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# *Eichleriella aculeobasidiata* sp. nov. (Auriculariales, Basidiomycota) evidenced by morphological characters and phylogenetic analyses in China

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**Summary.** A new wood-inhabiting fungal species, *Eichleriella aculeobasidiata* sp. nov. is described based on a combination of morphological features and molecular evidence. *E. aculeobasidiata* is characterised by a dimittic hyphal system with clamped generative hyphae, and narrowly ovoid to obconical, distinctly curved, hyaline, smooth, thin-walled basidiospores which contain oil droplets. Sequences of the ITS gene region of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and Bayesian inference methods. The phylogenetic analysis showed that *E. aculeobasidiata* belonged to *Eichleriella* and formed a monophyletic lineage with strong support (100% BS, 100% BP, 1.00 BPP), and then grouped with a clade comprised of *E. xinpingsensis* and *E. tenuicula*.

**Key Words.** phylogeny, taxonomy, wood-inhabiting fungi, Yunnan province.

## Introduction

*Eichleriella* Bres. (Auriculariales) was described by Bresadola (1903), belonging to the family Auriculariaceae, which is characterised by annual or short-living perennial, leathery to ceraceous basidiomata with smooth, pale coloured hymenophore (in some species covered by spines), monomitic to dimittic hyphal structure with clamped genitive hyphae, cystidia often present, longitudinally septate basidia with 2- or 4-celled, and hyaline, cylindrical to narrowly cylindrical basidiospores (Bresadola 1903; Malysheva & Spirin 2017). So far about 16 species have been accepted in this genus worldwide (Bresadola 1903; Roberts 2008; Malysheva & Spirin 2017; Liu *et al.* 2019), (<http://www.indexfungorum.org>) and (<https://www.mycobank.org>).

Weiss & Oberwinkler (2001), in a phylogenetic study of Auriculariales and related groups, showed that *Eichleriella* grouped with *Auricularia* Bull., *Exidia* Fr., *Exidiopsis* (Bref.) Möller and *Heterochaete* Pat. The classification and phylogeny of Auriculariales revealed that *Eichleriella* clustered with *Amphistereum* Spirin & Malysheva and *Auricularia*. Malysheva & Spirin (2017) introduced the taxonomy and phylogeny of the Auriculariales (Agaricomycetes, Basidiomycota) with stereoid basidiocarps, in which the genus *Eichleriella* was reinstated to encompass ten closely related species

with ellipsoid ovoid basidia, and the type of the genus, *E. incarnata* Bres., was placed in the synonymy of *E. leucophaea* Bres. Phylogenetically *Amphistereum* grouped closely with *Eichleriella*, with ten species nested within *Eichleriella*. Recently, Liu *et al.* (2019) described the new species *Eichleriella xinpingsensis* C.L.Zhao from southern China and suggested that *E. xinpingsensis* belonged to the Auriculariaceae and was closely related to *E. tenuicula* (Lév.) Spirin & Malysheva. *Heterochaetella ochracea* Viégas was moved to *Eichleriella* based on morphological evidence and DNA analyses (Alvarenga *et al.* 2019).

During studies on wood-inhabiting fungi in southern China, an additional taxon was found, which could not be assigned to any described species. In this study, a new species of *Eichleriella* is proposed based on a combination of macro-anatomical and molecular (internal transcribed spacer (ITS) fungal DNA barcode) characters.

## Materials and Methods

### Morphological studies

The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. Macromorphological

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descriptions are based on field notes. Colour terms follow Petersen (1996). Micromorphological data were obtained from dried specimens, and observed under a light microscope (Dai 2012). The following abbreviations are 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 for all spores), W = mean spore width (arithmetic average for 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.

### Molecular procedures and phylogenetic analyses

The EZNA HP Fungal DNA Kit (Omega Biotechnologies Co., Ltd, Kunming, Yunnan Province, P.R. China) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions with some modifications. The ITS region was amplified with primer pair ITS5 and ITS4 (White *et al.* 1990). 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 products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company, Kunming, Yunnan Province, P.R. China. All newly generated sequences were deposited at NCBI GenBank database (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequences. Sequences were aligned with MAFFT 7 (<http://mafft.cbrc.jp/alignment/server/>) using the "E-INS-I" strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (submission ID 26408). Sequences of *Amphistereum schrenkii* (Burt) Spirin & Malysheva and *A. leveilleanum* (Berk. & M.A.Curtis) Spirin & Malysheva obtained from GenBank were used as an outgroup to root trees following Malysheva & Spirin (2017) (Fig. 1).

Maximum parsimony analysis was applied to the ITS dataset. Approaches to phylogenetic analyses followed Zhao & Wu (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 1,000 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 1000 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 generated. The data matrix was also analysed using Maximum

Likelihood (ML) approach with RAxML-HPC2 through the CIPRES Science Gateway ([www.phylo.org](http://www.phylo.org); Miller *et al.* 2009). Branch support (BS) for ML analysis was determined by 1,000 bootstrap replicates.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). BI was calculated with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). Four Markov chains run for 2 runs from random starting trees for 700 thousand generations (Fig. 1) and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. The majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum likelihood bootstrap values (BS) >75%, maximum parsimony bootstrap values (BT) >75%, or Bayesian posterior probabilities (BPP) >0.95.

## Results

### Molecular phylogeny

The ITS dataset (Fig. 1) included sequences from 25 fungal specimens representing 14 species. The dataset had an aligned length of 517 characters, of which 387 characters are constant, 49 are variable and parsimony-uninformative, and 81 are parsimony-informative. Maximum parsimony analysis yielded 5 equally parsimonious trees (TL = 249, CI = 0.663, HI = 0.337, RI = 0.821, RC = 0.544). Best model for the ITS dataset estimated and applied in the Bayesian analysis was GTR+I+G (Iset 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 = 0.009996 (BI).

In the phylogeny (Fig. 1) inferred from ITS sequences obtained for related taxa of *Eichleriella*, *E. aculeobasidiata* formed a monophyletic lineage and grouped with *E. xipingensis* and *E. tenuicula* with strong support (100% BS, 100% BP, 1.00 BPP).

### Taxonomic Treatment

***Eichleriella aculeobasidiata* C.L.Zhao, sp. nov.** Type: China, Yunnan Province, Puer, Zhenyuan County, Heping Town, Damoshan, on fallen branch, 16 Jan. 2018, C. L. Zhao 6159 (holotype SWFC). MycoBank no.: MB 840260. GenBank no.: CLZhao 4422 (ITS MZ416783); CLZhao 6159 (ITS MZ416784); CLZhao 6473 (ITS MZ416785); CLZhao 11294 (ITS MZ416786).

*Basidiomata* annual, resupinate, ceraceous, first orbicular, later fusing together, without odour or taste when fresh, becoming ceraceous to corneous upon drying, up to 10 cm

**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		
<i>Amphistereum leveilleanum</i>	FP-106715	KX262119		Malysheva & Spirin 2017
<i>Amphistereum schrenkii</i>	HHB 8476	KX262130		Malysheva & Spirin 2017
<i>Eichleriella alliciens</i>	HHB 7194	KX262120		Malysheva & Spirin 2017
<i>Eichleriella bactriana</i>	TAAM 55071	KX262121		Malysheva & Spirin 2017
<i>Eichleriella bactriana</i>	TAAM 96698	KX262123		Malysheva & Spirin 2017
<i>Eichleriella bactriana</i>	TAAM 104431	KX262138		Malysheva & Spirin 2017
<i>Eichleriella crocata</i>	TAAM 101077	KX262100		Malysheva & Spirin 2017
<i>Eichleriella crocata</i>	TAAM 125909	KX262118		Malysheva & Spirin 2017
<i>Eichleriella desertorum</i>	LR 49350	KX262142		Malysheva & Spirin 2017
<i>Eichleriella flavida</i>	LR 49412	KX262137		Malysheva & Spirin 2017
<i>Eichleriella leucophaea</i>	KHL15277	KX262115		Malysheva & Spirin 2017
<i>Eichleriella leucophaea</i>	KHL 15299	KX262136		Malysheva & Spirin 2017
<i>Eichleriella leucophaea</i>	LE 303261	KX262111		Malysheva & Spirin 2017
<i>Eichleriella macrospora</i>	FP-101769	KX262129		Weiss & Oberwinkler 2001
<i>Eichleriella shearii</i>	LR 23258	KX262139		Malysheva & Spirin 2017
<i>Eichleriella shearii</i>	USJ 54609	AF291284		Weiss & Oberwinkler 2001
<i>Eichleriella tenuicula</i>	LR 17599	KX262141		Malysheva & Spirin 2017
<i>Eichleriella xinpingensis</i>	CLZhao 812	MK560878		Liu <i>et al.</i> 2019
<i>Eichleriella xinpingensis</i>	CLZhao 836	MK560879		Liu <i>et al.</i> 2019
<i>Eichleriella xinpingensis</i>	CLZhao 842	MK560880		Liu <i>et al.</i> 2019
<i>Eichleriella xinpingensis</i>	CLZhao 870	MK560881		Liu <i>et al.</i> 2019
<i>Eichleriella aculeobasidiata</i>	CLZhao 4422	MZ416783		Present study
<i>Eichleriella aculeobasidiata</i>	CLZhao 6159	MZ416784		Present study
<i>Eichleriella aculeobasidiata</i>	CLZhao 6473	MZ416785		Present study
<i>Eichleriella aculeobasidiata</i>	CLZhao 11294	MZ416786		Present study

long, 2.5 cm wide, 100 – 300 µm thick. Hymenial surface grandinioid, cream to buff when fresh, becoming clay-buff to fawn upon drying, covered by blunt spines, 0.1 – 0.3 mm long, 6 – 7 per mm. Margin sterile, cream to buff, 0.5 – 1 mm wide. *Hyphal structure.* Hyphal system dimitic, generative hyphae with clamp connections, IKI–, CB–; tissues unchanged in KOH. *Hymenium.* Generative hyphae with clamp connections, hyaline, thin-walled, unbranched, interwoven, 1.2 – 4.5 µm in diam.; skeletal hyphae hyaline, thick-walled with a narrow to wide lumen, rarely branched, interwoven, 2 – 4 µm in diam. Cystidia abundant, clavate, 17 – 42 × 4 – 11 µm. Basidia narrowly ovoid to obconical, longitudinally septate, embedded, two-celled, with oil drops, 14 – 47.5 × 5 – 14 µm. *Spores.* Basidiospores allantoid, hyaline, smooth, thin-walled, with oil drops, IKI–, CB–, 9 – 13.5 × 4.5 – 7.5 µm, L = 10.97 µm, W = 5.74 µm, Q = 1.78 – 2.15 (n = 120/4). Figs 2, 3.

**RECOGNITION.** *Eichleriella aculeobasidiata* differs from all its congeners by the following combination of traits: annual, resupinate basidiomata with cream to buff hymenophore, a dimitic hyphal system with clamped generative and thick-walled skeletal hyphae, and longitudinally septate, embedded, two-celled basidia, and allantoid basidiospores (9 – 13.5 × 4.5 – 7.5 µm).

**DISTRIBUTION.** Known only from the type locality.

**SPECIMENS EXAMINED.** CHINA. Yunnan Province, Puer, Jingdong County, Wuliangshan National Nature Reserve, on the angiosperm trunk, 6 Oct. 2017, C. L. Zhao 4422; Yuxi, Xinping County, Mopanshan Nation-

al Forestry Park, on the fallen branch of angiosperm, 19 Jan. 2018, C. L. Zhao 6473; Wenshan, Xichou County, Xiaoqiaogou National Nature Reserve, on the fallen branch of angiosperm, 16 Jan. 2019, C. L. Zhao 11294 (SWFC).

**HABITAT.** Lignicolous.

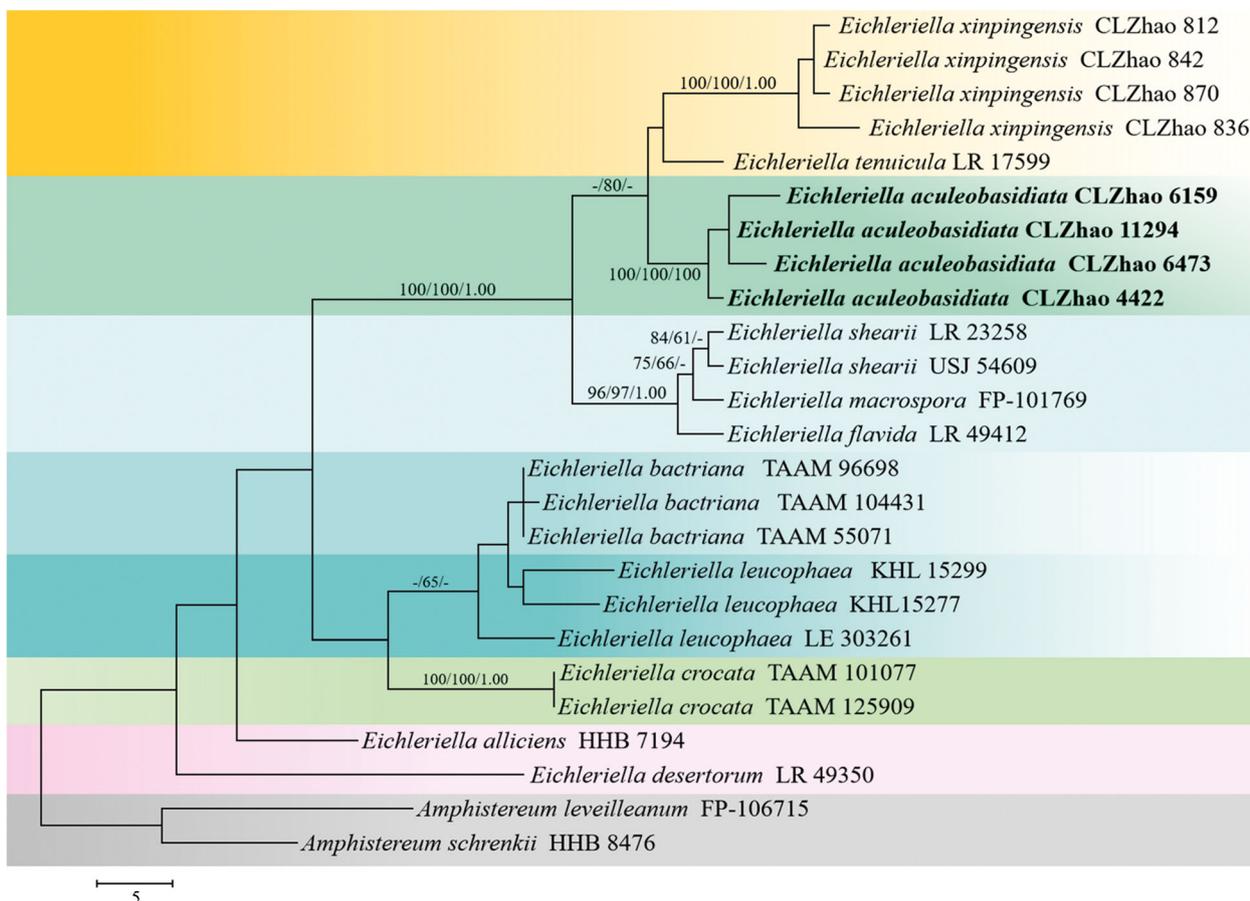
**CONSERVATION STATUS.** Not evaluated

**ETYMOLOGY.** Refers to the acute basidia of the type specimens.

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

Phylogenetically, *Eichleriella aculeobasidiata* is grouped with a clade including *E. xinpingensis* and *E. tenuicula* (Fig. 1). Morphologically, *E. tenuicula* differs from *E. aculeobasidiata* by the effused basidiomata with pale buff hymenial surface and larger basidiospores (16 – 21 × 5.5 – 6 µm, Roberts 2008); *E. xinpingensis* differs by the clay-pink to vinaceous hymenial surface and smaller basidiospores (6.5 – 10 × 3.5 – 4.5 µm, Liu *et al.* 2019).

Morphologically, *Eichleriella bactriana* Spirin & Malysheva, *E. shearii* (Burt) Spirin & Malysheva, *E. xinpingensis* and *E. aculeobasidiata*, share the similar character of a grandinioid or hydroid hymenial surface. However, *E. bactriana* differs from *E. aculeobasidiata* by having thicker basidiomata (up to 1 mm) with a dark brown hymenial surface (Malysheva & Spirin 2017); *E. shearii* differs in having larger basidiospores (11.4 – 16.2 × 5.1 – 7.1 µm,



**Fig. 1.** Maximum Parsimony strict consensus tree illustrating the phylogeny of *Eichleriella aculeobasidiata* and related species in *Eichleriella* based on ITS sequences. Branches are labelled with maximum likelihood bootstrap values > 70%, parsimony bootstrap values > 50% and Bayesian posterior probabilities > 0.95. Newly sequenced specimens are in bold.

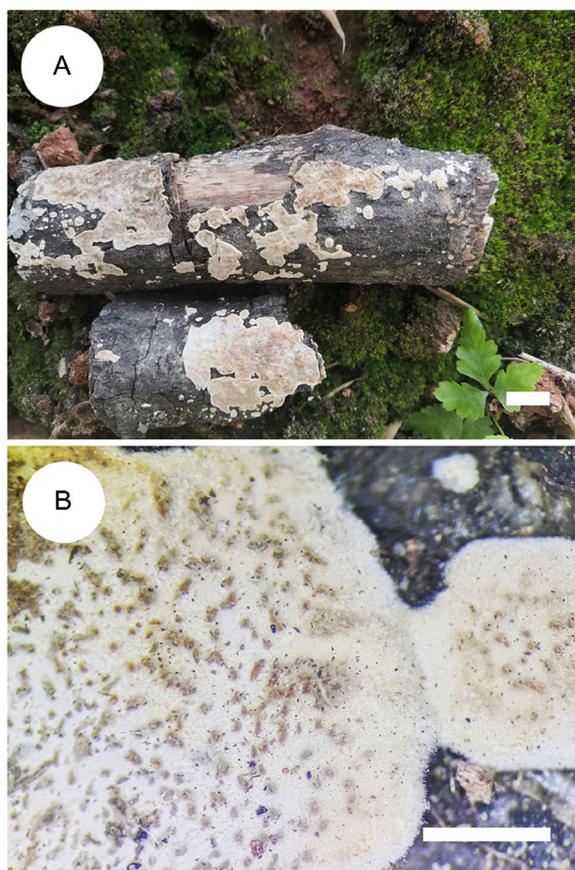
Malysheva & Spirin 2017); *E. xinpingensis* is separated from the new species by the pale brown to dark brown generative hyphae (Liu *et al.* 2019).

*Eichleriella alliciens* (Berk. & Cooke) Burt, *E. crocata* (Pat.) Spirin & Malysheva, *E. flavida* (Pat.) Spirin & Malysheva, *E. macrospora* (Ellis & Everh.) G.W.Martin, *E. shearii*, and *E. aculeobasidiata* are all characterised by having clavate cystidia. However, *E. alliciens* differs from *E. aculeobasidiata* by having a pinkish to pale ochraceous hymenial surface and four-celled basidia (Malysheva & Spirin 2017); *E. crocata* is separated from the new species by the smooth hymenial surface and four-celled basidia (Malysheva & Spirin 2017); *E. flavida* differs in having a monomitic hyphal system (Malysheva & Spirin 2017); *E. macrospora* differs in having larger basidiospores ( $12.3 - 16.5 \times 5.6 - 7.1 \mu\text{m}$ , Malysheva & Spirin 2017); *E. shearii* differs from *E. aculeobasidiata* by having larger basidiospores ( $11.4 - 16.2 \times 5.1 - 7.1 \mu\text{m}$ , Malysheva & Spirin 2017).

*Eichleriella desertorum* Spirin & Malysheva, *E. leucophaea* Bres., *E. sicca* Spirin & Miettinen and *E. aculeobasidiata* have allantoid basidiospores. Howev-

er, *E. desertorum* differs from *E. aculeobasidiata* by having a smooth hymenial surface (Malysheva & Spirin 2017); *E. leucophaea* differs in having the larger basidiospores ( $12.3 - 22.1 \times 9.0 - 12.3 \mu\text{m}$ , Malysheva & Spirin 2017); *E. sicca* is separated from the new species by the cyanophilous generative hyphae (Malysheva & Spirin 2017).

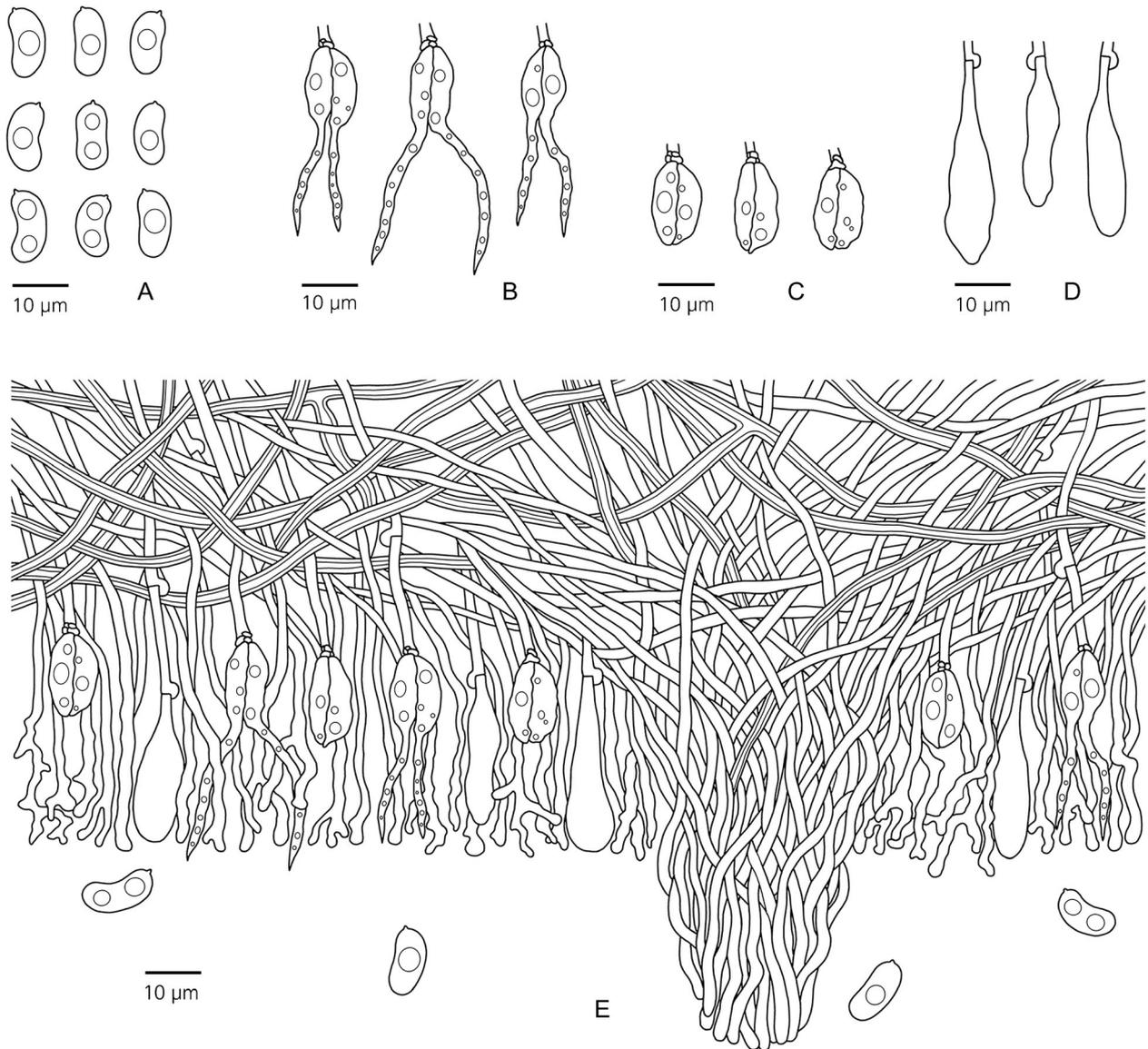
Wood-rotting fungi are an extensively studied group of Basidiomycota (Gilbertson & Ryvarden 1987; Núñez & Ryvarden 2001; Bernicchia & Gorjón 2010; Dai 2012; Ryvarden & Melo 2014; Chen *et al.* 2016; Han *et al.* 2016; Song *et al.* 2016; Cui *et al.* 2019; Guan *et al.* 2020; Wang *et al.* 2020), but the diversity of Chinese wood-rotting fungi is still not well known. The hosts of *Eichleriella* species are mainly distributed in hardwood forest (McNabb 1969; Malysheva & Spirin 2017; Alvarenga *et al.* 2019; Liu *et al.* 2019). Two *Eichleriella* species were reported from China prior to this study, *E. chinensis* Pilát and *E. xinpingensis* (Pilát 1940; Liu *et al.* 2019). However, *Eichleriella chinensis* differs from this new species by its reflexed margin and presence of gloeocystidia (Pilát 1940).



**Fig. 2.** Basidiomata of *Eichleriella aculeobasidiata* (holotype). Scale bars: A 1 cm; B 1 mm. PHOTOS: HUI WANG.

### Key to the known species of *Eichleriella* worldwide

- |  |                           |
|--|---------------------------|
| 1. Hymenophore smooth.....                                     | 2                         |
| 1. Hymenophore grandinioid or odontoid or tuberculate.....     | 7                         |
| 2. Basidiomata > 300 $\mu\text{m}$ in thickness.....           | 3                         |
| 2. Basidiomata < 300 $\mu\text{m}$ in thickness.....           | 4                         |
| 3. Cystidia > 25 $\mu\text{m}$ in length.....                  | <i>E. bactriana</i>       |
| 3. Cystidia < 25 $\mu\text{m}$ in length.....                  | <i>E. incarnata</i>       |
| 4. Hymenophore soft leathery.....                              | 5                         |
| 4. Hymenophore ceraceous.....                                  | 6                         |
| 5. Basidiospores > 5 $\mu\text{m}$ in width.....               | <i>E. alliciens</i>       |
| 5. Basidiospores < 5 $\mu\text{m}$ in width.....               | <i>E. crocata</i>         |
| 6. Subicular hyphae yellowish, slightly thick-walled, CB-..... | <i>E. desertorum</i>      |
| 6. Subicular hyphae hyaline, thin-walled, CB+.....             | <i>E. sicca</i>           |
| 7. Hyphal system dimittic.....                                 | 8                         |
| 7. Hyphal system monomitic.....                                | 10                        |
| 8. Hymenial surface clay-pink to vinaceous.....                | <i>E. ximpingensis</i>    |
| 8. Hymenial surface cream to buff.....                         | 9                         |
| 9. Basidiospores > 15 $\mu\text{m}$ in length.....             | <i>E. tenuicula</i>       |
| 9. Basidiospores < 15 $\mu\text{m}$ in length.....             | <i>E. aculeobasidiata</i> |
| 10. Margin detaching.....                                      | 11                        |
| 10. Margin adnate.....   | 12                        |
| 11. Hymenophore tuberculate .....                              | <i>E. shearii</i>         |
| 11. Hymenophore grandinioid to hydroid.....                    | <i>E. chinensis</i>       |



**Fig. 3.** Microscopic structures of *Eichleriella aculeobasidiata* (drawn from the holotype). A basidiospores; B cystidia; C basidia; D basidioles; E a section of hymenium. DRAWN BY HUI WANG.

12. Basidiospores > 17 µm in length.....	<i>E. hoheriae</i>
12. Basidiospores < 17 µm in length.....	13
13. Hymenophore ceraceous.....	14
13. Hymenophore soft leathery.....	15
14. Basidiomata > 300 µm in thickness.....	<i>E. pulvinata</i>
14. Basidiomata < 300 µm in thickness.....	<i>E. macrospora</i>
15. Spines < 100 µm in length.....	<i>E. flavida</i>
15. Spines > 100 µm in length.....	<i>E. ochracea</i>

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