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Research Article

Morphological Studies and Molecular Phylogenetic Evidence Unfold the New Generic Record of the Genus *Imleria* from India

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ABSTRACT

Boletes (Boletaceae) are important ectomycorrhizal mushrooms in tropical to subalpine forests of India. Imleria parva, which was collected from a subalpine forest of Uttarakhand, is described and illustrated for the first time in this country with conventional morphotaxonomy and molecular phylogeny. This report is also the first report of the genus Imleria from India.

Keywords: *Boletales, morphology, new record, phylogeny, taxonomy, wild mushroom*

INTRODUCTION

The genus *Imleria* Vizzini (2014), one of the small genera in the mushroom forming family Boletaceae (Boletales, Basidiomycota) was erected to accommodate *Boletus badiaus* Fr. (Farid et al., 2020). The position of *Boletus badius* has long been debated. The presence of its characteristic viscid pileus placed the species in *Suillus* Gray (Kuntze, 1898), whereas the subtomentose pileus (when dry) supported its placement in genus *Xerocomus* Quél. (Gilbert, 1931). However, molecular evidences confirmed that *B. badius* forms a distinct monophyletic lineage, well separated from both *Xerocomus* and *Xrocomellus* Šutara and was established as a distinct genus *Imleria*. Morphologically, this genus is distinct by the combination of the following features, chestnut brown pileus and stipe, viscid pileus surface when moist, cream to pale yellow hymenophore, context and hymenophore cyanescent on handling or exposure, ixotrichoderm nature of pileipellis and smooth basidiospores. Presently, *Imleria* is represented by eight species (www.indexfungorum.org) of which *I. obscurebrunnea* (Hongo) Xue T. Zhu & Zhu L. Yang, *I. parva* Xue T. Zhu & Zhu L. Yang and *I. subalpina* Xue T. Zhu & Zhu L. Yang are known to occur in Asia (Zhu et al., 2014).

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Repeated macrofungal forays to Uttarakhand since 2022, followed by morphological examination and molecular phylogenetic estimation of Boletoid mushrooms, revealed the first representative of the genus *Imleria* from this country and is reported herein as *I. parva*. This communication describes this species with macro- and micromorphology coupled with molecular phylogenetic estimation and illustrations.

MATERIALS AND METHODS

Morphological studies

While undertaking a routine macrofungal survey of Kedarnath Wildlife Sanctuary of Uttarakhand in 2023, several boletoid mushrooms were collected. Field characters were recorded in the forest or after returning to base camp. Color codes and terms used are after the Methuen Handbook of Color (Kornerup & Wanscher, 1978). After recording the macromorphological characters, the samples were dried using a field dryer. Micromorphological characters were observed under compound microscopes (Nikon Eclipse Ni-U and Olympus CX41). Free-hand sections from dry specimens were mounted in a mixture of 5% KOH, 1% Phloxine, and 1% Congo red or in distilled water. Micromorphological drawings were prepared with a drawing tube (attached to the Olympus CX 41 microscope) at 1000x. Basidiospore measurements were recorded in profile view from 30 basidiospores. Basidiospore measurements and length/width ratios (Q) are recorded here as minimum-mean-maximum. Herbarium codes are after Thiers (2025).

DNA extraction, PCR amplification 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 two nuclear loci, the internal transcribed spacer (ITS) and partial nuclear 28S region were done using the primer pairs ITS1-F and ITS4; LR0R and LR5; (White et al., 1990). The PCR protocol followed Das et al. (2023). The PCR products were purified using the QIAquick PCR purification kit (QIAGEN, Germany). Both strands of the PCR fragment were sequenced on a ABI 3500 DNA Analyzer (Applied Biosystems, USA) using the amplifying primers. 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, four sequences (two each for ITS and 28S) were generated from two separate collections (voucher no. KD 23-002 and KD 23-011) and subsequently deposited in GenBank (Table 1).

Phylogenetic analysis

Phylogenetic analysis using ITS and 28S, sequences data was carried out to establish the phylogenetic placement of our species. The ITS, 28S sequences of the newly described species and its close relatives were retrieved from BLASTn search in GenBank (www.ncbi.nlm.nih.gov/genbank) and relevant published phylogenies (Zhu et al., 2014, Farid et al., 2020). Two raw datasets (ITS and 28S) were created separately. All the datasets were aligned separately using the online version of the multiple sequence alignment program MAFFT ver. 7 (https://mafft.cbrc.jp/alignment/software) (Katoh et al., 2019) with L-INS-i strategy. The alignment was checked and trimmed manually with the conserved motifs with MEGA ver. 7 (Kumar et al., 2016). Species position was first examined using single locus phylogenies. When significant conflict was not observed among the single locus phylogenies, we concatenated the single-locus datasets (ITS and 28S) into a multi-locus dataset using BioEdit ver. 7.0.9 (Hall, 1999) and used it for the phylogenetic analyses. A total of 28 sequences were used of which four were newly generated in this study. Out of these sequences 25 were for ITS, 21 for 28S (Table 1). The multi-locus combined dataset consisting of 28 taxa and 1765 nucleotide sites (including gaps) for each taxon, of which 873 characters were from ITS, 892 from 28S was

prepared. In the analyzed 1765 nucleotides, 1276 were constant, 427 were variable, of which 344 were parsimony informative sites. The combined dataset was phylogenetically analysed using the maximum likelihood (ML) method. The ML was performed using raxmlGUI 2.0 (Edler et al., 2021) with the GTRGAMMA substitution model. ML analysis was executed using the rapid boot-strap algorithm with 1000 replicates to obtain nodal support values. Two of our collections are highlighted in the combined phylogenetic tree using bold red font. Maximum likelihood bootstrap (MLbs) values ≥ 70% are shown in the phylogenetic trees (Fig. 1).

RESULTS AND DISCUSSION

Molecular phylogenetic inference

The two-gene combined phylogenetic (ML) tree is consistent, and the phylogenetic analysis (Fig. 1), including the present Indian collections, resolved the genus *Imleria* as monophyletic with full support. The Indian collections of *Imleria* (represented by KD 23-002 and KD 23-016) were strongly supported (MLbs = 100) and well nested in "*Imleria parva* clade" clustering together with the Chinese collections of *Imleria parva*, yet there is negligible intraspecific divergence between specimens from the two countries (Fig. 1). This may be due to altitudinal and host variations.

Taxonomy

Imleria parva Xue T. Zhu & Zhu L. Yang, *Phytotaxa* 191: 91 (2015)

Figs. 2 & 3

GenBank: PQ657867 (ITS, CAL 2123), PQ656147 (28S); PQ657868 (ITS, CAL 2124), PQ656148 (28S).

Basidiomata are small to medium-sized. Pileus 32–65 mm in diam., convex when young, becoming planoconvex with maturity; surface viscid when wet, velvety when dry, minutely areolate near margin; brown to leather brown (6E5–7) when young, with maturity light brown (6D6) at centre, paler towards margin; margin entire, with a very narrow (up to 0.5 mm wide) sterile fap of tissue; turning Persian orange to deep orange (6A7–8) with KOH, brownish grey to brown (6E2–4) with $FeSO_4$. Pore surface pastel yellow to light yellow (2A4–5), becoming dull green (25D3–4) on bruising; pores angular, often compound, 1/mm. Tubes adnate, 5–7 mm long, light yellow (2A5), unchanging when bruised or exposed. Stipe 57–62 × 7–10 mm, more or less cylindrical; surface longitudinally striate, brownish grey to greyish brown (8D2–3) at apex, reddish brown (8D4) towards the middle downwards, basal mycelium white. The context in pileus up to 5–10 mm thick, dingy white; the context in stipe solid, dingy white; turning yellowish with KOH, greenish with $FeSO_4$. Odour mild. Spore print not obtained.

Basidiospores 10.2–11.69–12.9 ×3.5–4.40–4.9 µm, (n=30, Q=2.66–2.44–3.22), ellipsoid to fusoid and inequilateral in side view, hyaline, smooth under light microscope. Basidia 25–45 × 10–12.5 µm, clavate, 4-spored; sterigmata 3.5–5.5 × 0.5–1 µm. Pleurocystidia 23.5–55 × 5.5–10 µm, olive-green pigmented, ventricose, fusoid, or subcyllindric, thin-walled, hyaline but few with finely granular content, emergent up to 24 µm. Tube edge fertile. Cheilocystidia 41–90 × 6–7.5 µm, less frequent, similar to pleurocystidia, thin-walled, emergent up to 37 µm. Hymenophoral trama with thin-walled, septate, parallel hyphae; hyphae up to 4–5 µm wide, septate. Pileipellis 250–350 µm thick, an ixotrichodermium, composed of erect, closely packed, cylindrical, unbranched, thin-walled hyphae, few cells olive-green pigmented with granular content in 5% KOH; terminal elements 38–59.5 × 6–12 µm, subcylindrical, subventricose to fusiform. Stipitipellis up to 150 µm thick, fertile, composed of thin-walled, septate, parallelly arranged, repent hyphae and few tufts of basidia, basidioles and cystidia; caulocystidia 31.5–75 × 5.5–9 µm, subcylindric, ventricose to fusiform, few with encrustations; caulobasidia, rare, similar to tube basidia, 4-spored. Clamp connections absent in all tissues.

Specimens examined: INDIA, Uttarakhand, Rudraprayag district, Kedarnath Wildlife Sanctuary, 30° 29.350′ N 79° 12.203′ E, alt.

2924 m, subalpine mixed forests under *Quercus* sp., 2 August 2023, *K. Das*, KD 23-002 (CAL 2123); *ibid.*, subalpine mixed forests under *Quercus* sp., 2 August, 2023, *K. Das*, KD 23-016 (CAL 2124).

DISCUSSION

Xerocomus and *Imleria* appear similar in the field but can easily be distinguished by the presence of viscid pileus surface. *Xerocomus uttarakhandae* K. Das, Sud. Datta & A. Ghosh, a species originally discovered from the same state of India, closely resembles the present species in possessing medium-sized basidiomata, brownish pileus and yellow pore surface but can be easily separated from the latter by the absence of gluten and in showing bacillate ornamentation on spore surface (Das et al., 2023). Several other Indian *Xerocomus* species share morphological affinities with the present species. *Xerocomus doodhcha* K. Das, D. Chakr., A. Baghela, S.K. Singh & Dentinger is distinct by the "milk–tea" colour of pileus (Das et. al., 2016), whereas *X. longistipitatus* K. Das, A. Parihar, D. Chakr. & A. Baghela is separated from *I. parva* by exceptionally long and robust stipe (70–185 × 10–24 mm), pore surface that turns greenish grey to dull green slowly on bruising (Chakraborty et. al., 2017). *Xerocomus reticulostipitatus* Hembrom, D. Chakr., A. Parihar & K. Das shows very prominent brownish red to reddish brown reticulation on stipe and larger basidiospores (10.3–15.6 × 3.7–5.3 µm) (Das et. al., 2017). Another Asian species *X. rugosellus* (W.F. Chiu) F.L. Tai, has a distinct yellow pileus which clearly separates it from the present species of *Imleria* (Wu et. al., 2016). The Indian specimens are in conformity with the holotype (reported from China) but slightly vary in possessing a larger pileus in basidiomata (25–35 mm in diam. In type specimens) and also the occurrence under *Quercus* (*Castanopsis* and *Pinus* for type specimens).

Table 1. *Imleria parva* and allied sequences used in ML analyses of this study are shown in tabulated form. Newly generated sequences from Indian collections are presented in bold.

	Name	Voucher no.	Country	ITS	LSU
1.	*Imleria floridana*	Franck_4235	USA	MN535217	MN584689
2.	*Imleria floridana*	Franck_4234	USA	MN535216	MN584688
3.	*Imleria floridana*	Franck_3973	USA	MN535215	MN584687
4.	*Imleria obscurebrunnea*	HKAS52557	China	KC215207	KC215220
5.	*Imleria obscurebrunnea*	HKAS56083	China		KC215219
6.	*Imleria obscurebrunnea*	HKAS50477	China	KC215206	
7.	*Imleria subalpina*	HKAS74712	China	KC215208	KC215218
8.	*Imleria subalpina*	HKAS56375	China	KC215209	KC215217
9.	*Imleria parva*	HKAS55341	China	KC215202	KC215216
10.	*Imleria parva*	HKAS59437	China	KC215203	KC215215
11.	*Imleria badia*	HKAS74713	Italy	KC215205	KC215214
12.	*Imleria badia*	HKAS 74714	Germany		KC215212
13.	*Imleria badia*	HKAS53502	Germany	KC215204	KC215213
14.	*Imleria badia*	S.D. Russell ONT iNaturalist 137600257	USA	OP749849	
15.	*Imleria badia*	S.D. Russell ONT iNaturalist 136522948	USA	OP749234	
16.	*Imleria badia*	S-F119691	Sweden		KJ806971
17.	*Imleria pallida*	OMDL K. Canan iNaturalist # 178605519	USA	PP850509	
18.	*Imleria pallida*	FLAS-F-60971	USA	MH016920	
19.	*Imleria pallida*	FLAS-F-60585	USA	MH016795	

20.	*Imleria pallida*	S.D. Russell ONT iNaturalist 136441700	USA	OP749520	
21.	*Imleria pallida*	JLF2568	USA	KC812309	KC812310
22.	*Imleria pallida*	JLF2551	USA	KC812307	KC812308
23.	*Imleria pallida*	Mushroom Observer #244148	USA	MH225771	MH225770
24.	*Imleria pallida*	179/97	USA	DQ534564	AF457409
25.	*Spongiforma thailandica*	DED7873	Thailand	EU685113	EU685108
26.	*Spongiforma squarepantsii*	LHFB14	Borneo	HQ724511	HQ724509
27.	***Imleria parva***	**KD 23-002**	**India**	**PQ657867**	**PQ656147**
28.	***Imleria parva***	**KD 23-016**	**India**	**PQ657868**	**PQ656148**

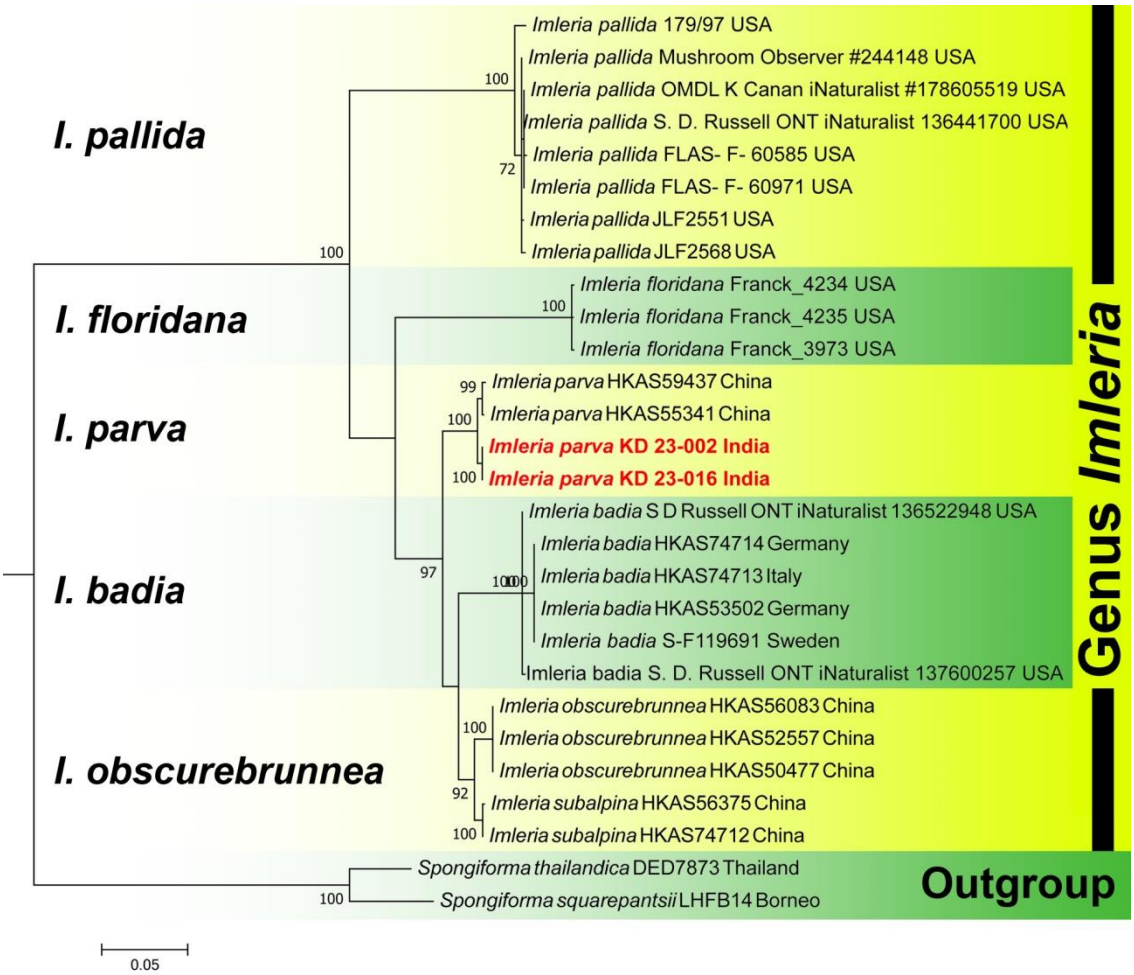


Fig. 1. Phylogram generated by Maximum Likelihood analysis based on nrITS-based sequence data for *Imleria parva* and allied species. Maximum likelihood bootstrap support values (MLbs) ≥70% are shown above or below the branches at nodes. *Imleria parva* is placed in bold red font to highlight its phylogenetic position in the tree.

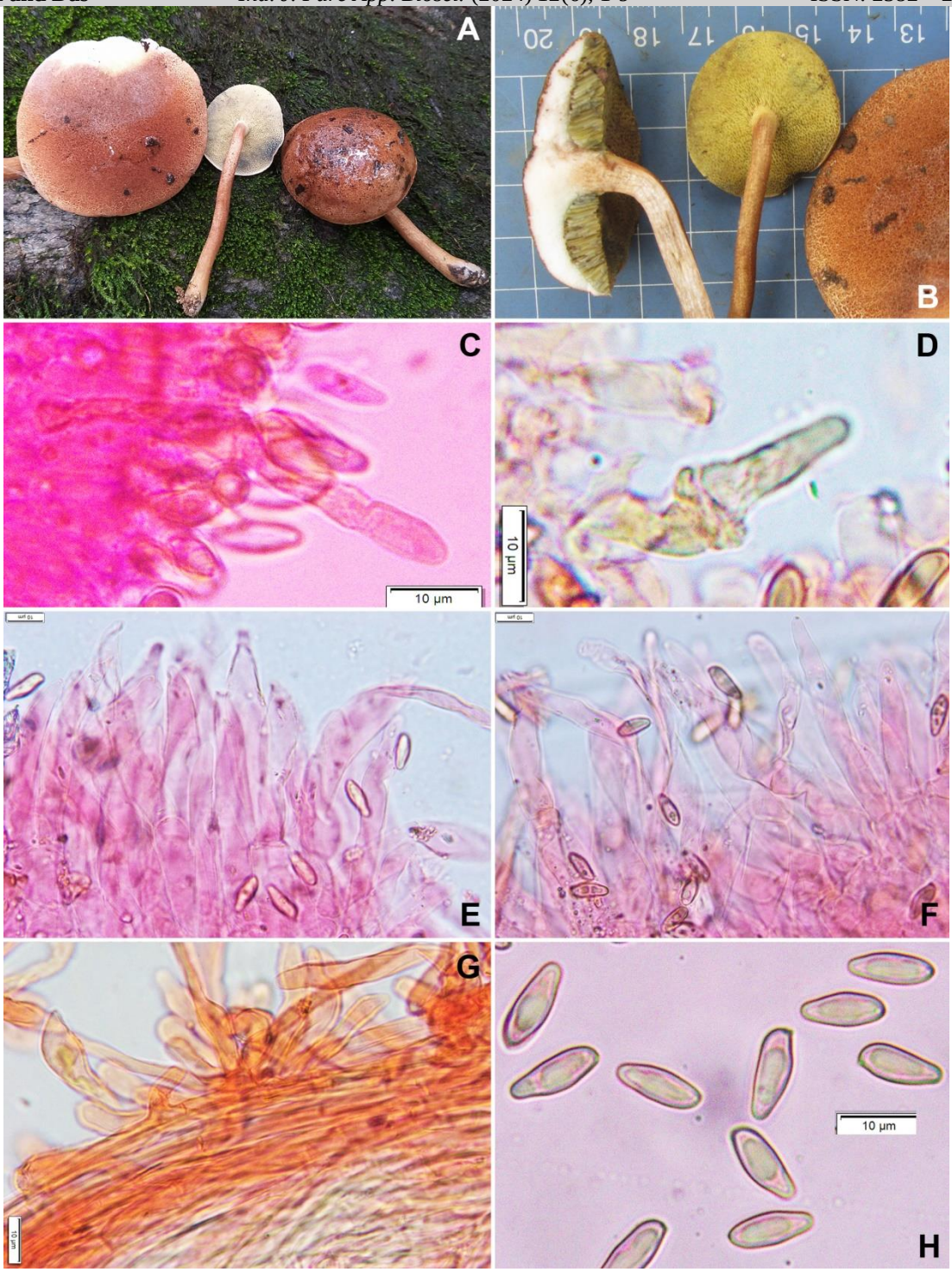
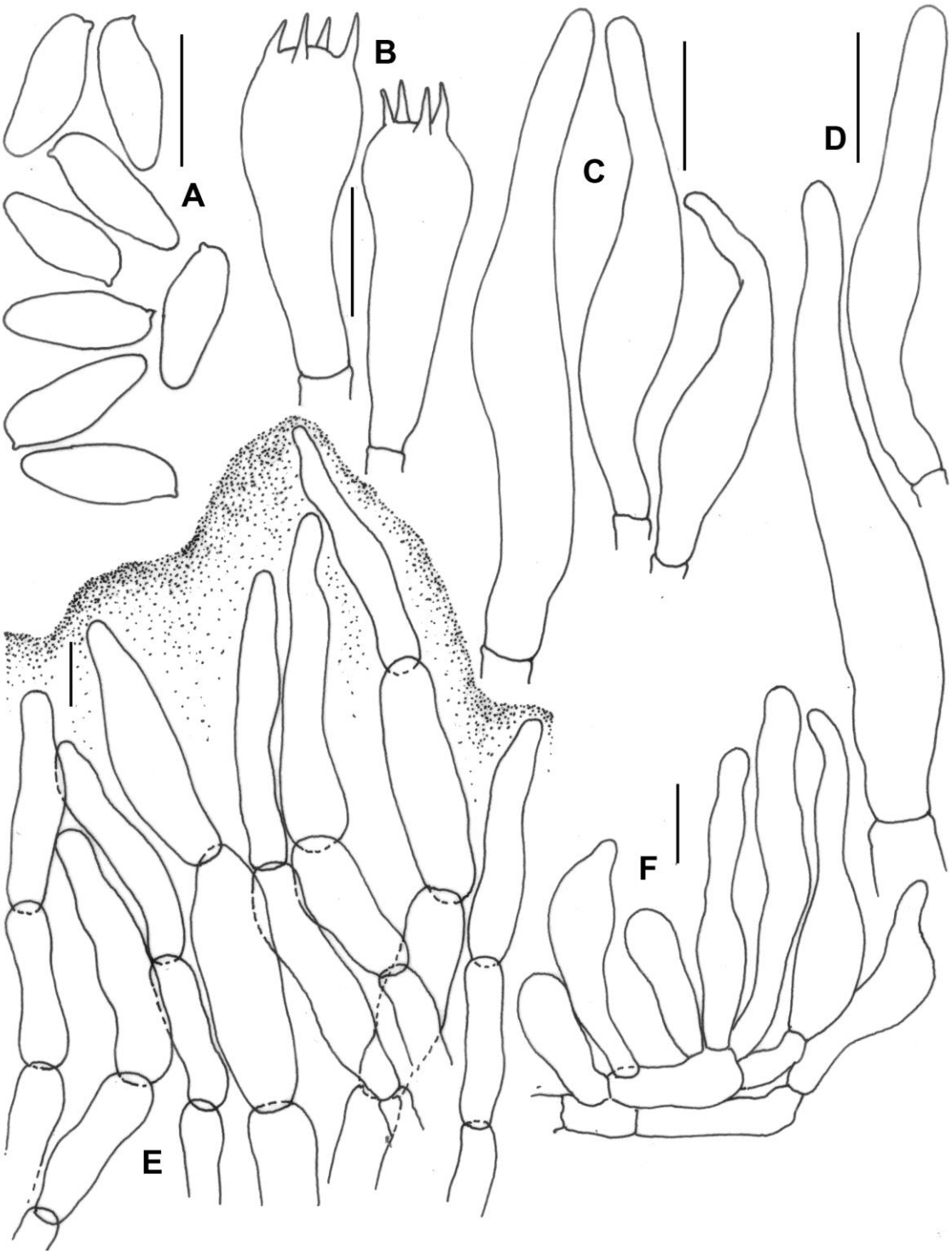


Fig. 2. Photoplate of *Imleria parva* (CAL 2123) A & B. Fresh and dissected basidiomata in the field and basecamp. C & D. Hymenial pleurocystidia. E & F. Transverse section through pileipellis showing its elements. G. Transverse section through stipitipellis showing its elements. H. Basidiospores. Scale bars: C–H = 10 µm.



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CONCLUSION

India harbors enormous diversity of wild mushrooms in general and boletes in particular. Unfortunately, most of this country's habitats (tropical to subalpine forests) are unexplored or seriously underexplored in terms of these mushrooms. Moreover, due to lack of trained manpower or boletologists diverse mycobiota of boletes are yet to be uncovered or identified. In the present backdrop, Botanical Survey of India has undertaken the work on survey, characterization, identification, discovery and documentation of these ecologically and

economically important group of wild mushrooms in project mode. It is anticipated that with the execution of this project treasure of Indian boletes will be unfolded with their distribution and correct identity.

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Conflict of Interest:

There is no such evidence of conflict of interest.

Author Contribution:

Both the authors have participated in writing the draft, critically revising the entire manuscript and approval of the final manuscript.

REFERENCES

Chakraborty, D., Parihar, A., Mehta, N., Baghela, A., & Das, K. (2017). A new species of Xerocomus (Boletaceae) from India. *Mycosphere 8*(1), 44–50.

Das, K., Chakraborty, D., Baghela, A., Singh, S.K., & Dentinger, B.T.M. (2016). New species of Xerocomoid Boletes (Boletaceae) from Himalayan India based on morphological and molecular evidence. *Mycol. 108*(4), 753–764.

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

Das, K., Ghosh, A., Chakraborty, D., Li, J., Qiu, L., Baghela, A., Halama, M., Hembrom, M.E., Mehmood, T., Parihar, A., Pencakowski, B., Bielecka, M., Reczyńska, K., Sasiela, D., Singh, U., Song, Y., Świerkosz, K., Szczęśniak, K., Uniyal, P., Zhang, J., & Buyck, B. (2017). *Cryptog. Mycol. 38*(3), 397.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Heled, J., Kearse, M., Moir, R., Stones, H.S., Sturrock, S., Thierer, T., & Wilson, A. (2010). *Geneious 5*(1), available from 485 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*, 373–377.

Farid, A., Franck, A. R., Bolin, J., & Garey, J. R. (2020). Expansion of the genus *Imleria* in North America to include *Imleria floridana*, sp. nov., and Imleria pallida, comb. nov. *Mycol.* DOI: 10.1080/00275514.2019.1685359

Gilbert, E.J. (1931). Les Bolets. Librairie le François, Paris, 256 pp.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analyses program for Windows 95/98/NT. *Nucleic Acids Symp. S. 41*, 95–98.

Katoh, K., Rozewicki, J., Yamada, K.D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform. 20*, 1160–1166.

Kornerup, A., & Wanscher, J.H. (1978). Methuen handbook of colour, 3rd Ed., reprinted. Eyre Methuen.

Kumar, S., Stecher, G., & Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis ver. 7.0 for bigger datasets. *Mol. Biol. Evol. 33*, 1870–1874.

Kuntze, O. (1898). Revisio Generum Plantarum. *3*, Felix, Leipzig, 576 pp.

Thiers, B. (2025) Index Herbariorum. A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. https://

sweet gum. nybg. org/ scien ce/ ih (Accessed 02 Jan 2025).

Vizzini, A. (2014). Imleria vizzini, gen. nov. *Index Fungorum*.

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

Wu, G., Li, Y-C., Zhu, X-T., Zhao, K., Han, L-H., Cui, Y-Y., Li, F ., Xu, J-P., & Yang, Z. L. (2016). One hundred noteworthy boletes from China. *Fungal Divers. 81*, 25–188.

Zhu, X., Li, Y., Wu, G., Feng, B., Zhao, K., Gelardi, M., Kost, G. W. & Yang, Z. L. (2014). The genus Imleria (Boletaceae) in East Asia. *Phytotaxa 191*(1), 81-98.