Mycological Progress (2020) 19:471–480
https://doi.org/10.1007/s11557-020-01574-8

DGfM

ORIGINAL ARTICLE

Two new species of *Neofavolus* (Polyporales, Basidiomycota) based on morphological characters and molecular evidence

Jia-Hui Xing[1] · Jun-Liang Zhou[1,2] · Bao-Kai Cui[1]

Received: 1 November 2019 / Revised: 28 February 2020 / Accepted: 2 March 2020

Abstract
During taxonomic studies of *Neofavolus*, two new species were discovered. *Neofavolus americanus* sp. nov. was collected in the USA and is characterized by a white to cream pileal surface when fresh, large pores, 1–3 mm long × 0.5–1 mm wide, and basidiospores 10.4–12 × 3.8–4.5 μm. *Neofavolus squamatus* sp. nov. was discovered in China and is characterized by bright squamae on the pileus and large pores, 0.7–3 mm long and 0.5–1.5 mm wide. Phylogenetic analyses carried out based on sequences from the internal transcribed spacer regions (ITS) – the large subunit of nuclear ribosomal RNA gene (nLSU), the small subunit of nuclear ribosomal RNA gene (nSSU), the small subunit of mitochondrial rRNA gene (mtSSU), the largest subunit of RNA polymerase II (*RPB1*), the second largest subunit of RNA polymerase II (*RPB2*), the β-tubulin gene (*TUB*), and the translation elongation factor 1-α gene (*TEF*) – confirmed affinities of the two new species within *Neofavolus*.

Keywords Multi-gene · Phylogeny · Polyporaceae · *Polyporus* · Taxonomy · White rot fungi

Introduction

Neofavolus Sotome & T. Hatt., which was previously treated as member of *Polyporus P. Micheli* ex Adans. (Núñez and Ryvarden 1995), was founded based on phylogenetical and morphological analyses (Sotome et al. 2013). It is characterized by a glabrous pileus with or without scales, a hyaline to brown cutis composed of hyaline to brown agglutinated generative hyphae. The segregation of *Neofavolus* from *Polyporus* was accepted in recent studies (Dai et al. 2014; Seelan et al. 2015; Sotome et al. 2016; Zhou et al. 2016; Zmitrovich and Kovalenko 2016; Zhou and Cui 2017; Cui et al. 2019; Luo et al. 2019; Palacio et al. 2019). Currently, six species are accepted in the genus *Neofavolus*: *N. alveolaris* (DC.) Sotome & T. Hatt. (known from North America, Europe, and East Asia, Sotome et al. 2013; Zhou and Cui 2017), *N. cremeoalbidus* Sotome & T. Hatt. (known from East Asia, Sotome et al. 2013), *N. mikawai* (Lloyd) Sotome & T. Hatt. (known from China and Japan, Palacio et al. 2019), *N. suavissimus* (Fr.) Seelan, Justo & Hibbett (known from the USA, Europe, and Japan, Seelan et al. 2015), *N. subpurpurascens* (Murrill) Palacio & Robledo (known from the neotropics, Palacio et al. 2019) and *N. yunnanensis* C.L. Zhao (known from China, Luo et al. 2019).

During the taxonomic and phylogenetic studies of polyporoid fungi, two new *Neofavolus* species were discovered and confirmed after morphological and molecular analyses. Their phylogenetic relationships were analyzed based on ITS+nLSU dataset and ITS+nLSU+nSSU+mtSSU+*TEF* + *TUB* + *RPB1* + *RPB2* dataset.

Materials and methods

Morphological studies

The examined specimens were mainly deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC, Beijing, China); duplicates will be sent to

Jia-Hui Xing and Jun-Liang Zhou contributed equally to this work.

This article is part of the "Topic collection on Basidiomycote Mycology in honor of Franz Oberwinkler who passed away in March 2018"

Section Editor: Marc Stadler

✉ Bao-Kai Cui
cuibaokai@yahoo.com

[1] Institute of Microbiology, School of Ecology and Nature Conservation, Beijing Forestry University, Beijing 100083, China

[2] International Exchange and Cooperation Department, Kunming University, Kunming 650214, Yunnan, China

Center for Forest Mycology Research, Northern Research Station, US Forest Service (CFMR, Madison, USA). Macromorphological characters were described based on field notes and dried specimens. Color descriptions follow Petersen (1996). Microscopic features were observed from free-hand sections mounted in 5% KOH solution after staining in 1% Congo red. Cotton Blue (CB) and Melzer's reagents were used to test the cyanophilous and amyloid reactions, respectively. Nikon Digital Sight DS-Fi1 microscope (Nikon Corporation, Tokyo, Japan) was used to observe and photograph the microscopic elements at magnifications of up to ×1000. Then, all the microscopic elements were measured using the Image-Pro Plus 6.0 (Media Cybernetics, Silver Spring, USA). The following abbreviations are used in this article: IKI– = neither amyloid nor dextrinoid, KOH = 5% potassium hydroxide, CB– = acyanophilous, L = mean spore length (arithmetic average of all basidiospores), W = mean spore width (arithmetic average of all basidiospores), Q = variation in the L/W ratios between the specimens studied, Qm = mean Q, *n* = number of basidiospores measured here.

Molecular phylogeny

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Co. Ltd., Beijing) was used to extract the total genomic DNA from dried specimens and performed the polymerase chain reaction (PCR) according to the manufacturer's instructions with some modifications (Han et al. 2016; Shen et al. 2019). Primer pairs used in this study were listed in Table 1.

Final polymerase chain reaction (PCR) reaction volumes were 50 μl, included 1.5 μl for each primer (10 pM), 2 μl DNA extract, 20 μl ddH_2O, and 25 μl 2 × EasyTaq PCR Supermix (TransGen Biotech Co., Ltd., Beijing, China). All DNA fragments were amplified in the S1000TM Thermal Cycler (Bio-Rad Laboratories, California, USA) and sequenced by the BGI (Beijing Genomics Institute, China) using the same primers. The PCR procedures for different DNA sequences followed those used by Zhou et al. (2016).

Besides the sequences generated in this study, other reference sequences for our phylogenetic analysis were selected from GenBank. The information of all the specimens used in this study are shown in Table 2. Sequences of *Trametes conchifer* (Schwein.) Pilát and *T. polyzona* (Pers.) Justo obtained from GenBank were selected as outgroups. Maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) phylogenetic analyses were performed as reported in Zhou et al. (2016) and Song and Cui (2017). RAxML v.7.2.8 was used to construct a maximum likelihood (ML) tree. Maximum parsimony (MP) analysis was applied to the combined multiple genes datasets, and the tree construction procedure was performed in PAUP* version 4.0b10. 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. 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. Bayesian

Table 1 The primers for each DNA fragment used in this study

DNA fragment	Primer	Sequence (5′-3′)	Reference
ITS	ITS4 ITS5	TCCTCCGCTTATTGATATGC GGAAGTAAAAGTCGTAACAAGG	White et al. 1990
nLSU	LR0R LR7	ACCCGCTGAACTTAAGC TACTACCACCAAGATCT	Vilgalys and Hester 1990
EF1-α	EF1-983F EF1-1567R	GCYCCYGGHCAYCGTGAYTTYAT ACHGTRCCRATACCACCRATCTT	Rehner and Buckley 2005
β-tubulin	Bt1a Bt1b	TTCCCCCGTCTCCACTTCTTCATG GACGAGATCGTTCATGTTGAACTC	Glass and Donaldson 1995
nSSU	PNS1 NS41	CCAAGCTTGAATTCGTAGTCATATGCTTGTC CCCGTGTTGAGTCAAATTA	Hibbett 1996
mtSSU	MS1 MS2	CAGCAGTCAAGAATATTAGTCAATG GCGGATTATCGAATTAAATAAC	White et al. 1990
RPB1	RPB1-Af RPB1-Cr	GARTGYCCDGGDCAYTTYGG CCNGCDATNTCRTTRTCCATRTA	Matheny et al. 2002
	iRPB1-2.2F	GAGTGTCCGGGGCATTTYGG	Binder et al. 2010
RPB2	fRPB2-5F bRPB2-6F	GAYGAYMGWGATCAYTTYGG TGGGGYATGGTNTGYCCYGC	Liu et al. 1999
	bRPB2-7.1R	CCCATRGCYTGYTTMCCCATDGC	Matheny 2005

Table 2 Species, specimens, and GenBank accession number of sequences used in this study

Species	Specimen no.	Country	GenBank accession no.							
			ITS	nLSU	EF1-α	mtSSU	β-tubulin	RPB1	RPB2	nSSU
Favolus acervatus	Cui 11053	China	KU189774	KU189805	KU189920	KU189956	KU189864	KU189889	KU189994	KU189835
Favolus emerici	Cui 10926	China	KU189776	KU189807	KU189922	–	KU189866	KU189890	KU189995	KU189837
Favolus niveus	Cui 11129 (T)	China	KX548955	KX548981	KX549045	KX549019	KX549035	KX549067	KX549074	KX549002
Favolus pseudoemerici	Cui 11079	China	KX548958	KX548984	KX549048	KX549022	KX549037	KX549069	KX549075	KX549004
Favolus spathulatus	Cui 8290	China	KX548969	KX548991	KX549055	KX549025	KX549038	–	–	KX549009
Favolus subtropicus	Lifang 1938	China	KX548971	KX548993	KX549057	KX549027	KX549039	KX549070	KX549076	KX549011
Neofavolus alveolaris	Cui 9900	China	KX548974	KX548996	KX549060	KX549030	KX549040	KX549072	KX549078	KX549014
Neofavolus alveolaris	Dai 11290	China	KU189768	KU189799	KU189913	KU189949	KU189859	KU189885	KU189982	KU189828
Neofavolus americanus	Dai 12761 (T)	USA	KX900072 [a]	KX900186 [a]	–	–	–	–	–	–
Neofavolus cremeoalbidus	Cui 12412	China	KX899982 [a]	KX900109 [a]	KX900330 [a]	KX900201 [a]	–	–	–	KX900259 [a]
Neofavolus cremeoalbidus	TUMH:50009 (T)	Japan	AB735980	AB735957	–	–	–	–	–	–
Neofavolus mikawai	Cui 11152	China	KU189773	KU189804	KU189919	KU189955	KU189863	KU189888	KU189986	KU189834
Neofavolus mikawai	Dai 12361	China	KX548975	KX548997	KX549061	KX549031	KX549041	–	KX549079	KX549015
Neofavolus squamatus	Cui 12175 (T)	China	KX900070 [a]	KX900184 [a]	KX900370 [a]	KX900250 [a]	KX899942 [a]	–	KX900317 [a]	KX900295 [a]
Neofavolus suavissimus	DSH2011	USA	KP283496	KP283525	–	–	–	KP325693	–	–
Neofavolus suavissimus	LE202237	USA	KM411460	KM411476	KM411491	–	–	–	–	–
Neofavolus subpurpurascens	CG6241	Brazil	MH544274	MH544276	–	–	–	–	–	–
Neofavolus subpurpurascens	CG6242	Brazil	MH544275	MH544277	–	–	–	–	–	–
Neofavolus sp.	MA672	USA	KP283506	KP283524	–	–	–	KP325696	–	–
Neofavolus yunnanensis	CLZhao1639 (T)	China	MK834523	MK834521	–	–	–	–	–	–
Neofavolus yunnanensis	CLZhao1633	China	MK834524	MK834522	–	–	–	–	–	–
Picipes baishanzuensis	Dai 13418 (T)	China	KU189762	KU189793	KU189907	KU189945	KU189855	KU189882	KU189977	KU189823
Picipes rhizophilus	Dai 11599	China	KC572028	KC572067	KU189933	KU189967	KU189874	KU189896	KU189992	KU189847
Polyporus mangshanensis	Dai 15151 (T)	China	KX851796	KX851797	KX851802	KX851798	KX851795	KX851800	KX851801	KX851799
Polyporus philippinensis	Cui 10941	China	KX548976	KX548998	KX549062	KX549032	KX549042	–	–	KX549016
Polyporus squamosus	Cui 10595	China	KU189778	KU189809	KU189925	KU189960	KU189868	KU189892	KU189988	KU189840
Polyporus tuberaster	Dai 11271	China	KU189769	KU189800	KU189914	KU189950	–	–	KU189983	KU189829
Polyporus umbellatus	Pen 13513	China	KU189772	KU189803	KU189917	KU189953	KU189862	KU189887	KU189985	KU189832
Trametes conchifer	FP106793	USA	JN164924	JN164797	JN164887	–	–	JN164823	JN164849	–
Trametes polyzona	Cui 11040	China	KR605824	KR605767	KR610760	KR606029	–	–	KR610849	KR605932

[a] indicates accession numbers for newly generated sequences; (T) indicates holotype specimen

inference (BI) was calculated with MrBayes 3.1.2. PhyloSuite v1.1.16 was used to determine the best-fit evolution model for the combined multi-gene dataset for Bayesian inference (BI).

Trees were viewed and derived by using of the FigTree 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/). The topology of the ML analysis was used, with ML bootstrap support > 50%, MP bootstrap support > 50%, and BI posterior probabilities > 0.95 shown on branches (Fig. 1 and 2).

Results

The combined ITS+nLSU dataset had an aligned length of 2017 characters, of which 1516 characters are constant, 125 are variable and parsimony-uninformative, and 376 are parsimony-informative. Maximum parsimony analysis yielded 12 equally parsimonious trees (TL = 1357, CI = 0.541, RI = 0.664, RC = 0.359, HI = 0.459). Best model for the combined ITS+nLSU dataset estimated and applied in the Bayesian analysis was GTR + F + I + G4, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.007016 (BI). The ML topology was shown in Fig. 1.

The combined dataset (ITS+nLSU+nSSU+mtSSU+*TEF* + *TUB* + *RPB1* + *RPB2*) had an aligned length of 7286 characters, of which 5098 characters are constant, 529 are variable and parsimony-uninformative, and 1659 are parsimony-informative. Maximum parsimony analysis yielded 2 equally parsimonious trees (TL = 6104, CI = 0.559, RI = 0.618, RC = 0.345, HI = 0.441). Best model for the combined 8-gene dataset estimated and applied in the Bayesian analysis was SYM + I + G4, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.002240 (BI). The ML topology was shown in Fig. 2.

In the two phylogenetic results, both *Favolus* and *Neofavolus* are well supported as individual monophyletic groups, and the materials examined are highly supported as two new species in the *Neofavolus* clade.

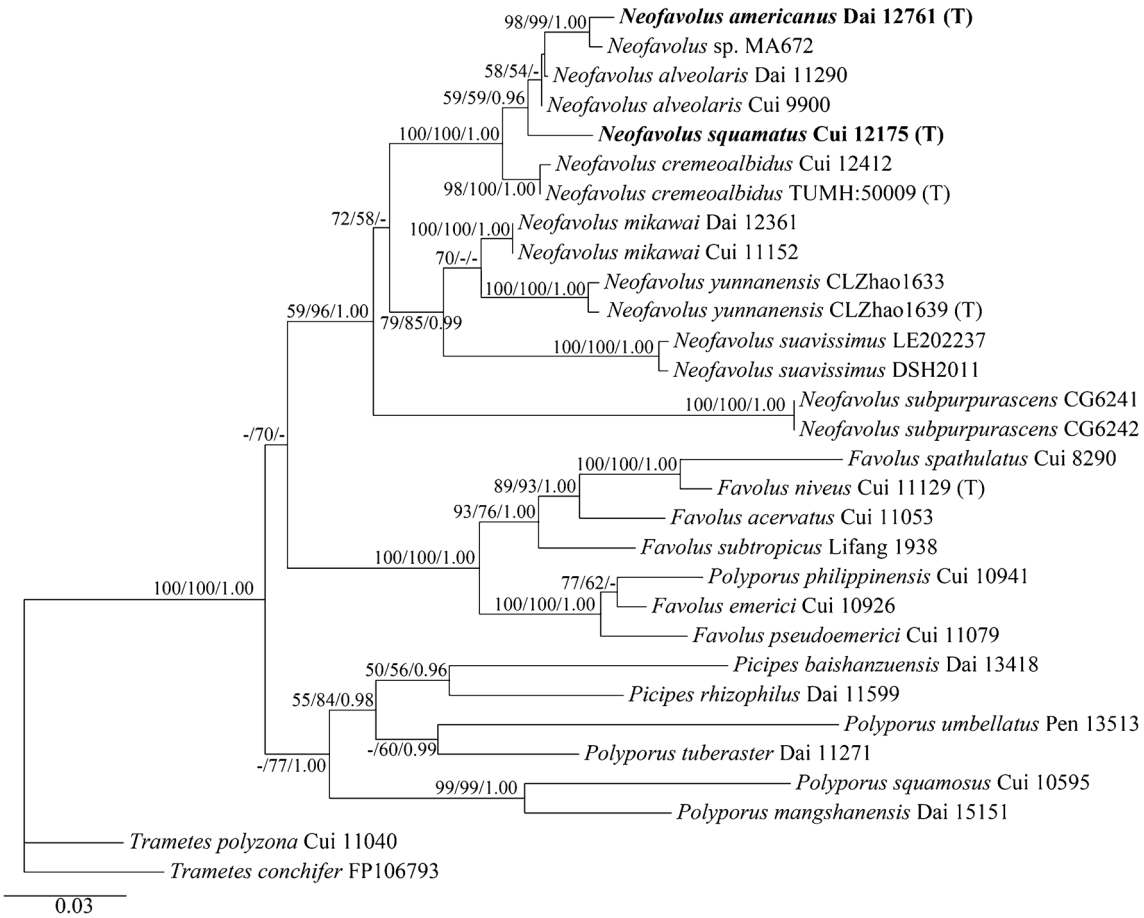

Fig. 1 Strict consensus tree illustrating the phylogeny of *Neofavolus* and related taxa generated by ML analysis based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 50%, parsimony bootstrap proportions higher than 50%, and Bayesian posterior probabilities more than 0.95. New species are indicated in bold

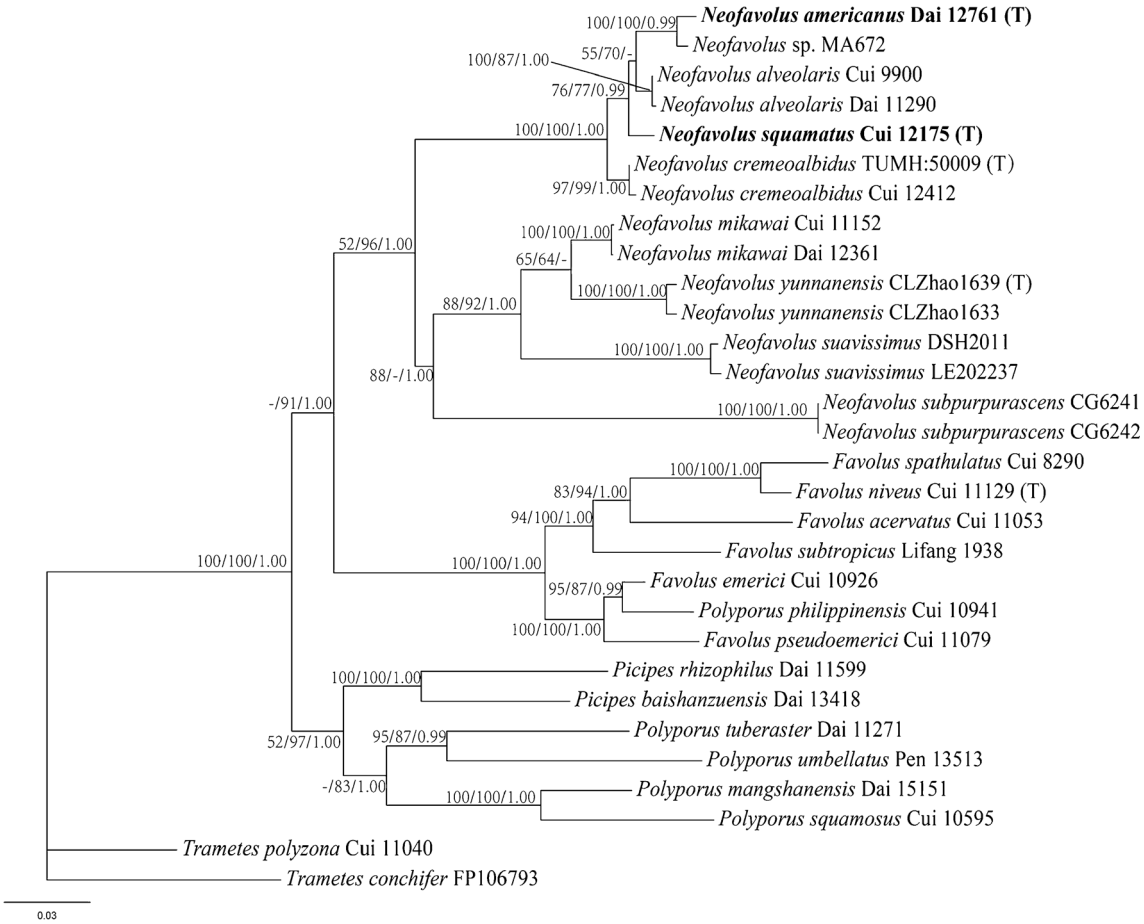

Fig. 2 Strict consensus tree illustrating the phylogeny of *Neofavolus* and related taxa generated by ML analysis based on ITS+nLSU+nSSU+ mtSSU+*TEF* + *TUB* + *RPB1* + *RPB2* sequences. Branches are labeled with maximum likelihood bootstrap higher than 50%, parsimony bootstrap proportions higher than 50%, and Bayesian posterior probabilities more than 0.95. New species are indicated in bold

Taxonomy

Neofavolus americanus J.H. Xing, J.L. Zhou & B.K. Cui, sp. nov. (Figs. 3 and 4)

MycoBank no.: MB 834716

Etymology. americanus (Lat.) referring to the geographic distribution in America(USA).

Fruiting body: Basidiomata annual, laterally stipitate, leathery when fresh, and hard when dry. Pilei semicircular, depressed toward the stipe, 1.8–2.3 cm long from base to margin, 2.8–3.5 cm wide, and up to 3 mm thick. Pileal surface glabrous, white to cream when fresh, buff to buff-yellow and with slightly radially aligned stripes on drying; margin plane when fresh and straight to slightly incurved upon drying. Pore surface white to cream when fresh, cream to saffron yellow when dry; pores angular to elongate, 1–3 mm long and 0.5–1 mm wide; dissepiments thin, entire to lacerate. Context white when fresh and ivory to cream when dry, woody hard upon drying, up to 1 mm thick. Tubes concolorous with pore surface, decurrent on one side of the stipe, up to 2 mm thick. Stipe short, glabrous, concolorous with pileal surface or slightly lighter when dry, up to 8 mm long and 7 mm in diameter.

Fig. 3 Basidiomata of *Neofavolus americanus* (*Dai 12761*). Scale bar = 1 cm

Hyphal structure: Hyphal system dimitic; generative hyphae bearing clamp connections; skeleto-binding hyphae IKI–, CB+; tissues unchanged in KOH.

Context: Generative hyphae frequent, hyaline, thin-walled, frequently branched, and 2–6.4 μm in diameter; skeleto-binding hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, frequently branched, interwoven, and 1.8–9.7 μm in diameter.

Tubes: Generative hyphae frequent, hyaline, thin-walled, frequently branched, and 2–3.8 μm in diameter; skeleto-binding hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, frequently branched, interwoven, and 2–4.5 μm in diameter. Cystidia and cystidioles absent. Basidia clavate, with a basal clamp and four sterigmata, 17.7–28 × 7–8.7 μm; basidioles in shape similar to basidia, but smaller.

Stipe: Generative hyphae frequent, hyaline, thin-walled, frequently branched, and 2–7 μm in diameter; skeleto-binding hyphae dominant, thick-walled with a narrow to wide lumen, frequently branched, interwoven, and 2–9 μm in diameter.

Spores: Basidiospores cylindrical, hyaline, thin-walled, smooth, frequently bearing small guttules, IKI–, CB–, (9.5–)10.4–12(−12.7) × (3.7–)3.8–4.5 μm, L = 11.18 ± 0.68 μm, W = 4.07 ± 0.18 μm, Q = 2.4–3.1, and Qm = 2.75 ± 0.17 (*n* = 53/1).

Rot type: A white rot.

Specimens examined: USA, CT, New Haven, Sleeping Giant State Park, on fallen branch of *Quercus*, 21 July 2012, Dai 12761 (holotype, BJFC).

Neofavolus squamatus J.H. Xing, J.L. Zhou & B.K. Cui, sp. nov. (Figs. 5 and 6)

MycoBank no.: MB 834717

Etymology: squamatus (Lat.) referring to the squamae on the pileal surface.

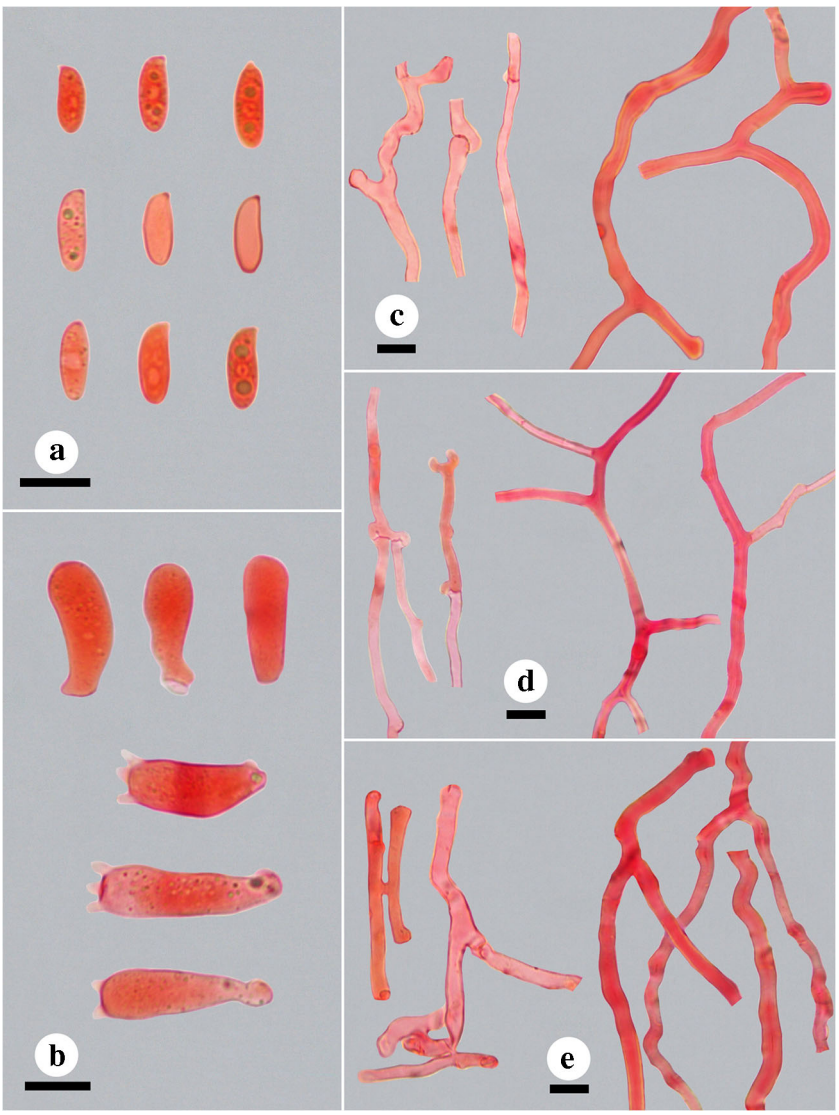

Fig. 4 Microscopic structures of *Neofavolus americanus*. **a** Basidiospores; **b** Basidia and basidioles; **c** Hyphae from context; **d** Hyphae from trama; **e** Hyphae from stipe. Scale bars = 10 μm

Fruiting body: Basidiomata annual, solitary, laterally stipitate, and soft-leathery when fresh and corky when dry. Pilei suborbicular, depressed toward the stipe, 1.1–2.7 cm long from base to margin, 3–3.8 cm wide, and up to 3 mm thick. Pileal surface white when fresh and buff to festucine when dry, covered by yellowish orange squamae when fresh, discoloring to apricot orange when dry, and more or less radially wrinkling on drying; margin incurved upon drying. Pore surface white to cream when fresh and buff when dry; pores angular, 0.7–3 mm long and 0.5–1.5 mm wide; dissepiments thin, entire. Context white, corky upon drying, up to 2.5 mm thick. Tubes white when fresh and light ivory to cream when dry, up to 1 mm thick, and decurrent on one side of the stipe. Stipe short, glabrous, white when fresh and buff yellow after drying, up to 6 mm long, and 5.5 mm in diameter.

Hyphal structure: Hyphal system dimitic; generative hyphae bearing clamp connections, hyaline, and thin-walled; skeleto-binding hyphae hyaline, thick-walled with a wide to narrow lumen, occasionally branched and with tapering ends, IKI–, and CB+; tissue unchanged in KOH.

Context: Generative hyphae frequent, hyaline, thin-walled, frequently branched, 2.8–9 μm in diameter, and occasionally inflated up to 27.7 μm in diameter at branched area; skeleto-binding hyphae dominant, hyaline, thick-walled with a wide lumen, occasionally branched, interwoven, 2.6–9.5 μm in diameter, and occasionally inflated up to 13.5 μm in diameter. Hyphae in squamae with buff inclusion inside, thin-walled hyphae bearing clamp connections, thick-walled hyphae simple-septate with a wide lumen, and 3.5–8.4 μm in diameter.

Tubes: Generative hyphae frequent, hyaline, thin-walled, frequently branched, and 2.2–4.2 μm in diam; skeleto-binding hyphae dominant, hyaline, thick-walled with a wide lumen, occasionally branched, interwoven, and 1.9–6.4 μm in diameter. Cystidia and cystidioles absent. Basidia clavate, with a basal clamp and four sterigmata, 27.7–51.7 × 6.5–9.8 μm; basidioles in shape similar to basidia, but smaller.

Stipe: Generative hyphae infrequent, hyaline, thin-walled, frequently branched, 2.2–6.3 μm in diameter, and occasionally inflated up to 16 μm in diameter at clamping area; skeleto-binding hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, occasionally branched, interwoven, and 2.6–8.6 μm in diameter.

Spores: Basidiospores cylindrical to navicular, hyaline, thin-walled, smooth, occasionally bearing one or two guttules, IKI–, CB–, (7.8–)8.9–12(−14.5) × 3.1–4.1(−4.3) μm, L = 10.34 ± 1.09 μm, W = 3.63 ± 0.29 μm, Q = 2.17–3.72, and Qm = 2.86 ± 0.31 (n = 70/1).

Fig. 5 Basidiomata of *Neofavolus squamatus* (*Cui 12175*). Scale bar = 1 cm

Rot type: A white rot.

Specimens examined: CHINA, Xizang, Linzhi County, Lulang, Sejila Mountain, on fallen angiosperm branch, 18 September 2014, Cui 12175 (holotype, BJFC).

Discussion

In the present study, *N. americanus*, from USA, and *N. squamatus*, from China, are described in *Neofavolus* based on morphological evidence and multi-gene phylogenetic inferences.

Neofavolus americanus is a species known so far from the temperate USA. It has glabrous basidiomata, large and elongate pores, and large basidiospores. Phylogenetically, *N. americanus* clustered with *N. alveolaris* (Figs. Fig. 1 and 2). However, the basidiospores of *N. americanus* are much larger than those of *N. alveolaris* (7–10 × 2.5–4 μm; Sotome et al. 2013). *Neofavolus cremeoalbidus* is similar to *N. americanus*; both share the light colored pileus and pore surface and short and lateral stipe, but based on our specimens collected from China, the former has much smaller pores (2–4 per mm) and basidiospores (8–10.7 × 3–3.8 μm). Moreover, *N. cremeoalbidus* is only known from East Asia, in China and Japan, while *N. americanus* is limited to northern–eastern USA. *Neofavolus mikawai* shares the cream pore surface and the short lateral stipe with *N. americanus*, but it differs in its smaller pores (3–5 per mm; Sotome et al. 2013) and basidiospores (6–9.5 × 2.3–3.6 μm; Sotome et al. 2013).

Neofavolus squamatus is a special species which in known from the Tibetan Plateau. It is characterized by its bright squamae on the pileus. It was initially thought to be a member of *Polyporus squamosus* (Huds.) Fr. for its squamae and large pores. However, phylogenetic analyses (Figs. Fig. 1 and 2) showed that it nested within the *Neofavolus* lineage. Morphologically, *N. squamatus* differs from other species in

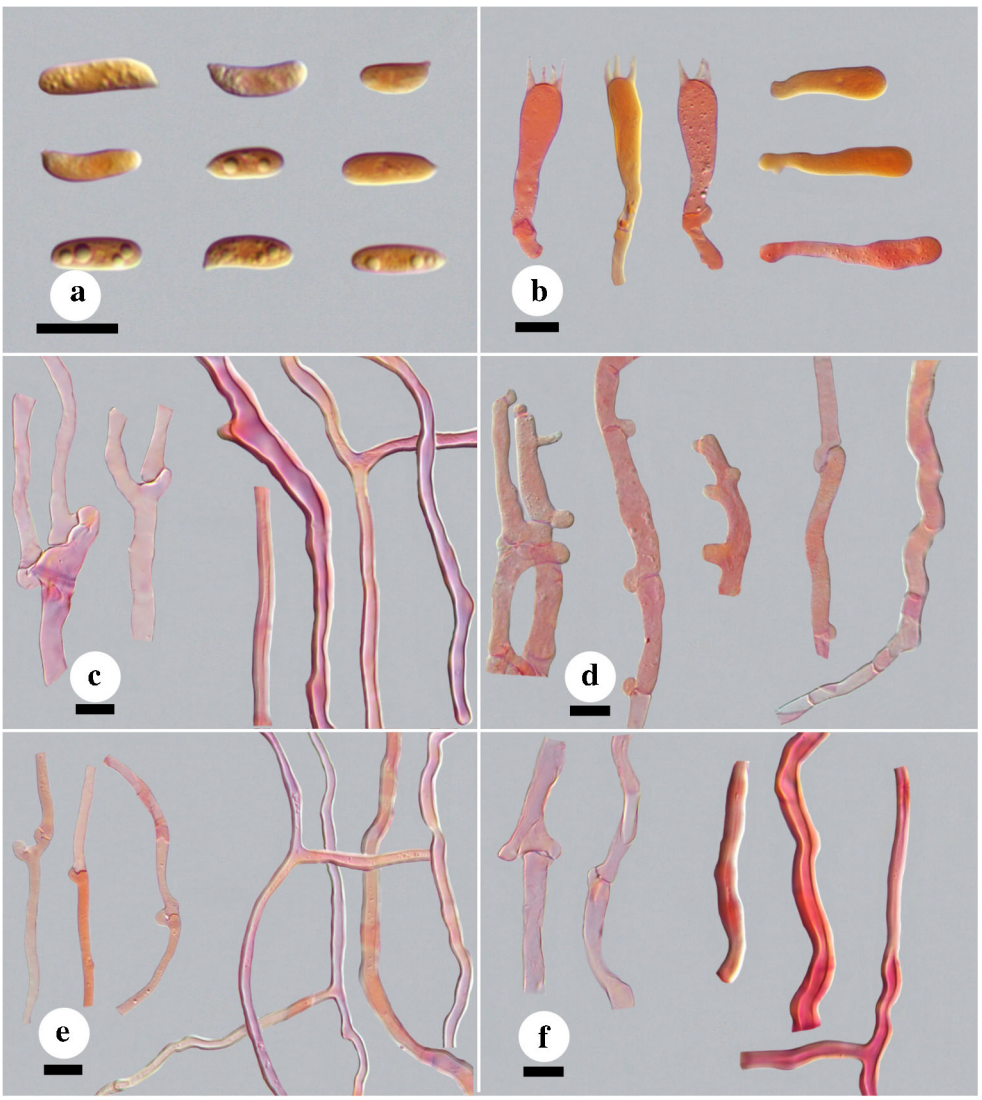

Fig. 6 Microscopic structures of *Neofavolus squamatus*. **a** Basidiospores; **b** Basidia and basidioles; **c** Hyphae from context; **d** Hyphae from pileal squamae; **e** Hyphae from trama; **f** Hyphae from stipe. Scale bars = 10 μm

the genus in having smaller basidiospores, thinner basidiomata, and a white stipe. *Neofavolus yunnanensis* is another *Neofavolus* species with scaled pileal surface and large angular pores, but it has light-colored pileus and much smaller basidiospores (5.5–7.5 × 2–3 μm, L = 6.4 μm, W = 2.65 μm; Luo et al. 2019) compared with *N. squamatus*. Although *N. alveolaris* occasionally has scales on the pileus, it has smaller basidiospores (7–10 × 2.5–4 μm, L = 8.29 μm, W = 3.01 μm; Sotome et al. 2013) and basidia (17.5–26 × 4–7 μm; Sotome et al. 2013) compared to those of *N. squamatus*. *Favolus roseus* Lloyd also has orangish basidiomata, large pores, and cylindrical basidiospores (7–12 × 2.4–4.2 μm), which are similar to *N. squamatus*, but its glabrous pileal surface, yellowish orange to brownish orange pore surface, and tropical distribution (Sotome et al. 2013) are different from *N. squamatus*.

Sotome et al. (2013) concluded that species of *Neofavolus* occur in temperate regions and are unknown from the tropics. We also previously suggested that temperature may be a critical variable affecting the distributions of *Neofavolus* species (Zhou and Cui 2017). However, several tropical specimens of *N. mikawai* and *N. alveolaris* collected from China (Zhou and Cui 2017; Luo et al. 2019) and the neotropical species (*N. subpurpurascens*) reported from Jamaica, Brazil (Coelho and Silveira 2014), and Bolivia (Palacio et al. 2019) may overthrow the inference, and it will be more convincing if sequences of *N. subpurpurascens* could be available from the type locality of Jamaica.

Acknowledgments We express our gratitude to Prof. Yu-Cheng Dai (Beijing Forestry University, China) for allowing us to study his specimen.

Funding information The research is supported by the National Natural Science Foundation of China (Nos. 31670016, 31870008) and Beijing Forestry University Outstanding Young Talent Cultivation Project (No. 2019JQ03016).

References

Binder M, Larsson KH, Matheny PB, Hibbett DS (2010) Amylocorticiales ord. nov. and Jaapiales ord. nov.: early diverging clades of Agaricomycetidae dominated by corticioid forms. Mycologia 102:865–880

Coelho G, Silveira RMB (2014) Taxonomy of *Polyporus* sensu lato and related genera (Basidiomycota) from Santa Maria, southern Brazil. Ciência e Natura 36:688–710. https://doi.org/10.5902/2179460X14939

Cui BK, Li HJ, Ji X, Zhou JL, Song J, Si J, Yang ZL, Dai YC (2019) Species diversity, taxonomy and phylogeny of Polyporaceae (Basidiomycota) in China. Fungal Divers 97:137–392. https://doi.org/10.1007/s13225-019-00427-4

Dai YC, Xue HJ, Vlasák J, Rajchenberg M, Wang B, Zhou LW (2014) Phylogeny and global diversity of *Polyporus* group *Melanopus* (Polyporales, Basidiomycota). Fungal Divers 64:133–144. https://doi.org/10.1007/s13225-013-0248-3

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330

Han ML, Chen YY, Shen LL, Song J, Vlasák J, Dai YC, Cui BK (2016) Taxonomy and phylogeny of the brown-rot fungi: *Fomitopsis* and its related genera. Fungal Divers 80:343–373. https://doi.org/10.1007/s13225-016-0364-y

Hibbett DS (1996) Phylogenetic evidence for horizontal transmission of group I introns in the nuclear ribosomal DNA of mushroom-forming fungi. Mol Biol Evol 13:903–917

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among Ascomycetes: evidence from an RNA polymerse II subunit. Mol Biol Evol 16:1799–1808

Luo KY, Ma X, Zhao CL (2019) *Neofavolus yunnanensis* sp. nov. (Polyporales, Basidiomycota) from China: evidence from morphology and DNA sequence data. Phytotaxa 408:109–116. https://doi.org/10.11646/phytotaxa.408.2.3

Matheny PB (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). Mol Phylogenet Evol 35:1–20

Matheny PB, Liu YJ, Ammirati JF, Hall BD (2002) Using RPB1 sequences to improve phylogenetic inference among mushrooms (*Inocybe*, Agaricales). Am J Bot 89:688–698

Núñez M, Ryvarden L (1995) *Polyporus* (Basidiomycotina) and related genera. Synopsis Fungorum 10:1–85

Palacio M, Silveira RMB, Robledo GL (2019) *Neofavolus subpurpurascens* comb. nov., with new records from the neotropics. Phytotaxa 405:180–186. https://doi.org/10.11646/phytotaxa.405.4.1

Petersen JH (1996) Farvekort. The Danish Mycological Society's color chart Greve: Foreningen til Svampekundskabens Fremme

Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97:84–98. https://doi.org/10.3852/mycologia.97.1.84

Seelan JSS, Justo A, Nagy LG, Grand EA, Redhead SA, Hibbett DS (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

Shen LL, Wang M, Zhou JL, Xing JH, Cui BK, Dai YC (2019) Taxonomy and phylogeny of *Postia*. Multi-gene phylogeny and taxonomy of the brown-rot fungi: *Postia* (Polyporales, Basidiomycota) and related genera. Persoonia 42:101–126. https://doi.org/10.3767/persoonia.2019.42.05

Song J, Cui BK (2017) Phylogeny, divergence time and historical biogeography of *Laetiporus* (Basidiomycota, Polyporales). BMC Evol Biol 17:102. https://doi.org/10.1186/s12862-017-0948-5

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

Sotome K, Matozaki T, Aimi T, Boonlue S (2016) *Polyporus thailandensis*, a new species of group *Polyporellus* in *Polyporus* (Polyporales, Agaricomycota) from northeastern Thailand. Mycoscience 57:85–89. https://doi.org/10.1016/j.myc.2015.07.006

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols, a guide to methods and applications. Academic Press, San Diego, pp 315–322

Zhou JL, Zhu L, Chen H, Cui BK (2016) Taxonomy and phylogeny of *Polyporus* group *Melanopus* (Polyporales, Basidiomycota) from China. PLoS One 11:e0159495. https://doi.org/10.1371/journal.pone.0159495

Zhou JL, Cui BK (2017) Phylogeny and taxonomy of *Favolus* (Basidiomycota). Mycologia 109:766–779. https://doi.org/10.1080/00275514.2017.1409023

Zmitrovich IV, Kovalenko AE (2016) Lentinoid and polyporoid fungi, two generic conglomerates containing important medicinal mushrooms in molecular perspective. Int J Med Mushrooms 18:23–38. https://doi.org/10.1615/IntJMedMushrooms.v18.i1.40