



***Radulotubus resupinatus* gen. et sp. nov. with a poroid hymenophore in Pterulaceae (Agaricales, Basidiomycota)**

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With 5 figures and 1 table

Abstract: A new poroid wood-inhabiting fungal genus, *Radulotubus*, is proposed to accommodate the type species *R. resupinatus* sp. nov. based on morphological and molecular evidence. The genus is characterized by an annual and resupinate basidiocarps, white to cream pores when fresh, darkening after bruising and drying, a monomitic hyphal system with clamped generative hyphae, pleural basidia, and globose, hyaline, thin- to slightly thick-walled, smooth, basidiospores without reactions in Melzer's reagent and Cotton Blue. The phylogenetic analyses based on ITS and nLSU sequences showed that *Radulotubus* belonged to the Pterulaceae clade in Agaricales, and was closely related to *Aphanobasidium* and *Radulomyces* that included species with smooth to hydroid hymenophore. *Radulotubus* is the sole genus with poroid hymenophore in Pterulaceae.

Key words: *Aphanobasidium*, phylogenetic analysis, *Radulomyces*, taxonomy, wood-rotting fungi.

Introduction

The Agaricales is the largest clade of mushroom-forming Agaricomycetes including 13, 233 described species level taxa in 413 genera and 33 families (Kirk et al. 2008). Pterulaceae Corner, typified by *Pterula* Fr., was established by Corner (1970), and the family is characterized by resupinate to effused or coralliform basidiocarps, a monomitic to dimittic hyphal structure, presence of pleural basidia in some genera, and globose, ellipsoid or fusiform, hyaline, thin- to slightly thick-walled, smooth, inamyloid, indextrinoid, acyanophilous basidiospores (Donk 1964, Corner 1970, Oberwinkler 1977, Bernicchia & Gorjon 2010). Species of Pterulaceae are of ecological and economic importance, for some are key players in the carbon cycle (Floudas et al. 2012), and some have potential application in biomedical engineering and biodegradation (Lang et al. 2006, Floudas et al. 2015).

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Recently, molecular studies involving Pterulaceae based on single- or multi-gene datasets have been carried out, grouping the family with Clavariaceae Chevall., Lachnellaceae Boud. and Stephanosporaceae Oberw. & E.Horak phylogenetically nested in Agaricales. Pterulaceae includes the type genus of *Pterula* and several other corticioid or clavarioid genera, such as *Aphanobasidium* Jülich, *Coronicium* J.Erikss. & Ryvardeen, *Merulicium* J. Erikss. & Ryvardeen, *Pterula* Fr. and *Radulomyces* M.P.Christ. (Larsson et al. 2004, Matheny et al. 2006, Hibbett et al. 2007, Larsson 2007, Oberwinkler 2012). Based on ITS and nLSU sequences, Larsson et al. (2004) showed that *Coronicium alboglaucum* (Bourdot & Galzin) Jülich, *Radulomyces confluens* (Fr.) M.P.Christ., *R. molaris* (Chaillet ex Fr.) M.P.Christ. and *R. rickii* (Bres.) M.P.Christ. were nested within the euagarics clade. Further studies employing a five-gene datasets have helped to confirm the close relationship of these corticioid species with *Pterula echo* D.J.McLaughlin & E.G.McLaughlin in Pterulaceae (Matheny et al. 2006). Later, *Aphanobasidium pseudotsugae* (Burt) Boidin & Gilles and *Merulicium fusisporum* (Romell) J. Erikss. & Ryvardeen were added to the Pterulaceae clade (Larsson 2007). Oberwinkler (2012) studied the evolutionary trends in basidiomycota and proposed that evolution was not a single one, but occurred several times convergently, that molecular techniques took over rapidly and were dominating now than traditionally comparative morphology.

The diversity of the corticioid fungi in China is not well known, though more than 500 species have been reported, including some new species described in the country (Dai & Li 2010, Dai 2011, He & Dai 2012, He & Li 2013, Zhou & Dai 2012, 2013). Recently, several wood-inhabiting fungal specimens collected from southern tropical China with strictly resupinate basidiocarps and poroid hymenophore, were re-studied and sequenced. Morphologically, they did not fit any of the known poroid genera. To confirm the affinity of this taxon, phylogenetic analyses were carried out based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences.

Materials and methods

The studied specimens are deposited at the herbarium of Beijing Forestry University (BJFC). 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 Zhao et al. (2013). The following abbreviations were used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB- = acyanophilous, 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.

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 primer pairs ITS5 and ITS4 (White et al. 1990). Nuclear LSU region was amplified with primer pairs LR0R and LR7 (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>). The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 58°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 48°C 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR products were purified and directly sequenced at Beijing Genomics Institute. All newly generated sequences were deposited at GenBank (Table 1).

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

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
<i>Aphanobasidium pseudotsugae</i> (Burt) Boidin & Gilles	HHB-822	GU187509	GU187567	Larsson 2007
<i>A. pseudotsugae</i>	UC 2023143	KP814192	–	Larsson 2007
<i>A. pseudotsugae</i>	UC 2022905	KP814258	–	Larsson 2007
<i>A. pseudotsugae</i>	UC 2022912	KP814259	–	Larsson 2007
<i>A. pseudotsugae</i>	UC 2023153	KP814353	AY586696	Larsson 2007
<i>Athelidium aurantiacum</i> (M.P.Christ.) Oberw.	KHL 11068	EU118606	–	Larsson 2007
<i>Athelia pyriformis</i> (M.P.Christ.) Jülich	Hjm 18581	EU118605	–	Larsson 2007
<i>Chondrostereum purpureum</i> (Pers.) Pouzar	AFTOL-ID 441	DQ200929	–	Larsson 2007
<i>Clavaria fumosa</i> Pers.	MR 00170	JN214482	–	Larsson 2007
<i>Clavulinopsis helvola</i> (Pers.) Corner	EL 111/04	EU118617	–	Larsson 2007
<i>C. laeticolor</i> (Berk. & M.A.Curtis) R.H.Petersen	EL 8/00	EU118618	–	Larsson 2007
<i>Coniophora olivacea</i> (Fr.) P.Karst.	CFMR:FP-104386	GU187516	GU187572	Larsson 2007
<i>C. puteana</i> (Schumach.) P.Karst.	CFMR:FP-105438	KJ995933	–	Larsson 2007
<i>Coronicium alboglaucum</i> (Bourdot & Galzin) Jülich	NH 4208	–	AY586650	Matheny et al. 2006
<i>Cristinia helvetica</i> (Pers.) Parmasto	Kristiansen s.n.	EU118620	–	Larsson 2007
<i>Cystostereum murrayi</i> (Berk. & M.A.Curtis) Pouzar	NFLI 2000-86/2	EU118623	EU118623	Larsson 2007
<i>Cystidiodontia lamini-fera</i> Berk. & M.A.Curtis) Hjortstam	KHL 13057	EU118622	–	Larsson 2007
<i>Gloeostereum incarnatum</i> S.Ito & S.Imai	KUC20131022-28	KJ668540	–	Larsson 2007
<i>Lichenomphalia umbellifera</i> (L.) Redhead, Moncalvo & Vilgalys	Rova 2501	EU118645	–	Larsson 2007
<i>Lindtneria trachyspora</i> (Bourdot & Galzin) Pilát	KGN 390/00	EU118646	–	Larsson 2007
<i>Merulicium fusisporum</i> (Romell) J.Erikss. & Ryvarden	Hjm s.n.	EU118647	EU118647	Larsson 2007
<i>Pterula echo</i> D.J.McLaughlin & E.G.McLaughlin	AFTOL-ID 711	DQ494693	AY629315	Larsson 2007
<i>P. echo</i>	DJM 302	–	AY458123	Larsson 2007
<i>Radulomyces confluens</i> (Fr.) M.P.Christ.	Cui 5977	KU535661	KU535669	In the present study
<i>R. confluens</i>	He 2224	KU535662	KU535670	In the present study
<i>R. confluens</i>	KHL 8792	AY463458	AY586704	Matheny et al. 2006

<i>R. copelandii</i> (Pat.) Hjortstam & Spooner	Dai 15061	KU535664	KU535672	In the present study
<i>R. copelandii</i>	Wu 9606-5	KU535663	KU535671	In the present study
<i>R. molaris</i> (Chaillet ex Fr.) M.P.Christ.	ML 0499	–	AY586705	Matheny et al. 2006
<i>Radulotubus resupinatus</i> Y.C.Dai, S.H.He & C.L.Zhao	Cui 8383	KU535660	KU535668	In the present study
<i>R. resupinatus</i>	Cui 8462	KU535657	KU535665	In the present study
<i>R. resupinatus</i>	Dai 15315	KU535658	KU535666	In the present study
<i>R. resupinatus</i>	Cui 8445	KU535659	KU535667	In the present study

^aNewly generated sequences for this study

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 18756). Sequences of *Coniophora olivacea* Sacc. and *C. puteana* (Schumach.) P.Karst. obtained from GenBank were used as outgroups to root tree in the ITS analyses (Larsson 2007). Sequences of *Athelidium aurantiacum* (M.P.Christ.) Oberw. and *Cristinia helvetica* (Pers.) Parmasto, obtained from GenBank, were used as outgroups to root trees in nLSU and ITS+nLSU analyses. Clade names follow Larsson (2007).

Maximum parsimony analyses were applied to the ITS, nLSU and ITS+nLSU datasets separately. Approaches to phylogenetic analyses followed Zhao et al. (2013), and the tree construction was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 on Abe through the Cipres Science Gateway (www.phylo.org; Miller et al. 2009). Branch support for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest2.3 (Posada & Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for each dataset 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 2 runs from random starting trees for 1 million generations (ITS, nLSU and 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) and maximum parsimony (MP) greater than or equal to 75%, and Bayesian posterior probabilities (BPP) greater than or equal to 0.95 were considered as significantly supported, respectively.

Results

The ITS dataset included sequences from 29 fungal samples representing 20 species. The dataset had an aligned length of 910 characters, of which 369 characters are constant, 124 are variable and parsimony-uninformative, and 417 are parsimony-

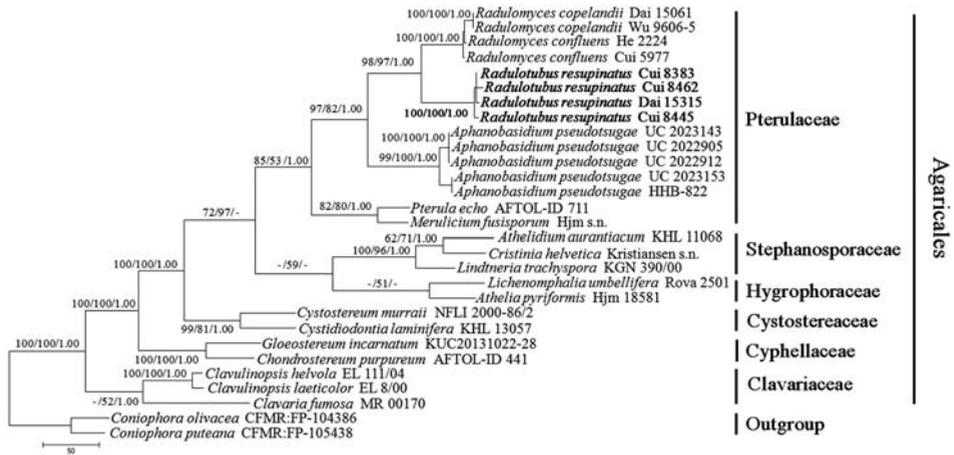


Fig. 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Radulotubus resupinatus* and related species in Agaricales based on ITS sequences datasets. Branches are labeled with maximum likelihood bootstrap (BS) higher than 70%, parsimony bootstrap proportions (BP) higher than 50% and Bayesian posterior probabilities (BPP) more than 0.95 respectively.

informative. Maximum parsimony analysis yielded 8 equally parsimonious trees (TL = 1824, CI = 0.342, RI = 0.623, RC = 0.170, HI = 0.452). Best model for the ITS dataset estimated and applied in the Bayesian analysis: 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 = 0.004478 (BI). The phylogeny (Fig. 1) inferred from ITS sequences demonstrated six major clades of the Agaricales. The new genus *Radulotubus* clustered into the Pterulaceae clade and was closely related to *Aphanobasidium* and *Radulomyces* with strong support (97% BS, 82% BP, 1.00 BPP).

The nLSU dataset included sequences from 18 fungal samples representing 10 species. The dataset had an aligned length of 1416 characters, of which 1195 characters are constant, 96 are variable and parsimony-uninformative, and 125 are parsimony-informative. Maximum parsimony analysis yielded 4 equally parsimonious trees (TL = 395, CI = 0.684, RI = 0.719, RC = 0.492, HI = 0.317). Best model for the nLSU dataset estimated and applied in the Bayesian analysis: 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 = 0.005738 (BI). The phylogeny (Fig. 2) inferred from nLSU sequences showed that the new genus formed a monophyletic lineage and grouped with related genera *Aphanobasidium* and *Radulomyces*, and then grouped with genera of *Coronicium*, *Merulicium* and *Pterula*.

The combined dataset (ITS+nLSU) included sequences from 19 fungal samples representing 10 species. The dataset had an aligned length of 2192 characters, of which 1627 characters are constant, 218 are variable and parsimony-uninformative,

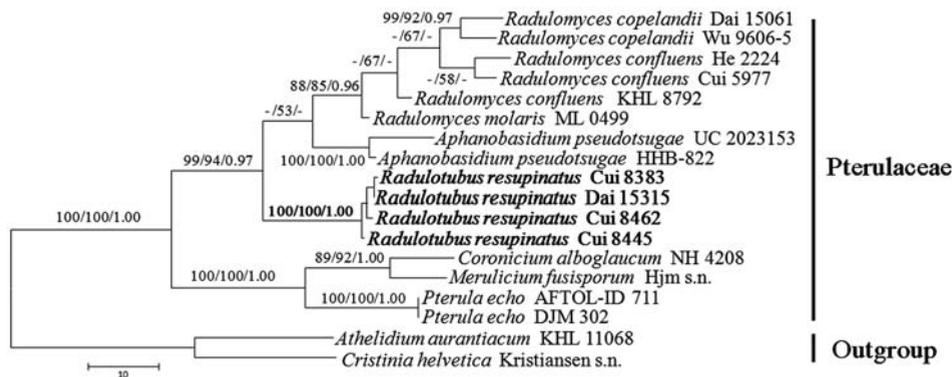


Fig. 2. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Radulotubus resupinatus* and related species in the Pterulaceae based on the nLSU sequence datasets. Branches are labeled with maximum likelihood bootstrap (BS) higher than 70%, parsimony bootstrap proportions (BP) higher than 50% and Bayesian posterior probabilities (BPP) more than 0.95 respectively.

and 347 are parsimony-informative. Maximum parsimony analysis yielded 2 equally parsimonious trees (TL = 1553, CI = 0.682, RI = 0.634, RC = 0.432, HI = 0.318). Best model for the combined ITS+nLSU estimated and applied in the Bayesian analysis: GTR+I+G, Iset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in the similar topology with an average standard deviation of split frequencies = 0.004006. The phylogeny (Fig. 3) inferred from the combined ITS+nLSU sequences dataset in the Pterulaceae demonstrated a similar topology with that of phylogenetic analyses of nLSU sequences.

Taxonomy

Radulotubus Y.C.Dai, S.H.He & C.L.Zhao, **gen. nov.**

Mycobank no.: MB 815760

DIAGNOSIS: Differs from other genera by a combination of resupinate basidiocarps with poroid surface, a monomitic hyphal system with clamp connections on generative hyphae, presence of pleural basidia, and hyaline, thin-to slightly thick-walled, smooth, IKI-, CB-, basidiospores.

TYPE SPECIES: *Radulotubus resupinatus*.

ETYMOLOGY: *Radulotubus* (Lat.): referring to scraped poroid hymenophore when bruised.

DESCRIPTION: Basidiocarps annual, resupinate, adnate, waxy to hygrophanous when fresh, becoming soft corky to slightly brittle when dry. Pore surface white to cream fresh pores, which become pale brown when bruised, and the bruised part becoming greyish brown to brown when dry. Hyphal system monomitic, generative hyphae

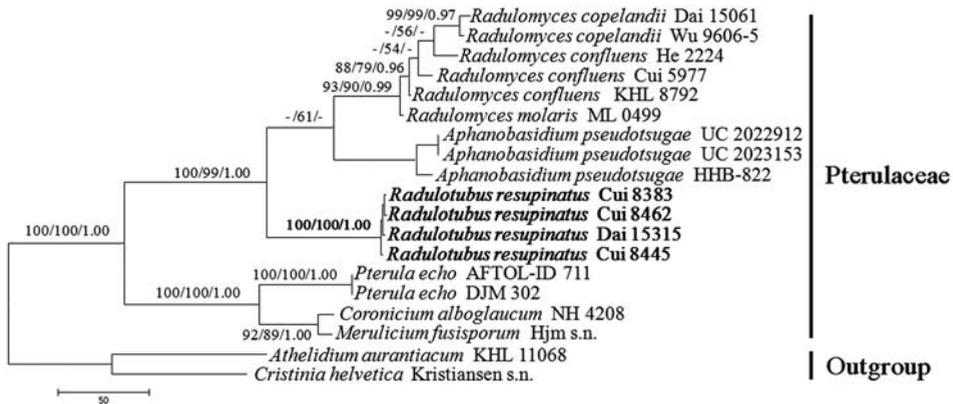


Fig. 3. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Radulotubus resupinatus* and related species in the Pterulaceae based on the combined ITS+nLSU sequence datasets. Branches are labeled with maximum likelihood bootstrap (BS) higher than 70%, parsimony bootstrap proportions (BP) higher than 50% and Bayesian posterior probabilities (BPP) more than 0.95 respectively.

hyaline, thin- to thick-walled with clamp connections, IKI-, CB-. Basidia pleural. Basidiospores hyaline, thin-to slightly thick-walled, globose, IKI-, CB-.

***Radulotubus resupinatus* Y.C.Dai, S.H.He & C.L.Zhao, sp. nov.**

Figs 4, 5

Mycobank no.: MB 815761

DIAGNOSIS: The species is distinct by its annual growth habit, poroid hymenophore with white surface, becoming pale brown to brown when bruised; a monomitic hyphal system with clamp connections, pleural basidia, and globose, hyaline, thin- to slightly thick-walled, smooth, IKI-, CB-, basidiospores.

HOLOTYPE: CHINA. Yunnan Prov., Mengla County, Lvshilin Forest Park, on fallen angiosperm trunk, 1 November 2009, Cui 8383 (BJFC).

ETYMOLOGY: *Resupinatus* (Lat.): referring to resupinate basidiocarps.

FRUITING BODY: Basidiocarps annual, resupinate, waxy to hygrophanous, without odor or taste when fresh, becoming soft corky to slightly brittle when dry, up to 8 cm long, 4 cm wide, 3 mm thick at center. Pore surface white when fresh, cream to buff when dry, the fresh pores becoming pale brown to brown when bruised, the bruised part becoming greyish brown to brown when dry; pores round to angular, 2–4 per mm; dissepiments thin, entire to lacerate. Sterile margin narrow, white to cream, slightly fimbriate, up to 1 mm wide. Subiculum cream, membranous, up to 0.5 mm thick. Tubes concolorous with pore surface, brittle, up to 2.5 mm long.

HYPHAL STRUCTURE: Hyphal system monomitic; generative hyphae hyaline, thin- to slightly thick-walled, bearing clamp connections, IKI-, CB-; tissues unchanged in KOH.

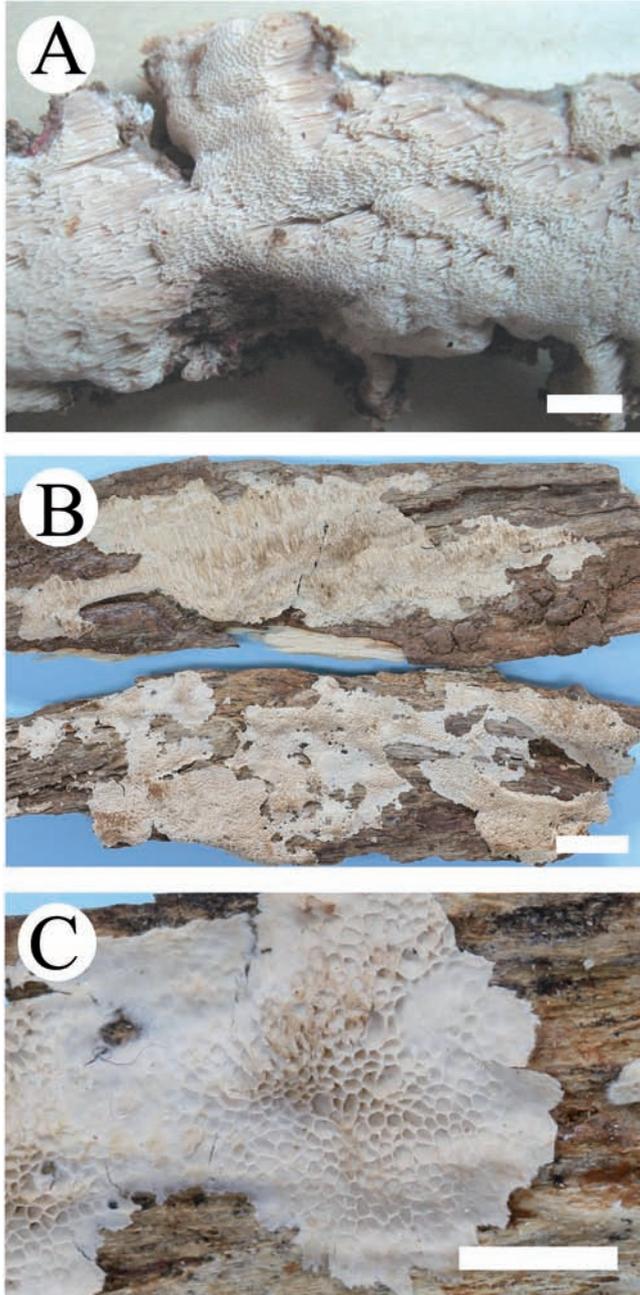


Fig. 4. Basidiocarps of *Radulotubus resupinatus* (A: Dai 15315; B & C: the holotype). Bars: A = 6 mm; B = 1 cm; C = 3 mm.

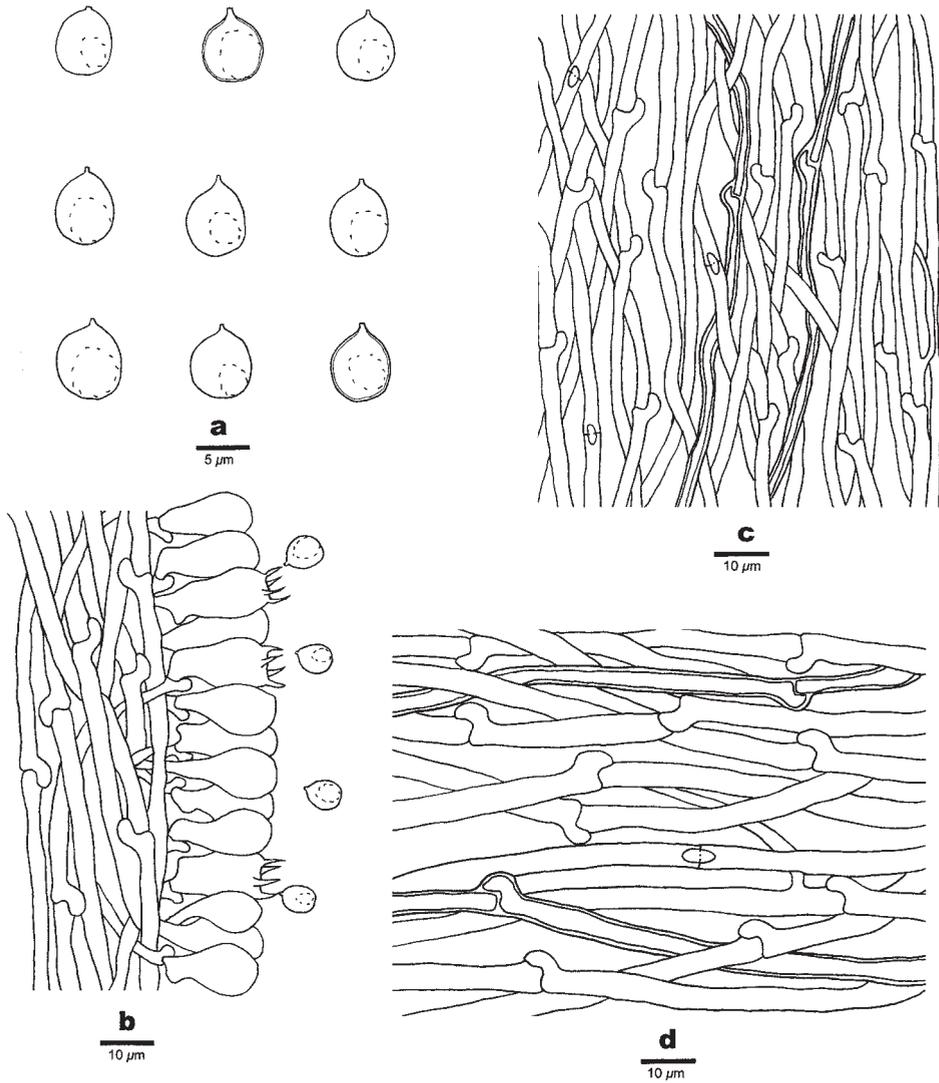


Fig. 5. Microscopic structures of *Radulotubus resupinatus* (drawn from the holotype). a. Basidiospores; b. A section of hymenium; c. Hyphae from trama; d. Hyphae from subiculum. Bars: A = 5 µm; B, C, D = 10 µm.

SUBICULUM: Generative hyphae hyaline, thin- to thick-walled, frequently with clamp connections, occasionally branched, more or less regularly arranged, 3–3.5 µm in diameter.

TUBES: Generative hyphae hyaline, thin- to thick-walled, frequently with clamp connections, occasionally branched, 2–3 μm in diameter. Cystidia and cystidioles absent; basidia distinctly pleural, pyriform to barrel-shaped, with four sterigmata and a basal clamp connection, 18–21 \times 8–10 μm ; basidioles dominant, in shape similar to basidia, but slightly smaller.

SPORES: Basidiospores globose, hyaline, thin-to slightly thick-walled, smooth, usually bearing one guttule and a distinct apiculus, IKI-, CB-, (5–)5.5–6(–6.5) \times (4.5–)5–5.5(–6) μm , L = 5.74 μm , W = 5.3 μm , Q = 1.01–1.09 (n = 120/4).

ROT TYPE: A white rot.

ADDITIONAL SPECIMENS (PARATYPES) EXAMINED: CHINA. Yunnan Prov., Mengla County, Lvshilin Forest Park, on fallen angiosperm trunk, 1 November 2009, Cui 8445 & Cui 8462 (BJFC); Guangxi Autonomous Region, Longchuan County, Nonggang Nature Reserve, 5 June 2015, Dai 15315 (BJFC).

Discussion

Larsson (2007) proposed a phylogenetic classification for corticioid fungi at the family level, in which *Coronicium* was as a sister to *Merulicium* and then grouped with *Aphanobasidium* in Pterulaceae on the basis of ITS+nLSU. But the present molecular phylogenetic analysis based on ITS+nLSU (Fig. 3) showed that *Coronicium* grouped with *Merulicium* and *Pterula echo*, and then clustered with *Aphanobasidium pseudotsugae*, *Radulomyces confluens* and *Radulotubus resupinatus*. Based on ITS sequences of Agaricales, *Radulotubus resupinatus* formed a distinct lineage in the Pterulaceae clade, and was closely related to *Aphanobasidium* and *Radulomyces* with strong support (97% BS, 82% BP, 1.00 BPP; Fig. 1). The phylogenetic analyses based on nLSU sequences of Pterulaceae yielded similar tree topology with ITS+nLSU datasets, and *Radulotubus resupinatus* also formed a distinct monophyletic lineage and clustered with *Aphanobasidium* and *Radulomyces* species with strong support in the phylogenetic trees (Figs 2, 3).

The type genus *Pterula* of Pterulaceae has become one of the largest among clavarioid fungi and surely most of them found in the tropics (Corner 1970). In present study, four sampled specimens were found in southern Yunnan Province and southwest of Guangxi Autonomous Region of China, between 20°09'–22°13'N and 101°05'–107°04'E, which are located in subtropics to tropics. The forests of our collections of *Radulotubus resupinatus* are subtropical to tropical vegetation, so *R. resupinatus* has more or less similar ecology as *Pterula*.

Kües and Navarro-González (2015) discussed the morphological aspects of fruiting bodies and their development in Agaricomycetes and other basidiomycetes, in which they concluded that: (1) genetic, physiological and environmental factors effecting morphological plasticity are addressed; (2) the shapes and features of wood-rotting fungi do not reveal close or distant phylogenetic relationships. Therefore, it is reasonable that basidiocarps with smooth, poroid, odontoid or grandinioid hymenophore grouped together in phylogenetic studies (Larsson et al. 2004, Matheny et al. 2006, Hibbett et al.

2007, Larsson 2007, Yurchenko & Wu 2013). In the present molecular analyses (Figs 1, 2, 3), the genus *Radulotubus* groups with *Aphanobasidium*, *Coronicium*, *Merulicium*, *Pterula* and *Radulomyces* inferred from the ITS, nLSU and ITS+nLSU analyses. However, morphologically the genus *Aphanobasidium* differs from *Radulotubus* by its smooth hymenophore (Jülich 1979, Bernicchia & Gorjon 2010). *Coronicium* is distinct from *Radulotubus* by smooth hymenophore, and presence of leptocystidia and ellipsoid to navicular basidiospores (Eriksson & Ryvarde 1975, Bernicchia & Gorjon 2010). *Merulicium* is separated from the new taxon by having merulioid hymenophore, a dimitic hyphal system and presence of leptocystidia and fusiform basidiospores (Eriksson & Ryvarde 1976, Bernicchia & Gorjon 2010). *Pterula* differs from *Radulotubus* by erect, branched to simple basidiomes, a dimitic hyphal system and ellipsoid to cylindrical basidiospores (Fries 1825, Senthilarasu 2013). *Radulomyces* differs from *Radulotubus* by its smooth, tuberculate, odontoid to hydroid hymenophore, and smooth or minutely ornamented basidiospores (Christiansen 1960, Bernicchia & Gorjon 2010). Although it is easy to distinct *Radulotubus* from all the other genera in Pterulaceae in macroscopy, they do have some similar characters in microscopy, for example, the globose spores in *Radulomyces* and pleural basidia in *Aphanobasidium*.

Morphologically, presence of resupinate basidiocarps and a monomitic hyphal system with generative hyphae bearing clamp connections reminds a few similar genera of polypores: *Ceriporiopsis* Domański, *Gelatoporia* Niemelä, *Obba* Miettinen & Rajchenb. and *Sebipora* Miettinen. *Ceriporiopsis* differs from *Radulotubus* by generative hyphae encrusted with pale-yellow crystals and frequently branched, and thin-walled, ellipsoid to subcylindrical basidiospores (Gilbertson & Ryvarde 1986, Núñez & Ryvarde 2001, Ryvarde & Melo 2014). *Gelatoporia* is separated from *Radulotubus* by its agglutinated generative hyphae which usually covered by crystals, and allantoid basidiospores (Miettinen & Rajchenberg 2012). *Obba* differs from *Radulotubus* by presence of a cartilaginous line on the subiculum with agglutinated generative hyphae, hyphal walls become swollen in KOH, and presence of coarse crystal rosettes in tramal structure (Miettinen & Rajchenberg 2012). *Sebipora* differs in its resupinate to pileate basidiocarps, generative hyphae encrusted with crystals and cylindrical basidiospores (Miettinen & Rajchenberg 2012).

Phlebiella P.Karst. and *Radulotubus* share the unique microscopic character of pleural basidia. However, *Phlebiella* differs from *Radulotubus* by having smooth hymenophore and warted basidiospores (Bernicchia & Gorjon 2010). Additionally, *Phlebiella* species are nested into the *Phlebiella* clade and was not close to Agaricales clade in the phylogeny presented by Larsson (2007).

In the present work, phylogenetic analysis uncovered six genera for eight species in Pterulaceae, but several other genera in this family, e.g. *Adustomyces* Jülich, *Allantula* Corner, *Chaetotyphula* Corner, *Deflexula* Corner, *Dimorphocystis* Corner, *Parapterulicium* Corner and *Pterulicium* Corner, lack molecular datasets. In order to fully resolve the phylogeny for Pterulaceae, evolutionary information from more conserved gene markers and type specimens of all genera in the family will likely be required.

A key to accepted genera of Pterulaceae

1. Basidiocarps resupinate to effused 2
1. Basidiocarps coralliform..... 6
2. Hymenophore poroid..... *Radulotubus*
2. Hymenophore smooth, tuberculate, odontoid to hydroid 3
3. Hyphal system dimitic, basidiospores fusiform *Merulicium*
3. Hyphal system monomitic, basidiospores globose, ellipsoid to navicular 4
4. Basidiospores thick-walled..... *Radulomyces*
4. Basidiospores thin-walled..... 5
5. Basidia pleural, leptocystidia absent..... *Aphanobasidium*
5. Basidia clavate to suburniform, leptocystidia present *Coronicium*
6. Gloeocystidia present, generative hyphae without clamp connections *Parapterulicium*
6. Gloeocystidia absent, generative hyphae with clamp connections 7
7. Basidiocarps simple or branched; cystidia dimorphous *Dimorphocystis*
7. Basidiocarps filiform to capitates; cystidia absent or simply conic-ventricose 8
8. Rhizomorphs present *Allantula*
8. Rhizomorphs absent 9
9. Basidiocarps decurved or inverted, basidiospores mostly > 12 µm in length..... *Deflexula*
9. Basidiocarps erect, basidiospores mostly < 12 µm in length..... 10
10. Presence of branched with resupinate corticioid patch *Pterulicium*
10. Absence of branched with resupinate corticioid patch..... *Pterula*

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References

- BERNICCHIA, A. & S.P. Gorjon 2010: Fungi Europaei 12: Corticiaceae I. – Edizioni Candusso, Lomazzo.
- CORNER, E.J.H. 1970: Supplement to a monograph of *Clavaria* and allied genera. – Beihefte Nova Hedwigia **33**: 1–299.
- CHRISTIANSEN, M.P. 1960: Danish resupinate fungi 2. Homobasidiomycetes. – Dansk Botanisk Arkiv **19**: 57–388.
- DAI, Y.C. 2011: A revised checklist of corticioid and hydroid fungi in China for 2010. – Mycoscience **52**: 69–79. doi:10.1007/S10267-010-0068-1
- DAI, Y.C. & H.J. LI 2010: Notes on *Hydnochaete* (Hymenochaetales) with a seta-less new species discovered in China. – Mycotaxon **111**: 481–487. doi:10.5248/111.481
- DONK, M.A. 1964: A conspectus of the families of Aphyllophorales. – Persoonia **3**: 199–324.
- ERIKSSON, J. & L. RYVARDEN 1975: The Corticiaceae of North Europe 3. – Fungiflora, Oslo.

- ERIKSSON, J. & L. RYVARDEN 1976: The Corticiaceae of North Europe 4. – Fungiflora, Oslo.
- FRIES, E.M. 1825: Systema orbis vegetabilis. – Typographia Academica, Lund.
- FELSENSTEIN, J. 1985: Confidence intervals on phylogenetics: an approach using bootstrap. – *Evolution* **39**: 783–791.
- FLOUDAS, D., M. BINDER, R. RILEY, K. BARRY, R.A. BLANCHETTE et al. 2012: The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. – *Science* **336**: 1715–1719. doi:10.1126/science.1221748
- FLOUDAS, D., B.W. HELD, R. RILEY, L.G. NAGY, G. KOEHLER et al. 2015: Evolution of novel wood decay mechanisms in Agaricales revealed by the genome sequences of *Fistulina hepatica* and *Cylindrobasidium torrendii*. – *Fungal Genet. Biol.* **376**: 78–92. doi:10.1016/j.fgb.2015.02.002
- GILBERTSON, R.L. & L. Ryvarden 1986: North American polypores 1. – Fungiflora, Oslo.
- HALL, T.A. 1999: Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – *Nucleic Acids Symp. Ser.* **41**: 95–98.
- HE, S.H. & Y.C. DAI 2012: Taxonomy and phylogeny of *Hymenochaete* and allied genera of Hymenochaetaceae (Basidiomycota) in China. – *Fungal Divers.* **56**: 77–93. doi:10.1007/s13225-012-0174-9
- HE, S.H. & H.J. LI 2013: *Veluticeps microspora* sp. nov. and *V. ambigua* new to Asia with a preliminary phylogenetic study on the genus. – *Mycol. Prog.* **12**: 367–374e. doi:10.1007/s11557-012-0842-x
- HIBBETT, D.S., M. BINDER, J.F. BISCHOFF, M. BLACKWELL, P.F. CANNON et al. 2007: A higher-level phylogenetic classification of the fungi. – *Mycol. Res.* **111**: 509–547. doi:10.1016/j.mycres.2007.03.004
- JÜLICH, W. 1979: Studies in resupinate Basidiomycetes 5. On some new taxa. – *Persoonia* **10**: 325–336.
- KATOH, K. & H. TOH 2008: Recent developments in the MAFFT multiple sequence alignment program. – *Briefings in Bioinformatics* **9**: 286–298.
- KIRK, P.M., P.F. CANNON, J.C. DAVID, D.W. MINTER & J.A. STALPERS 2008: Ainsworth and Bisby's dictionary of the fungi. 10th ed. – CAB International, Oxon.
- KÜES, U. & M. NAVARRO-GONZÁLEZ 2015: How do Agaricomycetes shape their fruiting bodies? 1. Morphological aspects of development. – *Fungal Biol. Rev.* **29**: 63–97. doi:10.1016/j.fbr.2015.05.001
- LANG, G., M.I. MITOVA, A.L.J. COLE, L.B. DIN & S. VIKINESWARY et al. 2006: Pterulamides I–VI, linear peptides from a Malaysian *Pterula* sp. – *J. Nat. Prod.* **69**: 1389–1393. doi:10.1021/np0600245
- LARSSON, K.-H. 2007: Re-thinking the classification of corticioid fungi. – *Mycol. Res.* **111**: 1040–1063. doi:10.1016/j.mycres.2007.08.001
- LARSSON, K.-H., E. LARSSON & U. KÖLJALG 2004: High phylogenetic diversity among corticioid homobasidiomycetes. – *Mycol. Res.* **108**: 983–1002. doi:10.1017/S0953756204000851
- MATHENY, P.B., J.M. CURTIS, V. HOFSTETTER, M.C. AIME & J.M. MONCALVO 2006: Major clades of Agaricales: a multilocus phylogenetic overview. – *Mycologia* **98**: 982–995. doi:10.3852/mycologia.98.6.982
- MIETTINEN, O. & M. RAJCHENBERG 2012: *Obba* and *Sebipora*, new polypore genera related to *Cinereomyces* and *Gelatoporia* (Polyporales, Basidiomycota). – *Mycol. Prog.* **11**: 131–147. doi:10.1007/s11557-010-0736-8

- MILLER, M.A., M.T. HOLDER, R. VOS, P.E. MIDFORD, T. LIEBOWITZ et al. 2009: The CIPRES Portals. CIPRES. URL: http://www.phylo.org/sub_sections/portal. 2009-08-04. (Archived by WebCite(r) at <http://www.webcitation.org/5imQlJeQa>)
- NÚÑEZ, M. & L. RYVARDEN 2001: East Asian polypores 2. – *Syn. Fung.* **14**: 165–522. – Fungiflora, Oslo.
- NYLANDER, J.A.A. 2004: MrModeltest v2. Program distributed by the author. – Evolutionary Biology Centre, Uppsala University.
- OBERWINKLER, F. 1977: Das neue System der Basidiomyceten. – In: FREY, W., HURKA, H., OBERWINKLER, F. (Eds.). *Beiträge zur Biologie der Niederen Pflanzen*. G. Fischer, pp. 59–105. Stuttgart, New York.
- OBERWINKLER, F. 2012: Evolutionary trends in Basidiomycota. – *Stafia* **96**: 45–104.
- PETERSEN, J.H. 1996: Farvekort. The Danish Mycological Society's colour-chart. Foreningentil Svampekundskabens Fremme, Greve.
- POSADA, D. & K.A. CRANDALL 1998: Modeltest: testing the model of DNA substitution. – *Bioinformatics* **14**: 817–818.
- RONQUIST, F. & J.P. HUELSENBECK 2003: MRBAYES 3: Bayesian phylogenetic inference under mixed models. – *Bioinformatics* **19**: 1572–1574. doi:10.1093/bioinformatics/btg180
- RYVARDEN, L. & I. MELO 2014: Poroid fungi of Europe. – *Syn. Fung.* **31**: 1–455. Fungiflora, Oslo.
- SENTHILARASU, G. 2013: Two interesting *Pterula* species from Maharashtra, India. – *Mycosphere* **4**: 766–771. doi:10.5943/mycosphere/4/4/13
- SWOFFORD, D.L. 2002: PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Massachusetts.
- WHITE, T.J., T. BRUNS, S. LEE & J. TAYLOR 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: INNIS, M.A., D.H.GELFAND, J.J. SNINSKY & T.J. WHITE (eds.): *PCR protocols: a guide to methods and applications*, pp. 315–322. Academic Press, San Diego.
- YURCHENKO, E. & S.H. WU 2013: Four new species of *Hyphodontia* (*Xylodons*.s. Hjortstam & Ryvarden, Basidiomycota) from Taiwan. – *Nova Hedwigia* **96**: 545–558. doi:10.1127/0029-5035/2013/0092
- ZHAO, C.L., B.K. CUI & Y.C. DAI 2013. New species and phylogeny of *Perenniporia* based on morphological and molecular characters. – *Fungal Divers.* **58**: 47–60. doi:10.1007/s13225-012-0177-6
- ZHOU, L.W. & Y.C. DAI 2012: Wood-inhabiting fungi in southern China 5. New species of *Theleporus* and *Grammothele* (Polyporales, Basidiomycota). – *Mycologia* **104**: 915–924. doi:10.3852/11-302
- ZHOU, L.W. & Y.C. DAI 2013: Taxonomy and phylogeny of hydroid Russulales: two new genera, three new species and two new combination species. – *Mycologia* **105**: 636–649. doi:10.3852/12-011.

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