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New Species

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Flavodontia rosea gen. & sp. nov. from southwestern China

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Abstract—A new white-rot corticioid wood-inhabiting fungal genus and species, *Flavodontia rosea*, collected from subtropical Yunnan, China, is proposed based on morphological and molecular evidence. *Flavodontia* is characterized by annual, resupinate basidiomes with a pink hymenial surface, a monomitic hyphal system with generative hyphae bearing simple septa, and ellipsoid basidiospores with thin hyaline smooth walls. Phylogenetic analyses of ITS and ITS + LSU nuclear RNA gene regions showed that *Flavodontia* formed a distinct, monophyletic lineage within a subclade that includes *Flavodon* and *Irpex.*

Key words—*Irpicaceae*, taxonomy, wood-rotting fungi

Introduction

Polyporales, a now well accepted and strongly supported order of *Agaricomycetes,* is one of the most intensively studied groups of fungi of special interest to fungal ecologists and applied scientists (Hibbett & al. 2014; Justo & al. 2017). The most recent Dictionary of the Fungi (Kirk & al. 2008) notes 1589 genera and more than 30,000 species of basidiomycetes (nearly 32% of all described fungal taxa; Dai & al. 2015), of which roughly 1800 described species are included in *Polyporales,* accounting for only about 1.5% of all known fungal species (Kirk & al. 2008). These taxa play a key role as wood-rotting fungi in addition to their pathogenicity and potential applications in biomedical

engineering and biodegradation (Dai & al. 2009, Levin & al. 2016, Bankole & al. 2020).

Polyporales has been sampled extensively in phylogenetic studies using ribosomal RNA (rRNA) genes (Hibbett & Vilgalys 1993, Hibbett & Donoghue 1995, Boidin & al. 1998, Larsson & al. 2004, Binder & al. 2005, Justo & al. 2017, Huang & al. 2020). Justo & al. (2017) included 18 families in their analyses of a 3-gene dataset (292 taxa), providing a phylogenetic overview of *Polyporales* as well as a framework for further taxonomic study. Among these, *Irpicaceae* Spirin & Zmitr. encompassed a great variation of basidiome and hymenophore types, with typical polypore and corticioid morphologies intermixing with each other. A survey of morphological, anatomical, physiological, and genetic traits supported 12 genera in this family.

During investigations of wood-inhabiting fungi in southern China, an unknown taxon was found that could not be assigned to any described genus. Here we expand samplings from other studies to examine the taxonomy and phylogeny of a new genus within the *Irpicaceae* based on the internal transcribed spacer (ITS) regions and the large subunit nrRNA gene (nLSU) sequences.

Materials & methods

The specimens are deposited at the herbarium of Southwest Forestry University, Kunming, P.R. China (SWFC). Macroscopical descriptions are based on field notes of fresh material. Colour terms follow Petersen (1996). Dried specimens were examined microscopically under a light microscope following Dai (2012). The following abbreviations are used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both inamyloid and nondextrinoid, 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 of specimens (b).

A Magen Biotech HiPure Fungal DNA Mini Kit II (Co., Ltd, Guangzhou, Guangdong, P.R. China) was used to extract genomic DNA from dried specimens according to the manufacturer's instructions with some modifications. A small piece (ca. 30 mg) of dried fungal material was ground to powder with liquid nitrogen, transferred to a 1.5 mL centrifuge tube, suspended in 0.4 mL of lysis buffer, and incubated at 65°C in a water bath for 60 min. After 0.4 mL phenol-chloroform (24:1) was added to each tube, the suspension was shaken vigorously. After centrifugation at 13,000 rpm for 30 s, 0.3 mL of supernatant was transferred to a new tube and mixed with 0.45 mL of binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation again for 30 s. Then,

Table 1. Sequences selected for ITS and nLSU phylogenetic analyses

Species name	Specimen no.	GenBank accession no.		References
		ITS	nLSU	
Byssomerulius corium	FP-102382	KP135007	KP135230	Justo & al. 2017
	Wu 1708-327	LC427007	LC427031	Chen & al. 2020a
Ceriporia reticulata	CBS 462.50	MH856710	MH868228	Vu & al. 2019
	RLG-11354-Sp	KP135041	KP135204	Justo & al. 2017
C. viridans	Cui 8012	KC182774	—	Jia & al. 2013
	TNM: GC 1708-211	LC427027	LC427049	Chen & al. 2020a
Emmia lacerata	FP-55521T	KP135024	KP135202	Floudas & Hibbett 2015
E. latemarginata	Dai 7165	KY131834	KY131893	Wu & al. 2017
	CBS 436.48	MH856427	MH867973	Vu & al. 2019
Flavodon flavus	TNM: WHC 1381	LC427029	LC427052	Chen & al. 2020a
	LE295997	KF856505	KF856510	Zmitrovich & Malysheva 2014
Flavodontia rosea	**CLZhao 18491 [T]**	**MW377575**	**MW377578**	**This study**
	CLZhao 18489	**MW377574**	**MW377577**	**This study**
Gloeoporus dichrous	CBS 446.50	MH856705	MH868222	Vu & al. 2019
	Dai 16370A	KU360399	KU360406	Yuan & al. 2016
G. pannocinctus	FCUG 2019	AF141612	AF141612	Yuan & al. 2016
	CBS 291.71	MH860130	MH871903	Vu & al. 2019
G. thelephoroides	BZ-2896	MG572757	MG572741	Jung & al. 2018
Hydnopolyporus fimbriatus	CBS 384.51	MH856910	MH868432	Vu & al. 2019
Irpex lacteus	FD-9	KP135026	KP135224	Justo & al. 2017
	CBS 431.48	MH856423	MH867969	Vu & al. 2019
Leptoporus mollis	RLG7163	KY948794	—	Justo & al. 2017
	TJV-93-174T	KY948795	EU402510	Justo & al. 2017
Meruliopsis leptocystidiata	TNM: Wu 1708-15	LC427012	LC427032	Chen & al. 2020a
	TNM: Wu 1708-43	LC427013	LC427033	Chen & al. 2020a
M. taxicola	CBS 455.48	MH856432	MH867978	Vu & al. 2019
	BU061013-38	MG572756	MG572740	Jung & al. 2018
Phanerochaete rhodella	FD-18	KP135187	KP135258	Justo & al. 2017
P. sordida	FD-241	KP135136	KP135252	Justo & al. 2017
Trametopsis aborigena	Robledo 1236	KY655336	KY655338	Gomez-Montoya & al. 2017
	Robledo 1238	KY655337	KY655339	Gomez-Montoya & al. 2017
T. brasiliensis	Meijer 3637	JN710510	JN710510	Gomez-Montoya & al. 2017
T. cervina	PRM900574	AY684175	AY855907	Tomšovský & al. 2006

0.5 mL of inhibitor removal fluid was added in AC for a centrifugation at 12,000 rpm for 30 s. After washing twice with 0.5 mL of 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 genomic DNA. The ITS region was amplified with primers ITS5 and ITS4 (White & al. 1990), and the nLSU region was amplified with primers LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The ITS PCR protocols involved initial denaturation at 95°C for 3 min, 35 cycles at 94°C for 40 s + 58°C for 45 s + 72°C for 1 min, and a final 10-min extension at 72°C. The nLSU PCR protocols involved initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s + 48°C for 1 min + 72°C for 1.5 min, and a final 10-min extension at 72°C. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited in GenBank (Table 1).

Sequences were aligned in MAFFT 7 (http://mafft.cbrc.jp/alignment/server/) using the "G-INS-I" strategy for nLSU and "E-INS-I" strategy for ITS + nLSU and manually adjusted in BioEdit (Hall 1999). Alignment datasets were deposited in TreeBase (submission ID 27155). *Phanerochaete sordida* and *P. rhodella* sequences obtained from GenBank were used as outgroup to root trees following Justo & al. (2017) in the ITS (Fig. 1) and ITS + nLSU (Fig. 2) phylogenetic trees.

Maximum parsimony analyses were applied to the ITS and ITS + nLSU dataset sequences following Zhao & Wu (2017), and the tree was generated in PAUP* v. 4.0b10 (Swofford 2002). All characters were equally weighted with gaps 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 1000 replicates (Felsenstein 1985). Tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree. The data matrix was also analyzed using Maximum Likelihood (ML) approach with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org; Miller & al. 2009). Branch support (BS) for ML analysis was determined by 1000 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 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 70,000 generations for ITS (Fig. 1), 200,000 generations for ITS + nLSU (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 were considered as significantly supported if they received maximum likelihood bootstrap (BS) >70%, maximum parsimony bootstrap (BT) >50%, or Bayesian posterior probability (BPP) >0.95.

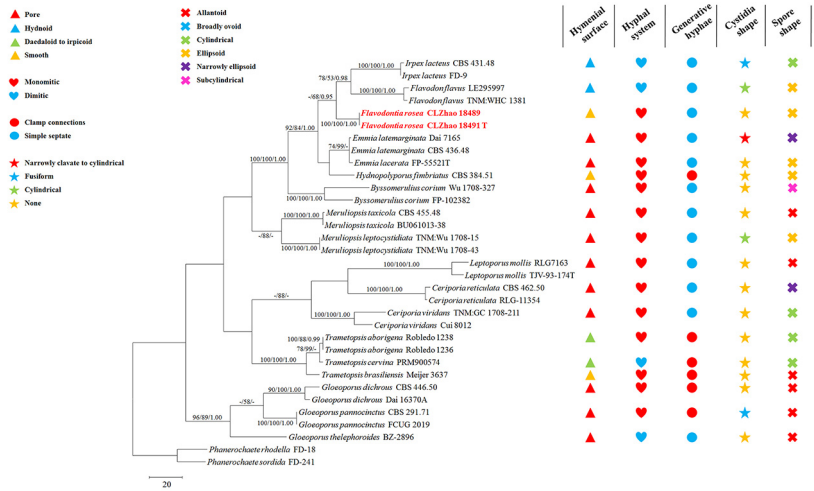

Fig. 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Flavodontia rosea* and related species in *Irpicaceae* based on ITS sequences. Branches are labelled with maximum likelihood bootstrap >75%, parsimony bootstrap >50%, and Bayesian posterior probability >0.95.

Phylogenetic results

The ITS dataset included sequences from 33 fungal specimens representing 20 taxa and with an aligned length of 666 characters, of which 358 characters were constant, 36 variable and parsimony-uninformative, and 272 parsimony-informative. Maximum parsimony analysis yielded 3 equally parsimonious trees (TL = 927, CI = 0.537, HI = 0.463, RI = 0.731, RC = 0.393). Best model for ITS estimated and applied in the Bayesian analysis: GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis produced a similar topology with an average standard deviation of split frequencies = 0.009412.

The phylogenetic tree (Fig. 1) inferred from ITS sequences obtained from related genera in *Irpicaceae* demonstrated that the new genus formed a monomitic lineage and then grouped with a clade including *Flavodon* Ryvarden and *Irpex* Fr.

The ITS + nLSU dataset (Fig. 2) included sequences from 33 fungal specimens, also representing 20 species. The dataset had an aligned length of 1702 characters, of which 1120 characters were constant, 146 variable and parsimony-uninformative, and 436 parsimony-informative. Maximum

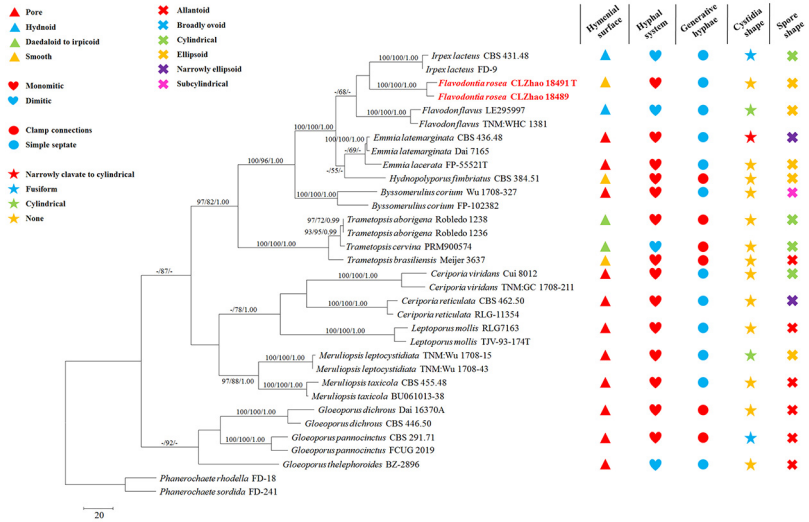

Fig. 2. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Flavodontia rosea* and related species in *Irpicaceae* based on ITS + nLSU sequences. Branches are labeled with maximum likelihood bootstrap >75%, parsimony bootstrap >50%, and Bayesian posterior probability >0.95.

parsimony analysis yielded 2 equally parsimonious trees (TL = 1382, CI = 0.587, HI = 0.413, RI = 0.754, RC = 0.442). Best model for the 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 and ML analyses produced a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.009828.

The additional phylogeny (Fig. 2) inferred from ITS + nLSU sequences grouped the new species with the related species *Flavodon flavus* (Klotzsch) Ryvarden and *Irpex lacteus* (Fr.) Fr.

Taxonomy

Flavodontia C.L. Zhao, **gen. nov.**

MB 838322

Differs from *Flavodon* by its resupinate basidiomata, smooth hymenial surface, monomitic hyphal system, and ellipsoid basidiospores.

Type species: *Flavodontia rosea* C.L. Zhao

Etymology: *Flavodontia* (Lat.): referring to the closely related genus, *Flavodon*.

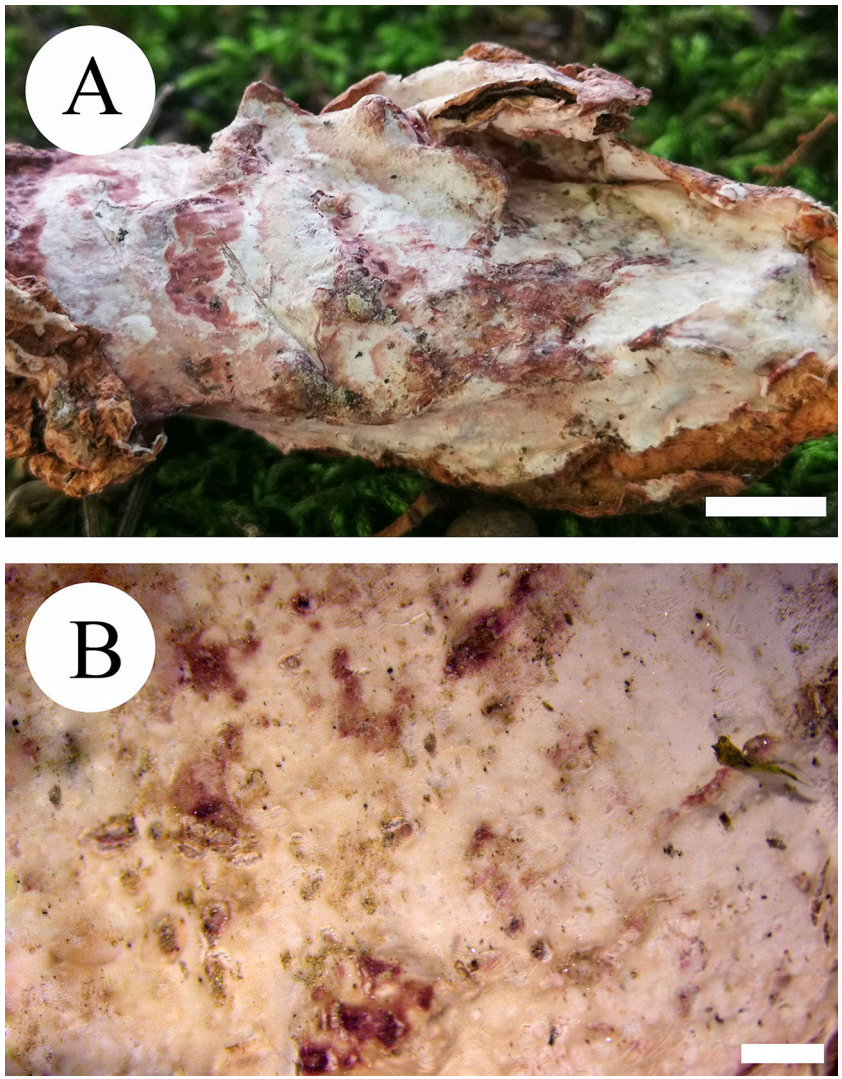

Fig. 3. *Flavodontia rosea* (holotype, SWFC 018491).
Basidiomata. Scale bars: A = 0.5 cm; B = 1 mm.

Basidiomata annual, resupinate, smooth hymenial surface, fragile to rigid upon drying. Hyphal system monomitic; generative hyphae bearing simple septa, IKI–, CB–; tissues unchanged in KOH. Cystidia and cystidioles absent.

Basidia clavate. Basidiospores ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–.

Type of rot: white.

Flavodontia rosea C.L. Zhao, **sp. nov**. Figs 3, 4

MB 838323

Differs from *Flavodon flavus* by its resupinate basidiomata with buff to slightly rose to rose hymenial surface, its monomitic hyphal structure, and its smaller basidiospores.

Type: China. Yunnan Province: Honghe, Pingbian County, Daweishan National Nature Reserve, 22.93°N 103.68°E, alt. 1714 m, on an angiosperm trunk, 3 August 2019, CLZhao 18491 (**Holotype**, SWFC 018491; GenBank MW377575, MW377578).

Etymology: From *rosea* (Lat.), referring to the rose hymenial surface of the basidiome.

Basidiomata annual, resupinate, coriaceous, without odour or taste when fresh, becoming fragile to rigid upon drying, ≤8 cm long, 3 cm wide, 500–800 μm thick. Hymenial surface smooth, buff to slightly rose to rose when fresh, becoming rose-coloured upon drying. Subiculum very thin, 0.2 mm thick, buff to slightly rose. Margin sterile, cream to buff, 2 mm wide.

Hyphal structure monomitic; generative hyphae with simple septa, colourless, thin to thick-walled, rarely branched, 3–4.5 μm diam., IKI–, CB–; tissues unchanged in KOH.

Hymenium cystidia and cystidioles absent; basidia clavate, 19–26.5 × 3–4 μm, with four sterigmata and a basal simple septum; basidioles dominant, in shape similar to basidia, but slightly smaller.

Basidiospores ellipsoid, colourless, thin-walled, smooth, IKI–, CB–, (3.1–)3.3–4.6(–4.8) × (2.5–)2.7–3.5 μm, L = 3.99 μm, W = 3.06 μm, Q = 1.29–1.30 (n = 60/2).

Type of rot: white rot.

Additional specimen examined: **CHINA**. **Yunnan province**. **Honghe:** Pingbian County, Daweishan National Nature Reserve, on the angiosperm trunk, 3 August 2019, CLZhao 18489 (SWFC 018489; GenBank MW377574, MW377577).

Comments: *Flavodon flavus* differs from *Flavodontia rosea* by pileate basidiomata with a poroid hymenophore surface that becomes hydnoid to irpicoid with time, a dimitic hyphal structure with thick-walled skeletal hyphae, and larger basidiospores (5.5–6.5 × 3–4 μm; Ryvarden 1973).

Discussion

Phylogenetic and morphological analyses strongly support *Flavodontia* as an independent genus that clusters with related taxa in *Irpicaceae*. Phylogenetically, *Flavodontia* is closely related to *Flavodon* and *Irpex* based

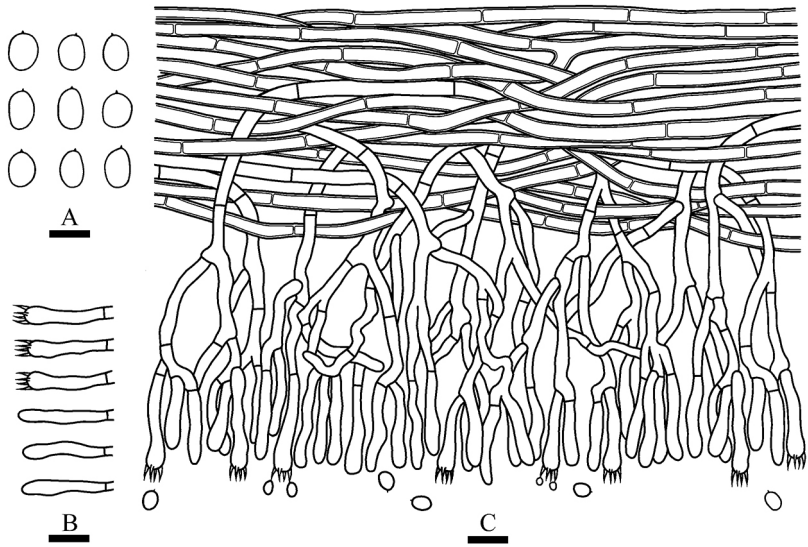

Fig. 4. *Flavodontia rosea* (drawn from the holotype, SWFC 018491).
A. Basidiospores; B. Basidia and basidioles; C. Section of hymenium.
Scale bars: A = 5 μm; B, C = 10 μm.

on ITS and ITS + LSU nRNA gene analyses (Figs 1 & 2) in agreement with multigene analyses by Justo & al. (2017). *Flavodon* is morphologically distinguished by pileate basidiomata with poroid to irpicoid hymenophores and encrusted cystidia (Ryvarden 1973), and *Irpex* differs from *Flavodontia* by its poroid to hydnoid hymenophore with irregular/irpicoid pores and dimitic hyphal system (Fries 1825, Bernicchia & Gorjón 2010).

The consensus phylogram of the comprehensive taxon dataset from 12 agaricomycete orders from ITS, LSU, mtSSU, atp6, tef1, and rpb2 grouped *Flavodon*, *Irpex*, and *Trametopsis* Tomšovský together in a clade labelled "*Byssomerulius* family" (Miettinen & al. 2012). A subsequent phylogenetic tree based on a 3-gene dataset and including more sequences and taxa in *Irpicaceae* supported *Emmia* Zmitr. & al. as sister to *Irpex* and clustered with *Trametopsis* (Justo & al. 2017). The relationships among different taxa in *Irpicaceae* from both studies (Miettinen & al. 2012, Justo & al. 2017) differed due to 3-gene and 6-gene datasets.

In the present study, the ITS (Fig. 1) and ITS + nLSU (Fig. 2) phylograms are very similar, clustering four genera together in agreement with previous studies and supporting *Flavodontia* as an independent genus. The different

symbols and colors in Figs 1 & 2 show that the macromorphological hymenophore characters are not consistent and regular across *Flavodontia* and related genera. However, the micromorphological data (hymenial surface, hyphal system, generative hyphae, and cystidial and spore shapes) show that four closely related genera *Emmia*, *Flavodon*, *Flavodontia*, and *Irpex* share the same character of simple septate generative hyphae, suggesting that micromorphology is less affected by the environment than macromorphology and thus more indicative of phylogenetic relationships.

Irpicioid fungi represent an extensively studied group of *Polyporales* (Núñez & Ryvarden 2001, Bernicchia & Gorjón 2010, Dai 2012, Ryvarden & Melo 2014, Dai & al. 2015), but the Chinese irpicioid fungus diversity is still not well known, especially in subtropics and tropics where many new taxa, including *Flavodontia,* have recently been described (Zhao & al. 2017, Shen & al. 2018, Cui & al. 2019, Ma & Zhao 2019, Chen & al. 2020b, Huang & al. 2020). We anticipate that more undescribed irpicioid taxa will be discovered throughout China after extensive collection combined with morphological and molecular analyses.

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