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Short communication

A new species of *Hyphodermella* (Polyporales, Basidiomycota) with a poroid hymenophore

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ABSTRACT

A new poroid wood-inhabiting fungal species, *Hyphodermella poroides*, is proposed based on morphological and molecular evidences. The species is characterized by resupinate basidiocarps with cream to orange pore surface, a monomitic hyphal system with generative hyphae bearing simple septa, and broadly ellipsoid, hyaline, thin-walled, smooth, IKI−, CB− basidiospores. The phylogenetic analyses based on ITS + nLSU sequences showed that *H. poroides* formed a single group with a strong support and was closely related to *H. corrugata* and *H. rosae*, and then grouped with *Pirex concentricus*. Both morphological and molecular evidences confirmed the placement of the new species in *Hyphodermella*.

Hyphodermella J. Erikss. & Ryvarden was established by Eriksson and Ryvarden (1976), and typified by *H. corrugata* (Fr.) J. Erikss. & Ryvarden. The genus is characterized by resupinate, crustose basidiomata, grandinioid to odontioid or hydnoid hymenophore with small aculei and a fibrillose apex, and a monomitic hyphal structure with simple septa on generative hyphae, the presence of encrusted hyphal ends, basidia clavate to suburniform, and ellipsoid to globose, smooth, thin-walled basidiospores, which are negative in Melzer's reagent and Cotton Blue (Eriksson and Ryvarden 1976; Bernicchia and Gorjón 2010; Duhem and Buyck 2011). The genus includes five species, *H. brunneocontexta* Duhem & Buyck described from France (Duhem and Buyck 2011), *H. corrugata* known from Asia, Europe, North America, South America (Volk et al. 1994; Maekawa 2002; Legon et al. 2005; Hjortstam and Ryvarden 2007; Ryvarden 2007), *H. maunakeaensis* Gilb. & Hemmes described from Hawaii (Gilbertson et al. 2001), *H. ochracea* (Bres.) Duhem found in Italy (Duhem 2010) and *H. rosae* (Bres.) Nakasone known from France, Italy, Portugal and Spain (Nakasone 2008; Bernicchia and Gorjón 2010).

Recently, molecular studies involving *Hyphodermella* based on single- or multi-gene datasets have been carried out and the type species or related taxon of *Hyphoderella* was phylogenetically placed in Phanerochaetaceae (Polyporales, Basidiomycota) (Larsson 2007; Binder et al. 2013; Floudas and Hibbett 2015; Miettinen et al. 2016). Miettinen et al. (2016)

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studies the polypores and genus concepts in Phanerochaetaceae based on the morphological and phylogenetic investigations. Their phylogenetic tree based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences showed that *H. corrugate* and *H. rosae* placed in the clade labeled as donkia clade together with *Donkia pulcherrima* (Berk. & M.A. Curtis) Pilát, *Phanerochaete deflectens* (P. Karst.) Hjortstam, *Pirex concentricus* (Cooke & Ellis) Hjortstam & Ryvarden and two species of *Phlebia* Fr.

During a study of Chinese wood-inhabiting fungi, two specimens collected from Hainan Province in southern China were characterized by resupinate, cream to orange basidiocarps with poroid hymenophore, a monomitic hyphal structure with simple septa, generative hyphae in subiculum distinctly wider than in trama, and hyaline, thin-walled, ellipsoid basidiospores which are negative in Melzer's reagent and Cotton Blue. These characters made them distinct from all the known wood-rotting fungal taxa, and here we propose a new species for the two specimens. To support this proposal, phylogenetic analyses on the position of the new species and related taxa were done based on ITS and nLSU sequences.

The specimens of *Hyphodermella poroides* sp. nov. were deposited at the herbarium of Beijing Forestry University (BJFC). Macro-morphological descriptions are based on field notes and indoor observation follows Dai (2010). Special color terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens, and observed under a light microscope following Dai (2010). 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 basidiospore length (arithmetic average of all basidiospores), 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 basidiospores (a) measured from given number (b) of specimens.

CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions with some modifications. 2 × EasyTaq PCR SuperMix (TransGen biotech, China) was used to amplify nLSU sequences with primers LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm) and ITS sequences with primers ITS5 and ITS4 (White et al. 1990). 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 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 sequenced at Beijing Genomics Institute, China, with primers LR0R and LR7 for nLSU region and primers ITS5 and ITS4 for ITS region. The newly generated sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank; Table 1).

Sequences were aligned in MAFFT 6 (Katoh and Toh 2008; http://mafft.cbrc.jp/alignment/server/) using "E-INS-I" strategy for ITS + nLSU, and manually adjusted in BioEdit (Hall 1999). Alignment datasets were deposited in TreeBase (submission ID 19081). *Phanerina mellea* (Berk. & Broome) Miettinen was selected as outgroup for phylogenetic analyses combined dataset of ITS and nLSU regions (Miettinen et al. 2016).

Maximum parsimony (MP) analysis was applied to the ITS + nLSU datasets. 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 5,000, branches of zero length were collapsed and all parsimonious trees were saved. A bootstrap analysis with 1000 replicates was assessed (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. Maximum Likelihood (ML) analysis with RAxML-HPC2 was conducted ITS + nLSU datasets on Abe through the Cipres Science Gateway (www.phylo.org; Miller et al. 2009). Branch support for MP and ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 (Posada and Crandall 1998; Nylander 2004) was used to determine the best-fit evolution model for each dataset for Bayesian inference (BI). Bayesian inference was calculated with MrBayes 3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution

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

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Donkia pulcherrima	Gothenburg 2022	KX752591	KX752591	Miettinen et al. 2016
Hyphodermella corrugata	MA-Fungi 26186	FN600379	JN939585	Floudas and Hibbett 2015
H. corrugata	MA-Fungi 24238	FN600378	JN939586	Floudas and Hibbett 2015
H. poroides	Dai 12045	KX008367	KX011852	Present study
H. poroides	Dai 10848	KX008368	KX011853	Present study
H. rosae	FP 150552	KP134978	KP135223	Floudas and Hibbett 2015
H. rosae	MA-Fungi 38071	FN600389	JN939588	Telleria et al. 2010
Phanerina mellea	Ryvarden 10132	KX752611	KX752611	Miettinen et al. 2016
Phlebia deflectens	FCUG 1568	AF141619	AF141619	Larsson 2007
P. firma	Edman K268	EU118654	EU118654	Larsson 2007
P. lilascens	FCUG 1801	AF141621	AF141621	Larsson 2007
Pirex concentricus	OSC-41587	KP134984	KP135275	Floudas and Hibbett 2015

rate variation across sites (Ronquist and Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 5 million generations (ITS + nLSU), 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 that received bootstrap support were considered as significantly supported for maximum likelihood (BL), maximum parsimony (BP) and Bayesian posterior probabilities (BPP) greater than or equal to 80% (BL), 75% (BP) and 0.95 (BPP) were considered as significantly supported, respectively.

The ITS + nLSU dataset included sequences from 12 fungal specimens or isolates representing 9 species (Table 1), in which the taxon sampling of this phylogenetic analyses except for outgroup are member of donkia clade. The dataset had an aligned length of 2093 characters, of which 1734 characters are constant, 199 are variable and parsimony-uninformative, and 160 are parsimony-informative. Maximum parsimony analysis yielded five equally parsimonious trees (TL = 585, CI = 0.763, HI = 0.238, RI = 0.556, RC = 0.424). Best model for the ITS + nLSU dataset estimated and applied in the Bayesian analysis as GTR + I + G. Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.003426 (BI).

The phylogeny (Fig. 1) inferred from ITS + nLSU sequences showed that the new species grouped with the related species *Hyphodermella corrugata* and *H. rosae* with a strong support (100% BS, 100% BP, 1.00 BPP).

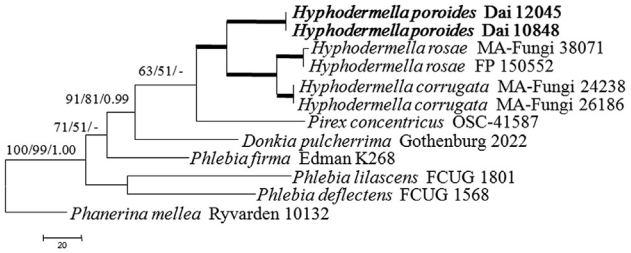

Fig. 1 – Maximum parsimony strict consensus tree illustrating the phylogeny of *Hyphodermella poroides* and related species based on ITS + nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively. Branches that receive 100/100/1.00 supports indicate in bold line without providing values.

Fig. 2 – Basidiocarps of *Hyphodermella poroides* (holotype). *Bar* 1 cm.

Hyphodermella poroides Y.C. Dai & C.L. Zhao, sp. nov. Figs. 2, 3. MycoBank no.: MB 820991.

The species has unique morphological characters by having an annual growth habit, a poroid hymenophore with cream to orange surface, a monomitic hyphal system with simple septa, generative hyphae in subiculum distinctly wider than in trama, and broadly ellipsoid, hyaline, thin-walled, smooth, IKI–, CB– basidiospores.

Type: CHINA, Hainan Province, Changjiang County, Bawangling Nature Reserve, on fallen trunk of *Alniphyllum fortunei* (Hemsl.) Makino, 25 Nov 2010, leg. Y.C. Dai, Dai 12045 (holotype, BJFC 009098).

rRNA gene sequence ex holotype: KX008367 (ITS), KX011852 (nLSU).

Etymology: *Poroides*, referring to the species having a poroid hymenophore.

Fruiting body: Basidiocarps annual, resupinate, adnate, soft, without odor or taste when fresh, ceraceous-crustaceous to hard corky when dry, up to 7 cm long, 5 cm wide, 2 mm thick at center. Pore surface white when fresh, cream to orange when dry; pores angular, 2–3 per mm; dissepiments thin, slightly lacerate. Sterile margin distinctly fimbriate, determinate, white, up to 2 cm wide. Subiculum white, ceraceous-crustaceous, up to 0.5 mm thick. Tubes concolorous with pore surface, ceraceous-crustaceous, up to 1.5 mm long.

Hyphal structure: Hyphal system monomitic; generative hyphae hyaline, thin- to slightly thick-walled, bearing simple septa, IKI–, slightly cyanophilous; tissues unchanged in KOH.

Subiculum: Generative hyphae hyaline, thin- to slightly thick-walled, frequently branched, interwoven, 4.5–7 μm diam.

Tubes: Generative hyphae hyaline, thin-walled, frequently branched, loosely interwoven to subparallel along the tubes, 2.5–4.5 μm in diam; hyphal ends occasionally capitated. Cystidia absent, but fusoid cystidioles occasionally present, hyaline, thin-walled, 20–23 × 3–4 μm; basidia long and clavate, with four sterigmata and a simple septum, 22–27 × 4–5 μm; basidioles dominant, in shape similar to basidia, but slightly smaller.

Spores: Basidiospores broadly ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (2.5–)3–3.5(–4) × 2.5–3 μm, L = 3.26 μm, W = 2.77 μm, Q = 1.07–1.21 (n = 60/2).

Associated wood-rot: White.

Additional specimen examined: CHINA, Hainan Province, Ledong County, Jianfengling Nature Reserve, on fallen angiosperm branch, 11 May 2009, leg. Y.C. Dai, Dai 10848 (BJFC 005090).

In the present study, a new species *Hyphodermella poroides* was described based on phylogenetic analyses and morphological characters. The species has unique morphological

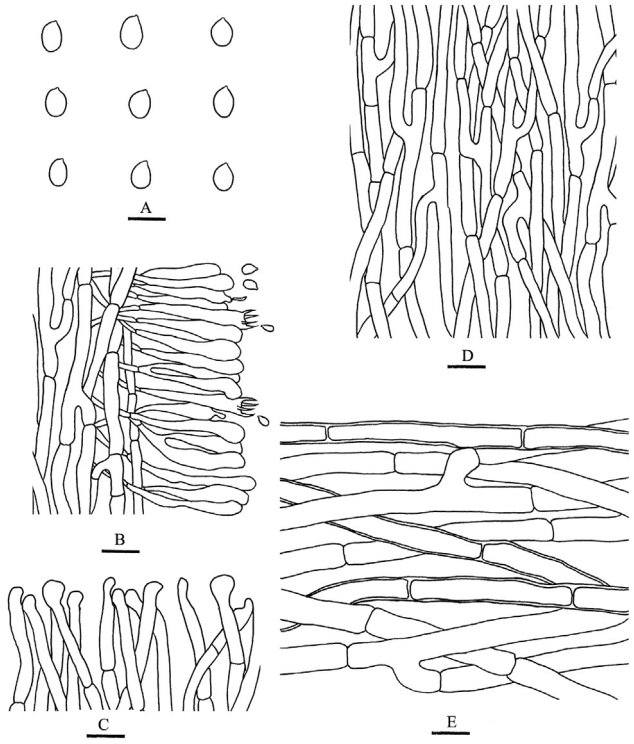

Fig. 3 – Microscopic structures of *Hyphodermella poroides* (drawn from the holotype). A: Basidiospores. B: A section of trama. C: Hyphal ends. D: Hyphal from trama. E: Hyphae from subiculum. *Bars*: A 5 μm; B–E 10 μm.

characters by having a poroid hymenophore in *Hyphodermella* and the new species grouped with the related species *H. corrugata* and *H. rosae* with a strong support (100% BS, 100% BP, 1.00 BPP).

Morphologically, *Hyphodermella brunneocontexta* is separated from *H. poroides* by an odontioid hymenophore, a brown subiculum and larger basidiospores (4.5–5 × 3.5–4 μm; Duhem and Buyck 2011). The type species of *Hyphoderella*, *H. corrugata* differs from *H. poroides* by its grandinioid hymenophore and larger basidia (35–50 × 6–7 μm; Eriksson and Ryvarden 1976; Bernicchia and Gorjón 2010). *Hyphodermella maunakeaensis* differs by a hydnoid hymenophore and larger basidiospores (4.5–5 × 3.5–4 μm; Gilbertson et al. 2001). *Hyphodermella ochracea* differs from *H. poroides* by its odontioid hymenophore, ochraceous subiculum and larger basidiospores (8–12 × 4–5.5 μm; Duhem 2010). *Hyphodermella rosae* differs from *H. poroides* by its odontioid hymenophore and cream to brown subiculum when drying (Bernicchia and Gorjón 2010).

Having resupinate basidiocarps with a poroid hymenophore and a monomitic hyphal system bearing simple septa reminds of the genus *Ceriporia* Donk. *Ceriporia* differs from *Hyphodermella* by having white to tan or brightly colored purple, orange, pink or greenish basidiocarps and cylindrical or allantoid basidiospores (Ryvarden and Melo 2014).

Hyphodermella poroides is put into the genus *Hyphodermella* mainly based on the phylogenetic analyses, in which three species of *H. corrugata*, *H. poroides* and *H. rosae* group together (Fig. 1). In addition, the morphological characters of *H. poroides* by having a poroid hymenophore is unique in this genus, and it shares the common characteristics with the genus *Hyphodermella* by having a monomitic hyphal system bearing simple septa with generative hyphae, the presence of hyphal ends, clavate to suburniform basidia and hyaline, thin-walled, smooth, IKI–, CB– basidiospores (Bernicchia and Gorjón 2010; Duhem and Buyck 2011). Hence, the generic concept of *Hyphodermella* is enlarged after *H. poroides* added in the genus, in which the hymenophore may be grandinioid to odontioid, hydnoid or poroid.

The poroid wood-rotting fungi is an extensively studied group of Basidiomycota (Gilbertson and Ryvarden 1986, 1987; Núñez and Ryvarden 2001; Ryvarden and Melo 2014), but the Chinese diversity is still not well known, especially in the subtropics and tropics, from where many taxa have recently been described (Dai 2012a,b; Li and Cui 2013; Chen et al. 2014; Zhao et al. 2014; Bian and Dai 2015; Han and Cui 2015). *Hyphodermella poroides* is from the subtropics, and it is likely that more new wood-rotting fungi will be found after further investigations.

Key to species of *Hyphodermella*

1a Basidiospores ≥8 μm in length.....2
1b Basidiospores <8 μm in length.....3
2a Hymenophore surface orange to yellow orange; basidia ≥35 μm in length..... *H. corrugata*
2b Hymenophore surface ochraceous; basidia <35 μm in length.....*H. ochracea*
3a Hymenophore poroid; basidiospores <4.5 μm in length.....*H. poroides*
3b Hymenophore odontioid to hydnoid; basidiospores ≥4.5 μm in length.....4
4a Hymenophore surface cream to pale orange yellow, margin fimbriate.....*H. rosae*
4b Hymenophore brownish, margin entire.....5
5a Hymenophore hydnoid; basidiospores narrowly ellipsoid.....*H. maunakeaensis*
5b Hymenophore odontioid; basidiospores broadly ellipsoid to subglobose.....*H. brunneocontexta*

Disclosure

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of the People's Republic of China.

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