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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