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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 dimitic 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. xinpingensis 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 dimitic hyphal structure with clamped generative 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 steroide 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 xinpingensis C.L.Zhao from southern China and suggested that E. xinpingensis 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
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 Amphisterum 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; 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; preset 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. xinpingensis 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
long, 2.5 cm wide, 100 – 300 μm thick. Hymenial surface granidinoid, 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. *Hymenial structure*. Hymal system dimitic, generative hyphae with clamp connections, IKI–, CB–; skeletal hyphae 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.


**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).

Morophologically, *Eichleriella bactriana* Spirin & Malyshvea, *E. shearii* (Burt) Spirin & Malyshvea, *E. xinpingensis* and *E. aculeobasidiata*, share the similar character of a granidinoid or hydnoid hymenial surface. However, *E. bactriana* differs from *E. aculeobasidiata* by having thicker basidiomata (up to 1 mm) with a dark brown hymenial surface (Malyshvea & Spirin 2017); *E. shearii* differs in having larger basidiospores (11.4 – 16.2 × 5.1 – 7.1 μm,
Eichleriella 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 & Malycheva, E. flavid (Pat.) Spirin & Malycheva, 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. flavid differs in having a monomitic hyphal system (Malycheva & Spirin 2017); E. macrospora differs in having larger basidiospores (12.3 – 22.1 × 9.0 – 12.3 μm, Malycheva & Spirin 2017); E. shearii differs from the new species by the cyanophilous generative hyphae (Malycheva & Spirin 2017).

Eichleriella desertorum Spirin & Malycheva, E. leucophaea Bres., E. sicca Spirin & Miettinen and E. aculeobasidiata have allantoid basidiospores. However, E. desertorum differs from E. aculeobasidiata by having a smooth hymenial surface (Malycheva & Spirin 2017); E. leucophaea differs in having the larger basidiospores (12.3 – 22.1 × 9.0 – 12.3 μm, Malycheva & Spirin 2017); E. sicca is separated from the new species by the cyanophilous generative hyphae (Malycheva & 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; Malycheva & 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).
Key to the known species of *Eichleriella* worldwide

1. Hymenophore smooth ........................................... 2
2. Hymenophore grandinioid or odontioid or tuberculate ........................................... 7
3. Basidiomata > 300 μm in thickness ........................................... 3
4. Basidiomata < 300 μm in thickness ........................................... 4
5. Cystidia > 25 μm in length ........................................... *E. bactriana*
6. Cystidia < 25 μm in length ........................................... *E. incarnata*
7. Hymenophore soft leathery ........................................... 5
8. Hymenophore ceraceous ........................................... 6
9. Basidiospores > 5 μm in width ........................................... *E. alliciens*
10. Basidiospores < 5 μm in width ........................................... *E. crocata*
11. Subicular hyphae yellowish, slightly thick-walled, CB– ........................................... *E. desertorum*
12. Subicular hyphae hyaline, thin-walled, CB+ ........................................... *E. sicca*
13. Hyphal system dimitic ........................................... 8
14. Hyphal system monomitic ........................................... 9
15. Hyphal system dimitic ........................................... 10
16. Hymeanal surface clay-pink to vinaceous ........................................... *E. xinpingensis*
17. Hymenial surface cream to buff ........................................... 11
18. Basidiospores > 15 μm in length ........................................... *E. tenuicula*
19. Basidiospores < 15 μm in length ........................................... *E. aculeobasidiata*
20. Margin detaching ........................................... 12
21. Margin adnate ........................................... 13
22. Hymenophore tuberculate ........................................... *E. shearii*
23. Hymenophore grandinioid to hydnoid ........................................... *E. chinensis*
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.
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References


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