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Gloeocystidiellum sinense sp. nov. (Stereaceae, Russulales), a wood-inhabiting fungus from Yunnan Province, China

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ABSTRACT

A new corticioid fungal species *Gloeocystidiellum sinense* from Yunnan Province, is described and illustrated based on morphological and molecular evidence. This species is characterised by its cream to pink-buff hymenial surface, a monomitic hyphal system, and ellipsoid basidiospores measuring as 3.7–4.4 × 2.8–3.2 μm. The phylogenetic analyses inferred from ITS + nLSU dataset indicated that the new species *G. sinense* nested within the genus *Gloeocystidiellum*, in which it formed a single lineage and grouped with *G. porosum* and *G. yunnanense* within the same clade. A full description, illustrations, and phylogenetic analysis results are provided. In addition, the new species is compared with closely related taxa.

Introduction

In forest ecosystems, wood-inhabiting fungi play an essential ecological role in driving carbon cycling in forest soils, mediating mineral nutrition of plants, and alleviating carbon limitations (Tedersoo et al. 2014; Lu et al. 2024; Zhang et al. 2024; Dong et al. 2024b). Corticioid fungi are an important group in the wood-inhabiting fungi and mainly a diverse and heterogeneous group in Basidiomycetes G. Winter, in which basidiomes are generally diverse configurations as smooth, grandinioid, odontioid or poroid hymenophore (Gorjón 2020; Zhao and Zhao 2023). Molecular phylogenetic studies revealed that the corticioid fungi included many different families and genera, in which they were known to be divided into more clades (Larsson and Larsson 2003; Larsson et al. 2004; Binder et al. 2005; Larsson 2007; Binder et al. 2010; Sulistyo et al. 2021; Zhao and Zhao 2023).

The corticioid genus *Gloeocystidiellum* Donk belonging to the order Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David, was established by Donk (1931) with *G. porosum* (Berk & MA Curtis) Donk as its type species. The genus is characterised by smooth, rarely grandinioid or odontioid hymenial surface, a monomitic hyphal system with nodose or simple-septate generative hyphae, gloeocystidia, subcylindrical to clavate basidia with four sterigmata and suballantoid, ellipsoid, subglobose or globose, verrucose or aculeate basidiospores (Donk 1931, 1957).

Recently, molecular studies involving the genus *Gloeocystidiellum* based on single-gene or multi-gene datasets have been carried out (Larsson and Hallenberg 2001; Gorjón and Hallenberg 2012; Jaramillo-Riofrío et al. 2023; Zhao and Zhao 2023). Phylogenetic relationships of russuloid basidiomycetes revealed that the species of the genus *Gloeocystidiellum* formed two clades based on phylogenetic analyses of nuclear 5.8S, ITS2 and nLSU rDNA genes, in which the generitype species *G. porosum* grouped into clade I (Larsson and Larsson 2003). The high phylogenetic diversity among corticioid homobasidiomycetes showed that the taxon *G. subasperisporum* (Litsch.) J. Erikss. & Ryvarden nested into the russuloid clade and closely grouped with *Gloeodontia discolor* (Berk. & M.A. Curtis) Boidin (Larsson et al. 2004). Molecular phylogenetic analyses based on ITS and nLSU sequences from these samples within the Peniophorales clade in the order Russulales, indicated that the genus *Gloeocystidiellum* clustered with Russulaceae Lotsy and *Gloeodontia* clade (Leal-Dutra et al. 2018). The analyses inferred from ITS + nLSU sequences showed that the genus *Gloeodontia* formed a single clade and then grouped with *Gloeocystidiellum* within the order Russulales (Chen et al. 2020; Zhao and Zhao 2023). In addition, Index Fungorum (2024, accessed on 25 October 2024) registers 85 specific and infraspecific names in this genus *Gloeocystidiellum*.

During investigations on wood-inhabiting fungi in southern China, many specimens were collected, and an additional taxon of the genus *Gloeocystidiellum* was found, which could not be assigned to any described species. In this study, the authors examine the taxonomy and phylogeny of a new species within the genus *Gloeocystidiellum*, based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences. Detailed description, illustrations, and phylogenetic analysis results of the new species *G. sinense* are provided.

Materials and methods

Sample collection and herbarium specimen preparation

The fresh fruiting bodies were collected on the fallen angiosperm branches which from Lincang, Yunnan Province, China. The samples were photographed in situ, and fresh macroscopic details were recorded. Photographs were recorded by a Nikon D7100 camera. All the photos were focus-stacked using Helicon Focus software. Macroscopic details were recorded and transported to a field station where the fruit body was dried on an electronic food dryer at 45 °C (Hu et al. 2022, Zhao et al. 2023). Once dried, the specimens were sealed in an envelope and zip-lock plastic bags and labelled (Rathnayaka et al. 2024; Dong et al. 2024a). The dried specimens were deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, China.

Morphology

The macromorphological descriptions were based on field notes and photos captured in the field and lab. The colour terminology followed Petersen . The micromorphological data were obtained from the dried specimens after observation under a light microscope with a magnification of 10×100 oil (Zhao et al. 2023; Dong et al. 2024a). Sections mounted in 5% KOH and 2% phloxine B ($C_{20}H_2Br_4C_{14}Na_2O_5$), and we also used other

reagents, including Cotton Blue and Melzer's reagent to observe micromorphology following as per Wu et al. (2021). To show the variation in spore sizes, 5% of measurements were excluded from each end of the range and shown in parentheses. At least thirty basidiospores from each specimen were measured. Stalks were excluded from basidia measurements, and the hilar appendage was excluded from basidiospores measurements. The following abbreviations are used: KOH = 5% potassium hydroxide water solution, CB – = acyanophilous, IKI – = both inamyloid and non-dextrinoid, L = mean spore length (arithmetic average for all spores), W = mean spore width (arithmetic average for all spores), Q = variation in the L/W ratios between the specimens studied.

Molecular phylogeny

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. The nuclear ribosomal of the internal transcribed spacer (ITS) region was amplified with ITS5 and ITS4 primers (White et al. 1990). The nuclear large subunit (nLSU) region was amplified with the LR0R and LR7 primer pair (Vilgalys and Hester 1990; Rehner and Samuels 1994). 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). The newly generated sequences were deposited in NCBI GenBank (Table 1).

The sequences were aligned in MAFFT version 7 using the G-INS-i strategy. The alignment was adjusted manually using AliView version 1.27. The dataset was aligned first, and then the sequences of ITS + nLSU were combined with Mesquite version 3.51. The combined ITS + nLSU sequences and ITS datasets were used to infer the position of the new species in the genus *Gloeocystidiellum* and related species. Sequences of *Asterostroma yunnanense* Y.L. Deng & C.L. Zhao was retrieved from GenBank and used as outgroup taxa in the ITS + nLSU analysis (Figure 1; Deng et al. 2024).

Maximum parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses were applied to the combined three datasets following a previous study (Zhao and Wu 2017), and the tree construction procedure was performed in PAUP* version 4.0b10 . All of the characters were equally weighted, and gaps were treated as missing data. Using the heuristic search option with TBR branch swapping and 1000 random sequence additions, trees were inferred. 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 1000 replicates (Felsenstein 1985). Descriptive tree statistics, tree length (TL), consistency index (CI), retention index (RI), 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 on XSEDE v 8.2.8 with default parameters (Miller et al. 2012). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

Table 1. Names, sample numbers, references and corresponding GenBank accession numbers of the taxa used in the phylogenetic analyses of this study. New species shown in bold.

Species Name	Sample No.	GenBank Accession No.		Country	References
		ITS	nLSU		
Asterostroma yunnanense	CLZhao 22781	OR048809	OR506285	China	Deng et al. (2024)
A. yunnanense	CLZhao 22786	OR048810	OR506286	China	Deng et al. (2024)
Gloeocystidiellum aspellum	He4262	–	KY860460	China	Jaramillo-Riofrío et al. (2023)
G. bisporum	CBS/961.96	AY048875	AY048875	Sweden	Jaramillo-Riofrío et al. (2023)
G. bisporum	KHL11135	AY048877	AY048877	Norway	Larsson and Larsson (2003)
G. clavuligerum	NH13159/2731	AF310083	AF310083	Russia	Larsson and Larsson (2003)
G. compactum	Wu880615-21	AF506434	AF506434	Taiwan	Jaramillo-Riofrío et al. (2023)
G. formosanum	Wu9404-19	AF506439	AF506439	Taiwan	Jaramillo-Riofrío et al. (2023)
G. heimii	LY/CBS321.66	AF506381	AF506381	Central African Republic	Jaramillo-Riofrío et al. (2023)
G. heimii	LY/CBS321.66	MH858805	MH858805	Central African Republic	Jaramillo-Riofrío et al. (2023)
G. kenyense	TFC/15278	FR878082	–	Portugal	Jaramillo-Riofrío et al. (2023)
G. lojanense	HUTPL(F)/2181	OP377059	OP377059	Ecuador	Jaramillo-Riofrío et al. (2023)
G. lojanense	HUTPL(F)/550	OP377083	OP377083	Ecuador	Jaramillo-Riofrío et al. (2023)
G. luridum	HK9808	AF506421	AF506421	Germany	Jaramillo-Riofrío et al. (2023)
G. porosum	CBS/51085	AF310097	AF310097	Netherlands	Jaramillo-Riofrío et al. (2023)
G. porosum	NH 10434	AF310094	AF310094	Denmark	Larsson and Hallenberg (2001)
G. purpureum	Wu9310-45	AF441338	AF441338	China	Larsson and Hallenberg (2001)
G. rajchenbergii	GB/NH16348	JQ734555	–	Chile	Jaramillo-Riofrío et al. (2023)
G. rajchenbergii	GB/NH16358	JQ734554	–	Chile	Jaramillo-Riofrío et al. (2023)
G. sinense	CLZhao 25040	PQ453516	PQ453518	China	Present study
G. sinense	CLZhao 25320	PQ453517	PQ453519	China	Present study
G. triste	KHL10334	AF506442	AF506442	Puerto Rico	Jaramillo-Riofrío et al. (2023)
G. yunnanense	CLZhao 7165	MZ710569	MZ710571	China	Zhao and Zhao (2023)
G. yunnanense	CLZhao 7202	MZ710570	MZ710572	China	Zhao and Zhao (2023)

jModelTest v2 (Darriba et al. 2012) was used to determine the best-fit evolution model for each dataset for the purposes of Bayesian Inference (BI), Bayesian Inference was performed using MrBayes 3.2.7a with a GTR + I + G model of DNA substitution and a gamma distribution rate variation across sites (Ronquist et al. 2012). The first one-fourth of all the generations were discarded as burn-ins. The majority-rule consensus tree of all the remaining trees was calculated. Branches were considered significantly supported if they received a Maximum Likelihood bootstrap value (BS) of ≥ 70%, a Maximum Parsimony bootstrap value (BT) of ≥ 50%, or Bayesian posterior probabilities (BPP) of ≥ 0.95.
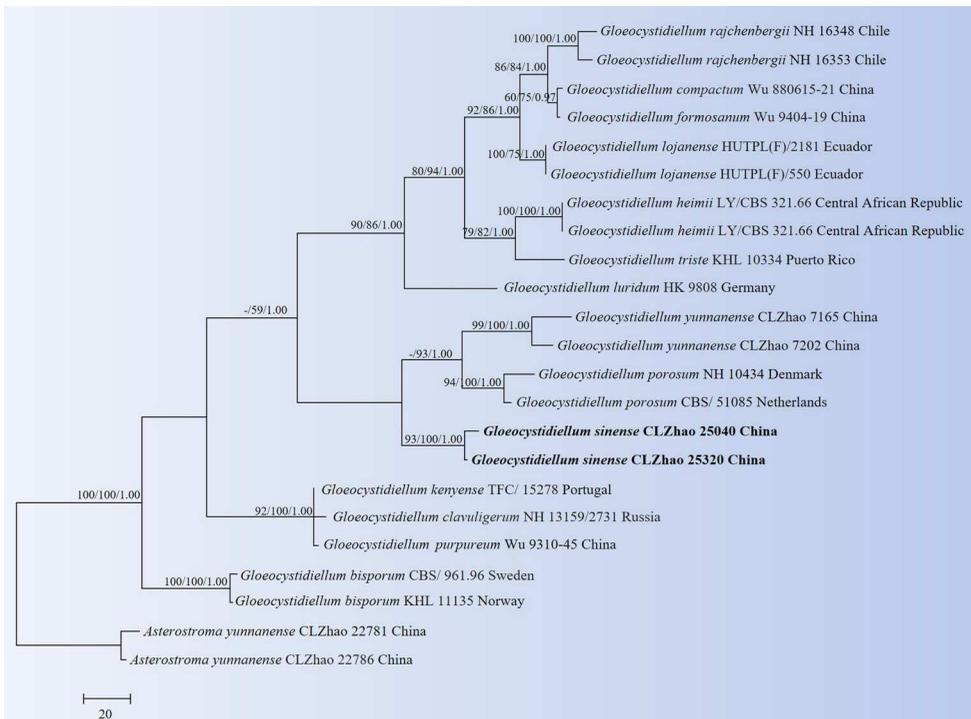
Figure 1. The combined ITS + nLSU sequences datasets were used to infer the position of the new species in the genus *Gloeocystidiellum* and related species. Branches are labelled with Maximum Likelihood bootstrap values equal to or above 70%, Maximum Parsimony bootstrap values equal to or above 50% and Bayesian posterior probabilities equal to or above 0.95. Newly generated specimens are shown in bold.

Results

Molecular phylogeny

The aligned dataset comprised 24 specimens representing 16 species (Figure 1). Four Markov chains were run for two runs from random starting trees, each for one million generations for the combined ITS + nLSU (Figure 1) data set with trees and parameters sampled every 1000 generations. The dataset had an aligned length of 1689 characters, of which 1297 characters are constant, 81 are variable and parsimony uninformative, and 311 are parsimony informative. Maximum parsimony analysis yielded 3 equally parsimonious trees (TL = 727, CI = 0.7378, HI = 0.2613, RI = 0.8426, and RC = 0.6224). The best model for the ITS + nLSU dataset, estimated and applied in the Bayesian analysis, was GTR + I + G (lset nst = 6, rates = invgamma); prset statefreqpr = dirichlet (1,1,1,1). Both Bayesian analysis and ML analyses resulted in a similar topology to MP analysis with an average standard deviation of split frequencies = 0.006392 (BI), and the effective sample size (ESS) for Bayesian analysis across the two runs is double the average ESS (avg. ESS) = 991.

The phylogram based on the combined ITS + nLSU sequences (Figure 1) indicated that the new species *Gloeocystidiellum sinense* was assigned to the genus *Gloeocystidiellum*, in which it formed a single lineage with a high support (93/100/1.00) and then grouped with *G. porosum* and *G. yunnanense* Y.L. Zhao & C.L. Zhao with a lower support rate.

Taxonomy

Gloeocystidiellum sinense S.C. Zhang & C.L. Zhao *sp. nov.* (Figures 2 and 3)

    MycoBank no: MB 856301.

    Etymology: *sinense* (Lat.): refers to the locality (China) of the holotype.

    Holotype: China, Yunnan Province, Lincang, Fengqing County, Lancangjiang Provincial Nature Reserve, 24°32′N, 99°58′E, altitude: 1748m asl., on a the fallen branch of angiosperm, leg. C.L. Zhao, 21 October 2022, CLZhao 25320 (SWFC).

    Basidiomata: Annual, resupinate, adnate but easily separable, coriaceous, without odour and taste when fresh and up to 4.5 cm long, 1.2 cm wide, 100–300 μm thick.
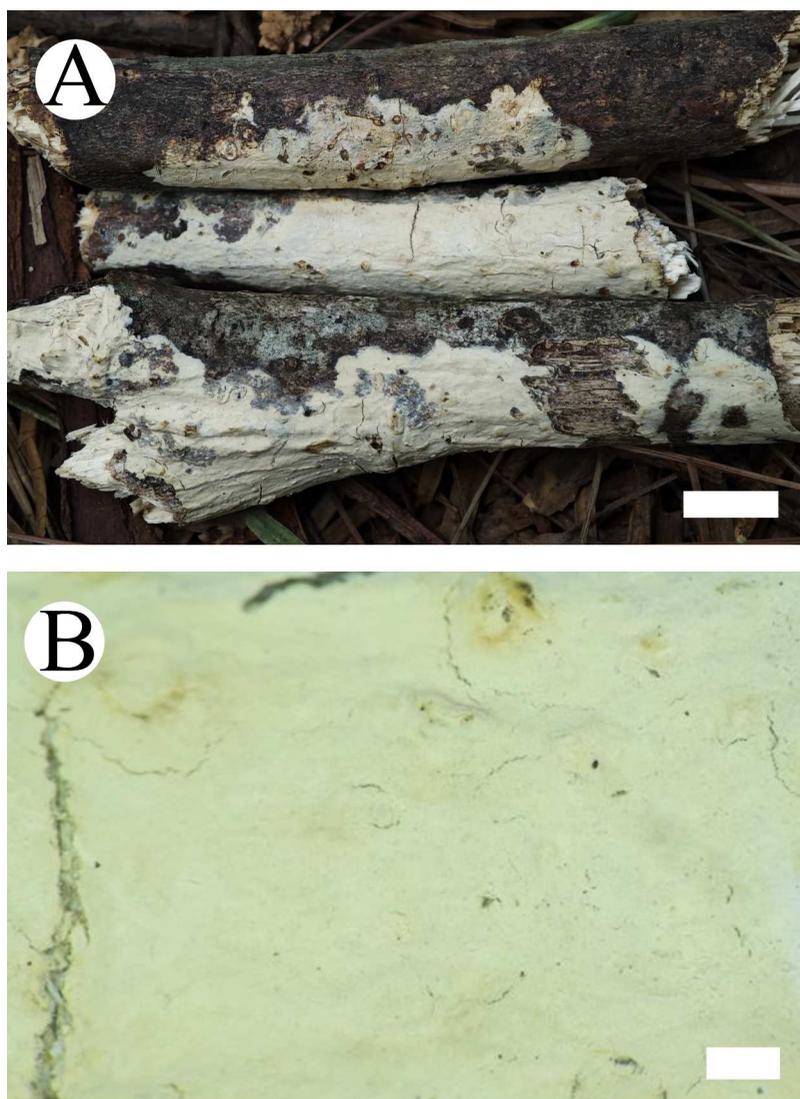

Figure 2. Basidiomata of *Gloeocystidiellum sinense* (CLZhao 25320, holotype). Scale bars: **A** = 1 cm, **B** = 1 mm.
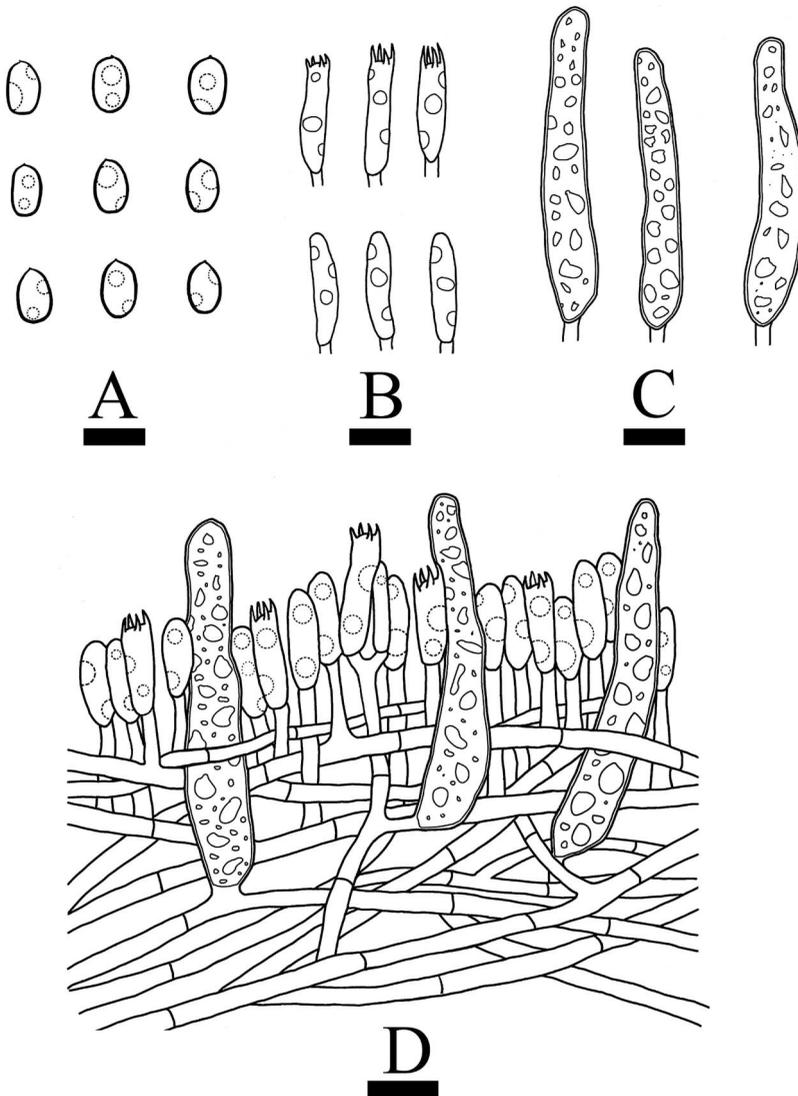
Figure 3. Microscopic structures of *Gloeocystidiellum sinense* (CLZhao 25320, holotype). **A,** Basidiospores. **B,** Basidia and basidioles. **C,** Gloeocystidia. **D,** A section of hymenium. Scale bars: **A** = 5 μm, **B** – **D** = 10 μm.

Hymenial surface smooth, cream to pink-buff when fresh, turn to flesh-pink upon drying. Sterile margin slightly cream and up to 1 mm wide.

Hyphal system: Monomitic; generative hyphae with simple-septa, colourless, thin-walled, smooth, branched, interwoven, 2–3 μm wide, IKI –, CB –, tissues unchanged in KOH.

Hymenium: Cystidia abundant, tubular, colourless, thick-walled, 44–52 × 7–9.5 μm; cystidioles absent. Basidia subcylindrical to subclavate, with a simple septum and four sterigmata, 17.5–20 × 3–4.5 μm; with oil drops, basidioles numerous, in shape similar to basidia but smaller.

Spores: Basidiospores ellipsoid, colourless, thin-walled, smooth, with oil drops, IKI –, CB –, (3.5 –)3.7–4.4 × 2.8–3.2 μm, L = 4.12 μm, W = 3.00 μm, Q = 1.31–1.37 μm (n = 60/2).

Additional specimen (paratype) examined: China, Yunnan Province, Lincang, Yun County, Maolan Town, Shiyan Village, GPS coordinates 24°16′N, 100°13′E, altitude: 1783m asl., on the fallen branch of angiosperm, leg. C.L. Zhao, 19 October 2022, CLZhao 25040 (SWFC).

Discussion

In the present study, one new species *Gloeocystidiellum sinense,* is described based on phylogenetic analyses and morphological characteristics.

The phylogenetic analyses inferred from ITS + nLSU dataset indicated that the new species *Gloeocystidiellum sinense* nested within the genus *Gloeocystidiellum*, in which it formed a single lineage and grouped with *G. porosum* and *G. yunnanense* within a clade. However, *Gloeocystidiellum porosum* differs from *G. sinense* by having the yellowish to isabelline hymenophore, and tubercular basidiospores, 85% (562/664 bp) differences in ITS sequence (Donk 1931; Larsson and Hallenberg 2001). The species *Gloeocystidiellum yunnanense* differentiates from *G. sinense* by having grandinoid hymenial surface, thin-walled gloeocystidia, and thick-walled, aculeate basidiospores, 84% (566/671 bp) differences in ITS sequence (3.9–4.7 × 3–3.5 μm vs. 3.7–4.4 × 2.8–3.2 μm; Zhao and Zhao 2023).

Morphologically, the species *Gloeocystidiellum sinense* resembles three taxa of *G. fimbriatum* Burds., Nakasone & G.W. Freeman, *G. lojanense* A. Jaram., D. Cruz & Decock and *G. rajchenbergii* Gorjón & Hallenberg in sharing the ellipsoid basidiospores. However, *Gloeocystidiellum fimbriatum* is different from *G. sinense* by having a pale yellow or yellowish white hymenial surface, and echinulate to granulose, larger basidiospores (5.5–6 × 3.5–4 vs. 3.7–4.4 × 2.8–3.2 μm; Burdsall et al. 1981). The species *G. lojanense* is different from *G. sinense* by having a bright grayish white hymenial surface, and larger, tubercular basidiospores (6.5–8 × 3.4–4.5 μm; Jaramillo-Riofrío et al. 2023). The species *G. rajchenbergii* differs from *G. sinense* by having tubular, thick-walled cystidia and longer basidiospores (6–7 × 3–3.5 vs. 3.7–4.4 × 2.8–3.2 μm; Gorjón and Hallenberg 2012).

China is an important reservoir of fungal diversity in Asia and the genus *Gloeocystidiellum* has 10 accepted species of this genus in China, accounting for 26% of the world's fungal (Larsson and Larsson 2003; Dai 2011; Zhao and Zhao 2023). Morphologically, *G. aspellum* differs from *G. sinense* by having a greyish orange to light orange, smooth or slightly tuberculate hymenial surface, longer gloeocystidia (150 × 5–10 μm) and larger basidiospores (7–8 × 3.5–4 vs. 3.7–4.4 × 2.8–3.2 μm; Hjortstam 1987). The species *G. clavuligerum* differs from *G. sinense* in its smooth, light buff to pinkish buff hymenial surface, pruinose to farinaceous margin, and larger, tubercular basidiospores (4.5–5.5 × 3.5–4 vs. 3.7–4.4 × 2.8–3.2 μm; Nakasone 1982).

Despite the discovery of high species diversity among corticioid fungi in recent years, systematic studies still need to be conducted for many groups (Bernicchia and Gorjón 2010; Dai 2011; Gorjón and Hallenberg 2012; Dai et al. 2015; Justo et al. 2017;

Jaramillo-Riofrío et al. 2023; Zhao and Zhao 2023). Corticioid fungi is an extensively studied group of Basidiomycota R.T. Moore, of which about 12 order and 26 families belong to this group (Bernicchia and Gorjón 2010; Dai 2011; Dai et al. 2015; Justo et al. 2017; He et al. 2019; He et al. 2024). However, the diversity of cortical fungi in China is not well known, especially in subtropical and tropical regions (Cui and Dai 2006; Cui 2009; Zhao et al. 2015; Yuan et al. 2016; Zhao et al. 2016; Wu et al. 2018; Cui et al. 2019; He et al. 2019; Shen et al. 2019; Zhao et al. 2019; Zhu et al. 2019; Wu et al. 2021; Dong et al. 2023a, 2023b; He et al. 2024, Yuan and Zhao 2024; Zhao et al. 2024; Dong et al. 2024a; 2024b). This paper enriches our knowledge of fungal diversity in this corticioid fungi, and likely, more new taxa will be found with further fieldwork and molecular analyses.

Disclosure statement

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