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Perenniporiopsis, a New Polypore Genus Segregated from *Perenniporia* (Polyporales)

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Perenniporiopsis, a new polypore genus segregated from *Perenniporia* (Polyporales)

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Abstract – *Perenniporiopsis gen. nov.* is described to accommodate *Perenniporiopsis minutissima* spec. et comb. nov. Phylogenetic analyses based on two data sets, [ITS+n28S nrRNA] and [ITS, n28S, mtSSU, *tef1*], and using Maximum Parsimony, Maximum Likelihood and Bayesian inferences, showed that specimens of *Perenniporia minutissima* form a monophyletic well-supported clade within the core polyporoid clade, The new genus grouped with the *Abundisporus*, *Perenniporiella* and *Perenniporia* s.s. lineages, and is phylogenetically distinct from the *Perenniporia* s.s. lineage (100% BS, 99% MP, 1.00 BPP). Morphologically, the consistency of the basidiocarps, waxy when fresh, drying rigidly osseous, as well as the large basidiospores characterize this species and distinguish it from *Perenniporia* s.s. The species seems to be endemic to temperate east Asia, and is known from temperate Japan and China.

Basidiomycota / Molecular phylogeny / polypore / Taxonomy / White-rot fungi

INTRODUCTION

Trametes minutissima Yasuda (1920) was described by Yasuda based on the combination of a pileate basidiocarp, a trimitic hyphal system and truncate, hyaline, thick-walled, smooth basidiospores (Yasuda, 1920). Hattori & Ryvarden (1994) transferred the species to the genus *Perenniporia* Murrill on the basis of the features of basidiospores, and this placement has been widely accepted (Núñez & Ryvarden, 2001; Dai *et al.*, 2002; Cui *et al.*, 2006; Dai, 2012). However, Zhao & Cui (2012) examined phylogenetic affinities within *Perenniporia* based on internal transcribed spacer (ITS) DNA sequence data and demonstrated that *T. minutissima* formed a monophyletic entity, distant from the *Perenniporia* s.s. lineage.

In order to resolve the placement of this taxon, multigene phylogenetic analyses have here been carried out using ITS, the large subunit nuclear ribosomal RNA gene (28S), the mitochondrial small ribosomal subunit DNA (mtSSU) and a segment of the gene encoding for the translation elongation factor 1-α (*tef1*).

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MATERIAL AND METHODS

Morphological studies

Specimens studied are deposited at the Beijing Forestry University (BJFC), the Institute of Applied Ecology, Chinese Academy of Sciences (IFP), the National Museum of Nature and Science (TNS) and the Forestry and Forest Products Research Institute (TFM). Macro-morphological descriptions were based on field notes and study of specimens. Colour terms followed Petersen (1996). Microscopic measurements were made from slide preparations of dried specimens stained with Cotton Blue and Melzer's reagent by light microscopy following Dai (2010). Sections were studied using an Olympus BX40 compound microscope. In presenting spore size variation, 5% of measurements were excluded from each end of the range and given in parentheses. The following abbreviations are used: KOH = 5% potassium hydroxide, CB = Cotton Blue, IKI = Melzer's reagent, IKI– = both inamyloid and indextrinoid, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = L/W ratio, n (a/b) = number of spores (a) measured from given number of specimens (b).

Molecular procedures and phylogenetic analyses

CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to extract DNA and obtain PCR products from dried specimens, according to the manufacturer's instructions. The ITS region was amplified with primer pairs ITS5 and ITS4 (White *et al.,* 1990). Nuclear 28S region was amplified with primer pairs LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The mitochondrial SSU region was amplified with primer pairs MS1 and MS2 (White *et al.,* 1990). *Tef1* was amplified with primer pairs EF1-983F and EF1-2218R (Rehner & Buckley, 2005). The PCR procedure for ITS and mtSSU was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 58°C for ITS and 55°C for mtSSU for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for n28S and *tef1* was as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 48°C for n28S and 59°C for *tef1* for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR products were purified and directly sequenced at Beijing Genomics Institute. All newly generated sequences were deposited at GenBank (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 6 (Katoh & Toh, 2008; http://mafft.cbrc.jp/alignment/server/) using the "G-INS-I" strategy and manually adjusted in BioEdit (Hall, 1999). Sequence alignment was deposited at TreeBase (submission ID 17712). Sequences of *Stereum hirsutum* (Willd.) Pers. and *Heterobasidion annosum* (Fr.) Bref. obtained from GenBank were used as outgroups to root trees following Binder *et al.*, (2013) in the ITS+nLSU analysis. Sequences of *Donkioporia expansa* (Desm.) Kotl. & Pouzar and *Pyrofomes demidoffi* (Lév.) Kotl. & Pouzar, obtained from GenBank, were used as outgroups to root trees in ITS+28S+mtSSU+*tef1* based inferences (Zhao *et al.*, 2013).

Phylogenetic inferences under the Parsimony hypothesis were inferred from the ITS+nLSU and the combined multi-gene dataset of ITS+nLSU+mtSSU+*tef1* using Paup (Swofford, 2002). The combinability of the individual data set was

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

Species name	*Sample no.*	*GenBank accessions*				*References*
		ITS	*LSU*	*mtSSU*	*TEF1*	
Abortiporus biennis (Bull.) Singer	TFRI 274	EU232187	EU232235	—	—	Larsson, 2007
A. biennis	EL65-03	JN649325	JN649325	—	—	Larsson, 2007
Abundisporus roseoalbus (Jungh.) Ryvarden	Dai 12269	KC415908	KC415910	KF051037	KF181131	Zhao *et al.*, 2014
A. pubertatis (Lloyd) Parmasto	Cui 5776	KC787565	KC787572	KF051029	KF181129	Zhao *et al.*, 2014
A. sclerosetosus Decock & Laurence	MUCL 41438	FJ411101	FJ393868	—	—	Robledo *et al.*, 2009
A. violaceus (Wakef.) Ryvarden	Ryvar10775	KF018126	KF018134	KF051058	KF181152	Zhao *et al.*, 2014
Amylocystis lapponica (Romell) Bondartsev & Singer	KHL 11755	EU118603	EU118603	—	—	Larsson, 2007
Antrodia albida (Fr.) Donk	CBS 308.82	DQ491414	—	—	—	Kim *et al.*, 2007
A. albida	FP 105979	EU232272	EU232272			Larsson, 2007
A. heteromorpha (Fr.) Donk	CBS 200.91	DQ491415	AY515350	—	—	Kim *et al.*, 2007
A. macra (Sommerf.) Niemelä	MUAF 887	EU340898	—	—	—	Binder *et al.*, 2013
Bjerkandera adusta (Willd.) P. Karst.	DAOM 215869	AB733156	AB733333	—	—	Binder *et al.*, 2013
Cinereomyces lindbladii (Berk.) Jülich	FBCC 177	HQ659223	HQ659223	—	—	Binder *et al.*, 2013
Climacocystis borealis (Fr.) Kotl. & Pouzar	KH 13318	JQ031126	JQ031126	—	—	Binder *et al.*, 2013
Coriolopsis caperata (Berk.) Murrill	LE(BIN)-0677	AB158316	AB158316	—	—	Tomšovský *et al.*, 2010
Donkioporia expansa (Desm.) Kotl. & Pouzar	MUCL 35116	FJ411104	FJ393872	—	—	Robledo *et al.*, 2009
Earliella scabrosa (Pers.) Gilb. & Ryvarden	PR1209	JN165009	JN164793	—	—	Justo & Hibbett, 2011
Fragiliporia fragilis Y.C. Dai, B.K. Cui & C.L. Zhao	Dai 13080	KJ734260	KJ734264	KJ734268	KJ790245	Zhao *et al.*, 2015
F. fragilis	Dai 13559	KJ734261	KJ734265	KJ734269	KJ790246	Zhao *et al.*, 2015
F. fragilis	Dai 13561	KJ734262	KJ734266	KJ734270	KJ790247	Zhao *et al.*, 2015
Ganoderma lingzhi Sheng H. Wu, Y. Cao & Y.C. Dai	Wu 1006-38	JQ781858	—	JX029989	JX029976	Zhao *et al.*, 2014
G. lingzhi	Dai 12479	JQ781864	—	JX029988	JX029975	Zhao *et al.*, 2014
G. australe (Fr.) Pat.	Cui 9511	JN048773	JN048792	—	—	Zhao *et al.*, 2013
G. sinense J.D. Zhao, L.W. Hsu & X.Q. Zhang	Wei 5327	KF494998	KF495008	—	KF494976	Zhao *et al.*, 2014
G. applanatum (Pers.) Pat.	Dai 12483	KF494999	KF495009	—	KF494977	Zhao *et al.*, 2014

Table I. A list of species, specimens, and GenBank accession number of sequences used in this study *(continued)*

Species name	*Sample no.*	*GenBank accessions*				*References*
		ITS	*LSU*	*mtSSU*	*TEF1*	
Gelatoporia subvermispora (Pilát) Niemelä	BRNU 592909	FJ496694	FJ496706	—	—	Tomšovský *et al.*, 2010
Grammetheliopsis subtropica B.K. Cui & C.L. Zhao	Cui 9041	JQ845096	JQ845099	KF051039	KF181133	Zhao *et al.*, 2014
Heterobasidion annosum (Fr.) Bref.	PFC 5252	KC492906	KC492906	—	—	Binder *et al.*, 2013
Hornodermoporus latissima (Berk.) Teixeira	Cui 6625	HQ876604	JF706340	KF051040	KF181134	Zhao *et al.*, 2014
H. martius (Berk.) Teixeira	Cui 7992	HQ876603	HQ654114	KF051041	KF181135	Zhao *et al.*, 2014
H. martius (Berk.) Teixeira	MUCL 41677	FJ411092	FJ393859	—	—	Robledo *et al.*, 2009
H. martius (Berk.) Teixeira	MUCL 41678	FJ411093	FJ393860	—	—	Robledo *et al.*, 2009
Hydnopolyporus fimbriatus (Cooke) D.A. Reid	LR 40855	JN649347	JN649347	—	—	Binder *et al.*, 2013
Hypochnicium lyndoniae (D.A. Reid) Hjortstam	NL 041031	JX124704	JX124704	—	—	Binder *et al.*, 2013
Lentinus tigrinus (Bull.) Fr.	DSH93-181	AY218419	AF518627	U27050	—	Justo & Hibbett, 2011
Microporellus violaceo-cinerascens (Petch) A. David & Rajchenb.	MUCL 45229	FJ411106	FJ393874	—	—	Robledo *et al.*, 2009
Obba rivulosa (Berk. & M.A. Curtis) Miettinen & Rajchenb.	KCTC 6892	FJ496693	FJ496710	—	—	Miettinen & Rajchenberg, 2012
Perenniporia hainaniana B.K. Cui & C.L. Zhao	Cui 6364	JQ861743	JQ861759	KF051044	KF181138	Zhao *et al.*, 2014
P. hainaniana	Cui 6365	JQ861744	JQ861760	KF051045	KF181139	Zhao *et al.*, 2014
*P. hainan*iana	Cui 6366	JQ861745	JQ861761	KF494996	KF494981	Zhao *et al.*, 2014
P. medulla-panis (Jacq.) Donk	MUCL 49581	FJ411088	FJ393876	—	—	Robledo *et al.*, 2009
P. medulla-panis	MUCL 43250	FJ411087	FJ393875	—	—	Robledo *et al.*, 2009
P. medulla-panis	Cui 3274	JN112792	JN112793	KF051043	KF181137	Zhao *et al.*, 2014
P. subacida (Peck) Donk	Cui 10053	KF495006	KF495017	KF218321	KF286327	Zhao *et al.*, 2014
P. subacida	MUCL 31402	FJ411103	AY333796	—	—	Robledo *et al.*, 2009
*P. substramin*ea B.K. Cui & C.L. Zhao	Cui 10177	JQ001852	JQ001844	KF051046	KF181140	Zhao *et al.*, 2014
P. substraminea	Cui 10191	JQ001853	JQ001845	KF051047	KF181141	Zhao *et al.*, 2014
*P. substr*aminea	Dai 10781	KF495007	KF495018	KF494995	KF494983	Zhao *et al.*, 2014
P. subadusta (Z.S. Bi & G.Y. Zheng) Y.C. Dai	Cui 8459	HQ876606	HQ654113	KF051042	KF181136	Zhao *et al.*, 2014

Perenniporiella chaquenia Robledo & Decock	MUCL 47647	FJ411083	FJ393855	—	HM467609	Robledo *et al.*, 2009
P. chaquenia	MUCL 47648	FJ411084	FJ393856	—	HM467610	Robledo *et al.*, 2009
P. micropora (Ryvarden) Decock & Ryvarden	MUCL43581	FJ411086	FJ393858	—	HM467608	Robledo *et al.*, 2009
P. neofulva (Lloyd) Decock & Ryvarden	MUCL 45091	FJ411080	FJ393852	—	HM467599	Robledo *et al.*, 2009
P. pendula Decock & Ryvarden	MUCL 46034	FJ411082	FJ393853	—	HM467601	Robledo *et al.*, 2009
***Perenniporiopsis minutissima* (Yasuda) C.L. Zhao**	TFM 16173	**KX962543**	**KX962550**	**KX962557**	**KX962562**	Present study
P. minutissima	TFM 16174	**KX962544**	**KX962551**	**KX962558**	**KX962563**	Present study
P. minutissima	TFM 16172	**KX962542**	**KX962549**	**KX96256**	**KX962564**	Present study
P. minutissima	TFM 16991	**KX962545**	**KX962552**	**KX962559**	**KX962565**	Present study
P. minutissima	TFM 15722	**KX962541**	**KX962548**	**KX962555**	**KX962566**	Present study
P. minutissima	Cui 10221	**KX962546**	**KX962553**	**KX962560**	**KX962567**	Present study
P. minutissima	Cui 6053	**JX978408**	**JX978409**	**KF218305**	**KF286311**	Present study
P. minutissima	Dai 6042	**KX962547**	**KX962554**	**KX962561**	**KX962568**	Present study
P. minutissima	Dai 11643	**HQ876602**	**KF495015**	**KF218303**	**KF286309**	Present study
P. minutissima	Dai 12457	**KF495004**	**KF495014**	**KF218302**	**KF286308**	Present study
P. minutissima	Cui 10979	**KF495003**	**KF495013**	**KF218304**	**KF286310**	Present study
Phanerochaete chrysosporium Burds.	BKM-F-1767	HQ188436	GQ470643	—	—	Tomšovský *et al.*, 2010
Phlebia unica (H.S. Jacks. & Dearden) Ginns	KHL 11786	EU118657	EU118657	—	—	Tomšovský *et al.*, 2010
Physisporinus sanguinolentus (Alb. & Schwein.) Pilát	BRNM 699576	FJ496671	FJ496725	—	—	Tomšovský *et al.*, 2010
Piloporia sajanensis (Parmasto) Niemelä	Mannine 2733a	HQ659239	HQ659239	—	—	Tomšovský *et al.*, 2010
Podoscypha venustula (Speg.) D.A. Reid	CBS 65684	JN649367	JN649367	—	—	Binder *et al.*, 2013
Polyporus tuberaster (Jacq. ex Pers.) Fr.	CulTENN 8976	AF516598	AJ488116	—	—	Sotome *et al.*, 2008
Postia alni Niemelä & Vampola	X 1400	KC595932	KC595932	—	—	Binder *et al.*, 2013
P. caesia (Schrad.) P. Karst.	CIEFAP 174	JX090109	JX090129	—	—	Binder *et al.*, 2013
P. guttulata (Sacc.) Jülich	KHL 11739	EU11865	EU11865	—	—	Pildain & Rajchenberg, 2013
P. venata (Rajchenb. & J.E. Wright) Rajchenb.	CIEFAP 346	JX090113	JX090133	—	—	Pildain & Rajchenberg, 2013
P. lacteal (Fr.) P. Karst.	X 1391	KC595939	KC595939	—	—	Binder *et al.*, 2013

Table I. A list of species, specimens, and GenBank accession number of sequences used in this study *(continued)*

Species name	*Sample no.*	*GenBank accessions*				*References*
		ITS	*LSU*	*mtSSU*	*TEF1*	
Pyrofomes demidoff i (Lév.) Kotl. & Pouzar	MUCL 41034	FJ411105	FJ393873	—	—	Robledo *et al.*, 2009
Sebipora aquosa Miett.	Miettinen 8680	HQ659240	HQ659240	—	—	Miettinen & Rajchenberg, 2012
Skeletocutis amorpha (Fr.) Kotl. & Pouzar	Miettinen 11038	FN907913	FN907913	—	—	Tomšovský *et al.*, 2010
Stereum hirsutum (Willd.) Pers.	NBRC 6520	AB733150	AB733325	—	—	Binder *et al.*, 2013
Trametes elegans (Spreng.) Fr.	FP105679	JN048766	JN048785	—	—	Zhao *et al.*, 2014
T. hirsuta (Wulfen) Lloyd	Cui 7784	JN048768	JN048787	—	—	Zhao *et al.*, 2014
T. hirsuta	RLG5133T	JN164854	JN164801	AF042154	JN164891	Justo & Hibbett, 2011
T. pubescens (Schumach.) Pilát	PRM 900586	AY684173	AY855906	—	—	Justo & Hibbett, 2011
*Truncospora ochroleuc*a (Berk.) Pilát	Dai 11486	HQ654105	JF706349	KF051048	KF181142	Zhao *et al.*, 2014
T. ochroleuca	MUCL 39726	FJ411098	FJ393865	—	—	Robledo *et al.*, 2009
T. ochroleuca	Cui 5671	JX941584	JX941602	KF218309	KF286315	Zhao *et al.*, 2014
T. ochroleuca	Cui 5673	JX941585	JX941603	KF218308	KF286314	Zhao *et al.*, 2014
*T. ornat*a Spirin & Bukharova	Cui 5714	HQ654103	HQ654116	KF051056	KF181150	Zhao *et al.*, 2014
T. ohiensis (Berk.) Pilát	MUCL 41036	FJ411096	FJ393863	—	—	Robledo *et al.*, 2009
Tyromyces chioneus (Fr.) P. Karst.	Cui 10225	KF698745	KF698756	—	—	Zhao *et al.*, 2015
T. kmetii (Bres.) Bondartsev & Singer	Penttila 13474	KF705040	KF705041	—	—	Zhao *et al.*, 2015
*Vanderbylia delavay*i (Bull.) D.A. Reid	Dai 6891	JQ861738	KF495019	KF218287	KF286293	Zhao *et al.*, 2014
V. fraxinea (Bull.) D.A. Reid	DP 83	AM269789	AM269853	—	—	Robledo *et al.*, 2009
V. fraxinea	Cui 7154	HQ654095	HQ654110	KF218288	KF286294	Zhao *et al.*, 2014
V. fraxinea	Cui 8885	HQ876611	JF706344	KF218289	KF286295	Zhao *et al.*, 2014
*V. fraxine*a	Cui 8871	JF706329	JF706345	KF051050	KF181144	Zhao *et al.*, 2014
*V. robiniophil*a (Bull.) D.A. Reid	Cui 5644	HQ876609	JF706342	KF051051	KF181145	Zhao *et al.*, 2014
V. vicina (Lloyd) D.A. Reid	MUCL 44779	FJ411095	AF518666	—	—	Robledo *et al.*, 2009
Yuchengia narymica (Pilát) B.K. Cui, C.L. Zhao & Steffen	Dai 10510	HQ654101	JF706346	KF051054	KF181148	Zhao *et al.*, 2014
Y. narymica	0809/3	JN641261	JN641268	KF051049	KF181143	Zhao *et al.*, 2014

evaluated with the incongruence length difference (ILD) test (Farris *et al.*, 1994) implemented in PAUP* 4.0b10 (Swofford, 2002), under a heuristic search and 1000 homogeneity replicates. The resulting P value of 1.000 is much greater than 0.01, which means there is no discrepancy among the four loci in reconstructing phylogenetic trees.

Phylogenetic analysis approaches followed Zhao *et al.* (2015). 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 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 each Maximum Parsimonious Tree (MPT) generated.

DNA sequence data were also analysed using Maximum Likelihood (ML) with RAxML-HPC2 on Abe through the Cipres Science Gateway (www.phylo.org; Miller *et al.*, 2009). Branch support for ML analysis was determined by 1000 bootstrap replicate.

MrModeltest 2.3 (Posada & Crandall, 1998; Nylander, 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). 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 & Huelsenbeck, 2003). Four Markov chains were run for 2 runs from random starting trees for 5 million generations for the [ITS+n28S] dataset and for 10 million generations for the [ITS+n28S+mtSSU+*tef1*]. Trees were sampled every 100 generations. The first quarter of the generations were by default discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum likelihood (BS), maximum parsimony (MP) and Bayesian posterior probabilities (BPP) greater than or equal to 75% (MP) and 0.95 (BPP) were considered as significantly supported, respectively.

RESULTS

Phylogenetic analyses

The ITS+n28S dataset included sequences from 67 fungal specimens representing 58 taxa. The dataset had an aligned length of 2216 characters, of which 1289 characters are constant, 244 are variable and parsimony-uninformative, and 683 are parsimony-informative. Maximum parsimony analysis yielded 4 equally parsimonious trees (TL = 4670, CI = 0.334, HI = 0.666, RI = 0.590, RC = 0.197).

The best estimated model for the ITS+ n28S dataset and applied in the Bayesian analysis is GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in a similar topology to the MP analysis, with an average standard deviation of split frequencies = 0.005675 (BI).

The phylogeny (Fig. 1) inferred from ITS+28S data set grouped the 58 species of the Polyporales into seven major clades. Collections of *P. minutissima*

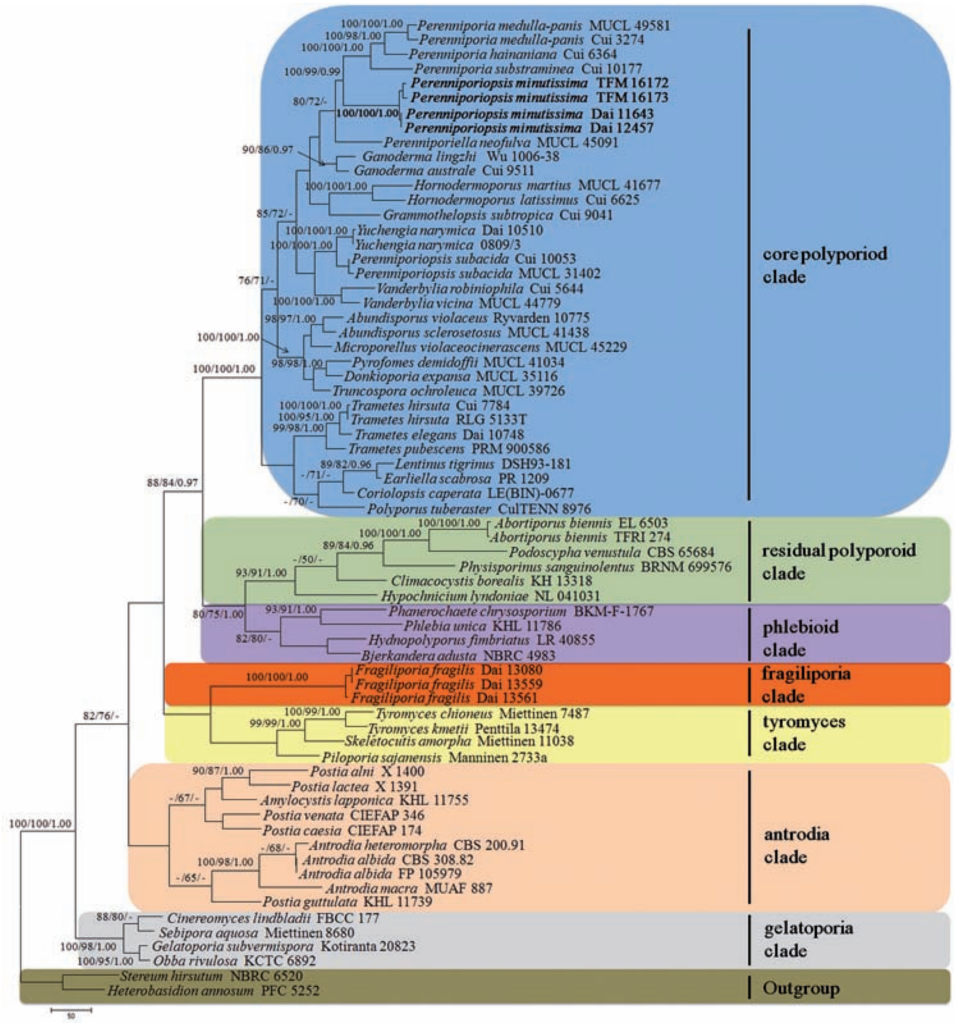

Fig. 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Perenniporiopsis minutissima*, and related species in Polyporales based on ITS+28S sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively. Clade names follow Binder *et al.* (2013).

formed a monophyletic group within core polyporoid clade. The *P. minutissima* clade grouped with *Perenniporiella* Decock & Ryvarden, *Ganoderma* P. Karst. and *Perenniporia* s.s.

The combined dataset included sequences from 55 specimens representing 31 species. The dataset had an aligned length of 3653 characters, of which 2544 characters are constant, 220 are variable and parsimony-uninformative, and

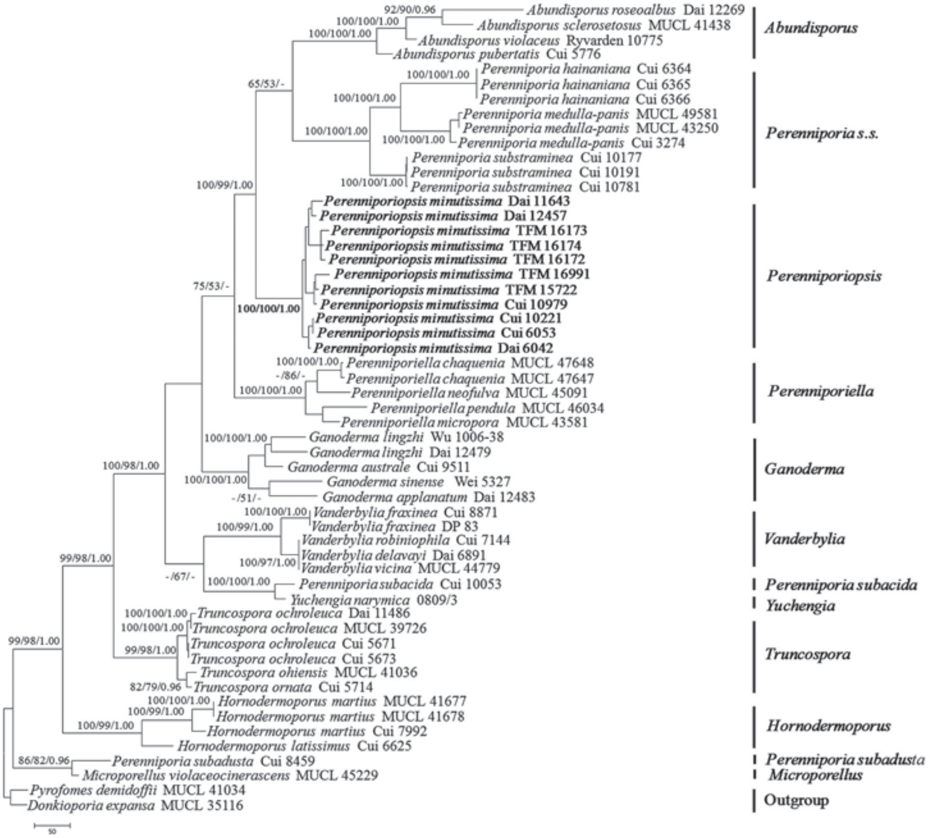

Fig. 2. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Perenniporiopsis minutissima*, and related species in *Perenniporia* s.l. based on the combined ITS+28S+mtSSU+*tef1* sequence datasets. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively. Clade names follow Zhao *et al.* (2013).

889 are parsimony-informative. Maximum parsimony analysis yielded 4 equally parsimonious trees (TL = 3303, CI = 0.497, HI = 0.503, RI = 0.744, RC = 0.370). Bayesian analysis and ML analyses resulted in a topology similar to the MP analysis, with an average standard deviation of split frequencies = 0.004301.

Additional phylogenetic inferences (Fig. 2) based on the combined ITS+28S+mtSSU+*tef1* sequences dataset and including more taxa of *Perenniporia* s.l. confirmed that *P. minutissima* forms a monophyletic lineage with strong support (100% BS, 99% BP, 1.00 BPP). This clade grouped with *Perenniporiella*, *Abundisporus* Ryvarden and *Perenniporia* s.s.

We concluded that *P. minutissima* is not congeneric with *P. medulla-panis* (Jacq.) Donk, but belongs to a distinct genus, here described as *Perenniporiopsis*.

Taxonomy

Perenniporiopsis C.L. Zhao**, gen. nov.**

MycoBank no.: MB 819846

Diagnosis: Differs from *Perenniporia* s.l. by having pileate, solitary or imbricate basidiocarps with orange brown to dark reddish brown surface and a trimitic hyphal system with dextrinoid skeletal hyphae and bigger, truncate, thick-walled, dextrinoid basidiospores.

Type species: Perenniporiopsis minutissima (Yasuda) C.L. Zhao.

Etymology: Perenniporiopsis (Lat.): referring to the similarity with the genus *Perenniporia*.

Basidiocarps annual, pileate; pileus solitary or imbricate, corky, inodor and without taste when fresh, becoming rigidly osseous upon drying. **Pileal surface** orange brown to dark reddish brown. **Pore surface** white when fresh, pale yellowish to ochraceous-tawny on drying. **Context** cream to buff-yellow, rigidly osseous on drying. **Tubes** white to cream buff, rigidly osseous on drying. **Hyphal system** trimitic; generative hyphae hyaline, thin-walled, with clamp connections; skeletal and binding hyphae dominant, hyaline, thick-walled, dextrinoid in Melzer's reagent, unchanged in KOH. **Cystidia** and cystidioles absent. **Basidiospores** oblong-ellipsoid, bigger, truncate, hyaline, thick-walled, smooth, dextrinoid, CB+.

Perenniporiopsis minutissima (Yasuda) C.L. Zhao, **spec. et comb. nov.** **Figs 3, 4**

Basionym: *Trametes minutissima* Yasuda, Bot. *Mag. Tokyo* 34: 29 (1920).

= *Perenniporia minutissima* (Yasuda) T. Hatt. & Ryvarden, *Mycotaxon* 50: 37 (1994).

MycoBank no.: MB 819847

Basidiocarps annual, pileate; **pileus** solitary or imbricate, corky, inodor and without taste when fresh, becoming rigidly osseous upon drying, applanate, projecting up to 6 cm, 8 cm wide, and 3 cm thick at base; **pileal surface** orange brown to dark reddish brown, azonate, verrucose; margin yellowish brown, obtuse; **pore surface** white when fresh, becoming pale yellowish to ochraceous-tawny when dry; pores round, 4–6 per mm; dissepiments thin, entire; **context** cream to buff-yellow, rigidly osseous, azonate, up to 2 cm thick; **tubes** white to cream buff, rigidly osseous, up to 1 cm long.

Hyphal system trimitic; generative hyphae hyaline, thin-walled, with clamp connections; skeletal and binding hyphae hyaline, thick-walled, dextrinoid in Melzer's reagent, CB– in the context, CB+ in the hymenophoral tramal, unchanged in KOH; in the context, generative hyphae infrequent, hyaline, thin-walled, rarely branched, 2.5–4.5 µm in diam; skeletal hyphae dominant, hyaline, thick-walled with a distinct lumen, infrequently branched, interwoven, 4–6.5 µm in diam; binding hyphae in context hyaline, thick-walled with a narrow lumen, frequently branched, interwoven, 1–2 µm in diam; in the hymenophoral tramal, generative hyphae infrequent, hyaline, thin-walled, usually unbranched, 2–4 µm in diameter; skeletal hyphae in trama dominant, hyaline, thick-walled with a narrow lumen, infrequently branched, interwoven, 3.5–5.5 µm in diameter; binding hyphae in trama hyaline, thick-walled with a narrow lumen, frequently branched, interwoven, 1–1.5 µm in diam. **Cystidia** and cystidioles absent. **Basidia** clavate, with four sterigmata and a basal clamp connection, 18.5–30 × 8–13 µm; basidioles dominant, in shape similar to basidia, **Basidiospores** oblong-ellipsoid, truncate, hyaline, thick-walled, smooth,

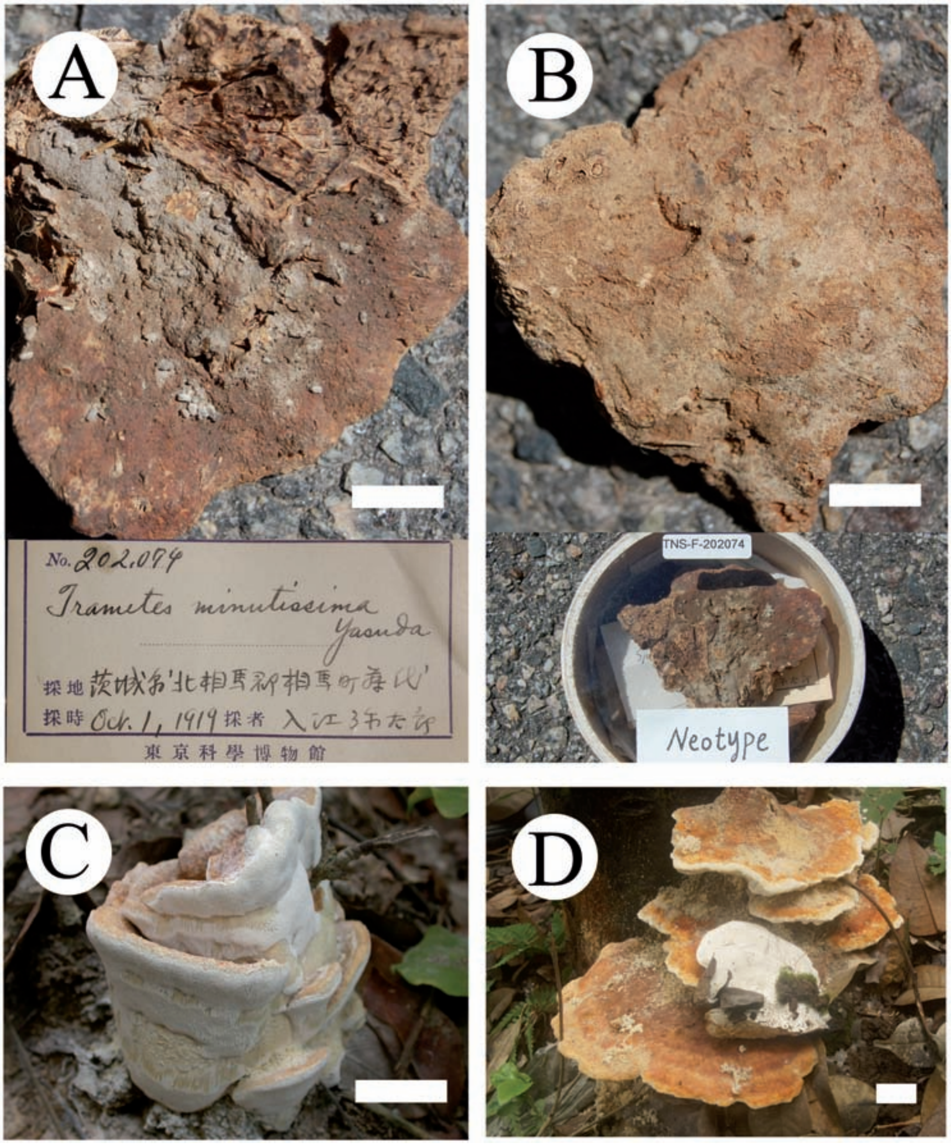

Fig. 3. Basidiocarps of *Perenniporiopsis minutissima*. **A–B.** Neotype, TNS 202074. **C.** Li 1147. **D.** Dai 12457. *Bars* = 1 cm.

dextrinoid, CB+, (11–)12–15(–16) × (5.5–)6.5–8(–8.5) µm, L = 13.3 µm, W = 7.1 µm, Q = 1.71–1.87 (n = 600/20).

Type of rot. White rot.

Specimens examined: **JAPAN.** Ibaraki, Fujishiro, on the *Symplocos* spp., 1 Oct. 1919, Y. Irie 202074 (**Neotype**, TNS); Tochigi Prefecture, Shiobara Yoshinuma, on the *Symplocos* spp., 18 Oct. 2002, T. Hattori (BJFC 009208); Tottori Prefecture,

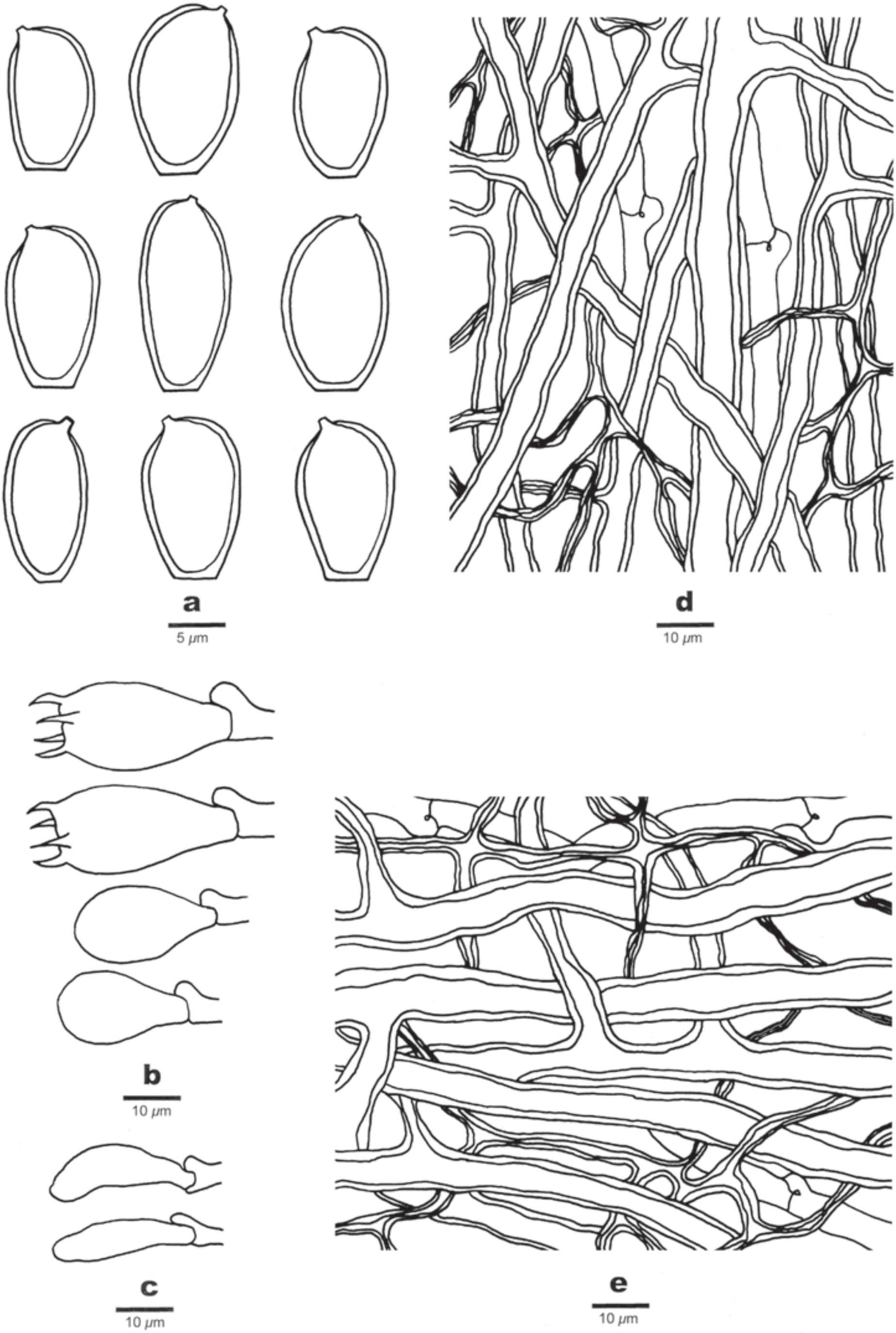

Fig. 4. Microscopic structures of *Perenniporiopsis minutissima* (drawn from the neotype). **A.** Basidiospores. **B.** Basidia and basidioles. **C.** Cystidioles. **D.** Hyphae from trama. **E.** Hyphae from context. *Bars*: A 5 µm; B–E = 10 µm.

Saihaku District, Daisen, on dead *Symplocos*, 5 August 1989, T. Hattori WD-679 (TFM 15722), 5 Aug. 1990, T. Hattori WD-526 (TFM 15118); Chiba Prefecture, Sakae, on dead *Pinus*, 28 Jul. 1991, T. Hattori WD-1525 (TFM 16172), T. Hattori WD-1526 (TFM 16173), T. Hattori WD-1527 (TFM 16174); Nagano Prefecture, Togakushi, on dead *Pinus*, 5 Oct. 1993, T. Hattori WD-1870 (TFM 16991); Ibakari Prefecture, Iwase, Tomiya, on dead *Symplocos*, 13 Aug. 1993, M. Izawa (TFM 18884). **CHINA.** Henan Province, Neixiang County, Baotianman Nature Reserve, on angiosperm stump, 25 Aug. 2006, J. Li, Li 1147 (IFP); Hunan Province, Changsha, Yuelushan Park, on *Symplocos* spp., 14 Jul. 2011, Y.C. Dai, Dai 12455, 12457 (BJFC); on angiosperm stump, 4 Dec. 2002, Y.C. Dai, Dai 3574 (IFP); Hubei Province, Wuhan, on rotten angiosperm stump, 8 Jul. 2010, Y.C. Dai, Dai 11643 (BJFC); Jiangsu Province, Nanjing, Zijin Mountain, on the *Symplocos* spp., 31 Jul. 2003, Y.L. Wei, Wei 1058, 1059 (IFP); on the *Pinus*, 11 Oct. 2003, *Dai 5257*, on the *Quercus*, 22 Aug. 2006, Y.C. Dai, Dai 4016 (IFP); Jiangxi Province, Jiujiang, Lushan County, on the *Symplocos* spp. 9 Oct. 2008, B.K. Cui, Cui 6053 (BJFC); Shandong Province, Taian, Tai Mountain, on the *Symplocos* spp., 4 August 2012, B.K. Cui, Cui 10979 (BJFC); Zhejiang Province, Lin'an County, Tianmushan Nature Reserve, on base of dead angiosperm tree, 15 Oct. 2004, Y.C. Dai, Dai 6402, on fallen angiosperm branch, 16 Oct. 2004, Y.C. Dai, Dai 6425; on angiosperm stump, 9 Oct. 2005, Y.C. Dai, Dai 2573, 12 Oct. 2005, B.K. Cui, Cui 2721, 2738 (IFP).

DISCUSSION

The placement of this species in *Perenniporia* has been widely accepted (Núñez & Ryvarden, 2001; Dai *et al.*, 2002; Cui *et al.*, 2006; Dai, 2012) and the combination of the skeletal and binding hyphae dominant in context and trama and hyaline, thick-walled, smooth, truncate, dextrinoid and CB+ basidiospores pointed towards *Perenniporia* as generally accepted (Núñez & Ryvarden, 2001). However, the waxy consistency of the basidiocarps when fresh, becoming rigidly osseous on drying and larger basidiospores deviate from *Perenniporia* s.s (Decock & Stalpers, 2006).

Zhao & Cui (2012) first showed that a specimen of this species formed a monophyletic well-supported clade distant from the *P. medulla-panis* clade based on ITS DNA sequence data. The present study confirmed that this species should be separated from *Perenniporia* based on phylogenetic analyses of two multigene ([ITS+n28S nrRNA] and [ITS, n28S, mtSSU, tef1]) datasets.

Molecular analyses showed that the genus *Perenniporiopsis* is closely related to *Perenniporiella*, *Ganoderma* and *Perenniporia* s.s. based on the ITS+n28S sequence data set (Fig. 1). In the combined ITS+28S+mtSSU+TEF1 sequences data set (Fig. 2), it still groups with *Perenniporiella*, *Perenniporia* s.s. and *Abundisporus*, but is distant from *Ganoderma*. Morphologically, *Perenniporiopsis* and *Perenniporiella* share the pileate basidiocarps. *Perenniporiella* differs especially by the non-truncate basidiospores (Decock & Ryvarden, 2003). *Ganoderma* mainly differs by its double-walled basidiospores with echinulae emerging from the endospore (Moncalvo & Ryvarden, 1997). *Abundisporus* is separated from *Perenniporiopsis* by its pale umber to deep purplish brown or greyish to umber brown context and a dimitic hyphal structure with yellow to pale brown skeletal hyphae, and pale yellowish and non-dextrinoid, non-truncate basidiospores (Ryvarden, 1998).

Hornodermoporus Teixeira, typified by *Hornodermoporus martius* (Berk.) Teixeira (Teixeira, 1993), *Truncospora* Pilát typified by *T. ochroleuca* (Berk.) Pilát (Pilát, 1953) share with *Perenniporiopsis minutissima* the pileate basidiocarps and truncate basidiospores. *Truncospora ochroleuca* differs by its relatively small, pileate basidiocarps with corky to wood corky consistency upon drying (Pilát, 1953; Decock & Ryvarden 1999; Decock 2011; Spirin *et al.*, 2015). *Hornodermoporus martius* differs in having a black crust on the pileus, strongly dextrinoid, unbranched skeletal hyphae and presence of cystidia (Berkeley, 1856; Teixeira, 1993).

Perenniporiopsis minutissima causes white rot of wood and is so far only known from Japan and China (Yasuda, 1923; Núñez & Ryvarden, 2001; Dai, 2012). Most collections have been made on *Symplocos spp*. but some specimens were collected on *Pinus densiflora*.

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