Species diversity of *Aspergillus* section *Versicolores* in clinical samples and antifungal susceptibility

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Abstract

Aspergillus section *Versicolores* includes species of clinical relevance and many others that have been poorly studied but are occasionally found in clinical samples. The aim of this study was to investigate, using a multi-locus phylogenetic approach, the spectrum of species of the section *Versicolores* and to determine their *in vitro* antifungal susceptibility. The study was based on a set of 77 clinical isolates from different USA

medical centers, which had been previously identified as belonging to this section. The genetic markers used were ITS, *BenA*, *CaM* and *RPB2*, and the drugs tested, following the CLSI guidelines, were amphotericin B, itraconazole, posaconazole, voriconazole, anidulafungin, caspofungin, micafungin, terbinafine and flucytosine. The most frequent species were *A. sydowii* (26%), *A. creber* (22%) and *A. amoenus* (18.2%), followed by *A. protuberus* (13%), *A. jensenii* (10.4%), and *A. tabacinus* (5.2%); while *A. cvjetkovicii*, *A. fructus*, *A. puulaauensis* and *A. versicolor* were represented by only one isolate each (1.3%). This is the first time that *A. jensenii* and *A. puulaauensis* have been reported from clinical samples. Considering the high number of isolates identified as belonging to this fungal group in this study, its clinical relevance should not be overlooked. *Aspergillus versicolor*, traditionally considered one of the most common species in this section in a clinical setting, was only rarely recovered in our study. The *in vitro* antifungal results showed that echinocandins and terbinafine were the most potent drugs, the azoles showed variable results, amphotericin B was poorly active, and 5-fluorocytosine was the less active.

Keywords: *Aspergillus*, section *Versicolores*, Multi-locus phylogeny, Taxonomy, Antifungal susceptibility.

1 Introduction

Aspergillus is one of the most ubiquitous genera of ascomycetes. It includes many species of biotechnological and industrial relevance (Houbraken et al. 2014). Some of them, particularly *Aspergillus fumigatus*, are involved in allergic diseases and severe infections in both animals and humans (de Hoog et al. 2011). Therefore, the correct identification of the fungal isolates is crucial for a better knowledge of the actual prevalence of the different species in their habitats and substrates. Traditionally, *Aspergillus* identification is based on macro- and micro-morphological characteristics,

and the species organized in groups or sections (Raper & Fennell 1965; Gams et al. 1985). Recent molecular studies have demonstrated that most of the *Aspergillus* sections are in fact monophyletic groups of closely related species. However, the boundaries of some sections still remain unclear (Houbraken & Samson 2011; Houbraken et al. 2014; Samson et al. 2014; Hubka et al. 2015). The section *Versicolores* is a clear example. It includes a group of relevant species but with a taxonomy not yet resolved. Some authors consider the delimitation of the members of this section from those of the section *Nidulantes* to be unresolved (Peterson 2008, Buzina 2013; Houbraken et al. 2014; Negri et al. 2014), while others treat *Versicolores* and *Nidulantes* as different sections (Jurjevic et al. 2012; Samson et al. 2014; Visagie et al.2014; Hubka et al. 2015). Despite their being closely related and being two monophyletic clades with low statistical support, both sections show some phenotypic characteristics that allow their distinction. Specifically, the *Versicolores* species are characterized by conidiophores with subglobose to pyriform vesicles, biseriate conidial heads, usually radiated, with greenish rough-walled usually globose to subglobose conidia (Raper & Fennel 1965; Klich 1993; Jurjevic et al. 2012). However, they are particularly difficult to distinguish among species because even though their cultural morphology is considerably different, their microscopic structures are very similar (Klich 1993, Jurjevic et al. 2012). The taxonomy of *Versicolores* has been investigated molecularly in recent years and 20 species have so far been accepted (Jurjevic et al. 2012; Samson et al. 2014; Visagie et al. 2014), *A. versicolor* and *A. sydowii* being the most well-known and studied species. The interest of the species of this section lies in their common occurrence in indoor environments (Zahradnik et al. 2013; Sharpe et al. 2015), the ability to produce sterigmatocystin, a carcinogenic and mutagenic precursor to aflatoxin $B_{1;}$ and in their different biotechnological applications (Schmitt et al. 2002;

Batista et al. 2003; Jurjevic et al. 2013; Dou et al. 2014; Li et al. 2015). Moreover, they have been reported as human and animal opportunistic pathogens (de Hoog et al. 2011; Buzina 2013) able to cause a variety of infections, including onychomycosis (Torres-Rodrígues et al. 1998; Takahata et al. 2007), endophthalmitis (Perri et al. 2005), ear infection (Rotoli et al. 2001), invasive pulmonary infections (Charles et al. 2011), aspergilloma (Kane et al. 2014), homograft valve infection (Huh et al. 2013), endodontic infection (Gomes et al. 2015) and vaginitis (Borsa et al. 2015); as well as infections in animals, such as dogs (Zhang et al. 2012) and horses (Ludwig et al. 2005; Lee et al. 2012). However, the spectrum of species of the section *Versicolores* in the clinical setting, considering modern taxonomic criteria proposed for *Aspergillus* (Jurjevic et al. 2012; Samson et al. 2014; Visagie et al. 2014), has not been fully explored. Additionally, the antifungal susceptibility of these species is practically unknown because it has only occasionally been reported (Torres-Rodríguez et al. 1998; Chavez et al. 2010; Negri et al. 2014). The aim of this study, therefore, was to investigate, using a multi-locus sequence analysis, the diversity of species of *Aspergillus* section *Versicolores* in clinical samples in the USA and to determine their *in vitro* susceptibility to the currently available antifungal drugs.

2 Materials and Methods

2.1 Fungal isolates

A total of 77 isolates of *Aspergillus* section *Versicolores* were investigated (Table 1), 69 from human origin, six from animal specimens and two from environmental source. These isolates were received at the Fungus Testing Laboratory of the University of Texas Health Science Center (USA) from other centers in the country to identify them and/or to determine their antifungal susceptibility. Most of the isolates had been

provisionally morphologically identified as *A. versicolor* (n = 74) and three as *Aspergillus* spp.

2.2 Morphological characterization

The fungal isolates were characterized morphologically following the criteria recommended by Samson et al. (2014). Briefly, the macro-morphology of the colonies and the growth rates were determined on Czapek Yeast Autolysate agar (CYA, Becton, Dickinson and Company®, Sparks MD, USA) and Malt Extract agar (MEA, Pronadisa®, Madrid, Spain) after 7 days of incubation at 25ºC and 37ºC. The microscopic structures were examined and measured on MEA cultures after 10-14 days of incubation at 25ºC, in wet mounts with 60% lactic acid. Photographs were taken with a Zeiss Axio Imager M1 light microscope (Zeiss, Oberkochen, Germany) with a mounted DeltaPix Infinity X digital camera using Nomarski differential interference contrast and phase contrast optics.

2.3 DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from MEA cultures after 7 days of incubation at 25ºC, using the FastDNA® Kit and the FastPrep® Instrument (MP Biomedicals, Irvine CA, USA), according to the manufacturer's specifications. Four genetic markers were amplified, i.e. the internal transcribed spacer (ITS) region of the rDNA, which comprises ITS1, the 5.8S gene and ITS2, and fragments of β-tubulin (*BenA*), calmodulin (*CaM*) and RNA polymerase II second largest subunit (*RPB2*) genes (Peterson 2008; Samson et al. 2014). The primers used were ITS5 and ITS4 for the ITS region (White et al. 1990), Bt2a and Bt2b for the *BenA* gene (Glass & Donaldson 1995), Cmd5 and Cmd6 for *CaM* gene (Hong et al. 2005), and 5F and 7CR for *RPB2* gene (Liu et al. 1999). PCR products were sequenced in both directions, using the same primers, at Macrogen Europe (Macrogen Inc., Amsterdam, the Netherlands). Sequences were

assembled and edited using Sequencher 4.1.4 (Gene Codes Corporation©, Ann Arbor MI, USA).

2.4 Molecular identification and phylogenetic analysis

The phylogenetic analyses were carried out first individually for each gene and after the topologies proved to be congruent, a concatenated study was then carried out. To give support to our analyses, sequences of the type strains of 19 species of the section *Versicolores* and of *Aspergillus multicolor* (outgroup) were obtained from GenBank and added to the analyses. For multiple sequence alignment, the ClustalW tool was used together with the MUSCLE tool inside MEGA v.6 software (Tamura et al. 2013), with manual adjustments for refinement. The Maximum Likelihood (ML) phylogenetic method was also run with MEGA v.6 software, as well as the estimation of the best nucleotide substitution method. Support of the internal branches was assessed by the Bootstrap method with 1,000 replications, where values ≥70 were considered significant. The Bayesian Inference (BI) method was performed using MrBayes version 3.1.2 software (Ronquist & Huelsenbeck, 2003). The evolutionary models that best fit each gene were assessed by MrModelTest software (Nylander 2004). Markov chain Monte Carlo (MCMC) sampling was performed with two simultaneous runs for 1 million generations, with samples taken every 100 generations. The 50% majority rule consensus trees and posterior probability values (pp) were calculated after removing the first 25% of the resulting trees for burn-in. A pp value of ≥0.95 was considered in the tree.

The type strain of *A. griseoaurantiacus* was not included in the final tree because the sequence for the *RPB2* gene was not available, although sequence comparison for the other three loci was done.

2.5 Antifungal susceptibility testing

A total of 73 isolates of the most frequent *Aspergillus* species identified here were tested against 9 antifungal drugs following the micro-dilution broth method, according to the document M38-A2 (CLSI, 2008). The antifungal agents, obtained as pure power, were amphotericin B (AMB) (Sigma Aldrich Quimica S.A., Madrid, Spain), itraconazole (ITC) (Jansen Pharmaceuticals, Beerse, Belgium), posaconazole (PSC) (Schering-Plough Res., Inst., NJ, EUA), voriconazole (VRC) (Pfizer S.A., Madrid, Spain), anidulafungin (AFG) (Pfizer S.A., Madrid, Spain), caspofungin (CFG) (Merk & Co., Inc., Rahway, EUA), micafungin (MFG) (Astellas Pharma, Madrid, Spain), terbinafine (TBF) and flucytosine (5FC) (Sigma Aldrich Química S.A., Madrid, Spain). The minimal inhibitory concentration (MIC) was defined as the lowest drug concentration that produced 100% inhibition of visible fungal growth for the AMB and the azoles (ITC, PSC and VRC) or 50% and 80% for 5FC and TBF, respectively. The minimum effective concentration (MEC) was determined for the echinocandins (AFG, CFG and MFG) and was defined microscopically as the lowest concentration of drug that would lead to the growth of small, rounded, compact hyphal forms as compared with the long, unbranched hyphal clusters that were seen in the growth control following 48 h of incubation. The incubation temperature was set to 30 ºC given the growth requirements of the most species of *Versicolores* (Jurjevic et al. 2012; Visagie et al. 2014). *Aspergillus flavus* (ATCC® 204304) and *Aspergillus fumigatus* (ATCC® MYA-3626) strains were used as quality controls. All tests were carried out in duplicate. Results were statistically analysed using the Prism software for Windows, version 6.0 (GraphPad Software, San Diego, CA).

2.6 Nucleotide sequence accession numbers

Sequences newly generated in this study were deposited in GenBank under accession numbers LN898664 to LN898740 (ITS), LN898818 to LN898894 (*BenA*), LN898741 to LN898817 (*CaM*) and LN898895 to LN898971 (*RPB2*) (Table 1).

3 Results

The single gene phylogenetic analyses proved that ITS, *BenA*, *CaM*, and *RPB2* were consistent for a concatenated study (see supplementary material). Therefore, a phylogenetic analysis combining the four mentioned markers was done for species recognition. The concatenated sequence alignment consisted of 2392 base pairs (ITS, 508 bp; *BenA*, 413 bp; *CaM*, 520 bp; *RPB2*, 951 bp), from which 486 were parsimony informative sites (ITS, 44; *BenA*, 76; *CaM*, 154; *RPB2*, 212). With only minor differences observed in the value of the supports of the internal nodes, the topologies of the trees obtained with ML and BI analyses were virtually the same. Based on that, our results showed that the 77 isolates included in the study clustered unambiguously with the type strains of 10 of the 20 species of the section *Versicolores* (Fig. 1). The majority of the strains nested to the *A. sydowii* (26%) clade, followed by *A. creber* (22%), *A. amoenus* (18.2%), *A. protuberus* (13%), *A. jensenii* (10.4%), *A. tabacinus* (5.2%), *A. cvjetkovicii* (1.3%), *A. fructus* (1.3%), *A. puulaauensis* (1.3%), and *A. versicolor* (1.3%).

The six isolates from animal specimens were identified as *A. amoenus*, *A. protuberus* and *A. sydowii*, with two isolates per species. The two environmental isolates belonged to *A. creber* and *A. cvjetkovicii*.

All isolates showed the typical morphological characteristics described for the *Versicolores* section. As expected, morphological identification at the species level was difficult to carry out due to the similarity of the features observed among the different

196 species of this section. Macro- and micro-morphological features of the most frequent
197 identified species are depicted in Fig 2.

198 The majority of human clinical isolates included in the study were from
199 bronchoalveolar lavage fluid (44.2%), followed by sputum (11.7%), nail (5.2%), sinus
200 (3.9%), lung biopsy (3.9%), pleural fluid (3.9%), and eye (2.6%).

201 Table 2 shows the antifungal susceptibility results of the isolates tested. In
202 general, all the drugs tested, with the exception of 5FC and AMB in some cases,
203 demonstrated potent activity. The drugs that exhibited the best results were the
204 echinocandins and TBF, with MIC values ranging from 0.03 to 0.125 µg/ml. The azoles
205 tested also showed potent activity, with MICs ranging from 0.6 to 4.0 µg/ml, but with
206 geometric means (GM) closer to the lowest MIC value (ITC, 0.283 µg/ml; PSC, 0.343
207 µg/ml; VRC, 0.88 µg/ml). The highest MICs were those of 5FC, ranging from 1.0 to
208 greater than 16.0 µg/ml, especially against *A. amoenus*, *A. creber* and *A. protuberus*,
209 with GM MICs higher than 11.0 µg/ml. For AMB, more variable results were observed
210 with MIC values ranging from 0.5 to 16.0 µg/ml. For this drug, the lowest GM MIC
211 values was observed against *A. jensenii* (0.6 µg/ml), and the highest was against *A.*
212 *sydowii* (4.7 µg/ml).

213 **4 Discussion**

214 Clinical interest in the species of *Aspergillus*, and particularly of those of the section
215 *Versicolores*, is increasing because of the reported number of infections that are
216 affecting not only humans but other mammals too (Arabatzis et al. 2011; Zhang et al.
217 2012; Huh et al. 2013; Kane et al. 2014; Negri et al. 2014; Borsa et al. 2015; Gomes et
218 al. 2015; Heo et al. 2015). However, all those reports include a single isolate, or just a
219 few, and, to date, no study has been conducted on a significant number of isolates.
220 Thus, the diversity and the relative frequency of the species of *Versicolores* in the
221 clinical setting is practically unknown. Here, using the molecular criteria proposed by

Samson et al. (2014), we found that, among the isolates belonging to that section that were received by a reference center in the USA, the most frequent species was *A. sydowii*, followed by *A. creber*, *A. amoenus, A. protuberus* and *A. jensenii*. Interestingly, this latter species together with *A. puulaauensis*, two species recently proposed by Jurjevic et al. (2012), have never been identified from clinical samples before. These results show a relative frequency and high diversity of the members of this section in this particular habitat. Although the high number of isolates recovered seems to suggest that these fungi might be opportunistic pathogens, further studies are needed to elucidate this because they might merely be contaminants or colonizers. Although *A. versicolor* has always been considered to be of some clinical relevance, its pathogenic importance might be overestimated.

The poor knowledge of the distribution and the habitat of the species of the *Versicolores* section is due to the difficulties in their morphological identification. According to Jurjevic et al. (2012), some phenotypic characteristics, such as conidial ornamentation, presence of soluble pigments, and the ability to grow at 37 ºC can be useful for differentiating some of these species. Although *A. amoenus* and *A. tabacinus* have been described with smooth conidia (Jurjevic et al. 2012), all the isolates in the present study identified molecularly as belonging to those species have finely roughened to rough conidia (Fig. 2). Only the stipe ornamentation of the conidiophores in *A. protuberus*, or growth at 37 º C in *A. amoenus*, *A. fructus*, *A. griseoaurantiacus*, *A. sydowii* and *A. versicolor* were useful for differentiating them from the rest. Our study, therefore, seems to confirm that reliable identification of these fungi is dependent on the use of molecular methods. However, in this sense, it is worth of mentioning that the analysis of ITS barcode, which is very useful for many other fungi, does not provide enough resolution for species recognition on this group of aspergilli (Jurjevic et al.

2012; Samson et al. 2014; Visagie et al. 2014). Jurjevicet al. (2012) proposed a multi-locus phylogenetic scheme to infer the phylogenetic relationship and identification of the members of the section *Versicolores*, which was based on the analysis of the markers *CaM*, *RPB2*, DNA replication licensing factor, and pre-rRNA processing protein. Samson et al. (2014) have since advocated the use of four different markers (ITS, *BenA*, *CaM* and *RPB2*) for *Aspergillus* identification in general. The combined use of these latter four genetic markers has allowed the successful identification of all the isolates investigated here.

The prevalence of *A. sydowii* in clinical samples demonstrated here has been reported previously in Czech isolates by Hubka et al. (2012). In that study, *A. sydowii* was the second most common species after *A. fumigatus*, with 17 of the 178 isolates (9.6%), and was involved mainly in superficial infections, affecting nails and skin, but also in ear and respiratory infections. Other studies have also reported this species to be an opportunistic pathogen (de Hoog et al. 2011; Nouripour-Sisakht et al. 2015, Sabino et al. 2014). In our case, *A. sydowii* was identified from very different human specimens, including superficial and deep tissues (Table 1). Although we are not able to demonstrate the pathogenic role of the isolates investigated, the high number of strains reinforces the importance of *A. sydowii* in the clinical setting.

Aspergillus creber, *A. amoenus* and *A. protuberus* represented here by 17, 14 and 10 of the isolates, respectively, have been recently reported as causal agents of infections in Brazil (*A. creber*, Negri et al. 2014) and in Turkey (*A. protuberus*, Borsa et al. 2015), while *A. amoenus*, a species previously identified as *A. versicolor*, was isolated from mammary gland in the USA (Jurjevic et al. 2012). Other species identified in our study, although with a lower frequency, were *A. tabacinus* with four isolates and *A. fructus* with one isolate. The former was previously isolated in Brazil from

respiratory secretions (Negri et al. 2014) and the latter in Portugal from a patient suspected to have allergic bronchopulmonary aspergillosis (Sabino et al, 2014).

The species *A. cvjetkovicii*, *A. jensenii* and *A. puulaauensis*, closely related to *A. creber* and *A. sydowii*, constituted together a well-supported clade that represent the 61% (47 of 77) of all the isolates identified. Due to the similarity among the species of this clade, some of them might have been misidentified in previous studies as *A. sydowii*, which may have hampered the significance of the other species.

The data available on the antifungal susceptibility of these fungi are very scarce and usually limited to occasional reports and with no confirmation of the correct identification of the species involved. In our study, the echinocandins and TBF showed the lowest MICs, and 5FC and AMB were the least potent. However, the data provided here are to some extent similar to those previously reported (Cuenca-Estrella & Rodriguez-Tudela 2010; Arabatzis et al. 2011; Buzina 2013). For instance, in the case of AMB, the MICs of our isolates were similar to those of the study of Heo et al. (2015), in which 6 strains of this section were studied and the range observed was from 1.0 to 2.0 µg/mL. Against *A. sydowii*, we observed lower potency for AMB than in previous reports, in which the isolates may have been misidentified (García-Martos et al. 2005; Buzina 2013; Heo et al. 2015). With respect to the azoles, the results were more variable, depending on the species and drugs tested; the less active being VRC against *A. sydowii.* In general, potent activity of these drugs has been reported (Pfaller et al. 2002; Arabatzis et al. 2011; Buzina, 2013). However, triazole resistance and elevated MIC values have also been reported for *A. versicolor* previously (Torres-Rodríguez et al. 1998; Baddley et al. 2009; Espinel-Ingroff et al. 2010).

In conclusion, the clinical relevance of the species of *Aspergillus* section *Versicolores* should not be overlooked, and it seems highly likely that apart from *A.*

297 *sydowii* other species of the section can also be responsible of human infections. Further
298 studies are needed, at least in animal models, to prove the pathogenic role of these
299 species and to evaluate the most appropriate therapies.

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Fig 1 − Maximum likelihood tree obtained from the combined ITS, *BenA*, *CaM* and *RPB2* sequences of the isolates. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The fully supported branches (100/1) and type strains are shown in bold. ■ indicates strain of animal origin, ● indicates the environmental strain. UTHSC, University of Texas Health Science Center (USA); FMR, Facultat de Medicina de Reus (Spain).

Fig 2 − Morphological features of *A. amoenus* (A−D), *A. creber* (E−H), *A. jensenii* (I−L), *A. protuberus* (M−P) and *A. sydowii* (Q−T). Colonies on CYA at 25 ºC after 7 days, front (A, E, I, M, Q) and reverse (B, F, J, N, R). Conidiophores (C, G, K, O, S) and conidia (D, H, L, P, T). Scale bars: C, D, G, H, K, L, O, P, T = 10 µm; S= 20 µm.

Table(s)

TABLE 1 − GenBank accession numbers of the sequences of each of the *Aspergillus* strains included in this study

Species	Isolate number	Origin	GenBank accession number			
			ITS	*BenA*	*CaM*	*RPB2*
A. amoenus (14)	UTHSC 05-2980	Animal	LN898664	LN898818	LN898741	LN898895
	UTHSC 06-1721	BAL	LN898665	LN898819	LN898742	LN898896
	UTHSC 07-1668	Sinus	LN898666	LN898820	LN898743	LN898897
	UTHSC 07-2785	Pleural fluid	LN898667	LN898821	LN898744	LN898898
	UTHSC 07-2881	Pleural fluid	LN898668	LN898822	LN898745	LN898899
	UTHSC 08-2366	---	LN898669	LN898823	LN898746	LN898900
	UTHSC 11-476	Sputum	LN898670	LN898824	LN898747	LN898901
	UTHSC 11-1419	BAL	LN898671	LN898825	LN898748	LN898902
	UTHSC 06-4284	BAL	LN898672	LN898826	LN898749	LN898903
	UTHSC 09-125	BAL	LN898673	LN898827	LN898750	LN898904
	UTHSC 12-340	Animal	LN898674	LN898828	LN898751	LN898905
	UTHSC 07-443	BAL	LN898675	LN898829	LN898752	LN898906
	UTHSC 07-3621	Chest	LN898676	LN898830	LN898753	LN898907
	UTHSC 09-2582	Lung biopsy	LN898677	LN898831	LN898754	LN898908
A. creber (17)	UTHSCDI 14-226	BAL	LN898678	LN898832	LN898755	LN898909
	UTHSCDI 14-228	Nail	LN898679	LN898833	LN898756	LN898910
	UTHSC 14-223	Arm	LN898680	LN898834	LN898757	LN898911
	UTHSC 03-2409	Environment	LN898681	LN898835	LN898758	LN898912
	UTHSC 05-2359	BAL	LN898682	LN898836	LN898759	LN898913
	UTHSC 09-1670	BAL	LN898683	LN898837	LN898760	LN898914
	UTHSC 09-3357	BAL	LN898684	LN898838	LN898761	LN898915
	UTHSC 14-188	BAL	LN898685	LN898839	LN898762	LN898916
	UTHSC 06-3435	BAL	LN898686	LN898840	LN898763	LN898917
	UTHSC 10-1327	Nail	LN898687	LN898841	LN898764	LN898918
	UTHSC 11-2813	Skin mucosa	LN898688	LN898842	LN898765	LN898919
	UTHSC 09-2679	BAL	LN898689	LN898843	LN898766	LN898920

	UTHSC 10-639	BAL	LN898690	LN898844	LN898767	LN898921
	UTHSC 04-799	Sputum	LN898691	LN898845	LN898768	LN898922
	UTHSC 07-2788	BAL	LN898692	LN898846	LN898769	LN898923
	UTHSC 04-434	Sputum	LN898693	LN898847	LN898770	LN898924
	UTHSC 10-582	BAL	LN898694	LN898848	LN898771	LN898925
A. cvjetkovicii	UTHSC 10-479	Environment	LN898695	LN898849	LN898772	LN898926
A. fructus	UTHSC 12-3194	Pericardium	LN898696	LN898850	LN898773	LN898927
A. jensenii (8)	UTHSCDI 14-220	Nail	LN898697	LN898851	LN898774	LN898928
	UTHSC 05-3600	Sputum	LN898698	LN898852	LN898775	LN898929
	UTHSC 09-2299	Sputum	LN898699	LN898853	LN898776	LN898930
	UTHSC 10-327	Sputum	LN898700	LN898854	LN898777	LN898931
	UTHSC 12-79	BAL	LN898701	LN898855	LN898778	LN898932
	UTHSC 07-3790	BAL	LN898702	LN898856	LN898779	LN898933
	UTHSC 10-71	BAL	LN898703	LN898857	LN898780	LN898934
	UTHSC 09-425	Nail	LN898704	LN898858	LN898781	LN898935
A. protuberus (10)	UTHSC 06-4104	BAL	LN898705	LN898859	LN898782	LN898936
	UTHSC 09-246	Animal	LN898706	LN898860	LN898783	LN898937
	UTHSC 11-269	BAL	LN898707	LN898861	LN898784	LN898938
	UTHSC 07-2433	BAL	LN898708	LN898862	LN898785	LN898939
	UTHSC 08-3392	BAL	LN898709	LN898863	LN898786	LN898940
	UTHSC 11-2175	Sputum	LN898710	LN898864	LN898787	LN898941
	UTHSC 12-338	Animal	LN898711	LN898865	LN898788	LN898942
	UTHSC 12-256	BAL	LN898712	LN898866	LN898789	LN898943
	UTHSC 06-2837	BAL	LN898713	LN898867	LN898790	LN898944
	UTHSC 08-1574	BAL	LN898714	LN898868	LN898791	LN898945
A. puulaauensis	UTHSC 11-1436	BAL	LN898715	LN898869	LN898792	LN898946
A. sydowii (20)	UTHSC 09-48	Blood	LN898716	LN898870	LN898793	LN898947
	UTHSC 11-204	Eye	LN898717	LN898871	LN898794	LN898948
	UTHSC 13-2518	Eye	LN898718	LN898872	LN898795	LN898949
	UTHSC 13-2630	Sinus	LN898719	LN898873	LN898796	LN898950

	UTHSC 06-2186	BAL	LN898720	LN898874	LN898797	LN898951
	UTHSC 06-2780	Bronchus	LN898721	LN898875	LN898798	LN898952
	UTHSC 06-4167	Sinus	LN898722	LN898876	LN898799	LN898953
	UTHSC 07-1018	Animal	LN898723	LN898877	LN898800	LN898954
	UTHSC 09-97	BAL	LN898724	LN898878	LN898801	LN898955
	UTHSC 12-934	BAL	LN898725	LN898879	LN898802	LN898956
	UTHSC 13-2674	BAL	LN898726	LN898880	LN898803	LN898957
	UTHSC 10-1222	---	LN898727	LN898881	LN898804	LN898958
	UTHSC 10-3180	Sputum	LN898728	LN898882	LN898805	LN898959
	UTHSC 11-2683	Spine	LN898729	LN898883	LN898806	LN898960
	UTHSC 06-727	BAL	LN898730	LN898884	LN898807	LN898961
	UTHSC 08-3215	Animal	LN898731	LN898885	LN898808	LN898962
	UTHSC 09-1708	Lung biopsy	LN898732	LN898886	LN898809	LN898963
	UTHSC 12-3109	Lung biopsy	LN898733	LN898887	LN898810	LN898964
	UTHSC 08-865	Hip joint	LN898734	LN898888	LN898811	LN898965
	FMR 14440	Ear exudate	LN898735	LN898889	LN898812	LN898966
A. tabacinus (4)	UTHSC 03-1197	Sputum	LN898736	LN898890	LN898813	LN898967
	UTHSC 07-2427	BAL	LN898737	LN898891	LN898814	LN898968
	UTHSC 10-1677	Pleural fluid	LN898738	LN898892	LN898815	LN898969
	UTHSC 08-2898	BAL	LN898739	LN898893	LN898816	LN898970
A. versicolor	UTHSC 03-3679	BAL	LN898740	LN898894	LN898817	LN898971

BAL, bronchoalveolar lavage fluid specimen; FMR, Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, USA.

TABLE 2 − Results of *in vitro* antifungal susceptibility test for 73 isolates of *Aspergillus* section *Versicolores*

Species (no. of isolates)	Parameter	MIC or MEC (μg/ml) for:								
		5FC	AFG	AMB	CFG	ITC	MFG	PSC	TBF	VRC
A. sydowii (20)	GM	6.616	0.03	4.757	0.03	0.334	0.03	0.595	0.0318	1.498
	MIC range	1.0–16	0.03	1.0–16.0	0.03	0.125–2.0	0.03	0.125–2.0	0.03–0.125	1.0–4.0
	MIC_{90}	8.0	0.03	8.0	0.03	0.5	0.03	2.0	0.125	4.0
A. creber (17)	GM	11.81	0.03	2.378	0.03	0.31	0.03	0.354	0.033	1.091
	MIC range	1.0–>16	0.03	1.0–8.0	0.03	0.125–1.0	0.03	0.125–0.5	0.03–0.125	0.5–2.0
	MIC_{90}	>16.0	0.03	8.0	0.03	0.5	0.03	0.5	0.03	2.0
A. amoenus (14)	GM	16.81	0.03	1.903	0.03	0.086	0.03	0.13	0.03	0.25
	MIC range	8.0–>16	0.03	1.0–4.0	0.03	0.06–0.125	0.03	0.06–0.25	0.03–0.03	0.125–0.5
	MIC_{90}	>16.0	0.03	4.0	0.03	0.125	0.03	0.25	0.03	0.5
A. protuberus (10)	GM	12.13	0.03	0.707	0.03	1.072	0.03	0.466	0.03	1.149
	MIC range	2.0–>16.0	0.03	0.5–1.0	0.03	0.5–4.0	0.03	0.25–0.5	0.03–0.03	1.0–2.0
	MIC_{90}	>16.0	0.03	1.0	0.03	2.0	0.03	0.5	0.03	2.0
A. jensenii (8)	GM	4.416	0.03	0.609	0.03	0.112	0.03	0.136	0.03	0.609
	MIC range	1.0–>16.0	0.03	0.5–1.0	0.03–0.06	0.06–0.25	0.03	0.06–0.25	0.03–0.03	0.25–1.0
A. tabacinus (4)	GM	4.595	0.03	2.297	0.03	0.6	0.03	0.66	0.03	1.149
	MIC range	2.0–8.0	0.03	2.0–4.0	0.03	0.25–1.0	0.03	0.5–1.0	0.03–0.03	1.0–2.0
Total (73)	GM	8.844	0.03	2.132	0.03	0.283	0.03	0.343	0.031	0.88
	MIC range	1.0–>16.0	0.03	0.5–16.0	0.03–0.06	0.06–4.0	0.03	0.06–2.0	0.03–0.125	0.06–2.0
	MIC_{90}	>16.0	0.03	8.0	0.03	1.0	0.03	1.0	0.03	2.0

5FC, flucytosine; AFG, anidulafungin; AMB, amphotericin B; CFG, caspofungin; ITC, itraconazole; MFG, micafungin; PSC, posaconazole; TBF, terbinafine; VRC, voriconazole; MIC, minimum inhibitory concentration; MEC, minimum effective concentration, for AFG, CFG, and MFG; GM, geometric mean.

Figure(s)

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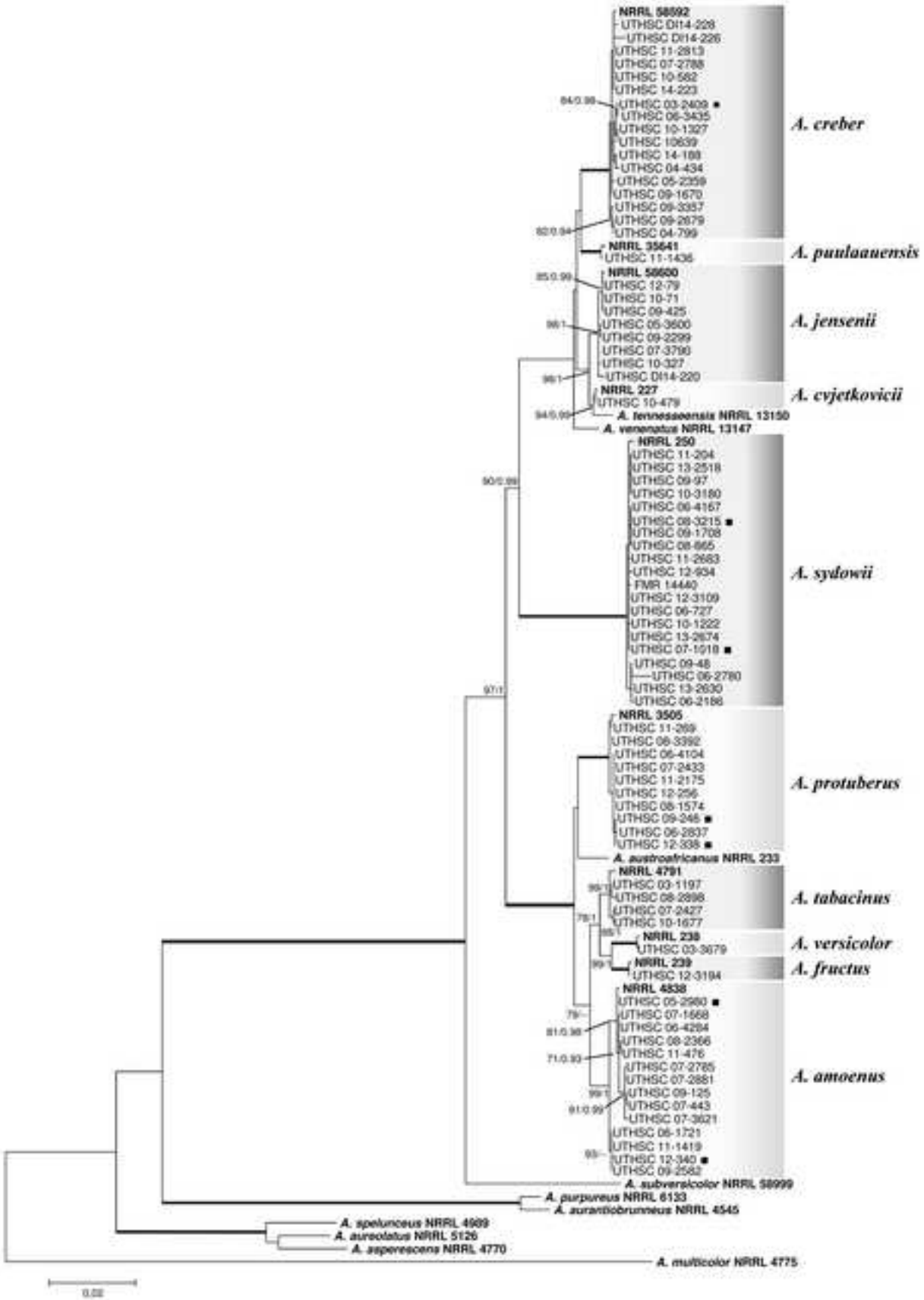

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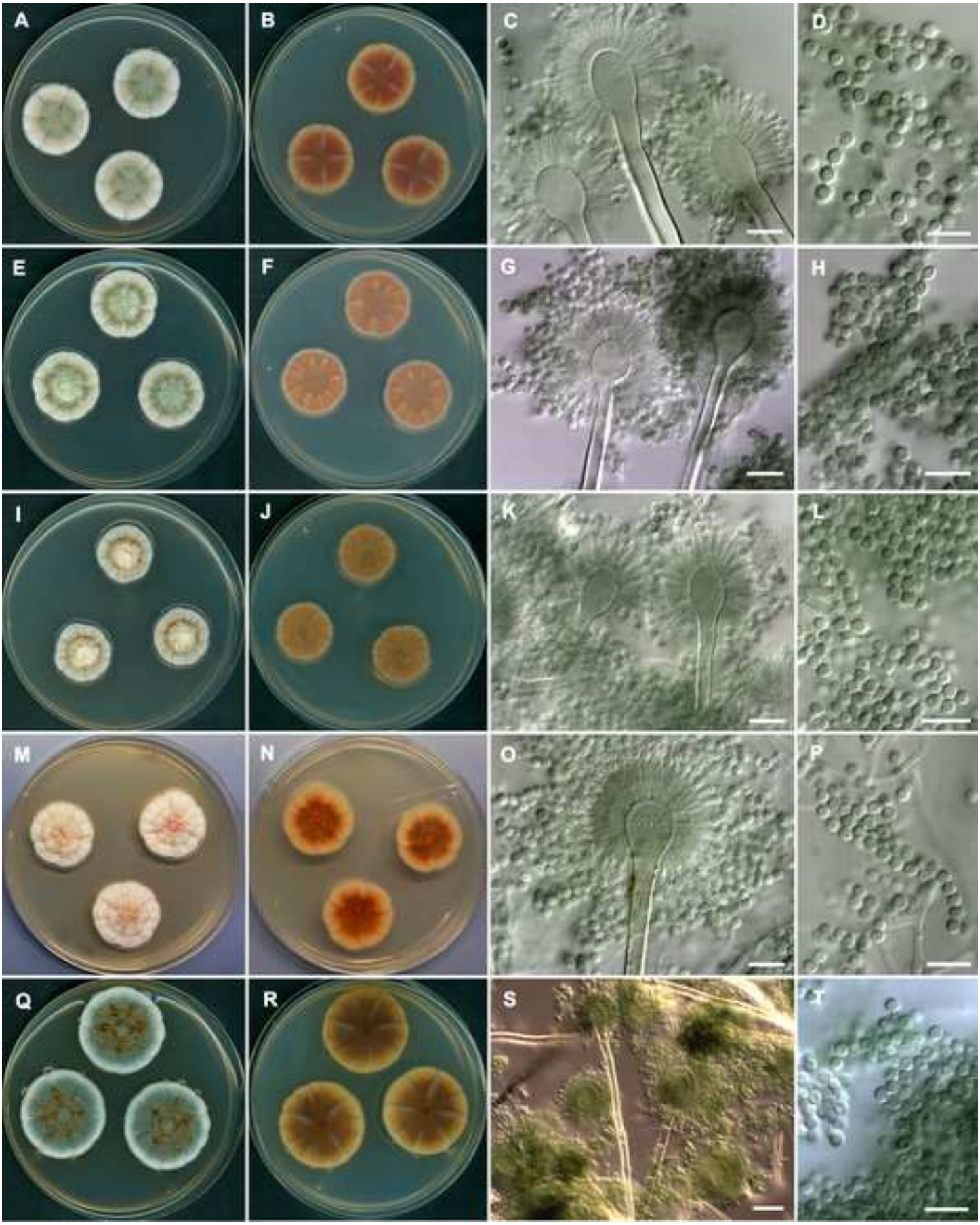

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