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ORIGINAL ARTICLE

Polyporus hapalopus sp. nov. (Polyporales, Basidiomycota) from China based on morphological and molecular data

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Abstract *Polyporus* sensu lato is understood to be a polyphyletic genus, including two narrowly defined genera (*Favolus* and *Neofavolus*), two subclades (Melanopus and Polyporellus), and some scattered species. In this study, *Polyporus hapalopus* is described and illustrated as a new species on the basis of morphological and molecular evidence. This species is characterized by laterally stipitate, imbricate and large basidiocarps (up to 40 cm diam.), grapefruit odor when fresh, angular pores with lacerate dissepiments, soft (when fresh) to tough (when dry) context, a dimitic hyphal system in context and stipe with variable wide skeleto-binding hyphae, a monomitic hyphal system in trama, and cylindrical basidiospores bearing one or two guttules. Phylogenetically, on the basis of the combined internal transcribed spacer and nuclear large subunit rDNA sequences, *P. hapalopus* is nested within the *Polyporus* sensu lato clade, but is separated from four previously established subclades and from other known species.

Keywords Polyporaceae · Polypore · *Polyporus* sensu lato · Taxonomy · Wood-decaying fungi

Introduction

Polyporus P. Micheli ex Adans. is a widespread polypore genus. It includes species with stipitate to substipitate basidiocarps, a dimitic hyphal system, thick-walled and arboriform skeleto-binding hyphae, thin-walled, smooth and cylindrical to subellipsoid basidiospores with negative reaction in Melzer's reagent, and causing a white rot (Gilbertson and Ryvarden 1987; Núñez and Ryvarden 1995).

Núñez and Ryvarden (1995) treated 32 species of *Polyporus* worldwide, arranged into six morphological groups. Subsequently, several *Polyporus* species have been reinstated (Zheng and Liu 2005), newly described (Buchanan and Ryvarden 1998; Dai et al. 2003, 2007d, 2009; Sotome et al. 2007; Xue and Zhou 2012) and emended (Dai 2012a). With the aid of molecular phylogeny from various genetic regions, *Polyporus* has been consistently characterized as a polyphyletic genus (Ko and Jung 2002; Krüger et al. 2006; Sotome et al. 2008). Recently, from both morphological and phylogenetic perspectives, Sotome et al. (2013) studied the taxonomy of morphological group Favolus as circumscribed in Núñez and Ryvarden (1995), and placed the species of this group in two genera *Favolus* Fr. and *Neofavolus* Sotome & T. Hatt., both belonging to *Polyporus* sensu lato. Meanwhile, Dai et al. (2014) recovered four clades (*Favolus*, *Neofavolus*, Melanopus and Polyporellus) and some scattered species in *Polyporus* sensu lato.

China has a high diversity of wood-decay fungi (Dai 2011, 2012b). Species of *Polyporus* have been repeatedly collected and documented from different regions (Dai et al. 2003, 2004, 2007a, b, c, d, 2009; Dai and Penttilä 2006; Cui et al. 2008; Li et al. 2008; Yuan and Dai 2008; Wang et al. 2011), and, to date, a total of 37 species of *Polyporus* have been recorded in China (Dai 2012b; Xue and Zhou 2012; Dai et al. 2014).

During a field trip in the Guangxi Autonomous Region of southern China in 2011, an unknown *Polyporus* specimen of giant size was collected. It was identified as a new species according to both morphological and phylogenetic analyses. We describe and illustrate it as *Polyporus hapalopus* in the present paper.

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Materials and methods

Morphological study

The studied specimen, Yuan 5809, is deposited in the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). The microscopic procedure follows Dai (2010). In this text, CB stands for Cotton Blue, CB+ for cyanophilous, CB– for acyanophilous, IKI for Melzer's reagent, IKI– for negative reaction in Melzer's reagent, and KOH for 5 % potassium hydroxide. The section was studied at magnification up to 1,000× using a Nikon Eclipse 80i microscope and phase contrast illumination. Measurements are made from sections stained with CB. In describing the variation in size of basidiospores, 5 % of measurements were excluded from each end of the range and given in parentheses. The meanings of abbreviations are as follows: L = mean basidiospore length (arithmetic average of all basidiospores), W = mean basidiospore width (arithmetic average of all basidiospores), Q = the ratio of L/W, and n = number of basidiospores measured from given number of specimens. Drawings are made with the aid of a drawing tube. Special color terms follow Petersen (1996).

Molecular procedures and phylogenetic analysis

Internal transcribed spacer (ITS) and nuclear large subunit rDNA (nLSU) sequences were obtained from the herbarium specimen using Phire® Plant Direct PCR Kit (Finnzymes Oy, Finland). The PCR procedure was conducted following Zhou and Xue (2012). For the genomic DNA extraction, a small piece of specimen Yuan 5809 was incubated in 20 μl Dilution Buffer for at least 3 min at room temperature. Then, 0.9 μl of the supernatant was used as template for a 30 μl PCR reaction. The ITS region was amplified and sequenced with the primer pair ITS5 and ITS4 (White et al. 1990), while nLSU sequence was amplified with the primers LR0R and LR7, and sequenced with the primers LR0R, LR3R, LR3, and LR7 (http://biology.duke.edu/fungi/mycolab/primers.htm). DNA sequencing was performed at Beijing Genomics Institute, China.

The newly generated sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov; Table 1). Other ITS and nLSU sequences for phylogenetic analysis (Table 1) were mainly adapted from Krüger et al. (2006), Sotome et al. (2009, 2011, 2013) and Dai et al. (2014). *Pycnoporus cinnabarinus* (Jacq.) P. Karst. and *Trametes orientalis* (Yasuda) Imazeki were selected as outgroup (Dai et al. 2014; Sotome et al.

Table 1 Data of ITS and nLSU sequences used in the phylogenetic analysis

Species	Voucher No.	Country of Origin	GenBank No.	
			ITS	LSU
Datronia mollis (Sommerf.) Donk	WD 794	Japan	AB587623	AB368063
Favolus acervatus (Lloyd) Sotome & T. Hatt.	WD 2373	Japan	AB735973	AB368091
F. spathulatus (Jungh.) Lév.	WD 1576	Japan	AB587633	AB587622
Neofavolus alveolaris (DC.) Sotome & T. Hatt.	TUMH 50002	Japan	AB735969	AB735947
N. cremeoalbidus Sotome & T. Hatt.	TUMH 50006	Japan	AB735979	AB735956
Polyporus arcularius (Batsch) Fr.	Dai 8159	China	KC572005	KC572044
P. badius (Pers.) Schwein.	Dai 10112	China	KC572008	KC572047
P. brumalis (Pers.) Fr.	Wei 2948	China	KC572021	KC572058
P. dictyopus Mont.	TENN 59385	Belize	AF516561	AJ487945
P. guianensis Mont.	TENN 58404	Venezuela	AF516566	AJ487948
P. guianensis	TENN 59093	Argentina	AF516564	AJ487947
P. hapalopus H.J. Xue & L.W. Zhou	Yuan 5809	China	KC297219	KC297220
P. leprieurii Mont.	TENN 58597	Costa Rica	AF516567	
P. melanopus (Pers.) Fr.	H 6003449	Finland	JQ964422	KC572064
P. squamosus (Huds.) Fr.	MUCL 30721	Belgium	AB587630	AB368094
P. subvarius C.J. Yu & Y.C. Dai	Yu 2	China	AB587632	AB587621
P. subvarius	WD 1872	Japan	AB587641	AB368092
P. tuberaster (Jacq.) Fr.	Dai 4662	China	KC572037	KC572074
P. tuberaster	DAOM 7997B	Italy	AY218420	AF393070
P. varius (Pers.) Fr.	Dai 12799	USA	KC572039	KC572076
P. varius	WD 2347	Japan	AB587636	AB368111
Outgroup				
Pycnoporus cinnabarinus (Jacq.) P. Karst.	WD 741	Japan	AB735965	AB735945
Trametes orientalis (Yasuda) Imazeki	WD 1660	Japan	AB735966	AB735946

2013). Following Dai et al. (2014), ITS and nLSU sequences were combined for phylogenetic analysis. The combined sequences were aligned by ClustalX 2.0 (Larkin et al. 2007) with default parameters. The resulting alignment was deposited in TreeBASE (http://www.treebase.org; accession number S14664). The best-fit evolutionary model was estimated by jModelTest (Guindon & Gascuel 2003; Posada 2008) based on corrected Akaike information criterion. Maximum likelihood (ML) analysis and Bayesian inference (BI) were performed using raxmlGUI 1.2 (Stamatakis 2006; Silvestro and Michalak 2012) and MrBayes 3.2 (Ronquist et al. 2012), respectively. The ML tree was constructed with the GTR model and auto FC option (Pattengale et al. 2010) in bootstrap (BS) replicates. For BI, two independent runs were launched. Each run performed a Metropolis-coupled Markov chain Monte Carlo analysis with four chains for 500,000 generations. Trees were saved every 1,000th generation. Chain convergence was determined using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/). The value of burn-in was set to discard the first 25 % trees, and the remaining trees were used to compute a 50 % consensus tree and for calculating Bayesian posterior probabilities (BPPs).

Results

Molecular phylogeny

The combined ITS and nLSU dataset, resulting in an alignment with 1,492 characters, includes 23 ITS and 22 nLSU sequences from 23 collections representing 19 species. The best-fit evolutionary model of this alignment for BI was estimated as HKY. The ML search stopped after 200 BS replicates. All chains for BI converged after 500,000 generations, which was indicated by the effective sample sizes of all

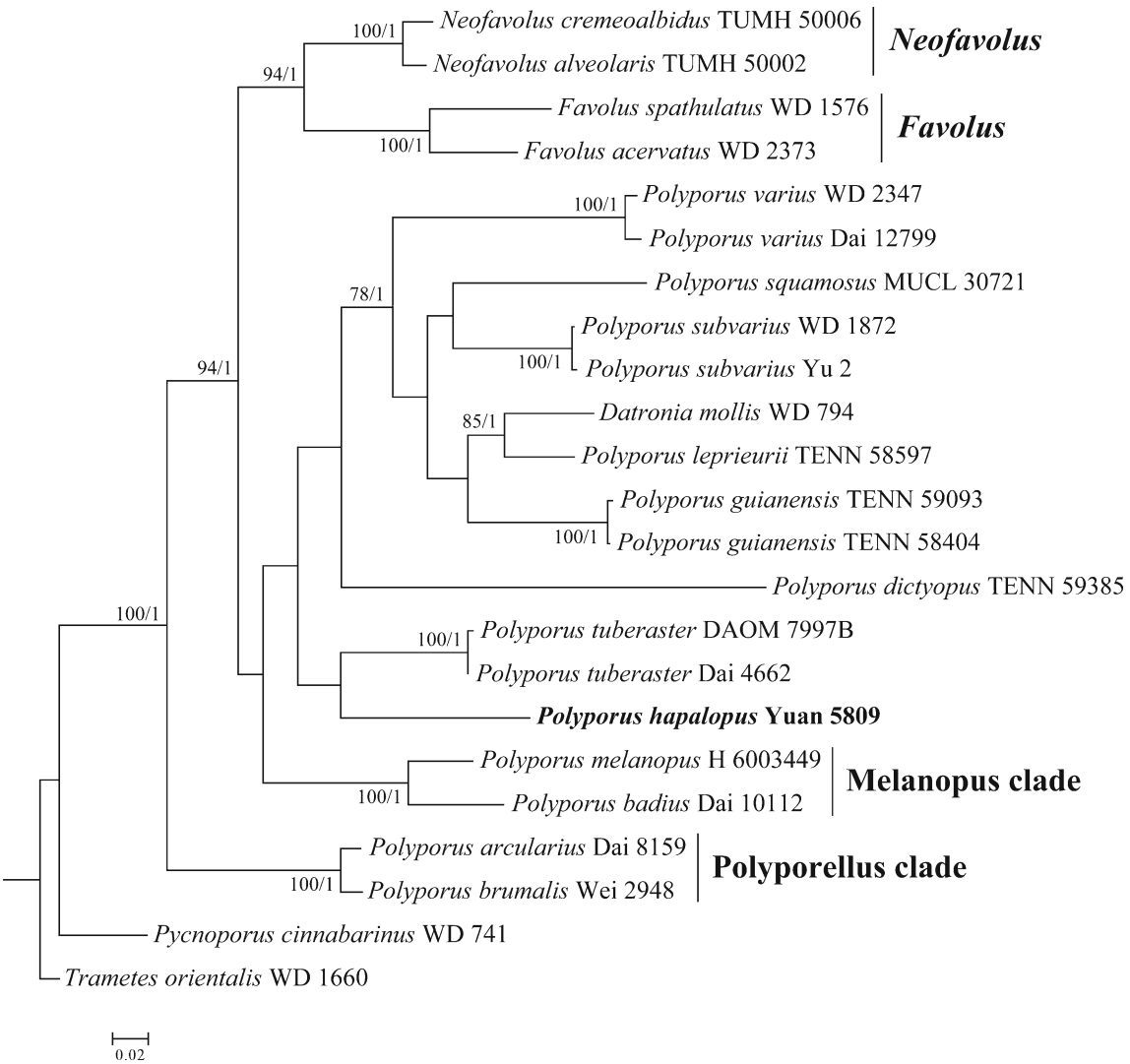

Fig. 1 The phylogenetic tree inferred from the combined ITS and nLSU sequences. Topological structure is from maximum likelihood analysis. Statistical values exceeding 50 % for bootstrap values and 0.95 for Bayesian posterior probabilities are indicated. The new species is in bold-face

parameters above 250 and the potential scale reduction factors close to 1.000. ML and BI analyses generated nearly congruent topologies, so both BS values and BPPs were shown at the nodes of the topology from ML (Fig. 1).

The current phylogeny (Fig. 1) is similar to that in Dai et al. (2014). Four subclades, *Favolus*, *Neofavolus*, Melanopus and Polyporellus, were recovered within the *Polyporus* sensu lato clade. The newly sequenced specimen, Yuan 5809, was nested within the *Polyporus* sensu lato clade, and formed a single branch separate from other sampled species and the four subclades.

Taxonomy

Polyporus hapalopus H.J. Xue & L.W. Zhou, sp. nov. (Figs. 2, 3)

MycoBank no.: MB 802658

Basidiocarps annual, laterally stipitate, imbricate. Pileus fan-shaped, up to 40 cm diam. Pileal surface cinnamon-buff to clay-buff, with more or less radial stripes. Pore surface white to cream; pores angular, 4–6 per mm. Stipe concolorous with the pileal surface. Hyphal system dimitic; generative hyphae with clamp connections. Basidiospores cylindrical, hyaline, IKI–, CB–, 6.1–6.9×2.2–2.4 μm.

Type. China, Guangxi Autonomous Region, Laibin, Jinxiu County, Dayaoshan Nature Reserve, on fallen angiosperm trunk, 23.VIII.2011, Yuan 5809 (Holotype in IFP 019116).

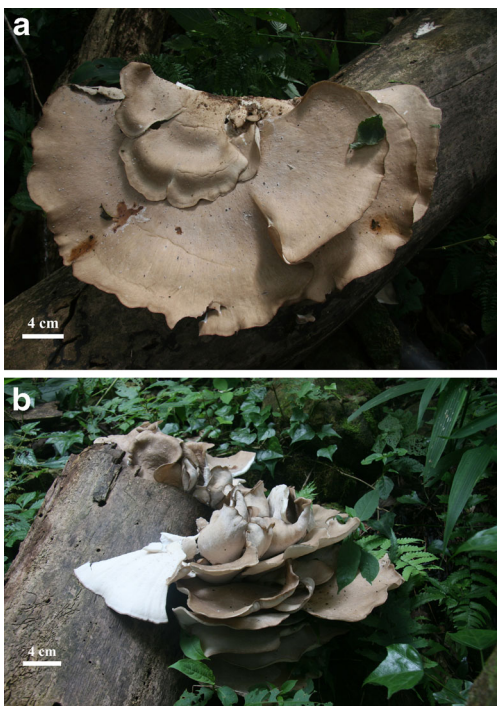

Fig. 2 Basidiocarps of *Polyporus hapalopus* (Holotype). **a** Vertically showing the basidiocarps. **b** Laterally showing the basidiocarps and pore surface

Etymology: *hapalopus* (Lat.): referring to the soft context when fresh.

Fruitbody. Basidiocarps annual, laterally stipitate, imbricate, fleshy and with grapefruit odor when fresh, becoming corky and light in weight upon drying. Pileus fan-shaped, up to 40 cm diam. when fresh. Pileal surface cinnamon-buff to clay-buff, bearing a cuticle, glabrous, with more or less radial stripes, wrinkled when dry; margin sharp. Pore surface white to cream when fresh, becoming cinnamon-buff to honey-yellow upon drying; pores angular, 4–6 per mm; dissepiments thin, lacerate. Context cream to straw-yellow, soft (when fresh) to tough (when dry), up to 4 mm thick. Tubes less than 1 mm long, decurrent along one side of the stipe, similar to pore surface in color. Stipe concolorous with the pileal surface, glabrous, corky, up to 2 cm long, up to 2.5 cm diam., bearing a concolorous cuticle on one side of the stipe.

Hyphal structure. Hyphal system dimitic in context and stipe, monomitic in trama; generative hyphae with clamp connections; skeleto-binding hyphae frequently branched, with straight proximal and central parts and tapering ends, rarely with arboriform branches, IKI–, CB+; tissue unchanged in KOH.

Context. Generative hyphae infrequent, hyaline, thin-walled, 4–5 μm diam.; skeleto-binding hyphae dominant, thick-walled with a distinct wide lumen, loosely interwoven, 1.5–7.5 μm diam. Hyphae in cuticle of pileal surface parallel; generative hyphae thin-walled to slightly thick-walled, sometimes branched, 2.5–9 μm diam.; skeleto-binding hyphae thick-walled with a distinct wide lumen, 3–8 μm diam.

Tubes. Generative hyphae hyaline, thin-walled, scarcely branched, subparallel along tubes, 2.6–4 μm diam. Cystidia and cystidioles absent; basidia clavate, with a basal clamp and four sterigmata, 15–17×5.5–6.5 μm; basidioles similar to basidia, but smaller.

Stipe. Hyphal structure similar to context; skeleto-binding hyphae dominant, thick-walled with a distinct wide lumen, 1.5–6 μm diam. Hyphae in cuticle gelatinized, interwoven; generative hyphae frequent, thin-walled, 2–4 μm diam.; skeleto-binding hyphae dominant, thick-walled, 1.5–6 μm diam.

Spores. Basidiospores cylindrical, hyaline, thin-walled, smooth, bearing one or two guttules, IKI–, CB–, (5.5–)6.1–6.9(–7.6)×(2–)2.2–2.4(–2.7) μm, L=6.32 μm, W=2.29 μm, Q=2.76 (n=30/1).

Discussion

Polyporus hapalopus is characterized by its laterally stipitate, imbricate and large basidiocarps (up to 40 cm diam.) with grapefruit odor when fresh, angular pores, lacerate dissepiments, soft (when fresh) to tough (when dry) context, a dimitic hyphal system in context and stipe with variable wide skeleto-binding hyphae, a monomitic hyphal system in trama, and

cylindrical basidiospores with one or two guttules. Its laterally stipitate basidiocarps, frequently branched skeleto-binding hyphae, cylindrical and IKI– basidiospores, and causing a white rot are features that fit the generic concept of *Polyporus*.

Polyporus hapalopus and *P. udus* Jungh. are both distributed in pantropical to warm-temperate areas (Núñez and Ryvarden 1995). *Polyporus udus*, belongs to the morphological group Polyporus, resembles *P. hapalopus* in sharing glabrous pilei and when dry a wrinkled pileal surface, angular pores, and generative hyphae dominant (or exclusive) in trama, but *P. udus* has much larger pores (1–2 per mm), smooth and grayish-brown cuticle with pink tints when fresh, and cylindrical to broadly ellipsoid basidiospores (10–15×4–6 μm, Núñez and Ryvarden 1995).

In the ITS plus nLSU based phylogenetic analysis (Fig. 1), *Polyporus hapalopus* nested within the *Polyporus* sensu lato clade separated from the subclade Melanopus and the genera *Favolus* and *Neofavolus*. *Polyporus tuberaster* (Jacq.) Fr., the

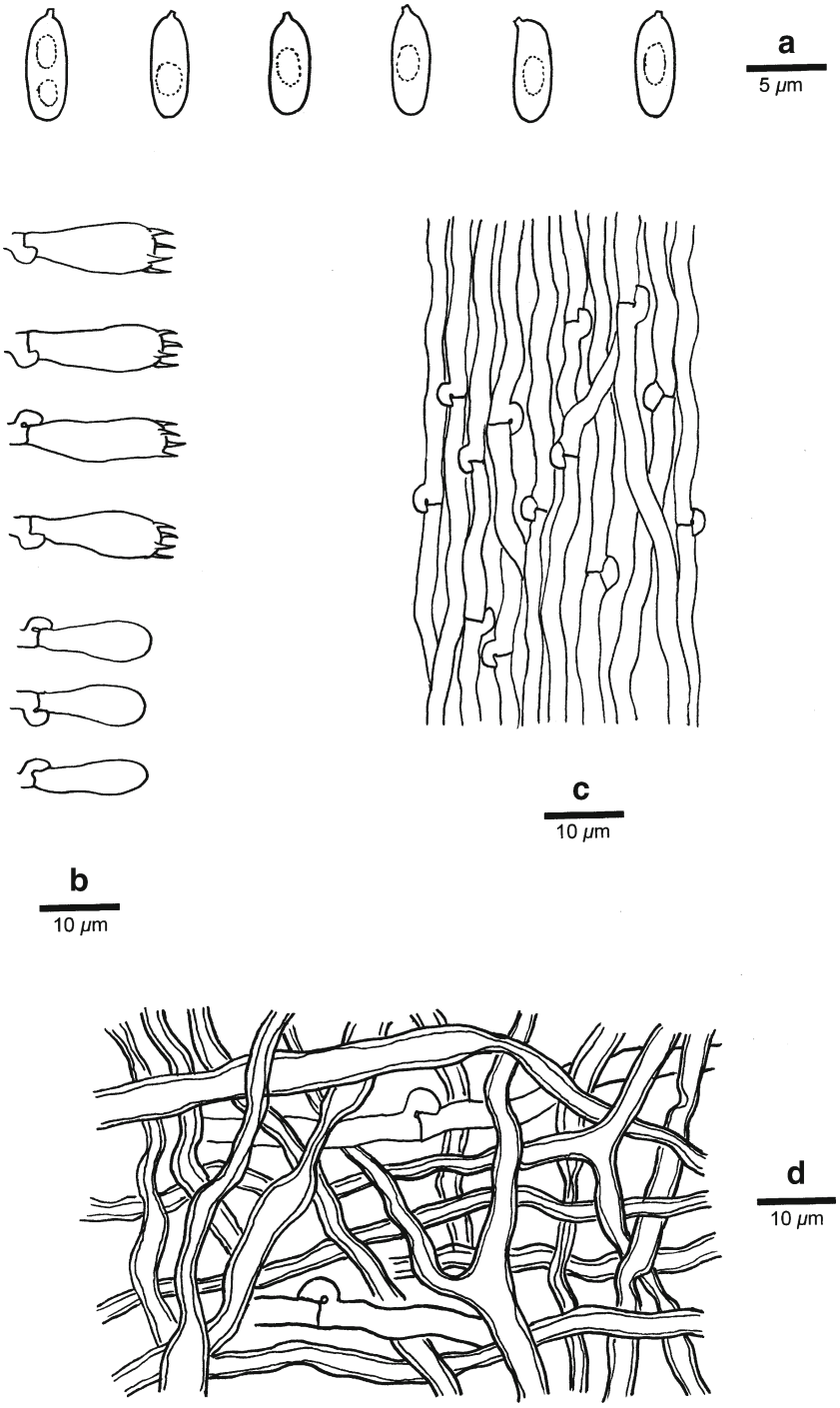

Fig. 3 Microscopic structures of *Polyporus hapalopus* (Drawn from holotype). **a** Basidiospores. **b** Basidia and basidioles. **c** Hyphae from trama. **d** Hyphae from context

generic type of *Polyporus*, seems close to *P. hapalopus* in phylogeny but without reliable statistical supports; however, its larger pores (< 2 per mm) and basidiospores (> 10 μm long) distinguish it from *P. hapalopus* (Núñez and Ryvarden 1995). In morphology, species within the subclade Melanopus often bear coriaceous basidiocarps and black cuticle on the stipe, which differs from *Polyporus hapalopus* (Dai et al. 2014). *Neofavolus* is characterized by the solitary basidiocarp (Sotome et al. 2013), which makes it different from *P. hapalopus* with imbricate basidiocarps. In addition, *P. hapalopus* has a radially striate pileal surface. *Favolus* also has a pileal surface with radial striate, but differs from *P. hapalopus* in its indistinct pileal cuticle (Sotome et al. 2013). In addition, *P. hapalopus* has a monomitic hyphal system in the trama, while *Favolus* and *Neofavolus* have a dimitic hyphal system in both context and trama (Sotome et al. 2013). Therefore, based on the evidence from morphological and phylogenetic perspectives, *Polyporus hapalopus* is a new member of the genus *Polyporus*, but its phylogenetic relationship with other species of *Polyporus* sensu lato is not resolved.

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