Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
7(2): 64–72 (2017) ISSN 2229-2225

www.creamjournal.org

Article
Doi 10.5943/cream/7/2/2

Russula sarnarii sp. nov. (Russulaceae, Basidiomycota) from Indian Himalaya

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Ghosh A, Das K, Bhatt RP –*Russula sarnarii* sp. nov. (Russulaceae) from Indian Himalaya. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 7(2), 64–72, Doi 10.5943/cream/7/2/2

Abstract

Russula sarnarii (*R.* Subg. *Russula* sect. *Polychromae* subsect. *Integriforminae*) growing under *Pinus* forest at Uttarakhand is presented in this paper as a novel species. It is characterized by small to medium sized basidiomata with pink rose to pastel pink colored lobed pileus, lamellae with different lengths of forkation and occational interveination, at least 4 lengths of lamellulae, dark yellow spore print, basidiospores with thick ridges, different types of cystidial apex (remarkably appendiculate cystidia with long appendages), branched, septate, erect, hair-like cuticular hyphae with attenuate or partially sharp hyphal extremites and occurrence under *Pinus roxburghii*. A detailed morphological description, illustrations, color photographs and ITS-based phylogeny are given for this new *Russula* species.

Key words – novel taxon, phylogeny, Russulales, taxonomy, Uttarakhand Himalaya

Introduction

Russula Pers., is one of the well known genera among larger gilled mushrooms has enormous diversity in worldwide (Kundsen & Borgen 1982, Rayner 1985, Singer 1986, Kibby & Fatto 1990, Buyck & Horak 1999, Buyck et al. 2006, 2008, Miller et al. 2012, Li et al. 2013, 2015) and with no exception in India where *ca* 136 taxa (Manjula 1983, Rawla 2001, Atri et al. 1994, Das & Sharma 2005, Das et al. 2006, 2008, 2010, 2013, 2014, Pradeep & Vrindra 2010, Manimohan & Latha 2011, Dutta et al. 2015, Paloi et al. 2015, 2016, Hyde et al. 2016, Ghosh et al. 2016) are known so far. *Russula* subsect. *Integriforminae* Bon (belonging to *R.* subg. *Russula*) is known by 5 series (Sarnari 1998): *R.* series *Decolorans*, *R.* series *Paludosa*, *R.* series *Romellii*, *R.* series aff. *Romellii* and *R.* series *Straminea*. Based on the European collections, *R.* series *Romellii* is typically featured by the combination of characteristics: medium sized basidiomata; context slightly changing to browning; spore print yellow to ochre; spore ornamentation mostly forming reticulum; dermatocystidia inconspicuous, aseptate to poorly septate, without incrustations and occurrence under broadleaf trees (Sarnari 1998, 2005).

During the survey of macrofungi from different parts of Uttarakhand (one of the biodiversity hotspots in India) we collected a number of wild mushrooms from a pure pine (*Pinus roxburghii* Sarg.) forest. Thorough macro- and micromorphological examination of these collections revealed an interesting unique species under the genus *Russula* series *Romellii*. This novel species is

Submitted 8 December 2016, Accepted 7 April 2017, Published online 17 April 2017
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formally proposed here as *Russula sarnarii* with ITS-based phylogeny and detailed morphological descriptions along with the illustrations and color photographs.

Materials & Methods

Morphological study

Fresh basidiomata were collected during a macrofungal foray to different temperate and subalpine forests (broadleaf/coniferous/mixed) at Uttarakhand during the rainy season (June–September) of 2015. Macro- and micromorphological characterization, SEM studies of basidiospores were done following Ghosh et al. 2016. All microstructures were observed under high magnification (1000X). Drawing of basidiospores and other micromorphological structures were made at a magnification of 2000X. Methuen Handbook of Color was used for color codes and terms (Kornerup & Wanscher, 1978). Samples were dried on a field drier (45° C–60° C) Spore-size measurements and length/width ratios (Q) are presented as: minimum–mean–maximum. Herbarium codes follow Thiers (continuously updated).

DNA extraction, polymerase chain reaction (PCR) and sequencing

Genomic DNA (for the molecular phylogenetic studies) was extracted from 100 mg of dried basidiome with the help of InstaGeneTM Matrix Genomic DNA isolation kit (Biorad, USA) following the manufacturer's instructions. The nrITS gene region was amplified with primer pairs ITS1F and ITS4B (White et al. 1990). PCR-amplification was performed on a thermal cycler (Eppendorf, Germany) programmed for 2 mins at 94°C, followed by 35 cycles of 45 secs at 94°C, 1 min at 55°C, 1 min at 72°C and a final stage of 10 mins 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 DNA sequence of the reverse strand was edited with Sequence Navigator version 1.0.1 (Applied Biosystems). The final consensus sequence was deposited at GenBank to procure the accession number (KY284154).

Phylogenetic analysis

Phylogenetic analysis based on ITS sequence data was carried out to establish the phylogenetic placement of our isolated taxon. Reference sequences and outgroup were selected from the relevant literature (Miller & Buyck 2002, Li et al. 2012, Kong et al. 2015) and GenBank. All sequences were aligned with MAFFT v. 7 (Katoh et al. 2002). No manual editing was done within the alignment. Phylogram was generated from maximum likelihood (ML) method based on the Kimura 2–parameter model (Kimura 1980). The tree with the highest log likelihood (-2077.9884) is shown. Evolutionary analysis was conducted in MEGA6 (Tamura et al. 2013). One-thousand bootstrap replicates were analyzed to obtain the nodal support values. The European materials of *Russula delica* Fr., *R. chloriodes* (Krombh.) Bres. (both from *R.* subg. *Brevipes*), *R. amoenicolor* Romagn. and *R. violeipes* Quél. (both from *R.* subg. *Amoenula*) were chosen as outgroup taxa.

Results

Phylogeny

The multiple ITS sequences of 36 different species of *Russula* (considering the sequences for all the relevant species of *Russula* subg. *Russula* available in GenBank and also those which are appeared in BLAST search including our isolate (AG 15–755) were analyzed. *Russula. romellii*, *Russula rubroalaba*, *R*ussula *flavida*, *Russula* cf. *rugulosa*, *Russula subsulphurea*, *Russula puellula*, *Russula jilinensis*, *Russula curtipes*, *Russula thindii*, *Russula nana*, *Russula sanguinea* from *R.* subg. *Russula* and *Russula vinosa*, *Russula claroflava*, *Russula buyckii*, *Russula. zvarae*, *Russula azurea*, *Russula subtilis*, *Russula lilacea* from *R.* subg. *Incrustatula* were also considered for this

analysis for better understanding the phylogenetic placement of our species. Our isolated sequence from AG 15–755 (*Russula sarnarii* sp. nov.) was recovered as a distinct taxon (marked with bold and red font) on a comparatively long branch in a strongly supported (96% BS) clade and clustered with GenBank sequences of other two available (in NCBI) species of *Russula* (belonging to *R.* subsect. *Integriforminae*). The phylogenetic tree is presented in Fig. 1.

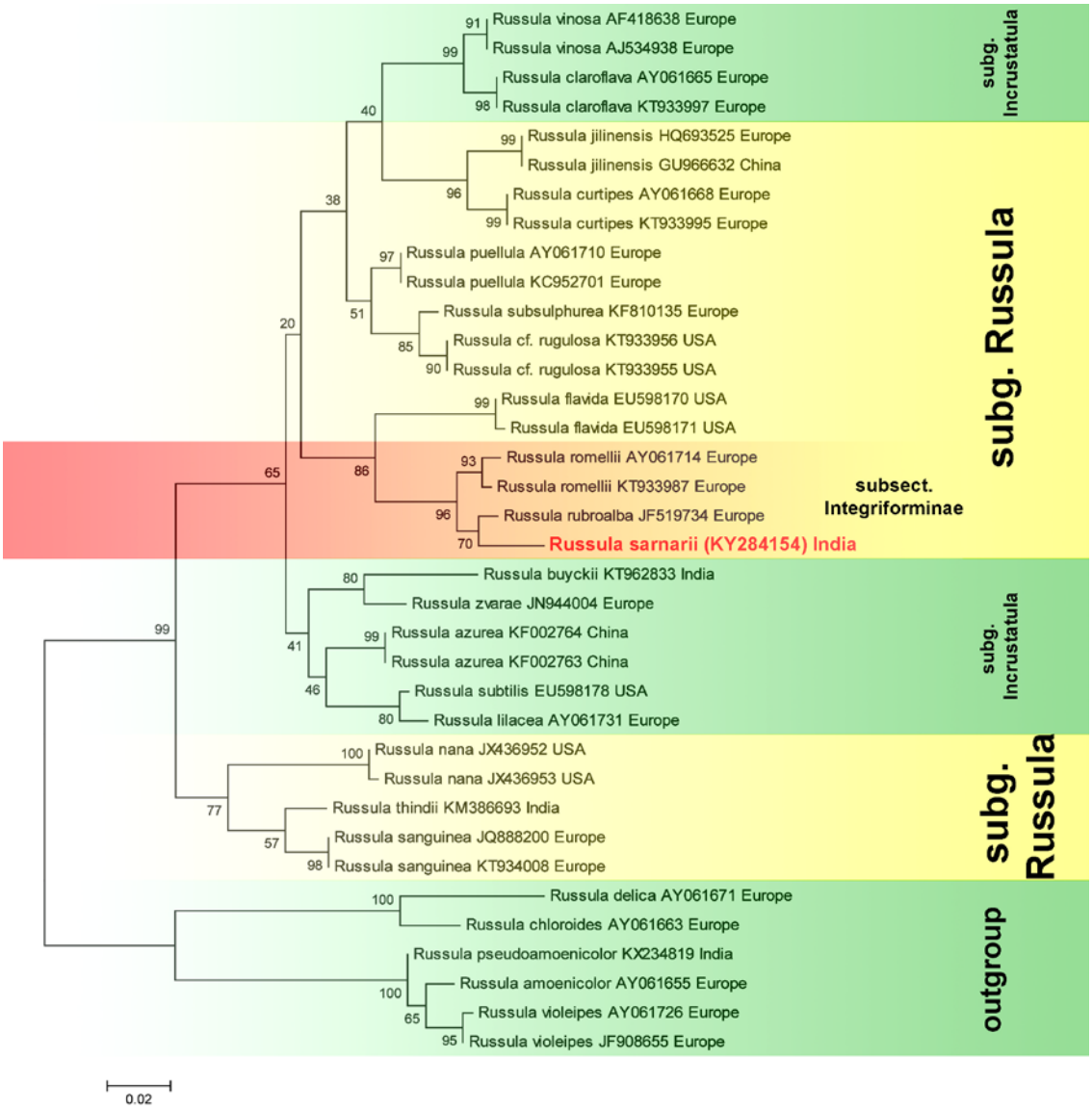


Fig. 1 – Phylogram of the new species AG 15–755 (*Russula sarnarii*, in bold, red font) inferred from Maximum Likelihood (ML) analysis of ITS sequences using MEGA 6.0.

Taxonomic description

Russula sarnarii A. Ghosh, K. Das & R.P. Bhatt sp. nov. Figs 2–18

MycoBank: MB 819419, Facesoffungi number: FoF03266

Type: India, Uttarakhand: Rudraprayag district, Hariyali Devi Forest, alt. 1651 m, N 30° 15.955' E 79° 03.719', 27th July 2015, *A. Ghosh*, AG 15–755 (holotype: CAL; isotype: GUH)

Diagnosis: differs from other known species of *Russula* subsect. *Integriforminae* by a pink rose to pastel pink colored irregularly wavy pileus, dark yellow spore print, lamellae with different lengths of forkation, subglobose to broadly ellipsoid basidiospores with thick ridges and few isolated warts forming partial reticulum and occurrence under pure pine forest.

Etymology: to commemorate Dr. Mauro Sarnari for his invaluable contribution to the systematics of the genus *Russula.*

Basidiomata up to 30–55 mm. in height, small to medium sized. *Pileus* 20–70 mm. in diam., hemispheric when young, then convex, planoconvex, expanding to applanate with broad but slight depression above the stipe on maturity, margin entire when young, margin irregularly wavy at maturity; surface dry, smooth, pitted at some places, cracked when mature, pale red (11–12A3), pink rose (11–12A4) to pastel pink (11A4–5), marginally pinkish white (11A2–3), greyish rose (11–12B4–6) over the center and around the depression, turning mustard yellow (3A5–6) with KOH; pileus context chalky white (1–2A1), spongy, unchanging when bruished but pale yellow (3A3) with KOH. *Lamellae* adnate to subdecurrent, close to rather crowded (7–10 per 10 mm at margin), occasionally interveined, forked near the stipe apex, middle of the cap and near the cap margin also, ochre or pale yellow (2–3A3), entire, with concolorous edges, lamellulae truncate, at least in 4 lengths. *Stipe* 33–55 × 15–22 mm., cylindrical, tapered at the base, brittle, central, dry, smooth, longitudinally striate, chalky white (1A1–2) with yellowish flush (3A4–5) present at the base turning pale yellow (3A3) with KOH and vivid red to high red (10A7–10A8) with guaiacol, context stuffed, becoming hollow with maturity, chalky white (1A1–2), unchanging when bruished. *Taste* mild. *Odor* indistinctive. *Spore print* dark yellow.

Basidiospores 7–7.8–8 × 6–6.6–7 µm, (n = 25, Q = 1.08–1.17–1.33), subglobose to broadly ellipsoid, rarely ellipsoid, ornamentation strongly amyloid, composed of thick ridges and warts, aligned or connected to form a partial reticulum, with few isolated warts (up to 0.9 µm), apiculi up to 2 µm high. *Basidia* 33–58 × 12–17 µm, subclavate to clavate, rarely cylindrical, 4-spored, sterigmata up to 8 µm long. *Subhymenium layer* up to 25 µm thick, pseudoparenchymatous. *Pleurocystidia* 47–87 × 11–16 µm, subclavate to clavate with appendiculate (appendages up to 16 µm high), capitate, moniliform, mucronate and rounded apex, emergent up to 32 µm. Lamellae edges fertile with basidia and cystidia. *Cheilocystidia* 32–53 × 8–10 µm, cylindrical to subclavate with appendiculate or rounded apex. *Pileipellis* up to 55 µm thick, an ixotrichoderm, composed of branched, septate, erect, hair-like hyphae (1.5–4 µm wide) with attenuate to partially sharp hyphal extremites and subterminal cells are elongated. *Pileocystidia* 3–5 µm broad, 0–1 septate, cylindrical. Stipitipellis a trichoderm, composed of branched, septate, tuft of erect, hair-like hyphae (2–5 µm wide) with attenuate hyphal extremites. *Caulocystidia* up to 3–6 µm broad, cylindrical, mostly rounded apex.

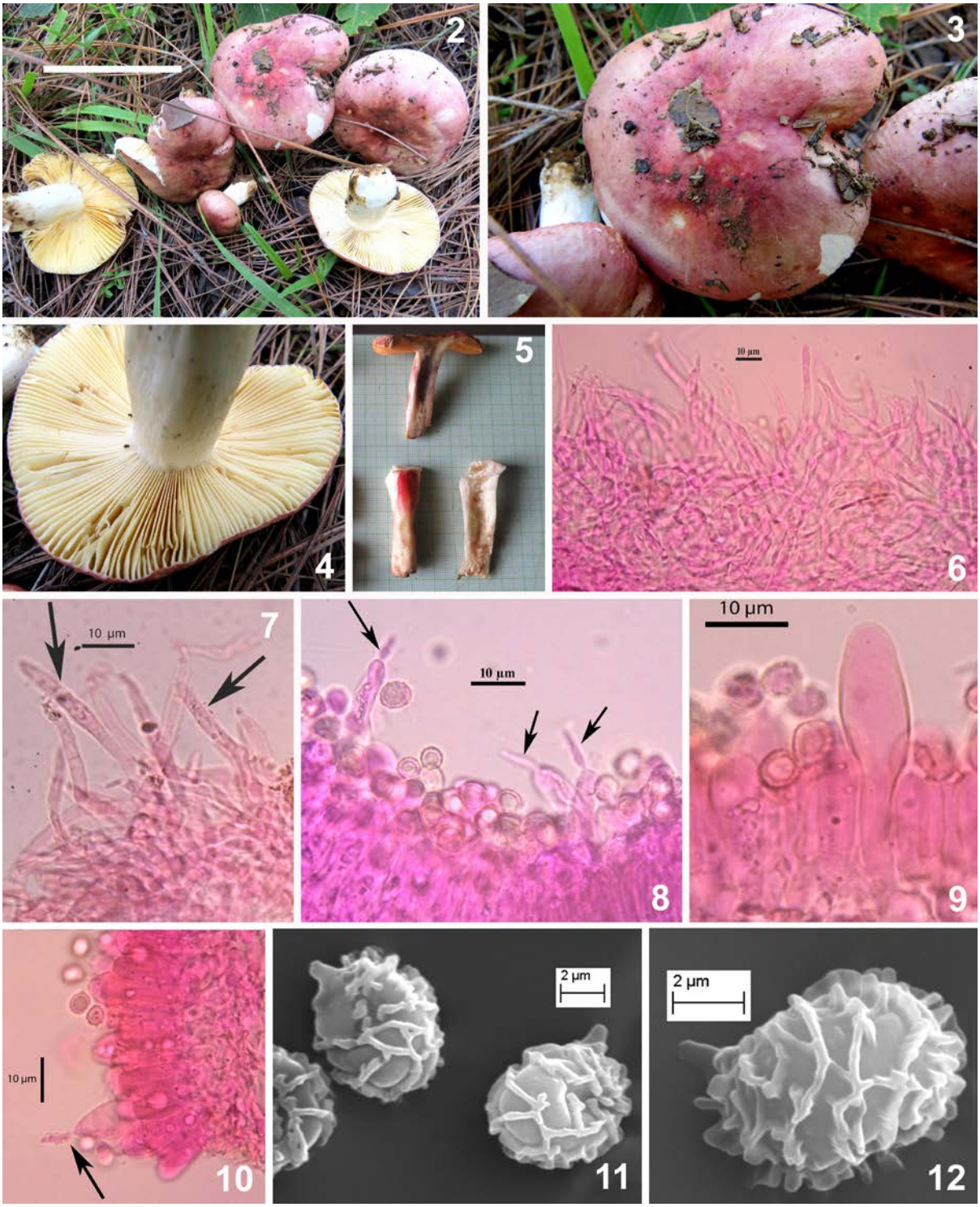
Material examined – India. Uttarakhand: Rudraprayag district, Hariyali Devi Forest, on ground, under *Pinus roxburghii* in temperate coniferous forest, alt. 1651 m, N30°15.955’ E 79°03.719’, 27 July 2015, *A. Ghosh*, AG 15–755.

Discussion

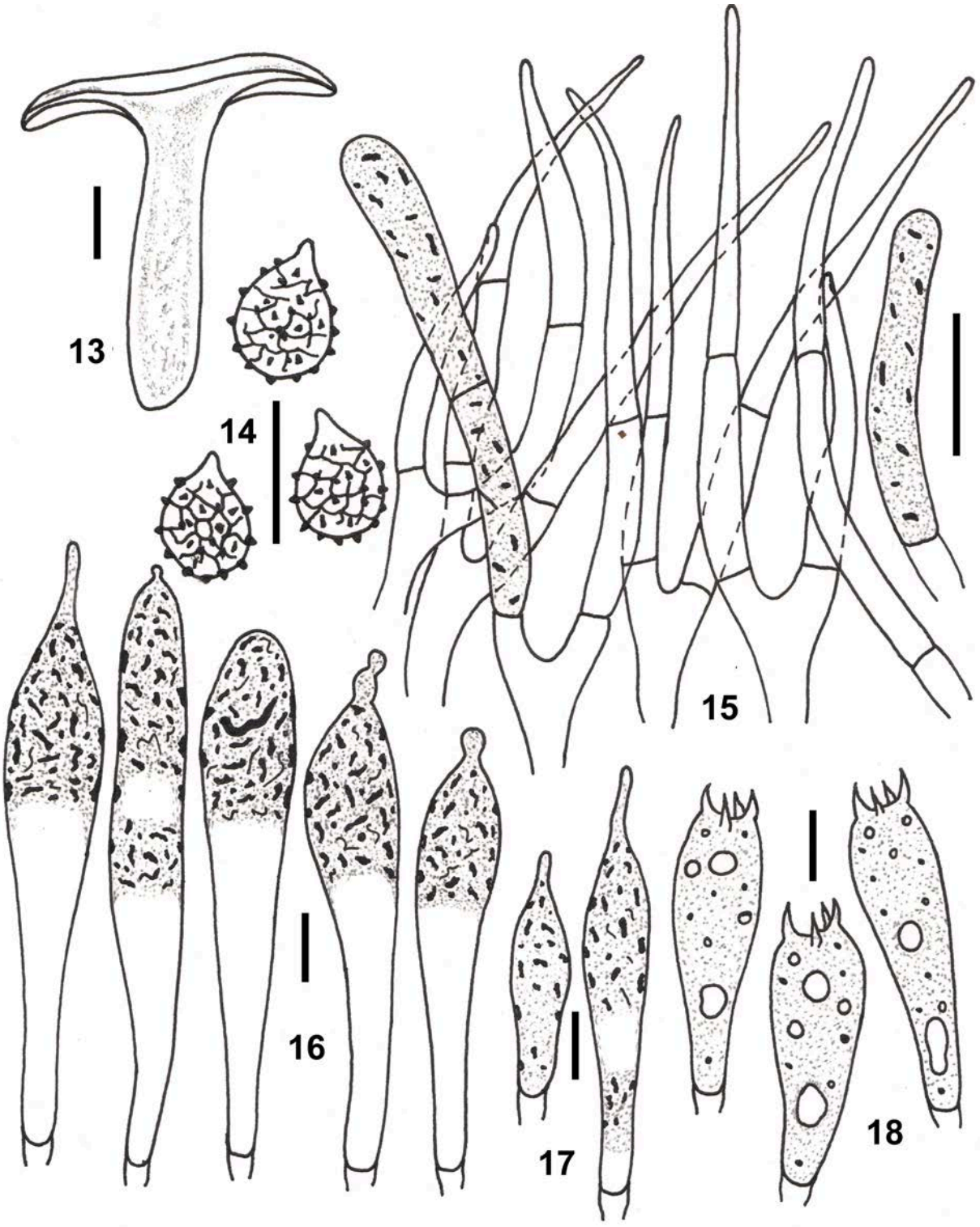
The combination of macro- and micromorphological features such as: pileipellis without primordial hyphae, 0–1 septate dermatocystidia without incrustations and dark yellow spore print undoubtedly place *Russula sarnarii* under subg. *Russula* sect. *Polychromae* subsect. *Integriforminae* (Sarnari 1998). In the field, *R. sarnarii* is distinct from other known species of this group by having its pink rose to pastel pink colored, irregularly wavy pileus that turns mustard yellow with KOH; lamellae with different lengths of forkation, presence of at least 4 lengths of lamellulae and dark yellow spore print. Similarly, micromorphologically, presence of subglobose to broadly ellipsoid (rarely ellipsoid) partially reticulate basidiospores with thick ridges and warts being aligned to form partial reticulum and with few isolated warts (up to 0.9 µm); noticeably appendiculate (appendages up to 16 µm high) cystidia along with mucronate, capitate and moniliform cystdial apex are also quite striking.

In this subsection and in series *Romellii*, two species *R. rubroalba* (Singer) Romagn. and *R. romellii* Marie (also appeared to be closest in ITS phylogeny) resemble with the proposed new taxon. *Russula rubroalba* (GenBank accession number JF519734 in Fig. 1) is somewhat similar to the present taxon with a buff to yellow lamellae, mild taste and attenuate hyphal extremites in cuticle but it segregates from present taxon in having shiny, bright scarlet, rust red to even purplish

red cap surface with pale ochre or whitish at the center; crowded lamellae, relatively roundish spores with lower ornamentations (0.5 µm) and occurrence under broadleaf forests (Sarnari 2005, Romagnesi 1967) whereas, *R. romellii* (GenBank accession number AY061714 & KT933987 in Fig. 1) differs from undescribed taxon by its larger [5–12(14) cm], smooth, shiny, purple to violaceous or olive colored pileus marked with yellowish areas at the center; deep yellow spore



Figs 2–12 – *Russula sarnarii* (from holotype, AG 15–755). 2–5 Fresh basidiomata in the field and base camp. 6 Radial section through pileipellis showing elements of pileipellis. 7 Pileipellis with pileocystidia. 8–9 Transverse section through lamellae showing pleurocystidia. 10 Transverse section through lamellae showing Cheilocystidia. 11–12 SEM images of basidiospores. Scale Bars: 2 = 75 mm. 6–10 = 10 µm. 11–12 = 2 µm.



Figs 13–18 – *Russula sarnarii* (from holotype, AG 15–755). 13 Longitudinal section through a fresh basidioma. 14 Basidiospores. 15 Radial section through pileipellis. 16 Pleurocystidia. 17 Cheilocystidia. 18 Basidia. Scale Bars: 13 =10 mm; 14–18 = 10 µm.

print; dermatocystidia with 1–2 septa, slightly larger spores [7.2–9 (9.6) × 5.6–7.2 µm].The European collections of *R. romellii* are strictly found under deciduous forests (Sarnari 2005, Rayner 1985). In Northeastern North America, it is mostly reported from deciduous forests (Kibby & Fatto 1990) although their occurrence under coniferous trees (Burlingham 1936) are also not unknown (collected from western part of North America in the month of Dec. 27, 1927 from pine woods at Pacific Grove, California and our present undescribed species *R. sarnarii* also grows under pure

pine forests). But, the conspecificity between North American and European collections are questionable.

Two more taxa *R. claroflava* Grove (GenBank accession number AY061665 & KT933997 in Fig. 1) and *R. vinosa* Lindblad (appeared also in BLAST search and represented by GenBank accession number AF418638 & AJ534938 in Fig. 1) are easily segregated from present species because, in those two taxa (belonging to subgenus *Incrustatula*) have moderately encrusted pilear hyphae without dermatocystidia (Sarnari 2005). Moreover *R. claroflava* has larger spores (8–9.6 × 6.4–7.8 µm) composed mostly of isolated warts (up to 0.6 µm) joined by occasional connectives and mild taste (or slightly hot when young) (Sarnari 2005, Galli 1996, Kibby & Fatto 1990, Rayner 1985) whereas, *R. vinosa* also differs from the proposed taxon due to comparatively larger spores (8.4–11.2 × 7–9 µm) with isolated cylindric to conical warts (up to 0.8 µm) and absence of dermatocystidia in pileipellis (Sarnari 2005, Rayner 1985, Beenken 2001). Another three taxa *R. flavida* Frost, *R. subsulphurea* Murrill and *R. puellula* Ebb., Moller & Schaffer (appeared in Blast search as well and and represented by GenBank accession numbers EU598170 & EU598171, KF810135 and AY061710 & KC952701 respectively in Fig. 1) are easily separated from present undescribed species in the field. *Russula flavida* can easily be distinguished from present taxon by the presence of brilliant to orange yellow colored granulose pileus with concolorous stipe and micromorphologically presence of thick walled (up to 11 µm broad) pileocystidia (Bills & Miller Jr. 1984). *Russula subsulphurea* (originally reported from Florida, USA) is segregated from present species by its large (9–10 cm), pallid to yellow-tinted to slightly rosy pileus and white spore print (Murrill 1945, Looney 2014). *Russula puellula* has bright scarlet to pinkish-red colored pileus, cream spore print and slender dermatocystidia with numerous septa (4–8) although Rayner states that dermatocystidia are 0–2 septate which is dubious since Sarnari and Galli both found multiseptate dermatocystidia (Sarnari 2005, Galli 1996, Rayner 1985).

In the field, *R. sarnarii* might be confused with *R. alutacea* (Fr.) Fr., but latter has larger (5–15 cm) purple to vinaceous pileus with often paler to straw or buff centre, usually white stipe with only flush of red at the base, distant thick strongly interveined lamellae and absence of dermatocystidia in pileipellis and grows under principally with *Fagus* on calcareous soils (Kibby & Fatto 1990, Galli 1996). Considering the cap coloration and habitat, the proposed undescribed species also shares the characters with *R. cessans* Pearson and *R. nauseosa* (Pers.) Fr., but *R. cessans* has dark purplish red, livid purple, vinaceous, very rarely brownish, green or pale yellow colored pileus; numerous dermatocystidia with 2–5 septa and comparatively larger spores [7.5–9(10) × 6.5–7.5 µm] with warts (0.75–1 µm high) forming partial to well-developed network (Sarnari 2005, Galli 1996, Rayner 1985) whereas, *R. nauseosa* differs from our new taxon by the presence of numerous dermatocystidia with 4–6 septa and lager spores (8–10 × 6.5–8 µm) with large (up to 1.2 µm high), more or less isolated spines (Sarnari 2005, Rayner 1985).

Keeping in view the combination of macro- and micromorphological features along with phylogenetic evidence *R. sarnarii* is distinct from any previously known species of *Russula*.

Acknowledgements

The authors are grateful to the Head, Department of Botany & Microbiology & USIC Dept. HNB Garhwal University, Srinagar Garhwal for providing all kinds of facilities during the present study and UGC for providing fellowship to Aniket Ghosh. Upendra Singh, Priyanka Uniyal and Tahir Mehmood (HNBGU) are thanked for assisting AG in field in many ways.

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