Phytotaxa

332

Magnolia Press, Auckland, New Zealand

Vol 332, No 1: 15 December 2017

Open Access Subscription or Fee Access

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Article

Correspondence

Phytotaxa 332 (1): 031–040
http://www.mapress.com/j/pt/

Article

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

https://doi.org/10.11646/phytotaxa.332.1.3

Morphological and molecular identification of a new species of *Atraporiella* (Polyporales, Basidiomycota) in China

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Abstract

A new poroid wood-inhabiting fungal species, *Atraporiella yunnanensis sp. nov.*, is proposed based on morphological and molecular characters. The species is characterized by resupinate, cream pore surface, and its fresh basidiocarp is easy to separate from substrate and very rapidly stained dark brown to black when bruised; a monomitic hyphal system with generative hyphae hyaline to pale brown, thin-walled, clamped, unbranched, interwoven; slightly allantoid basidiospores, 2.2–3 × 0.8–1.5 µm. The internal transcribed spacer (ITS) and the large subunit (LSU) regions of nuclear ribosomal RNA gene sequences of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and bayesian inference methods. The phylogenetic analysis based on molecular data of ITS+nLSU sequences showed that *Atraporiella yunnanensis* belonged to the residual polyporoid clade, formed a monophyletic lineage with a strong support (100% BS, 100% BP, 1.00 BPP) and was closely related to *A. neotropica*, and then grouped with other related genera: *Antrodiella*, *Pouzaroporia*, *Steccherinum* and *Xanthoporus*. Both morphological and molecular characters confirmed the placement of the new species in *Atraporiella*.

Key words: Phylogenetic analysis, Polypores, Taxonomy, Wood-rotting fungi

Introduction

Atraporiella Ryvarden (Meruliaceae, Polyporales) was erected by Ryvarden (2007). It is a small genus characterized by a combination of annual, resupinate basidiomata which are easily separable, soft and slightly waxy when fresh, brittle up on drying, pore surface very rapidly stained dark brown to black when bruised, and a monomitic hyphal structure with hyaline to pale brown generative hyphae bearing clamp connections, and hyaline, thin-walled, smooth, allantoid to ellipsoid basidiospores. In addition, its hyphae and basidiospores are acyanophilous and negative in Melzer's reagent.

Recently, the phylogenetic study of the new polypore genera *Obba* Miettinen & Rajchenb. and *Sebipora* Miettinen suggested that the genus *Atraporiella* was monophyletic, and *A. neotropica* was grouped with *Antrodiella* Ryvarden & I. Johans. and *Steccherinum* Gray on the base of the combined data of the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) gene sequences (Miettinen & Rajchenberg 2012). Binder *et al.* (2013) employed molecular study based on multi-gene datasets and demonstrated that the *A. neotropica* belonged to the residual polyporoid clade grouped with related genera *Antrodiella*, *Pouzaroporia* Vampola and *Steccherinum* when using ribosomal DNA sequences.

During investigations on the diversity of polypores in southwestern China, an additional undescribed species corresponding to *Atraporiella* was found. To confirm the affinity of the undescribed species of *Atraporiella*, phylogenetic analysis was carried out based on the ITS and nLSU sequences.

Accepted by Samantha Karunarathna: 4 Nov. 2017; published: 15 Dec. 2017

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. Special 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.—CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from dried specimens, according to the manufacturer's instructions with some modifications that a small piece of dried fungal specimen (about 30 mg) was ground to powder with liquid nitrogen. The powder was transferred to a 1.5 ml centrifuge tube, suspended in 0.4 ml of lysis buffer, and incubated in a 65 °C water bath for 60 min. After that, 0.4 ml phenol-chloroform (24:1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13 000 rpm for 5 min, 0.3 ml supernatant was transferred to a new tube and mixed with 0.45 ml binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation at 13 000 rpm for 0.5 min. Then, 0.5 ml inhibitor removal fluid was added in AC for a centrifugation at 12 000 rpm for 0.5 min. After washing twice with 0.5 ml washing buffer, the AC was transferred to a clean centrifuge tube, and 100 ml elution buffer was added to the middle of adsorbed film to elute the genome DNA. 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 Chen *et al*. (2015). 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 numbers of sequences used in this study.

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Abortiporus biennis (Bull.) Singer	TFRI 274	EU232187	EU232235	Binder *et al*. (2005)
Antrodia albida (Fr.) Donk	CBS 308.82	DQ491414	AY515348	Kim *et al*. (2007)
A. heteromorpha (Fr.) Donk	CBS 200.91	DQ491415	AY515350	Kim *et al*. (2007)
Antrodiella americana Ryvarden & Gilb.	Gothenburg 3161	JN710509	JN710509	Binder *et al*. (2013)
A. formosana T.T. Chang & W.N. Chou	TFRI 652	EU232184	EU232268	Binder *et al*. (2005)
A. pallasii Renvall, Johann. & Stenlid	Renvall 89a	AF126896	-	Binder *et al*. (2013)
A. semisupina (Berk. & M.A. Curtis) Ryvarden	FCUG 960	EU232182	EU232266	Binder *et al*. (2005)
Atraporiella neotropica Ryvarden	Ryvarden 44447	HQ659221	HQ659221	Miettinen & Rajchenberg (2012)
A. yunnanensis C.L. Zhao	CLZhao 603	MF962481	MF962484	Present study
A. yunnanensis	CLZhao 604	MF962482	MF962485	Present study
A. yunnanensis	CLZhao 605	MF962483	MF962486	Present study
Ceraceomyces serpens (Tode) Ginns	KHL 8478	AF090882	AF090882	Binder *et al*. (2005)
Ceriporia aurantiocarnescens (Henn.) B. Rivoire	Yuan 2066	JX623902	JX644042	Jia *et al*. (2014)
C. lacerata N. Maek., Suhara & R. Kondo	Dai 10734	JX623916	JX644068	Jia *et al*. (2014)
Ceriporiopsis alboaurantia B.K. Cui & Y.C. Dai	Cui 2877	KF845947	KF845954	Zhao & Cui (2014)
C. balaenae Niemelä	H7002389	FJ496669	FJ496717	Tomšovský *et al*. (2010)

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TABLE 1. (Continued)

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
C. consobrina (Bres.) Ryvarden	Rivoire 977	FJ496667	FJ496716	Tomšovský *et al*. (2010)
C. fimbriata C.L. Zhao & Y.C. Dai	Dai 11672	KJ698633	KJ698637	Zhao & Cui (2014)
C. fimbriata	Cui 1671	KJ698634	KJ698638	Zhao & Cui (2014)
C. gilvescens (Bres.) Domański	BRNM 710166	FJ496684	FJ496720	Tomšovský *et al*. (2010)
C. gilvescens	Yuan 2752	KF845946	KF845953	Zhao & Cui (2014)
C. gilvescens	BRNM 667882	FJ496685	FJ496719	Tomšovský *et al*. (2010)
C. guidella Bernicchia & Ryvarden	HUBO 7659	FJ496687	FJ496722	Tomšovský *et al*. (2010)
C. pseudogilvescens (Pilát) Niemelä & Kinnunen	TAA 168233	FJ496673	FJ496702	Tomšovský *et al*. (2010)
C. pseudogilvescens	BRNM 686416	FJ496679	FJ496703	Tomšovský *et al*. (2010)
C. pseudoplacenta Vlasák & Ryvarden	JV 050952	JN592499	JN592506	Vlasák *et al*. (2012)
C. pseudoplacenta	PRM 899297	JN592497	JN592504	Vlasák *et al*. (2012)
C. semisupina C.L. Zhao, B.K. Cui & Y.C. Dai	Cui 10222	KF845949	KF845956	Zhao & Cui (2014)
Cinereomyces lindbladii (Berk.) Jülich	KHL 12078	FN907906	FN907906	Binder *et al*. (2013)
Climacocystis borealis (Fr.) Kotl. & Pouzar	KH 13318	JQ031126	JQ031126	Binder *et al*. (2013)
Coriolopsis caperata (Berk.) Murrill	LE(BIN)-0677	AB158316	AB158316	Tomšovský *et al*. (2010)
Dacryobolus karstenii (Bres.) Parmasto	KHL 11162	EU118624	EU118624	Binder *et al*. (2005)
Daedalea quercina (L.) Pers.	DSM 4953	DQ491425	DQ491425	Kim *et al*. (2007)
Diplomitoporus flavescens (Bres.) Domański	X 84	FN907908	-	Binder *et al*. (2013)
Earliella scabrosa (Pers.) Gilb. & Ryvarden	PR 1209	JN165009	JN164793	Binder *et al*. (2005)
Fomitopsis pinicola (Sw.) P. Karst.	CBS 221.39	DQ491405	DQ491405	Kim *et al*. (2007)
F. rosea (Alb. & Schwein.) P. Karst.	ATCC 76767	DQ491410	DQ491410	Kim *et al*. (2007)
Fragiliporia fragilis Y.C. Dai, B.K. Cui & C.L. Zhao	Dai 13080	KJ734260	KJ734264	Zhao *et al*. (2015)
F. fragilis	Dai 13559	KJ734261	KJ734265	Zhao *et al*. (2015)
F. fragilis	Dai 13561	KJ734262	KJ734266	Zhao *et al*. (2015)
Ganoderma lingzhi Sheng H. Wu, Y. Cao & Y.C. Dai	Wu 1006-38	JQ781858	-	Zhao *et al*. (2015)
Gelatoporia subvermispora (Pilát) Niemelä	BRNU 592909	FJ496694	FJ496706	Tomšovský *et al*. (2010)
Gloeoporus pannocinctus (Romell) J. Erikss.	BRNM 709972	EU546099	FJ496708	Tomšovský *et al*. (2010)
G. dichrous (Fr.) Bres.	KHL 11173	EU118627	EU118627	Binder *et al*. (2005)
Grammothelopsis subtropica B.K. Cui & C.L. Zhao	Cui 9035	JQ845094	JQ845097	Zhao *et al*. (2015)
Heterobasidion annosum (Fr.) Bref.	PFC 5252	KC492906	KC492906	Binder *et al*. (2013)
Hornodermoporus martius (Berk.) Teixeira	MUCL 41677	FJ411092	FJ393859	Robledo *et al*. (2009)
Hypochnicium lyndoniae (D.A. Reid) Hjortstam	NL 041031	JX124704	JX124704	Binder *et al*. (2005)
Junghuhnia nitida (Pers.) Ryvarden	KHL 11903	EU118638	EU118638	Binder *et al.* (2005)
Mycoacia fuscoatra (Fr.) Donk	KHL 13275	JN649352	JN649352	Tomšovský *et al*. (2010)
M. nothofagi (G. Cunn.) Ryvarden	KHL 13750	GU480000	GU480000	Tomšovský *et al*. (2010)
Obba rivulosa (Berk.) Miettinen & Rajchenb	KCTC 6892	FJ496693	FJ496710	Miettinen & Rajchenberg (2012)

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TABLE 1. (Continued)

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
O. valdiviana (Rajchenb.) Miettinen & Rajchenb.	FF 503	HQ659235	HQ659235	Miettinen & Rajchenberg (2012)
Perenniporia medulla-panis (Jacq.) Donk	MUCL 49581	FJ411087	FJ393875	Robledo *et al*. (2009)
Perenniporiella neofulva Decock & Ryvarden	MUCL 45091	FJ411080	FJ393852	Robledo *et al*. (2009)
Phanerochaete chrysosporium Burds.	BKM-F-1767	HQ188436	GQ470643	Binder *et al*. (2005)
Phlebia livida (Pers.) Bres.	FCUG 2189	AF141624	AF141624	Tomšovský *et al*. (2010)
P. radiata Fr.	UBCF 19726	HQ604797	HQ604797	Binder *et al*. (2013)
P. subserialis (Bourdot & Galzin) Donk	FCUG 1434	AF141631	AF141631	Tomšovský *et al*. (2010)
P. unica (H.S. Jacks. & Dearden) Ginns	KHL 11786	EU118657	EU118657	Binder *et al*. (2013)
Piloporia sajanensis (Parmasto) Niemelä	Manninen 2733a	HQ659239	HQ659239	Tomšovský *et al*. (2010)
Podoscypha multizonata (Berk. & Broome) Pat.	Gothenburg 3005	JN710581	JN710581	Binder *et al*. (2013)
Polyporus tuberaster (Jacq. ex Pers.) Fr.	CulTENN 10197	AF516596	AJ488116	Binder *et al*. (2013)
Postia guttulata (Peck ex Sacc.) Jülich	KHL 11739	EU11865	EU11865	Kim *et al*. (2007)
Pouzaroporia subrufa (Ellis & Dearn.) Vampola	BRNM 710164	FJ496661	FJ496723	Tomšovský *et al*. (2010)
Skeletocutis amorpha (Fr.) Kotl. & Pouzar	Miettinen 11038	FN907913	FN907913	Tomšovský *et al*. (2010)
S. portcrosensis A. David	LY 3493	FJ496689	FJ496689	Tomšovský *et al*. (2010)
S. subsphaerospora A. David	Rivoire 1048	FJ496688	FJ496688	Tomšovský *et al*. (2010)
Steccherinum fimbriatum (Pers.) J. Erikss.	KHL 11905	EU118668	EU118668	Tomšovský *et al*. (2010)
S. ochraceum (Pers.) Gray	KHL 11902	JQ031130	JQ031130	Tomšovský *et al*. (2010)
S. ochraceum	Ryberg s.n.	EU118669	EU118670	Tomšovský *et al*. (2010)
Stereum hirsutum (Willd.) Pers.	NBRC 6520	AB733150	AB733325	Tomšovský *et al*. (2010)
Truncospora ochroleuca (Berk.) Pilát	MUCL 39726	FJ411098	FJ393865	Robledo *et al*. (2009)
Tyromyces chioneus (Fr.) P. Karst.	Cui 10225	KF698745	KF698756	Zhao *et al*. (2015)
Xanthoporus syringae (Parmasto) Audet	Gothenburg 1488	JN710607	JN710607	Tomšovský *et al*. (2010)

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 21519). Sequences of *Heterobasidion annosum* (Fr.) Bref. and *Stereum hirsutum* (Willd.) Pers. obtained from GenBank were used as outgroups to root trees following Binder *et al*. (2013) in the ITS+nLSU analysis.

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 through the Cipres Science Gateway (www.phylo.org; Miller *et al*. 2009). Branch support for ML analysis was determined by 1000 bootstrap replicate.

MrModeltest 2.3 (Posada & Crandall 1998, 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 5 million generations

(ITS+nLSU), 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 included sequences from 75 fungal specimens representing 65 species. The dataset had an aligned length of 2367 characters, of which 1339 characters are constant, 284 are variable and parsimony-uninformative, and 744 are parsimony-informative. Maximum parsimony analysis yielded four equally parsimonious trees (TL = 5936, CI = 0.294, HI = 0.706, RI = 0.575, RC = 0.169). Best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis: GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.001578 (BI).

The phylogeny (Fig. 1) inferred from ITS+nLSU sequences demonstrated seven major clades for 65 species of the Polyporales. The new species clustered into the residual polyporoid clade and was closely related to *Atraporiella neotropica* with a high support (100% BS, 100% BP, 1.00 BPP), and then grouped with other related genera: *Antrodiella*, *Pouzaroporia*, *Steccherinum* and *Xanthoporus* Audet.

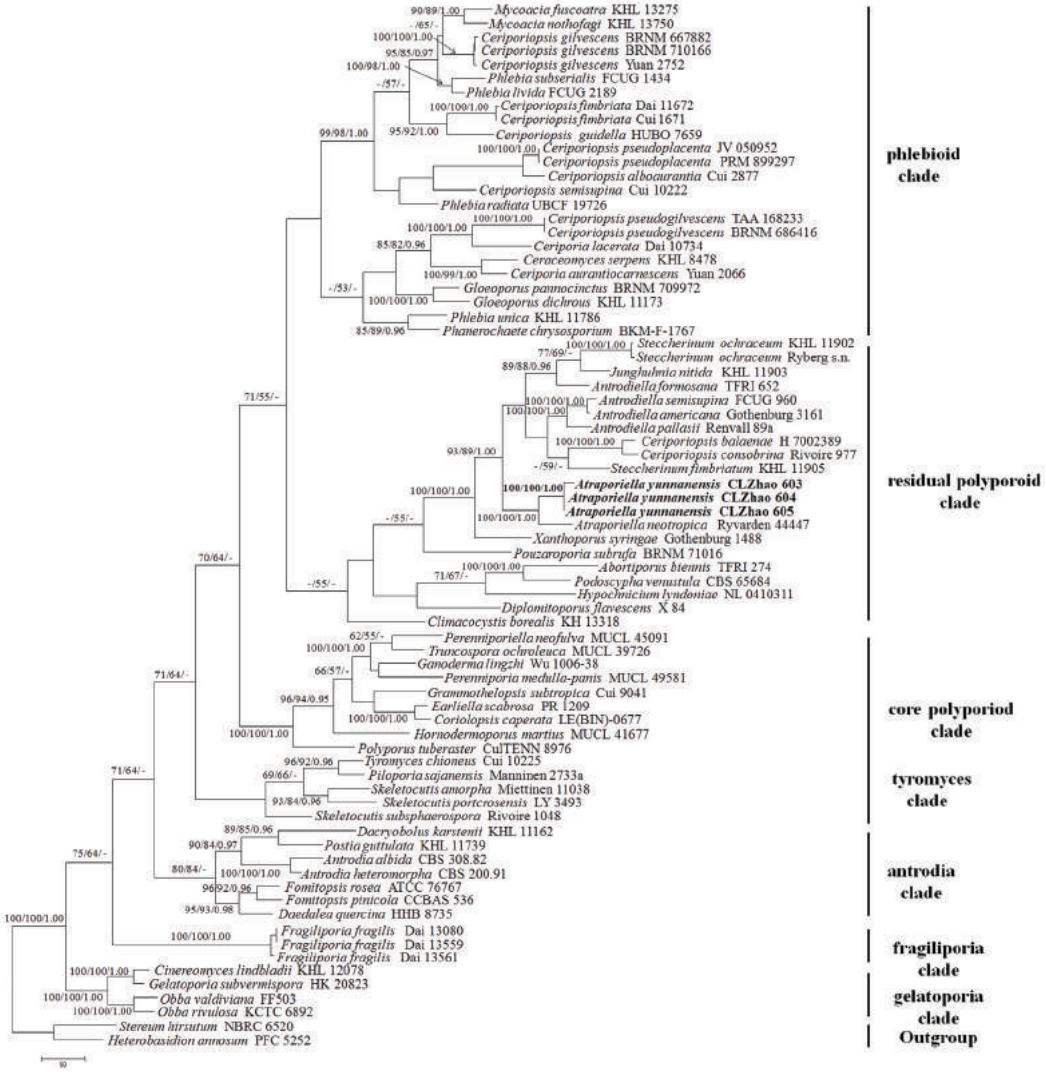

FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Atraporiella yunnanensis* and related species in Polyporales based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively. Clade names follow Binder *et al.* (2013).

Taxonomy

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

MycoBank no.: MB 823187

Type.—**China**. Yunnan Province, Jinghong, Sanchahe Nature Reserve, alt. 552 m, on fallen angiosperm trunk, 15 November 2016, *CLZhao 605* (holotype, SWFC!).

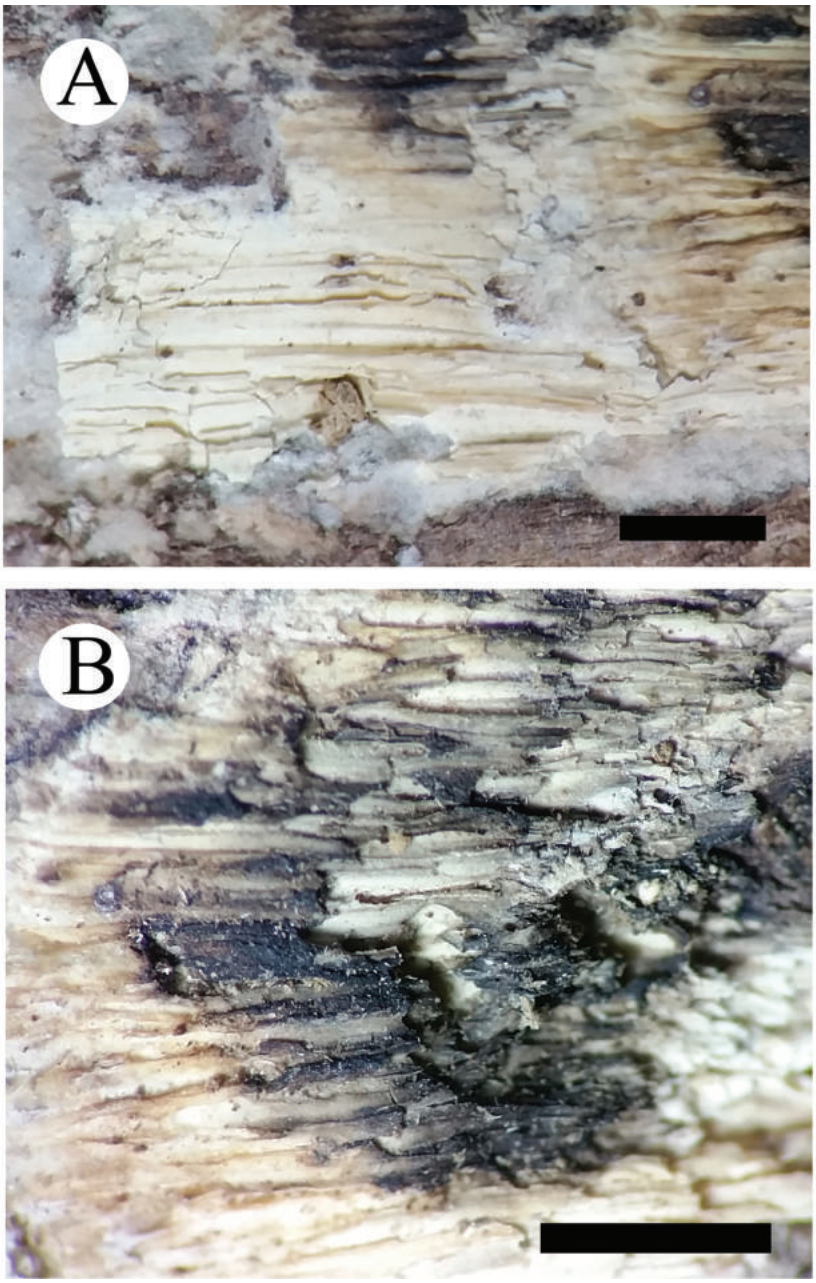

FIGURE 2. Basidiomata of *Atraporiella yunnanensis* (holotype). Scale bars: a—3 mm; b—2 mm.

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

Basidiomata.—Annual, resupinate, easy to separate from substrate, soft and slightly waxy when fresh, without odor or taste when fresh, becoming brittle and contracting up on drying, up to 2 cm long, 1 cm wide, 2 mm thick at centre. Pore surface white, very rapidly stained dark brown to black when bruised when fresh, turn to cream upon drying; pores angular, 3–4 per mm; dissepiments thin, lacerate. Sterile margin distinct, with raised loosened disc, white, up to 2 mm wide. Subiculum thin to almost invisible, pale brown, cottony, up to 0.2 mm thick. Tubes concolorous with pore surface, soft corky to fragile, up to 1.8 mm long.

Hyphal structure.—Hyphal system monomitic; generative hyphae with clamp connections, IKI–, CB–; tissues unchanged in KOH.

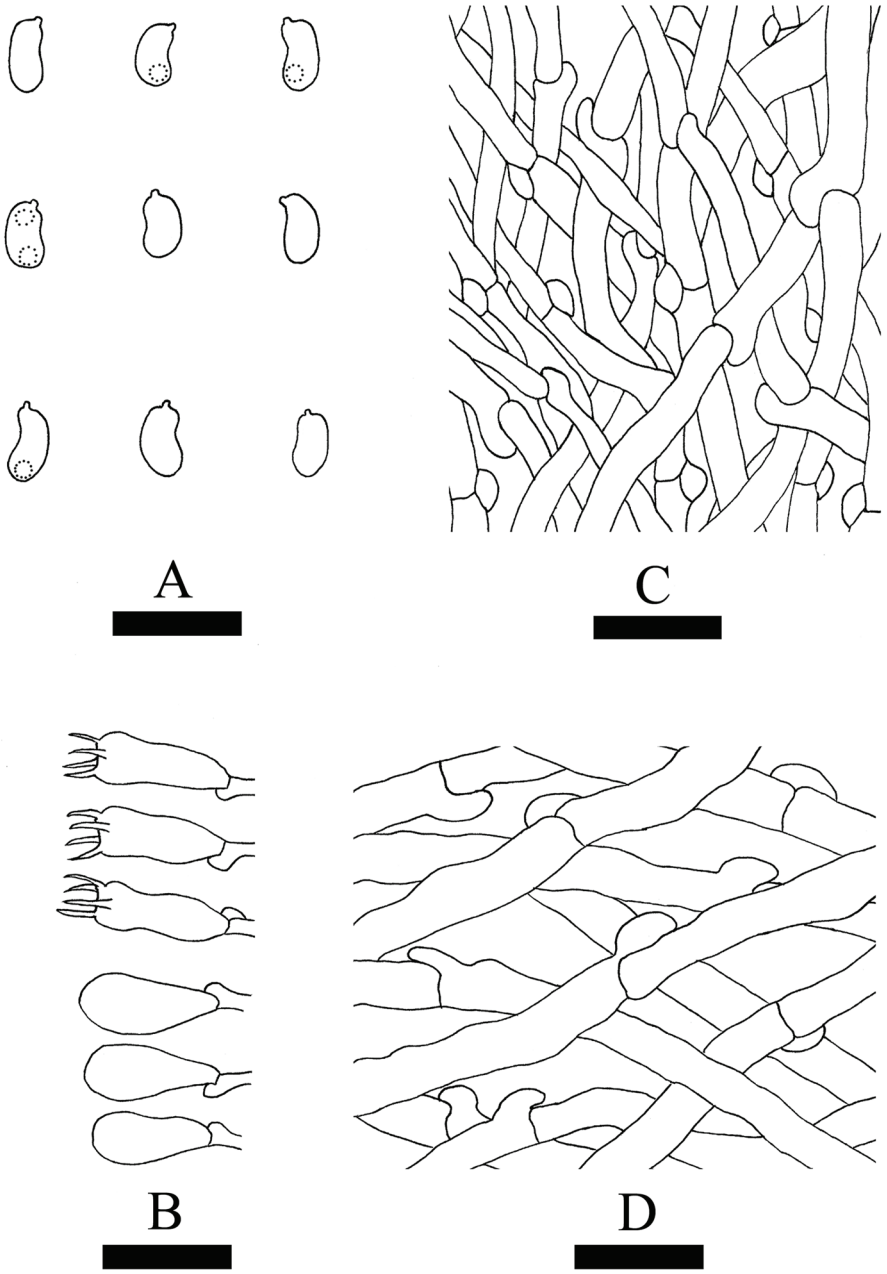

FIGURE 3. Microscopic structures of *Atraporiella yunnanensis* (drawn from the holotype). a basidiospores. b basidia and basidioles. c hyphae from trama. d hyphae from subiculum. Bars: a—5 µm; b–d—10 µm.

Subiculum.—Generative hyphae hyaline to pale brown, thin-walled, unbranched, interwoven, 2.5–4.5 µm in diameter.

Tubes.—Generative hyphae hyaline to pale brown, thin-walled, unbranched, interwoven, 3–6 µm in diameter. Cystidia and cystidioles absent; basidia clavate, with four sterigmata and a basal clamp connection, 9–13 × 3.5–5.5 µm; basidioles dominant, pyriform.

Spores.—Basidiospores slightly allantoid, hyaline, thin-walled, smooth, IKI–, CB–, (2–)2.2–3(–3.2) × 0.8–1.5 µm, L = 2.7 µm, W = 1.2 µm, Q = 1.84–2.52 (n = 90/3).

Additional specimens examined.—**China**. Yunnan Province, Jinghong, Sanchahe Nature Reserve, alt. 552 m, on fallen angiosperm trunk, 15 November 2016, *CLZhao 603, 604* (paratypes, SWFC!).

Discussion

In the present study, a new species, *Atraporiella yunnanensis*, is described based on phylogenetic analyses and morphological characters. In the ITS+nLSU analyses (Fig. 1), *Atraporiella yunnanensis* groups with *A. neotropica* with high statistical supports (100% BS, 100% BP, 1.00 BPP). However, morphologically *A. neotropica* differs from *A. yunnanensis* by its smaller pores (5–6 per mm) and both larger basidia (12–18×5–6 µm) and basidiospores (3–3.5 ×1.2–1.4 µm, Ryvarden 2007).

According to Binder *et al.* (2013), seven clades were found in the Polyporales, and *Atraporiella neotropica* was placed in the residual polyporoid clade, closely related to *Pouzaroporia subrufa* (Ellis & Dearn.) Vampola (= *Ceriporiopsis subrufa* (Ellis & Dearn.) Ginns). Our results support the placement of *Atraporiella* in the residual polyporoid clade as a monophyletic lineage, and now nested with *Antrodiella*, *Pouzaroporia*, *Steccherinum* and *Xanthoporus*.

Macroscopically *Antrodiella* differs from *Atraporiella* by the resupinate to pileate basidiomata and a di-trimitic hyphal system (Gilbertson & Ryvarden 1986, Dai & Niemelä 1997, Ryvarden & Melo 2014). *Pouzaroporia* is separated from *Atraporiella* by having the reddish to pale brown basidiomata and white subiculum, and hyaline, branched, thin- to thick-walled generative hyphae (Núñez & Ryvarden 2001). *Steccherinum* differs in its di-trimitic hyphal system and cyanophilous basidiospores (Bernicchia & Gorjon 2010). *Xanthoporus* differs from *Atraporiella* by its stipitate basidiomata with orange pore surface and larger basidiospores (L > 3.5 µm in length, Audet 2010).

Polypores are an extensively studied group of Basidiomycota (Gilbertson & Ryvarden 1986, 1987, Núñez & Ryvarden 2001, Ryvarden & Melo 2014), but the Chinese polypore diversity is still not well known, especially in subtropics and tropics, and many taxa of polypores are being described from these areas (Wu *et al*. 2017, Chen *et al*. 2016, 2017, Song *et al*. 2016b, Zhou *et al*. 2016, Ren & Wu 2017, Yuan *et al*. 2017a, b) as the new species in the present study, *Atraporiella yunnanensis*. Thus, it is possible that more new polypore taxa will be found after further investigations and molecular analyses.

Acknowledgements

We express our gratitude to Yong-He Li (Yunnan Academy of Biodiversity, Southwest Forestry University) for his support on molecular work. The research is supported by the National Natural Science Foundation of China (Project No. 31700023), and the Science Foundation of Southwest Forestry University (Project No. 111715) and the Science and Technology Talent Support Program of Three Areas in Yunnan Province (Project No. 21700329).

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