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Polyporus submelanopus sp. nov. (*Polyporales, Basidiomycota*) from Northwest China

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Abstract — *Polyporus submelanopus*, collected from Qinghai Province, Northwest China, is described and illustrated based on both morphological and phylogenetic analyses. It is characterized by a terrestrial habit, black-stipitate basidiocarps, circular to angular pores, two kinds of generative hyphae bearing simple septa and clamp connections respectively, and cylindrical smooth hyaline basidiospores. Morphologically, *P. submelanopus* belongs to the melanopus group and resembles *P. melanopus* in sharing similar pilei with a shallow central depression, circular to angular pores, basidiospores, and a terrestrial habit. However, *P. melanopus* has smaller pores, thicker and entire dissepiment, fusoid cystidioles, and clamped generative hyphae only. The differences between *P. submelanopus* and *P. badius, P. xinjiangensis,* and *P. tubaeformis* are discussed as well. Phylogenetic ITS sequence analysis supports *P. submelanopus* within the melanopus group but distinctly separate from other sampled species of this group.

Key words —*Polyporaceae,* polypore, taxonomy, wood-inhabiting fungi

Introduction

Polyporus P. Micheli ex Adans., type genus of the *Polyporaceae,* is a well-known polypore genus. It is characterized by typical formation of stipitate to substipitate basidiocarps, a dimitic hyphal system with arboriform skeleto-binding hyphae, smooth cylindrical to subellipsoid inamyloid basidiospores, and causing a white rot (Gilbertson & Ryvarden 1987, Núñez & Ryvarden 1995).

Núñez & Ryvarden (1995) divided all 32 species of *Polyporus* into six morphological groups without taxonomic rank: Admirabilis, Dendropolyporus [= *Dendropolyporus* (Pouzar) Jülich], Favolus (= *Favolus* Fr.), Polyporellus (= *Polyporellus* P. Karst.), Melanopus (= *Melanopus* Pat.), and Polyporus. The melanopus group is characterized by coriaceous and black-stipitate

basidiocarps, skeleto-binding hyphae mostly with narrow lumen but solid when mature, medium to large basidiospores (6–12 × 2–4 μm), and growth on dead woods except for the terrestrial *Polyporus melanopus* (Pers.) Fr. Since Núñez & Ryvarden (1995), some species have been newly described and/or emended based on morphology (Dai et al. 2003, 2007c, 2009; Sotome et al. 2007; Dai 2012a) or combined morphological and phylogenetic evidence (Sotome et al. 2011).

China is a vast area with varied landscapes and forests inhabited by a high diversity of wood-decaying fungi. More than 1,200 poroid, corticioid, and hydnoid taxa have been recorded in China (Dai 2011, 2012b). *Polyporus* species have been repeatedly documented from different parts of the country (Dai et al. 2003, 2004, 2007a,b,c,d, 2009; Dai & Penttilä 2006; Cui et al. 2008; Li et al. 2008; Yuan & Dai 2008; Wang et al. 2011), with 36 total species recorded before this study (Dai 2012b). However, many specimens deposited at the herbarium of Institute of Applied Ecology, Chinese Academy of Sciences (IFP), are still not identified to species level. During the re-examination of these specimens, we found two that match the *Polyporus* concept but were distinct from any known species based on both morphological and phylogenetic analyses. They are described and illustrated here as *Polyporus submelanopus*.

Materials & methods

The studied specimens are deposited at IFP, China, and Botanical Museum, Finnish Museum of Natural History (H), Helsinki. The microscopic procedure follows Dai (2010). Sections were studied at magnifications up to ×1000 using a Nikon Eclipse 80i microscope and phase contrast illumination. Measurements are made from sections stained with Cotton Blue. In this text, CB stands for Cotton Blue, CB+ for cyanophilous, CB– for acyanophilous, IKI for Melzer's reagent, IKI– for negative in Melzer's reagent, and KOH for 5% potassium hydroxide. In presenting the variation in the size of the basidiospores, 5% of measurements were excluded from each end of the range and given in parentheses. The meanings of other abbreviations are as follows: L = mean basidiospore length (arithmetic average of all basidiospores), W = mean basidiospore width (arithmetic average of all basidiospores), Q = variation in the L/W ratios between the specimens studied, 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 Anonymous (1969) and Petersen (1996).

ITS sequences were obtained from herbarium specimens using Phire® Plant Direct PCR Kit (Finnzymes Oy, Finland). The primers ITS5 and ITS4 (White et al. 1990) were used to perform PCR amplifications and subsequent sequencing reactions. Four newly generated sequences were deposited at GenBank (accession numbers JQ964422–JQ964425). Other ITS sequences were downloaded from GenBank (Table 1). *Polyporus grammocephalus* Berk. and *P. pseudobetulinus* (Murashk. ex Pilát) Thorn et al. were selected as outgroup (Krüger et al. 2006). The procedure of phylogenetic analysis was as below. Firstly, nucleotide sequences were aligned using ClustalX 2.0 (Larkin et al. 2007)

Table 1. ITS sequences from *Polyporus* used in the phylogenetic analysis

Species	Voucher No.[a]	Origin	GenBank No.
P. badius	–	Japan	AB587625
	–	USA	AF516558
	–	USA	AF516559
P. guianensis Mont.	–	Argentina	AF516564
	–	Venezuela	AF516566
P. leprieurii Mont.	–	Costa Rica	AF516567
P. melanopus	–	Argentina	AF516568
	–	Argentina	AF516569
	–	Austria	AF518759
	H 6003449	Finland	JQ964422
	H 6029190	Finland	JQ964423
P. mikawai Lloyd	–	China	AF516570
P. submelanopus	IFP 004953	China	JQ964424
— (holotype)	IFP 004946	China	JQ964425
P. tubaeformis	–	UK	AF511438
	–	UK	AF511439
	–	USA	AF511440
	–	USA	AF511441
	–	USA	AF511442
	–	Norway	AF511443
P. varius (Pers.) Fr.	–	Germany	AF516574
	–	Germany	AF516575
	–	Russia	AF516576
	–	USA	AF516577
	–	USA	AF516578
	–	USA	AF516579
	–	USA	AF516580
	–	Japan	AB587636
P. grammocephalus	–	Japan	AB587626
P. pseudobetulinus	–	Japan	AB587644

[a] Provided only for specimens newly sequenced in this study.

with defaulted parameters; secondly, the best-fit evolutionary models for maximum likelihood (ML) analysis and Bayesian analysis were estimated by jModelTest (Guindon & Gascuel 2003, Posada 2008) based on corrected Akaike information criterion; finally, following the optimal models, maximum likelihood (ML) and Bayesian trees were constructed using PhyML 3.0 (Guindon & Gascuel 2003) and Mrbayes3.2 (Ronquist et al. 2012), respectively. The ML tree was tested with 100 bootstrap replicates. For Bayesian analysis, two independent rums were employed. Each run performed a Metropolis-coupled Markov chain Monte Carlo analysis with four chains for 1,000,000 generations. Trees were saved per 100th generation. The value of burn-in was set to discard the first 25% trees, and other trees were used to compute a 50% consensus tree and calculate Bayesian posterior probability.

Taxonomy

Polyporus submelanopus H.J. Xue & L.W. Zhou, **sp. nov.** Fig. 1

MycoBank MB 800237

Differs from *Polyporus melanopus* in having two kinds of generative hyphae (one with simple septa and one with clamp connections), slightly larger pores, thinner and lacerate dissepiments, and no fusoid cystidioles.

Type: China, Qinghai Province, Huzhu County, Beishan Forest Farm, on ground in forest of *Picea*, 1.IX.2003 Dai 5015 (**holotype** IFP 004946; GenBank sequence JQ964425).

Etymology: *submelanopus* (Lat.): somewhat similar to *Polyporus melanopus*.

Basidiomes: Basidiocarps annual, terrestrial, centrally or laterally stipitate, solitary. Pilei sometimes slightly infundibuliform, up to 6.2 cm in diam, and 8 mm thick. Pileal surface cinnamon-buff to vinaceous, glabrous, sometimes wrinkled and faintly zonate when dry; margin acute, concolorous with pileus, curved down when dry. Pore surface straw-yellow to honey-yellow; pores circular to angular, 2–3 per mm; dissepiments thin, lacerate. Context white to cream, soft corky when dry, up to 6 mm thick. Tubes cream to straw-yellow, decurrent on about three fifths of one side of the stipe with obviously boundary, up to 2 mm long. Stipe bearing a fuscous to black cuticle, corky, up to 7 cm long, and 8 mm in diam.

Hyphal structure: Hyphal system dimitic; two kinds of generative hyphae with one type bearing only simple septa and the other with clamp connections at each septum; skeletal hyphae arboriform, IKI–, weakly CB+; tissue unchanged in KOH.

Context: Generative hyphae common, thin-walled, occasionally branched, 3.5–4 μm in diam; arboriform skeletal hyphae frequent, thick-walled with a wide lumen, moderately branched, aseptate, interwoven, up to 7 μm in diam.

Tubes: Generative hyphae frequent, hyaline, thin-walled, frequently branched and simple septate, clamp connection frequent on the generative hyphae near the basidia, 1.5–3.5 μm in diam; arboriform skeletal hyphae common, thick-walled with a wide to narrow lumen, moderately branched, aseptate, interwoven, 1.5–3.2 μm in diam; cystidia and cystidioles absent; basidia clavate, but narrower towards the base, hyaline, thin-walled, bearing a basal clamp connection and four sterigmata, 24–33 × 5–8 μm; basidioles in shape similar to basidia, but slightly smaller.

Stipe: Generative hyphae scanty; arboriform skeletal hyphae dominant, thick-walled with a narrow or wide lumen, moderately branched, aseptate, strongly interwoven, 1.5–7 μm in diam. Hyphae in black crust similar to those in stipe.

Spores: Basidiospores cylindrical, hyaline, thin-walled, smooth, bearing a large guttule. IKI–, CB–, (7.8–)8–10(–10.5) × 3–3.9(–4) μm, L = 8.95 μm, W = 3.38 μm, Q = 2.63–2.67 (n = 60/2).

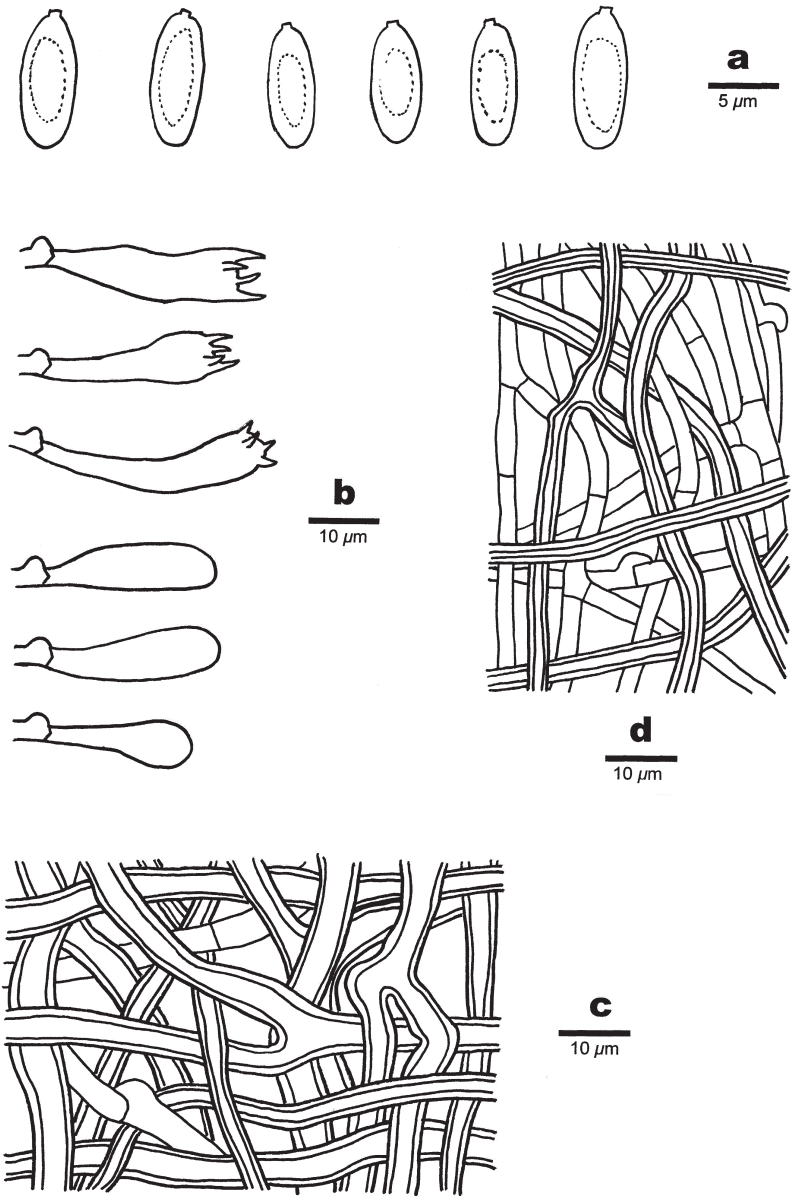


Fig. 1. *Polyporus submelanopus* (holotype).
a: Basidiospores. b: Basidia and basidioles.
c: Hyphae from context. d: Hyphae from trama.

Additional specimen examined: **CHINA**, **Qinghai Province**, Huzhu County, Beishan Forest Farm, on ground in forest of *Picea*, 1.IX.2003 Dai 4997 (IFP 004953; GenBank sequence JQ964424).

Remarks: *Polyporus submelanopus* is characterized by a terrestrial habit, centrally or laterally black-stipitate basidiocarps, straw-yellow to honey-yellow pore surface, circular to angular pores, two types of generative hyphae bearing either simple septa or clamp connections, and cylindrical basidiospores with a large guttule. Its black stipe and medium basidiospore size match the concept of the melanopus group (Núñez & Ryvarden 1995).

Polyporus melanopus resembles *P. submelanopus* in its similar pileus with a shallow central depression, circular to angular pore shape, basidiospores, and terrestrial habit. However, *P. melanopus* has slightly smaller pores (3–4 per mm, Gilbertson & Ryvarden 1987; 3–5 per mm, Bernicchia 2005; 3–4(–5) per mm, Boulet 2007), thicker and entire dissepiments, fusoid cystidioles, and generative hyphae bearing clamp connections only (Gilbertson & Ryvarden 1987).

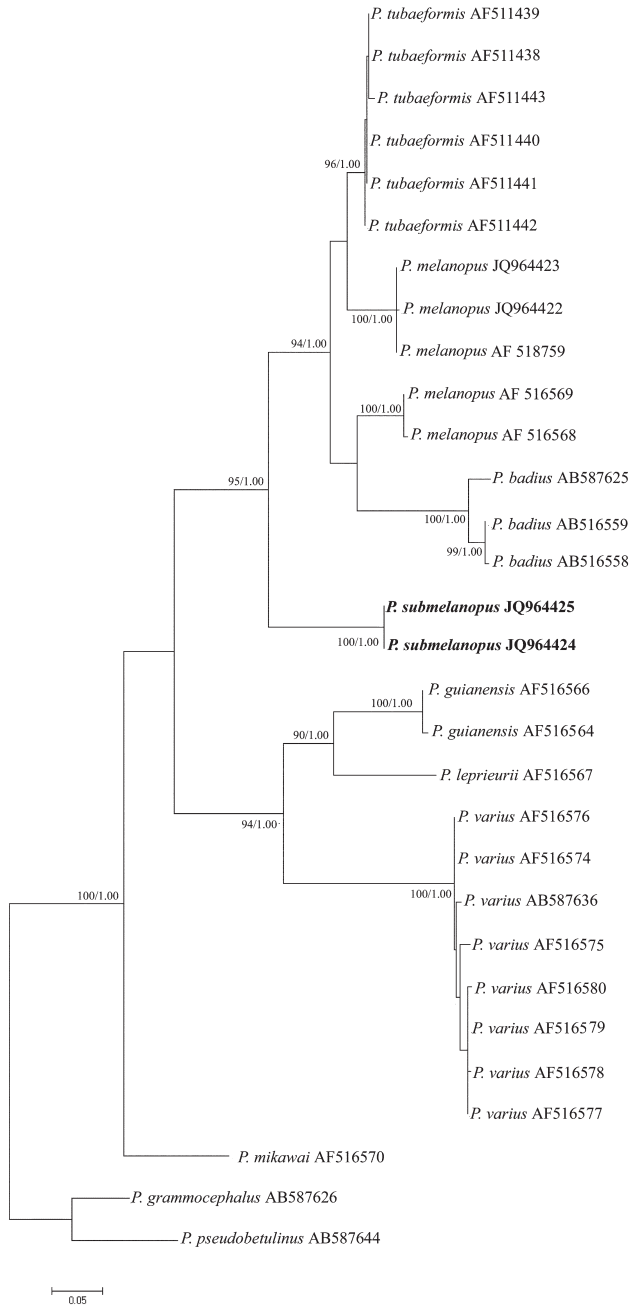
Polyporus badius (Pers.) Schwein., a species with simple-septate generative hyphae, is distinguished from *P. submelanopus* in darker pilei and smaller pores (5–8 per mm, Núñez & Ryvarden 1995). In addition, *P. badius* is a wood-inhabiting species rather than a terrestrial one.

Polyporus xinjiangensis J.D. Zhao & X.Q. Zhang, originally described from China, has generative hyphae bearing simple septa like *P. submelanopus* but produces no generative hyphae with clamp connections (Dai et al. 2007b); *P. xinjiangensis* further differs in its wood-inhabiting habit and slightly shorter basidiospores (7.1–8.5 μm long, Dai et al. 2007b).

Macroscopically, *P. submelanopus* shares similar infundibuliform pilei, a straw-yellow to pale brownish pore surface, and sharp margin with *P. tubaeformis* (P. Karst.) Ryvarden & Gilb., which is distinguished by possession of cystidioles and smaller pores [6–9(–10) per mm, (n = 150/5)] and basidiospores [(5.8–) 6–7.8(–8.2) × (2.1–)2.3–3.2(–3.5) μm, L = 6.49 μm, W = 2.75 μm, Q = 2.27–2.50 (n = 150/5), Dai 1996]. Besides, *P. tubaeformis* has only clamped generative hyphae and grows on wood rather than ground.

Our ITS dataset had 694 characters. Its best-fit evolutionary models for ML and Bayesian analyses were estimated as TrN + G and HKY + G, respectively. The phylogenetic tree (Fig. 2) formed of most members of melanopus group clusters *P. submelanopus* as a distinct clade within this group. Although we unfortunately failed to obtain an ITS sequence from *P. xinjiangensis,* the ecological habit and morphological differences above are enough to distinguish *P. submelanopus* from *P. xinjiangensis.*

Fig. 2. The phylogenetic tree inferred from ITS sequences of the members of Melanopus group. Topological structure was from ML analysis. The statistical values both above 75% of bootstrap value and 0.95 of Bayesian posterior probability are indicated.


P. tubaeformis AF511439
P. tubaeformis AF511438
P. tubaeformis AF511443
P. tubaeformis AF511440
96/1.00
P. tubaeformis AF511441
P. tubaeformis AF511442
P. melanopus JQ964423
P. melanopus JQ964422
100/1.00
P. melanopus AF 518759
94/1.00
P. melanopus AF 516569
100/1.00
P. melanopus AF 516568
95/1.00
P. badius AB587625
100/1.00
P. badius AB516559
99/1.00
P. badius AB516558
***P. submelanopus* JQ964425**
100/1.00
***P. submelanopus* JQ964424**
P. guianensis AF516566
100/1.00
P. guianensis AF516564
90/1.00
P. leprieurii AF516567
P. varius AF516576
94/1.00
P. varius AF516574
100/1.00
P. varius AB587636
100/1.00
P. varius AF516575
P. varius AF516580
P. varius AF516579
P. varius AF516578
P. varius AF516577
P. mikawai AF516570
P. grammocephalus AB587626
P. pseudobetulinus AB587644
0.05

The ITS tree also shows that the current concept of *P. melanopus* is not monophyletic. Five ITS sequences of *P. melanopus* formed two clades according to the specimens' origins. One clade comprised three European isolates, while two isolates from Argentina formed the other clade. Moreover, *P. melanopus* was described as a diverse species in pore and basidiospore size (Gilbertson & Ryvarden 1987, Bernicchia 2005, Niemelä 2005, Krüger et al. 2006, Boulet 2007). Therefore, more investigations are needed to clarify the exact circumscription of *P. melanopus*.

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