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Phellinidium asiaticum sp. nova (Hymenochaetales, Basidiomycota), the Asian kin of *P. fragrans* and *P. pouzarii*

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Phellinidium (Hymenochaetales, Basidiomycota) is characterized by a monomitic hyphal system, presence of hyphoid setae, and thin-walled, hyaline and acyanophilous basidiospores. The genus is polyphyletic and has at least three independent lineages. *Phellinidium asiaticum* Spirin, L.W. Zhou & Y.C. Dai is described as a new species based on four specimens from northeast China and the Russian Far East. In the nLSU-based phylogeny, *P. asiaticum* is nested within the clade including *P. ferrugineofuscum* (the generic type), and it is closely related to *P. fragrans* and *P. pouzarii*, known from North America and Europe, respectively. The morphological differences between *P. asiaticum* and *P. fragrans*, as well as *P. pouzarii*, are discussed.

Introduction

Phellinidium, belonging to the Hymenochaetaceae (Hymenochaetales, Basidiomycota), is characterized by a monomitic hyphal system, hyphoid setae dominating in trama and context (subiculum), and thin-walled, hyaline and acyanophilous basidiospores (Dai 1995). *Phellinidium* was first described as a subgenus of *Phellinus* and typified with *Poria ferrugineofusca* by Kotlaba (1968). Later, Fiasson and Niemelä (1984) raised it to generic rank, and included *Phellinus pouzarii* into it. Following that genus concept, *P. fragrans*, *P. noxium*, *P. orientale*, and *P. rufitinctum* were combined into *Phellinidium* by Nuss (1986) and Bondartseva *et al*. (1992). Dai (1995) provided a detailed study on the genus. He described a new species, *P. aciferum*, proposed three new combinations (*P. lamaënse*, *P. sulphurascens* and *P. weirii*), and more importantly, redefined the morphological concept of the genus. Subsequently *P. cryptocystidiatum* was described from western Russia (Spirin *et al*. 2006).

To date no phylogenetic studies focused on the genus *Phellinidium* have been published. However, the DNA studies on the Hymenochaetaceae *s. lato* by Wagner and Fischer (2002)

showed that *Phellinidium* is a polyphyletic grouping. In that analysis, *P. ferrugineofuscum* clustered with *P. fragrans* and *P. pouzarii*, while *P. sulphurascens* and *P. weirii* formed a separate clade. Dai (2010) showed that *P. noxium* represents a third independent lineage within *Phellinidium*, being closely related to *Pyrrhoderma adamantinum*.

Four specimens having typical characters of *Phellinidium* and representing a single species were collected recently in northeast China and the Russian Far East. Their affinity with *Phellinidium*, especially with *P. fragrans* and *P. pouzarii*, was confirmed based on morphology and nLSU-based phylogeny. However, no existing names were found for the species, therefore it is described here as new.

Material and methods

Morphological studies

The studied specimens are deposited at the herbaria of Institute of Applied Ecology, Chinese Academy of Sciences (IFP), Beijing Forestry University (BJFC), Botanical Museum, Finnish Museum of Natural History (H) and Center for Fundamental Materials Research (CFMR). The microscopic procedure follows Miettinen *et al.* (2006). The following abbreviations are used: *L* = mean basidiospore length (arithmetical average of all spores), *W* = mean basidiospore width (arithmetical average of all spores), *Q* = variation in the *L*/*W* ratios among the specimens studied (quotient of L and W of each specimen), *n* = the number of basidiospores measured from given number of specimens. KOH = 5% potassium hydroxide, IKI = Melzer's reagent, IKI– = inamyloid and indextrinoid, CB = Cotton Blue, CB+ = cyanophilous and CB– = acyanophilous. The microscopic characters were studied under Nikon 80i microscope at magnification up to 1000×. The measurements and drawings were made from slide preparations stained with CB. In presenting the size range of basidiospores, 5% of the measurements were excluded from each end of the range and are given in parentheses. The drawings were made with the aid of a drawing tube. The special colour terms follow Petersen (1996).

Phylogenetic studies

PCR products were directly obtained from herbarium specimens using Phire® Plant Direct PCR Kit (Finnzymes Oy, Finland) according to the manufacturer's instruction. Primers LR0R and LR7 (Vilgalys & Hester 1990) were used to amplify the nLSU region. The PCR procedure was as follows: initial denaturation at 98 °C for 5 min, followed by 39 cycles at 98 °C for 5 s, 48 °C for 5 s and 72 °C for 5 s, and a final extension at 72 °C for 1 min. The amplicons were sequenced in the Beijing Genomics Institute (Beijing, China) with the same primers as well as with LR3, LR3R and LR5 (Vilgalys & Hester 1990). The newly generated sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov).

Besides the newly obtained sequences, additional nLSU sequences (Fig. 1) from species of *Phellinidium* and *Pyrrhoderma* were included in the dataset for phylogenetic analysis. *Fuscoporia ferruginosa* was selected as outgroup following Wagner and Fischer (2002). The dataset was aligned using ClustalX 2.0 (Larkin *et al.* 2007) with default parameters. Maximum likelihood (ML) tree was constructed by raxmlGUI 1.2 (Stamatakis 2006, Silvestro & Michalak 2012) under GTR + I + G model and auto FC option (Pattengale *et al.* 2010) in bootstrap (BS) replicates. The best-fit evolutionary model for Bayesian Inference (BI) was selected by jModelTest (Guindon & Gascuel 2003, Posada 2008). MrBayes 3.2 (Ronquist & Huelsenbeck 2003) was used to perform BI. Two independent runs were employed, each starting from random trees and with four chains for 1 000 000 generations. Trees were sampled every 1000th generation. Chain convergence was determined using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/). The first quarter of sampled trees was discarded as burn-in, while the remaining trees were used to calculate a 50% majority consensus tree and posterior probabilities (PPs).

Results

Three specimens (*Cui 9947, Wei 5610* and *Spirin 5097*) were nLSU-sequenced (*see* below for their accession numbers at GenBank). Besides them,

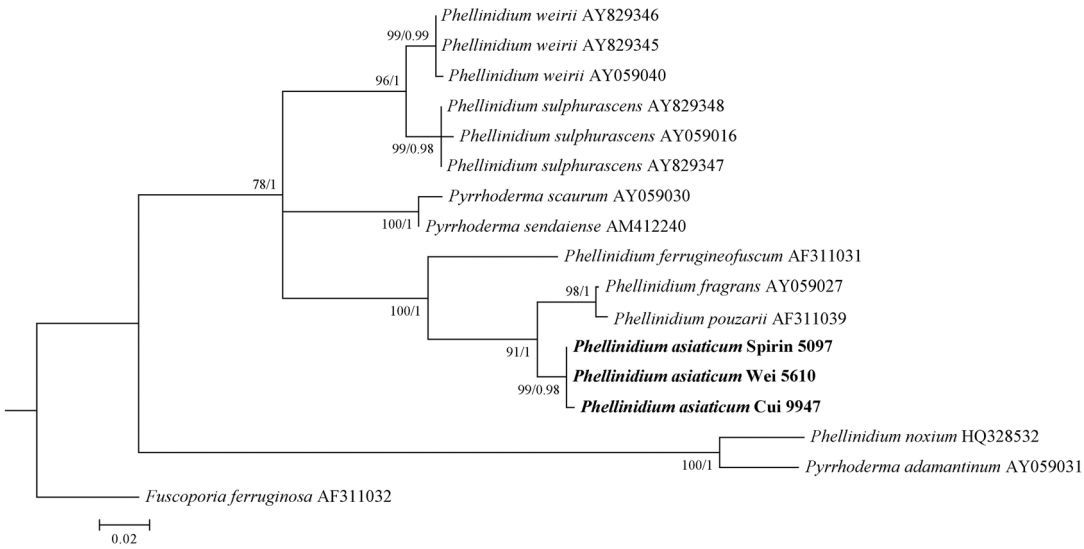

Fig. 1. Phylogeny of *Phellinidium* inferred from nLSU sequences. Topology is from ML, while statistical values (both BS above 70% and PP above 0.95) are indicated at the nodes. The species described in this paper are in boldface.

14 other nLSU sequences downloaded from GenBank were included in our data set. This dataset resulted in an alignment with 882 characters. ML tree was tested by 250 BS replicates. The best-fit model for BI was estimated as GTR + I + G. After running 1 000 000 generations, the values of all ESS (Estimated Sample Size) and PSRF (Potential Scale Reduction Factor) are more than 300 and close to 1, respectively, indicating the two runs converge.

The topologies from ML and BI were nearly congruent, and thus only the topology from ML is presented (Fig. 1). Both statistical values of BS and PP simultaneously above 70% and 0.95, respectively, are indicated at the nodes.

In the current phylogeny (Fig. 1), *Phellinidium* is polyphyletic. Two pathogens, *P. sulphurascens* and *P. weirii*, clustered together. *Phellinidium noxium* and *Pyrrhoderma adamantinum* formed a clade, which was separated from the clade being composed of *Pyrrhoderma sendaiense* (the generic type) and *P. scaurum*. The three newly sequenced specimens formed a distinct lineage, and clustered together with *Phellinidium fragrans*, *P. ferrugineofuscum* (the generic type) and *P. pouzarii* as *Phellinidium* core clade. Those specimens represent the new species described here.

Phellinidium asiaticum Spirin, L.W. Zhou & Y.C. Dai, *sp. nova* (Fig. 2)

MycoBank: MB 807075

Phellinidium fragrans cognatus, sed basidiomatis perennis et setae hyphoideae acutae.

Holotype. China. Jilin Prov.: Antu County, Changbaishan Nat. Res., fallen trunk of *Tilia*, 14 July 2010 *Wei 5610* (IFP; isotype H) (GenBank accession number KC859422).

Etymology. *Asiaticum* (Lat.): referring to the type provenance.

Basidiocarps perennial, resupinate, inseparable, corky and with strong fragrance when fresh, consistency woody hard and with weak fragrance odor when dry, up to 20 cm long, 7 cm wide and 10 mm thick. Pore surface even to step-wise (nodulose) on vertical substrates, snuff-brown to bright ferrugineous-brown, glancing; sterile margin distinct, yellowish to umber-brown or black, up to 10 mm wide; pores round or angular, on sloping positions elongated, 5–7 per mm; dissepiments thin, entire, occasionally lacerate. Subiculum rust-brown, woody hard when dry, azonate, up to 3 mm thick. Tubes greyish brown, paler than pore surface, fibrous to corky, stratified (up to 12 layers),

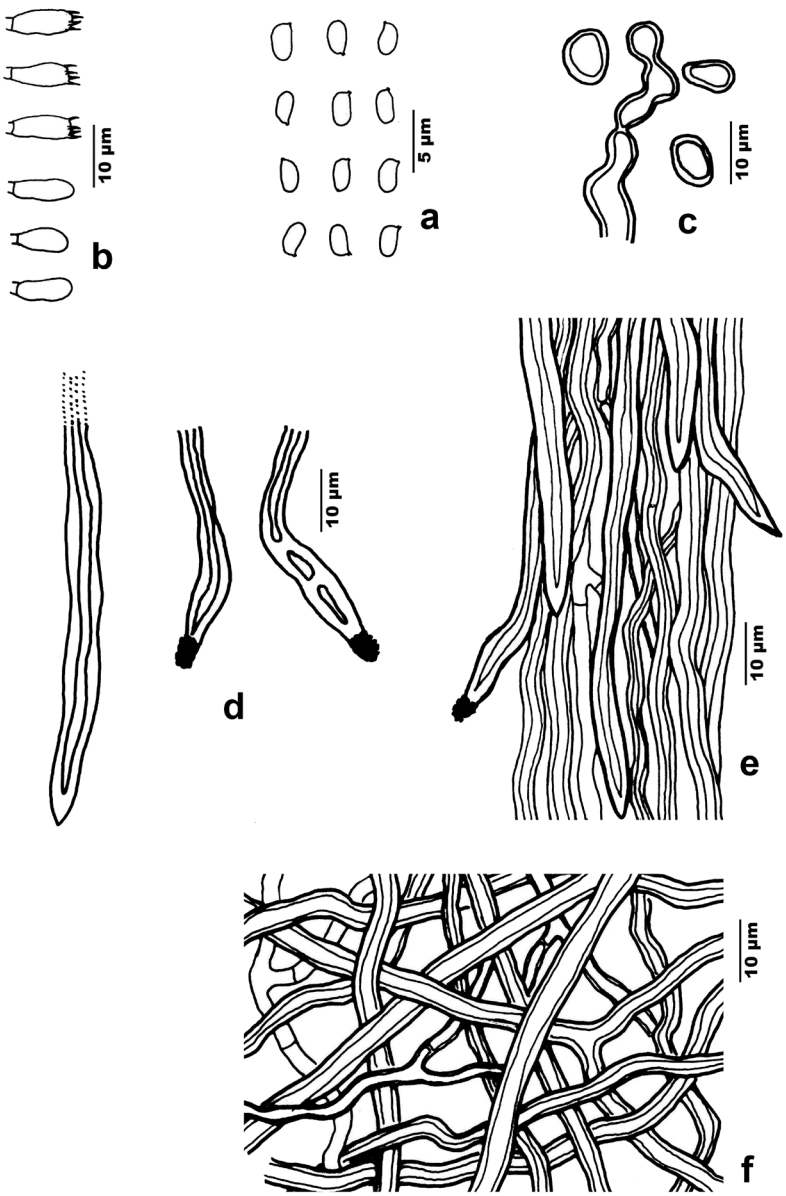

Fig. 2. Microscopic structures of *Phellinidium asiaticum* (from the holotype). — **a**: Basidiospores. — **b**: Basidia and basidioles. — **c**: Conidia. — **d**: Hyphoid setae. — **e**: Hyphae from trama. — **f**: Hyphae from subiculum.

annual layers distinct, each layer up to 4 mm long. Hyphal structure monomitic; generative hyphae simple septate; tissue darkening but otherwise unchanged in KOH. Generative hyphae of context varying from hyaline, thin-walled, frequently branched and septate, 2.2–3 μm in diam, to pale brown, fairly thick-walled, rarely branched, frequently septate, 3–4.5 μm in diam; all transitions present; hyphoid setae originating from pale brownish generative hyphae, rust-brown, thick-walled, unbranched, bearing a wide lumen, slightly broader at terminal part, usually pointed, several hundreds of μm long, 4.2–6.2 μm in diam; both the hyphae and hyphoid setae interwoven. Generative hyphae of tubes varying from hyaline to pale yellowish, thin- to slightly thick-walled, frequently branched and septate, 2–3.8 μm in diam; hyphoid setae dominating, dark brown, thick-walled, more or less parallel along the tubes, several hundred μm long, 4–6 μm in diam; some of them curved and penetrating into hymenium (resembling hymenial setae),

subulate, sharp-pointed, sometimes apically encrusted by small rosettes of crystals, 15–26 × 4–6 μm in hymenium. Subhymenium distinct, up to 10 μm thick, made up of delicate, thin-walled, hyaline hyphae, cells of hymenium and subhymenium CB+; cystidia and cystidioles absent; basidia short clavate to barrel-shaped, with four sterigmata and a simple septum at the base, 7.2–10 × 3.2–4.5 μm; basidioles in shape similar to basidia, but slightly smaller. Basidiospores oblong ellipsoid to thick cylindrical, hyaline, thin-walled, smooth, ventral side flattened or slightly convex, IKI–, CB–, (2.2–)2.4–3.2(–3.3) × (1.4–)1.5–2(–2.1) μm, L = 2.94, W = 1.77, Q = 1.64–1.69 (n = 60/2). Conidia mostly present in subiculum, ellipsoid, oblong-ellipsoid or irregular, yellowish brown, thick-walled, smooth, IKI–, CB–, 6–20 × 5–7 μm.

Discussion

Phellinidium asiaticum was tentatively treated as *P. fragrans* in China due to their similar morphological characters (Dai 2009, 2012). *Phellinidium fragrans* differs from *P. asiaticum* in having hyphoid setae with rounded apices, an indistinct subhymenium and by the presence of cystidia. It seems that *P. fragrans* produces annual or biennial basidiocarps (Larsen & Lombard 1976) while those of *P. asiaticum* are perennial. The basidiospores of *P. fragrans* are almost of the same size as in *P. asiaticum* but they are ellipsoid. *Phellinidium asiaticum* is an Asian species, while *P. fragrans* is so far known from North America (Gilbertson & Ryvarden 1987). Another similar species is *P. pouzarii*, originally described from Europe (Kotlaba 1968), which also has sharpened hyphoid setae and develops conidia in subiculum. However, the pores of *P. pouzarii* are distinctly larger (3–5 per mm), and its spores are narrowly cylindric and longer, 2.6–3.8 × 1.6–1.9 μm. In addition, *P. pouzarii* grows on coniferous trees, especially *Abies* (Kotlaba 1968), while *P. asiaticum* inhabits hardwood hosts.

According to our phylogenic analysis (Fig. 1), *Phellinidium asiaticum*, *P. fragrans* and *P. pouzarii* are closely related, which suggests that they may have originated in speciations. The occurrence of morphologically similar species in different geographic regions seems to be a common case among polypores. For example, two new species of *Antrodiella*, *A. chinensis* from China (Yuan 2013) and *A. niemelaei* from Europe (Vampola & Vlasák 2011), were separated from *A. americana*, originally described from USA. Therefore, taxonomic studies should be more careful and performed from both morphological and phylogenetic perspective, when comparing outwardly similar specimens collected in distant localities.

Phellinidium asiaticum, *P. fragrans* and *P. pouzarii* are confirmed to belong to the core of *Phellinidium*, being closely related to the generic type, *P. ferrugineofuscum* (Fig. 1). *Phellinidium noxium*, *P. sulphurascens* and *P. weirii* were not supported as members of *Phellinidium* in the current phylogeny (Fig. 1), as previous studies already showed (Wagner & Fischer 2002, Larsson *et al*. 2006, Dai 2010). The remaining five species currently placed in *Phellinidium*, but whose phylogenetic position is still unknown, should be studied before a taxonomic splitting of the genus.

Additional specimens examined of *Phellinus asiaticum* (paratypes). **China**. Jilin Prov.: Antu County, Changbaishan Nat. Res., fallen angiosperm trunk, 31 July 2008 *Dai 10058* (IFP), on living *Quercus*; 7 Aug. 2011 *Cui 9947* (BJFC, GenBank accession number KC859423). **Russia**. Khabarovsk Reg.: Khabarovsk Dist., Ilga, fallen decorticated log of *Quercus mongolica*, 10 Aug. 2012 *Spirin 5097* (H, GenBank accession number KC859424).

Other specimens examined. — *Phellinidium fragrans*. **USA**. Michigan: Cheboygan Co., Roxbury Creek, decayed log of *Acer saccharum*, 28 Oct. 1969 *Larsen 3832* (holotype, CFMR). — *Phellinidium pouzarii*. **Croatia**. Lika: Plitvička jezera Nat. Park, *Abies alba*, 9 Oct.1976 *Tortič 338-76* (H).

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