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Cover: Dorsal view of Mantis Shrimp *Cloridina ichneumon* (Fabricius, 1798) & *Gonodactylus demanii* (Henderson, 1893). © Fisheries Research Station, Junagadh Agricultural University, Sikka.

INTRODUCTION

Genus *Russula* is one of the dominant basidiomycetous fungi genus which grow in a wide variety of habitats. These are mostly found in mycorrhizal association with variety of plants including trees (Corrales et al. 2016). Studies on taxonomy and diversity of genus *Russula* are inviting more attention now a days primarily because of their importance in human welfare, ecosystem functioning and stability. These macro-fungi are important source of food, medicine, nutraceuticals and also play a pivotal role in ecosystem strengthening and maintenance as mycorrhizal associates (Manoharachary et al. 2005). It is reported that *Russula* spp. can form EcM with many temperate and tropical plant families, including *Leguminosae*, *Fagaceae*, *Cistaceae*, *Dipterocarpaceae*, *Salicaceae*, *Betulaceae*, *Nothofagaceae*, *Myrtaceae*, and *Pinaceae* (Tedersoo et al. 2010; Wang et al. 2017). The compounds derived from these mushrooms are reported to boost up immune system and avert diseases thereby improving human health (Wasser 2002). Different species of *Russula* are known to possess anti-inflammatory, antiviral, antibacterial, antiparasitic, antioxidant, hepatoprotective, anticancer, and antidiabetic properties (Wasser 2011).

To date, approximately 1,100 *Russula* species have been reported worldwide (Kirk 2014) and distributed across a wide range of habitats from the tropics to arctic zones (Rivière et al. 2007; Ba et al. 2012). *Russula* is one of the dominant ectomycorrhizal genera in Indian Himalaya (Saini & Atri 1984, 1989; Atri & Saini 1986; Atri et al. 1994; Kumar & Atri 2016, 2019; Sharma et al. 2016) and is represented by ca. 158 taxa from India (Sharma et al. 2017). While investigating the EcM diversity of Sal forest, two varieties of *R. camarophylla* Romagn. and *R. aurea* Pers. were documented, which upon investigation were found to be new to science based on detailed macro- and micro-morphological examination. In the present study sporocarps and their EcM colonised roots were collected by tracing the hyphal or rhizomorphs connections in association with *Shorea robusta* from pure Sal forests. These species are fully illustrated and described in this paper.

MATERIALS AND METHODS

Study area

Area selected for the present investigation is Sal forests of Shiwalik mountain range of northwestern India (Figure 1), which represent the geologically lowest and youngest mountain range of Himalaya. The study area

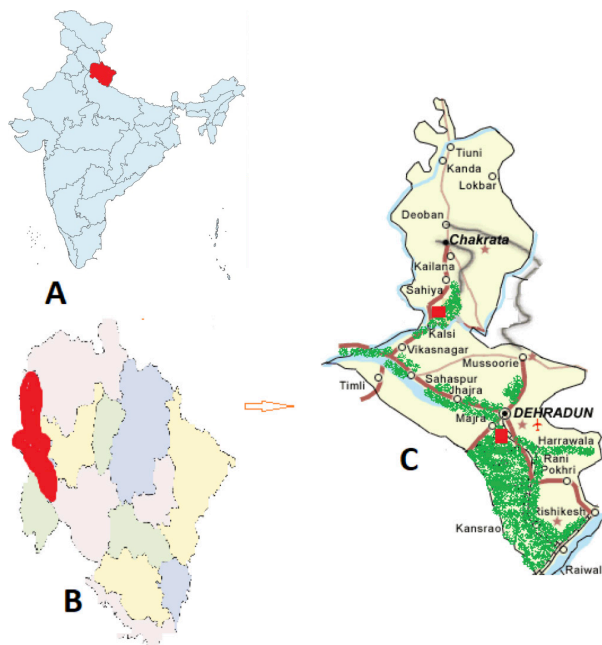


Figure 1. Location map of Study area (Red).

is located between 30.316N, 78.032E. Elevation range of the area is 400–1500 m and vegetation of the area is typical of tropical moist deciduous type (Champion & Seth 1968).

Sampling, identification and characterization

Sporocarps were collected from different localities of pure Sal forests, during the rainy season of 2013–2015. Macromorphological features were recorded from fresh collections in the field and colour codes used are that of Kornerup & 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 after bringing the specimens to the temporary laboratory setup. Sporocarps were air dried at 40–45 °C in a drier specially designed for drying mushroom specimens (Atri et al. 2005) which were finally packed in a cellophane paper packet for permanent preservation in Punjabi University Herbarium under PUN. The cross section of pileus and longitudinal section of stipe were stained in congo red for examination, drawn under a compound microscope and photographed under digital microscope (Leica DM4000 B LED). Observation of basidia, cystidia, and elements of pileipellis and stipitipellis were recorded for further use in taxonomic categorization. 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. 2000, 2017).

Scanning electron microscopy

Scanning electron microscopic (SEM) studies of basidiospores 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 3,000–15,000 X in high vacuum mode to observe pattern of spore ornamentation.

TAXONOMY

Russula aurea Pers. var. *minuta* var. nov.

(Image 1a–h, Figure 2A–G)

Mycobank number: MB834095

Diagnosis: *Russula aurea* Pers. var. *minuta* var. nov. is characterised by small golden to brightly yellow pileus with more darker brownish-yellow centre; dentate to wavy gill edges with golden deposition instead of smooth; sour taste, much smaller spores size and presence of pileocystidia.

Etymology: The variety name is based on the smaller size of sporophore and basidiospore as compared to *Russula aurea*.

Holotype: PUN 9112, Male, 27 July 2013, Rajban, Dehradun, Uttarakhand, India, 30.316N, 78.032E, 800 m, coll. J. Kumar.

Paratype: PUN 9113, 1 ex., Male, 21 August 2015, Kalsi, Dehradun, Uttarakhand, India, 30.316N, 78.032E, 1,190 m, coll. J. Kumar.

Taxonomic description

Sporophores 2.0–2.5 cm in height. Pileus 1.3–2.0 cm broad, convex to hemispherical when young, flattened depressed at maturity; centre umbonate when young, golden (6C7) to brightly yellow with more darker brownish-yellow centre; margin regular to slightly irregular, nonsplitting at maturity, moist, unchanging, apex depressed at maturity with slight umbo; cuticle half peeling; flesh 0.1 cm thick in the centre, almost absent along the margin, white (1A1), changes to light brown on bruising and cutting, brittle; taste sour, odour mild. Lamellae adnexed to slightly adnate, equal, moderately broad (2–3 mm), crowded (12–16 gills/cm), white with golden edges; gill edges not smooth, eroded or wavy.

Stipe central, 1.5–2.0 cm in length, 0.3–0.5 cm broad, cylindrical to slightly tapering downward, white (1A1) in the upper half, yellowish to pale white in the lower half, unchanging, first solid, then hollow, smooth. Spore deposit deep ochre.

Basidiopores 5.0–6.5 (7.5) × 4.0–5.0 (6.0) μm (excluding ornamentation), broadly ellipsoidal to ellipsoid (Q = 1.2–1.3), warty; warts up to 0.8 μm high, mostly connected by thick and thin lines to form partial to complete reticulum, ornamentation type IIIa, IIIb, IV, amyloid; plage hyaline, indistinct; apiculate, apiculus up to 1.6 μm long. Basidia 19.5–32.6 × 6.5–9.0 μm, clavate, bisporic to tetrasporic, hyaline, abundant; sterigmata up to 3.5 μm long; pleurocystidia 26.0–40.9 × 6.5–9.8 μm, clavate to ventricose granulated; cheilocystidia 22.5–37.4 × 4.1–13.1 μm, similar to pleurocystidia. Pileus cuticle clearly differentiated, epicutis gelatinised, heteromorous, palisade having interwoven projecting septate 3–5 μm broad hyphae mixed with 5–10 μm broad sphaerocyst and dermatocystidia, cuticle hyphae and cellular mass having dark yellow content throughout; pileocystidia

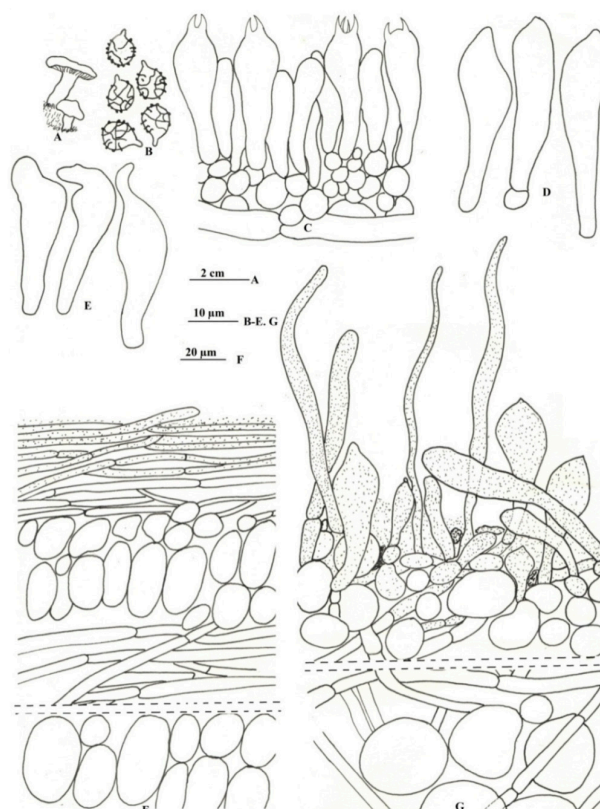


Figure 2. *Russula aurea* var. *minuta* var. nov.: A—Sporophores | B—Basidiospores | C—Hymenophore showing basidia | D—Pleurocystidia | E—Cheilocystidia | F—Cross section through stipe showing cuticular details and context | G—Cross section through pileus showing cuticular details and context.

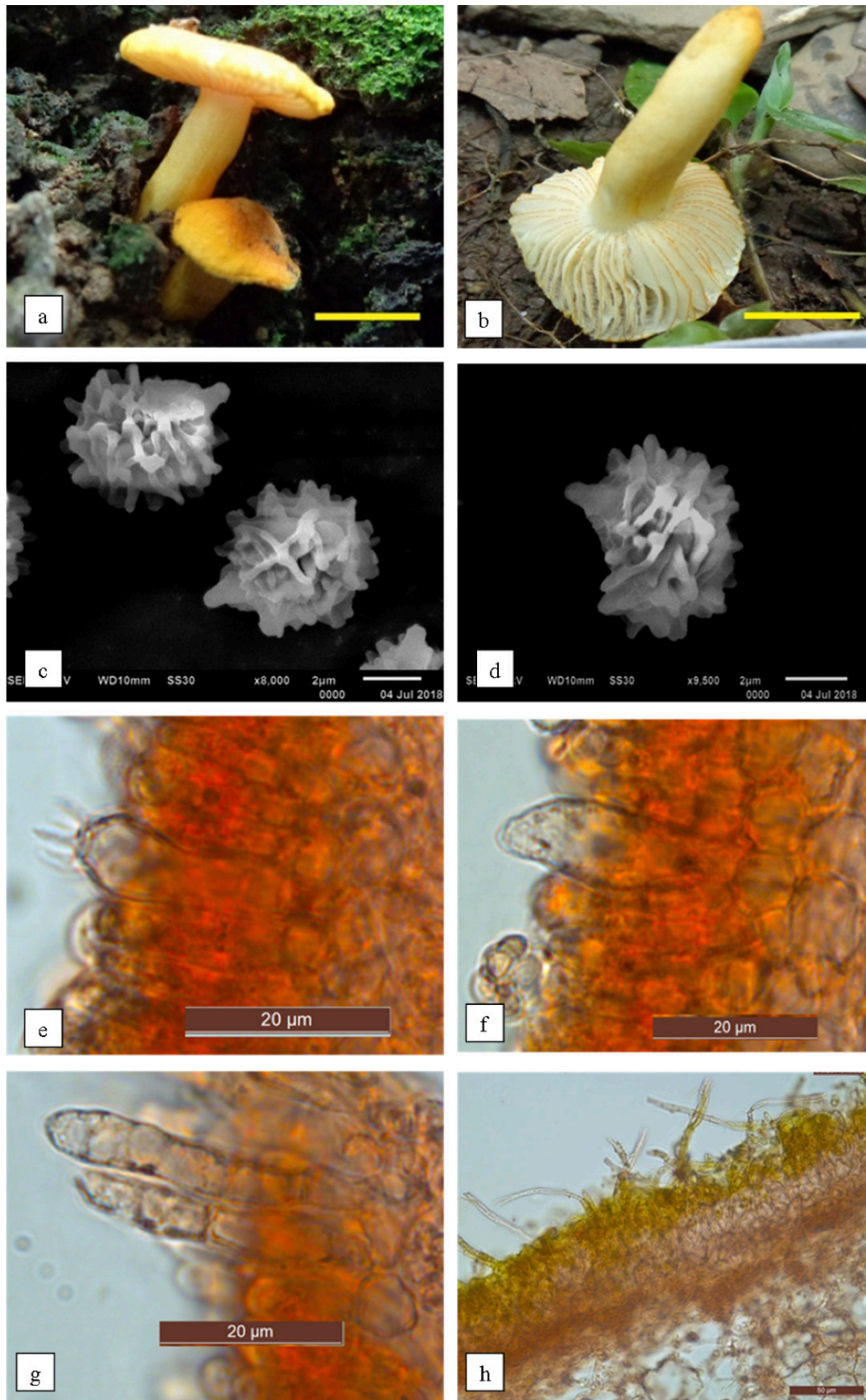


Image 1. *Russula aurea* Pers. var. *minuta* var. nov. a–b—Sporophores | c–d—Scanning electron photographs of basidiospores | e—Hymenophore showing basidia and cystidia | f—Pleurocystidia | g—Cheilocystidia | h—Cross section through pileus showing cuticular details and context. Scale bar a–b= 1 cm.

24.0–40.8 × 4.9–8.2 µm, thin walled, clavate, fusiform to fusoid ventricose with acute to blunt end; context heteromerous having multiseptate 3.0–6.5 µm broad hyphae intermingled with 8.0–36.0 × 8.0–32.6 µm rosettes of sphaerocysts. Hymenophoral trama 16–100 µm, heteromerous with up to 3.3–5.0 µm broad hyphae intermingled with 8.0–36.0 × 8.0–32.6 µm sphaerocysts. Stipe cuticle gelatinised with more or less parallel arranged 1.6–6.5 µm broad septate hyphae having yellowish content; context made up of 12–28 × 12–24 µm sized rosettes of sphaerocysts and 4–6 µm broad septate hyphae in alternate manner. Clamp connections absent.

Chemical colour reaction: Stipe surface pinkish with FeSO₄, Gills turns carmine red in Sulphovanillin.

Habitat: Sporophores directly attached to the roots at the base of *Shorea robusta* tree.

Collections examined: Uttarakhand: Dehradun, Rajban (800 m), in groups in Sal forest in association with *Shorea robusta*. Jitender Kumar, PUN 9112, 27 July 2013. Dehradun, Kalsi (1,190 m), solitary in Sal forest in association with *S. robusta* tree. Jitender Kumar PUN 9113, 21 August 2015.

Remarks: The overall diagnostic characters of the presently examined collection are in agreement with *Russula aurea* (Rayner 1970; Romagnesi 1967; Das & Marstad 2014) except that the carpophores are much smaller in size (2.0–2.5 cm instead of 4–9 cm), gill edges not smooth (dentate to wavy with golden or yellow deposition instead of smooth), much smaller spores size (5–7.5 × 4–6 µm instead of 7.5–10 × 6–8 µm) and presence of pileocystidia which are absent in case of *Russula aurea*. In view of the presence of some unique varied features in the presently examined collections in comparison to *R. aurea*, a new variety *minuta* has been named.

***Russula camarophylla* Romagn. var. *reticulospora* var. nov.**

(Image 2a–h, Figure 3A–G)

Mycobank number: MB834095

Diagnosis: *Russula camarophylla* Romagn. var. *reticulospora* var. nov. is characterised by larger sporophore with creamish-white pileus surface, distantly spaced lamellae, very hard and compact flesh and larger spore size. Also in basidiospores warts are connected to form mostly dense complete reticulum.

Etymology: The variety name is based on the densely reticulated basidiospores.

Holotype: PUN 9124, Male, 30 August 2013, Kalsi, Dehradun, Uttarakhand, India, 1,190 m, 30.316N, 78.032E, coll. J. Kumar.

Taxonomic description

Sporophore 7.5 cm in height. Pileus 10 cm broad, umbilicate with a depressed disc and irregular margin; pileus surface moist, glabrous, cream white to white (1A1), not peeling; flesh 5 mm thick in the centre, off white to slightly creamish, unchanging. Lamellae unequal broadly adnate to decurrent, distant (3–4 gills/cm), broad (11 mm at the centre), creamish-white to orange white (5A2), forked near the base, lamellulae present, gill edges smooth, normal. Stipe 2 cm long and up to 2 cm broad, central, solid, white, fleshy, concolorous with the pileus, unchanging on cutting and bruising; flesh taste spicy; odour fruity, spore deposit yellowish-white.

Basidiospores 6.5–8.0 (9.0) × 5.0–7.0 (7.5) µm, subglobose to broadly ellipsoid (Q= 1.12–1.33), densely ornamented, warty, warts up to 0.5 µm, connected to form mostly complete reticulum, superahilar area usually with low ornamentation, ornamentation type IIIa, IIIb; apiculate, apiculus up to 1.6 µm in size. Basidia 35–57 × 5.0–8.5 µm, clavate to subcylindric, 2–4 spored, sterigmata 6.5–9.8 µm long. Pleurocystidia 39.0–86.5

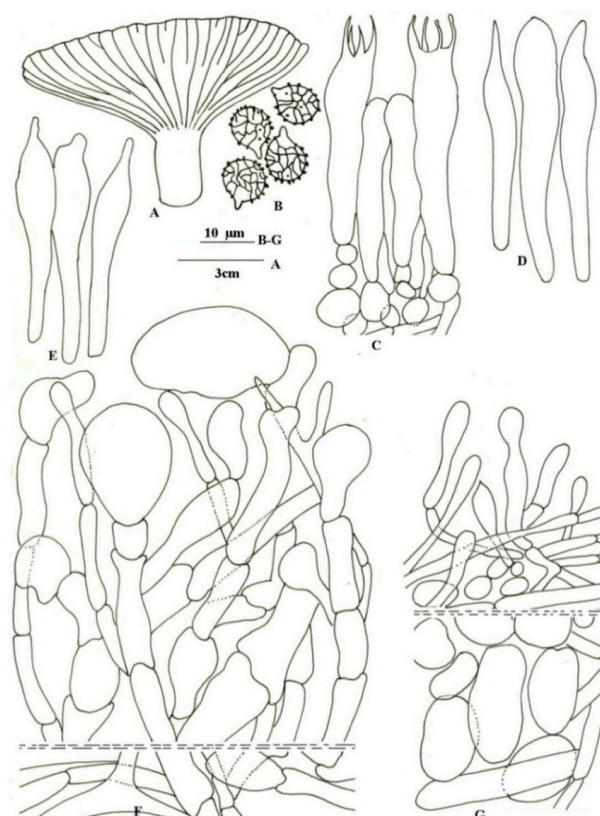


Figure 3. *Russula camarophylla* Romagn. var. *reticulospora* var. nov.: A—Sporophore | B—Basidiospores | C—Hymenophore showing basidia | D—Pleurocystidia | E—Cheilocystidia | F—Cross section through pileus showing cuticular details and context | G—Cross section through stipe showing cuticular details and context.

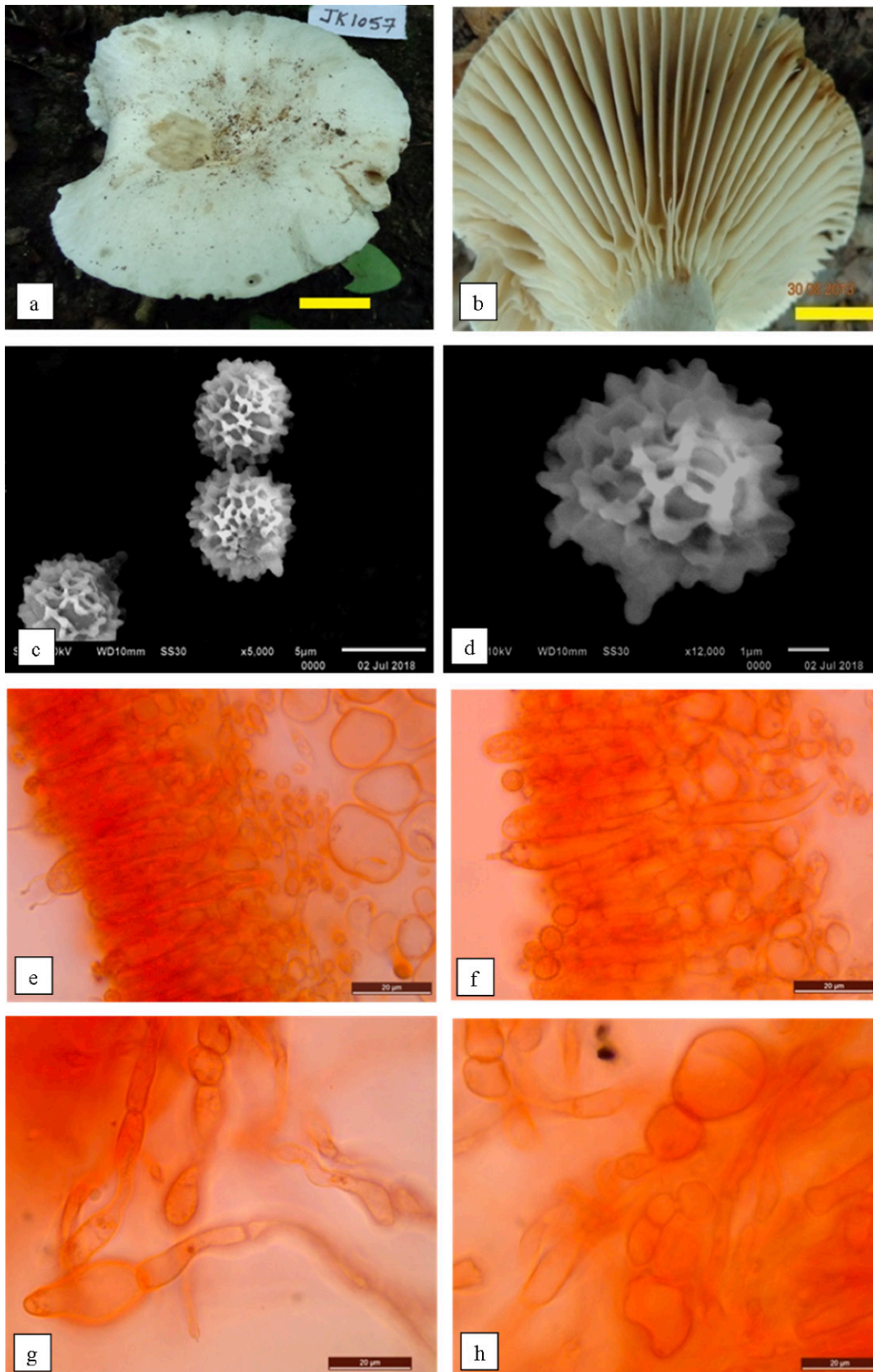


Image 2. *Russula camarophylla* Romagn. var. *reticulospora* var. nov.: a–b—Sporophores | c–d—Scanning electron microphotographs of basidiospores | e—Hymenophore showing basidia and cystidia | f—Pleurocystidia | g–h—Cross section through pileus showing cuticular details and context. Scalebar a–b= 2 cm.

× 4.8–9.8 µm, clavate, fusiform, fusoid clavate to fusoid ventricose, often acute to blunt ended, granulated to hyaline; arising usually from the subhymenium, nonprojecting. Cheilocystidia 35.8–48.9 × 4.8–9.0 µm, clavate to fusiform, apically acute to blunt, mostly hyaline, gill edges heteromorphic; subhymenium 31.4–47.2 µm, heteromerous with 3–13 µm broad hyphae interspersed with 3.0–11.5 × 3.0–10.0 µm sphaerocyst; hymenophoral trama 471–942 µm, mostly cellular with 6.5–11.5 µm hyphae and 6.5–42.5 × 4.9–42.5 µm sphaerocyst. Pileus cuticle up to 240 µm broad, trichoderm of nongelatinous, 6.5–18.2 µm interwoven septate projecting hyphae, hyphae ending attenuate or rounded and some ending with inflated or rounded cells, hyphal cells cylindric to ampullate at both sides of the septum, septa mostly constricted, some hyphae with large inflated cells in the intercalary position. Subcutis not clearly differentiated, made up of interwoven, nongelatinous, 6.5–18.5 µm broad hyphae with a few inflated scattered cells. Stipe composed of parallel arranged interwoven septate up to 4.9 µm broad hyphae; caulocystidia 21–41 × 3–5 µm, cylindric to clavate, rounded apically.

Chemical colour reactions: Stipe surface pinkish with FeSO₄, gills turns carmine red in Sulphovanillin.

Collection examined: Uttarakhand: Kalsi (1,190 m), in association with *Shorea robusta*. Jitender Kumar, PUN 9124, 30 August 2013.

Habitat: Solitary on the ground in monsoon under *Shorea robusta* tree.

Remarks: The external and internal characters of the presently examined collection are in agreement with *Russula camarophylla* (Romagnesi 1968) except that the carpophores are larger in size with cream white pileus surface and larger spore size (6.5–9 × 5–7.5 µm instead of 5–6.2 (7) × 3.6–4.8 µm). In basidiospores warts are connected to form mostly complete reticulum instead of mostly isolated warts in case of *Russula camarophylla* as documented in literature.

DISCUSSION

During the present study, *R. aurea* var. *minuta* and *R. camarophylla* var. *reticulospora* were found forming direct organic connection with *Shorea robusta*. The overall diagnostic characters of the presently examined collections of *R. aurea* var. *minuta* are in agreement with *Russula aurea* Pers. which is commonly known as the gilded brittle gill or golden *Russula* and is an uncommon species of mushroom found in deciduous woodland forests. Its specific epithet *aurea* has been

derived from the Latin word *aureum*, which means golden. Unlike many red-capped members of the genus, *Russula aurea* is edible and mild-tasting and is easily characterised in the field by its golden pileus, free to adnexed broad fairly distant golden gills, cylindrical smooth light yellow stipe and brittle yellow flesh. Mostly it is reported to grow solitary or scattered forming mycorrhizal association with pine trees (Romagnesi 1967; Rayner 1970; Das & Marstad 2014). *Russula aurea* var. *minuta* differs from *R. aurea* except in having small sized sporophores, dentate to wavy gill edges with golden or yellow deposition instead of smooth, much smaller spores and presence of pilocystidia which are absent in case of *Russula aurea*. In view of this a new variety *Russula aurea* var. *minuta* has been proposed. *Russula aurora* probably appears to be morphologically closest species to this undescribed taxon from which it differs in having fairly crowded pale cream lamellae with abundant forkation near the stipe, mild taste, and absence of dermatocystidia in pileipellis and low warted spores (0.25–0.5 µm) with few connections (Romagnesi 1967). Another close taxon is *Russula aurantiaca* which differs from *R. aurea* var. *minuta* in having usually brick-orange, copper to carmine coloured cap, widely spaced rather thick bright yellow ochre strongly interveined lamellae and presence of mild to slightly acid taste (Romagnesi 1967). Earlier *Russula aurea* was known as *R. aurata* and under this name it was documented from different localities of northwestern Himalaya from coniferous and angiospermic forest (Saini & Atri 1984, 1989; Atri & Saini 1986; Atri et al. 1994). The present collection is found in pure Sal forest in close vicinity to *Shorea robusta* tree from Uttarakhand.

Russula camarophylla, a rare western Mediterranean European representative of section *Archaeinae* is characterized by its camarophylloid habit, pale ochre or creamish sporophores with distant lamellae, very hard and compact flesh, hygrophoroid basidia and tiny spores with barely visible ornamentation (Romagnesi 1968). The present collection of *R. camarophylla* var. *reticulospora* is close to *Russula camarophylla* (Romagnesi 1968) except that the carpophores are larger in size with white cream pileus surface and larger spore size. In basidiospores warts are connected to form mostly complete reticulum instead of mostly isolated warts reported in case of *Russula camarophylla* (Romagnesi 1968). In view of this a new variety *Russula camarophylla* var. *reticulospora* has been proposed. The apical swelling of hyphal terminations in the pileipellis is an important feature that is very common within *Russula camarophylla* (Buyck et al. 2003) and presently examined collection. *R. camarophylla* var. *reticulospora* also resembles the recently described

Russula capillaris, by Buyck (in Wang et al. 2019) from Madagascar. The latter species is not only very similar in the field, but it also possesses similar apical swellings in the hyphal terminations of pileipellis. Spores, however, are much smaller with isolated and very low warts (0.1–0.2 µm) in *R. capillaris* and, again, the pileocystidia are not septate. *Russula camarophylla* is a very rare species and has been found only a few times in France (Buyck et al. 2003), northern Italy (Setti & Bigoni 1998; Boffelli 2012) and Austria (Pidlich-Aigner & Klofac 2018).

CONCLUSION

Two new varieties of *Russula* species, viz. *R. camarophylla* var. *reticulospora* var. nov. and *R. aurea* var. *minuta* var. nov. have been described based upon detailed macro- and micromorphological comparison with already existing *Russula* species. The newly proposed varieties are putative mycorrhizal associates of *Sal* and were found in direct organic connection with *Shorea robusta* roots.

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