



***Russula rajmahalensis*: A New Species of *Russula* (Russulaceae, Russulales) from Jharkhand State, India**

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

One new species of *Russula*, namely *R. rajmahalensis* belonging to subg. *Heterophyllidiae* sect. *Ingratae* is proposed herein based on its morphological features and nrITS-based phylogenetic inference. *Russula rajmahalensis* is characterized by pale yellow, light yellow to light orange pileus centrally with brown to dark brown; subdistant to close, adnexed, yellowish white lamellae; chalky white to yellowish white stipe with rusty brownish spots at the base; hymenial cystidia subcylindrical, subclavate to clavate with rounded, capitate, subcapitate or appendiculate apex;

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basidiopores subglobose to broadly ellipsoid, composed of amyloid obtuse warts, locally subreticulate, rarely isolated, fused in pairs or short chains, connected by occasional, fine, long line connections and occurrence under *Shorea robusta*. A detailed descriptions accompanied with colour photographs of the basidiomata, nrITS-based molecular phylogeny and comprehensive comparisons with similar species are also provided.

Keywords: Basidiomycota; *Shorea robusta*; nrITS; taxonomy.

1. INTRODUCTION

"*Russula*, the type genus of the family Russulaceae, is among the most taxonomically diverse groups of mushroom-forming fungi and ranks as the second-largest genus after *Cortinarius* (Pers.) Gray" (Kalichman et al., 2020). "It is distinguished by its often vividly coloured pileus, a brittle texture due to the abundance of sphaerocytes, the presence of amyloid spore ornamentation, and gloeoplerous elements. However, unlike the genera *Lactarius* and *Lactifluus*, it lacks a branching lactiferous system that terminates in pseudocystidia on the basidiome surface" (Buyck et al., 2018). "The genus *Russula* exhibits remarkable diversity across the Indian Himalayas, spanning habitats from tropical to subalpine regions, where it associates with both broadleaf and coniferous trees" (Adamčík et al., 2019). Buyck et al. (2018, 2020, 2008) demonstrated that the anatomy of ectomycorrhiza added support to a new infrageneric classification system of *Russula* based on a new multi-locus (nrLSU, mtSSU, *rpb2*, *rpb1* and *tef-1*) analysis.

Species belonging to *Russula* sect. *Ingratae* (Quél.) "Maire is typically characterized by a pileus that varies in colour from tawny, ochraceous, or ashy-grey to dark brown, often with a faintly or distinctly tuberculate-striate margin. Their lamellae are equal, sometimes forked, or intermixed with a few lamellulae. These species exhibit a taste ranging from mild to highly acrid, frequently accompanied by an unpleasant smell. They produce white to cream-colored spore prints, with basidiospores featuring a suprahilar area that is either inamyloid or partially amyloid. Additionally, they possess an abundance of gloeoplerous elements throughout their tissues, as well as small, often mucronate, unicellular pileocystidia on the pileus surface, interspersed with branched, short-celled hyphal ends within the pileipellis" (Singer, 1986, Sarnari, 1998).

During extensive macrofungal surveys conducted in *Shorea robusta* C.F.Gaertn. dominated

regions of Jharkhand, several noteworthy species from the genus *Russula*, were collected. Detailed morphological examinations and molecular phylogenetic analysis of recent collections revealed one previously undescribed species from subgen. *Heterophyllidiae*, sect. *Ingratae*. This species is introduced here as *Russula rajmahalensis* sp. nov. Comprehensive macro- and micromorphological descriptions of the new species are provided, supported by phylogenetic analysis based on nrITS sequences.

2. MATERIALS AND METHODS

2.1 Morphological Studies

"Macromorphological characterizations of the collected specimens were done from basidiomata either in the forest or in the respective basecamps. Images of the fresh basidiomata were captured with Sony DSC-RX100 and Canon Power Shot SX 50 HS. Colour codes and terms were used here mostly after the Methuen Handbook of Colour" (Kornerup and Wanscher, 1978). The methods for micromorphological characterizations and SEM work followed the protocols described by Ghosh et al., (2023). Specimens were deposited at the ASSAM herbarium, Shillong. The subgeneric classification used in this study followed Buyck et al., (2018, 2020). Herbarium acronyms follow Thiers (2018; <https://sweetgum.nybg.org/science/ih/>).

2.2 Molecular Studies

2.2.1 DNA extraction, PCR amplification and sequencing

The protocols for DNA extraction and sequencing of the ITS region followed Ghosh et al., (2023). The final consensus sequences were prepared using Geneious Pro v. 5.1 (Drummond et al., 2010) and deposited at GenBank to procure the accession numbers: PQ438401, PQ438402 for *Russula rajmahalensis* sp. nov.

2.2.2 Dataset assembly, alignment of the dataset and inferring phylogeny

To investigate the relationships of the nrITS sequences of the newly identified *Russula rajmahalensis* (ASSAM F015 and ASSAM F016), reference sequences showing the closest matches were retrieved from GenBank (<https://www.ncbi.nlm.nih.gov/genbank>), the UNITE database (<https://unite.ut.ee/>), as well as from relevant published phylogenies (Song et al., 2018, Adamčík et al., 2019, Yuan et al., 2019, Chen et al., 2021, Li et al., 2021, Ghosh et al., 2022). A multiple sequence alignment (MSA) of nrITS dataset was built with MAFFT ver. 7 (Katoh et al., 2019) using the L-INS-i algorithm, the 200PAM/k = 2 scoring matrix, a gap open penalty of 1.53 and an offset value of 0.123. The alignment was manually reviewed and trimmed based on conserved motifs using MEGA v. 7 (Kumar et al., 2016). Sites with 90% gaps were removed using trimAl v.1.2 program (Capella-Gutiérrez et al., 2009). Maximum likelihood (ML) analysis was performed using IQ-tree version 2.2.2.6 (Nguyen et al., 2015), with the optimal model for the nrITS locus (GTR+I+G) selected by ModelFinder (Kalyaanamoorthy et al., 2017) based on the Bayesian Information Criterion (BIC). Additionally, ultrafast bootstrap with 1,000 replicates was applied to obtain nodal support values. Maximum likelihood bootstrap (MLbs) values $\geq 70\%$ are shown in the phylogenetic trees (Fig. 1).

3. RESULTS AND DISCUSSION

3.1 Phylogenetic Inferences

In our ML phylogenetic analysis, the nrITS data matrix comprised of 98 taxa and 648 nucleotide sites (including gaps). The nrITS dataset contained 437 distinct patterns, 311 parsimony-informative, 70 singleton sites and 267 constant sites. The maximum likelihood (ML) tree obtained from the nrITS alignment with IQ-TREE analysis was constructed with log-likelihood of -7372.051. The rate parameters used were as follows: A-C: 1.50340, A-G: 7.16167, A-T: 1.50340, C-G: 1.00000, C-T: 7.16167 and G-T: 1.00000; base frequencies A: 0.220 C: 0.247 G: 0.228 T: 0.304; gamma distribution shape parameter $\alpha = 0.743$. Our nrITS based phylogenetic analysis (Fig. 1) showed that two sequences of *Russula rajmahalensis* (GenBank [PQ438401 (Holotype), PQ438402]) were placed in subg. *Heterophyllidia* sect. *Ingratae*, where it was part of a fully supported clade (MLbs = 94%)

comprising of unidentified *Russula* spp., *R. ombrophila* and *R. fluvialis*. However, our Indian collections are recovered as distinct species within the phylogenetic tree (Fig. 1).

3.2 Taxonomy

Russula rajmahalensis A. Ghosh, Hembrom & D. Chakr. sp. nov. Fig. 2

MycoBank: MB 856140

GenBank: PQ438401 (nrITS, Holotype), PQ438402 (nrITS)

Etymology: Referring to the Rajmahal hills of the state Jharkhand (India), the type locality.

Type: India: Jharkhand, Rajmahal hills, Sahibganj district, Durgapur Panchayat, Ulghutu area, village of Partee Pahar, 62 m elev., N24°48'06" E87°43'05", September 18, 2022, A. Ghosh, AGJH-037 (ASSAM F015).

Diagnosis: *Russula rajmahalensis* is mainly separated from closely related European *R. fluvialis* Taipale, Ruots. & Kälviäinen by combination of medium to large sized (40–100 mm in diam.) pileus, hymenial cystidia subcylindrical, subclavate to clavate with rounded, capitate, subcapitate or appendiculate apex, occurrence under *Shorea robusta* and nrITS sequence data.

Description: Pileus medium to large sized, 40–100 mm in diam., hemispherical when young, then convex, planoconvex to applanate when mature, somewhat depressed at the centre; margin tuberculate striate, decurved to plane with age; cuticle viscid, shiny when moist, hardly peeling, pale yellow to light yellow (4A3–4) to light orange (5A4–5), centrally brown to dark brown (6E–F6–8) with maturity. Pileus context up to 5 mm thick at the disc, thinning towards the margin, chalky white (1–2A1), unchanging after bruising or cutting; turning salmon pink (6A4) with FeSO₄ and deep to dark turquoise (24E–F7–8) in guaiacol. Lamellae subdistant to close (7–12/cm at the margin), adnexed, yellowish white (2–3A2), forked near the stipe apex, centre and margin; lamellulae absent; edge even and concolorous. Stipe 37–85 × 15–20 mm, cylindrical or sometimes tapered towards the base; surface dry, smooth, finely longitudinally striate, chalky white (1A1) to yellowish white (1A2) with rusty brownish spots at the base. Stipe context stuffed, chambered, chalky white

(1–2A1), unchanging after bruising or cutting; bald. Smell inconspicuous. Spore print not chemical reaction same as pileus context. Taste observed.

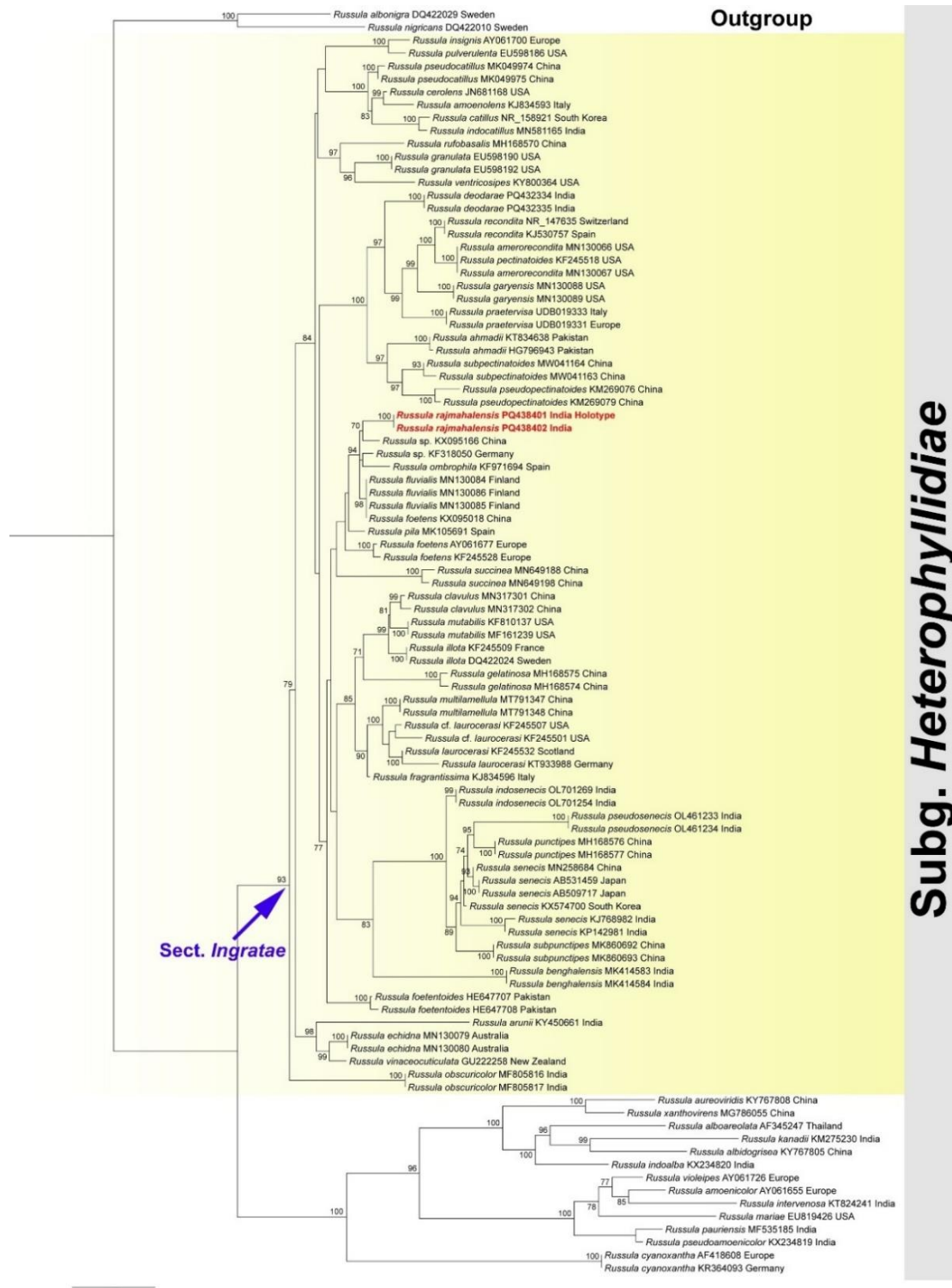


Fig. 1. Phylogram generated by means of maximum likelihood analysis based on nrITS sequence data of *Russula* spp. Maximum likelihood bootstrap support $\geq 70\%$ shown above or below the branches at nodes

Basidiopores subglobose to broadly ellipsoid, (6.5–)6.8–7.2–7.5(–8.0) \times (6.0–)6.2–6.5–6.9(–7.0) μm , [n=40, Q = (1.0–)1.04–1.10–1.16(–1.25)]; ornamentation composed of amyloid obtuse warts (up to 1.1 μm high), locally subreticulate, rarely isolated, fused in pairs or short chains, connected by occasional, fine, long line connections; apiculi up to 1.5 μm long; suprahilar plage amyloid. Basidia (41–)44–48–52(–55) \times (9–)9.5–10.6–11.6(–12.5) μm , clavate, 4-spored; sterigmata up to 5 μm long. Hymenial cystidia near the lamellae face, (45–)50–57–65(–75) \times (6.5–)7–8–9(–11.5) μm , subcylindrical, subclavate to clavate, apically rounded, capitate, subcapitate, appendiculate, often originating deep in lamellae context, thin-walled; contents

dense granulose, oil droplets present, weakly greying in sulfovanillin (SV); near the lamellae edges smaller, (28–)32–41–49(–55) \times (5–)5.5–6.4–7.4(–9) μm , mainly subclavate, rarely clavate, apically mostly obtuse-rounded, rarely subcapitate to capitate, contents dense granulose, oil droplets present. Lamellae edges fertile with basidia. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 140–220 μm deep, strongly gelatinized, vaguely divided into a 40–70 μm deep suprapellis, composed of branched, suberect to erect hyphal terminations, arranged in a trichoderm and dispersed pileocystidia and 100–150 μm deep subpellis of horizontally oriented densely packed hyphae.



Fig. 2. *Russula rajmahalensis* sp. nov. (A, B & C) Fresh basidiomata in the field and basecamp; (d) Transverse section through pileipellis showing elements; (E & F) Transverse section through lamellae showing hymenial cystidia; (G) SEM images of basidiospores. Scale bars: D = 25 μm ; E, F = 10 μm ; G = 1 μm

Acid-resistant incrustations absent. Hyphal terminations near the pileus margin thin-walled; terminal cells (11–)14–19–25(–34) × (2.5–)3–3.5–4(–4.5) µm, mostly cylindrical, sometimes tapered at the apex, apically mostly obtuse-rounded, sometimes attenuated; subterminal cells mostly cylindrical. Hyphal terminations near the pileus centre more densely arranged, with shorter terminal cells (8–)10–14–18(–21) × (2.5–)2.8–3.1–3.5 µm, usually cylindrical, sometimes tapered at the apex, apically obtuse-rounded and sometimes attenuated. Pileocystidia near the pileus margin frequent, always 1-celled, cylindrical, narrowly fusiform or lanceolate, rarely clavate, thin-walled, (20–)25–35–45(–55) × (3–)3.5–4.3–5(–6) µm, apically mucronate, subcapitate to capitate; contents with dispersed granulose, often with yellowish intracellular pigments, weakly greying in sulfovanillin (SV). Pileocystidia near the pileus centre similar in shape and contents, but in average shorter (15–)22–30–39 × (2–)3–4–4.6 µm, apically mostly mucronate to subcapitate, rarely moniliform. Clamp connections absent from all tissues.

Habit and habitat: Solitary or scattered, growing on soil in association with *Shorea robusta* in tropical deciduous forest.

Additional specimen examined: India: Jharkhand, Rajmahal hills, Sahibganj district, Borio block, Dhogada-Paharia burial ground forest, 110 m elev., N 25°02'23.7" E 87°39'35.8", 62 m elev., September 17, 2022, A. Ghosh, AGJH-032 (ASSAM F016).

Remarks: Considering the similar field appearance and grows in the Sal dominated tropical forests, the present taxon might be close to Indian *Russula benghalensis* S. Paloi & K. Acharya and *R. pseudosenecis* A. Ghosh, D. Chakr., K. Das & Buyck. However, *R. benghalensis* is distinct by the presence of small to medium sized (14–35 mm diam.) pileus, smaller (20–40 × 3–6 mm) stipe and presence of 1–2 tiers lamellulae (Yuan et al., 2020) whereas, *R. pseudosenecis* has brownish orange or light brown tinges on the lamellae edges, smaller stipe (20–45 × 9–15 mm), an ixopalysade type of pileipellis and very long (50–150 µm) single celled pileocystidia (Ghosh et al., 2022). Our phylogenetic analysis places our new species within the well-supported clade (MLbs = 94%) of sect. *Ingratae* (= subg. *Heterophyllidae*) comprising of *R. fluvialis*, *R. ombrophila* M.M. Gómez & L.C. Monedero and unidentified *Russula* sp. However, *R. fluvialis* is a common

boreal species in *Picea*-dominated forests, whereas, the spores of *R. ombrophila* have less crested spines that are rarely connected by line connections and pileocystidia are wider than 5 µm (Gomez and Monedero 2011, Adamčík et al., 2019).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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