Amylosporus sulcatus sp. nov. (Russulales, Basidiomycota) from Southern China

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ABSTRACT

Amylosporus sulcatus sp. nov. is described from Nonggang Nature Reserve, southern China, on the basis of morphological and molecular data. The morphological description and illustrations for the new species are provided. The species is characterized by pileate and stipitate basidiocarps. The pileus surface is obviously concentrically and radiately sulcate and tomentum, and the pore surface is snow white. Phylogenetic analyses based on sequences of the internal transcribed spacer and nuclear large subunit ribosomal DNA confirmed it to be a new species.

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1. Introduction

The genus Amylosporus was established and typified by Amylosporus campbellii (Berk.) Ryvarden, and it is characterized by both simple septate and multi-clamped generative hyphae, and finely asperulate and amyloid basidiospores [1]. The phylogenetic position of the genus in the family Wrightoporiaceae, with a sister clade Wrightoporia Pouzar, has been confirmed using the internal transcribed spacer (ITS) and nuclear large subunit ribosomal DNA (nLSU) sequence analyses [2]. Recently, a new species, A. guaraniticus Campi and Robledo, was added to the genus, and a total of 10 species are currently accepted worldwide [3]. Among the recognized species, four species, namely, A. casuarinicola (Y.C. Dai and B.K. Cui) Y.C. Dai, Jia J. Chen, and B.K. Cui; A. rubellus (Y.C. Dai) Y.C. Dai, Jia J. Chen, and B.K. Cui; A. daedaliformis G.Y. Zheng and Z.S. Bi; and A. succulentus JiaJ. Chen and L.L. Shen, have been reported from China [4–7].

During the macrofungal diversity research in southern China, we found an additional undescribed species of Amylosporus, and it was confirmed to be a new species on the basis of its morphological characteristics and phylogenetic analysis of combined sequence data of ITS and nLSU.

2. Materials and methods

2.1. Morphological examination

Specimens were found on dead angiosperm trunks in Nonggang Nature Reserve of Guangxi Autonomous Region in China. The specimens are deposited at the herbarium of Guangxi University (GXU). The description of macroscopic characters was based on both fresh and dried specimens. The following abbreviations were used: IKI = Melzer’s reagent, IKI− = negative in Melzer’s reagent, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB+ = cyanophilous, 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, and n = number of spores measured from given number of specimens. The sections were studied at a magnification of up to ×1500 by using a Nikon Eclipse 80i microscope and phase contrast illumination microscopy, and the basidiospores were also observed with a scanning electron microscope (SEM; SU-8020, Hitachi, Tokyo, Japan).

2.2. DNA extraction, amplification, and sequencing

DNA was extracted according to the conventional cetyl trimethylammonium bromide (CTAB) procedure. Nuclear ITS and nLSU regions were amplified with primer pairs ITS5/ITS4 and LR0R/LR5 [8,9]. The PCR procedure was as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 40 s, 56°C for 40 s, and 72°C for 1 min and a final extension at 72°C for 10 min. The PCR products were directly purified and sequenced by Beijing Genomics Institute (BGI) with the same
Table 1. Voucher and accession numbers for specimens from GenBank.

| Species                                      | Sample no. | GenBank no. |
|----------------------------------------------|------------|-------------|
| Aleurocystidiellum subcruentatum              | He2886     | KUS53941, KUS74847 |
| Amylosporus bracei                            | 1008/77    | KM267724, KJ807076 |
| Amylosporus campbellii                        | 0806/20a   | JF692200, KJ807077 |
| Amylosporus campbellii                        | Gilbertson 14806 KM107861 KM107879 |
| Amylosporus guananticus                       | M. Campi 106 MF377528 MF377529 |
| Amylosporus sp. UI-2014                       | IU29-1     | KMB81314, KMS93892 |
| Amylosporus sp. UI-2014                       | IU29-2     | KMB81315, KMS93893 |
| Amylosporus succulentes                       | Dai 7802   | KM213669, KM213671 |
| Amylosporus succulentes                       | Dai 7803   | KM213668, KM213670 |
| Amylosporus sulcatus                          | GXU 1084   | MG280818, MG280819 |
| Amylosporus sulcatus                          | GXU 1095   | MG280820, MG280821 |
| Bondarzewia montana                           | AFTOL-ID 452 | DQ200923, DQ234539 |
| Bondarzewia podocapri                         | Dai 9261   | KJS83207, KJS83221 |
| Heterobasidion annosum                       | 06129/6    | KJS83211, KJS83225 |
| Heterobasidion parvporum                      | 04121/3    | KJS83212, KJS83226 |
| Larssoniporia tropicales                      | TFM F-16446 | KBJ07072, KBJ07088 |
| Wighttoporia lenta                            | Cui 7804   | KJS13292, KBJ07081 |
| Wighttoporia lenta                            | Dai 10462  | KJS13291, KBJ07082 |
| Wighttoporia rubella                          | Dai 9233   | KBJ07071, KBJ07084 |
| Wighttoporia subavellanea                     | Dai 11484  | KJS13295, KBJ07085 |
| Wighttoporia subavellanea                     | Dai 11488  | KJS13296, KBJ07086 |

primers. Four newly generated sequences from this study have been submitted to GenBank; other related sequences obtained from GenBank were included in the analysis (Table 1).

2.3. Phylogenetic analyses

The sequence datasets for ITS and nLSU were aligned with MEGA 5.2 and ClustalX 1.83, respectively [10,11]. The sequence alignment was deposited at TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S22015) and performed by PAUP* version 4.0b10 [12]; MrMtgui 1.01 (http://www.gendrift.org/mtgui.php) and MrModeltest 2.3 [13,14] were used to find the best-fit model. The Bayesian analysis was performed using MrBayes 3.2.3 [15] with 300,000 generations. The phylogenetic tree was generated using maximum parsimony analyses performed with PAUP* version 4.0a151 by using tree-bisection reconnection branch-swapping algorithm, and clade robustness was assessed using bootstrap analysis with 1000 replicates. The following descriptive tree statistics were calculated: tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI).

3. Results and discussion

3.1. Taxonomy

**Amylosporus sulcatus**, F.C. Huang and Bin Liu, sp. nov. (Figures 1–3)

Mycobank: MB823782

Etymology: *sulcatus* (Lat.), referring to pileus surface obviously concentrically and radiately sulcate.

Type: China, Guangxi Autonomous Region, Chongzuo, Longzhou County, Nonggang Nature Reserve, on dead trunks of angiosperm tree, September 19, 2012, GXU 1095 (Holotype in GXU). rDNA sequence ex holotype: MG280820 (ITS), MG280821 (nLSU).

Basidiomata annual to perennial, pileate, eccentrically to laterally stipitate, fleshy and watery when fresh, corky when dry. Pileus up to 6.8 × 6.1 × 1.5 cm, nearly circular, tubes sometimes usually appearing on the surface of pileus, with some projected upward that then developed into small pilei – this was more obvious near the pileus margin; sterile margin absent. Pileus surface snow white, pale pinkish cinnamon, pale flesh color to liver brown when fresh; light ochraceous salmon, vinaceous cinnamon to mars brown when dry; obviously concentrically and radiately sulcate and tomentum. Pore surface snow white when fresh; salmon buff, cameo brown to liver brown when dry. Pores 3–5 per mm, angular, irregular to lacerate; dissepiments thin, entire to lacerate; tubes concolorous to pore surface, up to 0.5 mm deep; context white, Sanford’ brown to chestnut when fresh; salmon buff when dry; up to 1 cm thick at the base; a thin dense black layer near the bottom of the pileus context; stipe up to 2.9 × 3.9 cm, nearly cylindrical, thick, with poroid portions decurrently, white to snow white when fresh; salmon buff, cameo brown to liver brown when dry.

Hyphal system dimitic, tramal generative hyphae with simple septa and single clamp connection; contextual generative hyphae with simple septa but also with single, double, to multiple clamp connections (verticillate septa with up to five clamps); skeletal hyphae IKI–, CB+; tissue color unchanged in KOH. Contextual generative hyphae common to dominant, hyaline, thin- to thick-walled, frequently branched, 3.7–13.1 μm wide; contextual skeletal hyphae frequent, branched, thick-walled, with a wide to narrow lumen or solid, 1.6–7.4 μm wide. Gloeoplerous hyphae present in tissues of context, filled with refractive content, with more clamps and branched, some are simple septa, thin wall, 2.2–5.8 μm diameter; gloeoplerous hyphae also present in tissues of stipe, but with fewer clamps and more simple septa, fewer branched, thin- to thick-walled, 5.5–11.7 μm diameter. Tubes generative hyphae dominant, hyaline, thin-walled, moderately branched, 1.9–9.4 μm wide, tubes skeletal hyphae frequent, thick-walled, branched frequently with a wide to narrow lumen or solid, 1.6–4.8 μm wide. Some solid hyphae are easily destroyed and swollen in KOH. Cystidia and cystidioles absent; basidia long clavate, 27.1–79.9 × 4.6–11.9 μm, with basal simple septum and four sterigmata, basidioles short clavate to barrel shape, 8.1–27.8 × 3.2–8.3 μm. Basidiospores
Figure 1. Wild fresh basidiocarps and hyphal system of *Amylosporus sulcatus*. (A), (B): Basidiocarps; (C), (D), (E) (1), (F): contextual generative hyphae with simple septa and single or double to multiple clamp connections; (E) (2): contextual skeletal hyphae; (G), (H) (2): tramal skeletal hyphae; (H) (1): tramal generative hyphae with simple septa and single clamp connection. Scale bar: A, B, 1 cm; C, D, E, F, G, H, 10 μm.

Figure 2. Microscopic structure of *Amylosporus sulcatus*. (A), (B), (C): Basidia. (D): Basidioles. (E): Gloeoplerous hyphae from context. (F): Gloeoplerous hyphae from stipe. Scale bar: 10 μm.
ellipsoid to ovoid, hyaline, thin-walled, appearing smooth under a light microscope (observed in Melzer’s reagent) or with very fine warts (observed with SEM), most with one guttulate, IKI+, CB+, (2.9–) 3.6–5.7 (–6.0) × (2.4–) 2.7–3.6 (–4.0) μm, $L = 4.9$ μm, $W = 3.1$ μm, $Q = 1.58$ ($n = 65$).

Habitat: growing on dead trunks of angiosperm trees.

Additional specimen examined: China, Guangxi, Chongzuo, Nonggang Nature Reserve, on another fallen angiosperm trunk, September 19, 2012, GXU 1084. The basidiocarps were more mature, and from margin and side of pilei new pileus developed in a spiral arrangement; pileus surface tomentum and rough, tubes up to 5.5 mm deep.

Note: Differs from other Amylosporus species by pileate and stipitate basidiocarps. Pileus surface concentrically and radiately sulcate; gloeoplerous hyphae present in tissues of context and stipe, skeletal hyphae undextrinoid, tramal generative hyphae with simple septa and single clamp connection; basidiospores with very fine warts.

3.2. Phylogenetic analyses

The ITS + nLSU dataset included sequences from 21 fungal specimens that represent 15 taxa. The dataset had an aligned length of 1903 characters, including gaps (933 characters for ITS, 970 characters for nLSU), of which, 1142 characters were constant, 139 were variable and parsimony uninformative, and 622 were parsimony informative. The consensus tree is shown in Figure 4 (TL = 1580, CI = 0.714, RI = 0.823, RC = 0.588, and HI = 0.286). The best model for the ITS + nLSU dataset was estimated and applied using Bayesian analysis: GTR + I + G, Lset nst = 6 rates = invgamma; Prset statefreqpr = dirichlet (1, 1, 1, 1, 1, 1). The average standard deviation of split frequencies according to the Bayesian analysis is 0.001778.

Tree topology of the maximum parsimony analysis was the same as the tree topology of the Bayesian analysis.

The phylogenetic trees based on the combined ITS + nLSU sequences formed a well-supported lineage (Figure 4). The relationships among the species in the phylogeny were similar to those reported by Chen et al. [2]. Clade I included all test species from the genus Amylosporus; the species of clade II belonged to Wrightoporia, and species of Heterobasidion and Bondarzewia formed clade III. Amylosporus was closer with Wrightoporia than that of Heterobasidion and Bondarzewia. The new species A. sulcatus is genetically closer to the unnamed species Amylosporus

Figure 3. Basidiospores of Amylosporus sulcatus. (A), (B), (C): By SEM; (E): in KOH; (F): in Melzer’s reagent. Scale bar: 10 μm.
Amylosporus sp. IJ-2014, with strong support (BP = 100; BPP = 1.00); however, their distance in the entire ITS region is about 7%. Amylosporus sp. IJ-2014 was isolated and domesticated by Juma et al. [16], but the authors did not provide a detailed morphological description. We also successfully domesticated the new species *A. sulcatus*, and obtained the fully developed mature fruiting body; our fungus differs from *Amylosporus* sp. IJ-2014 by having pores extend decurrently on the surface of the stipe. Phylogenetically, *A. sulcatus* is also close to *A. campbellii* and *A. guaraniticus*, with strong support (BP = 91; BPP = 1.00). *A. campbellii* is distinguishable from the new species by its sterile surface smooth and tuberculate and gloeoplerous hyphae abundant in context [1,17,18]. *A. guaraniticus* differs from *A. sulcatus* by basidiomata sessile, imbricate, margin sterile, pores angular 2–3 per mm, and gloeoplerous hyphae present in context [3].

Morphologically, *A. auxiliadorae* Drechsler-Santos and Ryvarden [19], *A. campbellii*, *A. daedaliformis*, and *A. succulentus* are similar to *A. sulcatus* by having pileate and stipitate basidiocarps. However, *A. succulentus* differs from the new species by pore surface cream to pinkish violet when fresh; tramal generative hyphae with simple septa only; skeletal hyphae dextrinoid; gloeoplerous hyphae frequently in tubes; and cystidioles present.

**Table 2. Characteristics of the new species and other related species of *Amylosporus***

| Species                  | Basidiomata          | Pores                          | Hyphae                        | Basidiospores |
|--------------------------|----------------------|--------------------------------|-------------------------------|---------------|
| *Amylosporus sulcatus*   | Annual to perennial, stipitate | Size (pores/mm); shape 3–5; angular, irregular to lacerate | Septa of tramal generative hyphae Simple septa and single clamp | Surface and size (μm) Very fine warts, 3.6–5.7 × 2.7–3.6 |
| This work               |                      |                                | IKI–                          | In context and stipe |
| *Amylosporus campbellii* | Annual, stipitate to almost sessile | 2–4; round; to angular        | –                             | IKI– More abundant in context and stipe |
| [18]                    |                      |                                |                               | Finely echinulate, 4–5 × 2.5–4 |
| *Amylosporus guaraniticus* | Annual, pileate   | 2–3; angular                   | –                             | IKI– In context |
| [3]                     |                      |                                |                               | Finely asperulate, 4–5 × 3–4 |
| *Amylosporus auxiliadorae* | Annual, stipitate | 3–6; irregular                 | Simple septa only Dextrinoid | Not observed |
| [9]                     |                      |                                |                               | Finely asperulate, 4.0–5.0 × 2.5–4.0 |
| *Amylosporus daedaliformis* | Annual, sessile to stipitate | Pores daedaliform when mature | Simple septa and single clamp | Smooth |
| [4]                     |                      | 2–4; angular                   | Simple septa only Dextrinoid | 5–6 × 2.5–3.5 |
| *Amylosporus succulentus* | Annual, stipitate | 2–4; angular                   | Simple septa only Dextrinoid | 4.2–5.2 × 3–3.8 |

**Figure 4.** Phylogenetic tree was generated using maximum parsimony analyses based on the combined ITS + nLSU sequences. Bootstrap values (before the/) higher than 50% and Bayesian posterior probabilities (after the/) more than 0.90 are indicated along the branches.
A. auxiliadoraе is distinguishable from A. suлcatuе by having sterile margin, upper surface smooth; pore surface buff, clay buff to straw; skeletal hyphae slightly to dextrinoid; clamp connections absent in hymenium; and gloeoplerous hyphae not observed. A. daedaliformis was easily identified by character of pores daedaliform when mature, gloeoplerous hyphae present in tramal, and skeletal hyphae unbranched. Further differences between the new species and other related ones are listed in Table 2.

Disclosure statement
No potential conflict of interest was reported by the authors.

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