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Taxonomy and phylogeny of *Phellinidium* (Hymenochaetales, Basidiomycota): A redefinition and the segregation of *Coniferiporia* gen. nov. for forest pathogens

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ABSTRACT

Phellinidium, including 13 accepted polypore species mostly with resupinate basidiocarps, is one of the most aggressive forest pathogenic genera. This genus is characterized by the combination of a monomitic hyphal structure, abundant hyphoid setae in the context and trama, and hyaline and thin-walled basidiospores. To explore the relationships among the species of *Phellinidium*, especially those between forest pathogens and saprophytes, we examined 29 specimens representing all 13 previously known species from Asia, Europe and America from morphological and phylogenetic perspectives. A new genus, *Coniferiporia*, was found to segregate from *Phellinidium* for three aggressive forest pathogens, and three new combinations, viz. *Coniferiporia qilianensis* (the generic type), *Coniferiporia weirii* and *Coniferiporia sulphurascens*, were proposed. *Phellinidium cryptocystidiatum* was treated as a synonym of *C. sulphurascens*. The circumscription of *Phellinidium* was delimited to accommodate *Phellinidium asiaticum*, *Phellinidium ferrugineofuscum* (the generic type), *Phellinidium fragrans* and *Phellinidium pouzarii*. Accordingly, the concept of *Phellinidium* was emended to accommodate resupinate species bearing cylindrical to oblong-ellipsoid or allantoid basidiospores. No species of *Phellinidium* under the new circumscription has been reported to be a forest pathogen. *Phellinidium noxium* and *Phellinidium rufitinctum* were excluded from *Phellinidium*, while the taxonomical positions of *Phellinidium aciferum*, *Phellinidium lamaënse*, and *Phellinidium orientale* are still uncertain.

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Introduction

Hymenochaetaceae, which includes many valuable medicinal fungi (Dai *et al.* 2010) and forest pathogens (Dai *et al.* 2007), is one of the most economically important families of Hymenochaetales, Basidiomycota. Within this family, *Phellinus* Quél. is one of the largest genera and in a wide sense includes more than 220 species (Larsen & Cobb-Poulle 1990). With the aid of phylogenetic analyses, several more narrowly defined genera have been accepted to accommodate species previously placed in *Phellinus* (Niemelä *et al.* 2001; Wagner & Fischer 2001, 2002; Wagner & Ryvarden 2002; Dai 2010; Zhou 2015; Zhou *et al.* 2016). Among these, *Phellinidium* (Kotl.) Fiasson & Niemelä was raised to a generic level by Fiasson & Niemelä (1984) from a subgenus of *Phellinus*. Ryvarden (1991) argued that the transfer was not valid, but in fact Fiasson & Niemelä (1984) cited the basionym and its place of publication sufficiently to satisfy the requirements of the International Code of Botanical Nomenclature in force at the time (Voss *et al.* 1983; Dai 1995, 2010). Therefore, *Phellinidium* is a legitimate genus name and has been accepted by later mycologists.

Phellinidium undoubtedly belongs to the Hymenochaetaceae (Larsson *et al.* 2006) and, as defined by Fiasson & Niemelä (1984), is distinguished from other genera of this family by a combination of a monomitic hyphal structure, abundant hyphoid setae in the context and trama, and hyaline and thin-walled basidiospores. However, this small genus is polyphyletic and at least three lineages within Hymenochaetaceae were recovered in previous nuclear large subunit ribosomal DNA (nLSU)-based phylogenetic studies (Wagner & Fischer 2002; Larsson *et al.* 2006; Dai 2010; Zhou *et al.* 2014). The morphological differences between two of the three lineages, represented by only five species, were mentioned as the different shapes of basidiospores; however, no taxonomic change was proposed (Wagner & Fischer 2002).

Fiasson & Niemelä (1984) firstly selected *Phellinus ferrugineofuscus* (P. Karst.) Bourdot & Galzin as the generic type of *Phellinidium* and also put *Phellinus pouzarii* Kotl. in the genus. Later, seven other species of *Phellinus* were transferred to *Phellinidium* as *Phellinidium fragrans* (M.J. Larsen & Lombard) Nuss by Nuss (1986), *Phellinidium noxium* (Corner) Bondartseva & S. Herrera, *Phellinidium orientale* (Bondartseva & S. Herrera) Bondartseva & S. Herrera and *Phellinidium rufitinctum* (Berk. & M.A. Curtis ex Cooke) Bondartseva & S. Herrera by Bondartseva *et al.* (1992), and *Phellinidium lamaënse* (Murrill) Y.C. Dai, *Phellinidium sulphurascens* (Pilát) Y.C. Dai and *Phellinidium weirii* (Murrill) Y.C. Dai by Dai (1995). Meanwhile, *Phellinidium aciferum* Y.C. Dai by Dai (1995), *Phellinidium cryptocystidiatum* Spirin & Zmitr. by Spirin *et al.* (2006), *Phellinidium asiaticum* Spirin, L.W. Zhou & Y.C. Dai by Zhou *et al.* (2014) and *Phellinidium qilianense* B.K. Cui, L.W. Zhou & Y.C. Dai by Cui *et al.* (2015) were described, bringing the genus to a total of 13 species.

The species of *Phellinidium* are some of the most aggressive forest pathogens among the basidiomycetes. For example, *P. weirii* causes root and butt rot of western red cedar (*Thuja plicata*), while *P. sulphurascens* can kill several other types of conifers via laminated root rot in North America (Larsen *et al.* 1994). In northwestern China, laminated root rot of Qilian juniper (*Juniperus przewalskii*) is caused by *P. qilianense*, a species recently segregated from *P. weirii* (Cui *et al.* 2015). With regard to angiosperm trees, *P. noxium* was also demonstrated to be an aggressive pathogen (Hattori *et al.* 1996). Nevertheless, other species of *Phellinidium* are considered to be saprotrophs.

To explore the relationships among the species of *Phellinidium*, especially those between forest pathogens and saprophytes, 29 specimens representing all 13 previously known species of this genus from Asia, Europe, and America were examined. After critical morphological and phylogenetic analyses, we introduce a new genus segregated from *Phellinidium* for forest pathogens and redefine the circumscription of *Phellinidium*.

Materials and methods

Morphological examination

The studied specimens were deposited at the herbaria of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP); the Institute of Microbiology, Beijing Forestry University (BJFC); Botanical Museum, the Finnish Museum of Natural History (H); the Center for Forest Mycology Research, Forest Products Laboratory (CFMR); Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences (TAAM); and V. L. Komarov Botanical Institute (LE) as well as the private herbarium of J. Vlasák (JV).

The microscopic procedure followed that described by Zhou & Dai (2013). Sections were prepared in Melzer's reagent (IKI), Cotton Blue (CB) and 5 % potassium hydroxide (KOH) and then examined using a Nikon Eclipse 80i microscope at magnification up to 1000×. The following abbreviations are used in the text: IKI− = negative in Melzer's reagent, CB+ = cyanophilous, CB− = acyanophilous, Q = the ratio of mean basidiospore length and mean basidiospore width. When presenting basidiospore size variations, 30 basidiospores were measured from each specimen, and 5 % of measurements were excluded from each end of the range and are given in parentheses.

Molecular sequencing

The Phire® Plant Direct PCR Kit (Finnzymes Oy, Finland) was used to perform PCR amplifications from herbarium specimens according to the manufacturer's instructions. Internal transcribed spacer (ITS) and nLSU sequences were amplified using the primer pairs ITS5 and ITS4 (White *et al.* 1990) and LR0R and LR7 (Vilgalys & Hester 1990), respectively. The PCR procedure was as follows: initial denaturation at 98 °C for 5 min, followed by 39 cycles at 98 °C for 5 s, 59 °C for 5 s (ITS sequences)/48 °C for 5 s (nLSU sequences) and 72 °C for 5 s, and a final extension at 72 °C for 10 min. After purification, the PCR products were sequenced at the Beijing Genomics Institute, China, with the same primers used for PCR. Two internal primers, LR3 and LR3R (Vilgalys & Hester 1990), were also used for sequencing the nLSU region. All newly generated sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov; Table 1).