Three new species of Megasporia (Polyporales, Basidiomycota) from China

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Academic editor: C. Denchev | Received 15 January 2017 | Accepted 15 February 2017 | Published 7 March 2017


Abstract

Megasporia is a newly established polypore genus characterized by resupinate fruiting bodies with big pores, a dimitic hyphal structure with generative hyphae bearing clamp connections, more or less dextrinoid and cyanophilous skeletal hyphae, cylindrical, hyaline, thin-walled, smooth basidiospores, and growth mostly on fallen angiosperm branches. Species number is extremely rich in subtropical and tropical Asia. Three new species, namely M. rimosa, M. tropica and M. yunnanensis are described from China, and their illustrated descriptions are given. Differences between these new species and phylogenetically related and morphologically similar species are discussed. A key to the known species of Megasporia is provided.

Key words

Phylogeny, Polyporaceae, taxonomy, white-rot fungi

Introduction

Megasporia B.K. Cui et al. was recently derived from Megasporoporia Ryvarden & J.E. Wright, nested within the core polyporoid clade (Li and Cui 2013), both genera being members of Polyporaceae (Binder et al. 2005). Megasporia is morphologically similar to Megasporoporia, but they were phylogenetically distinct based on ITS and nLSU sequences (Li and Cui 2013). Megasporia is characterized by annual and resupinate basidiocarps, large pores, big basidiospores (mostly longer than 10 µm in length) which are mostly cylindrical, thin-walled, hyaline and negative in Cotton Blue and Melzer’s reagent, a dimitic hyphal system with clamped generative hyphae and more or less dextrinoid skeletal hyphae, the presence of tetrahedric or polyhedral crystals among
hymenial elements (Li and Cui 2013). The genus mainly grows on fallen or dead angiosperm branches which have not decayed much and causes a white rot (Li and Cui 2013). It used to be considered that only a few species were in *Megasporoporia* s.l. (Ryvarden et al. 1982), but the species diversity in the genus is very rich in subtropical and tropical Asia, with 12 species having been described from the region (Li and Cui 2013). In addition, four species, *Megasporoporia cavernulosa* (Berk.) Ryvarden, *M. hexagonoides* (Speg.) J.E. Wright & Rajchenb., *M. mexicana* Ryvarden and *M. setulosa* (Henn.) Rajchenb., have been found in subtropical and tropical America, but none has been recorded from Europe (Ryvarden et al. 1982, Ryvarden and Melo 2014).

During the study of polypores from southern China, six specimens collected on fallen angiosperm branches were examined, phylogenetic relationships were analyzed based on ITS and nLSU rDNA sequences data, and three new species of *Megasporia* were discovered. The aim of this work demonstrates the diversity of *Megasporia* in China. Illustrated descriptions of these species and a key to known species in the genus are provided in the present paper.

**Materials and methods**

**Morphological studies**

Specimens examined were deposited in the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Macro-morphological descriptions were based on field notes and herbarium specimens. Color terms follow Petersen (1996). Micro-morphological data were obtained from dried specimens, as observed under a light microscope. Sections were studied at a magnification of up to ×1000 using a Nikon E 80i microscope with phase contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic characters, measurements and drawings were made from slide preparations stained with Cotton Blue (CB) and Melzer’s reagent (IKI). Spores were measured from sections cut from the tubes. To represent variation in the size of spores, 5% of measurements were excluded from each end of the range, and are given in parentheses. The following abbreviations are used: KOH = 5% potassium hydroxide, IKI– = both non-amyloid and non-dextrinoid, CB– = acyanophilous, 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 a given number (b) of specimens.

**Molecular study and phylogenetic analysis**

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain total genomic DNA from dried specimens, according to the manufacturer’s instructions with some modifications (Chen et al. 2016). The DNA was
amplified with the primers: ITS5 and ITS4 for ITS (White et al. 1990), and LR0R and LR7 for nLSU (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, 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 1 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.

Sequences generated in this study were aligned with additional sequences downloaded from GenBank (Table 1) using ClustalX (Thompson et al. 1997) and manually adjusted in BioEdit (Hall 1999). Sequence alignment was deposited at TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:20432).

Maximum parsimony phylogenetic analysis followed Li and Cui (2013). It was applied to the combined dataset of ITS and nLSU sequences using PAUP* version 4.0b10 (Swofford 2002). Sequences of Cinereomyces lindbladii (Berk.) Jülich and Sebipora aquosa Miettinen were used as outgroups to root trees following Li and Cui (2013). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using 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 bootstrap 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 generated.

MrModeltest2.3 (Nylander 2004) was used to determine the best-fit evolution model for the combined dataset of ITS and nLSU sequences for estimating Bayesian inference (BI). Bayesian inference was calculated with MrBayes3.1.2 (Ronquist and Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 1 million generations, and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. Majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum parsimony (MP) and Bayesian posterior probabilities (BPP) greater than or equal to 75% (MP) and 0.95 (BPP), respectively, were considered as significantly supported.

**Results**

**Phylogenetic analysis**

The combined ITS and nLSU dataset included 45 sequences of ITS and 44 sequences of nLSU regions from 45 fungal samples representing 37 species. The dataset had an aligned length of 1919 characters in the dataset, of which 1330 characters are constant, 178 are variable and parsimony-uninformative, and 411 are parsimony-informative. Maximum parsimony analysis yielded 6 equally parsimonious trees (TL = 2082, CI = 0. 451, RI =...
Table 1. A list of species, specimens and GenBank accession numbers of sequences used in this study.

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New sequences are shown in bold.
Three new species of Megasporia (Polyporales, Basidiomycota) from China

0.625, RC = 0.282, HI = 0.549), and one of the maximum parsimonious trees is shown in Figure 1. The best model for the combined ITS and nLSU sequences dataset estimated and applied in the BI was GTR+I+G. BI resulted in a similar topology with an average standard deviation of split frequencies = 0.007691 to MP analysis, and thus only the MP tree is provided. Both bootstrap values (≥50%) and BPPs (>0.90) are shown at the nodes (Figure 1).
Samples of *Megasporia* clustered together (89% ML and 1 BPPs, Figure 1), then grouped with the sample of *Grammothelopsis subtropica* B.K. Cui & C.L. Zhao with low support (43% ML and 0.90 BPPs). Sampled specimens of the two new species *M. tropica* and *M. yunnanensis* formed well-supported lineages (100% MP and 1.0 BPPs, Figure 1), while *Megasporia rimosa* sp. nov. clustered with *M. hexagonoides* with low support (69% MP, 0.92 BPPs).

**Taxonomy**

*Megasporia rimosa* Y. Yuan, X.H. Ji & Y.C. Dai, sp. nov.

MycoBank: MB819610

Figure 2

**Diagnosis.** Differs from other *Megasporia* species by its extremely thin and cracked basidiocarp (less than 0.5 mm thick) when dry.

**Holotype.** CHINA. Guangxi Auto. Reg., Shangsi County, Shiwandashan Nature Reserve, on fallen angiosperm branch, 06 June 2015, Y.C. Dai 15357 (BJFC019468).

**Basidiocarps.** Annual, resupinate, corky, without odor or taste when fresh, becoming hard corky and cracked upon drying, up to 17 cm long, 4 cm wide, and 0.4 mm thick at centre. Sterile margin thinning out, white when fresh, cream when dry, very narrow to almost lacking. Pore surface white to cream when fresh, cream when dry; pores angular, 3–4 per mm; dissepiments thick, entire. Subiculum pale buff, corky, up to 0.1 mm thick. Tubes cream, paler than subiculum, corky, up to 0.3 mm long.

**Hyphal structure.** Hyphal system dimitic; generative hyphae bearing clamp connections; skeletal hyphae weakly dextrinoid, CB+; tissues unchanged in KOH.

**Subiculum.** Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, sometimes encrusted by crystals, 1.5–2.5 µm in diam; skeletal hyphae dominant, thick-walled with a narrow to medium lumen, moderately branched, mostly flexuous, interwoven, slightly gelatinized, sometimes encrusted by crystals, 2.5–3.5 µm in diam.

**Tubes.** Generative hyphae hyaline, thin-walled, occasionally branched, 1.5–2.5 µm in diam; skeletal hyphae dominant, thick-walled with a narrow lumen, unbranched, more or less straight, subparallel along the tubes, 2–3 µm in diam. Hyphal pegs absent, dendrohyphidia present along hymenium, cystidia absent; cystidioles present, mostly ventricose, thin-walled, smooth. Basidia broadly clavate to pear-shaped, with four stergmata and a basal clamp connection, 20–28 × 5–7.5 µm; basidioles in shape similar to basidia, but smaller. Small tetrahedric or polyhedric crystals frequently present among subhymenium and hymenium.

**Spores.** Basidiospores cylindrical, hyaline, thin-walled, smooth, sometimes with one or two guttula, IKI–, CB–, (16.5–)16.8–20.2 (–21) × (4.1–)4.3–5.5 (–5.9) µm, L = 18.49 µm, W = 4.88 µm, Q = 3.97 (n = 30/1).

**Etymology.** *Rimosa* (Lat.): referring to the cracked hymenophore when dry.
Three new species of *Megasporia* (Polyporales, Basidiomycota) from China

Figure 2. Microscopic structures of *Megasporia rimosa* sp. nov. (drawn from the holotype). a Basidiospores b Basidioles c Basidia d Cystidioles e Dendrohyphidia f Hyphae from trama. g Hyphae from subiculum.
Megasporia tropica Y. Yuan, X.H. Ji & Y.C. Dai, sp. nov.  
MycoBank: MB819611  
Figure 3

**Diagnosis.** Differs from other Megasporia species by strongly dextrinoid skeletal hyphae, and by lacking dendrohyphidia, cystidioles and hyphal pegs.

**Holotype.** CHINA. Hainan Prov., Wuzhishan County, Wuzhishan Nature Reserve, on fallen angiosperm branch, 10 Nov 2015, B.K. Cui 13660 (BJFC022532).

**Basidiocarps.** Annual, resupinate, corky, without odor or taste when fresh, becoming hard corky to leathery upon drying, up to 5 cm long, 3 cm wide, and 1.5 mm thick at centre. Sterile margin thinning out, cream when dry, up to 1 mm wide. Pore surface clay-pink to fawn when dry; pores round, 2–3 per mm; dissepiments thin, entire to lacerate. Subiculum cream, corky, up to 0.5 mm thick. Tubes clay-pink, slightly darker than subiculum, corky, up to 1 mm long.

**Hyphal structure.** Hyphal system dimitic; generative hyphae bearing clamp connections; skeletal hyphae strongly dextrinoid, CB+; tissues unchanged in KOH.

**Subiculum.** Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, 2.5–3 µm in diam; skeletal hyphae dominant, thick-walled with a narrow to medium lumen, unbranched, more or less flexuous, loosely interwoven, 3–4 µm in diam.

**Tubes.** Generative hyphae hyaline, thin-walled, occasionally branched, 1.5–2 µm in diam; skeletal hyphae dominant, thick-walled with a narrow lumen, occasionally branched, more or less straight, subparallel along the tubes, 2–3 µm in diam. Hyphal pegs, dendrohyphidia and cystidia absent; cystidioles present, subulate, thin-walled, smooth. Basidia broadly clavate, with four sterigmata and a basal clamp connection, sometimes with a big guttule, 20–25 × 7–9.5 µm; basidioles pear-shaped, slightly smaller than basidia. Small tetrahedric or polyhedric crystals frequently present among subhymenium and hymenium.

**Spores.** Basidiospores cylindrical, hyaline, thin-walled, smooth, mostly with a big guttula, IKI–, CB–, (14.2–)14.7–18.8(–19.7) × (4.9–)5–6.5(–7.1) µm, L = 16.55 µm, W = 5.65 µm, Q = 2.83–3.04 (n = 60/2).

**Additional specimen (paratype) examined.** CHINA. Hainan Prov., Ledong County, Jianfengling Forest Park, on fallen angiosperm branch, 21 Nov 2015, B.K. Cui 13740 (BJFC022533).

**Etymology.** Tropica (Lat.): referring to the species occurring in the tropics.

Megasporia yunnanensis Y. Yuan, X.H. Ji & Y.C. Dai, sp. nov.  
MycoBank: MB819612  
Figure 4

**Diagnosis.** Differs from other Megasporia species by brownish tints on pore surface and lacking tetrahedric or polyhedric crystals.
Three new species of *Megasporia* (Polyporales, Basidiomycota) from China

**Figure 3.** Microscopic structures of *Megasporia tropica* sp. nov. (drawn from the holotype). a Basidiospores  b Basidioles c Basidia d Cystidioles e Hyphae from trama f Hyphae from subiculum.

**Holotype.** CHINA. Yunnan Province, Kunming, Wild Duck Lake Park, on fallen angiosperm branch, 28 July 2014, Y.C. Dai 13870 (BJFC017600).

**Basidiocarps.** Annual, resupinate, corky, without odor or taste when fresh, becoming hard coryk upon drying, up to 3 cm long, 2 cm wide, and 2 mm thick at centre. Sterile margin thinning out, white when dry, up to 1 mm wide. Pore surface
Figure 4. Microscopic structures of *Megasporia yunnanensis* sp. nov. (drawn from the holotype). **a** Basidiospores **b** Basidioles **c** Basidia **d** Cystidioles **e** Hyphae from trama **f** Hyphae from subiculum.

White to cream but with brownish tints when dry; pores round, 2–3 per mm; dissepiiments thin, lacerate. Subiculum white, corky, up to 1 mm thick. Tubes cream, corky, up to 1 mm long.
Three new species of *Megasporia* (Polyporales, Basidiomycota) from China

**Hyphal structure.** Hyphal system dimitic; generative hyphae bearing clamp connections; skeletal hyphae weakly dextrinoid, CB+; tissues unchanged in KOH.

**Subiculum.** Generative hyphae frequent, hyaline, thin-walled, occasionally branched, 2–3 µm in diam; skeletal hyphae dominant, thick-walled with a wide to narrow lumen, occasionally branched, mostly flexuous, interwoven, 3–4 µm in diam.

**Tubes.** Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, 2–3 µm in diam; skeletal hyphae dominant, thick-walled with a wide to medium lumen, occasionally branched, flexuous, interwoven, 2.5–3.5 µm in diam. Hyphal pegs and cystidia absent, dendrohyphidia present; cystidioles present, mostly ventricose, thin-walled, smooth. Basidia broadly clavate, with four sterigmata and a basal clamp connection, 30–35 × 9–11 µm; basidioles in shape similar to basidia, but smaller. Tetrahedral or polyhedric crystals absent.

**Spores.** Basidiospores cylindrical, hyaline, thin-walled, smooth, IKI–, CB–, (15.1–)16.5–20.8(–21.5) × (5.1–)5.5–7.1(–7.5) µm, L = 18.38 µm, W = 6.19 µm, Q = 2.88–3.02 (n = 90/3).


**Etymology.** *Yunnanensis* (Lat.): referring to the locality (Yunnan Province, China) where the species was found.

**Discussion**

In this study, seven previously accepted species of *Megasporia* (*M. cystidiolophora* (B.K. Cui & Y.C. Dai) B.K. Cui & Hai J. Li, *M. ellipsoidea* (B.K. Cui & P. Du) B.K. Cui & Hai J. Li, *M. guangdongensis* B.K. Cui & Hai J. Li, *M. hengduanensis* B.K. Cui & Hai J. Li, *M. hexagonoides* (Speg.) B.K. Cui, Y.C. Dai & Hai J. Li, *M. major* (G.Y. Zheng & Z.S. Bi) B.K. Cui, Y.C. Dai & Hai J. Li and *M. violacea* (B.K. Cui & P. Du) B.K. Cui, Y.C. Dai & Hai J. Li) were referred to morphological examination and phylogenetic analysis. Three new *Megasporia* species, *M. rimosa*, *M. tropica* and *M. yunnanensis*, are described based on morphological differences and molecular phylogenetic analysis. Sampled specimens of *Megasporia* formed a well-supported lineage (89% ML and 1.0 BPPs), indicating that all are phylogenetically distinct from other genera, as suggested by the combined ITS and nLSU dataset (Figure 1).

Among the accepted *Megasporia* species, *M. hexagonoides* and *M. major* have big basidiospores (16.6–21.8 × 5.2–6.8 µm, 15.2–20 × 5.5–7.1 µm, Li and Cui 2013). The three new species described in the current paper have similar basidiospores as *M. hexagonoides* and *M. major*, but they have distinct smaller pores than those in *M. hexagonoides* and *M. major* (2–4 per mm vs. 0.5–1.5 per mm). Furthermore, hyphal pegs are present in *M. hexagonoides* and *M. major*, while they are absent in the three new species. In addition, *M. rimosa* differs from other species in *Megasporia* by its extremely
thin basidiocarp (less than 0.5 mm thick) and cracked when dry, while fruiting bodies in other species are more than 1 mm thick, and not cracked when dry (Dai and Wu 2004, Du and Cui 2009, Li and Cui 2013). *Megasporia tropica* is distinguished from other species in the genus by lacking dendrohyphidia, cystidioles and hyphal pegs. *Megasporia yunnanensis* has brownish tints on its pore surface and lacks tetrahedric or polyhedric crystals, while other species in *Megasporia* have abundant tetrahedric or polyhedric crystals but lack brownish tints on their pore surfaces.

It seems that species of *Megasporia* prefer small branches rather than big logs; all specimens of the genus having been collected mostly on fallen branches and dead branches on living trees, and such branches being not strongly decayed. The basidiocarps of the genus are usually not very big and usually form small patches, although some patches may be merged finally. All the species of *Megasporia* have been found on angiosperm wood (never on gymnosperms), and they have a distribution in subtropical and tropical forests, especially in open environments, e.g. fallen branches along roads or paths. In addition, the species diversity of the genus is very rich in subtropical and tropical Asia, many more undescribed taxa are found from our samples based on phylogenetic analyses, but all these samples are sterile as a common feature of the genus, and the best season for producing basidiospores on these taxa are unknown.

Although *Megasporoporiella*, *Megasporoporia* and *Megasporia* are very similar, we found some difference among these genera both in morphology and ecology. The main difference is that *Megasporoporia* has di-trimitic hyphal structure and strongly dextrinoid skeletal hyphae, while dimitic hyphal structure and weakly to moderately dextrinoid skeletal hyphae are in *Megasporoporiella* and *Megasporia*. In addition, *Megasporoporiella* has a distribution in temperate region, while *Megasporia* in subtropical to tropics.

**Key to known species of *Megasporia***

1. Pores 0.5–1.5 per mm................................................................. 2
   – Pores 2–7 per mm................................................................. 4
2. Basidiospores ellipsoid, gloeocystidia present ...................... *M. ellipsoidea*
   – Basidiospores cylindrical, gloeocystidia absent.................. 3
3. Pores 0.5–1 per mm, pore surface ash gray......................... *M. hexagonoides*
   – Pores 1–1.5 per mm, pore surface cream........................... *M. major*
4. Basidiospores < 15 µm in length, hyphal pegs present........... 5
   – Basidiospores > 15 µm in length, hyphal pegs absent........... 8
5. Pores 5–7 per mm, pores violet when fresh; dendrohyphidia present ........
   .......................................................................................... *M. violacea*
   – Pores 2–5 per mm, pores cream to buff when fresh; dendrohyphidia absent.... 6
6. Pores 2–3 per mm, skeletal hyphae moderately dextrinoid... *M. hengduanensis*
   – Pores 3–5 per mm, skeletal hyphae strongly dextrinoid................ 7
7. Basidiospores 3.4–4.5 µm in width, cystidioles collapsed .... *M. guangdongensis*
   – Basidiospores 4.1–5.6 µm in width, cystidioles not collapsed...........
   ....................................................................................... *M. cystidiolophora*
Three new species of *Megasporia* (Polyporales, Basidiomycota) from China

8  Basidiocarp < 0.5 mm thick and cracked when dry ........... *M. rimosa* sp. nov.
– Basidiocarp > 1 mm thick and not cracked when dry ......................... 9

9  Tetrahedric or polyhedric crystals present, dendrohyphidia absent ................................................. *M. tropica* sp. nov.
– Tetrahedric or polyhedric crystals absent, dendrohyphidia present ................ *M. yunnanensis* sp. nov.

**Acknowledgements**

We thank Prof. Bao-Kai Cui (Beijing) for providing important materials for our study. The research is supported by the Fundamental Research Funds for the Central Universities (Project No. 2016ZCQ04).

**References**


