Original Research Article

Russula deodarae sp. Nov., a ~~One~~ new species of *Russula* sect. *Ingratae* from India, based on morphology and molecular data

ABSTRACT

One new species of *Russula*, namely *Russula deodarae* belonging to subg. *Heterophyllidiae* sect. *Ingratae* is proposed herein based on its ~~their~~ morphological features and nrITS-based phylogenetic inference. *Russula deodarae* is characterized by brown, light brown to dark brown pileus; chalky white, adnate, subdistant lamellae; chalky white, yellowish white to dingy stipe with blood red base; hymenial cystidia with obtuse-rounded, subcapitate, mucronate or appendiculate apex; basidiospores composed of conical warts and ridges connected to give partial to incomplete reticulum, distinctly amyloid suprahilar plage and occurrence under *Cedrus deodara* in coniferous forests. A detailed descriptions accompanied with colour photographs of the basidiomata, illustrations of the main anatomical features, nrITS-based molecular phylogeny and comprehensive comparisons with similar species are also provided.

1. INTRODUCTION

The family Russulaceae harbours four predominantly agaricoid genera: *Lactifluus* Pers., *Lactarius* Pers., *Multifurca* Buyck & V. Hofst. and *Russula* Pers. (Buyck et al., 2008, 2010) and is one of the dominant and morphologically diverse ectomycorrhizal mushroom families in the Himalayas (Sharma et al., 2018, Ghosh et al., 2021, 2023a). *Russula*, the type genus of this family, is one of the most taxonomically diverse genera of mushroom-forming fungi and the second largest genus after *Cortinarius* (Pers.) Gray (Kalichman et al., 2020). It is characterized by a frequently colourful pileus, a brittle context because of the presence of abundant sphaerocytes, and the presence of an amyloid spore ornamentation and gloeoplerous elements, but is devoid of a branching lactiferous system ending in pseudocystidia at the basidiome surface as in the genera *Lactarius* and *Lactifluus* (Buyck et al., 2018). The genus *Russula* has an enormous diversity in the Indian Himalaya showing its wide range of distribution from tropical to subalpine areas and associates both with broadleaf and coniferous trees (Adamčík et al., 2019). Recently, Buyck et al., (2018) demonstrated that the anatomy of ectomycorrhiza added support to a new infrageneric classification system of *Russula*, which is followed here, based on a new multi-locus phylogenetic analysis.

Species belonging to *Russula* sect. *Ingratae* (Quél.) Maire are mostly characterized by a tawny, ochraceous or ashy-grey to dark brown coloured pileus with faintly or strong tuberculate striate margin, equal lamellae that are sometimes forked or intermixed with few lamellulae, a mild to very acrid taste and often unpleasant smell; white to cream coloured spore prints and basidiospores that are an inamyloid or partly amyloid suprahilar area; they have abundant gloeoplerous elements throughout their tissues and small, often mucronate, unicellular pileocystidia at the pileus surface mixed with branched, short-celled hyphal ends in the pileipellis (Singer, 1986,Sarnari, 1998). In the recently published ITS (Li et al., 2021, Ghosh et al., 2022) and multi-gene (Buyck et al., 2018, Chen et al., 2021, Han et al., 2022, Song et al., 2018) phylogenetic analyses shows that the members of sect. *Ingratae* forms a well-supported, monophyletic lineage within the subg. *Heterophyllidiae*Romagn. A total 10 species have been described from India such as: *Russulaabbotensis* K. Das & J. R. Sharma, *R. arunii*Paloi, A. K. Dutta & K. Acharya, *R. benghalensis* S. Paloi& K. Acharya, *R. dubdiana* K. Das, Atri&Buyck, *R. indocatillus* A. Ghosh, K. Das & R. P. Bhatt, *R. indosenecis* A. Ghosh, D. Chakr., K. Das &Buyck, *R. natarajanii* K. Das, J. R. Sharma &Atri, *R. obscuricolor* K. Das, A. Ghosh &Buyck, *R. pseudosenecis* A. Ghosh, D. Chakr., K. Das &Buyck and *R. tsokae* K. Das, Van de Putte&Buyck (Das et al., 2006, 2010, 2017,Crous et al., 2017, Ghosh et al., 2020, 2022, Yuan et al., 2019).

During extensive macrofungal surveys conducted in the Kalatop-Khajjiar Wildlife Sanctuary in Himachal Pradesh, several noteworthy species from the genus *Russula*, were collected. Detailed morphological examinations and molecular phylogenetic analyses of recent collections revealed one previously undescribed species from subg. *Heterophyllidiae*, sect. *Ingratae*. This species is introduced here as *Russuladeodarae* sp. nov. Comprehensive macro- and micromorphological description of the new species is provided, supported by phylogenetic analysis based on nrITS sequences.

2. MATERIAL AND METHODS

2.1 Sampling

The survey site was located in the northwestern Indian state of Himachal Pradesh. Mushroom forays were conducted during the rainy seasons (July to October) of 2020–2022. Fruiting bodies were collected from the Kalatop-Khajjiar Wildlife Sanctuary in Himachal Pradesh. Geographic coordinates were recorded using a Garmin e-trex30 handheld GPS receiver, and the presence of potential host trees was documented during field collection. The collected specimens were dried using a field dryer.

2.2 Morphological studies

Macromorphological characterizations of the collected specimens were done from young to mature 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 (KornerupandWanscher, 1978). The methods for micromorphological characterizations and SEM work followed the protocols described by Ghosh et al., (2023b). Specimens were deposited at the 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 (https://sweetgum.nybg.org/science/ih/).

2.3 Molecular studies

2.3.1 DNA extraction, PCR amplification and sequencing

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The protocols for DNA extraction and sequencing of the ITS region of the nuclear ribosomal DNA (nrDNA) marker followed Ghosh et al., (2023b). 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: PQ432334 and PQ432335 for *Russuladeodarae* sp. nov.

2.3.2 Dataset assembly, alignment of the dataset and inferring phylogeny

To investigate the relationships of the nrITS sequences of the newly identified *Russuladeodarae* (ASSAM F013 and ASSAM F014), 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% gapswere 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 (TPM2u+F+I+G4) 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 ≥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 94 taxa and 646 nucleotide sites (including gaps). The nrITS dataset contained 436 distinct patterns, 305 parsimony-informative, 64 singleton sites and 277 constant sites. The maximum likelihood (ML) tree obtained from the nrITS alignment with IQ-TREE analysis was constructed with log-likelihood of −7140.247. The rate parameters used were as follows:A-C: 1.50178, A-G: 7.02670, A-T: 1.50178, C-G: 1.00000, C-T: 7.02670 and G-T: 1.00000; base frequenciesA: 0.221 C: 0.247 G: 0.226 and T: 0.306; gamma distribution shape parameter α = 0.725. Our nrITS based phylogenetic analysis (Fig. 1) showed that two sequences of *Russuladeodarae* (GenBank [PQ432334 (Holotype), PQ432335]) were placed in subg. *Heterophyllidiae* sect. *Ingratae*, where it was part of a fully supported clade (MLbs = 99%) comprising of European *Russulapraetervisa*Sarnari, *R. recondita*Melera&Ostellari, American *R. amerorecondita* Avis & Barajas and *R. garyensis* Avis & Barajas. However, our Indian collections are recovered as distinct species within the phylogenetic tree (Fig. 1).
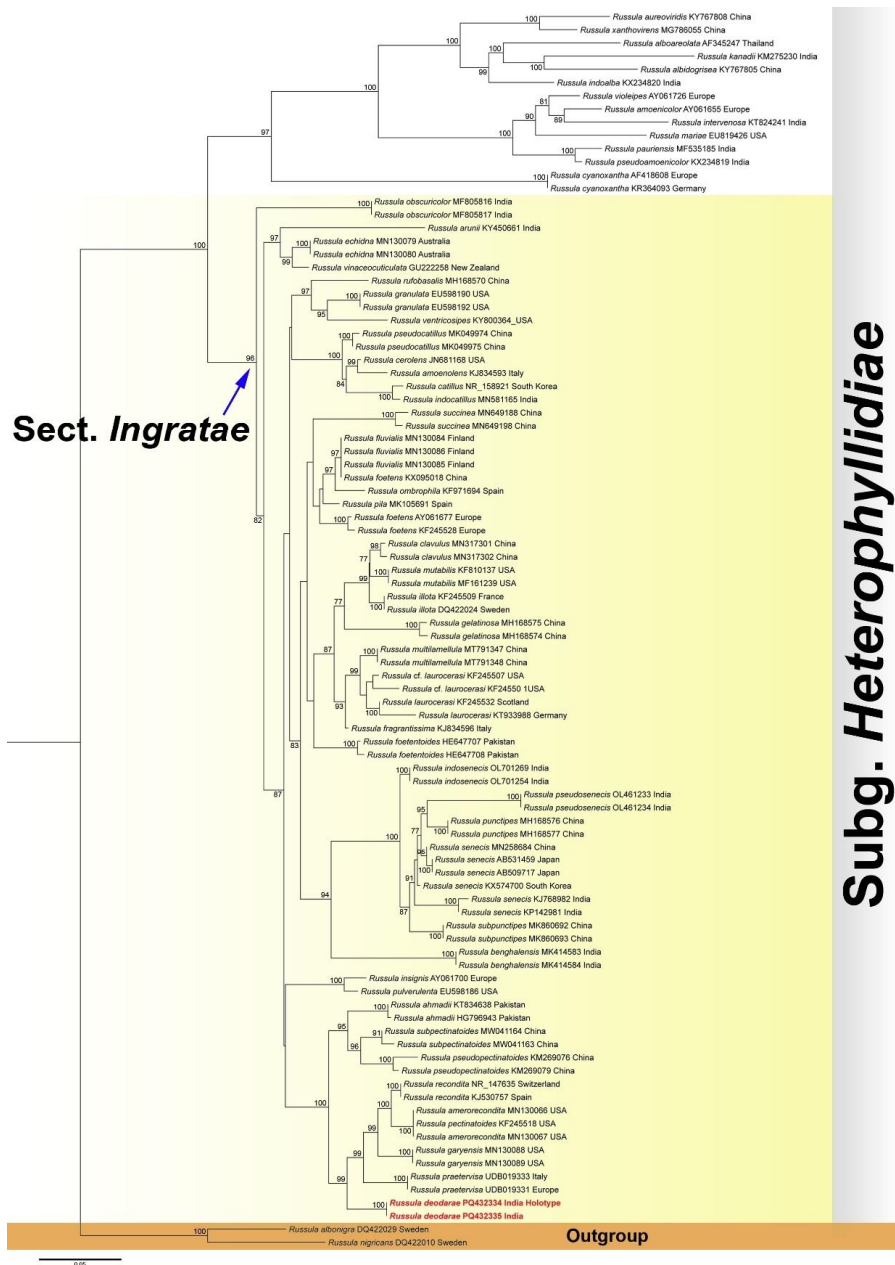
3.2 Taxonomy

Russuladeodarae D. Chakr. & A. Ghosh, sp. nov.   Figs. 2 & 3

MycoBank: MB 856121

GenBank: PQ432334 (nrITS, Holotype), PQ432335 (nrITS).

Etymology: '*deodarae*' refers to *Cedrusdeodara*(Pinaceae), the host tree.

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

Type: India: Himachal Pradesh, Chamba, Kalatop, 2423 m elev., N32°32.882' E76°01.143', July 22, 2021, D. Chakraborty, DC 21-74, (holotype!: ASSAM F013).

Diagnosis: *Russuladeodarae* is mainly separated from closely related Indian *R. obscuricolor* K. Das, A. Ghosh &Buyck by combination of mild taste, occurrence under *Cedrusdeodara*, hymenialcystidia with obtuse-rounded, subcapitate, mucronate or appendiculate apex and nrITS sequence data.

Description: Pileus medium to large sized, 32–112 mm in diam., hemispherical when young, then convex, planoconvex to applanate at maturity, broadly depressed at the center; margin incurved when young then gradually decurved to plane with age, strongly tuberculate striate; surface viscid when moist, glutinous; brown (6E4–5), light brown (6D4–5) when young, becoming orange grey (6B2), greyish brown, brown (6D–E3–4) to dark brown (6F5–7) at center then brownish grey (6C2) to orange grey (6B2) at margin with age. Lamellae adnate, subdistant (7–10/cm at pileus margin), chalky white (1A1), unchanging or light brown (6D4–5) on maturity or injured, forked at stipe apex, middle or at margin; lamellulae less, present in 3 series. Stipe 40–85 × 12–25 mm, central, subcylindrical, subclavate to clavate with broader ~~at~~ base; surface dry, smooth, chalky white to yellowish white (1A1–2) becoming dingy after handling, blood red at base; turning salmon pink (6A4) and deep to dark turquoise (24E–F7–8) with $FeSO_4$ and guaiacol respectively. Smell pleasant, slightly fruity. Taste mild. Spore print not obtained.

Basidiosporessubglobose to broadly ellipsoid, (6.0–)6.4–6.8–7.2(–7.6) × (5.0–)5.3–5.7–6.1(–6.5) μm, [n=40, Q = (1.06–)1.09–1.18–1.27(–1.48); ornamentation amyloid, composed of somewhat conical warts and ridges connected to give partial to incomplete reticulum; warts up to 0.9 μm high, suprahilarplageinamyloid; apiculi up to 1.2 μm long. Basidia (35–)40–45(–48) × (9–)10–11(–12) μm, 4-spored, subclavate to narrowly clavate, narrow base; sterigmata up to 5 μm high. Hymenial cystidia near the lamellae sides (66–)74–84(–100) × (8.8–)9–11(–11.2) μm, cylindrical to subclavate with obtuse-rounded, subcapitate or appendiculate apices; partly or completely filled with finely crystalline content, without reacting in sulfovanillin (SV). Lamellae edges fertile with basidia and cystidia. Hymenial cystidia near the lamellae edges usually smaller and narrower, measuring (35–)38–50(–52) × (5.8–)6–8(–8.3) μm, cylindrical to subclavate with obtuse-rounded or mucronate apices; filled with finely crystalline content, without reacting in sulfovanillin (SV). Subhymenium layer up to 30 μm thick, pseudoparenchymatous. Hymenophoraltrama mainly composed of large nests of sphaerocytes. Pileipellis orthochromatic in Cresyl blue, sharply delimited from the underlying sphaerocytes of the context, 380–400 μm thick, two-layered; vaguely divided in 90–120 μm thick suprapellis of relatively dense, composed of some erect or mostly decending hyphal terminations, arranged in a cutis and dispersed pileocystidia; subpellis 250–300 μm thick, composed of more or less dense, horizontally oriented hyphae. Hyphal terminations near the pileus margin often flexuous, thin-walled, composed of chain of 1–2 cells; terminal cells measuring (11–)15–23(–27) × (2–)2.8–3.2(–4) μm, mainly cylindrical, apically obtuse-rounded; subterminal cells cylindrical. Hyphal terminations near the pileus centre of similar structure, terminal cells slightly wider, measuring (17–)20–32(–35) × (2.8–)3–4(–4.5) cells mainly cylindrical. Pileocystidia near the pileus margin one-celled, flexuous, thin-walled; terminal cells (42–)50–62(–75) × (4–)4–4.5(–5) μm, cylindrical, apically mostly subcapitate, obtuse-rounded or mucronate; content finely crystalline, without reacting in sulfovanillin (SV). Pileocystidia near the pileus centre one-celled, thin-walled; slightly shorter terminal cells (28–)30–40(–42) × (3.2–)3.5–4(–4.5) μm, cylindrical, apically mucronate, capitate, subcapitate; content finely crystalline, without reacting in sulfovanillin (SV). Clamp connections absent from all tissues.
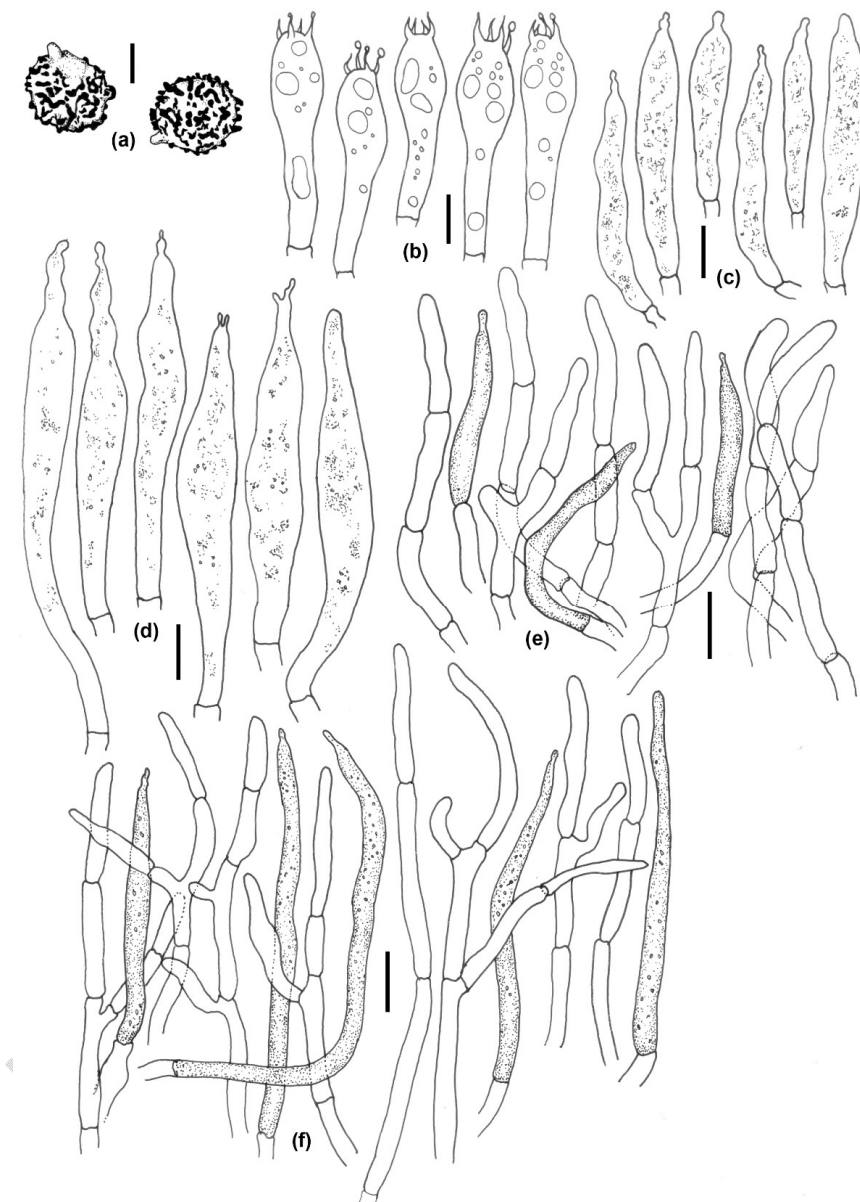
Fig. 2. *Russuladeodarae* sp. nov. (a, b) Fresh basidiomata in situ; (c, d) Transverse section through pileipellis showing elements; (e) Transverse section through lamellae showing hymenial cystidia near the lamellae sides and basidia; (f) SEM images of basidiospores. Scale bars: c = 100 μm; d, e = 10 μm; f = 3 μm

Fig. 3. Line drawings of *Russula deodarae* sp. nov. (a) Basidiospores (b) Basidia; (c) Hymenial cystidia near the lamellae edges; (d) Hymenial cystidia near the lamellae sides; (e) Elements of pileipellis: hyphal terminations and pileocystidia near the pileus margin; (f) Elements of pileipellis: hyphal terminations and pileocystidia near the pileus center. Scale bars: a = 3 μm; b–f = 10 μm

Habit and habitat: Solitary or scattered, growing on soil in association with *Cedrusdeodara* in temperate coniferous forest.

Additional specimen examined: India: Himachal Pradesh, Chamba, Kalatop, 2423 m elev., N32°32.882' E76°01.143', October 6, 2020, D. Chakraborty, NPDF917-07(ASSAM F014).

> **Comment [H16]:** Why not puting all examined specimens together and precise the one that is the holotype.

Remarks: The combination of macro- and micromorphological features of the present newly described species including medium to large sized basidiomata, tuberculate-striate pileus margin, stipe with many internal cavities, mild to slightly acrid taste, basidiospores with inamyloidsuprahilarplage place it undoubtedly in subg. *Heterophyllidiae*Romagnesi sect. *Ingratae*(Quel.) Maire. Our This placement is well-supported by thephylogenetic analysis (Fig. 1)that also positioned places our thisnew species within the well-supportedsame clade (MLbs = 96%) of sect. *Ingratae* (= subg. *Heterophyllidiae*) comprising of European *Russulapraetervisa*, *R. recondita*, North American *R. amerorecondita* and *R. garyensis*.

> **Comment [H17]:** What about Indian species that are morphologically close to this species.

Considering the purplish red spots at the base of the stipe, present taxon is close to the European *R. praetervisa* and *R. recondita*. But, *R. praetervisa* is distinct in having medium sized (35–70 mm in diam.) ochre-brown pileus, taste unpleasant and sometimes bitterish and presence of slightly larger (7.0–8.5 × 5.6–7.0 µm) basidiospores (Sarnari, 1998); whereas *R. recondita* differs by possessing medium sized (35–70 mm in diam.), ochraceous, ochre honey to ochre-grey-bistrecoloured pileus and slightly larger (7.0–8.5 × 5.5–7.0 µm) basidiospores (Melera et al., 2016). *Russulaamerorecondita* and *R. garyensis* have yellowish pileus, slightly acrid taste occasionally associated with mycoheterotrophic orchids in ofthe genus *Corallorhiza* (Adamčík et al., 2019).

However, our thepresent taxon may be confused with similarly coloured species *R. obscuricolor*(reported from India), but thelater is distinct by its a pale yellowish-white tinge in pileus margin, bitterish pungent taste, association with members of Fagaceae (*Castanopsis* sp.) and hymenial cystidia mostly with capitates, rounded and mucronate apex (Das et al., 2017). Several dark-coloured species from Asia such as: *Russulacatillus* H. Lee, M.S. Park & Y.W. Lim, *R. indocatillus* A. Ghosh, K. Das & R.P. Bhatt and *R. pseudocatillus* F. Yuan & Y. Song may be confused with the present taxon. However, *R. catillus* is easily distinguished by the absence of pileocystidia in the pileipellis (Lee et al., 2017). *Russulaindocatillus* is distinct in having small to medium sized pileus (20–52 mm in diam.), stipe base without blood red colouration and association with *Quercus* sp. (Ghosh et al., 2020); *R. pseudocatillus* has greyish-brown pileus centre, towards the margin very pale yellow and larger (7–9 µm in diam.) basidiospores (Yuan et al., 2019).

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4. CONCLUSION

> **Comment [H18]:** This conclusion needs to be revised fucusing mainly on the new species described and they fact that in India, they maybe many other new species to science or the country and then calling for more effort to document species

India, with its luxuriant forests of coniferous and deciduous trees, is immensely diverse in terms of fleshy mushrooms, and the genus *Russula*sare isnot an exception. *Russula* is by far the most speciose genus in the ectomycorrhizal family Russulaceae (Russulales, Basidiomycota), with about 2,000 species worldwide (Adamčík et al., 2019). To date, more than 183 taxa have been reported from India (Ghosh et al., 2023b). Major ectomycorrhizal host trees that support the growth and development of these mushrooms in India? belong to genera like *Quercus* L., *Castanopsis* (D. Don) Spach, *Cedrus* Mill., *Lithocarpus* Blume, *Hopea* L., *Shorea*Roxb. ex C.F. Gaertn., *Abies* Mill., *Picea* A. Dietr., *Cedrus*Trew, *Pinus* L., *Tsuga*(Endl.) Carrière, *Larix* Mill. etc. Compared with Europe (Romagnesi, 1985; Sarnari, 1998), detailed analyses of *Russula* sect. *Ingratae* in Asia began relatively late. In the past decade, rapid progress has been made in India, leading to the identification of six new *Russula* species within the Indian *Ingratae*, based on modern phylogenetic methods (Crous et al., 2017, Das et al., 2017, Ghosh et al., 2020, 2022, Yuan et al., 2019). Previously, many of these species were misidentified based on morphological characteristics, being confused with their European or North American counterparts. The present contribution with

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morphotaxonomyand molecular phylogeny is an initiative to uncover *Russula* species of sect. *Ingratae* in the Indian Himalayas. Moreover, the newly described ~~present~~ taxon (*Russuladeodarae* sp. nov.) is used as culinary value for local people but several species of *R.* sect. *Ingratae* may cause gastrointestinal problems if not properly pre-cooked (Dai et al., 2010,Bau et al., 2014, Chen et al., 2016).

Comment [H20]: This is good to be added in the result and discussion under the subtitle: «local uses» or «Ethnomycology), but with a paragraph in methos telling how you proceeded to know that they sre used.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

I, Aniket Ghosh, am 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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