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Cladosporium species recovered from clinical samples in the United States

RUNNING TITLE: *Cladosporium* from clinical samples

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ABSTRACT

Cladosporium species are ubiquitous, saprobic dematiaceous fungi, only infrequently associated with human and animal opportunistic infections. We have studied a large set of *Cladosporium* isolates recovered from clinical samples in the USA to ascertain the predominant species in that country, in light of recent taxonomic changes in this genus, and to determine if some could possibly be rare potential pathogens. A total of 92 isolates were identified using phenotypic and molecular methods, which included sequence analysis of the internal transcribed spacer (ITS) region, and a fragment of the large subunit (LSU) of the nuclear ribosomal DNA, as well as fragments of the translation elongation factor 1 alpha (*EF*-1α) and actin (*Act*) genes. The most frequent species was *Cladosporium halotolerans* (14.8%), followed by *C. tenuissimum* (10.2%), *C. subuliforme* (5.7%) and *C. pseudocladosporioides* (4.5%). However, 39.8 % of the isolates did not correspond to any known species comprising at least 17 new lineages for *Cladosporium*. The most frequent anatomic site of isolation was the respiratory tract (54.5%), followed by superficial (28.4%) and deep tissues and fluids (14.7%). Species of the two recently described cladosporium-like genera, *Toxicocladosporium* and *Penidiella,* are reported for the first time from clinical samples. *In vitro* susceptibility testing of 92 isolates against nine antifungal drugs showed variable results, but with a high overall activity for the azoles, echinocandins and terbinafine.

INTRODUCTION

Cladosporium species are among the most common fungal inhabitants worldwide, being isolated from almost any environmental source and geographic location (1). The genus is characterized by the typical form of its conidiophores, which are erect, straight or geniculate, producing abundant branched acropetal chains of smooth to roughened dry conidia, showing a distinct darkened coronate hilum, i.e, conidial scar characterized by a thick rim surrounding a central convex dome (2, 3). The relatively small conidia are easily detached and disseminated by the wind, *Cladosporium* being one of the most frequently isolated air-borne fungi (2, 4).

The most common *Cladosporium* species are primarily isolated from soil and plant material, where they are frequently encountered as saprobes or secondary invaders on follicular lesions, concomitant with other plant pathogenic fungi (1, 5, 6). However, several species are important pathogens of plants and some are also able to affect animals including humans (7-9). *Cladosporium* is usually associated with allergic rhinitis (10) or localized superficial or deep lesions (11-14), but rarely can cause disseminated infections (7, 15-17).

The genus *Cladosporium* has been shown to be both morphologically and phylogenetically heterogeneous (18). On the basis of molecular data, the true human-pathogenic species *C. bantiana*, *C. carrionii* and *C. devriesii*, characterized by their thermotolerance and the absence of conidiophores with pigmented conidial scars, were transferred to *Cladophialophora* (1, 7, 18). More recently, *Cladosporium* underwent extensive revisions based on polyphasic approaches (1, 3, 19-21), which resulted in the delimitation of 169 species currently accepted in *Cladosporium* sensu stricto (*Cladosporiaceae, Capnodiales*). On the other hand, a great number of taxa were excluded from that genus, now being considered as doubtful species or accommodated into several related new genera such as *Hyalodendriella*, *Ochrocladosporium*, *Rachicladosporium*, *Rhizocladosporium*, *Toxicocladosporium* and *Verrucocladosporium* (1, 3).

The diversity of *Cladosporium* associated with human disease is currently reduced to four species, i.e., *C. cladosporioides, C. herbarum, C. oxysporum* and *C. sphaerospermum* (7). Most of these data, however, are based on a reduced number of clinical cases with the identification of the etiological agents not confirmed by using reliable methods. Moreover, three of the clinically relevant species: *C. cladosporioides, C. herbarum* and *C. sphaerospermum* have been demonstrated to be species complexes (19-21), encompassing several morphologically sibling species only distinguished by means of phylogenetic analyses (1, 7). The clinical significance of these phylogenetic species, however, has yet to be evaluated (22).

The objective of this work was to assess the diversity of *Cladosporium* species associated with human and animal disease by analysing a large set of isolates from clinical specimens by means of phenotypic and DNA sequence data analyses. In addition, the *in vitro* susceptibility of these isolates was evaluated against nine clinically available antifungal drugs.

MATERIALS AND METHODS

Fungal isolates. A total of 92 isolates tentatively identified as *Cladosporium* spp. were included in this study (Table 1). All of the isolates were obtained from human and animal clinical specimens, mostly from the United States, received in the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (UTHSC) from different parts of the country mainly for identification purposes.

Phenotypic identification. The isolates were morphologically characterized following the procedures outlined in Bensch et al. (1), Crous et al. (23), Schubert et al. (19), and Zalar et al. (20). Briefly, all of the isolates were grown on Synthetic Nutrient-Poor Agar (SNA; KH_2PO_4 1 g, KNO_3 1 g, $MgSO_4.7H_2O$ 0.5 g, KCl 0.5 g, glucose 0.2 g, sucrose 0.2 g, water 1L) and Potato Dextrose Agar (PDA: Pronadisa, Spain) for 7d at 25 ºC. Microscopic observations were made from cultures on SNA mounted in Shear's solution (23). Colony characteristics were recorded from cultures on SNA and PDA. For the estimation of cardinal growth temperatures, the isolates were grown on PDA agar for 14d at temperatures ranging from 15 ºC to 35 ºC at intervals of 5 ºC, and 37 ºC.

DNA extraction, amplification and sequencing. Total genomic DNA was extracted from mycelia obtained from colonies growing on PDA, using FastPrep (MP Biomedicals, Santa Ana, California, USA) following the manufacturer's protocol. DNA was quantified using Nanodrop 3000 (Thermo Scientific, Madrid, Spain).

The primers ITS5 and ITS4 (24) were used to amplify a region spanning the internal transcribed spacers (ITS) 1 and 2 and the 5.8S gene of the rRNA, the primers pairs LR0R/LR5 (25, 26) were used to amplify a fragment of the LSU gene of the rRNA, and the EF-728F/EF-986R and ACT-512F/ACT-783R primers (27) were used for the translation elongation factor 1-α gene (*EF*-1α) and the actin gene (*Act*), respectively.

Sequencing was performed in both directions using the same PCR primers at Macrogen Europe (Macrogen Inc. Amsterdam, the Netherlands). Consensus sequences were obtained using SeqMan version 7.0.0 (DNAStar Lasergene, Madison, WI, USA).

Molecular identification and phylogenetic analyses. An initial presumptive generic identification of the isolates was performed based on BLAST searches of ITS and LSU sequences in the GenBank (http://www.ncbi.nlm.nih.gov/) and CBS (http://www.cbs.knaw.nl/) databases. Multiple sequence alignments of each locus were performed in MEGA version 6 (28) using the ClustalW application (29), refined with MUSCLE (30), and manually adjusted if necessary. Phylogenetic reconstructions were made using maximum-likelihood (ML) and Bayesian Inference (BI) under MEGA version 6 and MrBayes version 3.1.2 (31), respectively. The best nucleotide substitution model (GTR+G+I for the three independent datasets) was estimated using MrModelTest version 2.3 (32) following the Akaike criterion. Phylogenetic analyses using ML were at first made individually for each locus and compared in order to assess for any incongruent results between nodes with high statistical support. As no incongruences were observed, the four loci were combined as follows: ITS, *EF*-1α and *Act* for members of *Cladosporium* sensu stricto and ITS combined with LSU for members of other cladosporium-like genera. For the ML analysis, nearest-neighbour interchange (NNI) was used as the heuristic method for tree inference. Support for the internal branches was assessed by a search of 1,000 bootstrapped sets of data. A bootstrap support (bs) of ≥70 was considered significant. For BI analysis, two simultaneous runs of 10,000,000 generations were performed and samples were stored every 1,000 generations. The 50% majority-rule consensus tree and posterior probability values (pp) were calculated after discarding the first 25% of the samples. A pp value ≥0.95 was considered significant.

The first combined phylogenetic analysis with ITS, *EF*-1α and *Act* sequences of clinical isolates belonging to *Cladosporium* sensu stricto and all the available type and reference strains was carried out following the alignments of Bensch et al. (1) (data not shown). Only sequences of those species closely related (>95% similarity) with the clinical isolates tested here were included in the final analysis.

Antifungal Susceptibility. The antifungal susceptibility test was performed according to the CLSI M38-A2 standard (33) with slight modifications. The incubation temperature was set to 25 ºC given the optimal growth requirements of *Cladosporium* and related taxa (1, 33). Nine antifungal agents were tested: amphotericin B (AMB), 5-fluorocytosine (5FC), itraconazole (ITC), posaconazole (PSC), voriconazole (VRC), terbinafine (TRB), anidulafungin (AFG), caspofungin (CFG) and micafungin (MFG). The minimal effective concentration (MEC), defined as the lowest drug concentration at which short, stubby, highly branched hyphae were observed, was determined at 24 h for the echinocandins, and the minimal inhibitory concentration (MIC) was determined at 48 h for the remaining drugs. The MIC was defined as the lowest concentration exhibiting 100% visual inhibition of growth for AMB, ITC, PSC and VRC, or 50% and 80% reduction in growth for 5FC and TRB, respectively. *Paecilomyces variotii* ATCC MYA-3630 and *Aspergillus fumigatus* ATCC MYA-

3626 were used as quality control strains. Statistical analyses of the MIC/MEC data were performed using the Mann-Whitney test in Prism version 6.0 (GraphPad Software, San Diego, CA).

Nucleotide sequence accession numbers. DNA sequences determined in this study were deposited in GenBank under accession numbers LN834353 to LN834448 (rDNA), LN834449 to LN834536 (*EF*-1α) and LN834537 to LN834624 (*Act*) (Table 1).

RESULTS

Analysis of ITS and LSU sequences showed that 88 isolates (96%) belonged to *Cladosporium* sensu stricto, three isolates (3%) to the genus *Toxicocladosporium* and one isolate (1%) to the genus *Penidiella*.

The phylogenetic analysis of *Cladosporium* sensu stricto included 121 taxa and 1,002 bp (ITS 447 bp, *EF*-1α 337 bp and *Act* 218 bp) from which 485 bp were constant (ITS 347 bp, EF-1α 60 bp and Act 78 bp), 496 were variable (ITS 97 bp, EF-1α 260 bp and Act 139 bp) and 328 were parsimony informative (ITS 24 bp, EF-1α 197 bp and Act 107 bp) (Fig 1). The majority of isolates (57, 65%) nested into the *C. cladosporioides* complex: 28 belonged to nine species (i.e., *C. angustisporum, C. asperulatum, C. cladosporioides, C. flabelliforme, C. funiculosum, C. perangustum, C. pseudocladosporioides, C. subuliforme* and *C. tenuissimum*), while 29 isolates clustered into 12 terminal sub-clades genetically distant to any currently known species of the genus. A total of 14 isolates were related to the *C. herbarum* complex (16%), mostly corresponding to five species (i.e., *C. allicinum*, *C. herbarum*, *C. macrocarpum*, *C. ramotenellum*, and *C. subinflatum*), while five isolates clustered into four new lineages in the genus.

Seventeen isolates were nested within the *C. sphaerospermum* complex (19%) and mostly belonged to two species (i.e., *C. halotolerans* and *C. sphaerospermum*), while a single isolate represented a new lineage.

Distinct morphological features of isolates in the *C. cladosporioides* complex included the formation of mostly unbranched, cylindrical conidiophores, bearing ovoidal to ellipsoidal intercalary and terminal conidia, smooth or rarely showing fine ornamentation (Fig. 2 a-c); the maximum temperature for growth was 32 ºC for *C. cladosporioides*, *C. flabelliforme*, *C. perangustum* and *C. pseudocladosporioides*; and 35 ºC for *C. angustisporum*, *C. funiculosum*, *C. subuliforme* and *C. tenuissimum*. Isolates of the *C. herbarum* complex exhibited mostly nodulose conidiophores, bearing distinctly ornamented globose to subglobose terminal conidia (Fig. 2 d-f); none of the isolates of this complex were able to grow at temperatures above 32 ºC, while *C. allicinum* exhibited a maximum growth temperature of 30 ºC. Isolates of the *C. sphaerospermum* complex formed cylindrical and branched conidiophores, bearing globose to subglobose conidia, smooth or finely ornamented (Fig. 2 g-i); the maximum temperature for growth was 32 ºC for *C. sphaerospermum* and 35 ºC for *C. halotolerans*. None of the clinical isolates formed sexual morphs in culture.

Overall, the most commonly identified species was *C. halotolerans* (14.8%), followed by *C. tennuissimum* (10.2%) and *C. subuliforme* (5.7%). However, 39.8 % did not match with any known taxa, representing at least 17 putative new *Cladosporium* species (Fig. 1). The most common anatomical site of isolation was the respiratory tract (54.5%), mainly from bronchoalveolar lavage fluid (BAL) and nasal specimens, followed by superficial sites (28.4%);

these percentages were similar for all the identified species and species complexes.

Phylogenetic analysis of the *Toxicocladosporium* isolates included 15 taxa and 984 bp (LSU 530 bp, ITS 454 bp) from which 826 bp were constant (LSU 464 bp and ITS 362 bp), 155 were variable (LSU 66 bp and ITS 89 bp) and 129 were parsimony informative (LSU 56 bp and ITS 73 bp) (Fig 3). Two clinical isolates belonged to *T. irritans*, while the isolate UTHSC DI-13-172 formed an independent lineage, genetically related to *T. strelitziae*, but showing distinctive morphological features and probably corresponding to a new species. The main morphological characteristics of members of *Toxicocladosporium* were the presence of non-nodulose conidiophores with dark and thickened cell walls and septa, producing conidia without the typical coronate scars of *Cladosporium* (Fig. 2 j-l), and a maximum temperature for growth of 35 ºC.

According to the LSU sequence analysis, a single isolate (UTHSC DI-13-256), originally identified as *C. sphaerospermum*, was related to but distant (<98.2% sequence similarity) from members of the genus *Penidiella* (i.e., *P. aggregata* and *P. drakensbergensis*, sequence accession numbers JF499862 and KC005792, respectively) (data not shown). However, its final identification was not possible given the scarcity of DNA sequences of the latter species for comparison. This isolate was characterized by restricted growth (3-4 mm at 25 ºC for 7d) and the production of solitary penicillate conidiophores, composed of chains of ramoconidia with slightly pigmented and thickened conidiogenous scars.

Results of the antifungal susceptibility testing are summarized in Table 2. Overall results for *Cladosporium* species showed a geometric mean (GM) MIC and MIC_{90} values for AMB of 0.64 µg/ml and 2 µg/ml, respectively. Among the azoles, ITC and PSC were the most active, both drugs with a GM MIC of 0.43 µg/ml and a MIC_{90} of 0.5 µg/ml and 1 µg/ml, respectively; while VRC showed a GM MIC and MIC_{90} of 1.68 µg/ml and 4 µg/ml, respectively. Flucytosine showed variable activity with a GM MIC and MIC_{90} of 1.37 µg/ml and 4 µg/ml, respectively. TRB exhibited the most potent activity with a GM MIC and MIC_{90} of 0.09 µg/ml and 1 µg/ml, respectively. With the exception of CFG, the echinocandins exhibited strong *in vitro* activity with GM MIC values of 0.19 µg/ml and 0.12 µg/ml for AFG and MFG. All of the *Cladosporium* species tested showed similar susceptibility patterns except for *C. sphaerospermum*, where the three isolates tested exhibited higher MIC and MEC values, especially for the azoles, AMB, AFG and MFG ($P<0.001$). Comparison of antifungal susceptibility by species complex (Table 3) showed that AMB exhibited more potent activity against members of the *C. herbarum* complex with GM MIC and MIC_{90} values of 0.18 µg/ml and 1 µg/ml ($P<0.002$), while members of *C. sphaerospermum* complex exhibited higher GM MIC and MIC_{90} values for AMB, PSC, ITC and CSP ($P<0.003$). *Toxicocladosporium* and *Penidiella* isolates exhibited similar susceptibility patterns mostly with low GM MIC and MIC_{90} values against all antifungals tested but without statistically significant differences.

DISCUSSION

Members of *Cladosporium* are relatively easy to identify to genus and species complex based on their typical conidiogenous structures. However, morphological identification of *Cladosporium* species is difficult given the high morphological similarity between closely related species. In light of our results, it is strongly recommended that the phenotypic identification be confirmed with DNA sequencing. Several authors have demonstrated the usefulness of *EF*-1α and *Act* loci to allow a good species delimitation in *Cladosporium* (1, 19, 21). This is especially important for members of the *C. cladosporioides* complex, which demonstrated the greatest species diversity, the highest number of species associated with clinical samples, and also the greatest number of undescribed species. Moreover, we found that *C. cladosporioides*, the species most frequently cited as being clinically relevant, was poorly represented in our set of isolates, while *C. asperulatum*, *C. funiculosum*, *C. flabelliforme*, *C. pseudocladosporioides*, *C. subuliforme* and *C. tenuissimum* are described from the first time from clinical samples. Similarly, in the *C. sphaerospermum* complex, most of the isolates morphologically identified as *C. sphaerospermum* were genetically reidentified as belonging to the phenotypically similar species *C. halotolerans,* which according to our data, emerged as the most common species from clinical origin. The latter species has never been associated with human infection; however, some isolates had been reported from human or animal clinical samples (1). In the case of the *C. herbarum* complex, 13 of the 14 isolates morphologically identified as *C. herbarum*, also considered a clinically relevant species, were found to belong to other species of this complex (i.e., *C. allicinum*, *C. macrocarpum* and *C. ramotenellum*). While *C. macrocarpum* has been identified as the causative agent of human infections (17), *C. allicinum* and *C. ramotenellum* never have been reported before in the clinical setting, although some isolates have been recorded as obtained from human samples (1). However, due to the lack of clinical histories and histopathological findings, it was impossible for us to confirm the pathogenic role of the species reported here for the first time from clinical specimens.

It is remarkable that our phylogenetic analysis was unable to identify a high number of *Cladosporium* isolates at the species level (39.8%), which were originally considered as belonging to several common morphospecies. Instead, those unidentified isolates were grouped into 5 terminal clades and 12 monotypic lineages, representing a high variety of phylogenetic species. It is probable that many of these clades and monotypic lineages represent new species, however further studies combining phenotypic and molecular data would be necessary to confirm these findings. We report also for the first time the isolation of *Toxicocladosporium* and *Penidiella* species from clinical specimens. Isolates of such recently proposed genera were only known from leafs of several plants and environmental sources (3, 34). According to our data, the vast majority of isolates were obtained from respiratory specimens, including BAL, nasal and sputum samples. This is not rare, because *Cladosporium* is preponderant in the air-borne mycobiota (35), being considered as one of the most important respiratory allergenic fungi, after *Alternaria* (10, 34, 36).

Reports of invasive infections by *Cladosporium* are extremely rare. Bentz & Sautter (37) reported a mixed disseminated infection by *Aspergillus fumigatus* and *C. cladosporioides* in an immunocompromised patient. *Cladosporium cladosporioides* and *C. macrocarpum* have been reported from two clinical cases involving the central nervous system (15, 17), while *C. sphaerospermum* was isolated from an intrabronquial infection (38). However, in none of these cases was the etiology of the infection supported by histopathological studies. The isolation of *Cladosporium* species from deep tissues seems improbable considering the inability of these organisms to growth at temperatures exceeding 35 ºC, and thermotolerance being one of the most important virulence factors for invasive or disseminated infections (39). In our study less than half of the isolates exhibited very limited growth at 35 ºC, while none was able to grow at 37 ºC. However, surprisingly, several of our isolates were obtained from deep tissue samples, including bone marrow, CSF, lung and lymphatic tissue, among others. Isolation of these fungi from invasive infections may have been due to environmental contamination of the samples, however occasionally isolates that fail to grow in culture at 37 ºC have been reported to cause invasive disease in compromised individuals (40).

There is a paucity of information regarding antifungal susceptibility patterns for *Cladosporium* species. Most data are from a few reported clinical cases (7, 13, 15, 41). Our study provides the first *in vitro* data for a large set of clinical isolates including several species obtained from diverse anatomical sites and not previously reported from clinical samples. Case reports have shown a favourable outcome using azole-based therapies. ITC has shown efficacy in the treatment of superficial infections caused by *C. cladosporioides*, *C. sphaerospermum* and *C. oxysporum* (8, 14, 37, 41-44), while VRC was effective against *C. macrocarpum* in a brain abscess (17). This agrees with our *in vitro* data that demonstrated that the azoles, particularly ITC and PSC, have good activity against *Cladosporium* species, although VRC displayed variable activity. AMB has shown to be ineffective against *C. cladosporioides* (41) and *C. sphaerospermum* (38) in cases of skin and intrabronchial infections, respectively. Our results, however, suggest that this drug might be effective, especially against members of the *C. herbarum* complex. Kantarcioğlu & Yücel (45) reported potent *in vitro* activity of TRB against a set of unidentified *Cladosporium* species. Our data confirmed the results of that study, with TRB showing significant activity against all the species tested. Echinocandin activity against *Cladosporium* species has not been previously evaluated, however we observed that both AFG and MFG exhibited notable *in vitro* activity against all of our isolates, and could represent an important alternative for the treatment of infections by these fungi pending further confirmatory studies.

In conclusion, our study has significantly expanded the diversity of *Cladosporium* species seen in clinical specimens as a result of the molecular characterization of these isolates. We were unable, however, to document these organisms as etiologic agents in human or animal disease due to the lack of clinical information and/or histopathological findings. It is also important to note that most reported cases of *Cladosporium* infections lack molecular confirmation, and in those cases where they have been so characterized, the strains are not available. Given that many journals require the public availability of DNA sequence data, we recommend that clinical strains be deposited in international culture collections, thereby making them available for re-identification and further study.

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Table 1 Clinical isolates, type or reference strains and sequences included in this study.

Species	Strain/Isolate number	Origin (Country)	GenBank accession Nº			
			ITS	LSU	*EF*-1α	*Act*
Cercospora beticola	CBS 116456^{T}	*Beta vulgaris* (Italy)	NR121315	GU214404	AY840494	AY840458
Cercospora olivascens	CBS 253.67^{T}	Unknown	NR111773	-		
Cladosporium allicinum	CBS 121624^{T}	*Hordeum vulgare* (Belgium)	EF679350	-	EF679425	EF679502
	UTHSC DI-13-173	Human, lung (USA)	**LN834353**	-	**LN834449**	**LN834537**
	UTHSC DI-13-176	Human, skin (USA)	**LN834354**	-	**LN834450**	**LN834538**
	UTHSC DI-13-266	Canine, skin (USA)	**LN834355**	-	**LN834451**	**LN834539**
Cladosporium angustisporum	CBS 125983^{T}	*Alloxylon wickhamii* (Australia)	HM147995	-	HM148236	HM148482
	UTHSC DI-13-240	Human, toe nail (USA)	**LN834356**	-	**LN834452**	**LN834540**
Cladosporium asperulatum	CBS 126339	*Eucalyptus* leaf litter (India)	HM147997	-	HM148238	HM148484
	CBS 126340^{T}	*Protea susannae* (Portugal)	HM147998	-	HM148239	HM148485
	UTHSC DI-13-216	Feline, nasal (USA)	**LN834357**	-	**LN834453**	**LN834541**
Cladosporium cladosporioides	CBS 101367	Soil (Brasil)	HM148002	-	HM148243	HM148489
	CBS 112388^{T}	Indoor air (Germany)	HM148003	-	HM148244	HM148490
	UTHSC DI-13-204	Human, abdomen (USA)	**LN834358**	-	**LN834454**	**LN834542**
	UTHSC DI-13-209	Human, pleural (USA)	**LN834359**	-	**LN834455**	**LN834543**
	UTHSC DI-13-215	Human, sputum (USA)	**LN834360**	-	**LN834456**	**LN834544**
Cladosporium colocasiae	CBS 386.64^{T}	*Colocasia antiquorum* (Taiwan)	HM148067	-	HM148310	HM148555
	CBS 119542	*Colocasia esculanta* (Taiwan)	HM148066	-	HM148309	HM148554
Cladosporium cucumerinum	CBS 171.52^{T}	Fruit of *Cucumis sativus* (Netherlands)	HM148072	-	HM148316	HM148561
	CBS 173.54	Fruit of *Cucumis sativus* (Netherlands)	HM148074	-	HM148318	HM148563
Cladosporium flabelliforme	CBS 126345^{T}	*Melaleuca cajuputi* (Australia)	HM148092	-	HM148336	HM148581
	UTHSC DI-13-267	Human, sputum (USA)	**LN834361**	-	**LN834457**	**LN834545**
Cladosporium funiculosum	CBS 122128	Unknown	HM148093	-	HM148337	HM148582
	CBS 122129^{T}	Leaf of *Vigna umbellata* (Japan)	HM148094	-	HM148338	HM148583
	UTHSC DI-13-175	Human, BAL (USA)	**LN834362**	-	**LN834458**	**LN834546**

	UTHSC DI-13-223	Human, BAL (USA)	**LN834363**	-	**LN834459**	**LN834547**
	UTHSC DI-13-242	Human, nasal wash (USA)	**LN834364**	-	**LN834460**	**LN834548**
Cladosporium halotolerans	CBS 119416^T	Hypersaline water (Namibia)	DQ780364	-	JN906989	EF101397
	FMR 13493	Human, unknown (Spain)	**LN834365**	-	**LN834461**	**LN834549**
	UTHSC DI-13-164	Human, bone marrow (USA)	**LN834366**	-	**LN834462**	**LN834550**
	UTHSC DI-13-182	Marine mammal, dermis (USA)	**LN834367**	-	**LN834463**	**LN834551**
	UTHSC DI-13-183	Human, bronchus (USA)	**LN834368**	-	**LN834464**	**LN834552**
	UTHSC DI-13-206	Human, BAL (USA)	**LN834369**	-	**LN834465**	**LN834553**
	UTHSC DI-13-213	Human, lymph node (USA)	**LN834370**	-	**LN834466**	**LN834554**
	UTHSC DI-13-221	Human, bone marrow (USA)	**LN834371**	-	**LN834467**	**LN834555**
	UTHSC DI-13-231	Catheter tip (USA)	**LN834372**	-	**LN834468**	**LN834556**
	UTHSC DI-13-249	Human, nasal (USA)	**LN834373**	-	**LN834469**	**LN834557**
	UTHSC DI-13-250	Human, scalp (USA)	**LN834374**	-	**LN834470**	**LN834558**
	UTHSC DI-13-252	Human, toe nail (USA)	**LN834375**	-	**LN834471**	**LN834559**
	UTHSC DI-13-259	Human, BAL (USA)	**LN834376**	-	**LN834472**	**LN834560**
	UTHSC DI-13-263	Human, BAL (USA)	**LN834377**	-	**LN834473**	**LN834561**
Cladosporium herbaroides	CBS 121626^T	Hypersaline water (Israel)	EF679357	-	EF679432	EF679509
Cladosporium herbarum	CBS 121621^T	*Hordeum vulgare* (Netherlands)	EF679363	-	EF679440	EF679516
	UTHSC DI-13-220	Human, BAL (USA)	**LN834378**	-	**LN834474**	**LN834562**
Cladosporium iranicum	CBS 126346^T	Leaf of *Citrus sinensis* (Iran)	HM148110	-	HM148354	HM148599
Cladosporium iridis	CBS 138.40^T	Leaf of *Iris* sp. (Netherlands)	EF679370	-	EF679447	EF679523
Cladosporium macrocarpum	CBS 121623^T	*Spinacia oleracea* (USA)	EF679375	-	EF679453	EF679529
	UTHSC DI-13-191	Human, face (USA)	**LN834379**	-	**LN834475**	**LN834563**
Cladosporium oxysporum	CBS 125991	Soil (China)	HM148118	-	HM148362	HM148607
	CBS 126351	Indoor air (Venezuela)	HM148119	-	HM148363	HM148608
Cladosporium perangustum	CBS 125996^T	*Cussonia* sp. (South Africa)	HM148121	-	HM148365	HM148610
	UTHSC DI-13-208	Canine, BAL (USA)	**LN834380**	-	**LN834476**	**LN834564**
Cladosporium pseudocladosporioides	CBS 117153	Leaf of *Paeonia* sp. (Germany)	HM148157	-	HM148401	HM148646
	CBS 125993^T	Outside air (Netherlands)	HM148158	-	HM148402	HM148647
	UTHSC DI-13-187	Turtle, unknown (USA)	**LN834381**	-	**LN834477**	**LN834565**

	UTHSC DI-13-232	Human, shoulder (USA)	**LN834382**	-	**LN834478**	**LN834566**
	UTHSC DI-13-233	Human, BAL (USA)	**LN834383**	-	**LN834479**	**LN834567**
	UTHSC DI-13-261	Human, sputum (USA)	**LN834384**	-	**LN834480**	**LN834568**
Cladosporium ramotenellum	CBS 121628^{T}	Hypersaline water (Slovenia)	EF679384	-	EF679462	EF679538
	UTHSC DI-13-166	Human, nasal tissue (USA)	**LN834385**	-	**LN834481**	**LN834569**
	UTHSC DI-13-222	Animal, Nasal (USA)	**LN834386**	-	**LN834482**	**LN834570**
	UTHSC DI-13-224	Animal, Nasal (USA)	**LN834387**	-	**LN834483**	**LN834571**
Cladosporium sinuosum	CBS 121629^{T}	*Fuchsia excorticata* (New Zealand)	EF679386	-	EF679464	EF679540
Cladosporium sphaerospermum	CBS 193.54^{T}	Human, nail (Netherlands)	DQ780343	-	EU570261	EU570269
	UTHSC DI-13-184	Frog, abscess (USA)	**LN834388**	-	**LN834484**	**LN834572**
	UTHSC DI-13-229	Human, BAL (USA)	**LN834389**	-	**LN834485**	**LN834573**
	UTHSC DI-13-237	Human, BAL (USA)	**LN834390**	-	**LN834486**	**LN834574**
Cladosporium subinflatum	CBS 121630^{T}	Hypersaline water (Slovenia)	EF679389	-	EF679467	EF679543
	UTHSC DI-13-189	Human, toe nail (USA)	**LN834391**	-	**LN834487**	**LN834575**
Cladosporium subtilissimum	CBS 113754^{T}	Grape berry (USA)	EF679397	-	EF679475	EF679551
Cladosporium subuliforme	CBS 126500^{T}	*Chamaedorea metallica* (Thailand)	HM148196	-	HM148441	HM148686
	UTHSC DI-13-171	Human, CSF (USA)	**LN834392**	-	**LN834488**	**LN834576**
	UTHSC DI-13-180	Human, BAL (USA)	**LN834393**	-	**LN834489**	**LN834577**
	UTHSC DI-13-214	Human, BAL (USA)	**LN834394**	-	**LN834490**	**LN834578**
	UTHSC DI-13-254	Human, BAL (USA)	**LN834395**	-	**LN834491**	**LN834579**
	UTHSC DI-13-255	Human, toe nail (USA)	**LN834396**	-	**LN834492**	**LN834580**
Cladosporium tenuissimum	CBS 125995^{T}	Fruits of *Lagerstroemia* sp. (USA)	HM148197	-	HM148442	HM148687
	UTHSC DI-13-174	Marine mammal, lung (USA)	**LN834397**	-	**LN834493**	**LN834581**
	UTHSC DI-13-177	Human, skin (USA)	**LN834398**	-	**LN834494**	**LN834582**
	UTHSC DI-13-188	Human, BAL (USA)	**LN834399**	-	**LN834495**	**LN834583**
	UTHSC DI-13-205	Human, BAL (USA)	**LN834400**	-	**LN834496**	**LN834584**
	UTHSC DI-13-236	Human, nasal (USA)	**LN834401**	-	**LN834497**	**LN834585**
	UTHSC DI-13-239	Human, sputum (USA)	**LN834402**	-	**LN834498**	**LN834586**
	UTHSC DI-13-253	Human, BAL (USA)	**LN834403**	-	**LN834499**	**LN834587**

	UTHSC DI-13-258	Human, thorancentesis fluid (USA)	**LN834404**	-	**LN834500**	**LN834588**
	UTHSC DI-13-274	Human, toe (USA)	**LN834405**	-	**LN834501**	**LN834589**
Cladosporium sp.	UTHSC DI-13-165	Human, arm drainage (USA)	**LN834406**	-	**LN834502**	**LN834590**
	UTHSC DI-13-168	Human, BAL (USA)	**LN834407**	-	**LN834503**	**LN834591**
	UTHSC DI-13-169	Human, BAL (USA)	**LN834408**	-	**LN834504**	**LN834592**
	UTHSC DI-13-170	Human, toe nail (USA)	**LN834409**	-	**LN834505**	**LN834593**
	UTHSC DI-13-178	Animal, abscess (USA)	**LN834410**	-	**LN834506**	**LN834594**
	UTHSC DI-13-179	Human, hand (USA)	**LN834411**	-	**LN834507**	**LN834595**
	UTHSC DI-13-190	Human, CSF (USA)	**LN834412**	-	**LN834508**	**LN834596**
	UTHSC DI-13-207	Human, CSF (USA)	**LN834413**	-	**LN834509**	**LN834597**
	UTHSC DI-13-210	Human, skin (USA)	**LN834414**	-	**LN834510**	**LN834598**
	UTHSC DI-13-211	Human, BAL (USA)	**LN834415**	-	**LN834511**	**LN834599**
	UTHSC DI-13-212	Human, ethmoid sinus (USA)	**LN834416**	-	**LN834512**	**LN834600**
	UTHSC DI-13-217	Human, nasal (USA)	**LN834417**	-	**LN834513**	**LN834601**
	UTHSC DI-13-218	Human, BAL (USA)	**LN834418**	-	**LN834514**	**LN834602**
	UTHSC DI-13-219	Human, foot (USA)	**LN834419**	-	**LN834515**	**LN834603**
	UTHSC DI-13-225	Animal, BAL (USA)	**LN834420**	-	**LN834516**	**LN834604**
	UTHSC DI-13-226	Human, BAL (USA)	**LN834421**	-	**LN834517**	**LN834605**
	UTHSC DI-13-227	Human, sputum (USA)	**LN834422**	-	**LN834518**	**LN834606**
	UTHSC DI-13-228	Human, foot skin (USA)	**LN834423**	-	**LN834519**	**LN834607**
	UTHSC DI-13-234	Human, sputum (USA)	**LN834424**	-	**LN834520**	**LN834608**
	UTHSC DI-13-235	Human, BAL (USA)	**LN834425**	-	**LN834521**	**LN834609**
	UTHSC DI-13-238	Human, leg (USA)	**LN834426**	-	**LN834522**	**LN834610**
	UTHSC DI-13-241	Human, foot (USA)	**LN834427**	-	**LN834523**	**LN834611**
	UTHSC DI-13-244	Human, BAL (USA)	**LN834428**	-	**LN834524**	**LN834612**
	UTHSC DI-13-245	Human, toe (USA)	**LN834429**	-	**LN834525**	**LN834613**
	UTHSC DI-13-246	Human, BAL (USA)	**LN834430**	-	**LN834526**	**LN834614**
	UTHSC DI-13-247	Human, BAL (USA)	**LN834431**	-	**LN834527**	**LN834615**
	UTHSC DI-13-251	Human, BAL (USA)	**LN834432**	-	**LN834528**	**LN834616**

	UTHSC DI-13-257	Human, sputum (USA)	**LN834433**	-	**LN834529**	**LN834617**
	UTHSC DI-13-262	Dolphin, bronchus (USA)	**LN834434**	-	**LN834530**	**LN834618**
	UTHSC DI-13-265	Human, BAL (USA)	**LN834435**	-	**LN834531**	**LN834619**
	UTHSC DI-13-268	Human, toe nail (USA)	**LN834436**	-	**LN834532**	**LN834620**
	UTHSC DI-13-269	Human, BAL (USA)	**LN834437**	-	**LN834533**	**LN834621**
	UTHSC DI-13-270	Human, nail (USA)	**LN834438**	-	**LN834534**	**LN834622**
	UTHSC DI-13-271	Human, BAL (USA)	**LN834439**	-	**LN834535**	**LN834623**
	UTHSC DI-13-273	Human, toe nails (USA)	**LN834440**	-	**LN834536**	**LN834624**
Cladosporium variabile	CBS 121636^{T}	*Spinacia oleracea* (USA)	EF679402	-	EF679480	EF679556
Penidiella sp.	UTHSC DI-13-256	Human, nail (USA)	**LN834441**	**LN834445**	-	-
Toxicocladosporium banksiae	CBS 128215^{T}	Leaf of *Banksia emulata* (Australia)	HQ599598	HQ599599	-	-
Toxicocladosporium chlamydosporum	CBS 124157^{T}	Leaf of *Eucalyptus camaldulensis* (Madagascar)	FJ790283	FJ790301	-	-
Toxicocladosporium ficiniae	CBS 136406^{T}	Leaf of *Ficinia* sp. (South Africa)	KF777190	KF777241	-	-
Toxicocladosporium irritans	CBS 185.58^{T}	Mouldy paint (Suriname)	EU040243	EU040243	-	-
	UTHSC DI-13-181	Human, blood (USA)	**LN834442**	**LN834446**	-	-
	UTHSC DI-13-230	Human, finger nail (USA)	**LN834443**	**LN834447**	-	-
Toxicocladosporium pini	CBS 138005^{T}	Needles of *Pinus* sp. (China)	KJ869160	KJ869217	-	-
Toxicocladosporium posoqueriae	CBS 133583^{T}	Leaf of *Posoqueria latifolia* (Australia)	NR121555	KC005803	-	-
Toxicocladosporium protearum	CBS 126499^{T}	Leaf of *Protea burchellii* (South Africa)	HQ599586	HQ599587	-	-
Toxicocladosporium pseudoveloxum	CBS 128775^{T}	Leaf of *Phaenocoma prolifera* (South Africa)	JF499847	JF499867	-	-
Toxicocladosporium rubrigenum	CBS 124158^{T}	Leaf of *Eucalyptus camaldulensis* (Madagascar)	FJ790287	FJ790305	-	-
Toxicocladosporium sp.	UTHSC DI-13-172	Human, BAL (USA)	**LN834444**	**LN834448**	-	-
Toxicocladosporium strelitziae	CBS 132535^{T}	Leaf of *Strelitzia reginae* (South Africa)	NR111765	JX069858	-	-
Toxicocladosporium veloxum	CBS 124159^{T}	Leaf of *Eucalyptus camaldunensis* (Madagascar)	FJ790288	FJ790306	-	-

CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; 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; ^{T}, Ex-type strain. BAL, bronchoalveolar lavage fluid specimen; CSF, cerebrospinal fluid. Accession numbers in bold represent sequences generated in this study.

Table 2 Results of *in vitro* antifungal susceptibility testing of the 92 clinical isolates included in the study.

Genus	**Species (Nº of isolates tested)**	**MIC/MEC parameter**	**Result (µg/ml) for:**								
			AMB	**5FC**	**VRC**	**PSC**	**ITC**	**TBF**	**CFG**	**AFG**	**MFG**
Cladosporium	*C. cladosporioides* complex (57)	Range	0.06-2	0.06->32	0.25-16	<0.03-1	<0.03-2	<0.03-4	0.125-8	0.03-0.5	0.03-0.5
		GM	0.73	1.20	1.65	0.40	0.34	0.12	2.78	0.19	0.11
		MIC$_{90}$	1	4	4	0.5	0.5	1	8	0.5	0.25
	C. tenuissimum (9)	Range	0.5-1	1->16	1-4	0.25-0.5	0.25-0.5	0.06-1	4-8	0.125-0.5	0.125-0.25
		GM	0.93	2.72	1.85	0.37	0.29	0.18	4.67	0.37	0.15
		MIC$_{90}$	1.00	4.00	2.00	0.50	0.50	0.25	8.00	0.50	0.25
	C. subuliforme (5)	Range	1-2	0.25-2	0.25-2	0.25-0.5	0.25	0.06-1	4-8	0.06-0.5	0.06-0.25
		GM	1.15	1.00	0.66	0.29	0.25	0.28	5.28	0.16	0.12
		MIC$_{90}$	1.00	2.00	1.00	0.25	0.25	1.00	8.00	0.25	0.13
	C. pseudocladosporioides (4)	Range	0.5-1	0.5-1	2-4	0.25-0.5	0.5-1	0.03-2	0.25-8	0.03-0.25	0.03-0.125
		GM	0.59	0.71	2.38	0.42	0.59	0.21	2.38	0.15	0.07
		MIC$_{90}$	-	-	-	-	-	-	-	-	-
	C. cladosporioides (3)	Range	0.5-1	1-2	0.5-16	0.25-1	0.25-0.5	0.5-2	1-4	0.125-0.25	0.125
		GM	0.79	1.26	1.59	0.40	0.31	1.00	2.00	0.16	0.13
		MIC$_{90}$	-	-	-	-	-	-	-	-	-
	C. funiculosum (3)	Range	0.06-1	0.5-1	0.5-2	0.25-0.5	0.125-0.25	0.03-0.06	4	0.06-0.25	0.06-0.125
		GM	0.31	0.63	1.00	0.31	0.20	0.04	4.00	0.12	0.08
		MIC$_{90}$	-	-	-	-	-	-	-	-	-
	C. angustisporum (1)	Range	1.00	1.00	4.00	0.50	0.50	2.00	4.00	0.13	0.06
		GM	-	-	-	-	-	-	-	-	-

	MIC$_{90}$	-	-	-	-	-	-	-	-	-
C. asperulatum (1)	Range	1	0.25	2	0.25	0.5	0.03	8	0.25	0.125
	GM	-	-	-	-	-	-	-	-	-
	MIC$_{90}$	-	-	-	-	-	-	-	-	-
C. flabelliforme (1)	Range	2	2	2	0.5	0.25	0.03	4	0.25	0.125
	GM	-	-	-	-	-	-	-	-	-
	MIC$_{90}$	-	-	-	-	-	-	-	-	-
C. perangustum (1)	Range	0.5	4	4	0.5	0.5	0.03	4	0.125	0.06
	GM	-	-	-	-	-	-	-	-	-
	MIC$_{90}$	-	-	-	-	-	-	-	-	-
Cladosporium sp. (29)	Range	0.125-2	0.06->16	0.25-8	<0.03-1	<0.03-2	<0.03-4	0.125-8	0.03-0.5	0.03-0.5
	GM	0.67	1.10	1.73	0.43	0.36	0.09	2.00	0.18	0.10
	MIC$_{90}$	1.00	4.00	4.00	0.50	0.50	1.00	8.00	0.50	0.25
C. herbarum complex (14)	Range	<0.03-2	0.5->16	0.5-8	0.06-0.5	0.06-1	<0.03-0.125	0.125-8	0.06-1	0.06-0.5
	GM	0.18	2.97	1.81	0.37	0.35	0.05	0.67	0.23	0.15
	MIC$_{90}$	1	8	4	0.5	0.5	0.125	2	0.5	0.5
C. allicinum (3)	Range	<0.03-0.125	2-4	2-4	0.5	0.25-0.5	0.03-0.06	1.00	0.25-0.5	0.125-0.25
	GM	0.05	2.52	3.17	0.50	0.40	0.05	1.00	0.31	0.20
	MIC$_{90}$	-	-	-	-	-	-	-	-	-
C. ramotenellum (3)	Range	1-2	4->16	1-2	0.5	0.5-1	<0.03-0.125	0.5-8	0.25-1	0.125-0.5
	GM	1.26	4.00	1.59	0.50	0.63	0.05	2.00	0.40	0.20
	MIC$_{90}$	-	-	-	-	-	-	-	-	-

C. herbarum (1)	Range	0.06	8	2	0.5	0.5	0.03	0.5	0.125	0.125
	GM	-	-	-	-	-	-	-	-	-
	MIC_{90}	-	-	-	-	-	-	-	-	-
C. macrocarpum (1)	Range	0.5	2	1	0.25	0.5	0.125	1	0.125	0.5
	GM	-	-	-	-	-	-	-	-	-
	MIC_{90}	-	-	-	-	-	-	-	-	-
C. subinflatum (1)	Range	0.5	0.5	4	0.5	0.5	0.03	0.5	0.5	0.25
	GM	-	-	-	-	-	-	-	-	-
	MIC_{90}	-	-	-	-	-	-	-	-	-
Cladosporium sp. (5)	Range	0.06-0.5	2-4	0.5-8	0.06-0.5	0.06-0.5	<0.03-0.125	0.125-0.5	0.06-0.5	0.06-0.125
	GM	0.11	2.30	1.32	0.25	0.19	0.05	0.29	0.14	0.08
	MIC_{90}	1.00	4.00	4.00	0.50	0.50	1.00	8.00	0.50	0.25
C. sphaerospermum complex (17)	Range	0.125-2	0.06-4	0.5-16	0.06-4	0.25->16	<0.03-1	0.06-4	<0.03-1	0.06-1
	GM	1.13	1.13	1.70	0.64	1.13	0.06	1.27	0.15	0.13
	MIC_{90}	2	2	8	2	32	0.5	4	0.25	0.125
C. halotolerans (13)	Range	0.125-2	0.06-4	0.5-2	0.06-1	0.25-2	<0.03-1	0.06-4	<0.03-0.25	0.06-0.125
	GM	1.00	0.90	1.11	0.47	0.56	0.08	1.00	0.11	0.10
	MIC_{90}	2.00	2.00	2.00	1.00	2.00	1.00	4.00	0.25	0.13
C. sphaerospermum (3)	Range	1-2	2-4	8-16	2-4	>16	<0.03-0.125	2-4	0.25-1	0.125-1
	GM	1.59	2.52	10.08	2.52	>16	0.03	3.17	0.50	0.40
	MIC_{90}	-	-	-	-	-	-	-	-	-
Cladosporium sp. (1)	Range	2	2	2	0.5	0.5	<0.03	2	0.25	0.125
	GM	-	-	-	-	-	-	-	-	-

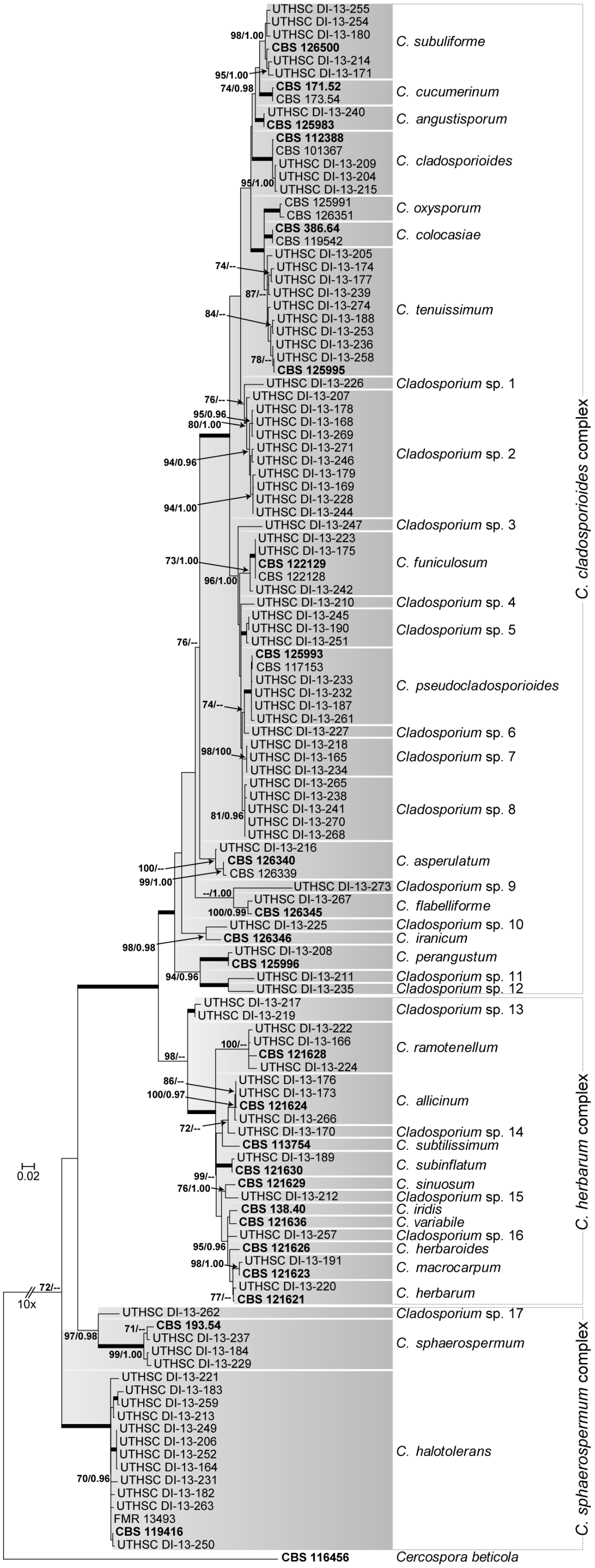
		MIC_{90}	-	-	-	-	-	-	-	-	-
	Overall (88)	Range	<0.03-2	0.06->16	0.25-16	<0.03-4	<0.03->16	<0.03-4	0.06-8	<0.03-1	0.03-1
		GM	0.64	1.37	1.68	0.43	0.43	0.09	1.91	0.19	0.12
		MIC_{90}	2.00	4.00	4.00	0.50	1.00	1.00	8.00	0.50	0.25
Toxicocladosporium	*T. irritans* (2)	Range	0.5-1	0.25-2	0.25	0.25-1	0.5	<0.03-0.06	0.125-2	0.06-0.25	0.06-0.5
		GM	0.71	0.71	0.25	0.50	0.50	0.03	0.50	0.12	0.17
		MIC_{90}	-	-	-	-	-	-	-	-	-
	Toxicocladosporium sp. (1)	Range	0.5	0.125	0.25	0.125	0.125	<0.03	1	0.125	0.06
		GM	-	-	-	-	-	-	-	-	-
		MIC_{90}	-	-	-	-	-	-	-	-	-
	Overall (3)	Range	0.5-1	0.125-2	0.25	0.125-1	0.125-0.5	<0.03-0.06	0.125-2	0.06-0.25	0.06-0.5
		GM	0.63	0.40	0.25	0.31	0.31	0.02	0.63	0.12	0.12
		MIC_{90}	-	-	-	-	-	-	-	-	-
Penidiella	*Penidiella* sp. (1)	Range	2	0.06	0.125	0.06	>0.03	>0.03	0.25	0.25	0.25
		GM	-	-	-	-	-	-	-	-	-
		MIC_{90}	-	-	-	-	-	-	-	-	-

GM, geometric mean; MIC_{90}, drug concentration that inhibited 90% of the isolates, shown only for species with ≥5 isolates. AMB, amphotericin B; 5FC, flucytosine; VRC, voriconazole; PSC, posaconazole; ITC, itraconazole; CFG, caspofungin; AFG, anidulafungin; MFG, micafungin; TRB, terbinafine. [a], these columns include MEC data.

Figure 1 Maximum likelihood tree inferred from combined ITS, *EF*-1α and *Act* sequences of *Cladosporium* isolates. Branch lengths are proportional to phylogenetic distance. ML bootstrap support (BS) values of ≥ 70% and posterior probability (PP) values of ≥ 0.95 are shown above the branches. Thickened branches indicate BS=100% and PP=1.00. *Cercospora beticola* (CBS 116456) was used to root the tree. Type strains are indicated in bold. CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; FMR, Facultat de Medicina i Ciències de la Salut, Reus, Spain; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, USA.

Figure 2 Conidiophores and conidia: (a to c) *C. cladosporioides* complex. (d to f) *C. herbarum* complex. (g to i) *C. sphaerospermum* complex. (j to l) *Toxicocladosporium* spp. White bars, 10 µm; black bars, 5 µm.

Figure 3 Maximum likelihood tree inferred from combined ITS and LSU sequences of *Toxicocladosporium* isolates. Branch lengths are proportional to phylogenetic distance. ML bootstrap support (BS) values of ≥ 70% and posterior probability (PP) values of ≥ 0.95 are shown above the branches. Thickened branches indicate BS=100% and PP=1.00. *Cercospora beticola* (CBS 116456) and *Cercospora olivascens* (CBS 253.67) were used to root the tree. Type strains are indicated in bold. CBS-KNAW, Fungal Biodiversity Centre culture collection, the Netherlands; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, USA.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95/1.00
74/0.98
CBS 171.52
CBS 173.54
C. cucumerinum
UTHSC DI-13-240
CBS 125983
C. angustisporum
CBS 112388
CBS 101367
UTHSC DI-13-209
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C. cladosporioides
95/1.00
CBS 125991
CBS 126351
C. oxysporum
CBS 386.64
CBS 119542
C. colocasiae
UTHSC DI-13-205
74/--
UTHSC DI-13-174
UTHSC DI-13-177
87/--
UTHSC DI-13-239
UTHSC DI-13-274
84/--
UTHSC DI-13-188
UTHSC DI-13-253
UTHSC DI-13-236
78/--
UTHSC DI-13-258
CBS 125995
C. tenuissimum
UTHSC DI-13-226
Cladosporium sp. 1
76/--
UTHSC DI-13-207
95/0.96
UTHSC DI-13-178
80/1.00
UTHSC DI-13-168
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94/0.96
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94/1.00
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Cladosporium sp. 3
UTHSC DI-13-223
UTHSC DI-13-175
73/1.00
CBS 122129
CBS 122128
96/1.00
UTHSC DI-13-242
C. funiculosum
UTHSC DI-13-210
Cladosporium sp. 4
UTHSC DI-13-245
UTHSC DI-13-190
UTHSC DI-13-251
Cladosporium sp. 5
76/--
CBS 125993
CBS 117153
UTHSC DI-13-233
UTHSC DI-13-232
74/--
UTHSC DI-13-187
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C. pseudocladosporioides
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UTHSC DI-13-218
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81/0.96
UTHSC DI-13-241
UTHSC DI-13-270
UTHSC DI-13-268
Cladosporium sp. 8
UTHSC DI-13-216
100/--
CBS 126340
99/1.00
CBS 126339
C. asperulatum
UTHSC DI-13-273
Cladosporium sp. 9
--/1.00
UTHSC DI-13-267
100/0.99
CBS 126345
C. flabelliforme
UTHSC DI-13-225
Cladosporium sp. 10
CBS 126346
C. iranicum
98/0.98
UTHSC DI-13-208
CBS 125996
C. perangustum
94/0.96
UTHSC DI-13-211
Cladosporium sp. 11
UTHSC DI-13-235
Cladosporium sp. 12
C. cladosporioides complex
UTHSC DI-13-217
UTHSC DI-13-219
Cladosporium sp. 13
UTHSC DI-13-222
100/--
UTHSC DI-13-166
CBS 121628
98/--
UTHSC DI-13-224
C. ramotenellum
86/--
UTHSC DI-13-176
100/0.97
UTHSC DI-13-173
CBS 121624
UTHSC DI-13-266
C. allicinum
72/--
UTHSC DI-13-170
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CBS 113754
C. subtilissimum
UTHSC DI-13-189
CBS 121630
C. subinflatum
99/--
CBS 121629
C. sinuosum
76/1.00
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CBS 138.40
C. iridis
CBS 121636
C. variabile
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Cladosporium sp. 16
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CBS 121626
C. herbaroides
98/1.00
UTHSC DI-13-191
CBS 121623
C. macrocarpum
UTHSC DI-13-220
77/--
CBS 121621
C. herbarum
C. herbarum complex
0.02
72/--
10x
UTHSC DI-13-262
Cladosporium sp. 17
71/--
CBS 193.54
97/0.98
UTHSC DI-13-237
99/1.00
UTHSC DI-13-184
UTHSC DI-13-229
C. sphaerospermum
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70/0.96
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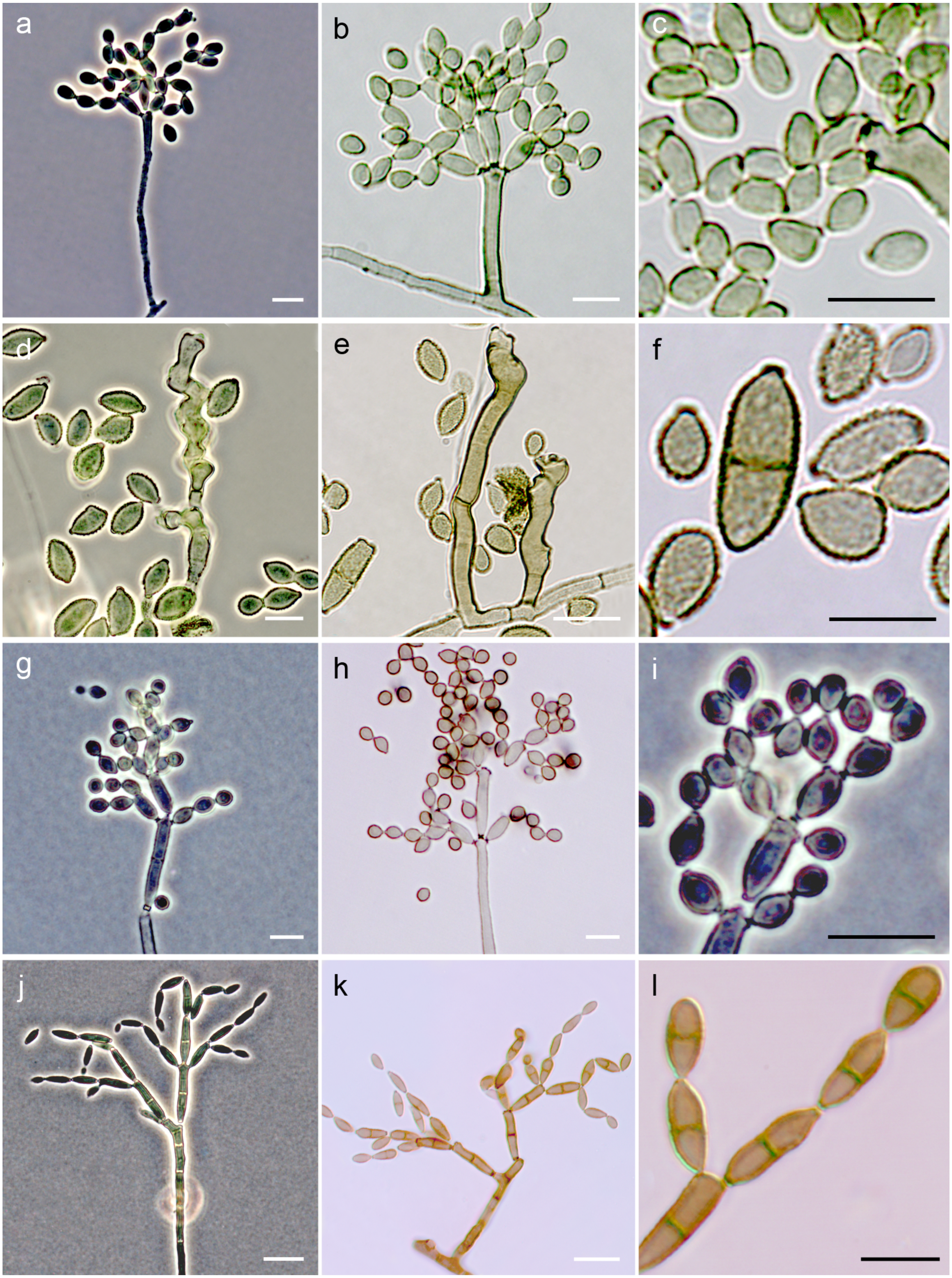
C. halotolerans
C. sphaerospermum complex
CBS 116456
Cercospora beticola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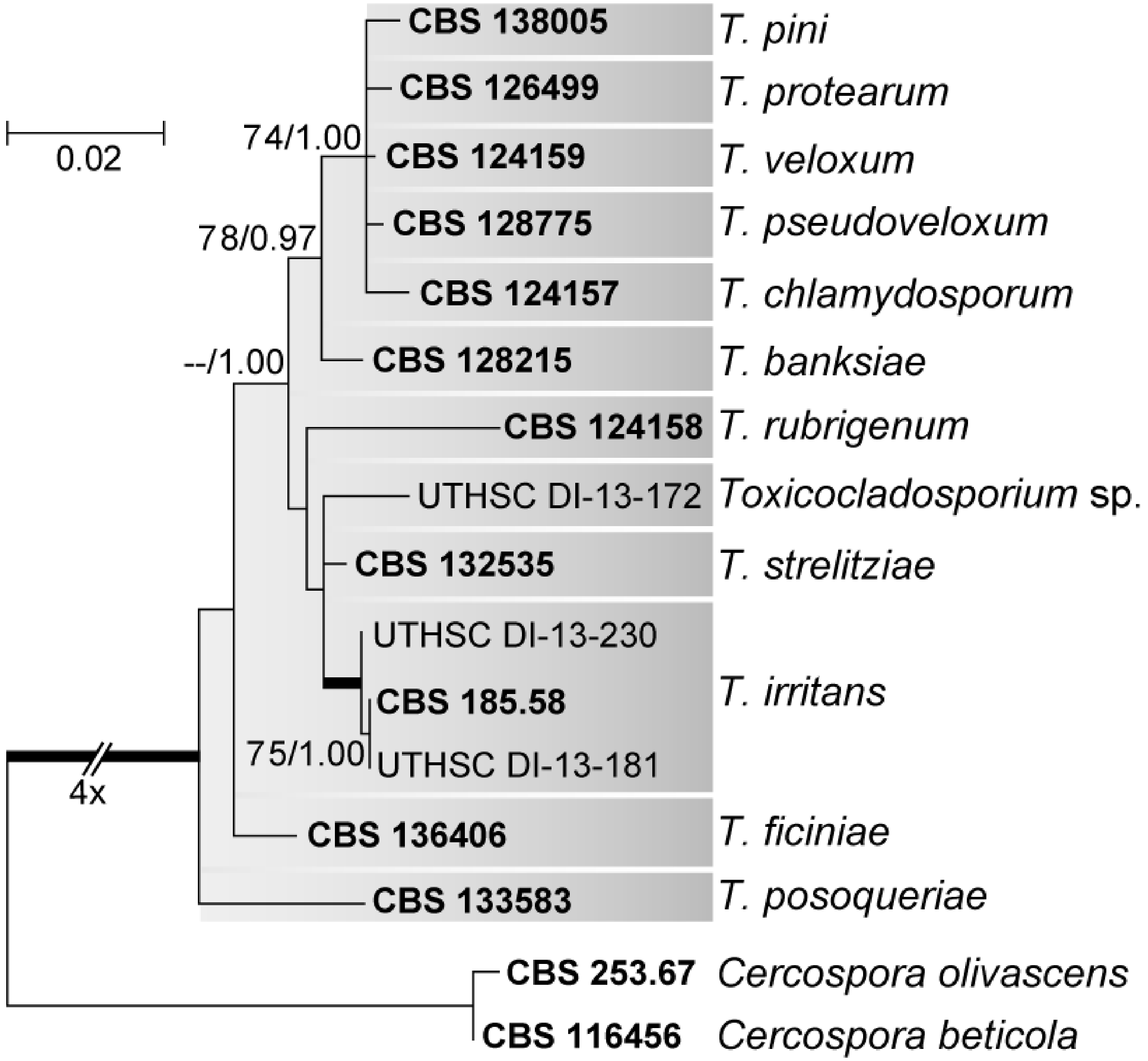
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CBS 124157
T. chlamydosporum
CBS 128215
T. banksiae
CBS 124158
T. rubrigenum
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Toxicocladosporium sp.
CBS 132535
T. strelitziae
UTHSC DI-13-230
CBS 185.58
UTHSC DI-13-181
T. irritans
CBS 136406
T. ficiniae
CBS 133583
T. posoqueriae
CBS 253.67
Cercospora olivascens
CBS 116456
Cercospora beticola