



# RESEARCH COMMUNICATION

# First report of *Caloboletus guanyui* (Boletaceae) in India: Taxonomic and phylogenetic insights from Mizoram

VL Thachunglura<sup>1\*</sup>, Prabhat Kumar Rai<sup>1</sup>, John Zothanzama<sup>2</sup>, Lallawmkima Bochung<sup>1</sup> & Zohmangaiha Chawngthu<sup>1</sup>

<sup>1</sup>Department of Environmental Science, School of Earth Science and Natural Resources Management, Mizoram University, Tanhril Aizawl 796004, India <sup>2</sup>Department of Biotechnology, School of Life Science, Mizoram University, Tanhril Aizawl 796004, India

\*Email: vlthachunglura@gmail.com



#### **ARTICLE HISTORY**

Received: 13 September 2024 Accepted: 03 November 2024

Available online

Version 1.0: 29 November 2024



#### **Additional information**

**Peer review:** Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

**Reprints & permissions information** is available at https://horizonepublishing.com/journals/index.php/PST/open\_access\_policy

**Publisher's Note**: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing\_abstracting

Copyright: © The Author(s). This is an openaccess article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (https://creativecommons.org/licenses/by/4.0/)

### **CITE THIS ARTICLE**

Thachunglura V L, Rai P K, Zothanzama J, Bochung L, Chawngthu Z. First report of *Caloboletus guanyui* (Boletaceae) in India: Taxonomic and phylogenetic insights from Mizoram . Plant Science Today (Early Access). https://doi.org/10.14719/pst.5046

#### **Abstract**

The family Boletaceae primarily consists of ectomycorrhizal fungi, which are essential for maintaining the health of forest ecosystems. These fungi form symbiotic relationships with trees, facilitating nutrient exchange and enhancing plant growth. In the present study, specimens of Caloboletus guanyui were collected from the Mizoram University campus during the rainy season of 2023. Identification was based on both morphological characteristics and molecular analysis of the ITS region of rDNA, with phylogenetic analysis further confirming the distinctiveness of Caloboletus guanyui from closely related species within the genus Caloboletus Vizzini. This mediumsized to large bolete is characterized by its dirty-white to pale-brown pileus and the basidiospores are elliptic-fusiform (spindle-shaped) to subfusiform  $9.5-11.5 \times 3.5-4.5 \mu m$ . This study represents the first recorded occurrence of Caloboletus quanyui in the tropical moist deciduous forests of Mizoram, India, thereby expanding the known distribution of this species. These new findings enhance our understanding of bolete diversity in India, particularly within the unique forest ecosystems of Mizoram.

# **Keywords**

Boletales; ectomycorrhizal fungi; macrofungi; phylogeny; taxonomy

# Introduction

Boletaceae Chevall., is a monophyletic group of fleshy, sequestrate, or pileate-stipitate macrofungi with a lamellate or tubular hymenophore (1). It has been widely studied worldwide, contributing valuable insights into their morphology, molecular phylogeny, and ecology. Advancements in molecular tools for studying Boletes have greatly increased the number of recognized genera in this family. Around 1,200 species and 100 genera of Boletaceae have been documented globally, with 65 genera proposed based on molecular evidence (2). However, due to their morphological complexity and the limited phylogenetic data on the numerous species and genera, our understanding of their systematics and evolution remains rather rudimentary (3). Macrofungi from the basidiomycete family Boletaceae Chevall., is a large, cosmopolitan family with abundant species and are ecologically and commercially significant. They are rich in nutrients and have potential health-promoting properties, making them valuable not only for their ecological roles but also as functional foods that can contribute to human health (4-6). They are integral components of the Indian ecosystem, contrib-

uting significantly to nutrient cycling and soil health through their roles as decomposers. Their ectomycorrhizal associations with various tree species enhance nutrient availability and plant resilience, thereby facilitating forest dynamics and promoting biodiversity (7). Currently, boletoid mushrooms comprising 96 species from 27 genera have been documented in India (8).

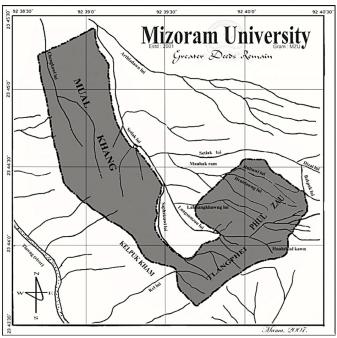
The genus Caloboletus Vizzini is characterized by yellow to olive-yellow tubes and occasionally orange to red pores that turn bluish when injured. It has a distinctly bitter taste due to specific chemical compounds, calopin and cyclocalopin (9). They are primarily found in subtropical and temperate zones within the Holarctic region and are commonly associated with plants from the Pinaceae and Fagaceae (10). The genus differs from all other genera of Boletaceae in its yellowish or reddish surface of the stipe, bluish color changed when bruised and pilleipellis composed of interwoven filamentous hyphae (11). Limited information is available on Caloboletus species in India. Caloboletus calopus (reported as Boletus calopus) and Caloboletus rubriceps (reported as Boletus rubripes) has been reported from Sikkim, India (12-13). This was further noted in a comprehensive checklist of 88 species within the Boletaceae family, which reported no additional Caloboletus species (14). Furthermore, based on the descriptions provided, Zhao et al. (10) suggested that the Indian specimen identified by Das (13) as B. rubripes likely corresponds to C. panniformis. The genus Caloboletus Vizzini display distinct geographical patterns as C. calopus and C. radicans remain confined to Europe, while C. firmus and C. inedulis are limited to North and Central America. In East Asia, several species, including C. panniformis, C. yunnanensis, C. taienus, and C. xiangtoushanensis, have been documented (9-10,15).

Mizoram is unique in many aspects such as rich floristic composition, high annual precipitation undulated topography and varying types of forest ecosystem which is complemented by a variety of macrofungi found throughout its different districts (16-18). More than 264 macrofungal species have been documented in Mizoram. Among these, the order Boletales includes two main families, Boletaceae (7 genera, 11 species) and Sclerodermataceae (2 genera, 3 species), making a total of 14 species within the order Boletales (19). Although macrofungi have been studied in particular areas over the past decades, there is still a lack of documented fungal species from Mizoram compared to other states in India. This highlights a knowledge gap, particularly regarding Caloboletus species in India, suggesting the potential for a wider distribution of this genus. In light of this, the present study describes and illustrates C. guanyui for the first time from the tropical moist deciduous forests of Mizoram University, Mizoram, India.

## **Materials and Methods**

Three specimens were collected during July-August 2023 from the campus of Mizoram University, Aizawl Mizoram, India, located between the coordinates 23° 45'25" N to

23° 43'37" N latitude and 92° 38'39" E to 92° 40'23" E longitude. The forested regions of the campus are characterized by porous, sandy loam, and humus-rich soils, which provide a fertile environment for vegetation. Mizoram University campus covers an area of approximately 980 acres (Fig. 1).



**Fig. 1.** Map of the Mizoram University campus [adapted from Lalchhuanawma (28)].

## Morphological studies

The collected specimens were maintained in paper bags for transport to the laboratory and identified using standard macroscopic (pileus, context, hymenophore, tubes, stipe, and odor) and microscopic (basidiospores, basidia, cystidia, pileipellis, and stipitipellis) characteristics in consultation with relevant literature (3,9-11,20). Photographs of the fruiting bodies were taken using Nikon-D7500. Location, date, temperature (°C), relative humidity (RH), elevation, coordinates and type of plants prevailing in the collection area of the specimen and their habits were documented. Color description was based on Kornerup and Wanscher (21). The macroscopic description, including shape, size, and color of the basidiocarps were documented from fresh sample in the fields. Microscopic characteristics were examined from both fresh and dried specimens using a light microscope (Carl ZEISS Axio Lab.A1) after sectioning and directly staining with 5% potassium hydroxide (KOH), Lactophenol cotton blue (LPCB) and Melzer's reagent (Hi-ARTM, India). Sections of the pileipellis were cut radially and perpendicularly halfway between the center and the margin of the pileus. For the stipitipellis, sections were taken from the middle part along the longitudinal axis of the stipe. Microscopic structures were drawn freehand to capture fine details observed under the microscope more precisely.

## DNA extraction, PCR and sequencing of the specimen

For molecular identification, following Zothanzama et al. (18) and reference therein, a small piece of tissue from the fruiting body of the specimen was added to a sterile 1.7 mL

micro centrifuge tube with glass beads and 500 µL of cetyltrimethylammonium bromide (CTAB) lysis buffer, then vortexed for 1 minute. After a brief centrifugation, the supernatant was transferred to a new tube and incubated at 65°C for 20 minutes. Following this, 500 μL of chloroform (Hi-ARTM, India) was added, mixed, and the mixture was centrifuged at 13,000 rpm for 5 minutes. The upper layer of the supernatant was transferred to another tube, and twothirds of the volume was mixed with isopropanol and stored at -20°C. After 5 minutes at room temperature, the mixture was centrifuged at 15,000 rpm for 7 minutes. The supernatant was removed, and the pellet was washed with 500 μL of 70% ethanol (Hi-ARTM, India), and then centrifuged at 15,000 rpm for 3 minutes. The ethanol was evaporated, and the DNA pellet was re-suspended in 100 µL of sterile water. PCR reactions were set up in 0.2 mL tubes with 12.5 μL GoTaq Green Mastermix (Promega), 9.5 μL nuclease-free water, 1 μL forward primer (5 μM), 1 μL reverse primer (5 μM), and 1 μL fungal DNA template, making up the volume up to 25.5 μL. PCR was performed with primers ITS1-F and ITS4-R using the following conditions: 94°C for 5 minutes, followed by 35 cycles of 94°C for 1 minute, 52°C for 1 minute, and 72°C for 1 minute, with a final extension at 72°C. PCR products were checked by electrophoresis on a 1% agarose gel with SYBR green and visualized using a Gel Documentation System. Sequencing was done using Sanger sequencing with both primers. Consensus sequences were trimmed and aligned using BioEdit and compared to GenBank entries using BLASTn. The aligned sequences were analyzed with Clustal W.

# Phylogenetic analyses

To construct the phylogeny of major lineages, representative taxa from the primary species were selected. Model testing and Maximum Likelihood (ML) phylogenetic analyses were performed using RAXMLGUI 2.0 (22), with recommended parameters to determine the best tree topology and bootstrap support values from 1000 search replicates, which were summarized in FigTree. Model testing was conducted using the inbuilt ModelTest-NG program (23). GTR+I+G was selected and found to be the optimal substitution model based on the corrected Akaike Information Criterion (24).

# **Results and Discussion**

The description of the specimen, including macroscopic and microscopic characteristics, habitat, known distribution, an illustration of the fruiting bodies and a phylogenetic tree of the specimen with related species obtained using the Maximum Likelihood method based on the ITS region is presented.

Caloboletus guanyui N.K. Zeng, H. Chai & S. Jiang

#### **Description of the specimen**

Macroscopic characters: Pileus 5.5–11 cm diam., convex to nearly flat; surface dry, finely tomentose, white to pale brown. Context 0.6–2 cm thick, white, bruising bluish when injured, then back to white. Hymenophore poroid, depressed around apex of stipe; pores subround, 0.3–0.6 mm

diam., reddish brown, then yellowish brown, bruising bluish black when injured; tubes 0.6-1.2 cm, yellowish then changing to bluish when injured. Stipe 5.5–9.5 x 0.8–1.6 cm, central, subcylindrical, solid, surface dry, covered with pale brown to reddish brown, minute squamules; context white; with white basal mycelium, Odor indistinct. Microscopic characters: Basidiospores 9.5-11.5 × 3.5-4.5 µm (n=30), elliptic-fusiform (spindle-shaped) to subfusiform, yellowish brown in KOH, smooth. Basidia 20–32 × 6.5–8.5 µm (n=30), clavate, thin-walled, yellowish in KOH; 4-spored, sterigmata 3.2-4 µm. Cheilocystidia 26-43 × 7-10 µm, fusiform, thin-walled, yellowish in KOH. Pleurocystidia 34-45 × 6-11.5 µm, fusiform, thin-walled, colorless to yellowish in KOH. Pileipellis a trichoderm about 100-200 µm thick, composed of slightly interwoven, 5-8.5 µm wide, thinwalled hyphae; terminal cells 26-35 × 5.5-10.5 µm, clavate or subclavate, with obtuse apex. Stipitipellis hymeniform, 80–100 µm, composed of thin-walled emergent hyphae, clavate, subclavate, fusiform, four-spored basidia. Clamp connections absent.

#### Habitat

Gregarious on the ground in tropical moist deciduous forests dominated by *Aporusa octandra* (Buch.-Ham. ex D.Don) A.R.Vickery, *Castanopsis tribuloides* (Sm.) A.DC., and *Saurauia punduna* Wall.; during summer and fall, in monsoon season.

# **Distribution and Ecology**

Japan (25), southeastern and southern China (20), and India (present report). This species grows on soil, typically in habitats with a mixed canopy of both understory and canopy trees, alongside fallen tree trunks and a layer of leaf litter covering the ground.

# **Specimen examined**

India, Mizoram, Aizawl district, Mizoram University campus, on soil, elevation 848 m (2782.15 ft), 26 July 2023 (24.3°C and 87.1% RH), 23°44'21.8"N 92°39'58.7"E, Thachunglura VL and Zohmangaiha C, MZU/JZT-VL/2022/036 (Fig. 2).

## **Notes**

Phylogenetic tree of *C. guanyui* and related species obtained with Maximum Likelihood method based on the ITS region. Numbers below the branches are bootstrap percentage values based on 1000 replicates, ML/MP bootstrap support values greater than 50% (Fig. 3). *Fomitopsis eucalypticola* was used as outgroup. The sequence of our fungal isolate *C. guanyui* from Mizoram, India was compared to 36 corresponding sequences of reference fungal taxa in the database and the list of species, voucher number, Gen-Bank accession number and locality used for the analysis is given in Table 1.

In the phylogenetic analysis, our species *C. guanyui* (PQ288749) is clustered within the *C. guanyui* clade, which is highlighted in blue colour. This clade also includes sequences of *C. guanyui* submitted by Chai et al. (20) such as MH885364, MH885365, and MH885366, indicating a close phylogenetic relationship among them. Additionally, this

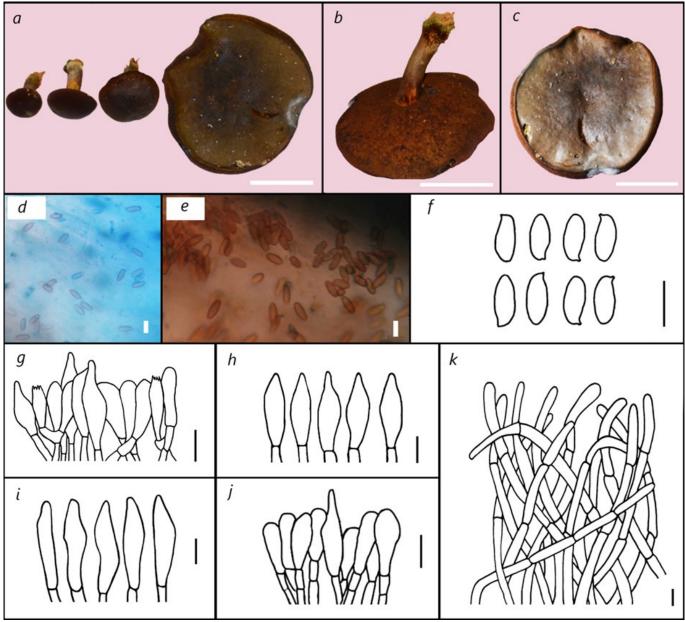


Fig. 2. Caloboletus guanyui . a-c: Basidiomata close-ups , d-f: Basidiospores, g: Basidia and Pleurocystidia, h: Cheilocystidia, i: Pleurocystidia, j: Stipitipellis and k: Pileipellis. (Scale bars: a-c: 5cm, d-k: 10 μm).

clade is distinct from other *Caloboletus* species (*C. calopus* and *C. panniformis*) which occupy separate branches in the phylogeny. The bootstrap support and clear clustering further confirm the genetic affinity of PQ288749 within the *C. guanyui* clade. Although some species, such as *C. griseoflavus*, *C. taienus*, *C. xiangtoushanensis* and *Neoboletus infuscatus* share several common morphological characteristics with our specimen (*C. guanyui*) such as the size of the pileus, pores, stipe, basidiospores, and basidia. Therefore, their distinct morphological features are compared in Table 2.

C. guanyui, characterized by medium to large basidiomata, was initially described by Hongo (25) under the name Boletus quercinus. However, this name was invalid because Schrader (26) had already used it for a different species. To resolve this, Chai et al. (20) proposed C. guanyui as the correct name for this species. Morphologically, C. taienus and C. xiangtoushanensis were also characterized by a reddish pore. However, C. guanyui can be easily distinguished from these two species by the colour of its pile-

us, which is characterized by dirty-white to pale-brown (20). Moreover, the pileus of *C. guanyui* is larger than that of both *C. taienus* and *C. xiangtoushanensis*. It is known that *C. guanyui* can be distinguished from *N. infuscatus* by its smaller basidiomata, the absence of a pruina layer on the pileus and stipe surfaces, a white context, a stipe densely covered with pale brown to reddish-brown squamules, and a stipe context that remains unchanged in color when injured (27). Moreover, it can be differentiated from *C. griseoflavus* by its stipe densely covered with pale brown to reddish-brown squamules and its smaller basidiospores.

Sequencing of the ITS region of rRNA and phylogenetic analysis further showed that the Mizoram sample matched GenBank accession *C. guanyui* from China (MH885366, MH885365 and MH885364) in a well-supported clade. Morphological evidence, molecular phylogenetic analyses, and ecological data collectively confirm that the specimen of *Caloboletus* from Mizoram is *C. guanyui*, a distinct species and separate from *C. griseoflavus*, *C.* 

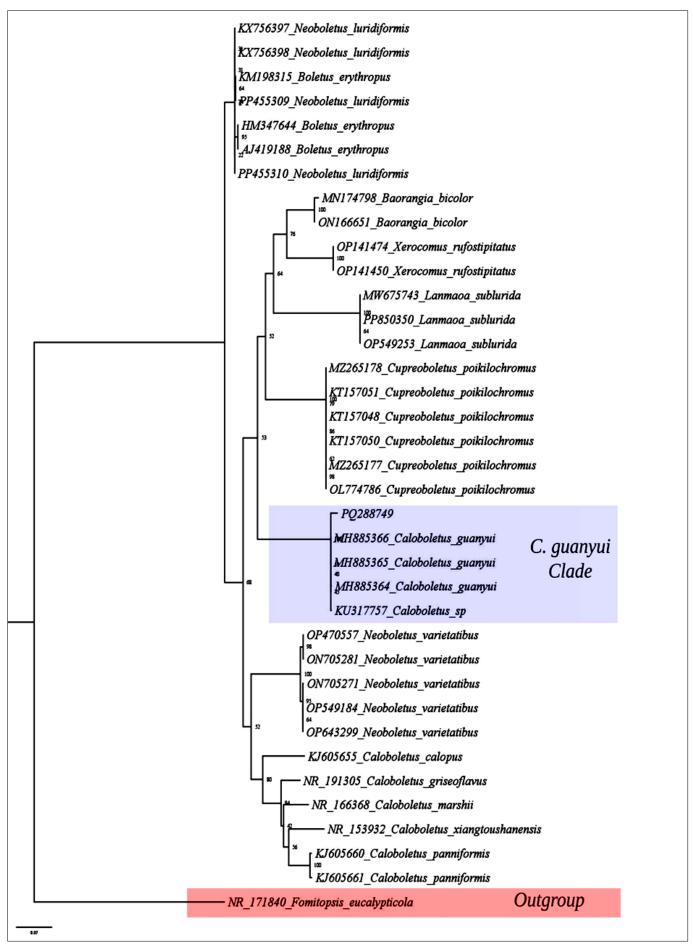


Fig. 3. Phylogenetic tree of Caloboletus guanyui and other related taxa.

taienus, C. xiangtoushanensis and N. infuscatus. Our collection broadens the known distribution of C. guanyui to India, aligning it with its presence in East Asian countries

such as Japan and China. This suggests that similar environmental factors may be promoting its spread across various regions. Documenting this occurrence in India not

 $\textbf{Table 1}. \ List of species, voucher, GenBank accession nos. and locality.$ 

No.	Voucher	Voucher Accession No. Species Nam		Locality
1	WA0000052272	KX756397	Neoboletus luridiformis	Poland
2	WA0000052289	KX756398	Neoboletus luridiformis	Poland
3	WU 36064	KM198315	Boletus erythropus	Austria
4	U3758	PP455309	Neoboletus luridiformis	Sweden
5	UF278	HM347644	Boletus erythropus	Portugal
6	-	AJ419188	Boletus erythropus	Spain
7	U3759	PP455310	Neoboletus luridiformis	Sweden
8	JLF6843	MN174798	Baorangia bicolor	USA
9	iNAT:15437268	ON166651	Baorangia bicolor	USA
10	PDD 101779	OP141474	Xerocomus rufostipitatus	New Zealand
11	JAC16762	OP141450	Xerocomus rufostipitatus	New Zealand
12	Farid_631	MW675743	Lanmaoa sublurida	USA
13	OMDL K. Canan iNaturalist # 131374134	PP850350	Lanmaoa sublurida	USA
14	S.D. Russell ONT iNaturalist 127411460	OP549253	Lanmaoa sublurida	USA
15	TUR-A 208927	MZ265178	Cupreoboletus poikilochromus	Italy
16	GS 10070	KT157051	Cupreoboletus poikilochromus	Italy
17	MG 271a	KT157048	Cupreoboletus poikilochromus	Italy
18	GS 11008	KT157050	Cupreoboletus poikilochromus	Italy
19	TUR-A 208926	MZ265177	Cupreoboletus poikilochromus	Italy
20	SOMF30350	OL774786	Cupreoboletus poikilochromus	Bulgaria
21	N.K.Zeng3079	MH885366	Caloboletus guanyui	China
22	N.K.Zeng3058	MH885365	Caloboletus guanyui	China
23	N.K.Zeng3263	MH885364	Caloboletus guanyui	China
24	HKAS74864	KU317757	Caloboletus sp.	China
25	S.D. Russell ONT iNaturalist # 129764007	OP470557	Neoboletus varietatibus	USA
26	#374573	ON705281	Neoboletus varietatibus	USA
27	#249632	ON705271	Neoboletus varietatibus	USA
28	S.D. Russell ONT iNaturalist 129866347	OP549184	Neoboletus varietatibus	USA
29	S.D. Russell ONT iNaturalist # 129907651	OP643299	Neoboletus varietatibus	USA
30	BR5020159063805	KJ605655	Caloboletus calopus	Belgium
31	BJTC FM2438	NR_191305	Caloboletus griseoflavus	China
32	SFSU Arora11118	NR_166368	Caloboletus marshii	USA
33	GDGM 44725	NR_153932	Caloboletus xiangtoushanensis	China
34	HKAS77506	KJ605660	Caloboletus panniformis	China
35	HKAS77530	KJ605661	Caloboletus panniformis	China
36	BJFC:029897	NR_171840	Fomitopsis eucalypticola	Australia

**Table 2.** Comparative morphological analysis of related *Caloboletus* species.

Species	Pileus	Pores (mm)	Stipe (cm)	Basidiospores (µm)	Basidia (µm)
Caloboletus griseoflavus	3–7.5 cm	Angular or subround; up to 0.5 mm	2.6-6 × 0.8-2 cm	12–15 × 4.5–5.5 μm	32-36 × 9-11 µm
Caloboletus guanyui	5.5–11 cm	Subround; 0.3–0.6 mm	5.5-9.5 × 0.8-1.6 cm	9.5-11.5 × 3.5-4.5 µm	20-32 × 6.5-8.5 μm
Caloboletus taienus	5–7 cm	Angular; 1–3 mm	6-8 × 1.5-2 cm	8-9 × 3-4 µm	25-31× 7-10 µm
Caloboletus xiangtoushanensis	4–9 cm	Angular; 2–3 mm	3-8 × 1-1.5 cm	9–13 × 4–5 µm	25-33× 7-10 µm
Neoboletus infuscatus	6–16 cm	Angular; 2 mm	9–11 × 1.8–2.5 cm	8.5-10.5× 3.5-4.5 µm	31.5-39.5 × 9.5-2.5 µm

only enhances our understanding of *C. guanyui* ecological preferences but also highlights the significance of India diverse forest ecosystems in supporting distinct and varied macrofungi.

# Conclusion

Our study indicates that the geographic distribution of *C. guanyui* now extends into India, showcasing its resili-

ence to a wide range of environmental circumstances. Characterized by medium-sized to large basidiomata, C. quanyui has been identified through morpho-molecular analysis, enriching the fungal diversity of the family Boletaceae in India and representing an especially significant addition to Mizoram. While Mizoram is known for its rich floral diversity, there remains limited information about its wild mushrooms, emphasizing the need for further studies to assess and document the broad range of mushrooms in this region. This situation emphasizes the critical need for comprehensive research initiatives to explore the full potential of these fungi. Such efforts could yield valuable insights into their ecological significance and possible contributions to health, industrial applications, and biotechnological innovations. By investigating the various characteristics and functions of wild mushrooms, we can deepen our understanding of their roles in ecosystems and the potential benefits they offer across different sectors.

# **Acknowledgements**

The authors thank the Department of Environmental Science and Department of Biotechnology, Mizoram University for their invaluable assistance and support. The authors express sincere gratitude to the anonymous reviewers of Plant Science Today for their valuable comments that improved the quality of the paper.

## **Authors' contributions**

VLT, LB, and ZC collected the samples, performed the morpho-molecular studies, contributed to the sequence alignment, constructing phylogenetic tree and prepared the manuscript draft. PKR and JZT assisted in the study design and manuscript review. All authors reviewed and approved the final manuscript.

# **Compliance with ethical standards**

**Conflict of interest**: Authors do not have any conflict of interests to declare.

Ethical issues: None.

# **Al Declaration**

During the preparation of this work, the author(s) used Artificial Intelligence [Curie] for language editing. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

## References

- Patil B, Gunasekaran P, Singh SK, Vaidya S. *Parvixerocomus matheranensis* (Boletaceae), a new species from India. Mycoscience. 2021;62(4):244-49. https://doi.org/10.47371/mycosci.2021.03.007
- Wu G, Li HJ, Horak E, Wu K, Li GM, Yang ZL. New taxa of Boletaceae from China. Mycosphere. 2023;14(1):45-76. https:// doi.org/10.5943/mycosphere/14/1/9

- Wu G, Feng B, Xu J, Zhu XT, Li YC, Zeng NK, et al. Molecular phylogenetic analyses redefine seven major clades and reveal 22 new generic clades in the fungal family Boletaceae. Fungal Diversity. 2014;69(1):93-115. https://doi.org/10.1007/s13225-014-0283-8
- Jaworska G, Pogoń K, Skrzypczak A, Bernaś E. Composition and antioxidant properties of wild mushrooms *Boletus edulis* and *Xerocomus badius* prepared for consumption. Journal of Food Science and Technology. 2015;52(12);7944-53. https:// doi.org/10.1007/s13197-015-1933-x
- Liu Y, Chen D, You Y, Zeng S, Li Y, Tang Q, et al. Nutritional composition of *Boletus* mushrooms from Southwest China and their antihyperglycemic and antioxidant activities. Food Chemistry. 2016;211:83-91.
  - https://doi.org/10.1016/j.foodchem.2016.05.032
- Tan Y, Zeng N-K, Xu B. Chemical profiles and health-promoting effects of porcini mushroom (*Boletus edulis*): A narrative review. Food Chemistry. 2022;390:133199. https://doi.org/10.1016/ j.foodchem.2022.133199
- Das K, Ghosh A, Datta S, Singh U, Chakraborty D, Tudu D, et al. Concordance of multigene genealogy along with morphological evidence unveils five novel species and two new records of boletoid mushrooms (fungi) from India. Scientific Reports. 2024;14:9298. https://doi.org/10.1038/s41598-024-59781-2
- Das K, Ghosh A, Chakraborty D, Datta S, Bera I, Layola MR, et al. Four novel species and two new records of boletes from India. Journal of Fungi. 2023;9(7):754. https://doi.org/10.3390/jof9070754
- 9. Vizzini A. Nomenclatural novelties. Index Fungorum. 2014;146:1-
- Zhao K, Wu G, Feng B, Yang ZL. Molecular phylogeny of Caloboletus (Boletaceae) and a new species in East Asia. Mycological Progress. 2014;13(4):1127-36. https://doi.org/10.1007/s11557-014-1001-3
- Wu G, Li Y-C, Zhu X-T, Zhao K, Han L-H, Cui Y-Y, et al. One hundred noteworthy boletes from China. Fungal Diversity. 2016;81 (1):25-188. https://doi.org/10.1007/s13225-016-0375-8
- Das K. Diversity and conservation of wild mushrooms in Sikkim with special reference to Barsey Rhododendron Sanctuary. NeBio. 2010;1:1-13.
- 13. Das K. *Boletus rubripes* Thiers, a new record of wild mushroom from Sikkim (India). Taiwania. 2013;58(2):136-39. https://doi.org/10.6165/tai.2013.58.136
- Verma RK, Pandro V. Distribution of Boleteceous mushrooms in India, Some new records from Sal forest of Central India. International Journal of Current Microbiology and Applied Sciences. 2018;7(06):1694-713.
  - https://doi.org/10.20546/ijcmas.2018.706.201
- 15. Zhang M, Li TH, Gelardi M, Song B, Zhong XJ. A new species and a new combination of *Caloboletus* from China. Phytotaxa. 2017;309(2):118. https://doi.org/10.11646/phytotaxa.309.2.2
- Thachunglura VL, Rai PK, Chawngthu Z, Renthlei L, Vanlalmalsawmi R, Bochung L, et al. Nutritional composition and mineral contents of common edible wild mushrooms from Mamit and Champhai districts of Mizoram, India. Food Agricultural Sciences and Technology. 2024;10(1):42-58.
- 17. Chawngthu Z, Thachunglura VL, Zothanzama J, Khumlianlal J. Risk assessment of heavy metals in wild mushroom from Mizoram, India. Environmental Pollution and Management. 2024. https://doi.org/10.1016/j.epm.2024.09.001
- Zothanzama J, Blanchette AR, Lalrinawmi H. Identification of the edible and poisonous mushrooms of Mizoram. Government of Mizoram. 2018.

 Thachunglura VL, Rai PK, Khumlianlal J, Zothanzama J. A checklist of wild mushrooms in Mizoram, Northeast India. Plant Pathology and Quarantine. 2024;14(1):125-42. https:// doi.org/10.5943/ppq/14/1/11

- Chai H, Liang ZQ, Xue R, Jiang S, Luo SH, Wang Y, et al. New and noteworthy boletes from subtropical and tropical China. Myco-Keys.2019;46:55-96. https://doi.org/10.3897/mycokeys.46.31470
- 21. Kornerup A, Wanscher J. Metheun hand book of colour. 3rd ed. Metheun London Ltd: London. 1978;144-48.
- Edler D, Klein J, Antonelli A, Silvestro D. raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML.
   Methods in Ecology and Evolution. 2020;12:373-77. https://doi.org/10.1111/2041-210x.13512
- Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T. ModelTest-NG: A new and scalable tool for the selection of DNA

- and protein evolutionary models. Molecular Biology and Evolution. 2020;37:291-94. https://doi.org/10.1093/molbev/msz189
- 24. Burnham KP, Anderson DR. Model selection and multimodel inference: A practical information-theoretic approach. 2nd ed. New York.: Springer; 2002.
- 25. Hongo T. Notulae mycologicae (6). Memoirs of Shiga University Press. 1967;17:89-95.
- 26. Schrader HA. Spicilegium Florae Germanicae. 1794;1-194
- 27. Jiang S, Mi HX, Xie HJ, Zhang X, Chen Y, Liang ZQ, et al. *Neoboletus infuscatus*, a new tropical bolete from Hainan, southern China. Mycoscience. 2021;62(3):205-11. https://doi.org/10.47371/mycosci.2021.03.001
- Lalchhuanawma. Ecological studies on plant diversity and productivity of herbaceous species in Mizoram University campus at Tanhril, Aizawl, Mizoram (N.E. India). Doctoral Thesis. Mizoram University. 2008;33.