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A new species of *Efibula* (Polyporales, Basidiomycota) from China

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Abstract

Efibula yunnanensis is proposed as a distinct new species based on morphological and molecular phylogenetic data. The species is characterized by an annual growth habit, resupinate basidiomata with smooth, cream to pale brown hymenial surface, a monomitic hyphal system with thin-walled, simple septate generative hyphae and ellipsoid, hyaline, thin-walled, smooth, IKI-, CB-basidiospores. The phylogenetic analyses based on ITS sequence data analyses revealed that *E. yunnanensis* was sister to a clade comprised *E. clarkii* and *E. gracilis*, and then grouped with *E. americana* and *E. tuberculata*.

Keywords: 1 new species, phylogeny, taxonomy, wood-inhabiting fungi, Yunnan Province

Introduction

Efibula Sheng H. Wu (1990: 21) is characterized by resupinate basidiomata with smooth hymenophore, a monomitic hyphal system; simple septate and compact subiculum; lack of cystidia; basidia cylindrical to clavate, and hyaline, thin-walled, smooth, ellipsoid to oblong, acyanophilous basidiospores (Wu 1990). So far 15 species have been accepted in the genus worldwide (Bourdot & Galzin1911, Cunningham 1954, Hjortstam & Ryvarden 1980, Wu 1990, Kotiranta & Saarenoksa 1993, Zmitrovich *et al*. 2006, Floudas & Hibbett 2015).

Revisiting the taxonomy of *Phanerochaete* (Polyporales, Basidiomycota) using a four gene dataset revealed that four *Efibula* species clustered together and then grouped *Byssomerulius corium* (Pers.) Parmasto (1967: 383) (Floudas & Hibbett 2015). Phylogenetic study of revising family-level classification of the Polyporales showed that *E. clarkii* Floudas & Hibbett (2015: 710) and *E. gracilis* Floudas & Hibbett (2015: 711) grouped together, in which *Efibula* species nested in the Irpicaceae family (Justo *et al*. 2017).

The corticoid 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 corticoid fungi diversity is still not well known, especially in southwest areas, and many recently described taxa of corticoid fungi were from these areas (Zhao & Wu 2017, Luo *et al*. 2019, Shen *et al*. 2018, Liu *et al*. 2019, Wu *et al*. 2019, Zheng *et al*. 2019). During our investigation on corticoid fungi in southern China, an interesting *Efibula* was found which on detailed study revealed it as an undescribed one which is detailed and discussed here.

Materials and methods

Morphological studies.—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 (2012). 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. The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China.

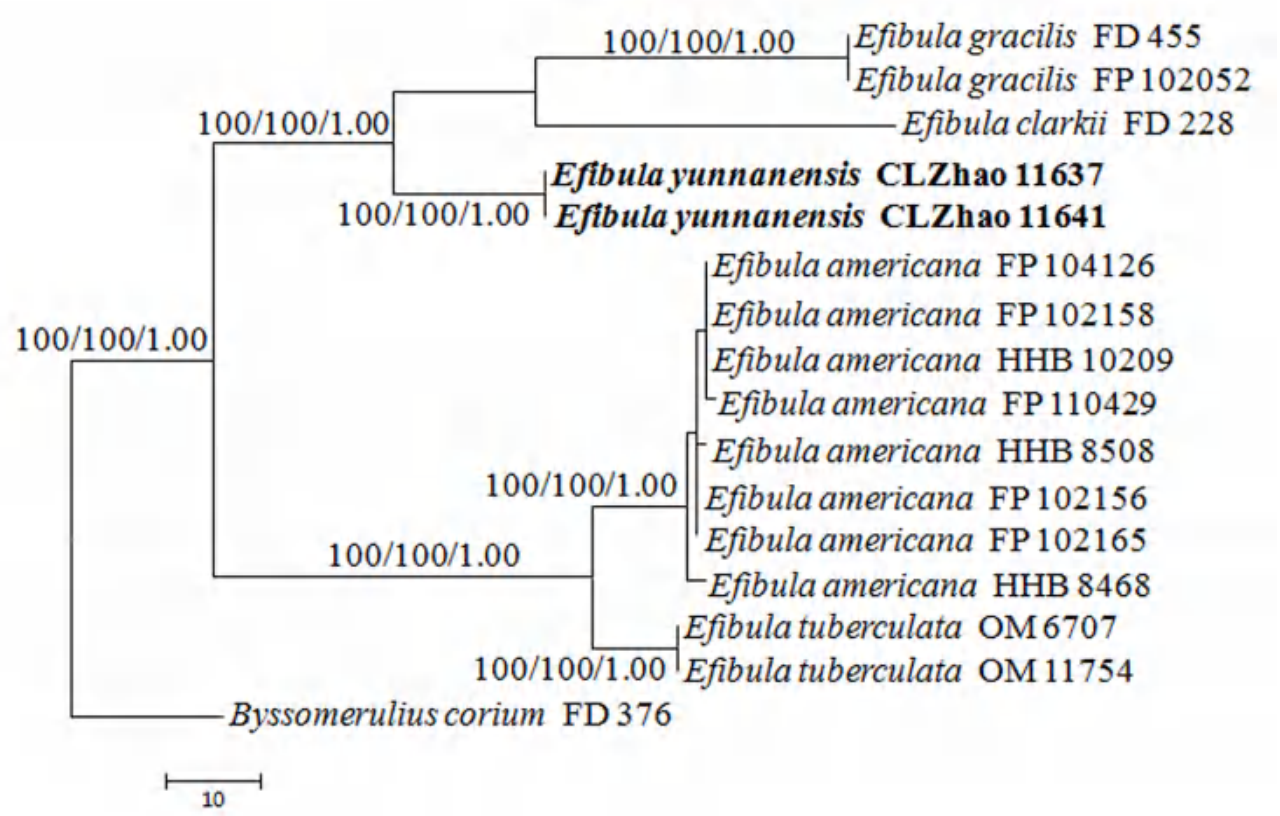

FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Efibula yunnanensis* and related species in *Efibula* based on ITS sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior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

Molecular procedures and phylogenetic analyses:—CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used for genomic DNA extraction from dried specimens, following the manufacturer's instructions with some modifications. A small piece of dried fungal specimen (about 30 mg) was ground to powder with liquid nitrogen. The powder was transferred to a 1.5 mL centrifuge tube, suspended in 0.4 mL of lysis buffer, and incubated in a 65 °C water bath for 60 min. After that, 0.4 mL phenol-chloroform (24:1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13 000 rpm for 5 min, 0.3 mL supernatant was transferred to a new tube and mixed with 0.45 mL binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation at 13, 000 rpm for 0.5 min. Then, 0.5 mL inhibitor removal fluid was added in AC for a centrifugation at 12, 000 rpm for 0.5 min. After washing twice with 0.5 mL washing buffer, the AC was transferred to a clean centrifuge tube, and 100 ml elution buffer was added to the middle of adsorbed film to elute the genome DNA. ITS region was amplified with primer pairs 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 GenBank (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 7 (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 (http://purl.org/phylo/treebase/phylows/study/25511). Sequences of *Byssomerulius corium* (Pers.) Parmasto was used as outgroup following Floudas & Hibbett (2015).

Maximum parsimony was applied to the ITS dataset sequences. Approaches to phylogenetic analysis 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 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 generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org, Miller *et al.* 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicate.

TABLE 1. List of species, specimens, and GenBank accession number of sequences used in this study.

Species name	Sample no.	GenBank accession no. ITS	References
Byssomerulius corium	FD 376	KP135005	Floudas *et al.* 2015
Efibula americana	FP 104126	KP135009	Floudas *et al.* 2015
E. americana	HHB 8508	KP135010	Floudas *et al*. 2015
E. americana	FP 102156	KP135011	Floudas *et al.* 2015
E. americana	HHB 8468	KP135012	Floudas *et al.* 2015
E. americana	FP 102158	KP135013	Floudas *et al.* 2015
E. americana	HHB 10209	KP135014	Floudas *et al.* 2015
E. americana	FP 110429	KP135015	Floudas *et al.* 2015
E. americana	FP 102165	KP135016	Floudas *et al.* 2015
E. clarkii	FD 228	KP135019	Floudas *et al.* 2015
E. gracilis	FD 455	KP135027	Floudas *et al.* 2015
E. gracilis	FP 102052	KP135028	Floudas *et al.* 2015
E. tuberculata	OM 6707	KP135017	Floudas *et al.* 2015
E. tuberculata	OM 11754	KP135018	Floudas *et al.* 2015
E. yunnanensis	CLZhao 11637	MT611528	Present study
E. yunnanensis	CLZhao 11641	MT611529	Present study

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes 3.1.2 with a general time reversible (GTR+I+G) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains run for 2 runs from random starting trees for 210,000 generations (Fig. 1) 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 were considered as significantly supported if they received maximum likelihood bootstrap (ML) >70%, maximum parsimony bootstrap (MP) >50%, or Bayesian posterior probabilities (PP) >0.95.

Results

Molecular phylogeny

The ITS dataset (Fig. 1) included sequences from 16 fungal specimens representing 6 species. The dataset had an aligned length of 660 characters, of which 519 characters are constant, 25 are variable and parsimony-uninformative, and 116 are parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious trees (TL = 236, CI = 0.7712, HI = 0.2288, RI = 0.8426, RC = 0.6498). Best model for the ITS dataset estimated and applied in the Bayesian analyses: 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 = 0.009999 (BI).

The phylogenetic tree (Fig. 1) inferred from ITS sequences revealed that the new species clustered with *Efibula clarkii* and *E. gracilis* Floudas & Hibbett.

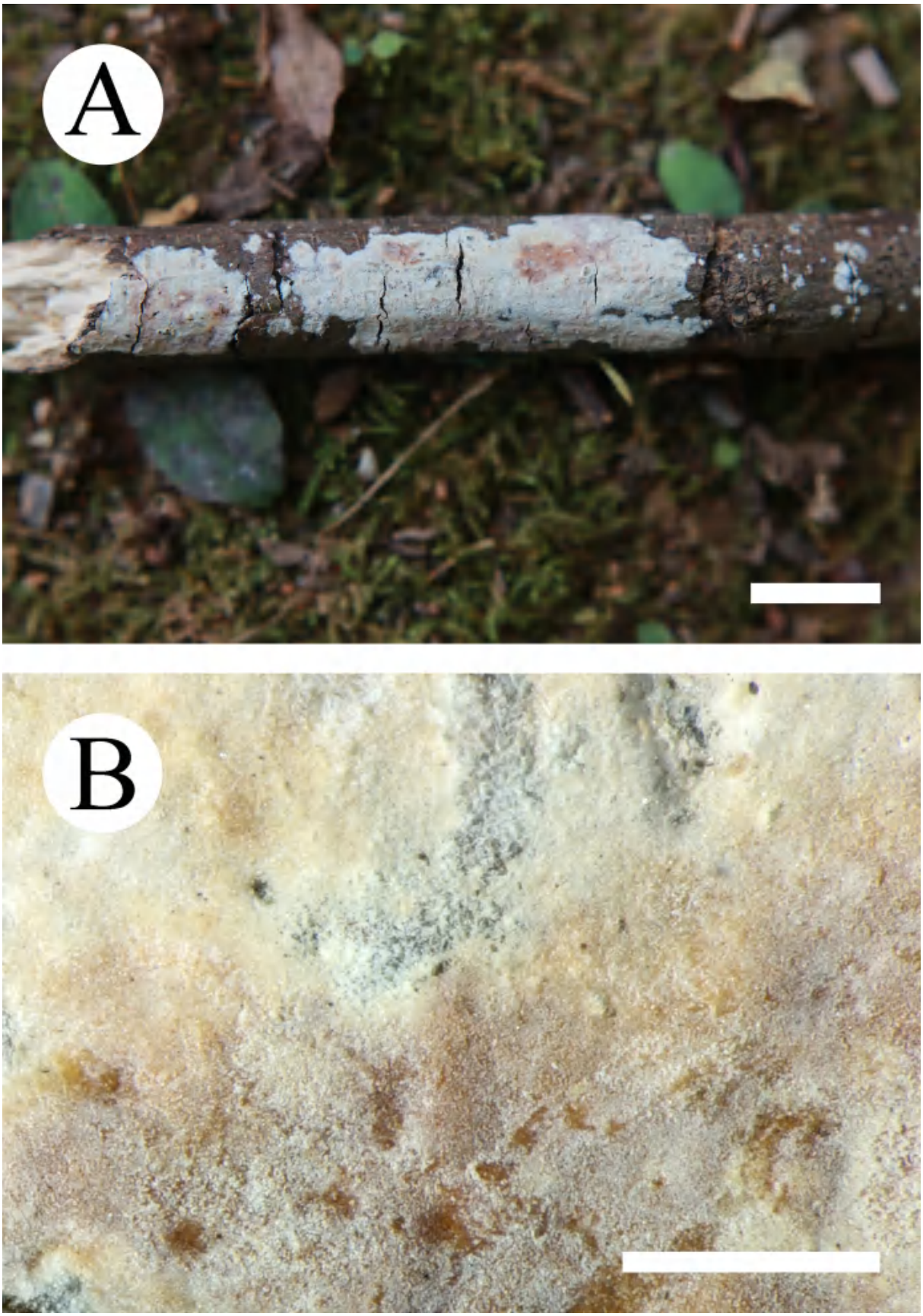

FIGURE 2. Basidiomata of *Efibula yunnanensis* (holotype). Bars: A = 1 cm, B = 1 mm.

Taxonomy

Efibula yunnanensis C.L. Zhao, *sp. nov.* (Figs. 2, 3)
MycoBank: MB 835877

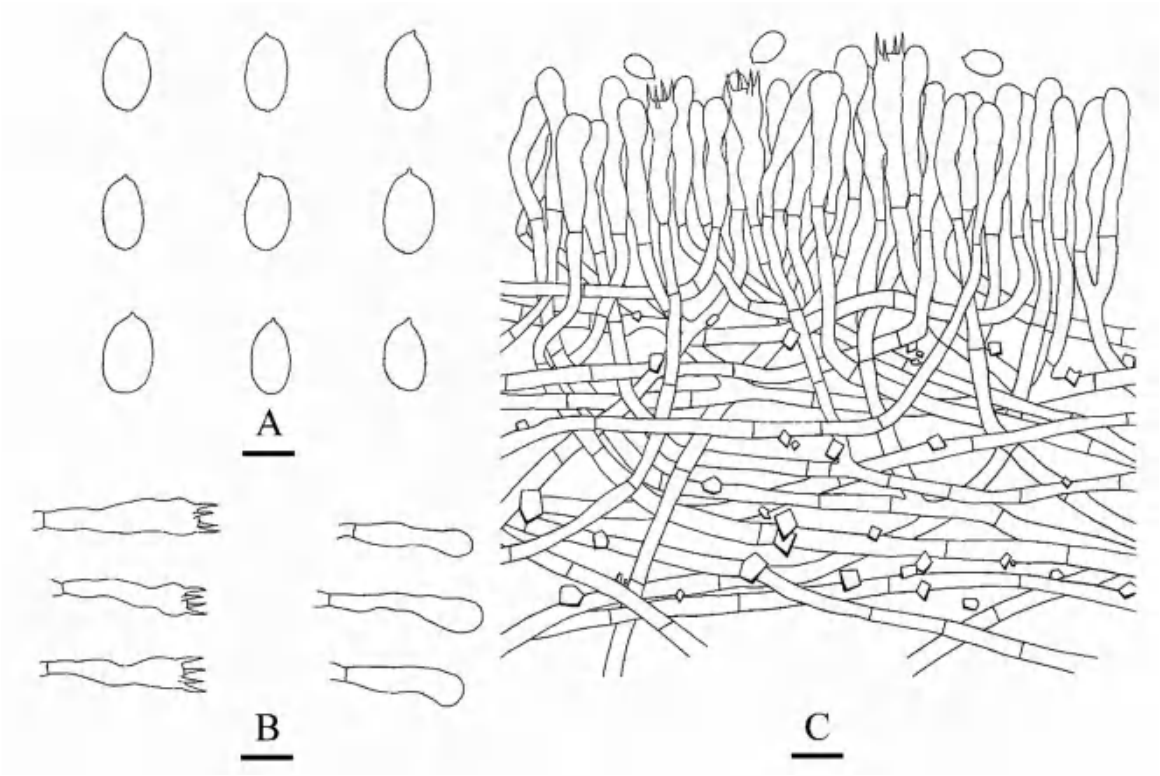

FIGURE 3. Microscopic structures of *Efibula yunnanensis* (drawn from the holotype). a. Basidiospores. b. Basidia and basidioles. c. A section of hymenium. Bars: a—5 µm, b, c—10 µm.

Diagnosis:—The species is characterized by an annual growth habit, resupinate basidiomata with smooth, cream to pale brown hymenial surface, a monomitic hyphal system with thin-walled, simple septate generative hyphae and ellipsoid, hyaline, thin-walled, smooth, IKI-, CB-basidiospores.

Holotype:—CHINA. Yunnan Province: Wenshan, Guangnan county, Babao town, on fallen branch of angiosperm, 19 January 2019, *CLZhao 11641* (SWFC).

Etymology:—*Yunnanensis*: refers to the type locality (Yunnan Province, P.R. China).

Basidiomata:—Annual, resupinate, leather, up to 10 cm long, 1.5 cm wide, 100–300 µm thick. Hymenial surface smooth, white to pale brown when fresh, cream to pale brown upon drying.

Hyphal structure:—Hyphal system monomitic; generative hyphae with simple septate, thin-walled, branched, 2–4 µm in diameter, IKI-, CB-; tissues unchanged in KOH.

Hymenium:—Cystidia and cystidioles absent; basidia clavate, four sterigmate, simple septate at the base, 25–31 × 6–7.5 µm, smooth, thin-walled, basidioles dominant, in shape similar to basidia, but slightly smaller.

Basidiospores:—ellipsoid, hyaline, thin-walled, smooth, IKI-, CB-, (5.2)5.5–7.5(–8.1) × (3.4)3.6–4.5(–4.7) µm, L = 6.68 µm, W = 4.26 µm, Q =1.53–1.62 (n = 60/2).

Ecology and distribution:—Lignicolous, causing a white rot. Known only from the type locality (Yunnan Province, P.R. China).

Additional specimen (paratype) examined.—CHINA. Yunan Province: Wenshan, Guangnan County, Babao Town, on fallen branch of angiosperm, 19 January 2019, *CLZhao 11637* (SWFC).

Discussion

Efibula yunnanensis is characterised by an annual growth habit, resupinate basidiomata with smooth, cream to pale

brown hymenial surface, a monomitic hyphal system with thin-walled, simple septate generative hyphae and ellipsoid, hyaline, thin-walled, smooth, IKI-, CB-basidiospores.

Phylogenetically, *Efibula yunnanensis* is closely related to *E. clarkii* and *E. gracilis* based on the phylogenetic result (Fig. 1). But morphologically *E. clarkii* differs in its slightly tuberculate hymenophore, very pale brown to brownish yellow hymenial surface and narrower basidiospores (6–7 × 3–3.5 µm, Floudas & Hibbett 2015). *Efibula gracilis* differs from *E. yunnanensis* by having the cracking hymenial surface and ellipsoid to oblong basidiospores (5.5–7 × 3.3–4 µm, Floudas & Hibbett 2015).

Morphologically, *Efibula yunnanensis* resembles three similar species in this genus, *E. americana* Floudas & Hibbett (2015: 711), *E. rosea* (Henn.) Kotir. & Saaren (1993: 217) and *E. verruculosa* (Hjortstam & Ryvarden 1980) Kotir. & Saaren (1993: 217). *Efibula americana* differs from *E. yunnanensis* by the smooth to reticulate hymenial surface and margin fibrillose with small hyphal cords (Floudas & Hibbett 2015). *Efibula rosea* differs by its grandinioid hymenophore with rosy-pinkish hymenial surface (Kotiranta & Saarenoksa 1993), while *E. verruculosa* by the grandinioid to odontoid hymenophore with cream hymenial surface and smaller basidiospores (4–4.5 × 2–2.5 µm, Kotiranta& Saarenoksa 1993).

Efibula lutea Sheng H. Wu (1990: 23) and *E. subodontoidea* (Sheng H. Wu) Zmitr. & Spirin (2006: 33) were earlier reported from China. However, *E. lutea* by its yellow hymenial surface and smaller basidiospores (5 × 2.5 µm, Wu 1990) and *E. subodontoidea* differs odontioid hymenial surface, and narrowly ellipsoid, larger basidiospores (6.5–8.5 × 3–3.7 µm, Wu 2000).

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