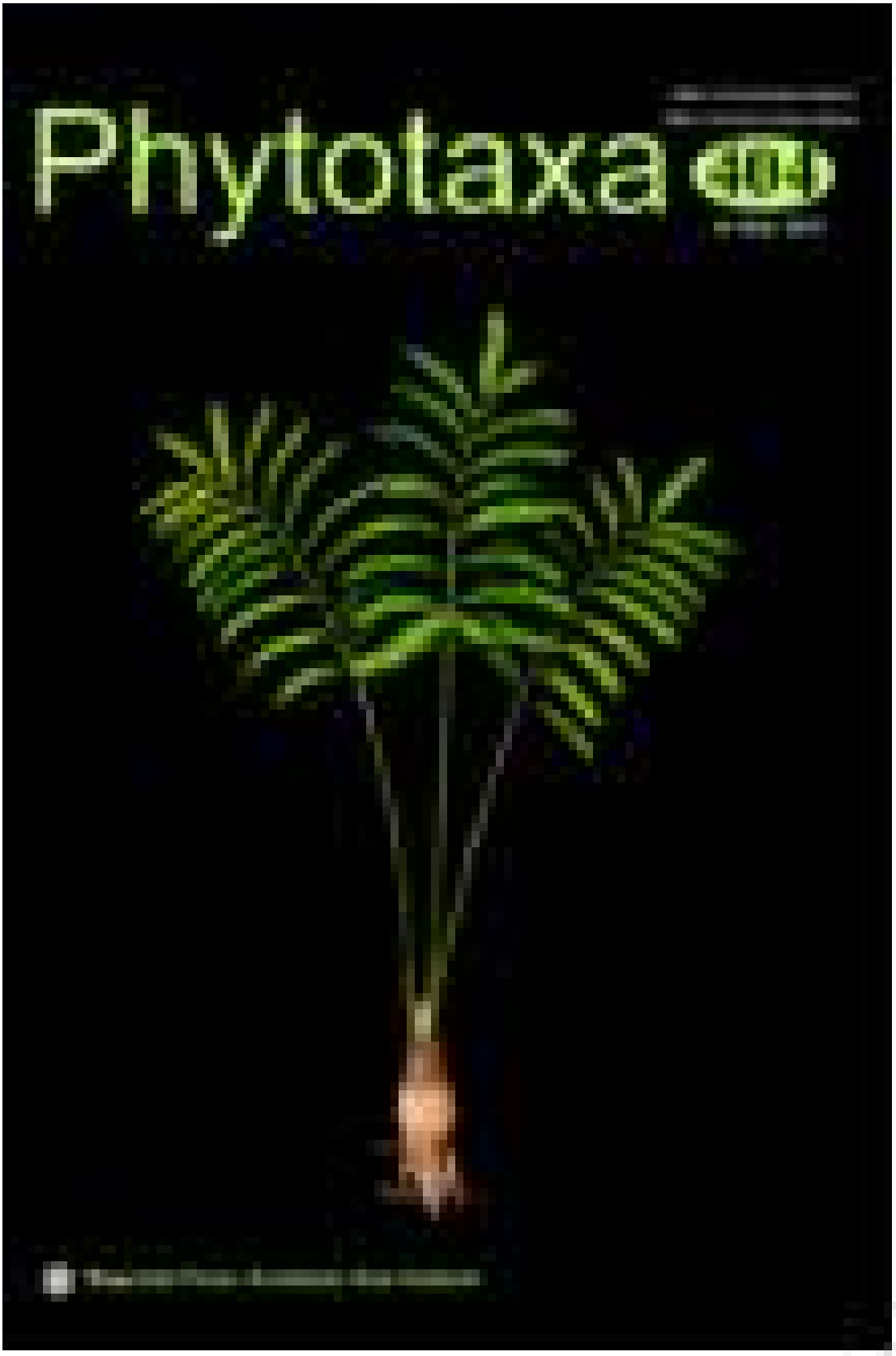
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Morphological and molecular identification of a new species of *Eichleriella* (Auriculariales, Basidiomycota) in China

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Abstract

A new wood-inhabiting fungal species, *Eichleriella xinpingensis*, is described based on a combination of morphological features and molecular evidence. The species is characterized by an annual, resupinate basidiocarps with soft leathery to ceraceous hymenial surface covered by blunt-pointed spines, a dimitic hyphal system with clamp generative hyphae, two-celled, narrowly ovoid to obconical basidia and broadly cylindrical, hyaline, thin-walled, smooth, basidiospores measuring as 6.5–10 ×3.5–4.5 µm. Sequences of ITS and LSU nrDNA regions of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and Bayesian inference methods. The phylogenetic analyses showed that *E. xinpingensis* belonged to the Auriculariaceae and was closely related to *E. tenuicula*.

Keywords: Auriculariaceae, Phylogeny, Taxonomy, Wood-inhabiting fungi

Introduction

Eichleriella Bres. (1903: 115) (Auriculariaceae, Auriculariales) was erected by Bresadola (1903), and it is a cosmopolitan genus characterized by a combination of basidiocarps annual or short-living perennial, cupulate or resupinate orbicular, leathery to ceraceous, hymenophore smooth, pale coloured, in some species covered by spines, hyphal structure monomitic to dimitic with clamp connections, cystidia often present, basidia longitudinally septate, 2- or 4-celled, and basidiospores hyaline, cylindrical to narrowly cylindrical (Bresadola 1903, Malysheva & Spirin 2017). So far about 15 species have been accepted in the genus worldwide (Bresadola 1903, Roberts 2008, Malysheva & Spirin 2017).

Recently, molecular studies involving Auriculariaceae Fr. have been carried out (Weiss & Oberwinkler 2001, Sotome *et al.* 2014, Malysheva & Spirin 2017). Phylogenetic study of Auriculariales and related groups suggested that *Eichleriella* grouped with *Auricularia* Bull. (1785: 20), *Exidia* Fr. (1822: 220), *Exidiopsis* Vuill. (1895: 82) and *Heterochaete* Pat. (1892: 120) (Weiss & Oberwinkler 2001). Malysheva & Spirin (2017) introduced the taxonomy and phylogeny of the Auriculariales and suggested that *Eichleriella* was grouped with *Amphistereum* Spirin & Malysheva (2017: 696) and *Auricularia*.

During investigations on wood-inhabiting fungi in south-western China, a new taxon belonging to *Eichleriella* was found and described and phylogeny of this new species within *Eichleriella* was carried out based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal DNA (nLSU) sequences.

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

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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 pairs ITS5 and ITS4 (White *et al*. 1990). Nuclear LSU region was amplified with primer pairs 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).

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	
Amphistereum leveilleanum	FP-106715	KX262119	KX262168	Malysheva & Spirin (2017)
A. schrenkii	HHB 8476	KX262130	KX262178	Malysheva & Spirin (2017)
Auricularia mesenterica	TUFC12805	AB915192	AB915191	Weiss & Oberwinkler (2001)
Eichleriella alliciens	HHB 7194	KX262120	KX262169	Malysheva & Spirin (2017)
E. bactriana	TAAM 96698	KX262123	KX262172	Malysheva & Spirin (2017)
E. bactriana	TAAM 55071	KX262121	KX262170	Malysheva & Spirin (2017)
E. bactriana	TAAM 104431	KX262138	KX262186	Malysheva & Spirin (2017)
E. crocata	TAAM 101077	KX262100	KX262147	Malysheva & Spirin (2017)
E. crocata	TAAM 125909	KX262118	KX262167	Malysheva & Spirin (2017)
E. tenuicula	TUFC33717	AB871766	AB871747	Sotome *et al*. (2014)
E. tenuicula	LR 17599	KX262141	KX262189	Malysheva & Spirin (2017)
E. desertorum	LR 49350	KX262142	KX262190	Malysheva & Spirin (2017)
E. flavida	LR 49412	KX262137	KX262185	Malysheva & Spirin (2017)
E. leucophaea	LE 303261	KX262111	KX262161	Malysheva & Spirin (2017)
E. leucophaea	KHL 15277	KX262115	KX262164	Malysheva & Spirin (2017)
E. leucophaea	KHL 15299	KX262136	KX262184	Malysheva & Spirin (2017)
E. macrospora	FP-101769	KX262129	—	Weiss & Oberwinkler (2001)
E. shearii	USJ 54609	AF291284	AF291335	Weiss & Oberwinkler (2001)
E. shearii	LR 23258	KX262139	—	Malysheva & Spirin (2017)
E. sicca	KHL 13785	KY264026	—	Malysheva & Spirin (2017)
E. sicca	OM 17349	KX262143	KX262191	Malysheva & Spirin (2017)
E. xinpingensis	CL Zhao 812	MK560878	MK560882	This study
E. xinpingensis	CL Zhao 836	MK560879	MK560883	This study
E. xinpingensis	CL Zhao 842	MK560880	MK560884	This study
E. xinpingensis	CL Zhao 870	MK560881	—	This study

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 23179). Sequences of *Amphistereum schrenkii* (Burt) Spirin & Malysheva (2017: 698) and *A. leveilleanum* (Berk. & M.A. Curtis) Spirin & Malysheva (2017: 697) obtained from GenBank were used as outgroups to root trees following Malysheva & Spirin (2017) in the ITS and ITS+nLSU analyses (Figs. 1, 2).

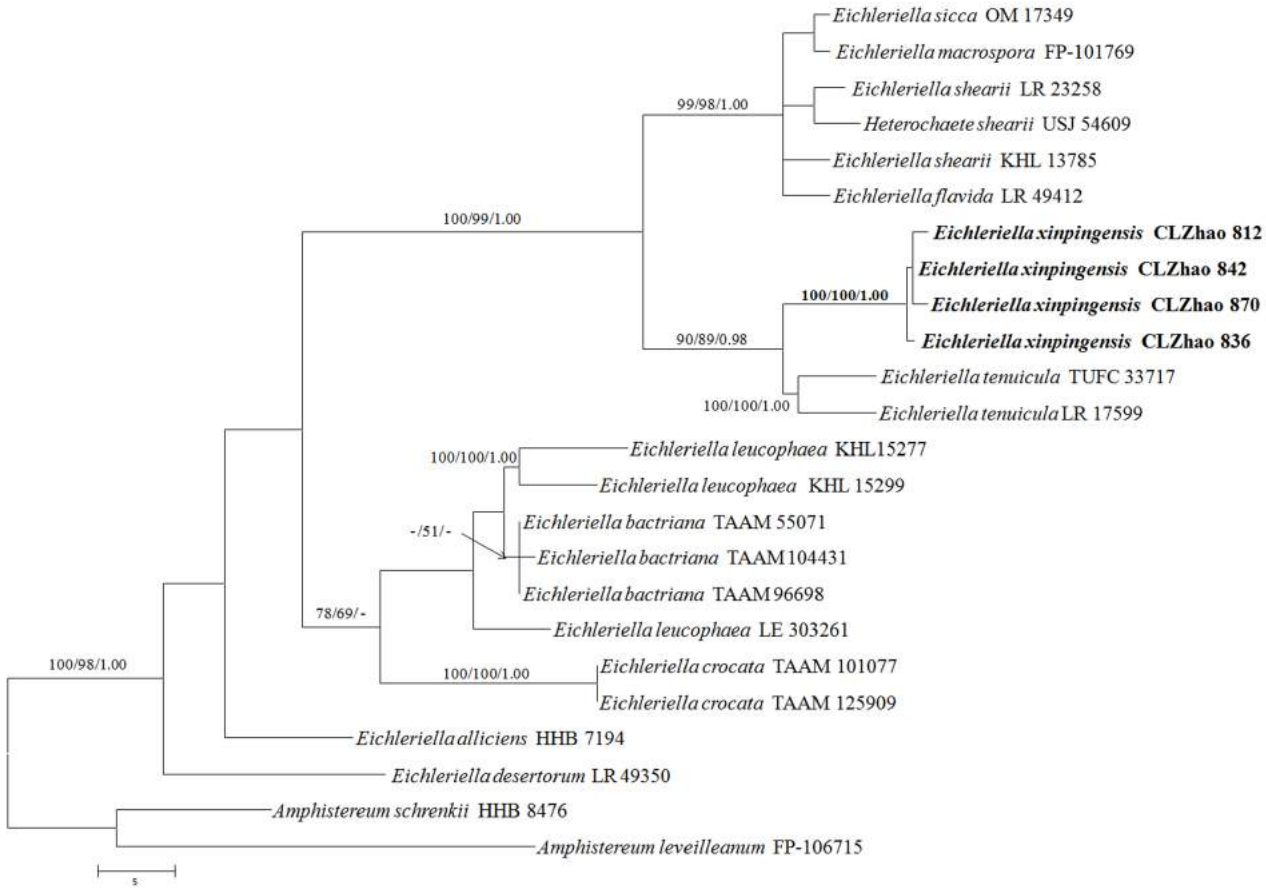

FIGURE 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Eichleriella xinpingensis* and related species in *Eichleriella* based on ITS sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively.

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analysis followed Song *et al*. (2016a) and Song & Cui (2017) 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.

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 3 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 (BP) and Bayesian posterior probabilities (BPP) greater than or equal to 75 % (BP) and 0.95 (BPP) were considered as significantly supported, respectively.

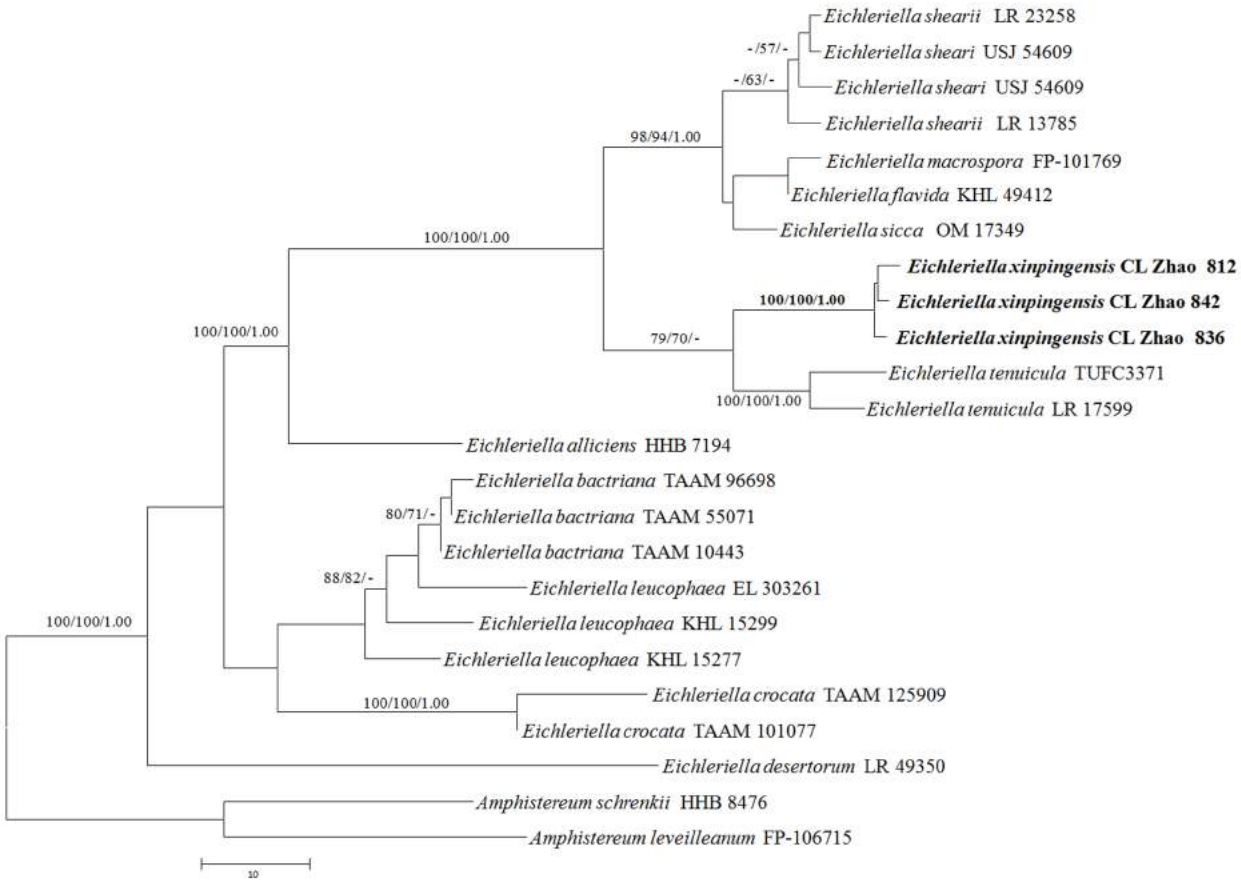

FIGURE 2. Maximum parsimony strict consensus tree illustrating the phylogeny of *Eichleriella xinpingensis* and related species in *Eichleriella* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian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

Results

Molecular phylogeny

The ITS dataset (Fig. 1) included sequences from 24 fungal specimens representing 13 species. The dataset had an aligned length of 571 characters, of which 433 characters are constant, 47 are variable and parsimony-uninformative, and 91 are parsimony-informative. Maximum parsimony analysis yielded 28 equally parsimonious trees (TL = 254, CI = 0.689, HI = 0.311, RI = 0.837, RC = 0.577). Best model for the ITS 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 = 0.003126 (BI).

The phylogenetic tree (Fig. 1) inferred from ITS sequences showed that the new species *Eichleriella xinpingensis* fell into the genus *Eichleriella* and it was closely related to *E. tenuicula* (Lév.) Spirin & Malysheva (2017: 121).

The ITS+nLSU dataset (Fig. 2) included sequences from 24 fungal specimens representing 13 species. The dataset had an aligned length of 1891 characters, of which 1664 characters are constant, 88 are variable and parsimony-uninformative, and 139 are parsimony-informative. Maximum parsimony analysis yielded 15 equally parsimonious trees (TL = 385, CI = 0.7068, HI = 0.294, RI = 0.805, RC = 0.568). 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 = 0.003342 (BI).

A further phylogeny (Fig. 2) inferred from the combined ITS+nLSU sequences demonstrated that the new species formed a monophyletic entity with a high 100% BS, 100% BP and 1.00 BPP and then grouped with *E. tenuicula*.

Taxonomy

Eichleriella xinpingensis C.L. Zhao, *sp. nov.* (Figs. 3, 4)

MycoBank no.: MB 830024

Type: CHINA, Yunnan Province, Yuxi, Xinping County, Mopanshan National Nature Reserve, on angiosperm trunk, 16 January 2017, *CLZhao 842/SWFC842* (holotype, SWFC!).

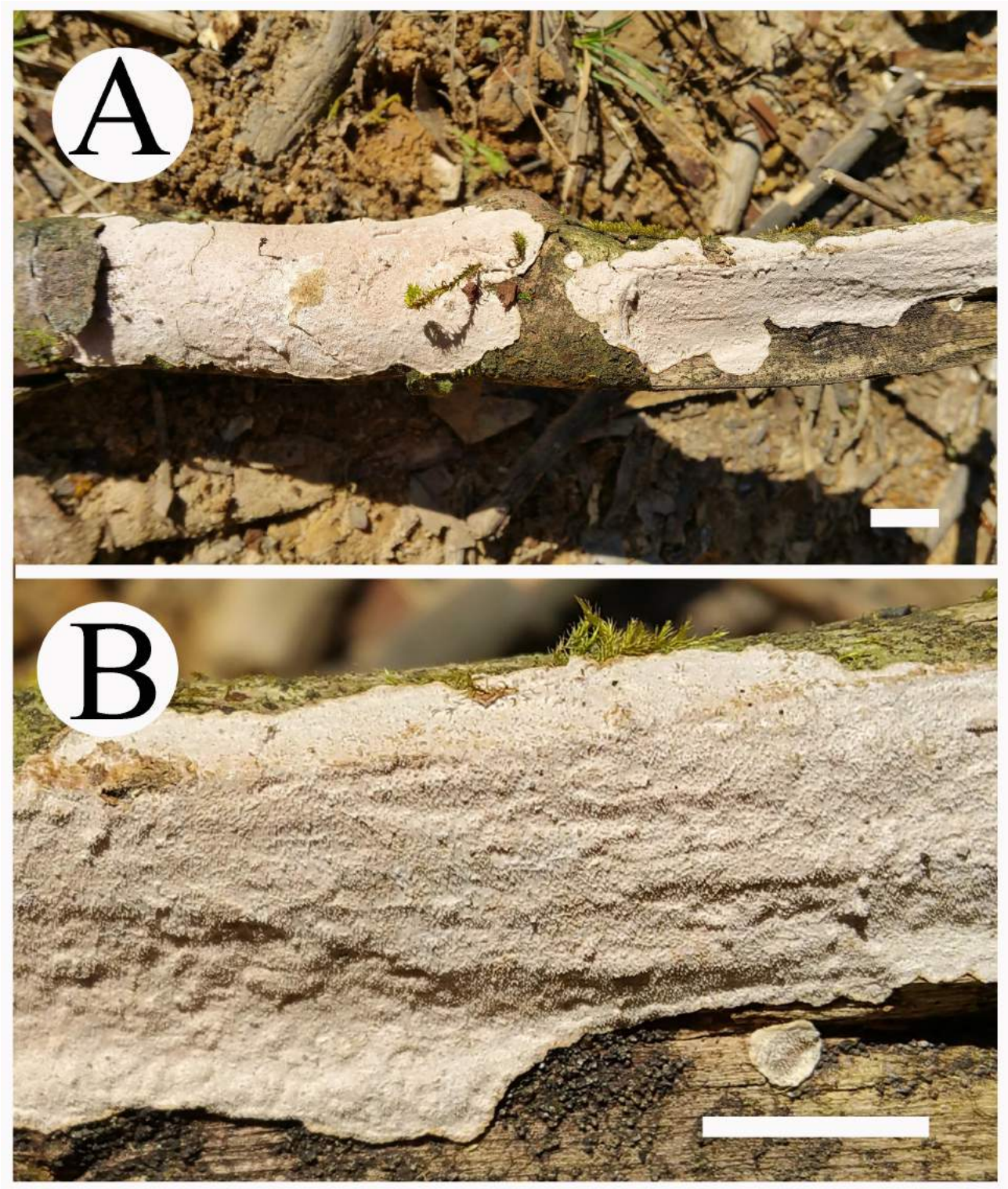

FIGURE 3. Basidiomata of *Eichleriella xinpingensis* (holotype). Scale bars: a–1 cm.

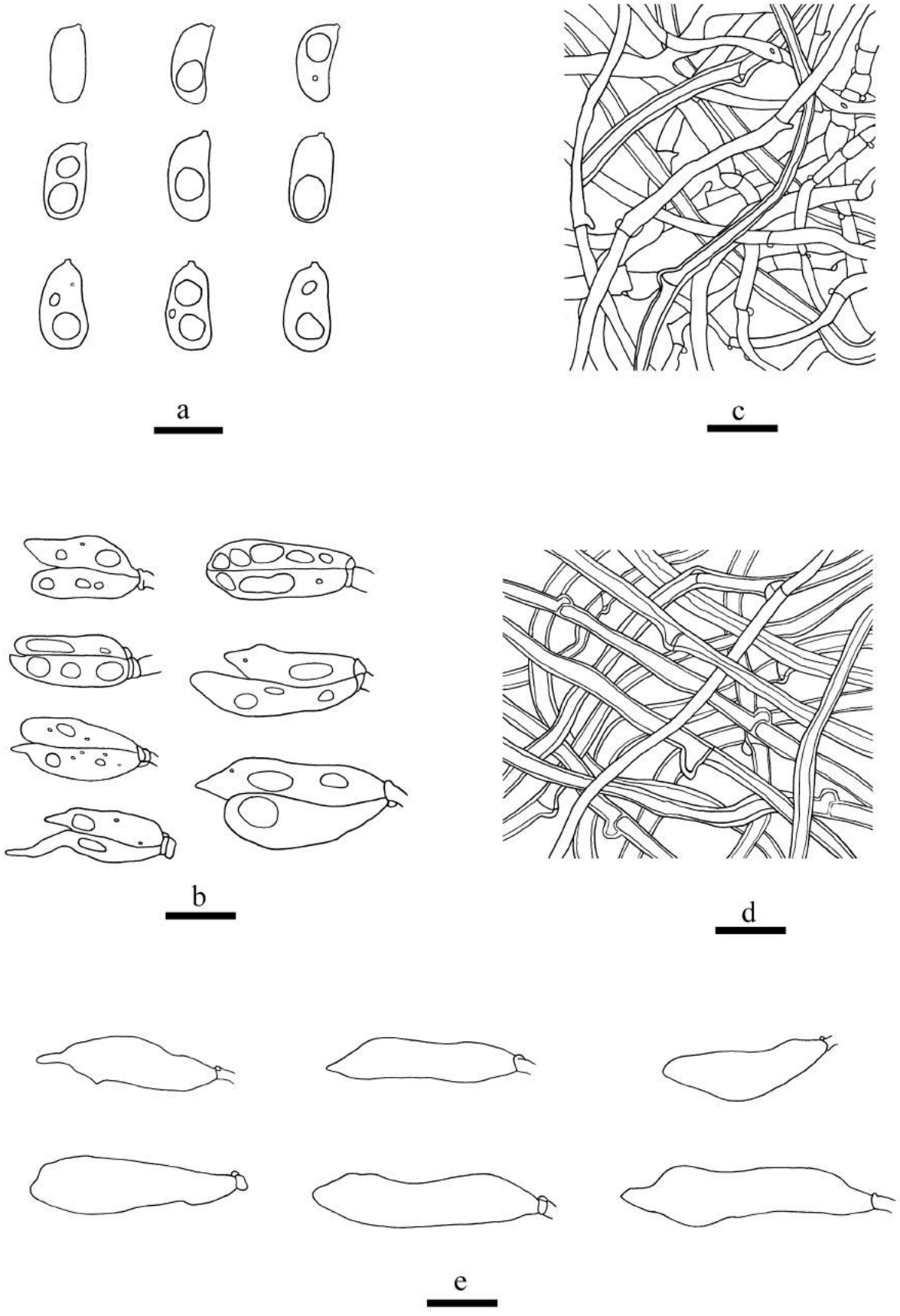

FIGURE 4. Microscopic structures of *Eichleriella xinpingensis* (drawn from the holotype). a. basidiospores. b. basidia. c. hyphae from trama. d. hyphae from context. e. cystidia. Bars: a, d–10 μm.

Etymology:—*Xinpingensis* (Lat.): referring to the locality (Xiping County) of the type specimens.

Basidiomata:—Basidiocarps annual, resupinate, soft leathery to ceraceous, without odor or taste when fresh, becoming leathery upon drying, about 2.5 cm in widest dimension and up to 8 cm long, 200–500 µm thick. Hymenial surface covered by blunt-pointed spines, 0.1–0.3 mm long, flesh-pink to clay-pink when fresh, turning to clay-pink to vinaceous upon drying.

Hyphal structure:—Hyphal system dimitic; generative hyphae with clamp connections, IKI–, CB–; tissues unchanged in KOH.

Subiculum:—Generative hyphae pale brown to dark brown, thin- to thick walled, unbranched, 2.5–4.5 µm in diam.; skeletal hyphae dark brown, thick-walled with a narrow to wide lumen, unbranched, interwoven, 3.5–5 µm in diam.

Hymenium:—Generative hyphae pale brown to dark brown, thin- to thick walled, infrequently branched, 2–4 µm in diam.; skeletal hyphae dark brown, thick-walled with a narrow to wide lumen, unbranched, interwoven, 3–4.5 µm. Cystidia abundant, clavate or tubular, often tapering, 20–40 × 6–9 µm. Basidia narrowly ovoid to obconical, two-celled, 15–28 × 5–9 µm, often with a short enucleate stalk, up to 4 × 3 µm.

Spores:—Basidiospores broadly cylindrical, hyaline, thin-walled, smooth, non-dextrinoid, CB–, (6–)6.5–10(–11) × (3–)3.5–4.5(–5) µm, L = 8.12 µm, W = 3.95 µm, Q = 1.93–2.13 (n = 240/4).

Additional specimens examined:—CHINA, Yunnan Province, Yuxi, Xinping County, Mopanshan National Nature Reserve, on angiosperm trunk, 16 January 2017, *CLZhao 812/SWFC812, 836/SWFC836, 870/SWFC870* (paratypes, SWFC!).

Discussion

In the present study, a new species, *Eichleriella xinpingensis*, is described based on phylogenetic analyses and morphological characters.

Eichleriella xinpingensis is closely related to *E. tenuicula* in the rDNA based phylogeny (Figs. 1, 2). But morphologically *E. tenuicula* produces the effused basidiocarps with pale buff hymenial surface, presence of hyphal pegs and larger basidiospores (16–21 × 5.5–6 µm, Roberts 2008).

Morphologically, eight species of *Eichleriella* are similar to *E. xinpingensis*: *E. alliciens* (Berk. & Cooke) Burt (1915: 746), *E. bactriana* Spirin & V. Malysheva (2017: 701), *E. crocata* (Pat.) Spirin & V. Malysheva (2017: 702), *E. desertorum* Spirin & V. Malysheva (2017: 702), *E. flavida* (Pat.) Spirin & V. Malysheva (2017: 703), *E. leucophaea* Bres. (1903: 116), *E. macrospora* (Ellis & Everh.) Martin (1915: 759) and *E. sicca* Spirin & Miettinen (2017: 709). However, *E. alliciens* differs from *E. xinpingensis* by having a monomitic hyphal structure and larger basidiospores (10.3–15.6 × 4.9–6.3 µm, Malysheva & Spirin 2017). *Eichleriella bactriana* differs in having thicker basidiocarps (up to 1 mm) with the dark brown hymenial surface and the four-celled basidia (Malysheva & Spirin 2017). *Eichleriella crocata* is separated from the new species by having a monomitic hyphal structure and larger basidiospores (10.3–15.8 × 4–5 µm, Malysheva & Spirin 2017). *Eichleriella desertorum* differs from *E. xinpingensis* by having smooth hymenial surface with greyish white colour (Malysheva & Spirin 2017). *Eichleriella flavida* is separated from the new species by the pale grey to light ochraceous-grey to almost white basidiocarps and presence of gloeocystidia (Malysheva & Spirin 2017). *Eichleriella leucophaea* differs in its cupulate to orbicular basidiocarps covered by scattered small warts and the larger basidiospores (12.5–20 × 4.4–6.1 µm, Malysheva & Spirin 2017). *Eichleriella macrospora* is separated from the new species by smooth hymenophore with greyish white to pale ochraceous hymenial surface and a monomitic hyphal structure (Malysheva & Spirin 2017). *Eichleriella sicca* is separated from the new species by white to pale cream hymenial surface and the cyanophilous generative hyphae (Malysheva & Spirin 2017).

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 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, Li *et al*. 2014, Song *et al*. 2014, 2016b, Chen *et al*. 2016, 2017, Zhou *et al*. 2016, Ren & Wu 2017, Wu *et al*. 2017, Yuan *et al*. 2017a, b, Xing *et al*. 2018). The new species in the present study, *Eichleriella xinpingensis*, is from subtropics, too. It is possible that new taxa will be found after further investigations and molecular analyses.

Acknowledgements

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