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FULL PAPER

Morphological and molecular evidence for a new species of *Perenniporia* (Basidiomycota) from Tibet, southwestern China

Bao-Kai Cui · Chang-Lin Zhao

Abstract A new polypore, *Perenniporia tibetica*, collected in Xizang (Tibet), southwestern China, is described and illustrated on the basis of morphological and molecular characters. *Perenniporia tibetica* is characterized by an annual growth habit, resupinate basidiocarps with a cream-buff to pinkish-buff pore surface and white to cream-colored rhizomorphs, a dimitic hyphal system with slightly dextrinoid and distinctly cyanophilous skeletal hyphae, and basidiospores that are ellipsoid, truncate or not, strongly dextrinoid, and cyanophilous, 6.7–8.7 × 5.3–6.8 µm. Its preliminary phylogenetic relationships are inferred based on sequence data from the ribosomal ITS and LSU regions, both suggesting that *P. tibetica* forms a distinct lineage in the genus *Perenniporia*.

Keywords Molecular phylogeny · Polypore · Taxonomy · White-rot fungi

Introduction

Perenniporia Murrill is a large, cosmopolitan genus, typified by *Polyprus medulla-panis* Jacq.: Fr. According to the modern definition, it is characterized by ellipsoid to distinctly truncate basidiospores, which are usually thick walled, having a cyanophilous and variable dextrinoid reaction; its hyphal structure is dimitic to trimitic with clamp connections on generative hyphae, and its vegetative hyphae are cyanophilous and variably dextrinoid (Decock and Stalpers 2006). *Loweporus* J.E. Wright was typified by *Polyporus lividus* Kalchbr.; it is closely related to *Perenniporia*, and the species differs from *Perenniporia* by having brownish basidiocarps. *Abundisporus* Ryvarden is another genus closely related to *Perenniporia*; it was established (Ryvarden 1998) to include species with colored and non-dextrinoid spores. Parmasto and Hallenberg (2000) made a detailed discussion of *Abundisporus*, *Loweporus*, and *Perenniporia*, and their opinion was that it was difficult to differentiate the three genera. Species in *Perenniporia* grow on living or dead hardwoods and conifers, are usually found in virgin forests, and all species in the genus are lignicolous and cause a white rot. Up to now, about 90 species have been described or transferred to the genus (Gilbertson and Ryvarden 1987; Ryvarden and Gilbertson 1994; Decock and Ryvarden 1999, 2000, 2003; Hattori and Lee 1999; Decock and Figueroa 2000; Decock et al. 2000, 2001; Decock 2001; Núñez and Ryvarden 2001; Dai et al. 2002; Cui et al. 2007).

Knowledge of *Perenniporia* in China was summarized by Dai et al. (2002). Since then, several new species and new records in the genus were discovered in China, and presently 30 species have been recorded from the country (e.g., Dai et al. 2003, 2004, 2007; Cui et al. 2006, 2007, 2008, 2010; Dai and Penttilä 2006; Xiong et al. 2008; Wang et al. 2009).

During an investigation of wood-inhabiting fungi in Tibet of southwestern China, an additional undescribed species of *Perenniporia* was found. To confirm the affinity of the new taxon and infer its evolutionary relationships among similar species of *Perenniporia*, phylogenetic analyses were performed based on internal transcribed spacer (ITS) and large subunit (LSU) sequences.

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Materials and methods

Morphological studies

The specimens studied are deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Macro-morphological descriptions are based on the field notes. Special color terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens, which were observed under a light microscope. The microscopic routine used in the study follows Dai (2010a). Sections were studied at magnifications up to ×1,000 using a Nikon Eclipse E 80i microscope and phase-contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic features, measurements, and drawings were made from slide preparations stained with cotton blue and Melzer's reagent. Spores were measured from sections cut from the tubes. In presenting the variation in the size of the spores, 5% of measurements were excluded from each end of the range and are given in parentheses. In the text, the following abbreviations are used: KOH = 5% potassium hydroxide, CB = cotton blue, CB+ = cyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, and n (a/b) = number of spores (a) measured from given number (b) of specimens.

Phylogenetic analysis

DNA isolation, polymerase chain reaction, and cycle sequencing analysis

The fungal taxa used in this study are listed in Table 1. The Phire Plant Direct PCR Kit (Finnzymes) procedure was used to extract total genomic DNA from the fruit bodies and to perform polymerase chain reaction (PCR). In the study, nuclear ribosomal RNA genes were used to determine the phylogenetic position of the new species. The internal transcribed spacer (ITS) regions were amplified with the primers ITS4 and ITS5 (White et al. 1990; Gardes and Bruns 1993), and the large subunit (nLSU) with the primers LROR and LR7 (Hopple and Vilgalys 1999). PCR amplification was confirmed on 1% agarose electrophoresis gels stained with ethidium bromide (Stöger et al. 2006). DNA sequencing was performed at Beijing Genomics Institute. All newly generated sequences have been submitted to GenBank and are listed in Table 1.

Sequence and phylogenetic analysis

Sequences were aligned with additional sequences from GenBank (Table 1) using BioEdit (Hall 1999) and ClustalX (Thomson et al. 1997). Alignment was manually adjusted to allow maximum alignment and minimize gaps. Sequence alignment was deposited at TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S12012?x-access-code=1ab94117bd2d22fb975ae1edc0980fee&format=html).

Maximum parsimony and Bayesian analysis were applied to the ITS and nLSU datasets. All characters were equally weighted, and gaps were treated as missing data. Maximum parsimony analysis was performed using PAUP* ver. 4.0b10 (Swofford 2002). Maximum parsimony trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max-trees were set to 5,000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for all trees generated under different optimality criteria. Bayesian analysis with MrBayes3.1.2 (Ronquist and Huelsenbeck 2003) implementing the Markov Chain Monte Carlo (MCMC) technique and parameters predetermined with MrMODELTEST2.3 (Posada and Crandall 1998; Nylander 2004) was performed, and the parameters in MrBayes were set as follows: lset nst = 6, and rates = gamma. Four simultaneous Markov chains were run with 2,000,000 generations, starting from random trees, and keeping one tree every 1,000th generation.

Results

Taxonomy

Perenniporia tibetica B.K. Cui & C.L. Zhao, sp. nov. Fig. 1

MycoBank no.: MB 561648

Basidiocarpium annuum, resupinatum. Facies pororum cremea, cremeo-bubalina vel roseo-bubalina; pori angulati, 2–3 per millimeter; marginatum rhizomorphum. Systema hypharum dimiticum, hyphae generatoriae fibulatae, hyphae skeletales subiculi 1.6–3.5 µm in diametro. Sporae hyalinae, ellipsoideae vel truncatae, dextrinoideae, CB+, 6.7–8.7 × 5.3–6.8 µm.

Typus: China. Xizang Autonomous Region (Tibet), Linzhi County, Tongmai, on fallen angiosperm trunk, 16.IX.2010 Cui 9457 (Holotypus in BJFC).

rDNA sequence ex holotype: JF 706326.

Etymology: *tibetica* (Lat.): referring to the locality of the type specimen.

Basidiocarps annual, resupinate, adnate, not easily separated from substrate, corky, without odor or taste when

Table 1 Species and sequences database accession numbers used in this study

Fungal taxon	Species no.	GenBank no.	
		ITS	LSU
Perenniporia corticola (Corner) Decock	Cui 1248	HQ848472	HQ848482
P. corticola	Dai 7330	HQ654094	HQ654108
P. corticola	Cui 2655	HQ654093	HQ848483
P. detrita (Berk.) Ryvarden	MUCL 42649	FJ411099[a]	FJ393866[a]
P. fergusii Gilb. & Ryvarden	Gilbertson 16116	HQ876607	–
P. fraxinea (Bull.) Ryvarden	Cui 7154	HQ654095	HQ654110
P. fraxinea	Cui 8871	JF706329	JF706345
P. fraxinea	Cui 8885	HQ876611	JF706344
P. fraxinea	DP 83	AM269789[a]	AM269853[a]
P. japonica (Yasuda) T. Hatt. & Ryvarden	Cui 7047	HQ654097	HQ654111
P. latissima (Bres.) Ryvarden	Cui 6625	HQ876604	JF706340
P. maackiae (Bondartsev & Ljub.) Parmasto	Cui 8929	HQ654102	JF706338
P. maackiae	Cui 5605	–	JN048780
P. martia (Berk.) Ryvarden	MUCL 41677	FJ411092[a]	FJ393859[a]
P. martia	MUCL 41678	FJ411093[a]	FJ393860[a]
P. martia	Cui 7992	HQ876603	HQ654114
P. medulla-panis (Jacq.) Donk	Dai 10780	HQ654099	JF713023
P. medulla-panis	MUCL 49581	FJ411088[a]	FJ393876[a]
P. minor Y.C. Dai & H.X. Xiong	Cui 5782	HQ883475	–
P. minor	Cui 5738	HQ848475	HQ848485
P. narymica (Pilát) Pouzar	Dai 10510	HQ654101	JF706346
P. narymica	Dai 7016	JF706331	JF706347
P. ochroleuca (Berk.) Ryvarden	Dai 11486	HQ654105	JF706349
P. ochroleuca	MUCL 39563	FJ411097[a]	FJ393864[a]
P. ochroleuca	MUCL 39726	FJ411098[a]	FJ393865[a]
P. ohiensis (Berk.) Ryvarden	MUCL 41036	FJ411096[a]	FJ393863[a]
P. ohiensis	Cui 5714	HQ654103	HQ654116
P. piceicola Y.C. Dai	Dai 4184	JF706328	JF706336
P. pyricola Y.C. Dai & B.K. Cui	Cui 9149	–	JN048782
P. pyricola	Dai 10265	–	JN048781
P. rhizomorpha B.K. Cui et al.	Cui 7507	HQ654107	–
P. rhizomorpha	Dai 7248	JF706330	–
P. robiniophila (Murrill) Ryvarden	Cui 5644	HQ876609	JF706342
P. robiniophila	Cui 7144	HQ876608	JF706341
P. robiniophila	Cui 9174	HQ876610	JF706343
P. straminea (Bres.) Ryvarden	Cui 8718	HQ876600	JF706335
P. straminea	Cui 8858	HQ654104	JF706334
P. subacida (Peck) Donk	Dai 8224	HQ876605	JF713024
P. subacida	Cui 3643	FJ613655	AY336753
P. subacida	MUCL 31402	FJ411103[a]	AY333796[a]
P. tenuis (Schwein.) Ryvarden	Cui 5523	HQ848474	HQ848480
P. tephropora (Mont.) Ryvarden	Cui 9029	HQ876601	JF706339
P. tephropora	Cui 6331	HQ848473	HQ848484
P. tibetica B.K. Cui & C.L. Zhao	Cui 9459	JF706327	JF706333
P. tibetica	Cui 9457	JF706326	JF706332
P. truncatospora (Lloyd) Ryvarden	Dai 5125	HQ654098	HQ848481
Trametes versicolor (L.) Lloyd	M 126	HM595570[a]	HM595617[a]

[a] Sequence downloaded from GenBank

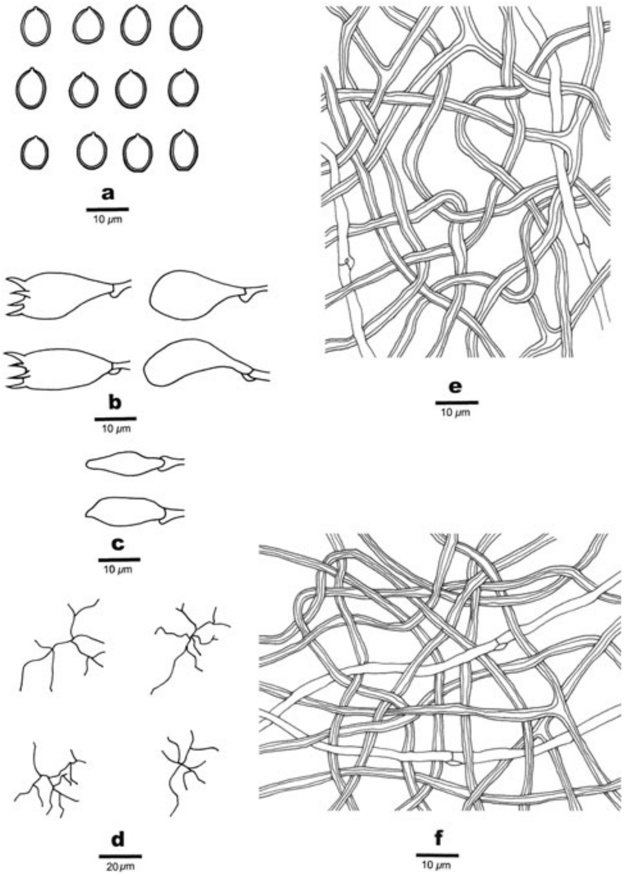

Fig. 1 Microscopic structures of *Perenniporia tibetica* (drawn from the holotype). **a** Basidiospores. **b** Basidia and basidioles. **c** Cystidioles. **d** Vegetative hyphae. **e** Hyphae from trama. **f** Hyphae from subiculum

fresh, becoming hard corky upon drying, up to 15 cm long, 5 cm wide, and 3 mm thick at center. Pore surface cream to cream buff when fresh, cream buff to pinkish buff upon drying; pores angular, 2–3 per millimeter; dissepiments thin, entire. Sterile margin distinct, white to cream, up to 1 mm wide, usually with white to cream-colored rhizomorphs. Subiculum cream, thin, up to 0.5 mm thick. Tubes concolorous with pore surface, hard corky, up to 2.5 mm long. Hyphal system dimitic; generative hyphae with clamp connections; skeletal hyphae weakly dextrinoid, CB+; hyphae unchanged in KOH. Generative hyphae in subiculum infrequent, hyaline, thin walled, usually unbranched, 1.5–3.2 µm in diameter; subicular skeletal hyphae dominant, hyaline, thick walled with a wide to narrow lumen, occasionally branched, interwoven, 1.6–3.5 µm in diameter; vegetative hyphae as arboriform skeletal hyphae present, hyaline, frequently branched, very thin, 0.1–0.8 µm in diameter. Tramal generative hyphae infrequent, hyaline, thin walled, unbranched, 1.5–2.5 µm in diameter; skeletal hyphae in trama dominant, hyaline, thick walled with a wide to narrow lumen, occasionally branched, interwoven, 1.2–3.6 µm in diameter. Cystidia absent; fusoid cystidioles present, hyaline, thin walled, 17–23 × 5–8.5 µm; basidia barrel shaped to capitate, with four sterigmata and a basal clamp connection, 15–25 × 8–12 µm; basidioles dominant, in shape similar to basidia, but slightly smaller. Basidiospores ellipsoid, truncate or not, hyaline, thick walled, smooth, dextrinoid, CB+, (6–)6.7–8.7(–9) × (5–)5.3–6.8(–7) µm, $L = 7.61$ µm, $W = 5.98$ µm, $Q = 1.23$–1.33 ($n = 60/2$).

Type of rot: White rot.

Known distribution: Linzhi, Xizang Autonomous Region (Tibet), China.

Additional specimen examined (paratype): China. Xizang Autonomous Region (Tibet), Linzhi County, Tongmai, on fallen angiosperm trunk, 16.IX.2010 Cui 9459 (BJFC).

Molecular phylogeny

The ITS dataset included sequences from 44 fungal specimens representing 23 taxa. The dataset had an aligned length of 579 base pairs with 332 parsimony-informative positions. Parsimony analysis yielded one parsimonious tree (TL = 505, CI = 0.491, RI = 0.766, RC = 0.376, HI = 0.509). Bayesian analysis resulted in average standard deviation of split frequencies = 0.006638 (Fig. 2).

The ITS + LSU dataset included sequences from 43 fungal specimens representing 22 taxa. The dataset had an aligned length of 2,064 base pairs with 394 parsimony-informative positions. Parsimony analysis yielded two parsimonious trees (TL = 779, CI = 0.519, RI = 0.768, RC = 0.398, HI = 0.481). Bayesian analysis resulted in average standard deviation of split frequencies = 0.002196 (Fig. 3). In phylogenetic reconstruction, the sequence of *Trametes versicolor* (L.) Lloyd obtained from GenBank was used as outgroup. The ITS and ITS + LSU strict consensus tree (Figs. 2, 3) generated by Bayesian analysis (Ronquist and Huelsenbeck 2003) and maximum parsimony analysis (Swofford 2002) showed sequences of *P. tibetica* were grouped together with other species of *Perenniporia* as a monophyletic cluster with strong support.

Discussion

The phylogenetic analysis showed that the two samples of *P. tibetica* formed a lineage phylogenetically distinct from other *Perenniporia* species (Figs. 2, 3). Both morphology and rDNA data confirmed that the two samples represent a new species in *Perenniporia*.

Perenniporia tibetica is characterized by an annual and resupinate basidiocarps with cream-buff to pinkish-buff pore surface and white to cream-colored rhizomorphs, a dimitic hyphal system with slightly dextrinoid and distinctly cyanophilous skeletal hyphae, and basidiospores

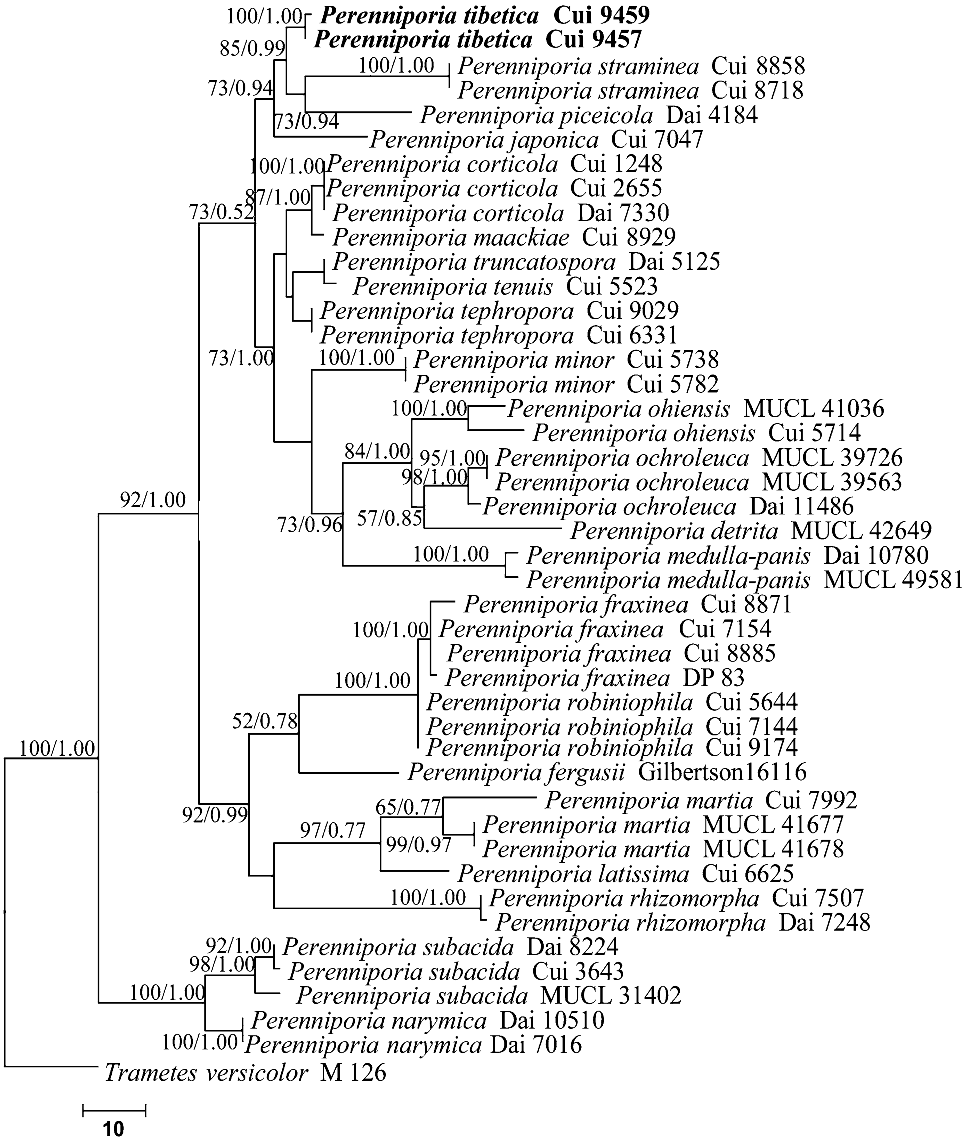

Fig. 2 Bayesian consensus tree illustrating the phylogeny of *Perenniporia tibetica* and related species generated by maximum parsimony and Bayesian analysis based on internal transcribed spacer (ITS) sequences. Parsimony bootstrap (before the *slash marks*) and Bayesian posterior probabilities (after the *slash marks*) greater than 50% are shown

that are ellipsoid, truncate or not, strongly dextrinoid, and cyanophilous, 6.7–8.7 × 5.3–6.8 µm.

Perenniporia tibetica in comparison with other *Perenniporia* species: *P. roseoisabellina* (Pat. & Gaillard) Ryvarden shares similar pores and basidiospores, but differs in its isabelline pore surface, nondextrinoid hyphae, distinctly truncate basidiospores, and lack of rhizomorphs (Ryvarden 1983). *Perenniporia subaurantiaca* (Rodway & Cleland) P.K. Buchanan & Ryvarden has resupinate basidiocarps and similar basidiospores, but differs in its pale brown pore surface, smaller pores (5–6 per millimeter), and lack of rhizomorphs (Buchanan and Ryvarden 1993). *Perenniporia pyricola* Y.C. Dai & B.K. Cui shares resupinate basidiocarps, cream-buff pore surface, and similar basidiospores, but it is distinguished by having perennial basidiocarps, smaller pores (3–5/per millimeter), and distinctly truncate basidiospores, and lacking rhizomorphs (Dai 2010b). *Perenniporia subacida* (Peck) Donk shares resupinate basidiocarps and a buff-colored pore surface (Núñez and Ryvarden 2001); however, it is distinguished by having perennial basidiocarps, smaller pores (4–6 per millimeter), and basidiospores (4.3–5.4 × 3.2–4.1 µm; Dai et al. 2002).

Five other *Perenniporia* species produce rhizomorphs at their margins, viz. *P. adnata* Corner (Corner 1989), *P. aurantiaca* (A. David & Rajchenb.) Decock & Ryvarden (Decock and Ryvarden 1999), *P. bambusicola* Choeyklin, T. Hatt. & E.B.G. Jones (Choeyklin et al. 2009), *P. japonica* (Yasuda) T. Hatt. & Ryvarden (Hattori and Ryvarden 1994; Núñez and Ryvarden 2001), and *P. rhizomorpha* B.K. Cui, Y.C. Dai & Decock (Cui et al. 2007). *Perenniporia adnata* has smaller and broadly ellipsoid to subglobose basidiospores (4–4.5 × 3.5 µm; Corner 1989), which separate it from *P. tibetica*. *Perenniporia aurantiaca* is distinguished by having a bright orange pore surface,

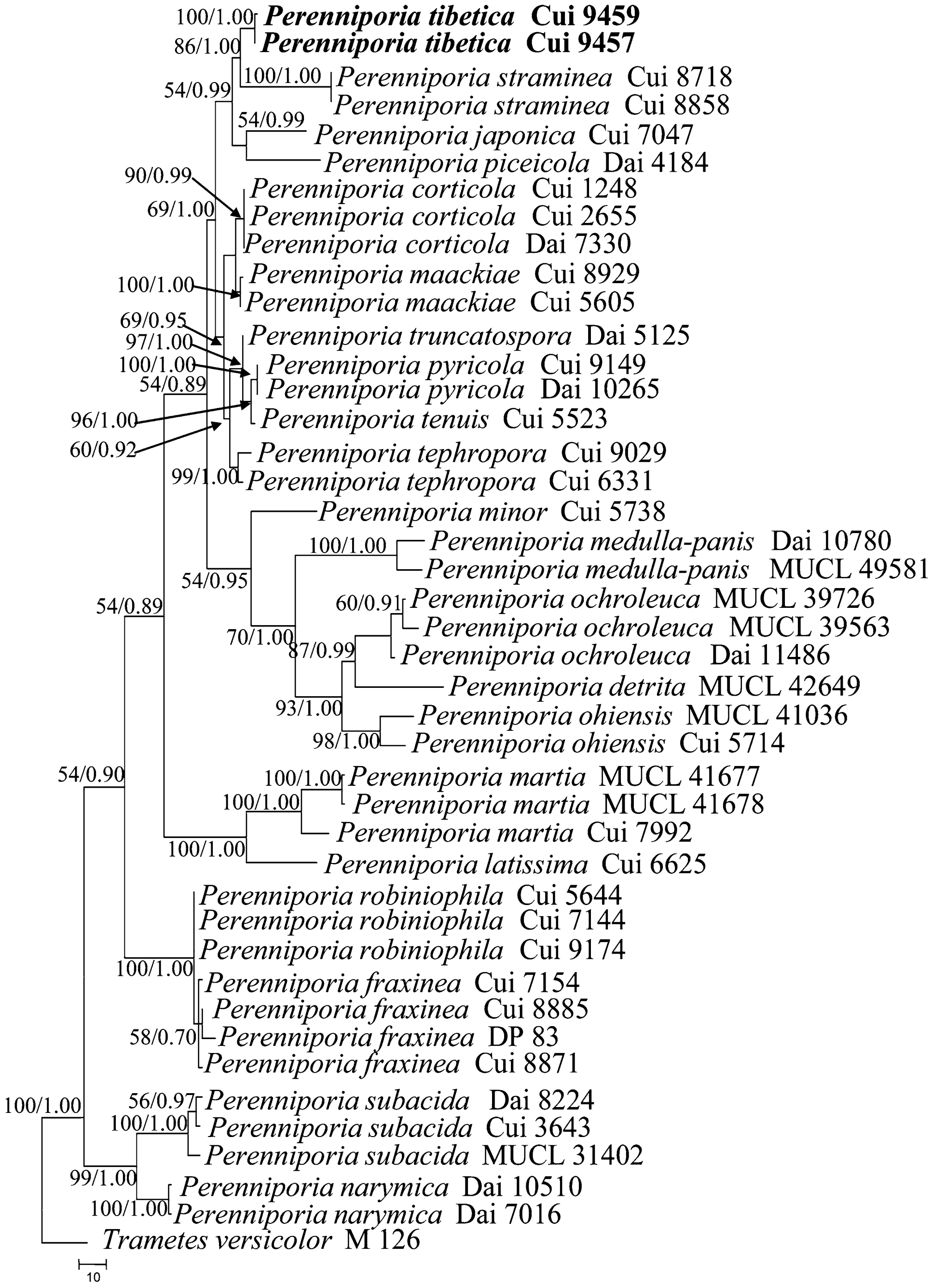

Fig. 3 Bayesian consensus tree illustrating the phylogeny of *Perenniporia tibetica* and related species generated by maximum parsimony and Bayesian analysis based on ITS + large subunit (LSU) sequences. The *arrows* are used to point out the place for the Parsimony bootstrap (before the *slash marks*) and Bayesian posterior probabilities (after the *slash marks*) greater than 50% are shown

smaller pores (7–8 per millimeter), and smaller basidiospores (4.2–5.5 × 3–4 µm; Decock and Ryvarden 1999). *Perenniporia bambusicola* is separated by its orange pore surface, smaller pores (6–8 per millimeter), and smaller basidiospores (3.8–5.8 × 1.8–2.5 µm; Choeyklin et al. 2009). *Perenniporia japonica* differs in having a whitish to isabelline pore surface, smaller pores (5–6 per millimeter), and smaller basidiospores (4–5.2 × 3–3.9 µm; Dai et al. 2002). *Perenniporia rhizomorpha* is separated by having smaller pores, unbranched and strongly dextrinoid skeletal hyphae, and non-truncate and smaller basidiospores (5.3–6.5 × 4.1–5.2 µm; Cui et al. 2007).

Phylogenetically, both ITS and ITS + LSU sequences supported *P. tibetica* as a distinct species, and resolved it as sister to both *P. straminea* (Bres.) Ryvarden and *P. piceicola* Y.C. Dai in the ITS dataset (85 PBS, 0.99 PP); in the

ITS + LSU dataset it is resolved with *P. straminea* only (86 PBS, 1.00 PP). However, *P. straminea* differs from *P. tibetica* in its distinctly smaller pores (6–7 per millimeter) and smaller basidiospores (3.2–4 × 2.4–3 µm; Cui et al. 2010). *Perenniporia piceicola* is distinguished from *P. tibetica* by having thick-walled and pyriform cystidia and distinctly truncate and larger basidiospores (11–14 × 5.4–7.5 µm; Dai et al. 2002).

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