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Monokariotic fruiting body and clamp cell formation in *Mycoleptodonoides aitchisonii* (Bunaharitake)

Rini Riffiani, Takayuki Wada, Norihiro Shimomura, Takeshi Yamaguchi, Tadanori Aimi

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Diversity of wood-inhabiting Agaricomycotina on wood of different size classes in riparian forests of Uruguay

Sebastián Martínez, Karen K. Nakasone, Lina Bettucci

Pages 156-164

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Xylodon kunmingensis sp. nov. (Hymenochaetales, Basidiomycota) from southern China

Zhong-Wen Shi, Xue-Wei Wang, Li-Wei Zhou, Chang-Lin Zhao

Pages 184-188

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Two new records of ascomycetes from Japan, *Pyrenopeziza protrusa* and *P. nervicola* (Helotiales, Dermateaceae *sensu lato*)

Hiyori Itagaki, Yasuhide Nakamura, Tsuyoshi Hosoya

Pages 189-196

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Efficient transformation of the entomopathogenic fungus *Lecanicillium muscarium* by electroporation of germinated conidia

Sergey Timofeev, Alexander Tsarev, Igor Senderskiy, Eugene Rogozhin, ... Viacheslav Dolgikh

Pages 197-200

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Establishment of monokaryotic and dikaryotic isolates of Hedgehog mushrooms (*Hydnum repandum* and related species) from basidiospores

Ryo Sugawara, Akiyoshi Yamada, Masataka Kawai, Kozue Sotome, ... Naoki Endo

Pages 201-209

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Short communication

Xylodon kunmingensis sp. nov. (Hymenochaetales, Basidiomycota) from southern China

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ABSTRACT

A new wood-inhabiting fungal species, *Xylodon kunmingensis*, is proposed based on morphological and molecular evidences. The species is characterized by an annual growth habit, resupinate basidiocarps with cream to buff hymenial, odontioid surface, a monomitic hyphal system with generative hyphae bearing clamp connections and oblong-ellipsoid, hyaline, thin-walled, smooth, inamyloid and indextrinoid, acyanophilous basidiospores, 5–5.8 × 2.8–3.5 μm. The phylogenetic analyses based on molecular data of ITS sequences showed that *X. kunmingensis* belongs to the genus *Xylodon* and formed a single group with a high support (100% BS, 100% BP, 1.00 BPP) and grouped with the related species *X. astrocystidiatus*, *X. crystalliger* and *X. paradoxus*. Both morphological and molecular evidences confirmed the placement of the new species in *Xylodon*.

Xylodon (Pers.) Gray (Schizoporaceae, Hymenochaetales) is a widespread genus typified by *X. quercinus* (Pers.) Gray (Gray, 1821). According to the modern definition, it is characterized by the resupinate basidiocarps with smooth, tuberculate, grandinioid, odontioid, coralloid, irpicoid or poroid hymenophore, a monomitic hyphal system with clamp connections on generative hyphae and bladder-like, bottle-shaped, capitate to subulate cystidia, suburniform basidia and globose to ellipsoid to cylindrical basidiospores and causing a white rot (Bernicchia & Gorjón, 2010). The morphological concept of *Xylodon* in its broad sense was outlined (Gray, 1821; Hjortstam & Ryvarden, 2009; Yurchenko & Wu, 2013, 2014). The MycoBank database (http://www.MycoBank.org) registered 192 specific and infraspecific names in *Xylodon* and the Index Fungorum (http://www.indexfungorum.org) registered 176, but the actual number of species is 95 (Chen, Zhou, Ji, & Zhao, 2016, 2018; Dai, 2011, 2012; Kan, Gafforov, Li, & Zhou, 2017a, b; Lee & Langer, 2012; Riebesehl & Langer, 2017; Wang & Chen, 2017; Wu, 1990, 2000, 2001, 2006; Xiong, Dai, & Wu, 2009, 2010; Yurchenko & Wu, 2013, 2014; Zhao, Cui, & Dai, 2014).

The genus *Hyphodontia* could be split into 13 genera and showed that *Xylodon* was a larger genus and included more species (Hjortstam & Ryvarden, 2009). Phylogenetic studies inferred from nuclear DNA sequence data for *Hyphodontia* s.l. demonstrated the polyphyletic genus consisted of six well-distinguished clades: *Lagarobasidium* clade, *Kneiffiella-Alutaceodontia* clade, *Hyphodontia* clade, *Hastodontia* clade, *Xylodon-Lyomyces-Rogersella* clade and *Xylodon-Schizopora-Palifer* clade, in which the genus *Xylodon* belonged to the *Xylodon-Schizopora-Palifer* clade (Yurchenko & Wu, 2014). Riebesehl and Langer (2017) studied the *Hyphodontia* s.l. based on the morphological and phylogenetic information and proposed 13 new combinations in *Xylodon*. Recently, many new species were found in *Hyphodontia* s.l. based on morphological and molecular evidences (Chen et al., 2016, 2018; Kan et al., 2017a, b; Wang & Chen, 2017; Zhao et al., 2014).

Wood-rotting fungi is a cosmopolitan group and it has a rich diversity on the basis of growing on boreal, temperate, subtropical, and tropical vegetations in southern China (Dai, 2011, 2012). During the investigations on wood-inhabiting fungi in Yunnan Province, an additional taxon was found which could not be assigned to any described species. In this study, the authors expand samplings from previous studies to examine taxonomy and phylogeny of this new species within the *Xylodon* based on the internal transcribed spacer (ITS) sequences.

The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC). Macro-morphological

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descriptions are based on field notes and indoor observation follows Dai (2010). Special colour 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 were used: KOH = 5% (w/v) potassium hydroxide, CB = Cotton Blue, CB- = acyanophilous, IKI = Melzer's reagent, IKI- = both inamyloid and indextrinoid, L = mean basidiospore length (arithmetic average of all basidiospores), 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 basidiospores (a) measured from given number (b) of specimens.

The EZNA HP Fungal DNA Kit (Omega Biotechnologies Co., Ltd, Kunming) 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, Bruns, Lee, & Taylor, 1990). 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 products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. The five new sequences from specimens of *Xylodon kunmingensis* were aligned with additional sequences of *Xylodon* downloaded from GenBank (Supplementary Table S1).

Sequences were aligned in MAFFT 6 (Katoh & Toh, 2008; http://mafft.cbrc.jp/alignment/server/) using the "G-INS-I" strategy for ITS, and manually adjusted in BioEdit (Hall, 1999). Alignment datasets were deposited in TreeBase (submission ID 23624). *Hastodontia hastata* (Litsch.) Hjortstam & Ryvarden was selected as

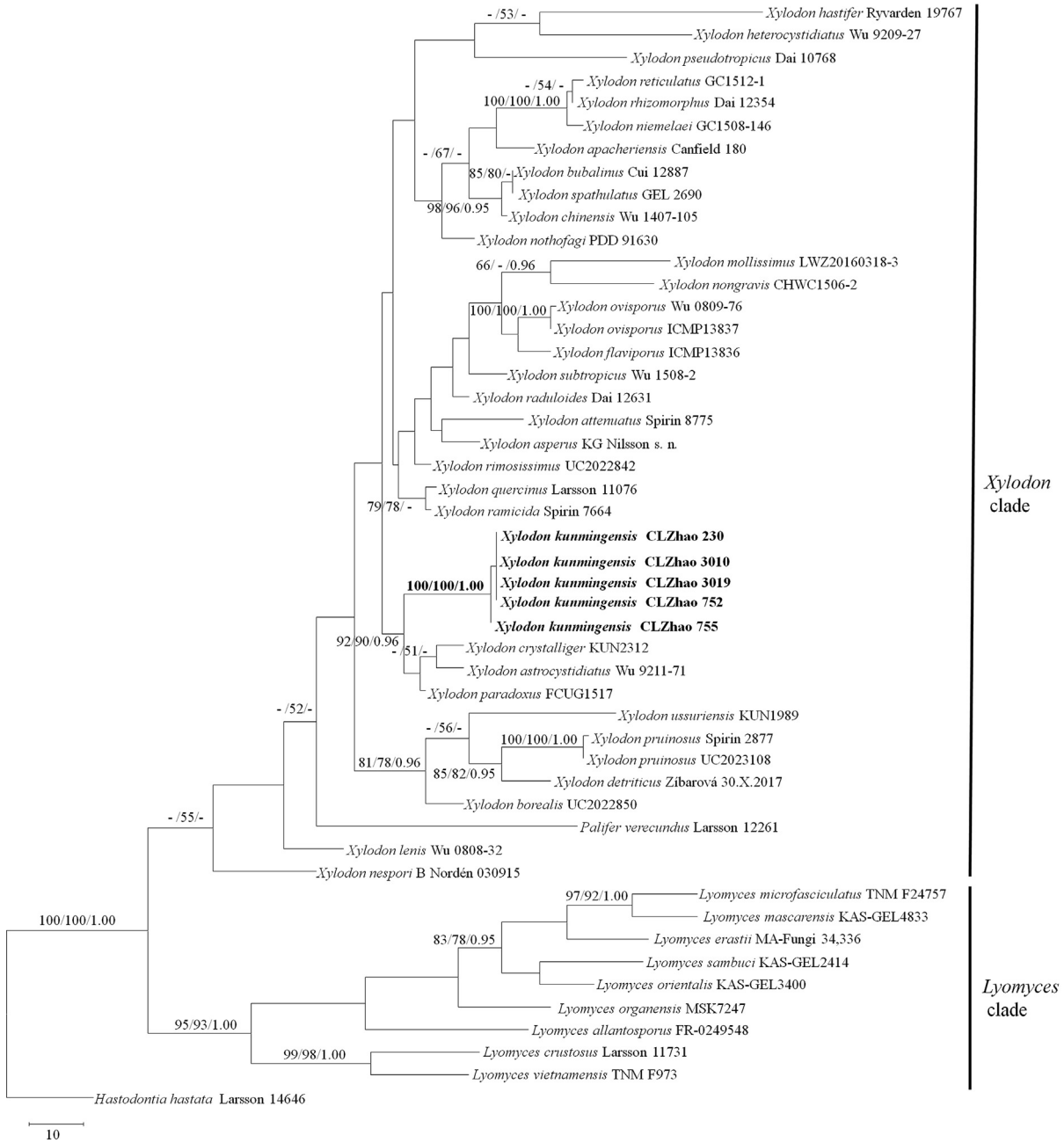

Fig. 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Xylodon kunmingensis* and related species in *Xylodon* based on ITS sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively.

outgroup for phylogenetic analysis of ITS (Viner, Spirin, Zíbarová, & Larsson, 2018).

Maximum parsimony analysis was applied to the ITS dataset sequences. 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 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 (BP) 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. Maximum Likelihood (ML) analysis with RAxML-HPC2 was conducted for ITS datasets on Abe through the Cipres Science Gateway (www.phylo.org; Miller et al., 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicate.

MrModeltest 2.3 (Nylander, 2004; Posada & Crandall, 1998) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes 3.1.2 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 6 million generations (ITS) 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), maximum parsimony (BP) and Bayesian posterior probabilities (BPP) greater than or equal to 80% (BL), 75% (BP) and 0.95 (BPP) were considered as significantly supported, respectively.

The ITS dataset included sequences from 36 fungal specimens or isolates representing 33 species. The dataset had an aligned length of 671 characters, of which 321 characters are constant, 140 are variable and parsimony-uninformative, and 210 are parsimony-informative. Maximum parsimony analysis yielded 12 equally parsimonious trees (TL = 387, CI = 0.486, HI = 0.514, RI = 0.429, RC = 0.209). Best model for the ITS dataset estimated and applied in the Bayesian analysis as GTR + I + G. Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.003541 (BI).

The phylogeny (Fig. 1) inferred from ITS sequences was obtained for more related taxa of *Xylodon* and showed that the new species formed a single group and grouped with the related species *X. astrocystidiatus* (Yurchenko & Sheng H. Wu) Riebesehl, Yurchenko & Langer, *X. crystalliger* Viner and *X. paradoxus* (Schrad.) Chevall.

Xylodon kunmingensis L.W. Zhou & C.L. Zhao, sp. nov. Figs. 2 and 3.

MycoBank no.: MB 829478.

Differs from other species by an odontioid hymenophore with cream to buff surface, a monomitic hyphal system with clamp connections, presence of the bladder-like cystidia and oblong-ellipsoid, IKI-, cyanophilous basidiospores measuring as 5–5.8 × 2.8–3.5 μm.

Type: CHINA, Yunnan Province, Kunming, Xishan Forestry Park, on fallen angiosperm branch, 17 Sep 2017, leg. C.L. Zhao, CLZhao

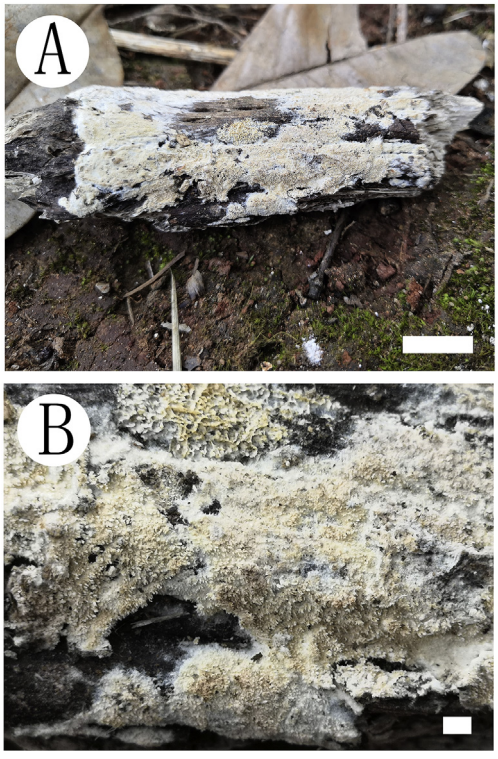

Fig. 2. Basidiocarps of *Xylodon kunmingensis* (holotype). *Bars*: A 1 cm; B 0.1 mm.

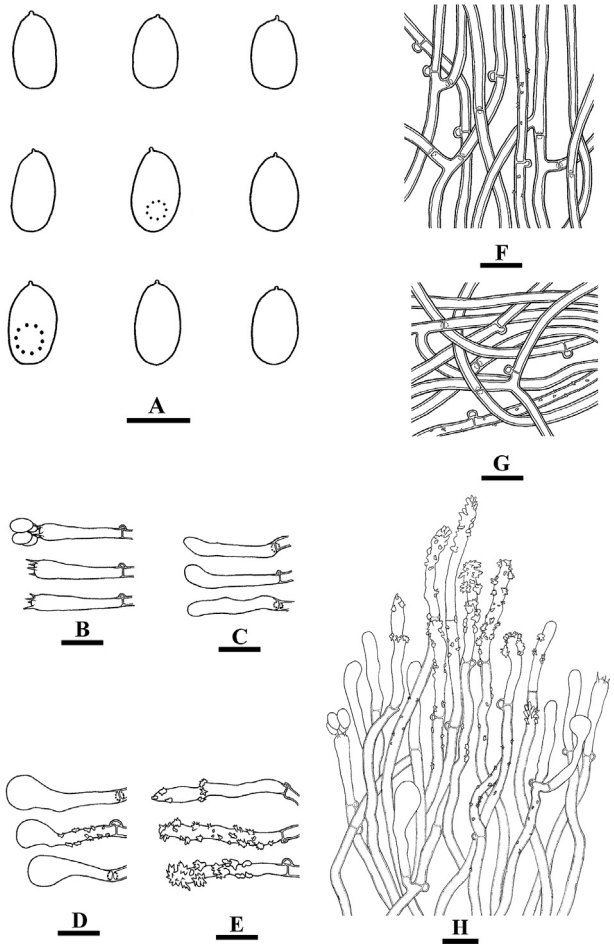

Fig. 3. Microscopic structures of *Xylodon kunmingensis* (drawn from the holotype). A: Basidiospores. B: Basidia. C: Subulate to ventricose cystidia. D: Capitate cystidia. E: Encrusted hyphal ends. F: Hyphae from aculei. G: Hyphae from subiculum. H: Section from tramal hyphae. *Bars*: A 5 μm; B-H 10 μm.

3019 (holotype, SWFC 003019).

rRNA gene sequence ex holotype: MK404532 (ITS).

Etymology: *kunmingensis* (Lat.): refers to the location where the species was collected.

Fruiting body: annual, resupinate, adnate, soft, without odor or taste when fresh, becoming corky upon drying, up to 8 cm long, 3 cm wide, 1 mm thick at centre. Hymenial surface odontioid, aculei conical, cream when fresh, cream to buff upon drying. Sterile margin distinct, white.

Hyphal structure: hyphal system monomitic; generative hyphae with clamp connections, IKI-, CB-; tissues unchanged in KOH.

Subiculum: generative hyphae hyaline, thick-walled, frequently branched, interwoven, 2.5–4.5 μm in diameter, occasionally encrusted with crystals.

Hymenium: cystidia or cystidium-like structures of several types: (1) subulate to ventricose cystidia, 20–25 × 3–5 μm; (2) capitate cystidia hyaline, thin-walled, 18–35 × 7–10 μm; (3) encrusted hyphal ends, thin-walled, 25–34 × 4–6 μm; basidia clavate to suburnifrom, with four sterigmata and a basal simple septa, 23–27 × 4–5 μm.

Spores: oblong-ellipsoid, hyaline, thin-walled, usually bearing one guttule, smooth, IKI-, CB-, (4.5–)5–5.8 (–6) × 2.8–3.5 μm, L = 5.45 μm, W = 3.22 μm, Q = 1.65–1.77 (n = 150/5).

Associated wood-rot: White.

Additional specimens examined: CHINA, Yunnan Province, Kunming, Panlong District, Yeyahu Forestry Park, on fallen angiosperm branch, 2 Aug 2016, leg. C.L. Zhao, CLZhao 230 (SWFC 000230); Xishan Forestry Park, on fallen angiosperm branch, 17 Sep 2017, leg. C.L. Zhao, CLZhao 3010 (SWFC 003010); on fallen branch of *Pinus yunnanensis* Franch., 11 Jan 2017, leg. C.L. Zhao, CLZhao 752 (SWFC 000752); on the angiosperm stump, 11 Jan 2017, leg. C.L. Zhao, CLZhao 755 (SWFC 000755).

In the present study, a new species *Xylodon kunmingensis* was described based on phylogenetic analyses and morphological characters.

Previously, a phylogenetic study inferred from nuclear DNA sequence data for *Hyphodontia* s.l. demonstrated the polyphyletic nature of this genus, consisting of six well-distinguished clades including *Lagarobasidium* clade, *Kneiffiella-Alutaceodontia* clade, *Hyphodontia* clade, *Hastodontia* clade, *Xylodon-Lyomyces-Rogersella* clade and *Xylodon-Schizopora-Palifer* clade (Yurchenko & Wu, 2014). According to our result based on the combined ITS sequence data (Fig. 1), *Xylodon kunmingensis* is nested into the *Xylodon* with strong support (100% BS, 100% BT, 1.00 BPP).

Phylogenetically, *Xylodon kunmingensis* singly formed a well-supported monophyletic lineage distinct from other *Xylodon* species and then grouped with the related species: *X. astrocystidiatus*, *X. crystalliger* and *X. paradoxus* in the rDNA based on the phylogeny (Fig. 1). But morphologically *X. astrocystidiatus* has the effused basidiocarps with the conical to cylindrical, peg-like hyphal aggregation, presence of the astrocystidia and broadly ellipsoid basidiospores (5–6 × 3.7–4.2 μm, Yurchenko & Wu, 2013). *Xylodon crystalliger* differs from *X. kunmingensis* by having the effuse basidiocarps with white, soft membranaceous hymenial surface and ellipsoid, smaller basidiospores (4.2–5.1 × 3.3–4.2 μm, Viner et al., 2018). *Xylodon paradoxus* differs in having the white to cream basidiocarps with tubulose to irpicoid hymenophore and a dimitic hyphal system and ovoid basidiospores (Donk, 1967).

Recently, three related species of *Xylodon* were found in China: *X. bubalinus* (Min Wang, Yuan Y. Chen & B.K. Cui) C.C. Chen & Sheng H. Wu, *X. dimiticus* (Jia J. Chen & L.W. Zhou) Riebesehl & Langer and *X. rhizomorphus* (C.L. Zhao, B.K. Cui & Y.C. Dai) Riebesehl, Yurchenko & Langer. However, all of them did not group closely in the phylogenetic trees based on the nuclear DNA sequence data (Chen, Wu, & Chen, 2018; Riebesehl & Langer, 2017; Wang & Chen, 2017). Morphologically, the species *X. bubaline* differs from *X. kunmingensis* by having cracking basidiocarps, cyanophilous generative hyphae and ellipsoid to subglobose basidiospores (4–5.3 × 3–4.2 μm, Wang & Chen, 2017). *Xylodon dimiticus* differs in having the poroid hymenophore, a dimitic hyphal system and smaller basidiospores (3.8–4.6 × 2.8–3.5, Chen et al., 2016). *Xylodon rhizomorphus* has the rhizomorphic basidiocarps with poroid hymenophore and broadly ellipsoid basidiospores (4.3–5.5 × 3.7–4.1 μm, Zhao et al., 2014).

Wood-rotting fungi is an extensively studied group of Basidiomycota (Gilbertson & Ryvarden, 1986, 1987; Núñez & Ryvarden, 2001; Ryvarden & Melo, 2014), but the Chinese wood-rotting fungi diversity is still not well known, especially in the subtropics and tropics, and many taxa were recently described from these areas (Bian & Dai, 2015; Chen et al., 2016, 2014; Dai, 2012; Han & Cui, 2015; Li & Cui, 2013; Wang & Chen, 2017). The new species, *Xylodon kunmingensis* is also from the subtropics, and it is likely that more new wood-rotting fungi will be found after further investigations and molecular analyses.

Disclosure

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of the People's Republic of China.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.myc.2019.02.002.

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