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Gloeodontia yunnanensis sp. nov. (Russulales, Basidiomycota) from China, evidenced by morphological characters and phylogenetic analyses

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Abstract

A new wood-inhabiting fungal species, *Gloeodontia yunnanensis*, is proposed based on a combination of morphological features and DNA data. The species is characterized by an annual, resupinate basidiomata with smooth hymenial surface, a monomitic hyphal system with thin-walled, clamped generative hyphae and obclavate cystidia and subglobose to globose, hyaline, thick-walled, asperulate, strongly amyloid, acyanophilous basidiospores measuring $3.3\text{--}4.3 \times 2.5\text{--}3.5 \mu\text{m}$. Sequences of ITS and 28S gene regions of the studied samples were generated and phylogenetic analyses were performed with Maximum Likelihood, Maximum Parsimony and Bayesian Inference methods. The analyses based on ITS+28S sequences showed that *G. yunnanensis* nested in the *Gloeodontia* clade and formed a monophyletic lineage with strong support (100% BS, 100% BP, 1.00 BPP).

Keywords: Gloeodontiaceae, *Gloeocystidiellum*, taxonomy, Yunnan Province

Introduction

The genus *Gloeodontia* Boidin (1966: 22) was typified by *G. discolor* (Berk. & M.A. Curtis) Boidin (1966: 22) (Boidin 1966), and characterized by a combination of resupinate or effused-reflexed basidiomata of ceraceous to membranaceous consistency, smooth to odontoid hymenophore, a monomitic or dimitic hyphal structure, clamped hyphae, presence of cystidia, cylindrical to urniform basidia, and hyaline to pale yellow, thick-walled, asperulate, ellipsoid to globose, acyanophilous and amyloid basidiospores (Boidin 1966, Bernicchia & Gorjón 2010). So far eight species have been accepted in the genus (Burdshall & Lombard 1976, Hjortstam 1987, Rajchenberg 1987, Telleria *et al.* 2008, Larsson & Larsson 2003, Gorjón & Jesus 2012, Zhou & Dai 2013).

Recently, molecular studies involving *Gloeodontia* have been carried out (Larsson & Larsson 2003, Zhou & Dai 2013, Leal-Dutra *et al.* 2018). Phylogenetic relationships of russuloid basidiomycetes with an emphasis on non-gilled taxa revealed that four species of *Gloeodontia* formed a monophyletic clade that grouped with Russulales and Stereales clades, and additionally, one new combination *G. subasperispora* (Litsch.) E. Larss. & K.H. Larss. (2003: 1062) was proposed (Larsson & Larsson 2003). Later, a taxonomic work on wood-inhabiting hydroid species in the Russulales showed that five *Gloeodontia* species grouped together as a well-supported clade (Zhou & Dai 2013). Leal-Dutra *et al.* (2018) studied Lachnocladiaceae and Peniophoraceae (Russulales) and showed that *Gloeodontia* formed a single clade and then grouped with the clades Amylostereaceae, Auriscalpiaceae, Bondarzewiaceae, *Gloeocystidiellum*, Russulaceae and Stereaceae.

During studies of wood-inhabiting fungi in southern China, an additional taxon resembling a *Gloeodontia* with a smooth hymenophore could not be assigned to any described species. In this paper, the authors examine the taxonomy and phylogeny of this new species, based on the internal transcribed spacer (ITS) regions and 28S sequences.

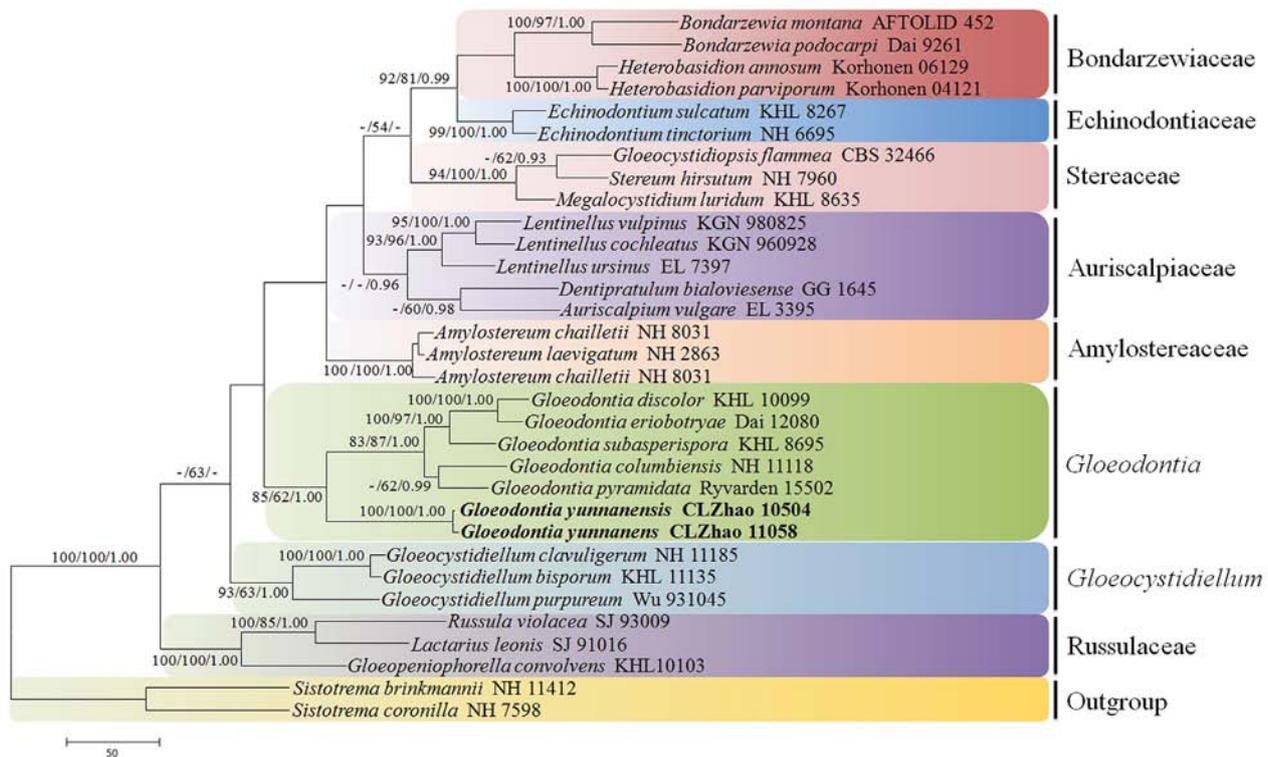


FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Gloeodontia yunnanensis* and related species in the Russulales based on ITS+28S sequences. Branches are labelled with maximum likelihood bootstrap values higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95, respectively.

Materials and methods

Morphological studies:—The two specimens studied are deposited at the herbarium of Southwest Forestry University, Kunming, Yunnan Province, P.R. China (SWFC). Macromorphology descriptions are based on field notes. Micromorphology was studied on dried specimens using a light microscope following Dai (2012). The following abbreviations were used: KOH = potassium hydroxide 5% water solution, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer’s reagent, IKI– = both inamyloid and non-dextrinoid, L = mean spore length (arithmetic average), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n = a/b—number of spores (a) measured from given number (b) of specimens.

Molecular procedures and phylogenetic analyses:—CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from dried specimens, according to the manufacturer’s instructions. The ITS region was amplified with the primer pair ITS5 and ITS4 (White *et al.* 1990). The 28S region was amplified with the primer pair LR0R and LR7 (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>). 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 28S was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 48 °C 1 min and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min (Shen *et al.* 2019). The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited at GenBank (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequences. Sequences were aligned in MAFFT 7 (<http://mafft.cbrc.jp/alignment/server/>) using the “G-INS-i” strategy and adjusted manually in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (ID 25437). Sequences of *Sistotrema brinkmannii* (Bres.) J. Erikss. (1948:134) and *S. coronilla* (Höhn.) Donk ex D.P. Rogers (1935: 23) obtained from GenBank were used as outgroup to root trees following Leal-Dutra *et al.* (2018).

Maximum parsimony analysis was applied to the ITS+28S dataset sequences. Procedure of this analysis followed Zhao & Wu (2017), and the tree construction was performed in PAUP* version 4.0b10 (Swofford 2002). All characters

were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 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 viz., tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree generated. The data matrix was also analyzed using the Maximum Likelihood (ML) approach with RAxML-HPC2 software through the Cipres Science Gateway (www.phylo.org, Miller *et al.* 2009). Branch support (BS) for ML analysis was determined through 1000 bootstrap replicates.

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

Species name	Specimen No.	GenBank accession No.		References
		ITS	28S	
<i>Amylostereum areolatum</i>	NH 8041 (NH)	AF506405	AF506405	Larsson & Larsson 2003
<i>A. chailletii</i>	NH 8031 (NH)	AF506406	AF506406	Larsson & Larsson 2003
<i>A. laevigatum</i>	NH 2863 (NH)	AF506407	AF506407	Larsson & Larsson 2003
<i>Auriscalpium vulgare</i>	EL 3395	AF506375	AF506375	Larsson & Larsson 2003
<i>Bondarzewia montana</i>	AFTOL ID 452 (DAOM)	DQ200923	DQ234539	Larsson & Larsson 2003
<i>B. podocarp</i>	Dai 9261	KJ583207	KJ583221	Chen & Shen 2014
<i>Dentipratulum bialoviesense</i>	GG 1645	AF506389	AF506389	Larsson & Larsson 2003
<i>Echinodontium sulcatum</i>	KHL 8267	AF506414	AF506414	Larsson & Larsson 2003
<i>E. tinctorium</i>	NH 6695 (NH)	AF506430	AF506430	Larsson & Larsson 2003
<i>Gloeocystidiellum bisporum</i>	KHL 11135	AY048877	AY048877	Larsson & Larsson 2003
<i>G. clavuligerum</i>	NH 11185 (NH)	AF310088	AF310088	Larsson & Hallenberg 2001
<i>G. purpureum</i>	Wu 9310-45	AF441338	AF441338	Larsson & Larsson 2003
<i>Gloeocystidiopsis flammea</i>	CBS 324.66 (CBS)	AF506437	AF506437	Larsson & Larsson 2003
<i>Gloeodontia columbiensis</i>	NH 11118 (NH)	AF506444	AF506444	Larsson & Larsson 2003
<i>G. discolor</i>	KHL 10099	AF506445	AF506445	Larsson & Larsson 2003
<i>G. eriobotryae</i>	Dai 12080	JQ349116	JQ349103	Zhou & Dai 2013
<i>G. pyramidata</i>	Ryvarden 15502 (LR)	AF506446	AF506446	Larsson & Larsson 2003
<i>G. subasperispora</i>	KHL 8695	AF506404	AF506404	Larsson & Larsson 2003
<i>G. yunnanensis</i>	CLZhao 10504	MN908252	MN908254	This study
<i>G. yunnanensis</i>	CLZhao 11058	MN908253	MN908255	This study
<i>Gloeopeniophorella convolvens</i>	KHL 10103	AF506435	AF506435	Larsson & Larsson 2003
<i>Heterobasidium annosum</i>	Korhonen 06129	KJ583211	KJ583225	Chen & Shen 2014
<i>H. parviporum</i>	Korhonen 04121	KJ583212	KJ583226	Chen & Shen 2014
<i>Lactarius leonis</i>	SJ 91016 (SJ)	AF506411	AF506411	Larsson & Larsson 2003
<i>Lentinellus cochleatus</i>	KGn 960928	AF506417	AF506417	Larsson & Larsson 2003
<i>L. ursinus</i>	EL 7397	AF506419	AF506419	Larsson & Larsson 2003
<i>L. vulpinus</i>	KGn 980825	AF347097	AF347097	Larsson <i>et al.</i> 2004
<i>Megalocystidium luridum</i>	KHL 8635	AF506473	AF506473	Larsson & Larsson 2003
<i>Russula violacea</i>	SJ 93009 (SJ)	AF506465	AF506465	Larsson & Larsson 2003
<i>Sistotrema brinkmannii</i>	NH 11412 (NH)	AF506473	AF506473	Larsson & Larsson 2003
<i>S. coronilla</i>	NH 7598 (NH)	AF506475	AF506475	Larsson & Larsson 2003
<i>Stereum hirsutum</i>	NH 7960 (NH)	AF506479	AF506479	Larsson & Larsson 2003

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference of the phylogeny (BI). BI was calculated with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 460,000 generations and trees were sampled every 100 generations. The first one-fourth of the generations was discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum

likelihood bootstrap (ML) >70%, maximum parsimony bootstrap (MP) >50%, or Bayesian posterior probability (PP) >0.95.

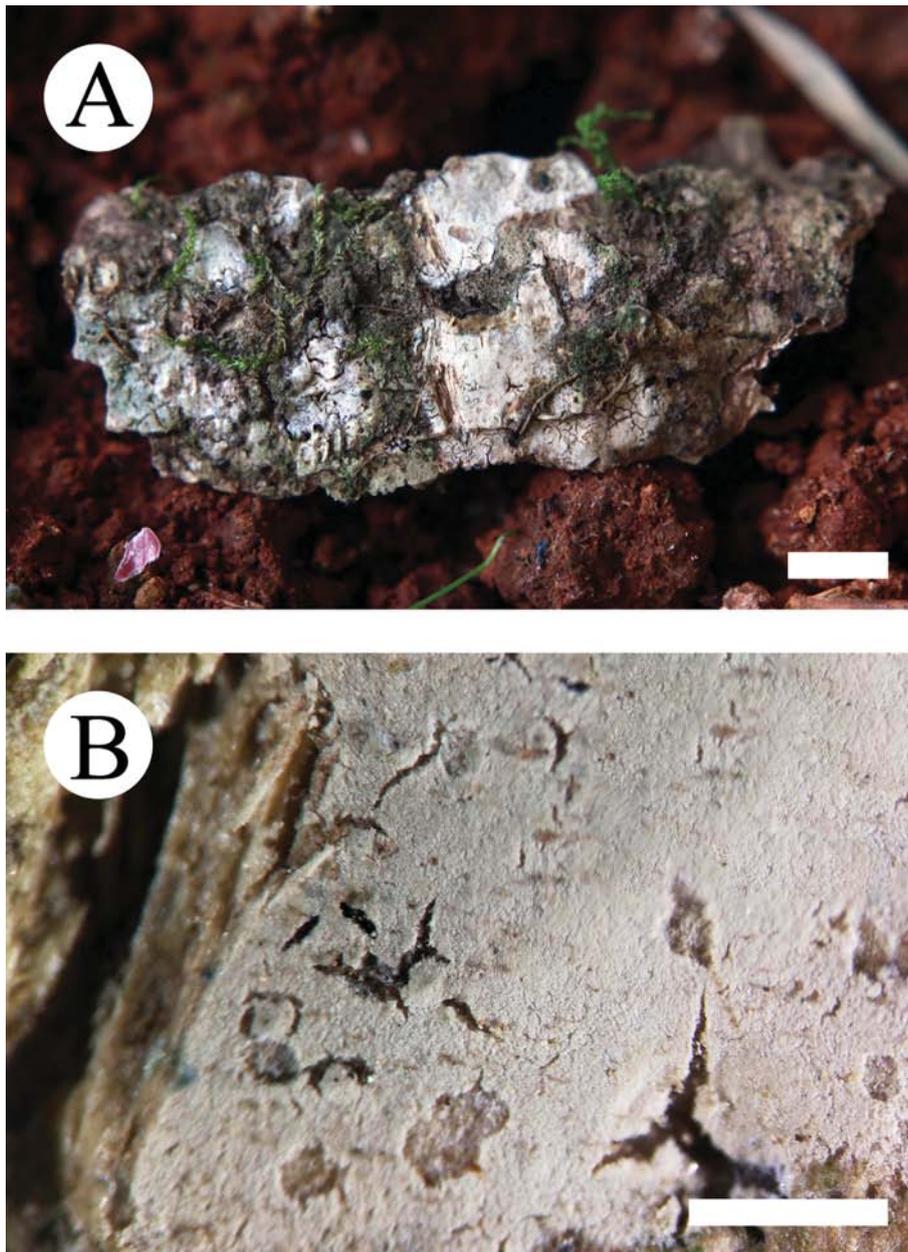


FIGURE 2. Basidiomata of *Gloeodontia yummanensis* (holotype). Bars: A = 0.5 cm, B = 1 mm. Photos by: Changlin Zhao.

Results

Molecular phylogeny

The ITS+28S dataset included sequences from 32 fungal specimens and 31 species. The dataset had the aligned length of 1999 characters, of which 1303 characters are constant, 243 are variable and parsimony-uninformative, and 453 are parsimony-informative. Maximum parsimony analysis yielded 2 equally parsimonious trees (TL = 2058, CI = 0.4956, RI = 0.5506, RC = 0.2729, HI = 0.5044). Best model for the ITS+28S dataset estimated and applied in BI was GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies in BI = 0.005526.

The phylogenetic tree (Fig. 1) revealed that the new species nested in the *Gloeodontia* clade and then formed a monophyletic lineage with a high support (100% BS, 100% BP and 1.00 BPP).

In addition, the results of BLAST queries in NCBI based on ITS and nLSU separately showed the sequences

producing significant alignments descriptions: in ITS blast results, the top one taxon is *Gloeodontia subasperispora* (Maximum record descriptions: Max score 523; Total score 523; Query cover 95%; E value 3e-144; Ident 83%). In nLSU blast results, the top one taxon is *G. subasperispora* (Maximum record descriptions: Max score 2281; Total score 2281; Query cover 98%; E value 0.0; Ident 96.85%).

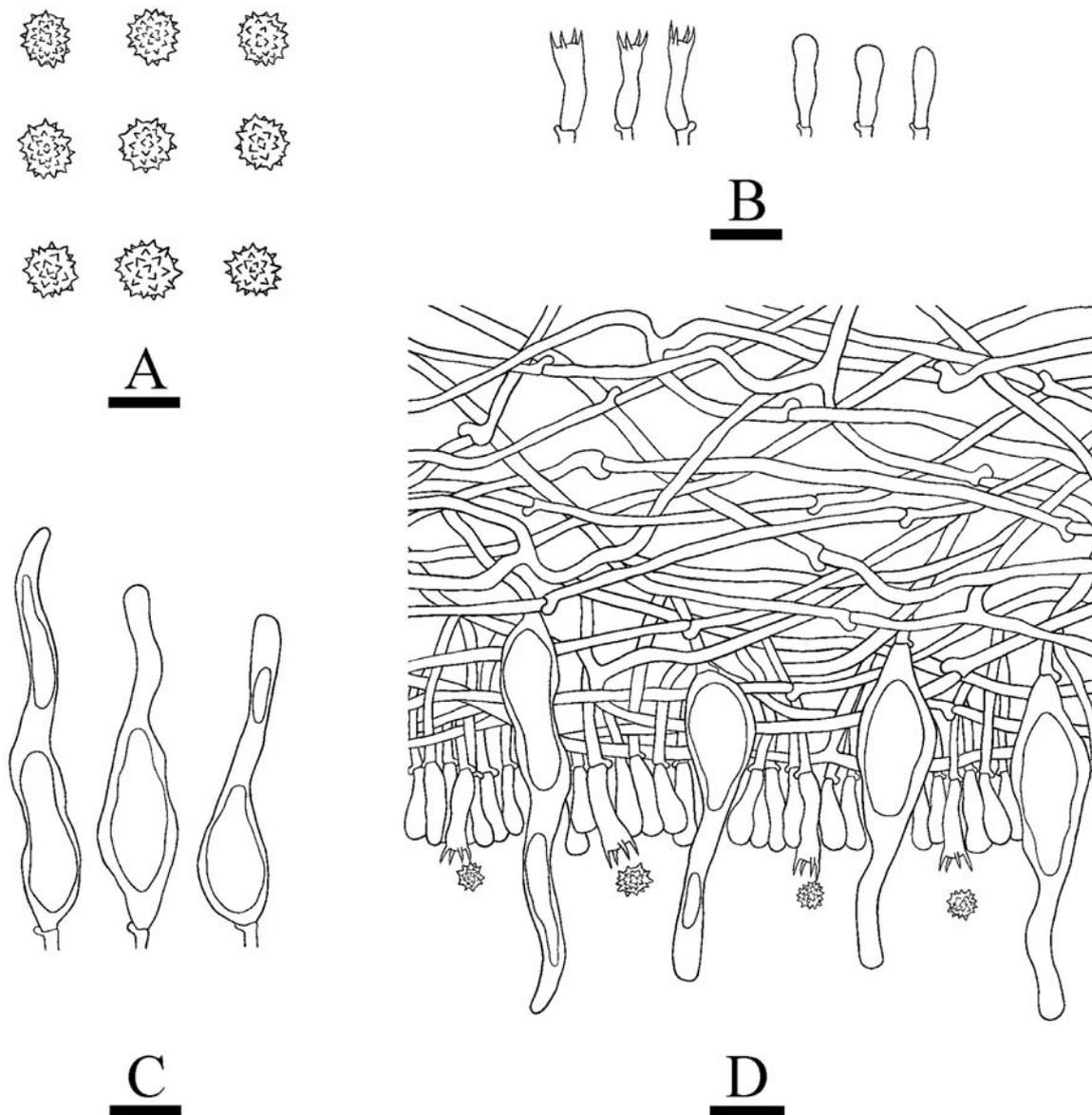


FIGURE 3. Microscopic structures of *Gloeodontia yunnanensis* (drawn from the holotype). a. Basidiospores. b. Basidia and basidioles. c. Cystida. d. A section of hymenium. Bars: a = 5 μ m; b, c, d = 10 μ m. Drawings by: Lu Chen.

Taxonomy

Gloeodontia yunnanensis C.L. Zhao *sp. nov.* (Figs. 2, 3)

Mycobank no.: MB 833888X

Holotype:—China. Yunnan Province: Dali, Nanjian County, Lingbaoshan National Forest Park, on angiosperm trunk, 10 January 2019, CLZhao 10504 (SWFC).

Etymology:—the epithet refers to the locality (Yunnan Province) of the type specimens.

Description:—*Basidiomata* annual, effused, ceraceous, up to 8 cm long, 4 cm wide, 400–800 μ m thick. Hymenial

surface smooth, cream to flesh pink when fresh, buff to brownish vinaceous upon drying. *Hyphal system* monomitic; generative hyphae with clamp connections, thin-walled, 1.5–2.5 µm in diameter, IKI–, CB–; tissues unchanged in KOH. *Hymenium* Gloeocystidia obclavate, widened in lower half and with flexuous-cylindrical apex, thin-walled, guttulate, 32–57 × 6–9 µm; basidia clavate, with four sterigmata and clamp connection at base, 9–14 × 3–4 µm, smooth, thin-walled; basidioles dominant, in shape similar to basidia, but slightly smaller. *Basidiospores* subglobose to globose, hyaline, thick-walled, asperulate, strongly amyloid, acyanophilous, 3.3–4.3 × 2.5–3.5 µm, L = 3.78 µm, W = 2.93 µm, Q = 1.22–1.38 (n = 60/2).

Additional specimen examined:—China. Yunnan Province: Wenshan, Xichou County, Xiaoqiaogou National Nature Reserve, on stump of angiosperm, 15 January 2019, CLZhao 11058 (SWFC).

Discussion

Phylogenetically, Leal-Dutra *et al.* (2018) showed that 19 clades were found in the Russulales, in which eight clades were related to the *Gloeodontia* clade. According to our result based on the combined ITS+28S sequence data (Fig. 1), *G. yunnanensis* is nested in the *Gloeodontia* clade with strong support (100% BS, 100% BT, 1.00 BPP).

Gloeodontia yunnanensis belong to the common clade with other *Gloeodontia* species, for which sequences are available: *G. columbiensis* Burt ex Burds. & Lombard (1976: 17), *G. discolor* (Berk. & M.A. Curtis) Boidin (1966: 22), *G. eriobotryae* Y.C. Dai & L.W. Zhou (2013: 644), *G. pyramidata* (Berk. & M.A. Curtis) Hjortstam (1987: 58) and *G. subasperispora* (Litsch.) E. Larss. & K.H. Larss. Morphologically *G. yunnanensis* is different from other *Gloeodontia* species for which DNA data are available. *G. columbiensis* differs by having an odontoid hymenophore with white to pale yellowish hymenial surface and larger basidiospores (5.5–7 × 3.5–4.5 µm, Bernicchia & Gorjón 2010). *Gloeodontia discolor* differs in its hydroid hymenophore with a white hymenial surface (Bresadola 1925). *Gloeodontia eriobotryae* differs from *G. yunnanensis* by having a dimitic hyphal system and encrusted cystidia (Zhou & Dai 2013). *Gloeodontia pyramidata* differs in its hydroid hymenophore and larger basidiospores (5–6.5 × 4–5 µm, Hjortstam 1987). *Gloeodontia subasperispora* differs from *G. yunnanensis* by having reniform basidiospores (Litschauer 1941).

Among the species for which DNA data are not available *Gloeodontia americana* Rajchenb. (1987: 557) differs from *G. yunnanensis* in having larger basidiospores (6–7.5 × 4–4.5 µm, Hjortstam 1987). *Gloeodontia halocystidiata* Gorjón (2012: 43) differs in its odontoid hymenophore, a dimitic hyphal system and presence of halocystidia and encrusted skeletocystidia (Gorjón & Jesus 2012). *Gloeodontia xerophila* Tellería, M. Dueñas, Rodr.-Armas, Beltrán-Tej. & Melo (2008: 673) differs from *G. yunnanensis* in having a tuberculate to odontoid hymenophore and a dimitic hyphal system (Tellería *et al.* 2008).

Gloeodontia is a group of ‘corticoid fungi’ (Bernicchia & Gorjón 2010, Dai 2011), but only one species has been reported from China. Many recently described taxa of corticoid fungi from China were from subtropical and tropical areas (Zhao & Wu 2017, Shen *et al.* 2018, Cui *et al.* 2019, Liu *et al.* 2019, Luo *et al.* 2019, Wu *et al.* 2019). The new species in the present study, *Gloeodontia yunnanensis*, is also from the subtropics. It is possible that new taxa will be found after further investigations.

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