Three new species of *Lactarius* (Russulaceae) from Sikkim, India

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Abstract – Since 2008, fungal explorations have been undertaken in a mycologically unexplored area: West district of Sikkim (India), located in the Esatern Himalaya. Three *Lactarius* species are proposed here as new taxa: *Lactarius elaioviscidus*, *L. ermineus* and *L. byssaceus*. Extended descriptions and illustrations are given; the taxonomic position and relation to allied species is discussed.

Macrofungi / India / Russulales / Lactarius / taxonomy

INTRODUCTION

Sikkim is a small (0.22% of geographical area of this country) hilly state located in the Eastern Himalaya in India. Keeping in view the enormous altitudinal (300-8585 m) and climatic variations supported by plenty of ectomycorrhizal hosts, the expected diversity of macrofungi in this mycologically almost unexplored area is high. Being a part of one (Himalaya Hotspot) of the 34 Global Biodiversity Hotspots (www.biodiversityhotspots.org), Sikkim is substantially diverse in plants and animals, and undoubtedly also in fungi, although, their diversity is seriously understudied. The West District, locating in the West of this state is stretched between N 27°07' to N 27°37' and E 88°01' to E88°22' and covers an area of 1166 sq. km. The elevation in the district ranges from 350 m to 7000 m. This district experiences a wide range of climatic zones: tropical (up to 500 m), subtropical (500-1500 m), temperate (1500-2700 m), sub-alpine (2700-5000 m) and alpine (above 5000 m) and is rich in the ectomycorrhizal host trees like, Lithocarpus pachyphyllus Rehder, Castanopsis tribuloides A. DC., C. hystrix A. DC., Quercus lamellosa Sm., Alnus nepalensis D. Don, Betula utilis D. Don, Schima wallichii Choisy, Abies densa Griff., A. spectabilis Spach, Tsuga dumosa Eichl., Pinus wallichiana A.B. Jacks. etc, that supports enormous growth and development of ectomycorrhizal mushrooms.

While concentrating on the West district of Sikkim, explorations have been undertaken regularly since 2008 to reveal the diversity of macrofungal flora (Das & Mishra 2009, Das & Sharma 2009-10, Das et al. 2010, Das et al. in press) with special reference to Russulales, one of the most important ectomycorrhizal groups. *Lactarius* is one of the common genera in the Himalayan forests and

13 new taxa namely, Lactarius princeps Berk., L. himalayanus Rawla & Sarwal, L. abbotanus K. Das & J.R. Sharma, L. mayawatianus K. Das & J.R. Sharma, L. dwaliensis K. Das, J.R. Sharma & Verbeken, L. maitlyensis K. Das, J.R. Sharma & Verbeken, L. dafianus V. Das, J.R. Sharma & Verbeken, L. sanjappae K. Das, J.R. Sharma & Montoya, L. mukteswaricus K. Das, J.R. Sharma & Montoya, L. verbekenae K. Das, J.R. Sharma & Montoya, L. montoyae K. Das & J.R. Sharma, L. capitatus K. Das, J.R. Sharma & Montoya and L. dhakurianus K. Das, Basso & J.R. Sharma have already been described from Eastern Himalaya (Berkeley 1852) and Western Himalaya (Rawla & Sarwal 1983, Sharma & Das 2003, Das et al. 2003, Das & Sharma 2004, Das et al. 2004a, Das et al. 2004b, Das et al. 2005) respectively. During a macrofungal foray to West district of Sikkim (Eastern Himalaya) in the year 2010, subtropical to subalpine areas like Yuksom, Dubdi, Khechiperi, Pemangtse, Bharen, Hilltok, Takredara, Hilley, Barsey and Tal were surveyed and a number of wild mushrooms were collected by one of us (KD). Out of these collections, several appeared to belong to undescribed taxa. In the present contribution, three of the *Lactarius* species are proposed as new to science: Lactarius elaioviscidus, L. ermineus and L. byssaceus. They were collected from two of the above mentioned sites namely, Hilltok and Yuksom. The first site is a temperate mixed forest (2200-2300 m) dominated mainly, by Castanopsis hystrix, Ilex dipyrena Wall., Eurya cerasifolia (D. Don) Kobuski, Tsuga dumosa, Pinus wallichiana, Cryptomeria japonica D. Don, Macaranga denticulata Müll. Arg. and Alnus sp. The second site is a subtropical to temperate broad-leaved forest (1690-1725 m) mainly dominated by Castanopsis tribuloides, C. hystrix, Prunus cerasoides D. Don, Michelia velutina Blume, Engelhardtia spicata Blume, Eurya cerasifolia (D. Don) Kobuski, Camellia kissi Wall., Macaranga denticulata, Ficus roxburghii Wall, and Alnus nepalensis.

MATERIALS & METHODS

Macromorphological characters were recorded from the fresh basidiomata. Colour codes and terms following Colour identification chart of the Flora of British fungi, edited by Her Majesty's Stationery Office, Edinburgh (1969) indicated in the descriptions as "a" and Kornerup & Wancher (1981), indicated in the descriptions as "b". Kränzlin (2005) was used for the colours of the spore prints and is referred to in the descriptions as "c". Field photographs of the fresh basidiomata were taken with Nikon D300s.

Micromorphological characters were observed from the dry samples mounted in a mixture of 5% KOH, 1% Phloxin, Congo red and 30% Glycerol and Melzer's reagent. Drawings of basidiospores were made mainly at 6000x & 2000x magnification and other micromorphological structures were drawn at an original magnification of 1000x. Basidium length excludes sterigmata, gill-density includes lamellae and lamellulae and spore-dimensions exclude the dimension of the ornamentations. Basidiospore measurements are based on twenty basidiospores. Spores are measured in side view and sizes are given as KDa-KDc-KDb \times KDx-KDz-KDy in which KDa = minimum value for the length of measured collections, KDb = maximum value for the length of measured collections, KDc = mean value for the length of measured collections, KDy = maximum value for the width of measured collections, KDy = maximum value for the width of measured

collections, KDz = mean value for the width of the measured collections. Quotient of spore indicates lenght-width ratio (Q = L/W) and is given as Qa-Qc-Qb where Qa = minimum quotient value amongst measured collections, Qb = maximum quotient value amongst measured collections, Qc = mean quotient value amongst measured collections. Scanning Electron Microscope (SEM) illustrations of basidiospores were obtained from dry spores from spore print that were directly mounted on a double-sided adhesive tape pasted on a metallic specimen-stub and then scanned with gold coating at different magnifications in high vacuum mode to observe patterns of spore-ornamentation. SEM work was carried out with a FEI's Quanta 200 model imported from The Netherlands and installed at the Bose Institute, Kolkata, India. Herbarium names follow Holmgren et al. (1990).

RESULTS

Lactarius elaioviscidus K. Das & Verbeken sp. nov.

Figs 1-8, 17-20

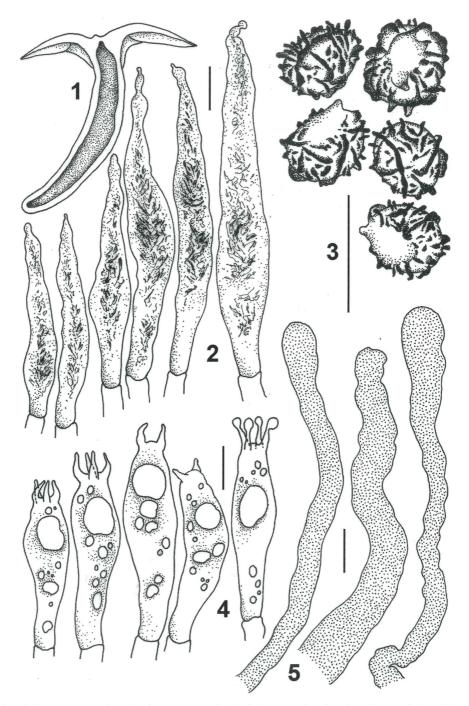
MycoBank: 561651

Ethymology: Referring the colour and nature of pileus i.e. olive and viscid to glutinous.

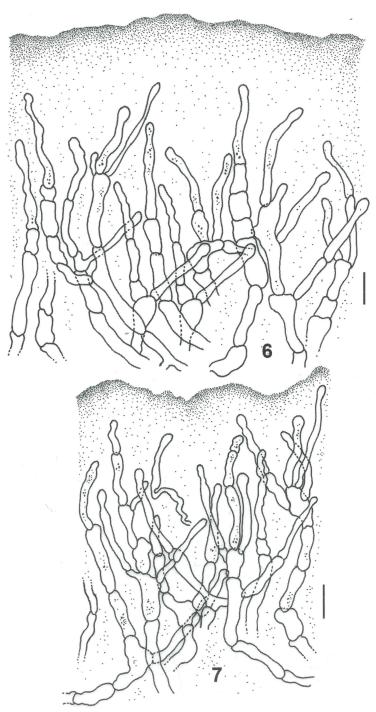
Pileus 35-53 mm diam., convexus ad planoconvexum, dein depressus, interdum papilla conica, glutinosus, viscidus, griseo-viridis ad pallide olivaceum. Lamellae densae, late adnatae vel decurrentes, luteoalbae. Stipes 45-62 \times 8-10 mm, subcylindricus, griseoluteus. Latex aquosus vel albus, immutabilis. Basidiosporae in cumulo subluteae, 7.0-8.1-8.7 \times 6.0-6.7-7.1 μ m, subglobosae vel ellipsoideae, amyloideae, reticulatae, cristis acutis usque ad 1 μ m altis ornamentae. Basidia 40-50 \times 11-14 μ m, bi- vel tetraspora. Pleuromacrocystidia 53-121 \times 10-13.5 μ m, abundantia, subfusiformia. Cheilomacrocystidia 50-77 \times 8-11 μ m, fusiformia vel subfusiformia. Pileipellis ex hyphis multiseptatis erectis trichodermium formantibus.

Typus: INDIA-SIKKIM - Hilltok, 3 September, 2010, *K. Das*, KD 10692 (holotypus BSHC, isotypus GENT).

Pileus 35-53 mm diam., at first convex with incurved margin, in centre depressed, with or without conical papilla, gradually planoconvex with depressed centre, sticky, very glutinous, slimy, greyish green (b: 30B5) to pale olive (b: 3C5), darker towards centre; papilla snuff brown (a: 17); margin non-striate. **Lamellae** broadly adnate to decurrent, crowded (17-21/cm at pileus margin), sometimes forked near the stipe, yellowish white (b: 4A2), with abundant lamellulae in 7 series; edge entire, concolorous. **Stipe** 45-62 \times 8-10 mm, subcylindrical to ventricose, tapering upwards and downwards, sticky, greyish yellow (b: 2B4 to 1B4), gradually yellowish white (b: 4A2) towards base and with a slightly paler and whitish zone just underneath the lamellae. **Context** watery hyaline, hollow in stipe, unchanging with FeSO₄ and KOH, but changing to dark green with guaiac after an exposure of 10 minutes; smell not remarkable;taste unknown. **Latex** watery white, abundant, initially unchanging after exposure, unchanging even with KOH, but changing to yellow to yellowish green or paler (b: 30A8) after nearly half an hour of drying. **Spore print** pale yellow (c: 20 Y 2 M).



Figs 1-5. *Lactarius elaioviscidus* sp. nov. 1. Basidiomata showing lamellae and lamellulae. 2. Cheilocystidia. 3. Basidiospores. 4. Two- and four-spored basidia. 5. Pleuropseudocystidia. Scale bars = $10~\mu m$. (KD 10692, drawings by K. Das)



Figs 6-7. Lactarius elaioviscidus sp. nov. 6-7. Radial section through pileipellis. Scale bars = $10~\mu m$. (KD 10692, drawings by K. Das)

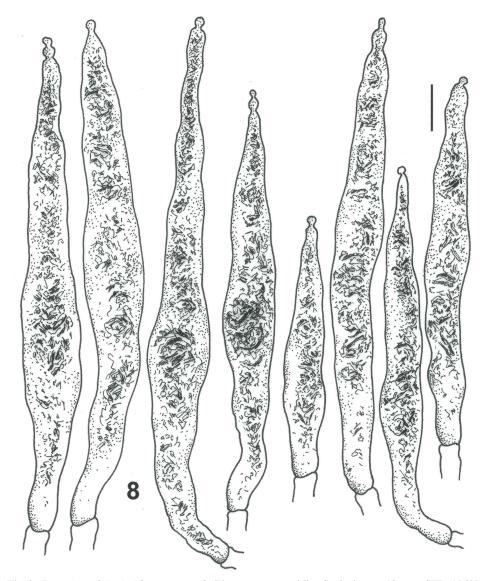


Fig 8. Lactarius elaioviscidus sp. nov. 8. Pleuromacrocystidia. Scale bar = $10~\mu m$. (KD 10692, drawings by K. Das)

Basidiospores $7.0-\underline{8.1}-8.7\times6.0-\underline{6.7}-7.1~\mu m$, (n = 20, Q = $1.10-\underline{1.21}-1.32$), subglobose to broadly ellipsoid; ornamentation amyloid, up to 1 μm high, composed of regular to irregular, rather broad ridges forming an irregular zebroid to occasionally reticulate pattern; short ridges and irregular warts present between long and parallel ridges; plage not amyloid. **Basidia** $40-50\times11-14~\mu m$, 2- to 4-spored, clavate to subclavate or ventricose; sterigmata $5-7\times2-3~\mu m$. **Pleuromacrocystidia** $53-121\times10-14~\mu m$, abundant, subfusiform with mucronate to moniliform or subcapitate apex, emergent up to $70~\mu m$; content dense, somewhat needle-like.

Pleuropseudocystidia subcylindric with rounded apex, mostly not emergent, 9-10 μm diam.; content refringent. Lamellar edge fertile. Cheilomacrocystidia 50-77 \times 8-11 μm , fusiform to subfusiform, often with mucronate to subcapitate or lageniform apex; content dense, needle-like. Hymenophoral trama with lactifers. Pileipellis 90-280 μm thick, an ixotrichoderm, composed of erect, multiseptate hyphae (up to 6 μm broad) which are embedded in a layer of slime (highly variable in thickness); underlying repent hyphae slightly narrower; terminal elements of erect hyphae mostly subcapitate. Clamp connections absent.

Studied material: INDIA-SIKKIM - Hilltok, alt. 2233 m, N 27°11'15.3" E 88°04'21.3", under *Tsuga dumosa*, temperate mixed forest, 3 September, 2010, K. Das, KD 10692 (holotype BSHC; isotype GENT).

Notes: The proposed species L. elaioviscidus is a clear representative of L. subgenus Piperites due to the extremely viscid pileipellis, a feature that is also reflected in the microscopy, where we observe a thick ixotrichoderm with a very distinct slime layer. This subgenus is well-represented in Asia, but this species is well-characterized by the smooth pileus without zonation or hairy margin and without scrobicules, the olivaceous pileus and the milk that dries yellowish green. Other Asian species with latex that dries greenish are L. lacteovirescens Verbeken & E. Horak from Papua New Guinea, L. akanensis S. Imai from Japan and L. maitlyensis from India. L. lacteovirescens is differing by the yellowish white to pale yellow cap with some zones near the margin and the spore ornamentation that lacks the zebroid pattern (Verbeken & Horak 2000). In the original descrption of L. akanensis it is mentioned that the spores are echinulate (Imai 1935), which is a striking difference with the species described here. The pileipellis in L. akanensis is also different (ixolattice) from L. elaioviscidus (Wang & Liu 2010). The Indian species Lactarius maitlyensis which was collected from Western Himalaya resembles through the light greenish yellow staining latex and the zebroid ornamentation of the spores, but the pileus is zonate, greyish to deep grey-reddish brown with pileipellis of ixocutis nature (Das et al. 2003).

Lactarius ermineus K. Das & Verbeken sp. nov.

Figs 9-16, 21-22

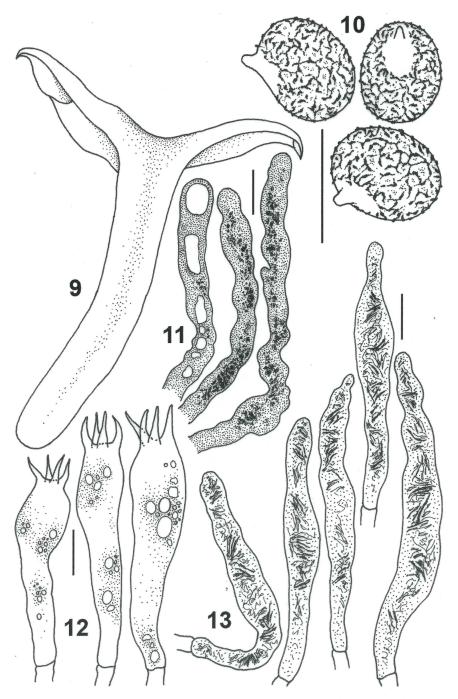
MycoBank: 561652

Etymology: Referring the colour of pileus i.e. white with slight yellow tinge.

Pileus 65-155 mm diam., convexus ad planoconvexum, dein depressus, papilla nulla, eburneus. Lamellae distantes, adnatosubdecurrentes, subflavae. Stipes 40-110 \times 20-32 mm, cylindricus, calceus. Latex albus, immutabilis. Basidiosporae in cumulo subflavae, 7.2-9.0-10.0 \times 6.9-7.8-9.3 µm, globosae vel ellipsoideae, amyloideae, subreticulatae. Basidia 35-50 \times 10-13 µm, tetraspora. Pleuromacrocystidia 70-118 \times 8-13 µm, abundantia, subcylindrica vel subfusiformia. Cheilomacrocystidia 50-77 \times 8-11 µm, subfusiformia. Pileipellis bistrata; suprapellis ex hyphis erectis, subpellis pseudoparenchymaticus.

Typus: INDIA-SIKKIM - Yuksom, 27 August, 2010, K. Das, KD 10622 (holotypus BSHC, isotypus GENT).

Pileus 65-155 mm diam., at first convex with incurved to inrolled margin, gradually planoconvex with depressed centre, without papilla; surface never sticky, white to pale yellow (a: 3C), gradually with small sienna (a: 11) to fulvous spots;



Figs 9-13. *Lactarius ermineus* sp. nov. 9. Basidiomata showing lamellae and lamellulae. 10. Basidiospores. 11. Pleuropseudocystidia. 12. Basidia. 13. Cheilocystidia. Scale bars = $10~\mu m$. (KD 10622, drawings by K. Das)

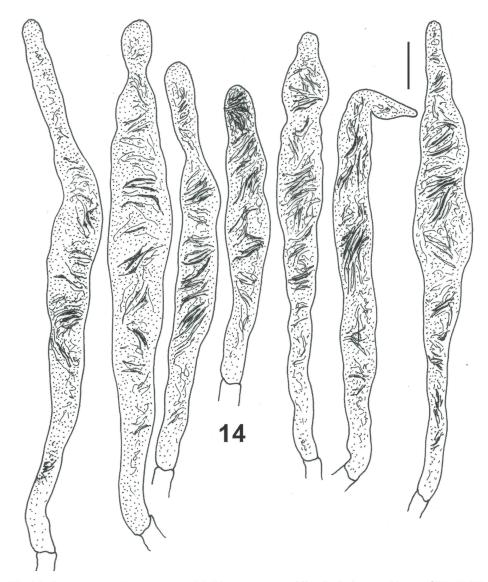
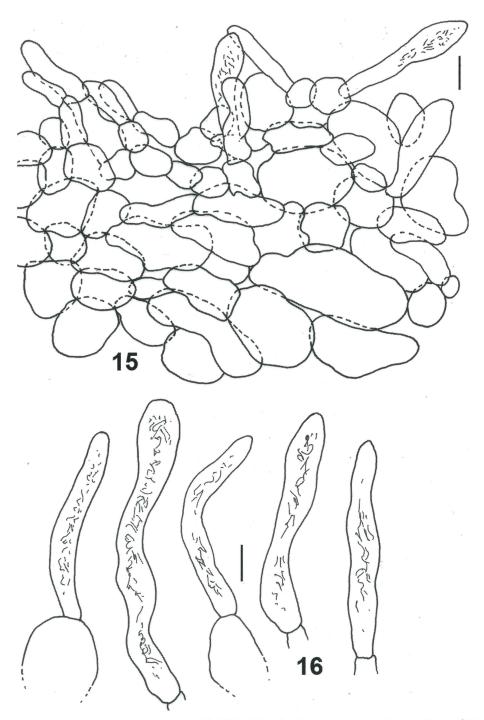
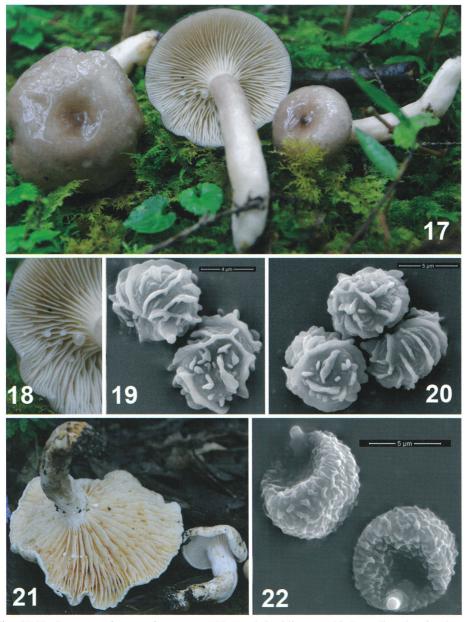


Fig 14. Lactarius ermineus sp. nov. 14. Pleuromacrocystidia. Scale bar = $10~\mu m$. (KD 10622, drawings by K. Das)

margin non-striate. **Lamellae** broadly adnate to subdecurrent, distant (5-6/cm at pileus margin), sometimes forked, pale yellow (a: 3C), with lamellulae in 6-7 series; edge entire, concolorous. **Stipe** 40-110 \times 20-32 mm, cylindrical, occasionally tapering towards base, never sticky, white (chalky), with rust (13) spots (but no scrobicules) at maturity. **Context** initially white, slowly yellowing, solid (never hollow) in stipe, changing to salmon or peach with FeSO₄, yellow with KOH and dark green with guaiac; smell not remarkable. **Latex** white, abundant, unchanging after exposure and when drying. **Spore print** pale yellow (a: 3C).



Figs 15-16. Lactarius ermineus sp. nov. 15. Pileipellis. 16. Pileocystidia. Scale bars = $10~\mu m$. (KD 10622, drawings by A. Verbeken)



Figs 17-22. *Lactarius elaioviscidus* sp. nov. 17. Fresh basidiomata. 18. Lamellae showing latex. 19-20. Scanning electron micrographs of basidiospores. 21-22. *Lactarius ermineus* sp. nov. 21. Fresh basidiomata. 22. Scanning electron micrographs of basidiospores.

Basidiospores $7.2-\underline{9.0}-10.0 \times 6.9-7.8-9.3 \ \mu m \ (n = 20, Q = 1.04-1.15-1.33),$ globose to broadly ellipsoid or rarely ellipsoid; ornamentation amyloid, up to 0.5 µm high, composed of irregular, mostly conical or blunt warts and ridges, aligned or connected and forming a broken or incomplete reticulum; plage not amyloid. **Basidia** 35-50 × 10-13 μm, 4-spored, clavate to subclavate; sterigmata long, $6.0-8.5 \times 2.5-3.5 \mu m$. Pleuromacrocystidia 70-118 \times 8-13 μm , abundant, subcylindrical to subfusiform, mostly tapering upwards, often with subcapitate, subfusoid, slightly attenuated or rounded apex, emergent up to 60 µm; content dense, somewhat needle-like. **Pleuropseudocystidia** cylindric with rounded apex, mostly not emergent, up to 8 µm diam., with refringent content. Lamellar edge fertile. Cheilomacrocystidia 50-77 × 8-11 µm, mostly subfusiform, often with mucronate to attenuated or lageniform apex; content dense, needle-like. Hymenophoral trama mixed, with sphaerocytes and with abundant lactifers. Pileipellis up to 120 µm thick, an undistinct palisade with a well-developed subpellis of sphaerocytes but a rather poorly developed suprapellis; terminal elements $15-45 \times 4-12 \,\mu m$, some thin-walled and hyaline, but others prominent as pileocystidia with a clearly needle-like content and sometimes slightly thickenedwall. **Clamp connections** absent.

Studied material: INDIA-SIKKIM - Yuksom, Near Forest Rest House, alt. 1693 m, N 27°22'06.0" E 88°13'29.3", under Castanopsis tribuloides, subtropical to temperate broad-leaved forest, 27 August, 2010, K. Das, KD 10622 (holotype BSHC, isotype GENT); ibid. 28 August, 2010, K. Das, KD 10635 (BSHC).

Notes: The white colour and dry aspect of pileus and stipe, distant lamellae, the lowly ornamented spores and the rather large pleurocystidia with needle-like content, all make this species distinct and argue for a position in the L. subg. Lactarius. Micromorphologically, the pileipellis of this subgenus is characterized by a distinct cellular layer (subpellis) with a narrow layer of hyaline hyphae (suprapellis) on top. Usually, this suprapellis is found to be arranged periclinally, whereas, present species is characterized by the presence of anticline elements, a bit aberrant in the subgenus. However, terminal elements are not very dense and many of them are typically pileocystidia with dense needle-like content and sometimes with a slightly thickened and refringent wall. These prominent pileocystidia make the species unique in the group. Other closely related Asian species in this group are L. leucophaeus Verbeken & E. Horak, L. paleus Verbeken & E. Horak and L. olivescens Verbeken & E. Horak, all described from Papua New Guinea (Verbeken & Horak 1999, 2000), L. dwaliensis K. Das, J.R. Sharma & Verbeken described from India (Das et al. 2003) and L. roseophyllus R. Heim and L. subpiperatus Hongo described from Thailand (Heim 1962) and Japan respectively. Micromorphologically, all these species differ from the present taxon by lacking pileocystidia in the pileipellis. Moreover, L. subpiperatus lacks pleuromacrocystidia in the hymenium (Hesler & Smith 1979).

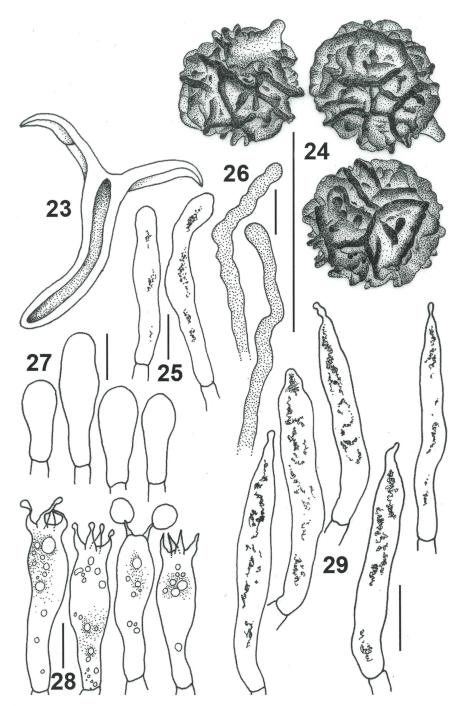
Lactarius byssaceus K. Das & Verbeken sp. nov.

Figs 23-34

MycoBank: 561653

Etymology: Referring to the whitish, cotonny margin of the pileus.

Pileus 50-60 mm diam., convexus ad planoconvexum, dein depressus, papilla nulla, viscidus, zonatus, subluteus vel subroseus. Lamellae densae, late adnatae vel subdecurrentes, luteolae. Stipes $35-52 \times 7-10$ mm, cylindricus, pileo



Figs 23-29. Lactarius byssaceus sp. nov. 23. Basidiomata showing lamellae and lamellulae. 24. Basidiospores. 25. Cheilocystidia. 26. Pleuropseudocystidia. 27. Marginal cells. 28. Two- and four-spored basidia. 29. Pleuromacrocystidia. Scale bars = $10 \,\mu m$. (KD 10628, drawings by K. Das)

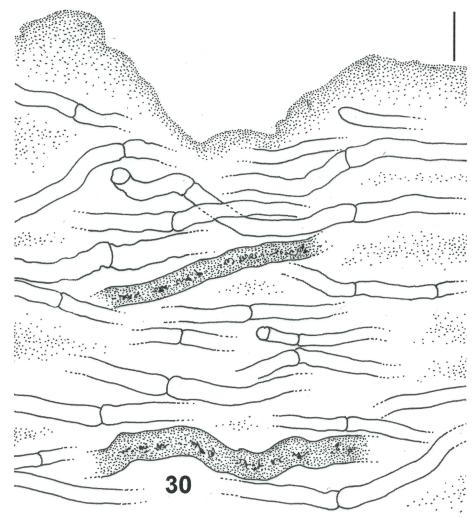


Fig 30. Lactarius byssaceus sp. nov. 30. Radial section through pileipellis. Scale bars = $10 \mu m$. (KD 10628, drawings by K. Das)

concoloris, scrobiculatus. Latex albus, immutabilis. Basidiosporae in cumulo subluteae, 5.8- $\underline{6.1}$ - 7.2×5.4 - $\underline{5.6}$ - $6.5 \mu m$, globosae vel subglobosae, amyloideae, subreticulatae vel reticulatae. Basidia 30- 40×8 - $9 \mu m$, bi- vel tetraspora. Pleuromacrocystidia 35- 47×5.5 - $6.5 \mu m$, fusiformia, ad apicem acuta vel moniliformia Cheilomacrocystidia 38- 50×7 - $8 \mu m$, subcylindrica vel cylindrica. Cellulae marginales 15- 35×7 - $10 \mu m$, clavatae vel subclavatae. Pileipellis ex hyphis hyalinis dense intertextis ixocutem formantibus.

Typus: INDIA-Sikkim - Yuksom, 28 August, 2010, K. Das, KD 10628 (holotypus BSHC, isotypus GENT).

Pileus 50-60 mm diam., at first convex with inrolled margin, becoming planoconvex to applanate with depressed centre, without papilla, sticky, with



Figs 31-34. Lactarius byssaceus sp. nov. 31-32. Fresh basidiomata. 33-34. Scanning electron micrographs of basidiospores.

distinct zonations, pale yellow to pinkish, cream-coloured (b: 3A3 to 4A3), darker towards centre; margin whitish and cottony when young, concolorous when mature, non-striate, slightly pubescent. **Lamellae** broadly adnate to subdecurrent, crowded (20/cm at margin), never forked, with lamellulae in 9 series, yellowish (c: 30 Y, 2 M), unchanging when bruised; edge entire, concolorous. **Stipe** 35-52 × 7-10 mm, cylindrical, concolorous with pileus, yellowish white (b: 4A2) to white beneath the juncture of lamellae, scrobiculate; scrobicules saffron. **Context** white (chalky), hollow in stipe, unchanging with FeSO₄, but changing to yellowish green (b: 30A7) with KOH and dark green (a: 60) with guaiac; smell not remarkable; taste not recorded. **Latex** white, unchanging after exposure. **Spore print** pale yellow (c: 20Y 2M).

Basidiospores $5.8-\underline{6.1}$ - $7.2\times5.4-\underline{5.6}$ -6.5 μm, (n = 20, Q = $1.01-\underline{1.03}$ -1.16), globose to subglobose or rarely broadly ellipsoid; ornamentation amyloid, up to 0.7 μm high, composed of regular to irregular, rather broad ridges and isolated warts forming an incomplete to complete reticulum; plage amyloid but often not distinguishable. **Basidia** $30-40\times8-9$ μm, 2- to 4-spored, clavate to subclavate; sterigmata $5-6\times2.5-3$ μm. **Pleuromacrocystidia** $35-47\times5-7$ μm, scattered, subfusiform with mucronate to moniliform or rarely acute apex, emergent up to 18 μm; content slightly dense but never needle like. **Pleuropseudocystidia** cylindric to tortuous with rounded apex, never emergent, 4-4.5 μm wide; contents refringent. **Lamellar edge** fertile. **Cheilomacrocystidia** $38-50\times7-8$ μm, rare, subcylindrical to cylindrical. **Marginal cells** $15-35\times7-10$ μm, clavate to subclavate, thin-walled, hyaline. **Hymenophoral trama** with abundant lactifers. **Pileipellis** an

ixocutis, up to 85 μ m thick, composed of repent hyaline hyphae (4-6 μ m broad) and lactifers which are embedded in a thick layer of slime. **Clamp connections** absent.

Studied material: INDIA-SIKKIM - Yuksom, Near Forest Rest House, alt. 1693 m, N 27°22'06.0" E 88°13'29.3", under Castanopsis tribuloides, subtropical to temperate broad-leaved forest, 28 August, 2010, K. Das, KD 10628 (holotype BSHC, isotype GENT).

Notes: The species belongs to L. subg. Piperites. The unchanging milk, zonate and sticky cap and the cotonny, slightly pubescent margin place the species L. in sect. Piperites Fr., reminding the European Lacarius pubescens Fr. (but, pileus less tomentose and beardy and without any zonation) and L. scoticus Berk. & Br. (but pileus without any zonation) (Heilmann-Clausen et al. 1998, Kränzlin 2005). Related species in Asia are L. austrotorminosus H.T. Le & Verbeken, described from Thailand (Le et al. 2007), differing by the drier and more scaly pileus and a more strigose margin, and L. strigosus Verbeken & E. Horak, described from Papua New Guinea, differing also by the distinctly fibrillose and strigose pileus (Verbeken & Horak 2000).

Acknowledgements. The authors are thankful to the Director, Botanical Survey of India (BSI), Kolkata (India) and the Dept. of Forest, Environment and Wild Life Management, Gangtok (India) for providing facilities during this study. Special thanks are also given to S.K. Rai, S. Pradhan and R.K. Ram of BSI, Gangtok for assisting the first author in many ways. The help rendered by S.C. Maikap of Bose Institute, Kolkata in obtaining the Scanning Electron Micrographs of basidiospores is also duly acknowledged.

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