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Full paper

Three new *Perenniporia* (Polyporales, Basidiomycota) species from China based on morphological and molecular data

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ABSTRACT

Three new *Perenniporia* species, *P. lacerata*, *P. luteola* and *P. tianmuensis*, are described based on morphological and molecular characters. *Perenniporia lacerata* is characterized by an annual habit, resupinate and papery basidiocarps with lacerate pores, a dimitic hyphal system with weakly dextrinoid skeletal hyphae, truncate and dextrinoid basidiospores. *Perenniporia luteola* is distinguished by a perennial habit, resupinate basidiocarps with buff-yellow pore surface, a dimitic hyphal system with dextrinoid skeletal hyphae, non-truncate and dextrinoid basidiospores. *Perenniporia tianmuensis* differs in its annual habit, pileate basidiocarps, a dimitic hyphal system with strongly dextrinoid skeletal hyphae, non-truncate and dextrinoid basidiospores. Phylogenetic analysis based on ITS and LSU-rDNA regions revealed five clades for 29 species of *Perenniporia* used in this study. Both morphological and molecular evidence confirmed the placement of three new species in *Perenniporia* and showed its relationships with similar species in the genus.

1. Introduction

Perenniporia Murrill is a large, cosmopolitan genus, and the genus is characterized by ellipsoid to distinctly truncate basidiospores, which usually are thick-walled, have cyanophilous and variable dextrinoid reactions; its hyphal structure is di- to trimitic with clamp connections on generative hyphae and its vegetative hyphae are cyanophilous, and variable dextrinoid (Decock and Stalpers 2006). Until now about 90 species have been described or transferred to the genus (Gilbertson and Ryvarden 1987; Ryvarden and Gilbertson 1994; Decock and Ryvarden 1999; Hattori and Lee 1999; Núñez and Ryvarden 2001; Choeyklin et al. 2009; Cui and Zhao 2012).

Taxonomic studies of *Perenniporia* in China have been carried out recently, and 41 species were recorded from the country (Dai 2012; Zhao et al. 2012), including several new species described from the country (Dai et al. 2002; Cui et al. 2007; Xiong et al. 2008; Dai 2010; Dai et al. 2011; Cui and Zhao 2012; Zhao and Cui 2012; Zhao et al. 2012). As a continuation of these surveys, three undescribed species matching the concepts of *Perenniporia* were found. To confirm the affinity of the three new taxa and infer the evolutionary relationships among representative species of *Perenniporia*, phylogenetic analysis was carried out based on ITS and nLSU sequences.

2. Materials and methods

2.1. Morphological studies

The studied specimens were deposited at the herbaria of the Institute of Microbiology, Beijing Forestry University (BJFC) and the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). The microscopic routine followed Dai et al.

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(2010). Sections were studied at magnification up to ×1000 using a Nikon Eclipse 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 were given in parentheses. In the text the following abbreviations were used: IKI = Melzer's reagent, 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, *n* = number of spores measured from given number of specimens. Special color terms followed Petersen (1996).

2.2. *Phylogenetic analysis*

2.2.1. *DNA isolation and PCR*

The fungal taxa used in this study were listed in Table 1. Phire® Plant Direct PCR Kit (Finnzymes) was used to obtain PCR products from dry specimens, according to the manufacturer's instructions. A small piece of dried fungal specimen was lysed in 30 μl dilution buffer for DNA extraction. After incubating 3 min at room temperature, 0.75 μl of the supernatant were used as template for a 30 μl PCR reaction. Nuclear ITS region was amplified with primer pairs ITS5 (GGA AGT AAA AGT CGT AAC AAG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC) (White et al. 1990), and LSU region was amplified with primer pairs LR0R (ACC CGC TGA ACT TAA GC) and LR7 (TAC TAC CAC CAA GAT CT) (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The PCR procedure for ITS was as follows: initial denaturation at 98 °C for 5 min, followed by 39 cycles at 98 °C for 5 s, 58 °C for 5 s and 72 °C for 5 s, and a final extension of 72 °C for 10 min. The only difference of the LSU amplification procedure was its annealing temperature was 48 °C. DNA sequencing was performed at Beijing Genomics Institute. All newly generated sequences have been submitted to GenBank and were listed in Table 1.

2.2.2. *Sequence and phylogeny analysis*

Sequences generated for this study were aligned with additional sequences downloaded from GenBank (Table 1) using BioEdit (Hall 1999) and ClustalX (Thomson et al. 1997).

In the study, nuclear ribosomal RNA genes were used to determine the phylogenetic position of the new species. Sequence alignment was deposited at TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S12899).

Maximum parsimony analysis was applied to the combined dataset of ITS and nLSU sequences. *Microporellus violaceo-cinerascens* (Petch) A. David & Rajchenb. was used as outgroup (Robledo et al. 2009). The tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 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 each Maximum Parsimonious Tree (MPT) generated.

MrMODELTEST2.3 (Posada and Crandall 1998; Nylander 2004) was used to determine the best-fit evolution model for each dataset for Bayesian inference (BY). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist and Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 2 million generations, and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum parsimony (MP) and Bayesian posterior probabilities (BPP) greater or equal than 75% (MP) and 0.95 (BPP) respectively were considered as significantly supported.

3. Results

3.1. *Taxonomy*

Perenniporia lacerata B.K. Cui & C.L. Zhao, sp. nov. Fig. 1.
MycoBank no.: MB 800937.

Differs from other *Perenniporia* species by an annual habit, resupinate and papery basidiocarps with lacerate pores, a dimitic hyphal system with weakly dextrinoid skeletal hyphae, truncate and dextrinoid basidiospores (6.1–7 × 5–5.7 μm).

Type: China, Henan Prov., Xiuwu County, Yuntaishan Park, on fallen angiosperm trunk, 3 September 2009, Cui 7220 (Holotypus in BJFC).

rDNA sequence ex holotype: JX141448.

Etymology: *Lacerata* (Lat.): referring to the lacerate pores.

Basidiocarps annual, resupinate, adnate, papery, without odor or taste when fresh, becoming corky upon drying, up to 9.5 cm long, 5.5 cm wide, 0.5 mm thick at center. Pore surface cream to buff when fresh, buff to yellowish buff upon drying; pores angular, 3–5 per mm; dissepiments thin, lacerate. Sterile margin narrow, cream, up to 0.5 mm wide. Subiculum cream, thin, up to 0.2 mm thick. Tubes concolorous with pore surface, corky, up to 0.3 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, 3–5.5 μm in diameter; subicular skeletal hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, frequently branched, interwoven, 1–3.9 μm in diameter. Tramal generative hyphae infrequent, hyaline, thin-walled, usually unbranched, 3.1–4.5 μm in diameter; skeletal hyphae in trama dominant, hyaline, thick-walled with a wide to narrow lumen, frequently branched, interwoven, 1–3.5 μm in diameter. Cystidia absent, but fusoid cystidioles present, hyaline, thin-walled, 16–17.5 × 5–6 μm; basidia clavate, with four sterigmata and a basal clamp

Table 1 – A list of species, specimens and GenBank accession number of sequences used in this study.

Fungal taxon	Specimen no.	GenBank no.	
		ITS	LSU
Perenniporia aridula	Dai 12398	JQ001855	JQ001847
P. aridula	Dai 12396	JQ001854	JQ001846
Perenniporia bannaensis	Cui 8560	JQ291727	JQ291729
P. bannaensis	Cui 8562	JQ291728	JQ291730
Perenniporia contraria	Knudsen 04-111	JQ861737	JQ861755
Perenniporia corticola	Cui 1248	HQ848472	HQ848482
P. corticola	Dai 7330	HQ654094	HQ654108
P. corticola	Cui 2655	HQ654093	HQ848483
Perenniporia fergusii	Gilbertson16116	HQ876607	JF706337
Perenniporia fraxinea	DP 83	AM269789	AM269853
P. fraxinea	Cui 7154	HQ654095	HQ654110
P. fraxinea	Cui 8871	JF706329	JF706345
P. fraxinea	Cui 8885	HQ876611	JF706344
Perenniporia japonica	Cui 7047	HQ654097	HQ654111
P. japonica	Cui 9181	JQ001856	JX141468[a]
Perenniporia lacerata	Cui 7220	JX141448[a]	JX141458[a]
P. lacerata	Dai 11268	JX141449[a]	JX141459[a]
P. lacerata	Wei 2208	JX141450[a]	JX141460[a]
Perenniporia latissima	Cui 6625	HQ876604	JF706340
Perenniporia luteola	K 333	JX141456[a]	JX141466[a]
P. luteola	K 433	JX141457[a]	JX141467[a]
Perenniporia maackiae	Cui 8929	HQ654102	JF706338
P. maackiae	Cui 5605	JN048760	JN048780
Perenniporia martia	Cui 7992	HQ876603	HQ654114
P. martia	MUCL 41677	FJ411092	FJ393859
P. martia	MUCL 41678	FJ411093	FJ393860
Perenniporia medulla-panis	MUCL 49581	FJ411088	FJ393876
P. medulla-panis	MUCL 43250	FJ411087	FJ393875
P. medulla-panis	Cui 3274	JN112792	JN112793
Perenniporia minor	Cui 5782	HQ883475	HQ654115
Perenniporia minor	Cui 5738	HQ848475	HQ848485
Perenniporia nanlingensis	Cui 7620	HQ848477	HQ848486
P. nanlingensis	Cui 7589	HQ848478	HQ848487
Perenniporia piceicola	Dai 4184	JF706328	JF706336
Perenniporia pyricola	Cui 9149	JN048762	JN048782
P. pyricola	Dai 10265	JN048761	JN048781
Perenniporia rhizomorpha	Cui 7507	HQ654107	HQ654117
P. rhizomorpha	Dai 7248	JF706330	JF706348
Perenniporia robiniophila	Cui 5644	HQ876609	JF706342
P. robiniophila	Cui 7144	HQ876608	JF706341
Perenniporia straminea	Cui 8718	HQ876600	JF706335
P. straminea	Cui 8858	HQ654104	JF706334
Perenniporia subacida	Dai 8224	HQ876605	JF713024
P. subacida	Cui 3643	FJ613655	AY336753
P. subacida	MUCL 31402	FJ411103	AY333796
Perenniporia substraminea	Cui 10177	JQ001852	JQ001844
P. substraminea	Cui 10191	JQ001853	JQ001845
Perenniporia tenuis	Wei 2783	JQ001858	JQ001848
P. tenuis	Wei 2969	JQ001859	JQ001849
Perenniporia tephropora	Cui 9029	HQ876601	JF706339
P. tephropora	Cui 6331	HQ848473	HQ848484
Perenniporia tibetica	Cui 9459	JF706327	JF706333
P. tibetica	Cui 9457	JF706326	JF706332
Perenniporia tianmuensis	Cui 2648	JX141453[a]	JX141463[a]
P. tianmuensis	Cui 2715	JX141454[a]	JX141464[a]
P. tianmuensis	Cui 2759	JX141455[a]	JX141465[a]
Perenniporia truncatospora	Cui 6987	JN048778	HQ654112
P. truncatospora	Dai 5125	HQ654098	HQ848481
Perenniporia vicina	MUCL 44779	FJ411095	FJ393862
Microporellus violaceo-cinerascens	MUCL 45229	FJ411106	FJ393874

a Sequences newly generated in this study.

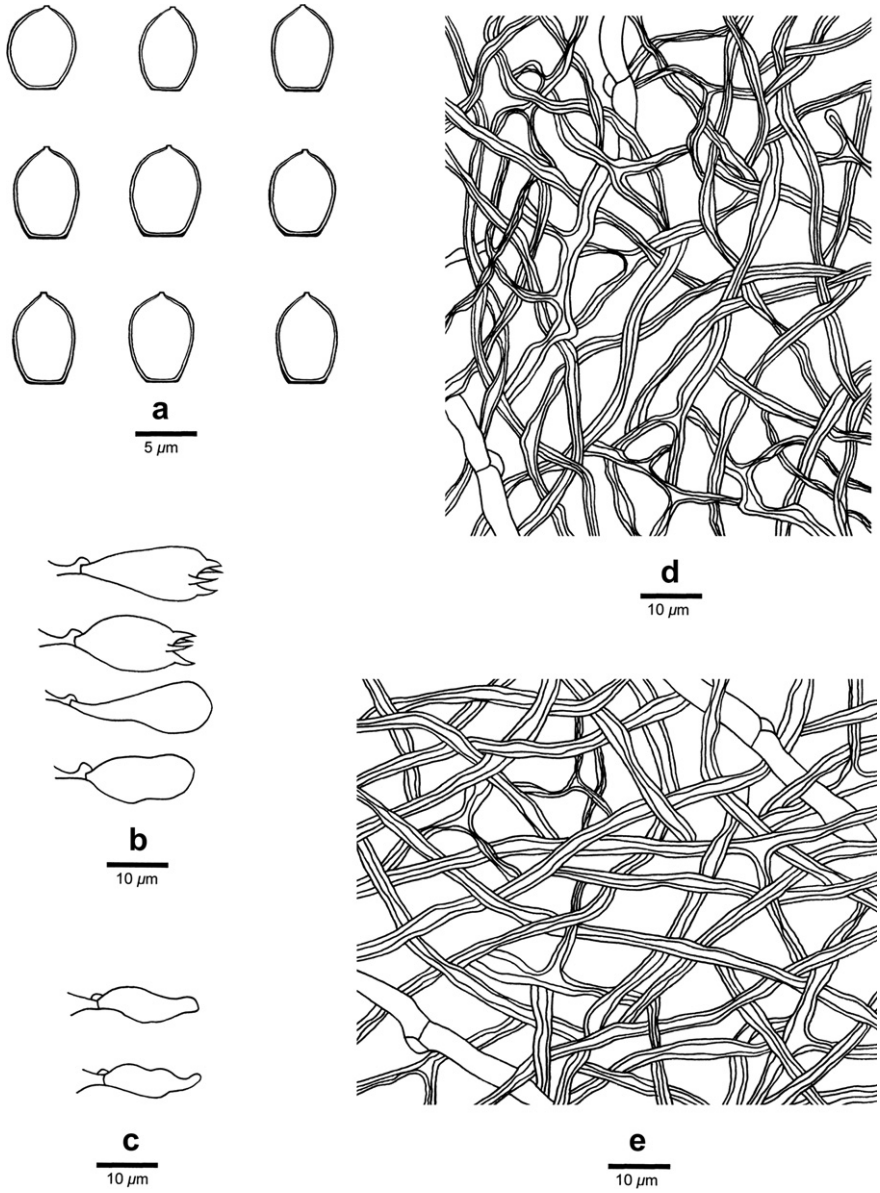

Fig. 1 – Microscopic structures of *Perenniporia lacerata* (drawn from the holotype). a: Basidiospores. b: Basidia and basidioles. c: Cystidioles. d: Hyphae from trama. e: Hyphae from subiculum.

connection, 16–20 × 8–9 μm; basidioles dominant, in shape similar to basidia, but slightly smaller. Basidiospores ellipsoid, truncate, hyaline, thick-walled, smooth, dextrinoid, CB+, (5.9–)6.1–7(–7.2) × (4.8–)5–5.7(–5.9) μm, L = 6.55 μm, W = 5.37 μm, Q = 1.13–1.29 (n = 90/3).

Type of rot: White rot.

Additional specimens examined (paratypes): China, Henan Prov., Neixiang County, Baotianman Nature Reserve, on rotten angiosperm wood, 22 September 2009, Dai 11268 (BJFC); Hubei Prov., Wufeng County, Houhe Nature Reserve, on fallen angiosperm trunk, 27 September 2004, Wei 2208 (IFP).

Remarks: *P. lacerata* is characterized by an annual habit, resupinate and papery basidiocarps with lacerate pores, a dimitic hyphal system with weakly dextrinoid skeletal hyphae, and ellipsoid, truncate, dextrinoid basidiospores (6.1–7 × 5–5.7 μm). *Perenniporia tenuis* (Schwein.) Ryvarden may be confused with *P. lacerata* by sharing resupinate basidiocarps and larger pores (3–5 per mm); however, *P. tenuis* is distinguished from *P. lacerata* by having subparallel tramal hyphae, and smaller basidiospores (5.5–6.5 × 4.5–5 μm; Dai et al. 2002). *Perenniporia pyricola* Y.C. Dai & B.K. Cui is similar to *P. lacerata* in producing resupinate basidiocarps, truncate and dextrinoid basidiospores (6.3–7.6 × 4.8–6.5 μm); however, *P. pyricola* differs in perennial and thick basidiocarps with entire pores (Dai 2010). *Perenniporia rosmarini* A. David & Malençon resembles *P. lacerata* by having truncate and dextrinoid basidiospores (6.5–7.5 × 5.5–6.5 μm), but it differs in having perennial basidiocarps with white to isabelline pore surface and smaller pores (6–7 per mm; Ryvarden and Gilbertson 1994). *Perenniporia medulla-panis* (Jacq.) Donk is similar to

P. lacerata by having resupinate basidiocarps and similar sized pores (4–5 per mm); however, *P. medulla-panis* has indextrinoid skeletal hyphae and smaller basidiospores (4.5–5.5 × 3.5–4.5 μm; Decock and Stalpers 2006).

Perenniporia luteola B.K. Cui & C.L. Zhao, sp. nov. Fig. 2.
MycoBank no.: MB 800938.

Differs from other *Perenniporia* species by a perennial habit, resupinate basidiocarps with buff-yellow pore surface, a dimitic hyphal system with dextrinoid skeletal hyphae, and ellipsoid, non-truncate, dextrinoid basidiospores (6.1–6.9 × 5.1–5.4 μm).

Type: China, Hunan Prov., Wugang County, Yunshan National Forest Park, on fallen angiosperm trunk, 19 September 2001, Harkonen 1308a (Holotypus in BJFC).

rDNA sequence ex holotype: JX141456.

Etymology: *Luteola* (Lat.): referring to the buff-yellow pore surface.

Basidiocarps perennial, resupinate, adnate, corky, without odor or taste when fresh, becoming hard corky upon drying, up to 5.5 cm long, 3 cm wide, 2 mm thick at center. Pore surface buff to buff-yellow when fresh, buff-yellow upon drying; pores round, 4–6 per mm; dissepiments thin, entire. Sterile margin wide, cream to buff, up to 3 mm wide. Subiculum cinnamon-buff, thin, up to 0.5 mm thick. Tubes concolorous with pore surface, corky, up to 1.5 mm long. Hyphal system dimitic; generative hyphae with clamp connections; skeletal hyphae dextrinoid, CB+; hyphae unchanged in KOH. Generative hyphae in subiculum infrequent, hyaline, thin-walled, usually unbranched, 2–3 μm in diameter; subicular skeletal hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, usually unbranched, interwoven, 2.5–3.5 μm in diameter. Tramal generative hyphae infrequent, hyaline, thin-walled, usually unbranched, 1.7–2.5 μm in diameter; skeletal hyphae in trama dominant, hyaline, thick-walled with a wide to narrow lumen, frequently

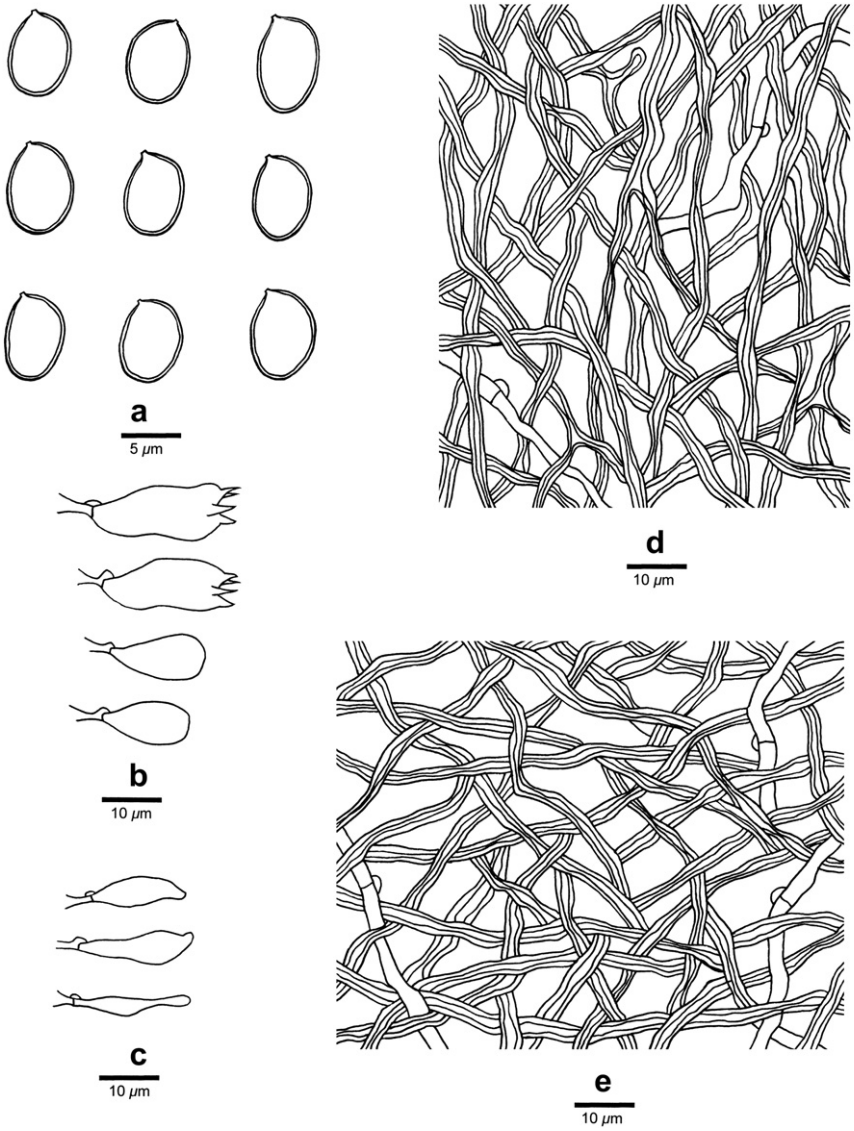

Fig. 2 – Microscopic structures of *Perenniporia luteola* (drawn from the holotype). a: Basidiospores. b: Basidia and basidioles. c: Cystidioles. d: Hyphae from trama. e: Hyphae from subiculum.

branched, interwoven, 2–3 μm in diameter. Cystidia absent, but fusoid cystidioles present, hyaline, thin-walled, 16–18 × 4–6 μm; basidia barrel-shaped, with four sterigmata and a basal clamp connection, 19–22 × 8–10 μm; basidioles dominant, in shape similar to basidia, but slightly smaller. Basidiospores ellipsoid, not truncate, hyaline, thick-walled, smooth, dextrinoid, CB+, (5.8–)6.1–6.9(–7) × (4.9–)5.1–5.4 (–5.6) μm, L = 6.38 μm, W = 5.16 μm, Q = 1.23–1.24 (*n* = 60/2).

Type of rot: White rot.

Additional specimen examined (paratype): China, Hunan Prov., Wugang County, Yunshan National Forest Park, on fallen angiosperm trunk, 19 September 2001, Harkonen 1308b (BJFC).

Remarks: *Perenniporia luteola* is characterized by a perennial habit, resupinate basidiocarps with buff-yellow pore surface, a dimitic hyphal system with dextrinoid skeletal hyphae, ellipsoid, non-truncate and dextrinoid basidiospores (6.1–6.9 × 5.1–5.4 μm). *Perenniporia bannaensis* B.K. Cui & C.L. Zhao may be confused with *P. luteola* by sharing a dimitic hyphal system with dextrinoid skeletal hyphae, ellipsoid, non-truncate and dextrinoid basidiospores; however, *P. bannaensis* is distinguished by its annual basidiocarps, smaller pores (6–8 per mm) and basidiospores (5.2–6 × 4–4.6 μm; Zhao et al. 2012). *Perenniporia chromatica* (Berk. & Broome) Decock & Ryvarden and *P. luteola* share similar sized pores (4–5 per mm), a dimitic hyphal system, and dextrinoid basidiospores (5.2–6.7 × 4.1–5.9 μm); but *P. chromatica* differs in having arboriform hyphae and truncate basidiospores (Decock and Ryvarden 1999). *Perenniporia subacida* (Peck) Donk is similar to *P. luteola*, and both have resupinate basidiocarps, a dimitic hyphal system, and non-truncate basidiospores; however, *P. subacida* has smaller basidiospores (4.5–6 × 3.5–4.5 μm, Ryvarden and Gilbertson 1994; 4.3–5.4 × 3.2–4.1 μm, Dai et al. 2002). *Perenniporia subaurantiaca* (Rodway & Cleland) P.K. Buchanan & Ryvarden is similar to *P. luteola* by producing similar sized pores (4–6 per mm), a dimitic hyphal system, and non-truncate, strongly dextrinoid basidiospores; however, it differs by having a cream to greyish orange pore surface and larger basidiospores (7.2–9.5 × 4.2–5.5 μm; Decock et al. 2000).

Perenniporia tianmuensis B.K. Cui & C.L. Zhao, sp. nov. Fig. 3.
MycoBank no.: MB 800939.

Differs from other *Perenniporia* species by an annual habit, pileate basidiocarps, a dimitic hyphal system with strongly dextrinoid skeletal hyphae, ellipsoid, non-truncate and dextrinoid basidiospores.

Type: China, Zhejiang Prov., Lin'an County, Tianmu Mountain, on base of dead angiosperm tree, 10 October 2005, Cui 2648 (Holotypus in BJFC).

rDNA sequence ex holotype: JX141453.

Etymology: *Tianmuensis* (Lat.): referring to the locality (Tianmu Mountain) of the type specimens.

Basidiocarps annual, pileate, solitary to imbricate, woody hard upon drying. Pileus usually fan-shaped, projecting up to 4.5 cm, 10 cm wide, and 1.5 cm thick at base. Pileal surface clay-buff to orange–brown, concentrically sulcate with distinctly zones, glabrous; margin obtuse. Pore surface buff to buff-yellow upon drying; pores round, 6–8 per mm; dissepiments thin, entire. Sterile margin narrow, cream to buff, up to 1 mm wide. Context cream to buff, corky, about 0.6 cm thick. Tubes concolorous with pore surface, woody hard, up to 9 mm thick. Hyphal system dimitic; generative hyphae with clamp connections; skeletal hyphae strongly dextrinoid, CB+; hyphae unchanged in KOH. Generative hyphae in context infrequent, hyaline, thin-walled, usually unbranched, 3.2–4 μm in diameter; skeletal hyphae in context dominant, hyaline, thick-walled with a wide lumen, frequently branched, interwoven, 1–5 μm in diameter. Tramal generative hyphae infrequent, hyaline, thin-walled, usually unbranched, 2.7–3.5 μm in diameter; skeletal hyphae in trama dominant, hyaline, thick-walled with a wide lumen, frequently branched, interwoven, 1–4.5 μm in diameter. Cystidia and cystidioles absent; basidia clavate to pear-shaped, with four sterigmata and a basal clamp connection, 15–18 × 5.5–7 μm; basidioles dominant, in shape similar to basidia, but slightly smaller. Basidiospores ellipsoid, not truncate, hyaline, thick-walled, smooth, dextrinoid, CB+, (4.8–)5–5.7(–5.9) × (3.8–)4–4.7(–4.9) μm, *L* = 5.3 μm, W = 4.31 μm, Q = 1.14–1.25 (*n* = 90/3).

Type of rot: White rot.

Additional specimens examined (paratypes): China, Zhejiang Prov., Lin'an County, Tianmu Mountain, on base of dead angiosperm tree, 11 October 2005, Cui 2715 (BJFC); on base of dead bamboo, 12 October 2005, Cui 2759 (BJFC).

Remarks: *Perenniporia tianmuensis* is characterized by an annual habit, pileate basidiocarps, a dimitic hyphal system with strongly dextrinoid skeletal hyphae, and its basidiospores are ellipsoid, not truncate, dextrinoid and cyanophilous. *Perenniporia subannosa* (Bres.) Decock, S. Herrera & Ryvarden and *P. tianmuensis* share pileate basidiocarps, similar sized basidiospores (3.7–5.5 × 2.7–4.5 μm); however, the former has large pores (4–5 per mm), and indextrinoid basidiospores (Decock et al. 2001). *Perenniporia truncatospora* (Lloyd) Ryvarden is similar to *P. tianmuensis*, and both have pileate basidiocarps and similar sized pores (6–8 per mm); however, *P. truncatospora* has larger and truncate basidiospores (6.5–8 × 5–6 μm; Núñez and Ryvarden 2001).

3.2. *Molecular phylogeny*

The ITS + LSU dataset included sequences from 60 fungal specimens representing 29 taxa. The dataset had an aligned length of 2038 characters in the dataset, of which 1581 characters are constant, 103 are variable and parsimony-uninformative, and 354 are parsimony-informative. Maximum Parsimony analysis yielded 10 equally parsimonious trees (TL = 908, CI = 0.469, RI = 0.771, RC = 0.362, HI = 0.531), and one of the maximum parsimonious trees was shown in Fig. 4. Best model for ITS + nLSU estimated and applied in the Bayesian analysis: GTR + I + G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis resulted in a same topology with an average standard deviation of split frequencies = 0.008879.

The phylogenetic tree (Fig. 4) inferred from ITS combined nLSU sequences demonstrates five clades for deep relationships among *Perenniporia* groups. Clade I includes the *Perenniporia* s.s. species, Clade II includes the *Perenniporia martia* (Berk.) Ryvarden complex, Clade III includes the *Perenniporia vicina* (Lloyd) D.A. Reid complex, Clade IV is composed of the *Perenniporia contraria* (Berk. & M.A. Curtis) Ryvarden group, and Clade V includes *P. subacida*. The ITS

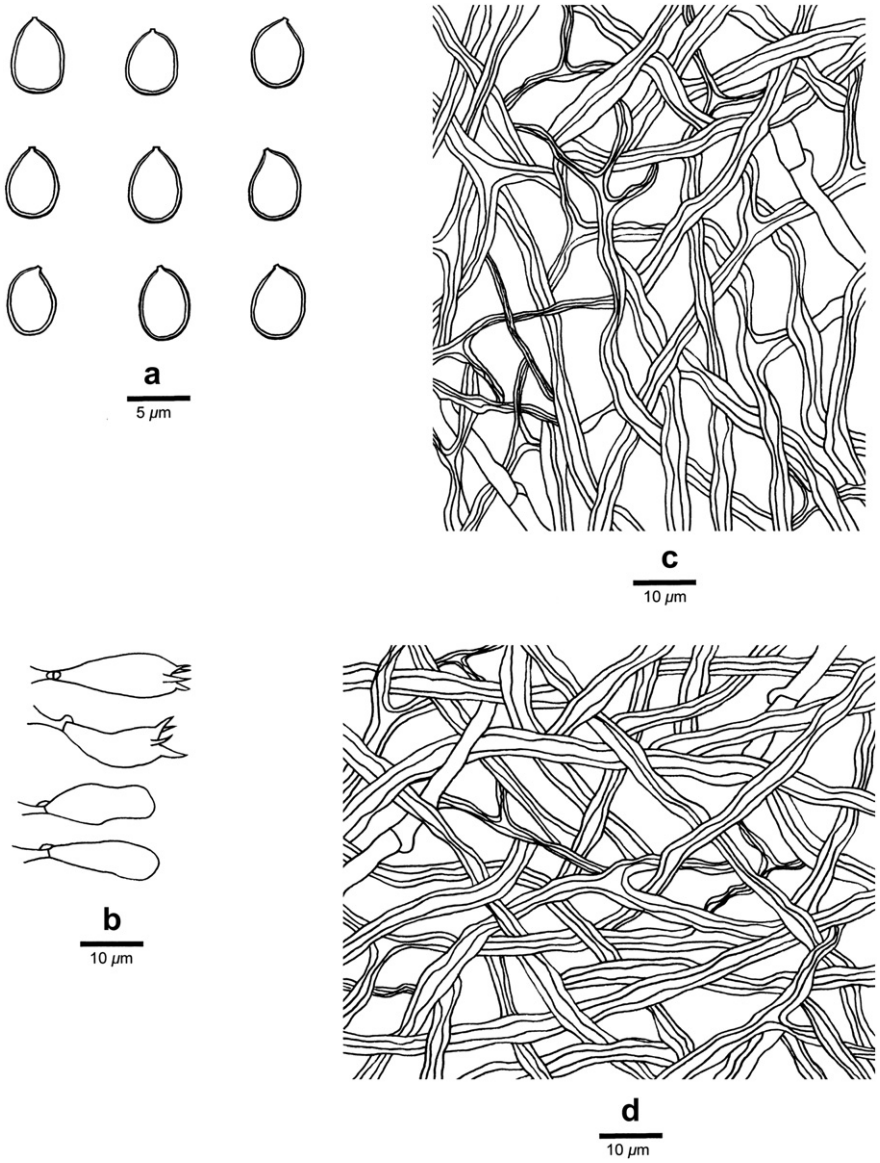

Fig. 3 – Microscopic structures of *Perenniporia tianmuensis* (drawn from the holotype). a. Basidiospores. b: Basidia and basidioles. c: Hyphae from trama. d: Hyphae from context.

combined nLSU sequences data suggested *P. lacerata* and *P. luteola* belonging to the *Perenniporia* s.s. clade, while *P. tianmuensis* belonging to the *P. contraria* group.

4. Discussion

Species of *Perenniporia* were usually described only based on morphological characters previously (e.g., Decock and Ryvarden 1999; Hattori and Lee 1999; Decock et al. 2000; Decock 2001; Dai et al. 2002; Cui et al. 2007; Xiong et al. 2008). Recently, molecular data have been used to confirm the taxonomic affinity of the new species and infer the evolutionary relationships among representative species of *Perenniporia* (Cui and Zhao 2012; Zhao and Cui 2012; Zhao et al. 2012).

In the present study, three new *Perenniporia* species: *P. lacerata*, *P. luteola* and *P. tianmuensis* are described based on morphological characters and rDNA sequence data. Molecular study based on sequence data from the ribosomal ITS and LSU regions (Fig. 4) confirmed the generic placement of the three new species, and all of them formed monophyletic lineages with strong support (100% BP, 1.00 BPP).

Phylogenetically (Fig. 4), *P. lacerata* sisters to *P. tibetica* B.K. Cui & C.L. Zhao, and these two species grouped together with strong support (89% BP, 0.99 BPP); however, *P. tibetica* produces a different morphology with white to cream colored rhizomorphs, larger both pores (2–3 per mm) and basidiospores (6.7–8.7 × 5.3–6.8 μm; Cui and Zhao 2012).

Perenniporia luteola is closely related to *Perenniporia rhizomorpha* B.K. Cui, Y.C. Dai & Decock according to the rDNA-based phylogeny (Fig. 4), and these two species grouped together with strong support (94% BP, 1.00 BPP); but morphologically, *P. rhizomorpha* is distinct by the cream to buff colored rhizomorphs and smaller basidiospores (5.5–6.5 × 4.1–5.2 μm; Cui et al. 2007).

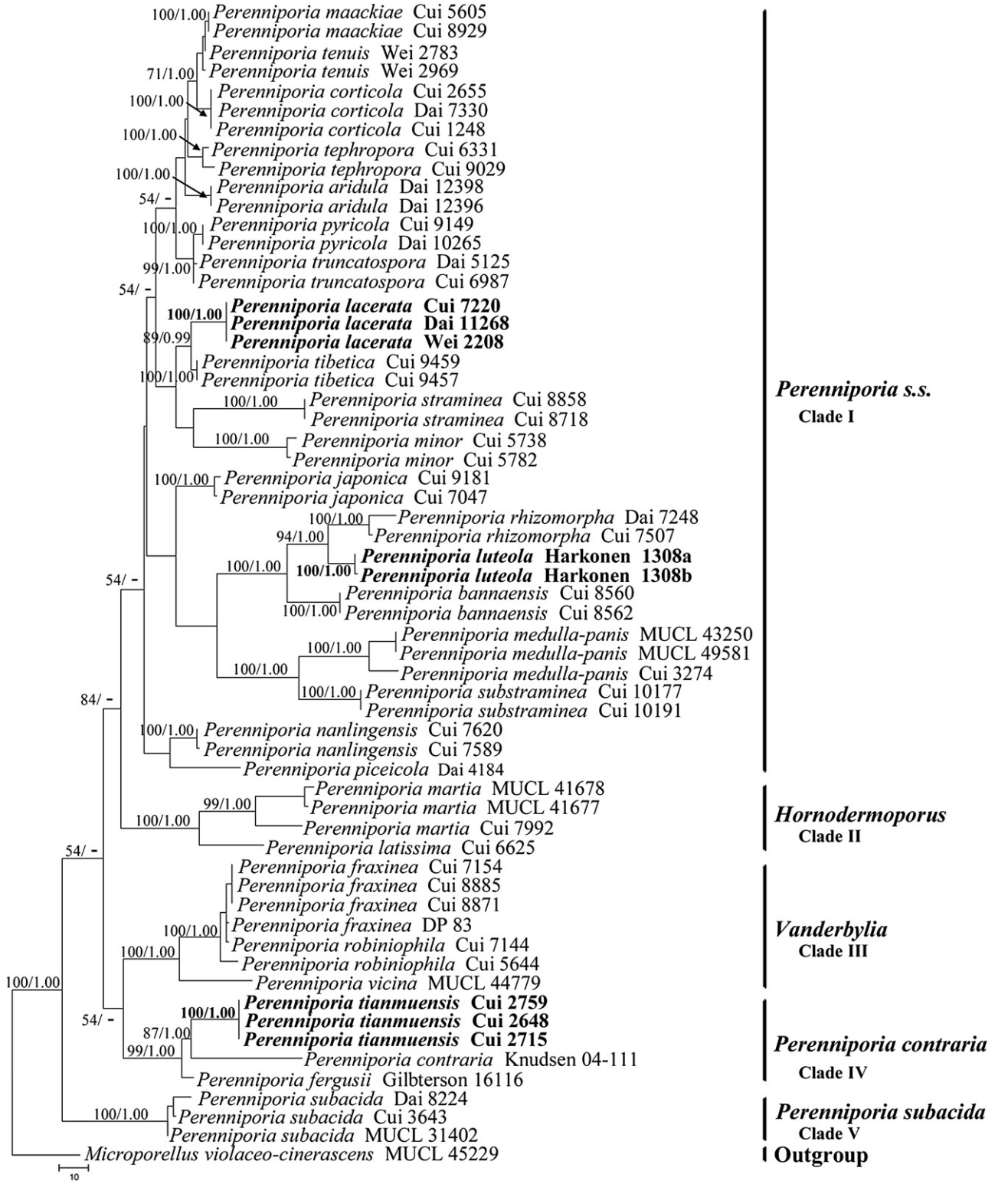

Fig. 4 – One of the maximum parsimonious trees illustrating the phylogeny of three new species and related species based on combined ITS + LSU sequences. Parsimony bootstrap proportions (before the slash markers) higher than 50% and Bayesian posterior probabilities (after the slash markers) more than 0.95 were indicated along branches.

Perenniporia tianmuensis clustered with *P. contraria* and *P. fergusii* Gilb. & Ryvarden with strong support (99% BP, 1.00 BPP, Fig. 4). *Perenniporia tianmuensis* somehow is easily confused with *P. contraria* by having pileate basidiocarps, smaller pores (6–8 per mm), strongly dextrinoid skeletal hyphae, and non-truncate basidiospores; however, *P. contraria* differs in its perennial basidiocarps, smaller and indextrinoid basidiospores (3.7–4.5 × 3–3.8 μm; Decock et al. 2001). *P. fergusii* can be easily

distinguished from *P. tianmuensis* by having resupinate basidiocarps with larger pores (4–6 per mm), and slightly truncate basidiospores (Gilbertson and Ryvarden 1987).

The preliminary phylogeny of *Perenniporia* s.l. was investigated with an analysis of nuclear ribosomal partial LSU and ITS DNA sequences data by Robledo et al. (2009); in their study, the differentiation of the hyphal system and the basidiospore morphology were outlined as critical features for the definition of genera in the *Perenniporia* complex. Zhao et al. (2012) carried out a phylogenetic study of *Perenniporia* s.l., and seven clades were recognized, among them, *P. ochroleuca* group, *P. vicina* group, *P. martia* group and *P. subacida* formed well supported monophyletic entities, which could be recognized as distinct genera.

In the present study, phylogenetic analysis revealed five clades for the 29 species of *Perenniporia*, among these clades, the *Hornodermoporus* clade (Clade II), the *Vanderbylia* clade (Clade III) and the *P. subacida* clade (Clade V) also formed well supported monophyletic entities (100% BP, 1.00 BPP; Fig. 4), which are identified to the previous study by Zhao et al. (2012).

Perenniporia lacerata and *P. luteola* were placed in the *Perenniporia* s.s. clade (Clade I) based on the phylogeny inferred from ITS combined nLSU sequence data (Fig. 4), this clade is composed of the core species of *Perenniporia*, and received only weak support (less than 50% BP and 0.95 BPP) in the present study. In order to fully resolve the phylogeny for this clade, evolutionary information from more conserved gene markers is needed in the future.

Robledo et al. (2009) mentioned that the *P. contraria* group (Decock et al. 2001) did not appear to belong to the core clade of *Perenniporia*, as regarding their hyphal system and basidiospore morphology. In our study, *P. tianmuensis* was recognized in the *P. contraria* clade (Clade IV), this clade is distant from the *Perenniporia* s.s clade (Clade I; Fig. 4). Species in the *P. contraria* clade usually have non-truncate basidiospores, while species in the *Perenniporia* s.s. clade usually have truncate basidiospores. Therefore, the *P. contraria* clade would be treated separately, and further phylogenetic study based on additional materials and multi-loci are needed.

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