Neofavolus yunnanensis sp. nov. (Polyporales, Basidiomycota) from China: evidence from morphology and DNA sequence data

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Abstract

A new wood-inhabiting species, Neofavolus yunnanensis, is proposed based on a combination of morphological features and molecular evidence. The species is characterized by an annual growth, laterally stipitate basidiomata with reniform to semicircular pileus, dimitic hyphal system with clamped generative hyphae, IKI–, CB–, and cylindrical, hyaline, thin-walled, smooth, IKI–, CB– basidiospores. Sequences of ITS and LSU nrRNA gene regions of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and Bayesian inference methods. The phylogenetic analyses showed that N. yunnanensis was nested into the genus Neofavolus and then was as a sister with N. mikawae and then grouped with N. alveolaris and N. cremeoalbidus.

Keywords: China, Polyporaceae, phylogeny, taxonomy, wood-inhabiting fungi

Introduction

Neofavolus Sotome & T. Hatt. (2013: 249) (Polyporaceae, Polyporales) is a small genus characterized by a combination of annual, laterally to rarely centrally stipitate basidiomata with reniform to semicircular pileus, in which the surface covered with flatted scales or smooth, white to cream or brownish and the stipe cylindrical, and hyphal system dimitic with the clamped generative hyphae and hyaline, IKI−, skeletal-binding hyphae, and the basidiospores cylindrical, thin-walled, smooth, hyaline, acyanophilous and negative in Melzer’s reagent (Sotome et al. 2013). So far four species have been accepted in the genus in the Northern Hemisphere: N. alveolaris (DC.) Sotome & T. Hatt. (generic species) (2013: 250), N. cremeoalbidus Sotome & T. Hatt. (2013: 250), N. mikawae (Lloyd) Sotome & T. Hatt. (2013: 251) and N. suavissimus (Fr.) J.S. Seelan, Justo & Hibbett (2015: 468) (Fries 1836, Sotome et al. 2013, Seelan et al. 2015).

Phylogenetic studies placed at least one species of Neofavolus (N. suavissimus= Lentinus suavissimus Fr.) in the core polyporoid clade (Binder et al.2013). Sotome et al. (2013) described Neofavolus as a new genus with three species, while Seelan et al. (2015) proposed a new combination and placed Neofavolus close to the clade of Favolus.

During investigations on wood-inhabiting fungi in southern China, specimens that could not be assigned to any described species were found. In this study, the authors expand samplings from previous studies to examine taxonomy and phylogeny of this new species within Neofavolus, based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences.

Materials and methods

Morphological studies.—The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC). Macro-morphological descriptions are based on field notes. Colour terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens, and observed under a light microscope following...
Dai (2010). The following abbreviations were used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer’s reagent, IKI– = both inamyloid and indextrinoid, 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 (a/b) = number of spores (a) measured from given number (b) of specimens.

**DNA extraction, amplification, sequencing and phylogenetic analyses.**—The EZNA HP Fungal DNA Kit (Omega Biotechnologies Co., Ltd, Kunming) was used to obtain PCR products from dried specimens, according to the manufacturer’s instructions with some modifications. ITS region was amplified with primer pairs ITS5 and ITS4 (White et al. 1990). Nuclear LSU region was amplified with primer pairs LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 48°C 1 min and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited at GenBank (Table 1).

<table>
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<th>Species name</th>
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Sequencer 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 6 (Katoh & Toh 2008, http://mafft.cbrc.jp/alignment/server/) using the “G-INS-I” strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (submission ID 23174). Sequences of *Lentinus tigrinus* (Bull.) Fr. and *Polyporus arcularius* (Batsch) Fr. obtained from GenBank were used as outgroup to root trees following Sotome et al. (2013) in the ITS+nLSU analyses (Fig. 1).

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analysis followed Song et al. (2016a), and 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 1,000 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. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 on Abe through the Cipres Science Gateway (www.phylo.org; Miller et al. 2009). Branch support for ML analysis was determined by 1000 bootstrap replicate.

**FIGURE 1.** Maximum Parsimony strict consensus tree illustrating the phylogeny of *Neofavolus yunnanensis* and related species in *Neofavolus* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). 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 & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for for 3 million generations (Fig. 1) 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 likelihood (BS), maximum parsimony (BP) and Bayesian posterior probabilities (BPP) greater than or equal to 75% (BP) and 0.95 (BPP) were considered as significantly supported, respectively.

**Results**

**Molecular phylogeny**

The ITS+nLSU dataset (Fig. 1) included sequences from 19 fungal specimens representing six species. The dataset had an aligned length of 1813 characters, of which 1643 were constant, 37 are variable and parsimony-uninformative, and 133 are parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious trees (TL = 212, CI = 0.939, HI = 0.061, RI = 0.976, RC = 0.916). The best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis was GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian and ML analyses resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.002113 (BI).

The phylogenetic tree (Fig. 1) inferred from the combined ITS+nLSU sequences showed that the new species formed a monophyletic entity with a high 100% BS, 100% BP and 1.00 BPP and then grouped with *N. cremeoalbidus* (2013: 250).
Taxonomy

*Neofavolus yunnanensis* C.L. Zhao, *sp. nov.* (Figs. 2, 3)

MycoBank no.: MB 830683

*Type.—* China. Yunnan Province, Kunming, Panlong District, Yeyahu Forestry Park, on the fallen branch of angiosperm, 23 June 2017, CLZhao 1639 (holotype, SWFC!)

**FIGURE 2.** Basidiomata of *Neofavolus yunnanensis* (holotype). Scale bars: a, b–5 mm.

Etymology.—Yunnanensis (Lat.): referring to the locality (Yunnan Province) of the type specimens.

Basidiomata.—Basidiomata annual, laterally stipitate, solitary. Pileus reniform to semicircular, circular in centrally stipitate, applanate to conchate, 0.5–1 cm from the base to margin, 1.5–2 cm wide, up to 4 mm thick; the surface radially striate, white to cream (60, 21), covered with appressed scales, azonate; the margin acute, entire. Pores radially elongated, 2–3 per mm, dissepiments thin, entire. Stipe cylindrical, up to 4 mm long, up to 2 mm in diam. Context fleshy tough to leathery in fresh condition, brittle or corky to leathery in dried condition, cream (21), thin, up to 1 mm thick. Tubes concolorous with poroid surface, corky, up to 3 mm long.

Hyphal structure.—Hyphal system dimitic; generative hyphae with clamp connections, IKI−, CB−; tissues unchanged in KOH.
Context.—Generative hyphae infrequent, hyaline, thin-walled, unbranched, 1.5–2 μm in diam.; skeletal-binding hyphae dominant, hyaline, thick-walled with a narrow to wide lumen, frequently branched, interwoven, 3–5 μm in diam.

Tubes.—Generative hyphae infrequent, hyaline, thin-walled, unbranched, 1–2.5 μm in diam.; skeletal-binding hyphae dominant, hyaline, thick-walled with a narrow to wide lumen, frequently branched, interwoven, 3–4 μm. Cystidia and cystidioles absent; basidia barrel-shaped, with four sterigmata and a basal clamp connection, 13–20 × 10–14 μm; basidioles dominant, mostly pear-shaped, but slightly smaller than basidia.

Spores.—Basidiospores cylindrical, hyaline, thin-walled, smooth, non-dextrinoid, CB–, (5–)5.5–7.5(–8) × 2–3(–3.5) μm, L = 6.4 μm, W = 2.65 μm, Q = 2.06–2.62 (n = 60/2).

Additional specimen examined.—China. Yunnan Province, Panlong District, Yeyahu Forestry Park, on the fallen branch of angiosperm, 23 June 2017, CLZhao 1633 (paratype, SWFC!).

Discussion

In the present study, a new species, *N. yunnanensis*, is described based on phylogenetic analyses and morphological characters.

*Neofavolus yunnanensis* is closely related to *N. mikawae* (Fig. 1), but morphologically *N. mikawae* produces larger, cream to brownish orange basidiomata with glabrous pileus surface, smaller basidia (12–16.5 × 5–8 μm) and larger basidiospores (6–9.5 × 2.3–3.6 μm), (Sotome et al. 2013).

From the other species of *Neofavolus*, *N. alveolaris* differs from by the larger pores (0.5–3 per mm) and basidiospores (7–10 × 2.5–4 μm), (Sotome et al. 2013), while *N. cremeoalbidus* differs in having the brownish orange to grayish orange basidiomata and larger basidiospores (8–12 × 3–4 μm, Sotome et al. 2013).

Macroscopically, *N. suavissimus* is separated from *N. yunnanensis* by its larger, subsessile basidiomata with subporoid hymenophore at the apex of the stipe (Fries 1836, Seelan et al. 2015).

Wood-rotting fungi is an extensively studied group of Basidiomycota (Gilbertson & Ryvarden 1986, 1987, Núñez & Ryvarden 2001, Dai 2012, Ryvarden & Melo 2014; Dai et al. 2015), but the Chinese wood-rotting fungi diversity is still not well known, especially in subtropics and tropics, many recently described taxa of wood-rotting fungi were from these areas (Zhou & Dai 2013, Zhao & Cui 2013, 2014, Song et al. 2016b, Zhou et al. 2016, Wu et al. 2017, Yuan et al. 2017a, b). The new species in the present study, *N. yunnanensis*, is from subtropics, too.

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