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Morphological and molecular identification of two new species of *Hyphodontia* (Schizoporaceae, Hymenochaetales) from southern China

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Abstract – Two new *Hyphodontia* species, *H. pseudotropica* and *H. rhizomorpha* spp. nov., are described based on morphological and molecular characters. *Hyphodontia pseudotropica* is characterized by poroid hymenophore with buff to buff-yellow pore surface, a monomitic hyphal system with thick-walled, frequently branched generative hyphae and oblong-ellipsoid basidiospores, and plenty of bladder-like cystidia. *Hyphodontia rhizomorpha* is distinguished by poroid hymenophores when juvenile, cracked with age; white pore surface, larger pores (1-2 per mm), bearing white rhizomorphs, a monomitic hyphal system with encrusted generative hyphae, and ellipsoid to broadly ellipsoid basidiospores (4.3-5.5 × 3.7-4.1 µm). Both morphological characters and phylogenetic analysis inferred from ITS sequences confirmed the placement of the two new species in *Hyphodontia* s.l. and showed its relationships with similar species in the genus.

Basidiomycota / ITS / Molecular phylogeny / Taxonomy / White-rot fungi

INTRODUCTION

Hyphodontia J. Erikss. (Schizoporaceae, Hymenochaetales) is a widespread genus typified by *H. pallidula* (Bres.) J. Erikss. According to the modern definition, it is characterized by resupinate basidiocarps mostly with smooth, poroid, odontioid or grandinioid hymenophore, a monomitic hyphal system with clamp connections on generative hyphae, suburniform basidia and broadly-ellipsoid basidiospores, and causing a white rot (Xiong *et al.*, 2010; Yurchenko & Wu, 2013a).

The morphological concept of *Hyphodontia* in its broad sense was outlined by Eriksson & Ryvarden (1976), Langer (1994), Vesterholt (1997). The MycoBank database (http://www.MycoBank.org) registered 184 specific and infraspecific names in *Hyphodontia* sensu lato and the Index Fungorum (http://www.indexfungorum.org) registered 163, but the actual number of species is much lower (Wu, 1990, 2000, 2001, 2006; Xiong *et al*., 2009, 2010; Dai, 2011, 2012; Lee & Langer, 2012; Yurchenko & Wu, 2013a, b).

Hjortstam & Ryvarden (2009) proposed that *Hyphodontia* could be split into 13 genera, of which *Xylodon* (Pers.) Gray and *Kneiffiella* (Pers.) Gray were the most species rich genera. Recently, a phylogenetic study inferred from nuclear DNA sequence data for *Hyphodontia* s.l. demonstrated the polyphyletic nature of

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this genus, consisting of six well-distinguished clades including *Lagarobasidium* clade, *Kneiffiella-Alutaceodontia* clade, *Hyphodontia* clade, *Hastodontia* clade, *Xylodon-Lyomyces-Rogersella* clade and *Xylodon-Schizopora-Palifer* clade, but obtained insufficient support for the definition of all the derivative genera (Yurchenko & Wu, 2013b). Awaiting support from multi-gene datasets, we still adopt here a broad concept of *Hyphodontia*.

The genus *Hyphodontia* in China has been extensively studied in the last twenty years, and 55 species were recorded from the country (Wu, 1990, 2000, 2001, 2006; Xiong *et al.*, 2009, 2010; Dai, 2011; Lee & Langer, 2012; Yurchenko & Wu, 2013a, b). During investigations on the diversity of polypores in southern China, two additional undescribed species corresponding to *Hyphodontia* s.l. were found. To confirm the affinity of the two new species in the genus, phylogenetic analysis was carried out based on nuclear ribosomal internal transcribed spacer (ITS) regions.

MATERIAL AND METHODS

Morphological studies

The studied specimens were deposited at the herbarium of Beijing Forestry University (BJFC). The microscopic examinations followed Zhao *et al.* (2013). Sections were studied at magnification up to × 1000 using a Nikon Eclipse 80i microscope and phase contrast illumination. Drawings were made with the aid of a drawing tube. Measurements and drawings of microscopic features, were made from slide preparations stained with Cotton Blue and Melzer's reagent. Spores were measured from sections cut from the tubes. In presenting the variation in spore size, 5% of measurements were excluded from each end of the range, and were given in parentheses. In the text the following abbreviations were used: KOH = 5% potassium hydroxide, IKI = Melzer's reagent, IKI- = negative in Melzer's reagent, CB = Cotton Blue, CB- = negative in Cotton Blue, CB+ = cyanophilous, 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 = number of spores measured from given number of specimens. Special color terms follow Petersen (1996).

Molecular procedures and phylogenetic analysis

The fungal taxa used in this study were listed in Table 1. Phire® Plant Direct PCR Kit (Finnzymes, Vantaa, Finland) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions. A small piece of dried fungal specimen was lysed in 30 µl dilution buffer for DNA extraction. After incubating 3 min at room temperature, 0.75 µl of the supernatant were used as template for a 30 µl PCR reaction. The ITS regions were amplified with the primers ITS4 and ITS5 (White *et al.*, 1990). The PCR procedure for ITS was as follows: initial denaturation at 98°C for 5 min, followed by 39 cycles at 98°C for 5 s, 58°C for 5 s and 72°C for 5 s, and a final extension of 72°C for 10 min. DNA sequencing was performed at Beijing Genomics Institute, China, with the same primers. All newly generated sequences were submitted to GenBank (Table 1).

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

Fungal taxon	*Specimen no.*	*GenBank no.*
		ITS
Fibrodontia gossypina Parmasto	GEL 5042	DQ249274
Hyphodontia abieticola (Bourdot & Galzin) J. Erikss.	GEL 2924	DQ340332
H. abieticola	KHL 12498	DQ873601
H. alutacea (Fr.) J. Erikss.	GEL 2284	DQ340340
H. alutacea	GEL 2937	DQ340338
H. alutaria	KHL 11889	DQ873603
H. alutaria	KHL 11978	EU118631
H. arguta (Fr.) J. Erikss.	Wu 0806-44	JN571548
H. aspera (Fr.) J. Erikss.	Nilsson s. n.	DQ873606
H. astrocystidiata Yurchenko & Sheng H. Wu	Wu 9211-71	JN129972
H. barba-jovis (Bull.) J. Erikss.	KHL 11730	DQ873609
H. breviseta (P. Karst.) J. Erikss.	KHL 12386	DQ873612
H. cineracea (Bourdot & Galzin) J. Erikss. & Hjortstam	GEL 4875	DQ340335
H. cineracea	GEL 4958	DQ340336
H. crustosa (Pers.) J. Erikss.	GEL 2325	DQ340313
H. crustosa	GEL 5336	DQ340314
H. crustosa	GEL 5360	DQ340315
H. crustosa	KHL 11731	DQ873614
H. flavipora (Berk. & M.A. Curtis ex Cooke) Sheng H. Wu	FCUG 1053	AF145575
H. flavipora	ICMP 13836	AF145585
H. floccosa (Bourdot & Galzin) J. Erikss.	Berglund 150-02	DQ873618
H. griseliniae (G. Cunn.) Langer	KHL 12971	DQ873651
H. hastate (Litsch.) J. Erikss.	GEL 2143	DQ340323
H. hastata	GEL 3124	DQ340311
H. heterocystidiata H.X. Xiong, Y.C. Dai & Sheng H. Wu	Wu 9209-27	JX175045
H. heterocystidiata	Wu 9312-20	JX175046
H. juniperi (Bourdot & Galzin) J. Erikss. & Hjortstam	GEL 4940	DQ340316
H. juniperi	Wu 0910-95	JX175047
H. microfasciculata Yurchenko & Sheng H. Wu	TNM F24757	JN129976
H. mollis Sheng H. Wu	Wu 0808-32	JX175043
H. nespori (Bres.) J. Erikss. & Hjortstam	GEL 3309	DQ340307
H. nespori	Nordon 030915	DQ873622
H. niemelaei Sheng H. Wu	GEL 4998	EU583422
H. nothofagi (G. Cunn.) Langer	ICMP 13842	AF145583
H. nothofagi	PDD 91630	GQ411524
H. pallidula (Bres.) J. Erikss.	GEL 2097	DQ340317
H. palmae Rick ex Langer	GEL 3456	DQ340333
H. paradoxa (Schrad.) Langer & Vesterh.	FCUG 1517	AF145572
H. paradoxa	FCUG 2425	AF145571
H. paradoxa	Miettinen 7978	FN907912
H. pruni (Lasch) Svrček	Ryberg 021018	DQ873624
H. pseudotropica C.L. Zhao, B.K. Cui & Y.C. Dai	Dai 10758	KF917542[a]
H. pseudotropica	Dai 10768	KF917543[a]
H. radula (Pers.) Langer & Vesterh.	ICMP 13832	AF145581
H. radula	PDD 91616	GQ411525
H. rhizomorpha C.L. Zhao, B.K. Cui & Y.C. Dai	Dai 12354	KF917544[a]
H. rhizomorpha	Dai 12367	KF917545[a]
H. rhizomorpha	Dai 12389	KF917546[a]
H. rimosissima (Peck) Gilb.	Ryberg 021031	DQ873627
H. sambuci (Pers.) J. Erikss.	GEL 3376	DQ340325
H. sambuci	GEL 3400	DQ340326
H. subalutacea (P. Karst.) J. Erikss.	GEL 2196	DQ340341
H. subalutacea	GEL 2142	DQ340334
H. tropica Sheng H. Wu	ICMP 13835	AF145586
H. tropica	ICMP 13837	AF145587
H. verecunda (G. Cunn.) Hjortstam & Ryvarden	KHL 12261	DQ873642
H. vietnamensis Yurchenko & Sheng H. Wu	TNM F 9073	JX175044

[a] Sequences newly generated in this study

Sequences generated for this study were aligned with additional sequences downloaded from GenBank (Table 1) using BioEdit (Hall, 1999) and ClustalX (Thompson *et al.*, 1997). In the study, nuclear ribosomal RNA genes were used to determine the phylogenetic position of the new species. Sequence alignment was deposited at TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S15036?x-access-code=29468f8fea03d88edcaf882e492a2534&format =html).

Phylogenetic analysis followed Li & Cui (2013). Maximum parsimony analysis was applied to the dataset of ITS sequences. Sequence of *Fibrodontia gossypina* Parmasto obtained from GenBank was used as outgroup to root trees following Yurchenko & Wu (2013b). 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 5,000, 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 (MPT) generated.

MrMODELTEST2.3 (Posada & Crandall, 1998; 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 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 3 million generations, 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 for maximum parsimony (MP) and Bayesian posterior probabilities (BPP) greater than or equal to 75% (MP) and 0.95 (BPP) were considered as significantly supported, respectively.

RESULTS

The ITS dataset included sequences from 55 fungal specimens representing 34 taxa. The dataset had an aligned length of 796 characters in the dataset, of which 287 characters are constant, 125 are variable and parsimony-uninformative, and 384 are parsimony-informative. Maximum parsimony analysis yielded 20 equally parsimonious trees (TL = 2209, CI = 0.422, RI = 0.631, RC = 0.267, HI = 0.578). 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 resulted in the same topology with an average standard deviation of split frequencies = 0.007840.

The phylogeny (Fig. 1) inferred from ITS sequences uncovers 34 species of *Hyphodontia* s.l., which demonstrates that sampled specimens of two new species, *H. pseudotropica* and *H. rhizomorpha* spp. nov., form well supported lineages distinct from other species. *Hyphodontia pseudotropica* singly formed a well supported monophyletic lineage distinct from other *Hyphodontia* species. *Hyphodontia rhizomorpha* sistered to *H. niemelaei* Sheng H. Wu with a strong support (100% MP, 1.00 BPP).

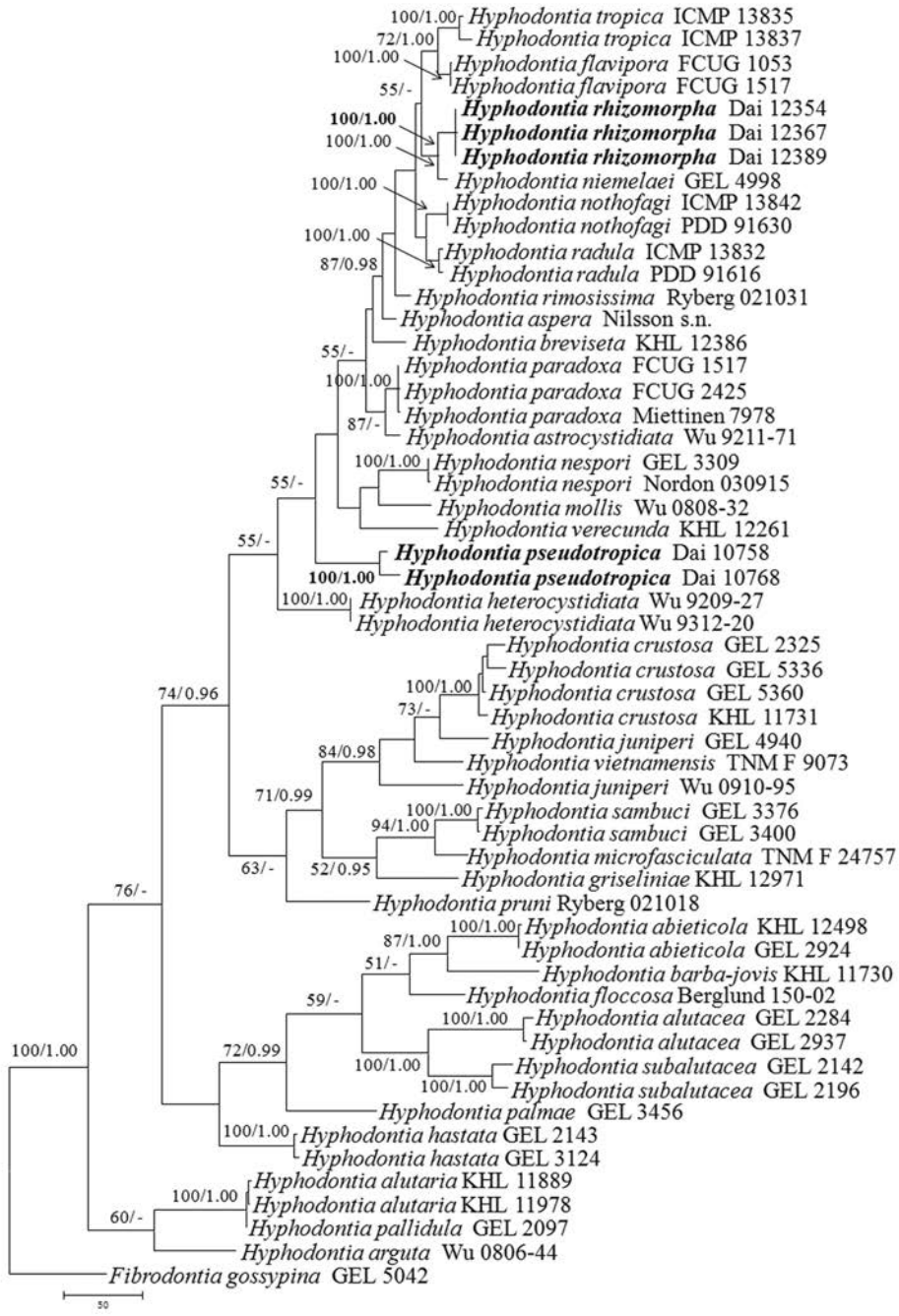

Fig. 1. One of the most parsimonious trees illustrating the phylogeny of the two *Hyphodontia* species, and related species based on ITS sequences. Parsimony bootstrap proportions (before the slash markers) higher than 50% and Bayesian posterior probabilities (after the slash markers) more than 0.95 are indicated along branches.

TAXONOMY

Hyphodontia pseudotropica C.L. Zhao, B.K. Cui & Y.C. Dai, **sp. nov.** Figs 2a, 3

MycoBank no.: MB 808054

Differs from other *Hyphodontia* species by buff to buff-yellow pore surface with angular, smaller pores (6-7 per mm), a monomitic hyphal system with thick-walled generative hyphae, and oblong-ellipsoid basidiospores measuring 4.3-4.9 × 2.8-3 µm.

HOLOTYPUS: **CHINA,** Hainan Province, Changjiang County, Bawangling Nature Reserve, on rotten angiosperm trunk, 8 May 2009, Y.C. Dai, Dai 10768 (BJFC).

Etymology. pseudotropica (Lat.): referring to similarity with *Hyphodontia tropica*.

Basidiocarps annual, resupinate, adnate, soft, without odor or taste when fresh, becoming corky upon drying, up to 18 cm long, 6 cm wide, 1.2 mm thick at centre. Pore surface cream when fresh, becoming buff to buff-yellow upon drying; pores angular, 6-7 per mm; dissepiments thin, entire; sterile margin white to cream, up to 0.5 mm wide. Subiculum cream to buff, up to 0.2 mm thick. Tubes concolorous with pore surface, corky, up to 1 mm long. **Type of rot.** White rot.

Hyphal system monomitic; generative hyphae with clamp connections, IKI-, CB+; tissues unchanged in KOH. **Subiculum** Generative hyphae hyaline, thick-walled, frequently branched, flexuous, interwoven, 3-4 µm in diameter. **Tubes** Generative hyphae hyaline, thick-walled, frequently branched, flexuous, interwoven, 2.5-3.5 µm in diameter. Bladder-like cystidia present, hyaline, thin-walled, 11-13.5 × 4-6 µm; basidia barrel-shaped to pyriform, with four sterigmata and a basal clamp connection, 9-12.5 × 3-5 µm; basidioles dominant, in shape similar to basidia, but slightly smaller. **Basidiospores** oblong-ellipsoid, hyaline, thin-walled, usually bearing one guttule, smooth, IKI-, CB-, (4.1-)4.3-4.9(-5.1) × (2.6-)2.8-3(-3.2) µm, L = 4.61 µm, W = 2.89 µm, Q = 1.61-1.63 (n = 60/2).

Additional specimen examined: *Hyphodontia pseudotropica* — CHINA. Hainan Province, Changjiang County, Bawangling Nature Reserve, on rotten wood of *Symplocos laurina*, 8 May 2009, Y.C. Dai, Dai 10758 (BJFC).

Hyphodontia rhizomorpha C.L. Zhao, B.K. Cui & Y.C. Dai, **sp. nov.** Figs 2b, 4

MycoBank no.: MB 808055

Differs from other *Hyphodontia* species by white poroid hymenophores when juvenile, cracked with age, large pores (1-2 per mm), bearing white rhizomorphs, and a monomitic hyphal system with generative hyphae encrusted with crystals and ellipsoid to broadly ellipsoid basidiospores measuring 4.3-5.5 × 3.7-4.1 µm.

HOLOTYPUS: **CHINA,** Yunnan Province, Puer, Taiyanghe National Forest Park, on rotten wood of *Castanea*, 9 Jun 2011, Y.C. Dai, Dai 12367 (BJFC).

Etymology. rhizomorpha (Lat.): referring to rhizomorphic basidiocarps.

Basidiocarps annual, resupinate, adnate, without odor or taste when fresh, becoming corky upon drying, up to 11 cm long, 7 cm wide, 1 mm thick at centre. Hymenophoral surface white when fresh, cream upon drying; poroid when juvenile, becoming cracked and odontioid with age; pores angular, 1-2 per mm; dissepiments thin, entire when juvenile, but distinctly lacerate to odontioid with

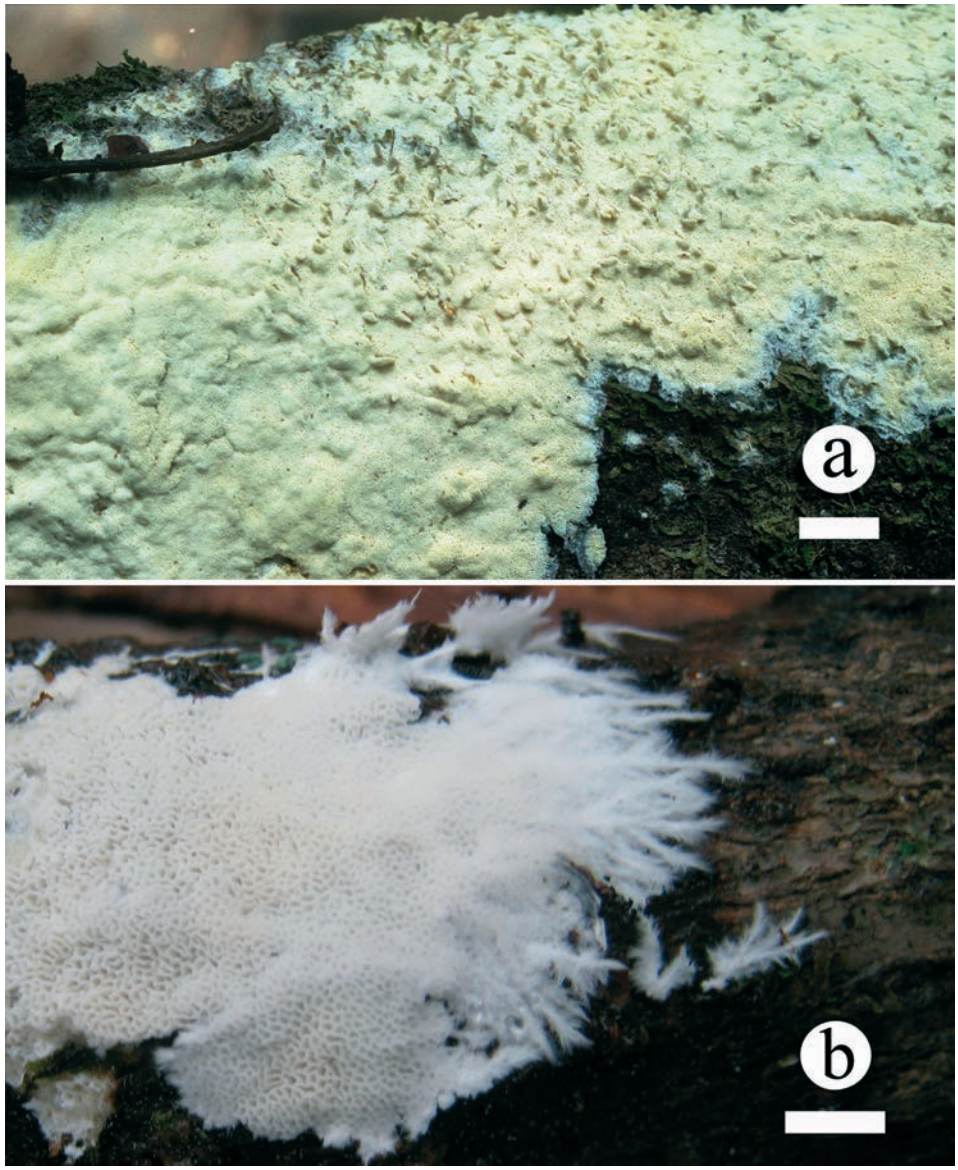

Fig. 2. Basidiocarp of two new *Hyphodontia* species. **A.** *H. pseudotropica*. **B.** *H. rhizomorpha*. *Scale bars* a = 1 cm, b = 0.5 cm.

age; sterile margin white, up to 0.2 mm wide. Subiculum white to cream, up to 0.2 mm thick. Tubes concolorous with pore surface, corky, up to 0.8 mm long. **Type of rot.** White rot.

Hyphal system monomitic; generative hyphae with clamp connections, IKI-, CB+; tissues unchanged in KOH. **Subiculum** Generative hyphae hyaline, thin to thick-walled, frequently branched, interwoven, flexuous, 3.5-5 µm in

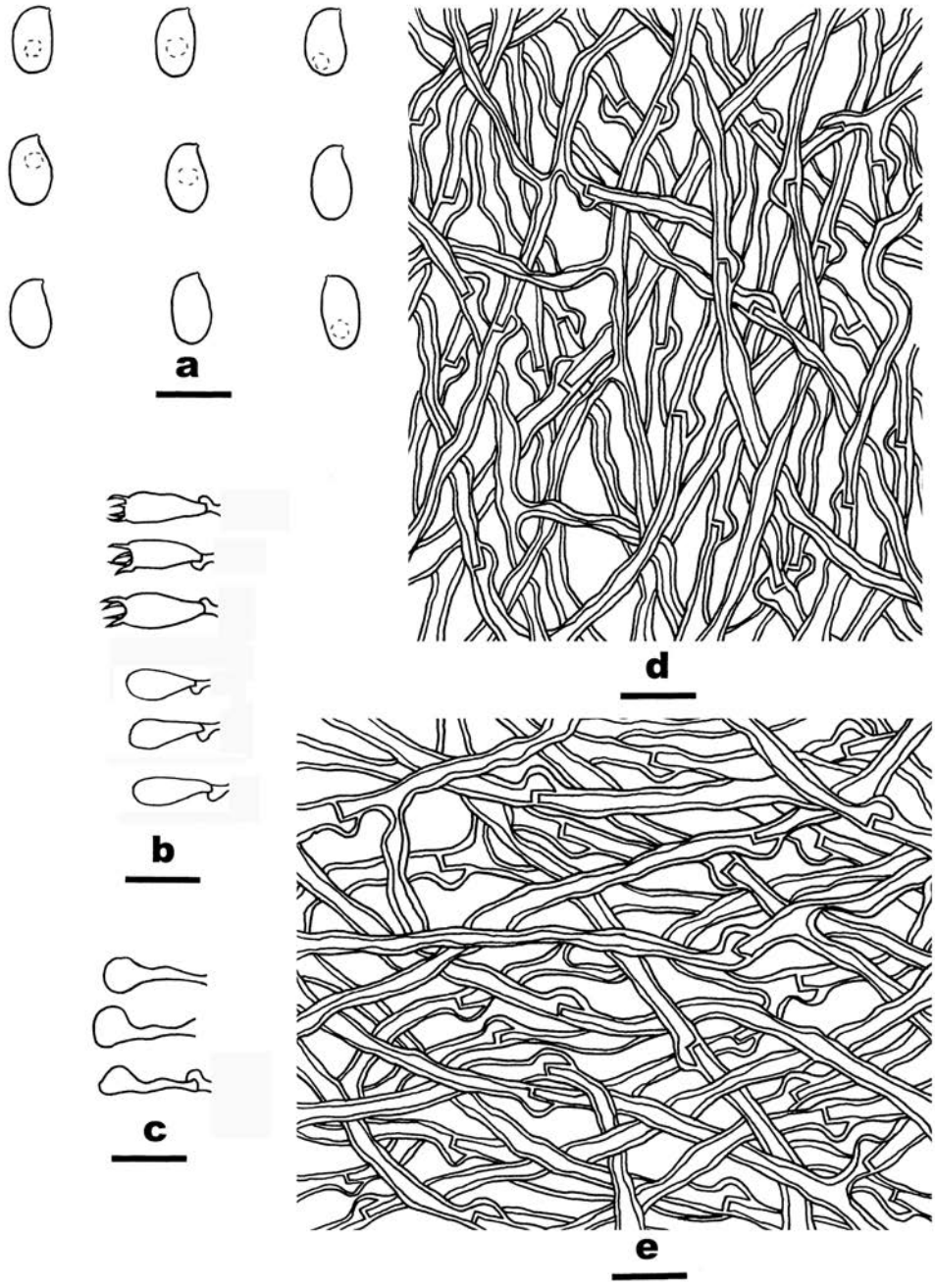

Fig. 3. Microscopic structures of *Hyphodontia pseudotropica* (drawn from the holotype). **A.** Basidiospores. **B.** Basidia and basidioles. **C.** Bladder-like cystidia. **D.** Hyphae from trama. **E.** Hyphae from subiculum. *Bars*: A 5 μm; B-F 10 μm.

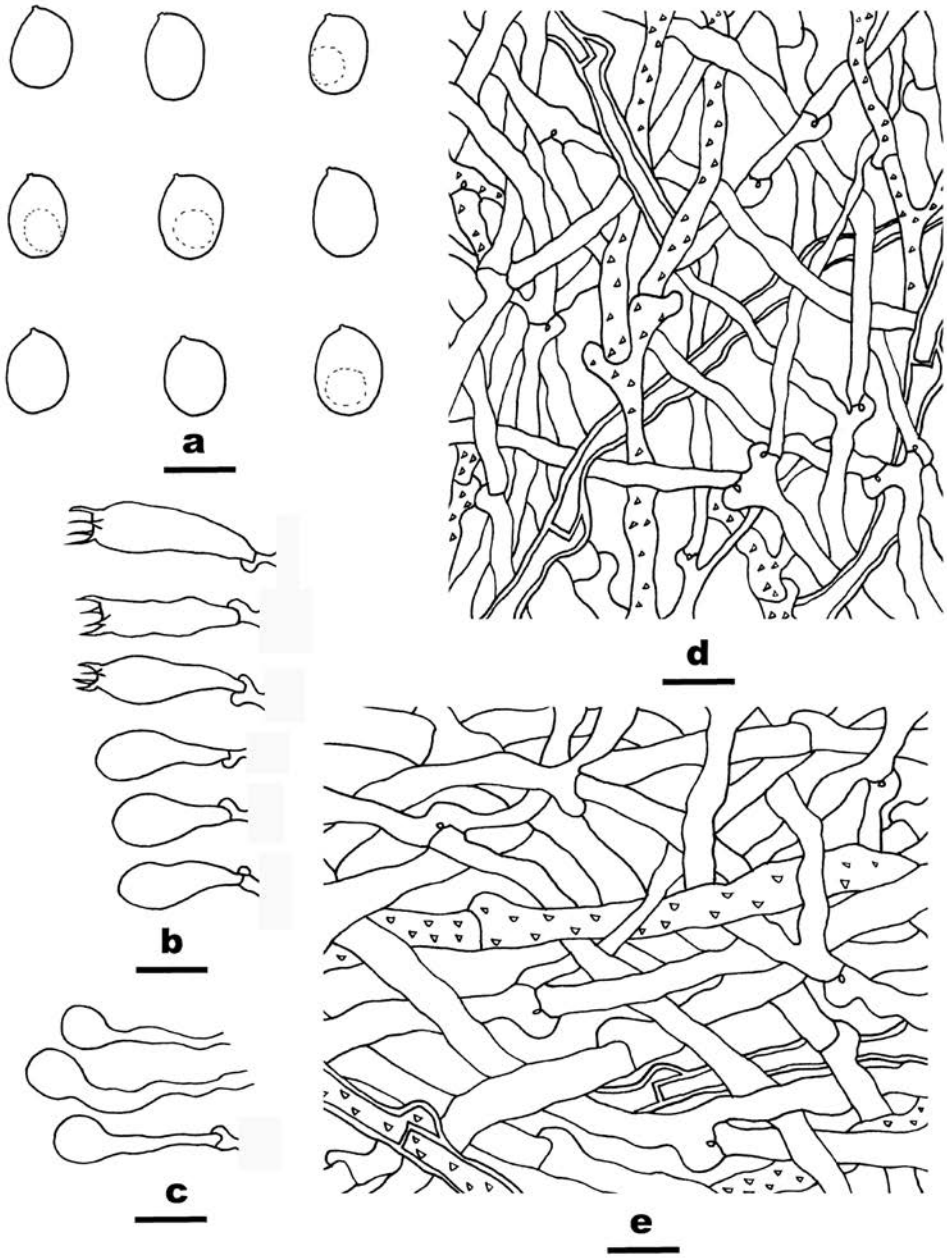

Fig. 4. Microscopic structures of *Hyphodontia rhizomorpha* (drawn from the holotype). **A.** Basidiospores. **B.** Basidia and basidioles. **C.** Bladder-like cystidia. **D.** Hyphae from trama. **E.** Hyphae from subiculum. *Bars*: A 5 μm; B-F 10 μm.

diameter, occasionally encrusted with fine crystals. **Tubes** Generative hyphae hyaline, thin to thick-walled, frequently branched, flexuous, interwoven, 3-4 μm in diameter, occasionally encrusted with fine crystals. Bladder-like cystidia present, hyaline, thin-walled, 20-27 × 6-7 μm; basidia clavate to pyriform, with four sterigmata and a basal clamp connection, 18-23 × 5-7 μm; basidioles dominant, pyriform, slightly smaller than basidia. **Basidiospores** ellipsoid to broadly ellipsoid, hyaline, thin-walled, usually bearing one guttule, smooth, IKI-, CB-, (4.1-)4.3-5.5(-5.9) × (3.5-)3.7-4.1(-4.3) μm, L = 4.94 μm, W = 3.9 μm, Q = 1.26-1.27 (n = 90/3).

Additional specimens examined: *Hyphodontia rhizomorpha* — **CHINA**. Yunnan Province, Puer, Taiyanghe National Forest Park, on fallen trunk of *Castanea*, 9 Jun 2011, Y.C. Dai, Dai 12354 & 12389 (BJFC).

DISCUSSION

In the present study, two new *Hyphodontia* species, *H. pseudotropica* and *H. rhizomorpha*, are described based on morphological differences and molecular phylogenetic analyses. Molecular study based on sequence data from the ribosomal ITS regions (Fig.) confirmed the generic placement of the two new species in *Hyphodontia* s.l., and they formed a monophyletic lineage with strong support (100 % MP, 1.00BPP).

Phylogenetically, *Hyphodontia pseudotropica* singly formed a well-supported monophyletic lineage distinct from other *Hyphodontia* species. *Hyphodontia tropica* Sheng H. Wu may be confused with *H. pseudotropica* in producing a buff to buff-yellow pore surface with pores of similar size (6-8 per mm); however, it differs from *H. pseudotropica* in having smaller basidiospores (3.7-4.3 × 2.8-3.3 μm, Wu, 2000). In addition, these two species are distant from each other in the ITS rDNA-based phylogeny (Fig. 1). *Hyphodontia flavipora* (Berk. & M.A. Curtis ex Cooke) Sheng H. Wu is similar to *H. pseudotropica* by buff pore surface and presence of bladder-like cystidia, but it is distinguished by larger pores (3-6 per mm) and wider basidiospores (4-5.1 × 2.9-3.7 μm, Wu, 2000). *Hyphodontia poroideoefibulata* Sheng H. Wu is similar to *H. pseudotropica* by having poroid hymenophore, thick-walled generative hyphae, but it differs in having simple-septate generative hyphae (Wu, 2001).

Hyphodontia rhizomorpha sisters to *H. niemelaei* in the phylogeny with strong supports (100% BP, 1.00 BPP, Fig. 1); but morphologically, *H. niemelaei* differs from *H. rhizomorpha* by its small pores (2-4 per mm), and large basidiospores (5.2-6.2 × 3.3-4 μm, Wu, 1990). *Hyphodontia astrocystidiata* Yurchenko & Sheng H. Wu and *H. rhizomorpha* share a monomitic hyphal system with thin to thick-walled, encrusted generative hyphae, and presence of bladder-like cystidia, but *H. astrocystidiata* differs in producing consistently odontioid hymenophores and larger basidiospores (5-6 × 3.7-4.2 μm, Yurchenko & Wu, 2013b). *Hyphodontia sinensis* H.X. Xiong, Y.C. Dai & Sheng H. Wu is similar to *H. rhizomorpha* by producing a monomitic hyphal system with branched generative hyphae, and similar basidiospores (4-6 × 3-4 μm); however, *H. sinensis* differs in having odontioid hymenophores, leptocystidia and thick-walled basidiospores (Xiong *et al.*, 2010). *Hyphodontia syringae* Langer is similar to *H. rhizomorpha* by having white to cream pore surface and larger pores (1-2 per mm), but differs by its larger basidiospores (8-9 × 3-3.5 μm, Langer & Dai, 1998).

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