Nova Hedwigia, Vol. 108 (2019), Issue 1–2, 265–279 **Article**
Published online October 12, 2018; published in print February 2019

Phlebiopsis yunnanensis sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analysis

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With 4 figures and 1 table

Abstract: A new wood-inhabiting fungal species, *Phlebiopsis yunnanensis* sp. nov., is proposed based on morphological and molecular characters. The species is characterized by resupinate, membranaceous to subceraceous basidiocarps, a monomitic hyphal system with simple separated generative hyphae, cystidia conical, thick-walled, heavily encrusted with large crystals in the apical part and ellipsoid basidiospores measuring 3.5–4.5 × 2.5–3.5 µm. The internal transcribed spacer (ITS) and the large subunit (LSU) regions of nuclear ribosomal RNA gene sequences of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and bayesian inference methods. The phylogenetic analyses based on molecular data of ITS+nLSU sequences showed that *P. yunnanensis* belonged to the Phanerochaetaceae and nested into the phlebioid clade. Further investigation was obtained for more representative taxa in the *Phlebiopsis* based on ITS+nLSU sequences, in which the result demonstrated that the species *P. yunnanensis* formed a monophyletic lineage with a strong support (100% BS, 100% BP, 1.00 BPP) and then grouped with *P. gigantea* and *P. lamprocystidiata.*

Key words: Phanerochaetaceae; phylogenetic analysis; polypores; taxonomy; wood-rotting fungi

Introduction

Phlebiopsis Jülich (Phanerochaetaceae, Polyporales) was erected by Jülich (1978), which is cosmopolitan genus characterized by a combination of resupinate to effused basidiocarps with a membranaceous to subceraceous consistencey when fresh, cracked when dry, hymenophore smooth to odontioid, a monomitic hyphal structure with simple-septate

 DOI: 10.1127/nova_hedwigia/2018/0508 www.borntraeger-cramer.de 0029-5035/2018/0508 $ 3.75

generative hyphae, cystidia hyaline, thick-walled, encrusted, basidia usually narrowly clavate, and basidiospores hyaline, thin-walled, smooth, cylindrical to ellipsoid, acyanophilous and negative in Melzer's reagent (Jülich 1978, Bernicchia & Gorjón 2010). So far about 21 species have been accepted in the genus worldwide (Jülich 1978, Hjortstam & Ryvarden 1980, Dhingra 1987, Gilbertson & Adaskaveg 1993, Ryvarden et al. 2005, Douanla-Meli & Langer 2009, Bernicchia & Gorjón 2010, Wu et al. 2010, Priyanka et al. 2011, Floudas & Hibbett 2015, Miettinen et al. 2016).

Molecular systematics has played a powerful role in inferring phylogenies within fungal groups since the early 1990s (White et al. 1990, Larsson 2007, Binder et al. 2013, Dai et al. 2015, Choia & Kima 2017). Recently, molecular studies involving *Phlebiopsis* have been carried out (Larsson 2007, Binder et al. 2013, Floudas & Hibbett 2015, Miettinen et al. 2016, Justo et al. 2017). Larsson (2007) introduced a new division for part of the Polyporales, effectively renaming the phlebioid and residual polyporoid clades and suggested that *Phlebiopsis flavidoalba* (Cooke) Hjortstam was nested into the family Phanerochaetaceae. Binder et al. (2013) employed molecular study based on multi-gene datasets demonstrated that the species of *Phlebiopsis flavidoalba* belongs to the phlebioid clade and appeared to be grouped with *Phanerochaete lutea* (Sheng H. Wu) Hjortstam and *Phlebia unica* (H.S. Jacks. & Dearden) Ginns. Phylogenetic study of revisiting the taxonomy of Phanerochaete by using a four gene dataset suggested that the species *Phlebiopsis* s.s. clustered into the phlebioid clade and grouped with *P. castanea (*Lloyd) Miettinen & Spirin and *P. flavidoalba* (Cooke) Hjortstam. Miettinen et al. (2016) explored DNA-phylogeny-based and morphology-based genus concepts can be reconciled in the basidiomycete family Phanerochaetaceae and the generic species *P. gigantean* was nested into the family Phanerochaetaceae and grouped with *Phaeophlebiopsis* Floudas & Hibbett and *Rhizochaete* Gresl., Nakasone & Rajchenb. By using the multi-gene datasets, Justo et al. (2017) revised family-level classification of the Polyporales (Basidiomycota), including eighteen families and showed that *Phlebiopsis gigantean* belongs to the family Phanerochaetaceae and grouped with *P. crassa* (Lév.) Floudas & Hibbett and *P. galochroa* (Bres.) Hjortstam & Ryvarden.

Wood-rotting fungi are a cosmopolitan group and they have a rich diversity on the basis of growing on boreal, temperate, subtropical, and tropical vegetations (Gilbertson & Ryvarden 1986, Núñez & Ryvarden 2001, Bernicchia & Gorjón 2010, Dai 2012, Ryvarden & Melo 2014, Dai et al. 2015). During investigations on wood-inhabiting fungi in southern China, an additional taxon was found which could not be assigned to any described species. In this study, the authors expand samplings from previous studies to examine taxonomy and phylogeny of this new species within the *Phlebiopsis*, based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences.

Materials and methods

The studied specimens are deposited at the herbarium of Southwest Forestry University (SWFC). Macro-morphological descriptions are based on field notes. Special colour terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens, and observed under a light microscope following Dai (2010). The following abbreviations were used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both inamyloid and indextrinoid, 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 between the specimens studied, n (a/b) = number of spores (a) measured from given number (b) of specimens.

The EZNA HP Fungal DNA Kit (Omega Biotechnologies Co., Ltd, Kunming) was used to obtain genomic DNA from dried specimens, according to the manufacturer's instructions with some modifications that a small piece of dried fungal specimen (about 30 mg) 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 that, 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 a 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 ml elution buffer was added to the middle of adsorbed film to elute the genome DNA. ITS region was amplified with primer pairs ITS5 and ITS4 (White et al. 1990). Nuclear LSU region was amplified with primer pairs 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 nLSU 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. 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 sequence. Sequences were aligned in MAFFT 6 (Katoh & Toh 2008; http://mafft.cbrc.jp/alignment/server/) using the "G-INS-I" strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (submission ID 22807). Sequences of *Heterobasidion annosum* (Fr.) Bref. and *Stereum hirsutum* (Willd.) Pers. obtained from GenBank were used as outgroups to root trees following Binder et al. (2013) in the ITS+nLSU analysis (Fig. 1) and *Phlebia unica* (H.S. Jacks. & Dearden) Ginns and *Ceraceomyces americanus* Nakasone, C.R. Bergman & Burds. obtained from GenBank were used as an outgroup to root trees following Miettinen et al. (2016) in the ITS+nLSU analyses (Fig. 2).

Table 1. A list of species, specimens, and GenBank accession number of sequences used in this study.

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Abortiporus biennis	TFRI 274	EU232187	EU232235	Binder et al. 2005
Antrodia albida	CBS 308.82	DQ491414	AY515348	Kim et al. 2007
A. heteromorpha	CBS 200.91	DQ491415	AY515350	Kim et al. 2007
Antrodiella americana	Gothenburg 3161	JN710509	JN710509	Binder et al. 2013
A. semisupina	FCUG 960	EU232182	EU232266	Binder et al. 2005
Ceraceomyces americanus	FP 102188	KP135409	KP135277	Floudas & Hibbett 2015
Ceraceomyces serpens	KHL 8478	AF090882	AF090882	Binder et al. 2005
Ceriporia lacerata	Dai 10734	JX623916	JX644068	Jia et al. 2014
Ceriporiopsis gilvescens	BRNM 710166	FJ496684	FJ496720	Tomšovský et al. 2010
Climacocystis borealis	KH 13318	JQ031126	JQ031126	Binder et al. 2013
Coriolopsis caperata	LE(BIN)-0677	AB158316	AB158316	Tomšovský et al. 2010
Dacryobolus karstenii	KHL 11162	EU118624	EU118624	Binder et al. 2005
Daedalea quercina	DSM 4953	DQ491425	DQ491425	Kim et al. 2007
Earliella scabrosa	PR 1209	JN165009	JN164793	Binder et al. 2005
Fomitopsis pinicola	CBS 221.39	DQ491405	DQ491405	Kim et al. 2007
F. rosea	ATCC 76767	DQ491410	DQ491410	Kim et al. 2007
Fragiliporia fragilis	Dai 13080	KJ734260	KJ734264	Zhao et al. 2015
F. fragilis	Dai 13559	KJ734261	KJ734265	Zhao et al. 2015
F. fragilis	Dai 13561	KJ734262	KJ734266	Zhao et al. 2015
Ganoderma lingzhi	Wu 1006-38	JQ781858	–	Zhao et al. 2015
Gelatoporia subvermispora	BRNU 592909	FJ496694	FJ496706	Tomšovský et al. 2010
Gloeoporus pannocinctus	BRNM 709972	EU546099	FJ496708	Tomšovský et al. 2010
G. dichrous	KHL 11173	EU118627	EU118627	Binder et al. 2005
Grammothelopsis subtropica	Cui 9035	JQ845094	JQ845097	Zhao et al. 2015
Heterobasidion annosum	PFC 5252	KC492906	KC492906	Binder et al. 2013
Hornodermoporus martius	MUCL 41677	FJ411092	FJ393859	Robledo et al. 2009
Hypochnicium lyndoniae	NL 041031	JX124704	JX124704	Binder et al. 2005
Junghuhnia nitida	KHL 11903	EU118638	EU118638	Binder et al. 2005
Obba rivulosa	KCTC 6892	FJ496693	FJ496710	Miettinen & Rajchenberg 2012
O. valdiviana	FF 503	HQ659235	HQ659235	Miettinen & Rajchenberg 2012

Table 1. cont.

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Phanerochaete chrysosporium	BKM-F-1767	KP135094	KP135246	Floudas & Hibbett 2015
Perenniporia medulla-panis	MUCL 49581	FJ411087	FJ393875	Robledo et al. 2009
Perenniporiella neofulva	MUCL 45091	FJ411080	FJ393852	Robledo et al. 2009
Phlebia floridensis	HHB-9905	KP135383	KP135264	Justo et al. 2017
P. radiata	AFTOL-ID 484	AY854087	AF287885	Binder et al. 2005
P. radiata	FD-85	KP135377	KP135377	Justo et al. 2017
P. setulosa	HHB-6891	KP135382	KP135267	Justo et al. 2017
P. unica	KHL 11786	EU118657	EU118657	Larsson 2007
Phlebiopsis brunneo-cystidiata	Chen 1143	–	GQ470639	Miettinen et al. 2016
P. castanea	Spirin 5295	KX752610	KX752610	Miettinen et al. 2016
P. crassa	KKN 86	KP135394	KP135215	Floudas & Hibbett 2015
P. crassa	FP 102496	AY219341	AY219341	Floudas & Hibbett 2015
P. crassa	HHB 8834	KP135393	KP135393	Floudas & Hibbett 2015
P. flavidoalba	FD 263	KP135402	KP135271	Floudas & Hibbett 2015
P. flavidoalba	OM 17897	KP135398	–	Floudas & Hibbett 2015
P. flavidoalba	ME 164	KP135399	–	Floudas & Hibbett 2015
P. flavidoalba	MR 4252	KP135400	–	Floudas & Hibbett 2015
P. flavidoalba	HHB 4617	KP135401	KP135401	Floudas & Hibbett 2015
P. flavidoalba	OM 17896	KP135397	–	Floudas & Hibbett 2015
P. flavidoalba	FD 407	KP135404	–	Floudas & Hibbett 2015
P. flavidoalba	FD 374	KP135403	–	Floudas & Hibbett 2015
P. galochroa	FP 102937	KP135391	KP135270	Floudas & Hibbett 2015
P. gigantea	FP 70857	KP135390	KP135272	Floudas & Hibbett 2015
P. gigantea	C 93318/1	AF087486	–	Binder et al. 2013

Table 1. cont.

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
P. gigantea	FP 101815	KP135389	–	Floudas & Hibbett 2015
P. gigantea	HHB-11416	KP135388	–	Floudas & Hibbett 2015
P. gigantea	Miettinen 15354	KX752605	–	Miettinen et al. 2016
P. lamprocystidiata	Wu 0109-14	–	GQ470648	Miettinen et al. 2016
P. laxa	Wu 9311-17	–	GQ470649	Miettinen et al. 2016
P. pilatii	Spirin 5048	KX752590	KX752590	Miettinen et al. 2016
P. yunnanensis	CLZhao 3958	MH744140	MH744142	This study
P. yunnanensis	CLZhao 3900	MH744141	MH744143	This study
Piloporia sajanensis	Mannine 2733a	HQ659239	HQ659239	Tomšovský et al. 2010
Podoscypha multizonata	Gothenburg 3005	JN710581	JN710581	Binder et al. 2013
Polyporus tuberaster	CulTENN 10197	AF516596	AJ488116	Binder et al. 2013
Postia guttulata	KHL 11739	EU11865	EU11865	Kim et al. 2007
Sebipora aquosa	Miettinen 8680	HQ659240	HQ659240	Miettinen & Rajchenberg 2012
Skeletocutis amorpha	Miettinen 11038	FN907913	FN907913	Tomšovský et al. 2010
S. jelicii	H 6002113	FJ496690	FJ496727	Tomšovský et al. 2010
S. portcrosensis	LY 3493	FJ496689	FJ496689	Tomšovský et al. 2010
S. subsphaerospora	Rivoire 1048	FJ496688	FJ496688	Tomšovský et al. 2010
Steccherinum fimbriatum	KHL 11905	EU118668	EU118668	Tomšovský et al. 2010
S. ochraceum	KHL 11902	JQ031130	JQ031130	Tomšovský et al. 2010
Stereum hirsutum	NBRC 6520	AB733150	AB733325	Tomšovský et al. 2010
Truncospora ochroleuca	MUCL 39726	FJ411098	FJ393865	Robledo et al. 2009
Tyromyces chioneus	Cui 10225	KF698745	KF698756	Zhao et al. 2015
Xanthoporus syringae	Gothenburg 1488	JN710607	JN710607	Tomšovský et al. 2010

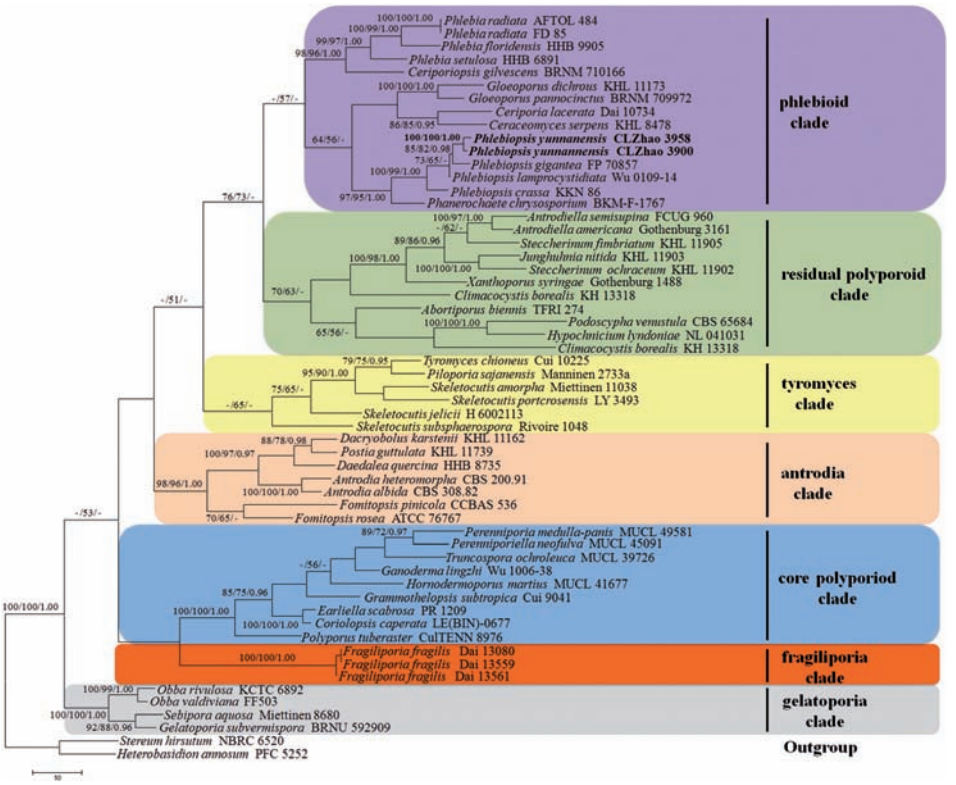

Fig. 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Phlebiopsis yunnanensis* and related species in Polyporales based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively. Clade names follow Binder et al. (2013).

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analysis followed Zhou et al. (2016), and the tree construction procedure 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 a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive 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 (MPT) generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org; Miller et al. 2009). Branch support for ML analysis was determined by 1000 bootstrap replicate.

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 MrBayes3.1.2 with a general time reversible (GTR) 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 5 million generations (ITS+nLSU) (Fig. 1), for 3 million generations (Fig. 2) and trees were sampled every 100 generations. The first one-fourth 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 as significantly supported, respectively.

Results

The ITS+nLSU dataset included sequences from 57 fungal specimens representing 53 species. The dataset had an aligned length of 2365 characters, of which 1271 characters are constant, 260 are variable and parsimony-uninformative, and 634 are parsimony-informative. Maximum parsimony analysis yielded two equally parsimonious trees (TL = 4807, CI = 0.3186, HI = 0.682, RI = 0.524, RC = 0.166). Best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis: GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.003248 (BI).

The phylogeny (Fig. 1) inferred from ITS+nLSU sequences demonstrated seven major clades for 53 species of the Polyporales. The new species clustered into the Phlebia clade and formed a monophyletic lineage with a high support (100% BS, 100% BP, 1.00 BPP), and was closely related to *Phlebiopsis gigantea* (Fr.) Jülich with a high support (85% BS, 82% BP, 0.98 BPP) and then grouped with *P. lamprocystidiata* (Sheng H. Wu) Sheng H. Wu & Hallenb.

The ITS+nLSU dataset (Fig. 2) included sequences from 26 fungal specimens representing 12 species. The dataset had an aligned length of 1945 characters, of which 1675 characters are constant, 121 are variable and parsimony-uninformative, and 149 are parsimony-informative. Maximum parsimony analysis yielded 100 equally parsimonious trees (TL = 438, CI = 0.742, HI = 0.258, RI = 0.824, RC = 0.612). 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). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.006157 (BI).

A further phylogeny (Fig. 2) inferred from the combined ITS+nLSU sequences was obtained for 26 fungal specimens representing 12 species within the genus *Phlebiopsis* and demonstrated that the new species formed a monophyletic entity with a high 100% BS, 100% BP and 1.00 BPP and then grouped with *P. gigantea* and *P. lamprocystidiata*.

Taxonomy

Phlebiopsis yunnanensis C.L. Zhao, **sp. nov.** Figs. 2, 3, 4

MycoBank no.: MB 827410

Diagnosis: The species is distinct by its membranaceous to subceraceous basidiocarps with white to cinnamon-buff hymenophores, a monomitic hyphal system with simple separated generative hyphae, cystidia apically encrusted with large crystals and basidiospores broadly ellipsoid, hyaline, thin-walled, smooth measuring 3.5–4.5 × 2.5–3.5 µm.

Holotypus: CHINA. Yunnan Prov., Puer, Jingdong County, Ailaoshan National Forest Park, on the angiosperm trunk, 5 October 2017, CLZhao 3958 (SWFC).

Etymology: *Yunnanensis* (Lat.): referring to the locality (Yunnan Province) of the type specimen.

Fruiting body: Basidiocarps annual, resupinate, very difficult to separate from substrate, membranaceous to subceraceous, without odour or taste when fresh, becoming cracked upon drying, up to 5 cm long, 100–500 µm thick. Hymenial surface smooth to odontioid, white when fresh, turn to buff to cinnamon-buff upon drying. Sterile distinct, cinnamon-buff.

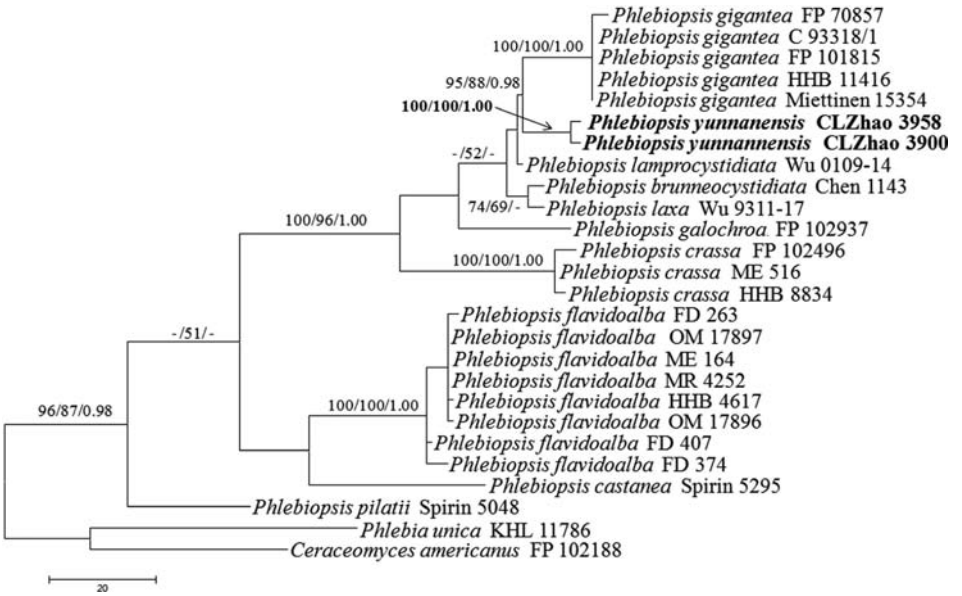

Fig. 2. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Phlebiopsis yunnanensis* and related species in *Phlebiopsis* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively.

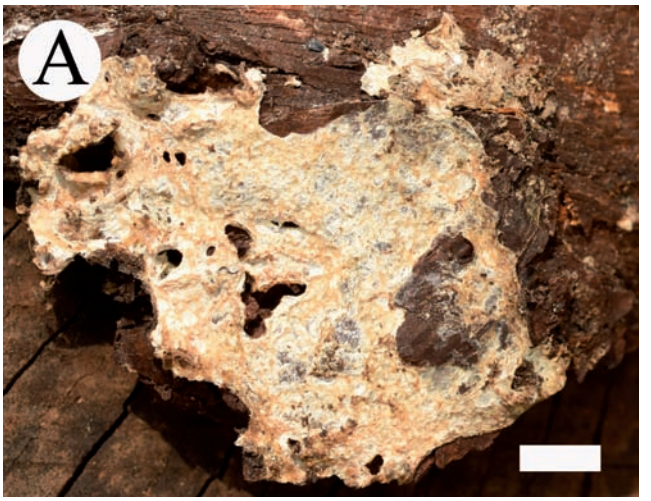

Fig. 3. A basidioma of *Phlebiopsis yunnanensis*. Bar: 5 mm (holotype)

Hyphal structure: Hyphal system monomitic; generative hyphae with simple septa, slightly thick-walled, branched, IKI–, CB–; tissues unchanged in KOH.

Hymenium: Cystidia conical, present in subiculum, thick-walled, apically encrusted with large and dense crystals, 36–41 × 8–11 µm, cystidioles absent; basidia narrowly clavate to subcylindrical, with four sterigmata and a simple septum, 10–21 × 3.5–4.5 µm; basidioles dominant, in shape similar to basidia, but slightly smaller.

Spores: Basidiospores broadly ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (3–) 3.5–4.5(–5.5) × (2–)2.5–3.5 µm, L = 3.98 µm, W = 2.91 µm, Q =1.31–1.42 (n = 60/2).

Rot type: A white rot.

Additional specimen (paratype) examined: CHINA. Yunnan Prov., Puer, Jingdong County, Ailaoshan National Forest Park, on the angiosperm trunk, 5 October 2017, CLZhao 3990 (SWFC).

Discussion

In the present study, a new species, *Phlebiopsis yunnanensis*, is described based on phylogenetic analysis and morphological characters. The species has unique morphological characters in *Phlebiopsis* and forms a monophyletic lineage within the phlebia clade.

Previously, seven clades were found in the Polyporales: antrodia clade, core polyporoid clade, fragiliporia clade, gelatoporia clade, phlebioid clade, residual polyporoid clade and tyromyces clade (Binder et al. 2013). According to our result based on the combined

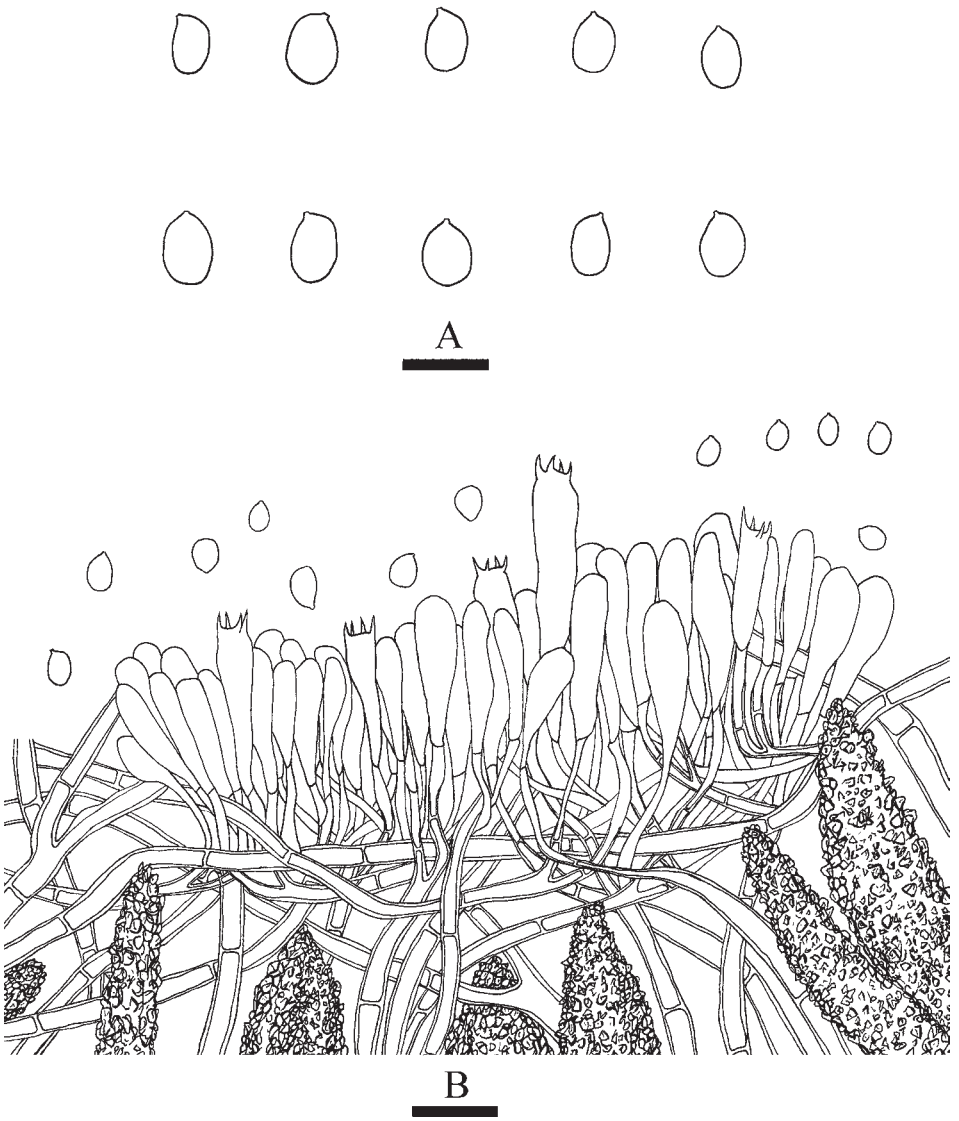

Fig. 4. Microscopic structures of *Phlebiopsis yunnanensis* (drawn from the holotype). A. Basidiospores; B. A section of basidiocarp. Bars: A = 5 µm; B = 10 µm.

ITS+nLSU sequence data (Fig. 1), *Phlebiopsis yunnanensis* is nested into the phlebioid clade with strong support (100% BS, 100% BP, 1.00 BPP).

In our analysis (Fig. 1), *Phlebiopsis yunnanensis* grouped with *P. gigantea* and *P. lamprocystidiata* inferred from the ITS+nLSU analysis, and then grouped with *P. brunneocystidiata* (Sheng H. Wu) Miettinen and *P. laxa* (Sheng H. Wu) Miettinen. However, morphologically *P. gigantea* differs from *P. yunnanensis* by its greyish white to buff basidiocarps, larger cystidia (50–80 × 10–15 µm) and narrowly ellipsoid, larger basidiospores (6.5–8 × 3–3.5 µm; Bernicchia & Gorjón 2010). *Phlebiopsis lamprocystidiata* can be distinguished by grayish yellow hymenial surface and broadly ellipsoid, larger basidiospores (5.5–7.5 × 3.8–4.8 µm; Wu 2004). The species *P. brunneocystidiata* differs from *P. yunnanensis* by pale brownish gray hymenial surface and narrowly ellipsoid, larger basidiospores (5.5–7.5 × 2.7–3.3 µm; Bernicchia & Gorjón 2010). *Phlebiopsis laxa* is separated from *P. yunnanensis* by its ivory colored hymenial surface, slightly darkening in KOH, filamentous margin and larger basidiospores (8–10 × 4–5 µm; Wu 2000).

Wood-rotting fungi is an extensively studied group of Basidiomycota (Gilbertson & Ryvarden 1987, Núñez & Ryvarden 2001, Bernicchia & Gorjón 2010, Dai 2012, Ryvarden & Melo 2014), but the Chinese polypore diversity is still not well known, especially in subtropics and tropics, many recently described taxa of wood-rotting fungi were from these areas (Cui & Dai 2006, 2011, Cui 2009, Dai et al. 2009, 2010, Zhou & Dai 2012, 2013, Yuan 2013, Song et al. 2014, 2016, Chen et al. 2015, 2016a, b, Zhao et al. 2015, 2016, Yuan et al. 2016, Zhou et al. 2016). The new species in the present study, *Phlebiopsis yunnanensis*, is from subtropics, too. It is possible that new polypore taxa will be found after further investigations and molecular analyses.

Acknowledgments

We express our gratitude to Yong-He Li (Yunnan Academy of Biodiversity, Southwest Forestry University, P.R. China) for his support on molecular work. The research is supported by the National Natural Science Foundation of China (Project No. 31700023) and Yunnan Agricultural Foundation Projects (2017FG001-042) and the Science Foundation of Southwest Forestry University (Project No. 111715) and the Science Foundation of Education Department in Yunnan (2018JS326).

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Manuscript received: May 28, 2018
Accepted: August 14, 2018