



New species of *Cladosporium* associated with human and animal infections

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Key words

Capnodiales
Cladosporiaceae
Dothideomycetes
phylogeny
taxonomy

Abstract *Cladosporium* is mainly known as a ubiquitous environmental saprobic fungus or plant endophyte, and to date, just a few species have been documented as etiologic agents in vertebrate hosts, including humans. In the present study, 10 new species of the genus were isolated from human and animal clinical specimens from the USA. They are proposed and characterized on the basis of their morphology and a molecular phylogenetic analysis using DNA sequences from three loci (the ITS region of the rDNA, and partial fragments of the translation elongation factor 1-alpha and actin genes). Six of those species belong to the *C. cladosporioides* species complex, i.e., *C. alboflavescens*, *C. angulosum*, *C. anthropophilum*, *C. crousii*, *C. flavovirens* and *C. xantochromaticum*, three new species belong to the *C. herbarum* species complex, i.e., *C. floccosum*, *C. subcinereum* and *C. tuberosum*; and one to the *C. sphaerospermum* species complex, namely, *C. succulentum*. Differential morphological features of the new taxa are provided together with molecular barcodes to distinguish them from the currently accepted species of the genus.

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INTRODUCTION

The genus *Cladosporium* (*Cladosporiaceae*, *Capnodiales*) is a large genus of the *Ascomycota*. It comprises 189 species, mostly saprobes with a worldwide distribution and isolated from a wide range of substrates (David 1997, Bensch et al. 2012, 2015, Crous et al. 2014). The genus also includes common endophytes, plant pathogens often causing leaf spots or other lesions, as well as hyperparasites of other fungi (Bensch et al. 2012). Certain species are relevant as potential biocontrol agents for plant diseases (Köhl et al. 2015) or, in the food industry, as fruit contaminants causing spoilage in low temperature storage or on cereals such as barley, oat, rye and wheat (Samson et al. 2010, Kulik et al. 2014, Frasz & Miller 2015). The role of cladosporia is not well understood in human pathology. Their small conidia are easily dispersed, making them one of the most common air-borne microorganisms (David 1997, De Hoog et al. 2011). They are among the most important allergenic fungi linked to allergic rhinitis and respiratory arrest in asthmatic patients (Black et al. 2000, Sellart-Altisent et al. 2007). Some species are described as a cause of opportunistic phaeoophomycosis, including subcutaneous and deep infections in humans and animals (De Hoog et al. 2011, Sandoval-Denis et al. 2015), although, their ubiquitous nature suggests that in some reports they may be mere colonizers.

Species identification in *Cladosporium* has always relied on the morphology of the conidiogenous apparatus together with data on host ranges (Crous et al. 2007b, Bensch et al. 2012). Traditionally, those dematiaceous fungi showing branched acropetal chains of aseptate to septate conidia were included in *Cladosporium*, which has made it a large and complex group of fungi difficult to differentiate (Bensch et al. 2012). However, recent phylogenetic studies have helped to clarify the taxonomy

of these fungi and demonstrated that most of the well-known morphologically-defined species comprises several phylogenetically cryptic species practically impossible to identify using morphological criteria alone (Braun et al. 2003, 2008; Crous et al. 2007b, Zalar et al. 2007, Schubert et al. 2007, 2009, Bensch et al. 2010, 2012, 2015). In its current circumscription, the genus *Cladosporium* includes dematiaceous fungi with solitary to fasciculate conidiophores, proliferating mostly sympodially and forming unbranched or branched acropetal conidial chains. However, the most characteristic feature is the presence of a thick refractive to darkened cladosporioid or coronate scar, defined as a raised periclinal rim with a central convex dome (Schubert et al. 2007, Bensch et al. 2012). The sexual morph (previously assigned to the genus *Davidiella*) is characterised by pseudothecial ascomata, 8-spored obovoid to subcylindrical asci, and hyaline, obovoid to ellipsoid ascospores showing irregular luminal inclusions (Schubert et al. 2007).

In recent years, the survey of unexplored habitats and sources by using molecular techniques has expanded our knowledge of fungal diversity. Similarly, clinical specimens have become an important source of undescribed fungi, including both true pathogens and/or also contaminants/colonizers (Gilgado et al. 2005, Perdomo et al. 2013, Giraldo et al. 2014, Guinea et al. 2015, Sandoval-Denis et al. 2015) that had not been recognizable previously because of their poor morphological differentiation (De Hoog et al. 2015).

In order to assess the real prevalence of *Cladosporium* in the clinical setting and the spectrum of species associated with clinical samples, we studied a large set of *Cladosporium* isolates from human and animal clinical origin using both molecular characterisation and phenotypic features (Sandoval-Denis et al. 2015). Surprisingly, we found that nearly 40 % of the isolates could not be assigned to any known species and probably represented new species for the genus. The objective of the present study is therefore to determine the phylogenetic relationships of those previously unidentified isolates by using the criteria currently accepted in the taxonomy of this genus.

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Table 1 Isolates and GenBank accession numbers of sequences included in this study.

Species ^a	Strain number ^b	Substrate ^c	GenBank accession numbers		
			ITS	<i>tef1</i>	<i>ActA</i>
<i>Cercospora beticola</i>	CBS 116456	<i>Beta vulgaris</i>	NR_121315	AY840494	AY840458
<i>Cladosporium acalyphae</i>	CBS 125982 ^T	<i>Acalypha australis</i>	HM147994	HM148235	HM148481
<i>Cladosporium aciculare</i>	CBS 140488 ^T	<i>Syzygium corynanthum</i>	KT600411	KT600509	KT600607
<i>Cladosporium aggregatocitricatatum</i>	CBS 140493 ^T	Culture contaminant	KT600448	KT600547	KT600645
<i>Cladosporium alboflavescens</i>	CBS 140690 ^T = UTHSC DI-13-225 = FMR 13338	Animal, BAL	LN834420	LN834516	LN834604
<i>Cladosporium allacinum</i>	CBS 121.47	Food, frozen <i>Phaseolus vulgaris</i>	KT600364	KT600461	KT600560
	CBS 121624 ^T	<i>Hordeum vulgare</i>	EF679350	EF679425	EF679502
	CBS 160.59	Human, sputum	KT600366	KT600463	KT600562
	CBS 374.53	<i>Centaurea rhapontica</i> = <i>Rhaponticum scariosum</i> subsp. <i>rhaponticum</i>	KT600368	KT600465	KT600564
	CPC 16759	<i>Alnus glutinosa</i>	KT600374	KT600471	KT600570
	UTHSC DI-13-170 = FMR 13295	Human, toenail	LN834409	LN834505	LN834593
	UTHSC DI-13-173 = FMR 13298	Human, lung	LN834353	LN834449	LN834537
<i>Cladosporium allii</i>	CBS 101.81	<i>Allium porrum</i>	JN906977	JN906983	JN906996
<i>Cladosporium angulosum</i>	CBS 140692 ^T = UTHSC DI-13-235 = FMR 13348	Human, BAL	LN834425	LN834521	LN834609
	CPC 11526	<i>Acacia mangium</i>	HM148127	HM148371	HM148616
	CPC 14566	<i>Corymbia foelscheana</i>	HM148147	HM148391	HM148636
	CPC 18494	<i>Ananas comosus</i>	KT600413	KT600511	KT600609
	CPC 18496	<i>Ananas comosus</i>	KT600414	KT600512	KT600610
<i>Cladosporium angustiterbarum</i>	CBS 140479 ^T	<i>Pinus ponderosa</i>	KT600378	KT600475	KT600574
<i>Cladosporium angustisporum</i>	CBS 125983 ^T	<i>Alloxylon wickhamii</i>	HM147995	HM148236	HM148482
	UTHSC DI-13-240 = FMR 13353	Human, nail	LN834356	LN834452	LN834540
<i>Cladosporium angustiterminale</i>	CBS 140480 ^T	<i>Banksia grandis</i>	KT600379	KT600476	KT600575
<i>Cladosporium antarcticum</i>	CBS 690.92 ^T	<i>Caloplaca regalis</i>	EF679334	EF679405	EF679484
<i>Cladosporium anthropophilum</i>	CBS 117483	Unknown	HM148007	HM148248	HM148494
	CBS 140685 ^T = UTHSC DI-13-269 = FMR 13382	Human, BAL	LN834437	LN834533	LN834621
	CPC 11122	<i>Phytolacca americana</i>	HM148019	HM148260	HM148506
	UTHSC DI-13-168 = FMR 13293	Human, BAL	LN834407	LN834503	LN834591
	UTHSC DI-13-169 = FMR 13294	Human, BAL	LN834408	LN834504	LN834592
	UTHSC DI-13-178 = FMR 13303	Animal, abscess	LN834410	LN834506	LN834594
	UTHSC DI-13-179 = FMR 13304	Human, hand	LN834411	LN834507	LN834595
	UTHSC DI-13-207 = FMR 13320	Human, CSF	LN834413	LN834509	LN834597
	UTHSC DI-13-226 = FMR 13339	Human, BAL	LN834421	LN834517	LN834605
	UTHSC DI-13-228 = FMR 13341	Human, foot skin	LN834423	LN834519	LN834607
	UTHSC DI-13-244 = FMR 13357	Human, BAL	LN834428	LN834524	LN834612
	UTHSC DI-13-246 = FMR 13359	Human, BAL	LN834430	LN834526	LN834614
	UTHSC DI-13-271 = FMR 13384	Human, BAL	LN834439	LN834535	LN834623
<i>Cladosporium aphidis</i>	CBS 132182 ^{ET}	<i>Echium pininana</i>	JN906978	JN906984	JN906997
<i>Cladosporium arthropodii</i>	CBS 124043 ^{ET}	Leaf lesions of rock lily	JN906979	JN906985	JN906998
<i>Cladosporium asperulatum</i>	CBS 126339	<i>Eucalyptus</i> leaf litter	HM147997	HM148238	HM148484
	CBS 126340 ^T	<i>Protea susannae</i>	HM147998	HM148239	HM148485
<i>Cladosporium australiense</i>	CBS 125984 ^T	<i>Eucalyptus moluccana</i>	HM147999	HM148240	HM148486
<i>Cladosporium austroafricanum</i>	CBS 140481 ^T	Leaf litter	KT600381	KT600478	KT600577
<i>Cladosporium austrohemisphaericum</i>	CBS 140482 ^T	<i>Lagunaria patersonia</i> , black mould on fruit surface	KT600382	KT600479	KT600578
<i>Cladosporium basiinflatum</i>	CBS 822.84 ^T	<i>Hordeum vulgare</i>	HM148000	HM148241	HM148487
<i>Cladosporium chalastosporioides</i>	CBS 125985 ^T	Fruiting bodies of <i>T. proteae-arborea</i> on leaves of <i>Protea arborea</i>	HM148001	HM148242	HM148488
<i>Cladosporium chubutense</i>	CBS 124457 ^T	Needles of <i>Pinus ponderosa</i>	FJ936158	FJ936161	FJ936165
<i>Cladosporium cladosporioides</i>	CBS 113738	Grape bud	HM148004	HM148245	HM148491
	CBS 112388 ^T	Indoor air	HM148003	HM148244	HM148490
	CPC 14292	Soil, pea field	HM148046	HM148287	HM148533
	UTHSC DI-13-215 = FMR 13328	Human, sputum	LN834360	LN834456	LN834544
<i>Cladosporium colocasiae</i>	CBS 386.64 ^T	<i>Colocasia antiquorum</i>	HM148067	HM148310	HM148555
	CBS 119542	Leaf of <i>Colocasia esculanta</i>	HM148066	HM148309	HM148554
<i>Cladosporium colombiae</i>	CBS 274.80B ^T	Dead leaf, Cortaderia	FJ936159	FJ936163	FJ936166
<i>Cladosporium crousii</i>	CBS 140686 ^T = UTHSC DI-13-247 = FMR 13360	Human, BAL	LN834431	LN834527	LN834615
<i>Cladosporium cucumerinum</i>	CBS 171.52 ^{ET}	Fruit of <i>Cucumis sativus</i>	HM148072	HM148316	HM148561
	CBS 173.54	Fruit of <i>Cucumis sativus</i>	HM148074	HM148318	HM148563
<i>Cladosporium cycadicola</i>	CPC 17251 ^T	Leaves of <i>Cycas media</i>	KJ869122	KJ869236	KJ869227
<i>Cladosporium delicatulum</i>	CBS 126342	Indoor building material	HM148079	HM148323	HM148568
	CBS 126344	Leaves of <i>Tilia cordata</i>	HM148081	HM148325	HM148570
<i>Cladosporium dominicanum</i>	CBS 119415 ^T	Hypersaline water	DQ780353	JN906986	EF101368
<i>Cladosporium echinulatum</i>	CBS 123191	Leaf of <i>Dianthus barbatus</i>	JN906980	JN906987	JN906999
<i>Cladosporium exasperatum</i>	CBS 125986 ^T	<i>Eucalyptus tintinnans</i>	HM148090	HM148334	HM148579
<i>Cladosporium exile</i>	CBS 125987 ^T	Chasmothecia of <i>P. guttata</i> on leaves of <i>Corylus avellana</i>	HM148091	HM148335	HM148580
<i>Cladosporium flabelliforme</i>	CBS 126345 ^T	<i>Melaleuca cajuputi</i>	HM148092	HM148336	HM148581
	UTHSC DI-13-267 = FMR 13380	Human, sputum	LN834361	LN834457	LN834545
<i>Cladosporium flavovirens</i>	CBS 140462 ^T = UTHSC DI-13-273 = FMR 13386	Human, toenails	LN834440	LN834536	LN834624
<i>Cladosporium floccosum</i>	CBS 140463 ^T = UTHSC DI-13-212 = FMR 13325	Human, ethmoid sinus	LN834416	LN834512	LN834600

Table 1 (cont.)

Species ^a	Strain number ^b	Substrate ^c	GenBank accession numbers		
			ITS	<i>tef1</i>	<i>ActA</i>
<i>Cladosporium funiculosum</i>	CBS 122128	<i>Ficus carica</i>	HM148093	HM148337	HM148582
	CBS 122129 ^T	Leaf of <i>Vigna umbellata</i>	HM148094	HM148338	HM148583
	UTHSC DI-13-175 = FMR 13300	Human, BAL	LN834362	LN834458	LN834546
<i>Cladosporium fusiforme</i>	CBS 119414 ^T	Hypersaline water	DQ780388	JN906988	EF101372
<i>Cladosporium gamsianum</i>	CBS 125989 ^T	<i>Strelitzia</i> sp.	HM148095	HM148339	HM148584
<i>Cladosporium globisporum</i>	CBS 812.96 ^T	Meat stamp	HM148096	HM148340	HM148585
<i>Cladosporium grevilleae</i>	CBS 114271 ^T	Leaves of <i>Grevillea</i> sp.	JF770450	JF770472	JF770473
<i>Cladosporium halotolerans</i>	CBS 119416 ^T	Hypersaline water	DQ780364	JN906989	EF101397
	UTHSC DI-13-250 = FMR 13363	Human, scalp	LN834374	LN834470	LN834558
<i>Cladosporium herbaroides</i>	CBS 121626 ^T	Hypersaline water	EF679357	EF679432	EF679509
<i>Cladosporium herbarum</i>	CBS 121621 ^{ET}	<i>Hordeum vulgare</i>	EF679363	EF679440	EF679516
	UTHSC DI-13-220 = FMR 13333	Human, BAL	LN834378	LN834474	LN834562
<i>Cladosporium hillianum</i>	CBS 125988 ^T	Leaves of <i>Grevillea</i> sp.	HM148097	HM148341	HM148586
<i>Cladosporium inversicolor</i>	CBS 143.65	Leaf of <i>Tilia</i> sp.	HM148100	HM148344	HM148589
	CBS 401.80 ^T	Leaf of <i>Triticum aestivum</i>	HM148101	HM148345	HM148590
<i>Cladosporium ipereniae</i>	CBS 140483 ^T	<i>Puya</i> sp.	KT600394	KT600491	KT600589
	CPC 16855	<i>Arctostaphylos pallida</i>	KT600395	KT600492	KT600590
<i>Cladosporium iranicum</i>	CBS 126346 ^T	Leaf of <i>Citrus sinensis</i>	HM148110	HM148354	HM148599
<i>Cladosporium iridis</i>	CBS 138.40 ^{ET}	Leaf of <i>Iris</i> sp.	EF679370	EF679447	EF679523
<i>Cladosporium langeronii</i>	CBS 189.54 ^{NT}	Man	DQ780379	JN906990	EF101357
<i>Cladosporium licheniphilum</i>	CBS 125990 ^{ET}	From <i>P. orbicularis</i> and <i>Physcia</i> sp.	HM148111	HM148355	HM148600
		on <i>Acer platanoides</i>			
<i>Cladosporium limoniforme</i>	CBS 113737	Grape berry	KT600396	KT600493	KT600591
	CBS 140484 ^T	<i>Musa acuminata</i>	KT600397	KT600494	KT600592
<i>Cladosporium longicatenatum</i>	CBS 140485 ^T	Unknown plant	KT600403	KT600500	KT600598
<i>Cladosporium longissimum</i>	CBS 300.96 ^T	Soil along coral reef coast	DQ780352	EU570259	EF101385
<i>Cladosporium lycoperdinum</i>	CBS 574.78C	<i>Aureobasidium caulivorum</i>	HM148115	HM148359	HM148604
	CBS 126347	From galls of <i>Apiosporina morbosa</i> on <i>Prunus</i> sp.	HM148112	HM148356	HM148601
<i>Cladosporium macrocarpum</i>	CBS 121623 ^{NT}	<i>Spinacia oleracea</i>	EF679375	EF679453	EF679529
	UTHSC DI-13-191 = FMR 13316	Human, face	LN834379	LN834475	LN834563
<i>Cladosporium montecillanum</i>	CBS 140486 ^T	Pine needles	KT600406	KT600504	KT600602
	CPC 15605	<i>Taraxacum</i> sp.	KT600407	KT600505	KT600603
<i>Cladosporium myrtacearum</i>	CBS 126350 ^{ET}	<i>Corymbia foelscheana</i>	HM148117	HM148361	HM148606
<i>Cladosporium ossifragi</i>	CBS 842.91 ^{ET}	<i>Nartheicum ossifragum</i>	EF679381	EF679459	EF679535
<i>Cladosporium oxysporum</i>	CBS 125991	Soil	HM148118	HM148362	HM148607
	CBS 126351	Indoor air	HM148119	HM148363	HM148608
<i>Cladosporium paracladosporioides</i>	CBS 171.54 ^T	Unknown	HM148120	HM148364	HM148609
<i>Cladosporium parapendelioides</i>	CBS 140487 ^T	<i>Eucalyptus</i> sp.	KT600410	KT600508	KT600606
<i>Cladosporium penidelioides</i>	CBS 140489 ^T	<i>Acacia verticillata</i>	KT600412	KT600510	KT600608
<i>Cladosporium perangustum</i>	CBS 125996 ^T	<i>Cussonia</i> sp.	HM148121	HM148365	HM148610
	CBS 126365	<i>Chasmothecia of Phyllactinia guttata</i> on leaves of <i>Corylus avellana</i>	HM148123	HM148367	HM148612
	CPC 11663	<i>Oncoba spinosa</i>	HM148128	HM148372	HM148617
	CPC 11815	<i>Chasmothecia of Phyllactinia guttata</i> on leaves of <i>Corylus</i> sp.	HM148130	HM148374	HM148619
	CPC 11819	<i>Chasmothecia of Phyllactinia guttata</i> on leaves of <i>Corylus</i> sp.	HM148131	HM148375	HM148620
	CPC 11821	<i>Chasmothecia of Phyllactinia guttata</i> on leaves of <i>Corylus</i> sp.	HM148132	HM148376	HM148621
	CPC 11831	<i>Chasmothecia of Phyllactinia guttata</i> on leaves of <i>Corylus</i> sp.	HM148133	HM148377	HM148622
	CPC 12216	<i>Morus rubra</i>	HM148135	HM148379	HM148624
	CPC 13727	<i>Teratosphaeria maculiformis</i>	HM148139	HM148383	HM148628
	CPC 13730	<i>Protea caffra</i>	HM148140	HM148384	HM148629
	CPC 13774	<i>Protea caffra</i>	HM148141	HM148385	HM148630
	CPC 13870	<i>Teratosphaeria fibrillosa</i>	HM148142	HM148386	HM148631
	UTHSC DI-13-208 = FMR 13321	Canine, BAL	LN834380	LN834476	LN834564
<i>Cladosporium phaenocoma</i>	CBS 128769 ^T	Leaf bracts of <i>Phaenocoma prolifera</i>	JF499837	JF499875	JF499881
<i>Cladosporium phlei</i>	CBS 358.69 ^{ET}	<i>Phleum pratense</i>	JN906981	JN906991	JN907000
<i>Cladosporium phyllactiniicola</i>	CBS 126353	<i>Chasmothecia of P. guttata</i> on leaves of <i>Corylus avellana</i>	HM148151	HM148395	HM148640
	CBS 126355 ^T	<i>Chasmothecia of P. guttata</i> on leaves of <i>Corylus avellana</i>	HM148153	HM148397	HM148642
<i>Cladosporium phyllophilum</i>	CBS 125992 ^{ET}	Fruits of <i>Prunus cerasus</i>	HM148154	HM148398	HM148643
<i>Cladosporium pini-ponderosae</i>	CBS 124456 ^T	<i>Pinus ponderosa</i>	FJ936160	FJ936164	FJ936167
<i>Cladosporium pseudiridis</i>	CBS 116463 ^T	<i>Iris</i> sp.	EF679383	EF679461	EF679537
<i>Cladosporium pseudochalastosporioides</i>	CBS 140490 ^T	Pine needles	KT600415	KT600513	KT600611
<i>Cladosporium pseudocladosporioides</i>	CBS 667.80	<i>Malus sylvestris</i>	HM148165	HM148409	HM148654
	CBS 125993 ^T	Outside air	HM148158	HM148402	HM148647
	CPC 13683	<i>Eucalyptus placita</i>	HM148173	HM148417	HM148662
	CPC 14020	Wheat	HM148185	HM148429	HM148674
	CPC 14295	Soil	HM148188	HM148432	HM148677
	UTHSC DI-13-165 = FMR 13290	Human, arm drainage	LN834406	LN834502	LN834590
	UTHSC DI-13-190 = FMR 13315	Human, CSF	LN834412	LN834508	LN834596
UTHSC DI-13-210 = FMR 13323	Human, skin	LN834414	LN834510	LN834598	

Table 1 (cont.)

Species ^a	Strain number ^b	Substrate ^c	GenBank accession numbers			
			ITS	<i>tef1</i>	<i>ActA</i>	
<i>Cladosporium pseudocladosporioides</i> (cont.)	UTHSC DI-13-218 = FMR 13331	Human, BAL	LN834418	LN834514	LN834602	
	UTHSC DI-13-227 = FMR 13340	Human, sputum	LN834422	LN834518	LN834606	
	UTHSC DI-13-234 = FMR 13347	Human, sputum	LN834424	LN834520	LN834608	
	UTHSC DI-13-238 = FMR 13351	Human, leg	LN834426	LN834522	LN834610	
	UTHSC DI-13-241 = FMR 13354	Human, foot	LN834427	LN834523	LN834611	
	UTHSC DI-13-245 = FMR 13358	Human, toe	LN834429	LN834525	LN834613	
	UTHSC DI-13-251 = FMR 13364	Human, BAL	LN834432	LN834528	LN834616	
	UTHSC DI-13-261 = FMR 13374	Human, sputum	LN834384	LN834480	LN834568	
	UTHSC DI-13-265 = FMR 13378	Human, BAL	LN834435	LN834531	LN834619	
	UTHSC DI-13-268 = FMR 13381	Human, toenail	LN834436	LN834532	LN834620	
	UTHSC DI-13-270 = FMR 13383	Human, nail	LN834438	LN834534	LN834622	
<i>Cladosporium psychrotolerans</i>	CBS 119412 ^T	Hypersaline water	DQ780386	JN906992	EF101365	
<i>Cladosporium puyae</i>	CBS 274.80A ^T	<i>Puya goudotiana</i>	KT600418	KT600516	KT600614	
<i>Cladosporium ramotenellum</i>	CBS 121628 ^T	Hypersaline water	EF679384	EF679462	EF679538	
	UTHSC DI-13-166 = FMR 13291	Human, nasal tissue	LN834385	LN834481	LN834569	
<i>Cladosporium rectoides</i>	CBS 125994 ^T	<i>Vitis flexuosa</i>	HM148193	HM148438	HM148683	
<i>Cladosporium rhusicola</i>	CBS 140492 ^T	<i>Rhus</i> sp.	KT600440	KT600539	KT600637	
<i>Cladosporium ruguloflabelliforme</i>	CBS 140494 ^T	<i>Diatrapaceae</i> sp. on <i>Aloe</i> sp.	KT600458	KT600557	KT600655	
<i>Cladosporium rugulovarians</i>	CBS 140495 ^T	Leaf sheaths of unidentified <i>Poaceae</i>	KT600459	KT600558	KT600656	
<i>Cladosporium salinae</i>	CBS 119413 ^T	Hypersaline water	DQ780374	JN906993	EF101390	
<i>Cladosporium scabrellum</i>	CBS 126358 ^T	<i>Ruscus hypoglossum</i>	HM148195	HM148440	HM148685	
<i>Cladosporium silenes</i>	CBS 109082	<i>Silene maritima</i>	EF679354	EF679429	EF679506	
<i>Cladosporium sinuosum</i>	ATCC 11285	Unidentified moss	KT600441	KT600540	KT600638	
	CBS 393.68	Air	KT600442	KT600541	KT600639	
	CBS 121629 ^T	<i>Fuchsia excorticata</i>	EF679386	EF679464	EF679540	
	CPC 14000	Wheat	KT600443	KT600542	KT600640	
	CPC 15454	<i>Crocus sativus</i>	KT600444	KT600543	KT600641	
	CPC 18365	<i>Iris pseudacorus</i>	KT600446	KT600545	KT600643	
	<i>Cladosporium soldaneliae</i>	CBS 132186 ^{NT}	<i>Soldanella alpina</i>	JN906982	JN906994	JN907001
	<i>Cladosporium sphaerospermum</i>	CBS 193.54 ^{NT}	Human, nail	DQ780343	EU570261	EU570269
		UTHSC DI-13-237 = FMR 13350	Human, BAL	LN834390	LN834486	LN834574
	<i>Cladosporium spinulosum</i>	CBS 119907 ^T	Hypersaline water	EF679388	EF679466	EF679542
<i>Cladosporium subinflatum</i>	CBS 121630 ^T	Hypersaline water	EF679389	EF679467	EF679543	
	UTHSC DI-13-189 = FMR 13314	Human, toenail	LN834391	LN834487	LN834575	
<i>Cladosporium subtilissimum</i>	CBS 113754 ^T	Grape berry	EF679397	EF679475	EF679551	
<i>Cladosporium subuliforme</i>	CBS 126500 ^T	<i>Chamaedorea metallica</i>	HM148196	HM148441	HM148686	
	UTHSC DI-13-214 = FMR 13327	Human, BAL	LN834394	LN834490	LN834578	
<i>Cladosporium subcinereum</i>	CBS 140465 ^T = UTHSC DI-13-257 = FMR 13370	Human, sputum	LN834433	LN834529	LN834617	
<i>Cladosporium succulentum</i>	CBS 140466 ^T = UTHSC DI-13-262 = FMR 13375	Dolphin, bronchus	LN834434	LN834530	LN834618	
<i>Cladosporium tenellum</i>	CBS 121634 ^T	Hypersaline water	EF679401	EF679479	EF679555	
<i>Cladosporium tenuissimum</i>	CBS 125995 ^{ET}	Fruits of <i>Lagerstroemia</i> sp.	HM148197	HM148442	HM148687	
	CPC 10882	<i>Gnaphalium affine</i>	HM148204	HM148449	HM148694	
	CPC 11555	<i>Citrus sinensis</i>	HM148205	HM148450	HM148695	
	CPC 11805	<i>Strelitzia</i> sp.	HM148207	HM148452	HM148697	
	CPC 12795	<i>Musa</i> sp.	HM148209	HM148454	HM148699	
	CPC 13222	<i>Callistemon viminalis</i>	HM148210	HM148455	HM148700	
	CPC 14250	<i>Magnolia</i> sp.	HM148211	HM148456	HM148701	
	UTHSC DI-13-258 = FMR 13371	Human, thoracentesis fluid	LN834404	LN834500	LN834588	
	<i>Cladosporium tuberosum</i>	CBS 140693 ^T = UTHSC DI-13-217 = FMR 13330	Human, nasal	LN834417	LN834513	LN834601
		UTHSC DI-13-219 = FMR 13332	Human, foot	LN834419	LN834515	LN834603
<i>Cladosporium variabile</i>	CBS 121635 ^{ET}	<i>Spinacia oleracea</i>	EF679402	EF679480	EF679556	
<i>Cladosporium varians</i>	CBS 126362 ^T	<i>Catalpa bungei</i>	HM148224	HM148470	HM148715	
<i>Cladosporium velox</i>	CBS 119417 ^T	Bamboo	DQ780361	JN906995	EF101388	
<i>Cladosporium verrucocladosporioides</i>	CBS 126363 ^T	<i>Rhus chinensis</i>	HM148226	HM148472	HM148717	
<i>Cladosporium versiforme</i>	CBS 140491 ^T	<i>Hordeum</i> sp.	KT600417	KT600515	KT600613	
<i>Cladosporium xantochromaticum</i>	CBS 140691 ^T = UTHSC DI-13-211 = FMR 13324	Human, BAL	LN834415	LN834511	LN834599	
	CBS 126364	<i>Erythrophleum chlorostachys</i>	HM148122	HM148366	HM148611	
	CPC 11133	<i>Eucalyptus</i> sp.	HM148126	HM148370	HM148615	
	CPC 11609	<i>Musa</i> sp.	EF679356	EF679431	EF679508	
	CPC 11806	<i>Strelitzia</i> sp.	HM148129	HM148373	HM148618	
	CPC 11856	<i>Acacia mangium</i>	HM148134	HM148378	HM148623	
	CPC 12792	<i>Musa</i> sp.	HM148136	HM148380	HM148625	
	<i>Cladosporium xylophilum</i>	CBS 125997 ^T	<i>Picea abies</i>	HM148230	HM148476	HM148721

^a New species described in this study are in **bold italic**.

^b ATCC, American Type Culture Collection, Manassas, VA, USA; CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; CPC, collection of Pedro Crous at CBS; FMR, Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, Texas, USA.

^c BAL fluid, bronchoalveolar lavage fluid specimen; CSF, cerebrospinal fluid.

^T Ex-type strain.

^{ET} Ex-epitype strain.

^{NT} Ex-neotype strain.

MATERIALS AND METHODS

Fungal isolates

A total of 48 isolates from clinical origin and belonging to the genus *Cladosporium* were included in this study, 35 of which corresponded to putatively undescribed species (Table 1). All the isolates were obtained from human and animal clinical specimens from the United States, submitted to the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (UTHSCSA) from different geographic regions of the country for either identification purposes and/or antifungal susceptibility studies.

Phenotypic studies

Macroscopic cultural characteristics of the isolates were recorded after incubation for 14 d at 25 °C, using oatmeal agar (OA) (30 g of filtered oat flakes, 20 g of agar, water 1 L), potato dextrose agar (PDA: Pronadisa, Spain) and synthetic nutrient-poor agar (SNA; KH₂PO₄ 1 g, KNO₃ 1 g, MgSO₄·x7H₂O 0.5 g, KCl 0.5 g, glucose 0.2 g, sucrose 0.2 g, agar 14 g, water 1 L) with and without pieces of sterilised paper as carbon source. In descriptions, colour notations of the colonies were from Kornerup & Wanscher (1978). Observations and measurements of the microscopic structures were carried out from colonies on SNA after incubation for 7 d at 25 °C, mounted on Shear's solution (Schubert et al. 2007, Zalar et al. 2007, Crous et al. 2009, Bensch et al. 2012). Photographs were made using 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. Scanning electron microscope (SEM) micrographs were obtained with a Jeol JSM-6400 apparatus, following the protocols described by Figueras & Guarro (1988). Cardinal temperatures of growth were determined culturing the isolates on PDA for 14 d at temperatures ranging from 15 °C to 35 °C at intervals of 5 °C.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted, amplified and sequenced in a previous work, using protocols described elsewhere (Bensch et al. 2012, Sandoval-Denis et al. 2015). Briefly, the primer pair ITS5/ITS4 (White et al. 1990) was used to amplify a region spanning the internal transcribed spacers 1 and 2 and the 5.8S gene of the rRNA (ITS), and the primer pairs EF-728F/EF-986R and ACT-512F/ACT-783R (Carbone & Kohn 1999) were used to amplify a partial fragment of the translation elongation factor 1- α gene (*tef1*) and the actin gene (*actA*), respectively.

Sequences were generated using the same PCR primers at Macrogen Europe (Macrogen Inc. Amsterdam, The Netherlands). Consensus sequences were assembled using SeqMan v. 7.0.0 (DNASStar Lasergene, Madison, WI, USA).

Sequence alignment and phylogenetic analyses

Multiple sequence alignments of each locus were performed with MEGA v. 6.06 (Tamura et al. 2013), using the ClustalW algorithm (Thompson et al. 1994) and refined with MUSCLE (Edgar 2004) or manually if necessary. The alignment included sequences from the clinical isolates complemented with sequences representing all the available ex-types and numerous reference strains of *Cladosporium* spp. retrieved from GenBank and mainly published by Bensch et al. (2012, 2015). These latter sequences were selected on the basis of sequence similarity with the putative new taxa as determined by BLAST searches on the NCBI database using ITS, *tef1* and *actA* loci (Table 1).

Phylogenetic reconstructions were performed using the maximum-likelihood (ML) and Bayesian Inference (BI) approaches under MEGA v. 6.06 and MrBayes v. 3.2 (Huelsenbeck &

Ronquist 2001), respectively. MrModelTest v. 2.3 (Nylander 2004) was used to determine the best nucleotide substitution model for each dataset (SYM+G for ITS and GTR+G+I for *tef1* and *actA*). Sequence alignments generated in this study were deposited in TreeBASE (<http://treebase.org>).

For the ML analyses, 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 analyses, four Markov chains were performed in two simultaneous runs for 10 000 000 generations with a sampling rate of 1 000 generations. Once checked for the convergence of the runs (average standard deviation of split frequencies parameter below 0.01), the 50 % majority-rule consensus tree and posterior probability values (pp) were calculated after discarding 2 500 trees for burn-in. A pp value ≥ 0.95 was considered significant. Phylogenetic concordance of the ITS, *tef1* and *actA* gene datasets was evaluated with the partition-homogeneity test implemented with PAUP v. 4.0b10 (Swofford 2003) and also by visual comparison of the individual phylogenies in order to assess for any incongruent results between nodes with high statistical support. Taxonomic novelties were deposited in MycoBank (Crous et al. 2004).

RESULTS

Phylogeny

The different partitions were congruent as determined by visual comparison of the individual phylogenies (data not shown) and by the partition homogeneity test ($p = 0.16$). Phylogenies obtained by ML and BI also showed topological congruence. The final combined analysis of the three mentioned loci datasets encompassed 197 sequences representing 101 taxa, including *Cercospora beticola* (CBS 116456) as the outgroup, and comprised 1 026 bp (ITS 448 bp, *tef1* 357 bp and *actA* 221 bp) from which 546 bp were variable (ITS 108 bp, *tef1* 291 bp and *actA* 147 bp) and 399 bp phylogenetically informative (ITS 42 bp, *tef1* 234 bp and *actA* 123 bp). Unique site pattern values for the Bayesian analyses were 92, 322 and 167 for ITS, *tef1* and *actA* datasets, respectively (Fig. 1). Of the 35 unidentified isolates, 21 clustered into ten groups that received strong statistical support with the exception of two monotypic lineages (CBS 140465 and CBS 140466), which, however, were genetically and morphologically differentiated from their closest phylogenetic relatives. The remaining 14 isolates were identified here as *C. pseudocladosporioides* (13 isolates) and *C. allicinum* (one isolate). The isolates representing putative new taxa grouped mainly in the *C. cladosporioides* species complex in which 16 isolates were distributed in three terminal clades and three monotypic lineages. Five isolates belonged to the *C. herbarum* species complex, two of them (CBS 140693 and UTHSC DI-13-219) grouped in a terminal clade, located in a basal position to the remaining species of the complex, while three isolates formed monotypic lineages. The *C. sphaerospermum* species complex included a single unidentified isolate (CBS 140466) forming a genetically and morphologically distinct lineage. The 10 phylogenetic groups are thus considered new species of *Cladosporium* and are described in the taxonomy section below.

TAXONOMY

Cladosporium alboflavescens Sandoval-Denis, Gené & Cano, sp. nov. — MycoBank MB815332; Fig. 2

Etymology. From Latin *albus* 'white' *flavus* 'yellow', referring to the colony colour of the species.

Colonies on OA attaining 20–23 mm diam after 14 d at 25 °C, white to grey-yellow (4A1/C4), flat, velvety, margin regular and with abundant submerged mycelium; reverse olive brown (4D5/F8),

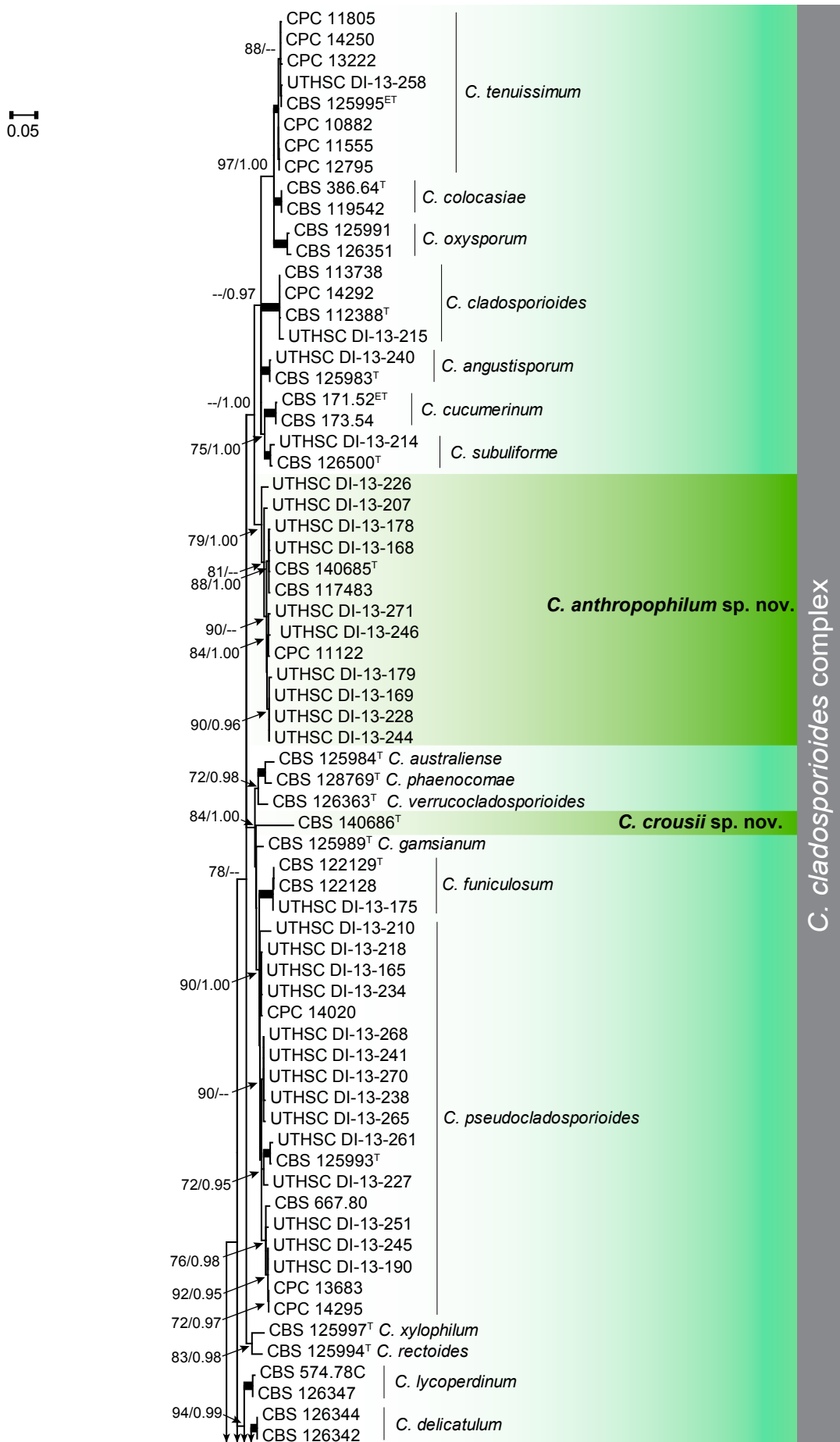
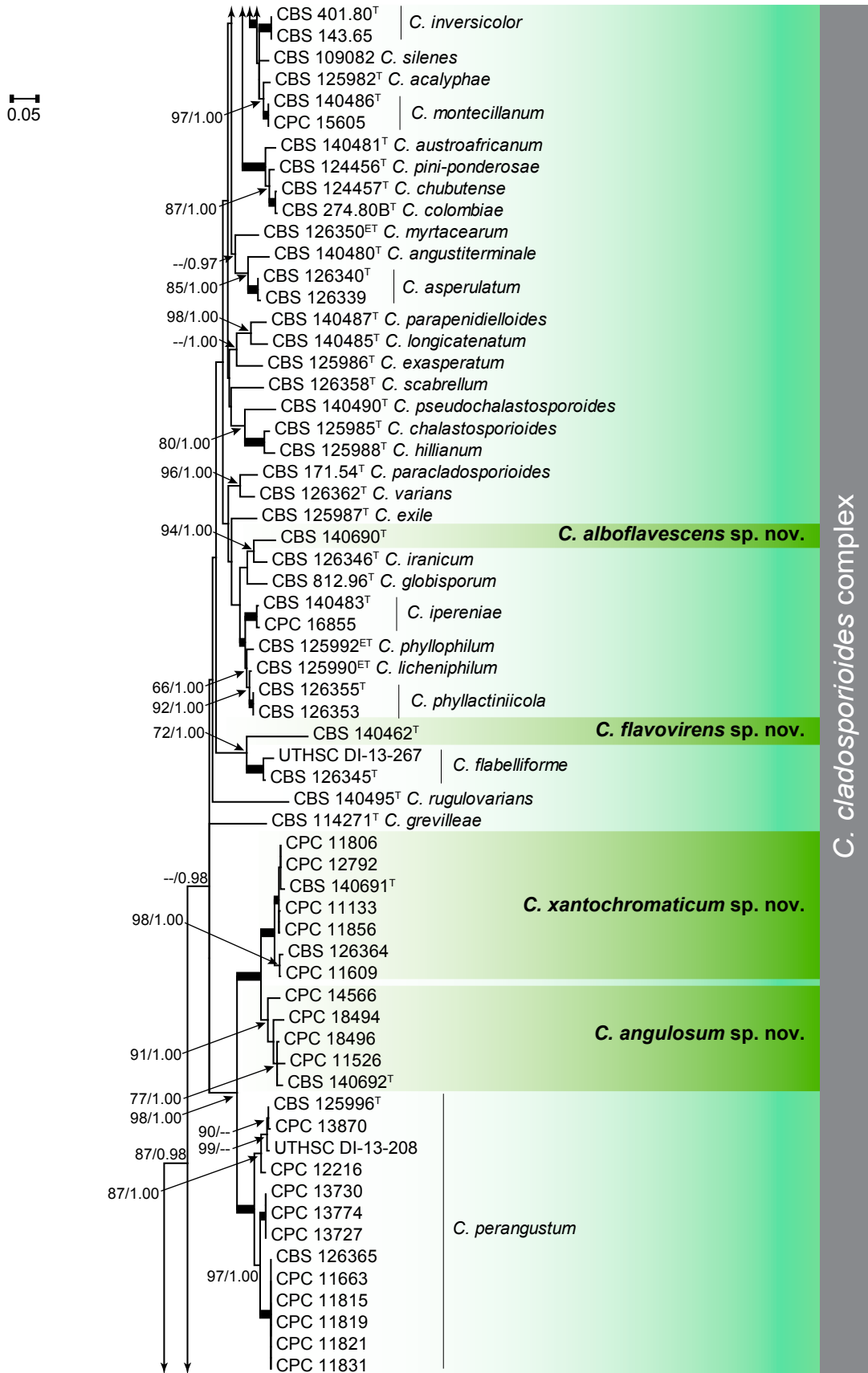


Fig. 1 Maximum likelihood (ML) tree obtained from the combined ITS, *tef1* and *actA* sequences of 196 strains from *Cladosporium* species. The tree is rooted with *Cercospora beticola* CBS 116456. Numbers on the branches represent ML bootstrap support values of 70 % and higher, followed by Bayesian posterior probabilities (pp) above 0.94. Fully supported branches are thickened and names of species newly described here are indicated in **bold**. Coloured blocks represent the species complex affinity of the novelties described here. Branch lengths are proportional to distance.
^T Ex-type strain. ^{ET} Ex-epitype strain. ^{NT} Ex-neotype strain.

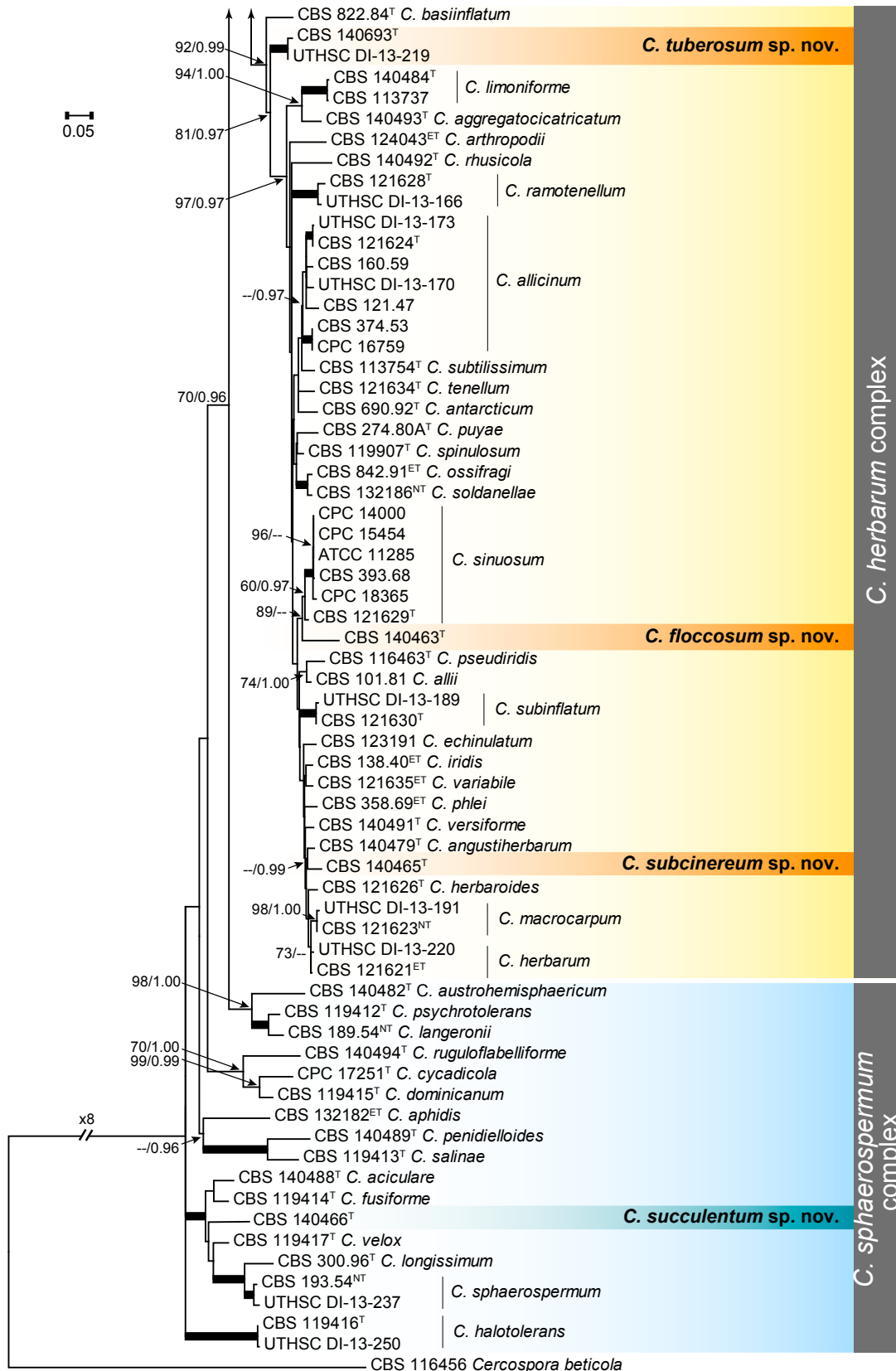
Fig. 1 (cont.)



without diffusible pigments. On PDA attaining 34–36 mm diam after 14 d at 25 °C, yellow-grey to olive brown (4B2/D4), with prominent light yellow (3A4) exudate, flat or umbonate, folded, margin regular; reverse grey-yellow to olive brown (4B4/F4) to black. On SNA reaching 22–25 mm after 14 d at 25 °C, obverse and reverse olive (3D5/E8), flat, velvety with granular centre, margin undulate and with abundant submerged mycelium. Mycelium superficial and immersed, composed of septate,

branched, 2.5–5 µm wide, subhyaline to pale brown, smooth to slightly roughened, thin-walled hyphae. Conidiophores erect, straight, cylindrical, non-nodulose, septate, simple or branched, up to 130 µm long, 2.5–4 µm wide, pale brown, smooth or sparingly verrucose with darkened and refractive scars. Conidiogenous cells terminal or intercalary, cylindrical, geniculate, 7–36 × 2–4 µm, with up to five apical loci of 1.5–2 µm diam, thickened and refractive. Ramoconidia aseptate, subcylindrical

Fig. 1 (cont.)



to cylindrical, 11–36 × 2–3 µm, pale brown, smooth-walled. *Conidia* forming branched chains with up to three conidia in the terminal unbranched part, pale brown, sparingly verrucose, with protuberant, somewhat darkened and refractive conidial hila; small terminal conidia aseptate, oval, 5–6.5 × 2–3.5 µm (av. (± SD) 5.9 (± 0.4) × 2.8 (± 0.4)); intercalary conidia aseptate, ellipsoidal to almost cylindrical with attenuated ends, 7–13 × 2.5–3 µm (av. (± SD) 10.6 (± 2.5) × 2.6 (± 0.2)); secondary ramoconidia 0–1-septate, ellipsoidal, 8.5–18 × 2–3 µm (av. (± SD) 14.3 (± 3.3) × 2.6 (± 0.5)).

Cardinal temperature for growth — Optimum 20–25 °C, maximum 30 °C, minimum 15 °C.

Specimen examined. USA, California, from animal bronchoalveolar lavage fluid, Mar. 2009, D.A. Sutton (holotype CBS H-22379, culture ex-type CBS 140690 = UTHSC DI-13-225 = FMR 13338).

Notes — *Cladosporium alboflavescens* is morphologically similar to *C. pini-ponderosae* and *C. verrucocladosporioides* (Schubert et al. 2009, Bensch et al. 2010). However, the new species differs mainly by its pale coloured vegetative struc-

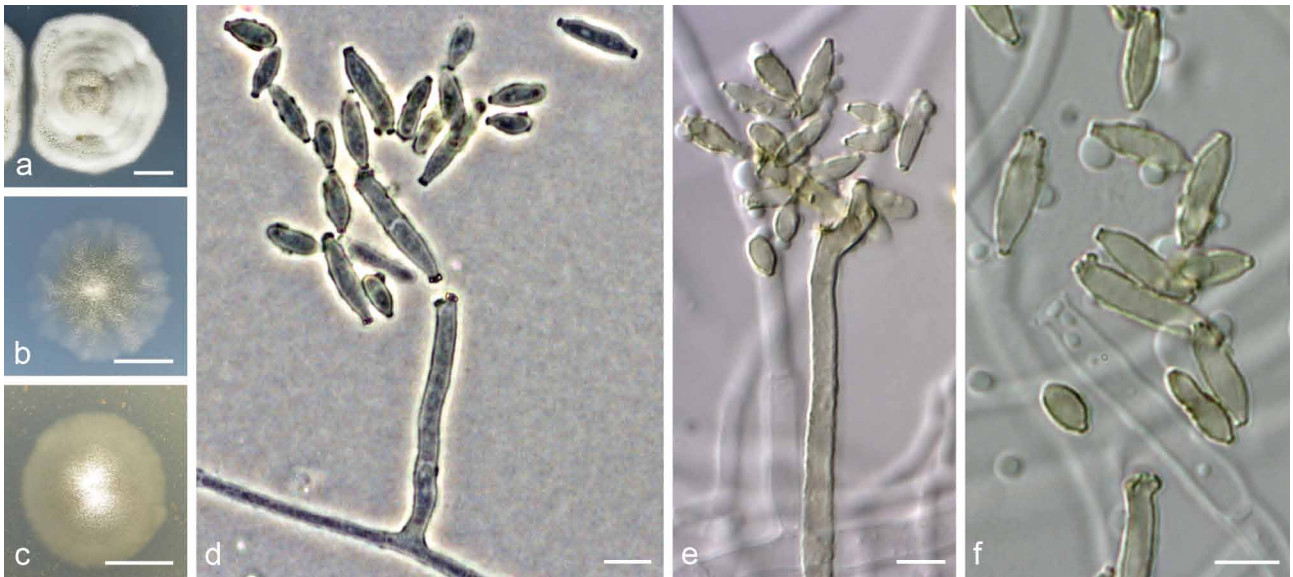


Fig. 2 *Cladosporium alboflavescens* CBS 140690. a–c. Colonies on (a) PDA, (b) SNA and (c) OA after 14 d at 25 °C; d–f. conidiophores and conidia. — Scale bars: a–c = 10 mm, d–f = 5 µm.

tures, and its yellow to pale olive colonies on OA and PDA vs olivaceous grey in the two latter species. The phylogenetically closely related species *C. iranicum* (Bensch et al. 2010) also shows similar micro-morphological characteristics to *C. alboflavescens*, but it differs in forming longer conidial chains with up to 10 conidia in the terminal unbranched part and often showing substrate intercalary conidia, while conidial chains of the novel species are much shorter and intercalary conidia ellipsoidal to cylindrical being also genetically well differentiated (99.8 %, 87.9 % and 90.1 % sequence similarity for ITS, *tef1* and *actA*, respectively).

Cladosporium angulosum Sandoval-Denis, Deanna A. Sutton & Guarro, *sp. nov.* — MycoBank MB815333; Fig. 3

Etymology. From Latin *angulosus* ‘full of corners’, referring to the shape of the conidiophore.

Colonies on OA reaching 52–55 mm after 14 d at 25 °C, olive brown (4E3/F8), flat, velvety to granular, with regular margin; reverse olive brown (4E3/F8) to black. On PDA attaining 50–56

mm diam after 14 d at 25 °C, olive brown (4F4/F8), with a raised or umbonate centre and radially folded towards the periphery, velvety to dusty or granular, with regular margin; reverse dark green (30F8) to black. On SNA reaching 37–40 mm after 14 d at 25 °C, olive brown (4D4/F6), flat, velvety, with lobulated margin; reverse olive brown (4D4/F6) to black. *Mycelium* superficial and immersed, composed of septate, branched, 1.5–3 µm wide, pale olivaceous brown, with smooth and thin-walled hyphae. *Conidiophores* erect, cylindrical, non-nodulose, septate, septa darkened, branched, frequently branching near the base in a 90° angle, up to 150 µm long, 3–4 µm wide, pale brown, smooth and thin-walled. *Conidiogenous cells* terminal or intercalary, cylindrical, 8–46 × 2–3.5 µm, bearing up to four conidiogenous loci of 1–1.5 µm diam, darkened and refringent. *Ramoconidia* aseptate, subcylindrical, straight, 24.5–46 × 2–3.5 µm, pale brown, finely roughened, with scars protuberant, thickened and darkened. *Conidia* forming long branched chains with up to 14 conidia in the terminal unbranched part, pale olivaceous brown, smooth and thin-walled, with protuberant conidial hila, not darkened; small terminal conidia aseptate, obovate to nearly

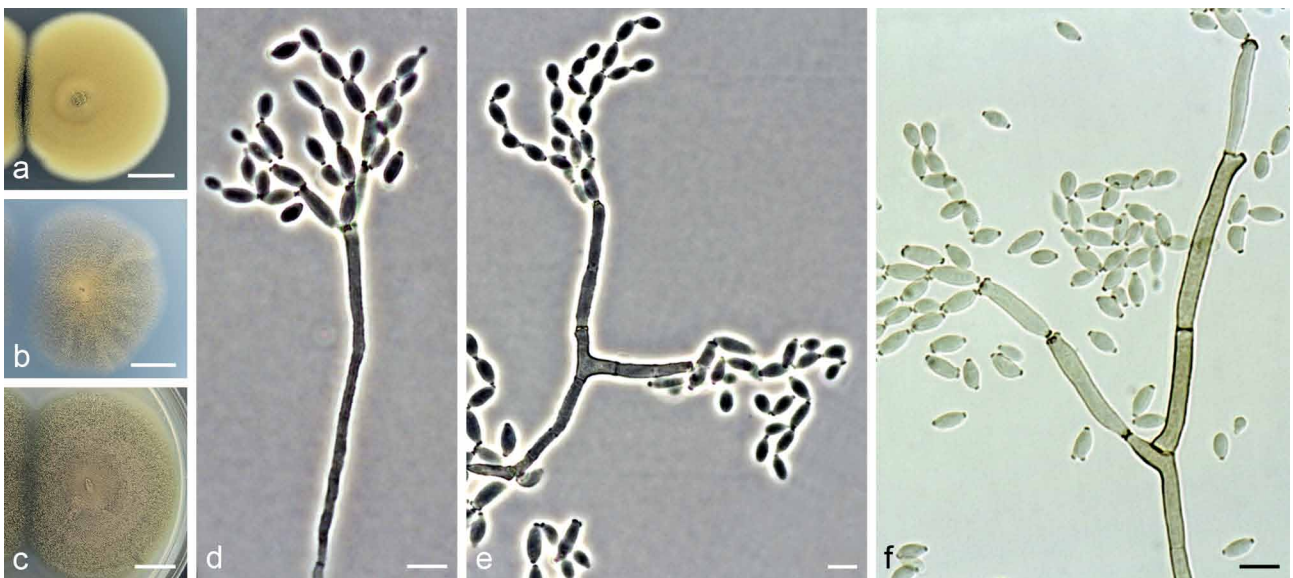


Fig. 3 *Cladosporium angulosum* CBS 140692. a–c. Colonies on (a) PDA, (b) SNA and (c) OA after 14 d at 25 °C; d–f. conidiophores and chains of conidia. — Scale bars: a–c = 10 mm, d–f = 5 µm.

cylindrical, $3.5\text{--}4.5 \times 2\text{--}2.5 \mu\text{m}$ (av. (\pm SD) $4.1 (\pm 0.3) \times 2.3 (\pm 0.3)$); intercalary conidia aseptate, ellipsoidal, $4\text{--}6 \times 2\text{--}3 \mu\text{m}$ (av. (\pm SD) $5.3 (\pm 0.6) \times 2.4 (\pm 0.4)$); secondary ramoconidia 0–1-septate, usually constricted at septum, subcylindrical, $8\text{--}17 \times 2.5\text{--}3 \mu\text{m}$ (av. (\pm SD) $12.2 (\pm 2.6) \times 2.8 (\pm 0.3)$).

Cardinal temperature for growth — Optimum $25 \text{ }^\circ\text{C}$, maximum $35 \text{ }^\circ\text{C}$, minimum $15 \text{ }^\circ\text{C}$.

Specimen examined. USA, Texas, from human bronchoalveolar lavage fluid, Sept. 2008, *D.A. Sutton* (holotype CBS H-22380, culture ex-type CBS 140692 = UTHSC DI-13-235 = FMR 13348).

Notes — The clade representative of *C. angulosum* includes several strains previously identified as *C. perangustum*, a species accepted with a considerable morphological and genetic diversity by Bensch et al. (2010, 2012, 2015). However, it shows a sufficient genetic distance (ITS, 100%; *tef1*, 77%; *actA*, 85.4% similarity) with respect to the ex-type strain of *C. perangustum* to be considered a distinct species. Morphologically, *C. angulosum* can be mainly differentiated from *C. perangustum* by its conidiophores, which are usually branched forming a 90° angle, while those of the latter are only occasionally branched. In addition, the new species produces smaller secondary ramoconidia and intercalary conidia (up to $17 \mu\text{m}$ and $6 \mu\text{m}$ long, respectively, vs $6\text{--}30\text{--}34 \mu\text{m}$ and $4\text{--}16\text{--}19 \mu\text{m}$ long, respectively, in *C. perangustum*) (Bensch et al. 2012). Another closely related species is *C. xanthochromaticum*, but it is genetically well differentiated from *C. angulosum* (99.1%, 81.1% and 90.8% similarity for ITS, *tef1* and *actA*, respectively), and morphologically it has longer conidiogenous cells (up to $32 \mu\text{m}$ long vs $27 \mu\text{m}$ long in *C. angulosum*), smaller ramoconidia (up to $39 \mu\text{m}$ long vs $46 \mu\text{m}$ long in *C. angulosum*) and does not grow at $35 \text{ }^\circ\text{C}$.

Cladosporium anthropophilum Sandoval-Denis, Gené & Wiederhold, *sp. nov.* — MycoBank MB815334, Fig. 4

Etymology. From the Greek *ánthrōpos* (άνθρωπος) ‘human’ and *philos* (φίλος) ‘fondness’, referring to the source of the ex-type, human clinical samples.

Colonies on OA attaining $27\text{--}32 \text{ mm}$ diam after 14 d at $25 \text{ }^\circ\text{C}$, olive to olive brown (3F2/4F8), flat, dusty or granular, aerial mycelium scarce, with fimbriate margin; reverse olive brown (4F8) to black, without diffusible pigment. On PDA attaining $17\text{--}39 \text{ mm}$ diam after 14 d at $25 \text{ }^\circ\text{C}$, grey-green to deep green

(28D7/D8), flat or folded, velvety to dusty or granular, aerial mycelium scarce, sometimes showing cottony to floccose white to grey cushions, with a regular margin; reverse dark green (28F8) to black. On SNA reaching $23\text{--}26 \text{ mm}$ after 14 d at $25 \text{ }^\circ\text{C}$, olive to olive brown (3F2/4F8), flat, dusty to cottony, aerial mycelium abundant, often with irregular to arachnoid margins; reverse olive to olive brown (3F2/4F8). *Mycelium* superficial and immersed, composed of septate, branched, $2\text{--}3 \mu\text{m}$ wide, subhyaline to pale green, smooth and thick-walled, anastomosing hyphae. *Conidiophores* erect, cylindrical, non-nodulose, geniculate, septate, usually branched, up to $550 \mu\text{m}$ long, $2\text{--}5 \mu\text{m}$ wide, pale green-brown, slightly roughened to verruculose toward the base, with a thickened and refractive wall. *Conidiogenous cells* terminal and intercalary, cylindrical or subcylindrical, $15\text{--}54 \times 3\text{--}5 \mu\text{m}$, often with a swollen apex, bearing $3\text{--}8\text{--}(10)$, protuberant, subdentate, $1\text{--}2.5 \mu\text{m}$ diam, thickened and somewhat darkened conidiogenous loci. *Ramoconidia* aseptate, cylindrical, $20\text{--}42 \times 2\text{--}5 \mu\text{m}$, pale green, smooth, with conidial scars protuberant, thickened and darkened. *Conidia* forming short branched chains with up to four conidia in the terminal unbranched part of the chain, aseptate, smooth or finely roughened, reticulate under SEM; small terminal conidia oval to ellipsoidal, $3.5\text{--}9 \times 2\text{--}3 \mu\text{m}$ (av. (\pm SD) $5.6 (\pm 1.2) \times 2.5 (\pm 0.4)$), subhyaline; intercalary conidia limoniiform to ellipsoidal, $4.5\text{--}11 \times 2\text{--}3 \mu\text{m}$ (av. (\pm SD) $6.9 (\pm 1.8) \times 2.7 (\pm 0.3)$), light green-brown; secondary ramoconidia 0–1-septate, ellipsoidal to subcylindrical, usually attenuated at the centre, $7\text{--}28 \times 2\text{--}5 \mu\text{m}$ (av. (\pm SD) $13.7 (\pm 4.8) \times 3.4 (\pm 0.6)$).

Cardinal temperature for growth — Optimum $25 \text{ }^\circ\text{C}$, maximum $35 \text{ }^\circ\text{C}$, minimum $5 \text{ }^\circ\text{C}$.

Specimens examined. USA, Minnesota, from human bronchoalveolar lavage fluid, Sept. 2012, *D.A. Sutton* (holotype CBS H-22381, culture ex-type CBS 140685 = UTHSC DI-13-269 = FMR 13382); from human bronchoalveolar lavage fluid, Sept. 2012, *D.A. Sutton*, UTHSC DI-13-168 = FMR 13293; California, from a hand, Oct. 2010, *D.A. Sutton*, UTHSC DI-13-179 = FMR 13304; Florida, from human bronchoalveolar lavage fluid, Jan. 2007, *D.A. Sutton*, UTHSC DI-13-271 = FMR 13384; from human bronchoalveolar lavage fluid, Mar. 2007, *D.A. Sutton*, UTHSC DI-13-246 = FMR 13359; from an animal abscess, Jan. 2012, *D.A. Sutton*, UTHSC DI-13-178 = FMR 13303; Massachusetts, from human bronchoalveolar lavage fluid, Mar. 2012, *D.A. Sutton*, UTHSC DI-13-169 = FMR 13294; Texas, from human cerebrospinal fluid, Mar. 2009, *D.A. Sutton*, UTHSC DI-13-207 = FMR 13320; from human bronchoalveolar lavage fluid, Jan. 2009, *D.A. Sutton*, UTHSC DI-13-226 = FMR 13339; from human foot skin, May 2008, *D.A. Sutton*, UTHSC DI-13-228 = FMR 13341; from human pleural fluid, Apr. 2008, *D.A. Sutton*, UTHSC DI-13-244 = FMR 13357.

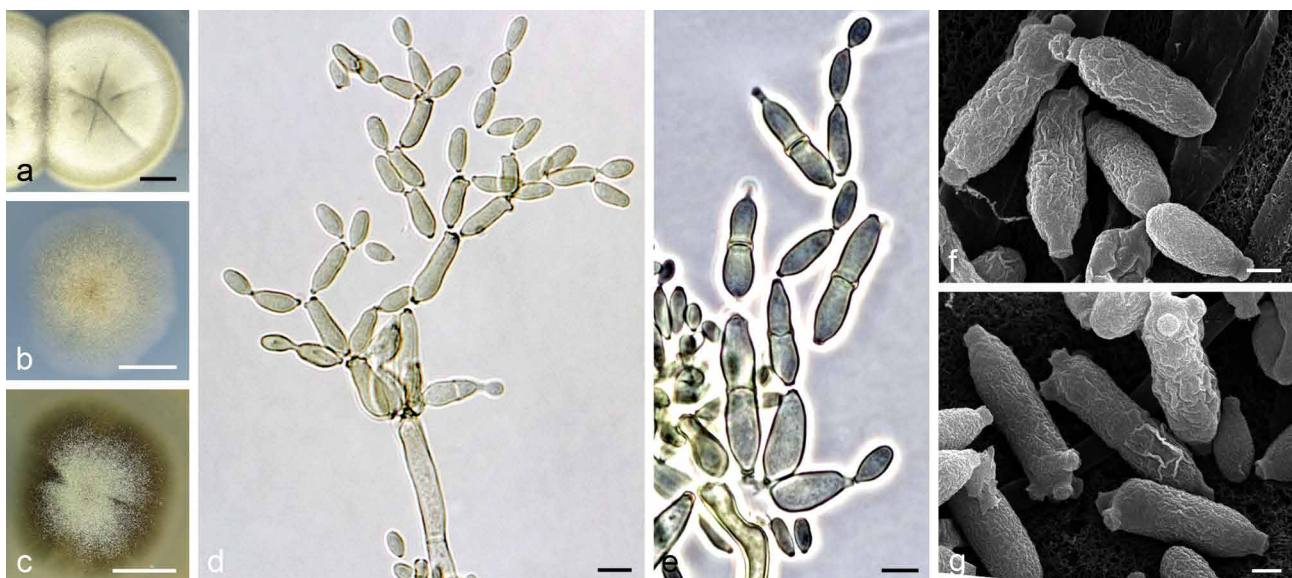


Fig. 4 *Cladosporium anthropophilum* CBS 140685. a–c. Colonies on (a) PDA, (b) SNA and (c) OA after 14 d at $25 \text{ }^\circ\text{C}$; d–e. conidiophores and chains of conidia; f–g. detail of conidial ornamentation. — Scale bars: a–c = 10 mm; d–e = 5 μm ; f–g = 1 μm .



Fig. 5 *Cladosporium crousii* CBS 140686. a–c. Colonies on (a) PDA, (b) SNA and (c) OA after 14 d at 25 °C; d–e. conidiophores and chains of conidia; f. conidia. — Scale bars: a–c = 10 mm, d–f = 5 µm.

Notes — *Cladosporium anthropophilum* is probably a common saprobic fungus, as determined by the number of isolates evaluated, and can also represent a clinically relevant fungus, being the second most prevalent species identified in a set of clinical isolates from the USA after *C. halotolerans* (Sandoval-Denis et al. 2015). The new taxon is morphologically similar to *C. cladosporioides* and *C. pseudocladosporioides*, but phylogenetically distant. Although the three species are difficult to separate morphologically, *C. anthropophilum* mainly differs by its longer (up to 550 µm) conidiophores and oval to ellipsoidal terminal conidia (3.5–9 µm long) showing a fine reticulation under SEM. The conidiophores of *C. cladosporioides* and *C. pseudocladosporioides* are 10–250 µm and 15–155 µm long, respectively, and their terminal conidia are subglobose to limoniform ((3–)4–8(–11) µm long) and with a irregularly reticulate or striped wall in the former, and obovoid to ellipsoidal (3–5.5 µm long) and smooth-walled or almost so in the latter species (De Vries 1952, Bensch et al. 2012). *Cladosporium anthropophilum* also resembles *C. tenuissimum*, a species previously described as human opportunistic pathogen (De Hoog et al. 2011). However both are genetically well differentiated (99.3 %, 87.7 % and 89.9 % similarity for ITS, *tef1* and *actA*, respectively) and, morphologically, *C. anthropophilum* shows longer terminal conidia (3.5–9 µm long (av. (± SD) 5.6 (± 1.2)) vs (2–)2.5–5(–6) µm long (av. (± SD) 3.7 (± 1.0)) in *C. tenuissimum*) and shorter intercalary conidia (4.5–11 µm long (av. (± SD) 6.9 (± 1.8)) vs 4–12(–17) µm long (av. (± SD) 8.1 (± 2.7)) in *C. tenuissimum*) (Bensch et al. 2012).

Cladosporium crousii Sandoval-Denis, Cano & Guarro, *sp. nov.* — MycoBank MB815341; Fig. 5

Etymology. In honour of Pedro W. Crous for his extensive work on *Cladosporium*.

Colonies on OA attaining 47–50 mm diam after 14 d at 25 °C, olive to dark green (3F8/30F8), flat, velvety to granular, aerial mycelium scarce, margin fimbriate and with abundant submerged mycelia; reverse olive to dark green (3F8/30F8) to black, without diffusible pigment. On PDA attaining 73–77 mm diam after 14 d at 25 °C, olive brown (4E3/E6), radially folded, velvety or granular with floccose centre and regular margin; reverse at first dark brown (7F8) turning black. On SNA reaching 39–41 mm after 14 d at 25 °C, olive brown (4D5/F8), flat, velvety with floccose centre, margin fimbriate and with abundant

submerged mycelium; reverse black. *Mycelium* superficial and immersed, composed of septate, branched, 2.5–3.5 µm wide, subhyaline hyphae, with slightly roughened walls. *Conidiophores* erect, cylindrical, septate, usually unbranched or sparingly branched, up to 230 µm long, 2–3.5 µm wide, pale green-brown, smooth-walled. *Conidiogenous cells* terminal and intercalary, cylindrical, sometimes geniculate toward the apex, 11–23 × 2.5–4 µm, bearing 1–4 conidiogenous loci of 1.5–2 µm diam, protuberant, black and refringent. *Ramoconidia* 0–1-septate, subcylindrical to cylindrical, 19–39 × 2–3 µm, pale brown, smooth. *Conidia* forming long branched chains with up to seven conidia in the terminal unbranched part of the chain, subhyaline, smooth, with protuberant, thickened and darkened conidial hila; small terminal conidia aseptate, ellipsoidal to subcylindrical, with a central constriction, 7–9 × 2–2.5 µm (av. (± SD) 7.8 (± 0.7) × 2.2 (± 0.2)); intercalary conidia aseptate, ellipsoidal to cylindrical, slightly curved, aseptate, 9–10 × 2–3 µm (av. (± SD) 9.5 (± 0.5) × 2.3 (± 0.4)); secondary ramoconidia 0–1-septate, cylindrical, 9.5–24 × 2.5–3.5 µm (av. (± SD) 15.7 (± 4.4) × 2.8 (± 0.3)).

Cardinal temperature for growth — Optimum 25 °C, maximum 30 °C, minimum 15 °C.

Specimen examined. USA, South Carolina, from human bronchoalveolar lavage fluid, May 2008, D.A. Sutton (holotype CBS H-22385, culture ex-type CBS 140686 = UTSHC DI-13-247 = FMR 13360).

Notes — *Cladosporium crousii* is closely related to *C. gamsianum*, but morphologically they are clearly differentiated. The first species is characterised by longer (up to 230 µm long) and pale coloured conidiophores with unthickened walls, and longer ellipsoidal terminal conidia (7–9 µm long). In contrast, *C. gamsianum* exhibits dark brown and thick-walled conidiophores of 10–146 µm long, and obovoid terminal conidia of 3–6 µm long (Bensch et al. 2010).

Cladosporium flavovirens Sandoval-Denis, Gené & Guarro, *sp. nov.* — MycoBank MB814508; Fig. 6

Etymology. From Latin *flavus* 'yellow' and *virens* 'green', referring to the colony colour on OA.

Colonies on OA attaining 53–55 mm diam after 14 d at 25 °C, olive yellow to olive (2D8/3F8) with olive grey to olive (2F2/E2) patches, flat, velvety to floccose, margin fimbriate and with abundant submerged mycelium; reverse olive yellow to olive



Fig. 6 *Cladosporium flavovirens* CBS 140462. a–c. Colonies on (a) PDA, (b) SNA and (c) OA after 14 d at 25 °C; d, e. conidiophores and chains of conidia; f. conidia. — Scale bars: a–c = 10 mm, d–f = 5 µm.

(2D8/3F8) to black, without diffusible pigment. On PDA attaining 63–65 mm diam after 14 d at 25 °C, obverse and reverse green-grey to dark green (30F2/F8), flat or umbonate and radially folded, velvety, with regular margin. On SNA reaching from 30–32 mm after 14 d at 25 °C, olive to olive brown (2E8/3E8), flat, velvety to granular, margin slightly irregular and with abundant submerged mycelium; reverse olive yellow (2D8) to black. *Mycelium* superficial and immersed composed of septate, branched, 2–3 µm wide, subhyaline to pale green-brown, rough- and thick-walled hyphae, with abundant anastomoses. *Conidiophores* erect, cylindrical, sometimes geniculate, non-nodulose, septate, simple or branched, up to 170 µm long, 4–5 µm wide, medium green-brown, slightly roughened to verruculose, with thick and refractive walls. *Conidiogenous cells* terminal or intercalary, subcylindrical or cylindrical, 15–54 × 3–5 µm, bearing up to four conidiogenous loci of 1–2 µm diam, darkened and refringent. *Ramoconidia* 0–1-septate, subcylindrical to cylindrical, often geniculate, 27–75 × 3–4 µm, smooth or finely verruculose. *Conidia* forming branched chains with up to five conidia in the terminal unbranched part,

pale green-brown, smooth- and thick-walled, with protuberant and darkened conidial hila; small terminal conidia aseptate, obovoidal to short ellipsoidal, 5–7 × 2.5–3 µm (av. (± SD) 5.9 (± 0.6) × 2.9 (± 0.2)); intercalary conidia aseptate, ellipsoidal, 7–10 × 3–3.5 µm (av. (± SD) 8.3 (± 0.9) × 3.2 (± 0.2)); secondary ramoconidia 0–2-septate, ellipsoidal to cylindrical, 9–30 × 3.5–4 µm (av. (± SD) 16.2 (± 6.7) × 3.8 (± 0.3)).

Cardinal temperature for growth — Optimum 25 °C, maximum 35 °C, minimum 15 °C.

Specimen examined. USA, Florida, from human toenail, Nov. 2006, D.A. Sutton (holotype CBS H-22326, culture ex-type CBS 140462 = UTHSC DI-13-273 = FMR 13386).

Notes — *Cladosporium flavovirens* is morphologically and phylogenetically related to *C. flabelliforme*. However, the new species is genetically well differentiated (99.8 %, 80.9 % and 81.8 % sequence similarity for ITS, *tef1* and *actA*, respectively) and produces somewhat longer secondary ramoconidia (up to 30 µm) which are often septate, in contrast to the aseptate secondary ramoconidia of *C. flabelliforme* which are up to 27 µm long (Bensch et al. 2012).



Fig. 7 *Cladosporium floccosum* CBS 140463. a–c. Colonies on (a) PDA, (b) SNA and (c) OA after 14 d at 25 °C; d–e. conidiophores and conidia; f. chain of conidia. — Scale bars: a–c = 10 mm, d–f = 5 µm.

Cladosporium floccosum Sandoval-Denis, Cano & Guarro, *sp. nov.* — MycoBank MB814509; Fig. 7

Etymology. From Latin *floccosus* 'spotted with small tufts', referring to the macroscopic characteristics of the colony.

Colonies on OA reaching 24–27 mm after 14 d at 25 °C, grey-beige to olive brown (4C1/F4), slightly umbonate and radially folded, velvety to dusty with regular margins; reverse olive brown (4D4/F4), without diffusible pigments. On PDA attaining 47–50 mm diam after 14 d at 25 °C, grey-green to dark green (30E5/F7), flat to umbonate and slightly folded, velvety with white cottony centre and regular margin; reverse olive brown (4D8/E8) with black patches. On SNA reaching 15–20 mm after 14 d at 25 °C, olive brown (4D2/F4), flat, velvety to floccose with abundant grey aerial mycelium, margin lobate and fimbriate with abundant submerged mycelium; reverse olive brown to dull green (4E4/30E4). *Mycelium* superficial and immersed composed of septate, branched, 1.5–4.5 µm wide, subhyaline to pale brown, verruculose and thin-walled hyphae. *Conidiophores* erect, flexuous, subcylindrical, distinctly geniculate, septate, mostly unbranched, up to 100 µm long, 4–5 µm wide, pale to medium olivaceous brown, smooth to slightly roughened, with thickened, darkened and refractive walls. *Conidiogenous cells* terminal, cylindrical, nodulose, 16–24 × 3–5 µm, smooth and thick-walled, bearing up to three conspicuous, refractive, slightly darkened conidiogenous loci of 1.5–2.5 µm diam. *Ramoconidia* not observed. *Conidia* forming unbranched chains with up to three conidia, pale brown, echinulate, with protuberant and darkened conidial hila; small terminal conidia 0–1-septate, sometimes slightly constricted at septa, obovoidal to ovoidal, 8–12.5 × 6–8.5 µm (av. (± SD) 10.7 (± 1.8) × 6.8 (± 0.9)); intercalary conidia 0–1-septate, ellipsoidal, 12–15 × 6–8.5 µm (av. (± SD) 13.7 (± 1.0) × 7.5 (± 0.8)); secondary ramoconidia not observed.

Cardinal temperature for growth — Optimum 25 °C, maximum 30 °C, minimum 15 °C.

Specimen examined. USA, Minnesota, from human ethmoid sinus, Sept. 2010, D.A. Sutton (holotype CBS H-22327, culture ex-type CBS 140463 = UTHSC DI-13-212 = FMR 13325).

Notes — *Cladosporium floccosum* is morphologically similar to *C. sinuosum*, which is also its closest phylogenetic relative; both species have distinctly geniculate conidiophores and do not form ramoconidia. However, *C. floccosum* has considerably smaller (up to 100 µm long) and rarely branched conidiophores

and slightly shorter terminal conidia (up to 12.5 µm long) respect to those of *C. sinuosum*, which has conidiophores up to 380 µm long and terminal conidia up to 15 µm long (Schubert et al. 2007, Bensch et al. 2015).

Cladosporium subcinereum Sandoval-Denis, Deanna A. Sutton & Gené, *sp. nov.* — MycoBank MB814511; Fig. 8

Etymology. From Latin *subcinereus* 'somewhat grey', referring to the colony colour.

Colonies on OA reaching 29–32 mm after 14 d at 25 °C, yellow-grey to olive grey (3B2/E2), flat, velvety to cottony, with regular margin, abundant crystalline exudates occasionally present; reverse yellow-grey to olive grey (3B2/E2) to black. On PDA attaining 34–37 mm diam after 14 d at 25 °C, yellow-grey to olive (3B2/F8), flat to radially folded, velvety to floccose, with regular margin; reverse dark green (30F8) to black. On SNA reaching 14–16 mm after 14 d at 25 °C, obverse and reverse white to olive (3A1/E3), flat, velvety to cottony, with regular margin. *Mycelium* superficial and immersed, composed of branched, septate, 2–5 µm wide, subhyaline hyphae with smooth or minutely verruculose and unthickened walls. *Conidiophores* erect, flexuous, geniculate and nodulose, septate, simple or branched, up to 140 µm long, 4–6 µm wide, pale to medium-brown, smooth to verruculose and thick-walled. *Conidiogenous cells* terminal, subcylindrical, nodulose, geniculate, 16–38 × 4–6 µm, thick-walled, bearing up to three conidiogenous loci of 2–3 µm diam, protuberant, darkened and refractive. *Ramoconidia* rarely formed, 0–2 septate, cylindrical, nodulose, 19–59 × 3–6 µm, pale brown, finely roughened. *Conidia* in branched chains, with up to three conidia in the terminal unbranched part, pale brown, echinulate, muricate to pustulate under SEM and thick-walled, with protuberant and not darkened conidial hila; small terminal conidia 0–1-septate, globose to subglobose, 5–7 × 4.5–6.5 µm (av. (± SD) 5.6 (± 0.7) × 5.3 (± 0.6)); intercalary conidia 0–1-septate, subglobose, obovoidal to ellipsoidal, 6–10 × 5–6.5 µm (av. (± SD) 8.9 (± 1.4) × 5.9 (± 0.6)); secondary ramoconidia 0–2-septate, sometimes constricted at septum, ellipsoidal to subcylindrical, often inflated at the apex, 8–27 × 4–7 µm (av. (± SD) 16.3 (± 5.6) × 5.0 (± 0.8)).

Cardinal temperature for growth — Optimum 25 °C, maximum 30 °C, minimum 15 °C.

Specimen examined. USA, Montana, from human sputum, Sept. 2007, D.A. Sutton (holotype CBS H-22329, culture ex-type CBS 140465 = UTHSC DI-13-257 = FMR 13370).

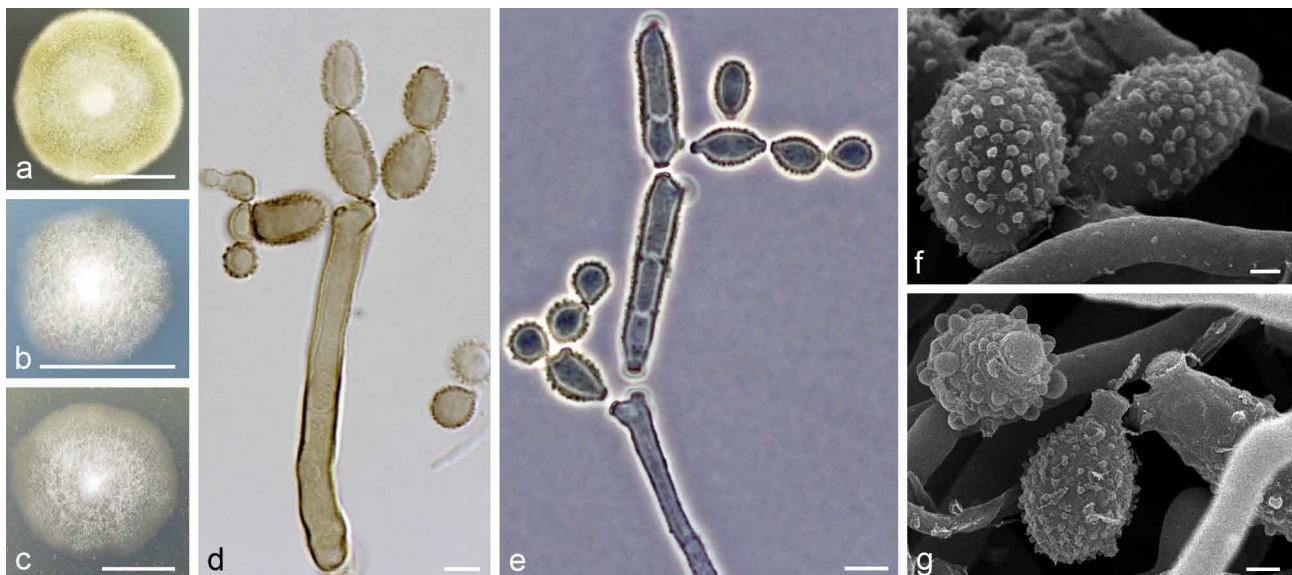


Fig. 8 *Cladosporium subcinereum* CBS 140465. a–c. Colonies on (a) PDA, (b) SNA and (c) OA after 14 d at 25 °C; d–e. conidiophores and chains of conidia; f–g. detail of conidial ornamentation. — Scale bars: a–c = 10 mm; d–e = 5 µm; f–g = 1 µm.

Notes — This species is phylogenetically related to *C. angustitherbarum* and *C. variabile*. However, *C. angustitherbarum* produces shorter and narrower conidiophores (up to 60 µm long and 4 µm wide) and does not form ramoconidia, while *C. variabile* produces multiseptate ramoconidia and long chains of broadly ellipsoidal conidia with a fine granulate ornamentation under SEM (De Vries 1952, Bensch et al. 2012). In *C. subcinereum* the ramoconidia are rarely formed and when present they are 0–2-septate, and its conidia are subglobose, obovoidal to ellipsoidal, exhibiting a much prominent muricate to pustulate ornamentation under SEM. *Cladosporium herbaroides* and *C. herbarum* are also morphologically similar to *C. subcinereum*, but they can be mainly differentiated by having larger/longer conidia (3–33 × (2–)3–6(–7) µm and 10–26(–35) × 2–3.5 µm respect to the two types of conidia described in *C. herbaroides*, and 4–10 × 3–5(–6) µm in *C. herbarum*) (Schubert et al. 2007, Bensch et al. 2012).

Cladosporium succulentum Sandoval-Denis, Deanna A. Sutton & Cano, *sp. nov.* — MycoBank MB814512; Fig. 9

Etymology. From Latin *succo* 'juice' and *ulentum* 'full of', referring to the abundant production of exudates on PDA.

Colonies on OA reaching 23–25 mm after 14 d at 25 °C, dark green (30F3/F8), flat, granular to floccose, with fimbriate margin; reverse olive to dark green (3F8/30F4) turning black. On PDA attaining 28–35 mm diam after 14 d at 25 °C, olive brown (4F4/F8), flat, velvety to granular, with regular margin, producing abundant dark green exudates after 20–25 d; reverse black-blue (20F8) to black. On SNA reaching 27–32 mm after 14 d at 25 °C, obverse and reverse olive to olive brown (3E8/4E8), flat, downy to granular, with regular margin. *Mycelium* superficial and immersed, composed of septate, branched, 1.5–3.5 µm wide, subhyaline, smooth- and thin-walled hyphae. *Conidiophores* erect, straight or flexuous, septate, highly branched, up to 190 µm long, 2.5–4 µm wide, subhyaline, pale green-brown, smooth to finely roughened and thin-walled. *Conidiogenous cells* terminal and intercalary, cylindrical, 13–30 × 2–4 µm, thin-walled, bearing 2–6 conidiogenous loci of 1–2.5 µm diam, darkened and refractive. *Ramoconidia* 0–1-septate, cylindrical to subcylindrical, flexuous, 20–36 × 2–4 µm, pale green-brown, smooth to finely roughened. *Conidia* in branched chains, with up to six conidia in the terminal unbranched part, aseptate, pale green-brown, smooth- and thin-walled, with protuberant and darkened

conidial hila; small terminal conidia oval to short clavate, 3–4 × 2–3 µm (av. (± SD) 3.6 (± 0.4) × 2.2 (± 0.4)), aseptate, with conspicuous and darkened conidial scars; intercalary conidia ovoid to limoniform, 4–6 × 2–3 µm (av. (± SD) 5.1 (± 0.6) × 2.3 (± 0.4)), with protuberant and not darkened conidial scars; secondary ramoconidia ellipsoidal to subcylindrical, 5–10 × 2–4.5 µm (av. (± SD) 8.2 (± 1.5) × 2.5 (± 0.4)).

Cardinal temperature for growth — Optimum 25 °C, maximum 35 °C, minimum 15 °C.

Specimen examined. USA, Florida, from a dolphin bronchus, July 2007, D.A. Sutton (holotype CBS H-22330, culture ex-type CBS 140466 = UTHSC DI-13-262 = FMR 13375).

Notes — *Cladosporium succulentum* is morphologically similar but genetically distant to *C. halotolerans* (98.4 %, 66.5 % and 79.8 % sequence similarity for ITS, *tef1* and *actA*, respectively) and *C. sphaerospermum* (97.5 %, 72.7 % and 83.8 % sequence similarity for ITS, *tef1* and *actA*, respectively). The latter two species can be differentiated from *C. succulentum* by having a maximum growth temperature at 30 °C (Zalar et al. 2007, Bensch et al. 2012) (35 °C in *C. succulentum*), and in the length and number of septa of their ramoconidia. In *C. halotolerans* and *C. sphaerospermum* these are 15–37 µm and (11.5–)20.5–40(–48) µm long, respectively, and they have up to five septa (Zalar et al. 2007, Bensch et al. 2012), while in *C. succulentum* the ramoconidia are 20–36 µm long with 0–1 septa. The phylogenetically closest species to *C. succulentum* are *C. fusiforme* and *C. velox* (sequence similarities less than 99.8 %, 80.7 % and 86.6 % for ITS, *tef1* and *actA*, respectively), but the new species can be differentiated by the abundant production of ramoconidia and by its oval to short clavate terminal conidia. Ramoconidia in *C. fusiforme* and *C. velox* are rarely formed and their terminal conidia are obovoid to fusiform in the first species and globose to ovoid in the latter one (Zalar et al. 2007).

Cladosporium tuberosum Sandoval-Denis, Cano & Wiederhold, *sp. nov.* — MycoBank MB815339; Fig. 10

Etymology. From Latin *tuberōsus* 'lumpy' (full of protuberances), because of the nodulose shape of its conidiophores.

Colonies on OA reaching 23–26 mm after 14 d at 25 °C, olive brown (4D5/F7), flat, velvety to floccose, margin regular and with abundant submerged mycelium; reverse olive brown (4D5/

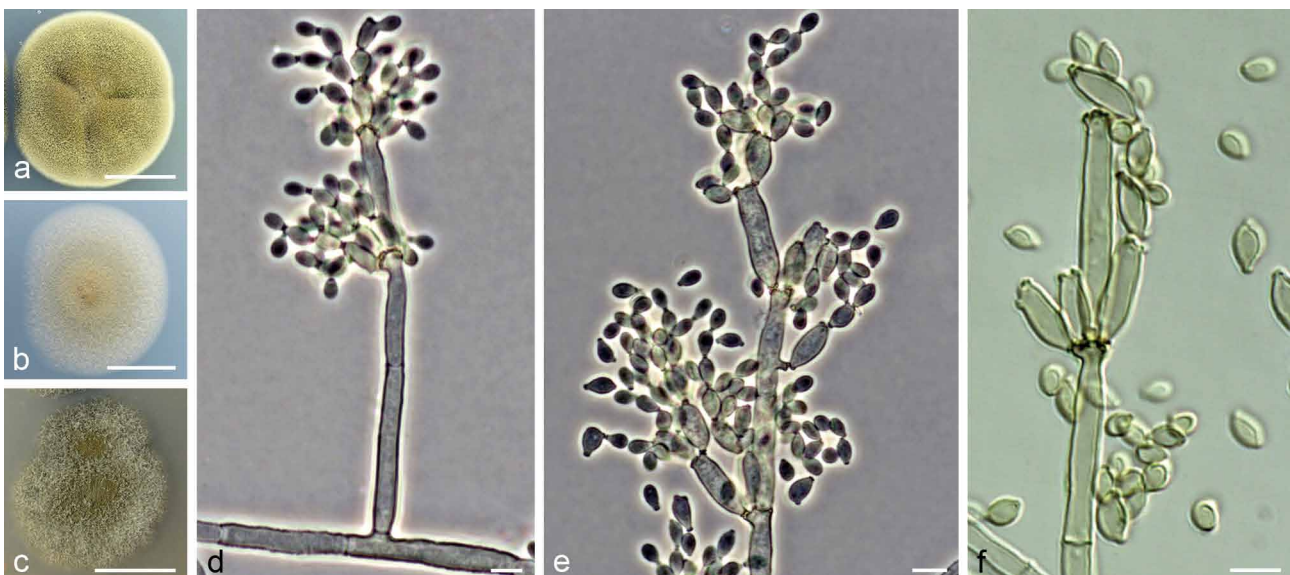


Fig. 9 *Cladosporium succulentum* CBS 140466. a–c. Colonies on (a) PDA, (b) SNA and (c) OA after 14 d at 25 °C; d–e. conidiophores and chains of conidia; f. conidia. — Scale bars: a–c = 10 mm, d–f = 5 µm.

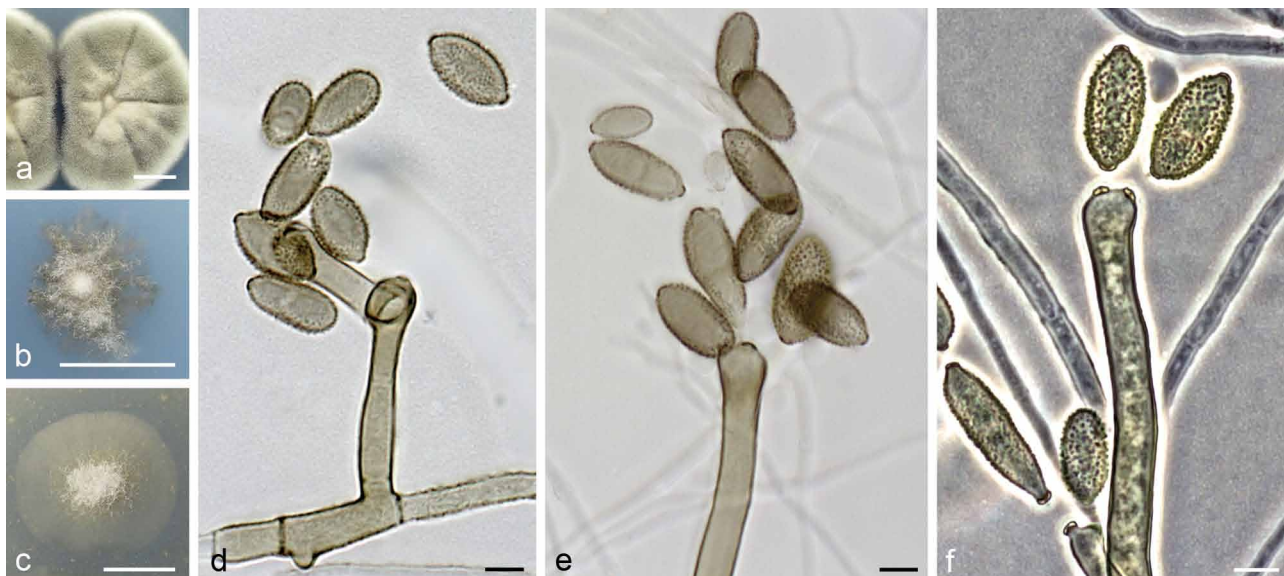


Fig. 10 *Cladosporium tuberosum* CBS 140693. a–c. Colonies on (a) PDA, (b) SNA and (c) OA after 14 d at 25 °C; d–f. conidiophores, conidiogenous cells and conidia. — Scale bars: a–c = 10 mm, d–f = 5 µm.

F7) to black. On PDA attaining 44–50 mm diam after 14 d at 25 °C, dull green to dark green (30E4/F7), flat and radially folded, velvety to dusty, margin regular and white; reverse olive brown (4E8) to black. On SNA reaching 13–20 mm after 14 d at 25 °C, olive brown (4E4/F4), flat, velvety with cottony patches, margin irregular and with abundant submerged mycelium; reverse olive brown (4E4/F4) to black. *Mycelium* superficial and immersed, composed of septate, branched, 3–4.5 µm wide, subhyaline, smooth and thin-walled hyphae. *Conidiophores* erect, flexuous, cylindrical-oblong, nodulose, or bent once or several times being geniculate, laterally swollen, septate, unbranched or rarely laterally branched, up to 390 µm long, 5–6 µm wide, pale brown to olivaceous brown, smooth and thick-walled. *Conidiogenous cells* terminal or intercalary, cylindrical or subnodulose, 15–38 × 4–5.5 µm, proliferating sympodially, forming lateral shoulders, bearing 1–2 conidiogenous loci at each shoulder, loci protuberant, 2–2.5 µm diam, darkened and refringent. *Ramoconidia* not observed. *Conidia* in branched chains, with up to three conidia in the terminal part, 0–1-septate, green-brown to yellow-brown, verrucose to echinulate and thick-walled with protuberant and darkened conidial hila; small terminal conidia oval, obovate or short ellipsoidal, 8–14 × 7–9 µm (av. (± SD) 13.1 (± 0.7) × 8.0 (± 0.8)); intercalary conidia ellipsoidal to limoniform, 11–16 × 7–10 µm (av. (± SD) 13.9 (± 1.7) × 8.5 (± 0.9)); secondary ramoconidia ellipsoidal to subcylindrical, 14–18 × 6–10 µm (av. (± SD) 16.1 (± 1.2) × 7.1 (± 1.3)).

Cardinal temperature for growth — Optimum 25 °C, maximum 30 °C, minimum 5 °C.

Specimens examined. USA, Florida, from human nasal biopsy, Dec. 2009, D.A. Sutton (holotype CBS H-22387, culture ex-type CBS 140693 = UTHSC DI-13-217 = FMR 13330); Washington, from human foot, Oct. 2009, D.A. Sutton, UTHSC DI-13-219 = FMR 13332.

Notes — This species is represented by two isolates of human clinical origin which cluster in a lineage clearly differentiated and together with *C. basinflatum* group in a position basal to the remaining species of the *C. herbarum* complex (Fig. 1). Despite this basal position, it shows the typical morphological features of the species of the complex. *Cladosporium tuberosum* morphologically resembles *C. sinuosum* in the production of short conidial chains and the absence of ramoconidia (Schubert et al. 2007). However, in *C. tuberosum* the conidiophores are not as geniculate as in *C. sinuosum* and the conidia are

always grouped forming short chains, while the conidia in *C. sinuosum* are often solitary although short chains can be also present (Bensch et al. 2015). In addition, *C. tuberosum* exhibits a faster growth rate on PDA, forming colonies almost black at the obverse rather than the olivaceous grey to pale olivaceous grey colonies of *C. sinuosum* (Bensch et al. 2015).

Cladosporium xanthochromaticum Sandoval-Denis, Gené & Cano, *sp. nov.* — MycoBank MB815340; Fig. 11

Etymology. From Greek *xanthós* (ξανθός) 'yellow' and *chrōma* (χρώμα) 'colour', referring to the production of a yellow diffusible pigment on PDA.

Colonies on OA reaching 40–50 mm after 14 d at 25 °C, obverse and reverse olive brown to grey-green (4F8/30E7), flat, granular, radiate, margin regular and with abundant submerged mycelium; diffusible pigment absent. On PDA attaining 60–67 mm diam after 14 d at 25 °C, olive brown (4E8/F8), flat or folded at centre, dusty or granular, velvety toward the periphery, margin regular, white to yellow, and with abundant submerged mycelium; reverse black, with a light yellow to grey-yellow (2A5/B5) diffusible pigment. On SNA reaching 35–37 mm after 14 d at 25 °C, olive brown (4E5/E8), flat, velvety to granular, radiate, margin regular and with abundant submerged mycelium; reverse olive brown (4E5/E8) to black, without diffusible pigment. *Mycelium* superficial and immersed, composed of septate, branched, 1.5–3 µm wide, pale brown, smooth and thin-walled hyphae. *Conidiophores* erect, flexuous, cylindrical, non-nodulose, septate, simple or branched typically immediately before a septum, up to 210 µm long, 2–4 µm wide, pale brown, smooth and thin-walled. *Conidiogenous cells* terminal, cylindrical, sometimes geniculate, 12–32 × 3–4 µm, bearing up to three conidiogenous loci of 1–1.5 µm diam, darkened and refringent. *Ramoconidia* aseptate, subcylindrical to cylindrical, 18–36 × 2–3.5 µm, pale brown, smooth or finely roughened. *Conidia* forming branched chains, with up to four conidia in the terminal unbranched part, pale green-brown, smooth- and thin-walled, with protuberant, not darkened conidial hila; small terminal conidia aseptate, obovate to short ellipsoidal 4–5 × 2–2.5 µm (av. (± SD) 4.3 (± 0.3) × 2.2 (± 0.2)); intercalary conidia aseptate, ellipsoidal to limoniform, 5–7 × 2.5–3.5 µm (av. (± SD) 5.8 (± 0.6) × 2.6 (± 0.3)); secondary ramoconidia 0–1-septate, subcylindrical, sometimes slightly constricted at the centre, 10–28 × 3–4 µm (av. (± SD) 15.7 (± 5.2) × 3.3 (± 0.4)).



Fig. 11 *Cladosporium xantochromaticum* CBS 140691. a–c. Colonies on (a) PDA, (b) SNA and (c) OA after 14 d at 25 °C; d–f. conidiophores and chains of conidia. — Scale bars: a–c = 10 mm, d–f = 5 µm.

Cardinal temperature for growth — Optimum 20 °C, maximum 30 °C, minimum 5 °C.

Specimen examined. USA, Texas, from human bronchoalveolar lavage fluid, Sept. 2010, *D.A. Sutton* (holotype CBS H-22388, culture ex-type CBS 140691 = UTHSC DI-13-211 = FMR 13324).

Notes — This species belongs to the *C. cladosporioides* species complex and clusters with *C. angulosum* and *C. perangustum*, forming a basal lineage characterised by narrow conidia and slightly roughened conidiophores and conidia. Bensch et al. (2012) considered *C. perangustum* a species with considerable genetic variability but morphologically uniform. The new species, however, is genetically (99.1 %, 75 % and 89.1 % sequence similarity for ITS, *tef1* and *actA*, respectively) and phenotypically well differentiated from *C. perangustum*. *Cladosporium xantochromaticum* has smaller ramoconidia (18–36 × 2–3.5 µm) and smooth-walled conidiophores, while in *C. perangustum* the ramoconidia are 25–45 × 2.5–3(–4.5) µm and the conidiophores are more or less rough-walled especially towards the base, asperulate-verruculose, and smooth to almost so at the apex (Bensch et al. 2010).

DISCUSSION

The genus *Cladosporium* has been extensively reviewed in recent years in efforts to clarify the phylogeny and taxonomic structure of its species and allied fungi, and has resulted in a modern redefinition of the genus (Crous et al. 2007a, b, Schubert et al. 2007, Zalar et al. 2007, Bensch et al. 2010, 2012, 2015). However, until recently, no attempt had been made to study the impact of these new approaches in the diversity of *Cladosporium* species of clinical interest.

In a previous study, we demonstrated that the species diversity of *Cladosporium* associated to clinical samples was underestimated (Sandoval-Denis et al. 2015). Furthermore, we found that species traditionally considered clinically relevant, identified by phenotypic criteria alone, were among the least represented. In fact, several morphologically similar sibling species were found to be more prevalent, including putative new taxa (De Hoog et al. 2011, Sandoval-Denis et al. 2015). Those previously undescribed lineages are characterised here using both molecular and phenotypic criteria and resulting in the proposal of 10 new *Cladosporium* species. Sampling for this study was limited to

isolates from the USA, and a wider sampling area is expected to provide a more precise reflection of the real distribution of these new species around the world.

The new species proposed here have been mostly isolated from human respiratory samples, which might be explained by the fact that *Cladosporium* conidia are easily dispersed by air (David 1997). However, the clinical relevance of the species of this genus, at least to produce invasive disease, has been questioned by their inability to grow at 37 °C (De Hoog et al. 2011, Sandoval-Denis et al. 2015), which was also confirmed with the new species. Nevertheless, despite the large number of species involved in this study, some of them were represented by numerous isolates, such as *C. anthropophilum*, which could be linked to a certain degree of specialisation towards colonisation of the human respiratory tract.

Within a given species complex, the different species of *Cladosporium* are often difficult to identify from morphological characters alone. However, some key differential features have been identified and have been detailed in a series of monographic papers (Schubert et al. 2007, Zalar et al. 2007, Bensch et al. 2012). We have followed the criteria from those papers in order to distinguish potentially new species from their closest phylogenetic and morphological relatives. As is usual in this genus, no sexual morphs were observed in any of them. In fact, sexual structures have been observed in vitro in only eight accepted species of *Cladosporium* (Bensch et al. 2012). Among the species described here, the most relevant differential morphological traits were the presence of ramoconidia, the length, complexity and ornamentation of the conidiophores, intercalary and terminal conidia. However, given the overlapping of these features, and the need for standardisation using special culture media and scanning electron microscopy procedures, the use of a molecular approach should be mandatory for correct identification of the species in this complex fungal group. With these studies, we have considerably expanded the list of *Cladosporium* species as potential human opportunistic fungi, which makes their identification difficult given their high morphological similarity (De Hoog et al. 2015). That said, distinguishing morphologically similar species of *Cladosporium* seems not to be as relevant from a clinical perspective because the in vitro antifungal response does not differ considerably between species of the same species complex (Sandoval-Denis et al. 2015). In contrast, in vitro antifungal susceptibilities do differ between

species complexes, with the *C. sphaerospermum* complex showing higher inhibitory concentrations against amphotericin B, azoles and caspofungin (Sandoval-Denis et al. 2015).

Our phylogenetic studies agree with previous revisions of the genus (Schubert et al. 2007, Zalar et al. 2007, Bensch et al. 2012). The most phylogenetic informative markers were *actA* and *tef1*, while ITS sequences were usually identical for species of the same complex as previously reported by Bensch et al. (2010). Although most of the taxa in the present study are consistently separated in terms of their genetic and morphological differences, a high genetic variability was observed in the clades representing the new species *C. anthropophilum* and *C. tuberosum*, as well in clades representing well-known species, i.e. *C. allacinum*, *C. perangustum*, *C. pseudocladosporioides*, *C. sinuosum* and *C. tenuissimum*. This might indicate an ongoing process of active divergence and speciation as it has been described for other fungi, which demands further study (Gao et al. 2015).

Several studies have shown a higher number of species in the *C. cladosporioides* complex (Bensch et al. 2010, 2012, 2015) and our results agree with them. Of the taxa that were newly described here, six species belonged to the *C. cladosporioides* complex, whereas only three and one, belonged to the *C. herbarum* and *C. sphaerospermum* species complexes, respectively. The *C. cladosporioides* complex is phylogenetically well defined and includes a large group of species characterised by unbranched or branched, almost cylindrical conidiophores, bearing ovoid to ellipsoidal intercalary and terminal conidia, smooth or rarely showing a fine ornamentation (Bensch et al. 2012). Although most of the known species of this complex do not tolerate high temperatures, our results showed that in the *C. cladosporioides* complex at least three of the new species (*C. angulosum*, *C. anthropophilum* and *C. flavovirens*), as well as several isolates identified as *C. pseudocladosporioides* are able to grow at 35 °C, which might explain their relatively high rate of isolation from homoeothermic hosts.

The *C. herbarum* species complex is also phylogenetically and morphologically well defined and contains a less diverse group of species characterised by nodulose conidiophores, bearing distinctly ornamented, globose to subglobose terminal conidia (Schubert et al. 2007). It is interesting that none of the new species of this complex were able to grow at temperatures higher than 30 °C. In contrast, the only new species described in the *C. sphaerospermum* complex was able to grow and sporulate, although poorly, at 35 °C. The members of the *C. sphaerospermum* species complex are morphologically homogeneous, characterised by conidiophores that are usually branched and lacking nodose inflations, producing both smooth-walled and ornamented conidia (Zalar et al. 2007). Most species currently included in this group exhibit a high degree of osmotic tolerance, but are unable to grow at temperatures exceeding 30 °C (Zalar et al. 2007, Bensch et al. 2012). However, it has been suggested previously that this complex does not represent a monophyletic group, but most likely represents various species complexes instead (Bensch et al. 2012). This was also suggested by our phylogenetic results which revealed that the species currently included in the *C. sphaerospermum* complex consistently grouped together as a polyphyletic arrangement in both combined and individual analyses, forming at least five different lineages with high statistical support and important genetic differences. The new species *C. succulentum* grouped in a lineage with *C. aciculare*, *C. fusiforme*, *C. longissimum*, *C. sphaerospermum* and *C. velox*. However, as previously described, there are no phenotypic differences to discriminate among these closely related taxa that would warrant the establishment of additional species complexes to accommodate these lineages (Zalar et al. 2007, Bensch et al. 2015).

In this study, the analysis of isolates from human and animal clinical specimens has allowed us to considerably increase the known diversity of species for the genus, expanding substantially the spectrum of species of potential clinical interest. Further studies are needed to fully understand the ecology and importance of these new species in the aetiology of infections in warm-blooded hosts.

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