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# New records of lamellate mushrooms associated with Sal from Shiwaliks, India

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## ABSTRACT

**This paper deals with four new records of fungal taxa from India. These were collected from Shiwalik range of North West, India, in association with *Shorea robusta*. These include *Amanita battarrae* (Boud.) Bon, *Asproinocybe lactifera* Heim., *Russula chlorinosma* Burl. and *R. nobilis* Velen. Morphological and microscopic characters are described and illustrated.**

**Key words:** Ectomycorrhiza, diversity, Sal forest, taxonomy.

## INTRODUCTION

Agarics are cosmopolitan basidiomycetous fungi which grow in a wide variety of habitats, from the tropics to arctic. These are mostly saprophytes and many of them form mycorrhizal association with variety of plants including trees. Studies on taxonomy and diversity of agarics are inviting more attention now a day's primarily because of their importance in human welfare and role in ecosystem functioning and stability. Although these macro-fungi are an integral part of the ecosystem, knowledge on their diversity in the tropical and subtropical regions including India are largely understudied (Lakhanpal, 2014). Many species are important as a source of food, medicine, nutraceuticals and also play a pivotal role in ecosystem strengthening and maintenance as mycorrhizal associates (Manoharachary *et al*., 2005). Present investigations are centred on identification of agaric diversity of Sal forests and ectomycorrhizal associates of *Shorea robusta* (Sal).

While investigating the EcM diversity of Sal forest, four taxa, *viz*. *Asproinocybe lactifera* Heim, *Russula chlorinosma* Burl., *Amanita battarrae* (Boud.) Bon, and *Russula nobilis* Velen were recorded for the first time from India (Upadhyay *et al*., 2017). These species are fully illustrated and described in this paper. Genus *Asproinocybe* forms a new genus record for India and earlier this genus was recorded from subtropical and tropical Africa (Heim, 1970; Heinemann, 1977), Malaysia, Mexico, Hungary (Guzmán *et al.,* 2004) and New Zealand and Australia (Lebel *et al*., 2020).

## MATERIALS AND METHODS

**Study area:** Area selected for the present investigation is Sal forests of Shiwalik mountain range of North West India, which are geologically the lowest and youngest mountain range of Himalaya. The study area is located between 290° 58/ -310° 2/ -Northern latitude and 770° 34/ -78° 18/ -Eastern longitudes. Average elevation of the area is 400-1500 m and vegetation of the area is typical of tropical moist deciduous forests (Champion and Seth, 1968). *Shorea robusta* purely dominates the Sal forests of Shiwalik Mountain.

**Sampling, identification and characterization:** Sporocarps were collected from different localities of pure Sal forests, during the rainy season of 2014-2016. Macromorphological features were recorded from fresh collections in the field and colour codes used are that of Kornerup and Wanscher (1978). After noting down morphological characters on the field key (Atri *et al*., 2005) some pieces of sporocarps from cap and stipe were preserved in liquid preservative [25 mL rectified alcohol (95%) + 5 mL formalin (37%) + 70 mL distilled water] for studying the microscopic characters. By adopting the standard procedures spore deposit was taken (Singer, 1986). Sporocarps were air dried at 40-45°C in a wooden drier especially designed for drying mushroom specimens (Atri *et al*., 2005). The dried specimens were finally packed in cellophane paper packets for permanent preservation in Punjabi University Herbarium under PUN. In each such packet small perforated sachets of Paradichlorobenzene was kept so as to check insect infestation. The cross sections of pileus and longitudinal section of stipe cut from wet/dried preserved material with the help of rajor blade were examined and details drawn under a compound microscope with the help of camera lucida and photographed under digital microscope (Leica DM4000 B LED). Line drawings and observation of basidia, cystidia and elements of pileipellis and stipitipellis were taken after staining the slides with Congo red. Melzer's reagent was used to observe the amyloidy in basidiospore ornamentation. The microscopic details were worked out as per standard methodology (Singer, 1986; Atri *et al*., 2017).

**Scanning electron microscopy (SEM):** Scanning electron microscopic (SEM) studies were carried out with JSM6610LV GEOL scanning electron microscope. For SEM examination basidiospores from spore print and lamellae tissue were mounted on a double-sided adhesive tape pasted on a metallic specimen holder or stub. The material was scanned at different magnification ranging from 3000-15000X in high vacuum mode to observe pattern of spore ornamentation.

## TAXONOMIC DESCRIPTION

***Asproinocybe lactifera*** Heim, *Revue de Mycologie* (Paris) **34** (4): 343, 1970. **Figs. 1(A-G) & 2(A-H)**

Basidiomata 8-13 cm in height. Pileus 8-13 cm diam., convex, flattened depressed to slightly infundibuliform at maturity; margin regular, non-splitting, surface greyish violet to deep violet (17B4/17D8) with orange white (6A2) tinge in the centre and dark violet (15F8) broken ring around the submarginal region of the pileus; margin feebly

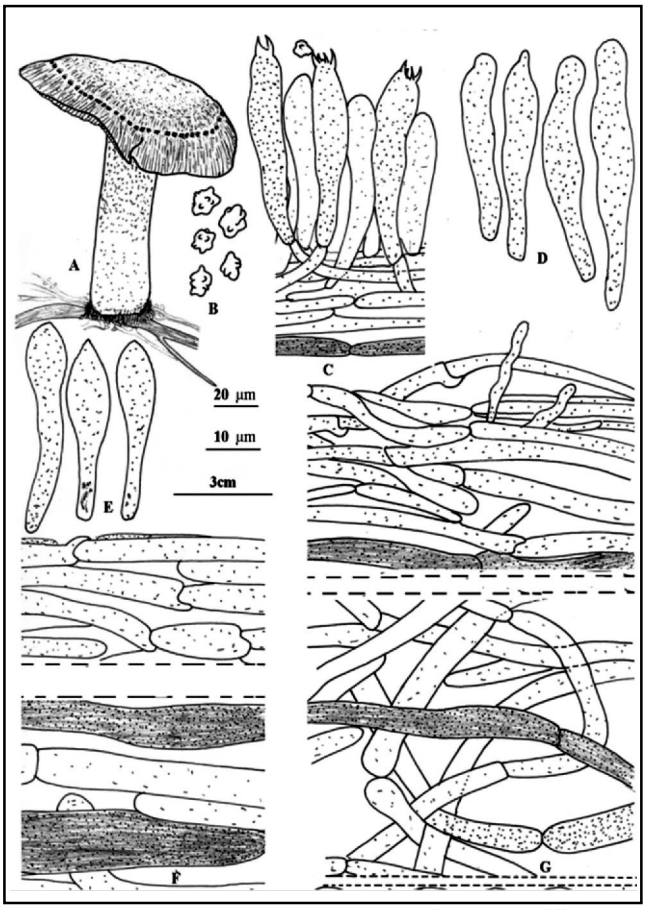


**Fig. 1(A-G).** ***Asproinocybe lactifera***: A. Sporophore, B. Basidiospores, C. Hymenophore showing basidia, D. Pleurocystidia, E. Cheilocystidia, F. Cross section through stipe showing cuticular details and context, G. Cross section through pileus showing cuticular details and context.

striate; cuticle not peeling. Lamellae adnexed, unequal, subdistant (8gills/cm at maturity and 14/cm when young), 6-9 mm broad, greyish violet (17B4), changes to deep violet (17D8) at maturity; lamella edge smooth, fragile. Stipe 7-8 × 2.0-2.8 cm broad, greyish violet (17B4), central, cylindric to slightly clavate, pruinose, unchanging, solid. Pileal trama 0.8-1.3 cm thick near the stipe, 0.5-0.9 cm in the centre and up to 0.1 cm along the margin, violet white changes to reddish grey (8B2) on bruising and cutting, exudes scanty hyaline fluid from the cut portion. Taste sour; odour fragrant. Spore deposit white.

Basidiospores 4.5-6.5 × 3.5-4.5 µm, stellate or nodulose, apiculate, apiculus up to 0.4 µm long, light brown, inamyloid. Basidia 32-43 × 5-8 µm, clavate, scattered, thin-walled, bisporic to tetrasporic, sterigmata up to 4.8 µm long; pleurocystidia 29-43 × 5-8 µm, abundant, thin-walled, clavate, cylindric to sub cylindric, granulated; cheilocystidia 19.6-32.5 × 5.7-8.2 µm broad, similar to pleurocystidia; gill edges heteromorphous. Pileus cuticle having 2.5-5 µm broad almost parallel running thin-walled hyphae with frequent clamp connections. Context homoiomerous, made up of 3-9 µm broad granulated hyphae intermingled with laticifers. Hymenophoral trama homoiomerous, 24-180 µm broad, regular with almost parallel running 5-10 µm broad septate,

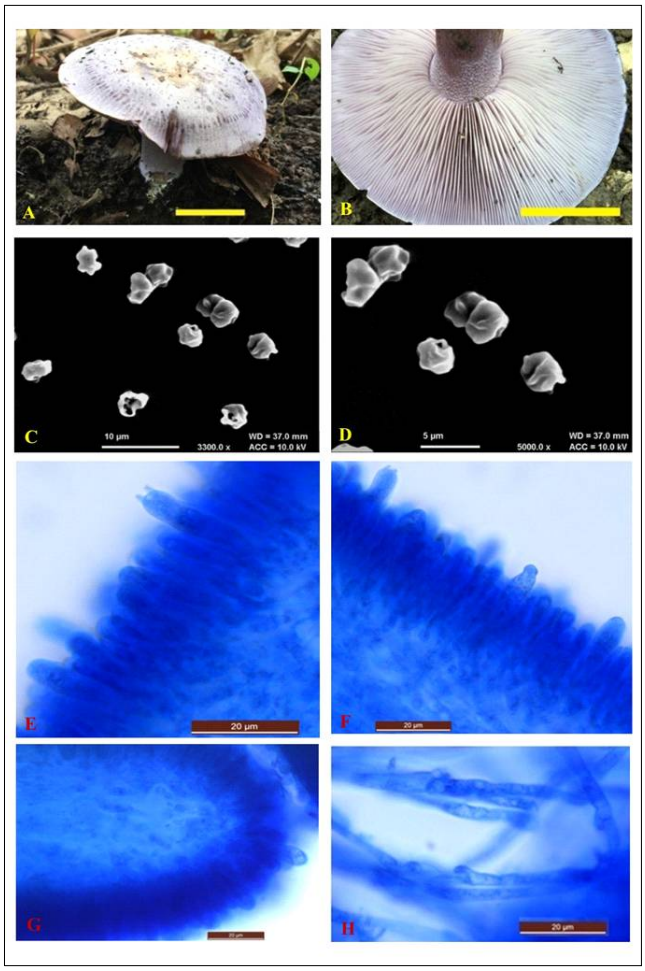


**Fig. 2(A-H)** ***Asproinocybe lactifera***: A-B. Sporophores, C-D. Scanning electron photographs of basidiospores, E. Hymenophore showing basidia and cystidia, F. Pleurocystidia, G. Cheilocystidia, H. Cross section through pileus showing cuticular hyphae with clamp connections. **Scale bar** A-B = 3 cm.

thin-walled hyphae. Subhymenium 16-24.5 µm thick, distinct, composed of interwoven 1.6-4.0 µm broad hyphae. Stipe cuticle regular with almost longitudinally parallel septate hyphae, 3.3-5.0 µm wide; stipe context homoiomerous, with almost longitudinally tangled parallel running hyaline hyphae measuring 3-13 µm in width; laticifers frequently present in the context. Clamp connections present in all tissues.

**Collection examined:** Uttrakhand: Lachhiwala (525 m), reported in association with *Shorea robusta*. 24 September 2015, Jitender Kumar, PUN 9167.

**Remarks:** *Asperoinocybe lactifera* is characterized by typically greyish violet to deep violet cap and lamellae, small nodulose basidiospores and presence of frequent laticifers throughout the context. These features agree well with the observations given by Heim (1970) except for spore size which is on the smaller side (4.5-6.5 × 3.5-4.5 µm instead of 5-8 × 4.5-5.8 µm) in the presently examined collection. The present collection seems to be a small spore variant of *A. lactifera* .

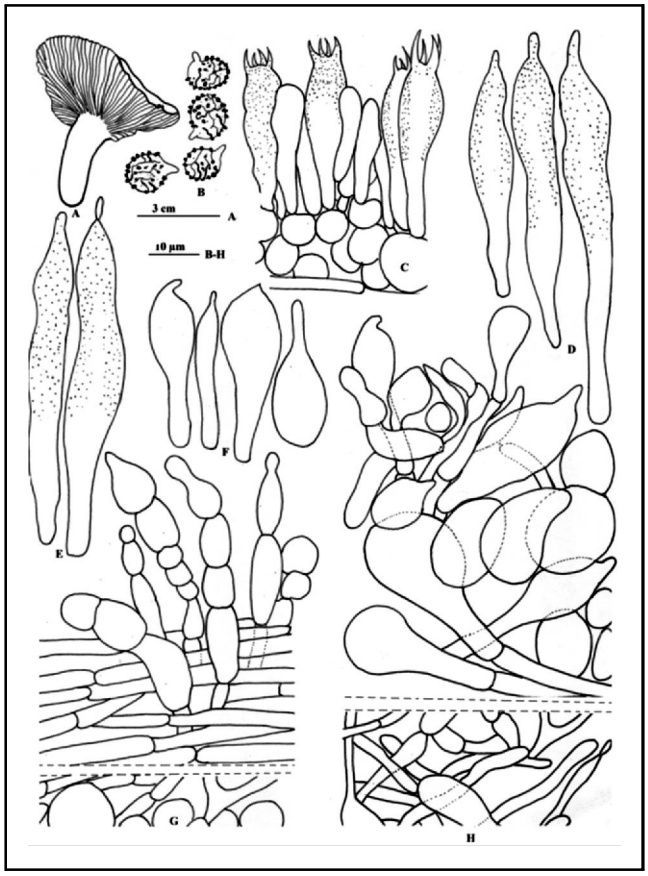


**Fig.3(A-H)** ***Russula chlorinosma***: A. Sporophore, B. Basidiospores, C. Hymenophore showing basidia, D. Pleurocystidia, E. Cheilocystidia, F. Caulocystidia, G. Cross section through stipe showing cuticular details and context, H. Cross section through pileus showing cuticular details and context.

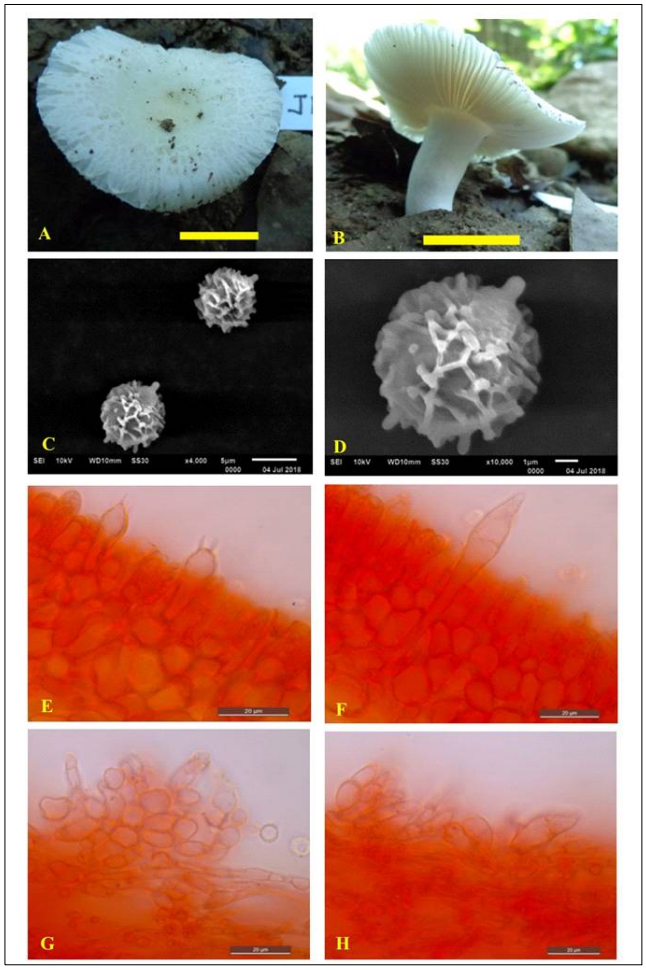


**Fig. 4(A-H)** ***Russula chlorinosma***: A-B. Sporophores, C-D. Scanning electron micrograph of basidiospores, E. Hymenophore showing basidia and cystidia, F. Pleurocystidia, G. Cross section through pileus showing cuticular details and context, H. Cross section through stipe showing cuticular details and context. **Scale bar** A = 2cm, B = 3cm.

***Russula chlorinosma*** Burl. *Mycologia* **16** (1): 22,1924.

**Figs. 3(A-H) & 4(A-H)**

Basidiomata 3.2-5.0 cm in height. Pileus 5.0-6.2 cm diam., umbilicate with flattened depressed centre; white (1A1) throughout, cracking along the margin; with some off white to cream colored squamulose structures seems to have been formed due to cracking of the pileal cuticle in the young stage. ; margin regular, non-splitting at maturity, moist, apex depressed; cuticle hardly peeling; Lamellae free to adnexed, equal, forked, moderately broad (2-4 mm), distant (10-12 gills/cm), pure white; gill edges smooth, normal. Stipe central, 2.0-2.5 × 1.2-1.5 cm, almost cylindrical, white (1A1), concolorous with the pileus, unchanging, solid, smooth. Context 0.1-0.3 cm thick in the centre, absent at the margin, white (1A1), unchanging, brittle; taste peppery, odour almost pungent. Spore deposit white.

Basidiospores 5.7-9.0 × 4.8-7.0 µm (excluding ornamentation), broadly ellipsoidal (Q =1.17-1.25), warty; warts up to 0.4 µm high, mostly connected to form partial to complete reticulum, ornamentation type IIIa, IIIb, amyloid; plage hyaline, indistinct; apiculate, apiculus up to 1 µm long. Basidia 19.5-35.8 × 6.5-9.0 µm, clavate, tetrasporic; sterigmata up to 6.5 µm long; pleuromacrocystidia 48.0-80.0 × 6.5-10.0 µm, fusoid ventricose to appendiculate, with acute to obtuse apex, granulated; cheilocystidia 48.9-73.2 × 6.5-9.8 µm, similar to pleurocystidia. Hymenophoral trama 57-130 µm broad, heteromerous with up to 4.8 µm broad hyphae intermingled with sphaerocysts; subhymenium 16-33 µm broad indistinct, cellular. Pileus cuticle 114-211 µm broad; made up of palisade consisting of chains of inflated 16.0-35.8 × 16.0-24.5 µm sized sphaerocysts intermixed with dermatocystidia. Pileocystidia 16.0-40.8 × 4.5-13.0 µm, thin-walled, clavate, fusiform to fusoid ventricose to obclavate with acute, blunt ended to flame shaped tips; subcutis 98-114 µm broad, with a zone of interwoven, septate, 1.6-6.5 µm broad hyphae; context heteromerous having multiseptate up to 5.71 µm broad hyphae intermingled with rosettes of sphaerocysts. Stipe cuticle consists of more or less parallel running 1.6-6.5 µm broad septate hyphae with caulocystidia; caulocystidia 16.5-48.7 × 4.9-9.8 µm, inflated to ampullate having attenuate to obtuse ends; context made up of sphaerocytes. Clamp connections absent.

**Chemical reaction:** Stipe surface pinkish with $FeSO_4$; lamellae turn carmine red in Sulphovanillin.

**Collections examined:** Himachal Pradesh: Sirmour, Paonta (380 m), Sal forest in association with *Shorea robusta*. 28 July 2013, Jitender Kumar, PUN 9108. Sirmour, Puruvala (425 m), Sal forest in association with *S. robusta*. 28 August 2013, Jitender Kumar, PUN 9109. Uttarakhand: Asharodi (700 m), Sal forest in association with *S. robusta*. 31 August 2013, N.S. Atri and Jitender Kumar, PUN 9110.

**Remarks:** External and internal details of the presently examined collections are in conformity with the description of *R. chlorinosma* as given by Burlingham (1924) except for slight variation in spore size. *R. chlorinosma* is characterised by its areolately cracked pure white pileus without any pigmentation, pungent smelling flesh and palisadic pileus cuticle having chains of sphaerocysts intermixed with dermatocystidial elements. Many of its features are quite close to *R. kanadii* (Dutta *et al.*, 2015). However, in *R. chlorinosma* unlike *R. kanadii* surface is neither creamy cinnamon nor rusty spotted and also lamellae are pure white instead of cream colored. In case of *R. chlorinosma* taste is peppery and not mild and the spores are larger in size in comparison to *R. kanadii*.

***Russula nobilis*** Velen. *České Houby* **1**: 138, 1920

**Figs. 5(A-G)& 6(A-H)**

Sporophore 6.5 cm in height. Pileus 5.5 cm broad, umbilicate, moist, glabrous, viscid, sticky, pastel red to reddish white with pink depressed centre; umbo absent; margin regular, non-splitting, striated; cuticle half peeling; flesh up to 4 mm thick, brittle, yellowish white, unchanging; taste slightly acrid, odour fruity. Lamellae adnate, equal, subdistant (12 gills/cm), forked at the margin, up to 0.2 cm broad; white (2A2), greyish brown on bruising; gill edges smooth. Stipe central, cylindric, 5 cm long, 1.0 cm broad, yellowish white (2A2) to reddish white towards the margin, unchanging, solid with persistent pith, smooth. Spore deposit white.

Basidiospores 5.7-9.0 × 4.9-7.0 µm (excluding ornamentation), subglobose to broadly ellipsoid (Q=1.14-1.16); warty, warts up to 0.8 µm high, conic to blunt ended, mostly connected to form almost complete reticulum, ornamentation type IIIa, amyloid; plage amyloid, distinct; apiculate, apiculus up to 2.0 µm long. Basidia 26-39 × 8.2-9.8 µm, clavate, thick walled, hyaline to granulated, bisporic to tetrasporic; sterigmata 3.3-4.8 µm long; pleurocystidia: macrocystidia 78.5-93.0 × 11.5-16.0 µm, fusiform to fusoid clavate to appendiculate with acute end, hyaline to granulated, usually with an irregularly dense granulated mass in the upper half; cheilocystidia 57.0-84.8 ×

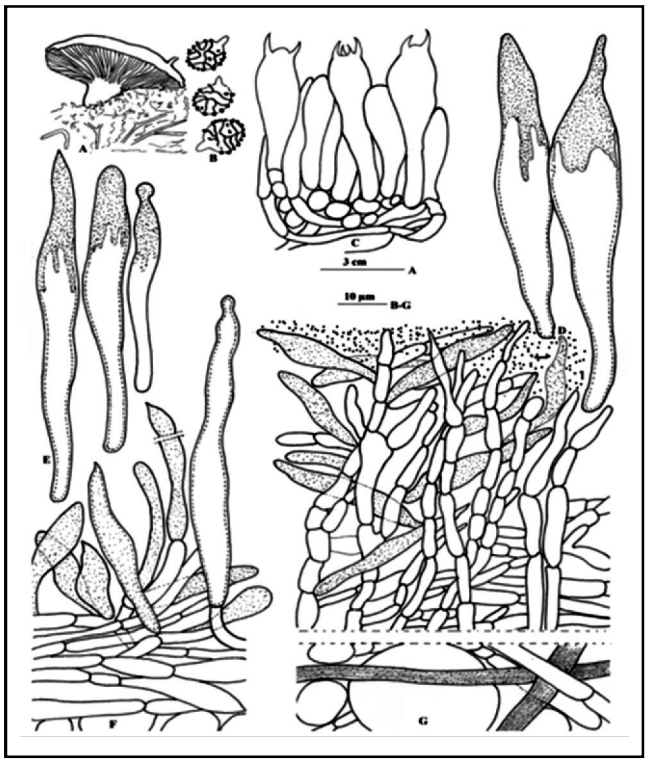


**Fig. 5(A-G).** ***Russula nobilis***: A. Sporophore, B. Basidiospores, C. Hymenophore showing basidia, D. Pleurocystidia, E. Cheilocystidia, F. Cross section through stipe showing cuticular details and context, G. Cross section through pileus showing cuticular details and context.

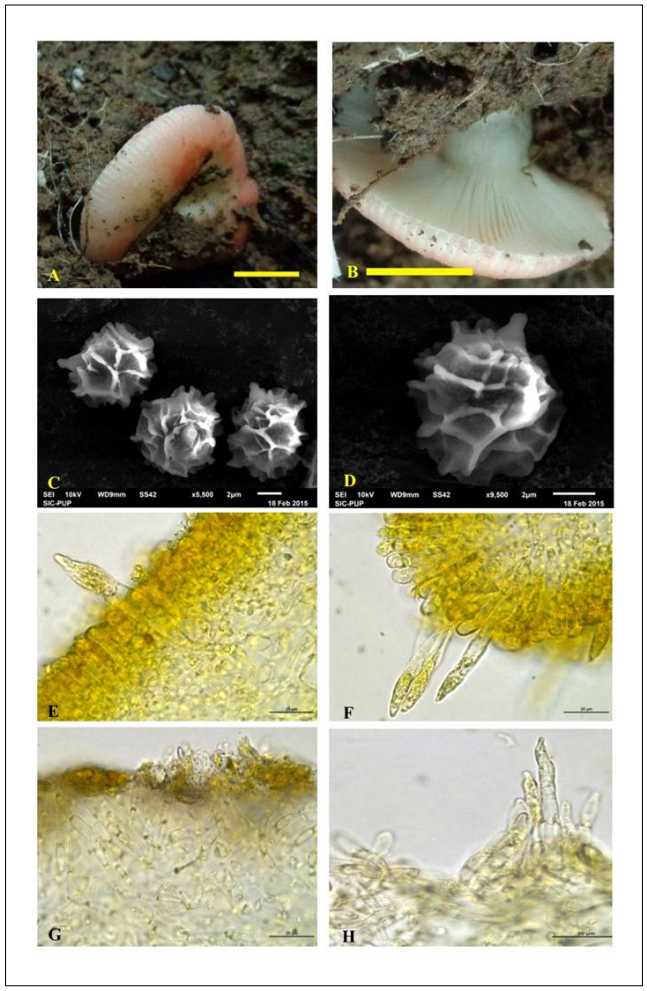


**Fig. 6(A-H).** ***Russula nobilis***: A-B. Sporophores, C-D Scanning electron micrographs of basidiospores, E. Hymenophore showing basidia and pleurocystidia, F. Cheilocystidia, G. Cross section through pileus showing cuticular details and context, H. Cross section through stipe showing cuticular details and context. **Scale bar** A-B = 2 cm.

4.9-9.2 µm broad, fusiform to subcylindric, with subacute to rounded apex, abundant, projecting up to 57 µm from the hymenial surface. Pileus cuticle 150-180 µm broad with interwoven moniliform to subcylindric hyphae and dermatocystidia in a gelatinised matrix. Pilocystidia 24.5-48.9 × 4.9-6.5 µm, thick walled densely granulated; epicutis 150-180 µm with 3.3-6.5 µm broad interwoven septate hyphae; context heteromerous, made up of rosettes of 29.4-57.0 × 24.5-53.8 µm broad sphaerocysts intermingled with 1.6-6.5 µm broad thin walled septate hyphae. Hymenophoral trama 32-320 µm broad, heteromerous, made up of 3.3-9.8 µm broad thin walled hyaline hyphae and 19.5-35.9 × 16.3-32.6 µm sized sphaerocysts; subhymenium distinct, 16-20 µm, cellular, cells 5-21 × 5-10 µm intermingled with 1.6-4.9 µm broad hyphae. Stipe cuticle composed of turf of thick walled numerous clavate to subclavate caulocystidia measuring 32.6-81.5 × 4.9-8.2 µm in size. Cuticular hyphae 1.6-8.2 µm broad, septate more or less parallel; context heteromerous, made up of alternate arrangement of cellular and hyphal layer, hyphae 6.5-11.5 µm broad, mixed with rosettes of 24.5-48.9 × 16.3-32.6 µm sized sphaerocysts. Clamp connections absent.

**Chemical colour reaction:** Gills turn greyish ruby (12E4) in Sulphovanillin, stipe surface pinkish with FeSO4.

**Collection examined:** Himachal Pradesh, Sirmour, Puruwala (425 m) reported in association with *Shorea robusta*. Jitender Kumar, PUN 9121, September 3, 2013.

**Remarks:** This species is typical of *R. nobalis*, which was recorded by Pala *et al*. (2012) from southern Kashmir and Kumar *et al*. (2014) from Nagaland. Present documentation is first of its type from the Sal forest of Shivaliks. This species was noted forming putative mycorrhizal association with *Shorea robusta* since there was a clear cut organic connection of mycelium emanating from the base of stipe of this mushroom with the Sal roots.

***Amanita battarrae*** (Boud.) Bon, *Documents Mycologiques* **16** (61): 16, 1985 **Figs. 7(A-E) &8(A-F)**

Basidiomata 15 cm in height. Pileus 8.5 cm broad, convex; surface light greyish brown at the margin, dark greyish brown to orange grey at the centre (8D3), viscid, glabrous; margin regular, non-splitting, striate; cuticle half peeling. Lamellae adnexed, unequal, soft, subdistant to close, up to 0.7 cm broad, white to creamy white (2A2); gill edge smooth, unchanging. Stipe central, white to orange grey (5B2) with grey brown scales (5E3), 13 × 0.8 cm, obclavate, solid becoming hollow, surface moist. Volva saccate, white in colour. Annulus absent. Context up to 0.2 cm thick, white, unchanging; odour fragrant. Spore deposit yellowish white (4A2).

Basidiospores 10-12 × 9-12 µm, globose (Q = 1-1.1), smooth, inamyloid, hyaline, thin-walled, apiculate, apiculus up to 1.6 µm long. Basidia 29-40 × 8-12 µm, clavate, tetrasporic, sterigmata up to 8.2 µm long, lacking clamp connections; gill edge fertile; cystidia absent. Pileus cuticle gelatinised with interwoven, septate, up to 6.5 µm broad hyphae, up to 8.2 µm broad oleiferous hyphae present in the context. Pileocystidia absent. Context homogenous with loosely tangled, septate, up to 24.5 µm broad hyphae. Hymenophoral trama divergent,

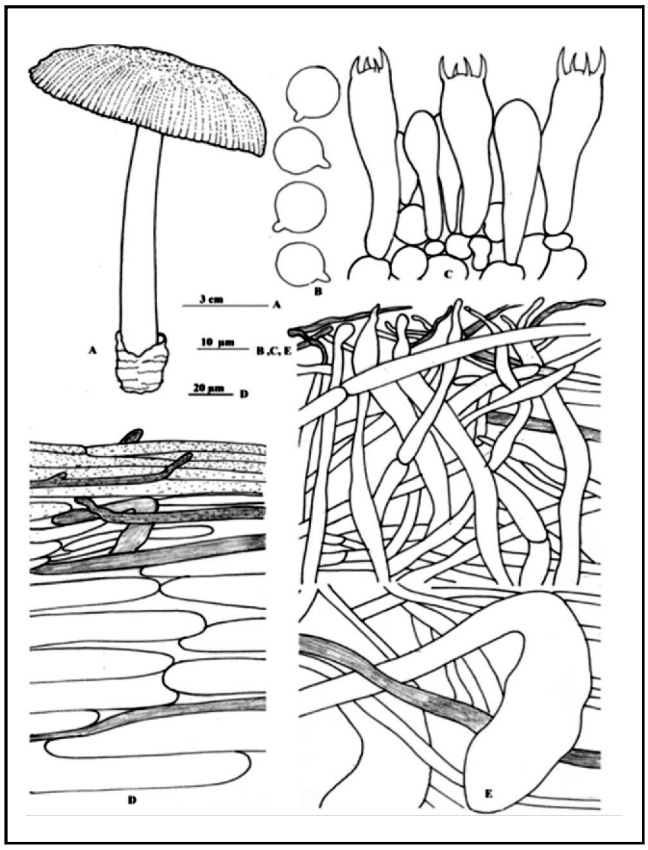


**Fig. 7(A-E)** ***Amanita battarrae***: A. Sporophore, B. Basidiospores, C. Hymenophore showing basidia, D. Cross section through stipe showing cuticular details and context, E. Cross section through pileus showing cuticular details and context.

with central hyphae up to 9.8 µm and divergent hyphae up to 32.6 µm broad. Stipe cuticle gelatinised with interwoven, septate hyphae up to 12 µm broad; context having septate hyaline parallel up to 32 µm broad hyphae. Oleiferous hyphae present in the context. Volva heteromerous, made up of hyphae intermingled with few cells. Clamp connections absent.

**Collection examined:** Himachal Pradesh: Dhaulakuan (425 m), Sirmour, in association with *Shorea robusta*. 28 August 2013. Jitender Kumar, PUN 9144.

**Remarks:** The external and internal details of the presently examined collection are in conformity with the description given by Bahram *et al*. (2006) except for spore size which are smaller and almost conforms to the spore size of *A. vaginata*. This fungus is characterised by its light greyish brown pileus margin and dark greyish brown to greyish orange centre, viscid and striated surface, white lamellae, white to orange grey stipe with grey brown scales and presence of white saccate volva.

## DISCUSSION

During the survey of Sal forest of Shiwalik range of North West India various species of agarics were collected, most of which were putatively ectomycorrhizal. Various genera such as *Russula, Agaricus, Amanita, Lactarius, Lactifluus, Xerula,*

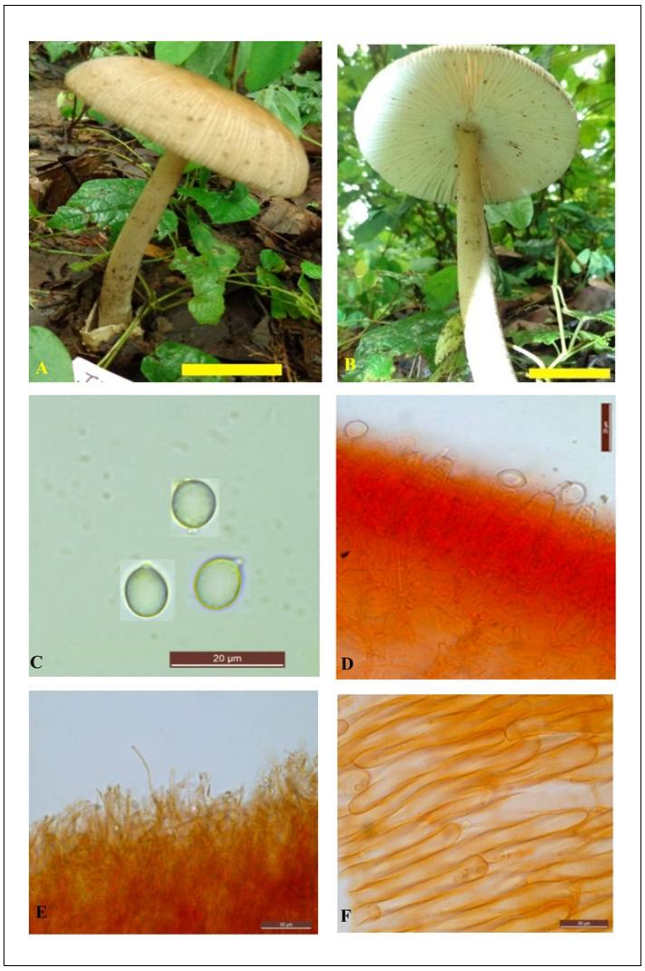


**Fig. 8(A-F)** ***Amanita battarrae***: A-B. Sporophores, C. Basidiospores, D. Hymenophore showing basidia, E. Cross section through pileus showing cuticular details and context, F. Cross section through stipe. **Scale bar** A-B = 3 cm.

*Termitomyces, Pholiota, Inocybe* and *Asperoinocybe* were documented during the present survey. There are earlier reports also about the documented genera from Sal forest (Bakshi, 1974; Natarajan *et al*., 2005; Pyasi *et al*., 2011, 2012; Kumar and Atri, 2016, 2019). However, genus *Asperoinocybe* was not documented prior to this study neither from this region nor from any other part of India. During the present study, *Asproinocybe lactifera* was found growing in association with *Shorea robusta* and looking into the organic connection of the mushroom mycelium with the roots of Sal it seems to form putative mycorrhizal association with the roots of this plant. *Asperoinocybe lactifera* is characterized by typically greyish violet to deep violet cap and lamellae, small nodulose basidiospores, presence of frequent laticifers throughout the context and exudation of scanty hyaline fluid from the cut portion of the sporophore. These features are in complete agreement with the observations recorded by Heim (1970). This species is quite close to *A. russuloides* and *A. brunneolilacina* except that hymenial cystidia are comparatively larger in *A. lactifera* and smaller in *A. russuloides*, however, these are altogether absent in *A. brunneolilacina*.

The mushroom genus *Russula* is a representative of the highly diverse EcM groups in *Agaricomycetes* which play a critical role in the maintenance and strengthening the forest ecosystems and biodiversity (Henkel *et al*., 2011, Corrales *et al*., 2016). To date, approximately 1150 *Russula* species have been reported worldwide (Kirk, 2016), and distributed across a wide range of habitats from the tropics to arctic zones. *Russula* is also reported to be one of the most common genus forming putative ectomycorrhizal association with members of Conifers, Oaks and Rhododendrons in North west Himalayas (Saini and Atri, 1984; Atri and Saini, 1986; Atri *et al*., 1997; Kaur *et al*., 2011; Sharma *et al*., 2016). In India this genus is represented by ca. 158 taxa from India (Sharma *et al*., 2018). During the present investigations the mycelium of *R. chlorinosma* was also found forming direct organic connection with the roots of *Shorea robusta*. From amongst the known species of this genus, *Russula chlorinosma* is very close to *R. virescens* and also to *R. kanadii*. Cracked patches in *R. chlorinosma* often remain whitish until age. Cuticle is hardly separable, and flesh is very thin and pungent smelling in case of *R. chlorinosma* but in comparison *R. virescens* have thick mild tasting flesh and easily separable cuticle. The species was for the first time recorded from North America from mixed forest by Burlingham (1924). Another *Russula* species, *R. nobalis*, is characterized by its viscid, sticky, pastel red surface with light brown centre, acrid taste, completely reticulated basidiospores and pileus cuticle having moniliform hyphal elements and embedded dermatocystidia in gelatinized matrix. Pala *et al*. (2012) listed it from southern Kashmir and Kumar *et al*. (2014) from Nagaland. Presently it was recorded for the first time forming putative mycorrhizal association with Sal in the Shivaliks of North western Himalayas.

Genus *Amanita* is also one of the dominant EcM genus, represented by ca. 80 taxa from India (Singh and Kaur, 2016; Upadhyay *et al*., 2017). During the present study *A. battarrae* was recorded for the first time forming putative mycorrhizal association with Sal in the Shiwaliks of Sirmour Discrict of Himachal Pradesh in India. This species is quite close to *A. umbrinoluta* with respect to having a different colour zone on the pileus surface but differ from it in having smaller spore size. *A. battarrae* is widely distributed in Europe and its range extends eastwards at least to North-Western Pakistan and Northern India and is reported mostly forming association with conifers (Bahram *et al*. 2006). From Iran the species is reported from the forest dominated by *Parrotia persica* and *Quercus castaneifolia* (Bahram *et al*., 2006).

## CONCLUSION

All the four presently investigated agarics, *viz. Asproinocybe lactifera , Russula chlorinosma, R. nobalis,* and *Amanita battarrae* were recorded forming direct organic connection with *Shorea robusta* roots, hence all the four species are putatively ectomycorrhizal associates of *Shorea robusta*.

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