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

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Phellinidium (Hymеноchaetales, 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 Hymenochaetaeae (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 ferrugineofuscus by Kotlaba (1968). Later, Fiasco 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. ruftinctum 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 Hymenochaetaeae 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. noxiuim* represents a third independent lineage within *Phellinidium*, being closely related to *Pyrrhodera adamanantium*.

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 LROR 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 *Pyrrhodera* 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,
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. ferrugineofascium* (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 hyphoidea acutae.


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 (nudulose) 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),
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 phylogenetic 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 polyposes. 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 noxiun, 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.


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References


