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ARTICLE

Two novel species of *Aspergillus* section *Nigri* from indoor air

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Abstract: *Aspergillus floridensis* and *A. trinidadensis* spp. nov. are described as novel uniseriate species of *Aspergillus* section *Nigri* isolated from air samples. To describe the species we used phenotypes from 7-d Czapek yeast extract agar culture (CYA), creatine agar culture (CREA) and malt extract agar culture (MEA), with support by molecular analysis of the β-tubulin, calmodulin, RNA polymerase II (*RPB2*), and translation elongation factor-alpha (*TEF*) gene amplified and sequenced from 56 air isolates and one isolate from almonds belonging to *Aspergillus* section *Nigri*. *Aspergillus floridensis* is closely related to *A. aculeatus,* and *A. trinidadensis* is closely related to *A. aculeatinus*. *Aspergillus brunneoviolaceus* (syn. *A. fijiensis*) and *A. uvarum* are reported for the first time from the USA and from the indoor air environment. The newly described species do not produce ochratoxin A.

Key words:
Aspergillus brunneoviolaceus
Aspergillus floridensis
Aspergillus trinidadensis
Aspergillus uvarum
Aspergillus violaceofuscus
black aspergilli
environment
phylogeny
ochratoxin A

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INTRODUCTION

Aspergillus section *Nigri* (Gams *et al*. 1985), commonly known as the black aspergilli, contains many common species in the environment (Klich 2009), and some have been implicated in human and animal diseases (de Hoog *et al.* 2000, Abarca *et al*. 2004, Klich 2009). They have a worldwide distribution and occur on a large variety of substrates including soil, grains, dairy and forage products, various fruit, vegetables, beans and nuts, cotton textiles and fabrics, and meat products (Raper & Fennell 1965, Pitt & Hocking 2007, 2009). Black aspergilli are used in the fermentation industry to produce various enzymes and organic acids (Raper & Fennell 1965, Varga *et al*. 2000). Some black aspergilli produce ochratoxin A (Abarca *et al.* 1994, 2003, 2004, Wicklow *et al*. 1996, Varga *et al*. 2000, Cabanes *et al*. 2002, Sage *et al.* 2004, Samson *et al*. 2004).

Although black aspergilli occur in clinical samples, they are much less frequent than *A. fumigatus*, *A. terreus,* or *A. flavus*. *Aspergillus* species are widely documented as causative pathogens in invasive and non-invasive infections as well as in allergic reactions especially Types III and IV (Richardson 2005). Indeed, the allergic forms of the disease appear to be "almost exclusively caused by *Aspergillus* species" (Moss 2002, Knutsen 2011). Some strains of black aspergilli are often misidentified as *A. niger* due to the difficulties of identifying the species in this group (Samson *et al.* 2007). Perrone *et al.* (2012a, b) recognized two new species of black aspergilli that may be involved in human disease from Sri Lanka: *A. brunneoviolaceus* (= *A. fijiensis*) in pulmonary aspergillosis, and *A. aculeatinus* in human dacryocystitis.

We collected 56 isolates of black uniseriate *Aspergillus* species from air (52 homes and four outside samples) from 17 states of the USA, Bermuda, Martinique, Trinidad &Tobago, and one from almonds in the Czech Republic. Using molecular data and macro- and micro-morphological observations, we discovered and describe here two new species related to *A. aculeatus* and *A. aculeatinus*.

MATERIALS AND METHODS

Fungal isolates

The provenance of fungal isolates examined is detailed in Table 1.

Culture methods

Observations were made on Czapek yeast extract agar (CYA), CYA with 20 % sucrose (CY20S), malt extract agar (MEA), oatmeal agar (OA), and creatine agar (CREA), (Pitt 1980, Samson *et al*. 2004) cultures incubated at 25 °C for 7 d in darkness, and CYA cultures incubated at 5 °C, 35 °C and 37 °C for 7 d. The cultures were grown on one plate as a three-point inoculation and on another plate as a single

Table 1. Provenance of fungal isolates characterized in this study.

*ITEM number	Provenance
Aspergillus aculeatus	
14807	USA: Georgia, iso. ex indoor air sample, 2010, *Ž. Jurjević*.
Aspergillus brunneoviolaceus (syn. *A. fijiensis*)	
14784	USA: Florida, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14785	USA: Florida, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14786	USA: Florida, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14790	Bermuda: isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14791	USA: Florida, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14794	USA: Texas, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14795	USA: Florida, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14796	USA: Florida, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14799	USA: Florida, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14802	USA: Arizona, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14804	Trinidad and Tobago: Tunapuna, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14806	USA: Alabama, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14809	USA: Florida, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14812	USA: Alabama, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14820	USA: Florida, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14825	USA: Missouri, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14831	USA: Alabama, isol. ex outside air sample, 2011, *Ž. Jurjević*.
14832	USA: Alabama, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
Aspergillus floridensis **sp. nov.**	
14783^{T}	USA: Florida, isol. ex indoor air sample, 2010, *Ž. Jurjević,* ex-type.
** (=NRRL 62478^{T})	
***CCF 4046	Czech Republic: Brno, isol. ex almonds in shells imported from USA, 2010, *V. Ostrý.*
CCF 4236	Martinique: Fort de France, isol. ex outside air sample, 2004, *N. Desbois.*
****CRI 323-04	Thailand: Phi Phi Islands, isol. ex *Xestospongia testudinaria*, 2006, *T.S. Bay*.
*****IFM 55703	Japan: isol. ex soil from grapery, 2007*, K. Yokoyama*.
Aspergillus violaceofuscus (syn. *A. japonicus*)	
14787	USA: Florida, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14788	USA: Kentucky, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14789	USA: New Jersey, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14792	USA: Florida, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14793	USA: Louisiana, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14797	USA: New York, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14798	USA: Florida, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14800	USA: Alabama, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14801	USA: Alabama, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14803	USA: Georgia, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14805	USA: Louisiana, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14808	USA: South Carolina, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14810	USA: Tennessee, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14811	USA: Hawaii, isol. ex indoor air sample, 2010, *Ž. Jurjević*.
14813	USA: Alabama, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14814	USA: Florida, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14815	USA: Delaware, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14816	USA: Maryland, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14817	USA: Alabama, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14818	USA: Alabama, isol. ex indoor air sample, 2011, *Ž. Jurjević*.

Table 1. (Continued)

*ITEM number	Provenance
14822	USA: Missouri, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14823	USA: Missouri, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14824	USA: Missouri, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14827	USA: Missouri, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14828	USA: Georgia, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14830	USA: Texas, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14834	USA: New Jersey, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14835	USA: North Carolina, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14836	USA: New York, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14837	USA: Texas, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
Aspergillus trinidadensis **sp. nov.**	
14821^{T} (=NRRL 62479^{T})	Trinidad and Tobago: Tunapuna, isol. ex indoor air sample, 2011, *Ž. Jurjević*, ex-type.
14829 (=NRRL 62480)	USA: California, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
Aspergillus uvarum	
14819	USA: Florida, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14826	USA: Missouri, isol. ex indoor air sample, 2011, *Ž. Jurjević*.
14833	USA: New Jersey, isol. ex outside air sample, 2011, *Ž. Jurjević*.

*ITEM, Agri-Food Toxigenic Fungi Culture Collection, Institute of Sciences of Food Production, Bari, Italy; **NRRL (Northern Regional Research Laboratory), the National Center For Agricultural Utilization Research, USA; ***CCF (Culture Collection of Fungi), Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic; ****CRI (Chulabhorn Research Institute), Vibhavadi-Rangsit Road, Laksi, Bangkok, Thailand; *****IFM (Medical Mycology Research Center) Chiba University, Chiba, Japan.

^{T}= ex-type strain

center-point inoculation on each medium in 9 cm diam Petri dishes. Colony diameters and appearance were recorded and photographs were made from 7 d culture plates incubated at 25 °C.

Microscopy

Microscopic examination was performed by gently pressing a *ca*. 20 × 5 mm piece of transparent tape onto a colony, rinsing the tape with one or two drops of 70 % ethanol and mounting the tape in lactic acid with fuchsin dye. Additional microscopic samples were made by teasing apart a small amount of mycelium in a drop of water containing 0.5 % Tween 20. A Leica DM 2500 microscope with bright field, phase contrast and DIC optics was used to view the slides. A Spot camera with Spot imaging software was mounted on the microscope and used for photomicrography. A Nikon digital SLR camera with a D70 lens was used for colony photography. Photographs were resized and fitted into plates using Microsoft PowerPoint 2010.

Ochratoxin A (OTA) assay

Aspergillus strains were grown in duplicate in 100-ml stationary liquid cultures (5 cm diam) of Czapek w/20 % Sucrose Broth (200 g l^{-1} Sucrose, 1 g l^{-1} K_2HPO_4, 3 g l^{-1} $NaNO_3$, 0.5 g l^{-1} KCl, 0.05 g $MgSO_4 \cdot 7 \bullet H_2O$, 0.01 g l^{-1} $FeSO_4$ $7 \bullet H_2O$) (Health Link®, Jacksonville, FL) and Yeast Extract Broth {2 g l^{-1} Yeast extract, 15 g l^{-1} Sucrose, 0.5 g l^{-1} $MgSO_4 \cdot 7 \bullet H_2O$, 1 ml l^{-1} trace metal solution (1 g l^{-1} $ZnSO_4 \cdot 7 \bullet H_2O$, 0.5 g l^{-1} $CuSO_4$ $5 \bullet H_2O$)} (Health Link®, Jacksonville, FL) in 250 ml flasks for 7 d at 25 °C± 0.2 °C in the dark. Each sample was inoculated with 10^6 spores counted by hemocytometer, previously grown on MEA.

Sample preparation (extraction and cleanup)

100 ml of liquid culture was homogenized (Waring®, USA) for 2 min. Two millilitre (ml) aliquots were diluted with 2 ml of acetonitrile/water (50/50, v/v) containing 0.5 % acetic acid, vortex mixed for 30 sec and then filtered through Acrodisc syringe filters with 0.45 µm PTFE membrane (Pall Corporation, http://www.pall.com/main/home.page) before LC/MS analysis.

Standard

Ochratoxin A standard was purchased from Sigma (http://www.sigmaaldrich.com/united-states.html) and stored at -20 °C.

LC/MS equipment and parameters

Analyses were performed on an Agilent 6330 series ion trap LC/MS system (http://www.home.agilent.com/), equipped with an ESI interface and an 1100 series LC system comprising a quaternary pump and an auto-sampler, from Agilent Technologies.

The analytical column was an Allure Bi-Phenyl column 30 mm x 2.1 mm with 5 µm particle sizes (http://www.restek.com/). The column oven was set at 40 °C. The flow rate of the mobile phase was 250 µl min l^{-1} and the injection volume was 10 µ l^{-1} . The column effluent was directly transferred into the ESI interface, without splitting.

Table 2. GenBank accession numbers of reference and ex-type strains. Sequence IDs in red are the sequences deposited for this manuscript.

Species	Source*	*RPB2*	*TEF*	*CaM*	*BenA*
Aspergillus acidus	ITEM 4507 = CBS 564.65	EF661052	FN665410	AM419749	AY585533
Aspergillus aculeatus	ITEM 7046^T = CBS 172.66^T	EF661046	**HE984381**	AJ964877	AY585540
Aspergillus 'aculeatus'	ITEM 4760 = CBS 620.78=NRRL 2053	EF661044	**HE984382**	EF661145	EU982087
Aspergillus 'aculeatus'	ITEM 15927 = NRRL 359	EF661043	**HE984383**	EF661146	EF661106
Aspergillus aculeatinus	CBS 121060^T = IBT 29077^T	**HF559233**	**HF559230**	EU159241	EU159220
Aspergillus aculeatinus	ITEM 13553	**HE984359**	**HE984385**	**HE984422**	**HE984407**
Aspergillus awamori	ITEM 4509^T = CBS 557.65^T	**HE984360**	FN665395	AJ964874	AY820001
Aspergillus brasiliensis	ITEM 7048 = CBS 101740^T	EF661063	FN665411	AM295175	AY820006
Aspergillus brunneoviolaceus	ITEM 7047^T = CBS 621.78^T	EF661045	**HE984384**	EF661147	EF661105
Aspergillus carbonarius	ITEM 4503^T = CBS 556.65^T	EF661068	FN665412	AJ964873	AY585532
Aspergillus costaricaensis	ITEM 7555^T = CBS 115574^T	**HE984361**	FN665409	EU163268	AY820014
Aspergillus ellipticus	ITEM 4505^T = CBS 482.65^T	EF661051	**HE984386**	AM117809	FJ629279
Aspergillus fijiensis	ITEM 7037^T = CBS 119.49^T	**HE984362**	**HE984387**	HE818081	HE818086
Aspergillus helicothrix	ITEM 4499 = CBS 677.79	**HE984363**	**HE984389**	AM117810	**FJ629279**
Aspergillus heteromorphus	ITEM 7045 = CBS 117.55	-	**HE984388**	AM421461	FJ629284
Aspergillus homomorphus	ITEM 7556^T = CBS 101889^T	**HE984365**	**HE984390**	AM887865	AY820016
Aspergillus ibericus	ITEM 4776^T = IMI 391429^T	EF661065	**HE984391**	AJ971805	AM419748
Aspergillus indologenus	ITEM 7038 = CBS 114.80	**HE984366**	**HE984392**	AM419750	AY585539
Aspergillus japonicus	ITEM 7034^T = CBS 114.51^T	EF661047	**HE984393**	AJ964875	HE577804
Aspergillus japonicus	ITEM 15926 = NRRL 35494	EU021639	**HE984394**	EU021690	EU021665
Aspergillus lacticoffeatus	ITEM 7559^T = CBS 101883^T	**HE984367**	FN665406	EU163270	AY819998
Aspergillus niger	ITEM 4501^T = CBS 554.65^T	XM_001395124	FN665404	AY585536	AJ964872
Aspergillus pulverulentus	ITEM 4510^T = CBS 558.65^T	**HE984368**	**HE984395**	**HE984423**	**HE984408**
Aspergillus saccharolyticus	ITEM 16159^T = CBS 127449	**HF559235**	**HF559232**	HM853554	HM853553
Aspergillus sclerotioniger	ITEM 7560^T = CBS 115572^T	**HE984369**	**HE984396**	EU163271	AY819996
Aspergillus tubingensis	ITEM 7040^T = CBS 134.48^T	EF661055	FN665407	AJ964876	AY820007
Aspergillus uvarum	ITEM 4834^T = IMI 388523^T	**HE984370**	**HE984397**	AM745755	AM457751
Aspergillus vadensis	ITEM 7561^T = CBS 113.365^T	**HE984371**	FN665408	EU163269	AY585531
Aspergillus violaceofuscus	ITEM 16177^T = CBS 102.23^T	**HF559234**	**HF559231**	FJ491698	HE577805

Eluent A was 95 % water: 5 % acetonitrile, and eluent B was 95 % acetonitrile: 5 % water, both containing 0.5 % acetic acid. Gradient elution was performed starting with 100 % eluent A, the proportion of eluent B was linearly increased to 100 % over a period of 5 min and then kept constant for 5 min. The column was re-equilibrated with 100 % eluent A for 5 min. For LC/MS analyses, the ESI interface was used in positive ion mode, with parameters set at: DRY TEMP 350 °C; NEBULIZER 40 psi, nitrogen, DRY GAS 10 l min^{-1}, Capillary voltage -3500 V. The mass spectrometer operated in MRM (multiple reaction monitoring) mode, by monitoring three transitions (1 quantifier, 2 qualifiers) for each compound, with a dwell time of 200 ms. Quantification of ochratoxin A was performed by measuring peak areas in the MRM chromatogram, and comparing them with the relevant calibration curve.

Tuning experiments were performed by direct infusion at a flow rate of 0.6 ml h $^{-1}$ of 1µg l^{-1} standard solutions in acetonitrile/water (50/50, v/v) containing 0.5 % acetic acid. The infusion was performed by using a model KDS100CE infusion pump (KDS Scientific Holliston, MA).

Interface parameters were: DRY TEMP 350 °C; NEBULIZER 10 psi nitrogen, DRY GAS 5L/min, Capillary voltage -3500 V, spacer was removed for flow infusion.

Fungal cultures, DNA extraction and DNA sequencing

Monoconidial isolates of each fungal strain were deposited at the ITEM Collection (CNR-ISPA, Bari, Italy) and received an ITEM accession number (Table 1). Supplemental information about the isolates can be recovered from the ITEM electronic catalogue (http: www.ispa.cnr.it/Collection).

For mycelium production, a suspension of spores from each fungal strain was grown in Wickerham's medium (glucose 40 g, peptone 5 g, yeast extract 3 g, malt extract 3 g and distilled water to 1 l). Mycelia were filtered and lyophilized for total DNA isolation. The fungal DNA was extracted with mechanical grinding using 5 mm iron beads in a Mixer Mill MM 400 (http://www.retsch.com/), and a "Wizard® Magnetic DNA Purification System for Food" kit (Promega, http://www.promega.com/), starting from 10 mg of lyophilized mycelium. The quality of genomic DNA was determined by electrophoresis and it was quantified using a ND-1000 (Nano Drop) spectrophotometer.

Table 3. GenBank accession numbers of *Aspergillus* strains isolated from air.

Species	Source	*RPB2*	*TEF*	*CaM*	*BenA*
Aspergillus aculeatus	ITEM 14807	HE984372	HE984398	HE984424	HE984409
Aspergillus brunneoviolaceus	ITEM 14784	HE984374	HE984400	HE984426	HE984411
Aspergillus brunneoviolaceus	ITEM 14785	HE984375		HE984427	
Aspergillus brunneoviolaceus	ITEM 14795			HE984428	
Aspergillus brunneoviolaceus	ITEM 14802		HE984401		
Aspergillus brunneoviolaceus	ITEM 14804		HE984402		
Aspergillus floridensis **sp. nov.**	ITEM 14783^T =NRRL 62478^T	HE984376	HE984403	HE984429	HE984412
Aspergillus violaceofuscus	ITEM 14787	HE984377	HE984404	HE984430	HE984413
Aspergillus violaceofuscus	ITEM 14788			HE984431	
Aspergillus violaceofuscus	ITEM 14789			HE984432	HE9844174
Aspergillus violaceofuscus	ITEM 14793				HE984415
Aspergillus violaceofuscus	ITEM 14801			HE984433	
Aspergillus violaceofuscus	ITEM 14805	HE984378			HE984416
Aspergillus violaceofuscus	ITEM 14814				HE984417
Aspergillus violaceofuscus	ITEM 14822				HE984418
Aspergillus violaceofuscus	ITEM 14834		HE984405		
Aspergillus violaceofuscus	ITEM 14835				HE984419
Aspergillus trinidadensis **sp.nov.**	ITEM 14821^T =NRRL 62479^T	HE984379	HE984406	HE984434	HE984420
Aspergillus trinidadensis	ITEM 14829 =NRRL 62480	HE984373	HE984399	HE984425	HE984410
Aspergillus uvarum	ITEM 14819	HE984380		HE984435	HE984421
Aspergillus uvarum	ITEM 14826	HE984364		HE984437	
Aspergillus uvarum	ITEM 14833			HE984436	

* The sequences were deposited only for the strains that differ in their sequences from the sequence of the type strain for a specific locus/gene.

Beta-tubulin (*BenA*, *ca*. 400 nt) was amplified using BT2a and BT2b primers and PCR conditions described by Glass & Donaldson (1995), calmodulin (*CaM*, *ca*. 650 nt) was amplified using CL1 and CL2A primers (O'Donnell *et al*. 2000), translation elongation factor-1 alpha (TEF-1α, *ca.* 700 nt) was amplified using A-EF_F/A-EF_R primers (Perrone *et al*. 2011) and RNA polymerase II (*RPB2*, *ca.* 950 nt) was amplified using primers 5F and 7CR (Liu *et al*. 1999). After amplification, the products were purified with the enzymatic mixture EXO/SAP (Exonuclease I, *Escherichia coli* / Shrimp Alkaline Phosphatase; Fermentas International, http://www.fermentas.com/en/home).

Bidirectional sequencing was performed for all loci and isolates. Sequence reactions were performed with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit for both strands, purified by gel filtration through Sephadex G-50 (Amersham Pharmacia Biotech) and analyzed on the "ABI PRISM 3730 Genetic Analyzer" (Applied Biosystems, http://www.appliedbiosystems.com/absite/us/en/home.html).

The preliminary alignments of sequences from each of the four loci was performed using the software package BioNumerics 5.1 from Applied Maths (http://www.applied-maths.com/bionumerics/bionumerics.htm) with manual adjustments where judged necessary.

Sequence Data Analysis

DNA sequences were aligned using the Clustal W algorithm (Thompson *et al*. 1994) in MEGA version 5 (Tamura *et al*. 2011). Sequences were deposited in GenBank (Tables 2 & 3). Each locus was aligned separately and then concatenated in a super-gene alignment used to generate the phylogenetic tree. Phylogenetic analysis was performed in MEGA5 using both Neighbor-Joining (NJ) (Saitou & Nei 1987) and Maximum Likelihood (ML) methods and the Tamura-Nei model (Tamura & Nei 1993). Evolutionary distances for NJ were computed using the Tamura-Nei method of the package and are in units of number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). Bootstrap values (Felsenstein 1985, 1995) were calculated from 1000 replications of the bootstrap procedure using programs within MEGA5.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model implemented in MEGA5. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically as follows. When the number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was

Table 4. Provenance of *Aspergillus* section *Nigri* isolates used as reference strains

Species	Source*	Provenance
Aspergillus acidus	ITEM 4507^T = IMI 104688^T = CBS 564.65^T = NRRL 4796^T	JAPAN: unknown, *R. Nakazawa.*
Aspergillus aculeatus	ITEM 7046^T = IMI 211388^T = CBS 172.66^T = NRRL 5094^T	USA: isol. ex tropical soil, 1962, *K. B. Raper.*
Aspergillus 'aculeatus'	ITEM 4760 = CBS 620.78 = NRRL 2053 = IMI 358696	New Guinea: isol. ex canvas tent, 1946, received from D. L. White
Aspergillus 'aculeatus'	ITEM 15927 = NRRL 359	*Thom and Raper 1945 received it from Dr. A. F. Blakeslee.*
Aspergillus brunneoviolaceus	ITEM 7047^T = CBS 621.78^T = IMI 312981^T = NRRL 4912^T	BRAZIL: culture contaminant, *A. C. Batista* and *H. Maia.*
Aspergillus aculeatinus	ITEM 16172^T = CBS 121060^T = IBT 29077^T	THAILAND: Chumporn Prov.: isol. ex arabica coffee, *P. Noonim.*
Aspergillus aculeatinus	ITEM 13553	SRI LANKA: isol. ex human dacryocystitis.
Aspergillus awamori	ITEM 4509^T = CBS 557.65^T = NRRL 4948^T = IMI 211394^T	Unknown - *Raper and Fennel 1965 received it from the Instituto Ozwaldo Cruz*
Aspergillus brasiliensis	ITEM 7048 = IMI 381727^T = CBS 101740^T = NRRL 26652^T	BRAZIL: Pedreira: isol. ex soil, *J. H. Croft.*
Aspergillus carbonarius	ITEM 4503^T = IMI 016136^T = CBS 556.65^T = NRRL 369^T	Unknown: paper, *A. F. Blakeslee*.
Aspergillus costaricaensis	ITEM 7555^T = CBS 115574^T	COSTA RICA: Taboga island: isol. ex soil, 2000, *M. Christensen.*
Aspergillus ellipticus	ITEM 4505^T = IMI 172283^T = CBS 482.65^T = NRRL 5120^T	COSTA RICA: isol. ex soil, 1962, *K. J. Kwon*.
Aspergillus fijensis	ITEM 7037^T = CBS 119.49^T	INDONESIA*:* Palembang: *Lactuca sativa,* 1949.
Aspergillus heteromorphus	ITEM 7045^T = CBS 117.55^T = IMI 172288^T = NRRL 4747^T	BRAZIL: Recife: culture contaminant, *A. C. Batista*.
Aspergillus homomorphus	ITEM 7556^T = CBS 101889^T	ISRAEL: isol. ex soil 2 km away from Dead Sea.
Aspergillus ibericus	ITEM 4776^T = CBS 121594^T = IMI 391429^T = NRRL 35644^T	PORTUGAL : Iberian Peninsula: isol. ex grapes, 2001, *R. Serra.*
Aspergillus indologenus	ITEM 7038^T = CBS 114.80^T = IBT 3679^T	INDIA: isol. ex soil.
Aspergillus japonicus	ITEM 7034^T = CBS 114.51^T	Unknown, *K. Saito.*
Aspergillus japonicus	ITEM 15926 = NRRL 35494	Unknown.
Aspergillus lacticoffeatus	ITEM 7559^T = CBS 101883^T	INDONESIA: South Sumatra: isol. ex coffee bean, *J. M. Frank.*
Aspergillus niger	ITEM 4501^T = IMI 050566^T = CBS 554.65^T = NRRL 326^T	USA: Connecticut: tannin-gallic acid fermentation, 1913, *A. Hollander*.
Aspergillus pulverulentus	ITEM 4510^T = CBS 558.65^T = NRRL 4851^T = IMI 211396^T	AUSTRALIA: Victoria: isol. ex *Phaseolus vulgaris*, ~1907, *D. McAlpine*.
Aspergillus sclerotioniger	ITEM 7560^T = CBS 115572^T = IBT 22905^T	INDIA: Karnataka: isol. ex green arabica coffee *J. M. Frank*.
Aspergillus tubingensis	ITEM 7040^T = CBS 134.48^T = NRRL 4875^T	Unknown: 1948, deposited by *R. Mosseray*.
Aspergillus uvarum	ITEM 4834^T = IMI 388523^T = CBS 127591^T = IBT 26606^T	ITALY: Brindisi: isol. ex grapes, 2001, *P. Battilani.*
Aspergillus uvarum	ITEM 4685 = IMI 387209	PORTUGAL: Régua, Douro Region: isol. ex grapes.
Aspergillus uvarum	ITEM 4962 = IMI 3888715	SPAIN: isol. ex grapes.
Aspergillus uvarum	ITEM 4997 = IMI 388670	ISRAEL: Lichron: isol. ex grapes.
Aspergillus uvarum	ITEM 5020 = IMI 388660	ITALY: Brindisi, Apulia: isol. ex grapes.
Aspergillus uvarum	ITEM 5321 = IMI 389195	FRANCE: Narbonne, Languedoc: isol. ex grapes.
Aspergillus uvarum	ITEM 5350 = IMI 389166	ISRAEL: Pdaya: isol. ex grapes.
Aspergillus vandensis	ITEM 7561^T = IMI 313493^T = CBS 113365^T	EGYPT: air, *A. H. Moubasher*.
Aspergillus violaceofuscus	ITEM 16159^T = CBS 102.23^T	FRANCE: Strassbourg: received by *D Borrel*, 1923
Aspergillus saccharolyticus	ITEM 16177^T = CBS 127449^T = IBT 28509^T	DENMARK: Gentofte: ex under a toilet seat made of treated oak wood, *P. J. Teller.*

*CBS, Centraalbureau voor Scimmelcultures, Utrecht, The Netherlands; IMI, CABI Bioscience Genetic Resource Collection, Egham, United Kingdom; ITEM, Agri-Food Toxigenic Fungi Culture Collection, Institute of Sciences of Food Production, Bari, Italy; NRRL (Northern Regional Research Laboratory), the National Center For Agricultural Utilization Research, USA.

^{T}= ex-type strain

Table 5. Sequence characteristics and phylogenetic information for *RPB2*, *TEF*, *CaM*, *BenA* and combined *MLS*.

Locus	Region	Sites	Net Sites	% GC	No. of variable sites	No. of informative sites	No. of mutations (Eta)	Nucleotide diversity
BenA	1-369	369	326	58	180	149	229	0.101
CaM	370-937	568	496	53	269	224	349	0.103
RPB2	938-1922	985	878	52	370	271	442	0.063
TEF	1923-2552	630	629	57	93	55	105	0.023
MLS	1-2560	2552	2329	55	912	699	1125	0.067

used; otherwise the BIONJ method with MCL distance matrix was used. A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories; +*G*, parameter = 0.2036). All positions containing gaps and missing data were eliminated. There were 2329 positions in the final dataset. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v3. 2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) for the combined sequences datasets. The analysis was run in duplicate with four MCMC chains and setting random trees for 10^7 generations sampled every 100 generations. A total of 15 738 trees were read in the two runs, 7869 for each, and the first 1967 trees (25 %) were discarded in each run as the burn-in phase of the analysis and posterior probabilities were determined from the remaining trees (5902 in each run).

Maximum Parsimony analysis (MP) was performed for all data sets using the heuristic search option and Close-Neighbor-Interchange algorithm (with search level 1 in which the initial trees were obtained with the random addition of sequences). To assess the robustness of the topology, 1000 bootstrap replicates were run. The tree is drawn to scale, with branch lengths calculated using the average pathway method and are in units of the number of changes over the whole sequence (Nei & Kumar 2000). The analysis involved data from 86 isolates and all positions containing gaps and missing data were eliminated. There were a total of 2329 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.* 2011).

RESULTS

Phylogenic analysis of sequence data

The multilocus analysis was performed on 56 isolates collected from air (52 homes and 4 outside samples) from 17 states of the United States, Bermuda, Martinique, Trinidad and Tobago, and one isolated from almonds in the Czech Republic (Table 1), along with 28 reference and ex-type strains from *Aspergillus* section *Nigri* (Table 4). The ex-type strain of *Aspergillus flavus* (ITEM 7526) was used as outgroup. The percentage of variable sites and parsimony informative sites for each locus differ, the *benA* sequences have the highest percentage of variable and parsimony informative sites, the *CaM* sequences have the highest nucleotide diversity, *TEF* sequences have the lowest variability and *RPB2* has lower sequence diversity than *CaM* and *benA* but the highest number of informative sites (Table 5). After a preliminary analysis using MEGA5 Neighbour-Joining, the best substitution model among the evolutionary models in MEGA5 was calculated. The best model was Tamura-Nei with Gamma distribution (TN93 + G). Evolutionary history was inferred using the Neighbor-Joining method. The tree with the highest log likelihood is shown (Fig 1). Bootstrap proportions are shown next to the branches. The tree is drawn to scale, with branch lengths reflecting evolutionary distance computed using the Maximum Composite Likelihood method as number of base substitutions per site (MEGA5). The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.3). Phylogenetic analysis was conducted first on the four single locus alignments and subsequently the combined alignment of the four loci. The single locus and four locus combined data trees contained the same topology fulfilling the requirements of genealogical concordance phylogenetic species recognition (GCPSR, Taylor *et al.* 2000).

Of the 56 strains collected from air, 30 strains were *A. violaceofuscus* (syn. *A. japonicus*), 18 *A. brunneoviolaceus* (syn. *A. fijiensis*), three *A. uvarum*, one (ITEM 14807) was *A. aculeatus*, two (ITEM 14821 and 14829) were grouped (high bootstrap) in a distinct cluster from *A. aculeatinus*, and three (ITEM 14783, CCF 4046 and CCF 4236) were phylogenetically isolated (with strong statistical support) from *A. aculeatus*, and are described as two new species here. In addition, two strains previously characterized by *CaM* analysis CRI 323-04 (sequence accession number FJ525444, Ingavat et al 2009) and IFM 55703 (sequences from Tetsuhiro Matsuzawa, Chiba University, Japan) resulted to have an homology > 99.5% with ITEM 14783. They also showed a different phylogenetic position from the uniseriate species decribed as *A. indologenus* (CBS 114.80), *A. brunneoviolaceus* (ITEM 7047) and from two atypical *Aspergillus "aculeatus"* (ITEM 4760 and ITEM 15927) strains, not yet well-defined and characterized as belonging in any of the known uniseriate species.

Bayesian inference analysis of the multilocus (*benA*, *CaM*, *TEF-1α*, *RPB2*) data set produced a phylogenetic tree (log likelihood 14835.94) with high PP values for the same monophyletic group obtained with Maximum Likelihood analysis, the atypical *A. aculeatus* isolate ITEM 4760 clustered together with ITEM 14873, while the two other atypical *A. aculeatus* isolates were placed in the *A. brunneoviolaceus* clade. The results obtained by the Maximum Parsimony analysis are represented by one of the 80 equally most parsimonious trees (Fig. 2). The consistency index is (0.491302), the retention

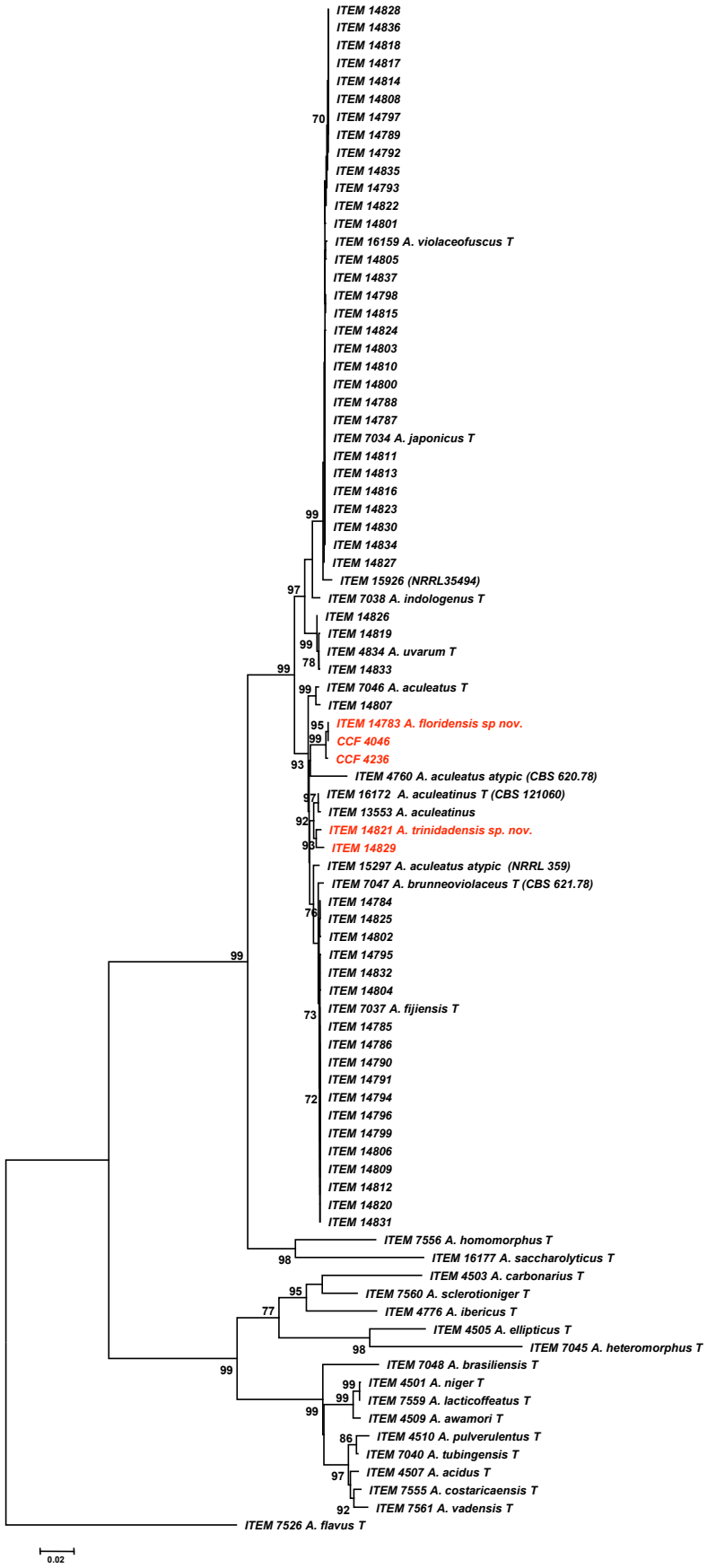

Fig. 1. Phylogenetic trees produced from the combined sequence data of four loci (*CaM*, *benA, RPB2* and *TEF*) of 57 strains of uniseriate black *Aspergillus,* 28 reference strains of species belonging to *Aspergillus* section *Nigri*, and *A. flavus* (ITEM 7526) as outgroup. Numbers above branches are bootstrap values**.** Only values above 70 % are indicated. The evolutionary history was inferred using the Neighbour-Joining method computed with the Maximum Likelihood Evolutionary method.

Fig. 2. Maximum parsimony phylogram derived from the combined sequence data of four loci (*CaM*, *benA, RPB2* and *TEF*) of 57 strains of uniseriate black *Aspergillus*, 28 reference strains of *Aspergillus* section *Nigri*, and *A. flavus* (ITEM 7526) as outgroup. Numbers at nodes are bootstrap values/Bayesian posterior probabilities**.** A dash indicates the support for the branch was < 70 % BP or < 0.80 PP.

index is (0.863662), and the composite index is 0.466789 (0.424319) for all sites and parsimony-informative sites (in parentheses). The MP phylogenetic analysis agreed with the evolutionary results obtained by the ML and Bayesian analysis. *A. violaceofuscus, A. brunneoviolaceus* and *A. uvarum* are the principal black aspergilli "uniseriate" species collected in these indoor air samples with the identification of two possible new species (ITEM 14821 and 14783). The topology of MP, Bayesian phylogenetic trees is concordant, and the two trees are represented together in Fig. 2. The phylogenetic tree obtained by ML analysis has also the same topology of the other two phylogenetic analyses with some minor exception regarding the clades of *A. ellipticus* and *A. heteromorphus* (Fig. 1). All three phylogenetic analyses performed give evidence with high bootstrap that the two new species belong to different monophyletic groups (Figs 1–2), and that the atypical strain (ITEM 15297) belongs to the *A. brunneoviolaceus* clade, and that ITEM 4760 needs further characterization as belonging alone in a clade close to the new species *A. floridensis* but with no high supported bootstrap (Figs 1–2).

TAXONOMY

Previously described species

Aspergillus brunneoviolaceus Batista & Maia, *Anais Soc. Biol. Pernambuco* **13:** 91 (1955).

Synonym: *Aspergillus fijiensis* Varga *et al., Stud. Mycol.* **69:** 9 (2011).

(Fig. 3a–f)

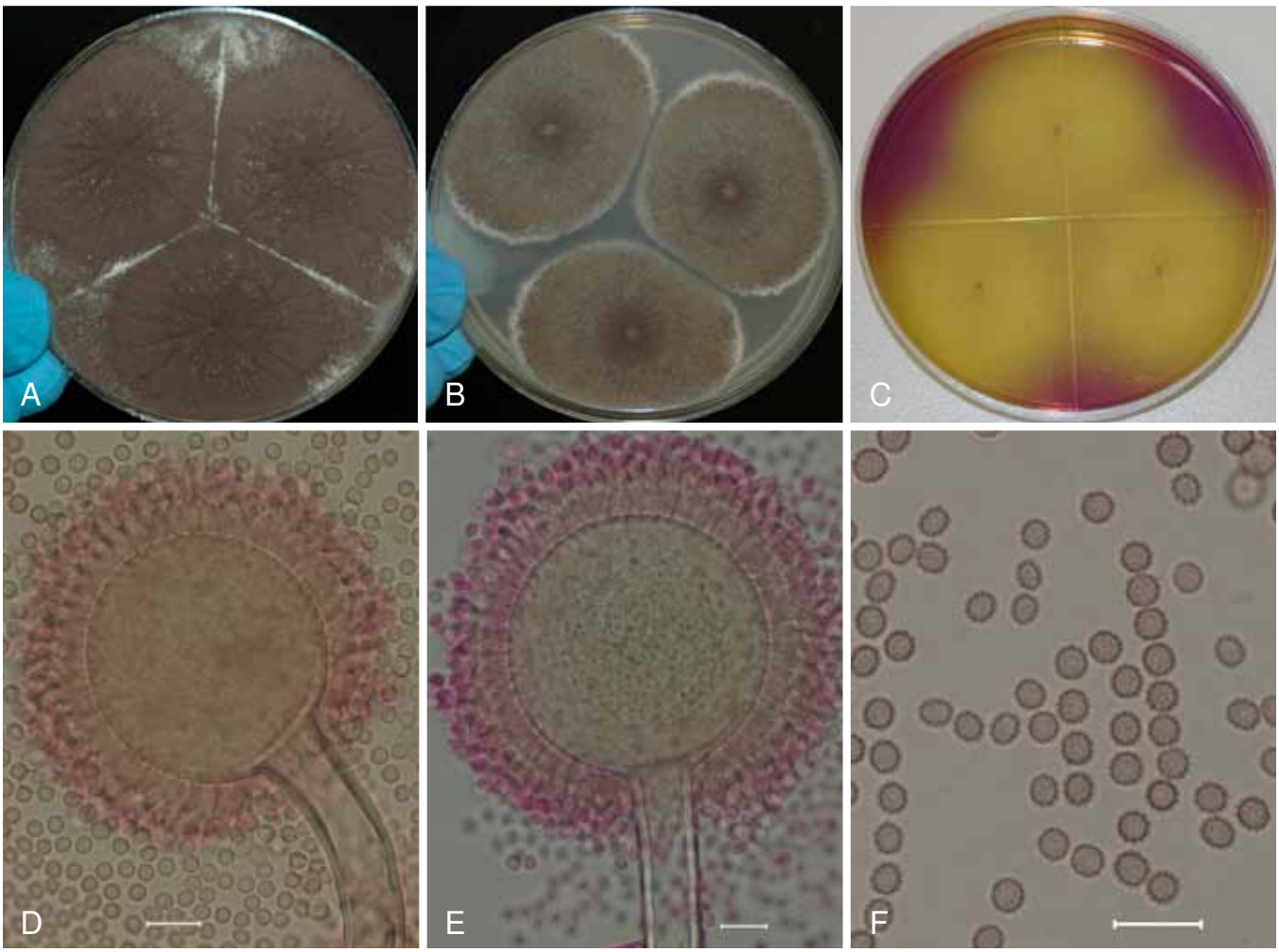

Fig. 3. *Aspergillus brunneoviolaceus* (syn. *A. fijiensis*; ITEM 7037), culture plates are 9 cm diam, colonies grown at 25 °C for 7 d. **A.** CYA colonies. **B.** MEA colonies. **C.** CREA colonies. **D–E.** Stipes smooth or with a limited surface granulation just below the vesicle, globose to ellipsoidal vesicle, and conidia. **F.** Globose to ellipsoidal, conidia, with echinulate surface. Bars = 10 µm.

Type: (CBS 621.78^{T} =NRRL 4912^{T}).

Description: Colony diameters after 7 d incubation at 25 °C on CYA (Fig. 3a) > 85 mm (50–75 mm 5 d), MEA (Fig. 3b) 45–75(< 85) mm, CY20S 50–65 mm, OA 55–70 mm, CREA (Fig. 3c) displayed poor sporulation but commonly good to very good acid production, conidial heads on CYA brown to dark brown near black, commonly abundant, velutinous to slightly floccose, white to buff mycelium, commonly moderate radial sulcation, exudate clear to brown, sparse to abundant, soluble pigment not seen, occasionally present and brown at 37 °C, if present sclerotia subglobose to elongate 250–800 µm long, buff to orange-brown, reverse buff to yellow. On MEA conidial heads are brown, sclerotia absent, mycelium white, reverse uncolored to yellowish-gray. Incubation for 7 d on CYA at 5 °C produced no growth or germination of conidia. Incubation for 7 d on CYA at 35 °C and 37 °C produced growth of 35–63 mm, and (12–)17–26 mm diam, respectively.

Stipes (Fig. 3d–e) smooth or with a limited surface granulation just below the vesicle, hyaline or pigmented just below the vesicle, (75–)200–800(–1600) × (8–)10–15(–21) µm, isolate ITEM 7037 has longer stipes (400–)800–2000 (–3400) × (8–)10–15(–18) µm than other *A. brunneoviolaceus* isolates, *vesicles* globose to elipsoidal, (30–)35–70(–90) µm diam, *conidial heads* uniseriate, *phialides* (6–)7–9(–10) × 3.5–4.5(–5) µm covering entire vesicle, *conidia* (Fig. 3e) globose to ellipsoidal, 3.5–4.5(–6) × 3.5–4.5(–5) µm, occasionally subglobose to angular 2.5–3.5 µm, brown near black, with coarsely roughened to echinulate surface.

Aspergillus uvarum G. Perrone *et al.*, *Int. J. Syst. Evol.Microbiol*. **58**: 1036 (2008).
MycoBank MB510962.
(Fig. 4a–f).

Type: **Italy**: Apulia, Brindisi, isol. ex grapes **(**ITEM 4834^{T}; = IMI 388523^{T}).

Description: Colony diameters after 7 d incubation at 25 °C on CYA (Fig. 4a) > 85 mm (47–88 mm at 5 d), MEA (Fig. 4b) > 85 mm (58–84 mm at 5 d), CY20S 55–65 mm, OA (Fig. 4c) 60-70 mm, CREA produced good growth, acid production ranged from good to very poor to none depending on the isolates, conidial heads on CYA brown to dark brown near black, sporulating abundantly, granular, mycelium white to buff, moderate to deep radial sulcation, exudate

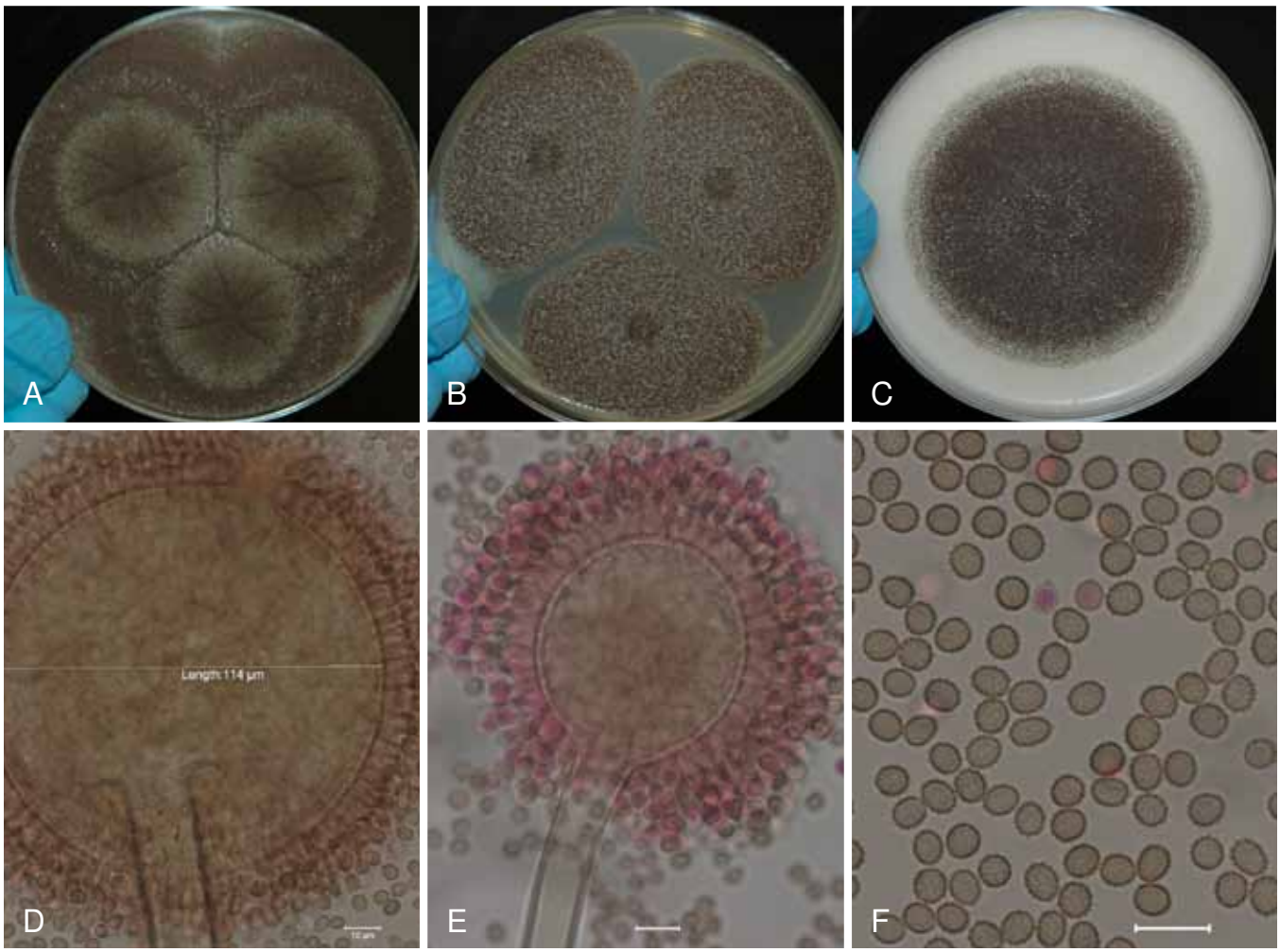

Fig. 4. *Aspergillus uvarum* (ITEM 4834^{T}), culture plates are 9 cm diam, colonies grown at 25 °C for 7 d. **A.** CYA colonies. **B.** MEA colonies. **C.** OA colony. **D–E.** Smooth stipes, globose to ellipsoidal vesicle, and conidia. **F.** Globose to ellipsoidal, conidia, with echinulate surface. Bars = 10 µm.

clear to brown, soluble pigments when present pale-yellow, brown at 35 °C, at 37 °C rarely present, brown, sclerotia when present abundant in the center of the colony, globose to elongate, buff to brown, reverse wrinkled, white to dull brown occasionally pink-orange. On MEA conidial heads are brown to dark brown, sporulating abundantly, mycelium white and commonly inconspicuous, reverse yellowish-grayish-green. Incubation for 7 d on CYA at 5 °C produced no growth or germination of conidia. Incubation for 7 d on CYA at 35 °C and 37 °C produced growth of 18–27(–46) mm, and (germinate)3–15(–21) mm diam, respectively.

Stipes (Fig. 4d–e) smooth, hyaline, becoming brown with age (250–)600–1800(–3600) × (8–)10–18(–24) µm, *vesicles* globose to ellipsoidal, (30–)45–100(–121) µm diam, *conidial heads* uniseriate, *phialides* 7–10(–12) × (3–)3.5–4.5(–7) µm covering entire vesicle, *conidia* (Fig. 4f) globose to ellipsoidal, (4–)4.5–7(–9) × 3.5–7 µm, with echinulate surface.

New species

Aspergillus floridensis *Ž.* Jurjević, G. Perrone & S. W. Peterson, **sp. nov.**
MycoBank MB802363.
(Fig. 5a–g)

Etymology: Isolated in Florida.

Type: **USA**: *Florida*: isol. ex air sample, August 2010, *Ž. Jurjević* (BPI 883907 – holotype; from dried colonies of ITEM 14783 (=NRRL 62478) grown 7 d at 25 °C on CYA and MEA)
.
Diagnosis: Stipes uniseriate, mycelium white to yellow on MEA, vesicles globose to subglobose occasionally ellipsoidal (14–)35–65(–105) µm diam, conidia globose to ellipsoidal, 4–5(–6) × 3.5–5.5 µm, with echinulate surface, incubation at 37 °C produced growth of 18–24 mm diam**.**

Description: *Colony* diameters after 7 d incubation at 25 °C on CYA (Fig. 5a) 80–85 (> 85) mm, MEA (Fig. 5c) 50–55 (> 85) mm, CY20S 32–53 mm, OA 55–60 mm, CREA (Fig. 5d) displayed poor sporulation but good acid production, conidial heads on CYA dark brown to black, abundantly produced, globose to subglobose at first and later radiate, developing into columns, mycelium white to buff-yellow, velutinous, moderate radial sulcation, exudate clear to brown, sparse to abundant, soluble pigment not seen, occasionally producing buff-yellowish sclerotia (Fig. 5b) subglobose to elongate (200–)400–700(–1100) µm long, reverse brownish-yellow to yellow-brown. On MEA conidial heads are brown, sclerotia absent, mycelium

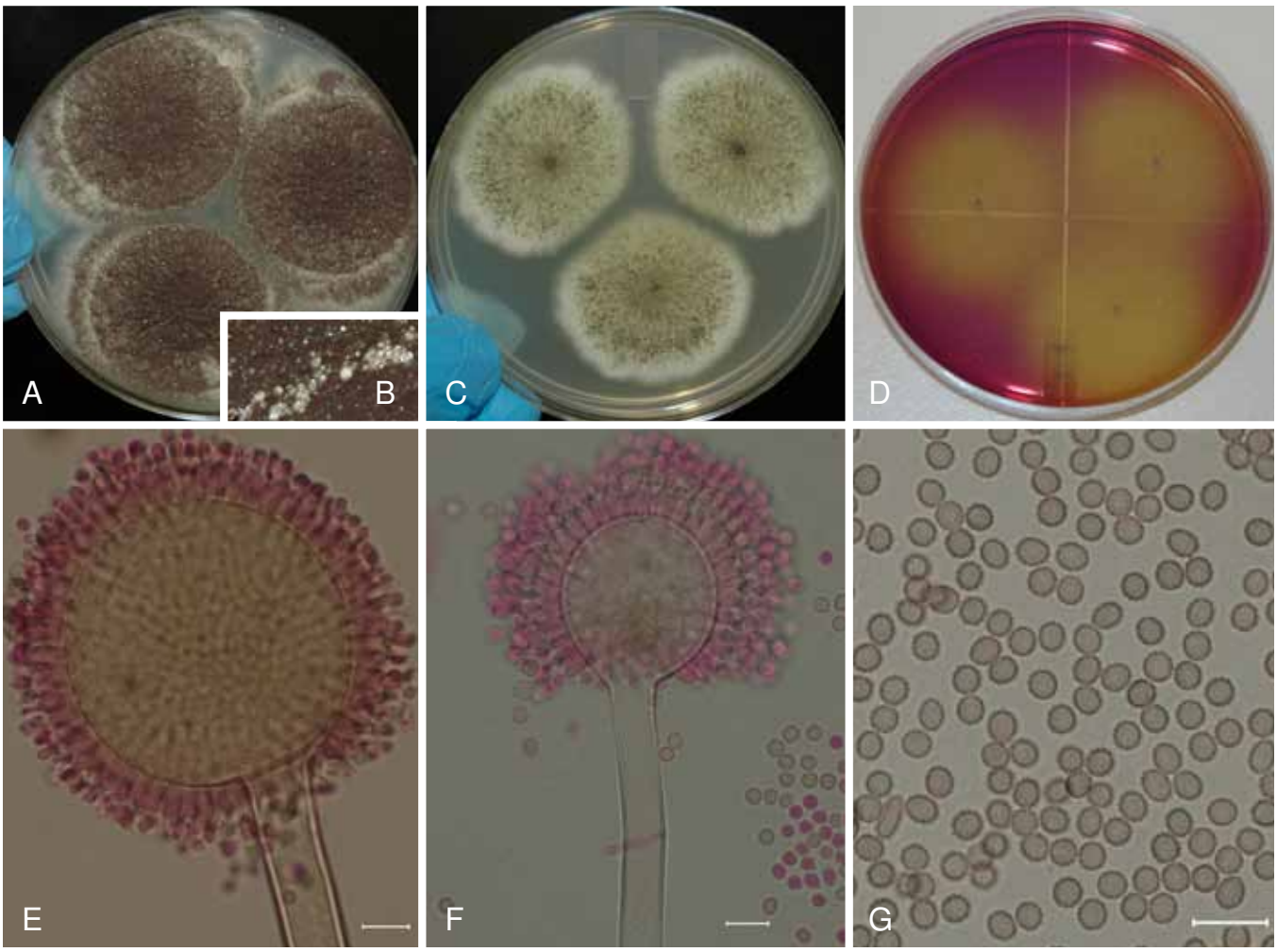

Fig. 5. *Aspergillus floridensis* (ITEM 14783^{T}) culture plates are 9 cm diam, colonies grown at 25 °C for 7 d. **A.** CYA colonies. **B.** CYA colony, buff-yellowish sclerotia, subglobose to elongate (200–)400–700(–1100) µm long, clear to brown exudates. **C.** MEA colonies. **D.** CREA colony. **E–F.** Smooth stipes, globose to ellipsoidal vesicle, and conidia. **G.** Globose to ellipsoidal, conidia, with echinulate surface. Bars = 10 µm.

white to yellow, reverse gray to grayish-yellow. Incubation for 7 d on CYA at 5 °C produced no growth or germination of conidia. Incubation for 7 d on CYA at 35 °C and 37 °C produced growth of 40–50 mm and 18–24 mm diam, respectively.

Stipes smooth, hyaline (50–)200–650(–950) × (8–)10–15(–21) µm, *vesicles* (Fig. 5e–f) globose to subglobose occasionally ellipsoidal (14–)35–65(–105) µm diam, *conidial heads* uniseriate, *phialides* (6–)7–9(–11) × 3.5–4.5(–5) µm covering entire vesicle, *conidia* (Fig. 5g) globose to ellipsoidal, 4–5(–6) × 3.5–5.5 µm, with echinulate surface. No ochratoxin A produced.

Aspergillus trinidadensis *Ž.* Jurjević, G. Perrone & S. W. Peterson, **sp. nov**.
MycoBank MB802364.
(Fig. 6a–g)

Etymology: Isolated in Trinidad.

Type: **Trinidad & Tobago:**Tunapuna, isol. ex air sample, July 2011, *Ž. Jurjević* (BPI 883908 – holotype; from dried colonies of ITEM 14821^{T} (=NRRL 62479^{T}) grown 7 d at 25 °C on CYA and MEA).

Diagnosis: Stipes uniseriate, mycelium white to orangish-yellow on CYA, vesicles globose to subglobose occasionally ellipsoidal (10–)30–70(–100) µm diam, conidia large 4–7(–8) × 3.5–7 µm, if borne from monophialides up to 13 × 10 µm, with finely spiny to echinulate surface, and range from no growth to 7 mm diam growth at 37 °C.

Description: *Colony* diameters after 7 d incubation at 25 °C on CYA (Fig. 6a) 65–78 mm, MEA (Fig. 6b) 57 to > 85 mm, CY20S 50–55 mm, OA 55–60 mm, CREA (Fig. 6c) showed poor sporulation and no acid production, conidial heads on CYA brown to dark brown, globose to subglobose initially, later radiate, then developing into columns, sporulating well, mycelium white to yellowish creamy or orangish-yellow toward the center of the colony, white at margins, floccose, moderate to deep radial sulcation, exudate clear to brownish, soluble pigments and sclerotia not seen, occasionally globose to elongate chlamydospores present, reverse brown to brownish-yellow. On MEA conidial heads brown to dark brown, sporulating well centrally, mycelium white to buff-yellowish-orange, reverse buff. Incubation for 7 d on CYA at 5 °C produced no growth or germination of conidia. Incubation for 7 d on CYA at 35 °C and 37 °C

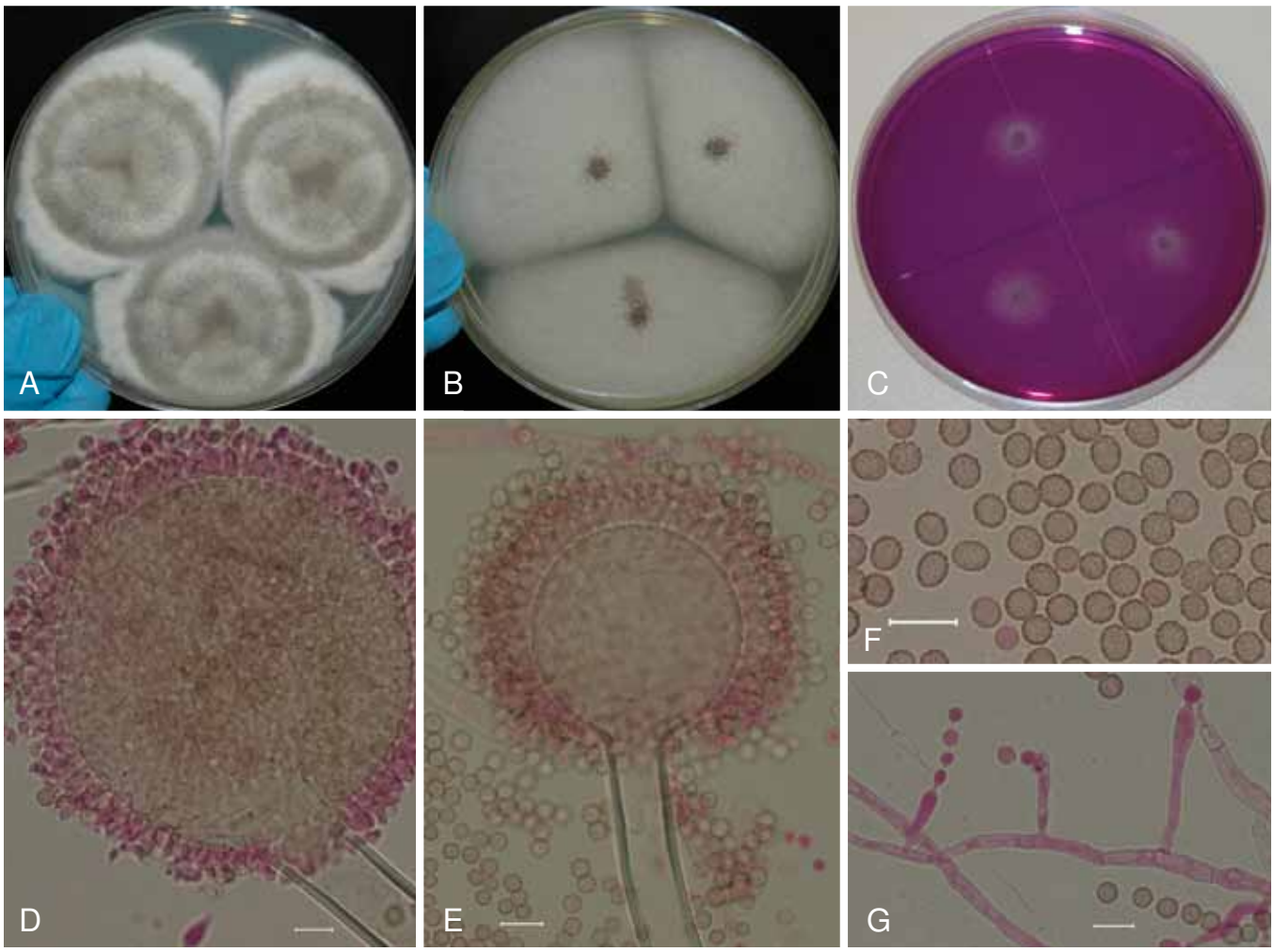

Fig. 6. *Aspergillus trinidadensis* (ITEM 14821^{T}) culture plates are 9 cm diam, colonies grown at 25 °C for 7 d. **A.** CYA colonies. **B**. MEA colonies. **C.** CREA colonies. **D–E**. Smooth stipes, globose to subglobose vesicle, and conidia. **F.** Globose to ellipsoidal, conidia, with echinulate surface. **G.** Monophialides and conidia. Bars = 10 µm.

produced growth 4–21 mm and from no growth to 7 mm diam, respectively.

Stipes (Fig. 6d–e) smooth or occasionally with a limited surface granulation just below the vesicle, hyaline or occasionally pigmented just below the vesicle, long if from substrate, short with small vesicles if borne from aerial hyphae (50–)150–800(–1800) × (5–)8–14(18) µm, *vesicles* globose to subglobose occasionally ellipsoidal (10–)30–70(–100) µm diam, *conidial heads* uniseriate, *phialides* (5-)7–9(–12) × (3–)3.5–4.5(–6) µm commonly covering entire vesicle, occasionally producing monophialides (Fig. 6g) 3–42 × 3.5–8 µm, *conidia* (Fig. 6f) globose to ellipsoidal, rarely pyriform, 4–7(–8) × 3.5–7 µm, if borne from monophialides up to 13 × 10 µm, with finely spiny to echinulate surface. No ochratoxin A produced.

DISCUSSION

In our studies of the indoor environment the dominant species of uniseriate *Aspergillus* section *Nigri* were *A. violaceofuscus* (syn. *A. japonicus*) (30 of 55 isolates) and *A.brunneoviolaceus* (syn. *A. fijiensis*) (18 of 55 isolates). *Aspergillus violaceofuscus* was isolated from 15 states in the USA, mainly from Southern and Mid-Atlantic states (Table 1). *Aspergillus violaceofuscus* (syn. *A. japonicas*) and *A. aculeatus* have previously only been found in the tropics (Nielsen *et al*. 2009). Nine isolates (ITEM 14800, 14803, 14805, 14810, 14827, 14828, 14830, 14834, and14837) of the 30 *A. violaceofuscus* isolates produced sclerotia on CYA or OA, buff to yellowish-orange or orange-brown, subspherical to elongate, 300–1000 µm long. Also, three isolates (ITEM 14794, ITEM 14799, and ITEM 14802) of *A. brunneoviolaceus* produced abundant buff to orange-brown sclerotia, 250–800 µm long. None of the three sclerotium producing *A. brunneoviolaceus* isolates produced ochratoxin A. *Aspergillus brunneoviolaceus* isolates commonly have good to very good acid production. However, two isolates ITEM 14806 and ITEM 14831, did not show acid reactions on CREA agar, nor were they sulcate on CYA. *Aspergillus brunneoviolaceus* (syn. *A. fijiensis*) was previously isolated from soil, Fiji (CBS 313.89), *Lactuca sativa*, Indonesia (CBS 119.49) (Varga *et al.* 2011), guano, Peru (IHEM 18675), corneal scraping keratitis, India (IHEM 22812), droppings of *Coenobita* sp., Bahamas (IHEM 4062) (Hendricks *at al*. 2011), and industrial material, China (CCF 108) (Hubka & Kolarik 2012). This is the first report of *A.*

brunneoviolaceus isolated from the indoor air environment and the first reported isolation in the United States. We found only one isolate of *A. aculeatus* and three isolates of *A. uvarum* (Table 1). *A. uvarum* was previously known only from grapes in the Mediterranean basin (Perrone *et al*. 2008). This is the first time that *A. uvarum* was isolated from the indoor air environment and its first isolation in the USA.

A. brunneoviolaceus, A. uvarum, and *A. violaceofuscus* are the uniseriate black aspergilli occurring in the indoor environment in the USA. The *A. brunneoviolaceus* clade (Fig. 1) showed the presence of two statistically supported subgroups, one included 15 strains and the ex-type strain of *A. fijiensis* ITEM 7037, while the other included 3 strain (ITEM 14802, 14825, and 14784). Two strains previously characterized as atypical *A. aculeatus* (ITEM 7047 – the ex-type strain of *A. brunneoviolaceus,* and NRRL 359) belong to the same subclade as *A. brunneoviolaceus* with high bootstrap in all the three phylogenetic analysis conducted (Figs 1–2). These findings confirm the data of Hubka & Kolarik (2012) that suggest treating *A. fijiensis* as a synonym of *A. brunneoviolaceus* because they are indistinguishable by multilocus sequence analysis and belong in the same highly supported clade. Then, as *A. brunneoviolaceus* has been previously described at species level, we suggest treating *A. fijiensis* as a synonym of it, in agreement with findings of Hubka & Kolarik (2012). The same should be done for *A. japonicus* and *A. violaceofuscus*, previously proposed as separate taxa (Varga *et al*. 2011), as our phylogenetic results do not support this separation and suggest they should be treated as the same taxon; i.e. *A. japonicus* should be treated as a synonym of *A. violaceofuscus* which was described earlier.

In the case of the atypical *A. aculeatus* isolate ITEM 4760, although the molecular difference suggests the possible recognition of further new species, there is no unique topology among the four single locus trees. Two loci indicate it belongs to *A. brunneoviolaceus* and the other two loci form a clade with the *A. floridensis* (data not shown). When the combined multilocus alignment was conducted, ML, MP, and PP criteria put it close to *A. floridensis* (Figs 1–2), but not with a high bootstrap/PP value.

Phenotypically, the atypical *A. aculeatus* (ITEM 4760) grows slower on CY20S (30 mm diam) and CYA (70-78 mm diam) after 7 d at 25 °C than *A. brunneoviolaceus* isolates that grow on CYA < 85 mm (50–75 mm diam 5 d), and CY20S 50–65 mm. ITEM 4760 also has slower growth on CYA when compared with *Aspergillus floridensis* that grows on CYA 80–85(> 85) mm diam after 7 d at 25 °C.

The phylogenetic analysis evidenced both in single locus and in a multilocus analysis showed that the two strains ITEM 14821 and 14829 of *A. trinidadensis* belong to the *A. aculeatinus* clade, a black *Aspergillus* species known only from Thai coffee beans (Noonim *et al*. 2008). Finally, the newly described *A. floridensis* was highly supported in both the MP, ML, and Bayesian analysis (Figs 1–2), and in particular the five strains (Table 1) isolated from different world geographic area belonging in the same group by phylogenetic calmodulin analysis (data not shown).

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