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

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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 forking and occasional 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 extremities 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

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 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 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 min 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. Amoenu) 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, Russula flavida, Russula cf. rugulosa, Russula subsulphurea, Russula puellula, Russula jilimensis, Russula curtipes, Russula thindii, Russula nana, Russula sanguinea from R. subg. Russula and Russula vinosa, Russula claraflava, Russula buyckii, Russula. zvarae, Russula azurea, Russula subtilis, Russula lilacea from R. subg. Incrustatula were also considered for this analysis for better understanding of 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 planar with broad but slight depression above the stipule 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 bruised 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 stipule 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 bruised. 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), capitulate, 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 extremities and subterminal cells are elongated. Pileocystidia 3–5 µm broad, 0–1 septate, cylindrical. Stipitpellis a trichoderm, composed of branched, septate, tuft of erect, hair-like hyphae (2–5 µm wide) with attenuate hyphal extremities. 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. Integriforinae (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, capitulate and moniliform cystidal 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 extremities 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,
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.

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

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. vinoso 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 Incurvatula) 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. vinoso 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 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 multisepate 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 larger 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.

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