

# Phylogeny and global diversity of *Polyporus* group *Melanopus* (Polyporales, Basidiomycota)

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**Abstract** *Polyporus* accommodates species with stipitate basidiocarps, a dimitic hyphal system with skeleto-binding hyphae, and hyaline, thin-walled, more or less cylindrical basidiospores. The species of *Polyporus* are divided into six morphological groups, of which *Melanopus* is characterized by coriaceous basidiocarps, thin context and a black cuticle on the stipe. In this study, we explore the phylogeny and species diversity of *Polyporus sensu lato* focusing on the group *Melanopus* based on global samples. Our combined ITS and nLSU dataset included 117 collections represented by 117 ITS and 94 nLSU sequences, of which 43 ITS and 37 nLSU sequences were newly generated. The resulting phylogenetic analysis recovered four clades, *Favolus*, *Neofavolus*, *Melanopus* and *Polyporellus* clades, while several species in the morphological groups *Melanopus* and *Polyporus* were outside these clades. Combined with morphological evidence, three new species in the *Melanopus* clade, *Polyporus americanus*, *P. austroandinus* and *P. conifericola*, are newly described and illustrated, and

their distinctive characters are discussed. The illegitimate name *Polyporus fraxineus* is renamed as *Polyporus fraxinicola*. The phylogeny and taxonomy of each species in group *Melanopus* (morphology) or *Melanopus* clade (phylogeny) and related species are briefly discussed.

**Keywords** ITS · nLSU · Morphology · Polypore · Group *Admirabilis* · Group *Polyporellus*

## Introduction

*Polyporus* P. Micheli ex Adans., the type genus of the Polyporaceae, was recognized early as a distinct fungal genus. The name was used for a group of several polypores by Micheli (1729) and later validated by Adanson (1763). *Polyporus brumalis* (Pers.) Fr. was first validly selected as the generic type (Clements and Shear 1931), but Donk (1933) rejected this typification for the reason that *P. brumalis* is not the original species referred to by Micheli (1729), which is an unreasonable principle, and assigned *Polyporus tuberaster* (Jacq. ex Pers.) Fr. as the type. Donk's opinion has been accepted by most subsequent mycologists (e.g., Donk 1960; Ryvarden 1991; Núñez and Ryvarden 1995), until Krüger and Gargas (2004) argued that *P. brumalis* should be the correct type of *Polyporus*. To maintain nomenclatural stability, we agree with Donk in this study.

*Polyporus* has a wide global distribution (Ryvarden and Johansen 1980; Gilbertson and Ryvarden 1987; Ryvarden and Gilbertson 1994; Núñez and Ryvarden 2001). It is characterized by stipitate basidiocarps, a dimitic hyphal system with so-called skeleto-binding hyphae (in which the proximal and central parts are straight, unbranched and regular, and the apex is divided into numerous, whip-like branches), and hyaline, thin-walled basidiospores (Núñez and Ryvarden 1995). Otherwise, however, it is a heterogeneous genus. Núñez and Ryvarden (1995) divided 32 members of *Polyporus sensu lato* into six morphological groups, viz. *Admirabilis*, *Dendropolyporus* [= *Dendropolyporus*

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(Pouzar) Jülich], *Favolus* (= *Favolus* Fr.), *Polyporellus* (= *Polyporellus* P. Karst.), *Melanopus* (= *Melanopus* Pat.) and *Polyporus*, according to macromorphological characters. Phylogenetically, *Polyporus* has been repeatedly reported as a polyphyletic genus (Krüger et al. 2006; Sotome et al. 2008, 2011), especially for species of group *Polyporellus* that cluster with *Lentinus* Fr. (Krüger and Gargas 2004; Sotome et al. 2009; Lee et al. 2010). With the aid of phylogenies inferred from RPB2, nLSU and ATP6 sequences, Sotome et al. (2008) recognized six well supported clades within *Polyporus*, but these did not entirely correspond to morphological groups.

Of the six morphological groups, the taxonomy of the group *Favolus* has been comprehensively explored. Phylogenetic analysis showed that sampled members of the group *Favolus* formed two clades, each having distinct characters (Sotome et al. 2013). One clade was proposed as a new genus, *Neofavolus* Sotome & T. Hatt., while the other was *Favolus sensu stricto* (Sotome et al. 2013).

The group *Melanopus* accommodates species with coriaceous basidiocarps, thin context and a black cuticle on the stipe (Núñez and Ryvarden 1995). Núñez and Ryvarden (1995) proposed *Polyporus badius* (Pers.) Schwein., *P. centroafricanus* Núñez & Ryvarden, *P. dictyopus* Mont., *P. guianensis* Mont., *P. leprieurii* Mont., *P. mikawai* Lloyd, *P. melanopus* (Pers.) Fr., *P. nigrocristatus* E. Horak & Ryvarden, *P. tubaeformis* (P. Karst.) Ryvarden & Gilb., *P. varius* (Pers.) Fr. and *P. virgatus* Berk. & M.A. Curtis as members of this group. Recently, Xue and Zhou (2012) described a new species *P. submelanopus* H.J. Xue & L.W. Zhou belonging to group *Melanopus*. Krüger et al. (2006) showed that *P. badius*, *P. melanopus* and *P. tubaeformis* formed a clade, but the relationships among them remained ambiguous and only limited samples from group *Melanopus* and related groups were analyzed. Sotome et al. (2008) reported that three species of group *Melanopus*, *Polyporus badius*, *P. dictyopus* and *P. tubaeformis*, clustered together, while *P. leprieurii* and *P. varius* formed another clade with species from other groups.

Based on global samples mostly from the Northern Hemisphere and references to previous studies (Krüger et al. 2006; Sotome et al. 2008, 2013), we conducted a taxonomic study of *Polyporus* focusing on group *Melanopus*. With the aid of morphological and phylogenetic (ITS+nLSU) analyses, three new species of group *Melanopus* were described and illustrated.

## Materials and methods

### Morphology

Specimens examined are held in herbaria of the Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China (IFP), Muzeum Vysočiny, Jihlava, Czech Republic (MJ), the private herbarium of J. Vlasák (JV) with duplicates

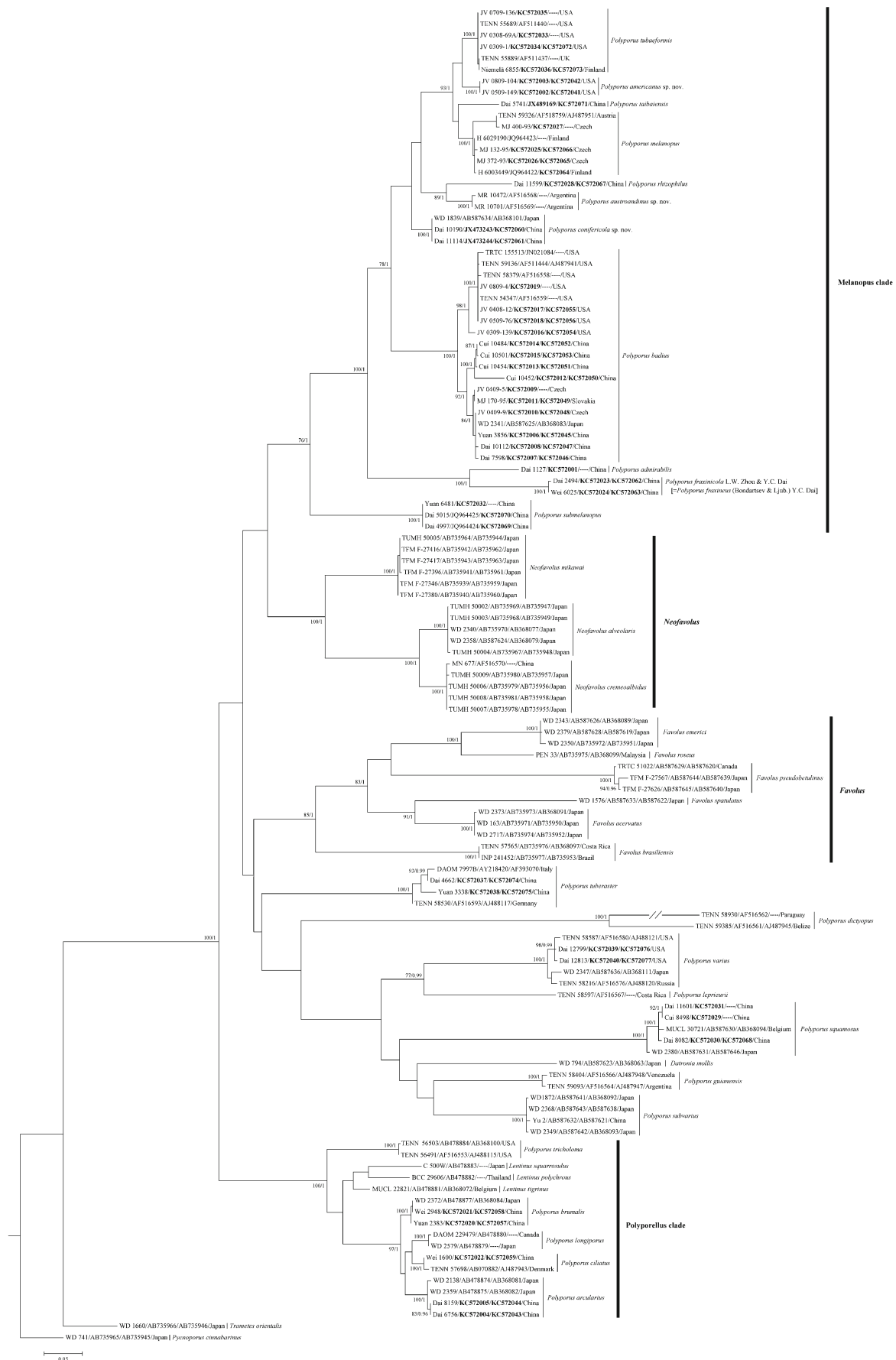
**Fig. 1** Phylogeny of *Polyporus* inferred from combined ITS and nLSU dataset. Topology is from ML analysis with statistical values from both ML (former) and BI (latter). For each collection, its voucher number, GenBank accession number (ITS/nLSU) and locality are provided. The accession numbers for the newly generated sequences in this study are shown in *bold*. “—” means nLSU sequences are unavailable and not used in this analysis. The species name in square brackets is illegitimate

in the Prague Museum Herbarium, Czech Republic (PRM), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina (BAFC), the mycological herbarium at Centro Forestal CIEFAP in Esquel, Argentina (CIEFAP), Botanical Museum, Finnish Museum of Natural History, Helsinki, Finland (H), and Forestry and Forest Products Research Institute, Ibaraki, Japan (FFPRI). The meanings of abbreviations are as follows: CB = Cotton Blue, CB+ = cyanophilous, CB− = acyanophilous, IKI = Melzer’s reagent, IKI− = negative in Melzer’s reagent, KOH = 5 % potassium hydroxide, 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. The microscopic procedure follows Zhao and Cui (2012). Sections were studied at magnifications up to ×1,000 using a Nikon Eclipse 80i microscope and phase contrast illumination. Measurements were made from sections stained with CB. In presenting the variation in size of basidiospores, 5 % of measurements were excluded from each end of the range and given in parentheses. Drawings are made with the aid of a drawing tube. Special color terms follow Petersen (1996).

### Molecular phylogeny

Herbarium specimens were employed as the source of ITS and nLSU sequences using Phire® Plant Direct PCR Kit (Finnzymes, Finland) according to the manufacturer’s procedure. Primers ITS5 and ITS4 (White et al. 1990) were used to amplify ITS sequences, and LR0R and LR7 (Vilgalys and Hester 1990) to amplify nLSU sequences. PCR procedure was as follows: initial denaturation at 98 °C for 5 min., followed by 39 cycles at 98 °C for 5 s., 59 °C (for ITS)/49 °C (for nLSU) for 5 s. and 72 °C for 5 s., and a final extension of 72 °C for 1 min. The PCR products were directly sequenced in the Beijing Genomics Institute, China or in Genomics laboratory of the Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice. Besides the primers used in the amplifications, LR3R, LR3 and LR5 (Vilgalys and Hester 1990) were also used as sequencing primers.

All newly generated sequences were submitted to GenBank (<http://www.ncbi.nlm.nih.gov>; Fig. 1). Other related sequences used in phylogenetic analysis were downloaded from GenBank (Fig. 1). *Trametes orientalis* (Yasuda) Imazeki and *Pycnoporus cinnabarinus* (Jacq.) P. Karst.



were selected as outgroup (Sotome et al. 2013). Firstly, the ITS dataset was analyzed, and the resulting tree received low statistical support (not shown). Then, the nLSU sequences were combined with ITS sequences for analysis, although for a few collections nLSU sequences failed to amplify (Fig. 1).

The combined dataset was aligned using MAFFT 6.935 (Kato et al. 2002; Kato and Toh 2008) with auto option, and the resulting alignment is deposited at TreeBASE (<http://www.treebase.org>; accession number S14040). raxmlGUI 1.2 (Stamatakis 2006; Silvestro and Michalak 2012) was used to construct a maximum likelihood (ML) tree with GTR + G + I model and auto FC option (Pattengale et al. 2010) in bootstrap (BS) replicates. Bayesian inference (BI) was conducted using MrBayes 3.2 (Ronquist and Huelsenbeck 2003) under the best-fit evolutionary model selected by jModelTest (Guindon and Gascuel 2003; Posada 2008). Two independent runs, each starting from random trees, were employed with four chains. Trees were sampled every 1000th generation. Chain convergence was determined using Tracer v1.5 (<http://tree.bio.ed.ac.uk/software/tracer/>). The first quarter of sampled trees was discarded as burn in, while the remaining trees were used for calculating a 50 % majority consensus tree and Bayesian posterior probabilities (BPPs). The tree topology from ML analysis is presented along with both BS (above 75 %) and BPP (above 0.95) values.

## Results

A total of 43 ITS and 37 nLSU sequences were newly generated in this study (Fig. 1). The combined dataset, including 117 collections represented by 117 ITS and 94 nLSU sequences, resulted in an alignment with 1,473 characters. The best evolutionary model of this alignment for BI was estimated as SYM + I + G. After 5,000,000 generations all chains converged, where the effective sample sizes of all parameters were greater than 250 and the potential scale reduction factors approached 1.000. The ML search stopped after 200 BS replicates. The tree inferred from ML analysis of the combined dataset (Fig. 1) had nearly congruent topology with that from ITS sequences but received higher support.

In the current phylogeny (Fig. 1), all sampled species of *Polyporus* formed a strongly supported clade that included related species from other genera: *Datronia* Donk, *Favolus*, *Lentinus* and *Neofavolus*. Within this clade, 1) the genera *Favolus* and *Neofavolus* were each supported as monophyletic; 2) *Polyporus admirabilis* Peck, *P. badius*, *P. fraxineus* (Bondartsev & Ljub.) Y.C. Dai, *P. melanopus*, *P. rhizophilus* (Pat.) Sacc., *P. submelanopus*, *P. taibaiensis* Y.C. Dai, *P. tubaeformis*, and three previously undescribed taxa formed a well supported subclade, named as Melanopus clade; 3) *Polyporus arcularius* (Bastch) Fr., *P. brumalis*, *P. ciliatus* Fr.,

*P. longiporus* Audet, Boulet & Sirard, *P. tricholoma* Mont. and three species of *Lentinus* clustered with strong support as Polyporellus clade; 4) *Polyporus dictyopus*, *P. guianensis*, *P. lepieurii*, *P. squamosus* (Huds.) Fr., *P. subvarius* C.J. Yu & Y.C. Dai, *P. tuberaster*, *P. varius* and *Datronia mollis* (Sommerf.) Donk were scattered.

The three undescribed taxa in Melanopus clade have distinct morphological characters that separate them from other species of *Polyporus*. They are described and illustrated below.

## Taxonomy

### *Polyporus americanus* Vlasák & Y.C. Dai, sp. nov. Figs. 2 and 3

Mycobank no.: MB 803796

Basidiocarps annual, stipitate, solitary. Pilei nearly circular, flat, with shallow central depression. Pileal surface cinnamon-buff to blackish brown, faintly radially striate. Pore surface buff to honey yellow, shining; pores angular, 5–6 per mm. Stipe with a black cuticle. Hyphal system dimitic; generative hyphae with clamp connections. Basidiospores cylindrical, hyaline, IKI–, CB–, 7–9 × 2.5–3.1 μm. On angiosperm wood, associated with a white-rot.

*Type*. USA. New York, Fish Creek Pond, on fallen angiosperm twig, 18 September 2005, *JV 0509/149-J* (holotype in PRM, isotype in IFP).

*Etymology*. *americanus* (Lat.): referring to the geographic distribution in the USA.

*Fruitbody*. Basidiocarps annual, centrally to laterally stipitate, solitary. Pilei nearly circular, up to 4.5 cm wide and 2.7 mm thick, flat, with shallow central depression. Pileal surface cinnamon-buff when juvenile, to blackish brown with age, radially striate to faintly radially ridged on drying; margin sharp. Pore surface buff to honey yellow upon drying, shining; pores angular, 5–6 per mm; dissepiments thin, lacerate. Context white to cream, becoming woody hard upon drying, up to 1.5 mm thick. Tubes concolorous with pore surface, brittle, up to 1.2 mm long. Stipe bearing a fuscous to black cuticle, glabrous at first, wrinkled on drying, up to 2.7 cm long, 4 mm diam.

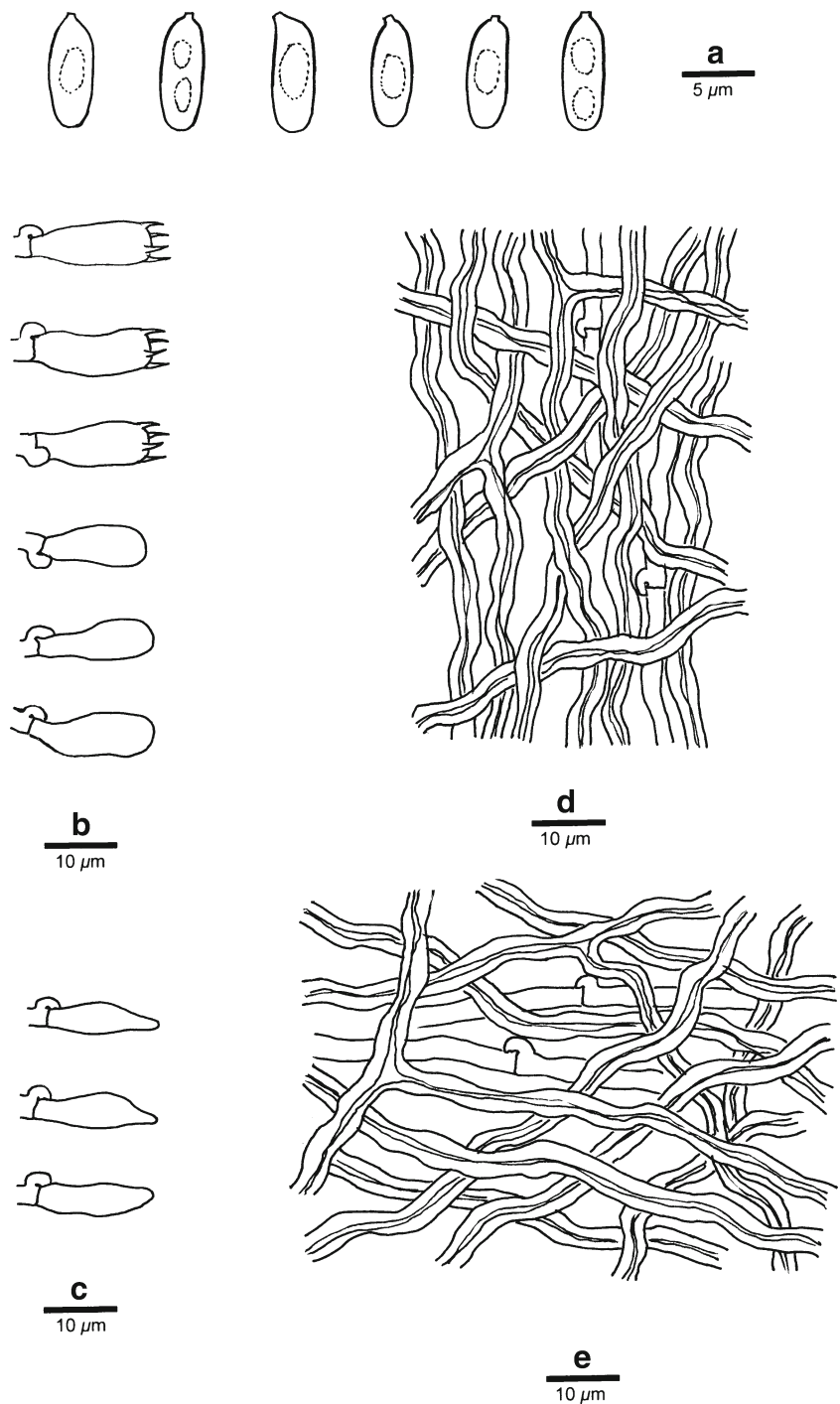
*Hyphal structure*. Hyphal system dimitic; generative hyphae bearing clamp connections, thin-walled, hyaline; skeleto-binding hyphae thick-walled, with arboriform branches and tapering ends, IKI–, CB+; tissue unchanged in KOH.

*Context*. Generative hyphae frequent, 3–4 μm diam.; skeleto-binding hyphae dominant, thick-walled to subsolid, moderately branched, strongly interwoven, 2–4 μm diam. Hyphae in cuticle thick-walled with a wide lumen, branched, hyaline or brownish, regularly arranged into a palisade, 1.5–6.5 μm diam.

*Tubes*. Generative hyphae usually present near hymenium, 2–3 μm diam.; skeleto-binding hyphae dominant, thick-



**Fig. 2** Microscopic structures of *Polyporus americanus* (drawn from the holotype). **(a)**: Basidiospores; **(b)**: Basidia and basidioles; **(c)**: Cystidioles; **(d)**: Hyphae from trama; **(e)**: Hyphae from context



walled to subsolid, moderately branched, strongly interwoven, often gelatinized, 1–4  $\mu\text{m}$  diam. Cystidia absent; cystidioles frequent, subulate; basidia clavate, with a basal clamp and four sterigmata, 16–20  $\times$  5–7  $\mu\text{m}$ ; basidioles in shape similar to basidia, smaller than basidia.

**Stipe.** Hyphal structure similar to that in context; generative hyphae slightly thick-walled, 3–5  $\mu\text{m}$  diam.; skeleto-binding hyphae thick-walled, 1.5–6  $\mu\text{m}$  diam. Hyphae in cuticle hyaline to brown; skeleto-binding hyphae thick-walled, up to 8  $\mu\text{m}$  diam.

**Spores.** Basidiospores cylindrical, thin-walled, hyaline, smooth, bearing one or two guttules, IKI–, CB–, (6.9–)7–9(–9.2)  $\times$  2.5–3.1(–3.2)  $\mu\text{m}$ , L=7.92  $\mu\text{m}$ , W=2.91  $\mu\text{m}$ , Q=2.68–2.77 ( $n=60/2$ ).

**Rot type.** A white rot.

**Additional specimen (paratype) examined. USA.** New Hampshire, White Mountains, Sawyer Pond, on fallen angiosperm twig, 18 September 2008, JV 0809/104 (JV, PRM, IFP).

**Remarks.** *Polyporus americanus* and *P. tubaeformis* share a brown to fuscous upper surface, gelatinized tramal hyphae,



**Fig. 3** A basidiocarp of *Polyporus americanus* (JV 0509/149-J)

similarly sized pores and basidiospores, presence of cystidioles, and growth on angiosperm wood (Ryvarden and Gilbertson 1994). However, *P. tubaeformis* has a deeply funnel-shaped pileus, entire dissepiments and less abundant cystidioles (Ryvarden and Gilbertson 1994). *P. americanus* is also similar to *P. melanopus*; however, the latter differs in having a superficially terrestrial habit—growing from underground roots, more robust and fleshy basidiocarps often with several basidiocarps growing from the same base, and a glabrous pileal surface when dry. *Polyporus americanus* and *P. conifericola* are also alike, but the latter has entire dissepiments, smaller pores, and grows on gymnosperm wood.

***Polyporus austroandinus*** Rajchenb. & Y.C. Dai, sp. nov.

Fig. 4

Mycobank no.: MB 803797

Basidiocarps annual, laterally to eccentrically stipitate, rarely subcentral. Pilei nearly circular. Pileal surface greyish brown to fuscous, wrinkled upon drying. Pore surface white cream, shining; pores angular, 4–5 per mm. Stipe with a fuscous to black cuticle, glabrous. Hyphal system dimitic; generative hyphae with clamp connections. Basidiospores cylindrical to obclavate, hyaline, IKI–, CB–,  $9\text{--}11.5 \times 3\text{--}3.8 \mu\text{m}$ . Associated with a white-rot.

**Type.** ARGENTINA. Chubut, Futaleufú, ‘La 106’ Ranch, on stump of *Austrocedrus chilensis*, 30 April 1992, M. Rajchenberg 10700 (holotype in BAFC 52287).

**Etymology.** *austro* + *andinus* (Lat.): referring to the geographical distribution of the species in the southern forests of Andes Cordillera.

**Fruitbody.** Basidiocarps annual, laterally to eccentrically stipitate, rarely subcentral, solitary or clustered. Pilei flabellate to nearly circular, small to large, up to  $13 \times 18 \times 0.9 \text{ cm}$ . Pileal surface glabrous to slightly pubescent, greyish brown, chocolate brown, dark chestnut to dark reddish chestnut to fuscous, wrinkled upon drying; margin sharp, involute. Pore surface white cream when fresh, pinkish buff to honey-yellow when dry, shining; sterile margin distinct, clay-buff to grayish brown, up to 6 mm wide; pores angular, 4–5 per mm;

dissepiments thin, lacerate. Context white cream when fresh to straw yellow upon drying, corky, up to 6.5 mm thick. Tubes concolorous with pore surface, brittle, up to 4 mm long, decurrent on the upper part of the stipe with distinct boundary. Stipe bearing a fuscous to black cuticle, glabrous, up to 4 cm long and 1.6 cm diam.

**Hyphal structure.** Hyphal system dimitic, generative hyphae bearing clamp connections, thin-walled to slightly thick-walled, hyaline; skeleto-binding hyphae thick-walled, IKI–, CB+; tissue unchanged in KOH.

**Context.** Generative hyphae common, thin-walled to slightly thick-walled, occasionally branched,  $3\text{--}4\text{--}6 \mu\text{m}$  diam.; skeleto-binding hyphae dominant, thick-walled with a narrow lumen, moderately branched, tightly interwoven,  $2\text{--}6 \mu\text{m}$  diam. Cuticle  $40\text{--}100 \mu\text{m}$  thick, hyphae thick-walled to subsolid, hyaline to brownish,  $6\text{--}12 \mu\text{m}$  diam.

**Tubes.** Generative hyphae frequent, usually present near hymenium, hyaline, thin-walled, commonly branched,  $2\text{--}3 \mu\text{m}$  diam.; skeleto-binding hyphae dominant, thick-walled with a narrow to wide lumen, frequently branched, strongly interwoven,  $1.5\text{--}6 \mu\text{m}$  diam. Cystidia absent; cystidioles present, subulate; basidia clavate, with a basal clamp and four sterigmata,  $15\text{--}25 \times 7\text{--}10 \mu\text{m}$ ; basidioles similar in shape to basidia, smaller than basidia.

**Stipe.** Hyphal structure similar to that in context; skeleto-binding hyphae thick-walled with a narrow lumen to solid, commonly branched,  $2\text{--}5 \mu\text{m}$  diam. Hyphae in cuticle hyaline to pale brown; skeleto-binding hyphae thick-walled,  $5\text{--}7 \mu\text{m}$  diam.

**Spores.** Basidiospores cylindrical to obclavate, thin-walled, hyaline, smooth, bearing one or two guttules, IKI–, CB–,  $(8\text{--}9)\text{--}11.5\text{--}(13.2) \times 3\text{--}3.8\text{--}(4) \mu\text{m}$ ,  $L=10.17 \mu\text{m}$ ,  $W=3.3 \mu\text{m}$ ,  $Q=3.08$  ( $n=30/1$ ).

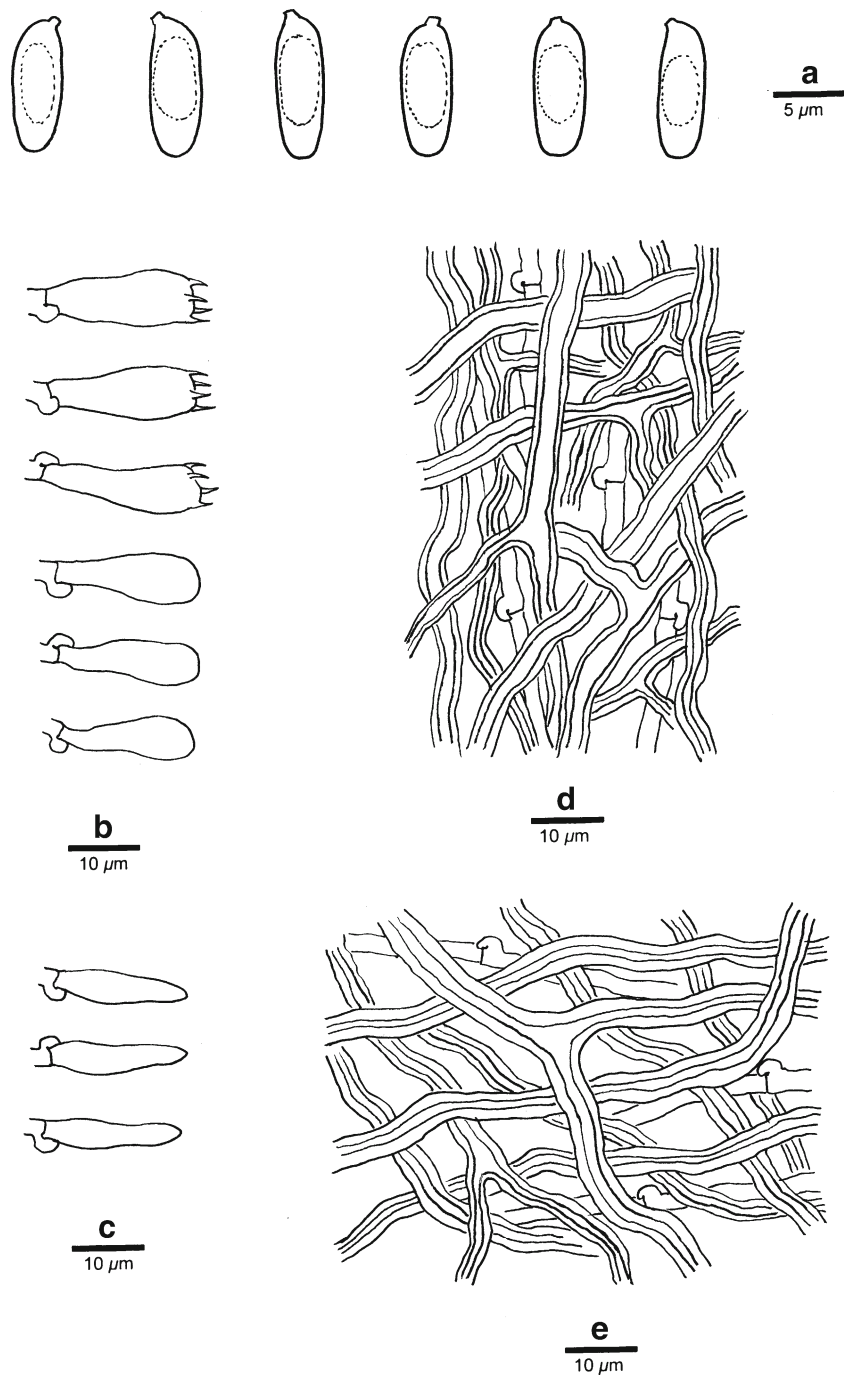
**Rot type.** A white rot.

**Substrates and habitat.** A saprophyte on fallen small branches of *Nothofagus dombeyi*, *N. pumilio* and *N. antarctica* and on stumps, dead stems and roots of *Austrocedrus chilensis*, *Araucaria araucana*, *Guevinia avellana* and *Aristotelia chilensis*.

**Additional specimens (paratypes) examined.** ARGENTINA. Chubut, Futaleufú, ‘La 106’ ranch, on stump of *Austrocedrus chilensis*, 30 April 1992, M. Rajchenberg 10701 (BAFC 52288 and IFP). Chubut, *Ibid.*, on a branch on soil in *Austrocedrus chilensis* and *Lomatia hirsuta* mixed forest, 9 May 1991, M. Rajchenberg 10472 (CIEFAP). Chubut, Cushamen, Epuyén Ms. Ponolef property, on stump of *A. chilensis*, 20 May 1996, M. Rajchenberg 11186 (CIEFAP). For further specimens cfr. Wright and Deschamps (1972, 1975) and Rajchenberg (2006).

**Remarks.** *Polyporus austroandinus* is a well known taxon that was previously treated as *Polyporus dictyopus* (Wright and Deschamps 1972, 1975) and as *P. melanopus* (Hjortstam

**Fig. 4** Microscopic structures of *Polyporus austroandinus* (drawn from Rajchenberg 10701). **(a)**: Basidiospores; **(b)**: Basidia and basidioles; **(c)**: Cystidioles; **(d)**: Hyphae from trama; **(e)**: Hyphae from context



and Ryvar den 1985; Rajchenberg 2006). *Polyporus dictyopus* is a pantropical species that differs in having smaller pores [5–7(–10) per mm] and smaller elliptical basidiospores [(6–)7–8.5(–9)×2.5–4 μm] (Núñez and Ryvar den 1995). *Polyporus melanopus* differs in having a terrestrial habit, entire dissepiments, and smaller basidiospores [7–9(10)×3–4 μm] (Ryvar den and Gilbertson 1994). *Polyporus submelanopus* resembles *P. austroandinus* in having brown and black-stipitate basidiocarps, but the former has smaller basidiospores (8–10×3–3.9 μm),

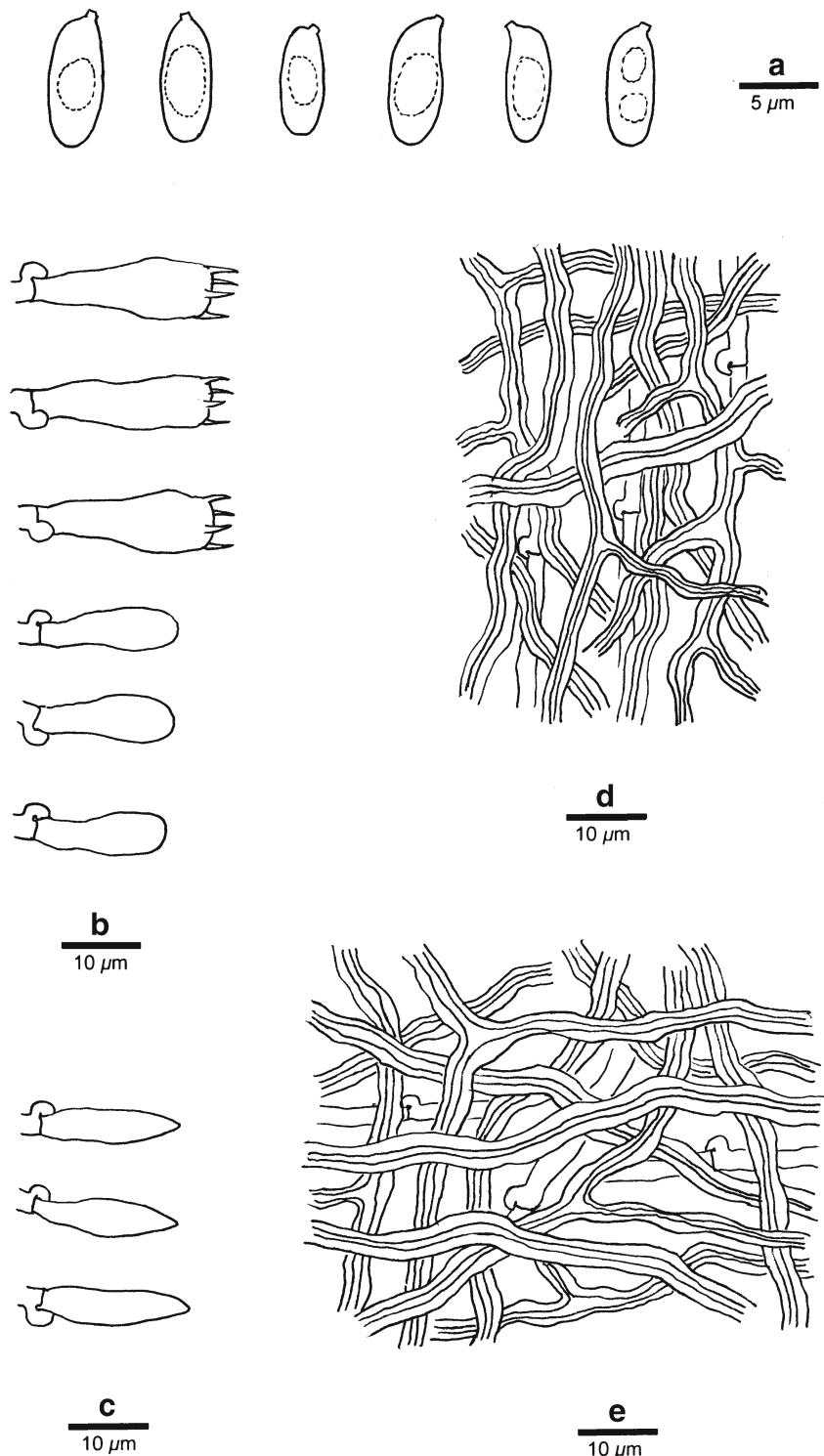
generative hyphae bearing both simple septa and clamp connections, and absence of cystidioles (Xue and Zhou 2012).

*Polyporus conifericola* H.J. Xue & L.W. Zhou, sp. nov. Figs. 5 and 6

Mycobank no.: MB 801216

Basidiocarps annual, centrally or eccentrically stipitate. Pilei circular to infunduliform. Pileal surface orange brown to fuscous, glabrous, with a cuticle. Pore surface cream, straw-yellow to pale brownish, shining; pores angular to round, 7–10 per mm. Stipe with a fuscous to black cuticle,

**Fig. 5** Microscopic structures of *Polyporus conifericola* (drawn from the holotype). **(a)**: Basidiospores; **(b)**: Basidia and basidioles; **(c)**: Cystidioles; **(d)**: Hyphae from trama; **(e)**: Hyphae from context



glabrous. Hyphal system dimittic; generative hyphae with clamp connections. Basidiospores cylindrical, hyaline, IKI-, CB-,  $6\text{--}8 \times 2.3\text{--}3.1 \mu\text{m}$ . Associated with a white-rot.

*Type*. **CHINA**. Inner Mongolia Autonomous Region, Genhe, on fallen trunk of *Larix*, 29 August 2009, *Dai 11114* (holotype in IFP).

*Etymology*. *conifericola* (Lat.): referring to growth on conifer wood.

*Fruitbody*. Basidiocarps annual, centrally or eccentrically stipitate, solitary or in clusters, coriaceous when fresh, becoming hard upon drying. Pilei circular to infundibuliform, up to 7 cm wide and 3 mm thick; margin sharp. Pileal surface





**Fig. 6** Basidiocarp of *Polyporus conifericola* (Dai 10091)

orange brown to fuscous, glabrous, with a cuticle bearing indistinctly concentric zones when fresh, azonate and more or less radially wrinkled upon drying. Pore surface cream, straw-yellow to pale brownish when dry, shining; sterile margin distinct, orange brown to fuscous; pores angular to round, 7–10 per mm; dissepiments thin to fairly thick, entire to slightly lacerate. Context cream, corky, up to 2 mm thick. Tubes concolorous with pore surface, brittle, up to 1 mm long, sometimes decurrent on the upper part of the stipe with distinct boundary. Stipe bearing a fuscous to black cuticle, glabrous, slender, up to 5 cm long and 6 mm diam.

**Hyphal structure.** Hyphal system dimitic; generative hyphae with clamp connections, hyaline, thin-walled; skeleto-binding hyphae thick-walled, with arboriform branches and tapering ends, IKI–, CB+; tissue unchanged in KOH.

**Context.** Generative hyphae infrequent, 2–4.5  $\mu\text{m}$  diam.; skeleto-binding hyphae dominant, thick-walled to subsolid, moderately branched, strongly interwoven, 1.5–5  $\mu\text{m}$  diam. Hyphae in cuticle thick-walled to solid, brown, arranged into a palisade, 3–6  $\mu\text{m}$  diam.

**Tubes.** Generative hyphae usually present near to hymenium, 2–3.5  $\mu\text{m}$  diam.; skeleto-binding hyphae dominant, thick-walled to subsolid, moderately branched, interwoven, often gelatinized, 1–4  $\mu\text{m}$  diam. Hyphae at dissepiment edges a little swollen. Cystidia absent; cystidioles scanty to frequent, subulate; basidia clavate, with a basal clamp and four sterigmata, 15–20  $\times$  6.5–8  $\mu\text{m}$ ; basidioles in shape similar to basidia, or pyriform, smaller than basidia.

**Stipe.** Hyphal structure similar to that in context; skeleto-binding hyphae thick-walled, 1.5–4.5  $\mu\text{m}$  diam.; binding hyphae (i.e. binding sections of the skeleto-binding hyphae) abundant. Hyphae in cuticle hyaline to pale brown; generative hyphae mostly infrequent, but frequent in some specimens, 2–4  $\mu\text{m}$  diam.; skeleto-binding hyphae thick-walled, 5–7  $\mu\text{m}$  diam.

**Spores.** Basidiospores cylindrical, thin-walled, hyaline, smooth, bearing one or two guttules, IKI–, CB–, (5.8–)6–

8(–8.2)  $\times$  (2.1–)2.3–3.1(–3.3)  $\mu\text{m}$ , L=6.86  $\mu\text{m}$ , W=2.81  $\mu\text{m}$ , Q=2.2–2.59 ( $n=120/4$ ).

**Rot type.** A white rot

**Additional specimens (paratypes) examined.** **CHINA.** Jilin Province, Fusong County, on rotten wood of *Abies*, 21 July 1993, *Dai 626* (IFP 015260); Jilin Province, Antu County, Changbaishan Nature Reserve, on rotten wood of *Pinus*, 5 September 1993, *Dai 1081* (IFP 015261); rotten gymnosperm wood, 1 August 2008, *Dai 10091* (IFP 008255); on rotten wood of *Picea*, 3 August 2008, *Dai 10190* (IFP 008350).

**Remarks.** *Polyporus conifericola* is morphologically similar to *P. tubaeformis*, both species sharing infundibuliform and orange brown to reddish brown basidiocarps, straw-yellow to ochraceous pore surface, commonly gelatinized and interwoven tramal hyphae, and presence of cystidioles. However, *P. tubaeformis* has larger pores (5–7 per mm, Ryvarden and Gilbertson 1994) and slightly larger basidiospores (7–9  $\times$  3–3.5  $\mu\text{m}$  in Ryvarden and Gilbertson 1994; 7–9  $\times$  2.5–3.5  $\mu\text{m}$  in Krüger et al. 2006). Moreover, *P. tubaeformis* mostly grows on dead angiosperm wood and very rarely on *Picea* (Ryvarden and Gilbertson 1994), while *P. conifericola* is exclusively found on dead gymnosperm wood.

*Polyporus conifericola* is similar to *P. americanus* in sharing gelatinized tramal hyphae and the presence of cystidioles. However, *P. conifericola* has entire dissepiments, smaller pores and growth on gymnosperm wood.

*Polyporus submelanopus* resembles *P. conifericola* in having similar infundibuliform pilei, straw-yellow pore surface and sharp margin, but *P. submelanopus* has a terrestrial habit, larger pores (2–3 per mm) and basidiospores (8–10  $\times$  3–3.9  $\mu\text{m}$ ), and both simple septate and clamped generative hyphae (Xue and Zhou 2012).

*Polyporus badius* and *P. melanopus* share with *P. conifericola* solitary or clustered black-stipitate basidiocarps, a blackish brown pileal surface, angular to circular pores and presence of cystidioles. However, compared to *P. conifericola*, *P. badius* has slightly larger pores (5–8 per mm), wider basidiospores (3.3–5  $\mu\text{m}$  wide) and simple septate generative hyphae (Ryvarden and Gilbertson 1994). *P. melanopus* is distinguished from *P. conifericola* by its mostly terrestrial habit, larger pores (3–4 per mm), wider basidiospores (3–4  $\mu\text{m}$  wide), and more loosely interwoven and nongelatinous tramal hyphae (Ryvarden and Gilbertson 1994; Dai 1996).

***Polyporus fraxinicola*** L.W. Zhou & Y.C. Dai, nom. nov.  
Mycobank no.: MB 803799

Basionym: *Piptoporus fraxineus* Bondartsev & Ljub., Novosti Sist. Nizsh. Rast. 1965:135, 1965.

Synonym: *Polyporus fraxineus* (Bondartsev & Ljub.) Y.C. Dai, Fungal Science, 14(3, 4): 69, 1999.

Non *Polyporus fraxineus* (Bull.) Fr., Syst. mycol. (Lundae) 1: 374, 1821.

**Remarks.** *Polyporus fraxineus* (Bondartsev & Ljub.) Y.C. Dai was based on the name *Piptoporus fraxineus* Bondartsev & Ljub. (Dai 1999). However, the former is illegitimate because of its earlier homonym *Polyporus fraxineus* (Bull.) Fr. In Index Fungorum (<http://www.indexfungorum.org/>) and MycoBank, *P. fraxineus* (Bondartsev & Ljub.) Y.C. Dai was treated as a synonym of *P. admirabilis*, but our phylogenetic analysis indicates that they are different although closely related (Fig. 1). Morphologically, *P. fraxineus* (Bondartsev & Ljub.) Y.C. Dai differs from *P. admirabilis* in having entire dissepiments, absence of cystidioles and shorter basidiospores ( $6.5\text{--}8 \times 2.8\text{--}3.7 \mu\text{m}$ , Dai 1999), whereas *P. admirabilis* has lacerate dissepiments, cystidioles and longer basidiospores ( $7.8\text{--}9 \times 3\text{--}3.5 \mu\text{m}$ , Dai 1999). Therefore, we propose *Polyporus fraxinicola* as a new name.

## Discussion

In this study, four clades were distinguished within *Polyporus sensu lato*. They are *Favolus*, *Neofavolus*, *Melanopus* clade and *Polyporellus* clade. The former two clades have been treated recently as genera (Sotome et al. 2013). The other two clades and several scattered species are briefly discussed below.

At the generic rank, *Melanopus* was erected by Patouillard (1887) to accommodate species with a black stipe. This genus was later accepted as a synonym of *Polyporus* (Donk 1960). Species of *Melanopus* included members of the groups *Melanopus* and *Polyporus* as defined by Núñez and Ryvarden (1995). Donk (1960) assigned *Polyporus melanopus* as the generic type of *Melanopus*. However, Ryvarden (1991) argued against this typification and selected *P. squamosus* as type. In our current phylogeny (Fig. 1), *P. melanopus* clustered within *Melanopus* clade along with several other *Polyporus* species, some considered to be within and others outside group *Melanopus*. *Polyporus squamosus*, on the other hand, was separated from the four well-supported clades. We failed to identify any distinctive morphological characters linking species within the *Melanopus* clade, and thus we will not currently promote the clade as an independent genus or combine names of its members.

### Species in *Melanopus* clade

Three newly described species, *Polyporus americanus*, *P. austroandinus* and *P. conifericola* share typical characters of the group *Melanopus*, which was supported by both morphological and phylogenetic evidence. Inclusion of *Polyporus badius*, *P. melanopus*, *P. submelanopus* and *P. tubaeformis* in the group *Melanopus* (Núñez and Ryvarden 1995) was also supported by the current phylogeny (Fig. 1).

*Polyporus xinjiangensis* J.D. Zhao & X.Q. Zhang was originally described from Xinjiang, northwestern China based on a single specimen (Zhao et al. 1981). Dai and Yuan (2010) reexamined this specimen and found it is sterile. From another specimen of *P. xinjiangensis* (Dai 7598) collected also in Xinjiang, this species was redescribed and illustrated (Dai et al. 2007a). In the current phylogeny, the specimen Dai 7598 clustered with *Polyporus badius* (Fig. 1). Morphologically, the two species share a dark-colored pileal surface and stipe, generative hyphae bearing simple septa, and similarly sized basidiospores (Dai et al. 2007a). So we propose *P. xinjiangensis* as a synonym of *P. badius*.

At least three subclades were recovered within *Polyporus badius* clade (Fig. 1). Among the three subclades, their genetic distances indicated by branch lengths were relatively close and morphological differences were not found. As a result, we treat the three subclades as a single species, *P. badius*.

*Polyporus taibaiensis* Y.C. Dai is the revised name for *Polyporus rhododendri* Y.C. Dai & H.S. Yuan (Dai 2012), since the latter is a later homonym of *Polyporus rhododendri* Schwein. and thus illegitimate. Here we formally register the name *Polyporus taibaiensis* Y.C. Dai (MB 803798) at MycoBank (<http://www.mycobank.org/>). This species morphologically belongs to group *Melanopus* (Dai et al. 2009) and phylogenetically is within the *Melanopus* clade (Fig. 1).

*Polyporus rhizophilus* was previously classified in group *Polyporellus* (Núñez and Ryvarden 1995); our analyses, however, place it within *Melanopus* clade, not *Polyporellus* clade (Fig. 1). This species has a terrestrial habit like *P. melanopus* and *P. submelanopus*, and produces a dark brownish-black stipe (Núñez and Ryvarden 1995), which makes it different from other members of group *Polyporellus* and more similar to species of group *Melanopus*.

*Polyporus admirabilis* was identified as a species of group *Admirabilis*, also including *P. gayanus* Lév. and *P. pseudobetulinus* (Murashk. ex Pilát) Thorn, Kotir. & Niemelä (Núñez and Ryvarden 1995). Our phylogeny suggests *P. admirabilis* is a member of *Melanopus* clade (Fig. 1), and supports the combination of *Polyporus pseudobetulinus* as *Favolus pseudobetulinus* (Murashk. ex Pilát) Sotome & T. Hatt. (Sotome et al. 2013). *Polyporus gayanus* was not included in our phylogenetic study.

### Species from group *Melanopus* that are excluded from *Melanopus* clade

*Polyporus mikawai*, previously considered to be a species of group *Melanopus* (Núñez and Ryvarden 1995), has been transferred to *Neofavolus* as *N. mikawai* (Lloyd) Sotome & T. Hatt. (Sotome et al. 2013).

*Polyporus dictyopus*, *P. guianensis*, *P. lepieurii* and *P. varius* that were placed in group *Melanopus* (Núñez and Ryvarden

1995) are phylogenetically separate from *Melanopus* clade (Fig. 1).

*Polyporus subvarius*, described from eastern Tibet, south-western China (Dai et al. 2007b) is phylogenetically separate from *Melanopus* clade and not aligned with any other well-supported clade (Fig. 1).

All six of the above species have morphological characters typical of group *Melanopus*, indicating that these characters do not adequately define natural groups of species within *Polyporus sensu lato*.

#### Species from group Polyporus

Two members of group Polyporus, *P. squamosus* and *P. tuberaster*, were included in our study (Fig. 1) and in an earlier study by Sotome et al. (2008). Both species share brittle basidiocarps when dry, and large pores (< 2 per mm) and basidiospores (> 10 µm long, Núñez and Ryvarden 1995), but they are phylogenetically separated from each other. The monophyly of group Polyporus should be reevaluated by phylogenetic study of more members of this group, such as *Polyporus austroafricanus* Núñez & Ryvarden, *P. craterellus* Berk. & M.A. Curtis, *P. radicans* Schwein. and *P. udus* Jungh. Till now, no species have been identified that are phylogenetically related to *Polyporus tuberaster*, the generic type species of *Polyporus*.

#### Species from group Polyporellus

All species sampled from group Polyporellus, except *Polyporus rhizophilus*, clustered together as Polyporellus clade (Fig. 1). It is well known that species of this morphological group are more closely related phylogenetically to several species of *Lentinus* than to other *Polyporus* groups (Fig. 1, Krüger and Gargas 2004; Sotome et al. 2008, 2009; Lee et al. 2010). Further phylogenetic analysis and morphological examinations of *Lentinus* species are needed before considering this group at generic rank.

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