

---

# MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2020

January–March 2020—Volume 135, pp. 103–117

<https://doi.org/10.5248/135.103>

---

## Morphological and molecular identification of *Phlebia wuliangshanensis* sp. nov. in China

RUO-XIA HUANG<sup>1,2</sup>, KAI-YUE LUO<sup>3</sup>, RUI-XIN MA<sup>3</sup>, CHANG-LIN ZHAO<sup>1,2,3\*</sup>

<sup>1</sup>Key Laboratory of State Forestry Administration for Highly Efficient Utilization of  
Forestry Biomass Resources in Southwest China,

<sup>2</sup>Key Laboratory for Forest Resources Conservation and Utilization in the  
Southwest Mountains of China, Ministry of Education, and

<sup>3</sup>College of Biodiversity Conservation,

<sup>1,2,3</sup>Southwest Forestry University, Kunming 650224, P.R. China

\* CORRESPONDENCE TO: [fungichanglinz@163.com](mailto:fungichanglinz@163.com)

**ABSTRACT** —A new white-rot fungus, *Phlebia wuliangshanensis*, is proposed based on a combination of morphological features and molecular evidence. The species is characterized by an annual growth habit, resupinate basidiocarps with a smooth to tuberculate hymenial surface, a monomitic hyphal system with thin- to thick-walled generative hyphae bearing simple septa, presence of cystidia, and narrow ellipsoid to ellipsoid basidiospores (5–6 × 3–3.7 μm). Our phylogenetic analyses of ITS and LSU nrRNA sequences performed with maximum likelihood, maximum parsimony, and Bayesian inference methods support *P. wuliangshanensis* within a phlebioid clade in *Meruliaceae* (*Polyporales*). ITS+nLSU sequence analyses of additional *Phlebia* taxa strongly support *P. wuliangshanensis* within a monophyletic lineage grouped with *P. chrysocreas* and *P. uda*.

**KEY WORDS**—*Basidiomycota*, *Ceriporiopsis*, taxonomy, wood-inhabiting fungi, Yunnan Province

### Introduction

*Phlebia* Fr. (*Meruliaceae*, *Polyporales*) is typified by *P. radiata* Fr. (Fries 1821). Basidiocarps are resupinate or rarely pileate with a subceraceous to subgelatinous consistency when fresh and membranaceous to coriaceous when dry. The hymenophore may be smooth, tuberculate, phlebioid,

odontoid, merulioid, or poroid. Microscopic characters include a monomitic (rarely dimitic) hyphal structure with clamp connections or simple-septa, narrowly clavate basidia, and hyaline basidiospores that are thin-walled, smooth, allantoid to ellipsoid, acyanophilous, and negative in Melzer's reagent (Bernicchia & Gorjón 2010). So far about 100 species have been accepted in the genus worldwide (Fries 1821; Ginns 1969; Nakasone & Burdsall 1984, 1995; Dhingra 1989; Nakasone 1997, 2002, 2003, 2009; Roberts 2000; Gilbertson & Hemmes 2004; Duhem & Michel 2007; Duhem 2009, 2013; Bernicchia & Gorjón 2010; Singh & al. 2010; Westphalen & al. 2018; Gorjón & Greslebin 2012; Kaur & al. 2017; Shen & al. 2018).

Recent, molecular studies have elucidated the classification of *Phlebia* among corticioid homobasidiomycetes (Larsson & al. 2004; Larsson 2007; Tomšovský & al. 2010; Binder & al. 2013, Justo & al. 2017; Shen & al. 2018). Larsson & al. (2004) showed that *Phlebia* clustered into a phlebioid clade and grouped with *Ceriporia* Donk and *Gloeoporus* Mont. Larsson (2007) emended part of *Polyporales* and demonstrated that *Phlebia* was polyphyletic and nested within *Meruliaceae*. A phylogenetic study of European taxa of *Ceriporiopsis* Domański showed that *Phlebia radiata* and the generic type of *Ceriporiopsis*, *C. gilvescens* (Bres.) Domański, grouped closely on the basis of combined nuclear ribosomal large subunit RNA (nLSU) and mitochondrial ribosomal small subunit rRNA (mtSSU) gene sequences (Tomšovský & al. 2010). Binder & al. (2013) multi-gene sequence analyses placed *P. radiata* within a phlebioid clade and apparently grouped with *Ceriporiopsis* and *Climacodon* P. Karst. Also using multi-gene datasets, Justo & al. (2017) revised the family-level classification of *Polyporales*, including eighteen families. They showed that *P. radiata* belonged to *Meruliaceae* and grouped with *Aurantiporus* Murrill and *Ceriporiopsis gilvescens*. Shen & al. (2018) described a new *Phlebia* species based on morphological characters and rDNA sequences. This species belonged within the phlebioid clade and was related to *P. radiata*.

During our investigations of wood-inhabiting fungi in southern China, we found an additional taxon that could not be assigned to any described species. In examining the taxonomy and phylogeny of this new species, we employed a two-gene molecular phylogenetic approach using internal transcribed spacer (ITS) and long subunit (nLSU) plus an expanded sampling of *Phlebia* isolates.

## Materials & methods

The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC). Macro-morphological descriptions are based on field notes. Colour terms follow Petersen (1996). Micro-morphological data were obtained

TABLE 1. Species, specimens, and sequence data used in this study.  
New sequences in **bold**.

SPECIES NAME	SAMPLE NO.	GENBANK ACCESSION NO.		REFERENCES
		ITS	LSU	
<i>Abortiporus biennis</i>	TFRI 274	EU232187	EU232277	Unpublished
<i>Antrodia albida</i>	CBS 308.82	DQ491414	AY515348	Kim & al. 2007
<i>A. heteromorpha</i>	CBS 200.91	DQ491415	AY515350	Kim & al. 2007
<i>Antrodiella americana</i>	Gothenburg 3161	JN710509	JN710509	Binder & al. 2013
<i>A. semisupina</i>	FCUG 960	EU232182	EU232266	Binder & al. 2005
<i>Ceriporiopsis gilvescens</i>	BRNM 710166	FJ496684	FJ496720	Tomšovský & al. 2010
<i>Climacocystis borealis</i>	KH 13318	JQ031126	JQ031126	Binder & al. 2013
<i>Corioloropsis caperata</i>	LE(BIN)-0677	AB158316	AB158316	Tomšovský & al. 2010
<i>Dacryobolus karstenii</i>	KHL 11162	EU118624	EU118624	Binder & al. 2005
<i>Daedalea quercina</i>	HHB 8735	FJ403214	FJ403214	Lindner & al. 2011
<i>Earliella scabrosa</i>	PR 1209	JN165009	JN164793	Binder & al. 2005
<i>Fomitopsis pinicola</i>	CCBAS 536	FJ608588	—	Unpublished
<i>F. rosea</i>	ATCC 76767	DQ491410	DQ491410	Kim & al. 2007
<i>Fragiliporia fragilis</i>	Dai 13080	KJ734260	KJ734264	Zhao & al. 2015a
	Dai 13559	KJ734261	KJ734265	Zhao & al. 2015a
	Dai 13561	KJ734262	KJ734266	Zhao & al. 2015a
<i>Ganoderma lingzhi</i>	Wu 1006-38	JQ781858	—	Zhao & al. 2013
<i>Gelatoporia subvermispora</i>	BRNU 592909	FJ496694	FJ496706	Tomšovský & al. 2010
<i>Gloeoporus dichrous</i>	KHL 11173	EU118627	EU118627	Binder & al. 2005
<i>G. pannocinctus</i>	BRNM 709972	EU546099	FJ496708	Tomšovský & al. 2010
<i>Grammothelopsis subtropica</i>	Cui 9041	JQ845094	JQ845099	Zhao & al. 2013
<i>Heterobasidion annosum</i>	PFC 5252	KC492906	KC492906	Binder & al. 2013
<i>Hornodermoporus martius</i>	MUCL 41677	FJ411092	FJ393859	Robledo & al. 2009
<i>Hydnophlebia chrysohiza</i>	FD-282	KP135338	KP135216	Floudas & Hibbett 2015
<i>Hypochnicium lyndoniae</i>	NL 041031	JX124704	JX124704	Unpublished
<i>Junghuhnia nitida</i>	KHL 11903	EU118638	EU118638	Binder & al. 2005
<i>Obba rivulosa</i>	KCTC 6892	FJ496693	FJ496710	Miettinen & Rajchenberg 2012
				Miettinen & Rajchenberg 2012
<i>O. valdiviana</i>	FF 503	HQ659235	HQ659235	
<i>Phanerochaete chrysosporium</i>	BKM-F-1767	HQ188436	GQ470643	Wu & al. 2010
<i>P. velutina</i>	HHB-15343	KP135184	—	Floudas & Hibbett 2015
<i>Perenniporia medulla-panis</i>	MUCL 49581	FJ411087	FJ393875	Robledo & al. 2009
<i>Perenniporiella neofulva</i>	MUCL 45091	FJ411080	FJ393852	Robledo & al. 2009
<i>Phlebia acanthocystis</i>	FP 150571	KY948767	KY948844	Justo & al. 2017
<i>P. acerina</i>	FD-301	KP135378	KP135378	Justo & al. 2017
	HHB-11146	KP135372	—	Floudas & Hibbett 2015
	FP-135252	KP135371	—	Floudas & Hibbett 2015
	DR-60	KP135375	KF691615	Floudas & Hibbett 2015

Table 1, continued on next page

Table 1, continued

SPECIES NAME	SAMPLE NO.	GENBANK ACCESSION NO.		REFERENCES
		ITS	LSU	
<i>P. ailaoshanensis</i>	CLZhao 3996	MH784926	MH784936	Shen & al. 2018
	CLZhao 4036	MH784927	MH784937	Shen & al. 2018
<i>P. aurea</i>	DLL 2011-100	KJ140614	—	Unpublished
	FCUG 2767	HQ153409	HQ153409	Binder & al. 2013
	NH-14434	AY463445	AY586691	Larsson & al. 2004
	RLG-5075	KY948759	KY948918	Justo & al. 2017
<i>P. centrifuga</i>	HHB-9239	KP135380	KP135262	Floudas & Hibbett 2015
	L-15541	KP135381	—	Floudas & Hibbett 2015
	GB-1013	KP135379	—	Floudas & Hibbett 2015
<i>P. chrysocreas</i>	HHB-6333	KP135358	KP135263	Floudas & Hibbett 2015
	HHB 3946	KP135357	AY586695	Floudas & Hibbett 2015
	FP-102161	AY219367	—	Binder & al. 2005
	KUC 20121123-24	KJ668482	KJ668335	Floudas & Hibbett 2015
<i>P. floridensis</i>	HHB-9905	KP135383	KP135264	Justo & al. 2017
	HHB-6466	KP135385	—	Floudas & Hibbett 2015
	HHB-7175	KP135384	—	Floudas & Hibbett 2015
	FP-102562-T	KP135386	—	Floudas & Hibbett 2015
<i>P. fuscoatra</i> ≡ <i>Mycoacia fuscoatra</i>	HHB-10782	KP135364	KP135265	Floudas & Hibbett 2015
	HHB 15354T	KP135367	KP135363	Floudas & Hibbett 2015
	HHB 18642	KP135366	—	Floudas & Hibbett 2015
	FP-102173	KP135364	—	Floudas & Hibbett 2015
	KHL 13275	JN649352	JN649352	Tomšovský & al. 2010
<i>P. hydnoides</i>	HHB-1993	KY948778	KY948853	Justo & al. 2017
<i>P. lindneri</i>	GB-501	KY948772	KY948847	Justo & al. 2017
<i>P. livida</i>	FCUG 2189	AF141624	AF141624	Tomšovský & al. 2010
<i>P. ludoviciana</i>	FD-427	KP135342	—	Floudas & Hibbett 2015
<i>P. nantahaliensis</i>	HHB-2816	KY948777	KY948852	Justo & al. 2017
<i>P. nothofagi</i> ≡ <i>Mycoacia nothofagi</i>	HHB-4273	KP135369	KP135266	Floudas & Hibbett 2015
	HHB-6906	KP135368	—	Floudas & Hibbett 2015
	HHB-12067	KP135370	—	Floudas & Hibbett 2015
<i>P. radiata</i>	KHL 13750	GU480000	GU480000	Tomšovský & al. 2010
	UBCF 19726	HQ604797	HQ604797	Binder & al. 2013
	AFTOL-ID 484	AY854087	AF287885	Binder & al. 2005
	FD-85	KP135377	KP135377	Justo & al. 2017
	FD-121	KP135376	—	Floudas & Hibbett 2015
<i>P. rufa</i>	HHB-14924	KP135374	KX065989	Floudas & Hibbett 2015
<i>P. setulosa</i>	HHB-6891	KP135382	KP135267	Justo & al. 2017
	AH 31879	GQ259417	GQ259417	Binder & al. 2005
	PH 11749	GU461312	—	Binder & al. 2005
<i>P. subochracea</i>	HHB 8715	KY948770	KY948846	Floudas & Hibbett 2015
<i>P. subserialis</i>	FCUG 1434	AF141631	AF141631	Tomšovský & al. 2010
<i>P. uda</i>	FP-101544	KP135361	KP135232	Floudas & Hibbett 2015
	FCUG 2452	AF141614	—	Unpublished
	USDA Kropp-1	AB084621	—	Suhara & al. 2002
<i>P. wuliangshanensis</i>	CLZhao 3475 T	MK881787	MK881897	Present study
	CLZhao 3639	MK881788	MK881898	Present study
	CLZhao 3645	MK881789	MK881899	Present study

Table 1, concluded

SPECIES NAME	SAMPLE NO.	GENBANK ACCESSION NO.		REFERENCES
		ITS	LSU	
<i>Piloporia sajanensis</i>	Mannine 2733a	HQ659239	HQ659239	Tomšovský & al. 2010
<i>Podoscypha venustula</i>	CBS 65684	JN649367	JN649367	Sjoekvist & al. 2012
<i>Polyporus tuberaster</i>	CuLTENN 10197	AF516596	AJ488116	Binder & al. 2013
<i>Postia guttulata</i>	KHL 11739	EU11865	EU11865	Kim & al. 2007
<i>Pouzaroporia subrufa</i>	BRNM 710164	FJ496661	FJ496723	Tomšovský & al. 2010
<i>Sebipora aquosa</i>	Miettinen 8680	HQ659240	HQ659240	Miettinen & Rajchenberg 2012
<i>Skeletocutis amorphia</i>	Miettinen 11038	FN907913	FN907913	Tomšovský & al. 2010
<i>S. jelicii</i>	H 6002113	FJ496690	FJ496727	Tomšovský & al. 2010
<i>S. portcrosensis</i>	LY 3493	FJ496689	FJ496689	Tomšovský & al. 2010
<i>S. subsphaerospora</i>	Rivoire 1048	FJ496688	FJ496688	Tomšovský & al. 2010
<i>Steccherinum fimbriatum</i>	KHL 11905	EU118668	EU118668	Tomšovský & al. 2010
<i>S. ochraceum</i>	KHL 11902	JQ031130	JQ031130	Tomšovský & al. 2010
<i>Stereum hirsutum</i>	NBRC 6520	AB733150	AB733325	Tomšovský & al. 2010
<i>Truncospora ochroleuca</i>	MUCL 39726	FJ411098	FJ393865	Robledo & al. 2009
<i>Tyromyces chioneus</i>	Cui 10225	KF698745	KF698756	Zhao & al. 2013
<i>Xanthoporus syringae</i>	Gothenburg 1488	JN710607	JN710607	Tomšovský & al. 2010

from the dried specimens and observed under light microscopy following Dai (2012). Abbreviations are: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB- = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios among the specimens studied, n (a/b) = number of spores (a) measured from given number (b) of specimens.

We extracted genomic DNA from dried specimens using HiPure Fungal DNA Mini Kit II (Magen Biotech Co.) according to the manufacturer's instructions with some modifications. A small piece (c. 30 mg) of dried fungal material was ground to powder with liquid nitrogen. The powder was transferred to a 1.5 ml centrifuge tube, suspended in 0.4 ml of lysis buffer, and incubated in a 65 °C water bath for 60 min, after which 0.4 ml phenol-chloroform (24:1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13,000 rpm for 5 min, 0.3 ml supernatant was transferred to a new tube and mixed with 0.45 ml binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation at 13,000 rpm for 0.5 min. Then, 0.5 ml inhibitor removal fluid was added in AC for centrifugation at 12,000 rpm for 0.5 min. After washing twice with 0.5 ml washing buffer, the AC was transferred to a clean centrifuge tube, and 100 µl elution buffer was added to the middle of adsorbed film to elute the genomic DNA. The ITS region was amplified with primer pairs ITS5 and ITS4 (White & al. 1990). Nuclear LSU region was amplified with primer pairs LR0R and LR7 (Vilgalys 2018). The ITS was amplified by 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 nLSU was amplified by 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. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited in GenBank (TABLE 1).

DNA sequences were edited using Sequencher 4.6. Sequences were aligned in MAFFT 7 (Katoh & Toh, 2008) using the “G-INS-I” strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment (ID 24249) was deposited in TreeBase. In FIG. 1, *Heterobasidion annosum* (Fr.) Bref. and *Stereum hirsutum* (Willd.) Pers. were used as outgroup to root the tree following Binder & al. (2013). In FIG. 2, *Hydnophlebia chrysohiza* (Eaton) Parmasto and *Phanerochaete velutina* (DC.) P. Karst. were used as outgroup to root the tree following Floudas & Hibbett (2015).

Phylogenetic analyses of the ITS+nLSU sequences were performed using maximum parsimony, maximum likelihood, and Bayesian inference methods. Maximum parsimony (MP) analyses followed Zhao & Wu (2017), and 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 (BP) analysis with 1,000 replicates (Felsenstein 1985). Tree statistics 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. Sequences were analyzed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (Miller & al. 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 (Posada & Crandall 1998; Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes\_3.1.2 using a general time reversible (GTR+G) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 8 million generations (ITS+nLSU) in FIG. 1, for 5 million generations (ITS+LSU) in FIG. 2 and trees were sampled every 100 generations. The first 25% of the generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum likelihood (BS), maximum parsimony (BP) and Bayesian posterior probabilities (BPP) greater than or equal to 75 % (BP) and 0.95 (BPP) were considered significantly supported, respectively.

## Molecular phylogeny

The first ITS+nLSU dataset (FIG. 1) included sequences from 57 fungal specimens representing 49 species of *Polyporales* plus the outgroup had an aligned length of 2104 characters, of which 1236 characters were constant, 248 variable and parsimony-uninformative, and 620 parsimony-informative.

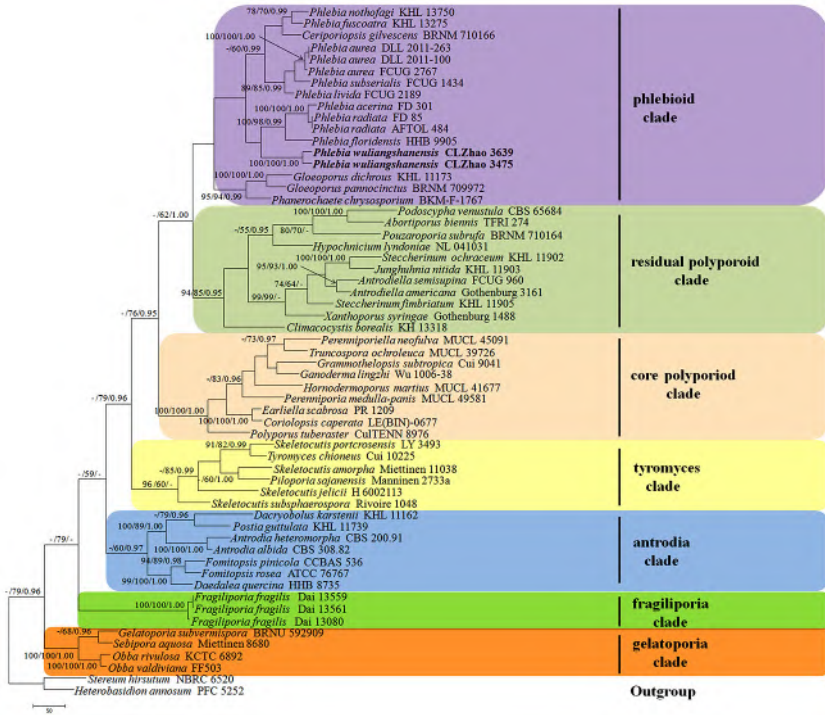


FIG. 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Phlebia wuliangshanensis* and related species in *Polyporales* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap >70%, parsimony bootstrap proportions >50% and Bayesian posterior probabilities >0.95. Clade names follow Binder & al. (2013).

Maximum parsimony analysis yielded 3 equally parsimonious trees (TL = 4783, CI = 0.307, HI = 0.693, RI = 0.526, RC = 0.162). Best model for this dataset estimated and applied in the Bayesian analysis: GTR+I+G, Iset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis produced a similar topology as MP analysis (average standard deviation of split frequencies = 0.003241 (BI)). The tree clustered the 57 polypore species into seven major clades, placing our new species, *Phlebia wuliangshanensis*, in the phlebioid clade (FIG. 1).

The second ITS+nLSU dataset (FIG. 2) comprising sequences from 51 fungal specimens representing 22 *Phlebia* species plus the outgroup had an aligned length of 2102 characters, of which 1588 characters were constant, 142 variable



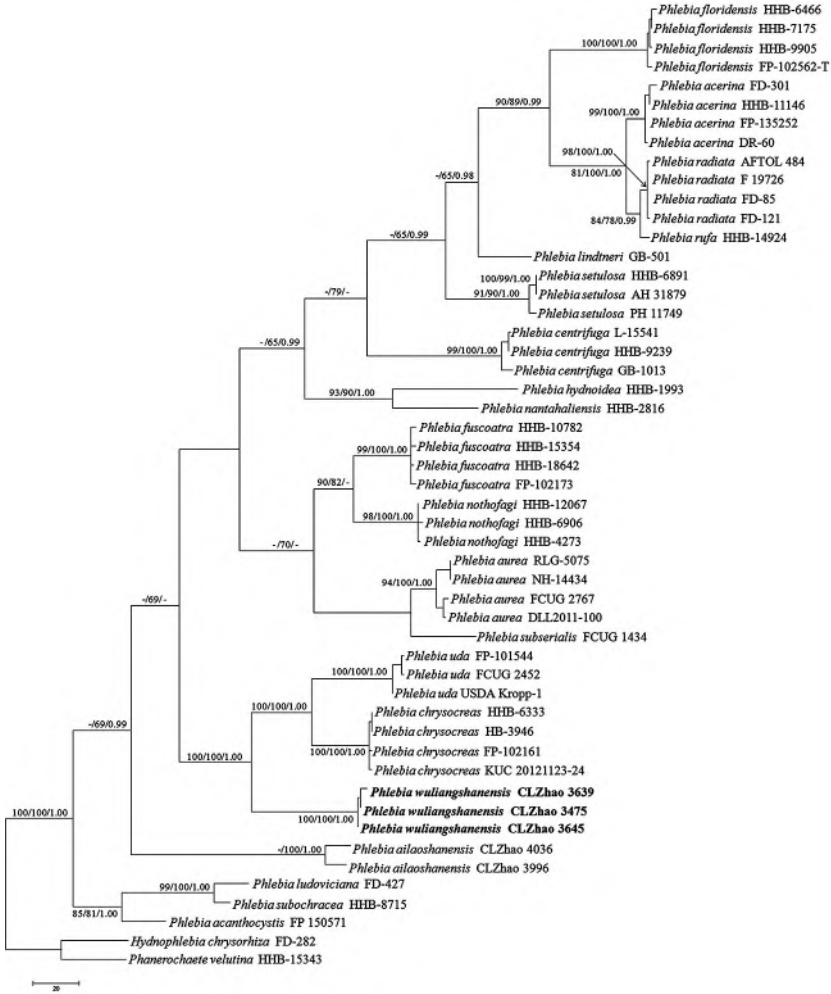


FIG. 2. Maximum parsimony strict consensus tree illustrating the phylogeny of *Phlebia wuliangshanensis* and related species in *Phlebia* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap >70%, parsimony bootstrap proportions >50% and Bayesian posterior probabilities >0.95.

and parsimony-uninformative, and 372 parsimony-informative. Maximum parsimony analysis yielded 10 equally parsimonious trees (TL = 1113, CI = 0.518, HI = 0.482, RI = 0.873, RC = 0.403). Best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis: GTR+I+G, lset nst = 6,



rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Here also Bayesian analysis and ML analysis generated a similar topology as MP analysis (average standard deviation of split frequencies = 0.002221 (BI). The phylogeny (FIG. 2) inferred from the combined ITS+nLSU sequences from 49 *Phlebia* isolates grouped the new species with *P. chrysocreas* (Berk. & M.A. Curtis) Burds. and *P. uda* (Fr.) Nakasone.

### Taxonomy



FIG. 3. *Phlebia wuliangshanensis* (holotype, SWFC 003475). Scale bar = 1 cm.

*Phlebia wuliangshanensis* C.L. Zhao, sp. nov.

Figs 3, 4

MB 830801

Differs from *Phlebia chrysocreas* by its narrower ellipsoid basidiospores and from *P. uda* by its smooth to tuberculate hymenium and generative hyphae with simple septa.

TYPE: China. Yunnan Province: Puer, Jingdong County, Wuliangshan National Nature Reserve, on angiosperm trunk, 2 October 2017, CLZhao 3475 (Holotype, SWFC 003475; GenBank MK881787, MK881897).

ETYMOLOGY: The specific epithet *wuliangshanensis* (Lat.) refers to the locality (Wuliangshan) of the type specimen.

**BASIDIOMATA** annual, resupinate, easily separable from the substratum, ceraceous to gelatinous, without odor or taste when fresh, becoming membranaceous upon drying,  $\leq 12$  cm long, 200–700  $\mu\text{m}$  thick. Hymenial

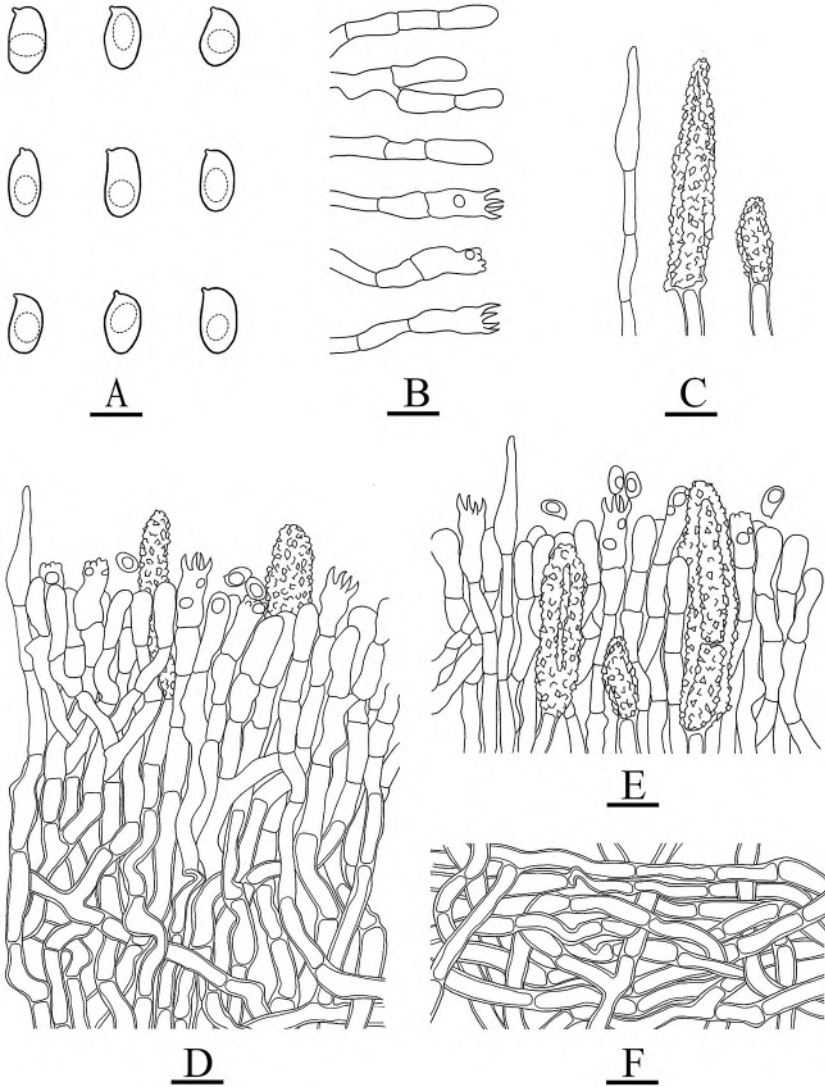


FIG. 4. Microscopic structures of *Phlebia wuliangshanensis* (drawn from the holotype, SWFC 003475). A. Basidiospores; B. Basidia and basidioles; C. Cystidia; D. Basidiocarp section; E. Hymenium section; F. Hyphae from subiculum. Scale bars: A = 5  $\mu$ m; B–F = 10  $\mu$ m.

surface smooth to tuberculate, white to cream to pale brown when fresh, cream to pale brown upon drying. Sterile margin distinct, white.

**HYPHAL STRUCTURE** monomitic; generative hyphae with simple septa, negative in Melzer's reagent, CB-; tissues unchanged in KOH.

**SUBICULUM** generative hyphae hyaline, thin- to thick-walled, branched, 3–5.5  $\mu\text{m}$  in diam.

**HYMENIUM** cystidia of two kinds: 1) hyaline, cylindrical, numerous, thick-walled, strongly encrusted, 20–55  $\times$  6–10.5  $\mu\text{m}$ ; 2) hyaline, lanceolate, few, thin-walled, 12–18  $\times$  3–4.5  $\mu\text{m}$ ; basidia barrel-shaped, with four sterigmata and a simple basal septum, 10–16  $\times$  4–5  $\mu\text{m}$ ; basidioles dominant, in shape similar to basidia, but slightly smaller.

**BASIDIOSPORES** narrowly ellipsoid to ellipsoid, more or less curved, hyaline, thin-walled, smooth, negative in Melzer's reagent, CB-, 5–6(–6.5)  $\times$  3–3.7  $\mu\text{m}$ , L = 5.42  $\mu\text{m}$ , W = 3.32  $\mu\text{m}$ , Q = 1.64–1.73 (n = 180/3).

**TYPE OF ROT:** white.

**ADDITIONAL SPECIMENS EXAMINED:** CHINA. YUNNAN PROVINCE. Puer: Jingdong County, Wuliangshan National Nature Reserve, on angiosperm trunk, 2 October 2017, CLZhao 3639 (SWFC 003639; GenBank MK881788, MK881898), CLZhao 3645 (SWFC 003645; GenBank MK881789, MK881899).

## Discussion

We describe a new species, *Phlebia wuliangshanensis*, based on phylogenetic analyses and morphological characters.

Previously, seven clades were found in *Polyporales*: /antrodia, /core polyporoid, /fragiliporia, /gelatoporia, /phlebioid, /residual polyporoid, and /tyromyces (Binder & al. 2013, Zhao & al. 2015). Our combined ITS+nLSU sequence analysis (FIG. 1) strongly supports *Phlebia wuliangshanensis* (100% BS, 100% BP, 1.00 BPP) within the phlebioid clade and related to the type species, *P. radiata*.

*Phlebia wuliangshanensis* is closely related to *P. chrysocreas* and *P. uda* in the ITS+nLSU phylogenetic tree (FIG. 2). Morphologically, *P. chrysocreas* differs from *P. wuliangshanensis* by having ochraceous-buff to yellow ochre color hymenophore and narrowly ovoid basidiospores (4–6  $\times$  2–2.5  $\mu\text{m}$ , Lombard & al. 1975), while *P. uda* presents an odontoid hymenophore, generative hyphae bearing clamp connections, and smaller basidiospores (5–5.5  $\times$  2–2.5  $\mu\text{m}$ , Bernicchia & Gorjón 2010).

*Phlebia wuliangshanensis* morphologically resembles other *Phlebia* species: *P. bispora* (Stalpers) Nakasone, *P. capitata* Bernicchia & Gorjón, *P. coccineofulva* Schwein., *P. fensjoeensis* (Litsch. & S. Lundell) J. Erikss.

& Hjortstam, *P. livida* (Pers.) Bres., *P. nothofagi* (G. Cunn.) Nakasone, *P. radiata*, *P. rufa* (Pers.) M.P. Christ., *P. segregata* (Bourdot & Galzin) Parmasto, and *P. subserialis* (Bourdot & Galzin) Donk. These species differ from *P. wuliangshanensis* as follows: *P. bispora* by a hydroid hymenophore, dimitic hyphal system, and smaller basidiospores ( $4\text{--}5 \times 2.5\text{--}3 \mu\text{m}$ , Nakasone 2002); *P. capitata* by an odontoid hymenophore, generative hyphae with clamps, and the presence of capitate cystidia (Bernicchia & Gorjón 2010); *P. coccineofulva* by a granular hymenophore with vivid yellow radiating margin and metuloid cystidia (Schweinitz 1832); *P. femsjoeensis* by orange to violaceous basidiocarps (Eriksson & al. 1981); *P. livida* by its reddish hymenophore and a monomitic hyphal system with generative hyphae with clamps (Bernicchia & Gorjón 2010); *P. nothofagi* by effused basidiocarps with a hydroid hymenophore, a monomitic hyphal system with generative hyphae with clamps, and narrower basidiospores ( $4\text{--}6 \times 2\text{--}3 \mu\text{m}$ , Nakasone 1997); *P. rufa* by its effused basidiocarps with the reticulate or subporoid hymenophore (Christiansen 1960); *P. segregata* by its smooth hymenophore and cylindrical basidiospores ( $6\text{--}7 \times 2\text{--}2.5 \mu\text{m}$ , Parmasto 1967); and *P. subserialis* by a white hymenium, clamped generative hyphae, and allantoid basidiospores ( $6\text{--}7 \times 1.5\text{--}2 \mu\text{m}$ , Bernicchia & Gorjón 2010).

Although wood-rotting fungi are an extensively studied group in *Basidiomycota* (Gilbertson & Ryvarden 1987, Núñez & Ryvarden 2001, Bernicchia & Gorjón 2010, Dai 2012, Ryvarden & Melo 2014, Dai & al. 2015), Chinese wood-rotting fungi diversity is still not well known, especially in the subtropics and tropics. The new species *Phlebia wuliangshanensis* is from the Chinese subtropics, where many new taxa in the *Polyporales* have been described (Cui & al. 2007, 2009, 2010, 2011; Cui & Dai 2008; Du & Cui 2009; Li & Cui 2010; He & Li 2011; Jia & Cui 2011; Yu & al. 2013; Yang & He 2014; Chen & al. 2015; Zhao & Wu 2017; Zhao & Ma 2019). We anticipate that more new polypore taxa will be found in China after further investigations and molecular analyses.

#### **Acknowledgments**

Special thanks are due to Jason Karakehian (Harvard University, USA) and Dr. Mei-Ling Han (Langfang Normal University, P.R. China) who reviewed the manuscript. The research was supported by the National Natural Science Foundation of China (Project No. 31700023) and the Key Laboratory of State Forestry Administration for Highly Efficient Utilization of Forestry Biomass Resources in Southwest China (Southwest Forestry University) (Project No. 2019-KF10).

#### Literature cited

- Bernicchia A, Gorjón SP. 2010. Fungi Europaei 12: *Corticaceae* l. Edizioni Candusso, Lomazzo. 1007 p.
- Binder M, Justo A, Riley R, Salamov A, López-Giráldez F, Sjökvist E, Copeland A, Foster B, Sun H, Larsson E, Larsson KH, Townsend J, Grigoriev IV, Hibbett DS. 2013. Phylogenetic and phylogenomic overview of the *Polyporales*. *Mycologia* 105: 1350–1373. <https://doi.org/10.3852/13-003>
- Christiansen MP. 1960. Danish resupinate fungi. Part II. *Homobasidiomycetes*. *Dansk botanisk Arkiv* 19: 57–388.
- Chen JJ, Shen LL, Dai YC. 2015. *Dentipellucula austroafricana* sp. nov. supported by morphological and phylogenetic analyses. *Mycotaxon* 130: 17–25. <https://doi.org/10.5248/130.17>
- Cui BK, Dai YC. 2008. *Skeletocutis luteolus* sp. nov. from southern and eastern China. *Mycotaxon* 104: 97–101.
- Cui BK, Dai YC, Decock C. 2007. A new species of *Perenniporia* (*Basidiomycota*, *Aphylophorales*) from eastern china. *Mycotaxon* 99: 175–180.
- Cui BK, Dai YC, Bao HY. 2009. Wood-inhabiting fungi in southern China 3. A new species of *Phellinus* (*Hymenochaetales*) from tropical China. *Mycotaxon* 110:125–130. <https://doi.org/10.5248/110.125>
- Cui BK, Dai YC, Yuan HS. 2010. Two new species of *Phylloporia* (*Basidiomycota*, *Hymenochaetales*) from China. *Mycotaxon* 113:171–178. <https://doi.org/10.5248/113.171>
- Cui BK, Zhao CL, Dai YC. 2011. *Melanoderma microcarpum* gen. et sp. nov. (*Basidiomycota*) from China. *Mycotaxon* 116:295–302. <https://doi.org/10.5248/116.295>
- Dai YC. 2012. Polypore diversity in China with an annotated checklist of Chinese polypores. *Mycoscience* 53: 49–80. <https://doi.org/10.1007/s10267-011-0134-3>
- Dai YC, Cui BK, Si J, He SH, Hyde KD, Yuan HS, Lui XY, Zhou LW. 2015. Dynamics of the worldwide number of fungi with emphasis on fungal diversity in China. *Mycological Progress* 14: 62. <https://doi.org/10.1007/s11557-015-1084-5>
- Dhingra GS. 1989. Genus *Phlebia* Fr. in the eastern Himalaya. *Journal of the Indian Botanical Society* 84: 111–117.
- Duhem B. 2009. *Phlebia pyrenaica* sp. nov., une nouvelle espèce méditerranéenne. *Cryptogamie Mycologie* 30: 319–328.
- Duhem B. 2013. *Phlebia rhodana* sp. nov. et *Phlebia jurassica* sp. nov. (*Agaricomycotina*). *Cryptogamie Mycologie* 34: 291–301. <https://doi.org/10.7872/crym.v34.iss4.2013.291>
- Duhem B, Michel H. 2007. Une espèce nouvelle de *Phlebia* possédant des fibres arboriformes. *Cryptogamie Mycologie* 28: 29–38.
- Du P, Cui BK. 2009. Two new species of *Megasporoporia* (*Polyporales*, *Basidiomycota*) from tropical China. *Mycotaxon* 110: 131–138. <https://doi.org/10.5248/110.131>
- Eriksson J, Hjortstam K, Ryvarden L. 1981. The *Corticaceae* of North Europe. *Synopsis Fungorum* 6: 1048–1276.
- Felsenstein J. 1985. Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* 39: 783–791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
- Floudas D, Hibbett DS. 2015. Revisiting the taxonomy of *Phanerochaete* (*Polyporales*, *Basidiomycota*) using a four gene dataset and extensive ITS sampling. *Fungal Biology* 119: 679–719. <https://doi.org/10.1016/j.funbio.2015.04.003>
- Fries EM. 1821. *Systema mycologicum*. Greifswald 1: 1–520.
- Ginns JH. 1969. The genus *Merulius* 2. Species of *Merulius* and *Phlebia* proposed by Lloyd. *Mycologia* 61: 357–372. <https://doi.org/10.1080/00275514.1969.12018737>



- Gilbertson RL, Hemmes DE. 2004. New species of lignicolous basidiomycetes from Hawaii. *Memoirs of the New York Botanical Garden* 89: 81–92.
- Gilbertson RL, Ryvarden L. 1987. North American polypores 2. *Fungiflora*, Oslo. 448 p.
- Gorjón SP, Greslebin AG. 2012. Type studies of the species of *Odontia* described by G.H. Cunningham. *New Zealand Journal of Botany* 50: 289–301.  
<https://doi.org/10.1080/0028825X.2012.681385>
- Hall TA. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98
- He SH, Li HJ. 2011. *Hymenochaete* in China. 2. A new species and three new records from Yunnan Province. *Mycotaxon* 118: 411–422. <https://doi.org/10.5248/118.411>
- Jia BS, Cui BK. 2011. Notes on *Ceriporia* (*Basidiomycota*, *Polyporales*) in China. *Mycotaxon* 116: 457–468. <https://doi.org/10.5248/116.457>
- Justo A, Miettinen O, Floudas D, Ortiz-Santana B, Sjökvist E, Lindner D, Nakasone K, Niemelä T, Larsson KH, Ryvarden L, Hibbett DS. 2017. A revised family-level classification of the *Polyporales* (*Basidiomycota*). *Fungal Biology* 121: 798–824. <https://doi.org/10.1016/j.funbio.2017.05.010>
- Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298. <https://doi.org/10.1093/bib/bbn013>
- Kaur G, Singh AP, Dhingra GS. 2017. *Phlebia brevivasidia* sp. nov. from India. *Mycotaxon* 132: 95–97. <https://doi.org/10.5248/132.95>
- Larsson KH. 2007. Re-thinking the classification of corticioid fungi. *Mycological Research* 111: 1040–1063. <https://doi.org/10.1016/j.mycres.2007.08.001>
- Larsson KH, Larsson E, Kõljalg U. 2004. High phylogenetic diversity among corticioid homobasidiomycetes. *Mycological Research* 108: 983–1002.  
<https://doi.org/10.1017/S0953756204000851>
- Li HJ, Cui BK. 2010. A new *Trametes* species from Southwest China. *Mycotaxon* 113: 263–267. <https://doi.org/10.5248/113.263>
- Lombard FF, Burdsall HH, Gilbertson RL. 1975. Taxonomy of *Corticium chrysocreas* and *Phlebia livida*. *Mycologia* 67: 495–510. <https://doi.org/10.1080/00275514.1975.12019775>
- Miller MA, Holder MT, Vos R, Midford PE, Liebowitz T, Chan L, Hoover P, Warnow T. 2009. The CIPRES Portals. CIPRES. [http://www.phylo.org/sub\\_sections/portal](http://www.phylo.org/sub_sections/portal). 2009-08-04. (Archived by WebCite(r) at <http://www.webcitation.org/5imQJJeQa>).
- Nakasone KK. 1997. Studies in *Phlebia*. Six species with teeth. *Sydowia* 49: 49–79.
- Nakasone KK. 2002. *Mycoaciella*, a synonym of *Phlebia*. *Mycotaxon* 81: 477–490.
- Nakasone KK. 2003. Type studies of resupinate hydneaceous hymenomycetes described by Patouillard. *Cryptogamie Mycologie* 24: 131–145.
- Nakasone KK. 2009. Type studies of corticioid hymenomycetes (*Basidiomycota*) with aculei. *Sydowia* 61: 273–285.
- Nakasone KK, Burdsall Jr HH. 1984. *Merulius*, a synonym of *Phlebia*. *Mycotaxon* 21: 241–246.
- Nakasone KK, Burdsall Jr HH. 1995. *Phlebia* species from eastern and southeastern United States. *Mycotaxon* 54: 335–359.
- Núñez M, Ryvarden L. 2001. East Asian polypores 2. *Polyporaceae* s. lato. *Synopsis Fungorum* 14: 165–522.
- Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Parmasto E. 1967. *Corticaceae* U.R.S.S. IV. Descriptiones taxorum novarum. *Combinaciones novae*. *Eesti NSV Teaduste Akadeemia Toimetised* 16: 377–394.

- Petersen JH. 1996. Farvekort. The Danish Mycological Society's colour-chart. Foreningen til Svampekundskabens Fremme, Greve.
- Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818. <https://doi.org/10.1093/bioinformatics/14.9.817>
- Roberts P. 2000. Corticioid fungi from Korup National Park, Cameroon. *Kew Bulletin* 55: 803–842. <https://doi.org/10.2307/4113628>
- Ryvarden L, Melo I. 2014. Poroid fungi of Europe. *Synopsis Fungorum* 31:1–455.
- Schweinitz LD. 1832. Synopsis fungorum in America boreali media degentium. *Transactions of the American Philosophical Society* 4: 141–316. <https://doi.org/10.2307/1004834>
- Shen S, Ma X, Xu TM, Zhao CL. 2018. *Phlebia ailayshanensis* sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analyses. *Phytotaxa* 373: 184–196. <https://doi.org/10.11646/phytotaxa/373.3.2>
- Singh AP, Dhingra GS, Singla N. 2010. A new species of *Phlebia* (Basidiomycetes) from India. *Mycotaxon* 112: 21–24. <https://doi.org/10.5248/112.21>
- Swofford DL. 2002. PAUP\*: phylogenetic analysis using parsimony (\*and other methods). Version 4.0b10. Sinauer Associates, Massachusetts.
- Tomšovský M, Menkis A, Vasaitis R. 2010. Phylogenetic relationships in European *Ceriporiopsis* species inferred from nuclear and mitochondrial ribosomal DNA sequences. *Fungal Biology* 114: 350–358. <https://doi.org/10.1016/j.funbio.2010.02.004>
- Vilgalys R. 2018. Conserved primer sequences for PCR amplification of fungal rDNA. Retrieved from: [https://sites.duke.edu/vilgalyslab/rdna\\_primers\\_for\\_fungi/](https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/) (Duke University, Vilgalys Lab).
- Westphalen MC, Rajchenberg M, Tomsovsky M, Gugliotta AM. 2018. A re-evaluation of Neotropical *Junghuhnia* s. lat. (Polyporales, Basidiomycota) based on morphological and multigene analyses. *Persoonia* 41: 130–141. <https://doi.org/10.3767/persoonia.2018.41.07>
- Yang J, He SH. 2014. *Hymenochaete* in China. 8. *H. biformisetosa* sp. nov. with a key to species with denticulate setae. *Mycotaxon* 128: 137–144. <https://doi.org/10.5248/128.137>
- Yu HY, Zhao CL, Dai YC. 2013. *Inonotus niveomarginatus* and *I. tenuissimus* spp. nov. (Hymenochaetales), resupinate species from tropical China. *Mycotaxon* 124: 61–68. <https://doi.org/10.5248/124.61>
- Zhao CL, Cui BK, Song J, Dai YC. 2015. *Fragiliporiaceae*, a new family of Polyporales (Basidiomycota). *Fungal Diversity* 70: 115–126. <https://doi.org/10.1007/s13225-014-0299-0>
- Zhao CL, Wu ZQ. 2017. *Ceriporiopsis kunmingensis* sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analysis. *Mycological Progress* 16: 93–100. <https://doi.org/10.1007/s11557-016-1259-8>
- Zhao CL, Ma X. 2019. *Perreniporia mopanshanensis* sp. nov. from China. *Mycotaxon* 134: 125–137. <https://doi.org/10.5248/134.125>