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Phlebiopsis lacerata sp. nov. (Polyporales, Basidiomycota) from southern China

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Abstract

A new white-rot species, *Phlebiopsis lacerata sp. nov.*, is proposed based on morphological and molecular characters. It is characterized by resupinate to effuse-reflexed basidiomata, a monomitic hyphal system with simple septate generative hyphae, conical cystidia that are thick-walled, heavily encrusted with large crystal in the apical part, and ellipsoid basidiospores (4–5.5 × 3–3.5 µm). Phylogenetic analyses of the ITS and LSU nrRNA gene regions showed that *P. lacerata* belongs to the Phanerochaetaceae and is nested in the phlebioid clade. Further investigation based on ITS+nLSU sequences with more representative taxa in *Phlebiopsis* demonstrated that *P. lacerata* forms a monophyletic lineage with a strong support (100% BS, 100% BP, 1.00 BPP) and then groups with *P. crassa*.

Key words: Phanerochaetaceae, Phylogenetic analyses, Taxonomy, Wood-rotting fungi

Introduction

Phlebiopsis Jülich (1978: 137) (Phanerochaetaceae, Polyporales), typified with *P. gigantea* (Fr.) Jülich (1978: 137) is a genus characterized by a combination of resupinate to effused-reflexed basidiomata with a membranaceous to subceraceous consistencey when fresh, cracked when dry, a smooth to odontioid to poroid hymenophore, a monomitic hyphal structure with simple-septate generative hyphae, hyaline cystidia that are thick-walled and encrusted, usually narrowly clavate basidia, and basidiospores that are hyaline, thin-walled, smooth, cylindrical to ellipsoid, acyanophilous and negative in Melzer's reagent (Jülich 1978, Bernicchia & Gorjón 2010). So far, about 22 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, Zhao *et al.* 2019).

The systematics of *Phlebiopsis* has benefited from numerous molecular studies (Larsson 2007, Binder *et al.* 2013, Floudas & Hibbett 2015, Miettinen *et al.* 2016, Justo *et al.* 2017, Zhao *et al.* 2019). Larsson (2007) presented the classification of corticioid fungi and suggested that *Phlebiopsis flavidoalba* (Cooke) Hjortstam (1987: 58) was nested into the family Phanerochaetaceae. Nevertheless, Binder *et al.* (2013) published a multigene, molecular phylogenetic study showed that *Phlebiopsis flavidoalba* belonged to the phlebioid clade and appeared to be grouped with *Phanerochaete lamprocystidiata* Sheng H. Wu (2004: 426). A phylogenetic study of *Phanerochaete* P. Karst. (1889: 426) using a four gene dataset by Floudas & Hibbett (2015) suggested that *Phlebiopsis* s.s. clustered into the phlebioid clade and grouped with *Phaeophlebiopsis* Floudas & Hibbett (2015: 707) and *Rhizochaete* Gresl., Nakasone & Rajchenb. (2004: 261). Miettinen *et al.* (2016) also showed that the generic type species *P. gigantea* grouped with *Phaeophlebiopsis* and *Rhizochaete*. By using a multi-gene dataset, Justo *et al.* (2017) proposed a revised family-level classification of the

Polyporales and confirmed that *P. gigantea* belongs to the family Phanerochaetaceae and grouped with *Phlebiopsis crassa* (Lév.) Floudas & Hibbett (2015: 710) and *P. galochroa* (Bres.) Hjortstam & Ryvarden (1980: 285). Zhao *et al*. (2019) uncovered a new species *Phlebiopsis yunnanensis* C.L. Zhao (2019: 273) based on morphological characters and rDNA sequences, in which this species was related to *P. gigantea* and *P. lamprocystidiata*.

Recently, we collected an undescribed taxon from southern China that could not be assigned to any described species. We present morphological and molecular phylogenetic evidence that support the recognition of this new species in the genus *Phlebiopsis*.

Materials and methods

Morphological studies.—The studied specimens were deposited at the herbarium of Southwest Forestry University (SWFC). Macro-morphological descriptions were based on field notes. Special colour terms followed Petersen (1996). Micro-morphological data were obtained from the dried specimens, and observed under a light microscope following Dai (2010) and Han *et al.* (2016). 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 pair ITS5 and ITS4 (White *et al*. 1990). Nuclear LSU region was amplified with primer pair LROR 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 at TreeBase (submission ID 24453). Sequences of *Heterobasidion annosum* (Fr.) Bref. (1888: 154) and *Stereum hirsutum* (Willd.) Pers. (1800: 90) obtained from GenBank were used as an outgroup to root trees following, Binder *et al*. (2013) in the ITS+nLSU analysis (Fig. 1) and *Phlebia unica* (H.S. Jacks. & Dearden) Ginns (1984: 329) and *Ceraceomyces americanus* Nakasone, C.R. Bergman & Burds. (1994: 56) obtained from GenBank were used as an outgroup to root trees following, Miettinen *et al*. (2016) in the ITS+nLSU analyses (Fig. 2).

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analysis followed Zhao & Wu (2017) and Shen *et al*. (2019) 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 (BS) 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 6 million generations (Fig. 1), for 5 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 (BT) and Bayesian posterior probabilities (BPP) greater than or equal to 80 % (BL), 75 % (BP) and 0.95 (BPP) were considered as significantly supported, respectively.

TABLE 1. List of species, specimens and GenBank accession numbers 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
C. jelicii	H6002113	FJ496690	FJ496727	Tomšovský *et al*. 2010
C. subrufa	BRNM 710172	FJ496662	FJ496724	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
D. quercina	HHB 8735	FJ403214	—	Binder *et al*. 2013
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
G. subtropica	Cui 9041	JQ845096	JQ845099	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
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

...continued on the next page

TABLE 1. (Continued)

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Phlebiopsis brunneocystidiata	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. crassa	ME 516	KP135395	KP135395	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
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. lacerata	CLZhao 3692	MT180946	MT180950	This study
P. lacerata	CLZhao 3705	MT180947	MT180951	This study
P. lacerata	CLZhao 3721	MT180948	MT180952	This study
P. lacerata	CLZhao 3747	MT180949	MT180953	This study
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	Zhao *et al*. 2019
P. yunnanensis	CLZhao 3900	MH744141	MH744143	Zhao *et al*. 2019
Piloporia sajanensis	Mannine 2733a	HQ659239	HQ659239	Tomšovský *et al*. 2010
P. venustula	CBS 656.84	JN649367	JN649367	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. 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	FJ411098	Robledo *et al*. 2009
Tyromyces chioneus	Cui 10225	KF698745	KF698756	Zhao *et al*. 2015
Xanthoporus syringae	Gothenburg 1488	JN710607	JN710607	Tomšovský *et al*. 2010

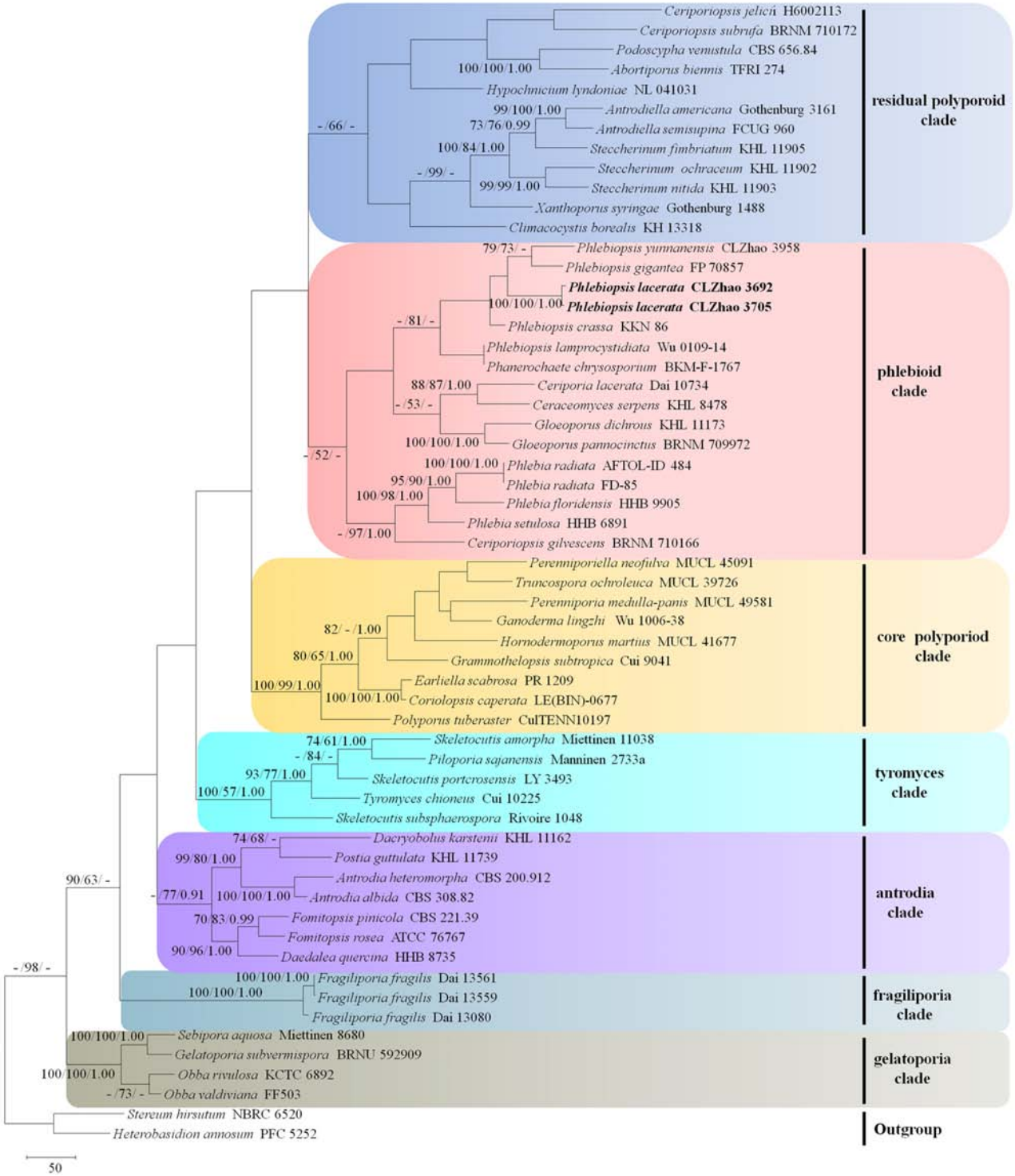

FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Phlebiopsis lacerata* and related species in Polyporales based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior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

Results

Molecular phylogeny

The ITS+nLSU dataset (Fig. 1) included sequences from 58 fungal specimens representing 56 species. The dataset had an aligned length of 2337 characters, of which 1364 characters are constant, 281 are variable and parsimony-

uninformative, and 692 are parsimony-informative. Maximum parsimony analysis yielded four equally parsimonious trees (TL = 5285, CI = 0.312, HI = 0.687, RI = 0.516, RC = 0.161). 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 = 0.001515 (BI).

The phylogeny (Fig. 1) inferred from ITS+nLSU sequences demonstrated seven major clades for 56 species of the Polyporales. The new species clustered into the phlebia clade and grouped with the generic species *Phlebiopsis gigantea*.

The ITS+nLSU dataset (Fig. 2) included sequences from 30 fungal specimens representing 13 species. The dataset had an aligned length of 2057 characters, of which 1724 characters are constant, 146 are variable and parsimony-uninformative, and 187 are parsimony-informative. Maximum parsimony analysis yielded 100 equally parsimonious trees (TL = 550, CI = 0.734, HI = 0.266, RI = 0.842, RC = 0.618). 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 = 0.002128 (BI).

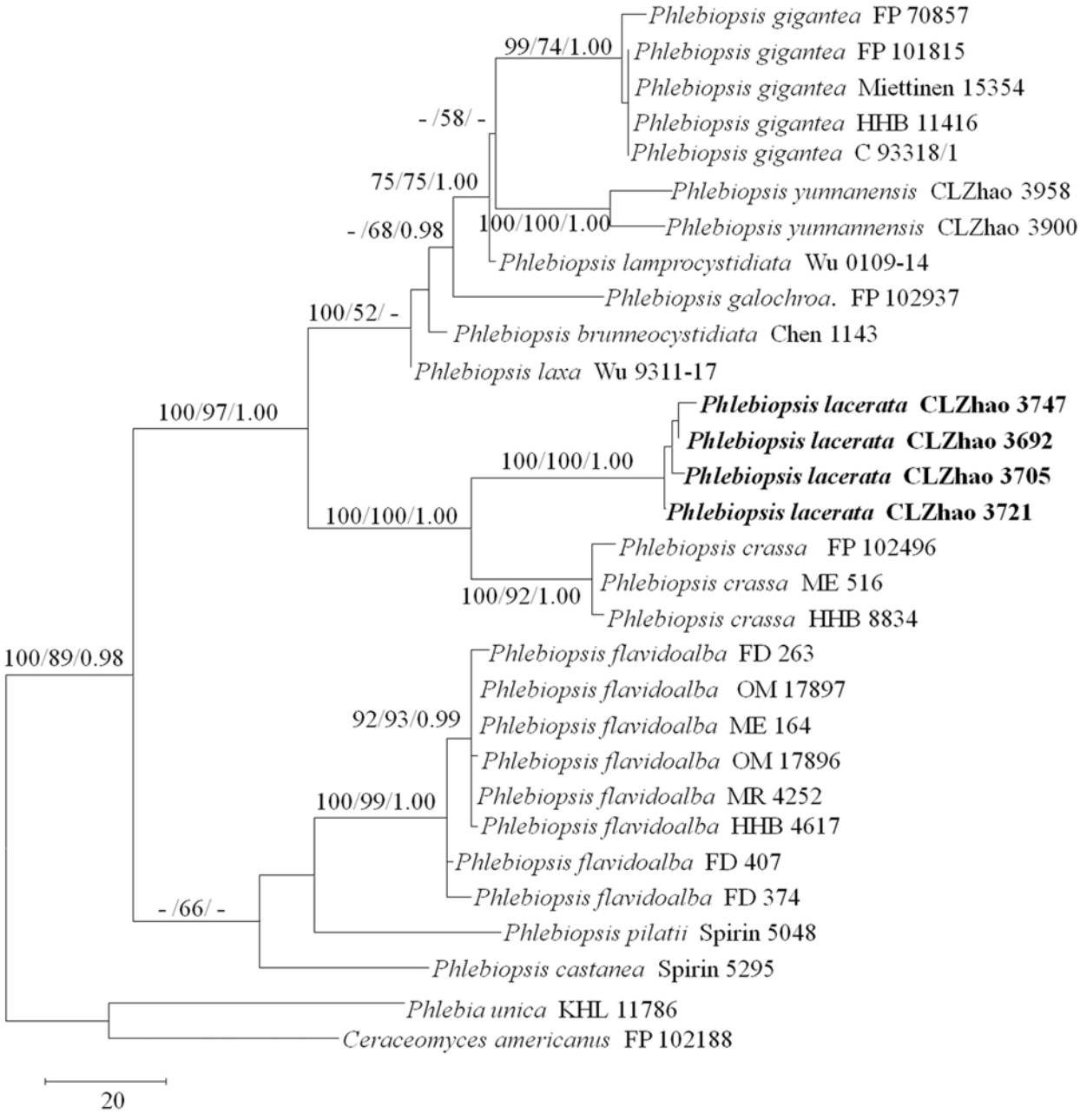

FIGURE 2. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Phlebiopsis lacerata* and related species in *Phlebiopsis* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian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

A further phylogeny (Fig. 2) inferred from the combined ITS+nLSU sequences was obtained for 11 species (ingroup) within the genus *Phlebiopsis* and showed that the new species formed a monophyletic lineage with a strong support (100% BS, 100% BP and 1.00 BPP) and was sister to *P. crassa*.

Taxonomy

Phlebiopsis lacerata C.L. Zhao, *sp. nov.* (Figs. 3, 4, 5)

MycoBank no.: MB 834941

Type: CHINA, Yunnan Province, Puer, Jingdong County, Wuliangshan National Nature Reserve, on angiosperm fallen branch, 3 October 2017, *CLZhao 3692/SWFC 3692* (holotype, SWFC!).

Etymology.—*Lacerata* (Lat.): referring to the lacerate hymenophore.

Basidiomata.—Annual, resupinate to effuse-reflexed, subceraceous when fresh, turning to leather upon drying, up to 15 cm long, 4 cm wide, 3 mm thick. Pilei often laterally fused. Pileal surface tomentose, buff to ochraceous when fresh and grey ochraceous upon drying. Pores poroid when juvenile in margin, 1–2 per mm, dissepiments thin, lacerated and splitting to spines or odontioid with age, white to cream when fresh, turning to cream to violaceous upon drying. Sterile margin distinct, white.

Hyphal structure.—Hyphal system monomitic; generative hyphae simple septate, thick-walled, branched, 3–5.5 µm diam, IKI–, CB–; tissues unchanged in KOH.

Hymenium.—Cystidia conical, thick-walled, apically encrusted with large and heavy crystal, 50–85 × 3–5.5 µm, cystidioles absent; basidia narrowly clavate, with four sterigmata and a simple septum, 18–26.5 × 3.5–5.5 µm; basidioles dominant, in shape similar to basidia, but slightly smaller.

Spores.—Basidiospores ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, 4–5.5(–6) × (2.5)3–3.5 µm, L = 4.45 µm, W = 3.19 µm, Q =1.31–1.48 (n = 240/4).

Associated wood-rot: White.

Additional specimens examined.—CHINA, Yunnan Province, Puer, Jingdong County, Wuliangshan National Nature Reserve, on angiosperm fallen branch, 3 October 2017, *CLZhao 3705/SWFC 3705, 3721/SWFC 3721, 3747/SWFC 3747* (paratypes, SWFC!).

Discussion

In the present study, a new species, *Phlebiopsis lacerata*, is described based on phylogenetic analysies and morphological characters.

Binder *et al*. (2013) showed that six clades are found in the Polyporales: antrodia clade, core polyporoid clade, gelatoporia clade, phlebioid clade, residual polyporoid clade and tyromyces clade. According to our results based on the combined ITS+nLSU sequence data (Fig. 1), *Phlebiopsis lacerata* is nested into the phlebioid clade with strong support (100% BS, 100% BP, 1.00 BPP).

In our analysis (Fig. 2), *Phlebiopsis lacerata* grouped with *P. crassa* inferred from the ITS+nLSU sequences. However, morphologically, *P. crassa* differs from *P. lacerata* by its brown basidiomata with smooth to tuberculate hymenophore surface and narrowly ellipsoid, larger basidiospores (6–7 × 3–4 µm; Bernicchia & Gorjón 2010). *Phlebiopsis castanea* differs in its cinnamon to salmon pink hymenophore surface and broadly allantoid, slightly thick-walled basidiospores (Núñez & Ryvarden 2001).

In geographical distribution, *Phlebiopsis yunnanensis* C.L. Zhao and *P. lacerata* were found in China. However, *P. yunnanensis* can be distinguished by smooth to odontioid hymenophoral surface and smaller cystidia (36–41 × 8–11 µm; Zhao *et al*. 2019). In addition, both species did not group closely in the phylogenetic tree (Fig 2).

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 wood-rotting fungal diversity is still not well known, especially in the subtropics and tropics, and many recently described taxa of wood-rotting fungi were from these areas (Zhao & Cui 2013, 2014, Ren & Wu 2017, Wu *et al*. 2017, Yuan *et al*. 2017a, b, Xing *et al*. 2018, Zhao *et al*. 2019). The new species *Phlebiopsis lacerata* is from the subtropics, too.

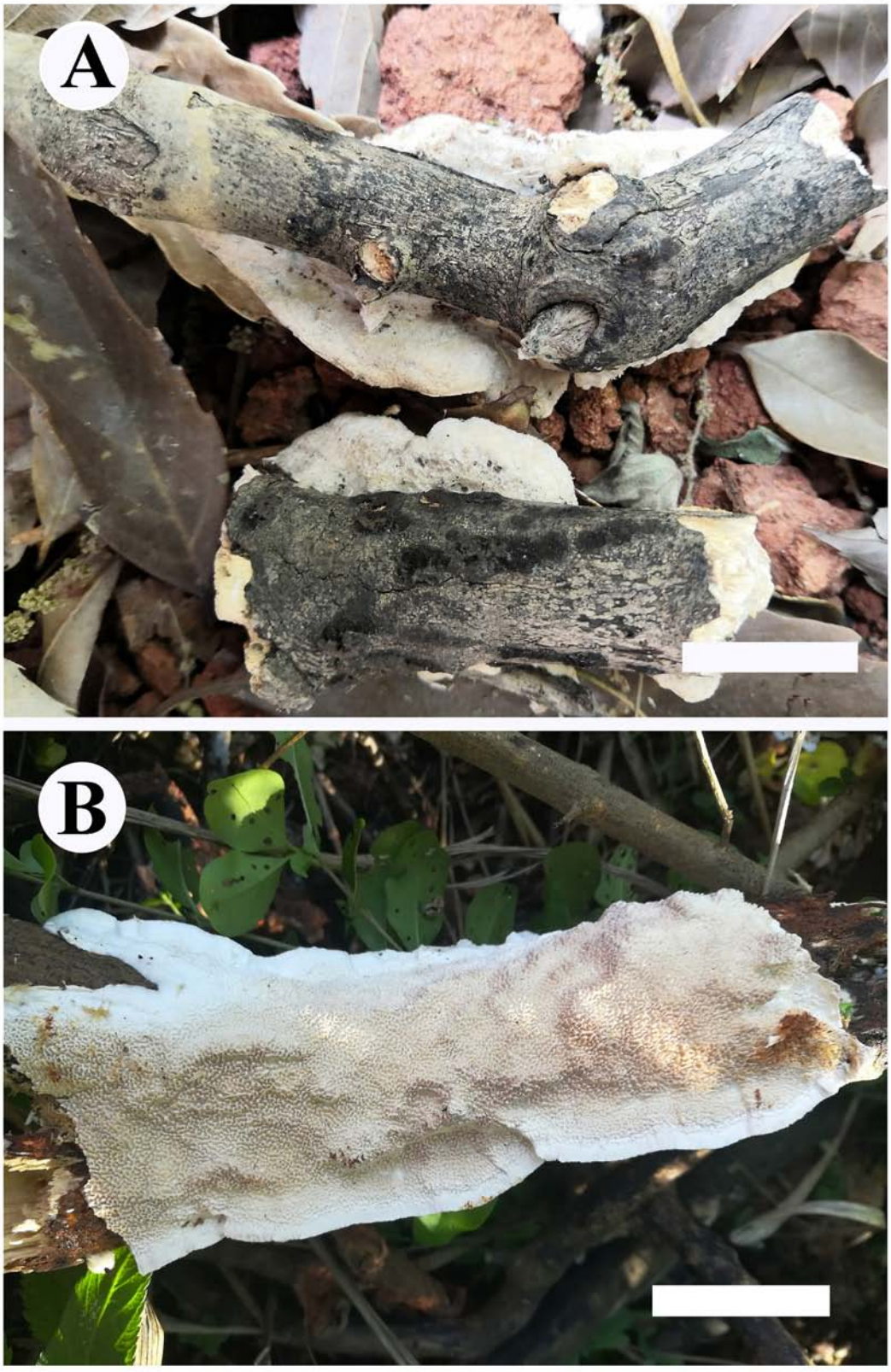

FIGURE 3. Basidiomata of *Phlebiopsis lacerata* (holotype). Scale bars: a, b– 2 cm.

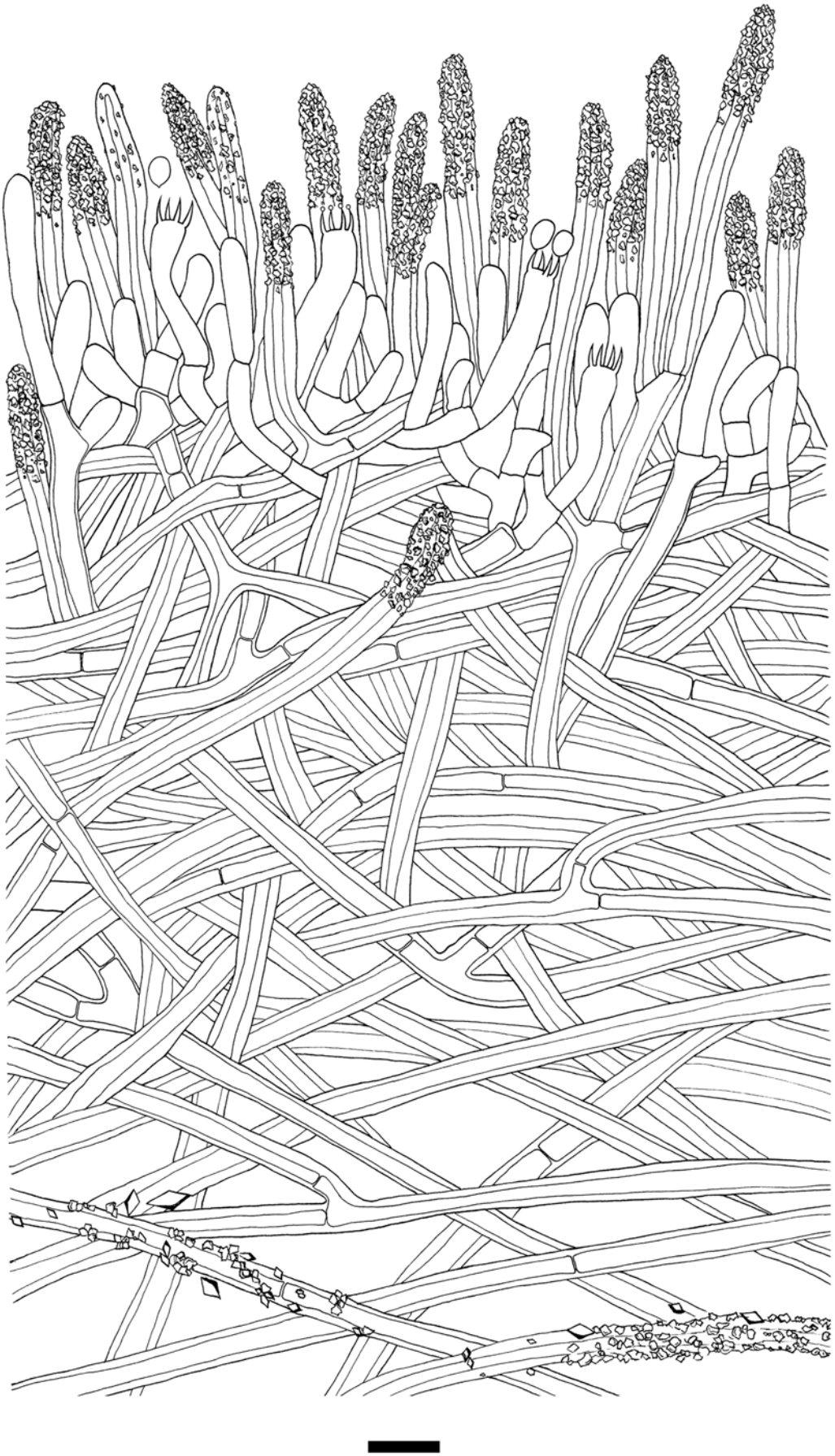

FIGURE 4. A section of hymenium of *Phlebiopsis lacerata* (drawn from the holotype). Bars: a–10 μm.

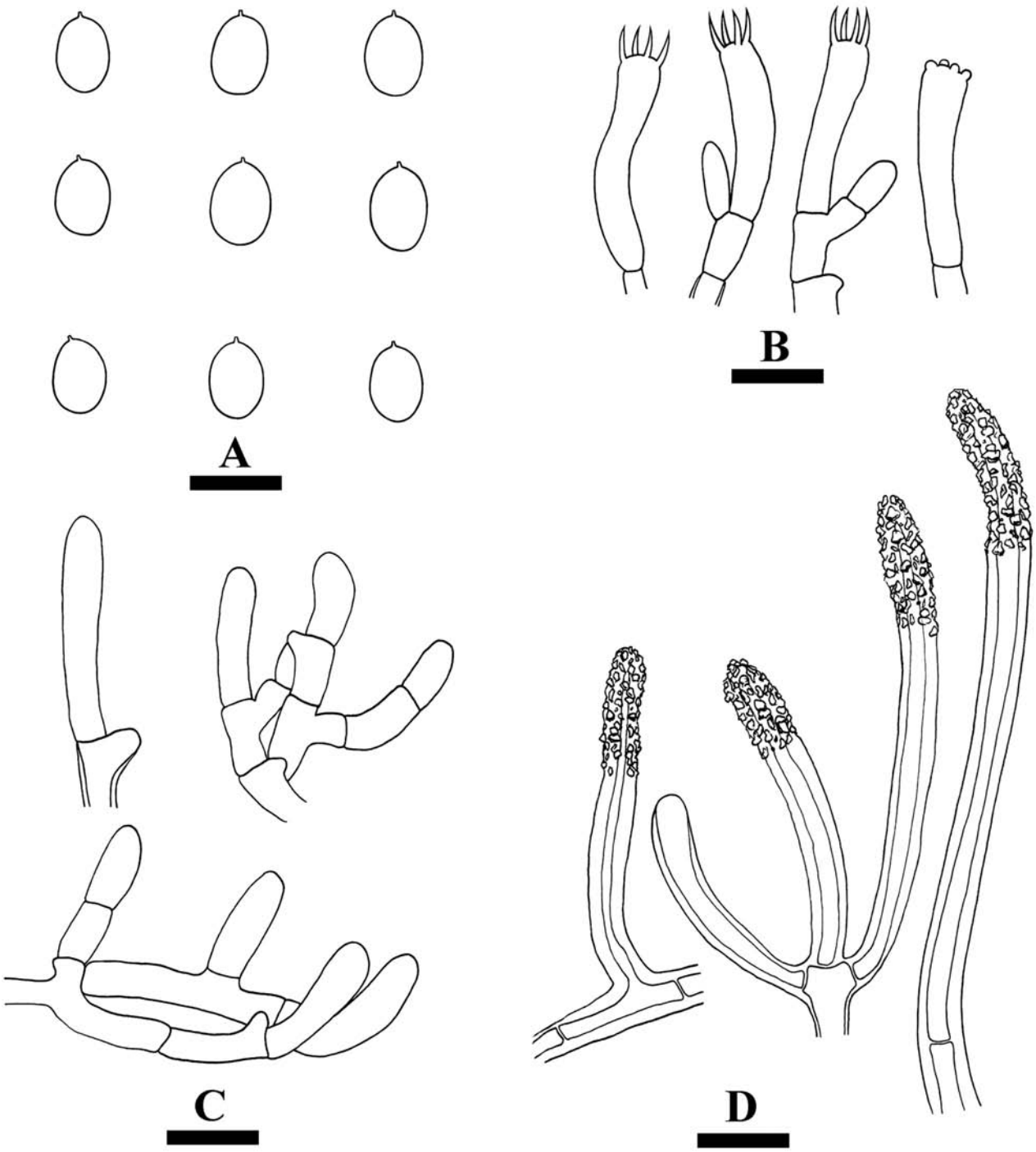

FIGURE 5. Microscopic structures of *Phlebiopsis lacerata* (drawn from the holotype). a. basidiospores. b. basidia. c. basidioles. d. cystidia. Bars: a–5 μm; b, c–10 μm.

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