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Journal of Threatened Taxa

40 **zoo**reach
Zoo Outreach Organisation
Years

10.11609/jott.2025.17.4.26763-26938

www.threatenedtaxa.org

26 April 2025 (Online & Print)

17(4): 26763-26938

ISSN 0974-7907 (Online)

ISSN 0974-7893 (Print)



Open Access





ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

Publisher

Wildlife Information Liaison Development Society

www.wild.zooreach.org

Host

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www.zooreach.org

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Cover: Nilgiri Large Burrowing Spider *Haploclostus nilgirinus*. Acrylic on canvas. © Aakanksha Komanduri.



Extended distribution of the rare basidiolichen *Sulzbacheromyces yunnanensis* (Lichenized Basidiomycota) from Mizoram, India

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Abstract: Basidiolichens are rare and understudied in India due to their confinement to specialized niches. *Sulzbacheromyces yunnanensis* D.Liu, Li S.Wang & Goffinet, a clavarioid basidiolichen is described herein for its expanded distribution from Mizoram, India based on morpho-anatomical characteristics and ITS sequence based phylogenetic analysis. A detailed account of the habitat preference and comparison with related taxa is also provided.

Keywords: *Crustose thallus*, diversity, Indo-Burma hotspot, Lepidostromataceae, lichen, morpho-molecular identification, new distribution, photobiont, soil habitat, taxonomy.

Editor: Avneet Pal Singh, Punjabi University, Patiala, India.

Date of publication: 26 April 2025 (online & print)

Citation: Thachunglura, V.L., P.K. Rai, Z. Chawngthu, L. Bochung, P.C. Vanlalhluna & J. Zothanzama (2025). Extended distribution of the rare basidiolichen *Sulzbacheromyces yunnanensis* (Lichenized Basidiomycota) from Mizoram, India. *Journal of Threatened Taxa* 17(4): 26887–26892. <https://doi.org/10.11609/jott.9399.17.4.26887-26892>

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Funding: MoTT UGC-NFST.

Competing interests: The authors declare no competing interests.

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Author contributions: VLT, ZC, and LB collected the samples, carried out the identification, and conducted the phylogenetic analysis. JZT and PKR supervised the research and provided support in methodology and study design. VLT, PKR, PCV and JZT participated in writing, reviewing, and editing the manuscript.

Acknowledgements: The authors would like to thank the Ministry of Tribal Affairs, Government of India, for providing financial assistance in the form of the National Fellowship for Scheduled Tribes (NFST) (202021-NFST-MIZ-03084) to Mr. VL Thachunglura, research scholar - Department of Environmental Science, Mizoram University. We are grateful to DBT-NER (No. DBTNER/AAB/64/2017; dated 14.10.2019) for providing the essential laboratory facilities crucial for the successful completion of this study.



INTRODUCTION

The vast majority of lichenized fungi are classified within the phylum Ascomycota, while several smaller clades containing lichenized species are also present in Basidiomycota (Hodkinson et al. 2012). Basidiolichens exhibit a variety of growth forms, including crustose, squamulose (scale-like), foliose, or coral-like structures. Some resemble mushrooms with decurrent gills, while others appear as bracket fungi or crust-like resupinate basidiomata (Ertz et al. 2008). They were once considered to have a relatively small number of species, but recent studies have broadened our understanding of their diversity, with new species being discovered in various regions, particularly in the Neotropics (Lücking et al. 2014; Sulzbacher et al. 2016). They can be found in cosmopolitan to subcosmopolitan ranges as well as in regional to continental distributions (Oberwinkler 2012). Basidiolichens are comparatively understudied due to their confinement to specific ecological niches (Gasulla et al. 2020).

Among different families of the basidiolichens, Lepidostromataceae comprises three major genera, viz., *Lepidostroma* Mägd. & S.Winkl., having a squamulose thallus with medulla, *Ertzia* B.P.Hodk. & Lücking, with a microsquamulose thallus without medulla, and *Sulzbacheromyces* B.P.Hodk. & Lücking being distinguished by a crustose and membranaceous thallus (Hodkinson et al. 2014; Liu et al. 2019). *Sulzbacheromyces* is a pantropical genus of basidiolichens with 10 recognized species distributed across Africa, South America, and Asia: *S. arunachalensis* A.Debnath & Nayaka; *S. bicolor* Dong Liu, Li S.Wang & Goffinet; *S. caatingae* (Sulzbacher & Lücking) B.P.Hodk. & Lücking; *S. chocoensis* Coca, Lücking & B.Moncada; *S. fossicolus* (Corner) D.Liu & Li S.Wang; *S. leucodontius* Coca, Gómez-Gómez, Guzmán-Guillermo & Dal Forno; *S. miomboensis* DeKesel & Ertz; *S. sinensis* (R.H. Petersen & M. Zang) D.Liu & Li S.Wang; *S. tutunendo* Coca, Lücking & B.Moncada; and *S. yunnanensis* D.Liu, Li S.Wang & Goffinet (Liu et al. 2019; Coca et al. 2023; Debnath et al. 2025).

Initially, there were few reports of the genus *Sulzbacheromyces* from Asia, with *S. sinensis* found to have a broad distribution across Korea, China, Japan, Taiwan, Singapore, and Philippines. In contrast, other species, such as *S. bicolor*, *S. yunnanensis*, and *S. fossicolus* have much narrower distributions (Liu et al. 2019; Coca et al. 2023). The studies on the genus *Sulzbacheromyces* from India are limited. *S. fossicolus* (= *Multiclavula fossicola*) was reported from India by Petersen & Zang (1986). More recently, *S. arunachalensis*

has been described as a new species from India, while *S. bicolor* and *S. yunnanensis* have been reported as new records. Mizoram, a small state in northeastern India, is well-known for its rich biodiversity (Zothanzama et al. 2016; Chawngthu et al. 2024), and the Hlimen forest is no exception to this. However, there are only a few reports of fungi from the state (Lalrinawmi et al. 2018; Thachunglura et al. 2024). Therefore, to address this gap, the present studies were taken up and a rare species of basidiolichen *Sulzbacheromyces yunnanensis* is being described from Mizoram, India based on morphological characteristics and molecular phylogenetic analyses.

MATERIALS AND METHODS

Collection site

Hlimen Forest is located approximately 7 km south of Aizawl, the capital of Mizoram, between 23.6824 °N and 92.7164 °E. The landscape is hilly, with altitude ranging 1,100–1,200 m. The region is primarily composed of tertiary rocks from the Bhuban sub-group. The region experiences an average temperature of 26.1 °C and provides ideal conditions for the growth of fungi. The Hlimen Forest is highly diverse and plays a crucial role in maintaining ecological stability. The common tree species in Hlimen Forest include *Aporosa octandra*, *Lithocarpus xylocarpa*, *Macaranga indica*, *Callicarpa arborea*, *Albizia chinensis*, *Anogeissus acuminata*, *Archidendron monadelphum*, *Emblica officinalis*, *Lithocarpus elegans*, *Litsea monopetala*, and *Morus macrourea*.

Morphological observation, DNA extraction, PCR, and sequencing

Basidiomata were collected from Hlimen Forest, Aizawl, Mizoram and were initially cleaned from forest debris and identified using standard mycotaxonomical methods and authenticated by referring Liu et al. (2017) and Suwannarach et al. (2019). The colour description was based on Kornerup & Wanscher (1978). The microscopic characteristics of the specimens were recorded using the compound microscope (Carl ZEISS Axio Lab.A1). For microscopic observations, sections of basidioma were mounted in Melzer's reagent, 5% (w/v) potassium hydroxide (KOH) solution after staining with 1% (w/v) Congo red solution. For molecular analysis following Zothanzama et al. (2016) and reference therein, DNA was extracted using the Cetyltrimethylammonium bromide (CTAB) method, amplified with polymerase chain reaction (PCR) using internal transcribed spacer (ITS1 and ITS4) primers, and sequenced using Sanger sequencing.

Phylogenetic analysis

To construct phylogeny of major lineages, representative taxa of members from the major species were chosen. Model testing and maximum likelihood (ML) phylogenetic analyses were conducted in RAxMLGUI 2.0 (Edler et al. 2020) with the recommended parameters to determine the best tree topology and bootstrap support values from 1,000 search replicates, which are summarized in the phylogenetic tree. Model testing is carried out using the inbuilt program ModelTest-NG (Darriba et al. 2020) to select the best substitution model based on the corrected Akaike information criterion (AIC; Burnham & Anderson 2002).

RESULTS AND DISCUSSION

Taxonomy

Sulzbacheromyces yunnanensis D.Liu, Li S.Wang & Goffinet

Image 1

Description: Thallus crustose, covering an area of 0.5–45 mm diameter, distinct, dark green in shaded areas or depressions, yellow-green, indistinguishable from soil on exposed ground; forming a thin layer on the substrate, containing clusters of single-celled chlorococcoid algae, without prothallus. Photobiont 4–9.5 µm in diameter, globose to sub globose, smooth, contiguous, surrounded by a single layer of hyaline hyphae. Basidioma solid, clavarioid, fusiform, simple, with two conspicuous, wide, longitudinal depressions or grooves, never circled by transverse cracks at maturity, apex obtuse to narrowly obtuse to truncate, 24–60 × 0.5–2.5 mm, with hymenium covering the upper part, surface pruinose, without tomentum at the base, orange or yellow when exposed to direct sunshine, base dark ochraceous, ochraceous upon drying. Tramal hyphae 2–7.5 µm in diameter, parallel, clamped, slightly thick-walled, with bulbous apex. Hymenium 70–80 µm thick. Basidia 50–75 × 5–6.5 µm, oblong when young, subclavate to clavate with age, thin-walled, hyaline, 4-spored, with basal clamp connections, fragile sterigmata 4.5–5 µm long. Basidiospores 8.3–12.5 × 3.5–5.8 µm (n = 50), Q = 2.0–2.4, ellipsoid to slightly reniform, thin-walled, hyaline, smooth, hilar appendix present, guttulate or not.

Specimens examined—India, Mizoram, Aizawl district, Hlimen, on soil, elev. 1,128 m, 24 May 2022, Thachunglura VL, Chawngthu Z & Bochung L, MZU/JZT-VL/2022/004; MZU/JZT-VL/2022/006, GenBank PQ222572 & PQ222573.

Distribution and Ecology: China (Liu et al. 2017), Thailand (Suwannarach et al. 2019), India – Arunachal Pradesh (Debnath et al. 2025), Mizoram – Hlimen, Aizawl District (Present study). *Sulzbacheromyces yunnanensis* grows on soil near an informal path, in an area characterized by thick sandstones interbedded with thin shale. It thrives in this environment alongside *Chromolaena odorata*, indicating its preference for exposed substrates and moderate disturbance.

Remarks: Phylogenetic analysis was conducted using ITS sequence data from the collected specimens, along with 42 strains obtained through BLAST search (NCBI) and recent publications. *Ganoderma enigmaticum* (NR_132918) was selected as the outgroup and the best-scoring RA × ML tree is presented in Figure 1. The tree topologies derived from ML and maximum parsimony (MP) methods were consistent with earlier investigations (Liu et al. 2017). *Sulzbacheromyces* species were segregated into seven clades, grouping alongside *Leptosporomyces* and *Lepidostroma* in the phylogenetic tree. Our sequences (PQ222572 and PQ222573) formed an independent clade, representing a distinct lineage when compared with other *S. yunnanensis* specimens, and clustered together with 99% ML and MP support.

The identified species *S. yunnanensis* closely resembles other species within *Sulzbacheromyces*, such as *S. caatingae*, *S. chocoensis*, *S. sinensis*, and *S. tutunendo*. However, it is distinguished by comparatively longer basidia and basidiospores (Table 1). The tramal hyphae, hymenium structure, and basidiospores dimensions were found to be nearly identical to those reported by Liu et al. (2017) and Suwannarach et al. (2019). Additionally, the ITS sequence analysis confirmed the distinctness of *S. yunnanensis* from other species of the genus and supports the morphology based identification of the collected specimens. This finding is ecologically significant as it extends the known range of *S. yunnanensis*, suggesting greater adaptability to diverse habitats within the Asian region. Our collection extended

Table 1. Comparison of basidia and basidiospores sizes in *Sulzbacheromyces yunnanensis* and related taxa.

Species	Basidia (µm)	Basidiospores (µm)	References
<i>S. caatingae</i>	23–45 × 4–7	6.5 × 3.9	Sulzbacher et al. 2016
<i>S. chocoensis</i>	25–40 × 5–7	4 × 6	Coca et al. 2018
<i>S. sinensis</i>	13–50 × 2.5–8	5.9–11.5 × 4.5–7	Liu et al. 2017
<i>S. tutunendo</i>	25–40 × 5–7	4 × 6	Coca et al. 2018
<i>S. yunnanensis</i>	50–75 × 5–6.5	8.3–12.5 × 3.5–5.8	This study

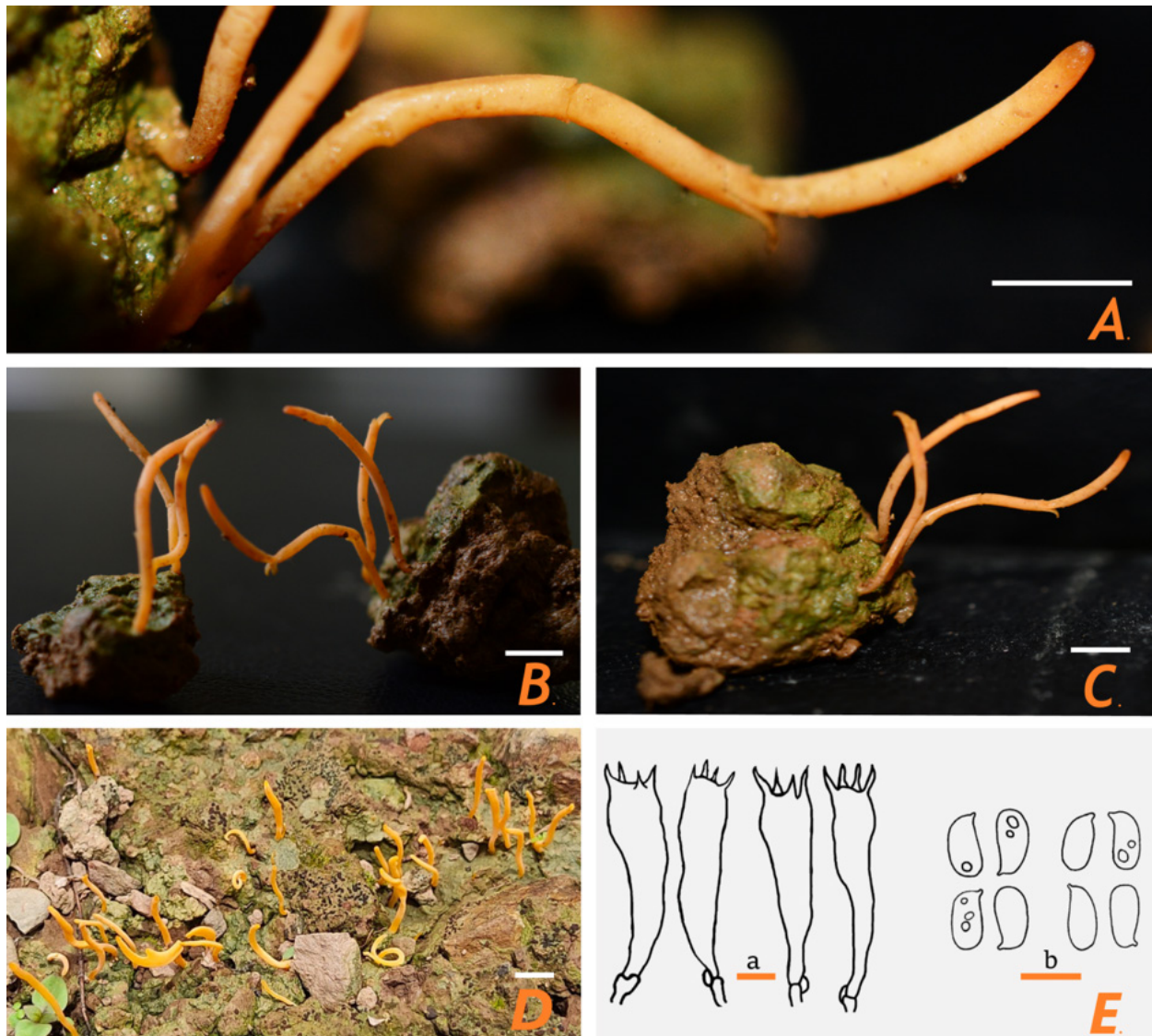


Image 1. Field and laboratory photographs of *Sulzbacheromyces yunnanensis* (MZU/JZT-VL/2022/004) collected from Hlimen Forest: A–C—basidiomes closed-up | D—basidiomes in field | E—(a) basidia (b) basidiospores. Scale bars: 10 mm (A–D) | 10 μ m (E—a,b). © Zohmangaiha Chawngthu

the distribution of the genus *Sulzbacheromyces* and the presence of *S. yunnanensis* in Hlimen forest, India aligns with its occurrence in subtropical forests of China and Thailand. This finding corroborates the recent report of *S. yunnanensis* from northeastern India (Debnath et al. 2025) and provides additional data on its habitat, morphology, and phylogenetic placement, further enhancing our understanding of this rare basidiolichen in India. However, only around 264 species of macrofungi have been reported from Mizoram (Thachunglura et al. 2024), which is relatively low considering the rich forest ecosystems and favorable climatic conditions. This indicates that the fungal diversity of the region remains largely unexplored. Therefore, it is essential to conduct

regular and extensive surveys to document the full range of macrofungal species. Such studies are crucial not only for biodiversity conservation but also for understanding the ecological roles of these fungi and their potential applications in food, medicine, and biotechnology. The favorable environmental conditions of northeastern India may support its growth, highlighting the need for further research within this important region of the Indo-Burma global biodiversity hotspot.

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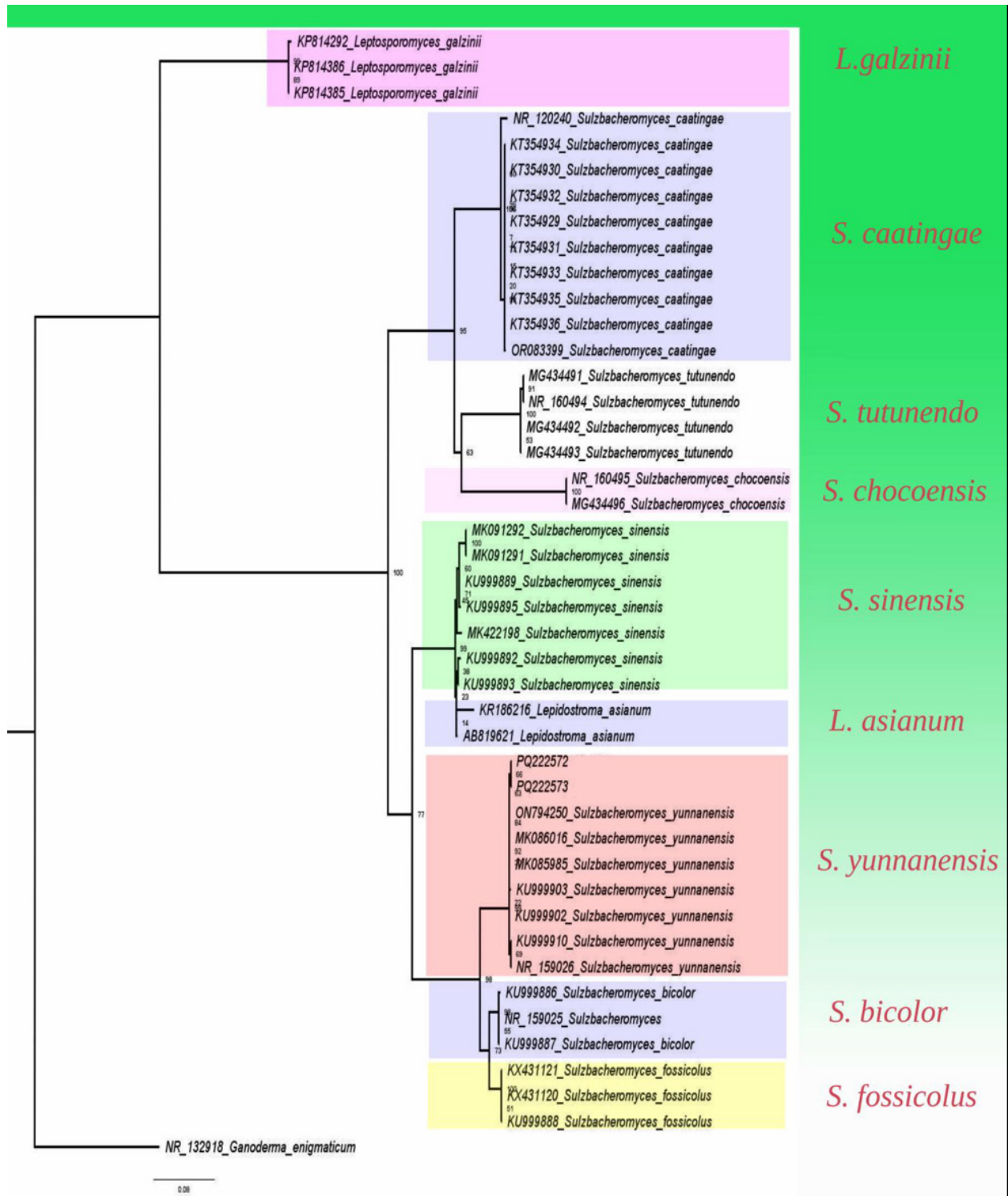


Figure 1. Phylogenetic tree of *Sulzbacheromyces yunnanensis* and related species obtained with maximum likelihood method based on the ITS region. Numbers below the branches are bootstrap percentage values based on 1,000 replicates, ML/MP bootstrap support values greater than 50%.

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Journal of Threatened Taxa is indexed/abstracted in Bibliography of Systematic Mycology, Biological Abstracts, BIOSIS Previews, CAB Abstracts, EBSCO, Google Scholar, Index Copernicus, Index Fungorum, JournalSeek, National Academy of Agricultural Sciences, NewJour, OCLC WorldCat, SCOPUS, Stanford University Libraries, Virtual Library of Biology, Zoological Records.

NAAS rating (India) 5.64



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ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

April 2025 | Vol. 17 | No. 4 | Pages: 26763–26938

Date of Publication: 26 April 2025 (Online & Print)

DOI: 10.11609/jott.2025.17.4.26763-26938

www.threatenedtaxa.org

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