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ORIGINAL ARTICLE

Leifiporia rhizomorpha gen. et sp. nov. and *L. eucalypti* comb. nov. in Polyporaceae (Basidiomycota)

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Abstract A new poroid wood-inhabiting fungal genus, *Leifiporia*, is proposed, based on morphological and molecular evidence, which is typified by *L. rhizomorpha* sp. nov. The genus is characterized by an annual growth habit, resupinate basidiocarps with white to cream pore surface, a dimitic hyphal system with generative hyphae bearing clamp connections and branching mostly at right angles, skeletal hyphae present in the subiculum only and distinctly thinner than generative hyphae, IKI–, CB–, and ellipsoid, 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 analysis based on molecular data of ITS + nLSU sequences showed that *Leifiporia* belonged to the core polyporoid clade and was closely related to *Diplomitoporus overholtsii* and *Lopharia cinerascens*, and then grouped with *Pycnoporus* and *Trametes*. Further investigation was obtained for more representative taxa in the Polyporaceae based on nLSU sequences, in which the results demonstrated that the genus *Leifiporia* formed a monophyletic lineage with a strong support (100 % BS, 100 % BP, 1.00 BPP). Both morphological and molecular evidence confirmed the placement of the new genus in the core polyporoid clade. In addition, a new combination, *Leifiporia eucalypti*, is proposed based on examination of its type material and phylogeny.

Keywords Phylogenetic analysis · Polypores · Taxonomy · Wood-rottingfungi

Introduction

Polypores are a very important group of wood-inhabiting fungi which have been extensively studied Among them, the Polyporaceae is a diverse group of Polyporales, including species having annual to perennial, resupinate, pileate and stipitate basidiocarps, a monomitic to dimitic or trimitic hyphal structure with simple septa or clamp connections on generative hyphae, and thin- to thick-walled, smooth to ornamented, cyanophilous to acyanophilous basidiospores (Ryvarden and Johansen 1980; Gilbertson and Ryvarden 1986, 1987; Dai 2012; Ryvarden and Melo 2014).

Molecular systematics is a powerful tool to infer phylogenies within fungal groups including polypores (White et al. 1990; Binder et al. 2005, 2013; James et al. 2006; Hibbett et al. 2007). By using ribosomal DNA sequences, Binder et al. (2005) resolved the taxonomic structure of Polyporales and divided the polyporoid clade into three main groups—the core polyporoid clade, the antrodia clade, and the phlebioid clade—together with some 'residual' taxa not assigned to any group. Hibbett et al. (2007) analyzed a higher-level phylogenetic classification of the fungi, and showed that Polyporales formed a monophyletic lineage within Basidiomycota. Recently, molecular studies employing multi-gene (5.8S, nrLSU, nrSSU, rpb1, rpb2, tef1) datasets have helped to investigate the phylogenetic overview of the Polyporales, in which 35 families were recognized in Polyporales, and the Polyporaceae species were mainly nested in the core polyporoid clade (Binder et al. 2013; Zhao et al. 2015). Overviews of studies on fungal diversity worldwide have been carried out based on increasing specialized knowledge and new techniques (Hawksworth 2012; Dai et al. 2015),

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and new genera of polypores have recently been found (Miettinen and Larsson 2011; Cui 2013; Li et al. 2013, 2014; Sotome et al. 2013; Spirin et al. 2013; Chen et al. 2014, 2016; Ariyawansa et al. 2015; Ghobad-Nejhad 2015; Westphalen et al. 2015; Zhou et al. 2016).

During a study of Chinese polypores, two samples collected from eastern China were characterized by resupinate, white to cream basidiocarps with distinct rhizomorphic margins, a monomitic hyphal structure in tube trama, but few skeletal hyphae in the subiculum, and hyaline, thin-walled, ellipsoid basidiospores, which are negative in Melzer's reagent and Cotton Blue, and causing a white rot. These characters distinguished them from all the known polypore taxa, and we have proposed a new genus for the two collections. To support our proposal, phylogenetic analyses on the position of the new genus and related taxa were carried out based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences.

Materials and methods

Morphology

The studied specimens are deposited at the herbaria of Beijing Forestry University (BJFC) and the University of Oslo (O). Macro-morphological descriptions are based on field notes. Special color 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 have been 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.

Molecular phylogeny

CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies, Beijing, China) 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). The 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 Beijing Genomics Institute. All newly generated sequences were deposited at GenBank (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 6 (Katoh and 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 18818). 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. Sequences of *Ceriporiopsis gilvescens* (Bres.) Domański and *Phlebia radiata* Fr., obtained from GenBank, were used as outgroups to root trees in nLSU tree.

Maximum parsimony analysis was applied to the nLSU and ITS + nLSU dataset sequences. Approaches to phylogenetic analysis followed Zhao et al. (2013, 2014), and the tree construction procedure was performed in PAUP* v.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.

MrModeltest 2.3 (Posada and 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 and Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 5 million generations (ITS + nLSU), and for 2 million generations (nLSU), and trees were sampled every 100 generations. The first one-quarter of the 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.

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	
Abundisporus fuscopurpureus	Cui 10950	KC456254	KC456256	Zhao et al. 2015
Antrodia albida	CBS 308.82	DQ491414	AY515348	Kim et al. 2007
A. heteromorpha	CBS 200.91	DQ491415	AY515350	Kim et al. 2007
Antrodiella americana	Gothenburg 3161	JN710509	JN710509	Binder et al. 2013
A. semisupina	FCUG 960	EU232182	EU232266	Binder et al. 2005
Ceriporiopsis gilvescens	BRNM 710166	FJ496684	FJ496720	Tomšovský et al. 2010
C. gilvescens	Yuan 2752	KF845946	KF845953	Zhao et al. 2015
C. guidella	HUBO 7659	FJ496687	FJ496722	Tomšovský et al. 2010
Cinereomyces lindbladii	KHL 12078	FN907906	FN907906	Binder et al. 2013
Dacryobolus karstenii	KHL 11162	EU118624	EU118624	Binder et al. 2005
Daedalea quercina	DSM 4953	DQ491425	DQ491425	Kim et al. 2007
Datronia scutellata	RLG 9584 T	JN165004	JN164792	Binder et al. 2005
Dichomitus squalens	Cui 9639	JQ780407	JQ780426	Li and Cui 2013
D. squalens	Cui 9725	JQ780408	JQ780427	Li and Cui 2013
D. squalens	CBS 472.89	FJ349622	—	Binder et al. 2005
Diplomitoporus overholtsii	HHB 9465	—	AY333813	Binder et al. 2013
Donkioporia expansa	MUCL 35116	FJ411104	FJ393872	Robledo et al. 2009
Earliella scabrosa	PR 1209	JN165009	JN164793	Binder et al. 2005
Fomes fomentarius	ES 2008-3	JX109860	—	Binder et al. 2005
F. fomentarius	Cui 8020	JX290073	JX290070	Li and Cui 2013
Fomitopsis pinicola	CBS 221.39	DQ491405	DQ491405	Kim et al. 2007
F. rosea	ATCC 76767	DQ491410	DQ491410	Kim et al. 2007
Fragiliporia fragilis	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
F. fragilis	Yuan 5516	KJ734263	KJ734267	Zhao et al. 2015
Ganoderma lingzhi	Wu 1006-38	JQ781858	—	Zhao et al. 2015
Gelatoporia subvermispora	BRNU 592909	FJ496694	FJ496706	Tomšovský et al. 2010
Grammothele fuligo	PCT.08	HQ248224	—	Binder et al. 2005
Grammothelopsis subtropica	Cui 9035	JQ845094	JQ845097	Zhao et al. 2013
Heterobasidion annosum	PFC 5252	KC492906	KC492906	Binder et al. 2013
Hornodermoporus martius	MUCL 41677	FJ411092	FJ393859	Robledo et al. 2009
Hypochnicium lyndoniae	NL 041031	JX124704	JX124704	Binder et al. 2005
Junghuhnia nitida	KHL 11903	EU118638	EU118638	Binder et al. 2005
Leifiporia eucalypti	Oslo 910674	JQ780412	JQ780441	Li and Cui 2013
L. rhizomorpha	Dai 13380	KU599925	KU599927	Present study
L. rhizomorpha	Dai 13432	KU599926	KU599928	Present study
lenzites betulinus	Cui 7095	JX290075	JX290072	Binder et al. 2005
Lentinus tigrinus	DSH93-181	AY218419	AF518627	Binder et al. 2005
Lignosus hainanensis	Dai 10670	GU580884	GU580886	Li and Cui 2013
Lopharia cinerascens	FP 105043	JN165019	JN164813	Binder et al. 2005
Megasporia hengduanensis	Cui 8076	JQ780392	JQ780433	Li and Cui 2013
Megasporoporia bannaensis	Dai 12306	JQ314362	JQ314379	Li and Cui 2013
Megasporoporiella lacerata	Yuan 3880	JQ314377	JQ314395	Li and Cui 2013
Melanoderma microcarpum	Dai 9811	HQ678173	HQ678175	Zhao et al. 2013
Microporus xanthopus	Cui 8242	JX290074	—	Li and Cui 2013
Mycoacia fuscoatra	KHL 13275	JN649352	JN649352	Tomšovský et al. 2010
M. nothofagi	KHL 13750	GU480000	GU480000	Tomšovský et al. 2010
Obba rivulosa	KCTC 6892	FJ496693	FJ496710	Miettinen and Rajchenberg 2012
O. valdiviana	FF 503	HQ659235	HQ659235	Miettinen and Rajchenberg 2012
Pachykytospora tuberculosa	KA 11	JX124705	JX124705	Binder et al. 2005
Perenniporia hainaniana	Cui 6364	JQ861743	JQ861759	Zhao et al. 2013
P. medulla-panis	MUCL 43250	FJ411087	FJ393875	Robledo et al. 2009
P. medulla-panis	Cui 3274	JN112792	JN112793	Zhao et al. 2013
P. substraminea	Dai 10781	KF495007	KF494983	Zhao et al. 2013
Phlebia livida	FCUG 2189	AF141624	AF141624	Tomšovský et al. 2010
P. radiata	UBCF 19726	HQ604797	HQ604797	Binder et al. 2013
P. subserialis	FCUG 1434	AF141631	AF141631	Tomšovský et al. 2010

Table 1 (continued)

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Piloporia sajanensis	Mannine 2733a	HQ659239	HQ659239	Tomšovský et al. 2010
Polyporus tuberaster	CulTENN 10197	AF516596	AJ488116	Binder et al. 2013
Postia guttulata	KHL 11739	EU11865	EU11865	Kim et al. 2007
Pouzaroporia subrufa	BRNM 710164	FJ496661	FJ496723	Tomšovský et al. 2010
Pseudofavolus cucullatus	WD 2157	AB587637	AB368114	Binder et al. 2013
Pycnoporus cinnabarinus	KHL 8557	AY586703	AY586703	Binder et al. 2013
Sebipora aquosa	Miettinen 8680	HQ659240	HQ659240	Miettinen and Rajchenberg 2012
Skeletocutis amorpha	Miettinen 11038	FN907913	FN907913	Tomšovský et al. 2010
S. jelicii	H 6002113	FJ496690	FJ496727	Tomšovský et al. 2010
S. portcrosensis	LY 3493	FJ496689	FJ496689	Tomšovský et al. 2010
Sparsitubus nelumbiformis	402038	—	DQ887631	Binder et al. 2013
Steccherinum fimbriatum	KHL 11905	EU118668	EU118668	Tomšovský et al. 2010
S. ochraceum	KHL 11902	JQ031130	JQ031130	Tomšovský et al. 2010
Stereum hirsutum	NBRC 6520	AB733150	AB733325	Tomšovský et al. 2010
Trametes elegans	FP105679	JN048766	JN048785	Justo and Hibbett 2011
T. hirsuta	L 12964	JN164935	—	Binder et al. 2013
T. hirsuta	RLG5133T	JN164854	JN164801	Justo and Hibbett 2011
T. ochracea	HHB 13445	JN164954	JN164812	Justo and Hibbett 201
Truncospora ochroleuca	MUCL 39726	FJ411098	FJ393865	Robledo et al. 2009
Tyromyces chioneus	Cui 10225	KF698745	KF698756	Zhao et al. 2013
Vanderbylia vicina	MUCL 44779	FJ411095	AF518666	Robledo et al. 2009
Xanthoporus syringae	Gothenburg 1488	JN710607	JN710607	Tomšovský et al. 2010
Yuchengia narymica	Dai 6998	JN048775	JN048794	Zhao et al. 2013

Results

The ITS + nLSU dataset included sequences from 75 fungal specimens representing 65 species. The dataset had an aligned length of 2240 characters, of which 1326 characters are constant, 245 are variable and parsimony-uninformative, and 669 are parsimony-informative. Maximum parsimony analysis yielded four equally parsimonious trees (TL = 5510, CI = 0.287, HI = 0.713, RI = 0.529, RC = 0.152). 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 analysis and ML analysis resulted in a similar topology as the MP analysis, with an average standard deviation of split frequencies = 0.007467 (BI).

The phylogeny (Fig. 1) inferred from ITS + nLSU sequences demonstrated seven major clades for 65 species of the Polyporales. The new genus *Leifiporia* clustered in the core polyporoid clade and was closely related to *Diplomitoporus overholtsii* (Pilát) Gilb. & Ryvarden and *Lopharia cinerascens* (Schwein.) G. Cunn, and then grouped with *Pycnoporus* P. Karst. and *Trametes* Fr. with a high support (98 % BS, 87 % BP, 0.98 BPP).

The nLSU dataset included sequences from 35 fungal specimens representing 32 species. The dataset had an aligned length of 1370 characters, of which 1129 characters are constant, 76 are variable and parsimony-uninformative, and 165 are parsimony-informative. Maximum parsimony analysis yielded 12 equally parsimonious trees (TL = 693, CI = 0.414, HI = 0.586, RI = 0.418, RC = 0.173). The best model for the 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 analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.002783 (BI).

A further phylogeny (Fig. 2) inferred from nLSU sequences was obtained for more representative taxa in the Polyporaceae and showed that the new genus formed a monophyletic lineage and grouped with the related species *Diplomitoporus overholtsii* and *Lopharia cinerascens* with strong support (100 % BS, 100 % BP, 1.00 BPP).

Taxonomy

Leifiporia Y.C. Dai, F. Wu & C.L. Zhao, gen. nov.

MycoBank no.: MB 817362

Differs from other genera by its resupinate, brittle basidiocarps with poroid surface, a dimitic hyphal system with clamp connections on generative hyphae which are frequently branched at right angles, skeletal hyphae present in the subiculum only, distinctly thinner than generative hyphae, IKI–, CB–, and basidiospores ellipsoid, hyaline, thin-walled, smooth, usually bearing one or two guttules, IKI–, CB–.

Type species. Leifiporia rhizomorpha Y.C. Dai, F. Wu & C.L. Zhao.

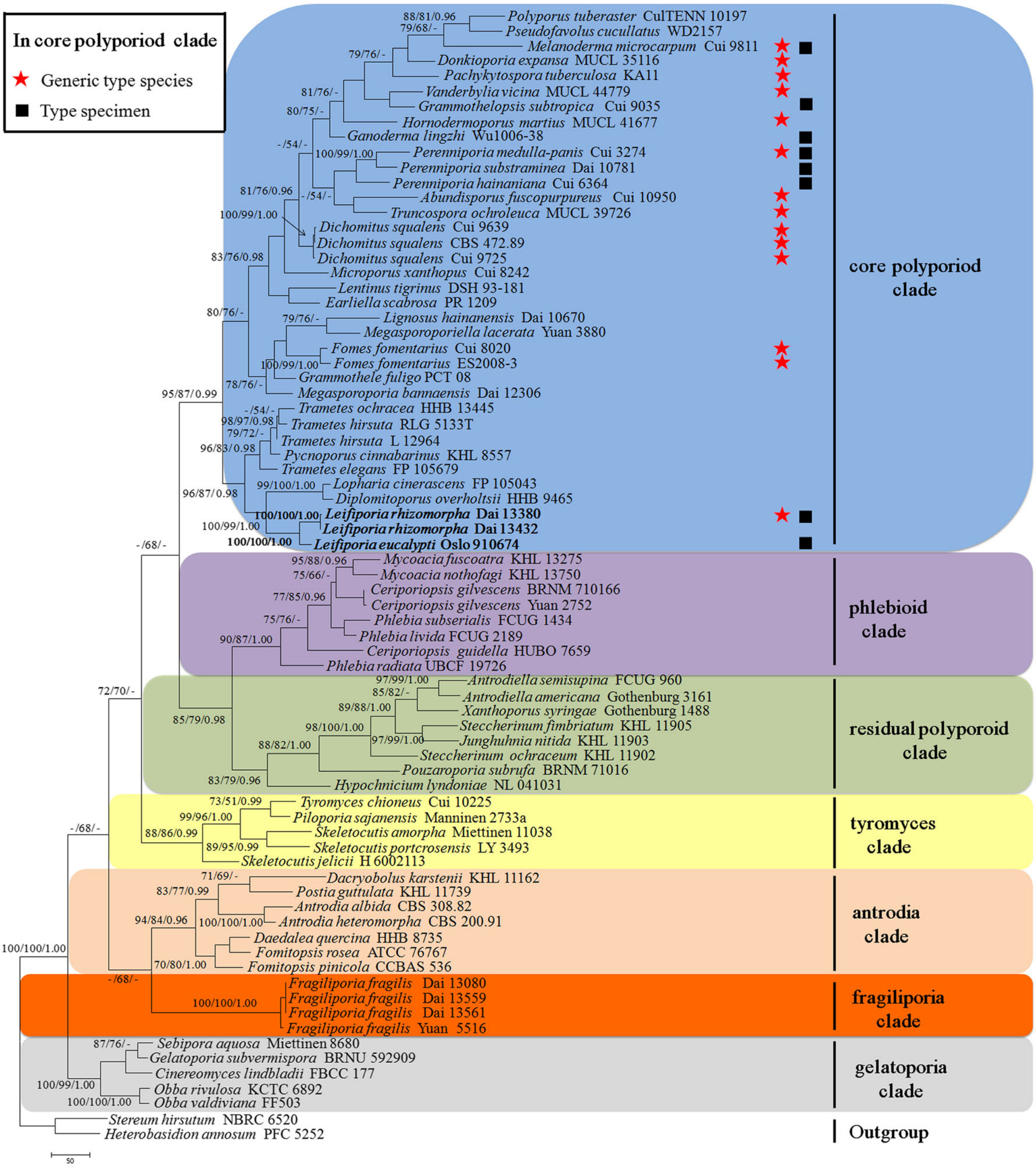

Fig. 1 Maximum Parsimony strict consensus tree illustrating the phylogeny of *Leifiporia* and related species in Polyporales based on ITS + nLSU sequences. *Branches* are labeled with maximum likelihood bootstrap higher than 70 %, parsimony bootstrap proportions higher than 50 % and Bayesian posterior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

Etymology. Leifiporia (Lat.): in honour of the Norway mycologist Prof. Leif Ryvarden.

Basidiocarps annual, resupinate, adnate, soft when fresh, brittle when dry. Pore surface white to cream. Pores angular; dissepiments thin, entire to slightly lacerate. Hyphal system dimitic, generative hyphae hyaline, thin-walled with clamp connections, frequently branched at right angles; skeletal hyphae present in subiculum only, interwoven, distinctly thinner than generative hyphae, IKI–, CB–; tissues unchanged in KOH. Basidia barrel-shaped to pyriform. Basidiospores ellipsoid, hyaline, thin-walled, smooth, usually bearing one or two guttules, IKI–, CB–.

Leifiporia rhizomorpha Y.C. Dai, F. Wu & C.L. Zhao, sp. nov. Figs. 3a-b and 4

MycoBank no.: MB 817363

Holotype. **CHINA**. Anhui Prov., Qimen County, Guniujiang Nature Reserve, on fallen angiosperm trunk, 9 August 2013, *Dai 13380* (BJFC).

Etymology. Rhizomorpha (Lat.): referring to the species bearing a rhizomorphic margin.

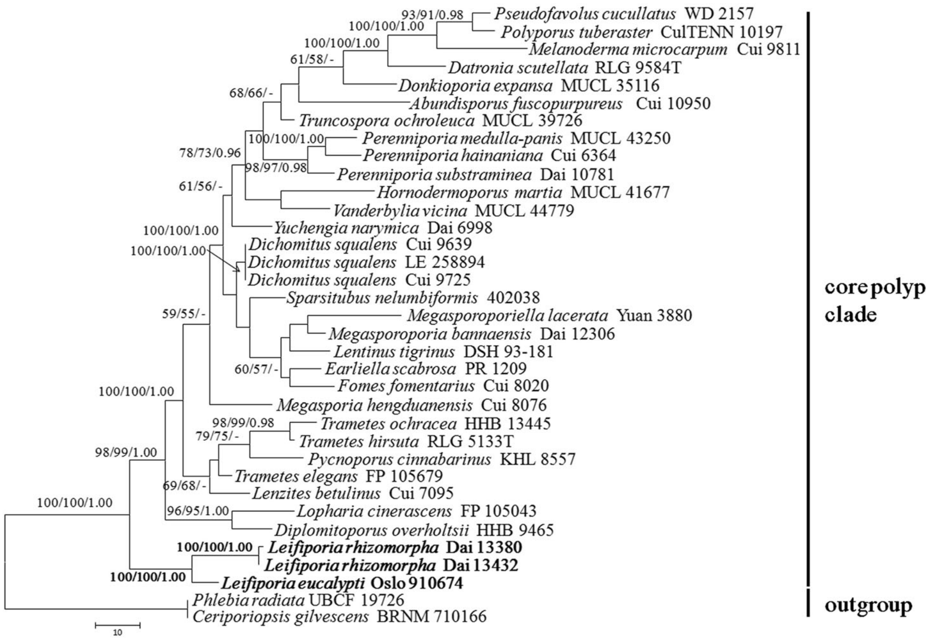

Fig. 2 Maximum Parsimony strict consensus tree illustrating the phylogeny of *Leifiporia* and related species obtained for more representative taxa in the Polyporaceae, based on the nLSU sequence datasets. *Branches* are labeled with maximum likelihood bootstrap higher than 70 %, parsimony bootstrap proportions higher than 50 % and Bayesian posterior probabilities more than 0.95 respectively. Clade names follow Binder et al. (2013)

Fruiting body. Basidiocarps annual, resupinate, soft, without odor or taste when fresh, brittle when dry, up to 20 cm long, 4 cm wide, 1.5 mm thick at center (measured in two hitherto available specimens). Pore surface white to cream when fresh, cream when dry; pores angular, 3–4 per mm; dissepiments thin, entire to slightly lacerate. Sterile margin distinctly rhizomorphic, white, up to 4 mm wide. Subiculum white, soft corky, up to 0.2 mm thick. Tubes concolorous with pore surface, brittle, up to 1.3 mm long.

Hyphal structure. Hyphal system dimitic; generative hyphae hyaline, thin-walled, bearing clamp connections; skeletal hyphae present in the subiculum only, IKI–, CB–; tissues unchanged in KOH.

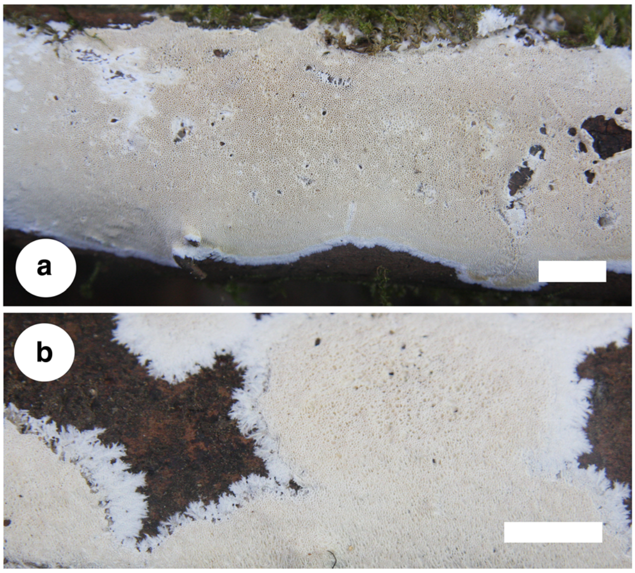

Fig. 3 Basidiomata of *Leifiporia rhizomorpha*. *Bars*: (**A**, **B**) 1 cm (holotype)

Subiculum. Generative hyphae dominant, hyaline, thin-walled, frequently branched at right angles, interwoven, 2.5–4 μm in diameter; skeletal hyphae infrequent, hyaline, thick-walled with a narrow lumen or subsolid, occasionally branched, interwoven, distinctly thinner than generative hyphae, 1.5–2.5 μm in diameter.

Tubes. Generative hyphae hyaline, thin-walled, frequently branched at right angle, interwoven, 2–3.5 μm in diameter. Cystidia absent, but fusoid cystidioles occasionally present, hyaline, thin-walled, 11–13 × 5–6 μm; basidia barrel-shaped to pyriform, with four sterigmata and a basal clamp connection, 14–17 × 5.5–8 μm; basidioles dominant, in shape similar to basidia, but slightly smaller.

Spores. Basidiospores mostly ellipsoid, hyaline, thin-walled, smooth, usually bearing one or two guttules, IKI–, CB–, (4.5–)5–6(−6.5) × 2–3 μm, L = 5.68 μm, W = 2.51 μm, Q = 2.15–2.38 (*n* = 60/2).

Type of rot. White rot.

Additional specimen (paratype) examined: **CHINA**. Zhejiang Prov., Qingyun County, Baishanzu Nature Reserve, on fallen angiosperm branch, 14 August 2013, *Dai 13432* (BJFC).

Leifiporia eucalypti (Ryvarden) Y.C. Dai, F. Wu & C.L. Zhao, comb. nov. Fig. 5

MycoBank no.: MB 817364

Basionym: *Dichomitus eucalypti* Ryvarden, Trans Br Mycol Soc 85: 539 (1985).

Fruiting body. Basidiocarps annual, resupinate, brittle when dry, up to 2 cm long, 1 cm wide, 1.5 mm thick at center. Pore surface white to pale cream when dry; pores angular, 2–3 per mm; dissepiments thin, entire to slightly lacerate. Sterile margin narrow, white, up to 1 mm wide. Subiculum white, soft corky, up to 0.3 mm thick. Tubes concolorous with pore surface, brittle, up to 1.2 mm long.

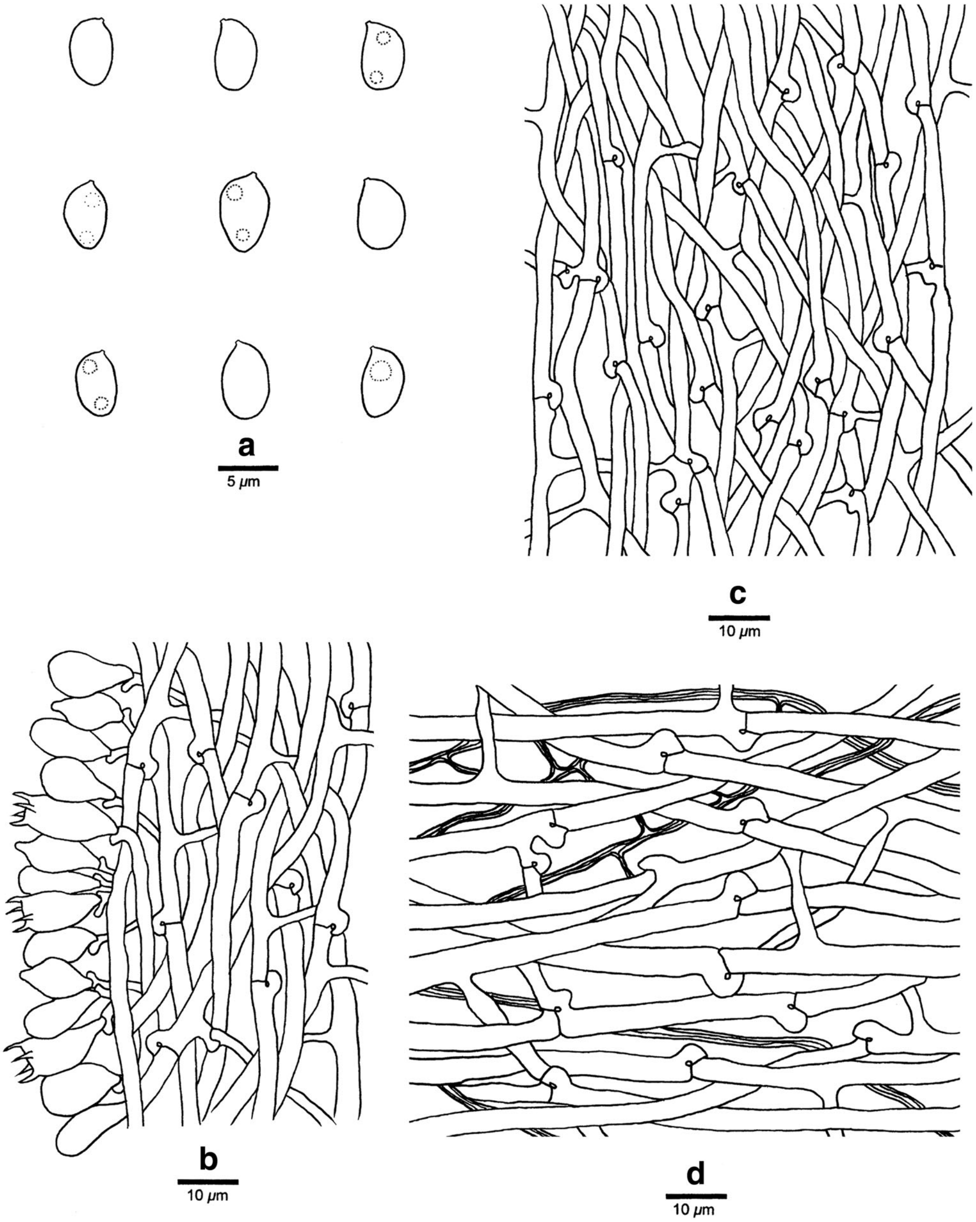

Fig. 4 Microscopic structures of *Leifiporia rhizomorpha* (drawn from the holotype). **a** Basidiospores; **b** section of hymenium; **c** hyphae from trama; **d** hyphae from subiculum. *Bars* (**a**) 5 µm; (**b**–**d**) 10 µm

Hyphal structure. Hyphal system dimitic; generative hyphae hyaline, thin-walled, bearing clamp connections; skeletal hyphae present in subiculum only, IKI–, CB–; tissues unchanged in KOH.

Subiculum. Generative hyphae dominant, hyaline, thin-walled, frequently branched mostly at right angle, interwoven, 2.5–4 µm in diameter; skeletal hyphae infrequent, hyaline, thick-walled with a narrow lumen to subsolid, frequently branched, interwoven, distinctly thinner than generative hyphae, 1.5–2.5 µm in diameter.

Tubes. Generative hyphae hyaline, thin-walled, frequently branched at right angle, interwoven, 2–3.5 µm in diameter. Cystidia absent, but fusoid cystidioles present, hyaline, thin-walled, 10–13 × 4–5.5 µm; basidia barrel-shaped to pyriform, with four sterigmata and a basal clamp connection, 15–20 × 5–9 µm; basidioles dominant, in shape similar to basidia, but slightly smaller.

Spores. Basidiospores ellipsoid, hyaline, thin-walled, smooth, usually bearing one guttule, IKI–, CB–, (6.5–) 7–8(−8.5) × (3–)3.5–4.5(−5) µm, L = 7.67 µm, W = 3.95 µm, Q = 1.94 (*n* = 30/1).

Type of rot. White rot.

Specimen (isotype) examined: **AUSTRALIA**, Northern Territory, Stokes Creek, George Gill Ranges, on *Eucalyptus camaldulensis*, 10 August 1981, leg. A. C. Kalotas, 910674 (O).

Discussion

In the present study, a new genus, *Leifiporia*, was described, based on phylogenetic analyses and morphological characters. The

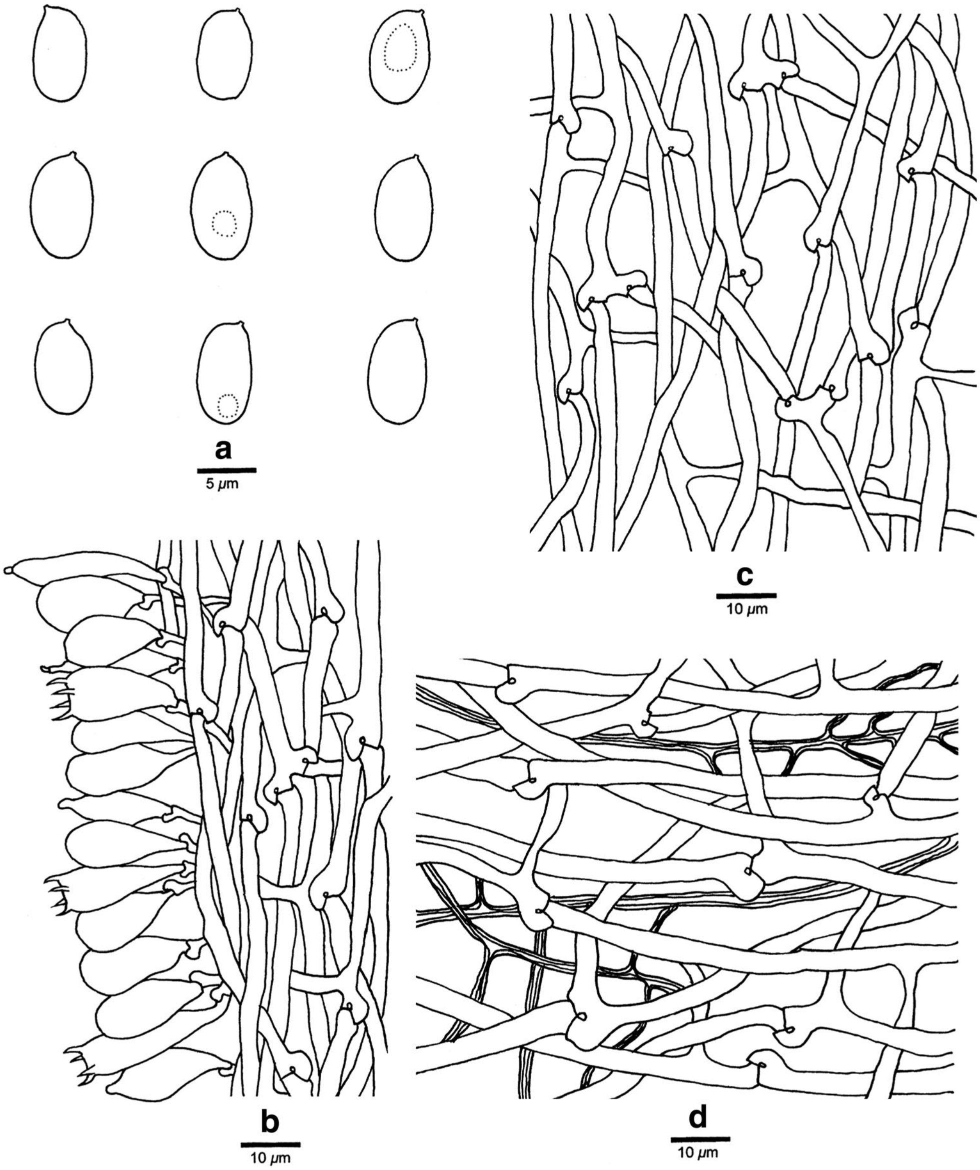

Fig. 5 Microscopic structures of *Leifiporia eucalypti* (drawn from the isotype). **a** Basidiospores; **b** section of hymenium; **c** hyphae from trama; **d** hyphae from subiculum. *Bars* (**a**) 5 μm; (**b**–**d**) 10 μm

genus had unique morphological characters in Polyporaceae and formed a monophyletic lineage within core polyporoid clade.

Previously, seven clades were found in the Polyporales: antrodia clade, core polyporoid clade, fragiliporia clade, gelatoporia clade, phlebioid clade, residual polyporoid clade and tyromyces clade (Binder et al. 2013; Zhao et al. 2015). According to our results based on the combined ITS + nLSU sequence data (Fig. 1), the new genus is nested in the core polyporoid clade with strong support (100 % BS, 99 % BP, 1.00 BPP). In the nLSU analysis, *Leifiporia* grouped with *Diplomitoporus overholtsii* and *Lopharia cinerascens* based on phylogenetic analysis with high support (Fig. 1), which was mentioned in a previous study by Li and Cui (2013).

The species *Dichomitus eucalypti* Ryvarden was described by Ryvarden based on resupinate, brittle basidiocarps and a dimitic hyphal system with clamp connections (Ryvarden 1985). However, it grouped with *Pycnoporus* and *Trametes* in ITS analysis, and clustered with *Diplomitoporus overholtsii* and *Lopharia cinerascens* in nLSU analysis (Li and Cui 2013). In the present study, *Dichomitus eucalypti* was the sister species to *L. rhizomorpha*, and not related to the generic type of *Dichomitus* D.A. Reid, *D. squalens* (P. Karst.) D.A. Reid, which was mentioned by Li and Cui (2013). Binder et al. (2013) employed multi-gene datasets to investigate the phylogenetic overview of the Polyporales and showed that the type species of *Dichomitus*, *D. squalens*, was the sister species to *Sparsitubus nelumbiformis* L.W. Hsu & J.D. Zhao and then grouped with *Perenniporia* Murrill species. According to our inference with LSU sequences (Fig. 2), *D. squalens* also grouped with *S. nelumbiformis* and then was closely related to some species of *Perenniporia* s.l.; a similar result was indicated by Binder et al. (2013). Thus, the new combination, *Leifiporia eucalypti*, is proposed.

Morphologically, *Dichomitus*, *Diplomitoporus* Domański, *Lopharia* Kalchbr. & MacOwan, *Pycnoporus* and *Trametes* are different from *Leifiporia* (see the key to these genera, below). *Dichomitus* is separated from the new genus by hard and woody basidiocarps, and the presence of arboriform skeletal hyphae, which are occasionally cyanophilous and dominated in trama and context (Gilbertson and Ryvarden 1986; Núñez and Ryvarden 2001; Yuan 2013; Ryvarden and Melo 2014). *Diplomitoporus* differs from *Leifiporia* by its corky basidiocarps, weakly amyloid skeletal hyphae dominated in whole basidiocarps, and allantoid basidiospores (Ryvarden and Melo 2014). *Lopharia* differs from *Leifiporia* by a smooth or hydnoid hymenophore and the presence of thick-walled cystidia, cyanophilous skeletal hyphae and large basidiospores (10–16 × 6–7.5 μm; Hjortstam and Ryvarden 1989). *Pycnoporus* differs in pileate, hard corky and reddish basidiocarps and a trimitic hyphal system (Núñez and Ryvarden 2001). *Trametes* differs from *Leifiporia* by its pileate, corky basidiocarps and a trimitic hyphal system (Ryvarden and Melo 2014).

Fibroporia Parmasto is similar to *Leifiporia* in having infrequent skeletal hyphae in the subiculum only, but it has slightly thick-walled basidiospores, and causes a brown rot (Ryvarden and Melo 2014).

Having resupinate basidiocarps with brittle consistency is similar to two similar genera in the Polyporales: *Fragiliporia* Y.C. Dai, B.K. Cui & C.L. Zhao and *Physisporinus* P. Karst. The former differs from *Leifiporia* by a completely monomitic hyphal system with thick-walled generative hyphae and allantoid basidiospores (Zhao et al. 2015), while the latter is separated from *Leifiporia* by its ceraceous basidiocarps which change to red when bruised, a completely monomitic hyphal system with simple septa and globose basidiospores (Gilbertson and Ryvarden 1987; Núñez and Ryvarden 2001; Ryvarden and Melo 2014). In addition, the three genera were nested in different clades based on previous phylogenetic analyses (Binder et al. 2013).

Polypores are an extensively studied group of Basidiomycota (Gilbertson and Ryvarden 1987; Núñez and Ryvarden 2001; Ryvarden and Melo 2014), but the Chinese polypore diversity is still not well known, especially in the subtropics and tropics; many recently described genera of polypores have been from these areas (Cui 2013; Li et al. 2013, 2014; Chen et al. 2014, 2016; Zhao et al. 2015). The new genus in the present study, *Leifiporia*, is also from the subtropics. It is possible that new polypore taxa will be found after futher investigations and molecular analyses.

A key to *Leifiporia* and related genera

1 Hyphal structure trimitic, binding hyphae present 2
1* Hyphal structure dimitic, binding hyphae absent 3
2 Basidiocarps cinnabar red *Pycnoporus*
2* Basidiocarps white, gray, pale *Trametes*
3 Hymenophore smooth to irregularly irpicoid; cystidia present *Lopharia*
3* Hymenophore poroid; cystidia absent 4
4 Basidiocarps resupinate to pileate; arboriform skeletal hyphae present *Dichomitus*
4* Basidiocarps resupinate; arboriform skeletal hyphae absent 5
5 Hyphal structure monomitic· 6
5* Hyphal structure dimitic at least in subiculum 7
6 Clamp connection present *Fragiliporia*
6* Simple septa present *Physisporinus*
7 Skeletal hyphae present in both trama and subiculum, basidiospores allantoid *Diplomitoporus*
7* Skeletal hyphae present in subiculum only, basidiospores ellipsoid 8
8 Basidiospores thin-walled, skeletal hyphae thinner than generative hyphae *Leifiporia*
8* Basidiospores thick-walled, skeletal hyphae thicker than generative hyphae *Fibroporia*

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