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Aspergillus trisporus: A new Jani section species from Brazilian soil

Souza SC[1], Pereira VM[1], Moreira SI[2], Costa SS[2], Moreira GM[6], Mendes WO[3], Nery EM[2], Alves E[2], Chalfoun SM[4], Moreira FMS[5], Batista LR[3]*

[1] *Departamento de Biologia, Universidade Federal de Lavras, Lavras, MG 37200-000, Brazil*
[2] *Departamento de Fitopatologia, Universidade Federal de Lavras, Lavras, MG 37200-000, Brazil*
[3] *Departamento de Ciência dos Alimentos, Universidade Federal de Lavras, Lavras, MG 37200-000, Brazil*
[4] *Empresa de Pesquisa Agropecuária de Minas Gerais, Lavras, MG 37200-000, Brazil*
[5] *Departamento de Ciências do Solo, Universidade Federal de Lavras, Lavras, MG 37200-000, Brazil*
[6] *Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG 36570-000, Brazil*

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Abstract

Phenotypic characters and phylogenetic analyses based on *ITS*, β-tubulin (*BenA*) and the second largest subunit of RNA polymerase II (*RPB2*) gene sequences were applied to identify *Aspergillus trisporus*, a third member of the *Aspergillus Jani* section isolated from soil in the Ferriferous Quadrangle, Brazil. This new species has, as morphological markers, stipes of white conidiophores markedly smaller than the other species of the section and slow growth in all culture media evaluated. *A. trisporus* is phylogenetically and phenotypically more related to *A. brevijanus*, but it can be differentiated by non-growth at 37 °C and by the shorter stipes of the white conidiophores and predominantly clavated vesicles. This report contributes to the knowledge of Brazilian soils' fungal diversity, especially in poorly explored habitats such as the Ferriferous Quadrangle.

Key words – 1 new species – multilocus phylogeny – soil-borne fungi – taxonomy

Introduction

One of the oldest genera discovered, *Aspergillus* was first described almost 300 years ago by Micheli (1729). Since then, species of this genus have become increasingly well-known and much studied due to their beneficial characteristics, such as production of antibiotics, organic acids and enzymes. In addition, they are also commonly recognized for their negative impact on human activities, associated with food spoilage and mycotoxin production; some species are also described as pathogens of humans (Samson et al. 2014).

The genus *Aspergillus* is quite diverse, considered cosmopolitan and able to colonize an extensive variety of substrates, especially soils and decaying vegetation. This ability is related to its rapid growth over a wide temperature range (6 to 55 °C), its high production of conidia, enzymes and other secondary metabolites (Klich 2002, Bennett 2010, Krijgsheld et al. 2013).

Advances in the analysis techniques for molecular biology and metabolites culminated in changes within the species classification of *Aspergillus*, and currently it is carried out by a

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Corresponding Author: Luis Roberto Batista – e-mail – luisrb@dca.ufla.br

polyphasic approach, based on colony characteristics, micromorphology, phylogeny and, in some cases, metabolite production (Samson et al. 2014, Tanney et al. 2014). Important modifications in the classification of *Aspergillus* can be mentioned, such as the division of the extensive family Trichocomaceae into three distinct families, Trichocomaceae, Aspergillaceae and Thermoascaceae, based on multigenic phylogeny (Houbraken & Samson 2011). After this, the recent changes in the International Code of Nomenclature for Algae, Fungi and Plants (McNeill et al. 2012), mainly to support the principle of "one fungus: one name", led to discussions about whether to divide teleomorphs and anamorphs of *Aspergillus* into distinct genera or to keep them as a single genus. Thus, the currently accepted nomenclature is the maintenance of the name *Aspergillus* for the two states of life (Houbraken et al. 2014, Pitt & Taylor 2014, Samson et al. 2014, Kocsubé et al. 2016). Finally, many studies have been carried out in order to reorganize the *Aspergillus* subgenera and sections based on phylogeny. Examples of this are the species *A. janus* and *A. brevijanus*, previously classified in the *Versicolores*, *Terrei* and *Flavipedes* sections, but which now belong to the *Jani* section (Hubka et al. 2015, Jurjević et al. 2015).

The *Jani* section comprises two species to date often associated with soil in many countries (Hubka et al. 2015). The *Aspergillus janus* Raper & Thom neotype strain was isolated from soils in Panama, and the *Aspergillus brevijanus* S.W. Peterson neotype strain, formerly known as *Aspergillus janus* var. *brevis* Raper & Thom, was obtained from soils in Mexico (Raper & Thom 1944, Peterson 2008). The first reports of *A. janus* and *A. brevijanus* were based only on morphological markers, but the more recent works demonstrated that both species represent monophyletic groups, strongly supported by phylogenetic data using DNA sequences of the *ITS* region of rDNA and partial sequences of *BenA*, calmodulin, and *RPB2* genes (Peterson 2008, Hubka et al. 2012, 2015).

A strain with the typical morphological characters of the *Jani* section, but morphologically different from the two described species in this group, was isolated from collections made in soils of the Ferriferous Quadrangle, Brazil. This area is recognized for its mineral wealth and as a biodiversity hotspot, due to important deposits of iron and gold and its unique environment, which has a number of endemic species that face a risk of extinction. In this study we describe and illustrate this identified strain, based on multilocus phylogeny and morphological characterization, as a new species within the *Jani* section.

Materials & Methods

Fungal isolation

Two strains were obtained from soil samples collected at a depth of 20 cm under cultivation of *Eucalyptus* sp. in the VALE Biodiversity Center (VALE S.A. Company) in the Ferriferous Quadrangle, Minas Gerais, Brazil. Isolations were carried out using the soil dilution plate (10^1 to 10^4) on Dichloran Glycerol Agar (DG18) and Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) media. After fungal growth, the monoconidial purification of the strains were done on Malt Extract Agar (MEA) and the fungi were deposited in the Coleção de Culturas do Departamento de Ciências dos Alimentos (CCDCA) and in the Coleção Micológica de Lavras (CML) in the Plant Pathology Department (Table 1). Both collections are located at the Universidade Federal de Lavras - UFLA, Minas Gerais, Brazil.

DNA extraction, PCR and Phylogenetic analyses

The strains were grown on potato-dextrose-agar and the biomass was harvested into 1.5 mL centrifuge tube. The total DNA of the strains were extracted using CTAB method (Doyle & Doyle 1987). Portions of the internal transcribed spacer region (*ITS*, ITS1 and ITS4, White et al. 1990), the second largest subunit of RNA polymerase II (*RPB2*, fRPB2-5F and fRPB2-7cR, Liu et al. 1999), β-tubulin (*BenA*, TUB2FD and TUB4RD, Aveskamp et al. 2009), and calmodulin (*CaM*, CMD5 and CMD6, Hong et al. 2005) were amplified using a GoTaq Colorless Master Mix (Promega) in T100 Thermal Cycler (Bio-Rad). Cycling conditions for *ITS RPB2* and *CaM* were

according to Visagie et al. (2014) and for *BenA* were: 94 C for 2 min, 40 cycles of 94 C for 30 s, 54 C for 30 s and 72 C for 40 s, and a final extension at 72 C for 4 min. Gene regions were sequenced on a 3500 XL (Applied Biosystems) sequencer.

Bidirectional DNA sequences for each region were assembled by software SeqAssem (Hepperle 2004). The sequences were compared with those deposited in BLAST (Altschul et al. 1997), and additional sequences of type isolates of other species of *Aspergillus* were obtained from GenBank, NCBI (Table 1). The sequences were aligned by using ClustalW implemented in the MEGA X (Kumar et al. 2018). The alignments were deposited in TreeBASE (submission ID 21412, http://purl.org/phylo/treebase/phylows/study/TB2:S21412). Maximum parsimony (MP) and Maximum likelihood (ML) analyses were performed using MEGA X for each of the three gene regions and for the combined data sets. Clade stability was assessed with 1000 bootstrap replications (Alfaro et al. 2003). ML analysis was performed under the Tamura-Nei model of nucleotide substitution, with gamma rate heterogeneity and a proportion of invariant sites (TN93+G+I). Sequences of *A. campestris* (NRRL 13001) and *A. candidus* (NRRL 303), from the *Candidi* section, were selected as outgroup taxa. The DNA sequences generated in this study have been deposited in GenBank (Table 1).

Morphological characterization

The strains were grown at 25 °C in darkness on Czapek Yeast Extract Agar (CYA), Malt Extract Agar (MEA), Yeast Extract Sucrose Agar (YES) and Creatine Agar (CREA) for macro-morphological observations. Plates with CYA were also incubated at 30 and 37 °C (Samson et al. 2014, Hubka et al. 2015). The cultures were grown in triplicate as a three-point inoculation on each medium in 90 mm diameter Petri dishes. Macro-morphology was described after 14 days of incubation when the colony color was fully expressed and all typical features were present (Hubka et al. 2015).

Microscopic observations were made using mounts in lactic acid from colonies grown on YES; alcohol 70% was sprayed to remove air bubbles and excess of conidia. An inverted bright field Zeiss Axio Observer Z1 microscope with differential interference contrast (DIC) (Zen 2012 software) and a Nikon SMZ1500 stereoscope microscope (NIS Elements D3.2 software) were employed for micro-morphological examinations. When possible, the morphological structure measures were based on 30 units of each structure. To verify the occurrence of Hülle cells, subsequent examinations were done at 21 and 28 days of incubation. The images obtained were edited using Corel Draw X7.

Fragments of colonies grown on MEA 25 °C for 14 days were fixed in Karnovsky solution (pH 7.2), post-fixed with 1% osmium tetroxide solution and dehydrated in an acetone series for observation under Scanning Electron Microscopy (SEM) (Bozzola & Russell 1999). Drying of the specimen was performed on the Critical Point apparatus (Bal-tec CPD 030 Balzers) followed by gold coating on Sputter Coater evaporator (Bal-tec SCD 050 Balzers). The acquisition of the images was performed with the Zeiss LEO EVO 40 XVP MEV in the UFLA Electronic Microscopy and Ultrastructural Analysis Laboratory, using Smart Sem software, at 20 Kv and a working distance of 6 mm. The images acquired were edited using Corel Draw X7.

Results

Phylogenetic analyses

Sequences from fragments of *RPB2*, *BenA*, *ITS*, and *CaM* were compared with sequences of type isolates of other species from *Aspergillus* subgroups in GenBank (Hubka et al. 2015). The aligned *RPB2* sequences consisted of 1014 bp (353 variable sites of which 326 were phylogenetically informative), *BenA* of 610 bp (333/294), *ITS* of 477 bp (96 / 84), and *CaM* of 633bp (348/302). There were no conflicts among the topologies obtained using ML and MP, once the ML tree is shown in Fig. 1. In the phylogenetic analyses, a strongly supported clade was formed

only with strains CCDCA FI15 and CCDCA 11948 within the *Jani* section, distinct from other species in this section, and it has *A. brevijanus* as sister species (Fig. 1).

Based on phylogenetic and morphological analysis the strains CCDCA FI15 and CCDCA 11948 were described as *Aspergillus trisporus* sp. nov.

Table 1 *Aspergillus* isolates and reference sequences of *ITS*, *RPB2*, *BenA* and *CaM* regions used in the phylogenetic analyses.

Species	**Code(s)***	**Origin**	**Reference**	**GenBank Accession Number**			
				ITS	***RPB2***	***BenA***	***CaM***
A. trisporus sp. nov.	CCDCA FI 15/ CML3603	Soil, Brazil	This work	MF616388	MF616389	MF616387	MN013146
A. trisporus sp. nov.	CCDCA 11948	Soil, Brazil	This work	MK995631	MN013143	MN013144	MN013145
A. ardalensis	CCF 4031	Soil, Spain	Hubka et al. 2015	FR733808	HG916704	HG916683	HG916725
A. flavipes	NRRL 302	Bainier's culture of *Sterigmatocystus flavipes*, France	Thom & Church 1926	EF669591	EF669633	EU014085	EF669549
A. iizukae	NRRL 3750	Soil, Japan	Sugiyama 1967	EF669597	EF669639	EU014086	EF669555
A. mangaliensis	CCF 4698	Soil, Romania	Hubka et al. 2015	HG915902	HG916716	HG916695	HG916738
A. frequens	NRRL 4578	Soil, Haiti	Hubka et al. 2015	EF669602	EF669644	EU014082	EF669560
A. neoflavipes	NRRL 5504	Cellulosic material buried in forest soil, Thailand	Hubka et al. 2015	EF669614	EF669656	EU014084	EF669572
A. spelaeus	CCF 4425	Cave sediment, Spain	Hubka et al. 2015	HG915905	HG916719	HG916698	HG916741
A. polyporicola	NRRL 58570	Basidioma of *Earliella scabrosa*, USA	Hubka et al. 2015	EF669595	EF669637	EU014088	LM644252
A. movilensis	CCF 4410	Soil, Romania	Hubka et al. 2015	HG915904	HG916718	HG916697	HG916740
A. luppii	NRRL 6326	Natural truffle soil, France	Hubka et al. 2015	EF669617	EF669659	EU014079	EF669575
A. neoniveus	NRRL 5299	Forest soil, Thailand	Samson et al. 2011	NR137474	EF669654	EU014098	EF669570
A. ambiguus	NRRL 4737	Savannah soil, Somalia	Sappa 1955	NR135400	EF669648	EF669534	EF669564
A. microcysticus	NRRL 4749	Savannah soil, Somalia	Sappa 1955	NR135401	EF669649	EF669515	EF669565
A. allahabadii	NRRL 4539	Soil, India	Mehrotra & Agnihotri 1962	NR135399	EF669643	EF669531	EF669559
A. carneus	NRRL 527	Culture contaminant, USA	Blochwitz 1933	NR135402	EF669653	EF669529	EF669569
A. neoindicus	NRRL 6134	Soil, India	Samson et al. 2011	NR131296	EF669658	EF669532	EF669574
A. aureoterreus	NRRL 1923	Wheat flour, India	Samson et al. 2011	EF669580	EF669622	EF669524	EF669538
A. alabamensis	NRRL 29810	Soil, USA	Balajee et al. 2009	EF669589	EF669631	EF669522	EF669547
A. terreus	NRRL 255	Soil, USA	Thom & Church 1926	EF669586	EF669628	EF669519	EF669544
A. pseudoterreus	NRRL 4017	Soil, Argentina	Samson et al. 2011	NR137472	EF669640	EF669523	EF669556

Table 1 Continued.

Species	Code(s)*	Origin	Reference	GenBank Accession Number			
				ITS	*RPB2*	*BenA*	*CaM*
A. brevijanus	NRRL 1935	Soil, Mexico	Hubka et al. 2015	EF669582	EF669624	EU014078	EF669540
A. janus	NRRL 1787	Soil, Panama	Hubka et al. 2015	NR131295	EF669620	EU014076	EF669536
A. campestris	NRRL 13001	Soil, USA	Christensen 1982	EF669577	EF669619	EU014091	EF669535
A candidus	NRRL 303	Unknown	Link 1809	EF669592	EF669634	EU014089	EF669550

*Abbreviations of culture collections: CCDCA = Coleção de Culturas do Departamento de Ciências dos Alimentos, Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais, Brazil; CML = Coleção Micológica de Lavras, UFLA, Lavras, Minas Gerais, Brazil; NRRL = Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois, USA; CCF = Culture Collection of Fungi, Charles University, Prague, Czech Republic.

Taxonomy

Aspergillus trisporus Souza, Pereira, Moreira & Batista, sp. nov. Fig. 2

Index Fungorum number: IF 556635; Facesoffungi number: FoF 06279; MycoBank number: MB822378

Etymology – The epithet '*trisporus*' refers to the three distinct conidia produced by the species.

Holotype – CCDCA FI15.

Asexual morph *Conidiophores* in three different types. *Conidiophore* in green heads radiate, biseriate, stipes uncolored to pale brown, smooth-walled, usually non-septate, 84–138 µm ($\bar{x}$=97±12) µm (n=25) × 4– 8 µm ($\bar{x}$=6±0.6) µm (n=25). *Vesicles* pyriform to subclavate, 9–12 µm ($\bar{x}$=10±1) µm (n=28) long and 5–8 µm ($\bar{x}$=6±0.7) (n=28) wide. *Metulae* 5–9 µm ($\bar{x}$=7±0.8) (n=29) long. *Phialides* 5–7 µm ($\bar{x}$=6±0.6) (n=30) long. *Conidia* green, markedly echinulate and globose to subglobose, 3–4.3 ($\bar{x}$=3.6±0.5) µm diam. (without echinules). *Conidiophore* in white heads radiate, biseriate, stipes uncolored, smooth walled, usually non-septate, 325–740 µm ($\bar{x}$=514±114) (n=24) × 5–9 µm ($\bar{x}$=7±1) (n=24). *Vesicles* clavate, 19–25 µm ($\bar{x}$=23±1.9) (n=17) long and 13–20 µm ($\bar{x}$=16±1.9) (n=17) wide. *Metulae* 6–10 µm ($\bar{x}$=8±0.8) (n=30) long. *Phialides* 5–8 µm ($\bar{x}$=6±0.8) (n=30) long. *Conidia* hyaline, smooth and globose to subglobose, elliptical or ovoid, 2.2–3.8 µm ($\bar{x}$=2.7±0.5) (n=25) diam. *Conidiophore* micro- to semi-macronematous hyaline. *Conidia* hyaline, globose to subglobose, 3.3–5 µm ($\bar{x}$=4.2±0.5) (n=30) diam. Sexual morph not observed. Hülle cells were not present.

Culture characteristics – Colonies on CYA at 25 °C reach 22–27 mm diameter in 14 d (Fig. 2A), floccose to granular, raised, wrinkled, irregular margin, sporulation greyish green, surface white to yellowish, white segments in margin, white and gray sterile mycelium in the colony center, reverse brownish. On CYA at 30 °C colonies attain 25–33 mm diameter (Fig. 2B), moderately raised, irregularly wrinkled, velutinous, light gray bluish coloration, with a lower field dark green and point white mycelium masses, reverse light brown. Colonies on CREA attain 17–21 mm diameter (Fig. 2C), floccose, white coloration, no acid production. On YES colonies reaching 24–27 mm diameter (Fig. 2D), raised, velutinous, greyish-dark green and white sporulation near the margin, white and gray sterile mycelium in the colony center, reverse light brownish in center and dark green and pale

near the margin. Colonies on MEA attain 18–21 mm diameter (Fig. 2E), flat, low, velutinous, irregular margin, green sporulation near the margin and white gray covering the colony, mycelium in margin white to yellowish pale, reverse light brown. No growth on CYA at 37 °C. On YES two different growing lines of sporulation were observed, green and white (Fig. 2F, G). The white conidiophores were taller than the green one (Fig. 2H, I).

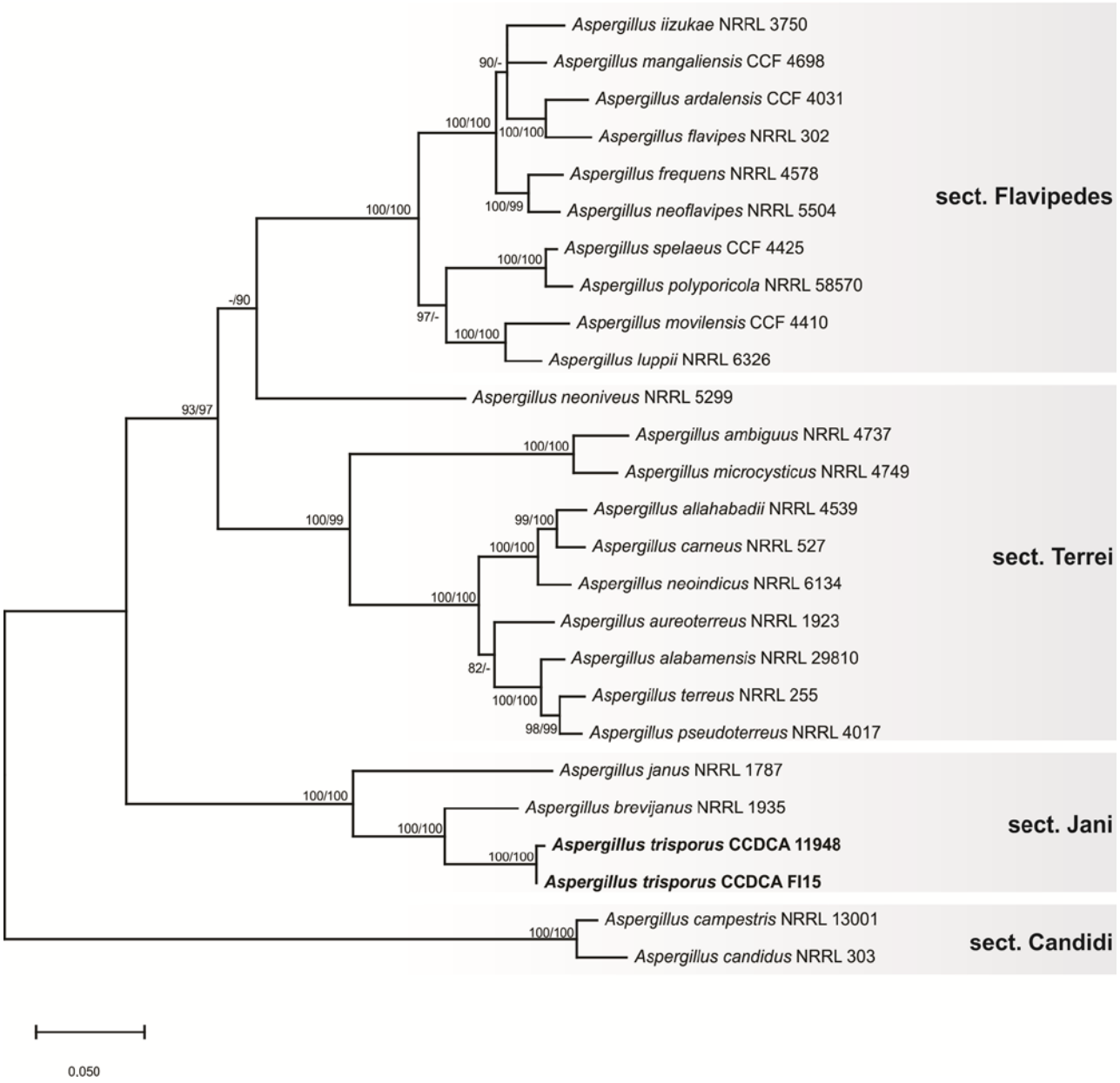

Fig. 1 – Maximum likelihood phylogenetic tree inferred from combined *RPB2*, *BenA*, *ITS* and *CaM* sequences showing the phylogenetic relationships of strain CCDCA FI15 and CCDCA 11948 with species of *Aspergillus*. The TN93 model of nucleotide substitution, with gamma rate heterogeneity and a proportion of invariant sites, was used as the model for nucleotide substitution. ML/MP bootstrap values > 70 % are in the nodes. This tree is rooted with *A. campestris* (NRRL 13001) and *A. candidus* (NRRL 303). Abbreviations of culture collections: CCDCA= Coleção de Culturas do Departamento de Ciências dos Alimentos, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; NRRL = Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria Illinois, USA; CCF = Culture Collection of Fungi, Charles University, Prague, Czech Republic.

Material examined – Brazil, Minas Gerais State, Sabará, Ferriferous Quadrangle soils, on soil, February 2014, Vanessa M. Pereira (Holotype CCDCAFI 15, CML 3603 – ex-type culture).

Notes – *Aspergillus trisporus* differs morphologically from *A. janus* and *A. brevijanus* in its shorter stipes of the white conidiophores and predominantly clavated vesicles. Shorter green conidiophores and the absence of Hülle cells differentiate it from *A. janus*, and no growth on CYA at 37 °C distinguishes it from *A. brevijanus*. The macro and microscopic characteristics of *A. trisporus* compared to the characteristics of *A. janus* and *A. brevijanus* are shown in Table 2. This new species differs from its closest phylogenetic species, *A. brevijanus* and *A. janus*, by ML and MP analyses, based on alignments of the concatenate sequences of *ITS* (MF616388), *RPB2* (MF616389), *BenA* (MF616387), *CaM* (MN013146) and deposited in TreeBASE (S21412).

Discussion

Aspergillus trisporus nov. sp. belongs to the *Jani Aspergillus* section, which is composed of two other species, *A. janus* and *A. brevijanus*. *Aspergillus trisporus* presented typical characteristics of the section (Raper & Thom 1944, Hubka et al. 2015), with green and white sporulation and grey and white sterile mycelia on CYA, YES and MEA media incubated at 25 °C. Besides, it exhibits the typical conidiogenesis of the *Jani* section (Hubka et al. 2015), with at least three types of conidiophores: short biseriate conidiophores producing green echinulated conidia; long biseriate conidiophores with white and smooth conidia; and micro- to semi-macronematous conidiophores producing white and smooth accessory conidia (Fig. 2). This species differs phylogenetically from *A. janus* and *A. brevijanus*, with more proximity to *A. brevijanus* (Fig. 1). This closer kinship level can also be observed when considering the morphological aspects, since it has more characteristics in common with *A. brevijanus* than *A. janus*. The presence of shorter green conidiophores and the absence of Hülle cells are characteristics shared by *A. trisporus* and *A. brevijanus*, contrasting with *A. janus*, with longer green conidiophores and the presence of Hülle cells (Table 2). However, the white conidiophores of *A. trisporus* differ from *A. brevijanus* and *A. janus*, with shorter stipes and predominantly clavated vesicles (Table 2). In addition, *A. trisporus* and *A. janus* can be differentiated from *A. brevijanus* by the lack of ability to develop in CYA medium at 37 °C (Table 2).

Other species of the *Jani Aspergillus* section were also reported associated with soils. Strains of *A. brevijanus* were isolated from soils in Mexico (Raper & Thom 1944) and Turkey (Azaz & Pekel 2002). On the other hand, isolates of *A. janus* were associated with soils in many countries, such as Brazil (in sand) (Gomes et al. 2008), Czech and Slovak Republics (Nováková et al. 2012), Egypt (in sand) (Migahed 2003), Pakistan (Mirza & Bajwa 2005), Panama (Raper & Thom 1944), Turkey (Asan 2004) and the USA (Hodges 1962). Besides that, strains of *A. janus* were found either in other environments, such as in blue pine seed in Pakistan (Farooq 2000), in a cave in Israel (Grishkan et al. 2004), in air and hospital air in India (Singh & Singh 1999), in foodstuffs in Turkey (Asan 2004), in aeciospores of *Cronartium comandrae* in Canada (Powell 1971), in the digestive tract of *Panstrongylus megistus* in Brazil (Moraes et al. 2001), in a keyboard in Italy (Piccoli et al. 2001) and in carton material in the USA (Narciso & Parish 1997).

The Ferriferous Quadrangle represents one of the most important mining regions in Brazil, containing large iron and gold mines (Azevedo et al. 2012). This region is also well known for its natural aspects and great beauty (Lamounier et al. 2011). It stands out, nationally, due to its unique environment, and it hosts many endemic species and some that are under risk of extinction, so it is considered a region of “extreme biological importance” (Costa 1998, Drummond et al. 2005). Thus, the description of *A. trisporus* contributes to the knowledge of the under-estimated fungal diversity, especially in Brazilian soils, and the species-rich *Aspergillus* genus, confirming the necessity of protecting natural habitats, such as those found in the Ferriferous Quadrangle.

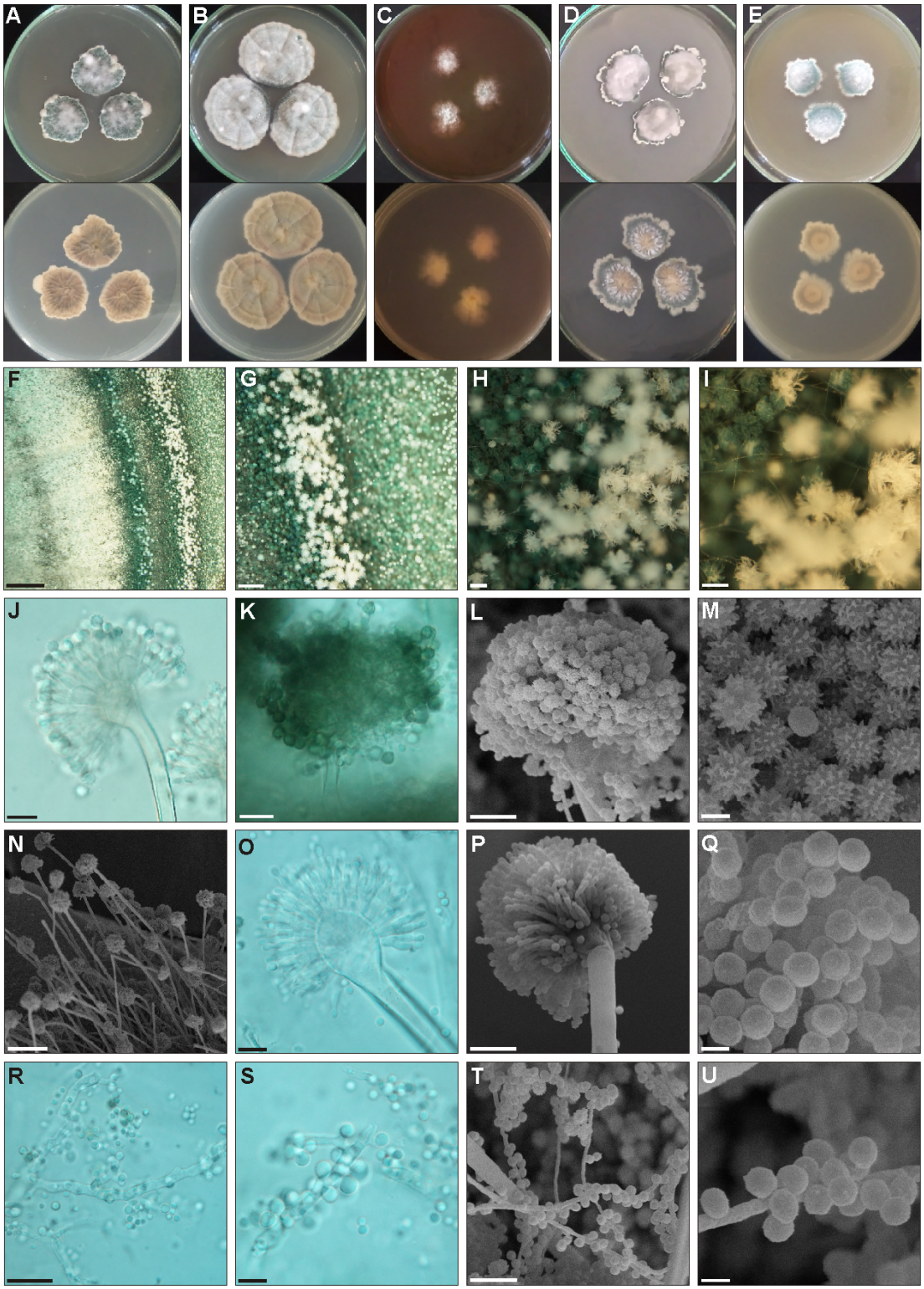

Fig. 2 – *Aspergillus trisporus* morphology. A–E Surface and reverse of colonies incubated 14 days in different culture media: (A) CYA 25 ºC, (B) CYA 30 ºC, (C) CREA 25 ºC, (D) YES 25 ºC, (E) MEA 25 ºC. F–I Details of the colony growth in the YES medium observed using a stereomicroscope. J–U Micro-morphological characters of *A. trisporus* incubated at 25 ºC on YES medium in darkness for 14 days: (J–M) green and echinulate sporulation in biseriate conidiophores, (N–Q) white sporulation and smooth walled conidia in long stipe biseriate conidiophores, (R–U) micro- to semi-macronematous conidiophores producing accessory conidia. J, K, O, R, S: bright field microcopy with DIC. L, M, N, P, Q, T, U: scanning electron microcopy. L, P, T: 3k× magnification. M, Q, U: 10k× magnification. Scale bars: F–G = 500 µm, H–I, N = 100 µm, J–L, O–P, R–T = 10 µm. M, Q, U = 2 µm.

Table 2 *Aspergillus trisporus* sp. nov. macro- and micro-morphology characteristics analyzed and compared with *A. janus* and *A. brevijanus*.

		***A. trisporus* sp. nov.**	***A. janus***	***A. brevijanus***
Colony Diameter (mm)	CYA 25 ºC	22–27	32–52	28–44
	CYA 30 ºC	25–33	–	–
	CYA 37 ºC	Not growth	Not growth	10–15
	CREA 25 ºC	17–21	20–36	21–22
	YES 25 ºC	24–27	–	–
	MEA 25 ºC	18–21	33–42	28–35
Green conidiophore structures (µm)*	Stipe length	84–138	500	55–120
	Vesicle length	9–12	(4.5–) 9–16 (–21)	7.5–12
	Vesicle shape	Pyriform to subclavate	Globose, pyriform or spatulate	Pyriform
	Metule length	(5–) 7 (–9)	(3–) 4.5–6 (–7.5)	4–7.5
	Phialide length	5–7	(3–) 4–5.5 (–6.5)	4.5–5.5
	Conidia diameter	(3–) 3.6 (–4.3)	2.5–3.4	3.1–4
	Conidia surface	Markedly echinulate	Markedly echinulate	Echinulate
	Conidia shape	Globose to subglobose	Globose to subglobose	Globose to subglobose
	Conidial heads	Radiate	Radiate to short columnar	Radiate
White conidiophore structures (µm)*	Stipe length	(325–) 514 (–740)	+1000	+1000
	Vesicle length	(19–) 23 (–25)	(10–) 45 (–75)	9–20
	Vesicle shape	Clavate	Spatulate, clavate, globose or pyriform	Pyriform
	Metule length	(6–) 8 (–10)	(4–) 6.5 (–8)	(5.5–) 7 (–9)
	Phialide length	(5–) 6 (–8)	(4–) 6 (–7)	5–6.5
	Conidia diameter	(2.2–) 2.7 (–3.8)	2.1–3	(2.2–) 2.5–3.1 (–3.4)
	Conidia surface	Smooth	Smooth	Smooth
	Conidia shape	Globose, subglobose, ovoid or elliptical	Globose, subglobose, or elliptical	Globose, subglobose, ovoid or elliptical
	Conidial heads	Radiate	Loosely radiate to radiate	Loosely radiate to radiate
Micro- to semi-macronematous conidiophore structures (µm)*	Conidia diameter	(3.3–) 4.2 (–5)	(3–) 4.5 (–5.5)	3–5
	Conidia shape	Globose to subglobose	Globose, subglobose, elliptical or clavate	Globose, subglobose, elliptical or clavate
Hülle cell (µm)*	Conidia diameter	-	5–15	-

**Aspergillus trisporus* sp. nov. measures obtained from colonies grown on YES medium at 25 ºC in darkness. Measurement data from *A. janus* and *A. brevijanus* were obtained from Hubka et al. (2015) with the neotype strains.

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References

Alfaro ME, Zoller S, Lutzoni F. 2003 – Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Molecular Biology and Evolution 20, 255–266.

Altschul SF, Madden TL, Schäffer AA, Zhang J et al. 1997 – Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–402.

Asan A. 2004 – *Aspergillus*, *Penicillium*, and related species reported from Turkey. Mycotaxon 89, 155–157.

Aveskamp MM, Verkley GJM, Gruyter J, Murace MA et al. 2009 – DNA phylogeny reveals polyphyly of *Phoma* section *Peyronellaea* and multiple taxonomic novelties. Mycologia 101, 363–382.

Azaz AD, Pekel O. 2002 – Comparison of soil fungi flora in burnt and unburnt forest soils in the vicinity of Karicak (Alanya, Turkey). Turkish Journal of Botany 26, 409–416.

Azevedo UR, Machado MMM, Castro PTA, Renger FE et al. 2012 – Geoparque Quadrilátero Ferrífero (MG). In: Schobbenhaus C, Silva CR. (Eds.) Geoparques do Brasil/Propostas v. 1. CPRM, Rio de Janeiro, pp. 183–220.

Balajee SA, Baddley JW, Peterson SW, Nickle D et al. 2009 – *Aspergillus alabamensis*, a new clinically relevant species in the section Terrei. Eukaryotic Cell 8, 713–722.

Bennett JW (Ed.). 2010 – An overview of the genus *Aspergillus*. Caister Academic.

Blochwitz A. 1933 – Die Gattung *Aspergillus*. Neue Species, Synonyme und Nachträge. Annales Mycologici 3, 73–83.

Bozzola JJ, Russell LD. 1999 – Electron microscopy. Jones and Bartlett, Boston.

Christensen M. 1982 – The *Aspergillus ochraceus* group: two new species from western soils and a synoptic key. Mycologia 74, 210–225.

Costa CMR. 1998 – Biodiversidade em Minas Gerais: Um atlas para sua conservação 1a Ed. Fundação Biodiversitas, Belo Horizonte.

Doyle JJ, Doyle JL. 1987 – A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19, 11–15.

Drummond GM, Martins CS, Machado ABM, Sebaio FA, Antonini Y. 2005 – Biodiversidade em Minas Gerais: Um Atlas para sua Conservação. Fundação Biodiversitas, Belo Horizonte.

Farooq A. 2000 – Mycoflora associated with blue pine seed. The Pakistan Journal of Forestry 50, 25–31.

Gomes D, Cavalcanti M, Fernandes M, Lima D, Passavante J. 2008 – Filamentous fungi isolated from sand and water of “Bairro Novo” and “Casa Caiada” beaches, Olinda, Pernambuco, Brazil. Brazilian Journal of Biology 68, 577–582.

Grishkan I, Nevo E, Wasser SP. 2004 – Micromycetes from the saline arubotaim cave: Mount Sedom, the Dead Sea southwestern shore, Israel. Journal of Arid Environments 57, 431–443.

Hepperle D. 2004 – SeqAssem©: A sequence analysis tool, contig assembler and trace data visualization tool for molecular sequences. http://www.sequentix.de (accessed 07 February 2017).

Hodges CS. 1962 – Fungi isolated from southern forest tree nursery soils. Mycologia 54, 221–229.

Hong SB, Go SJ, Shin HD, Frisvad JC, Samson RA. 2005 – Polyphasic taxonomy of *Aspergillus fumigatus* and related species. Mycologia 97:1316–1329.

Houbraken J, De Vries RP, Samson RA. 2014 – Modern taxonomy of biotechnologically important *Aspergillus* and *Penicillium* species. Advances in Applied Microbiology 86, 199–249.

Houbraken J, Samson RA. 2011 - Phylogeny of *Penicillium* and the segregation of Trichocomaceae into three families. Studies in Mycology 70:1–51.

Hubka V, Kubatova A, Mallatova N, Sedlacek P et al. 2012 – Rare and new etiological agents revealed among 178 clinical Aspergillus strains obtained from Czech patients and characterized by molecular sequencing. Medical Mycology 50, 601–610.

Hubka V, Novakova A, Kolarik M, Jurjevic Z, Peterson SW. 2015 – Revision of *Aspergillus* section Flavipedes: seven new species and proposal of section Jani sect. nov. Mycologia 107, 169–208.

Jurjević Ž, Kubátová A, Kolarik M, Hubka V. 2015 - Taxonomy of *Aspergillus* section Petersonii sect. nov. encompassing indoor and soil-borne species with predominant tropical distribution. Plant Systematics and Evolution 301:2441–2462.

Klich MA. 2002 – Biogeography of *Aspergillus* species in soil and litter. Mycologia 94, 21–27.

Kocsubé S, Perrone G, Magistà D, Houbraken J et al. 2016 – *Aspergillus* is monophyletic: Evidence from multiple gene phylogenies and extrolites profiles. Studies in Mycology 85, 199–213.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018 – MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.

Krijgsheld P, Bleichrodt RJ, van Veluw GJ, Wang F et al. 2013 – Development in *Aspergillus*. Studies in Mycology 74, 1–29.

Lamounier WL, Carvalho VLM, Salgado AAR. 2011 – Serra do Gandarela: Possibilidade de Ampliação das Unidades de Conservação no Quadrilátero Ferrífero-MG. Revista do Departamento de Geografia – USP 22: 171–192.

Link HF. 1809 – Observationes in ordines plantarum naturales. Der Gesellschaft Naturforschender Freunde 3, 3–42.

Liu YJ, Whelen S, Hall BD. 1999 – Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Molecular Biology and Evolution 16, 1799–1808.

McNeill J, Barrie FR, Buck WR, Demoulin V et al. 2012 – International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). http://www.iapt-taxon.org/nomen/main.php (acessed 03 March 2017).

Mehrotra BS, Agnihotri VP. 1962 – Two New Species of Aspergillus from India. Mycologia 54, 400–406.

Micheli PA. 1729 – Nova Plantarum Genera. B. Paperninii, Florence.

Migahed FF. 2003 – Distribution of fungi in the sandy soil of Egyptian beaches. Mycobiology 31, 61–67.

Mirza J, Bajwa R. 2005 – New records of Fungi Imperfecti from Pakistan. Research Bulletin - 1st Fungal Culture Bank of Pakistan 1, 1–15.

Moraes AML, de Figueiredo AR, Junqueira ACV, da Costa GL et al. 2001 – Fungal flora of the digestive tract of *Panstrongylus megistus* (Reduviidae) used for experimental xenodiagnosis of *Trypanosoma* (Schizotripanum) cruzi Chagas, 1909. Revista Iberoamericana de Micología 18, 79–82.

Narciso J, Parish M. 1997 – Endogenous mycoflora of gable —top carton paperboard used for packaging fruit juice. Journal of Food Science 62, 1223–1239.

Nováková A, Šimonovičvá A, Kubátová A. 2012 – List of cultivable microfungi recorded from soils, soil related substrates and underground environment of the Czech and Slovak Republics. Mycotaxon 119, 1–186.

Peterson SW. 2008 – Phylogenetic analysis of Aspergillus species using DNA sequences from four loci. Mycologia 100, 205–226.

Piccoli B, Assini R, Gambaro S, Pastoni F et al. 2001 – Microbiological pollution and ocular infection in CAD operators: an on-site investigation. Ergonomics 44, 658–667.

Pitt JI, Taylor JW. 2014 – *Aspergillus*, its sexual states and the new International Code of Nomenclature. Mycologia 106:1051–1062.

Powell J. 1971 – Fungi and bacteria associated with *Cronartium comandrae* on lodgepole pine in Alberta. Phytoprotection 52, 45–51.

Raper KB, Thom C. 1944 – New Aspergilli from Soil. Mycologia 36, 555–575.

Samson RA, Varga J, Frisvad JC. 2011 – Taxonomic studies on the Genus *Aspergillus*. Studies in Mycology 69, 1–97.

Samson RA, Visagie CM, Houbraken J, Hong SB et al. 2014 – Phylogeny, identification and nomenclature of the genus *Aspergillus*. Studies in Mycology 78, 141–73.

Sappa F. 1955 – Nuove specie di *Aspergillus* dei terreni della savana spinosa somala. Allionia 2, 247–257.

Singh A, Singh AB. 1999 – Aspergillus spp. as an important occupational risk factor among susceptible individuals. Aerobiologia 15, 233–240.

Sugiyama J. 1967 – Mycoflora in core samples from stratigraphic drillings in middle Japan. The genus *Aspergillus*. Journal of the Faculty of Science University of Tokyo, Section III, Botany 9, 377–405.

Tanney JB, Varga J, Kocsubé S, Szigeti G et al. 2014 – Phylogeny, identification and nomenclature of the genus *Aspergillus*. Studies in Mycology 78, 141–173.

Thom C, Church MB. 1926 – The Aspergilli. Williams & Wilkins, Baltimore.

Visagie CM, Houbraken J, Frisvad JC, Hong SB et al. 2014 – Identification and nomenclature of the genus *Penicillium*. Stud. Mycol. 78, 343–371.

White TJ, Bruns T, Lee S, Taylor JW. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. NewYork, USA. AcademicPress.pp.315–322.