Phytotaxa 408 (2): 109–116
https://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.408.2.3

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

KAI-YUE LUO[3], XIANG MA[3] & CHANG-LIN ZHAO[1,2,3]*

[1]*Key Laboratory of State Forestry and Grassland Administration for Biodiversity Conservation in Southwest China, Southwest Forestry University, Kunming 650224, P.R. China*
[2]*Key Laboratory for Forest Resources Conservation and Utilization in the Southwest Mountains of China, Ministry of Education, Southwest Forestry University, Kunming 650224, P.R. China*
[3]*College of Biodiversity Conservation, Southwest Forestry University, Kunming 650224, P.R. China*
** Corresponding author's e-mail: fungichanglinz@163.com*

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

Accepted by Masoomeh Ghobad-Nejhad: 22 Jun. 2019; published: 28 Jun. 2019

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 1. A list of species, specimens and GenBank accession number of sequences used in this study.

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Favolus acervatus	F 27443	AB735975	—	Sotome *et al*. (2008)
F. acervatus	F 14764	AB735972	—	Sotome *et al*. (2008)
Lentinus tigrinus	MUCL 22821	AB478881	AB368072	Sotome *et al*. (2008)
Neofavolus alveolaris	TUFC 14556	AB735969	AB735947	Sotome *et al*. (2013)
N. alveolaris	TUFC 14544	AB735968	AB735949	Sotome *et al*. (2013)
N. alveolaris	WD 2340	AB735970	AB368077	Sotome *et al*. (2013)
N. alveolaris	WD 2358	AB368079	AB587624	Sotome *et al*. (2013)
N. alveolaris	TUFC 14286	AB735967	AB735948	Sotome *et al*. (2013)
N. cremeoalbidus	TUFC 14497	AB735955	AB735978	Sotome *et al*. (2013)
N. cremeoalbidus	TUFC 14528	AB735956	AB735979	Sotome *et al*. (2013)
N. cremeoalbidus	TUFC 14541	AB735958	AB735981	Sotome *et al*. (2013)
N. cremeoalbidus	TUFC 14529	AB735957	AB735980	Sotome *et al*. (2013)
N. mikawae	TUFC 14501	AB735944	AB735964	Sotome *et al*. (2013)
N. mikawae	TUFC 14360	AB735960	AB735963	Sotome *et al*. (2013)
N. mikawae	TUFC 14359	AB735942	AB735962	Sotome *et al*. (2013)
N. mikawae	TUFC 14350	AB735941	AB735961	Sotome *et al*. (2013)
N. mikawae	TFM F-27380	AB735940	AB735960	Sotome *et al*. (2013)
N. mikawae	TFM F-27346	AB735949	AB735959	Sotome *et al*. (2013)
N. yunnanensis	CLZhao 1633	MK834521	MK834523	Present study
N. yunnanensis	CLZhao 1639	MK834522	MK834524	Present study
Polyporus arcularius	WD 2359	AB368082	AB478875	Sotome *et al*. (2008)

Sequencher 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.

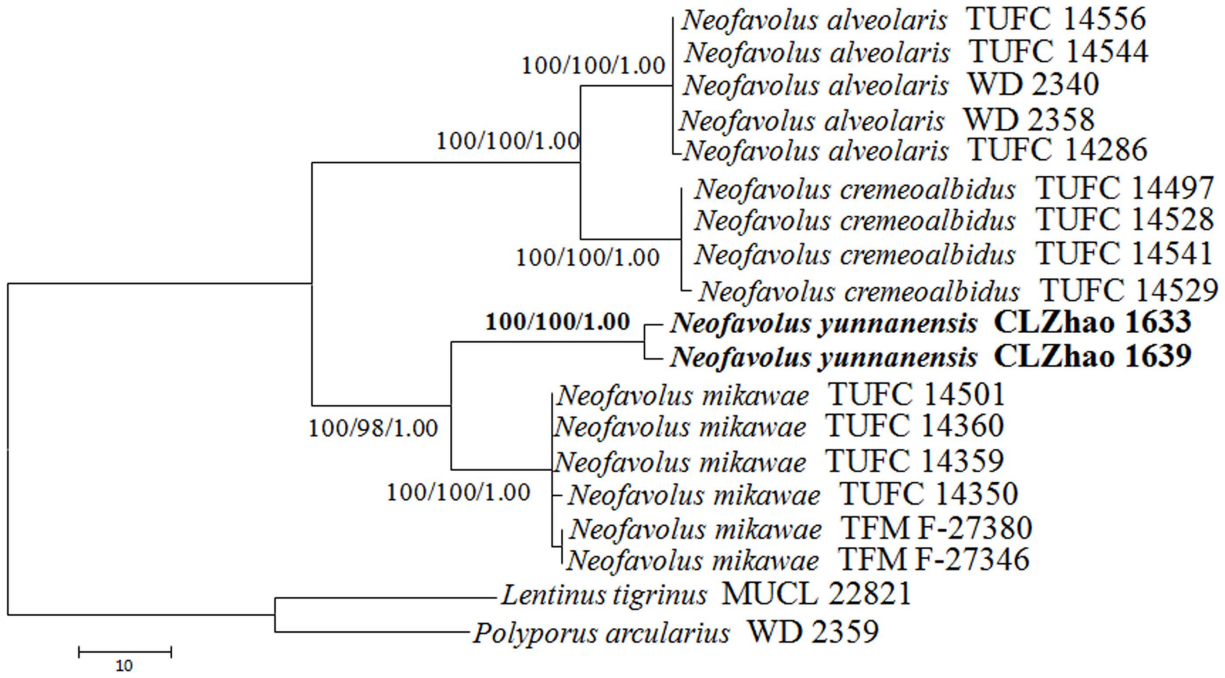

FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Neofavolus yunnanensis* and related species in *Neofavolus* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian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

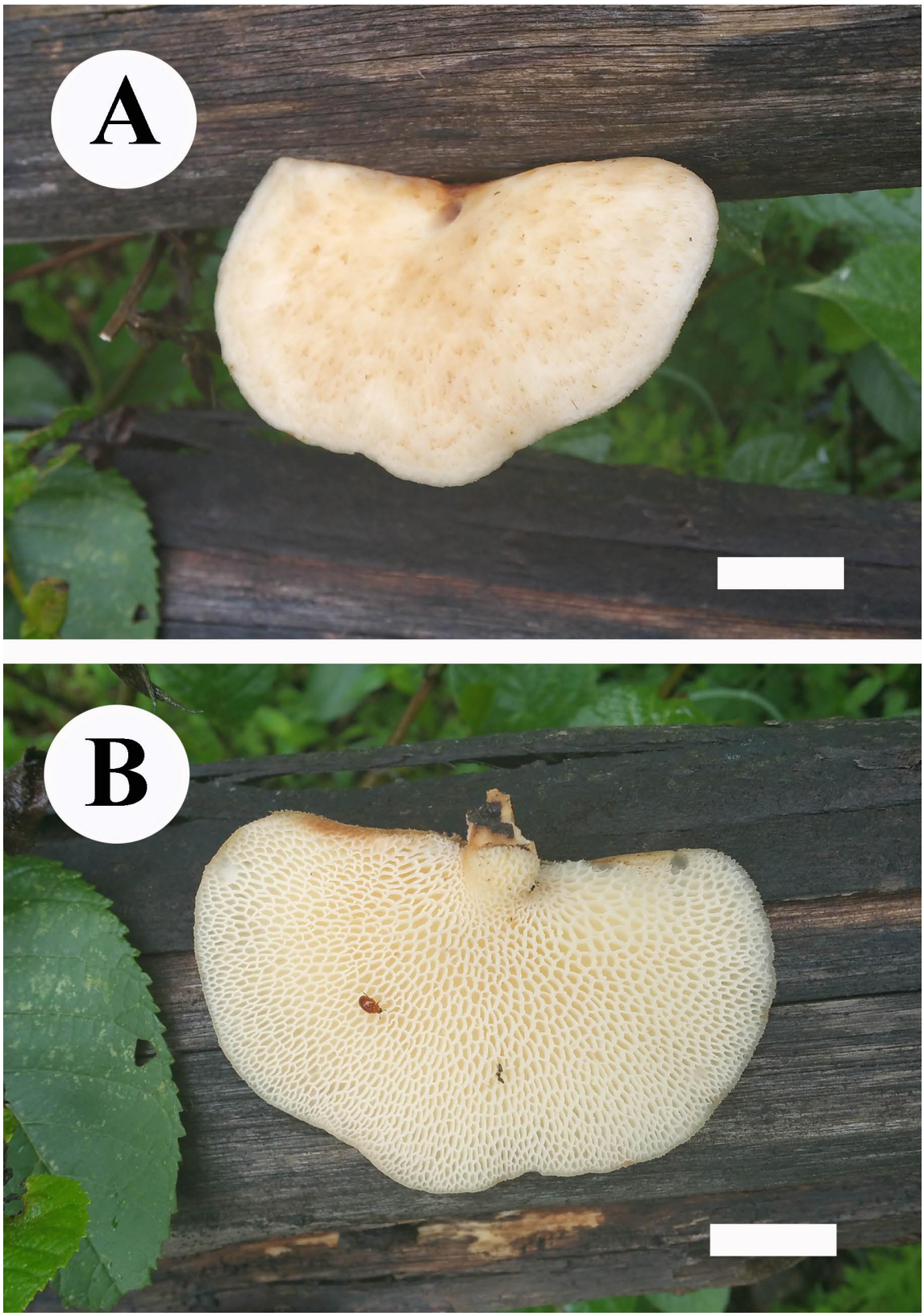

FIGURE 2. Basidiomata of *Neofavolus yunnanensis* (holotype). Scale bars: a, b–5 mm.

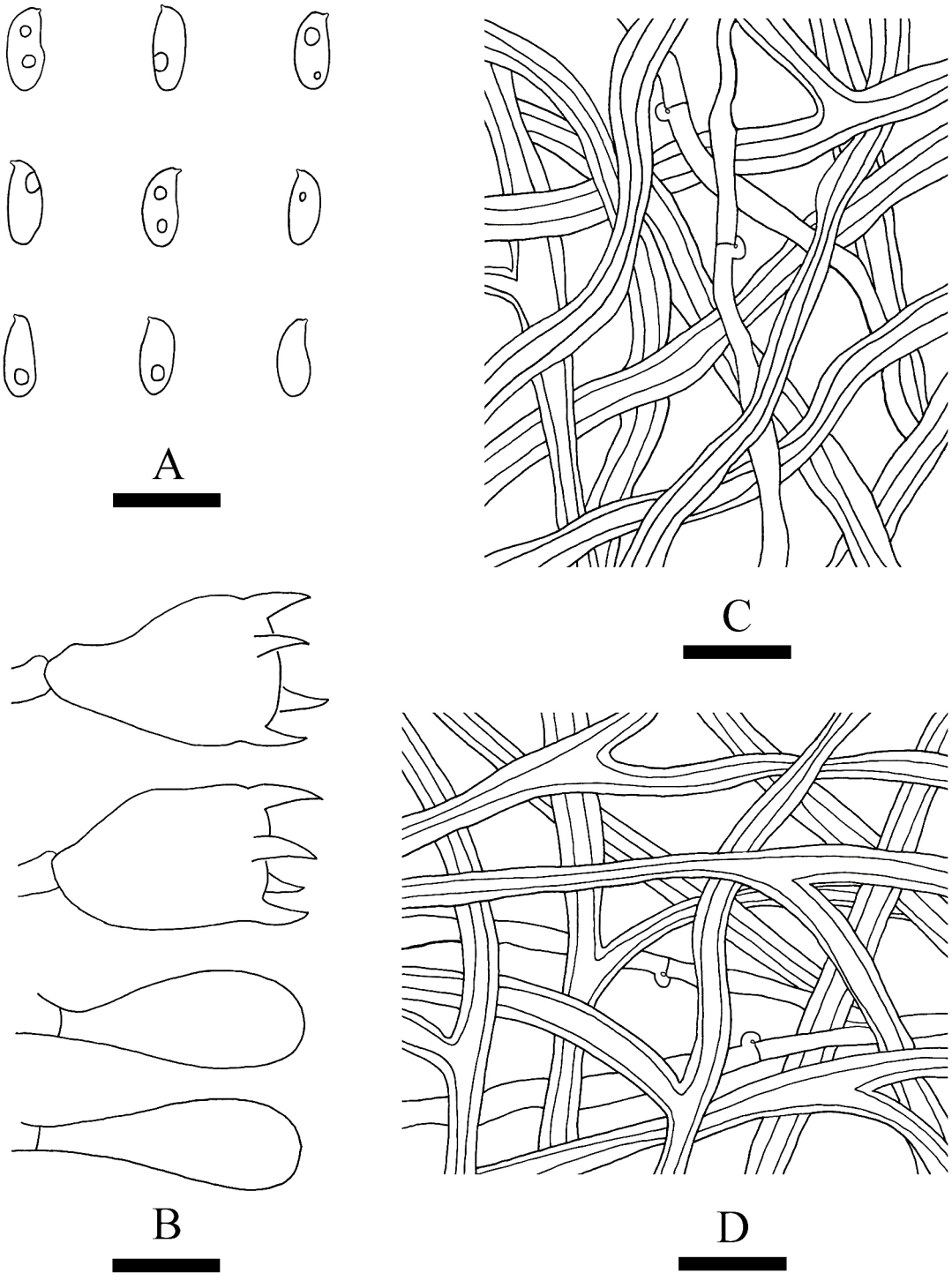

FIGURE 3. Microscopic structures of *Neofavolus yunnanensis* (drawn from the holotype). a. basidiospores. b. basidia and basidioles. c. hyphae from trama. d. hyphae from context. Bars: a, b, c, d–10 μm.

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 sub-poroid 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.

Acknowledgements

The research is supported by the National Natural Science Foundation of China (Project No. 31700023), Yunnan Agricultural Foundation Projects (2017FG001-042), the Science Foundation of Southwest Forestry University (Project No. 111715) and the Yunnan Provincial Innovation Team on Kapok Fiber Industrial Plantation (2018HC014).

References

Binder, M., Justo, A., Riley, R., Salamov, A., López-Giráldez, F., Sjökvist, E., Copeland, A., Foster, B., Sun, H., Larsson, E., Larsson, K.H., Townsend, J., Grigoriev, I.V. & Hibbett, D.S. (2013) Phylogenetic and phylogenomic overview of the Polyporales. *Mycologia* 105: 1350–1373.
https://doi.org/10.3852/13-003

Dai, Y.C. (2010) Hymenochaetaceae (Basidiomycota) in China. *Fungal Diversity* 45: 131–343.
https://doi.org/10.1007/s13225-010-0066-9

Dai, Y.C. (2012) Polypore diversity in China with an annotated checklist of Chinese polypores. *Mycoscience* 53: 49–80.
https://doi.org/10.1007/s10267-011-0134-3

Dai, Y.C., Cui, B.K., Si, J., He, S.H., Hyde, K.D., Yuan, H.S., Lui, X.Y. & Zhou, L.W. (2015) Dynamics of the worldwide number of fungi with emphasis on fungal diversity in China. *Mycological Progress* 14: 62.
https://doi.org/10.1007/s11557-015-1084-5

Felsenstein, J. (1985) Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* 39: 783–791. https://doi.org/10.2307/2408678

Fries, E.M. (1836) Synopsis generis *Lentinorum*. *Sylloge Fungorum* 610: 1–16.

Gilbertson, R.L. & Ryvarden, L. (1986-1987) North American polypores 1–2. Fungiflora, Oslo. pp. 1–433.

Hall, T.A. (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

Katoh, K. & Toh, H. (2008) Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298. https://doi.org/10.1093/bib/bbn013

Miller, M.A., Holder, M.T., Vos, R., Midford, P.E., Liebowitz, T., Chan, L., Hoover, P. & Warnow, T. (2009) The CIPRES Portals. CIPRES. URL: http://www.phylo.org/sub_sections/portal. 2009-08-04. (Archived by WebCite(r) at http://www.webcitation.org/5imQlJeQa).

Núñez, M. & Ryvarden, L. (2001) East Asian polypores 2. *Synopsis Fungorum* 14: 165–522.

Nylander, J.A.A. (2004) *MrModeltest v2. Program distributed by the author*. Evolutionary Biology Centre, Uppsala University.

Petersen, J.H. (1996) Farvekort.*The Danish Mycological Society's colour-chart*. Foreningen til Svampekundskabens Fremme, Greve. 6 pp.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes3: bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Ryvarden, L. & Melo, I. (2014) Poroid fungi of Europe. *Synopsis Fungorum* 31:1–455.

Seelan, J.S., Justo, A., Nagy, L.G., Grand, E., Redhead, S.A. & Hibbett, D.S. (2015) Phylogenetic relationships and morphological evolution in *Lentinus*, *Polyporellus* and *Neofavolus*, emphasizing southeastern Asian taxa. *Mycologia* 107: 460–474. https://doi.org/10.3852/14-084

Song, J., Chen, J.J., Wang, M., Chen, Y.Y. & Cui, B.K. (2016a) Phylogeny and biogeography of the remarkable genus *Bondarzewia* (Basidiomycota, Russulales). *Scientific Reports* 6: 34568. https://doi.org/10.1038/srep34568

Song, J., Xing, J.H., Decock, C., He, X.L. & Cui, B.K. (2016b) Molecular phylogeny and morphology reveal a new species of *Amauroderma* (Basidiomycota) from China. *Phytotaxa* 260: 47–56. https://doi.org/10.11646/phytotaxa.260.1.5

Sotome, K., Hattori, T., Ota, Y., To-anun, C., Salleh, B. & Kakishima, M. (2008) Phylogenetic relationships of *Polyporus* and morphologically allied genera. *Mycologia* 100: 603–615. https://doi.org/10.3852/07-191R

Sotome, K., Akagi, Y., Lee, S.S., Ishikawa, N.K. & Hattori, T. (2013) Taxonomic study of *Favolus* and *Neofavolus* gen. nov. segregated from *Polyporus* (Basidiomycota, Polyporales). *Fungal Diversity* 58: 245–266. https://doi.org/10.1007/s13225-012-0213-6

Swofford, D.L. (2002) *PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10*. Sinauer Associates, Massachusetts.

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR Protocols: A guide to methods and applications*. Academic Press, San Diego, pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wu, F., Zhou, L.W., Yuan, Y. & Dai, Y.C. (2017) *Aporpium miniporum*, a new polyporoid species with vertically septate basidia from southern China. *Phytotaxa* 317: 137–143. https://doi.org/10.11646/phytotaxa.317.2.6

Yuan, H.S., Mu, Y.H. & Qin, W.M. (2017a) A new species of *Postia* (Basidiomycota) based on morphological and molecular characteristics. *Phytotaxa* 292: 287–295. https://doi.org/10.11646/phytotaxa.292.3.9

Yuan, Y., Ji, X.H., Wu, F. & Chen, J.J. (2017b) *Ceriporia albomellea* (Phanerochaetaceae, Basidiomycota), a new species from tropical China based on morphological and molecular evidences. *Phytotaxa* 298: 20–28. https://doi.org/10.11646/phytotaxa.298.1.2

Zhao, C.L. & Cui, B.K. (2013) *Truncospora macrospora* sp. nov. (Polyporales) from Southwest China based on morphological and molecular data. *Phytotaxa* 87: 30–18. https://doi.org/10.11646/phytotaxa.87.2.2

Zhao, C.L. & Cui, B.K. (2014) Phylogeny and taxonomy of *Ceriporiopsis* (Polyporales) with descriptions of two new species from southern China. *Phytotaxa* 164: 17–28.

https://doi.org/10.11646/phytotaxa.164.1.2

Zhou, L.W. & Dai, Y.C. (2013) Taxonomy and phylogeny of wood-inhabiting hydnoid species in Russulaes: two new genera, three new species and two new combinations. *Mycologia* 105: 636–649.
https://doi.org/10.3852/12-011

Zhou, J.L., Zhu, L., Chen, H. & Cui, B.K. (2016) Taxonomy and phylogeny of *Polyporus* group *Melanopus* (Polyporales, Basidiomycota) from China. *PLoS ONE* 11 (8): e0159495.
https://doi.org/10.1371/journal.pone.0159495