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Research Article

THREE INTERESTING SPECIES OF WILD MUSHROOMS FROM SIKKIM (INDIA)

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ABSTRACT
In the present paper three species of wild mushrooms are described macro- and micromorphologically from North Sikkim with required illustrations. *Leccinum insigne* is a new record for Indian mycobiota, whereas, other two: *L. scabrum* and *Agaricus abruptibulbus* show new distributional/ state records for India.

Keywords: *Macrofungi, India, Taxonomy, Agaricaceae, Boletaceae*

INTRODUCTION
North district of Sikkim spreading over 4226 sq. km. including various climatic zones like tropical (up to 500 m), subtropical (500- 1500 m), temperate (1500-2700 m), sub-alpine (2700-5000 m) and alpine (above 5000 m). Routine macrofungal explorations of this district have been undertaken by the senior author since 2009. During recent forays to Dombang, Shingba Rhododendron Sanctuary, Shibmandir, Zero, Zema I-IV, Kalep, Thangu, etc. a large number of wild mushrooms were collected. Thorough morphological examination followed by literature study of those samples resulted in a number of novel or interesting taxa (Putte *et al.*, 2012; Das *et al.*, 2012, 2013; Das 2013a, 2013b; Das and Chakraborty 2013, 2014; Das and Zhao 2013). Three of those species are dealt in the present paper.
Leccinum insigne A.H. Sm., Thiers and Watling, which was collected from Kalep (Subalpine mixed forest dominated by Abies and Salix) and Zema-IV (Subalpine mixed forest dominated by *Abies*, *Betula* and *Juniperus*) is being reported for the first time from India, whereas, *L. scabrum* (Bull.) Gray (Kalep and Zema-IV) and *Agaricus abruptibulbus* (collected from Dombang, a mixed subalpine forest dominated by *Picea, Tsuga, Larix and Abies*) are new records for the state of Sikkim. Their macro- and micromorphological descriptions along with required illustrations are given and comparative notes with their allied taxa are supplemented.

MATERIALS AND METHODS
Collected fresh specimens were characterized macromorphologically in the base camp. Field photography was made with the help of Olympus C-5060 (wide zoom) camera. Colour codes and terms follow Methuen Handbook of Colour (Kornerup and Wanscher, 1978). After recording the macromorphological features specimens were dried in a field drier.
Micromorphological features were recorded with the aid of a light microscope: Olympus CX 41 based on the dry samples mounted in a mixture of 3–5 % KOH and phloxin, lactophenol, glycerin, cotton blue and Melzer's reagent. Measurements of basidiospores are recorded based on that of twenty. Basidiospores are measured in side view. Measurements of spore-size and length/width ratios (Q) are given as: minimum–mean–maximum. Herbarium name follows Holmgren *et al.*,

Taxonomy
Leccinum insigne A.H. Sm., Thiers and Watling Figure 1.
Pileus 45–110 mm diam., broadly convex, surface areolate surrounding the center and fibrillose-squamulose towards the margin, pale orange to greyish red (7B4) when young, gradually becoming darker or brownish orange (7C8) after maturity. Margin entire, decurved with sterile flap of tissue, often interrupted with several segment, dark brown to rusty brown. Pore surface depressed near the stipe, white to cream buff, becoming olivaceous then greyish orange to brown (6B4 to 6D7) after bruising, becoming slowly yellowish brown (5E6 to 5E5); pore rounded, 2–3/mm. Tubes sinuate, 10 mm long, concolorus to

pore surface, slightly olivaceous before becoming blackish on exposure. Stipe 45–100 × 12–28 mm, cylindric with bulbous base, chalky white; surface with dense scabers, white, then light orange to brown, becoming blackish in latter half; beneath the scabers often greyish green to bluish green (25D5 to 25D6) at base. Partial veil and annulus absent. Context solid, chalky white, becoming bluish grey (19B2) to greyish violet (16E5 to 18B3), then blackish without an intermediate reddish coloration, turning greyish violet (17B3) with guiacol, yellowish white (1A2) with KOH, greenish grey (1B2 to 1C2) in $FeSO_4$. Pileus surface turning dull green (27E3 to 27E4) with $FeSO_4$. Spore print reddish brown (9E5), becoming olive brown (4D8 to 4E8) when dry.

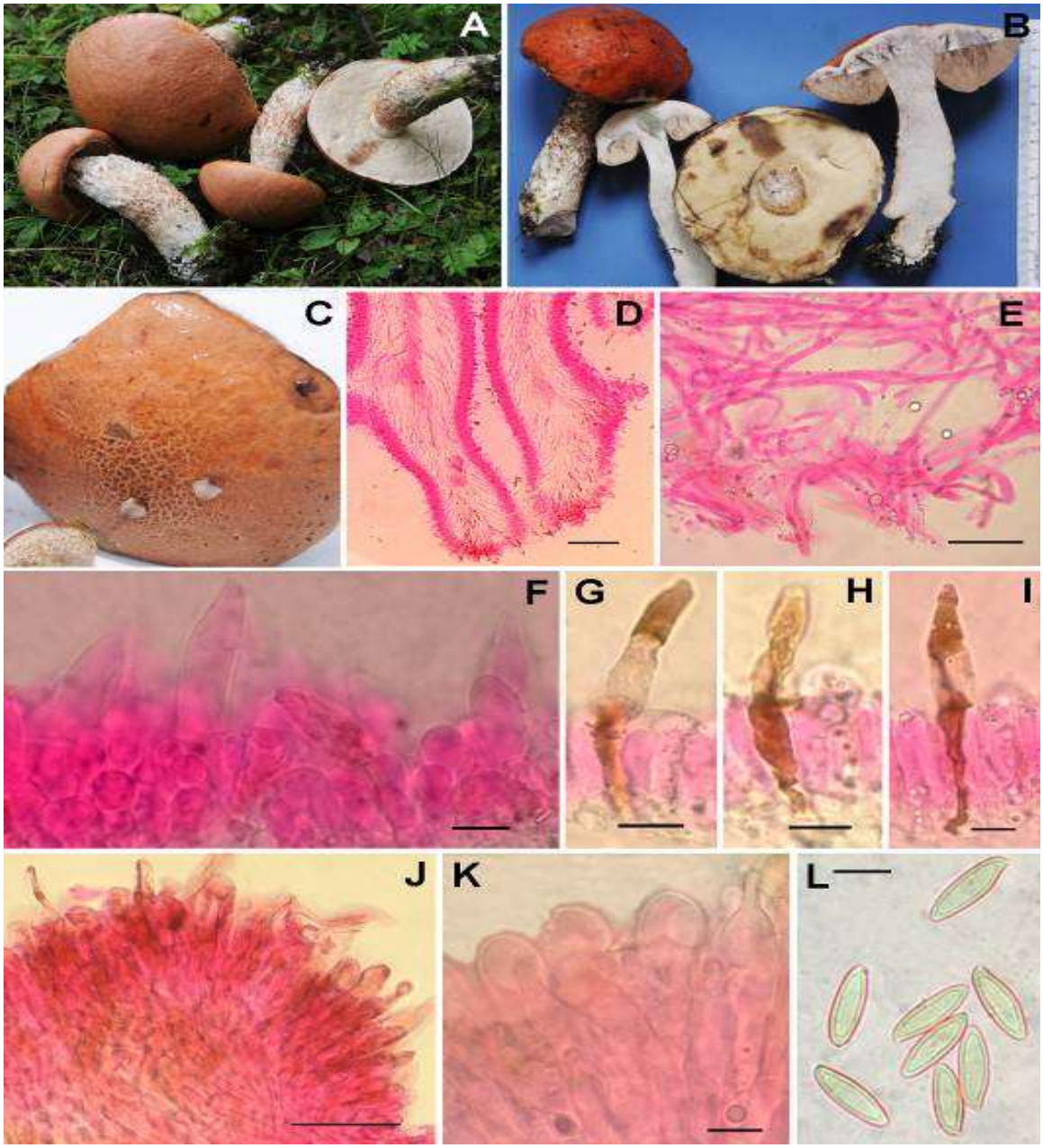

Figure 1: *Leccinum insigne.* A and B. Fresh basidiomata showing pileus, stipe and pore surface and context. C. areolate to fibrillose-squamulose pileus surface. D. Divergent tube trama. E. Cross section through pileipellis. F–I. Pleurocystidia. J and K. Caulocystidia. L. Basidiospores. Scale bars: D = 100 µm. E and J = 50 µm. F–I, K and L = 10 µm

Basidiospores 13–15.5–17 × 4–4.6–5 µm, (n = 20, Q = 1.79–2.63–3.16), cylindric to subfusiform, smooth, thin-walled. Basidia 26–35 × 8–10.5 µm, 4-spored, clavate; sterigmata 2–8 × 1–3 µm. Pleuromacrocystidia two types; first one: Brown pigmented, emergent up to 31 µm, cylindrical to ventricose, often with tortuous base, thin walled with granular content; second one: Non pigmented; emergent up to 27 µm, ventricose to appendiculate with mostly fusoid to subfusoid apex, thin walled. Tube edge sterile with cystidia and marginal cell; Cheilocystidia 25–45 × 7–9.5 µm, of two types, brown pigmented and nonpigmented, clavate, ventricose-rostrate, obclavate with rounded, subfusoid to fusoid apex, content slightly dense; marginal cell abundant, cylindrical to clavate, branched and often multiseptate. Tube trama divergent, slightly gelatinous. Pileipellis to 220 µm thick, composed of loosely packed erect hyphae (42–46 × 5–7.5 µm), somewhat intermingled near apex; hyphae branched, septate; terminal cell cystidoid to subfusoid with rounded to subacute apex, content dense, granular, vacuolated, no significant coloration with Melzer's reagent, Pilear trama with few hyphae darker (black) in Melzer's reagent. Stipitipellis fertile, composed of hyphae, cystidia and basidia; hyphae to 7 µm wide, suberect to interwoven, branched septate thin-walled, with protuberance. Basidia 22 × 11µm, clavate. Caulocystidia in clusters, 34–50 × 6.5–14 µm, cylindrical, clavate to ventricose with subacute, fusiform to appendiculate apex, terminal cell cylindrical with rounded apex.

Specimen examined: INDIA, Sikkim, North district, Kalep, alt. 3710 m, N27°51'58.7" E88°32'49.2", under *Abies densa*, subalpine mixed forest (broad-leaf and coniferous), 29 July 2013, K. Das, KD 13-063, (CAL); *ibid*., North district, Kalep, alt. 3710 m, N27°51'58.7" E88°32'49.2", under *Abies densa*, subalpine mixed forest (broad-leaf and coniferous), 29 July 2013, K. Das, KD 13-065, (CAL); *ibid*., North district, Zema-IV, alt. 3238 m, N27°48'28.1" E88°33'09.8", under *Abies densa*, subalpine mixed forest (broad-leaf and coniferous), 31 August 2012, K. Das, KD 12-220 (CAL); *ibid*., North district, Samthang, alt. 3406 m, N27°49'48.3" E88°32'54.7", under *Abies densa* and *Salix* sp., subalpine mixed forest (broad-leaf and coniferous), 29 July 2013, K. Das, KD 13-062, (CAL).

Note: *Leccinum insigne* A.H. Sm., Thiers and Watling, Michigan Bot. 5: 160 (1966) is commonly known as Aspen Bolete, and also known as *Krombholziella insignis* (A.H. Sm., Thiers and Watling) Šutara, Česká Mykol. 36(2): 81 (1982). It is an edible mushroom and consumed by the local people of North Sikkim (local name is Pakchu Syamo) with delicacy.

Considering the macro- and micromorphological characters, like, pileus margin that extends beyond the tubes as sterile flap of tissue, exposed context turning black from initial chalky white without an intermediate reddish coloration, circular pore and absence of reticulum on stipe surface, these specimens are placed under *Leccinum insigne* (Smith *et al.*, 1971). Morphological features of the present specimen almost agree with that of its American counterpart but, unlike American collections where specimens grow mainly under *Populus*, Indian specimens were found to grow under different host-trees.

Leccinum atrostipitatum A.H. Sm., Thiers and Watling, *L. largentii* Thiers (never reported from India) and *L. aurantiacum* (Bull.) Gray, appear to be quite similar with this species but *L. atrostipitatum* and *L. aurantiacum* can easily be separated by the presence of an intermediate reddish coloration of the exposed context. Moreover, *Leccinum atrostipitatum* differ from *L. insigne* by the presence of black scabers from the very beginning and *L. largentii* has angular pore and stipe surface with conspicuous reticulum (Smith *et al.*, 1971; Bessete *et al.*, 2010). Further determination can be made through molecular systematics in due course.

Leccinum scabrum (Bull.) Gray Figure 2

Pileus 45–110 mm diam., convex to planoconvex, flat at maturity, surface sticky when wet in young basidiomata, becoming dry to slightly velvety or subvelvety (downy), brown (6E6) in centre, gradually becoming paler to light brown (6D4) towards margin. Margin entire, incurved to decurved, often with a narrow flap of tissue. Pore surface depressed near the junction of stipe, yellow white to greyish yellow (4A2–4C5) when mature, becoming slowly orange white (5A2) after bruising; pore rounded to irregular, 2–3/mm. Tubes sinuate, yellowish white, slowly becoming slightly pinkish after exposure. Stipe 120× 22 mm, cylindric with slightly bulbous base, mostly covered with whitish mycelium, surface with black scabers forming reticulum on basal half, yellowish, turning pale red (8A3) when bruised. Context solid to

partly hollow (where infested by larvae) in stipe, white initially, slowly becoming yellowish, turning reddish white (11A2) with guiacol, pale yellow to light yellow (4A3–4A4) becoming light orange to greyish orange (5A4–5B4) after sometime with KOH, greyish green (29E5–28E6) in $FeSO_4$. Spore print brown (7E5).

Basidiospores 15.2–18.1–22 × 4–5.3–6.2 µm (n = 20, Q = 3–3.44–4.6), subfusiform to elliptic, inequilateral, smooth, slightly thick-walled, cyanophilous. Basidia 16.5–34 × 10–13 µm, 4-spored, clavate; sterigmata to 3.2 µm long, to 2 µm wide at base; Pleurocystidia rare, 35–44 × 7–12 µm, ventricose, appendiculate, emergent 23–30 µm. Tube trama divergent. Pileipellis to 250 µm thick, composed of suberect to parallel septate hyphae, terminal cells 29–96 × 6–11 µm, cystidoid, content dense, brown, of various shapes, cylindric to fusoid or appendiculate. Stipitipellis to 100 µm thick, fertile, composed of basidia, cystidia and hyphae; caulocystidia 36–66 × 6.5–11.2 µm, subcylindric to subfusoid, mucronare to subcapitate or ventricose to appendiculate, with brown pigmentation; hyphae erect, septate, terminal cells 23–51 × 5.5–10.5 µm, cylindric to flask- or bottle-shaped, with rounded apices, mostly with wider base. Clamp connections absent.

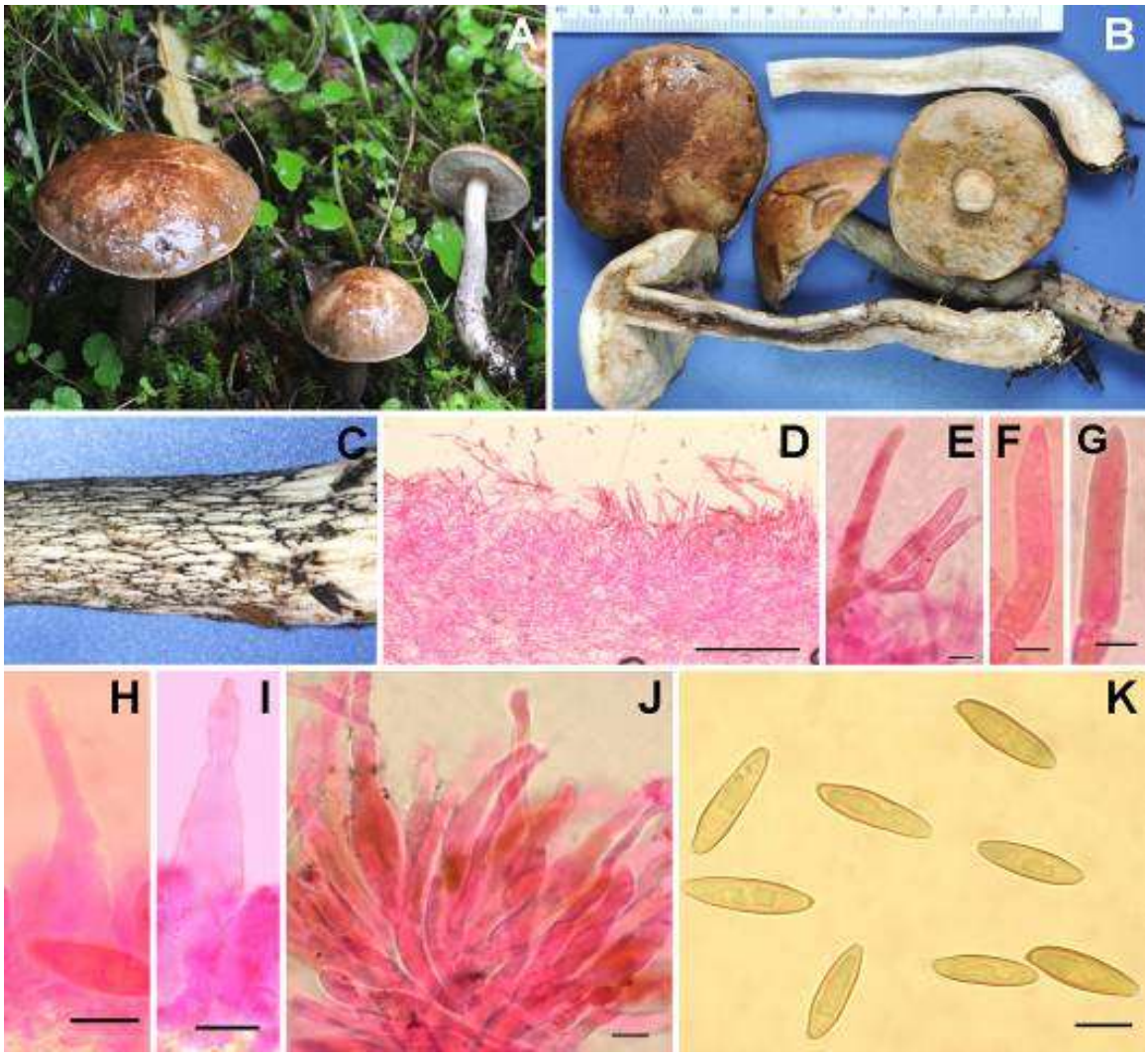

Figure 2: *Leccinum scabrum*. A and B. Fresh basidiomata showing pileus, stipe and pore surface and context. C. Reticulum on stipe surface. D. Cross section through pileipellis. E–G. Terminal cells of hyphae in pileipellis. H and I. Pleurocystidia. J. Caulocystidia. K. Basidiospores. Scale bars : D = 100 µm. E–L = 10 µm

Specimen examined: INDIA, Sikkim, North district, Zema-IV, alt. 3238 m, N27°48'28.1" E88°33'09.8", under *Abies densa*, subalpine mixed forest (broad-leaf and coniferous), 28 July

2013, K. Das, KD 13-054 (CAL); *ibid*., North district, Kalep, alt. 3710 m, N27°51'58.7" E88°32'49.2", under *Abies densa*, subalpine mixed forest (broad-leaf and coniferous), 29 July 2013, K. Das, KD 13-068, (CAL); *ibid*., North district, Zema-IV, alt. 3238 m, N27°48'28.1" E88°33'09.8", under *Abies densa*, subalpine mixed forest (broad-leaf and coniferous), 30 July 2013, K. Das, KD 13-070, (CAL); *ibid*., North district, Zema-IV, alt. 3238 m, N27°48'28.1" E88°33'09.8", under *Abies densa*, subalpine mixed forest (broad-leaf and coniferous), 2 September 2012, K. Das, KD 12-246 (CAL).

Note: *Leccinum scabrum* (Bull.) Gray, Nat. Arr. Brit. Pl. (London) 1: 646 (1821) is also known as *Boletus albus* Gillet, Revue mycol., Toulouse 3: 5 (1881), *Boletus scaber* Bull., Herb. Fr. 3: tab. 132 (1783) [1782-83], *Gyroporus scaber* (Bull.) Quél., Enchir. fung. (Paris): 162 (1886), *Suillus albus* Henn., in Engler and Prantl, Nat. Pflanzenfam., Teil. I (Leipzig) 1: 190 (1898) [1900], *Krombholzia oxydabilis* Singer, Revue Mycol., Paris 3: 189 (1938), *Boletus oxydabilis* (Singer) J. Blum, Bull. trimest. Soc. mycol. Fr. 85(4): 560 (1970) [1969], *Leccinum oxydabile* (Singer) Singer, Am. Midl. Nat. 37: 123 (1947). Considering macro- and micromorphological features, like, absence of sterile flap of tissue in pileus of young basidiomata, absence of inflated terminal cells in pileipellis, yellowish (on exposure) stipe context (Smith *et al.*, 1971), blackish scabers form reticulum on stipe surface (Noordeloos, 2006), the present specimen is placed under the *Leccinum scabrum*.

Leccinum subpulchripes A.H. Sm. and Thiers, *L. montanum* Thiers, are quite similar with that of *L. scabrum* which is also reported from another state (Himachal Pradesh) of India (Lakhanpal, 1996). But, both of them differ from this specimen in absence of reticulum on stipe surface. Morover *L. subpulchripes* shows only rounded pore, absence of pleurocystidia, moreover *L. montanum* has angular pore. Furthermore, *Leccinum subpulchripes* is found to be associated with species of *Pinus* along with *Alnus* and *L. montanum* is found under species of *Populus* (Bessete *et al.*, 2010).

Agaricus abruptibulbus Peck Figure 3, 4

Pileus 35–90 mm diam., ovate when young, gradually convex to planoconvex; surface dry, with few silky scales, white, slowly becoming pale yellow (2A2–2A3) then pale yellow to light yellow (3A4–4A5) with maturity and / or after bruising; margin with veilar remnants, lamellae free, edge wavy, white when young, gradually pinkish, reddish grey to greyish brown (7B2, 8B2, 8E3), then dark brown with age; lamellulae 18–20/10 mm. Stipe 60–132 × 10–15 mm, mostly cylindric with an abruptly-bulbous base, concolorous with pileus or yellowish white to pale yellow (3A2–4A2 or 4A3) after maturity, surface dry, annulus white, of double membrane, split into a star shape around the stipe. Context thick in pileus and pithy in stipe, white then slowly becoming pinkish, turning yellow (2A7) with KOH, reddish white (7A2) with guiacol and unchanging in $FeSO_4$. Spore print dark ruby (12F4).

Basidiospores 5–6.3–7.6 × 3.5–3.6–4 µm (n = 20, Q = 1.42–1.62 –1.84), smooth, ellipsoid to oblong, slightly wider towards the hilum, one or two guttulate, dark brown to grey-brown at maturity; thick-walled, to 0.8µm thick. Basidia 14–18 × 5–6 µm, 4-spored, clavate; sterigmata 1–1.6 × 0.2 µm. Lamellar edge fertile, composed of basidia, cystidia, and marginal cell; cheilocystidia 14–22 × 5.5–7 µm present but not clearly distinguished, clavate to subclavate; marginal cell 8–12 × 4–6 µm, cylindrical to narrowly clavate. Subhymenium 14–17 µm thick, cellular, composed of irregularly swollen inamyloid cells. Hymenial trama hyphal, of parallel to interwoven pattern; hyphae septate, branched, to 8.5 µm wide. Pileipellis to 338 µm thick, of erect to interwoven pattern; hyphae regularly septate, constricted at septa, branched, clamp connections absent. Stipitipellis hyphal; hyphae 1.2–3 µm wide, regularly septate with rounded apex. Stipe trama to 150 µm thick, pseudoparenchymatous, divided into several chambers towards centre, cells irregular 3–14 × 2.5–7.5 µm.

Specimen examined: INDIA, Sikkim, North district, Dombang, alt. 2975 m, N27°44'08.7" E88°45'58.3", under *Abies densa*, subalpine mixed forest (broad-leaf and coniferous), 24 July 2013, K. Das, KD 13-015, (CAL).

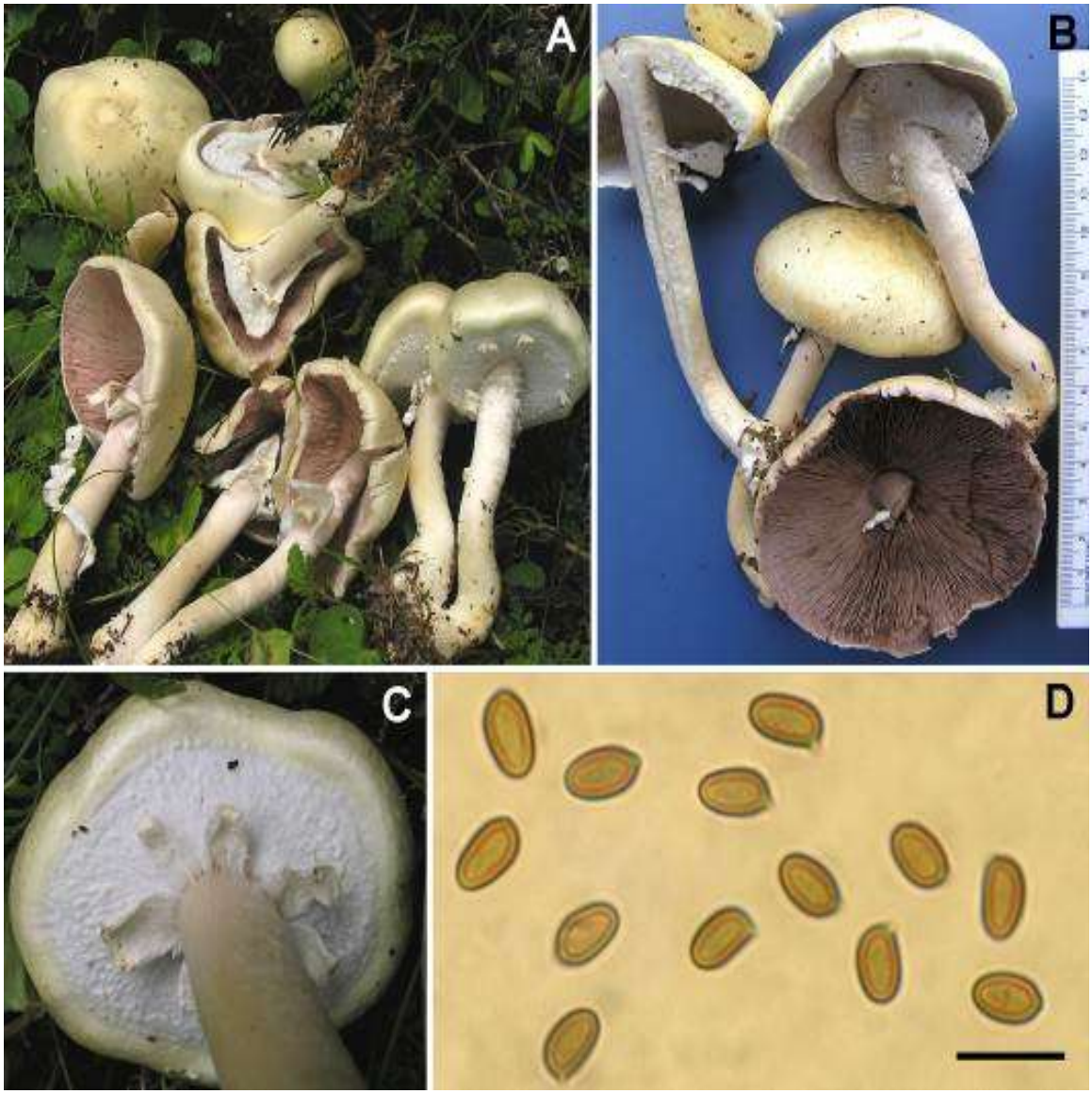

Figure 3: *Agaricus abruptibulbus*. A and B. Fresh basidiomata showing pileus, stipe and lamellae and context. C. Annulus with double membrane. D. Basidiospores. Scale bar : D = 10 µm

Notes: *Agaricus abruptibulbus* is one of the rare (collected once in three repeated forays) species found in the forested areas (explored so far) of North Sikkim and commonly known as 'woodland mushroom'. It is also reported from two other states (Maharashtra and Uttarakhand) of India (Bilgrami *et al.*, 1991; Vishwakarma *et al.*, 2012). Taxonomically, it is placed under sect. *Arvenses* of the genus *Agaricus*. *Agaricus arvensis* Schaeff. and *A. sylvicola* (Vittad.) Peck resemble in macro- and micromorphological characters with this species. But, the earlier two differ from the latter one by their continuous bulbous base (without any margin) as stated by Miller and Miller (2006) and Bessette *et al.*, (1997). Moreover, *A. arvensis* distinguishes from the present species by its more robust (pileus 80–200 mm diam., stipe 50–200 × 10–30 mm) stature, larger (7–9.2 × 4.4–5.5 µm) spores, as after Miller and Miller (2006), and presence of clamp connections in hyphal elements of pileipellis (Wasser 2000), whereas, *A. sylvicola* has shorter (5–6.8 × 3.5–4.4 µm) spores, as after Miller and Miller (2006). In spite of having the morphological similarities, these three species are now phylogenetically well established with the aid of molecular taxonomy (Geml and Royse, 2002).

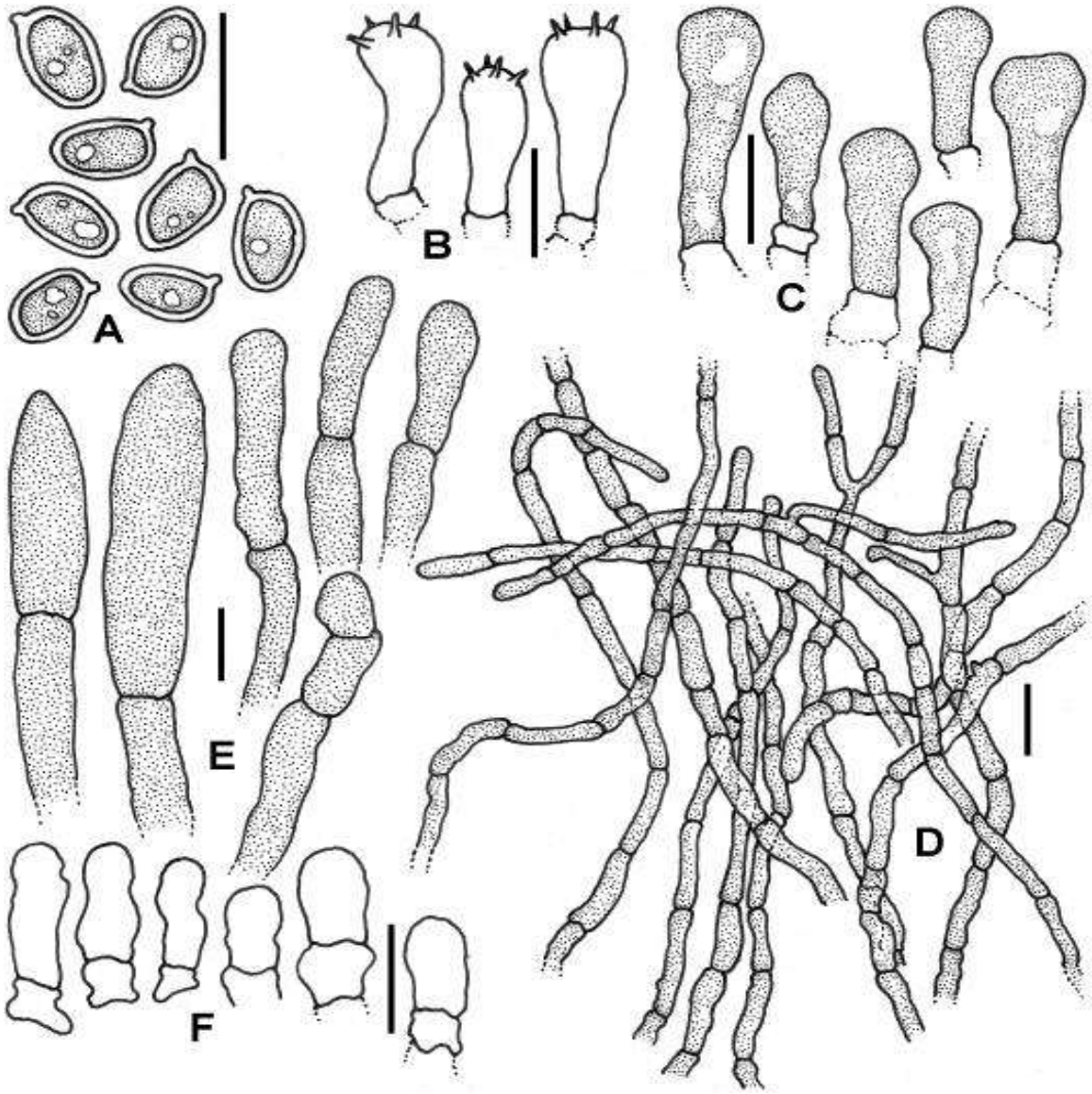

Figure 4: *Agaricus abruptibulbus*. A. Basidiospores, B. Basidia, C. Hymenial cystidia, D. Pileipellis, E. Terminal and subterminal cells of hyphae in pileipellis, F. Marginal cells of lamellar edge. Scale bars: A–C, E and F = 10 µm; D = 25 µm

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