

Phellinopsis helwingiae (Hymenochaetales, Basidiomycota), a new species from China and a brief note on *P. junipericola*

Wen-Min Qin^{1,2,*} & Li-Wei Zhou²

¹⁾ Institute of Microbiology, Beijing Forestry University, Beijing 100083, China (*corresponding author's e-mail: wenminqin@sina.com)

²⁾ State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110164, China

Received 2 Apr. 2013, final version received 24 Sep. 2013, accepted 15 Oct. 2013

Qin, W. M. & Zhou, L. W. 2013: *Phellinopsis helwingiae* (Hymenochaetales, Basidiomycota), a new species from China and a brief note on *P. junipericola*. — *Ann. Bot. Fennici* 50: 408–412.

Phellinopsis was recently erected for *P. conchata*, the generic type, and *P. occidentalis*. Later on *P. junipericola* and *P. resupinata* were described as new species. In this study, more specimens of *Phellinopsis* were examined and a phylogenetic analysis based on ITS sequences was carried out. *Phellinopsis helwingiae* L.W. Zhou & W.M. Qin *sp. nova* is described and illustrated from Sichuan Province, southwest China as the fifth species in this genus. In addition, information on the hosts of *P. junipericola* is elaborated.

Introduction

Phellinopsis, belonging to the Hymenochaetales, was recently (Dai 2010) split from *Phellinus s. lato* because of the former's hymenial setae arising from tramal hyphae and colorless basidiospores becoming pale yellowish with age. Dai (2010) placed *P. conchata* and *P. occidentalis* in this genus. The former, designated as the generic type, has a wide distribution, while the latter is known only from USA (Zhou & Qin 2013). Recently, two more species, *P. junipericola* and *P. resupinata*, were described from China by Zhou and Qin (2013). They also provided a key to the four species of *Phellinopsis*.

Quite a few new species in the Hymenochaetales have recently been described from China (Dai 1995, Dai & Cui 2005, Dai *et al.* 2008, Dai & Li 2010, Zhou & Jia 2010, He & Dai 2012, Zhou & Dai 2012, Zhou & Qin 2012, Zhou &

Xue 2012, Tian *et al.* 2013). During the examination of the specimens collected in the east of the Qinghai–Tibetan Plateau in 2012, three specimens of *Phellinopsis* were recognized. Among them, two specimens were identified as *P. junipericola*, and the third one represented an undescribed species.

Material and methods

The studied specimens are deposited in the herbaria of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP) and Beijing Forestry University (BJFC). The microscopic procedure follows Dai (2010). We use the following abbreviations: *L* = mean spore length (arithmetical average of all spores), *W* = mean spore width (arithmetical average of all spores), *Q* = the ratio of *L/W*, *n* = the number of spores

measured from given number of specimens. In presenting the size range of spores, 5% of the measurements were excluded from each end of the range, and the measurements were given in parentheses. IKI stands for Melzer's reagent, IKI- for negative in Melzer's reagent, KOH for 5% potassium hydroxide, CB for Cotton Blue, CB+ for cyanophilous, and CB- for acyanophilous. Drawings were made with the aid of a drawing tube. The color terms follow Petersen (1996).

Three herbarium specimens were directly amplified using Phire[®] Plant Direct PCR Kit (Finnzymes Oy, Finland) according to the manufacturer's instruction. Primers ITS5 and ITS4 (White *et al.* 1990) were used for amplifying ITS sequences. The PCR procedure was as follows: initial denaturation at 98 °C for 5 min, followed by 39 cycles at 98 °C for 5 s, at 59 °C for 5 s and at 72 °C for 5 s, and a final extension at 72 °C for 10 min. The PCR products were sequenced in Beijing Genomics Institute, China with ITS5 and ITS4. Specimens *Yuan 6606* & *6614* were successfully sequenced by both ITS5 and ITS4, while for specimen *Dai 12936* only the result from ITS4 was readable.

The three newly generated ITS sequences (deposited at GenBank; <http://www.ncbi.nlm.nih.gov>; *Dai 12936*: KC843784, *Yuan 6606*: KC843785 and *Yuan 6614*: KC843786) were added to the dataset of Zhou and Qin (2013), resulting in a new dataset for the current phylogenetic analysis. The new dataset was aligned using MAFFT 6.935 (Katoh *et al.* 2002, Katoh & Toh 2008) with auto option. The resulting alignment was analyzed by maximum likelihood (ML) and maximum parsimony (MP) methods using raxmlGUI 1.2 (Stamatakis 2006, Silvestro & Michalak 2012) and PAUP* 4.0b10 (Swofford 2002), respectively. ML tree was constructed under GTR + G model and auto FC option (Pattengale *et al.* 2010) for bootstrap (BS) replicates. Heuristic searches for MP tree were conducted as follows: starting tree obtained via stepwise addition, tree-bisection-reconnection branch swapping, steepest descent option not in effect, and 'multrees' option in effect. All characters had equal weights and gaps were set as missing data. One thousand BS searches were performed to test the MP tree.

Results

The new dataset including 20 ITS sequences generated an alignment with 835 characters, of which 285 were parsimony-informative. The ML tree was tested by 300 BS replicates, and four most parsimonious trees of 758 steps (CI = 0.776, RI = 0.763) were retained. The ML tree is presented with BS values from both ML and MP analyses (Fig. 1) since they generated highly similar topologies. In the current phylogeny (Fig. 1), *Phellinopsis* was recovered as a strongly supported clade, within which *Dai 12936* formed a distinct lineage, while *Yuan 6606* & *6614* clustered together with *P. junipericola*.

Phellinopsis helwingiae L.W. Zhou & W.M. Qin, sp. nova (Figs. 2a and 3)

Mycobank No.: MB 803931.

HOLOTYPE: China. Sichuan Province, Mianning County, Lingshan temple, on living tree of *Helwingia* sp., 18 Sep. 2012 *Dai 12936* (BJFC 013194).

ETYMOLOGY: *helwingiae* (Lat.) refers to the host plant *Helwingia*.

FRUITBODY. Basidiocarps perennial, usually effused-reflexed to pileate, occasionally resupinate; pileus solitary to imbricate, irregular in shape, hard corky and without odor or taste when fresh, woody hard and light-weight when dry. Pilei projecting up to 3 cm, 5 cm wide, and 15 mm thick at base. Pileal surface dark brown to black with age, concentrically sulcate with narrow zones, delicately velutinate, becoming glabrous and forming a crust with age; margin acute. Pore surface yellowish brown, unglancing; sterile margin distinct, yellow, paler than pores, 1 mm wide; pores circular, 6–7 per mm; dissepiments thick, entire. Context dark brown, 0.5 mm thick. Tubes yellowish brown, hard corky, single layer up to 5 mm thick; white mycelial strands present in old tubes.

HYPHAL STRUCTURE. Hyphal system dimitic; generative hyphae simple-septate, CB-; tissue darkening but otherwise unchanged in KOH.

CONTEXT. Generative hyphae infrequent, hyaline, thin-walled, frequently branched and simple-septate, 1–2 µm in diam; skeletal hyphae dominant, yellowish brown, thick-walled with a

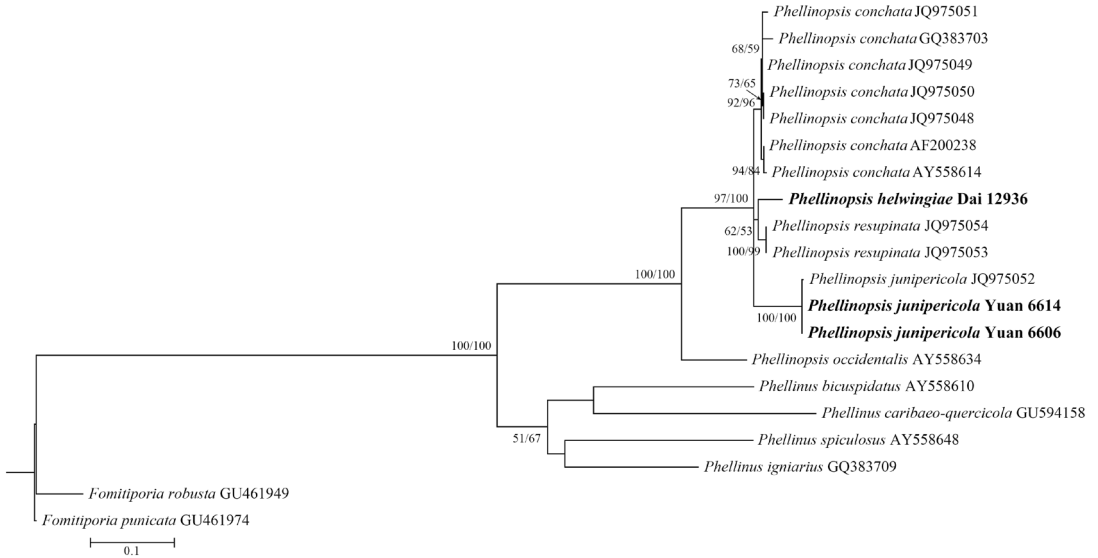


Fig. 1. Phylogenetic position of *Phellinopsis helwingiae* inferred from ITS sequences. ML tree is presented with BS values from MP analysis after those from ML analysis. The three specimens first sequenced for the present study are in boldface.



Fig. 2. Basidiocarps of *Phellinopsis*. — **a:** *P. helwingiae* (holotype). — **b:** *P. junipericola* (Yuan 6606). — **c:** *P. junipericola* (Yuan 6614).

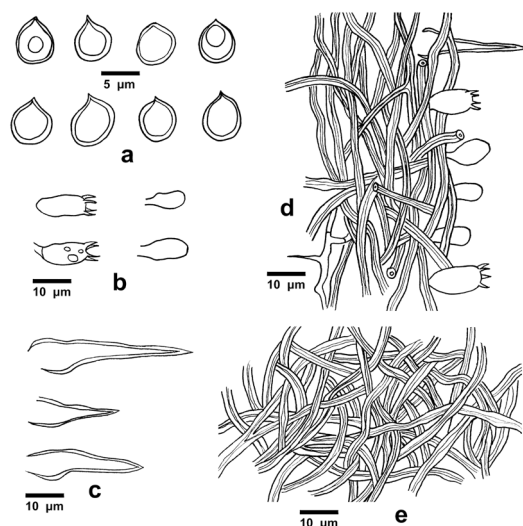


Fig. 3. Microscopic structures of *Phellinopsis helwingiae* (from the holotype). — **a:** Basidiospores. — **b:** Basidia and basidioles. — **c:** Setae. — **d:** Vertical section through trama. — **e:** Hyphae from context.

narrow lumen, rarely branched, interwoven, 2–4 μm in diam.

TUBES. Generative hyphae infrequent, hyaline, thin-walled, frequently branched and septate, 1–2 μm in diam; skeletal hyphae dominant, yellowish brown, slightly thick to thick-walled with a wide to narrow lumen, rarely branched, interwoven, 1.5–2.3 μm in diam. Setae infrequent, subulate, brown to dark brown, thick-walled, 23–50 \times 3.5–8 μm , a few of them shorter or wider. Cystidia and cystidioles absent. Basidia more or less barrel-shaped, with four sterigmata and a simple septum at the base, 9–13 \times 5–6 μm ; basidioles in shape similar to basidia, but slightly smaller.

SPORES. Basidiospores broadly ellipsoid to subglobose, colorless, becoming pale yellowish with age, thick-walled, smooth, IKI–, CB– or weakly CB+, (5–)5.2–6 \times (4.5–)4.7–5.5(–5.8) μm , $L = 5.64 \mu\text{m}$, $W = 5.03 \mu\text{m}$, $Q = 1.12$ ($n = 30/1$).

Discussion

Phellinopsis helwingiae has typical characters of the genus, and clusters together with other

species of *Phellinopsis* (cf. Dai 2010, Zhou & Qin 2013). Compared with the four previously known species, *P. helwingiae* is most similar to *P. conchata* by the effused-reflexed to pileate basidiocarps and similar pore size (6–8 per mm in *P. conchata*), but the latter has unbranched skeletal hyphae and more importantly narrower basidiospores (4–4.5 μm wide; Gilbertson 1979, Ryvarden & Gilbertson 1994, Zhou & Qin 2013). *Phellinopsis resupinata* produces exclusively resupinate basidiocarps and small basidiospores (Zhou & Qin 2013), which are different from those of *P. helwingiae*. *Phellinopsis junipericola* and *P. occidentalis* can be easily distinguished from *P. helwingiae* by having larger pores (< 6 per mm, Gilbertson 1979, Zhou & Qin 2013). Moreover, *P. junipericola* has longer basidiospores (5.7–6.6 μm ; Zhou & Qin 2013) and *P. occidentalis* is restricted to USA (Gilbertson 1979). Our analysis also shows that *P. helwingiae* forms a distinct lineage from other known species of *Phellinopsis*. Therefore, both morphological and phylogenetic evidence supports *P. helwingiae* as a distinct species in *Phellinopsis*.

Phellinopsis junipericola was originally described from Qinghai Province, the northeast of Qinghai-Tibetan Plateau in western China, based on a single type specimen (Zhou & Qin 2013). That specimen grew on an endemic gymnosperm tree, *Junipericola przewalskii* (Zhou & Qin 2013). We found two additional specimens of *P. junipericola* (Yuan 6606 & 6614; cf. Fig. 2b and c), collected from Ningxia Autonomous Region, which is also located in the northeast Qinghai-Tibetan Plateau. However, the two cited specimens (*see below*) inhabit angiosperm wood.

OTHER SPECIMENS STUDIED. — *Phellinopsis conchata*. **China.** Henan Province, Neixiang County, Baotianman Nature Reserve, on living *Juglans* sp., *Dai 11291* (IFP 016033, BJFC 007265); on living *Toxicodendron* sp., *Dai 11299* (IFP 016034, BJFC 007273). Shanxi Province, Jiaocheng County, Panguangou Nature Reserve, on dead branch of living *Salix* sp., *Yuan 840* (IFP 004167, BJFC 001666), *Yuan 926* (IFP 004185, BJFC 001667), *Yuan 2492* (IFP 004187, BJFC 001664). — *Phellinopsis junipericola*. **China.** Qinghai Province, Huzhu County, Beishan Forest Farm, on bark of *Juniperus przewalskii*, *Dai 5057* (holotype IFP 016007). Ningxia Autonomous Region, Jingyuan County, Liupanshan National Forest Park, on fallen angiosperm branch, *Yuan 6606* (IFP 018590; Fig. 2b), *Yuan 6614* (IFP

018598; Fig. 2c). Henan Province, Neixiang County, Baotianman Nature Reserve, on fallen trunk of *Quercus* sp., *Dai 11318* (holotype IFP 016035, isotype BJFC 007464), *Dai 11329* (IFP 016036, BJFC 007475).

Acknowledgements

Yu-Cheng Dai (BJFC, China) is thanked for improving the manuscript. This research was financed by the National Natural Science Foundation of China (project no. 31200015).

References

- Dai, Y. C. 1995: Changbai wood-rotting fungi 3. The genus *Phellinidium* (Basidiomycetes) and a new species, *P. aciferum*. — *Annales Botanici Fennici* 32: 63–73.
- Dai, Y. C. 2010: Hymenochaetaceae (Basidiomycota) in China. — *Fungal Diversity* 45: 131–343.
- Dai, Y. C. & Cui, B. K. 2005: Two new species of Hymenochaetaceae from eastern China. — *Mycotaxon* 94: 341–347.
- Dai, Y. C. & Cui, B. K. 2011: *Fomitiporia ellipsoidea* has the largest fruiting body among the fungi. — *Fungal Biology* 115: 813–814.
- Dai, Y. C., Cui, B. K. & Decock, C. 2008: A new species of *Fomitiporia* (Hymenochaetaceae, Basidiomycota) from China based on morphological and molecular characters. — *Mycological Research* 112: 375–380.
- Dai, Y. C. & Li, H. J. 2010: Notes on *Hydnochaete* (Hymenochaetales) with a seta-less new species discovered in China. — *Mycotaxon* 111: 481–487.
- Gilbertson, R. L. 1979: The genus *Phellinus* (Aphyllphorales: Hymenochaetaceae) in western North America. — *Mycotaxon* 9: 51–89.
- He, S. H. & Dai, Y. C. 2012: Taxonomy and phylogeny of *Hymenochaete* and allied genera of Hymenochaetaceae (Basidiomycota) in China. — *Fungal Diversity* 56: 77–93.
- Katoh, K., Misawa, K., Kuma, K. & Miyata, T. 2002: MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. — *Nucleic Acids Research* 30: 3059–3066.
- Katoh, K. & Toh, H. 2008: Recent developments in the MAFFT multiple sequence alignment program. — *Briefings in Bioinformatics* 9: 286–298.
- Pattengale, N. D., Alipour, M., Bininda-Emonds, O. R. P., Moret, B. M. E. & Stamatakis, A. 2010: How many bootstrap replicates are necessary? — *Journal of Computational Biology* 17: 337–354.
- Petersen, J. H. 1996: *Farvekort. The Danish Mycological Society's colour-chart*. — Foreningen til Svampekundskabens Fremme, Greve.
- Ryvarden, L. & Gilbertson, R. L. 1994: European polypores 2. *Meripilus*–*Tyromyces*. — *Synopsis Fungorum* 7: 394–743.
- Silvestro, D. & Michalak, I. 2012: raxmlGUI: a graphical front-end for RAXML. — *Organisms Diversity & Evolution* 12: 335–337.
- Stamatakis, A. 2006: RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. — *Bioinformatics* 22: 2688–2690.
- Swofford, D. L. 2002: *PAUP*: Phylogenetic analysis using parsimony (*and other methods)*, version 4.0b10. — Sinauer Associates, Sunderland.
- Tian, X. M., Yu, H. Y., Zhou, L. W., Decock, C., Vlasák, J. & Dai, Y. C. 2013: Phylogeny and taxonomy of the *Inonotus linteus* complex. — *Fungal Diversity* 58: 159–169.
- White, T. J., Bruns, T. D., Lee, S. & Taylor, J. W. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. — In: Innis, M. A., Gelfand, D. H., Sninsky, J. & White, T. J. (eds.), *PCR Protocols: a guide to methods and applications*: 315–322. Academic Press, San Diego.
- Zhou, L. W. & Dai, Y. C. 2012: Phylogeny and taxonomy of *Phylloporia* (Hymenochaetales) with the description of five new species and a key to worldwide species. — *Mycologia* 104: 211–222.
- Zhou, L. W. & Jia, B. S. 2010: A new species of *Phellinus* (Hymenochaetaceae) growing on bamboo in tropical China. — *Mycotaxon* 114: 211–216.
- Zhou, L. W. & Qin, W. M. 2012: *Inonotus tenuicontextus* sp. nov. (Hymenochaetaceae) from Guizhou, southwest China with a preliminary discussion on the phylogeny of its kin. — *Mycological Progress* 11: 791–798.
- Zhou, L. W. & Qin, W. M. 2013: Phylogeny and taxonomy of the recently proposed genus *Phellinopsis* (Hymenochaetales, Basidiomycota). — *Mycologia* 105: 689–696.
- Zhou, L. W. & Xue, H. J. 2012: *Fomitiporia pentaphylacis* and *F. tenuitubus* spp. nov. (Hymenochaetales, Basidiomycota) from Guangxi, southern China. — *Mycological Progress* 11: 907–913.