European Journal of Taxonomy 782: 157–172
https://doi.org/10.5852/ejt.2021.782.1595
ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2021 · Ghosh A. *et al*

Research article

Two new species in the *Russula* (Russulaceae, Basidiomycota) crown clade from Indian Himalaya

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Abstract. Two species, namely *Russula adwanitekae* A.Ghosh, K.Das & Buyck sp. nov. and *Russula purpureozonata* K.Das, A.Ghosh & Buyck sp. nov. are proposed herein as new mushroom taxa from the Indian Himalaya based on their morphological features and ITS-based phylogenetic inferences. Both species belong to the crown clade of *Russula* subgenus *Russula* but with affinities to different subsections, viz. subsect. *Laricinae* Romagn. and subsect. *Decolorantes* Maire, respectively. *Russula adwanitekae* sp. nov. was collected in mixed temperate forests where it is most likely associated with conifers. It is distinct from several similarly looking, small, mild species with dark spore print and reddish lilac, orchid purple or greyish to deep magenta colored pileus in subsect. *Laricinae* by its sequence data (nrITS) or geographic distribution. *Russula purpureozonata* sp. nov. associates with *Abies densa* Griff., and possesses all typical features of *Decolorantes*, viz. the amyloid suprahilar spot on the spores, presence of pileogloeocystidia, the reddening then blackening context, equal lamellae and colored spore print. It reminds of North American *R. californiensis* Burl. and *R. magna* Beardslee under the microscope but has distinctly smaller spores and differs further in the unique coloration and concentrically zonated pileus margin. Macro- and micromorphological features are illustrated for both species. Their habitats, distributions and relationships with allied species are discussed.

Keywords. *Decolorantes*, *Laricinae*, nrITS, phylogeny, Russulales.

Ghosh A., Das K. & Buyck B. 2021. Two new species in the *Russula* (Russulaceae, Basidiomycota) crown clade from Indian Himalaya. *European Journal of Taxonomy* 782: 157–172. https://doi.org/10.5852/ejt.2021.782.1595

Introduction

The genus *Russula* Pers., erected in 1796, is one of the most speciose ectomycorrhizal (ECM) genera with about 2000 species worldwide and has a cosmopolitan distribution ranging from the arctic tundra

to tropical rain forests (Adamčík *et al*. 2019). Species within *Russula* are recognized by the combination of their often colourful fragile pileus, amyloid spore ornamentation, a brittle, not fibrous context mainly composed of sphaerocytes, and presence of gloeoplerous elements that do not form a branching lactiferous system ending in pseudocystidia at the basidiome surface as in the genera *Lactarius* Pers. and *Lactifluus (*Pers.) Roussel (Buyck *et al*. 2018). All members of *Russula* are obligatory root-mutualistic species which can form ectomycorrhiza with diverse plants, playing important ecological roles in sustaining forest biodiversity and as nutrient sources for some animals (Yuan *et al*. 2019). Recently, Buyck *et al*. (2018) demonstrated that the anatomy of ectomycorrhiza added support to a new infrageneric classification system of the genus based on a new multi-locus analysis of five house-keeping genes, which is followed in this study.

Extensive macrofungal forays have been undertaken in different parts of Eastern and Western Himalaya (Sikkim and Uttarakhand) for the past two decades and a number of interesting specimens of genus *Russula* were collected. Using morphological examinations and molecular phylogenetic analyses, we revealed the existence of two undescribed species proposed herein as *R. purpureozonata* sp. nov. and *R. adwanitekae* sp. nov. Their affinities are with species of subsect. *Decolorantes* Maire and subsect. *Laricinae* Romagn., respectively, both belonging to *R*. subg. *Russula*.

Detailed macro- and micromorphological description coupled with the illustrations are given along with a phylogenetic tree based on nrITS sequences.

Material and methods

Morphology

Fresh basidiomata (young to mature) were collected during mushroom forays to different parts of Sikkim and Uttarakhand Himalaya during the rainy season (June–October) of 2014–2019. Macromorphological characters were recorded in the forest or basecamp from the fresh and dissected basidiomata. Images of the basidiomata were taken with the help of Canon SX 120 cameras and Canon Power Shot SX 50 HS. Color codes and terms mostly follow *Methuen Handbook of Color* (Kornerup & Wanscher 1978). Samples were dried with a field drier. Spores were observed in Melzer's reagent. All other microscopic observations were made in ammoniacal Congo red, after a short treatment in warm, aqueous 5% KOH solution to dissolve the gelatinous matrix and improve tissue dissociation. Cresyl Blue, sulfovanillin and carbolfuchsin were used to observe the presence and color changes of incrustations and cystidial contents (Adamčík *et al*. 2019). Drawings of micromorphological features were made with the help of a drawing tube (attached to Olympus CX 41 at 1000× magnification) and a camera lucida (attached to Olympus CH20i at 2000× magnifications). Microscopic photography was done with the respective dedicated cameras attached to the compound microscopes: Olympus CX 41 and CX21iLED. Basidiospores were measured in lateral view excluding the height of ornamentations. Basidiospore measurements are represented as: (MIN–)AV-SD–AV–AV+SD(–MAX) spore-length × (MIN–)AV-SD–AV–AV+SD(–MAX) spore-width and Q = (MIN–)AV-SD–AV–AV+SD(–MAX), in which MIN = the minimum value, MAX = the maximum value, AV = AVERAGE value of total measured collections, SD = standard deviation and Q = corresponds to basidiospore 'length/width ratio'. Statistics of other microscopic characters are expressed as the mean ± standard deviation with extreme values in parentheses. Basidium length excludes the length of sterigmata. Hyphal terminations and pileocystidia were observed near the pileus margin and the pileus centre. Scanning Electron Microscope (SEM) images of basidiospores were obtained from dry spores that were directly mounted on a double-sided adhesive tape pasted on a metallic specimen-stub and then scanned with silver coating at different magnifications in high vacuum mode (20 KV) to observe patterns of spore-ornamentation. SEM work was carried out with a ZEISS EVO 18 SPECIAL EDITION model imported from Germany and installed at The University Science Instrumentation Centre (USIC) Dept., Hemvati Nandan Bahuguna Garhwal University (HNBGU)

Srinagar (Garhwal) India. Herbarium codes follow Index Herbariorum (Thiers, continuously updated). Classification used in this study followed Buyck *et al*. (2018).

DNA extraction, polymerase chain reaction (PCR) and sequencing

Genomic DNA was extracted from 100 mg of dried basidiome (for two species) with the InstaGeneTM Matrix Genomic DNA isolation kit (Biorad, USA) following the manufacturer's instructions. The nrITS gene region was amplified with primer pairs ITS-1F and ITS-4R (White *et al*. 1990). PCR amplification was performed on a thermal cycler (Eppendorf, Germany) programmed for 2 min at 94°C, followed by 35 cycles of 45 s at 94°C, 1 min at 55°C, 1 min at 72°C and a final stage of 10 min at 72°C. The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Germany). Both strands of the PCR fragment were sequenced on the 3730xl DNA Analyzer (Applied Biosystems, USA) using the amplifying primers. The sequence quality was checked using Sequence Scanner Software ver. 1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious ver. 5.1 (Drummond *et al*. 2010). The final consensus sequences were deposited at GenBank to procure the accession numbers: MN263242 and MN263243 for *R. adwanitekae* sp. nov., MN267570 and MN269951 for *R. purpureozonata* sp. nov.

Phylogenetic analysis

The newly generated nrITS sequence of the two new species of *Russula* (*R. adwanitekae* sp. nov. and *R. purpureozonata* sp. nov.) plus similar ones acquired from nBLAST search (Altschul *et al*. 1997), GenBank (Clark *et al*. 2016), UNITE database (Kõljalg *et al*. 2013) and relevant published phylogenies (Kong *et al*. 2015; Liu *et al*. 2017; Adamčík *et al*. 2019), were aligned using the online version (https://mafft.cbrc.jp/alignment/software/) of the multiple sequence alignment program MAFFT ver. 7, with the L-INS-i strategy (Katoh *et al*. 2019). The aligned sequences were trimmed with the conserved motifs 5′-(…aagg)atcatta… and …ttgacct(caaa…)-3′. To eliminate ambiguously aligned positions in the alignment as objectively as possible, the on-line program Gblocks 0.91b (Talavera & Castresana 2007) was used. The alignment can be consulted at https://www.researchgate.net/publication/356505316_Aligned_sequence_file_of_Russula_adwanitekae_and_Russula_purpureozonata. The program was run with settings allowing for smaller blocks, gaps within these blocks and less strict flanking positions. Maximum likelihood (ML) was computed in raxmlGUI 2.0 (Edler *et al*. 2021) with the GTRGAMMA substitution model. ML analysis was executed applying the rapid bootstrap algorithm with 1000 replicates to obtain nodal support values. Branches with bootstrap values (BS) exceeding 70% were considered to be significantly supported. Sequences of nrITS were phylogenetically analysed using ML.

Results

Molecular phylogeny

The final dataset consisted of 92 nrITS sequences including our consensus sequence for each species. The final alignment comprised 521 characters including gaps. Phylogenetic tree (showing two novel species in bold red font) is presented in Fig.1.

ML analysis of rDNA ITS sequences places *R. adwanitekae* sp. nov. (GenBank accession numbers MN263242 and MN263243) in a clade with high bootstrap support (MLBS = 97%) composed of species of subsect. *Laricinae* sensu Vidal *et al.* (2019). On the other hand, our second species *R. purpureozonata* sp. nov. (GenBank accession numbers MN267570 and MN269951) is a blackening species that remains of uncertain phylogenetic position as our ITS-based phylogeny lacks significant support to place it firmly in a particular subsection; yet, it is placed without support in a larger clade that comprises several other Asian or European blackening species of *Russula* (see Fig. 1).

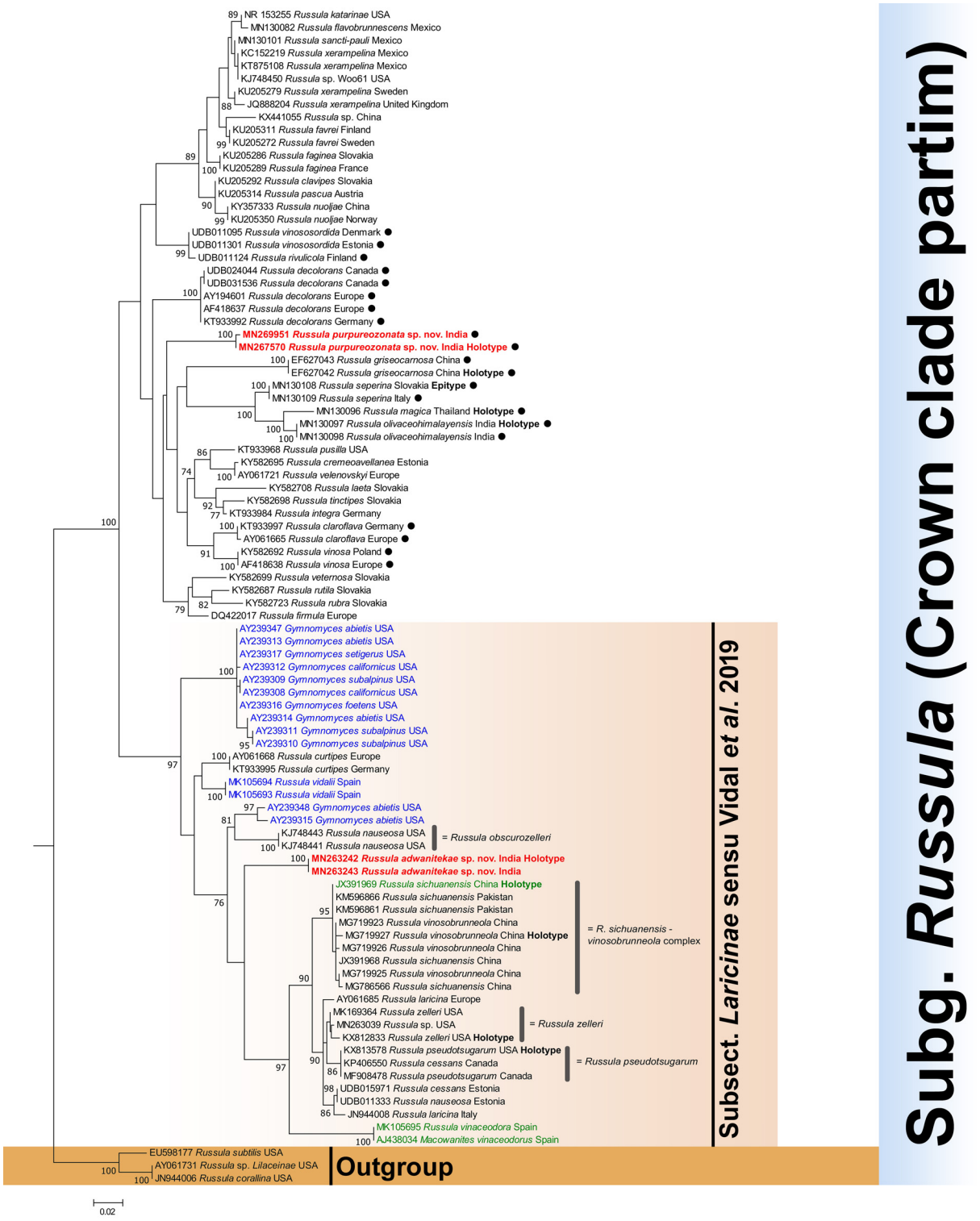

Fig. 1. Phylogram generated from rDNA ITS sequences: the evolutionary history was inferred by using the maximum likelihood (ML) method in raxmlGUI 2.0. Bootstrap support values (> 70%) obtained from the ML analysis are shown above or below the branches at nodes. Collections of the two novel Indian species described below are shown in bold red font. Secotioid taxa are shown in green font, hypogeous ones are indicated in blue font and blackening species followed by black dots.

Taxonomic treatments

Phylum Basidiomycota R.T.Moore
Class Agaricomycetes Doweld
Order Russulales Kreisel ex P.M.Kirk, P.F.Cannon & J.C.David
Family Russulaceae Lotsy
Genus *Russula* Pers.

Russula adwanitekae A.Ghosh, K.Das & Buyck sp. nov.
Figs 2–3

Diagnosis

Russula adwanitekae sp. nov. is mainly separated from similarly looking species with reddish lilac, orchid purple or greyish to deep magenta colored pileus in subsect. *Laricinae* by its sequence data (nrITS) and geographic distribution.

Etymology

After the name of the forest locality, Adwani-Teka.

Material examined

Holotype
INDIA • Uttarakhand, Pauri Garhwal, Adwani-Teka forest; 30°05.681′ N, 78°43.890′ E; alt. 1989 m a.s.l.; in temperate mixed forest; 3 Oct. 2016; *A. Ghosh*, *AG 16-1430*; GenBank: MN263242 (ITS); CAL[1821].

Paratype
INDIA • Uttarakhand, Pauri Garhwal, Adwani-Teka forest; 30°05.675′ N, 78°43.880′ E; alt. 1953 m a.s.l.; in temperate mixed forest; 5 Oct. 2016, *A. Ghosh*, *AG 16-1435*; GenBank: MN263243 (ITS); CAL[1822].

MycoBank: MB 838571; **Index Fungorum number**: IF558126; **Facesoffungi number**: FoF 09581

Description

Pileus small to medium-sized, 36–53 mm diam., convex when young, becoming plano-convex, applanate to uplifted with maturity, broadly depressed centre, near the margin becoming tuberculately striate and more or less straight with age, entire; pileipellis peeling to mid-radius; surface dry, shiny and viscid when moist, glabrous, reddish lilac (14C3–5), orchid purple (14C8) or greyish magenta (14D5–7) to deep magenta (14D8) and centrally dark magenta (13–14F4–8). Pileus context firm, brittle, yellowish white (1–2A2), unchanging after bruising, on exposure or with age. Lamellae adnexed to subdecurrent, equal but mixed with some dispersed lamellulae of different length, close (10–12/cm at pileus margin), cream to pale yellow or light yellow (4A2–4), up to 6 mm broad, occasionally forked near the stipe apex; lamella edge even and concolorous. Stipe 35–51 × 7–14 mm, cylindrical to subclavate, tapered at the apex, central, solid but not firm; surface dry, finely longitudinally venose, chalky white (1–2A1) but with yellowish white to pale yellow (4A2–3) flush near its middle portion, changing greyish white (1–2B1) after bruising. Stipe context solid, chalky white (1–2A1), changing dirty white or greyish white (1–2B1) after bruising or on exposure; turning reddish brown (8E7–8) to dark brown (8F7–8) with application of guaiacol. Odour indistinctive. Taste mild. Spore print not obtained.

Fig. 2. *Russula adwanitekae* A.Ghosh, K.Das & Buyck sp. nov. (from holotype, *AG 16-1430*). **A–C.** Fresh and dissected basidiomata in the field and basecamp. **D.** Transverse section through pileipellis showing elements. **E–H.** Transverse section through lamellae showing hymenial cystidia near the lamellae sides and basidia. **I.** SEM images of basidiospores. Scale bars: D–H = 10 µm; I = 2 µm.

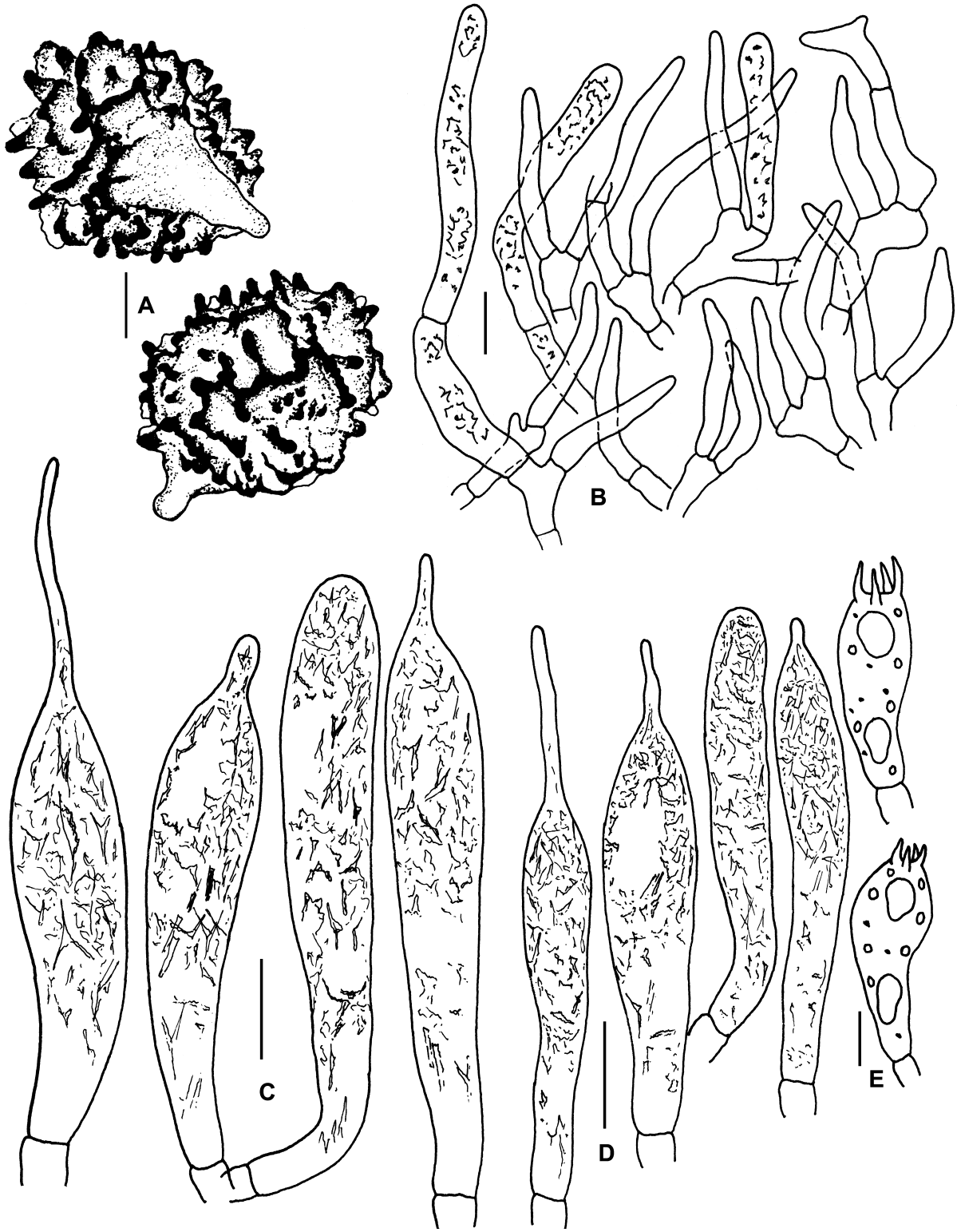

Fig. 3. *Russula adwanitekae* A.Ghosh, K.Das & Buyck sp. nov. (from holotype, *AG 16-1430*). **A.** Basidiospores. **B.** Elements of pileipellis: hyphal terminations and pileocystidia. **C.** Hymenial cystidia near the lamellae sides. **D.** Hymenial cystidia near the lamellae edges. **E.** Basidia. Scale bars: A = 2 μm; B–E = 10 μm.

Basidiospores subglobose to broadly ellipsoid, rarely ellipsoid, (7–)7.73–8.23–8.72(–9.5) × (6–)6.6–6.93–7.3(–8) µm, Q = (1.07–)1.13–1.19–1.25(–1.33), ornamentation amyloid (up to 1.7 µm high), consisting of thick ridges and warts forming incomplete reticulum, with some isolated intermediate warts; suprahilar plage amyloid; apiculi up to 2 µm high. Basidia (29–)32–35–38(–40) × (10–)10–11–13(–15) µm, 4-spored, subclavate to clavate; sterigmata up to 8 µm long. Subhymenium layer up to 37 µm thick, pseudoparenchymatous. Hymenial cystidia on lamellar sides (55–)61.5–71.5–81(–92) × (7–)8–9.5–10.5(–11) µm, cylindrical, subclavate to fusiform with frequent lageniform (up to 23 µm long) or appendiculate or few rounded-obtuse apices, emergent up to 32 µm beyond the basidiole tips; contents crystalline, without reaction in sulfovanillin. Lamellae edges fertile with basidia and cystidia. Hymenial cystidia on lamellar edges (37–)42.5–52.5–62.5(–66) × (6–)6–7–8(–9) µm, cylindrical to subclavate with lageniform (up to 20 µm long) or appendiculate or rounded apex; contents crystalline, without reaction in sulfovanillin. Hymenophoral trama mainly composed of large nests of sphaerocytes and few hyphal elements. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying sphaerocytes of the context, 100–120 µm thick, two-layered, distinctly divided in 40–50 µm deep suprapellis composed of erect or ascending hyphal terminations, arranged in a trichodermal structure and dispersed pileocystidia, and subpellis 60–70 µm deep, composed of more or less dense, horizontally oriented hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin usually branched at the subterminal cells or the cells just below, thin-walled; terminal cells (11–)19–28.5–37.5(–50) × 3–4–4.5(–6) µm, mainly subulate, sometimes cylindrical to subcylindrical, apically obtuse or slightly narrowed towards tips and wider near base or sometimes attenuated; subterminal cells usually equal in size, sometimes with lateral branches; near the pileus centre with slightly shorter and less wide terminal cells, measuring (15–)18.5–25–32(–44) × (2–)2.5–3–3.5(–4) µm, but equally wide subterminal cells. Pileocystidia near the pileus margin 1–4-celled, cylindrical, usually originating deep in subpellis and often originating from branched subterminal cells, thin-walled; terminal cells (23–)40.5–62–83(–110) × (4–)4.5–5–6(–7) µm, cylindrical or sometimes slightly tapered towards tips, rounded-obtuse apex, without any incrustations; contents crystalline, without reaction in sulfovanillin; those near the pileus centre with 0–3 septa and shorter terminal cells (15–)25.5–38–51(–70) × (3.5–)4–5–5.5(–7) µm. Clamp connections absent from all tissues.

Russula purpureozonata K.Das, A.Ghosh & Buyck sp. nov.
Figs 4–5

Diagnosis

Russula purpureozonata sp. nov. is a blackening species with a dark purplish concentric zonation on the pileus surface and otherwise typical features of subsect. *Decolorantes*; it differs from the microscopically similar, North American *R. burkei* Burl. in its mild taste and ectomycorrhizal association with *Abies densa*, and from North American *R. californiensis* and *R. magna* in its distinctly smaller spores.

Etymology

Refers to the dark purplish concentric zones on the pileus surface.

Material examined

Holotype

INDIA • Sikkim, East district, Memeinchu; 27°21.108′ N, 88°49.660′ E; alt. 3539 m a.s.l.; on the soil under *Abies densa*; 2 Aug. 2018; *K. Das*, *KD 18-003*; GenBank: MN267570 (ITS); CAL[1817].

Additional material

INDIA • Sikkim, East district, opposite to Gnathang firing range forest; 27°18.605′ N, 88°48.794′ E; alt. 3885 m a.s.l.; on the soil under *Abies densa*; 5 Aug. 2018; *K. Das*, *KD 18-15* (CAL 1818); GenBank: MN269951 (ITS); CAL[1818].

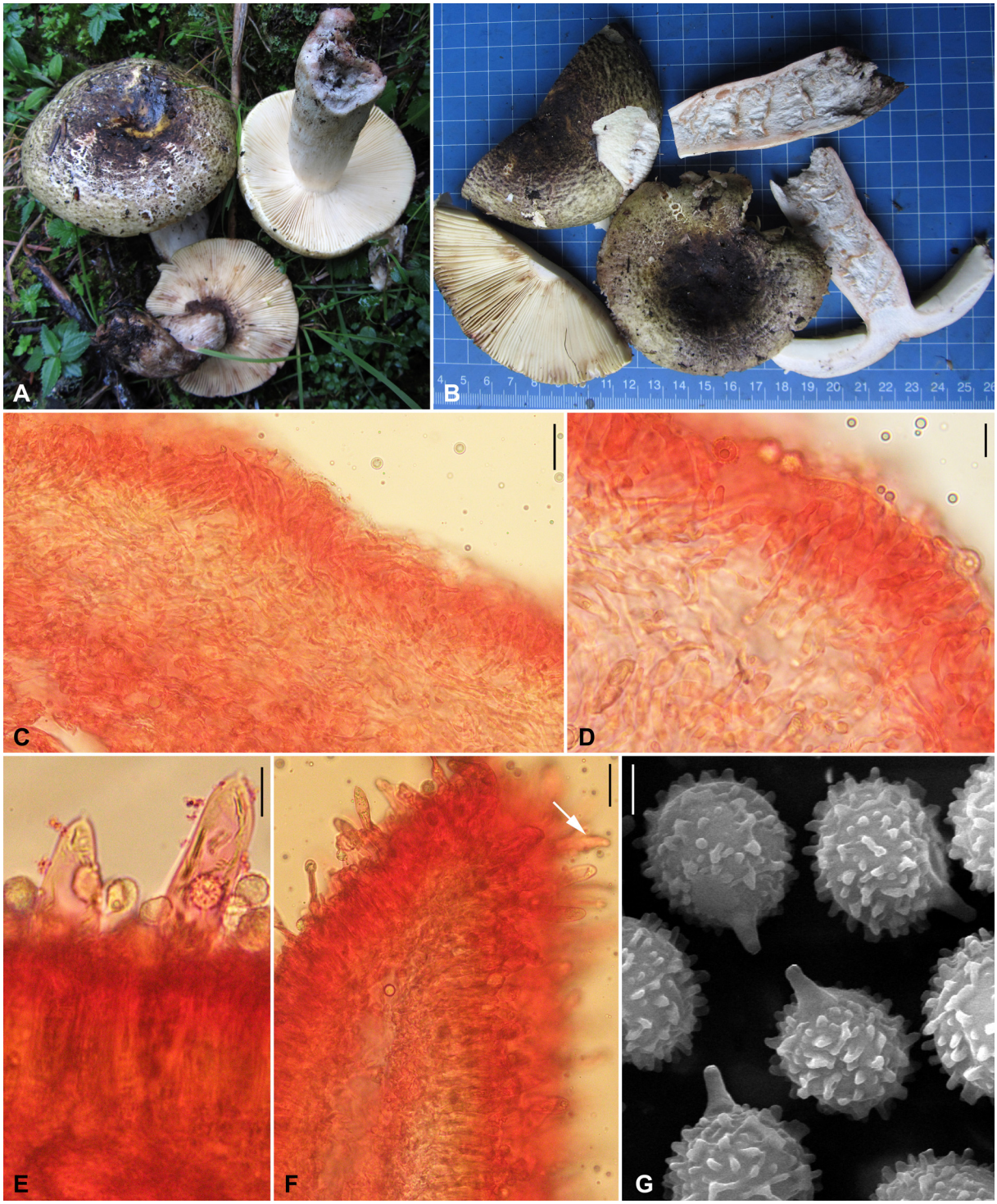

Fig. 4. *Russula purpureozonata* K.Das, A.Ghosh & Buyck sp. nov. (from holotype, *KD 18-003*). **A–B.** Fresh and dissected basidiomata in the field and basecamp. **C–D.** Transverse section through pileipellis showing elements. **E.** Transverse section through lamellae showing hymenial cystidia near the lamellae sides. **F.** Transverse section through lamellae showing hymenial cystidia near the lamellae edges (indicated with white arrow). **G.** SEM images of basidiospores. Scale bars: C = 100 µm; D–E = 10 µm; F = 50 µm; G = 2 µm.

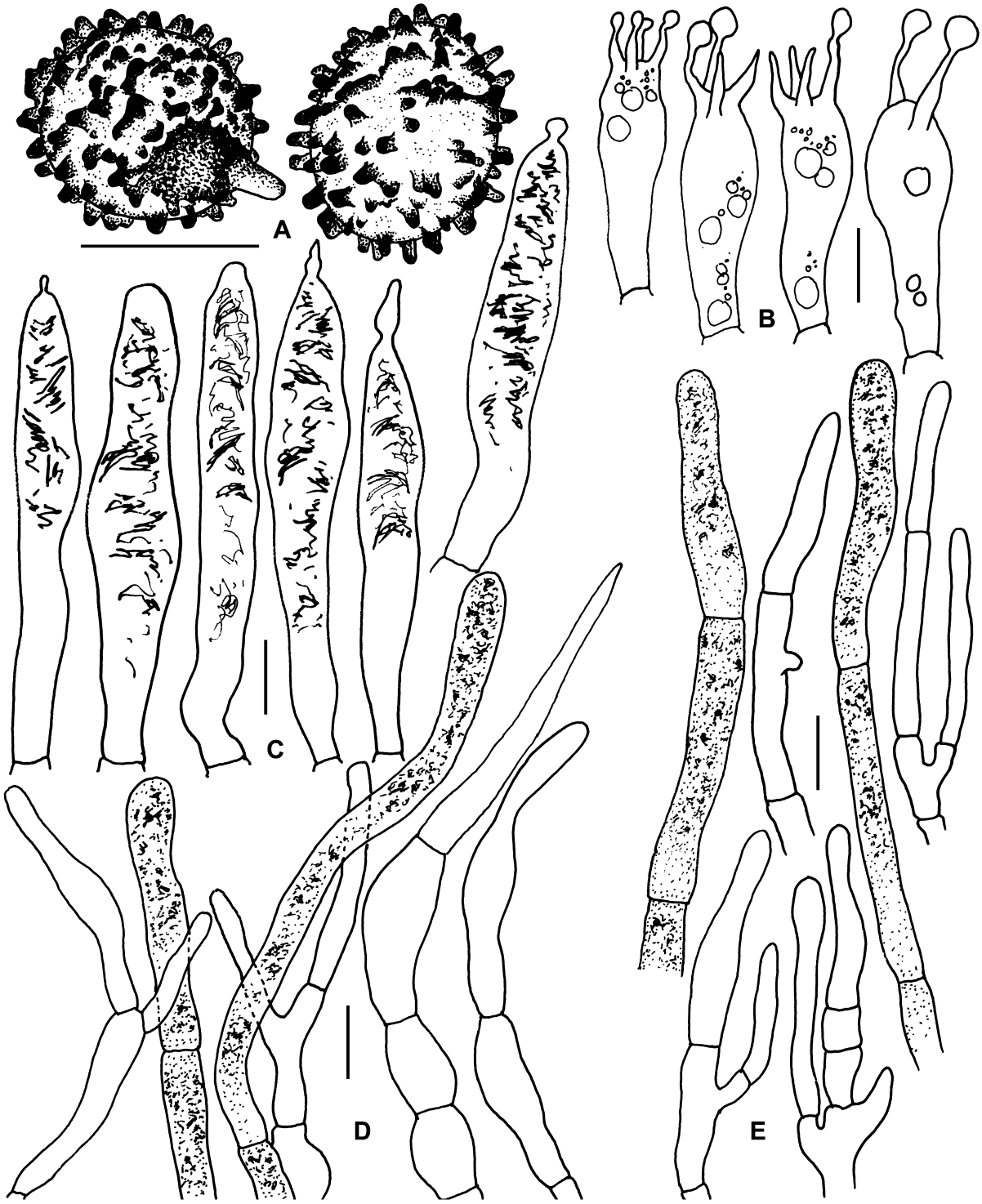

Fig. 5. *Russula purpureozonata* K.Das, A.Ghosh & Buyck sp. nov. (from holotype, *KD 18-003*). **A.** Basidiospores. **B.** Basidia. **C.** Hymenial cystidia near the lamellae sides. **D.** Elements of the pileipellis near the pileus centre: hyphal terminations and pileocystidia. **E.** Elements of the pileipellis near the pileus margin: hyphal terminations and pileocystidia. Scale bars: A = 5 μm; B–E = 10 μm.

MycoBank: MB 838572; **Index Fungorum number**: IF558127; **Facesoffungi number**: FoF 09580

Description

Pileus large-sized, 85–105 mm diam., hemispherical when young, then convex, plano-convex to applanate, broadly but shallowly depressed in the centre when mature; margin decurved to plane with maturity, entire; surface viscid when moist, then dry, finely areolate, peeling to ½ of the radius, greyish yellow (2C3–4) or olive to linden green (2D5–6), centrally dark brown (7F4–5) to dark purple or purple-black with pastel yellow to light yellow patches (3A4–5), fading towards mid in distinctive concentric zones. Pileus context up to 8 mm thick, thinning towards margin, firm, brittle, chalky white (1–2A2), changing first orange-red, then black when cut or bruised; turning greyish red to reddish brown (8C–D5) and dull green (26E3–4) with guaiacol and $FeSO_4$, respectively. Lamellae adnexed to free, subdistant (6–7/cm at pileus margin), yellowish white (2–3A2), forked near the stipe apex; edge even and concolorous. Stipe 70–100 × 20–30 mm, subclavate to clavate, tapered at the apex, central, solid; surface dry, finely longitudinally venose, chalky white (1–2A1) with yellowish white to pale yellow (4A2–3) flush at one side of the centre, changing first orange-red, then black when cut or bruised. Stipe context solid, chalky white (1–2A1), changing first orange-red, then black when cut or bruised; turning greyish red to reddish brown (8C–D5) and dull green (26E3–4) with guaiacol and $FeSO_4$, respectively. Odour indistinctive. Taste mild. Spore print yellowish white (3A2).

Basidiospores globose, subglobose to broadly ellipsoid, (7.4–)7.6–8.02–8.4(–8.9) × (6.5–)6.8–7.2–7.7 (–8) µm, Q = (1.04–)1.07–1.11–1.15(–1.23), ornamentation amyloid, composed of somewhat cylindric (mostly with rounded or obtuse apices) isolated very small (0.3 µm) to high (up to 1.2 µm) spines and most of spines fused laterally and connected by thin to thick ridges (like small crests) forming partial reticulum; suprahilar plage amyloid; apiculi up to 2 µm high. Basidia (35–)41–49–57(–68) × 10–11–12(–14) µm, 4-spored, subclavate to clavate. Subhymenium layer 20–25 µm thick, made up of pseudoparenchymatous cells. Hymenial cystidia on lamellar sides (63–)71.5–86–101(–134) × (9–) 9.5–11–12.5(–14) µm, subcylindrical, cylindrical to ventricose with capitate, mucronate, moniliform or appendiculate (up to 7 µm long appendage) apex, emergent up to 55 µm beyond the basidiole tips; contents dense, heteromorphous and partly or completely filled with fibrous to somewhat crystalloid components, hardly staining in sulfovanillin. Lamellae edges fertile with frequent basidia. Hymenial cystidia on lamellar edges (72–)75–84–92.5(–98) × (9–)9.6–10.5–11.5(–12) µm, subcylindrical, cylindrical to ventricose with obtuse-rounded, capitate, mucronate or appendiculate apex; contents dense, heteromorphous and partly or completely filled with fibrous to somewhat crystalloid components, hardly staining in sulfovanillin. Hymenophoral trama composed of numerous sphaerocytes and connecting hyphae; sphaerocytes globose to elliptical. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying sphaerocytes of the context, 120–180 µm thick, two-layered, distinctly divided in 50–80 µm deep suprapellis composed of erect or ascending hyphal terminations, arranged in a densely turf of trichodermal structure and dispersed pileocystidia, and subpellis 70–100 µm deep, composed of more or less horizontally irregularly oriented, moderately dense, 2.5–3.5 µm wide pilear hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin usually branched at the subterminal cells or the cells just below, occasionally slightly flexuous, thin-walled; terminal cells (13–)15.5–24.5–34(–53) × 3–3.5–4.5(–5) µm, mainly subulate to tapering towards tips or cylindrical to subcylindrical, apically obtuse or slightly narrowed towards tips and wider near base; subterminal cells usually equal in size, rarely with lateral branches or nodulose, equally wide or more or slightly wider. Hyphal terminations near the pileus centre with shorter but slightly wider terminal cells measuring (9–)17–22.5–28.5(–35) × (2.5–)3–4–4.5(–6) µm, mainly tapering to subulate towards tips or cylindrical or ventricose or occasionally lageniform, apically obtuse or slightly narrowed towards tips and wider near base; subterminal cells equally wide, sometimes slightly wider or ventricose, rarely with nodulose or with lateral branches. Pileocystidia near the pileus margin 1–4-celled, numerous, cylindrical, usually originating deep in subpellis and often originating from branched subterminal cells, thin-walled; terminal

cells (30–)32.5–52–71(–108) × (4–)4.5–5–6(–6.5) µm, cylindrical or sometimes slightly tapered towards tips, rounded-obtuse, without any incrustations; contents heteromorphous-crystalline, without reaction in sulfovanillin. Pileocystidia near the pileus centre with often more septa (1–7); terminal cells (14–) 22.5–38–54(–73) × (4–)4.5–5–6(–7) µm, cylindrical or slightly tapered towards tips, rounded-obtuse apex. Clamp connections absent from all tissues.

Discussion

Russula adwanitekae sp. nov. is probably typical for either higher latitudes or mountain habitats as our nrITS phylogeny (Fig. 1) places it with significant bootstrap support (MLBS = 97%) with the other species of subsect. *Laricinae* sensu Vidal *et al.* (2019). This recent redefinition of the latter subsection was based on a multigene phylogenetic approach, and although *Laricinae* is still almost exclusively composed of species in the northern hemisphere that associate with conifers, the enlarged concept now includes very few species (in our Fig. 1, *R. vidalii* Trappe & T.F.Elliott and *R. curtipes* F.H.Møller & Jul. Schäff.) that associate with deciduous trees (mostly Fagaceae Dumort.) in the lowland Mediterranean area. Vidal *et al.* (2019) also demonstrated the abundant occurrence in *Laricinae* of secotioid (or '*Macowanites*'-like taxa, indicated in green in Fig.1) to fully hypogeous (or '*Gymnomyces*'-like taxa, indicated in blue in Fig.1) as already suggested by Whitbeck (2003).

Most *Laricinae* are small *Russula* with an often very variable pileus color. They are not easy to identify but are believed to have frequently distinct preferences for a particular habitat or host tree which orients the identification (Sarnari 2005). Moreover, some more or less yellowing species still placed in *Laricinae*, such as the European *R. sapinea* Sarnari, are reminiscent of the much more diverse, but phylogenetically closely related *Puellarinae* Singer, a subsection composed of equally small species that are often strongly yellowing and most frequently occur under deciduous trees. The exact color of the spore print is considered an important feature for identification, but reliable sequence data are urgently needed to fix the interpretation of the various European species.

The identity of the host tree for our new species was impossible to determine in the field, but its phylogenetic placement as part of *Laricinae* suggests that it is probably a conifer. From Asia, the epigeous to semi-hypogeous *R. sichuanensis* G.J.Li & H.A.Wen, originally described as a principally two-spored species with very large basidiospores from subalpine *Picea* A.Dietr. forests in China, clearly belongs here (Li *et al*. 2013). It was later reported on the basis of near-identical ITS sequences (99.83 similarity for 100% coverage with the *R. sichuanensis* holotype sequence) obtained from specimens growing with *Pinus wallichiana* A.B.Jacks in Pakistan (Saba & Khalid 2015), but with a macro- and micromorphology that corresponds clearly to the more recently described epigeous *R. vinosobrunneola* G.J.Li & R.L.Zhao (Li *et al*. 2018), another *Laricinae* that associates with *Pinus* L. – and perhaps also *Abies* Mill. – in northern (arctic) China. One could conclude that these Pakistani collections belong to the more recently described *R. vinosobrunneola*, but the fact that their ITS sequences are (near-)identical to the holotype of *R. sichuanensis* is strange, as ITS performed very well so far to differentiate among the ca 1000 described species of *Russula* worldwide. It might have been preferable to include at least the *R. sichuanensis* holotype in the multigene comparison with *R. vinosobrunneola,* but Li *et al.* (2018) used two newly gathered collections to represent *R. sichuanensis,* and the LSU used for one of these two new 'sichuanensis'-specimens clearly suggests it might be a different taxon when viewing the fast minimum evolution tree for nBLAST results. Additionally, nBLAST of sequences for the single protein-coding gene (*tef1*) used in the latter paper are labeled 'unverified' in GenBank, meaning there might perhaps be an issue with the reading frame, and none of these new *tef1* sequences are showing up in BLAST results. In our opinion, providing additional illustrated morphological analyses and sequence data for distinctly secotioid specimens of *R. sichuanensis* might solve this issue.

Very few other extra-European species have been assigned to this subsection (all are placed in our Fig.1). Among these, Bazzicalupo *et al*. (2017) described a few years ago the very similarly colored *R. pseudotsugarum* Bazzical., D.Mill. & Buyck, associated with its host *Pseudotsuga* Carrière, from the American Pacific North West (PNW). From that same area, also *R. obscurozelleri* Bazzical., D.Mill. & Buyck, growing with *Pseudotsuga*, *Tsuga* (Endl.) Carrière and *Pinus*, belongs in *Laricinae*, while first sequences produced for the very similar *R. zelleri* Burl., equally occurring in the PNW but associated with *Picea*, also placed this species in the same subsection.

As for the here newly described *R. purpureozonata* sp. nov., all of the typical features suggest affinities with subsect. *Decolorantes* in the crown clade of *Russula* subg. *Russula* (Buyck *et al*. 2018). These include the amyloid suprahilar spot on the spores, presence of pileogloeocystidia, the reddening then blackening context, equal lamellae and colored spore print. Sequence data for most of the northern hemisphere species presently attributed to this subsection, do not firmly place our new species in subsect. *Decolorantes* for the moment as it lacks at least significant support to group it with the European *R. decolorans* (Fr.) Fr., the type species of this subsection. The blackening context after bruising or on exposure is by far the most remarkable field character and it is shared with a number of other species that appear more or less closely related. Most of these blackening species are represented in our nrITS phylogeny (see black dots in Fig.1). The European *R. seperina* Dupain, the recently described Asian *R. olivaceohimalayensis* A.Ghosh, K.Das & R.P. Bhatt and *R. griseocarnosa* X.H.Wang, Zhu L.Yang & Knudsen, as well as the European *R. claroflava – R. vinosa* clade all differ from our new species because pileigloeocystidia are either ill-differentiated or completely lacking (Wang *et al*. 2009). Among the blackening species that do possess these pileigloeocystidia, there are those placed in subsect. *Decolorantes,* a subsection which is believed to hold few species in Europe. These are essentially from arctic-(sub)alpine habitats, including the Scandinavian *R. rivulicola* Ruots. & Vauras and circum-arctic *R. vinososordida* Ruots. & Vauras. Both latter taxa have similar spores as our Indian species, but are again very different in field habit (see Sarnari 2005). Judging from the numerous near-identical sequences to those of *R. decolorans* and *R. vinososordida* in GenBank or UNITE sequence databases, including many sequences obtained from Asian specimens, these appear to be typical species of arctic zones in the northern hemisphere and (sub) alpine habitats at lower latitudes. *Russula decolorans* differs considerably from our new species in its much larger spores with isolated spines, different pileus color, and in its association with *Pinus* or, more rarely, with other conifers, or even with *Betula* L. at those altitudes where birch replaces pine near the tree limit.

In North America, blackening species in subg. *Russula* are more diverse compared to Europe. Their type specimens have been the subject of modern, microscopic revisions (Adamčík & Buyck 2011; Adamčík *et al*. 2015), but reliable ITS sequence data are still missing. *Russula subdensifolia* Murrill has been shown to be a good member of *Decolorantes* in a recent multigene phylogeny (Buyck *et al*. 2018) and differs equally from our new species in its more isolated spines on the spores (Adamčík & Buyck 2011). A similar spore ornamentation distinguishes also the North American *R. rubriceps* (Kauffman) Singer and *R. rubescens* Beardslee, two additional, supposedly good members of *Decolorantes*. American *Decolorantes* with subreticulate, low spore ornamentation, similar to our new species, comprise *R. magna* Beardslee, *R. burkei* and *R. cinerascens* Beardslee, all described from the southeastern United States. *Russula magna* can be discarded on the basis of its much larger spores, while *R. burkei* is surely the morphologically most similar species, also in pileus coloration, but it differs from our species in the taste becoming slowly very acrid (Adamčík & Buyck 2011). Unlike all these American taxa, the pileus of our novel species shows a distinct dark brown to purple-black concentric zonation, making it unmistakable in the field.

Other blackening species of *Russula* are very distantly related, such as most members of subg. *Compactae* (Fr.) Bon, *R. gossypina* Buyck in subg. *Archaeae* Buyck & V. Hofst., as well as some atypical African

species of subg. *Malodorae* Buyck & V. Hofst.; these all differ from the blackening taxa discussed above in having unequal gills in the hymenophore.

Acknowledgements

The authors are grateful to the Director of the Botanical Survey of India (BSI), Kolkata for providing facilities. Entire forest department of Govt of Sikkim is thanked for kindly issuing the permit for the macrofungal exploration to the restricted areas. One of us (AG) is grateful to the Head of the Department of Botany & Microbiology & USIC Dept. HNB Garhwal University, Srinagar Garhwal for providing facilities and to UGC for providing a fellowship to him. Dr Tahir Mehmood, Dr Upendra Singh and Dr Priyanka Uniyal (HNBGU) are thanked for assisting AG in the field.

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Manuscript received: 22 April 2021
Manuscript accepted: 5 August 2021
Published on: 16 December 2021
Topic editor: Frederik Leliaert
Desk editor: Connie Baak