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Research

Hyphodermella zixishanensis (Polyporales, Basidiomycota), a new species with reddish hymenial surface

Hui Wang, Zi-Rui Gu and Chang-Lin Zhao

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A new corticioid fungal species, *Hyphodermella zixishanensis*, is described based on a combination of morphological features and molecular evidence. The species is characterized by an annual growth habit, resupinate basidiomata with reddish hymenial surface, a monomitic hyphal system with generative hyphae bearing simple septa, IKI–, CB– and ellipsoid, colorless, thin-walled, smooth basidiospores. Sequences of ITS and nLSU gene regions were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and Bayesian inference methods. The phylogenetic analyses showed that the species belongs to *Hyphodermella* and is placed as sister to *H. aurantiaca*.

Keywords: Phanerochaetaceae, phylogeny, taxonomy, wood-inhabiting fungi, Yunnan Province

Introduction

The polypore genus *Hyphodermella* J. Erikss. & Ryvarden (Polyporales, Basidiomycota) is characterized by resupinate, crustose basidiomata, smooth to grandinioid to odontioid or hydnoid or poroid hymenophore and a monomitic hyphal structure with simple septa in generative hyphae, presence of encrusted hyphal ends, clavate to suburniform basidia and ellipsoid to globose, smooth, thin-walled basidiospores (Eriksson and Ryvarden 1976, Bernicchia and Gorjón 2010, Duhem and Buyck 2011). The genus is typified by *H. corrugata* (Fr.) J. Erikss. & Ryvarden and so far eight species have been accepted in the genus worldwide (Gilbertson et al. 2001, Hjortstam and Ryvarden 2007, Nakasone 2008, Duhem 2010, Wang and Zhao 2021).

Hyphodermella has been included in several recent molecular phylogenetic analyses. A study focusing on the classification of corticioid fungi showed that *H. corrugata* grouped with *Phlebia firma* J. Erikss. & Hjortstam in the Phanerochaetaceae, based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences (Larsson 2007). Binder et al. (2013) in studies employing multi-gene (5.8S, nLSU, TEF1, mtSSU, RPB1, RPB2) datasets to investigate the phylogenetic relationships within Polyporales, recovered *H. corrugata* nested in the phlebioid clade and placed next to *Terana coerulea* (Lam.) Kuntze. Floudas and Hibbett

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(2015) analyzed the genus *Phanerochaete* P. Karst using a four gene dataset and revealed that *Hyphodermella rosae* (Bres.) Nakasone grouped with *Pirex concentricus* (Cooke & Ellis) Hjortstam & Ryvarden in the phlebioid clade. A revised family-level classification of the Polyporales showed that *H. rosae* clustered with *Donkia pulcherrima* (Berk. & M.A. Curtis) Pilát and *P. concentricus* (Cooke & Ellis) Hjortstam & Ryvarden (Justo et al. 2017). Based on morphological and molecular evidence, Zhao et al. (2017) proposed a new species, *H. poroides* Y.C. Dai & C.L. Zhao, which grouped together with *H. corrugata* and *H. rosae*. Recently, Wang and Zhao (2021) introduced *H. aurantiaca* C.L. Zhao, a species closely related to *H. corrugata*, *H. poroides* and *H. rosae*.

During investigations on wood-inhabiting fungi in southern China, a new taxon with affinities to *Hyphodermella* was found. In this study, we expand the sampling from previous studies in order to examine the taxonomy and phylogeny of this new species, based on sequence from the ITS and nLSU genes.

Material and methods

Morphological studies

The studied specimens were 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 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.

Molecular techniques and phylogenetic analyses

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from dried specimens. 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 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 the adsorbed film to elute the DNA. The ITS region was amplified with the primer pair ITS5 and ITS4 (White et al. 1990). The nuclear LSU region was amplified with the primer pair LR0R and LR7 (<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, Kunming, Yunnan Province, P.R. China. All newly generated sequences were deposited at GenBank (Table 1).

Sequences were aligned in MAFFT 6 (Katoh and Toh 2008; <http://mafft.cbrc.jp/alignment/server/>) using the 'G-INS-I' strategy for nLSU, the 'E-INS-I' strategy for ITS+nLSU, and manually adjusted in BioEdit (Hall 1999). Alignment datasets were deposited in TreeBase (submission ID 28053). *Candelabrochaete africana* Boidin obtained from GenBank was used as an outgroup to root the LSU tree (Fig. 1) following Justo et al. (2017) and *Pirex concentricus* was selected as an outgroup for phylogenetic analyses of the ITS+nLSU region (Fig. 2) following Zhao et al. (2017).

Maximum parsimony analyses were applied to the ITS+nLSU and nLSU dataset sequences. Approaches to phylogenetic analysis followed Chen et al. (2016), and the tree construction procedure was performed in PAUP* ver. 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 was 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). The 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/sub_sections/portal>). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicate.

MrModeltest ver. 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 MrBayes3.1.2 (Ronquist and Huelsenbeck 2003). Four Markov chains were run for two runs from random starting trees for 250 000 generations for nLSU (Fig. 1) and 100 000 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 ML bootstrap value >

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

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Bjerkandera adusta	CBS 371.52	–	MH868620	Vu et al. 2019
B. adusta	HHB 12826	–	KP135198	Floudas and Hibbett 2015
Candelabrochaete africana	FP 102987	–	KP135199	Floudas and Hibbett 2015
Hapalopilus rutilans	FO 29328	–	AF291333	Weiss and Oberwinkler 2001
H. rutilans	CBS 422.48	–	MH867966	Vu et al. 2019
Hyphodermella aurantiaca	CLZhao 10487	MW209023	MW209012	Wang and Zhao 2021
H. aurantiaca	CLZhao 10500	MW209025	MW209014	Wang and Zhao 2021
H. aurantiaca	CLZhao 10521	MW209029	MW209018	Wang and Zhao 2021
H. corrugata	MA-Fungi 26186	FN600379	JN939585	Telleria et al. 2010
H. corrugata	MA-Fungi 24238	FN600378	JN939586	Telleria et al. 2010
H. poroides	Dai 12045	KX008367	KX011852	Zhao et al. 2017
H. poroides	Dai 10848	KX008368	KX011853	Zhao et al. 2017
H. rosae	FP 150552	KP134978	KP135223	Floudas and Hibbett 2015
H. rosae	MA-Fungi 38071	FN600389	JN939588	Telleria et al. 2010
H. zixishanensis	CLZhao 7124	MZ305276	–	Present study
H. zixishanensis	CLZhao 7129	MZ305277	MZ305286	Present study
H. zixishanensis	CLZhao 7159	MZ305278	MZ305287	Present study
H. zixishanensis	CLZhao 7204	MZ305279	MZ305288	Present study
H. zixishanensis	CLZhao 7206	MZ305280	MZ305289	Present study
H. zixishanensis	CLZhao 7228	MZ305281	MZ305290	Present study
H. zixishanensis	CLZhao 7412	MZ305282	MZ305291	Present study
H. zixishanensis	CLZhao 7433	MZ305283	MZ305292	Present study
H. zixishanensis	CLZhao 7535	MZ305284	–	Present study
H. zixishanensis	CLZhao 7718	MZ305285	MZ305293	Present study
Phanerochaete ericina	HHB 2288	–	KP135247	Floudas and Hibbett 2015
P. laevis	HHB 15519	–	KP135249	Floudas and Hibbett 2015
P. magnoliae	HHB 9829	–	KP135237	Floudas and Hibbett 2015
P. pseudosanguinea	FD 244	–	KP135251	Floudas and Hibbett 2015
P. rhodella	FD 18	–	KP135258	Floudas and Hibbett 2015
Phlebiopsis gigantea	FP 70857	–	KP135272	Floudas and Hibbett 2015
Pirex concentricus	OSC 41587	KP134984	KP135275	Floudas and Hibbett 2015
Porostereum spadiceum	CBS 476.48	–	MH867985	Vu et al. 2019
Rhizochaete brunnea	MR 229	–	AY219389	Greslebin et al. 2004
R. fouquieriae	KKN 121	–	AY219390	Greslebin et al. 2004
Terana caerulea	CBS 163.56	–	MH869102	Vu et al. 2019
T. caerulea	FP 104073	–	KP135276	Floudas and Hibbett 2015

75%, maximum parsimony bootstrap value (MP) > 75% or Bayesian posterior probabilities (PP) > 0.95.

Results

Molecular phylogeny

The nLSU dataset (Fig. 1) included sequences from 33 fungal specimens representing 19 species. The dataset had an aligned length of 1207 characters, of which 1044 characters were constant, 67 were variable and parsimony-uninformative and 96 were parsimony-informative. Maximum parsimony analysis yielded three equally parsimonious trees (TL = 316, CI = 0.627, HI = 0.373, RI = 0.787, RC = 0.493). Best model for the nLSU dataset estimated and applied in the Bayesian analysis: a general time reversible (GTR+I+G) model of DNA substitution and a gamma distribution rate variation across sites, 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 = 0.009816 (BI).

The phylogeny (Fig. 1) inferred from nLSU sequences showed that the new species nested in *Hyphodermella*, in which it grouped with *H. aurantiaca*, *H. corrugata*, *H. poroides* and *H. rosae*, forming its own lineage that supported it as a new taxon.

The ITS+nLSU dataset (Fig. 2) included sequences from 11 fungal specimens representing six species. The dataset has an aligned length of 2068 characters, of which 1870 characters were constant, 68 were variable and parsimony-uninformative and 130 were parsimony-informative. Maximum parsimony analysis yielded a single most parsimonious tree (TL = 248, CI = 0.887, HI = 0.113, RI = 0.908, RC = 0.806). The 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 = 0.009768 (BI).

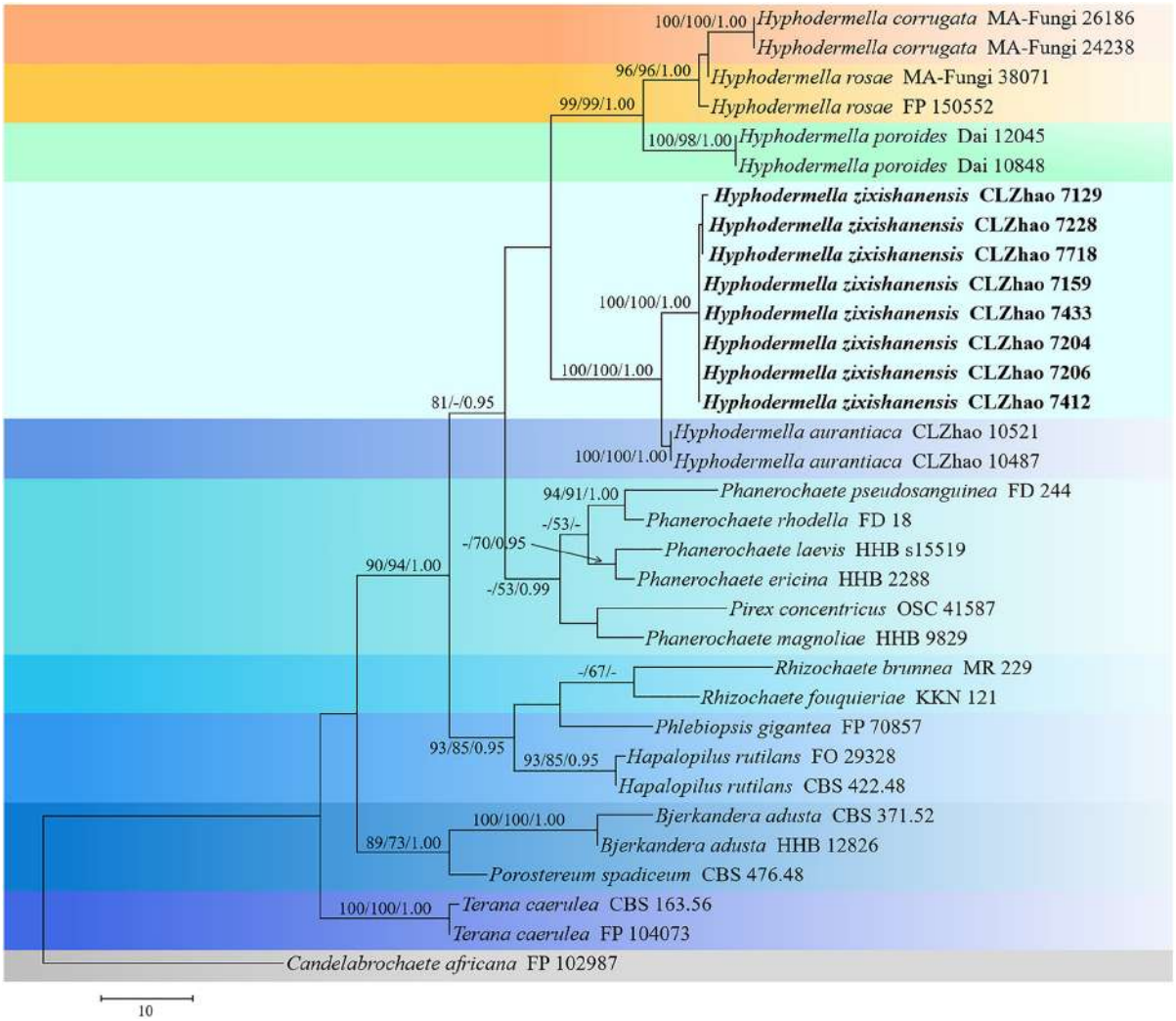

Figure 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Hyphodermella zixishanensis* and related species in *Hyphodermella* based on nLSU sequences. Branches are labeled with maximum likelihood bootstrap value higher than 70%, parsimony bootstrap value higher than 50% and Bayesian posterior probabilities more than 0.95, respectively.

The phylogeny inferred from ITS+nLSU sequences (Fig. 2) was obtained to study the relationship among taxa of *Hyphodermella*, which strongly supported our taxon as a new species and as a monophyletic lineage resolved as sister to *H. aurantiaca* with strong support (100% BS, 100% BP, 1.00 BPP).

Taxonomy

Hyphodermella zixishanensis C.L. Zhao, sp. nov. (Fig. 3, 4)

Holotype: China, Yunnan Province: Chuxiong, Zixishan National Forestry Park, on fallen branch of angiosperm, 24°9'°N, 101°22'E, 1 Aug 2018, C.L. Zhao 7718 (SWFC). MB 839869

Basidiomata annual, resupinate, soft ceraceous when fresh, turning ceraceous to crustose upon drying, up to 20 cm long, 6 cm wide, 500–800 µm thick. Hymenial surface tuberculate, slightly reddish when fresh, reddish to brown when dry, cracking. Sterile margin distinctly fimbriate, narrow, slightly rose.

Hyphal system monomitic; generative hyphae bearing simple septa, IKI–, CB–, tissues unchanged in KOH. Hymenial generative hyphae colorless, more or less interwoven, thin- to slightly thick-walled, frequently branched, 2.0–3.5 µm in diameter. Subiculum absent or indistinct, subicular hyphae colorless, thick-walled, occasionally branched, more or less parallel, 3–5 µm wide. Hymenial cystidia and cystidioles absent; basidia clavate, with four sterigmata and a simple septum, 17.5–27.0 × 3.0–5.5 µm; basidioles dominant, similar in shape to basidia, but slightly smaller.

Basidiospores ellipsoid, colorless, thin-walled, smooth, IKI–, CB–, with oily inclusions, (3.5–)3.7–5.8(–6.0) × (2.4–)2.6–4.0(–4.2) µm, L = 4.72 µm, W = 3.24 µm, Q = 1.40–1.60 (n = 300/10).

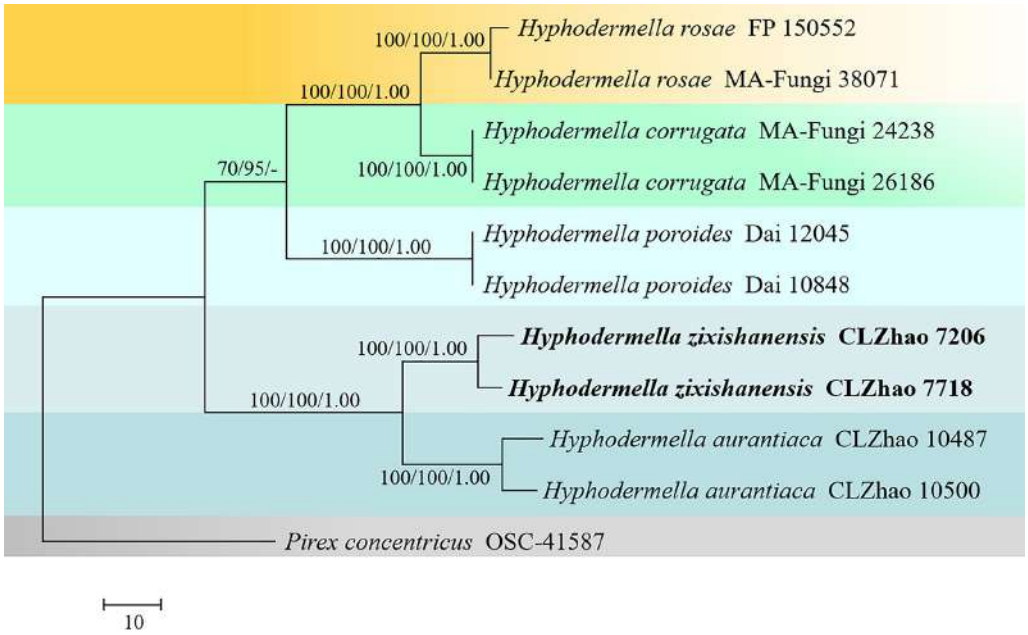

Figure 2. Maximum parsimony strict consensus tree illustrating the phylogeny of *Hyphodermella zixishanensis* and related species in *Hyphodermella* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap value higher than 70%, parsimony bootstrap value higher than 50% and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

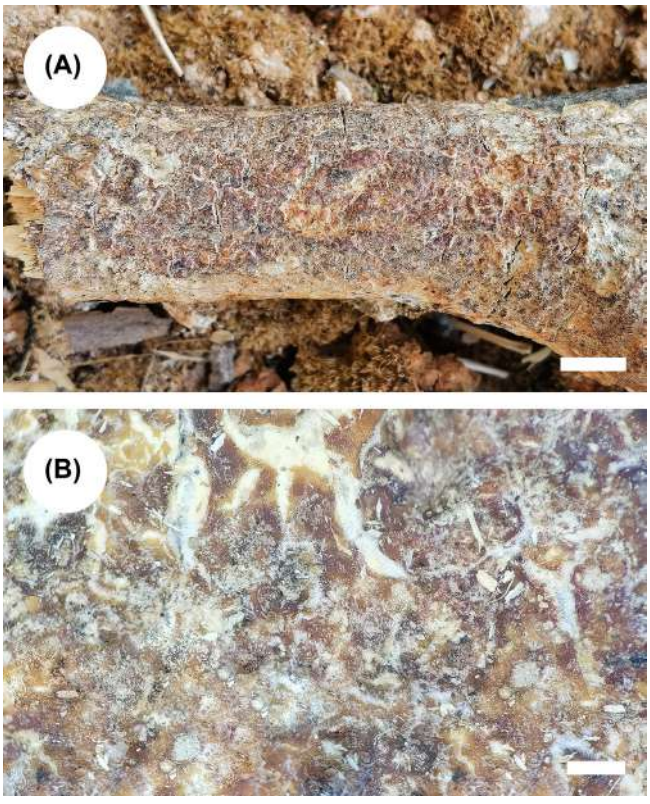

Figure 3. Basidiomata of *Hyphodermella zixishanensis*. Bars: (A) = 1 cm; (B) = 1 mm (holotype).

Etymology

Zixishanensis (Lat.): referring to the provenance (Zixishan National Forestry Park) of the type specimens.

Ecology and distribution

Lignicolous, causing a white rot. Found in China.

Additional specimens examined (paratypes)

China, Yunnan Province: Chuxiong, Zixishan National Forestry Park, on fallen branch of angiosperm, 24°9'N, 101°21'E, 1 Jul 2018, C.L. Zhao 7204, C.L. Zhao 7206, C.L. Zhao 7124, C.L. Zhao 7129, C.L. Zhao 7159, C.L. Zhao 7228; 2 Jul 2018 C.L. Zhao 7412, C.L. Zhao 7433, C.L. Zhao 7535 (SWFC).

Discussion

Two *Hyphodermella* species were reported from China prior to this study, *H. aurantiaca* and *H. poroides* (Zhao et al. 2017, Wang and Zhao 2021). The new species grouped with *H. aurantiaca* based on the combined ITS+nLSU (Fig. 1) and nLSU sequence data (Fig. 2). However, morphologically, *H. aurantiaca* differs from *H. zixishanensis* by its saffron to orange hymenial surface (Wang and Zhao 2021).

Morphologically, *Hyphodermella brunneocontexta*, *H. corrugata*, *H. maunakeaensis*, *H. rosae* and *H. zixishanensis* share ellipsoid basidiospores. However, *H. brunneocontexta* differs by having a brown and odontoid hymenophore surface (Duhem and Buyck 2011); *H. corrugata* by larger basidiospores (7.5–9.0 × 5.0–5.5 µm, Nakasone 2008); *H. maunakeaensis* differs from *H. zixishanensis* by an hydnoid

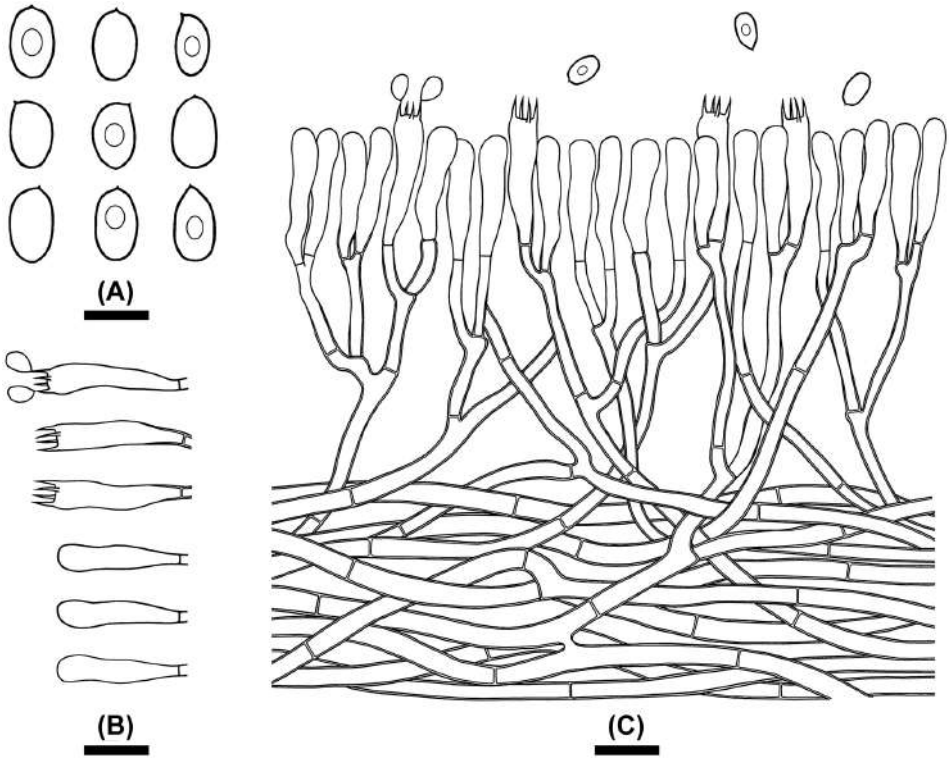

Figure 4. Microscopic structures of *Hyphodermella zixishanensis* (drawn from the holotype). (A) Basidiospores, (B) basidia and basidioles, (C) a section of hymenium. Bars: (A) = 5 µm; (B–C) = 10 µm.

hymenophore and presence of cystidia (Gilbertson et al. 2001); *H. rosae* is separated from the new species by an odontoid hymenophore surface and larger basidiospores (6.0–8.0 × 4.3–5.0 µm, Nakasone 2008).

Hyphodermella poroides and *H. zixishanensis* both have a fimbriate margin. However, *H. poroides* differs from *H. zixishanensis* by having cyanophilous generative hyphae (Zhao et al. 2017).

China's special ecological system, especially in some provinces located in the tropics and subtropics, has proved to be particularly favorable to the development of this group of fungi, allowing the description of many new taxa (Cui et al. 2011, Cui 2013, Li and Cui 2013, Zhao et al. 2013, 2019, Chen et al. 2016, Yuan and Dai 2008, Zong et al. 2021), therefore it is possible that new taxa of *Hyphodermella* species will be found after further field studies and molecular analyses.

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Author contributions

Hui Wang: Data curation (equal); Formal analysis (equal); Investigation (equal); Writing – original draft (lead). **Zi-Rui Gu**: Data curation (equal). **Chang-Lin Zhao**: Data curation (lead); Formal analysis (lead); Methodology (lead); Project administration (lead); Writing – review and editing (lead).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.8sf7m0cnq> (Wang et al. 2021).

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