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Three species of wood-decaying fungi in *Polyporales* new to China

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Abstract—Three wood-decaying fungi, *Ceriporiopsis lagerheimii*, *Sebipora aquosa*, and *Tyromyces xuchilensis*, are newly recorded in China. The identifications were based on morphological and molecular evidence. The phylogenetic tree inferred from ITS+nLSU sequences of 49 species of *Polyporales* nests *C. lagerheimii* within the phlebioid clade, *S. aquosa* within the gelatoporia clade, and *T. xuchilensis* within the residual polyporoid clade. The three species are described and illustrated based on Chinese material.

Key words—*Basidiomycota*, polypore, taxonomy, white rot fungus

Introduction

Wood-decaying fungi play a key role in recycling nutrients of forest ecosystems by decomposing cellulose, hemicellulose, and lignin of the plant cell walls (Floudas et al. 2015). *Polyporales*, a large order in *Basidiomycota*, includes many important genera of wood-decaying fungi. Recent molecular studies employing multi-gene datasets have helped to provide a phylogenetic overview of *Polyporales*, in which thirty-four valid families are now recognized (Binder et al. 2013).

The diversity of wood-decaying fungi is very high in China because of the large landscape ranging from boreal to tropical zones. More than 1200 species of wood-decaying fungi have been found in China (Dai 2011, 2012), and some

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important genera have been extensively investigated (Jia & Cui 2011; He & Dai 2012; Zhou & Dai 2012, 2013; Li & Cui 2013; Li et al. 2014; Song et al. 2014; Chen et al. 2015, 2016; Zhou 2015; Zhou et al. 2015, 2016a,b,c). Recently, three additional species in *Polyporales*—*Ceriporiopsis lagerheimii*, *Sebipora aquosa*, and *Tyromyces xuchilensis*—were found in Yunnan Province, southwestern China, and are described and illustrated here. In addition to morphological analysis, the phylogenetic positions of these Chinese specimens were inferred from ITS+nLSU sequences.

Materials & methods

Morphological study

The studied specimens are deposited at the herbaria of Beijing Forestry University, Beijing, China (BJFC) and University of Oslo, Oslo, Norway (O). Macro-morphological descriptions are based on field notes. Special colour terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens under a light microscope. The following abbreviations are used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = neither amyloid nor dextrinoid, L = mean spore length, W = mean spore width, Q = variation in the L/W ratios, n = number of spores (a) measured from number of specimens (b).

Molecular phylogeny

A CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions with some modifications. ITS region was amplified with the primer pair ITS5 and ITS4 (White et al. 1990), and the nuclear LSU region was amplified with the primer pair LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The ITS PCR began with 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 at 72°C for 10 min. The nLSU PCR began with initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 48°C for 1 min, and 72°C for 1.5 min, and a final extension at 72°C for 10 min. The PCR products were purified and sequenced at Beijing Genomics Institute. The sequences were deposited at GenBank (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor) was used to edit the DNA sequences. The original ITS and nLSU sequences were combined directly and then 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 concatenated alignment was subjected to the incongruence length difference (ILD) test (Farris et al. 1994) implemented in PAUP* 4.0b10 (Swofford 2002) with a heuristic search and 1000 bootstrap (BS) replicates. The ILD test generated a P value of 1.000 much greater than 0.01, indicating that there was no incongruence between the ITS and nLSU regions. The sequence alignment was deposited in TreeBase (submission ID 19179). *Heterobasidion annosum* (Fr.) Bref. and *Stereum hirsutum* (Willd.) Pers. were selected as outgroup (Binder et al. 2013). Clade names follow Binder et al. (2013).

Table 1. Species and sequences used in the phylogenetic analyses. Newly generated sequences are set in bold.

Species	Voucher no.	GenBank no.	
		ITS	nLSU
Abortiporus biennis (Bull.) Singer	TFRI 274	EU232187	EU232235
Antrodia albida (Fr.) Donk	CBS 308.82	DQ491414	AY515348
Antrodia heteromorpha (Fr.) Donk	CBS 200.91	DQ491415	AY515350
Antrodiella americana Ryvarden & Gilb.	Gothenburg 3161	JN710509	JN710509
Antrodiella semisupina (Berk. & M.A. Curtis) Ryvarden	FCUG 960	EU232182	EU232266
Ceriporiopsis balaenae Niemelä	H7002389	FJ496669	FJ496717
Ceriporiopsis consobrina (Bres.) Ryvarden	Rivoire 977	FJ496667	FJ496716
Ceriporiopsis fimbriata C.L. Zhao & Y.C. Dai	Dai 11672	KJ698633	KJ698637
	Cui 1671	KJ698634	KJ698638
Ceriporiopsis gilvescens	BRNM 667882	FJ496685	FJ496719
	BRNM 710166	FJ496684	FJ496720
	Yuan 2752	KF845946	KF845953
Ceriporiopsis guidella	HUBO 7659	FJ496687	FJ496722
Ceriporiopsis lagerheimii	**Ryvarden 58240**	**KX081077**	**KX161652**
	Dai 12304	**KX161647**	**KX161651**
Climacocystis borealis	KH 13318	JQ031126	JQ031126
Coriolopsis caperata (Berk.) Murrill	LE(BIN)-0677	AB158316	AB158316
Dacryobolus karstenii (Bres.) Oberw. ex Parmasto	KHL 11162	EU118624	EU118624
Daedalea quercina (L.) Pers.	DSM 4953	DQ491425	DQ491425
Earliella scabrosa (Pers.) Gilb. & Ryvarden	PR1209	JN165009	JN164793
Fomitopsis pinicola (Sw.) P. Karst.	CBS 221.39	DQ491405	DQ491405
Fomitopsis rosea (Alb. & Schwein.) P. Karst.	ATCC 76767	DQ491410	DQ491410
Fragiliporia fragilis Y.C. Dai et al.	Dai 13080	KJ734260	KJ734264
	Dai 13559	KJ734261	KJ734265
	Dai 13561	KJ734262	KJ734266
Ganoderma lingzhi Sheng H. Wu et al.	Wu 1006-38	JQ781858	—
Gelatoporia subvermispora	BRNU 592909	FJ496694	FJ496706
Grammothelopsis subtropica B.K. Cui & C.L. Zha**O**	Cui 9041	JQ845096	JQ845099
Heterobasidion annosum	PFC 5252	KC492906	KC492906
Hornodermoporus martius (Berk.) Teixeira	MUCL 41677	FJ411092	FJ393859

Table 1, concluded

Species	Voucher no.	GenBank no.	
		ITS	nLSU
Hypochnicium lyndoniae (D.A. Reid) Hjortstam	NL 041031	JX124704	JX124704
Junghuhnia nitida (Pers.) Ryvarden	KHL 11903	EU118638	EU118638
Obba rivulosa (Berk. & M.A. Curtis) Miettinen & Rajchenb.	KCTC 6892	FJ496693	FJ496710
Obba valdiviana (Rajchenb.) Miettinen & Rajchenb.	FF 503	HQ659235	HQ659235
Perenniporia medulla-panis (Jacq.) Donk	MUCL 49581	FJ411088	FJ393876
Perenniporiella neofulva (Lloyd) Decock & Ryvarden	MUCL 45091	FJ411080	FJ393852
Phlebia livida (Pers.) Bres.	FCUG 2189	AF141624	AF141624
Phlebia radiata Fr.	UBCF 19726	HQ604797	HQ604797
Phlebia subserialis (Bourdot & Galzin) Donk	FCUG 1434	AF141631	AF141631
Piloporia sajanensis (Parmasto) Niemelä	Mannine 2733a	HQ659239	HQ659239
Podoscypha venustula (Speg.) D.A. Reid	CBS 65684	JN649367	JN649367
Polyporus tuberaster (Jacq. ex Pers.) Fr.	CulTENN 8976	AF516598	AJ488116
Postia guttulata (Sacc.) Jülich	KHL 11739	EU11865	EU11865
Sebipora aquosa	Miettinen 8868	HQ659242	—
	Miettinen 8680	HQ659240	HQ659240
	Miettinen 9265	HQ659243	—
	Dai 13268	**KX161648**	**KX161661**
	Dai 13592	**KU376422**	**KX161660**
Skeletocutis amorpha (Fr.) Kotl. & Pouzar	Miettinen 11038	FN907913	FN907913
Skeletocutis jelicii Tortič & A. David	H 6002113	FJ496690	FJ496727
Skeletocutis portcrosensis A. David	LY 3493	FJ496689	FJ496689
Skeletocutis subsphaerospora A. David	Rivoire 1048	FJ496688	FJ496688
Steccherinum fimbriatum (Pers.) J. Erikss.	KHL 11905	EU118668	EU118668
Steccherinum ochraceum (Pers.) Gray	KHL 11902	JQ031130	JQ031130
Stereum hirsutum	NBRC 6520	AB733150	AB733325
Truncospora ochroleuca (Berk.) Pilát	MUCL 39726	FJ411098	FJ393865
Tyromyces chioneus (Fr.) P. Karst.	Cui 10225	KF698745	KF698756
Tyromyces xuchilensis	**Dai 12234**	**KX161649**	**KX161658**
	Ryvarden 44669	**KX161650**	**KX161659**
Xanthoporus syringae (Parmasto) Audet	Gothenburg 1488	JN710607	JN710607

Maximum parsimony analysis was applied to the ITS+nLSU sequences dataset, and performed in PAUP* version 4.0b10 (Swofford 2002) according to Zhao et al. (2013). 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 1000 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 generated Maximum Parsimonious Tree (MPT). Sequences were also analyzed using Maximum likelihood (ML) with RAxML-HPC2 on Abe through the Cipres Science Gateway (www.phylo.org). 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 Bayesian inference (BI). BI 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 10 million generations, and trees were sampled every 100 generations. The first quarter generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated.

Results

Phylogenetic analyses

The combined dataset (ITS+nLSU) included sequences from 62 samples representing 51 species. The dataset had an aligned length of 2202 characters, of which 1294 characters are constant, 232 are variable and parsimony-uninformative, and 676 are parsimony-informative. MP analysis yielded 13 equally parsimonious trees (TL = 4893, CI = 0.313, HI = 0.687, RI = 0.578, RC = 0.181). Best model estimated and applied in the Bayesian analysis was: GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). The average standard deviation of split frequencies in BI was 0.001416. BI and ML analyses resulted in similar topologies as that of MP analysis.

The MP phylogenetic tree supported seven major clades for 49 species of *Polyporales* with *Ceriporiopsis lagerheimii* nested within the phlebioid clade, *Sebipora aquosa* within the gelatoporia clade, and *Tyromyces xuchilensis* within the residual polyporoid clade (Fig. 1). For all these three species, specimens from China clustered with authentic specimens from elsewhere with strong support (Fig. 1).

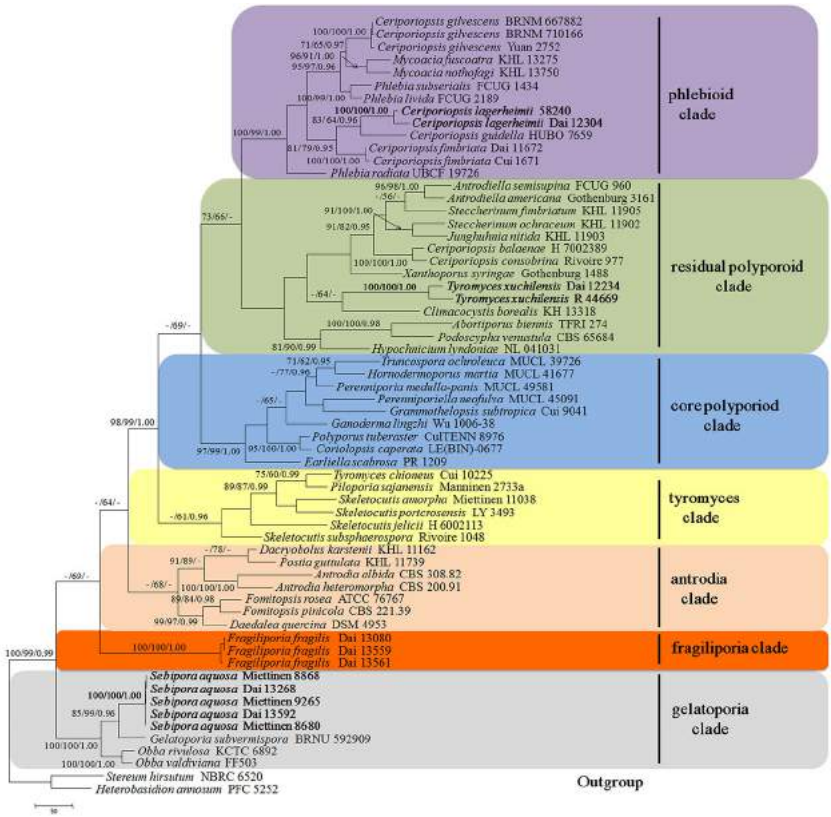

Fig. 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Ceriporiopsis lagerheimii*, *Sebipora aquosa*, *Tyromyces xuchilensis*, and related species in *Polyporales* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap ≥70%, parsimony bootstrap proportions ≥50% and Bayesian posterior probabilities ≥0.95.

Taxonomy

Ceriporiopsis lagerheimii Læssøe & Ryvarden, Syn. Fung. 27: 44, 2010. Figs 2a, 3

Basidiome Annual, resupinate, adnate, soft corky, without odor or taste when fresh, corky when drying; ≤2 cm long, 1.5 cm wide, 2 mm thick at center. Pore surface cream when fresh, cream to buff upon when drying; pores angular, 5–6 per mm; dissepiments thin, entire. Subiculum thin, white, ≤0.2 mm thick. Tubes concolorous with pore surface, ≤1.8 mm long.

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

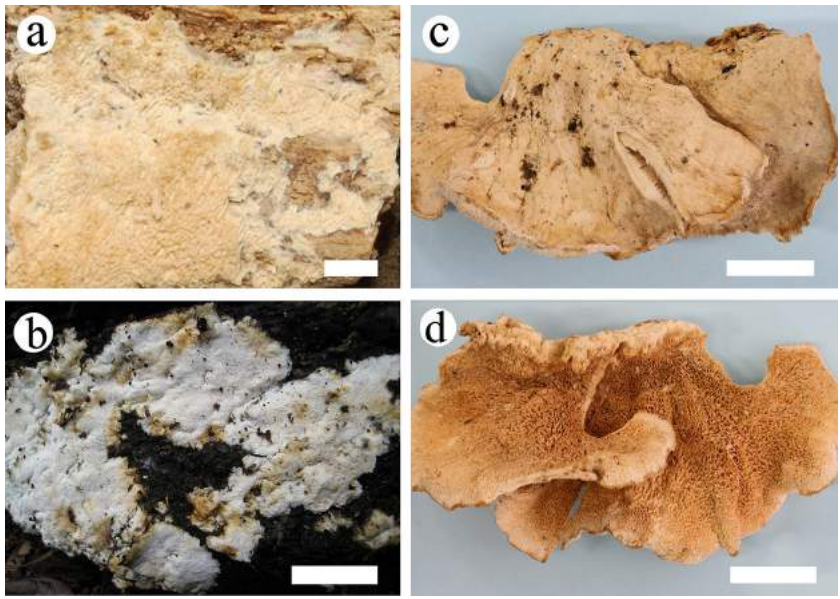

Fig. 2. Basidiocarps: a. *Ceriporiopsis lagerheimii* (Dai 12304); b. *Sebipora aquosa* (Dai 13592); c, d. *Tyromyces xuchilensis* (Dai 12234). Scale bars: a = 2 mm; b = 2 cm; c, d = 1 cm.

Subiculum Generative hyphae hyaline, thin-walled, branched, flexuous, interwoven, 3–5 µm in diam.

Tubes Generative hyphae hyaline, thin-walled, branched, flexuous, interwoven, 3.5–4.5 µm in diam. Cystidia and cystidioles absent. Basidia barrel-shaped to pyriform, hyaline, thin-walled, with four sterigmata and a basal clamp connection, 9–14 × 5–7 µm; basidioles similar to basidia, but slightly smaller.

Spores Basidiospores cylindrical, tapering toward apiculus, hyaline, thin-walled, smooth, IKI–, CB–, (3–)3.2–3.7(–4) × (1.3–)1.5–1.8(–2) µm, L = 3.5 µm, W = 1.7 µm, Q = 2.1 (n = 30/1).

Type of rot: White rot.

Specimens examined: **CHINA. Yunnan Province**: Jinghong, Xishuangbanna Nature Reserve, Sanchahe, on rotten angiosperm wood, 7 June 2011, Dai 12304 (BJFC010586). **ECUADOR. Napo Province**, Cuyuja, 4 May 2002, Ryvarden 58240 (isotype in O).

Comments: The Chinese specimen has cream to buff pore surface after drying, while the isotype specimen has white to cream pore surface. Otherwise, they have same morphological characters.

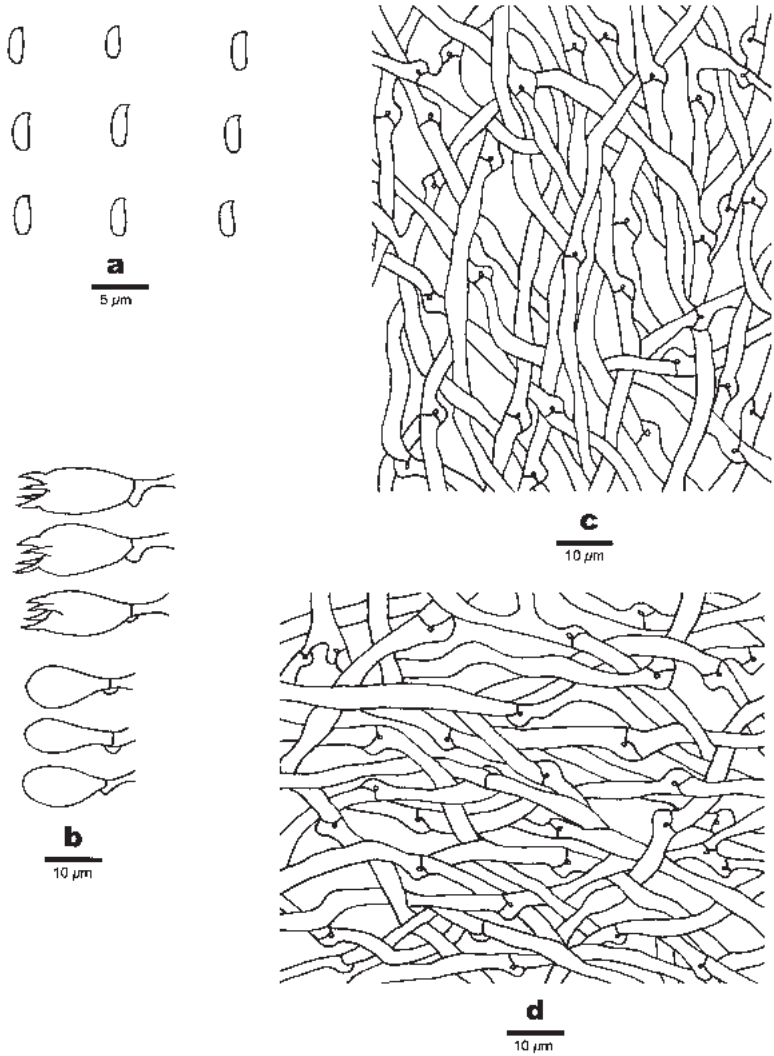

Fig. 3. *Ceriporiopsis lagerheimii* (drawn from Dai 12304).
a. Basidiospores; b. Basidia and basidioles; c. Tramal hyphae; d. Subicular hyphae.

Sebipora aquosa Miettinen, Mycol. Prog. 11: 144, 2012. Figs 2b, 4

Basidiome Annual, resupinate, watery and soft when fresh, becoming hard corky when drying; ≤9 cm long, 5 cm wide, 8 mm thick at center. Pore surface white when fresh, cream to yellow upon drying, more or less tallowing; pores

angular, 4–6 per mm; dissepiments thin, entire. Sterile margin cream to pale brown, ≤1 mm wide. Context white to cream, soft corky, ≤1 mm thick. Tubes concolorous with pore surface, hard corky, ≤7 mm long.

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

Subiculum Generative hyphae hyaline, thin- to slightly thick-walled, occasionally branched, more or less parallel to substrate, 3–5 µm in diam.

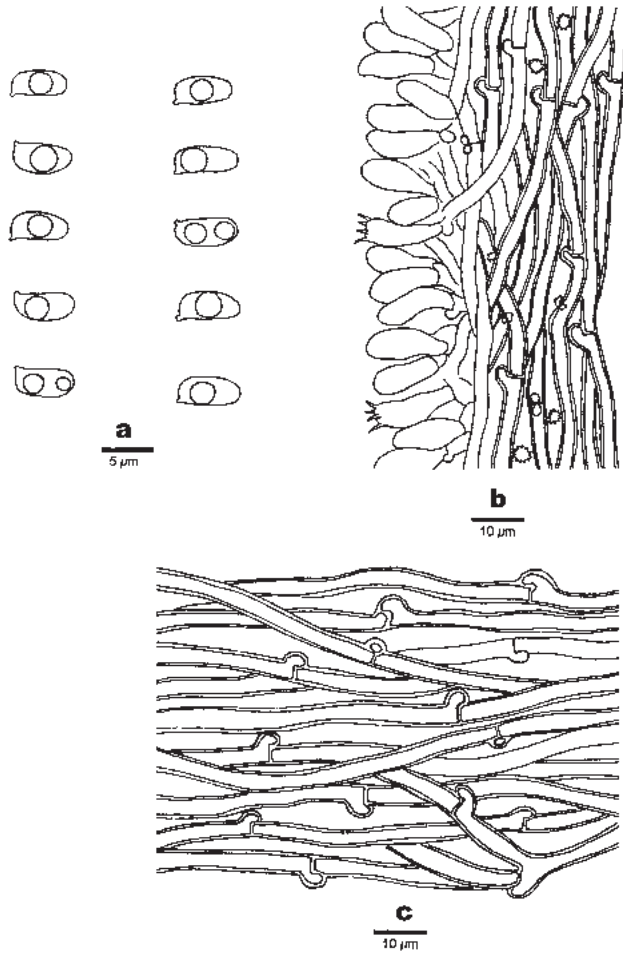

Fig. 4. *Sebipora aquosa* (drawn from Dai 13592).
a. Basidiospores; b. Tramal section; c. Subicular hyphae.

Tubes Generative hyphae hyaline, thin- to thick-walled, unbranched, subparallel to the tubes, 2.5–4 μm in diam. Small pale-yellow resinous granules occasionally present. Cystidia and cystidioles absent. Basidia clavate to pyriform, with four sterigmata and a basal clamp connection, 16–24 × 5–6 μm; basidioles similar to basidia, but slightly smaller.

Spores Basidiospores cylindrical to oblong-ellipsoid, often slightly curved, hyaline, thin-walled, smooth, usually bearing one or two guttules, IKI–, CB–, (5.5–)6–6.5(–6.7) × (2–)2.2–2.8 μm, L = 6.2 μm, W = 2.5 μm, Q = 2.3–2.5 (n = 60/2).

Type of rot: White rot.

Specimens examined: **CHINA. Yunnan Province**: Jinghong, Xishuangbannan Nature Reserve, Wangtianshu, on fallen angiosperm trunk, 19 October 2013, Dai 13592 (BJFC015054); Nanhua County, Dazhongshan Nature Reserve, on rotten angiosperm wood, 15 July 2013, Dai 13268 (BJFC014755).

Comments: The Chinese material conforms closely to the original description, which cites slightly longer basidiospores (5.7–7.6 × 2.1–2.7 μm; Miettinen & Rajchenberg 2012).

Tyromyces xuchilensis (Murrill) Ryvarden, Mycotaxon 23: 175, 1985. Figs 2c,d, 5

Basidiome Annual, pileate, soft and sappy when fresh, shrinking and becoming corky when drying. Pilei more or less semicircular, projecting ≤3 cm long, 4 cm wide, and 5 mm thick at center. Pileal surface white when fresh, becoming cream to pale ochraceous upon drying, glabrous. Pore surface white when fresh, buff to ochraceous upon drying; sterile margin cream, ≤1 mm wide; pores angular, 3–5 per mm; dissepiments thin, entire. Context white to cream, soft corky, ≤1 mm thick. Tubes concolorous with pore surface, corky, ≤4 mm long.

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

Context Generative hyphae hyaline, thin- to slightly thick-walled, occasionally branched, interwoven, 4–7 μm in diam.

Tubes Generative hyphae hyaline, thin- to slightly thick-walled, unbranched, interwoven, more or less parallel to tubes, 3–5 μm in diam. Cystidia and cystidioles absent; basidia clavate, with four sterigmata and a basal clamp connection, 15–18.5 × 5–7 μm; basidioles similar in shape to basidia, but slightly smaller.

Spores Basidiospores subglobose to broadly ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (3.3–)3.5–4.4(–4.7) × (2.3–)2.5–3.5(–3.8) μm, L = 4 μm, W = 3.3 μm, Q = 1.2 (n = 30/1).

Type of rot: White rot.

Specimens examined: **CHINA**. **Yunnan Province**: Pu'er County, Laiyanghe Forest Park, on fallen angiosperm trunk, 6 June 2011, Dai 12234 (BJFC010517). **ECUADOR**. **Orellana Province**, Yasuni National Park, 9 March 2002, Ryvarden 44669 (O).

Comments: The Chinese material conforms closely to the Ecuadorian specimen and the original description, which cites slightly narrower basidiospores (3.5–4.5 × 2.5–3 μm; Ryvarden 1985).

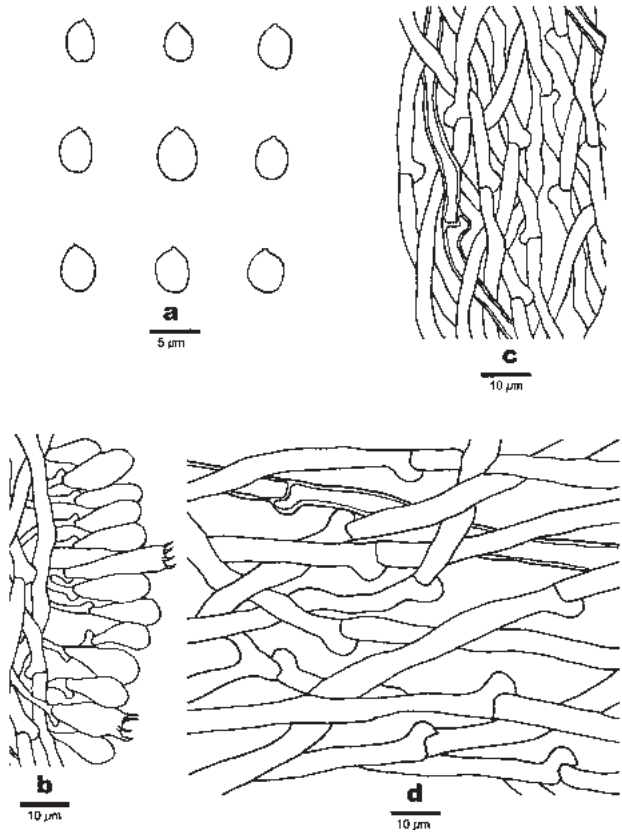

Fig. 5. *Tyromyces xuchilensis* (drawn from Dai 12234).
a. Basidiospores; b. Tramal section; c. Tramal hyphae; d. Context hyphae.

Discussion

The ITS+nLSU sequence analyses revealed seven major clades of *Polyporales*: antrodia clade, core polyporoid clade, fragiliporia clade, gelatoporia clade, phlebioid clade, residual polyporoid clade, and tyromyces clade (Fig. 1). The phylogenetic placement of the sequences from the three new Chinese records

was consistent with previous studies (Miettinen & Rajchenberg 2012; Binder et al. 2013).

In the phylogenetic tree, *Ceriporiopsis lagerheimii* was closely related to *C. guidella* Bernicchia & Ryvarden; however, *C. guidella* produces a dull yellow to green pore surface and has larger pores (4–5 per mm) and basidiospores (4–5 × 2.1–2.4 µm; Bernicchia & Ryvarden 2003; Ryvarden & Melo 2014). *Ceriporiopsis flavilutea* (Murrill) Ryvarden is similar to *C. lagerheimii* in having resupinate basidiocarps with white to cream pore surface, but it has larger pores (3–5 per mm) and ellipsoid basidiospores (3.5–4.5 × 2–2.5 µm; Ryvarden 1985). *Ceriporiopsis gilvescens* (Bres.) Domański resembles *C. lagerheimii* by having similar basidiospores (3.5–4.5 × 1.5–2 µm); however, *C. gilvescens* has an orange-brown pore surface, larger pores (4–5 per mm), and hyphae frequently covered by small, rod-like crystals (Ryvarden & Melo 2014). *Ceriporiopsis lagerheimii* was described from its type locality in Ecuador (Læssøe & Ryvarden 2010). The Chinese specimen (Dai 12304) clustered with the isotype (O, Ryvarden 58240) with strong support. This is the first report of *C. lagerheimii* from China.

Two *Sebipora aquosa* specimens from China (Dai 13592, Dai 13268) clustered with the holotype (ANDA, Miettinen 8868) and two other samples from Indonesia with strong support to form a sister lineage with *Gelatoporia subvermispora* (Pilát) Niemelä within the gelatoporia clade. Morphologically, *G. subvermispora* differs from *S. aquosa* by larger pores (2–4 per mm) and smaller allantoid basidiospores (4.5–5.5 × 1–1.5 µm; Miettinen & Rajchenberg 2012; Ryvarden & Melo 2014). This is the first report of *S. aquosa* from China.

Two *Tyromyces xuchilensis* specimens (Dai 12234 from China; Ryvarden 44669 from Ecuador) formed a strongly supported lineage not in the tyromyces clade but in the residual polyporoid clade. This unexpected result was also noted by Binder et al. (2013). The Mexican type specimen of *T. xuchilensis* (Murrill 1171) should be reexamined in order to resolve its taxonomic status. In the tree, *T. xuchilensis* appeared close to *Climacocystis borealis* (Fr.) Kotl. & Pouzar. However, *C. borealis* is easily distinguished from *T. xuchilensis* by its duplex context, larger pores (1–2 per mm), acute cystidia, larger basidiospores (4.5–6.5 × 3–4.5 µm), and habit on gymnosperms (Song et al. 2014). *Tyromyces xuchilensis* was described from Mexico, and later found in Ecuador (Ryvarden 1985); this is the first report of the species from China.

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