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A new species of *Antrodia* (Basidiomycota, Polypores) from China

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Abstract

A new species, *Antrodia monomitica* sp. nov., is described and illustrated from China based on morphological characters and molecular evidence. It is characterized by producing annual, fragile and nodulose basidiomata, a monomitic hyphal system with clamp connections on generative hyphae, hyaline, thin-walled and fusiform to mango-shaped basidiospores (6–7.5 × 2.3–3 µm), and causing a typical brown rot. In phylogenetic analysis inferred from ITS and nLSU rDNA sequences, the new species forms a distinct lineage in the Antrodia s. l., and has a close relationship with *A. oleracea*.

Key words – Fomitopsidaceae – phylogenetic analysis – taxonomy – wood-decaying fungi

Introduction

Antrodia P. Karst., typified with *Polyporus serpens* Fr. (=*Antrodia albida* (Fr.) Donk (Donk 1960, Ryvarden 1991), is characterized by a resupinate to effused-reflexed growth habit, white or pale colour of the context, a dimitic hyphal system with clamp connections on generative hyphae, hyaline, thin-walled, cylindrical to very narrow ellipsoid basidiospores which are negative in Melzer's reagent and Cotton Blue, and causing a brown rot (Ryvarden & Melo 2014).

Antrodia is a highly heterogeneous genus which is closely related to *Fomitopsis* P. Karst., *Daedalea* Pers. and *Oligoporus* Bref (Bernicchia et al. 2012, Kim et al. 2003, Rajchenberg et al. 2011, Spirin et al. 2012, Yu et al. 2010). Recently studies have divided *Antrodia* sensu lato into three genera: *Antrodia* sensu stricto, *Fibroporia* Parmasto and *Amyloporia* Bondartsev & Singer (Bernicchia et al. 2012, Chen et al. 2015b, Chen & Cui 2016, Cui 2013, Cui & Dai 2013, Rajchenberg et al. 2011, Spirin et al. 2013, Yu et al. 2010), but there are still several unrelated lineages spread among other brown rot polypores (Han et al. 2016, Ortiz-Santana et al. 2013, Spirin et al. 2013, 2015, 2016).

A recent investigation of *Antrodia* has been carried out in China, two specimens are identified belonging to *Antrodia* s. l., but they are distinguished from any known species. Phylogenetic analysis was performed using the internal transcribed spacer (ITS) regions and nuclear large subunit (nLSU) ribosomal RNA gene regions. Combined with the morphological characters, a new species is described in this study.

Materials & Methods

Morphological studies

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The studied specimens are deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). The microscopic procedure follows Li et al. (2014) and Han et al. (2016). Color terms followed Petersen (1996). Spores were measured from sections cut from the tubes. 5% of measurements were excluded from each end of the range, and were given in parentheses. The following abbreviations were used: KOH = 5% potassium hydroxide; IKI = Melzer's reagent; IKI– = neither amyloid nor dextrinoid; CB = Cotton Blue; CB– = acyanophilous; L = mean spore length (arithmetic average of all spores); W = mean spore width (arithmetic average of all spores); Q = the ratios of L/W; n (a/b) = number of spores (a) measured from given number (b) of specimens.

Molecular study

A CTAB rapid plant genome extraction kit (Demeter Biotech Co., Ltd., Beijing, China) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions with some modifications (Chen et al. 2015a, 2016a). ITS4 and ITS5 were used as primers for ITS. LR0R and LR7 were used as primers for nLSU. The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles at 94 °C for 40 s, 54 °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 5 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 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 sequenced in Beijing Genomics Institute, China, with the same primers.

Phylogenetic analysis

To explore the phylogenetic position of the two newly sequenced specimens, their ITS + nLSU sequences were compiled to part of the ITS + nLSU dataset from Ortiz-Santana et al. (2013). In addition, sequences from Chen & Cui (2016) are also used in the current analysis. *Fibroporia albicans* B.K. Cui & Yuan Y. Chen and *F. vaillantii* (DC.) Parmasto were selected as outgroups.

The dataset was aligned by BioEdit (Hall 1999) and then manually adjusted with ClustalX (Thompson et al. 1997). Sequence alignments were deposited at TreeBase (http://www.treebase.org; submission ID 20524). Phylogenetic analysis was done as in Zhao et al. (2013, 2015) and Chen et al. (2016b). Maximum parsimony (MP) analysis was applied to the ITS + nLSU dataset. 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 1,000 random sequence additions. Max-trees were set to 5,000, 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.

RAxML v.7.2.8 was used to construct maximum likelihood (ML) trees with GTR+G+I model of site substitution including estimation of Gamma-distributed rate heterogeneity and a proportion of invariant sites (Stamatakis 2006). The branch support was evaluated with bootstrapping method of 1000 replicates (Hillis & Bull 1993).

MrModeltest 2.3 (Posada & Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for the dataset for Bayesian inference (BI). BI was calculated with MrBayes3.1.2 (Ronquist & Huelsenbeck 2003) with a general time reversible (GTR) model of DNA substitution and an invgamma distribution rate variation across sites. Four Markov chains were run for 2000,000 generations until the split deviation frequency value < 0.01, and sampled every 100th generations. The burn-in was set to discard the first 25 % of the trees. A majority rule consensus tree of all remaining trees was calculated.

Phylogenetic trees were visualized using Treeview (Page 1996). Branches that received bootstrap support for maximum likelihood (BS), maximum parsimony (MP) and Bayesian posterior

probabilities (BPP) greater than or equal to 75 % (BS and MP) 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	Specimen/Strain No.	Locality	GenBank no.	
			ITS	LSU
Amyloporia carbonica	DAOM-F-8281-T	Canada	KC585239	KC585061
Amyloporia carbonica	FP-105585-Sp	USA	KC585240	KC585062
Antrodia favescens	Vlasák 0412/4J	USA	KC543129	KC543129
Antrodia heteromorpha	Niemelä 2621	Canada	KC543149	KC543149
Antrodia heteromorpha	Dai 12755	USA	KP715306	KP715322
Antrodia hyalina	Spirin 2772	Russia	JQ700283	JQ700283
Antrodia infirma	Niemelä 7637	Finland	KC595894	KC595894
Antrodia infirma	Niemelä 7644	Finland	KC595895	KC595895
Antrodia juniperina	SRM-403-T	USA	KC585285	KC585109
Antrodia juniperina	FP-105489-Sp	USA	KC585282	KC585105
Antrodia leucaena	Pennanen 927	Finland	JQ700278	JQ700278
Antrodia leucaena	Dai 11398	China	KX958179	—
Antrodia macra	Eriksson 1967	Unknown	KR605810	KR605749
Antrodia macra	Hottola 2729	Finland	KC543135	KC543135
Antrodia malicola	Miettinen 10595.1	China	KC595896	KC595896
Antrodia malicola	Cui 7754	China	KX958180	KX958184
Antrodia mappa	Niemelä 2669	Canada	KC543130	KC543130
Antrodia mappa	Penttila 11756	Finland	KC543113	KC543113
Antrodia mellita	Spirin 3315	Russia	KC543140	KC543140
Antrodia minuta	Miettinen X1317	Russia	KC595900	KC595900
Antrodia minuta	Spirin 2680	Russia	KC595898	KC595898
Antrodia monomitica	Dai 10630	China	KY421732 [a]	KY421734 [a]
Antrodia monomitica	Dai 16894	China	KY421733 [a]	KY421735 [a]
Antrodia oleracea	HHB-5988-Sp	USA	KC585293	KC585117
Antrodia oleracea	Mad-4907	USA	KC585295	KC585119
Antrodia primaeva	Miettinen 177	Russia	JQ700272	JQ700272
Antrodia pulvinascens	Miettinen 7488	Finland	JQ700274	JQ700274
Antrodia ramentacea	Marstad 274-09	Norway	KC543138	—
Antrodia ramentacea	Dai 6118	China	KC951178	—
Antrodia serialiformis	Miettinen 14816	USA	JQ700290	—
Antrodia serialiformis	JK-2009a	USA	FJ788412	—
Antrodia serialis	Miettinen 12401	Finland	JQ700271	JQ700271
Antrodia serialis	Dai 7626	China	KR605812	KR605751
Antrodia serpens	Dai 7465	Luxemburg	KR605813	KR605752
Antrodia serpens	Miettinen X1370	Slovakia	KC543143	KC543143
Antrodia subserpens	Cui 8310	China	KP715310	KP715326
Antrodia subserpens	Dai 13233	China	KP715309	KP715325
Antrodia tanakae	Dai 11770	China	KR605815	KR605754
Antrodia tanakae	Yuan 1106	China	KP715313	KP715329
Antrodia tropica	Cui 6471	China	JQ837939	—
Antrodia tropica	Dai 13434	China	KX958181	KX958185
Antrodia variiformis	Spirin 8406_2	USA	KT995137	KT995159
Daedalea dickinsii	Yuan 2685	China	KP171201	KP171223

Daedalea dickinsii	Yuan 2707	China	KP171202	KP171224
Daedalea quercina	Dai 12152	Czech	KP171207	KP171229
Daedalea quercina	Dai 12659	Finland	KP171208	KP171230
Fibroporia albican	Cui 9464	China	KC456250	KR605758
Fibroporia vaillantii	FP-90877-R	USA	KC585345	KC585170
Fomitopsis pinicola	Cui 10312	China	KR605781	KR605720
Fomitopsis subtropica	Cui 10578	China	KR605787	KR605726
Postia fragilis	Vlasák 0610_8	Czech	JF950573	—
Postia fragilis	Cui 10088	China	KF699120	KJ684977
Postia lacteus	Miettinen X1378	Finland	KC595938	KC595938
Postia lacteus	Miettinen X1391	Finland	KC595939	KC595939
Postia placenta	Niemelä 7609 (GB)	Finland	JX109846	JX109846
Postia placenta	Miettinen X1351	Finland	KC595950	KC595950

a Sequences newly generated in this study.

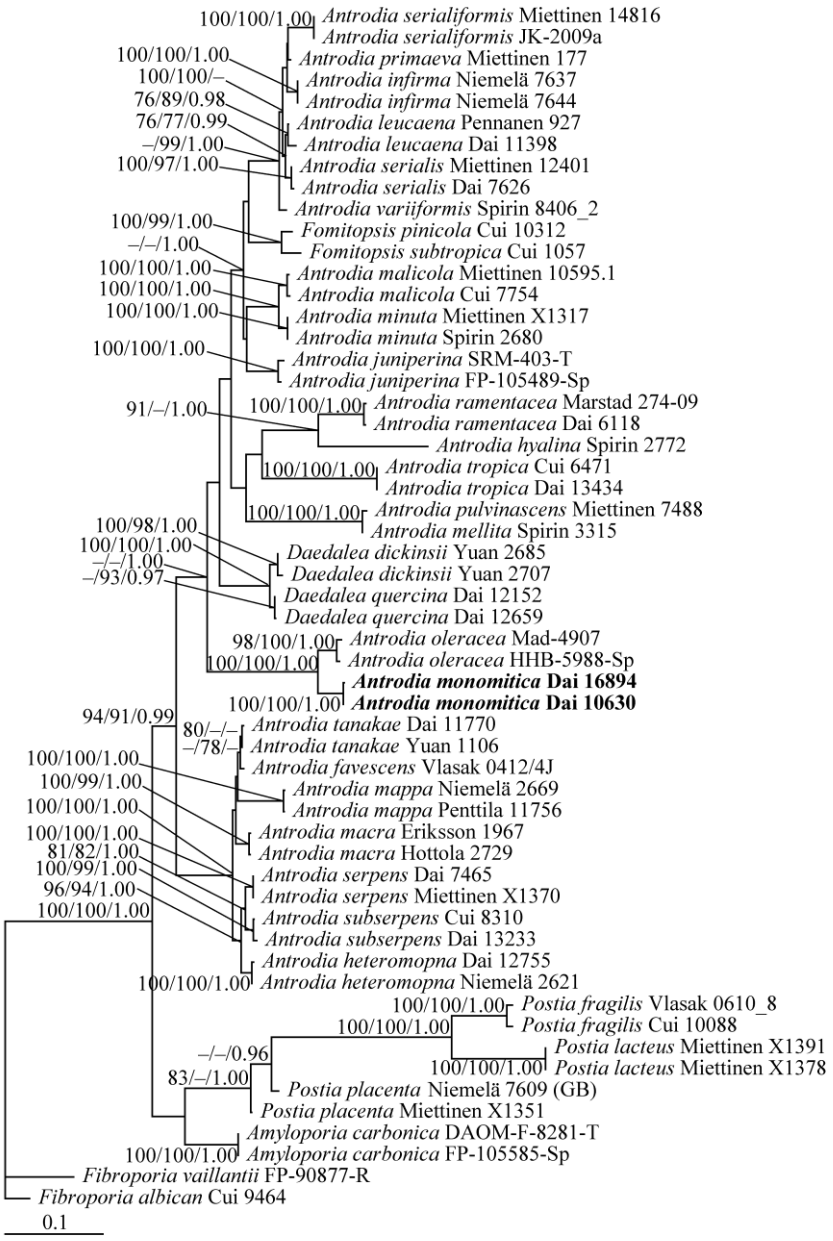

Fig. 1 – Phylogeny of *Antrodia* inferred from ITS + nLSU dataset.

Results

Phylogenetic analyses

Two ITS and two nLSU sequences were newly generated for this study and their accession numbers are available in GenBank (Table 1).

The ITS + nLSU dataset included 48 specimens resulted in an alignment of 1613 characters, of which, 1127 characters are constant, 72 are variable and parsimony-uninformative, and 414 are parsimony informative characters. Maximum parsimony analysis yielded only one tree (TL = 1299, CI = 0.580, RI = 0.819, RC = 0.475, HI = 0.420). "GTR+ I + G" was the best-fit evolutionary model in the Bayesian analysis. The ML search stopped after 1000 BS replicates. All chains in BI converged after 2 million generations. ML and BI analyses yielded similar tree topologies with an average standard deviation of split frequencies = 0.008918, and only the ML tree was provided. Both bootstrap values (≥75%) and BPPs (≥0.95) were showed at the nodes (Fig 1).

The phylogeny inferred from the ITS + nLSU dataset shows that the two newly sequenced specimens formed a terminal lineage within *Antrodia* s. l. with full statistical supports (100% BS, 1 BPP) and clustered with *A. oleracea* (R.W. Davidson & Lombard) Ryvarden.

Taxonomy

Antrodia monomitica Yuan Y. Chen, sp. nov. Figs 2–3

MycoBank number: MB 819955; Facesoffungi number: FoF 03384

Etymology – *Monomitica* (Lat.): referring to the monomitic hyphal structure of the species.

Known distribution –A rare species spread in subtropical and temperate regions of China.

Material examined – CHINA, Heilongjiang Prov., Harbin, the Campus of Harbin University of Science and Technology, on rotten wood of *Morus*, 6 Aug 2016, Y.C. Dai, Dai 16894 (BJFC 22529, holotype).

Fruiting body – Basidiomata annual, resupinate, nodulose without real cap, up to 10 cm long, 6 cm wide and 3 cm thick at centre; fleshy when fresh, chalky and fragile upon drying. Pore surface white to cream when fresh, cream, buff or cinnamon buff upon drying; sterile margin white, very narrow to almost lacking; pores round to angular, 3–4 per mm; dissepiments thin, entire to slightly lacerate. Subiculum white, fleshy when fresh, chalky to fragile upon drying, up to 2 cm thick. Tubes concolorous with the pore surface, chalky, up to 1 cm long.

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

Fig. 2 – Basidiomata of *Antrodia monomitica* (BJFC 22529). – Bars = 10 mm.

Subiculum – Generative hyphae hyaline, thin- to slightly thick-walled, frequently branched some bearing fine crystals, flexuous, interwoven, 3–8 µm in diam, some inflated up to 11 µm in diam.

Tubes – Generative hyphae hyaline, thin- to slightly thick-walled, occasionally branched, interwoven, 2–6 µm in diam. Cystidia absent; cystidioles present, fusoid, thin-walled, 12–15 × 3.5–4 µm. Basidia clavate, with four sterigmata and a basal clamp connection, 14–20 × 5–6.5 µm; basidioles in shape similar to basidia, but slightly smaller.

Spores – Basidiospores fusiform to mango-shaped, hyaline, thin-walled, smooth, sometimes with one guttule, IKI–, CB–, 6–7.5(–8) × 2.3–3 µm, L = 6.62 µm, W = 2.68 µm, Q = 2.44–2.51 (n = 60/2).

Additional specimen examined – CHINA, Jiangxi Prov., Nanchang, Forest Park of Jiangxi Academy of Forestry, on rotten angiosperm wood, 24 Sept 2008, Y.C. Dai, Dai 10630 (BJFC 004879, paratype).

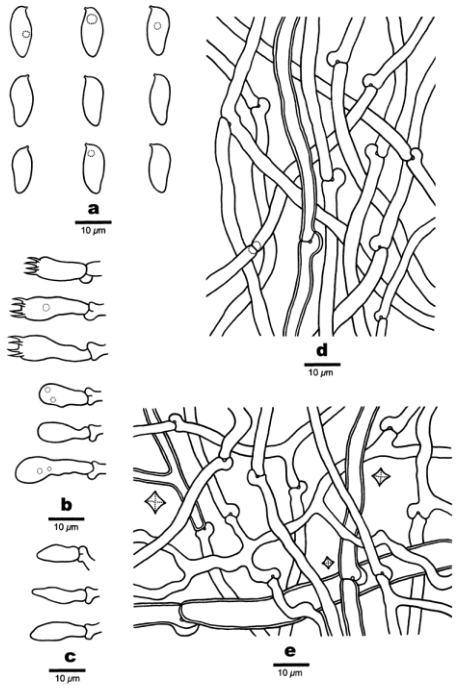

Fig. 3 – Microscopic structures of *Antrodia monomitica* (draw from the holotype). a: Basidiospores; b: Basidia and basidioles; c: Cystidioles; d: Hyphae from trama; e: Hyphae from subiculum. – Bars = 10 µm.

Discussion

Antrodia monomitica is characterized by annual, resupinate to nodulose basidiomata which becoming fragile upon drying, a monomitic hyphal structure with clamp connections on generative hyphae, hyaline, thin-walled and fusiform to mango-shaped basidiospores (6–7.5 × 2.3–3 µm), and causing a typical brown rot.

Morphologically, *Antrodia monomitica* is similar to species of *Postia* Fr. because of its annual fruiting bodies, a monomitic hyphal system with clamp connections on generative hyphae and causing a brown rot. However, *Postia* species have allantoid to cylindrical basidiospores, and they belong to different lineages in the phylogenetic analysis (Shen & Cui 2014, Shen et al. 2014, 2015).

Phylogenetic analysis based on ITS and nLSU regions indicated *Antrodia monomitica* belongs to *Antrodia* s. l. with full statistical supports and has a closer relationship with *A. oleracea*. However, *A. oleracea* is different in having much thinner basidiomata, a dimitic hyphal system and cylindrical to oblong ellipsoid basidiospores (Ryvarden & Johansen 1980).

Amyloporia carbonica (Overh.) Vampola & Pouzar and *Antrodia leucaena* Y.C. Dai & Niemelä have white basidiomata and similar pore size, and they somehow resemble *Antrodia*

monomitica in morphology, but *Amyloporia carbonica* has distinctly amyloid skeletal hyphae and wider cylindrical basidiospores (6–7.5 × 2.8–4 µm) and growing on dead conifer wood (Henrici & Ryvarden 1997, Ryvarden & Melo 2014); *Antrodia leucaena* is distinguished from *Antrodia monomitica* in having thinner basidiomata, a dimitic hyphal system and narrowly ellipsoid to subcylindrical basidiospores (Dai & Niemelä 2002, Ryvarden & Melo 2014). Besides, these three species clustered in different lineages in the phylogenetic analysis.

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