fresh/dissected basidiomata. **5.** Watery white latex oozing out from the cut lamellae. **6.** Cut lamellae after long exposure. **7** & **8.** Radial section through pileipellis. **9.** Marginal cells at lamellae edge. **10.** Pseudocystidia. **11.** Transverse section through stipitipellis. **12** & **13.** Basidiospores under SEM. Scale bars **7** & **11** = 50 µm; **8**, **9** & **10** = 10 µm; **12** = 5 µm; **13** = 3 µm.

Basidiospores 8.7-**9.3**-10 × 8.1-**8.9**-9.5 µm, (n = 20, Q = 1.00-**1.03**-1.09), globose to subglobose; ornamentation amyloid, 2-2.8 µm high, composed of irregular to regular ridges which are aligned or connected and forming a complete reticulum; plage distinct and strongly distally amyloid; under SEM, margin of ridges with irregular spine-like extremities. **Basidia** 35-57 × 12-15 µm, 2- to 4-spored, clavate to subclavate to subventricose; sterigmata 7-9 × 2-3 µm. **Pleuromacrocystidia** absent. **Pleuropseudocystidia** abundant, irregularly cylindrical to tortuous, often branched at apex, thin-walled, mostly not emergent, 3-6 µm wide, with olivaceous or darker content. **Lamellae edge** sterile. **Cheilomacrocystidia** absent. **Cheilopseudocystidia**



Figs 14-19. *Lactarius ferruginascens* sp. nov. (drawings from holotype: KD 16-051). **14.** Basidiospores. **15.** Basidia. **16.** Pleuropseudocystidia. **17.** Marginal cells at lamellar edge. **18.** Transverse section through pileipellis. **19.** Transverse section through stipitipellis. Scale bars **14-19** = 10 µm.

present, same as pleuropseudocystidia. **Marginal cells** abundant, multiseptate, with terminal elements 12-32 × 4-11 µm, cylindrical to subclavate or subventricose, thin- to slightly thick-walled, often with brown intracellular pigmentation. **Hymenophoraltrama** with lactifers. **Pileipellis** an ixohymeniderm, 70-90 µm thick (including 6-15 µm thick gluten layer); terminal elements cylindrical to subclavate or clavate, 11-37 × 7-18 µm, thin- to slightly thick-walled, with brown intracellular pigmentation. **Stipitipellis** a palisade, 60-75 µm thick, with terminal elements cylindrical to subventricose and thin-walled, with brown intracellular pigmentation. **Stipe trama** with abundant nests of sphaerocytes. **Clamp connections** absent.

Other collection examined: INDIA: **Sikkim**, North district, between Lachung and Yumthang, 27°46'42.4''N, 88°42'47.4''E, 3522 m asl., 27th Aug. 2011, *K. Das*, KD 16-119 (CAL).

Comments: The combination of dark pigmented, often brown to grey pileus and stipe, basidiospores with high ornamentation, absence of macrocystidia and presence of abundant dark pigmented marginal cells fit well for a placement in *L.* subg. *Plinthogalus*. An ixohymeniderm is exceptional in this group, but also occurs in the African *L. sulcatulus* Verbeken.

Morphologically, *L. crenulatus* K. Das & Verbeken, which was also reported from Sikkim, reminds *L. ferruginascens* in the field. The former can be separated by the smaller pileus with absence of glutinous or sticky surface, the smaller stipe (40-57 × 3.5-5 mm), watery white latex drying pinkish on cut lamellae, a hymeniderm without glutinous layer as pileipellis structure and the occurrence under *Castanopsis* sp., a broadleaf tree (Das &Verbeken 2012).

Three species from this same subgenus, *L. fumosus* Peck, *L. subvernalis* var. *albo-ochraceous* Hesler & A.H. Sm., and *L. subvernalis* var. *himalayensis* Atri, Siani & M.K. Saini were reported earlier from this subcontinent and appear to be close to *L. ferruginascens* (though for the first two species the conspecificity between North American and Indian taxa have not yet been confirmed through molecular studies). Morphologically, all three species can be distinguished from *L. ferruginascens* as they do not have an ixohymeniderm as a pileipellis, which is also reflected in the macroscopical aspect because they are not glutinous or sticky. Moreover, *L. fumosus* (Rawla 2002) has hyaline cheilocystidia and unbranched filamentous pleuropseudocystidia, whereas both *L. subvernalis* var. *albo-ochraceus* and *L. subvernalis* var. *himalayensis* possess pleuro- as well as cheilomacrocystidia, a rare character in *L.* subg. *Plinthogalus* (Atri *et al.* 1990; Atri *et al.* 1993).

Lactarius lignyotus Fr. and *L. fallax* A.H. Sm. & Hesler (both showing 97% identity under 100% query coverage) appeared as closest relatives of the new species in our phylogenetic analysis. Both in *L. lignyotus* and *L. fallax*, the injured lamellae turn dirty pinkish to reddish as in our new species, but they are separated from it in the field by the more robust nature of the basidiomata and the dry (not sticky) aspect of the pileus surface (always glutinous and sticky in *L. ferruginascens*) (Heilmann-Clausen *et al.* 1998; Kränzlin 2005). Micromorphologically, the hymeno-epithelium structure of the pileipellis and the basidiospores with comparatively low ornamentation are distinct for *L. lignyotus*, whereas *L. fallax* has a trichoderm structure as pileipellis and stipitipellis of "vertical-interwoven hyphae" (Hesler & Smith 1979).

Lactarius indoaquosus K. Das, Verbeken & A. Baghela, **sp. nov.** **Figs 20-38**

MycoBank: MB820869.

Diagnosis: Distinct from the morphologically similar *L. aquosus* by the larger azonate pileus, absence of pleuromacrocystidia and a hymeniderm to trichopalisade as pileipellis structure.

Holotype: INDIA: **Sikkim**, South district, Maenam Wild Life Sanctuary, 27°18’44.1’’N, 88°21’53.6’’E, 2096 m asl., 16th Aug. 2016, *K. Das*, KD 16-006 (CAL).

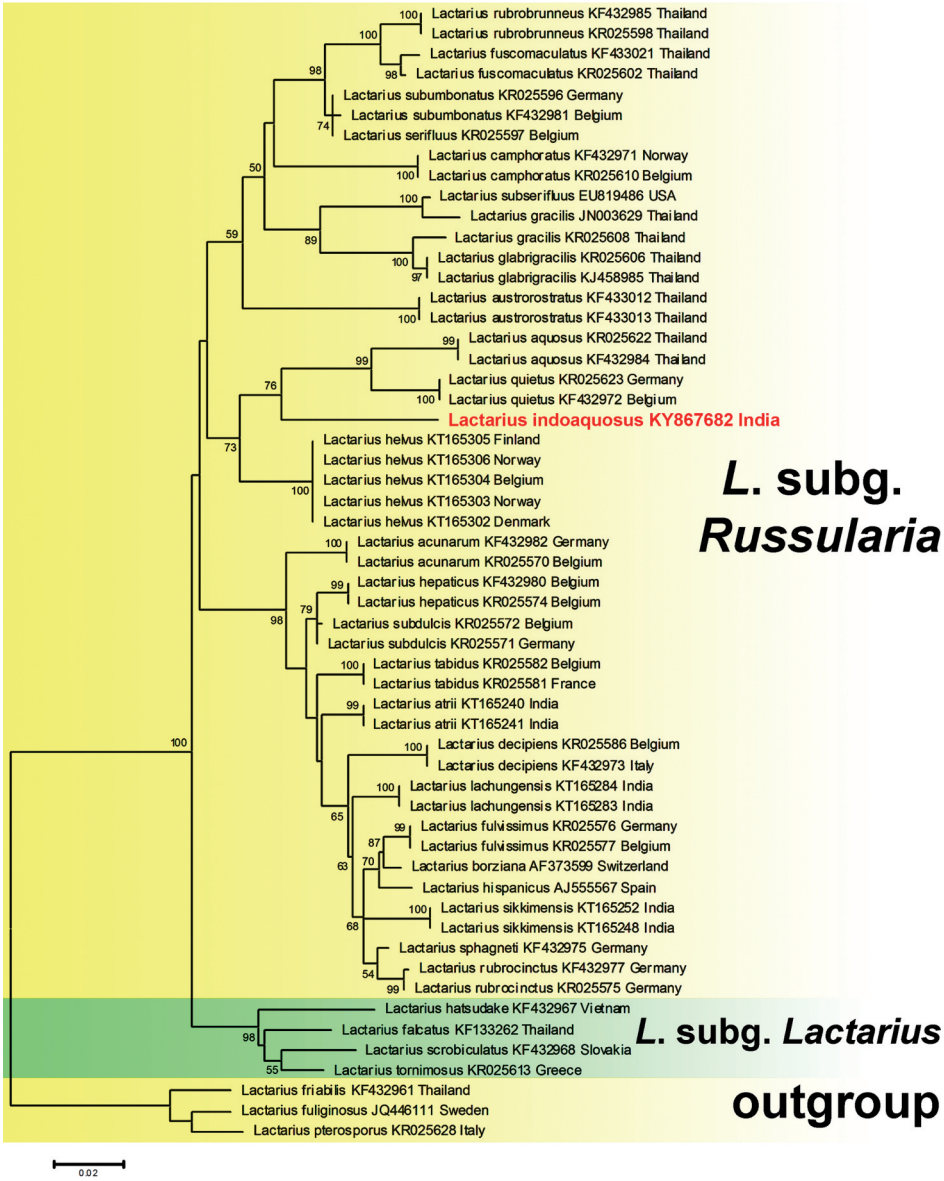
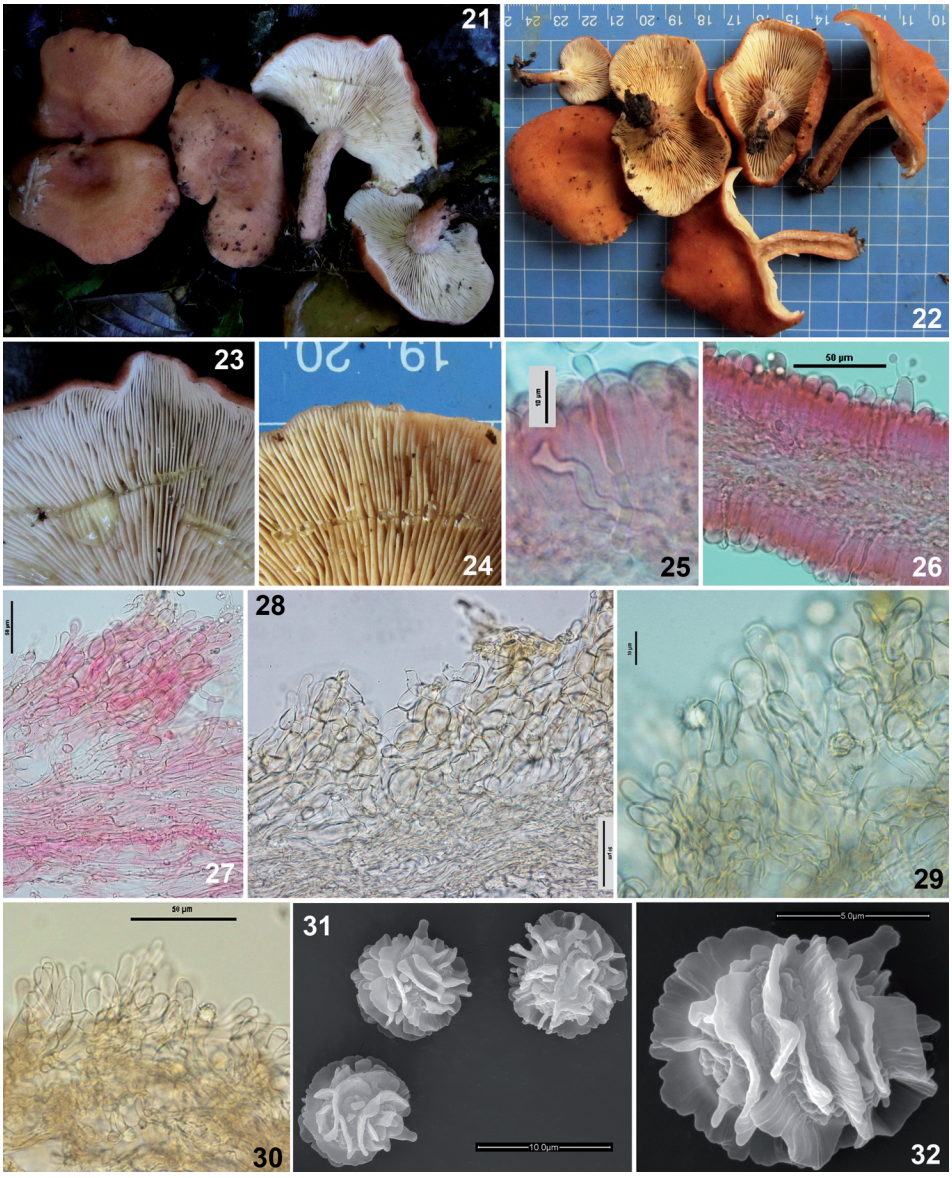


Fig. 20. Phylogram generated from ITS-rDNA sequences: The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [Kimura M. 1980]. The tree with the highest log likelihood (-4211.5117) is shown. *Lactarius indoaquosus* sp. nov. (KD 16-006) having GenBank accession number KY867682 is shown in red color. The *Lactarius friabilis*, *L. fuliginosus*, and *L. pterosporus* were considered as the out group. Evolutionary analysis was conducted in MEGA6 (Tamura *et al.* 2013).

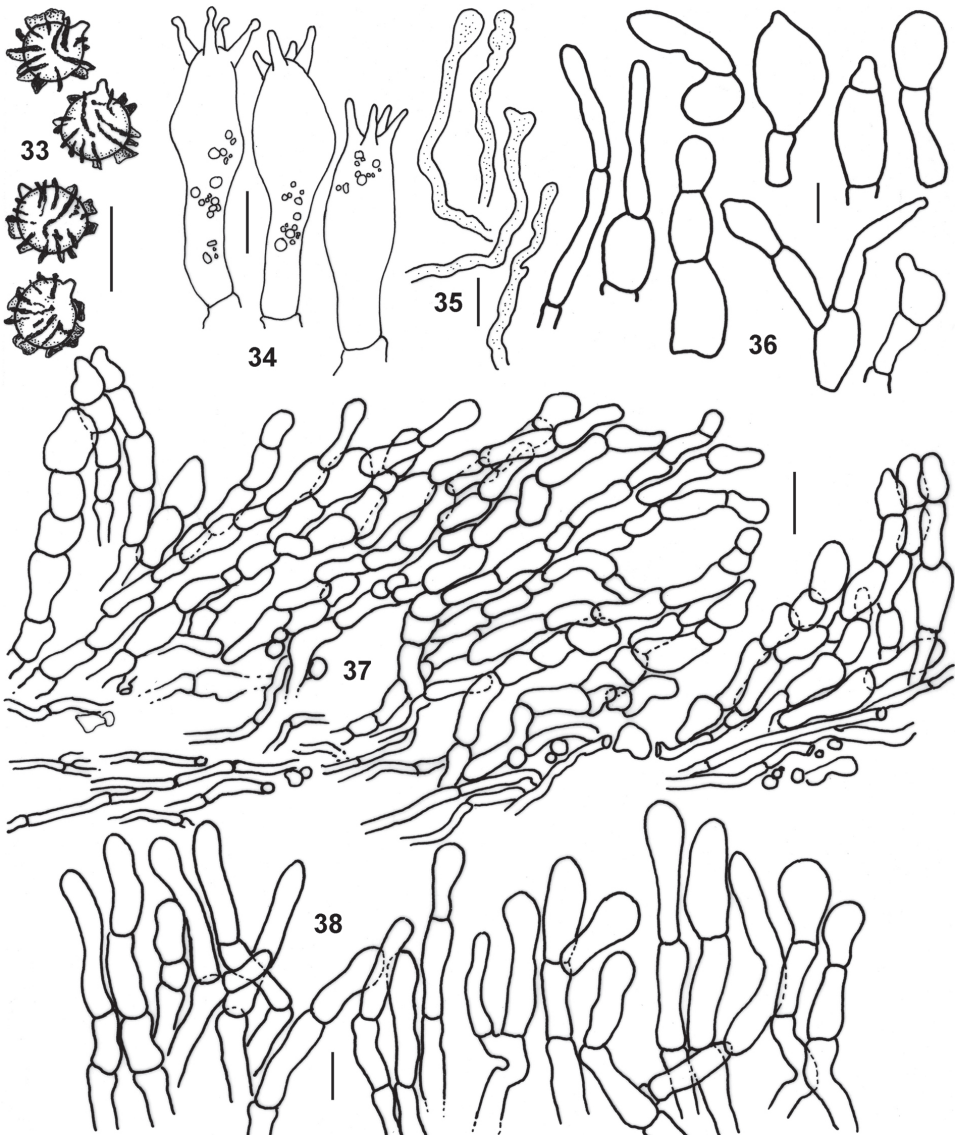
Etymology: Referring to the locality (indo-) and the completely transparent latex (aquosus).

Pileus 25-65 mm diam., convex with slightly depressed center when young, gradually more depressed and often with a small umbo at the center but never



Figs 21-32. *Lactarius indoaquosus* sp. nov. (KD 16-006). **21** & **22.** Fresh/dissected basidiomata. **23** & **24.** Transperant (colorless) latex oozing out from the cut lamellae. **25** & **26.** Hymenium layer showing pleuropseudocystidia, basidia & basidioles. **27** & **28.** Radial section through pileipellis. **29** & **30.** Transverse section through stipitipellis. **31** & **32.** Basidiospores under SEM. Scale bars **25**, **29** & **31** = 10 µm; **26**, **27**, **28** & **30** = 10 µm; **32** = 5 µm.

infundibuliform; margin incurved to decurved but irregularly wavy or lobed; surface smooth, moist, never viscid or sticky, brownish orange, light brown to reddish brown (7-8D6-7). **Lamellae** adnexed or rarely subdecurrent, crowded (20-25/cm at pileus margin), initially white (1A1), becoming pale orange (5A3) after sometime or with age, with lamellulae in 9-10 series; edge entire, concolorous. **Stipe** 26-40 × 4-9 mm, mostly eccentric, slender, brittle, cylindrical or gradually tapered towards base;



Figs 33-38. *Lactarius indoaquosus* sp. nov. (drawings from holotype: KD 16-006). **33.** Basidiospores. **34.** Basidia. **35.** Pleuropseudocystidia. **36.** Terminal and subterminal cells of the elements in pileipellis. **37.** Transverse section through pileipellis. **38.** Transverse section through stipitipellis. Scale bars **33-36**, **38** = 10 μm; **37** = 25 μm.

surface smooth, dry, strigose (hairy) at base, brownish orange to light brown (7C-D5), changing greenish grey to dull green (28D2-3) with guaiac and grey (28D1) with $FeSO_4$. **Context** pithy in stipe, brownish orange (6C4), slightly darker sometimes after exposure. **Latex** abundant, watery, colorless (transparent), unchanging when isolated but, slightly yellowish shortly after exposure. **Odor** not distinctive. **Spore print** pale cream (3A2).

Basidiospores 7.1-**7.95**-9.0 × 6.8-**7.62**-8.4 µm, [Q = 1.00-1.04-1.09(1.13)], globose to subglobose; ornamentation amyloid, 2-2.3 µm high, composed mostly of parallel to subparallel ridges which are aligned and form a zebroid pattern; under SEM with short or medium, irregular to regular ridges which are aligned or connected forming a clear zebroid pattern, with some isolated small warts; plage indistinct and inamyloid. **Basidia** 30-44 × 9-13 µm, 4-spored, subclavate to ventricose; sterigmata 5-8.5 × 1.5-3 µm. **Pleuromacrocystidia** absent. **Pleuropseudocystidia** 5-6 µm wide, cylindrical with subclavate, subcapitate or irregular apex, sometimes with frequent constrictions towards apex, 5-8 µm emergent. **Lamellae edge** fertile, mostly with basidioles, basidia and cheilopseudocystidia. **Cheilomacrocystidia** absent. **Cheilopseudocystidia** filamentous. **Subhymenium** 18-25 µm thick, pseudoparenchymatous. **Marginal cells** 11-20 × 7-11 µm, mostly clavate to subclavate, often multiseptate. **Hymenophoral trama** with lactifers. **Pileipellis** a hymeniderm to trichopalisade, 130-250 µm thick, composed mostly of hyphal to inflated cells arranged in chains; terminal cells 10-50 × 8-39 µm, cylindrical to appendiculate, pyriform, clavate to conical, thick-walled; wall 1-1.5 µm thick. **Pileus** trama with numerous nests of sphaerocytes. **Stipitipellis** 90-120 µm thick, a trichopalisade, composed of erect, frequently septate, thin- to slightly thick-walled hyphae; terminal cells cylindrical, narrowly to broadly clavate or bulbous. **Clamp** connections absent.

Comments: The orange to brown colored pileus and stipe, unchanging latex and hymeniderm to trichopalisade structure of the pileipellis fit well for *L.* subg. *Russularia*.

Lactarius aquosus H.T. Le & K.D. Hyde, reported from Thailand and sharing the exudation of transparent latex, looks similar to *L. indoaquosus* in the field but the former differs in a smaller (21-37 mm diam.) zonate pileus, presence of pleuromacrocystidia and a cutis as pileipellis structure (hymeniderm to trichopalisade in the present species) (Wisitrassameewong *et al.* 2015).

Lactarius helvus, a European species with transparent latex appears to be closest (showing only 94% identity under 100% query coverage) to the present undescribed species in our phylogenetic analysis but is morphologically very different. *Lactarius helvus* has distinctly larger basidiomata (pileus 25-130 mm diam., stipe 15-100 × 6-22 mm) with faintly zonate, pinkish buff to yellowish brown, very dry pileus, basidiospores with low (0.5-1.0 µm) ornamentations, presence of pleuro- and cheilomacrocystidia and a trichoderm to cutis as a pileipellis structure (Heilmann-Clausen *et al.* 1998). Furthermore, *L. helvus* is recognized by the strong odour of fenugreek, celery or maggi while our new species has a mild spicy odour.

Two Asian species possessing reddish-brown pileus and zebroid ornamentation of basidiospores, e.g. *Lactarius verbekenae* K. Das, J.R. Sharma & Montoya and *L. chichuensis* W.F. Chiu may be confused with *L. indoaquosus*. But, unlike the latter one both *L. verbekenae* (originally reported from India) and *L. chichuensis* (originally reported from China) have colored (never transparant) latex ("yellowish-white" in *L. verbekenae* and "milk white" in *L. chichuensis*), smaller basidiospores with distinctively lower ornamentations ["6-7.8 × 5.8-7 µm" with ornamentations "up to 1.5 µm" high in *L. verbekenae* and "(5.7-)6.2-7.5(-8.5)

× (5.2-)5.7-6.6(-8.0) µm" with ornamentations "up to 1 µm" high in *L. chichuensis*] and presence of pleuromacrocystidia in the hymenium layer (Wang & Liu 2002; Das *et al.* 2004).

Two other Asian species with a zebroid spore ornamentation but with a more brownish and less reddish cap are *L. corrugatus* Verbeken & E. Horak and *L. castanopsis* Hongo. *Lactarius corrugatus* (originally reported from Papua New Guinea) has basidiospores with remarkably abundant short ridges and warts between the wings, which is not the case in *L. indoaquosus*; it furthermore differs by the completely cellular pileipellis and sterile lamellae edge. In the field, *L. corrugatus* is recognized by the strong *L. quietus*-like smell. *L. castanopsis* has larger spores and remarkably large and fusoid to mucronate cheilocystidia.

Several Indian *Lactarius*, namely *L. sikkimensis* Verbeken & K. Das, *L. atrii* Van de Putte & K. Das, *L. lachungensis* Verbeken & Van de Putte and *L. flavigalactus* Verbeken & K. Das, also appear to be genetically close to our species but these four species have white to whitish latex. Moreover, *L. sikkimensis* has an ixotrichopalisade, *L. atrii* and *L. flavigalactus* have an ixocutis and *L. lachungensis* has an ixohyphoepithelium to ixotrichoepithelium as pileipellis structure (Wisitrassameewong *et al.* 2016).

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REFERENCES

ATRI N.S., SAINI S.S. & MANN D.K., 1990 — Studies on North West Indian Agarics: The genus *Lactarius*. *Indian Phytopathology* 44: 185-192.

ATRI N.S., SAINI S.S., SAINI M.K. & GUPTA A.K., 1993 — Systematic studies on Russulaceous fungi – The genus *Lactarius* Pers. *Journal of Indian Botanical Society* 72: 155-158.

ATRI N.S., SHARMA S., SAINI M.K. & DAS K, 2016 — Researches on Russulaceous Mushrooms-An Appraisal. *Kavaka* 47: 63-82.

ALTSCHUL S.F., MADDEN T.L., SCHÄFFER A.A, ZHANG J., MILLER W. & LIPMANN D.J., 1997 — Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389-3402.

BHUPATHY S., CHETTRI B. & BAUER A.M., 2009 — Rediscovery and revalidation of *Takydromus sikkimensis* (Günther, 1888) (Squamata: Lacertidae) from Sikkim, India. *Journal of Herpetology* 43(2): 267-274.

CLARK K., KARSCH-MIZRACHI I., LIPMAN D.J., OSTELL J. & SAYERS E.W., 2016 — GenBank. *Nucleic Acids Research* 44: D67-D72.

DAS K. & CHAKRABORTY D., 2014 — *Lactarius vesterholtii*, a new species from India. *Mycotaxon* 129(2): 477-484.

DAS K., GHOSH A., CHAKRABORTY D., LI J., QIU L., BAGHELA A., HALAMA M., HEMBROM M.E., MEHMOOD T., PARIHAR A., PENCAKOWSKI B., BIELECKA M., RECZYNSKA K., SASIELA D., SINGH U., SONG Y., ŚWIERKOSZ K., SZCZĘŚNIAK K., UNIYAL P., ZHANG J. & BUYCK B., 2017 — Fungal Biodiversity Profiles 31-40. *Cryptogamie, Mycologie* 38(3): 353-406.

DAS K., SHARMA J.R. & MONTOYA L., 2004 — *Lactarius* (*Russulaceae*) in Kumaon Himalaya. 1. New species of subgenus *Russularia*. *Fungal Diversity* 16: 23-33.

DAS K., VAN DE PUTTE K. & BUYCK B., 2010 — New or interesting *Russula* from Sikkim Himalaya (India). *Cryptogamie, Mycologie* 31(4): 373-387.

DAS K. & VERBEKEN A., 2011 — Three new species of *Lactarius* (Russulaceae) from Sikkim, India. *Cryptogamie, Mycologie* 32(4): 365-381.

DAS K. & VERBEKEN A., 2012 — New species of *Lactarius* subg. *Plinthogalus* and new records of *Lactifluus* subg. *Gerardii* (Russulaceae) from Sikkim, India. *Taiwania* 57(1): 37-48.

DAS K., VERBEKEN A. & NUYTINCK J., 2015 — Morphology and phylogeny of four new *Lactarius* species from Himalayan India. *Mycotaxon* 130(1): 105-130.

DE CROP E., NUYTINCK J., VAN DE PUTTE K., WISITRASSAMEEWONG K., HACKEL J., STUBBE D., HYDE K.D., ROY M., HALLING R.E. & MOREAU P.A., 2017 — A multi-gene phylogeny of *Lactifluus* (Basidiomycota, Russulales) translated into a new infrageneric classification of the genus. *Persoonia* 38: 58-80.

GEML J., LAURSEN G.A., HERRIOTT I.C., MCFARLAND J.M., BOOTH M.G., LENNON N., NUSBAUM H.C. & TAYLOR D.L., 2009 — Phylogenetic and ecological analyses of soil and sporocarp DNA sequences reveal high diversity and strong habitat partitioning in the boreal ectomycorrhizal genus *Russula* (Russulales; Basidiomycota). *Molecular Ecology* 18: 2213-2227.

GEML J., TIMLING I., ROBINSON C.H., LENNON N., NUSBAUM H.C., BROCHMANN C., NOORDELOOS M.E. & TAYLOR, D.L., 2012 — An arctic community of symbiotic fungi assembled by long-distance dispersers: Phylogenetic diversity of ectomycorrhizal basidiomycetes in Svalbard based on soil and sporocarp DNA. *Journal of Biogeography* 39(1): 74-88. DOI: 10.1111/j.1365-2699.2011.02588.x

HEILMANN-CLAUSEN J., VERBEKEN A. & VESTERHOLT J., 1998 — The genus *Lactarius*. The Danish Mycological Society, Denmark.

HESLER L.R. & SMITH A.H., 1979 — *North American species of Lactarius*. The University of Michigan Press, Ann Arbor.

HYDE K.D., HONGSANAN S., JEEWON R., BHAT D.J., MCKENZIE E.H.C., JONES E.B.G., PHOOKAMSAK R., ARIYAWANSA H.A., BOONMEE S., ZHAO Q., ABDEL-AZIZ F.A., ABDEL-WAHAB M.A., BANMAI S., CHOMNUNTI P., CUI B.K., DARANAGAMA D.A., DAS K., DAYARATHNE M.C., DE SILVA N.I., DISSANAYAKE A.J., DOILOM M., EKANAYAKA A.H., GIBERTONI T.B., GÓES-NETO A., HUANG S.K., JAYASIRI S.C., JAYAWARDENA R.S., KONTA S., LEE H.B., LI W.J., LIN C.G., LIU J.K., LU Y.Z., LUO Z.L., MANAWASINGHE I.S., MANIMOHAN P., MAPOOK A., NISKANEN T., NORPHANPHOUN C., PAPIZADEH M., PERERA R.H., PHUKHAMSAKDA C., RICHTER C., SANTIAGO A.L.C.M. de A., DRECHSLER-SANTOS E.R., SENANAYAKE I.C., TANAKA K., TENNAKOON T.M.D.S., THAMBUGALA K.M., TIAN Q., TIBPROMMA S., THONGBAI B., VIZZINI A., WANASINGHE D.N., WIJAYAWARDENE N.N., WU H.X., YANG J., ZENG X.Y., ZHANG H., ZHANG J.F., BULGAKOV T.S., CAMPORESI E., BAHKALI A.H., AMOOZEGAR A.M., ARAUJO-NETA L.S., AMMIRATI J.F., BAGHELA A., BHATT R.P., BOJANTCHEV S., BUYCK B., DA SILVA G.A., DE LIMA C.L.F., DE OLIVEIRA R.J.V., DE SOUZA C.A.F., DAI Y.C., DIMA B., DUONG T.T., ERCOLE E., MAFALDA-FREIRE F., GHOSH A., HASHIMOTO A., KAMOLHAN S., KANG J.C., KARUNARATHNA S.C., KIRK P.M., KYTOVUORI I., LANTIERI A, LIIMATAINEN K, LIU ZY, LIU XZ, LÜCKING R., MEDARDI G., MORTIMER P.E., NGUYEN T.T.T., PROMPUTTHA I., RAJ K.N.A., RECK M.A., LUMYONG S., SHAHZADEH-FAZELI S.A., STADLER M., SOUDI M.R., SU H.Y., TAKAHASHI T., TANGTHIRASUNUN N., UNIYAL P., WANG Y., WEN T.C., XU J.C., ZHANG Z.K., ZHAO Y.C., ZHOU J.Z.& ZHU L., 2016 — Fungal diversity notes 367-490: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* 80(1): 1-270.

KIMURA M., 1980 — A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16(2): 111-120.

KORNERUP A. & WANSCHER J.H., 1981 — Methuen handbook of colour. 3rd Ed. London, Methuen.

LE H.T., STUBBE D., VERBEKEN A., NUYTINCK J., LUMYONG S. & DESJARDIN D.E., 2007 — *Lactarius*in Northern Thailand: 2. *Lactarius* subgenus *Plinthogali*. *Fungal Diversity* 27: 61-94.

LEE H., PARK M.S., JUNG P.E., FONG J.J., OH S-Y., VERBEKEN A. & LIM Y.W., 2015 — *Lactarius cucurbitoides* (Russulales, Basidiomycota), a new species from South Korea supported by molecular and morphological data. *Phytotaxa* 205(3): 168-176.

MITTERMEIER R.A., GIL P.R., HOFFMAN M., PILGRIM J., BROOKS T., MITTERMEIER C.G., LAMOREAUX J. & DA-FONSECA G.A.B., 2005 — *Hotspots revisited*. [Earth's biologically richest and most endangered terrestrial ecor-egions]. Conservation International, Arlington.

RAWLA G.S., 2002 — *Lactarius* DC ex S.F. Gray in India – list and critical review. In: Pullaiah T (ed) Biodiversity in India. Regency Publications, New Delhi, India, pp 221-255.

STUBBE D. & VERBEKEN A., 2012 — *Lactarius* subg. *Plinthogalus*: the European taxa and American varieties of *L. lignyotus* re-evaluated. *Mycologia* 104: 1490-1501.

TAMURA K., NEI N. & KUMAR S., 2004 — Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences of the United States of America* 101(30): 11030-1135.

TAMURA K., STECHER G., PETERSON D., FILIPSKI A. & KUMAR S., 2013 — MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725-2729.

THIERS B., (continuously updated) Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium [online]. Available at http://sweetgum.nybg.org/ih/.

VAN DE PUTTE K., NUYTINCK J., DAS K. & VERBEKEN A., 2012 — Exposing hidden diversity by concordant genealogies and morphology—a study of the *Lactifluus volemus* (Russulales) species complex in Sikkim Himalaya (India). *Fungal Diversity* 55: 171-194.

VERBEKEN A. & NUYTINCK J., 2013 — Not every milkcap is a *Lactarius. Scripta Botanica Belgica* 51: 162-168.

WANG X-H. & LIU P-G., 2002 — *Lactarius chichuensis* and *L. hirtipes*, two easily confused species. *Mycotaxon* 84: 391-400.

WHITE T.J., BRUNS T., LEE S. & TAYLOR J., 1990 — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds), PCR Protocols: a guide to method and applications. Academic Press, San Diego, pp 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* 30: 1-28.

WISITRASSAMEEWONG K., NUYTINCK J., LE H.T., DE CROP E., HAMPE F., HYDE K.D. & VERBEKEN A., 2015 — *Lactarius* subgenus *Russularia* (Russulaceae) in South-East Asia: 3. new diversity in Thailand and Vietnam. *Phytotaxa* 207(3): 215-241.