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Multigene genealogy and morphology unveil one new genus, one new species and one new combination of boletoid mushrooms (Boletaceae) from Indian Himalaya

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ABSTRACT: The present study from the state of Uttarakhand (India) describes the new genus *Singeroboletus* in the subfamily Suillelloideae (="*Pulveroboletus* group") (Boletaceae, Boletales). This genus is supported by morphological and multigene molecular data. At present this novel genus is represented only by two Asian species, *Singeroboletus hainanensis* comb. nov. (based on *Butyriboletus hainanensis*) and *S. himalayanus* sp. nov. Detailed morphology, illustrations, micromorphological drawings and multigene molecular phylogenetic inferences are presented for *S. himalayanus*.

KEY WORDS: Agaricomycetes, Fagaceae, molecular phylogeny, *Singeroboletus*, taxonomy, Uttarakhand.

INTRODUCTION

Being ectomycorrhizal partners of angiospermous and gymnospermous trees, boletoid mushrooms (Boletaceae, Boletales, Agaricomycetes, Basidiomycota) are one of the key functional groups of tropical to subalpine ecosystems. The morphological complexity among its genera, less availability of boletologists across the continents and their poor phylogenetic information kept this important mushroom family unresolved for many years in terms of its systematics and evolution. Only ca 50 genera and 800 species were known in this family by the Dictionary of Fungi (Kirk *et al.*, 2008). Polyphyly prevailed in most of its large and historic genera like *Boletus* Fr., *Leccinum* Gray, *Pulveroboletus* Murrill, *Tylopilus* P. Karsten, *Xerocomus* Quél., etc., because many traditionally used phenotypic characters in these mushrooms do not necessarily predict phylogenetic relatedness and hence, morphology-based generic delimitation hardly supports phylogenetic estimations. The proper framework of Boletaceae only came into the light once the family was treated with a combined approach of morphology and multi-locus molecular data involving protein-coding genes. These advanced studies led to the discovery of more than 100 genera and 1200 species (Das *et al.*, 2023a; Wu *et al*., 2023a) across the world. This redefined framework revealed eight major clades within this family namely, subfamilies Austroboletoideae, Boletoideae, Chalciporoideae, Leccinoideae, Phylloboletelloideae, Suillelloideae (= the *Pulveroboletus* group sensu Wu *et al*., 2014), Xerocomoideae, and Zangioideae (Wu *et al.*, 2014, 2023b; Tremble *et al*., 2023, 2024).

Like the boletes (pileate stipitate fleshy poroid mushrooms) of many other countries, most of the boletes found in India are unknown or poorly known. Several taxa (genera and species) have been overlooked by mushroom taxonomists or boletologists. As boletoid mushrooms are exceptionally diverse and cryptic (group of morphologically close species that belong to different evolutionary lineages), several genera are known incorrectly by their respective European and North American lookalikes (genera and species) and due to their morphological resemblance, cryptic taxa (genera and species) have remained undescribed and undiscovered for decades in this country. In the poorly explored India, western Himalaya has been relatively well explored in terms of bolete diversity. Presently, 86 species belonging to 25 genera are known from Indian Himalaya (Chakraborty 2022; Mushtaq *et al*., 2022; Das *et al*., 2023a,b).

Recently, during the rainy season in 2022 and 2023, repeated macrofungal explorations to Rudraprayag district of the state of Uttarakhand were undertaken by the authors (KD & AG). A large number of boletoid mushrooms was collected. Thorough examination of field characters, morphological features followed by multigene molecular phylogenetic inferences of the collected specimens uncovered a new genus and a new species in the family Boletaceae from this country. *Singeroboletus himalayanus* gen. and sp. nov. are proposed herein with morphological and molecular data.

MATERIAL AND METHODS

Morphology

Fresh basidiomata were collected during the rainy seasons 2022–2023 (July to August) from various locations in Uttarakhand. Macromorphological and field characters were noted in the field or in the basecamp.

Images of the fresh and dissected basidiomata were captured with Canon Power Shot SX50 HS and Canon Power Shot SX220 HS cameras. Colour codes and terminology primarily followed Kornerup and Wanscher (1978). After recording all the macromorphological characters, dissected samples were placed for drying in an aluminium field drier. Micromorphological characters were observed after mounting the freehand sections of dried samples in a solution of 5% KOH, 1% phloxine, and 1% ammoniacal Congo red under an Olympus CX 41 compound microscope. Drawings of the micromorphological features were made with the help of a drawing tube at 1000× magnification. Microscopic photographs were captured with a camera attached to an Olympus BX 53 microscope. The basidiospores were measured in lateral view. Basidiospore measurements and length/width ratios (Q) are recorded as: minimum–mean–maximum. Basidium length excludes the length of sterigmata. Field emission scanning electron microscope (FESEM) images of basidiospores were obtained by mounting spore prints on a double-sided adhesive tape pasted on a metallic specimen stub and then scanned with a gold coating at different magnifications in high vacuum mode to observe patterns of spore ornamentation. This work was carried out with an FEI Quanta FEG 250 model installed at Centre for Research in Nanoscience and Nanotechnology (CRNN) in University of Calcutta, India. Herbarium acronyms follow Thiers (https://sweetgum.nybg.org/science/ih/).

DNA extraction, polymerase chain reaction (PCR) and sequencing

Genomic DNA was extracted from 100 mg of dried basidiome with the HiPurA Fungal DNA Purification Kit (HIMEDIA) following the manufacturer's instructions. The PCR amplification of four nuclear loci, the internal transcribed spacer (ITS1-5.8S-ITS2 = ITS), and the genes coding for the partial nuc. 28S rDNA D1-D2 regions (28S), region between conserved domains 6 and 7 of second largest subunit of RNA polymerase II (*rpb*2), and translation elongation factor 1-α (*tef* 1) were done using the primer pairs ITS1-F and ITS4, LR0R and LR5, b*rpb*2-6F and f*rpb*2-7cR, ef1-983F and ef1-1567R, respectively (White *et al*., 1990; Gardes and Bruns, 1993; Liu *et al*., 1999; Matheny *et al*., 2005; Rehner and Buckley, 2005). PCR amplification for these loci was carried out in a ProFlex PCR system (Applied Biosystems) programmed for an initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 50 °C for 30 sec, and extension at 72 °C for 1 minute. The final extension was kept at 72 °C for 7 minutes. The PCR products were purified using the QIAquick PCR purification kit (QIAGEN, Germany). The cycle sequencing products were run on ABI 3500 automated DNA analyzer (Applied Biosystems, USA). The sequence quality was checked using Sequence Scanner Software ver. 1 (Applied Biosystems). Sequence alignment, required editing and contig preparation of the obtained sequences were carried out using Geneious Pro ver. 5.1 (Drummond *et al*., 2010). In this study, eight sequences (two each for ITS, 28S, *rpb*2 and *tef* 1-α) were generated from two separate collections of *Singeroboletus* (voucher nos. KD 22-018 and KD 23-006) and subsequently deposited in GenBank (Table 1).

Phylogenetic analysis

The ITS, 28S, *rpb*2 and *tef* 1-α sequences of the newly described *Singeroboletus* species plus close relatives were retrieved from BLASTn search against GenBank (https://www.ncbi.nlm.nih.gov/genbank) and relevant published phylogenies (Biketova *et al*., 2022; Wu *et al*., 2023a, Tremble *et al*., 2024). Four raw datasets (ITS, 28S, *rpb*2 and *tef* 1-α) were created separately. All the datasets were aligned separately using the online version of the multiple sequence alignment program MAFFT v. 7 (https://mafft.cbrc.jp/alignment/software/) with L-INS-i strategy. The alignment was checked and trimmed with the conserved motifs manually with MEGA v. 7 (Kumar *et al*., 2016). Intron regions of protein-coding genes (*rpb*2 and *tef* 1-α) were removed in the final analyses. Sites with 90% gaps (100 positions in total) were removed using trimAl v.1.2 program (Capella-Gutiérrez *et al*., 2009) from ITS alignment. Furthermore, four alignments (ITS, 28S, *rpb*2 and *tef* 1-α) were concatenated into multi-locus dataset using BioEdit v. 7.0.9 (Hall, 1999) and used for the phylogenetic analyses. The combined dataset was phylogenetically analysed using both Maximum Likelihood (ML) and Bayesian inference (BI) methods. The ML was performed using raxmlGUI 2.0 (Edler *et al*., 2021) with the GTRGAMMAI substitution model. ML analysis was executed applying the rapid bootstrap algorithm with 1000 replicates to obtain nodal support values. In combined dataset, four partitions (ITS-28S-*rpb*2-*tef* 1-α) were assigned for Bayesian inference (BI) analysis. PartitionFinder2 was used to find the best nucleotide substitution models using the Bayesian information criterion (BIC) with a greedy search over all models (Lanfear *et al*., 2017). For the concatenated analyses of *Singeroboletus himalayanus*, each locus was considered a partition and assigned its own best-fitting substitution model. For *S. himalayanus*, the models were HKY+I+G for ITS, SYM+I+G for 28S, *rpb*2 and *tef* 1-α. Bayesian inference (BI) was performed with MrBayes v.3.2.6 (Ronquist *et al*., 2012) as an additional method for determining branch support. Two MCMC runs of four chains were executed simultaneously from a random starting tree for 3,000,000 generations. Average standard deviation of split frequency (ASDSF) value was lower than 0.01 at the end of the generations. Trees were sampled every 100th generation. The first 25% of trees were discarded as burn-in. Chain convergence was determined using Tracer 1.5 (Rambaut *et al*., 2014) to

Table 1. List of species used for phylogenetic analyses of this study, with voucher nos. and GenBank accession numbers. Newly generated sequences are in bold.

Name of the species	Voucher no.	ITS	28S	*tef* 1-α	*rpb2*
Acyanoboletus controversus	HKAS126560	OQ888701	OQ888714	OQ873451	OQ873490
Acyanoboletus controversus	HKAS101248	—	OQ888715	OQ873452	OQ873491
Acyanoboletus dissimillis	ZT:14030	—	NG_242127	OQ873453	OQ873492
Amoenoboletus granulopunctatus	HKAS56280	MZ708840	KF112418	KF112265	KF112708
Amoenoboletus granulopunctatus	MHHNU9490	MW520189	MW520186	MW566747	MW560081
Amoenoboletus mcrobbii	PDD97418	MZ708841	—	—	—
Amoenoboletus miraculosus	ZT14046	MZ708842	MW520188	MW566745	
Amoenoboletus weberi	FLAS-F-61525	MH211950	—	—	—
Amoenoboletus weberi	FLAS-F-68076	OL960512	—	—	—
Baorangia major	OR0486	—		MG897433	MG897443
Baorangia pseudocalopus	HKAS:75081	—	KF112356	KF112168	KF112678
Baorangia rufomaculata	4414	—	KF030248	KF030406	
Boletaceae sp.	TRTC168574-SWK386		PRJNA1022813 (from whole genome)		
Butyriboletus appendiculatus	BR 50200893390-25	KT002598	KT002609	KT002633	—
Butyriboletus appendiculatus	BR 50200892955-50	KJ605668	KJ605677	KJ619472	—
Butyriboletus fechtneri	AT2003097	KC584784	KF030270	—	—
Butyriboletus hainanensis	N.K.Zeng1197	KU961653	KU961651	—	KU961658
Butyriboletus hainanensis	N.K.Zeng2418	KU961654	KU961652	KU961656	KX453856
Butyriboletus pseudoroseoflavus	HMJAU59470	OL604164	OL587853	OL739124	OL739126
Butyriboletus pseudoroseoflavus	HMJAU59471	OL604165	OL587852	OL739123	OL739125
Butyriboletus roseoflavus	HKAS63593	KJ909517	KJ184559	KJ184571	—
Butyriboletus roseoflavus	HKAS54099	KJ909519	KF739665	KF739779	—
Cacaoporus pallidicarneus	HKAS:52601	—	KF112469		KF112732
Cacaoporus tenebrosus	OR0654	—		MK372275	MK372288
Caloboletus aff. *calopus*	HKAS:74739	—	KF112335	KF112166	KF112667
Caloboletus panniformis	HKAS:55444	—	KF112334	KF112165	KF112666
Caloboletus peckii	Mushroom Observer #246697	—	MH220330	MH318614	—
Costatisporus cyanescens	Henkel 9067	—	LC053662		LC053664
Crocinoboletus laetissimus	FHMU:2030	—	MK850935	MK850948	MK850944
Crocinoboletus rufoaureus	HKAS:59820	—	KF112434	—	KF112709
Crocinoboletus rufoaureus	HKAS:53424	—	KF112435	KF112206	KF112710
Cupreoboletus poikilochromus	GS 10070	—	KT157060	KT157072	KT157068
Cyanoboletus bessettei	ARB1393A	—	—	MW737482	MW737457
Cyanoboletus brunneoruber	HKAS80579-1	—	KT990568	KT990763	KT990401
Cyanoboletus cyaneitinctus	Farid_920	MW675744	MW662579	—	MW737465
Cyanoboletus fagaceophilus	HKAS126556	OQ888702	OQ888718	OQ873455	OQ873494
Cyanoboletus instabilis	HKAS:59554	—	KF112412	KF112186	KF112698
Cyanoboletus pulverulentus	MG 628a	—	KT157064	KT157073	KT157069
Cyanoboletus sinopulverulentus	HKAS:59609	—	KF112366	KF112193	KF112700
Erythrophylloporus aurantiacus	REH7271	—	—	MH614715	MH614761
Erythrophylloporus cinnabarinus	GDGM70536	—	MH374045	MH378802	MH374035
Exsudoporus floridanus	FLAS-F-59069	OL960514	OL960488	OL960496	OL960503
Exsudoporus floridanus	FLAS-F-61008	OL960516	OL960489	OL960497	OL960504
Exsudoporus frostii	NY815462	—	JQ924342	KF112164	KF112675
Exsudoporus frostii	FLAS-F-60742	MH016833	OL960493	OL960499	OL960506
Exsudoporus permagnificus	IB 19800750	OL960522	—	—	—
Exsudoporus permagnificus	AB B15-254	OL960524	—	—	—
Exsudoporus ruber	KUN-HKAS 106891	—	MN930518	MT063123	MT063120
Exsudoporus ruber	KUN-HKAS 103513	—	MN930519	MT063124	MT063121
Hongoboletus ventricosus	TNS-F-44611	OQ888710	OQ888732	—	OQ873507
Hongoboletus ventricosus	HKAS122793	OQ888709	OQ888734	—	—
Imperator torosus	MB000258	—	—	MW566748	MW560082
Lanmaoa angustispora	HKAS 74759	—	KM605140	KM605155	KM605178
Lanmaoa asiatica	HKAS63516	—	KT990584	KT990780	KT990419

Neoboletus antillanus	JBSD127417	—	MK388302	—	MK488082
Neoboletus brunneissimus	HKAS:52660	—	KF112314	KF112143	KF112650
Neoboletus brunneorubrocarpus	HKAS76660	OQ888703	KF112328	KF112180	KF112731
Neoboletus ferrugineus	HKAS77617	—	KT990595	KT990788	KT990430
Neoboletus hainanensis	HKAS:59469	—	KF112359	KF112175	KF112669
Neoboletus luridiformis	AT2001087	—	JQ326995	JQ327023	
Neoboletus magnificus	HKAS:54096	—	KF112324	KF112149	KF112654
Neoboletus obscureumbrinus	HKAS63498	—	KT990598	KT990791	KT990433
Neoboletus rubriporus	HKAS83026	—	KT990601	KT990795	KT990437
Neoboletus sanguineoides	HKAS57766	—	KT990605	KT990799	KT990440
Neoboletus sanguineus	HKAS80849	—	KT990609	KT990803	KT990443
Pulveroboletus brunneopunctatus	HKAS74926	—	KT990621	KT990815	KT990456
Pulveroboletus macrosporus	HKAS:58860	—	KF112408	KF112263	KF112714
Pulveroboletus subrufus	N.K.Zeng1857	—	KX453837	KX453855	KX453841
Rubroboletus esculentus	HKAS:68679	—	KF112333	KF112147	KF112662
Rubroboletus flammeus	FHMU6927	—	OM514334	OM525826	OM525824
Rubroboletus flavus	HKAS90906	OQ888704	OQ888722	OQ873459	OQ873497
Rubroboletus latisporus	HKAS80358	KJ951990	KP055023	KP055020	KP055029
Rubroboletus serpentiformis	HKAS126557	OQ888705	OQ888723	OQ873460	OQ873498
Rubroboletus sinicus	HKAS 68620	KJ951991	KY418896	KF112146	KF112661
Rugiboletus brunneiporus	HKAS 83009	—	KM605133	KM605146	KM605169
Rugiboletus extremiorientalis	HKAS 76663	—	KM605135	KM605147	KM605170
Singeroboletus himalayanus	**KD 23-006**	**PP133249**	**PP133251**	**PP188019**	**PP157640**
Singeroboletus himalayanus	**KD 22-018**	**PP133250**	**PP133252**	**PP188020**	**PP157641**
Singerocomus atlanticus	ACM 1275	—	KY926777	—	—
Singerocomus rubriflavus	GAS 900	—	KY926779	—	—
Suillellus amygdalinus	112605ba	—	JQ326996	JQ327024	—
Suillellus flaviporus	HKAS123826	OQ888706	OQ888726	OQ873463	OQ873501
Suillellus pinophilus	HKAS126550	OQ888707	OQ888729	OQ873466	OQ873504
Suillellus yunnanensis	HKAS126548	OQ888708	OQ888730	OQ873467	OQ873505
Sutorius australiensis	REH9441	—	JQ327006	JQ327032	MG212652
Sutorius eximius	REH9400	—	JQ327004	JQ327029	MG212653
Sutorius subrufus	N.K.Zeng3043	—	MH879698	MH879728	MH879745
Zangia citrina	HKAS52677	—	HQ326940	HQ326871	—
Zangia citrina	HKAS52684	—	HQ326941	HQ326872	—

ensure sufficiently large effective sample size (ESS) values (>200). Our novel taxon (with two collections) is highlighted in the combined phylogenetic tree using bold red font (Fig. 1 & S1).

RESULTS

Phylogenetic inferences

In our present phylogenetic analysis, four-locus dataset (ITS + 28S + *rpb*2 + *tef* 1-α) of *Singeroboletus* consisted of 90 taxa and 2,937 nucleotide sites, including gaps (900 bp for ITS, 906 bp for 28S, 653 bp for *rpb*2 and 478 bp for *tef* 1-α) with *Zangia citrina* Yan C. Li & Zhu L. Yang as outgroup. The combined dataset contained 1591 distinct patterns, 1004 parsimony-informative, 276 singleton sites and 1657 constant sites. Based on the multi-gene molecular phylogenetic analysis (Fig. 1& S1), our target samples (KD 22-018 and KD 23-006) formed a strongly supported clade (MLbs = 100%, BPP = 1) along with the previously described *Butyriboletus hainanensis* and a possibly undescribed species represented by a single sequence from Malaysia (Boletaceae sp. TRTC168574-SWK386). This clade is recognized as a new genus *Singeroboletus* K. Das, Su. Datta, A. Ghosh & Vizzini (see below). An unidentified collection from Malaysia (Boletaceae sp. TRTC168574-SWK386) within the *Singeroboletus* clade (Fig. 1 & S1) represents an unpublished new species of *Singeroboletus*. It is well supported as sister to the two genera *Butyriboletus* and *Exsudoporus* by Maximum Likelihood analysis (MLbs = 87%), but the branch could not be recovered in the Bayesian analysis.

TAXONOMIC TREATMENTS

Singeroboletus K. Das, Su. Datta, A. Ghosh & Vizzini, ***gen. nov.***

MycoBank: MB852039

Generic Type: *Singeroboletus himalayanus* K. Das, Su. Datta, A. Ghosh & Vizzini, see below in this publication

***Diagnosis*:** *Singeroboletus* is characterised by medium to large basidiomata, brown to dark brown, smooth to

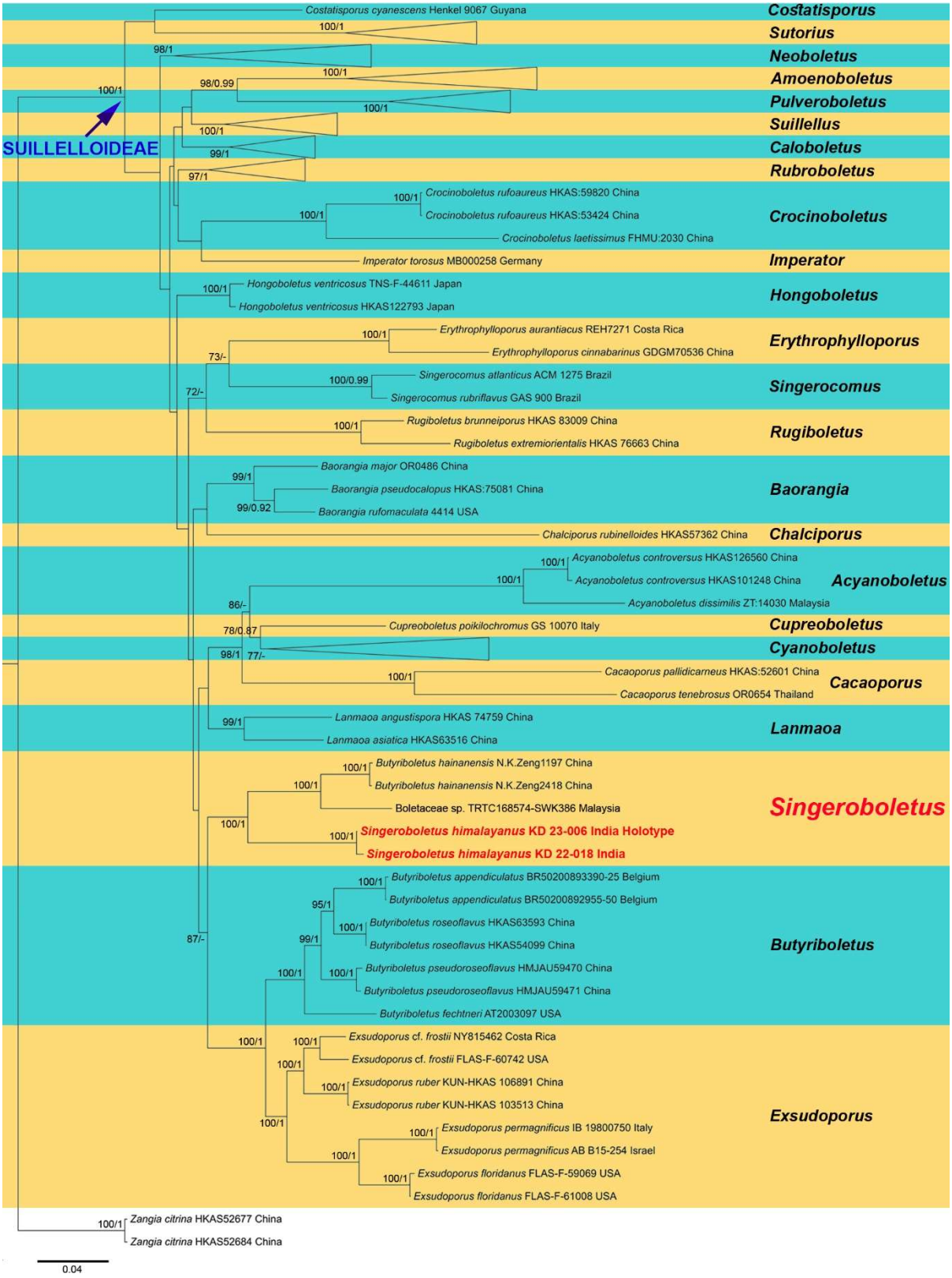

Fig.1. Maximum likelihood phylogenetic tree inferred from the four-gene dataset (ITS, 28S, *rpb*2 and *tef* 1-α), showing position of the new genus ***Singeroboletus*** in Suillelloideae. Maximum likelihood bootstrap support values (MLbs) ≥70% are shown on the left of "/" and Bayesian posterior probabilities (BPP) ≥0.95 are shown on the right above or below the branches at nodes. Sequences of ***Singeroboletus himalayanus*** is placed in bold red font to highlight their phylogenetic positions in the tree.

areolate pileus with very thin hymenophore and thick pileus context; yellow hymenophore turning instantly blue-black, then slowly dark brown when bruised, stipe yellow to reddish brown, smooth to finely cracked; smooth basidiospores; an interwoven trichodermal pileipellis, and trichodermal stipitipellis showing hyphae with inflated to cystidoid terminal elements, caulohymenium absent. Clamp connections absent.

Generic description: Basidiomata epigeous, stipitate-pileate. Pileus brown to dark brown; surface dry, finely velvety, smooth to areolate. Hymenophore very thin, tubular; pore surface yellow to greyish yellow or greyish orange, becoming slightly greenish yellow with maturity but instantly turning blue-black, then slowly dark brown; pores circular to angular, not stuffed. Context white, changing blue quickly, then turning red and finally black when exposed. Stipe subclavate, upper yellow then reddish brown to brownish red towards below; surface smooth or finely cracked towards apex at maturity. Basal mycelium white. Odour indistinct. Taste unknown. Basidiospores subfusiform to ellipsoid, smooth. Hymenial cystidia subfusoid to fusoid. Pileipellis a trichoderm, composed of erect to suberect interwoven hyphae. Stipitipellis composed of interwoven subparallel to suberect hyphae; terminal elements cystidioid to inflated or appendiculate. Caulocystidia and caulohymenium absent. Clamp connections absent.

Etymology: "*Singero*-" commemorates Rolf Singer for his invaluable contribution to mushroom taxonomy and "-*boletus*" refers to the resemblance to the genus *Boletus*, representing the iconic 'fleshy poroid mushroom' group.

***Distribution*:** India and China.

***Ecology*:** Tropical and temperate forests, under broadleaf trees (Fagaceae) from 300 to 2500 m alt.

Singeroboletus hainanensis (N.K. Zeng, Zhi Q. Liang & S. Jiang) K. Das, Su. Datta, A. Ghosh & Vizzini, ***comb. nov.***

MycoBank: MB852041

Basionym: *Butyriboletus hainanensis* N.K. Zeng, Zhi Q. Liang & S. Jiang, Phytotaxa 267(4): 257 (2016)

Singeroboletus himalayanus K. Das, Su. Datta, A. Ghosh & Vizzini, ***sp. nov.*** **Figs. 2 & 3**

***MycoBank*:** MB852040

***Type*:** India, Uttarakhand, Rudraprayag district, Baniakund, elev. 2557 m, N 30°28.187′ E 79°13.115′, 3 Aug 2023, *K. Das*, KD 23-006 (**holotype**! CAL 1976).

***GenBank*:** PP133249 (ITS, holotype), PP133250 (ITS); PP133251 (28S, holotype), PP133252 (28S); PP157640 (*rpb*2, holotype), PP157641 (*rpb*2); PP188019 (*tef* 1-α, holotype), PP188020 (*tef* 1-α).

***Diagnosis*:** Distinguished from the closely allied species in this proposed genus, i.e. *Singeroboletus hainanensis*, by presence of cracked to areolate pileus surface in mature specimens, reddish brown colouration of middle to lower part of stipe, distinctively larger basidiospores 9.1–14.3 × 4.3–5.1 μm, occurrence in tropical forests and the combined molecular data (ITS, 28S, *rpb*2 and *tef* 1-α sequences).

***Description*:** Pileus 45–175 mm diam., initially hemispherical, then convex to plano-convex, finally applanate at maturity; surface dry, smooth when young, cracked or areolate at maturity exposing context, dark brown (7F4) near centre, brown (7D4) near margin when young, becoming paler up to greyish brown (6D3) near centre and dark brown (7F4) towards margin when mature, becoming darker on bruising but turning violet brown (10F8) with KOH and dull green (25D3) with NH_4OH; margin entire, incurved when young, decurved to upturned with narrow sterile flap of tissue up to 1 mm. Pore surface yellowish white (2A2) when young, gradually light yellow (4A4) to greyish yellow or greyish orange to brownish orange 4–5(B–C)5 with maturity, tuning instantly greyish green (25E5) to blue-black on bruising then slowly changing to brown (7E5); pores rounded to angular, 1/mm, not stuffed. Tubes thin, 1–7 mm long, adnate, concolourous to pore surface. Stipe 65–123 × 10–25 mm, clavate, broad towards base, greyish yellow or corn (4A4–5) to reddish brown (9E7) at apex, just below the tube, brown (6F4) or darker all along, smooth but finely cracked towards apex at maturity. Context thick, up to 22 mm wide in pileus, white, immediately becoming greyish blue (21C5) on exposure, then becoming dull red (10C4), but turning light orange (5A5) with KOH; stipe context in young basidiomata white in upper half, greyish turquoise (24E5) in lower half, instantly becoming brick red (7D8) and then darker on exposure. Odour indistinct. Taste unknown. Spore print olive brown.

Basidiospores 9.1–11.68–14.3 × 4.3–4.57–5.1 μm (n = 30, Q = 1.77–2.56–3.19), ellipsoidal, thin-walled, smooth under light microscope and SEM. Basidia 22–38 × 9–12 μm, clavate, hyaline, 4-spored; sterigmata 4–7 × 0.5–1 μm. Pleurocystidia 38–54 × 5–9 μm, abundant, subcylindrical to ventricose-fusoid with appendiculate apex, thin-walled, with olive-brown intracellular pigmentation, few with granular content, emergent up to 25 μm. Subhymenial layer 10–20 μm thick. Tube edge sterile; cheilocystidia 20–35 × 5–7 μm, subcylindrical, clavate to subventricose or appendiculate, emergent up to 18 μm, with olive brown intracellular pigmentation. Hymenophoral trama divergent, composed of thin-walled, septate, loosely arranged hyphae (4–7 μm wide). Pileipellis 174–290 μm thick, a trichodermium, composed of suberect, loosely arranged interwoven hyphae; terminal cells 16–58 × 3–13 μm, subcylindrical to subclavate, with olive brown intracellular pigmentation. Stipitipellis composed of subparallel to suberect, thin-walled, loosely arranged, pigmented hyphae forming an interwoven trichodermium; terminal elements 17–45 × 6–13 μm, inflated to subclavate, ventricose or cystidioid or appendiculate, broadly subclavate to cylindro-clavate,

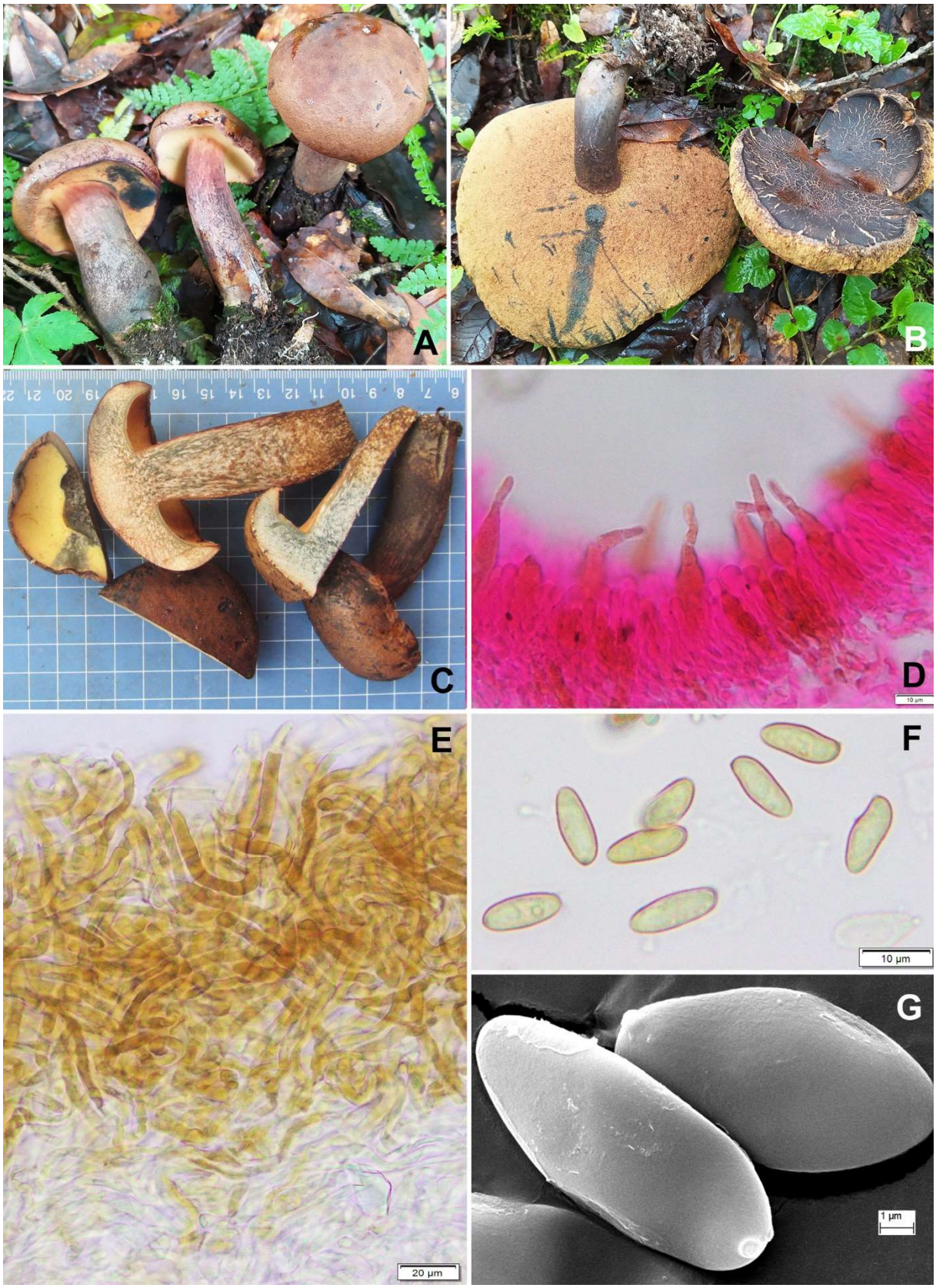

Fig. 2. Photographic illustrations of ***Singeroboletus himalayanus*** (from CAL 1976) **A–C**. Fresh basidiomata. **D**. Pleurocystidia. **E**. Pileipellis. **F**. Basidiospores under light microscope. **G**. Basidiospores under SEM. Scale bars: **D** & **F =** 10 µm, **E** = 20 µm, **G** = 1 µm.

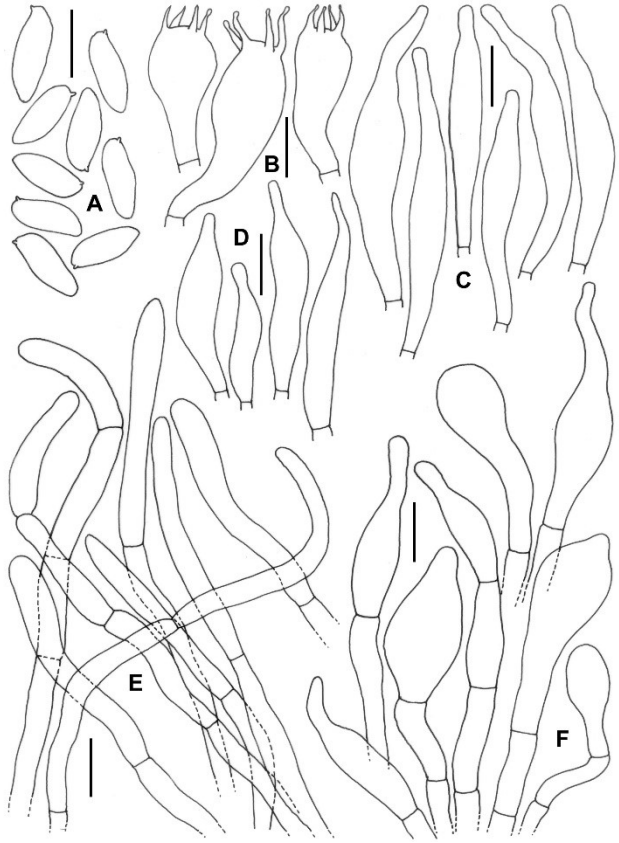

Fig. 3. Drawing illustrations of ***Singeroboletus himalayanus*** (from CAL 1976) **A**. Basidiospores. **B**. Basidia. **C**. Pleurocystidia. **D**. Cheilocystidia. **E**. Elements of pileipellis. **F**. Elements of stipitipellis. Scale bars: **A–F** = 10 µm.

ventricose. Caulohymenium absent. Clamp connections absent.

***Etymology*:** referring to the Himalaya where the type locality is located.

***Habitat*:** solitary or in groups, under *Quercus* sp. (Fagaceae) in temperate Himalaya.

***Additional specimens examined*:** India, Uttarakhand, Rudraprayag district, Baniyakund, elev. 2622 m, N 30°29.000′ E 79°10.743′, 19 Aug 2022, *K. Das*, *KD 22-018* (CAL 1978); ibid., elev. 2563 m, N 30°10.146′ E 78°52.107′, 19 Aug 2022, *K. Das*, *KD 22-022* (CAL 1979).

DISCUSSION

Exudoporus Vizzini, Simonini & Gelardi and *Butyriboletus* D. Arora & J.L. Frank are morphologically or phylogenetically close to our newly proposed genus *Singeroboletus*. However, *Exudoporus* is easily distinguished in the field itself by overall bright red coloured basidiomata and reddish pore surface which is typically beaded with golden droplets, whereas, *Butyriboletus* is distinguished by basidiocarps with comparatively long tube layer that often bruises blue (always blue in *Singeoboletus*), yellow reticulate stipe and firm yellow-tinged flesh that may or may not turn blue when exposed (Arora and Frank, 2014; Biketova *et al*., 2022).

Singeroboletus himalayanus, our newly proposed species is characterized by a set of morphological features: medium to large basidiomata, brown pileus with thick context and distinctively thin hymenophore, light yellow to greyish yellow or greyish orange pore surface that instantly becoming blue-black on bruising, subclavate stipe with yellow (apex) to reddish brown (middle to lower) colouration and faint reticulation, white basal mycelium, smooth basidiospores, trichodermium pattern of pileipellis and stipitipellis hyphae with inflated to cystidioid terminal cells and occurrence under broadleaf trees. Based on our molecular phylogenetic estimation, it appears that *Singeroboletus hainanensis* (≡ *Butyriboletus hainanensis* N.K. Zeng, Zhi Q. Liang & S. Jiang), originally reported from China, was the only so far known taxon in *Singeroboletus* (Fig. 1). However, *S. hainanensis* can be readily distinguished from the proposed Indian species by its smooth (never areolate or cracked) pileus surface, yellow colouration of 1/3 of stipe, brownish red colouration of lower 2/3 of stipe, smaller basidiospores [(7.5–10(–11) × 4–5 µm)] and occurrence in tropical (380–850 m) broadleaf forests dominated by Fagaceae (Liang *et al*., 2016; Zeng *et al*., 2024).

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LITERATURE CITED

Arora, D., Frank, J.L. 2014 Clarifying the butter Boletes: a new genus, *Butyriboletus*, is established to accommodate *Boletus* sect. *Appendiculati*, and six new species are described. Mycologia **106(3)**: 464–480.

Biketova, A.Yu., Gelardi, M., Smith, M.E., Simonini, G., Healy, R.A., Taneyama, Y., Vasquez, G., Kovács, Á., Nagy, L.G., Wasser, S.P., Peintner, U., Nevo, E., Bunyard, B.A., Vizzini, A. 2022 Reappraisal of the genus *Exsudoporus* (Boletaceae) worldwide based on multi-gene phylogeny, morphology and biogeography, and insights on *Amoenoboletus*. J. Fungus **8(2)**: 101.

Capella-Gutiérrez, S., Silla-Martínez, J.M., Gabaldón, T. 2009 trimAl, a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics **25(15)**: 1972–1973.

Chakraborty, D. 2022 First record of *Boletus himalayensis* (Basidiomycota, Boletaceae) from Kalatop Wildlife Sanctuary, Himachal Pradesh, India. Kavaka **58(2)**: 17–21.

Das, K., Datta, S., Singh, U., Ghosh, A., Chakraborty, D. 2023b Multigene molecular phylogeny and morphological evidence reveal one new species and three new records of boletoid fungi from India. Nelumbo **65(2)**: 1–18

Das, K., Ghosh, A., Chakraborty, D., Datta, S., Bera, I., Layola, M.R., Banu, F., Vizzini, A., Wisitrassameewong, K. 2023a Four novel species and two new records of Boletes from India. J. Fungus **9(7)**: 754.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T., Wilson, A. 2010 Geneious v5.1. Available from http://www.geneious.com

Edler, D., Klein, J., Antonelli, A., Silvestro, D. 2021 raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. Methods Ecol. Evol. **12(2)**: 373–377.

Gardes, M., Bruns, T.D. 1993 ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mole. Ecol. **2(2)**: 113–118.

Hall, T.A. 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp. Ser. **41**: 95–98.

Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A. 2008 Ainsworth & Bisby's Dictionary of the Fungi (10th edn). CABI, UK, 771 pp.

Kornerup, A., Wanscher, J.H. 1978 Methuen handbook of colour, 3rd ed., Eyre Methuen Ltd., London, UK.

Kumar, S., Stecher, G., Tamura, K. 2016 MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol. **33(7)**: 1870–1874.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B. 2017 PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. **34(3)**: 772–773.

Liang, Z-Q., Dong-Yu, A.N., Jiang, S., Su, M-S., Zeng, N.K. 2016 *Butyriboletus hainanensis* (Boletaceae, Boletales), a new species from tropical China. Phytotaxa **267(4)**: 256–262.

Liu, Y.J., Whelen, S., Hall, B.D. 1999 Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol. Biol. Evol. **16()**: 1799–1808.

Matheny, P.B. 2005 Improving phylogenetic inference of with *RPB*1 and *RPB*2 nucleotide sequences (*Inocybe*; Agaricales). Molecular. Phylogenetics and. Evol. **35(1)**: 1–20.

Mushtaq, F., Malik, W.S., Chakraborty, D., Bhat, M.H., Vyas, A., Das, K. 2022 *Veloporphyrellus latisporus*, a new generic record for India. Mycotaxon **137(4)**: 953–962.

Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J. 2014 Tracer version 1.6. Available from: http://beast.bio.ed.ac.uk/tracer.

Rehner, S.A., Buckley E. 2005 A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia **97(1)**: 84–98.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P. 2012 MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. **61(3)**: 539–542.

Tremble, K., Halling, R.E., Henkel, T. W., Moncalvo, J-M., Dentinger, B.T.M. 2023 Nomenclatural novelties. Index Fungorum **555**: 1.

Tremble, K., Henkel, T. W., Bradshaw, A., Domnauer, C., Brown, L., Thám, L.X., Furci, G., Aime, C., Moncalvo, J-M., Dentinger, B.T.M. 2024 A revised phylogeny of Boletaceae using whole genome sequences. Mycologia **116(3)**: 392–408.

White, T.J., Bruns, T., Lee S., Taylor, J. 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., D. H. Gelfand, J. J. Sninsky and T. J. White. (eds.), PCR Protocols: a guide to method and applications. Academic Press, San Diego. pp. 315–322.

Wu, G., Feng, B., Xu, J. P., Zhu, X.T., Li, Y. C., Zeng, N. K., Hosen M.I., Yang, Z.L. 2014 Molecular phylogenetic analyses redefine seven major clades and reveal 22 new generic clades in the fungal family Boletaceae. Fungal Divers. **69(1)**: 93–115.

Wu, G., Li, H-J., Horak, E., Wu, K., Li, G-M., Yang, Z. L. 2023a New taxa of Boletaceae from China. Mycosphere **14(1)**: 745–776.

Wu, G., Wu, K., Halling, R.E., Horak, E., Xu, J., Li, G-M., Lee, S., Pecoraro, L., Flores, R. A., Ebika, S.T.N., Aouali, S., Persiani, A.M., Yorou, N.S., Xu, X., Feng, B., Li, Y-C., Yang, Z.L. 2023b The rapid diversification of Boletales is linked to Early Eocene and Mid-Miocene Climatic Optima. bioRxiv preprint. https://doi.org/10.1101/2023.10.24.563795

Zeng, Y-P., Huang, J-Y., Tu, L., Zhao, K. 2024 Complete mitochondrial genome sequence of *Butyriboletus hainanensis* (Boletales, Basidiomycota). Mitochondrial DNA, Part B. Resour. **9(1)**: 46–49.

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