Phytotaxa

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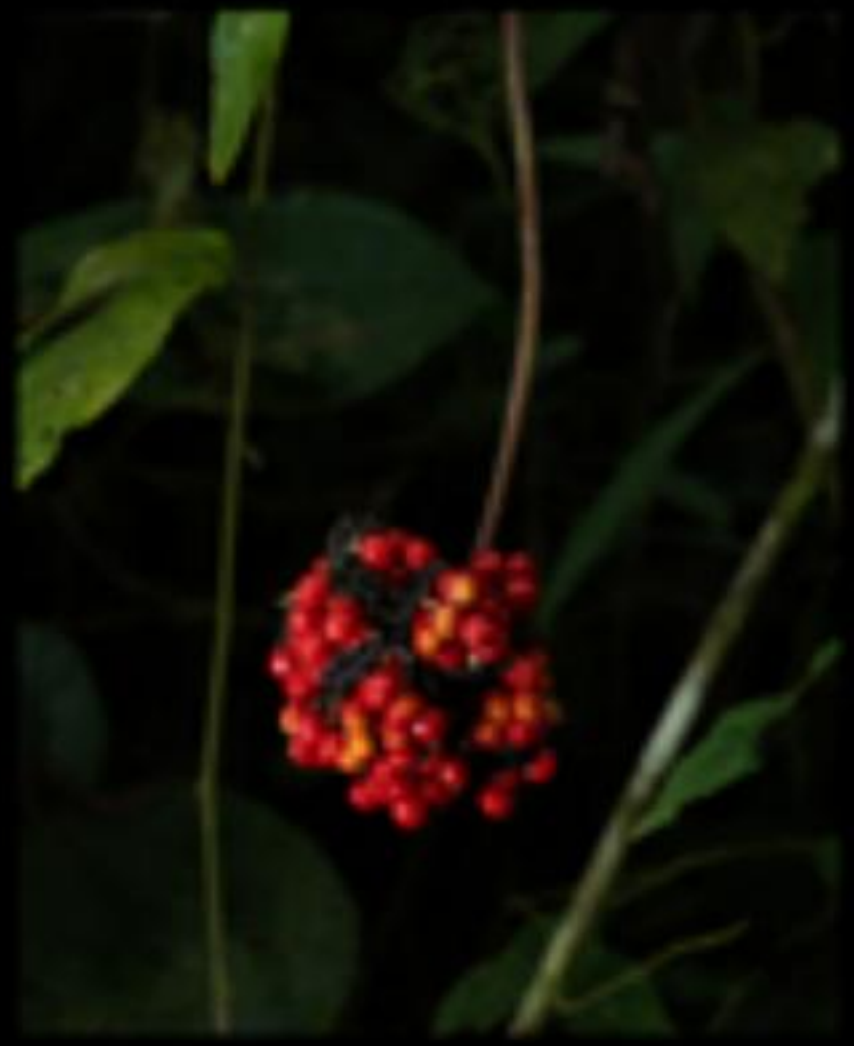

Magnolia Press, Auckland, New Zealand

A nomenclator botanicus for the bryophyllous and related species published in, or transferred to, *Bryophyllum*, now included in *Kalanchoe* (Crassulaceae subfam. Cotyledonoideae)

GIDEON F. SMITH

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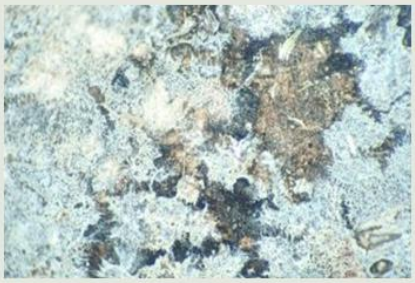

Molecular phylogeny and morphology reveal a new wood-inhabiting fungal species, *Mycobernardia tenuis* (Corticiaceae, Corticiales) from China

HONGJUAN WANG, YINGLIAN DENG, YU LIU, LINFENG LIU, MENG CHEN, CHANGLIN ZHAO

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PEDRO HENRIQUE CARDOSO, GABRIEL BARROS DA SILVA, MARCELO TROVÓ, FÁTIMA REGINA GONÇALVES SALIMENA

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Dracula colombiana (Pleurothallidinae: Orchidaceae), a new orchid species with a history of misidentification in trade and collections

LUIS E. BAQUERO R., ESTEBAN DOMINGUEZ VARGAS, SANTIAGO MESA, EDICSON PARRA-SANCHEZ

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Molecular phylogeny and morphology reveal a new wood-inhabiting fungal species, *Mycobernardia tenuis* (Corticiaceae, Corticiales) from China

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Abstract

Taxonomy plays a central role in understanding the diversity of life, translating the products of biological exploration and discovery—specimens and observations—into systems of names that establish a "classification home" for taxa. In this study, a new species, *Mycobernardia tenuis* is proposed based on a combination of morphological features and molecular evidence. *Mycobernardia tenuis* is characterized by membranaceous, reticular basidiomata with white to slightly cream hymenial surface, a monomitic hyphal system with clamped generative hyphae, subcylindrical to subclavate basidia, allantoid basidiospores measuring 6–8.5 × 2–3.5 µm. The phylogenetic tree inferred from ITS+nLSU sequences revealed that *Mycobernardia tenuis* is nested within the family Corticiaceae of the order Corticiales, where it forms a sister species to *M. incrustans* and is closely related to *M. yunnanensis.* A full description, illustrations, and phylogenetic analysis results of the new species are provided.

Keywords: Basidiomycota, Classification, Molecular systematics, Wood-decaying fungi, Yunnan province

Introduction

Fungi are crucial components of life on Earth with essential ecological and economic impacts. Their interactions with other organisms significantly contribute to the proper functioning of terrestrial ecosystems, and previous studies indicate that they have promoted the evolution of many terrestrial clades (Berbee *et al*. 2017, Loron *et al*. 2019, Gan *et al*. 2021, Yuan *et al*. 2023, Hyde *et al*. 2024).

The phylum Basidiomycota R.T. Moore (1980: 371) is one of the major branches in the fungal tree of life (Tedersoo *et al*. 2021, Baldrian *et al*. 2022, He *et al*. 2022, Niskanen *et al*. 2023). The order Corticiales K.H. Larss. (2007: 540) is one of the major lineages of Basidiomycota with a corticioid type of basidiomata, which was originally introduced by Hibbett *et al.* (2007) for the "corticioid clade" comprising a few taxa exclusively with a corticioid-type of basidiomata (Larsson *et al*. 2004, Binder *et al*. 2005). Boidin *et al*. (1998) introduced the order Vuilleminiales Boidin, Mugnier & Canales (1998: 486), with Vuilleminiaceae Maire (1902: 80) as its type family. Justification for the synonymization of Vuilleminiales under Corticiales was already provided by Hibbett *et al*. (2007). Currently, the order Corticiales contains four families, viz. Corticiaceae Herter (1910: 70), Dendrominiaceae Ghobad-Nejhad (2015: 224), Punctulariaceae Donk (1964: 287), and Vuilleminiaceae, and about 29 genera (He *et al*. 2024), and the order and its four families were well-established and recovered in molecular phylogenetic studies (Ghobad-Nejhad *et al*. 2021, He *et al*. 2024). Corticiales taxa are widespread and inhabit woody plants, grasses, mosses, and lichen thalli, in which most of the species are saprotrophic on hardwood plants. Economic aspects of Corticiales were mainly related to the

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phytopathogenic species in *Erythricium* J. Erikss. & Hjortstam (1970: 165), *Laetisaria* Burds. (1979: 420), and *Waitea* Warcup & P.H.B. Talbot (1962: 503), which caused diseases such as pink disease, brown ring patch, red thread sheath spot, and pink patch disease (Jayawardena *et al*. 2019).

Hibbett *et al*. (2007) retained a single family Corticiaceae for the order Corticiales. The family Corticiaceae was first described by Herter (1910) as encompassing the vast group of aphyllophoroid fungi characterized by a corticioid or crust-like form of the fruiting body (Ghobad-Nejhad *et al*. 2021). Ghobad-Nejhad *et al*. (2021) employed multigene phylogenetic analyses based on extensive original specimens to investigate the circumscription of genera in this family and provided a well-supported phylogenetic backbone for the family Corticiaceae, which comprises 13 genera, namely *Basidiodesertica* Maharachch., Wanas. & Al-Sadi (2021: 31), *Capillosclerotium* Prameela & Deeba (2013: 90), *Corticium* Pers. (1794: 110), *Disporotrichum* Stalpers (1984: 29), *Erythricium* J. Erikss. & Hjortstam (1970: 165), *Galzinia* Bourdot (1922: 577), *Giulia* Tassi (1904: 92), *Laetisaria* Burds. (1979: 420), *Lawreymyces* Lücking & Moncada (2017: 15), *Marchandiomyces* Diederich & D. Hawksw. (1990: 311), *Mycobernardia* Ghobad-Nejhad (2021: 7), *Tretopileus* B.O. Dodge (1946: 223), and *Waitea* (He *et al*. 2024). The genus *Mycobernardia* is monotypic, characterized by the curved, allantoid basidiospores and internally repetitive basidia (Ghobad-Nejhad *et al.* 2021). Taxonomic and phylogenetic disentanglements in Corticiaceae s.s. (Corticiales, Basidiomycota) and evolution of nutritional modes showed that the molecular studies have helped to assign many corticioid fungi to diverse families and orders, in which the deep analyses supported the recognition of ten monophyletic genera in the family Corticiaceae and revealed that the genus *Mycobernardia* was described as new to science (Ghobad-Nejhad *et al.* 2021). According to Index Fungorum (www.indexfungorum.org; accessed on 22 May 2025), and Mycobank (https://www.mycobank.org/; accessed on 22 May 2025), the genus *Mycobernardia* only has specific and registered names, with two species being accepted worldwide (Ghobad-Nejhad *et al*. 2021, Li *et al.* 2024).

During surveys of wood-inhabiting fungi, we collected a new species of *Mycobernardia* from the order Corticiales in Yunnan Province, China, which did not match any known species of wood-inhabiting fungi. We present the morphological characteristics and molecular analyses with ITS and nLSU DNA markers that support the taxonomy and phylogenetics of *Mycobernardia* species.

Materials and methods

Morphological studies

Fresh fruiting bodies of fungi growing on angiosperm branches in Lincang, Yunnan Province, China, were photographed *in situ*. After the collection information was recorded (Rathnayaka *et al*. 2024), the specimens were taken to the laboratory and dried in an electric food dehydrator at 50 °C (Hu *et al.* 2022), then sealed and stored in an envelope bag and deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. The macro-morphological descriptions were based on field notes and photos captured in the field and lab. The color terminology followed Petersen (1996) and was confirmed in general terms according to the CMYK color code (Deep White Printing Team 2022).

The micro-morphological data were obtained from dried specimens observed under a light microscope, following the method described by Zhao *et al.* (2023). The following abbreviations were used: KOH = 5% potassium hydroxide water solution, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, L = mean spores' length (arithmetic average for all spores), W = mean spores' width (arithmetic average for all spores), Q = variation in the L/W ratios between the specimens studied, and n = a/b (number of spores (a) measured from a given number (b) of specimens), Q = the average of Q (arithmetic average for all spores).

Molecular procedures and phylogenetic analyses

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to obtain genomic DNA from the dried specimens according to the manufacturer's instructions (Zhao *et al.* 2023). The nuclear ribosomal internal transcribed spacer (ITS) region was amplified with the primer pair ITS5/ITS4 (White *et al.* 1990), the nuclear large subunit (nLSU) region with the primer pair LR0R/LR7 (Vilgalys & Hester 1990). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s, and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for nLSU was as follows:

initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 48 °C for 1 min, and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min. The PCR products were purified and sequenced at Kunming Tsingke Biological Technology Limited Company (Yunnan Province, P.R. China). All of the newly generated sequences were deposited in GenBank (Table 1).

TABLE 1. List of species, specimens and GenBank accession numbers of sequences used in this study.

Species name	Sample no.	GenBank accessions no.		References
		ITS	LSU	
Corticium boreoroseum	MG 42	MW805842	MW805816	Ghobad-Nejhad *et al*. 2021
Corticium boreoroseum	MG 47	MW805846	HM046920	Ghobad-Nejhad *et al*. 2021
Corticium canfieldii	ERC-72-11	MW805850	MW805821	Ghobad-Nejhad *et al*. 2021
Corticium lombardiae	MG 147	MW805848	-	Ghobad-Nejhad *et al*. 2021
Corticium lombardiae	MG 148	MW805849	-	Ghobad-Nejhad *et al*. 2021
Corticium malagasoroseum	PC 0094401	MW805856	MW805822	Ghobad-Nejhad *et al*. 2021
Corticium roseum	MG 252	MW805872	MW805836	Ghobad-Nejhad *et al*. 2021
Corticium silviae	S.Feusi05.06.2017	MH520061	MH520061	Diederich *et al*. 2018
Corticium thailandicum	MG 242	MW805868	MW805831	Ghobad-Nejhad *et al*. 2021
Erythricium laetum	MG 72	GU590875	GU590878	Ghobad-Nejhad *et al*. 2021
Erythricium laetum	MG 73	GU590874	GU590879	Ghobad-Nejhad *et al*. 2021
Laetisaria agaves	RLG-10805	MW805851	-	Ghobad-Nejhad *et al*. 2021
Laetisaria fuciformis	CBS:182.49	MH856485	MH868023	Vu *et al*. 2019
Lawreymyces palicei	Palice 2509	AY542864	AY542864	Ghobad-Nejhad *et al*. 2021
Lawreymyces palicei	Palice 4369	AY542865	AY542865	Ghobad-Nejhad *et al*. 2021
Marchandiomyces corallinus	JL128-98	AY583327	AY583331	DePriest *et al*. 2005
Marchandiomyces lignicola	MYA 299	AY583328	AY583332	DePriest *et al*. 2005
Mycobernardia incrustans	CBS:172.36	MH855759	MH867272	Ghobad-Nejhad *et al*. 2021
Mycobernardia incrustans	CBS:173.36	MH855760	MH867273	Ghobad-Nejhad *et al*. 2021
Mycobernardia tenuis	CLZhao 25372	PQ654707	PQ654708	Present study
Mycobernardia yunnanensis	CLZhao 26048	OR844493	OR891521	Li *et al*. 2024
Mycobernardia yunnanensis	CLZhao 26082	PP092993	OR891522	Li *et al*. 2024
Mycobernardia yunnanensis	CLZhao 26091	OR844494	OR891523	Li *et al*. 2024
Punctularia strigosozonata	CBS:345.34	MH855559	MH867064	Vu *et al*. 2019
Punctulariopsis obducens	MG 70	HM046918	HM046933	Ghobad-Nejhad *et al*. 2021
Waitea circinata	CBS:472.82	MH861518	MH873265	Vu *et al*. 2019
Waitea circinata	299-G-17	MK817577	MN121346	Vojvodic *et al*. 2023

To determine the phylogeny, we compiled an ITS+nLSU dataset. In the combined dataset, Sequences of *Punctularia strigosozonata* (Schwein.) P.H.B. Talbot (1958: 143) and *Punctulariopsis obducens* (Hjortstam & Ryvarden) Ghob.-Nejh. (2010: 1529) were selected as an outgroup in the ITS + nLSU analysis (Figure 1) as inspired by a previous study (Li *et al.* 2024). The sequences were aligned initially by using MAFFT (https://mafft.cbrc.jp/alignment/server/) using the "G-INS-I" strategy (Katoh *et al.* 2019), and then manually optimized in AliView version 1.27 (Larsson 2014). Finally, the two gene fragments were concatenated with Mesquite v3.70 (Maddison & Maddison 2021; https://www.mesquiteproject.org/) for further phylogenetic analyses.

Maximum parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses were applied to the combined datasets following a previous study (Dong *et al*. 2024), and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All of the characters were equally weighted, and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max trees were set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics, tree length (TL), the consistency index (CI), the retention index (RI), the rescaled consistency index (RC), and the homoplasy index (HI) were calculated for each maximum parsimonious tree generated. The multiple sequence

alignment was also analyzed using maximum likelihood (ML) in RAxML-HPC2 (Miller *et al.* 2012). Branch support (BS) for ML analysis was determined by 1,000 bootstrap replicates.

JModelTest2 on ACCESS was used to determine the best-fit evolution model for each data set in the CIPRES Science Gateway (Miller *et al*. 2012). The best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis was GTR+I+G. Bayesian Inference (BI) phylogenies were inferred using MrBayes v3.2.7a with a general time reversible model of DNA substitution and a gamma distribution rate variation across sites (Ronquist *et al.* 2012). Four Markov chains were run twice from a random starting tree, for 0.8 million generations of the datasets (Figure 1) with trees and parameters sampled every 1,000 generations. The first one-fourth of all generations was discarded as burn-in. The majority rule consensus tree of all remaining trees was calculated. Branches were considered significantly supported if they received a maximum likelihood bootstrap value (BS) ≥70%, a maximum parsimony bootstrap value (BT) ≥70%, or a Bayesian posterior probability (BPP) ≥0.95.

Genealogical concordance phylogenetic species recognition (GCPSR) analysis

We employed the genealogical concordance phylogenetic species recognition analysis (GCPSR) to detect significant recombination events (Quaedvlieg *et al.* 2014). The data were analyzed using the pairwise homoplasy index (PHI) test in SplitsTree 4 to determine the recombination level with closely related species (Bruen *et al*. 2006, Huson & Bryant 2006, Quaedvlieg *et al*. 2014). Multilocus datasets (ITS+nLSU) with closely related species were used for the analysis. The pairwise homoplasy index lower than 0.05 ($\Phi w < 0.05$) indicates significant recombination in the dataset. The relationships between closely related taxa were visualized by constructing split graphs from the concatenated datasets using the LogDet transformation and split decomposition options.

Results

Phylogenetic analyses

Based on ITS+nLSU (Figure 1), the dataset comprised sequences from 28 fungal specimens representing 19 species. The dataset had an aligned length of 2,080 characters, of which 1,465 characters were constant, 121 were variable and parsimony-uninformative, and 494 were parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 1,592, CI = 0.5854, HI = 0.4146, RI = 0.7431, RC = 0.4350). Bayesian analysis and ML analysis yielded a similar topology to the MP analysis, with an average standard deviation of split frequencies of 0.006002 (BI), and the effective sample size (ESS) across the two runs was double the average ESS (avg ESS) = 747. The phylogenetic tree inferred from ITS+nLSU sequences revealed that the new species *Mycobernardia tenuis* was nested within the genus *Mycobernardia* within the family Corticiaceae (Corticiales), forming a sister species to *M. incrustans* (Parmasto) Ghobad-Nejhad, and was related to the species *M. yunnanensis* Q. Li & C.L. Zhao.

Applying the pairwise homoplasy index (PHI) test to the combined partial ITS+nLSU data tree revealed no recombination level within phylogenetically related species. No significant recombination events were observed between *Mycobernardia tenuis* and phylogenetically closely related species *M. incrustans* and *M. yunnanensis* (Figure 2). The test results of the combined partial ITS+nLSU sequences dataset show $\Phi w = 0.4128$ ($\Phi w > 0.05$), no recombination is present in the new species with *M. incrustans*, and *M. yunnanensis* (Figure 2).

Taxonomy

Mycobernardia tenuis Y.L. Deng & C.L. Zhao, *sp. nov.* Figs. 3 and 4
MycoBank no.: MB 856784

Etymology:—*tenuis* (Lat.) refers to the thin basidiomata of the type specimen.

Diagnosis:—Differs from *Mycobernardia incrustans* by having membranaceous, reticular basidiomata with white to slightly cream hymenial surface, thin-walled generative hyphae, and larger basidiospores measuring 6–8.5 × 2–3.5 µm.

Holotype:—CHINA. Yunnan Province, Lincang, Fengqing County, Lancangjiang Provincial Nature Reserve,

GPS coordinates 23°48' N, 100°05' E, altitude 2,900 m asl., on the fallen branch of an angiosperm, leg. C.L. Zhao, 21 October 2022, CLZhao 25372 (SWFC).

Basidiomata:—Annual, resupinate, membranaceous, reticular, without odor or taste when fresh, and up to 9 cm long, 2.5 cm wide, 50–100 µm thick. Hymenial surface smooth, white when fresh, turning to white to a slightly cream upon drying. Sterile margin thin, white, up to 1 mm.

Hyphal structure:—Hyphal system monomitic; generative hyphae with clamp connections, colorless, thin-walled, moderately branched, interwoven, 3–4 µm in diameter; IKI–, CB–, tissues unchanged in KOH.

Hymenium:—Cystidia and cystidioles absent. Basidia subcylindrical to subclavate, with 4 sterigmata and a basal clamp connection, 13–17 × 4–5 µm; basidioles dominant, in shape similar to basidia, but slightly smaller.

Spores:—Basidiospores allantoid, colorless, smooth, thin-walled, IKI–, CB–, 6–8.5 × 2–3.5 µm, L = 7.21 µm, W = 2.91 µm, Q = 2.41 (n = 30/1).

Type of rot:—White rot

Notes:—The phylogenetic tree (Figure 1) inferred from ITS and nLSU sequences revealed that *Mycobernardia tenuis* was grouped closely with *M. incrustans* and *M. yunnanensis*. However, morphologically, the new species is easily separated from other known species of the genus *Mycobernardia*. The species *M. incrustans* can be delimited from *M. tenuis* by having ceraceous basidiomata, cream-colored hymenial surface with a faint rose tint, thick-walled generative hyphae, and smaller basidiospores measuring 4.5–6 × 1.5–2.5 µm (Hjortstam & Ryvarden 2007, Ghobad-Nejhad *et al*. 2021). The taxon *M. yunnanensis* can be distinguished from *M. tenuis* by its membranaceous to hard, cracked basidiomata, slightly olivaceous to olivaceous hymenial surface, thin- to thick-walled generative hyphae, and shorter basidiospores measuring 4.8–6 × 2–3 µm (Li *et al*. 2024).

Discussion

The genus *Mycobernardia* was established by Ghobad-Nejhad (2021), typed by *M. incrustans* based on extensive type studies and sequences of nLSU, ITS, IGS, nSSU, and mtSSU regions of the family Corticiaceae (Ghobad-Nejhad *et al*. 2021). The species *Galzinia incrustans* Parmasto (1965: 225) as basionym of species *M. incrustans* (Ghobad-Nejhad *et al*. 2021). *Mycobernardia* and its type were previously assigned to *Galzinia*, typified by *G. pedicellata* Bourdot (1922: 577), because of its curved, allantoid basidiospores and internally repetitive basidia. In contrast, basidiomata in *Mycobernardia* are thicker, distinct, and ceraceous, with a cream-colored appearance (Ghobad-Nejhad *et al*. 2021). The present study proposes a new species, *M. tenuis,* based on a combination of morphological features and molecular evidence.

Mycobernardia is parasitic on decorticated, decayed wood, mainly from frondose trees, typically found in wet localities (Eriksson & Ryvarden 1975). The biotopes are as a rule fertile deciduous forests, characterized by a field vegetation of herbs and ferns (Wang *et al*. 2023, 2024, Zhao *et al*. 2024). The phylogenetic position of *M. tenuis* was evaluated based on combined ITS and nLSU (Figure 1). The pairwise homoplasy index (PHI) test (Figure 2) confirmed no recombination in the new species compared to the closely related taxa. Based on phylogenetic and morphological evidence, the taxon *M. tenuis* is proposed as a new species. Morphologically, *M. tenuis* resembles two similar species, viz., *M. incrustans* and *M. yunnanensis*, and their morphological characteristics are presented in Table 2.

TABLE 2. A morphological comparison of *Mycobernardia XXX* and two closely related species in the genus *Mycobernardia*.

Species	Basidiomata	Hymenial surface	Generative hyphae	Basidia	Basidiospores	References
Mycobernardia incrustans	Effused, ceraceous	cream-colored with a faint rose tint	thick-walled	Subcylindrical to suburniform, 15-20 x 4-5 µm	Allantoid, 4.5-6 x 1.5-2.5 µm	Hjortstam & Ryvarden 2007, Ghobad-Nejhad *et al*. 2021
Mycobernardia yunnanensis	Resupinate, membranaceous	cracked, olivaceous	thin- to thick-walled	Subcylindrical to clavate, 13–23 x 3.5–5.5 µm	Allantoid, 4.8–6 x 2–3 µm	Li *et al*. 2024
Mycobernardia tenuis	Resupinate, membranaceous, reticular	white to purple grey to yellowish white	thin-walled	Subcylindrical to clavate, 13–17 x 4–5 µm	Allantoid, 6–8.5 x 2–3.5 µm	Present study

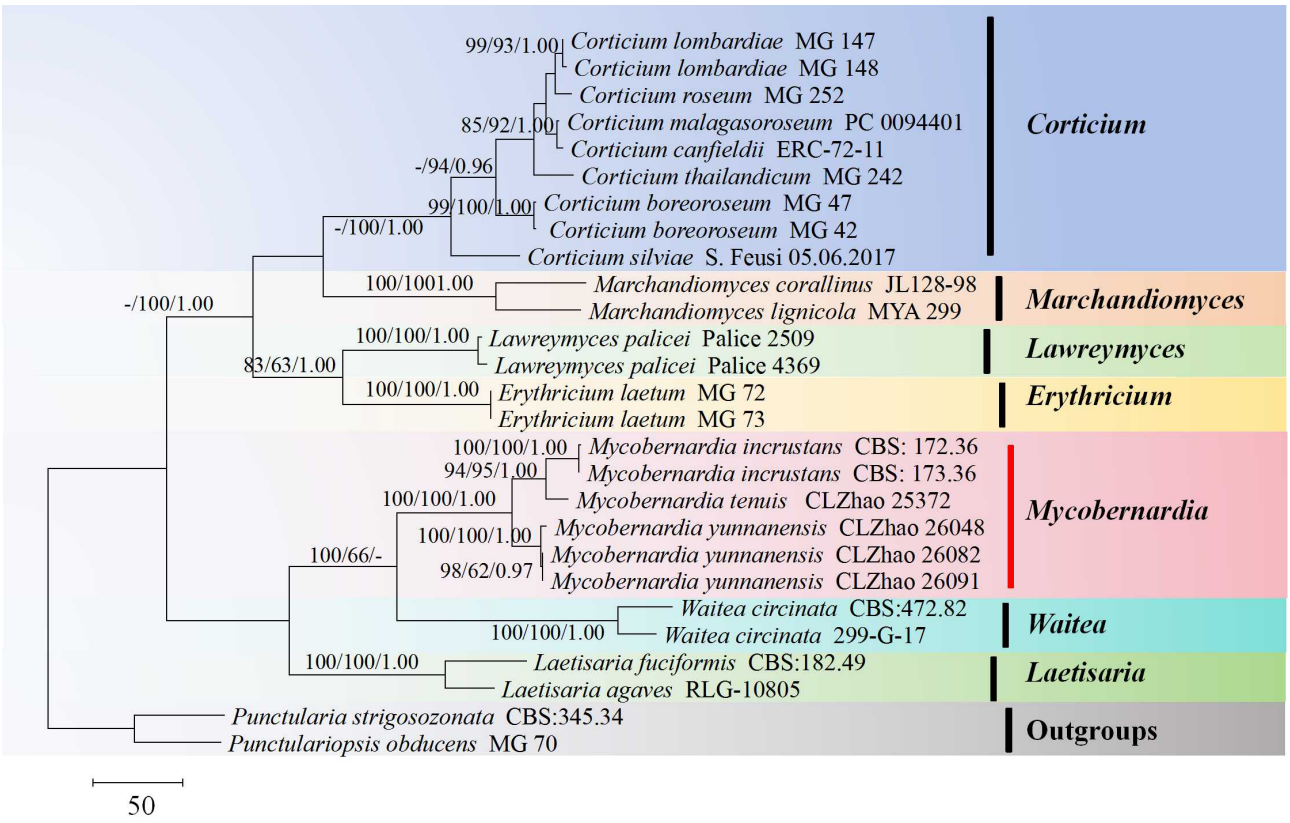

FIGURE 1. Maximum parsimony strict consensus tree illustrating the phylogeny of the new species *Mycobernardia tenuis* and related species in the family Corticiaceae within the order Corticiales based on ITS+nLSU sequences. The branch is labeled with a maximum likelihood lead value equal to or greater than 70%, a reduced lead value equal to or greater than 50%, and a Bayesian posterior probability equal to or greater than 0.95.

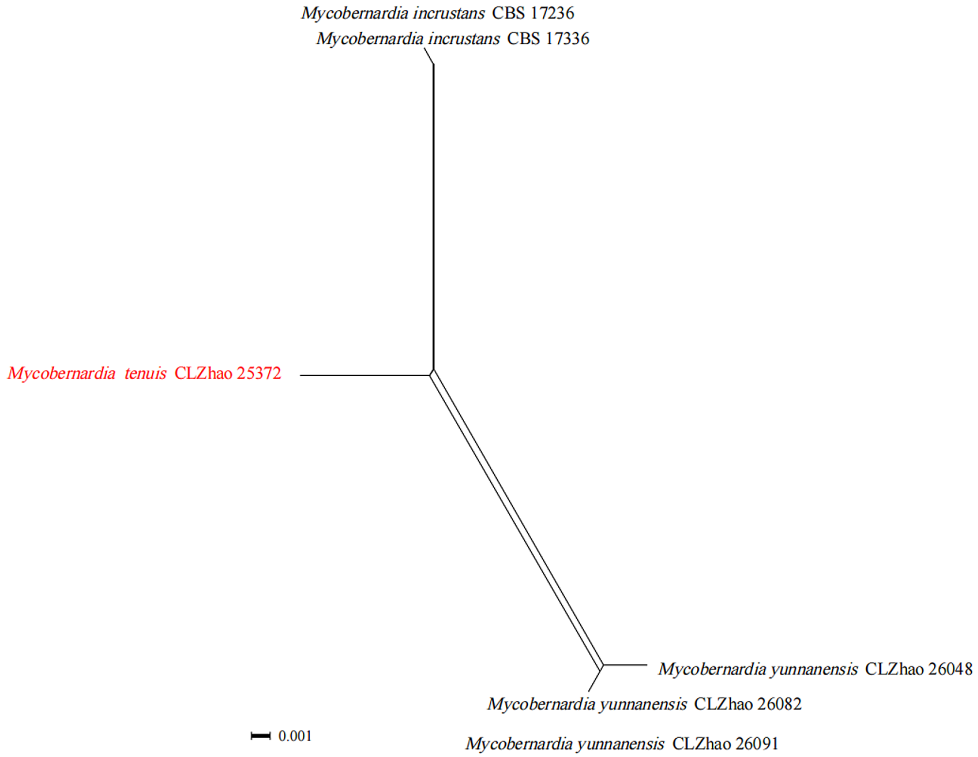

FIGURE 2. The pairwise homoplasy index (PHI) test results for the combined partial ITS+nLSU sequences data of *Mycobernardia tenuis* and closely related taxa using LogDet transformation and splits decomposition. PHI test results $\Phi w \leq 0.05$ indicate a significant recombination within the dataset. New taxa are in red, while closely related species to the new species are in other colors.

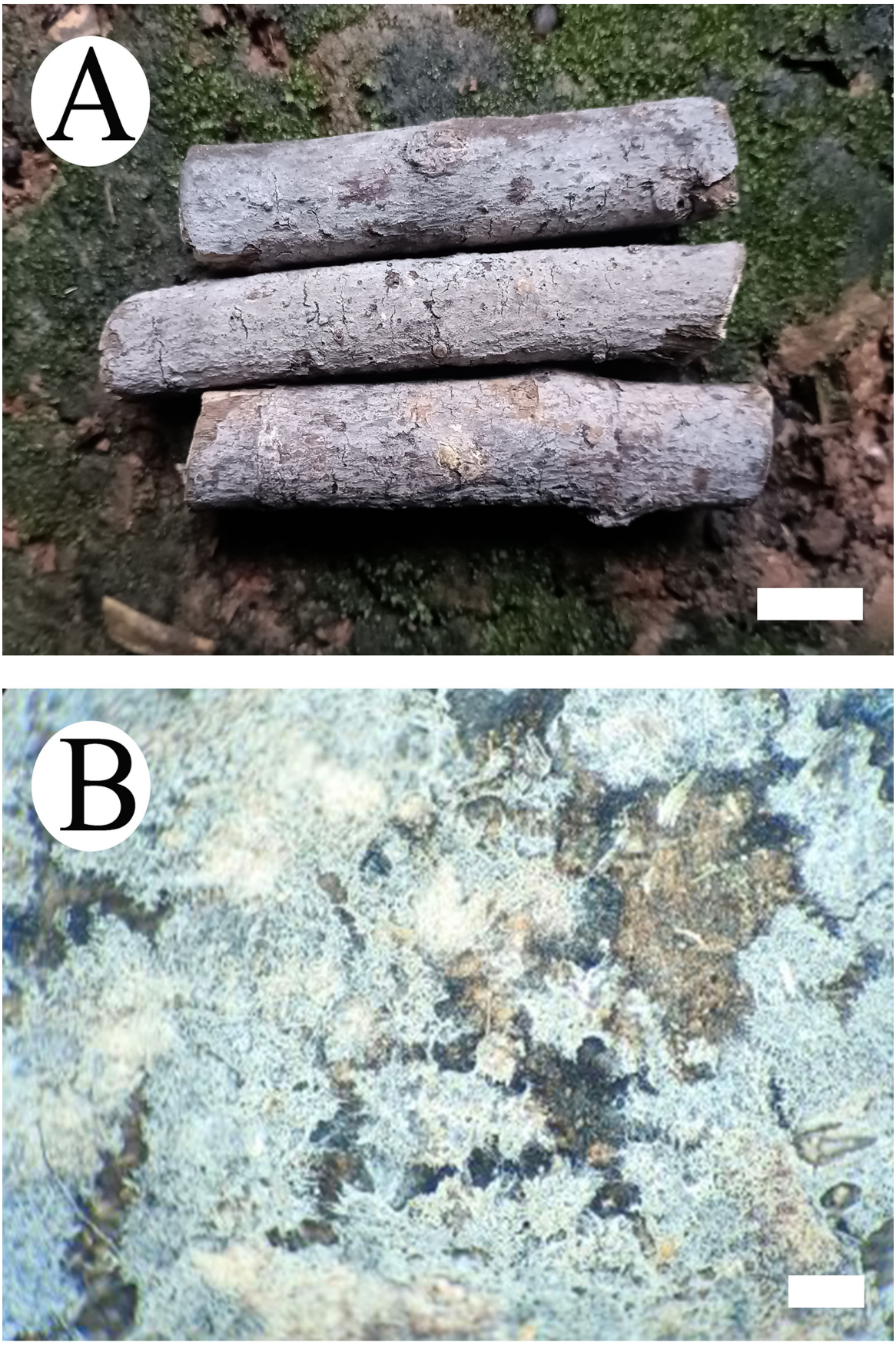

FIGURE 3. *Mycobernardia tenuis* (holotype, CLZhao 25372): basidiomata on the substrate (A), macroscopic characteristics of hymenophore (B). Bars: (A) = 1 cm and (B) = 1 mm.

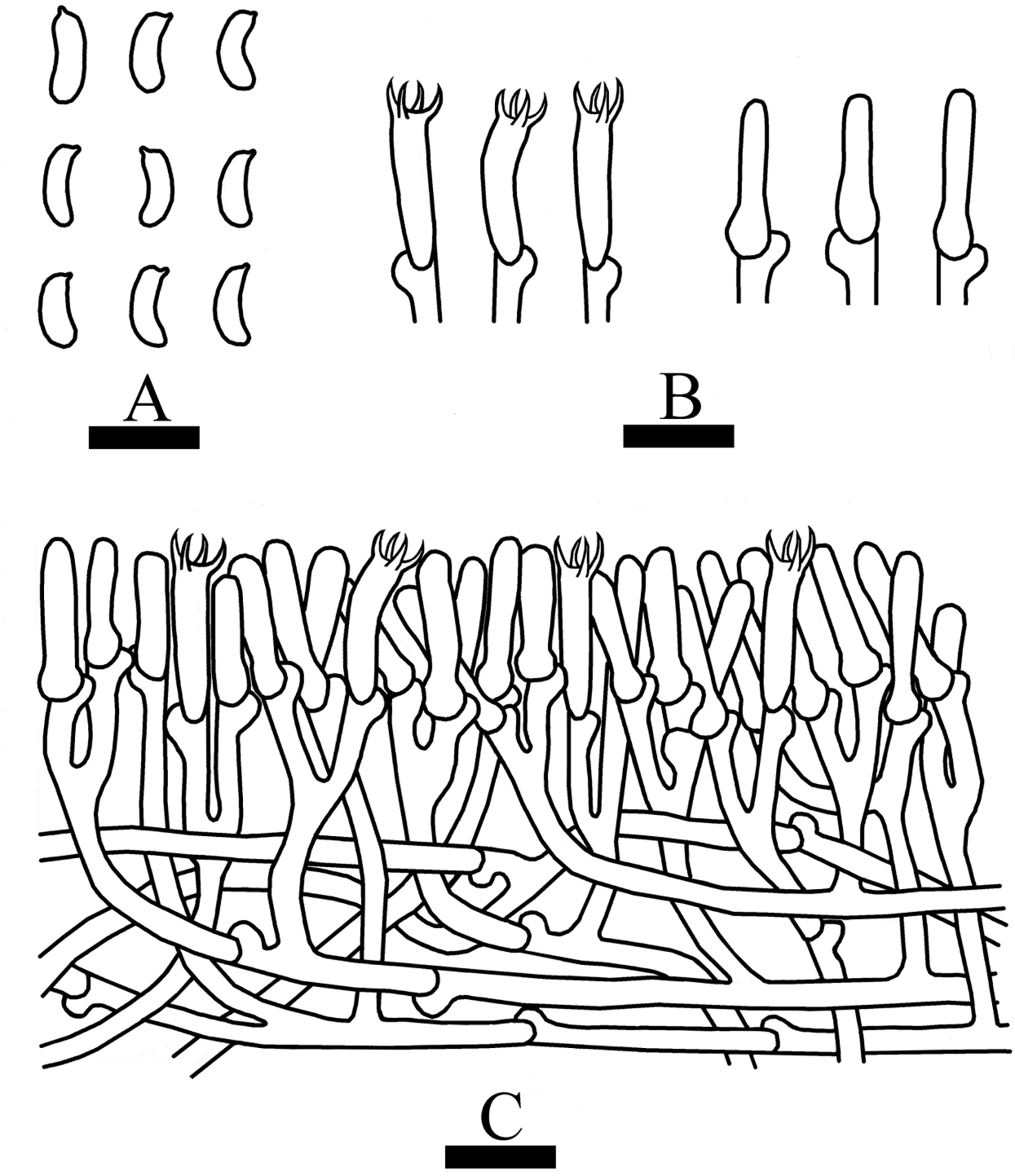

FIGURE 4. Microscopic structures of *Mycobernardia tenuis* (holotype, CLZhao 25372): basidiospores (A), basidia and basidioles (B), a section of the hymenium (C). Bars: A–C= 10 µm.

In ecology and biogeography, the macrofungi are an extensively studied group of Basidiomycota, which are an important group in the forest ecosystem (Wu *et al*. 2022, Deng & Zhao 2023, Yang *et al*. 2023, Dong *et al*. 2024, Li *et al*. 2024, Zhang *et al*. 2024, Zhou *et al*. 2024). To date, only two species of *Mycobernardia* have been identified; however, the diversity of *Mycobernardia* in China remains largely unknown, particularly in subtropical and tropical areas. This paper reports a new species, *M. tenuis*, which enriches our knowledge of fungal diversity in this area. The discovery of *M. tenuis* highlights the need for additional fieldwork and molecular analyses to identify new taxa and deepen our understanding of fungal diversity.

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