



Three new species of *Russula* (Russulales) from India

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Abstract

Since 2008, macrofungal explorations have been undertaken in a mycologically under-explored West district of Sikkim (India), located in the Eastern Himalaya. Three species, namely *Russula sharmae*, *R. dubdiana* and *R. sikkimensis* are proposed herein as new taxa. Macro- and micromorphological illustrated descriptions of these species are given along with their taxonomic positions and relations to allied species.

Key words – Macrofungi – Russulaceae – Sikkim – taxonomy

Introduction

Barsey Rhododendron Sanctuary (BRS) is one of seven wildlife sanctuaries of the small state Sikkim which covers only 0.22 % of the geographical area of India, one of the megadiverse countries of the world. Situated in the West district of Sikkim, it is one of the important wildlife sanctuaries. It has Nepal as its western border and is connected to Khanchanjunga Biosphere Reserve in the north and Shingalila National Park of West Bengal (another state of India) in the south. BRS has an area of about 104 km² with an altitudinal variation ranging between 2200–4100 m, which supports the wide range of biodiversity distributed from temperate to subalpine forests and meadows. This area is rich in *Rhododendron*, which is represented by ten species out of 37 known from Sikkim Himalaya, but has also ectomycorrhizal trees belonging to several plant families: *Lithocarpus pachyphyllus* Rehder, *Castanopsis tribuloides* A. DC., *C. hystrix* A. DC., *Quercus lamellosa* Sm. (Fagaceae); *Alnus nepalensis* D. Don, *Betula utilis* D. Don (Betulaceae); *Abies densa* Griff., *A. spectabilis* Spach, *Tsuga dumosa* Eichl., *Pinus wallichiana* A.B. Jacks. (Pinaceae), etc. When accounting also for the altitudinal and climatic variations, the expected diversity of macrofungi in this mycologically under-explored area is potentially very high (Das 2009).

The diversity of Russulaceae, one of the most important ectomycorrhizal families in the area, has regularly been explored in different parts of the West district of Sikkim since 2008 by the senior author (Das 2010, Das et al. 2010, Das & Verbeken 2011, 2012). The present paper proposes three new species of the genus *Russula*. Two of these (*R. sharmae* and *R. sikkimensis*) have been collected from two localities of BRS: Takredara (a temperate forest area dominated by *Lithocarpus pachyphyllus* and *Tsuga dumosa*) and the trail to Tal (dominated by *Tsuga dumosa*, *Abies densa*

and *Betula utilis*). The third species, *R. dubdiana*, was collected from Dubdi, close to the well-known locality Yuksom and mainly dominated by *Castanopsis* spp.).

Materials & Methods

Macromorphological and field characters were recorded in the forest and base camp from the fresh basidiomata. Colour codes and terms following *Flora of British Fungi: Colour Identification Chart* (1969) indicated in the descriptions as “a”, and Kornerup & Wancher (1981), indicated in the descriptions as “b”. Field photographs of the fresh basidiomata were taken with a Nikon D300s camera. After recording the macromorphological characters specimens were dried in a field drier.

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 1000× and 6000× magnification and other micromorphological structures were drawn at an original magnification of 1000×. Basidium length excludes sterigmata, gill-density includes lamellae and lamellulae and spore dimensions exclude the dimension of the ornamentations. Basidiospore measurements are based on 20 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 the measured collections, KDb = maximum value for the length of measured collections, KDc = mean value for the length of measured collections and KDx = minimum 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 length-width ratio ($Q = L/W$) and is given as $Qa-Qc-Qb$ where Qa = minimum quotient value amongst the measured collections, Qb = maximum quotient value amongst the measured collections, Qc = mean quotient value amongst the 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 spattered 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).

Taxonomic treatment

Russula sharmae K. Das, Atri & Buyck, **sp. nov.**

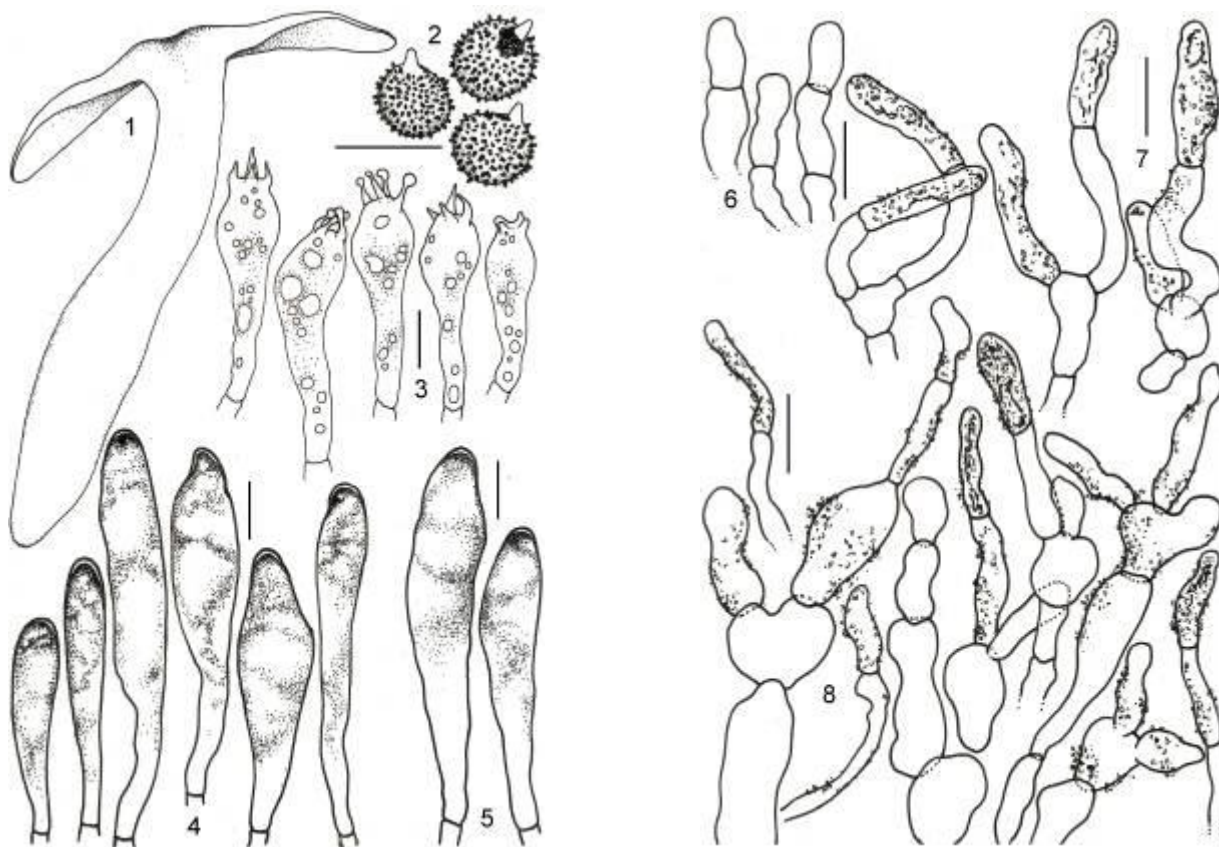
Figs 1–8, 24, 27–28

Mycobank – MB 804167

Etymology – after the name of J.R. Sharma for his contribution to Indian macrofungi.

Pileus 70–140 mm diam., convex when young, mostly planoconvex to slightly uplifted (infundibuliform) with maturity, centre often unbonate, later depressed. Pileipellis viscid to glutinous with strongly sulcate margin; completely red (a: 42) when young, gradually turning scarlet (a: 43) with yellow (a: 5E) blotches in the centre; margin incurved when young, gradually decurved with maturity, paler (b: 10A4–10A3) or slowly fading. Lamellae free to narrowly adnexed, subdistant (5–6/cm at pileus margin) after maturity, forked near the pileus margin, white when young, turning saffron (a: 49) at maturity, unchanging after bruising; edge concolourous but reddish near pileus margin (up to 15 mm from pileus margin); lamellulae absent. Stipe 80–145 × 12–25 mm, centrally broadened and gradually tapering towards base, white (b: 1A1) with pink-flush or with tinge of rose (a: 39). Context solid to stuffed in stipe, white (b: 1A1) chalky, unchanging after bruising or with KOH, turning pale saffron (b: 4A5) with $FeSO_4$, reddish orange (b: 7A7) with guaiac; context of dry sample turning lobster (red) (b: 9B8–10C8) with sulfovanillin. Taste not recorded. Odour indistinctive. Spore print pale yellow (b: 3A3).

Basidiospores 6.8–7.3–7.9 × 6.0–6.6–7.0 μm ($Q = 1.04–1.11–1.18$), globose to subglobose or broadly ellipsoid; ornamentation amyloid, up to 1 μm high, composed of isolated warts; high warts spinoid with rounded apices, rarely fused with each other (but, no connectives or veins in between); some low conical warts between high warts; suprahilar plage distinct, amyloid. Basidia 26–44 × 8–12 μm , subclavate, 4-spored; sterigmata 5–9 × 1.5–2.5 μm . Subhymenium up to 22 μm thick,



Figs 1–8. *Russula sharmae* sp. nov. 1 Fresh basidiomata. 2 Basidiospores. 3 Basidia. 4 Pleuromacrocystidia. 5 Cheilomacrocystidia. 6 Marginal cells. 7 Cystidioid elements. 8 Radial section through pileipellis. Scale bars = 10 μ m. (KD 11536, drawings by K. Das).

cellular; cells globose to ellipsoid, 4–14 \times 3.5–9 μ m. Pleuromacrocystidia 35–80 \times 7–14 μ m, emergent up to 20 μ m, abundant, fusiform to subclavate, with rounded apices, thick-walled (wall up to 1 μ m thick towards apex), content dense. Lamellae edge of two patterns: up to 15 mm from the pileus margin (reddish part) distinctly heteromorphous, appendiculate and sterile, with marginal cells and cystidioid elements exactly as at the pileus surface; rest (concolourous part) fertile with basidia, marginal cells and cystidia. Cheilocystidioid elements 13–25 \times 4–6.6 μ m, encrusted, thick-walled (0.6 μ m thick); marginal cells slightly fusoid, cylindrical to narrowly clavate, up to 6 μ m wide without encrustations, slightly thick-walled. Cheilomacrocystidia 40–60 \times 10–14 μ m, also abundant, fusiform to subclavate, thick-walled (same as pleuromacrocystidia), content distinct; marginal cells cylindrical to clavate. Lamellar trama composed of abundant oleiferous hyphae and numerous sphaerocytes surrounded by connective hyphae (up to 7 μ m wide, septate). Pileipellis thick, up to 80 μ m, a palisade to trichopalisade, composed of globose to polymorphic inflated cells bearing cylindrical to fusoid thick-walled cells with encrustations, septate encrusted primordial hyphae and mostly encrusted pileocystidia; primordial hyphae 2–4.5 μ m wide, slightly thick-walled; pileocystidia up to 6.5 μ m wide, cylindrical to subclavate or slightly fusoid, thick-walled (wall up to 0.7 μ m thick). Stipitipellis a palisade, up to 125 μ m thick, suprapellis of repent to erect hyphae and caulocystidia; hyphal ends 2.5–4 μ m wide, septate, moderately to heavily incrustated; caulocystidia abundant, up to 6.5 μ m wide, moderately incrustated, thick-walled. Stipe trama composed of connective hyphae and nested sphaerocytes. Clamp connections absent in all tissues.

Materials examined – India, Sikkim, West district, Takredara, alt. 2516 m, N 27°09'47.9" E 88°07'01.7", on ground, under *Lithocarpus pachyphyllus* Rehder, temperate mixed (broad-leaf and coniferous) forest, 15 July 2010, K. Das, KD 11536, BSHC 44148 (holotype), PC 0086207 (isotype); ibid., Chittarey, alt. 2546 m, N 27°16'31.7" E 88°02'55.3", on ground, under *L.*

pachyphyllus Rehder, temperate mixed (broad-leaf and coniferous) forest, 13 September 2008, K. Das, KD 084, BSHC 44149.

Notes – *Russula sharmae* can be easily recognized by the bright red pileus, pink-flushed stipe that tapers towards the base, the partly concolourous, partly reddish lamellar edge, basidiospores with isolated warts and the particular structure of the lamella edge.

Considering the colouration of pileus, nature of the context (turning red with sulfovanillin) and nature of pileipellis, our species is similar to *R. rosea* Pers. and *R. minutula* var. *robusta* Saini, Atri & Singer, both reported from India. However, both *R. rosea* (Romagnesi 1985, Saini et al. 1989, Kränzlin 2005) and *R. minutula* var. *robusta* (Saini et al. 1982) can be distinguished from the present species by the absence of an umbo on the pileus, and a spore ornamentation that is more typical of *Roseinae* (partial reticulum or warts connected by veins).

This is a very puzzling species in several respects. The context turning red with SV and the structure of the pileipellis are typical features of *Roseinae* (subgenus *Incrustatula*), yet the yellow spore print produced by gills that are absolutely white for a very long time, as well as the spore ornamentation of very dense isolated warts are against such a placement. The dark spore print might be suggestive of a placement in *Amethystinae* (subgenus *Incrustatula*), but then again the structure and composition of the pileipellis are not.

In several respects, *R. sharmae* reminds one of *R. hixsonii* Murrill from the Southeastern United States (Buyck et al. 2011), which shares the white young gills that finally produce a yellow spore print, the robust stature, decolouring cap center and well developed spore ornamentation. Whereas, the context of *R. hixsonii* turns strongly grey in some collections as in *Russula* subsect. *Decolorantinae* (Adamcik & Buyck 2011), the context of our species is reddening on drying. Nevertheless, the pileipellis structure of both is quite different, even if both possess incrustated elements on the cap surface.

For the moment, *R. sharmae* remains difficult to place in a classification that is based on European species.

Russula dubdiana K. Das, Atri & Buyck, **sp. nov.**

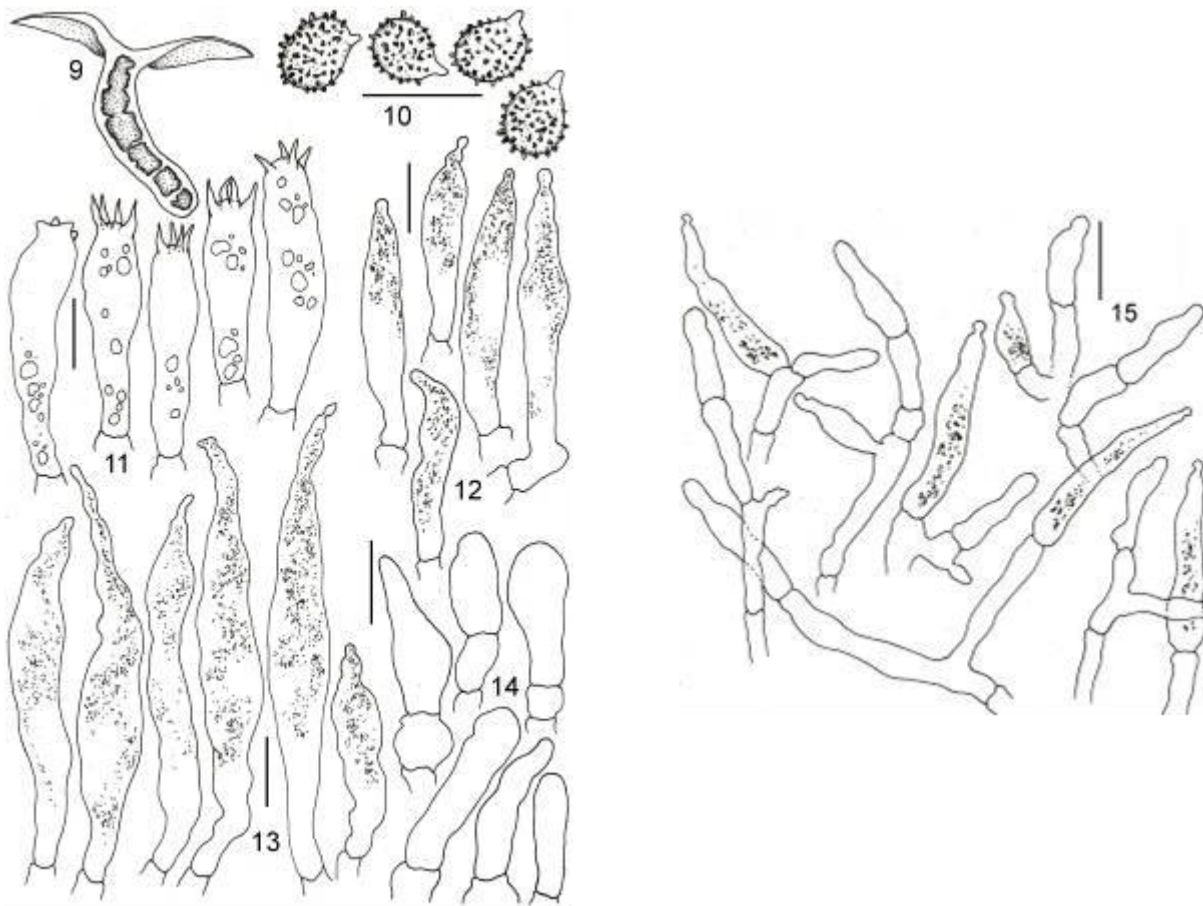
Figs 9–15, 25, 29–30

MycoBank – MB 804169

Etymology – after the name of the place, Dubdi, the type locality.

Pileus 48–65 mm diam., convex with depressed centre when young, mostly planoconvex with depressed centre when mature. Pileipellis viscid to glutinous with strong tuberculate striations, sulcate towards margin; saffron (a: 49) or paler (b: 3B5) or fulvous (a: 12), gradually paler towards margin, often even snuff brown (a: 17) at the centre, becoming paler up to camel (b: 6D4) or buff (a: 52) towards margin. Lamellae adnexed, close to subdistant (6–8/cm at pileus margin), few forked, white (b: 1A1) when young, sienna (a: 11) after bruising, often becoming spotted sienna (a: 11) to rust (a: 13) coloured after maturity. Stipe 32–50 × 9–15 mm, mostly cylindrical, finely longitudinally venose, white when young, faintly greying in places at maturity or hazel (a: 27), often fulvous (a: 12) to cinnamon (a: 10) towards base when old or on bruising, solid when young, multichambered at maturity. Context white (b: 1A1), unchanging after bruising, turning salmon (a: 45) with FeSO₄, quickly dark green (a: 60) with guaiac (not blue-green). Taste acrid. Spore print cream.

Basidiospores 5.2–6.1–7.0 × 4.2–4.8–5.5 μm (Q = 1.17–1.27–1.45), broadly ellipsoid to ellipsoid; ornamentation amyloid, up to 1 μm high, composed mostly of cylindrical warts and very few ridges; high warts cylindrical with rounded apices, few connected with each other by lower lines, some low conical warts between high warts; suprahilar plage indistinct, not amyloid. Basidia 27–43 × 7–10 μm, mainly subclavate, 4-spored (rarely 2-spored); sterigmata 4–6 × 2–3 μm. Subhymenium 18–22 μm thick, cellular; cells 6–13 × 6–11 μm. Pleuromacrocytidia 31–85 × 8–11 μm, abundant, fusiform, mostly constricted apically or with an appendage, moniliform or often with capitate apex projecting up to 30 μm beyond basidioles, content not very distinct. Lamellae edge fertile with basidia, marginal cells and cystidia. Cheilomacrocytidia 29–48 × 7–8 μm, abundant, fusiform or similar to pleuromacrocytidia. Marginal cells 10–25 × 5–8 μm cylindrical to



Figs 9–15 *Russula dubdiana* sp. nov. 9 Fresh basidiomata. 10 Basidiospores. 11 4-spored basidia. 12 Cheilomacrocystidia. 13 Pleuromacrocystidia. 14 Marginal cells. 15 Radial section through pileipellis. Scale bar = 10 μ m. (KD 10620, drawing by K. Das).

fusiform or clavate. Lamellar trama composed of nested sphaerocytes surrounded by connective hyphae. Pileipellis thick, up to 150 μ m, an ixocutis, composed of branched extremities of septate hyphae and pileocystidia; pileocystidia 9–30 \times 4–5 μ m, fusiform, mostly capitate, content distinct; hyphae up to 5 μ m broad, hyphal end narrower towards tip. Stipitipellis a cutis, composed of cylindrical hyphal ends; hyphae up to 5 μ m broad; caulocystidia not found. Stipe trama composed of connective hyphae and few sphaerocytes. Clamp connections absent in all tissues.

Materials examined – India, Sikkim, West district, Dubdi, near Yuksom, alt. 1940 m, - N27°22'06.0" E88°13'29.3", on ground, under *Castanopsis hystrix* A. DC., subtropical to temperate broad-leaf forest, 27 August 2010, K. Das, KD 10620, BSHC 44147 (holotype), PC 0086208 (isotype).

Notes – The combination of macro- and micromorphological characters clearly place *Russula dubdiana* in the series *Ingratae* (Quél.) Maire (subgenus *Ingratula*). It can be easily recognized by the combination of saffron to brown tuberculately striate glutinous pileus, venose multichambered stipe becoming yellowish to brownish towards the base when old, cream spore print and small ellipsoid basidiospores with mainly isolated warts (up to 1 μ m high).

It partly resembles *R. senecis* Imai, another species frequently occurring in Sikkim Himalaya. Though both possess a glutinous, tuberculately-striate pileus, a yellowish multichambered stipe, the latter can be separated from the former species by the presence of a brown-dotted stipe (Das 2009), and basidiospores with high ridges (up to 2.2 μ m high) and warts (with winged pattern of spore-ornamentation).

R. amoenolens Romagn., originally described from Europe appears to be quite close to *R. dubdiana* and shares the acrid taste. But, *R. amoenolens* is more robust and has darker greyish

colours and basidiospores are somewhat larger ($6.3\text{--}8.4 \times 4.7\text{--}6.4 \mu\text{m}$) (Kränzlin 2005, Sarnari 1998),

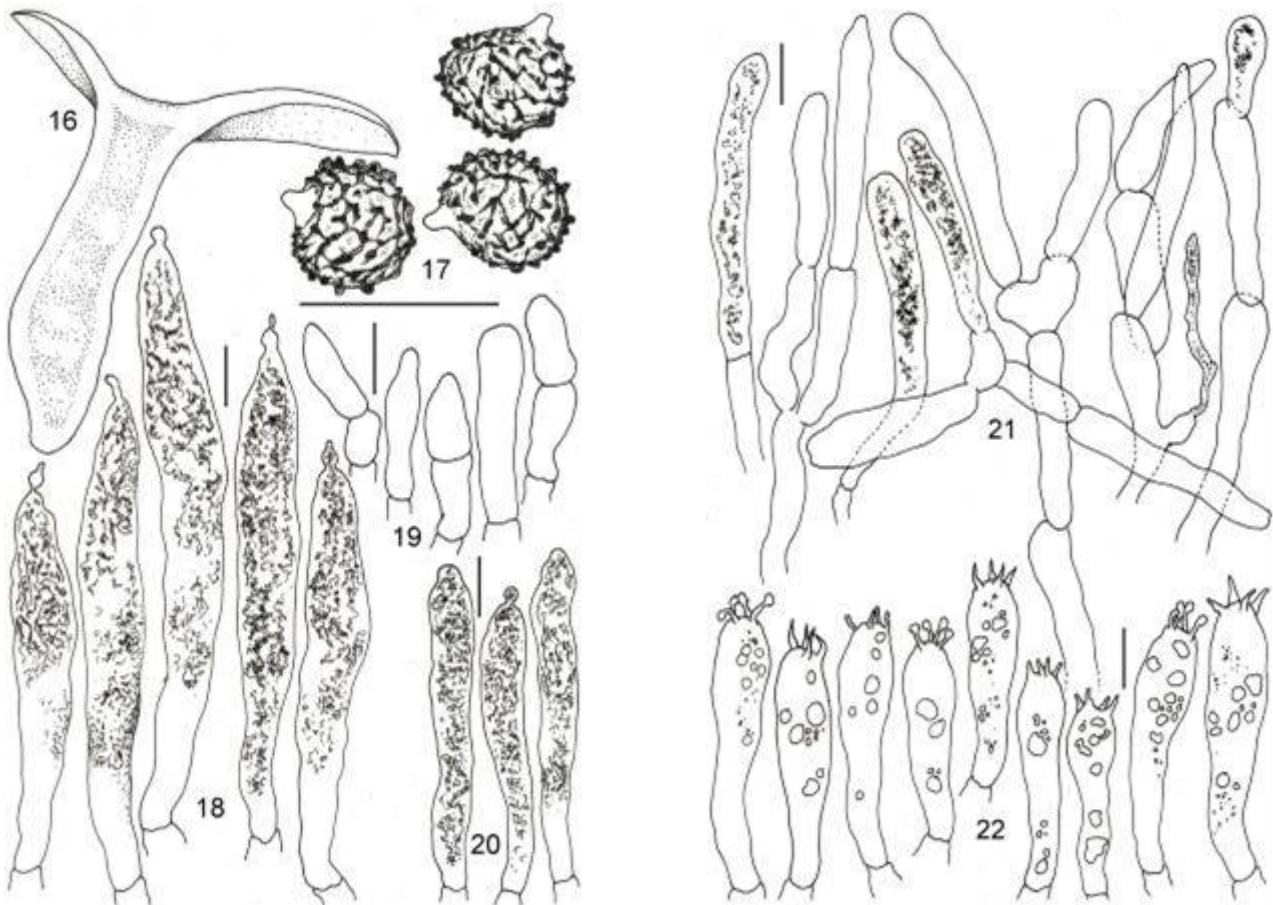
R. praetervisa Sarnari and *R. pectinatoides* Peck, both reported from India as well, differ from *R. dubdiana* in their mild taste and both are usually tinged reddish at the extreme stipe base. *R. pectinatoides* sensu European authors has a blue-green (dark green in the present taxon) reaction with guaiac and basidiospores are larger ($6.6\text{--}8.5 \times 5.2\text{--}6.5 \mu\text{m}$) and darker i.e. pale yellow (b: 3A3) in spore print (Wanscher & Kornerup 1981). *R. praetervisa*. can be easily distinguished by the larger, subreticulate basidiospores ($6.9\text{--}8.3 \times (5\text{--})5.5\text{--}6.3 \mu\text{m}$) (Sarnari 1998, Sharma et al. 2005).

***Russula sikkimensis* K. Das, Atri & Buyck, sp. nov.**
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Figs 16–23, 26, 31–32

Etymology – After the name of the small Indian state, Sikkim, from where the specimens were collected.

Pileus 55–85 mm diam., convex to planoconvex with broadly depressed centre. Pileipellis smooth, never glutinous, never striate or sulcate, parrot green (b: 30E8) with dark green (b: 29F8) centre, sometimes paler to olive towards margin. Lamellae adnexed, close (8–9/cm at pileus margin), many forked towards the juncture of stipe, yellowish white, unchanging when bruised, lamellulae absent. Stipe 55–75 \times 17–21 mm, cylindrical with tapering base, white (b: 1A1) to chalky, changing yellowish when bruised, solid. Context white (b: 3A2) when young, turning to pale yellow (a: 4A3) with maturity, unchanging after bruising, turning orange white (a: 5A2) with FeSO₄, leaf green (a: 59) with guaiac and unchanging with KOH. Taste slightly acrid to mild (at maturity). Spore print yellowish white to cream.



Figs 16–22 *Russula sikkimensis* sp. nov. 16 Fresh basidiomata. 17 Basidiospores. 18 Pleuromacrocystidia. 19 Marginal cells. 20 Cheilomacrocystidia. 21 Radial section through pileipellis. 22 Basidia. Scale bar = 10 μm . (KD 10770, drawings by K. Das).

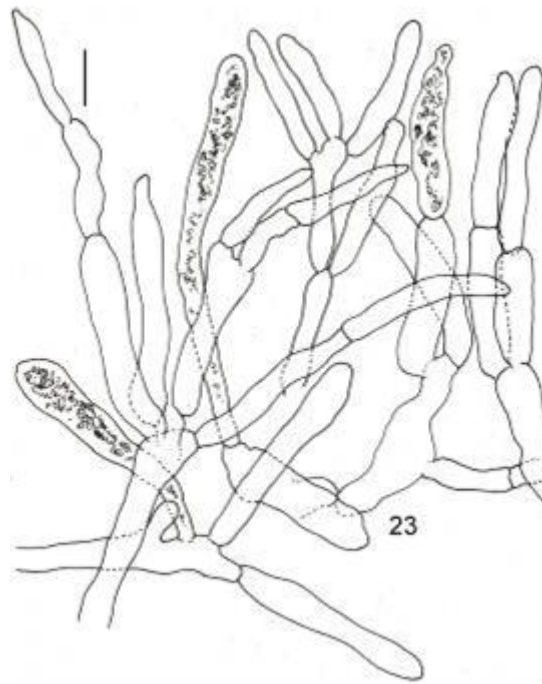
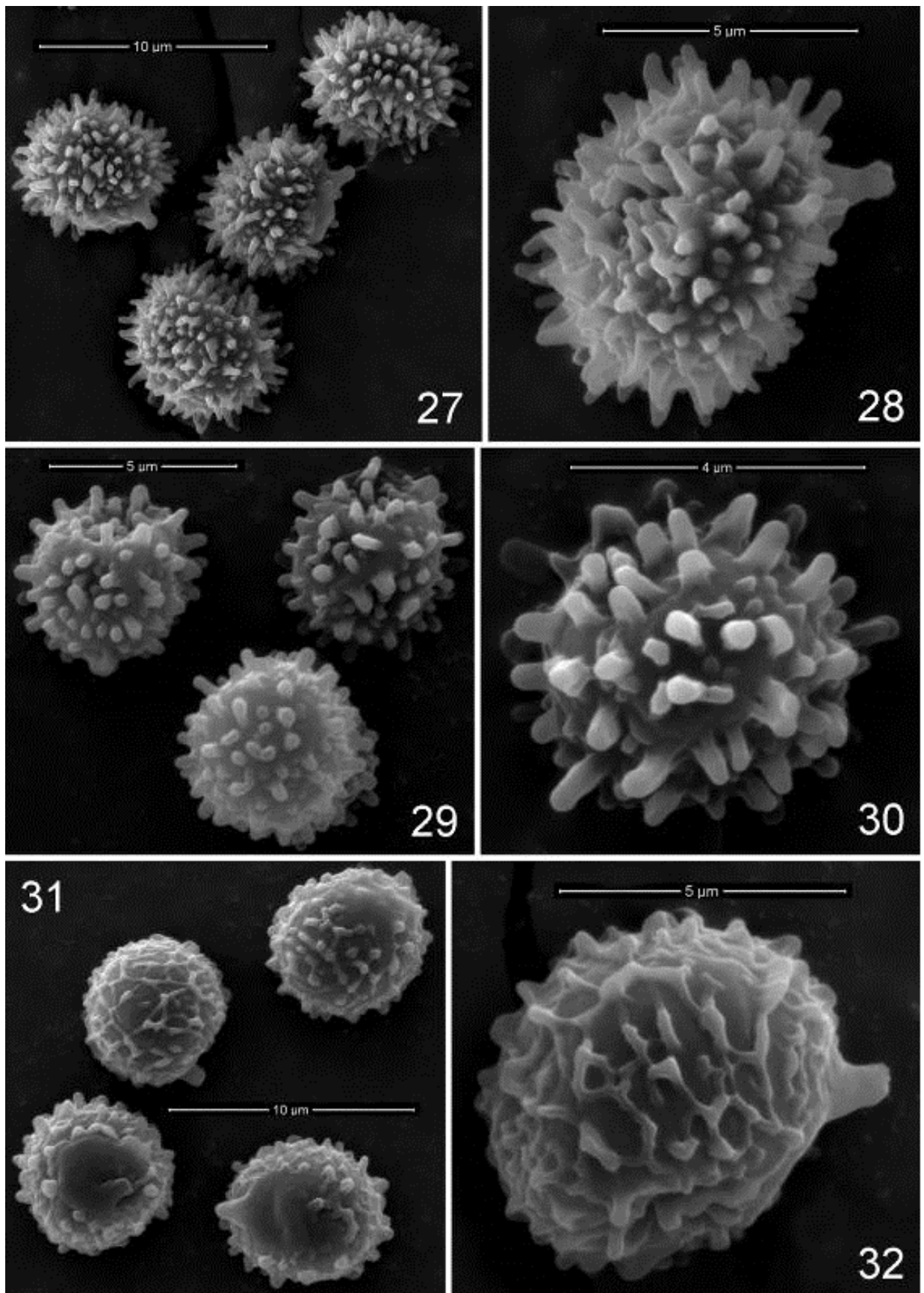


Fig. 23 – *Russula sikkimensis* sp. nov. 23 Radial section through pileipellis. Scale bar = 10 μ m. (KD 10770, drawing by K. Das).



Figs 24–26 – Fresh basidiomata (dorsal and ventral view). 24 *Russula sharmae* sp. nov. 25 *Russula dubdiana* sp. nov. 26 *Russula sikkimensis* sp. nov. (photographs by K. Das).



Figs 27–32 – Scanning electron micrographs of basidiospores. 27–28 *Russula sharmae* sp. nov. 29–30. *R. dubdiana* sp. nov. 31–32. *R. sikkimensis* sp. nov. Scale bars: 27, 31 = 10 µm; 28, 29, 32 = 5 µm; 30 = 4 µm.

Basidiospores $7.2\text{--}7.7\text{--}8.7 \times 6.2\text{--}6.9\text{--}7.8 \mu\text{m}$ ($Q = 1.03\text{--}1.12\text{--}1.21$), globose to broadly ellipsoid; ornamentation weakly amyloid, composed of very low ridges and warts that are mostly lower than $0.9 \mu\text{m}$ and forming an irregular, interrupted to partially complete or rarely complete reticulum; suprahilar plage indistinct, not amyloid. Basidia $40\text{--}54 \times 7.5\text{--}10 \mu\text{m}$, slender, mostly subclavate (never clavate), 4-spored; sterigmata short, $3\text{--}5 \times 1.5\text{--}2.5 \mu\text{m}$. Subhymenium $25\text{--}35 \mu\text{m}$ thick, cellular. Pleuromacrocystidia $64\text{--}110 \times 9.5\text{--}13.5 \mu\text{m}$, not abundant, fusiform to lanceolate with capitate, moniliform or appendiculate apex, emergent up to $35 \mu\text{m}$, content dense. Lamellar edge fertile with basidia, cheilomacrocystidia and marginal cells. Cheilomacrocystidia $48\text{--}58 \times 7\text{--}9 \mu\text{m}$, not abundant, fusiform, sometimes with capitate apex, emergent up to $26 \mu\text{m}$, content dense. Marginal cells fusiform to narrowly clavate. Lamellar trama composed of nested sphaerocytes surrounded by connective hyphae. Pileipellis thick, up to $180 \mu\text{m}$, cutis to trichoderm, composed of branched septate extremities of bottle shaped to elongate hyphae and pileocystidia; hyphal ends $3\text{--}6 \mu\text{m}$, wide, fusoid to cylindrical, thin-walled; pileocystidia $18\text{--}70 \times 6\text{--}8 \mu\text{m}$, unicellular, narrowly clavate to clavate, rarely subclavate or even slightly fusiform, content dense. Stipitipellis up to $50 \mu\text{m}$ thick, mostly a cutis, composed of cylindrical to subclavate hyphal ends (up to $6 \mu\text{m}$ broad) and caulocystidia measuring $35\text{--}60 \times 6\text{--}8 \mu\text{m}$, cylindrical to narrowly clavate or lanceolate to fusiform with rounded apex and dense contents. Stipe trama composed of numerous nested sphaerocytes surrounded by connective hyphae. Clamp connections absent in all tissues.

Materials examined – India, Sikkim, West district, near Tal, alt. 3022 m, N $27^{\circ}12'24.8''$ E $88^{\circ}06'55.8''$, on ground, under *Abies densa* Griff., subalpine mixed (broad-leaf and coniferous) forest, 8 September 2010, K. Das, KD 10770, BSHC 44146 (holotype), PC 0086209 (isotype).



Figs 33–35 – Habitat. 33–34 *Lithocarpus* dominated forested areas from where basidiomata of *Russula sharmae* were collected. 35 *Abies* dominated forested area from where basidiomata of *Russula sikkimensis* were collected. (photographs by K. Das).

Notes – The combination of macro- and micromorphological characters suggest that *Russula sikkimensis* belongs in subsect. *Griseinae* J. Schaeffer (sect. *Heterophyllae*, subgenus *Heterophyllidia*) (Sarnari 1998). The species can be easily recognized by the combination of the parrot green (b: 30E8) non-striate pileus, white stipe, context yellowing but never turning bright orange with FeSO₄, cream spore print, subreticulate basidiospores with weakly amyloid ornamentations and pileipellis with numerous bottle-shaped elongate terminal cells (with hyphal 3–6 µm wide ends) and single celled (nonseptate) pileocystidia.

Other *Griseinae* with predominantly green cap and (sub)reticulate spores are some forms of *R. grisea* (Batsch) Fr., *R. parazurea* J. Schaeffer, *R. pseudoaeruginea* (Romagn.) Romagn. and *R. atroglauca* Einhellinger

R. grisea, a species which is also reported from India, can be separated from the present species by its more variegated colours, darker spore print (pale yellow), context that reacts stronger with FeSO₄ and less reticulate spores. Moreover, its association with *Abies densa* is also very distinct.

R. pseudoaeruginea has colours that are of a softer green and the pileipellis is composed of more isodiametric cells and it seems to be associated with broad-leaved trees only (Sarnari 1998, Kränzlin 2005).

R. parazurea has a comparatively darker spore print, typically very elongated terminal cells in the pileipellis, as well as smaller, more elongate spores (6–8 × 5–6.4 µm, Sarnari 1998; 6–7.5 × 4.9–6.1 µm, Kränzlin 2005).

R. atroglauca is most probably the closest species to *R. sikkimensis*, with a very similar colouration and, in addition, a very similar composition of the pileipellis, similar FeSO₄ reaction and it also has been reported in association with conifers, although mostly with *Betula*. It differs in the less reticulate and distinctly smaller (6.4–8 × 5.6–6.4 µm) spores (Sarnari 1998).

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