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Amylosporus guaraniticus sp. nov. (Wrightoporiaceae, Russulales) a new neotropical species from Paraguay

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Abstract
Amylosporus guaraniticus is described as a new species from Paraguay based on morphological characters and molecular evidence based on ITS and LSU markers. The species is characterized by its annual, pileate basidiomata, poroid hymenophore, a dimitic hyphal system with simple septate and double to multiple clamped generative hyphae and asperulated, amyloid basidiospores (4–6.5 × 3–4.5 µm). An identification key to *Amylosporus* species is presented.

Key words – fungal diversity, molecular phylogeny, neotropical polypores, taxonomy

Introduction

Amylosporus Ryvarden was erected to accommodate *A. campelli* (Berk.) Ryvarden characterized by having finely asperulate amyloid basidiospores, and a dimitic hyphal system with generative hyphae presenting both simple and multi-clamped septa (Ryvarden 1973).

The most recent and comprehensive phylogenetic work placed the genus within *Wrightoporiaceae* Jülich as a sister clade of *Wrightoporia* Pouzar (Chen et al. 2016). Only five species have been included in phylogenetic analyses, *i.e. A. bracei* (Murrill) A. David & Rajchenb., *A. campbellii* (Berk.) Ryvarden, *A. casuarinicola* (Y.C. Dai & B.K. Cui) Y.C. Dai, Jia J. Chen & B.K. Cui, *A. rubellus* (Y.C. Dai) Y.C. Dai, Jia J. Chen & B.K. Cui and *A. succulentus* Jia J. Chen & L.L. Shen, and they conform a monophyletic clade (Chen et al. 2016). This phylogenetic circumscription of the genus maintains the original diagnostic morphological characters (Ryvarden 1973).

Amylosporus campbelli has been considered a "pantropical" species, widely distributed in tropical and subtropical regions of the world (Ryvarden & Johansen 1980). However it has been shown that *A. campbelli* involves more than one species, and new taxa have been segregated as *A. succulentus* (Dai 2007, Chen & Shen 2014). *Amylosporus campbellii* has been recorded in South America from Argentina and Guyana (David & Rajchenberg 1985, Robledo & Rajchenberg 2007). Morphological and phylogenetic analysis of specimens collected in Paraguay purportedly

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corresponding to *A. campbellii* showed to represent a new species here described as *Amylosporus guaraniticus* sp. nov.

Materials & Methods

Morphological analyses

Studied specimens are kept at Laboratorio de Análisis de Recursos Vegetales, Área Micología, Universidad Nacional de Asunción *FACEN* herbarium and Mycoteca of Fundación FungiCosmos (FCOS). For the study of the specimens, macroscopic and microscopic observations were made on fresh and dried materials following Gómez Montoya et al. (2017). Microscopic features, measurements and drawings were made from slide preparations rehydrated with KOH 3–5%, stained with congo red, phloxine and melzer's reagent under light microscope. Measurements of basidiospores followed a standard sampling of thirty measurements (n = 30). The following abbreviations were used for basidiospore measurements X= arithmetic mean, Q = arithmetic mean the ratio of length/width, N= number of specimens.

DNA extraction, amplification and sequencing

Total DNA was extracted from small pieces of dried basidiomata triturated with LyserMjolnir 1.0 Franchi-Robledo TM 2012 for 2 o 3 min. The powder was transferred to an Eppendorf-tubes and mixed with lysis buffer consisting of 2% CTAB, at 65C for at least 1 hour. Chloroform extraction was done once, and DNA precipitated with isopropanol (Doyle 1991). Amplification of the ITS and nucLSU regions was performed using ITS8/ITS6 (including ITS1, 5.8S and ITS2) and LR0R/LR5 that for nLSU (White et al. 1990, Gardes & Bruns 1993, Hopple & Vilgalys 1999). PCR was performed following the programs and protocols indicated by Gomez Montoya et al. 2017. PCR products were visualized with 1.5% agarose gel electrophoresis. Amplified products were purified and then sequenced in both directions. In all cases, the same primer used in the amplification was used for sequencing. The cycle-sequencing was performed in an Applied Biosystems 3730xl DNA Analyser in MacroGen Ltd. (South Korea). For the analyzes were used the newly generated sequences, and including related sequences downloaded from GenBank (Table 1).

Two sequences of interest were obtained for our analysis. Additionally 26 sequences obtained from GenBank were included in the analysis. DNA sequences were edited with the software program BioEdit sequence alignment editor, version 7.0.5.3 (Hall 1999) and assembled using a text editor. The dataset was aligned using Muscle (Thompson et al. 1997). The alignment obtained was manually examined and adjusted with MEGA 6 (Tamura et al. 2013) and finally deposited at TreeBase (Submission ID 21248). To select the best phylogenetic model MEGA 6 was used (Tamura et al. 2013). A bootstrap analysis was performed with 1000000 replications. Bayesian inference under the K2 + G model (Nei & Kumar 2000) was calculated with MrBayes v. 3.2.5 (Huelsenbeck & Ronquist 2001). Two Markov chains were run from random starting trees for 50 million generations. The first 5000 generations were discarded as burn-in.

Results

Phylogenetic analyses

The complete alignment of the ITS region included 947 positions, including gaps, whereas LSU region included 571 positions including gaps. According to the preceding analyses, K2+G was chosen as the best-fit substitution model to the ITS dataset in the Bayesian Inference, with the following base frequencies A (0.250), C (0.250), G (0.250), T (0.250) and a gamma distribution shape parameter of 0.31. The nucleotide substitution rates estimated according to this model were A/G=0.176, A/T=0.037, C/G=0.37, C/T=0.176 and G/T=0.37. According to the preceding analyses, K2+G was chosen as the best-fit substitution model to the LSU dataset in the Bayesian Inference, with the following base frequencies A (0.250), C (0.250), G (0.250), T (0.250), proportion of

invariable sites of 0.025. The nucleotide substitution rates estimated according to this model were A/C=0.012, A/G=0.226, A/T=0.012, C/G=0.012, C/T=0.226, G/T=0.12. The two independent runs of Bayesian analysis converged to stable values after 50000000 generations (average standard deviation of split frequencies = 0.001267; average potential scale reduction factor = 1.000), a total of 25% of the sampled trees were discarded as burn-in. The remaining trees were used to construct the 50% majority-rule consensus trees (Figure 1).

The studied materials grouped in a strongly supported clade (BPP = 1.0) constituting an independent phylogenetic lineage which we describe below as a new species, *Amylosporus guaraniticus*. *Amylosporus campbellii*, represented by four neotropical specimens grouped with the new species conforming a strongly supported clade (BPP=1). A group of three paleotropical species consisting of *Amylosporus succulentus* from China and two unnamed species from Tanzania and India respectively conform a sister group of neotropical species. These sisters clades are strongly supported clade (BPP=1), and hereafter called 'Amylosporus campbelli clade'. *Amylosporus casuarinicola*, *A. bracei* and *A. rubellus*, relationships are not well resolved, however.

Amylosporus guaraniticus Campi & Robledo sp. nov. Figs 2–3

MycoBank number MB821985; Facesoffungi number: FoF 03568

Etymology— Guaraniticus refers to the area where Guaraní indigenous ethnicity inhabited, mainly Eastern Paraguay and NE Argentina.

Holotypus. Paraguay, San Lorenzo, University Campus, 03.III.2016, leg. M. Campi 106 (FACEN 3422).

Basidiomata annual, sessile, imbricate, semicircular, broadly adhered to the surface, 23 × 12 × 5 cm, fleshy and soft when fresh, corky when dry. **Pileal surface** glabrous to velvety, soft when fresh, whitish to pale brown, with chestnuts areas towards the base which become dark brown or blackish at maturity. Margin sterile, round, whitish to cream when fresh, acute and light brown when dry. **Pores** angular 2–3 per mm, dissepiments entire occasionally lacerate, pore surface whitish when fresh becoming cream to light brown when dry. **Context** homogeneous, fleshy, white to cream when fresh, corky and light brown when dry, up to 42 mm. Tube layer concolorous with the context up to 10 mm thick. **Hyphal system** dimitic, generative hyphae thin-walled, 4–6.5 µm diam; the context composed of with generative hyphae 6–14 µm diam., with simple septa and double or multiple clamp connections, gloeopleurous hyphae present. Skeletal hyphae restricted to the tubes, thick-walled, 3–8 µm diam, branched, flexuous, IKI–. **Basidia** clavate, 10– 25 × 5–8 µm, with four sterigmata 2–6 µm long. **Basidiospores** ellipsoid, (3.8)4–5.5(6.5) × (2.5)3–4 (4.5) µm, X=4.6 × 3.4 µm, Q= 1.4, N=2, hyaline, thin- to slightly thick-walled, finely asperulate, with a central guttule, strongly amyloid.

Known distribution – So far know from type locality. Records of *A. campbellii* in South America must be reviewed in order to confirm their identity, see discussion.

Material examined – Paraguay, Central Department, San Lorenzo city, Universidad Nacional de Asunción, Facultad de Ciencias Químicas FCQ, inside a sump, 25°23´21.55´´ S 57°10´56.1´´ W, 245 m a.s.l., 03 March 2016, M. Campi, 106 (FACEN 003422, HOLOTYPE). Ibid, Facultad de Arquitectura, Diseño y Arte, coming out from between the brick board against the floor , 25°33´38.47´´ S 57°51´64.04´´ W 245 m a.s.l., 2 Feb 2016, M. Campi, 105 (FACEN 003421).

A key to *Amylosporus* species

1. Basidiomata stipitate **2**
1'. Basidiomata resupinate to pileate. **4**

2. Skeletal hyphae non-dextrinoid. ..... ***A. campbellii***
2'. Skeletal hyphae dextrinoid. **3**

3. Pores 3–6/mm; known from NE Brazilian semiarid región .....***A. auxiliadorae***

3'. Pores large, 2–3/mm; known from China .....***A. succulentus***

4. Basidiomata pileate, demediate, imbricated .....***A. guaraniticus***

4'. Basidiomata resupinate **5**

5. Skeletal hyphae IKI– ..... ***A. ryvardenii***
5'. Skeletal hyphae dextrinoid..... **6**

6. Pores 6–8/mm **7**
6'. Pores 2–4/mm **8**

7. Multiple clamp connections present ..... ***A. bracei***
7'. Multiple clamp connections absent.....***A. efibulatus***

8. Clamps present..... ***A. iobapha***
8'. Clamp absent..... **9**

8. Basidiospores subglobose, 3.5–3.9 × 2.7–3.2 µm ..... ***A. casuarinicola***
8'. Basidiospores ellipsoid, 4–6 × 3–4.2 µm.....***A. rubellus***

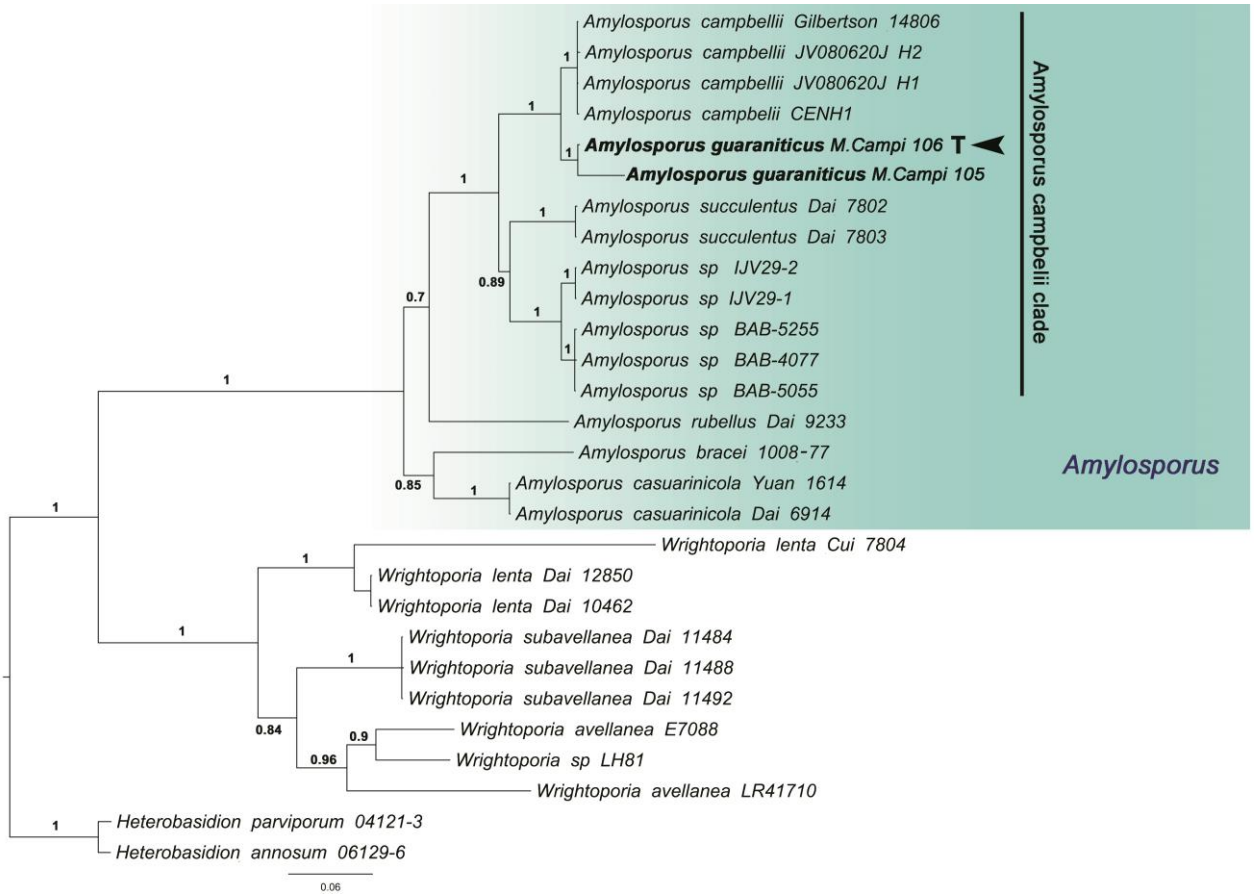

Figure 1. Strict consensus tree from Bayesian inference of the combined ITS-LSU dataset illustrating the phylogenetic relationships of *Amylosporus* within Wrightoporiaceae. The new species specimens from which new sequences were obtained in this work are in boldface. **T**◄= type specimen.

Table 1 List of species, collections, and GenBank accession numbers for the ITS and LSU sequences used in the phylogenetic analyses**.** n/d= no described; n/i= no identified

Species	Substrate	GenBank accesion N°	
Specimen N°, Locality		ITS	LSU
Amylosporus bracei (Murrill) A. David & Rajchenb.			
1008/77, USA	Angiosperm	KM267724	KJ807076
Amylosporus casuarinicola (Y.C. Dai & B.K. Cui) Y.C. Dai et al.			
Dai 6914/BJFC 2760, China, Guangxi Autonomous Region, Beihai,	Living tree of *Casuarina equisetifolia*	KJ807068	
Yuan 1614/IFP 12866, China, Yunnan Province, Xi-Shuang-Banna	Fallen angiosperm trunk	KM107862	
Amylosporus campbelli (Berk.) Ryvarden			
JV080620J-H1, Jamaica	Angiosperm	JF692200	KJ807077
JV080620J-H2, Jamaica	Angiosperm	JF692201	KJ807077
Gilbertson 14806, USA	n/d	KM107861	KM107879
CENH1, Puerto Rico,	Orchid roots	KU847468	
Amylosporus guaraniticus Campi & Robledo			
M. Campi 106 (FACEN 3422), Paraguay, Asunción (**T**)	n/i	MF377528	MF377529
M. Campi 105 (FACEN 3421), Paraguay, Asunción	n/i	MF377530	
Amylosporus rubellus (Y.C. Dai) Y.C. Dai, Jia J. Chen & B.K. Cui			
Dai 9233/IFP 12318, China, Beijing, Xiangshan	Rotten wood of *Koelreuteria sp*	KJ807071	KJ807084
Amylosporus succulentus Jia J. Chen & L.L. Shen 2014			
Dai 7802/BJFC 15731 China	Lawn	KM213669	KM213671
Dai 7803/BJFC 15732, China	Lawn	KM213668	KM213670
Amylosporus sp.			
IJV29-1, Tanzania	n/d	KM851314	KM593892
IJV29-2, Tanzania	n/d	KM851315	KM593893
BAB-5255, India	n/d	KT186196	
BAB-4077, India	n/d	KJ670296	
BAB-5055, India	n/d	KR155100	
Wrightoporia avellanea (Bres.) Pouzar			
LR 41710, Jamaica	n/d	AF506488	AF506488
E 7088, Indonesia	n/d	AJ537507	AJ53750

Table 1 List of species, collections, and GenBank accession numbers for the ITS and LSU sequences used in the phylogenetic analyses**.** n/d= no described; n/i= no identified

Species	Substrate	GenBank accesion N°	
Specimen N°, Locality		ITS	LSU
Wrightoporia lenta (Overh. & J. Lowe) Pouzar			
Dai 10462/ BJFC 4711, China, Jiangxi Province, Fenyi County	Rotten wood of *Cunninghamia*	KJ513291	KJ807082
Dai 12850/BJFC 13139, Heilongjiang Province,	Stump of Picea sp	KJ513295	KJ807085
Cui 7804, China, Jiangxi Province, Fenyi County	Stump of *Cunninghamia sp*	KJ513292	KJ807081
Wrightoporia subavellanea Jia J. Chen & B.K. Cui			
Dai 11484/ BJFC 7352, China, Guangxi Autonomous Region	Rotten wood of *Pinus*	KJ513295	KJ807085
Dai 11488/ BJFC 7356, China, Guangxi Autonomous Region	Rotten wood of *Pinus*	KJ513296	KJ807086
Dai 11492/ BJFC 7360, China, Guangxi Autonomous Region	Rotten wood of *Pinus*	KJ513297	KJ807087
Wrightoporia sp.			
LH81, Malasya, Sarawak Lambir Hills National Park	Ectomycorrhizal root tip	GQ268628	
Heterobasidion annosum (Fr.) Bref.		KJ583211	KJ583225
06129/6, Russia	*Pinus sylvestris*		
Heterobasidion parviporum Niemelä & Korhonen		KJ583212	KJ583226
04121/3, Finland	*Picea abies*		

Discussion

Amylosporus guaraniticus is characterized by fleshy, whitish pileate basidiomata, with both simple septate and double to multiple clamped generative hyphae, hyaline skeletal hyphae without reaction in Melzer´s reagent, presence of gloeoplerous hyphae; and ovoid to ellipsoid, asperulate strongly amyloid basidiospores, 4–6.5 × 3–4.5 µm.

Morphologically and phylogenetically *A. guaraniticus* is related to *A. campbellii* and *A. succulentus* within the 'A. campbellii clade'. *Amylosporus campbellii* presents similar basidiospore size, simple septate and double to multiple clamped generative hyphae, the presence of gloeoplerous hyphae and skeletal hyphae without reaction in Melzer´s reagent (Table 2). However, *A. guaraniticus* develops sessile basidiomata, broadly attached and imbricated while *A. campbellii* develops stipitate to subestipitate basidiomata single or in clusters

Species within 'A. campbellii clade' present particular substrata. *Amylosporus cambellii* has been described with a terrestrial growth on grasslands, being a possible grass parasite (Ryvarden & Johansen 1980). One of the specimens of *A. cambpelii* included in our analyses [CENH1, from Puerto Rico] stands out as it has been isolated from orchids roots (Table 1). *Amylosporus succulentus* [previously recorded as *A. campbelli* (Dai 2007)] grow on grass. *Amylosporus guaraniticus* was collected inside a sump, arising from the concrete wall (Fig. 2A-B), located in a corridor of a building distanced at least 30 meters from the nearest open place with grass and trees.

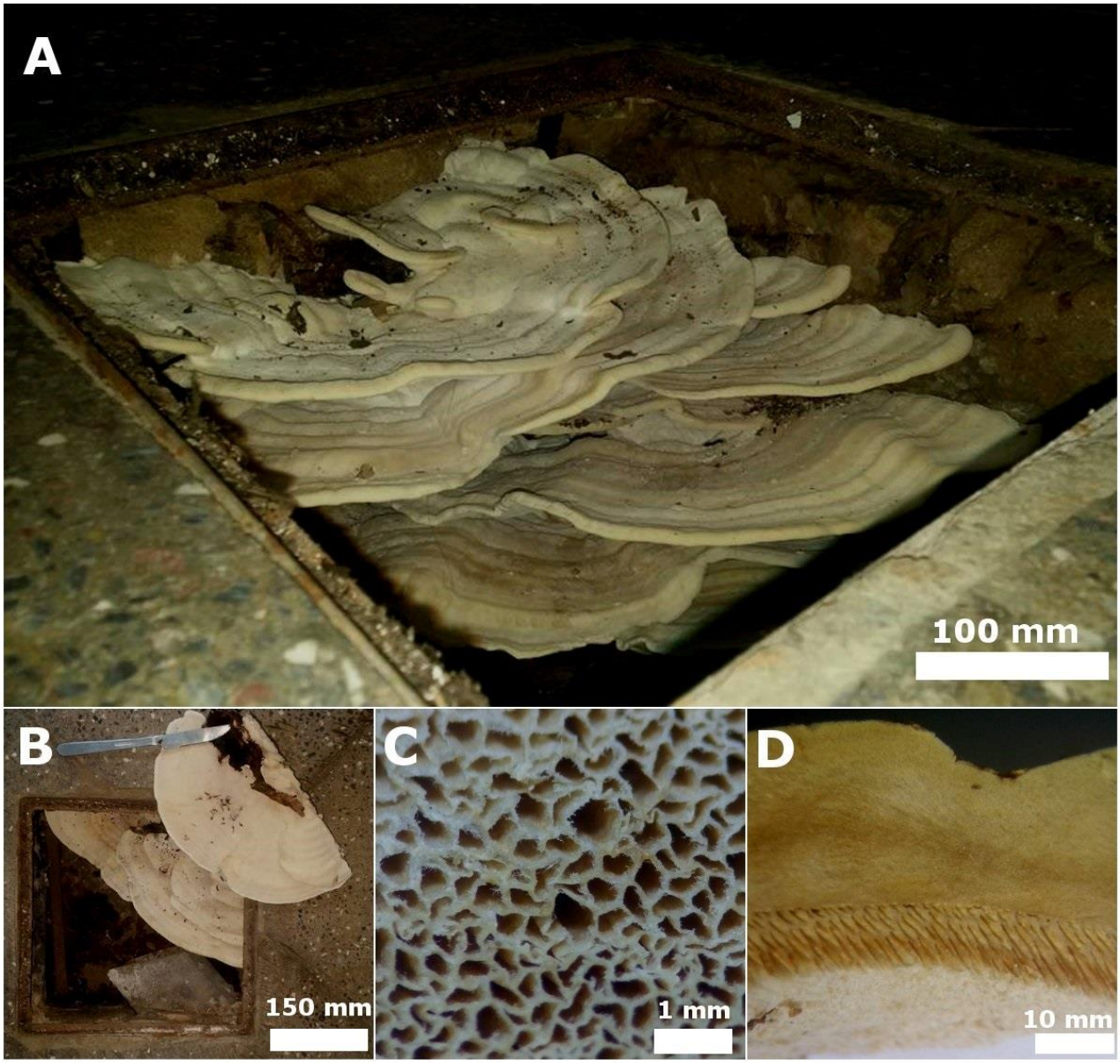

Figure 2. *Amylosporus guaraniticus* (FACEN 003422, HOLOTYPE) macroscopical features. **A-B** general view inside a sump, **C** close up of pores, **D** longitudinal section showing context ant tube layer

The mycelium could be living on a buried log or trunk, decaying organic matter, or be related to living tree roots that follow pipes, however it could not be verified so far. The definition of *Amylosporus* as a white rot genus (Chen et al. 2016) has to be reconsidered.

Our phylogenetic reconstructions recovered *Amylosporus* as monophyletic clade within the Wrightoporiaceae (Figure 1), according with previous results (Chen et al. 2016). However, five species are currently combined in *Amylosporus* so far not included into phylogentice analyses, *i.e. A. auxiliadorae* Drechsler-Santos & Ryvarden, *A. daedaliformis* G.Y. Zheng & Z.S. Bi, *A. efibulatus* (I. Lindblad & Ryvarden) Y.C. Dai, Jia J. Chen & B.K, *A. iobaphus* (Pat.) A. David & Rajchenb. and *A. ryvardenii* Stalpers. *Amylosporus auxiliadorae* presents morphological characteristics and habit (growing on ground) suggesting a relationship with 'Amylosporus campbelli clade'. *Amylosporus daedaliformis* has been described with basidiospores IKI–, suggesting phylogenetic relationships outside *Amylosporus*. *Amylosporus efibulatus* and *A. ryvardenii* (≡ *Rigidoporiopsis amylospora* Ryvarden) develop resupinate and adnate basidiomata with mono- to dimitic simple septate hyphal system (Ryvarden & Johansen 1980, Ryvarden 2000). *Amylosporus iobaphus* develops resupinate and widely effused basidiomata, with a dimitic hyphal system composed by simple septate and multi-clamped generative hyphae and dextrinoid skeletal

hyphae (David & Rajchenberg 1985). Further molecular evidence is desirable to depict the relationships of these species within Wrightoporiaceae.

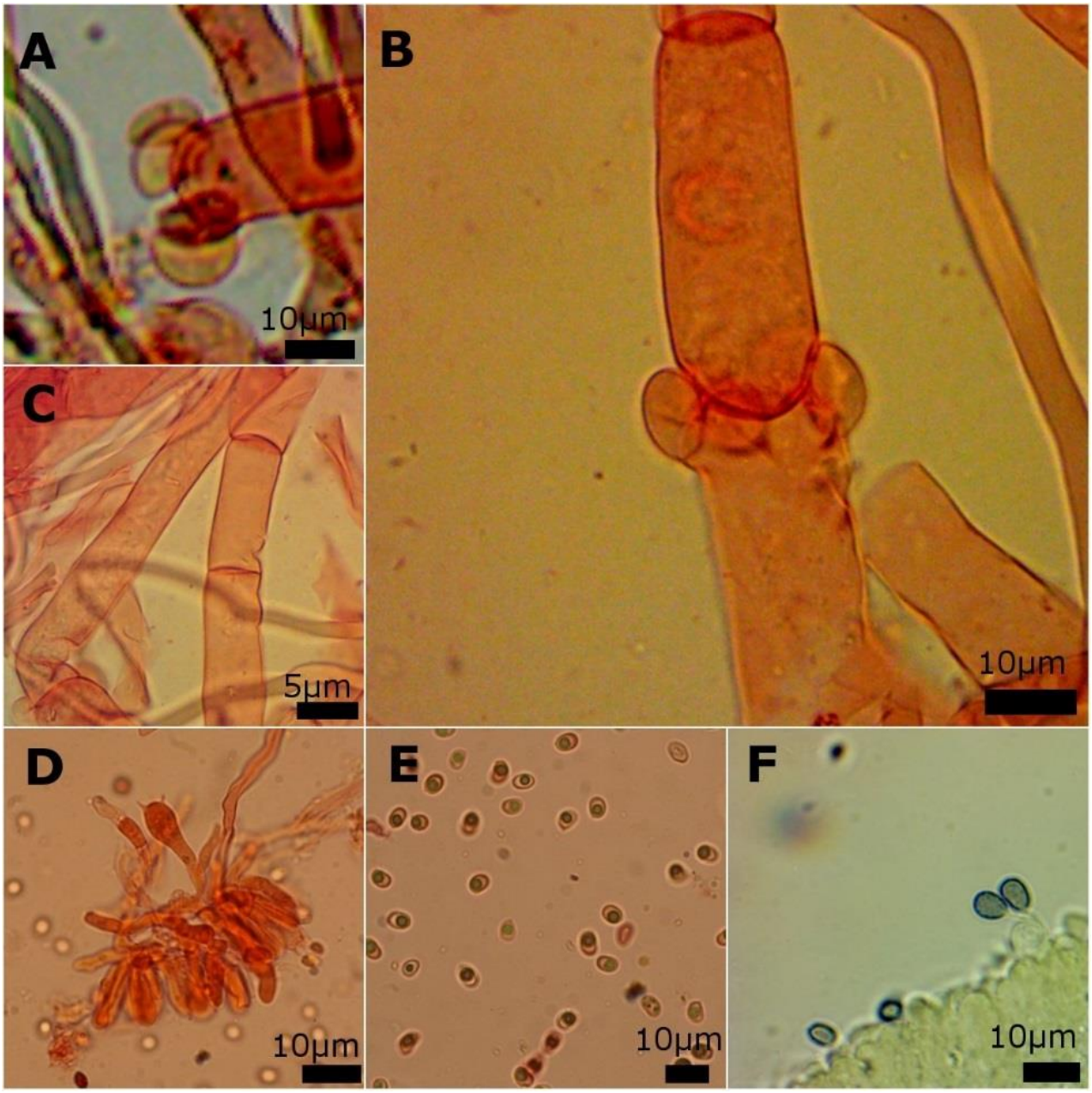

Figure 3 – *Amylosporus guaraniticus* (FACEN 003422, HOLOTYPE) microscopical features **A**-**B** multiple clamped generative hyphae. **C** simple septate generative hyphae. **D** Basidia. **E** Basidiospores in KOH + Floxine, showing refringent central guttula. **F** Basidiospores in Melzer´s reagent.

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TABLE 2. Comparative table between *Amylosporus* species, based on specimens studied and literature. **T**= description based on the type specimen, S=simple septa present, MC= multiple clamp connections present, n/d= no described, * size range based on 30 measurements.

Species	**Basidiomata**	**Pores**	**Hyphae**			**Basidiospores**
Specimens / Reference		**Size (p/mm); shape**	**septa**	**skeletal**	**GH**	**Size (µm)**
Amylosporus campbellii						
Ryvarden & Johansen (1980)	A, Stipitate	2–4, round to angular	S, MC	IKI–	+	4–5 × 2.5–4
Amylosporus guaraniticus						
This work (**T**)	A, Pileate	2–3; angular	S, MC	IKI–	+	4–5.5 × 3–4 *
Amylosporus succulentus						
Chen & Shen (2014) (**T**)	A, Stipitate	2–4, angular	S, MC	Dextrinoid	+	4.2–5.2 × 3–3.8
Amylosporus bracei						
Rajchenberg (1983) as *A. wrightii*	A, P Sessile-Resupinate	7–8, n/d	S, MC	Dextrinoid	–	3.1–3.6 × 2.6
Amylosporus casuarinicola						
Dai & Cui (2006)	P, Sessile-Resupinate	3–4, round to angular	S	Dextrinoid	–	3.5–3.9 × 2.7–3.2
Amylosporus efibulatus						
Lindblad & Ryvarden (1999) (**T**)	A, Sessile-Resupinate	6–8, round	S	IKI–	–	3–3.5
Amylosporus auxiliadorae						
Drechsler-Santoset al. (2016) (**T***)*	A, Stipitate	3–6, irregular	S, MC	Dextrinoid	+	4.0–5.0 × 2.5–4.0
Amylosporus iobaphus						
Ryvarden (1983) (**T**)	A, Sessile-Resupinate	3–4, irregular, angular	S, MC	Dextrinoid	–	3–4.5 × 3–4
Amylosporus ryvardenii						
Johansen & Ryvarden (1979) as *Rigidoporopsis amylospora* (**T**)	A, Sessile-Resupinate,	4–6, circular	S	IKI–	–	4–5 × 2.5–3
Amylosporus rubellus						
Dai (1995)	A, Sessile-Resupinate to EF	3–4, round to angular	S	Dextrinoid	+	4–6 × 3–4.2

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