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Phlebia ailaoshanensis sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analyses

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Abstract

A new wood-inhabiting fungus, *Phlebia ailaoshanensis*, is proposed based on a combination of morphological features and molecular evidence. The species is characterized by an annual growth habit, resupinate basidiocarps with tuberculate to phlebioid hymenial surface, a monomitic hyphal system with slightly thick-walled generative hyphae bearing simple septa, IKI–, CB– and ellipsoid, hyaline, thin-walled, smooth, IKI–, CB– basidiospores measuring as $5.7\text{--}8.5 \times 3\text{--}4.3 \mu\text{m}$. Sequences of ITS and LSU nrRNA gene regions 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. ailaoshanensis* belonged to the Meruliaceae and nested into the phlebioid clade. Further investigation was obtained for more representative taxa in the *Phlebia* based on ITS+nLSU sequences, in which the result demonstrated that the species *P. ailaoshanensis* formed a monophyletic lineage with a strong support (100% BS, 100% BP, 1.00 BPP) and then grouped with *P. acanthocystis*, *P. chrysocreas*, *P. ludoviciana*, *P. subcretacea* and *P. uda*.

Key words: *Ceriporiopsis*, Meruliaceae, Phylogeny, Yaxonomy, Wood-inhabiting fungi

Introduction

Phlebia Fr. (Meruliaceae, Polyporales) was erected by Fries (1821), which is a large, cosmopolitan genus characterized by a combination of resupinate or rarely pileate basidiocarps with a subceraceous to subgelatinous consistency when fresh, membranaceous to coriaceous when dry, hymenophore smooth, tuberculate, phlebioid, odontoid, meruloid or poroid, a monomitic hyphal structure (rare dimitic) with clamp connections or simple-septate, narrowly clavate basidia and basidiospores hyaline, thin-walled, smooth, allantoid to ellipsoid, acyanophilous and negative in Melzer's reagent (Bernicchia and 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 *et al.* 2010, Westphalen *et al.* 2010, Gorjón & Greslebin 2012, Duhem 2014, Kaur *et al.* 2017).

Molecular systematics has played a powerful role in inferring phylogenies within fungal groups since the early 1990s (White *et al.* 1990, Hibbett *et al.* 2007, Larsson 2007, Miettinen *et al.* 2012, Binder *et al.* 2013, Dai *et al.* 2015, Choia & Kima 2017). Recently, molecular studies involving Meruliaceae P. Karst. have been carried out (Binder *et al.* 2005, 2013, Miettinen & Larsson 2011, Miettinen & Rajchenberg 2012, Hibbett *et al.* 2016, Miettinen *et al.* 2016, Justo *et al.* 2017). Phylogenetic study of the high phylogenetic diversity among corticioid homobasidiomycetes suggested that the genus *Phlebia* clustered into the phlebioid clade and grouped with *Ceriporia* Donk and *Gloeoporus* Mont. (Larsson *et al.* 2004). Larsson (2007) introduced a new division for part of the Polyporales, effectively renaming the phlebioid and residual polyporoid clades and suggested that the genus was polyphyletic, and its type species *P. radiata* was nested into the Meruliaceae. Phylogenetic study of European *Ceriporiopsis* taxa showed that the generic species *Phlebia radiata* and the type *C. gilvescens* were grouped closely on the base of the combined data of the large subunit nuclear ribosomal RNA gene (nLSU) sequences and mitochondrial small subunit rRNA (mtSSU) gene

sequences (Tomšovský *et al.* 2010). Binder *et al.* (2013) employed molecular study based on multi-gene datasets demonstrated that the type species of *Phlebia* (*P. radiata*) belonged to the phlebioid clade and appeared to be grouped with *Ceraceomyces* Jülich, *Ceriporia* and *Ceriporiopsis*. By using the multi-gene datasets, Justo *et al.* (2017) revised family-level classification of the Polyporales (Basidiomycota), including eighteen families and showed that *P. radiata* belonged to the family Meruliaceae and grouped with *Aurantiporus* Murrill and *C. gilvescens*.

Wood-rotting fungi is a cosmopolitan group and it has a rich diversity on the basis of growing on boreal, temperate, subtropical, and tropical vegetations (Gilbertson & Ryvarden 1987, 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 *Phlebia*, based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences.

Materials and methods

Morphological studies.—The specimens studied 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.

DNA extraction, amplification, sequencing and phylogenetic analyses.—The EZNA HP Fungal DNA Kit (Omega Biotechnologies Co., Ltd, Kunming) was used to obtain PCR products from dried specimens, according to the manufacturer’s instructions with some modifications. 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).

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	
<i>Antrodia albida</i>	CBS 308.82	DQ491414	AY515348	Kim <i>et al.</i> 2007
<i>A. heteromorpha</i>	CBS 200.91	DQ491415	AY515350	Kim <i>et al.</i> 2007
<i>Antrodiella americana</i>	Gothenburg 3161	JN710509	JN710509	Binder <i>et al.</i> 2013
<i>A. semisupina</i>	FCUG 960	EU232182	EU232266	Binder <i>et al.</i> 2005
<i>Ceraceomyces serpens</i>	KHL 8478	AF090882	AF090882	Binder <i>et al.</i> 2005
<i>Ceriporia lacerata</i>	Dai 10734	JX623916	JX644068	Jia <i>et al.</i> 2013
<i>Ceriporiopsis gilvescens</i>	BRNM 710166	FJ496684	FJ496720	Tomšovský <i>et al.</i> 2010
<i>Climacocystis borealis</i>	KH 13318	JQ031126	JQ031126	Binder <i>et al.</i> 2013
<i>Coriolopsis caperata</i>	LE(BIN)-0677	AB158316	AB158316	Tomšovský <i>et al.</i> 2010
<i>Dacryobolus karstenii</i>	KHL 11162	EU118624	EU118624	Binder <i>et al.</i> 2005
<i>Daedalea quercina</i>	DSM 4953	DQ491425	DQ491425	Kim <i>et al.</i> 2007
<i>Earliella scabrosa</i>	PR 1209	JN165009	JN164793	Binder <i>et al.</i> 2005
<i>Fomitopsis pinicola</i>	CBS 221.39	DQ491405	DQ491405	Kim <i>et al.</i> 2007

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TABLE 1. (Continued)

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
<i>Fragiliporia fragilis</i>	Dai 13080	KJ734260	KJ734264	Zhao <i>et al.</i> 2015a
<i>F. fragilis</i>	Dai 13559	KJ734261	KJ734265	Zhao <i>et al.</i> 2015a
<i>F. fragilis</i>	Dai 13561	KJ734262	KJ734266	Zhao <i>et al.</i> 2015a
<i>Ganoderma lingzhi</i>	Wu 1006-38	JQ781858	—	Zhao <i>et al.</i> 2013
<i>Gelatoporia subvermispora</i>	BRNU 592909	FJ496694	FJ496706	Tomšovský <i>et al.</i> 2010
<i>Gloeoporus pannocinctus</i>	BRNM 709972	EU546099	FJ496708	Tomšovský <i>et al.</i> 2010
<i>G. dichrous</i>	KHL 11173	EU118627	EU118627	Binder <i>et al.</i> 2005
<i>Grammothelopsis subtropica</i>	Cui 9035	JQ845094	JQ845097	Zhao <i>et al.</i> 2013
<i>Heterobasidion annosum</i>	PFC 5252	KC492906	KC492906	Binder <i>et al.</i> 2013
<i>Hornodermoporus martius</i>	MUCL 41677	FJ411092	FJ393859	Robledo <i>et al.</i> 2009
<i>Hydnophlebia chrysorhiza</i>	FD-282	KP135338	KP135216	Floudas & Hibbett 2015
<i>Junghuhnia nitida</i>	KHL 11903	EU118638	EU118638	Binder <i>et al.</i> 2005
<i>Mycoacia fuscoatra</i>	KHL 13275	JN649352	JN649352	Tomšovský <i>et al.</i> 2010
<i>M. nothofagi</i>	KHL 13750	GU480000	GU480000	Tomšovský <i>et al.</i> 2010
<i>Obba rivulosa</i>	KCTC 6892	FJ496693	FJ496710	Miettinen & Rajchenberg 2012
<i>O. valdiviana</i>	FF 503	HQ659235	HQ659235	Miettinen & Rajchenberg 2012
<i>Phanerochaete chrysosporium</i>	HHB 6251	KP135094	KP135246	Floudas & Hibbett 2015
<i>Phanerochaete velutina</i>	HHB-15343	KP135184	—	Floudas & Hibbett 2015
<i>Perenniporia medulla-panis</i>	MUCL 49581	FJ411087	FJ393875	Robledo <i>et al.</i> 2009
<i>Perenniporiella neofulva</i>	MUCL 45091	FJ411080	FJ393852	Robledo <i>et al.</i> 2009
<i>Phlebia acerina</i>	FD-301	KP135378	KP135378	Justo <i>et al.</i> 2017
<i>P. acerina</i>	HHB-11146	KP135372	—	Floudas & Hibbett 2015
<i>P. acerina</i>	FP-135252	KP135371	—	Floudas & Hibbett 2015
<i>P. acerina</i>	DR-60	KP135375	KF691615	Floudas & Hibbett 2015
<i>P. acanthocystis</i>	FP150571	KY948767	KY948844	Floudas & Hibbett 2015
<i>P. ailaoshanensis</i>	CLZhao 3882	MH784919	MH784929	Present study
<i>P. ailaoshanensis</i>	CLZhao 3953	MH784925	MH784935	Present study
<i>P. ailaoshanensis</i>	CLZhao 3996	MH784926	MH784936	Present study
<i>P. ailaoshanensis</i>	CLZhao 3904	MH784922	MH784932	Present study
<i>P. ailaoshanensis</i>	CLZhao 3905	MH784923	MH784933	Present study
<i>P. ailaoshanensis</i>	CLZhao 3942	MH784924	MH784934	Present study
<i>P. ailaoshanensis</i>	CLZhao 3879	MH784918	MH784928	Present study
<i>P. ailaoshanensis</i>	CLZhao 3893	MH784920	MH784930	Present study
<i>P. ailaoshanensis</i>	CLZhao 4036	MH784927	MH784937	Present study
<i>P. ailaoshanensis</i>	CLZhao 3897	MH784921	MH784931	Present study
<i>P. aurea</i>	DLL2011-263	KJ140747	KJ140747	Binder <i>et al.</i> 2013
<i>P. aurea</i>	DLL2011-100	KJ140614	AY586691	Binder <i>et al.</i> 2013
<i>P. aurea</i>	FCUG2767	HQ153409	HQ153409	Binder <i>et al.</i> 2013
<i>P. centrifuga</i>	HHB-9239	KP135380	KP135262	Floudas & Hibbett 2015
<i>P. centrifuga</i>	L-15541	KP135381	—	Floudas & Hibbett 2015
<i>P. centrifuga</i>	GB-1013	KP135379	—	Floudas & Hibbett 2015
<i>P. chrysocreas</i>	HHB-6333	KP135358	KP135263	Floudas & Hibbett 2015
<i>P. chrysocreas</i>	HHB-3946	KP135357	AY586695	Floudas & Hibbett 2015
<i>P. chrysocreas</i>	FP-102161	AY219367	—	Binder <i>et al.</i> 2005

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TABLE 1. (Continued)

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
<i>P. floridensis</i>	HHB-9905	KP135383	KP135264	Justo <i>et al.</i> 2017
<i>P. floridensis</i>	HHB-6466	KP135385	—	Floudas & Hibbett 2015
<i>P. floridensis</i>	HHB-7175	KP135384	—	Floudas & Hibbett 2015
<i>P. floridensis</i>	FP-102562-T	KP135386	—	Floudas & Hibbett 2015
<i>P. fuscoatra</i>	HHB-10782	KP135364	KP135265	Floudas & Hibbett 2015
<i>P. fuscoatra</i>	HHB-15354-T	KP135367	KP135363	Floudas & Hibbett 2015
<i>P. fuscoatra</i>	HHB-18642	KP135366	—	Floudas & Hibbett 2015
<i>P. fuscoatra</i>	FP-102173	KP135365	—	Floudas & Hibbett 2015
<i>P. hydnoidea</i>	HHB-1993	KY948778	KY948853	Justo <i>et al.</i> 2017
<i>P. lindtneri</i>	GB-501	KY948772	KY948847	Justo <i>et al.</i> 2017
<i>P. livida</i>	FCUG 2189	AF141624	AF141624	Tomšovský <i>et al.</i> 2010
<i>P. ludoviciana</i>	FD-427	KP135342	—	Floudas & Hibbett 2015
<i>P. nantahaliensis</i>	HHB-2816	KY948777	KY948852	Justo <i>et al.</i> 2017
<i>P. nothofagi</i>	HHB-4273	KP135369	KP135266	Floudas & Hibbett 2015
<i>P. nothofagi</i>	HHB-6906	KP135368	—	Floudas & Hibbett 2015
<i>P. nothofagi</i>	HHB-12067	KP135370	—	Floudas & Hibbett 2015
<i>P. radiata</i>	UBCF 19726	HQ604797	HQ604797	Binder <i>et al.</i> 2013
<i>P. radiata</i>	AFTOL-ID 484	AY854087	AF287885	Binder <i>et al.</i> 2005
<i>P. radiata</i>	FD-85	KP135377	KP135377	Justo <i>et al.</i> 2017
<i>P. rufa</i>	HHB-14924	KP135374	KX065989	Floudas & Hibbett 2015
<i>P. setulosa</i>	HHB-6891	KP135382	KP135267	Justo <i>et al.</i> 2017
<i>P. setulosa</i>	AH 31879	GQ259417	GQ259417	Binder <i>et al.</i> 2005
<i>P. setulosa</i>	PH 11749	GU461312	—	Binder <i>et al.</i> 2005
<i>P. subserialis</i>	FCUG 1434	AF141631	AF141631	Tomšovský <i>et al.</i> 2010
<i>P. subochracea</i>	HHB-8715	KY948770	KY948846	Floudas & Hibbett 2015
<i>P. uda</i>	AY787676	AY787676	—	Floudas & Hibbett 2015
<i>P. uda</i>	FP-101544	KP135361	KP135232	Floudas & Hibbett 2015
<i>Piloporia sajanensis</i>	Mannine 2733a	HQ659239	HQ659239	Tomšovský <i>et al.</i> 2010
<i>Polyporus tuberaster</i>	CuITENN 10197	AF516596	AJ488116	Binder <i>et al.</i> 2013
<i>Postia guttulata</i>	KHL 11739	EU11865	EU11865	Kim <i>et al.</i> 2007
<i>Sebipora aquosa</i>	Miettinen 8680	HQ659240	HQ659240	Miettinen & Rajchenberg 2012
<i>Skeletocutis amorpha</i>	Miettinen 11038	FN907913	FN907913	Tomšovský <i>et al.</i> 2010
<i>S. jelicii</i>	H 6002113	FJ496690	FJ496727	Tomšovský <i>et al.</i> 2010
<i>S. porterosensis</i>	LY 3493	FJ496689	FJ496689	Tomšovský <i>et al.</i> 2010
<i>S. subsphaerospora</i>	Rivoire 1048	FJ496688	FJ496688	Tomšovský <i>et al.</i> 2010
<i>Steccherinum fimbriatum</i>	KHL 11905	EU118668	EU118668	Tomšovský <i>et al.</i> 2010
<i>S. ochraceum</i>	KHL 11902	JQ031130	JQ031130	Tomšovský <i>et al.</i> 2010
<i>Stereum hirsutum</i>	NBRC 6520	AB733150	AB733325	Tomšovský <i>et al.</i> 2010
<i>Truncospora ochroleuca</i>	MUCL 39726	FJ411098	FJ393865	Robledo <i>et al.</i> 2009
<i>Tyromyces chioneus</i>	Cui 10225	KF698745	KF698756	Zhao <i>et al.</i> 2013
<i>Xanthoporus syringae</i>	Gothenburg 1488	JN710607	JN710607	Tomšovský <i>et al.</i> 2010

Seqencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 6 (Kato & 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 22766). 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 *Hydnophlebia chrysorrhiza* (Torr.) Parmasto and *Phanerochaete velutina* (DC.) P. Karst. obtained from GenBank were used as an outgroup to root trees following Floudas & Hibbett (2015) in the ITS+nLSU analyses (Fig. 2).

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analysis followed Song *et al.* (2016a), 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 on Abe through the Cipres Science Gateway (www.phylo.org; Miller *et al.* 2009). Branch support for ML analysis was determined by 1000 bootstrap replicate.

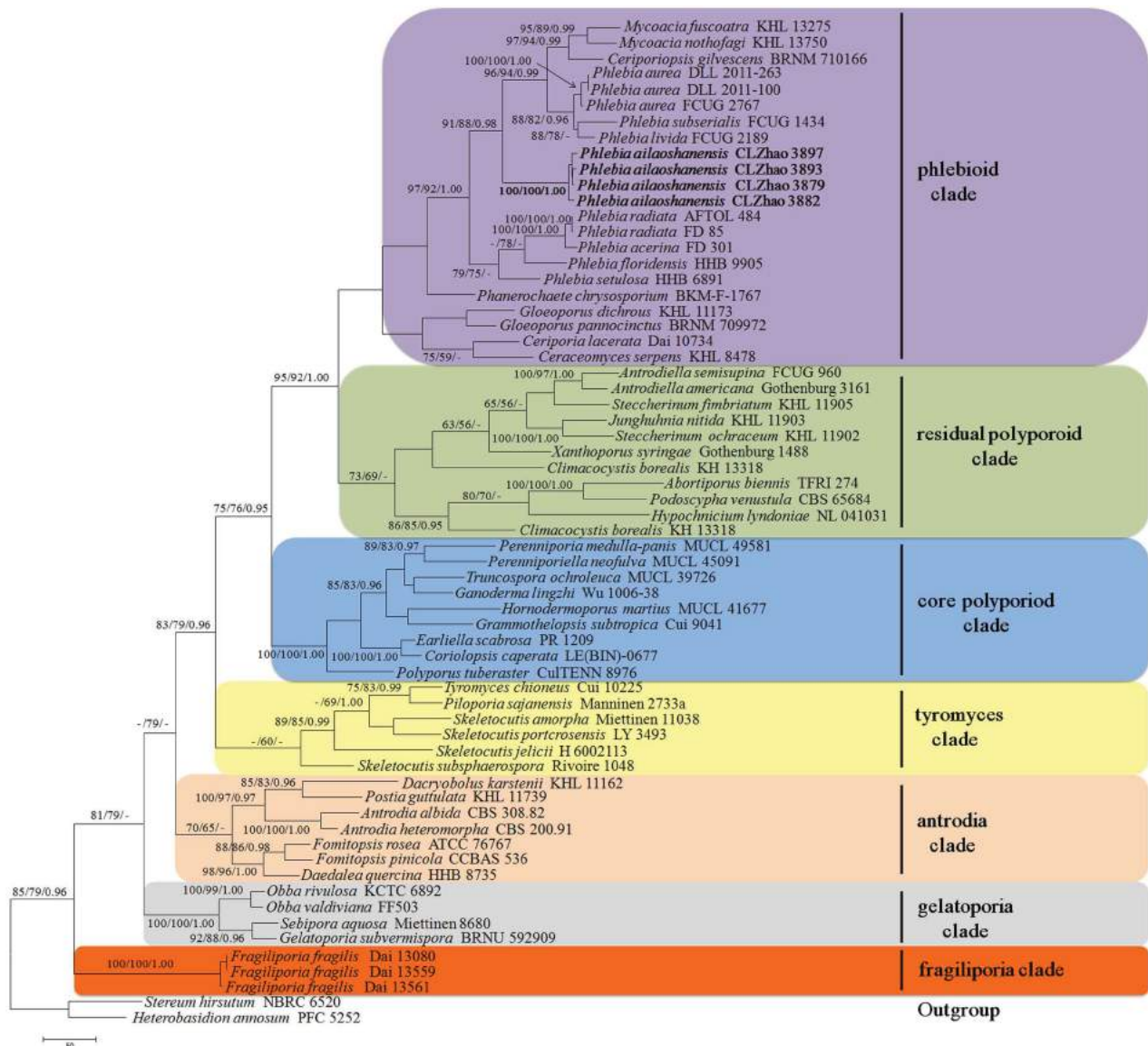


FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Phlebia ailaoshanensis* and related species in Polyporales based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian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

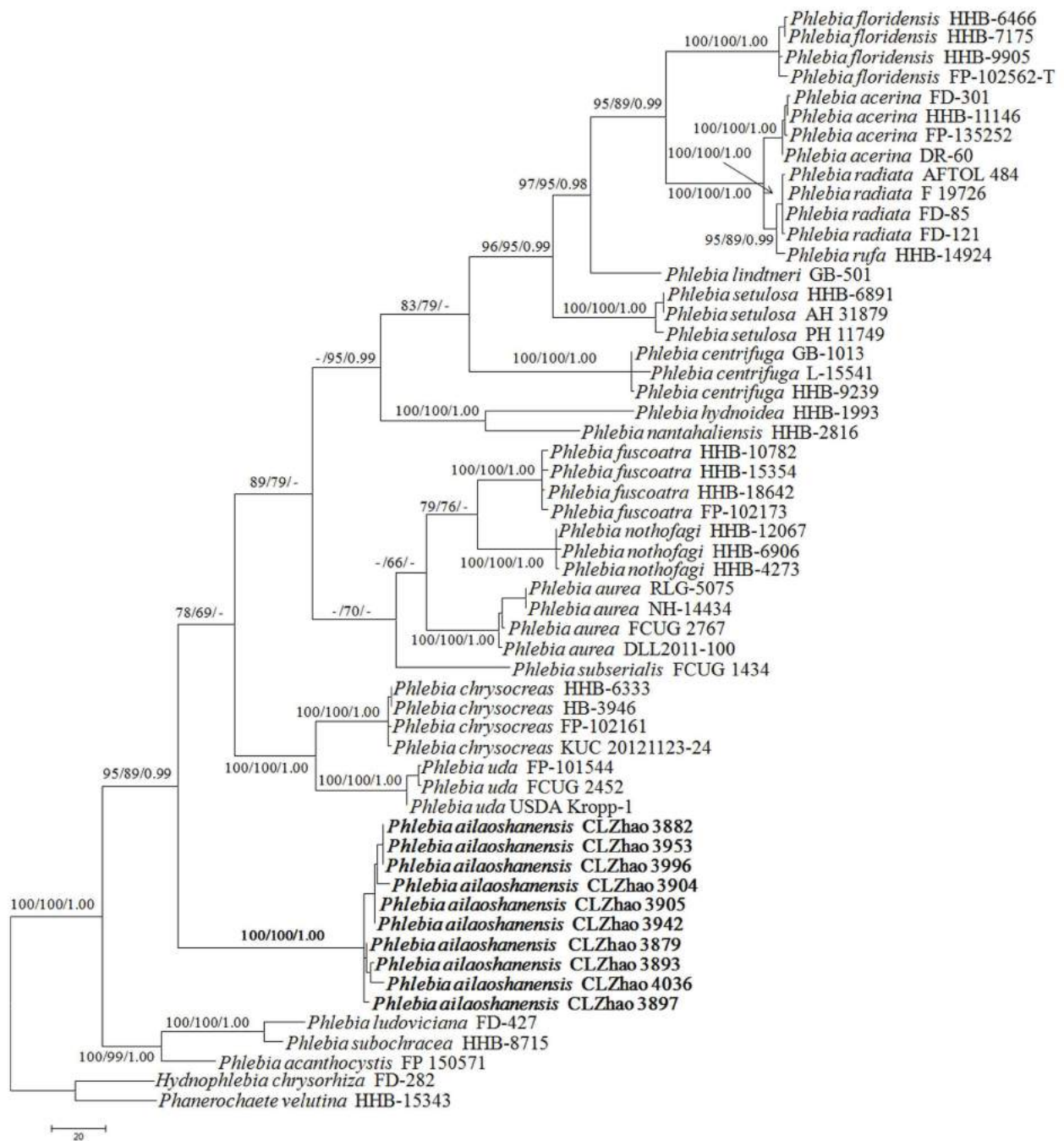


FIGURE 2. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Phlebia ailaoshanensis* and related species in *Phlebia* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior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

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 (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

Molecular phylogeny

The ITS+nLSU dataset (Fig. 1) included sequences from 64 fungal specimens representing 56 species. The dataset had an aligned length of 2137 characters, of which 1247 characters are constant, 253 are variable and parsimony-uninformative, and 637 are parsimony-informative. Maximum parsimony analysis yielded 2 equally parsimonious trees (TL = 5058, CI = 0.298, HI = 0.702, RI = 0.532, RC = 0.159). 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 = 0.007863 (BI).

The phylogenetic tree (Fig. 1) inferred from ITS+nLSU sequences, demonstrated seven major clades for 56 sampled species of the Polyporales. The new species *Phlebia ailaoshanensis* fell into the Meruliaceae within the phlebioid clade. The genus *Phlebia* was closely related to *Ceriporiopsis* and *Phanerochaete* P. Karst.

The ITS+nLSU dataset (Fig. 2) included sequences from 56 fungal specimens representing 21 species. The dataset had an aligned length of 2017 characters, of which 1524 characters are constant, 123 are variable and parsimony-uninformative, and 370 are parsimony-informative. Maximum parsimony analysis yielded 100 equally parsimonious trees (TL = 1233, CI = 0.578, HI = 0.422, RI = 0.854, RC = 0.494). 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 = 0.002615 (BI).

A further phylogeny (Fig. 2) inferred from the combined ITS+nLSU sequences was obtained for 56 fungal specimens representing 21 taxa within the genus *Phlebia* 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. chrysocreas* (Berk. & M.A. Curtis) Burds. and *P. uda* (Fr.) Nakasone.

Taxonomy

Phlebia ailaoshanensis C.L. Zhao, *sp. nov.* (Figs. 2, 3)

Mycobank no.: MB 827601

Type.—**China**. Yunnan Province, Puer, Jingdong County, Ailaoshan National Nature Reserve, on the angiosperm trunk, 4 October 2017, *CLZhao 4036* (holotype, SWFC!)

Etymology.—*Ailaoshanensis* (Lat.): referring to the locality (Ailaoshan) of the type specimens.

Basidiomata.—Basidiocarps annual, resupinate, ready to separate from substrate, subceraceous to subgelatinous, without odor or taste when fresh, becoming membranaceous to coraceous upon drying, up to 21 cm long, 100–500 µm thick. Hymenial surface tuberculate to phlebioid with aculei, cream to buff-yellow to orange-yellow when fresh, buff to yellowish brown upon drying. Sterile margin distinct, white, fimbriate.

Hyphal structure.—Hyphal system monomitic; generative hyphae with simple septa, IKI–, CB–; tissues unchanged in KOH.

Hymenium.—Cystidia and cystidioles absent; numerous crystals present among the hyphae, basidia clavate, with four sterigmata and a basal simple septa, 15.5–25.5 × 5–7 µm; basidioles dominant, in shape similar to basidia, but slightly smaller. Basidiospores ellipsoid, more or less curved, hyaline, thin-walled, smooth, IKI–, CB–, (5.5–)5.7–8.5(–8.8) × 3–4.3(–4.5) µm, L = 7.15 µm, W = 3.62 µm, Q = 1.63–2.22 (n = 300/10).

Additional specimens examined.—**China**. Yunnan Province, Puer, Jingdong county, Ailaoshan National Nature Reserve, on the angiosperm trunk, 4 October 2017, *CLZhao 3882, 3897, 3905, 3942, 3953* (SWFC!), on the fallen angiosperm branch, 4 October 2017, *CLZhao 3879, 3893, 3996* (SWFC!), on the fallen branch of *Pinus*, 4 October 2017, *CLZhao 3904* (paratypes, SWFC!).

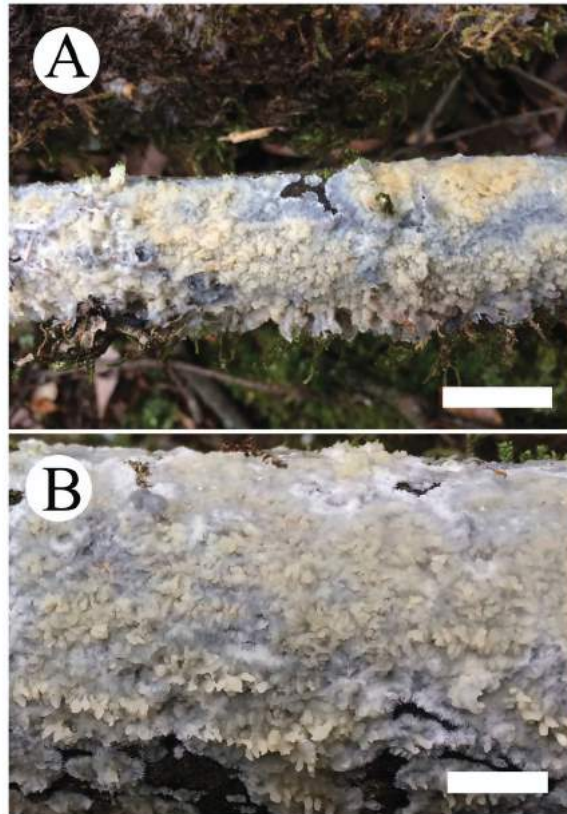


FIGURE 3. Basidiomata of *Phlebia ailaoshanensis* (holotype). Scale bars: a–1 cm, b–5 mm.

Discussion

In the present study, a new species, *Phlebia ailaoshanensis*, is described based on phylogenetic analyses and morphological characters. The species has an unique morphological characters in the genus *Phlebia*.

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, Zhao *et al.* 2015). According to our result based on the combined ITS+nLSU sequence data (Fig. 1), *Phlebia ailaoshanensis* is nested into the phlebioid clade with strong support (100% BS, 100% BP, 1.00 BPP).

Kües & Navarro-González (2015) discussed the morphological aspects of fruiting bodies and their development in Agaricomycetes and other basidiomycetes, in which they concluded that: (1) genetic, physiological and environmental factors effecting morphological plasticity are addressed; (2) the shapes and features of wood-rotting fungi do not reveal close or distant phylogenetic relationships. Therefore, it is reasonable that basidiocarps with smooth, poroid, odontoid or grandinioid hymenophore grouped together in phylogenetic studies (Binder *et al.* 2005, 2013, Hibbett *et al.* 2007, Larsson 2007). In present molecular analyses (Fig. 1), *Phlebia* groups with *Ceriporiopsis* inferred from the ITS+nLSU analyses. However, morphologically *Ceriporiopsis* differs from *Phlebia* by its poroid hymenophore (Ryvarden & Melo 2014)

Phlebia ailaoshanensis is closely related to *P. acanthocystis* Gilb. & Nakasone, *P. chrysocreas* (Berk. & M.A. Curtis) Burds., *P. ludoviciana* (Burt) Nakasone & Burds., *P. subcretacea* (Litsch.) M.P. Christ. and *P. uda* (Fr.) Nakasone. in the rDNA based on the phylogeny (Fig. 2). But morphologically *P. acanthocystis* has clamp connections and presence of leptocystidia (Nakasone & Gilbertson 1998). *Phlebia chrysocreas* differs from *P. ailaoshanensis* by having smooth to slightly warted hymenial surface with ochraceous-buff to yellow ochre color and smaller, narrowly ovoid basidiospores (Lombard *et al.* 1975). *Phlebia ludoviciana* differs in having the broadly effused basidiocarps with cinnamon-buff to tawny-olive or isabella hymenial surface, encrusted cystidia and smaller basidiospores ($5.5\text{--}6.5 \times 2\text{--}2.5 \mu\text{m}$, Nakasone *et al.* 1982). *Phlebia subcretacea* is separated from the new species by white hymenophore and allantoid basidiospores (Bernicchia & Gorjón 2010). *Phlebia uda* differs from *P. ailaoshanensis* by having the odontoid hymenophore, a generative hyphae bearing clamp connections and smaller basidiospores ($5\text{--}5.5 \times 2\text{--}2.5 \mu\text{m}$, Bernicchia & Gorjón 2010).

Having generative hyphae with simple septa reminds of four similar species in the genus *Phlebia*: *P. badia* (Pat.) Nakasone, *P. deflectens* (P. Karst.) Ryvarden, *P. griseolivens* (Bourdot & Galzin) Parmasto. and *P. viridesalebrosa* J. Erikss. & Hjortstam. *Phlebia badia* differs from *P. ailaoshanensis* by the effused to effused-reflexed basidiocarps with hydroid hymenophore and a dimitic hyphal system (Nakasone 2002, Bernicchia & Gorjón 2010). *Phlebia deflectens* is separated from *P. ailaoshanensis* by its smooth hymenophore with ochraceous to pale brown color and smaller basidiospores ($4\text{--}5 \times 2.5\text{--}3 \mu\text{m}$, Bernicchia & Gorjón 2010). *Phlebia griseolivens* differs in its pruinose to smooth hymenophore, thin-walled generative hyphae and the ovoid to oblong basidiospores (Bernicchia & Gorjón 2010). *Phlebia viridesalebrosa* differs from *P. ailaoshanensis* by the yellowish-ochraceous hymenial surface and smaller, narrowly ellipsoid basidiospores ($6\text{--}7 \times 3\text{--}3.5 \mu\text{m}$, Bernicchia & Gorjón 2010).

Wood-rotting fungi are an extensively studied group of Basidiomycota (Gilbertson & Ryvarden 1987, Núñez & Ryvarden 2001, Bernicchia & Gorjón 2010, Dai 2012, Ryvarden & Melo 2014), but the Chinese wood-rotting fungi diversity is still not well known, especially in subtropics and tropics, many recently described taxa of wood-rotting fungi were from these areas (Zhou & Dai 2012, 2013; Zhao & Cui 2013, 2014, Ren & Wu 2017, Wu *et al.* 2017, Yuan *et al.* 2017a, b, Song *et al.* 2014, 2016b, Zhou *et al.* 2016). The new species in the present study, *Phlebia ailaoshanensis*, is from subtropics, too. It is possible that new taxa will be found after further investigations and molecular analyses.

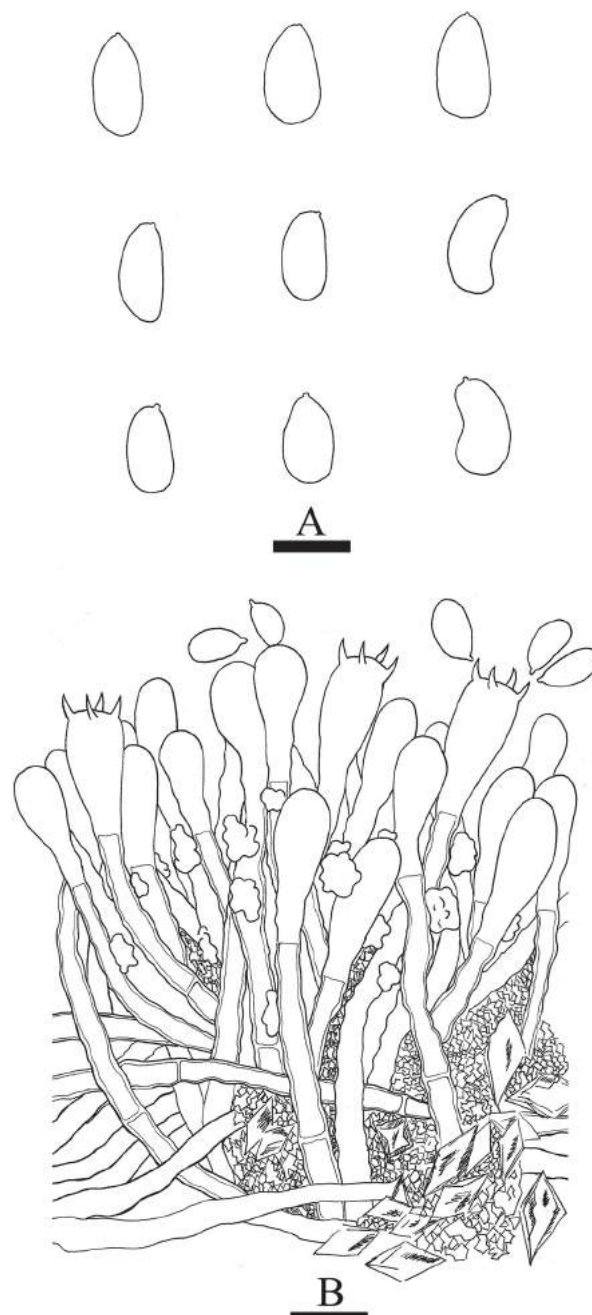


FIGURE 4. Microscopic structures of *Phlebia ailaoshanensis* (drawn from the holotype). a. Basidiospores. b. A section of basidiocarps. Bars: a–5 μm ; b–10 μm .

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