

# Taxonomic study on a threatened polypore, *Polyporus pseudobetulinus*, and a morphologically similar species, *P. subvarius*

Kozue Sotome · Tsutomu Hattori · Yuko Ota

Received: 4 October 2010 / Accepted: 3 February 2011 / Published online: 27 February 2011  
© The Mycological Society of Japan and Springer 2011

**Abstract** A taxonomic study based on morphological and phylogenetic characters revealed that the specimens referred to as '*Polyporus pseudobetulinus*' in Japan actually represent two distinct species, namely, *P. pseudobetulinus* and *P. subvarius*. These can be distinguished by characteristics of the pileus surface, the presence/absence of clamp-connections on generative hyphae, and the shape of the basidiospores. *P. subvarius* is newly reported from Japan following the original description from China. The phylogenetic positions, detailed descriptions, and illustrations are provided for both species.

**Keywords** Pathogen · Phylogeny · Red list · Taxonomy

## Introduction

*Polyporus* Fr. (Polyporales, Basidiomycota) is a cosmopolitan genus with mostly stipitate basidiocarps, a dimitic

hyphal system with arboriform skeletal binding hyphae, and cylindrical and smooth basidiospores, and it characteristically causes a white rot (Gilbertson and Ryvarden 1987; Núñez and Ryvarden 1995a). This genus accommodates variable species in terms of macro-morphology and is polyphyletic (Sotome et al. 2008).

*Polyporus pseudobetulinus* (Murashk. ex Pilát) Thorn, Kotir. & Niemelä is a rare polypore distributed in boreal regions of North America and the northern part of Eurasia (Bondartsev 1953; Domański et al. 1967; Thorn et al. 1990; Boulet 2003; Schigel and Toresson 2005). This species is characterized by basidiocarps with a short, rounded, and undifferentiated stipe and a thin cuticle that becomes areolate and lacks clamp-connections on generative hyphae (Thorn et al. 1990; Núñez and Ryvarden 1995a). It occurs on dead or living *Populus* spp. in North America and Europe (Domański et al. 1967; Thorn et al. 1990).

*Polyporus subvarius* C.J. Yu & Y.C. Dai is similar to *P. pseudobetulinus* in its macro-morphology but is distinct in having clamp-connections on generative hyphae. This species grows on *Salix* spp. and is at the present time known only from a boreal area of eastern Tibet (Dai et al. 2007a).

Núñez and Ryvarden (1995b) first reported '*P. pseudobetulinus*' on *Salix* spp. from a boreal area of Hokkaido in Japan based on two specimens. However, our provisional examination of one of the voucher specimens cited by Núñez and Ryvarden (1995b) revealed clamp-connections on generative hyphae. Some additional specimens of '*Polyporus pseudobetulinus*' with clamp-connections were collected in a limited location of Hokkaido in Japan. We report here our taxonomic study of '*P. pseudobetulinus*' in Japan based on a comparison with specimens of *P. pseudobetulinus* examined by Thorn et al. (1990), including a detailed comparison of the *P. pseudobetulinus* holotype and the holotype of *P. subvarius*. Their phylogenetic relationships were inferred based on

K. Sotome (✉)

Tokyo Department of Botany, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba, Ibaraki 305-0005, Japan  
e-mail: ksotome@kahaku.go.jp

K. Sotome

Kanagawa Prefectural Museum of Natural History, Iryuda 499, Odawara, Kanagawa 250-0031, Japan

T. Hattori

Forestry and Forest Products Research Institute, Kansai Research Center, 68 Nagai-Kyutaro, Momoyama/Fushimi, Kyoto, Kyoto 612-0855, Japan

Y. Ota

Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan

the sequences of 5.8S-internal transcribed spacer 2 (ITS2) and the nuclear large-subunit (nuLSU) rRNA regions together with other *Polyporus* and related species.

## Materials and methods

### Morphological studies

The procedure for morphological observation follows that of Sotome et al. (2007). Color descriptions are given according to the Munsell color system (Munsell Color Co., Boston, MA). Herbaria holding specimens are abbreviated according to the system of Holmgren et al. (1990). The cultures examined have been deposited in the culture bank of the Forestry and Forest Products Research Institute, Tsukuba, Japan (FFPRI) and Genbank, National Institute of Agrobiological Sciences, Japan (MAFF). Basidiospores were measured on materials mounted in Melzer's reagent. The following abbreviations are used for basidiospore measurements: L = mean basidiospore length, W = mean basidiospore width, R = the ratio of length/width of a basidiospore,  $\bar{r}$  = arithmetic mean of R; ( $n = x/y$ ) means  $x$  measurement of basidiospores from  $y$  specimens. Cultural characteristics were studied on specimens grown on malt agar (Difco Laboratories, Detroit, MI) plates at 25°C and described according to the system devised by Nobles (1965). The mycelial growth rate,  $K_r$ , at 25°C was calculated as follows:  $R_1 = R_0 + K_r(t_1 - t_0)$ , where  $R_1$  = colony radius at time  $t_1$ , and  $R_0$  = colony radius at time  $t_0$ . Extracellular oxidase reactions were tested according to Käärik (1965).

### Molecular techniques and phylogenetic analyses

DNA was extracted from cultured mycelia or dried specimens using the DNeasy Plant Mini kit (QIAGEN, Hilden, Germany) or EZNA Fungal DNA kit (Omega Bio-Tek,

Norcross, GA). The nuLSU and ITS1-5.8S-ITS2 regions were amplified by the PCR following the method described by Sotome et al. (2008, 2009). All PCR products were purified using the MonoFas DNA Purification kit (GL Sciences, Tokyo, Japan). DNA sequences were determined using a Big Dye Terminator ver. 3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) with the ABI 3100 DNA sequencer. Sequences newly generated in this study were submitted to GenBank (accession no. AB587619–AB587646, AB588989; Tables 1, 2).

The ITS1-5.8S-ITS2 and nuLSU sequences were initially aligned using the Fast Statistical Alignment program (Bradley et al. 2009). Unalignable regions, including the ITS1, were excluded from the analyses. Sequence alignment data were deposited at TreeBASE under study accession number S10847. Maximum parsimony (MP) analysis was performed in PAUP\* 4.0b10 (Swofford 2003) with gaps treated as missing data. The most parsimonious trees were identified using heuristic searches with random addition sequences (1000), with the maxtree option set to auto increase, and tree-bisection-reconnection (TBR) branch swapping was employed. A bootstrap (BS) analysis was performed with 1000 replicates, with ten random taxon sequence additions per replicate and branch swapping set to TBR.

The maximum likelihood (ML) trees were obtained using the PhyML 3.0 program (Guindon and Gascuel 2003) under the general time-reversible (GTR) substitution model with gamma distributed rates among sites and a proportion of invariant sites. Bootstrap support values were obtained on the basis of 1000 replicates analyzed using the same maximum likelihood procedure. Dataset congruence was tested with the incongruence length difference (ILD) test (Farris et al. 1994) as implemented in PAUP\* 4.0b10 ('partition-homogeneity test'). A  $P$  value of <0.05 was considered statistically significant.

**Table 1** List of '*Polyporus pseudobetulinus*' in Japan and their GenBank accession numbers

Isolates/voucher specimens	Locality	Culture bank (number)	GenBank accession no.	
			nuLSU	ITS
WD1872/TFM F-16868	Hokkaido, Japan	MAFF (420838)	AB368092 <sup>b</sup>	AB587641
WD2349/TFM F-21701	Hokkaido, Japan	MAFF (420805)	AB368093 <sup>b</sup>	AB587642
WD2368/TFM F-27548	Hokkaido, Japan	FFPRI (420876)	AB587638	AB587643
-/TFM F-27567 <sup>a</sup>	Hokkaido, Japan	–	AB587639	AB587644
-/TFM F-27626 <sup>a</sup>	Hokkaido, Japan	–	AB587640	AB587645

nuLSU nuclear large subunit, ITS internal transcribed spacer

<sup>a</sup> Specimens used for the DNA extraction

<sup>b</sup> Sequences determined by Sotome et al. (2008) as *Polyporus cf. pseudobetulinus*

**Table 2** List of species and their GenBank accession numbers

Species	Strain or Specimen no.	Locality	Culture bank (number)	GenBank accession no.	
				nucLSU	ITS
<i>Datronia mollis</i>	WD794	Fukushima, Japan	MAFF (420817)	AB368063 <sup>c</sup>	AB587623
<i>Ganoderma lucidum</i>	WD565	Ibaraki, Japan	MAFF (420210)	AB368068 <sup>c</sup>	AB462322 <sup>c</sup>
<i>G. tsunodae</i>	WD2034	Ibaraki, Japan	MAFF (420822)	AB368069 <sup>c</sup>	AB588989
<i>Lentinus tigrinus</i>	MUCL22821	Belgium	MUCL (22821)	AB368072 <sup>c</sup>	AB478881 <sup>c</sup>
<i>Perenniporia tephropora</i>	WD1618	Okinawa, Japan	MAFF (420826)	AB368076 <sup>c</sup>	AB462323 <sup>c</sup>
<i>Polyporus alveolaris</i>	WD2358	Chiba, Japan	MAFF (420828)	AB368079 <sup>c</sup>	AB587624
<i>P. arcularius</i>	WD2359	Ibaraki, Japan	MAFF (420830)	AB368082 <sup>c</sup>	AB478875 <sup>c</sup>
<i>P. badius</i>	WD2341	Ibaraki, Japan	MAFF (420797)	AB368083 <sup>c</sup>	AB587625
<i>P. brumalis</i>	WD2372	Miyagi, Japan	MAFF (420834)	AB368084 <sup>c</sup>	AB478877 <sup>c</sup>
<i>P. grammocephalus</i>	WD2343	Okinawa, Japan	MAFF (420799)	AB368089 <sup>c</sup>	AB587626
<i>P. grammocephalus</i>	WD2351	Ibaraki, Japan	MAFF (420807)	AB368090 <sup>c</sup>	AB587627
<i>P. grammocephalus</i>	WD2379	Okinawa, Japan	MAFF (429836)	AB587619	AB587628
<i>P. pseudobetulinus</i>	TRTC51022 <sup>a</sup>	Ontario, Canada	–	AB587620	AB587629
<i>P. squamosus</i>	MUCL30721	Belgium	MUCL (30721)	AB368094 <sup>c</sup>	AB587630
<i>P. squamosus</i>	WD2380	Shizuoka, Japan	–	AB587646	AB587631
<i>P. subvarius</i>	IFP Yu 2 <sup>a,b</sup>	Tibet, China	–	AB587621	AB587632
<i>P. tenuiculus</i>	WD1576	Okinawa, Japan	MAFF (420842)	AB587622	AB587633
<i>P. tubaeformis</i>	WD1839	Nagano, Japan	MAFF (420843)	AB368101 <sup>c</sup>	AB587634
<i>P. varius</i>	WD691	Tottori, Japan	MAFF (420234)	AB368110 <sup>c</sup>	AB587635
<i>P. varius</i>	WD2347	Aomori, Japan	MAFF (420813)	AB368111 <sup>c</sup>	AB587636
<i>Pseudofavolus cucullatus</i>	WD2157	Kanagawa, Japan	MAFF (420850)	AB368114 <sup>c</sup>	AB587637

<sup>a</sup> Specimens used for the DNA extraction

<sup>b</sup> Holotype

<sup>c</sup> Sequences determined by Sotome et al. (2008) or (2009)

## Results

### Phylogenetic analyses

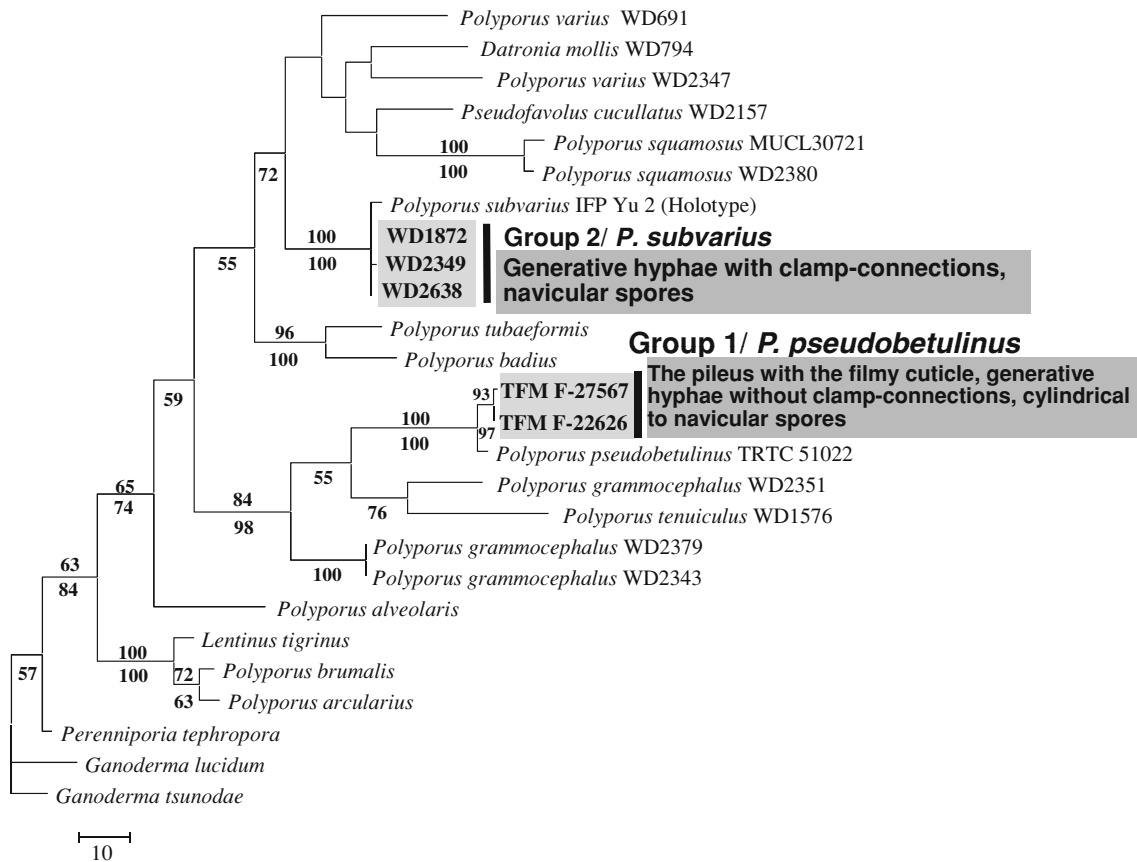
The ILD test showed no significant incongruence between the nucLSU and 5.8S-ITS2 datasets ( $P = 0.07$ ). The combined nucLSU and 5.8S-ITS2 dataset consisted of 1024 characters, of which 146 characters were phylogenetically informative for parsimony analysis. Four trees with equal MP in 458 steps [confidence interval (CI) = 0.49, RI = 0.71] were constructed by the MP analysis. The MP tree and ML tree showed similar topologies and no inconsistency in any supported clades. The resulting topology is represented in Fig. 1. In the phylogenetic tree, Japanese '*P. pseudobetulinus*' collections were divided into two clades labeled as Group 1 and Group 2 (Fig. 1). Group 1 and Canadian *P. pseudobetulinus* formed a distinct monophyletic group with a strong support (MPBT 100%; MLBT 100%) and comprised a single clade with *P. grammocephalus* Berk. and *P. tenuiculus* (P. Beauv.) Fr. (MPBT 84%; MLBT 98%). Group 2 and the holotype of *P. subvarius* formed a strongly supported clade (MPBT 100%; MLBT

100%), which included the clade containing *Datronia mollis* (Sommerf.) Donk, *P. varius* (Pers.) Fr. and *Pseudofavolus cucullatus* (Mont.) Pat. (MLBT 72%).

### Characterization of the two clades

Specimens included in Group 1 share a brownish-orange to yellowish-brown pileus covered by a filmy cuticle, generative hyphae without clamp-connections, and middle-sized and cylindrical to slightly navicular basidiospores ( $7.2\text{--}10.8 \times 2.6\text{--}3.7 \mu\text{m}$ ). A specimen of *Polyporus pseudobetulinus* from Canada (TRTC 51022) clustering with Group 1 has slightly larger basidiospores ( $8.8\text{--}11.6 \times 2.8\text{--}3.6 \mu\text{m}$ ), but other morphological characters agree with those of Group 1.

Group 2 included specimens with a pileus lacking a filmy cuticle, generative hyphae with clamp-connections, and large and navicular basidiospores ( $10\text{--}14 \times 4\text{--}6 \mu\text{m}$ ). Basidiocarps of the type of *P. subvarius* are imbricate, whereas those of Group 2 are solitary, but the other morphological characteristics are identical. Additionally, they commonly occur only on *Salix* spp. Consequently, we



**Fig. 1** One of the four equally parsimonious trees obtained from nuclear large-subunit (nucLSU) and 5.8S-internal transcribed spacer 2 (5.8S-ITS2) sequences. Support values above branches are from

maximum parsimony bootstrap searches, and values below branches are from bootstrap analyses with PhyML 3.0

concluded that the appropriate species names for Group 1 and Group 2 are *P. pseudobetulinus* and *P. subvarius*, respectively.

## Taxonomy

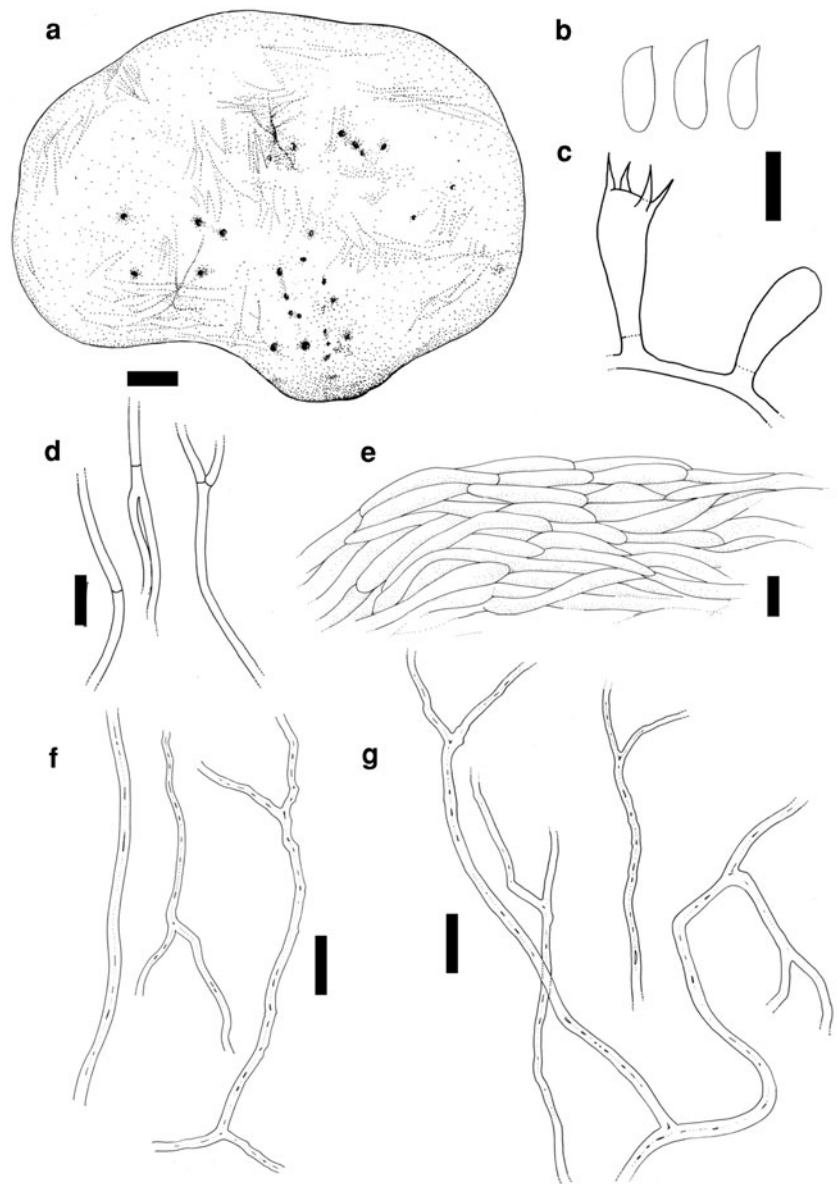
*Polyporus pseudobetulinus* (Murashk. ex Pilát) Thorn, Kotir. & Niemelä, Mycologia 82:583, 1990

### Fig. 2

Basidiocarps annual, attached by a short, rounded and undifferentiated lateral stipe to almost sessile, solitary. Pileus dimidiate, semicircular to orbicular, convex, up to 7.5 cm from the base to margin, up to 10.5 cm in width; surface glabrous, covered by smooth to slightly wrinkled and filmy and easily separable cuticle, brownish orange to yellowish brown (10YR6–8/4–8) in dry condition, occasionally pellicle breaks up and forms small and appressed scales, azonate; margin acute, entire, incurved in dry condition. Stipe, if present, up to 15 mm long, up to 25 mm thick, usually covered with pores on underside. Context fleshy in

fresh condition, corky upon drying, white to cream (10YR9/2–4), up to 2.5 cm thick. Pore surface brownish orange to grayish brown (10YR5–7/4–8), pores angular, 1–2/mm, dissepiments thin, entire to slightly lacerate. Tubes concolorous with context, up to 5 mm deep. Hyphal system dimitic with generative hyphae and skeletal binding hyphae. Contextual generative hyphae thin-walled, hyaline, without clamp-connections, 3–4(–5)  $\mu$ m in diameter. Contextual skeletal binding hyphae dominant, thick-walled to solid, occasionally to moderately branched, interwoven, hyaline, non-dextrinoid, 3.5–5  $\mu$ m in diameter at basal stalk, tapering to 1.5–2  $\mu$ m in diameter. Tramal generative hyphae, without clamp-connections, thin-walled, hyaline, (2–)2.5–4.5  $\mu$ m in diameter. Tramal skeletal binding hyphae dominant, thick-walled to solid, moderately branched, interwoven, non-dextrinoid, 3.5–5  $\mu$ m in diameter at basal stalk, taper to 1.7  $\mu$ m in diameter at apex. Pileipellis 85–140  $\mu$ m thick, a cutis of parallel hyphae easily squashed in KOH; hyphae short-celled, thin-walled, yellow to hyaline, non-dextrinoid, 6–7.5  $\mu$ m in diameter. Basidia clavate, 4-sterigmate, 17.5–26  $\times$  4–7  $\mu$ m. Basidiospores cylindrical to slightly

**Fig. 2** *Polyporus pseudobetulinus* (TFM F-27625). **a** Basidiocarp, **b** basidiospores, **c** basidium and basidiole, **d** generative hyphae from trama, **e** cutis of parallel hyphae, **f** skeleto-binding hyphae from context, **g** skeleto-binding hyphae from trama. Bars: (a) 1 cm, (b–e) 10  $\mu$ m, (f, g) 20  $\mu$ m



navicular, hyaline, non-dextrinoid,  $(6.8\text{--}7.2\text{--}11.6 \times 2.6\text{--}3.7\text{--}4) \mu\text{m}$ ,  $L = 9.35 \mu\text{m}$ ,  $W = 3.14 \mu\text{m}$ ,  $R = 2.3\text{--}3.6\text{--}3.8$ ,  $r = 3$  ( $n = 198/5$ ).

**Substrates** This species is to date restricted to *Salix* spp. in Japan, while it grows on huge trunks of *Populus* spp. in Europe and North America (Thorn et al. 1990) except for a limited occurrence on *Salix* in Sweden (Schigel and Toresson 2005).

**Distribution** Reported from Canada in North America (Thorn et al. 1990; Boulet 2003), China, Finland, Sweden, and Russia from the Eurasian continent (Bondartsev 1953; Domański et al. 1967; Thorn et al. 1990; Schigel and Toresson 2005; Dai et al. 2010). In Japan, this species is

restricted to some localities within the Taisetsu Mountains of Hokkaido.

**Specimens examined** Canada, Ontario, Ottawa-Carleton Co., on dead standing and fallen trunks of *Populus balsamifera* (fallen trunks with fruiting bodies had fallen this spring) in wooded swamp with dead *Ulmus*, R.G. Thorn, 19 June 1988 (TRTC 51022); Ontario, Dufferin Co., on dead *P. balsamifera*, J.R. Parkin, 24 June 1987 (TRTC 51026); Ontario, Durham Co., on dead standing *P. balsamifera* in wet woods., R.G. Thorn, 28 June 1987 (TRTC 51028); Ontario, Grey Co., on dead *P. balsamifera*, J. R. Parkin, 24 June 1987 (TRTC 51029); Ontario, Grey Co., R.G. Thorn, 23 May 1987 (TRTC 51031). Japan, Hokkaido, Kamikawa, Ginsendai, on *Salix* sp. A. Horoya, 2 July 1989 (TFM

F-16136); the same place, on *Salix* sp., S. Sato, 2 Aug. 2003 (TFM F-27567); Hokkaido, Kamikawa, Akadake, on *Salix* sp., S. Sato and A. Horoya, 1 July 1989 (TFM F-27625); the same place, 900 m a.s.l., on dead *Salix* sp., S. Sato, 7 July 1991 (TFM F-27626).

**Remark** The holotype of *P. pseudobetulinus* (PRM 603630) was not available at the National Museum, Prague, Czech Republic (PRM). All Canadian specimens examined here were previously examined together with the holotype by Thorn et al. (1990). Núñez and Ryvar den (1995b) erroneously cited a specimen of *P. subvarius* (TFM F-16868) as a new record of *P. pseudobetulinus* from Japan. *Polyporus pseudobetulinus* is distinct from other *Polyporus* species by the pseudostipitate basidiocarps that almost lack stipes, a filmy cuticle on the pileus surface, simple septate generative hyphae, and slightly navicular basidiospores.

***Polyporus subvarius*** C.J. Yu & Y.C. Dai, Ann. Bot. Fennici. 44:142, 2007

### Fig. 3

Basidiocarps annual, laterally stipitate (attached to the substrate by a narrow base), solitary or imbricate. Pileus dimidiate to circular, usually convex, 5.7–15 cm in diam, up to 2.3 cm thick; surface glabrous, radially striate, yellowish brown (10YR5–6/4–8) in fresh condition, color unchanged in dried condition, often with dark brown (10YR4/4) spots, azonate; margin acute, entire (undulating according to the original description), slightly incurved when dry. Stipe up to 10 mm long, up to 23 mm thick in Japanese specimens (2.5 cm long, 1.5 cm thick according to the original description), partly covered with a black crust. Context fleshy to tough fleshy in fresh condition, drying hard corky, white to cream (10YR9/4), up to 2.3 mm thick. Pore surface light brown to grayish brown (10YR6/4) in fresh condition, drying dark brown (10YR3–4/2–4), pores angular, 2–4/mm, dissepiments thin, entire to slightly lacerate. Tubes yellowish brown (10YR6–8/4–8), up to 1.5 mm deep in Japanese specimens (5 mm according to the original description) deep. Hyphal system dimitic with generative hyphae and skeletal binding hyphae. Contextual generative hyphae thin-walled, hyaline, with clamp-connections, 2.5–5 µm in diameter. Contextual skeletal binding hyphae thick-walled, moderately branched, interwoven, hyaline, non dextrinoid, 3–8 µm in diameter at basal stalk. Tramal generative hyphae same as in context. Tramal skeletal binding hyphae dominant, thick-walled to solid, moderately branched, interwoven, hyaline, non-dextrinoid, (2–)2.5–6.5 µm in diameter. Pileipellis 24–40 µm thick, a cutis of parallel hyphae not easily squashed in KOH solution; hyphae thin-walled, yellow to brown, non-dextrinoid, with clamp-connections,

7–9.2 µm in diameter. Basidia clavate, 4-sterigmate, 20–26 × 6–8(–10) µm. Basidiospores navicular, hyaline, non-dextrinoid, (9.6–)10–14.8 × 4–6 µm, L = 11.9 µm, W = 4.8 µm, R = 2–3.2, r = 2.5 (n = 152/3).

**Substrates** On living and dead *Salix* spp.

**Distribution** Currently known from one locality in Tibet (China) and restricted areas of the Taisetsu Mountains in Hokkaido (Japan).

**Specimens examined** China, Xizang Autonomous Region (Tibet), Lasha, Luobulinka Park, on living tree of *Salix* sp., 2 Aug. 2004 (HOLOTYPE; IFP Yu 2). Japan, Hokkaido, Kamikawa, Sounkyo, on *Salix* sp., S. Sato, 28 Aug. 1993 (TFM F-16868); Hokkaido, Kamikawa, Ginsendai, on *Salix* sp., S. Sato, 12 Aug 2003 (TFM F-21701); Hokkaido, Shintoku, Tomuraushi, H. Shirayama, 23 Sep. 2007 (TFM F-27548).

**Cultural characters** Growth extremely slow, 0.2–0.3 mm/day. Advancing zone even, appressed, some mycelia submerged in the agar, brown (10YR3–5/4). Mat at first light yellow (10YR9/4), then light brown (10YR5–7/4) to dark brown (10YR2–4/2–4), aerial mycelium woolly to flat, mostly light brown (10YR5–7/4) to dark brown (10YR2–4/2–4), felty to velvety. Reverse brown (10YR4–5/4–8). Odor none. Hymenophore development not seen within 6 weeks. Generative hyphae from advancing zone thin-walled, with clamp-connections light brown, 2.5–4 µm in diameter. Generative hyphae from aerial mycelium thin- to thick-walled, with clamp-connections, hyaline to brown, 3–5 µm in diameter. Fiber hyphae not seen. Chlamydo spores absent.

Extracellular peroxidase activities; 1-naphthol, +; tyrosine, –.

**Species code** 2, 3, 37, 39, 47, 53, 54.

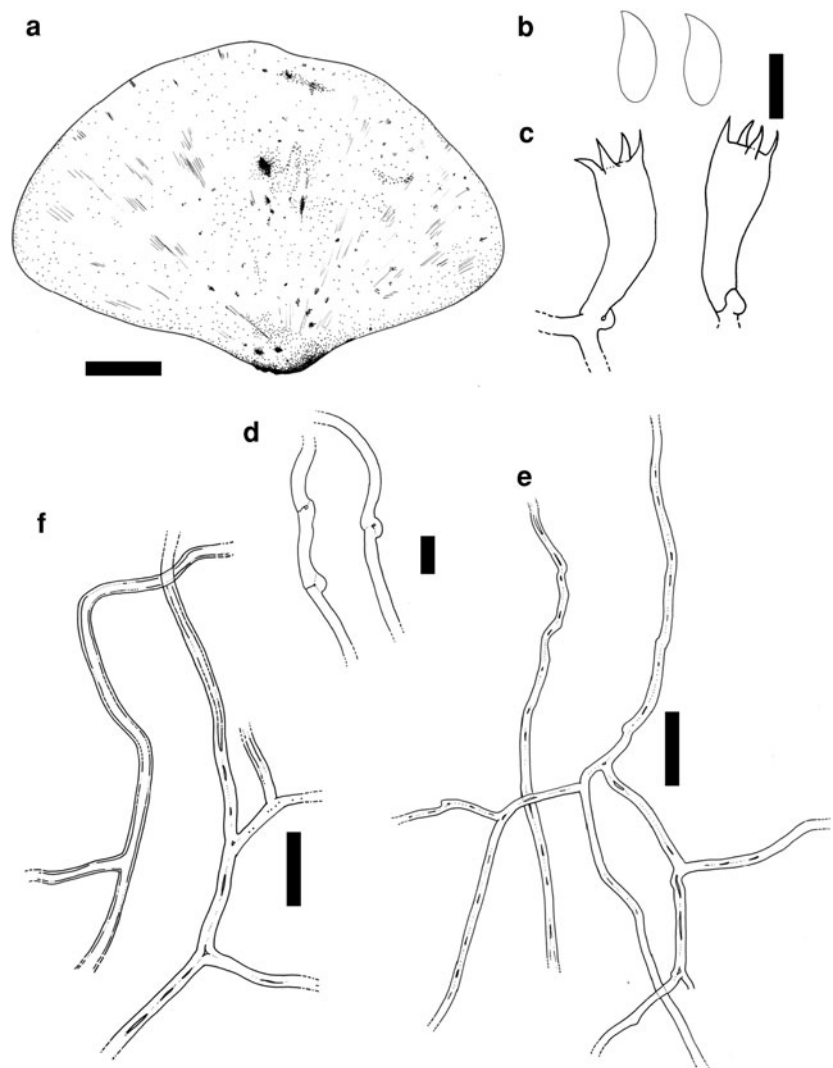
**Cultures examined** WD1872, isolated from tissue of basidiocarp, TFM F-16868.

**Remarks** This species is characterized by the circular to dimidiate basidiocarps with a short lateral stipe and navicular basidiospores. The basidiocarps are variable in shape.

## Discussion

The results of this taxonomic study revealed that the specimens referred to as '*Polyporus pseudobetulinus*' in Japan actually represent two distinct species, *P. pseudobetulinus* and *P. subvarius*. They are distributed in the same subalpine area in Hokkaido exclusively on *Salix* spp. and produce basidiocarps from August to September. Their macro-morphology, distribution, and host are similar, but

**Fig. 3** *Polyporus subvarius*. **a** Basidiocarp (TFM F-21701), **b** basidiospores (TFM F-27548), **c** basidia (TFM F-27548), **d** generative hyphae from trama (TFM F-27548), **e** skeleto-binding hyphae from context (TFM F-27548), **f** skeleto-binding hyphae from trama (TFM F-27548). Bars: (a) 1 cm, (b–d) 10  $\mu$ m, (e, f) 20  $\mu$ m



*P. pseudobetulinus* is distinct from *P. subvarius* by the thin and filmy and easily separable cuticle on the pileus surface, the absence of clamp-connections on generative hyphae, and the smaller and less navicular basidiospores. Furthermore, contextual skeletal binding hyphae of *P. pseudobetulinus* are less branched than those of *P. subvarius*. The present phylogenetic tree based on the combined data of the nucLSU and ITS regions also showed that they are phylogenetically distinct species.

*Polyporus pseudobetulinus* was included in the clade with *P. grammacephalus* and *P. tenuiculus*, which are mainly distributed in tropical areas. *P. pseudobetulinus* was placed in group Admirabilis, while *P. grammacephalus* and *P. tenuiculus* were in group Favolus (Núñez and Ryvarden 1995a), but all of these species commonly produce similar basidiocarps with a pileus lacking distinctive scales and a lateral and often inconspicuous stipe without a crust (Núñez and Ryvarden 1995a). The lack of clamp-connections is one of the distinctive characteristics of

*P. pseudobetulinus*. De (1996) considered the lack of a clamp-connection to be a genus-level characteristic and described *Royoporos* A.B. De to accommodate ‘*Polyporus*’ spp. without clamp-connections. In a later publication, De (1998) proposed the combination *Royoporos pseudobetulinus* (Murashk. ex Pilát) A.B. De. However, the lack of a clamp-connection is also seen in *P. badius* (Pers.) Schwein. and in one collection of *P. grammacephalus* (WD2351) (Sotome et al. 2007). *P. badius* is phylogenetically related to *P. dictyopus* Mont., *P. melanopus* (Pers.) Fr. and *P. tubaeformis* (P. Karst.) Ryvarden & Gilb., both of which have clamp-connections on generative hyphae and similar ecology (Dai 1996; Krüger et al. 2006; Sotome et al. 2007). Our study showed that *P. pseudobetulinus* is also related to *P. tenuiculus* with clamp-connections. Thus, the lack of clamp-connections can be considered to be a valid characteristic only at the species level among *Polyporus* spp.

*Polyporus subvarius* is characterized by a circular to dimidiate basidiocarp with a short lateral stipe and

navicular and middle- to large-sized basidiospores. Dai et al. (2007b) reported it as a pathogenic fungus for *Salix*, but all of the Japanese materials were on dead trees of *Salix* spp., and these authors stated that this species forms imbricate basidiocarps up to 15 cm with a short stipe, whereas those of Japanese collections are always solitary, smaller (up to 7.2 cm), and attached to the substrates by a narrow and round base. Despite the specimens' different macro-morphology, we conclude that they are conspecific, with similar micro-morphology and sequences, and we suggest that this is a variable species with regard to the shape of basidiocarps.

Phylogenetic analyses showed that *P. subvarius* is included in the same clade as *Datronia mollis*, *Pseudofavolus cucullatus*, and two other *Polyporus* species, *P. varius* and *P. squamosus*. This clade corresponds with Clade 1 detected by Sotome et al. (2008). Species included here together with those in Clade 1 (Sotome et al. 2008) have basidiocarps covered by a crust on the pileus and/or part of the stipe, and a dimitic hyphal system with skeletal binding hyphae and cylindrical basidiospores, but these species are morphologically variable, including *D. mollis* with effused–reflexed to resupinate basidiocarps, dark-brown context, and dark-brown tomentose to hispid pileus.

*Polyporus pseudobetulinus* is considered to be an endangered species in China, Finland, and Sweden (Schigel and Toresson 2005; Dai et al. 2010). *P. pseudobetulinus* is also very rare in Japan, with a restricted distribution in subalpine areas of one region, and the species is red-listed in the Japan Integrated Biodiversity Information System ([http://www.biodic.go.jp/rdb/rdb\\_f.html](http://www.biodic.go.jp/rdb/rdb_f.html)). *P. subvarius* shows a similar host range and distribution pattern in Japan and should also possibly be red-listed because their existing localities can be easily lost due to climate changes and/or deforestation.

**Acknowledgments** We would like to thank Ms. H. Shirayama and Mr. S. Sato for kindly providing specimens. We are grateful to Dr. J.-M. Moncalvo (Natural Mycological Herbarium of Canada, DAOM) and Prof. Y.-C. Dai (Beijing Forestry University) for the loan of Canadian specimens of *P. pseudobetulinus* and the type of *P. subvarius*, respectively. We are also grateful to Drs. T. Hosoya and K. Hosaka (National Museum of Nature and Science, Tokyo) and T. Kasuya (University of Tsukuba) for their kind help with this study. This study was supported in part by a research grant of the Institute for Fermentation, Osaka, Japan.

## References

- Bondartsev AS (1953) The Polyporaceae of the European USSR and Caucasia (translated from Russian). Israel Program for Scientific Translation, Jerusalem
- Boulet B (2003) Les champignons des arbres de l'est de l'Amérique du Nord. Les Publications du Québec, Québec
- Bradley RK, Roberts A, Smoot M, Juvekar S, Do J, Dewey C, Holmes I, Pachter L (2009) Fast statistical alignment. *PLoS Comput Biol* 5(5):e1000392
- Dai YC (1996) Changbai wood-rotting fungi 5. Study on *Polyporus mongolicus* and *P. tubaeformis*. *Annales Botanici Fennici* 33:153–163
- Dai Y-C, Yu C-J, Wang H-C (2007a) Polypores from eastern Xizang (Tibet), western China. *Ann Bot Fennici* 44:135–145
- Dai Y-C, Cui B-K, Yuan H-S, Li B-C (2007b) Pathogenic wood-decaying fungi in China. *For Pathol* 37:105–120
- Dai Y-C, Cui B-K, Yuan H-S, Wei Y-L (2010) A red list of polypores in China (in Chinese). *Mycosystema* 29:164–171
- De AB (1996) *Royoporus*—a new genus for *Favolus spathulatus*. *Mycotaxon* 60:143–148
- De AB (1998) Taxonomy of *Royoporus pseudobetulinus* comb. nov. *Mycotaxon* 69:137–143
- Domański S, Orłowski H, Skirgiełło A (1967) Fungi. Polyporaceae II (pileatae) Mucronoporaceae II (pileatae) Ganodermataceae, Bondarzewiaceae, Boletopsidaceae, Fistulinaceae (Grzyby), revised edn (translated from Polish). Foreign Scientific Publications Department of the National Center for Scientific, Technical, and Economic Information, Warsaw
- Farris JS, Källersjö M, Kluge AG, Bult C (1994) Testing significance of incongruence. *Cladistics* 10:315–319
- Gilbertson RL, Ryvarden L (1987) North American polypores, vol 2. *Fungiflora*, Oslo
- Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52:696–704
- Holmgren PK, Holmgren NH, Barnett LC (1990) Index herbariorum part 1: the herbaria of the world, 8th edn. New York Botanical Garden, New York
- Käärik A (1965) The identification of the mycelia of wood-decay fungi by their oxidation reactions with phenolic compounds. *Biblioth Mycol* 81:1–151
- Krüger D, Petersen RH, Hughes KW (2006) Molecular phylogenies and mating study data in *Polyporus* with special emphasis on group “*Melanopus*” (Basidiomycota). *Mycol Prog* 5:185–206
- Nobles MK (1965) Identification of cultures of wood-inhabiting Hymenomycetes. *Can J Bot* 43:1097–1139
- Núñez M, Ryvarden L (1995a) *Polyporus* (Basidiomycotina) and related genera. *Synopsis Fungorum* 10:1–85
- Núñez M, Ryvarden L (1995b) *Polyporus* new to Japan 1. Species of *Polyporus*, with a note on *P. hartmanni*. *Mycoscience* 36:61–65
- Schigel DS, Toresson HG (2005) New records of *Polyporus pseudobetulinus*, a rare polypore fungus (Basidiomycota, Aphyllophorales) in Scandinavia, and notes on associated beetles. *Mem Soc Pro Fauna Flora Fennica* 81:102–107
- Sotome K, Hattori T, Makoto K (2007) *Polyporus phyllostachydis* sp. nov. with notes on other rhizophilic species of *Polyporus* (Basidiomycota, Polyporaceae). *Mycoscience* 48:42–46
- Sotome K, Hattori T, Ota Y, To-anun C, Salleh B, Kakishima M (2008) Phylogenetic relationships of *Polyporus* and morphologically allied genera. *Mycologia* 100:603–615
- Sotome K, Hattori T, Ota Y, Kakishima M (2009) Second report of *Polyporus longiporus* and its phylogenetic position. *Mycoscience* 50:415–420
- Swofford DL (2003) PAUP 4.0b10: phylogenetic analysis using parsimony. Sinauer Assoc, Sunderland
- Thorn G, Kotiranta H, Niemelä T (1990) *Polyporus pseudobetulinus* comb. nov.: new records in Europe and North America. *Mycologia* 82:582–594