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ARTICLE

New endophytic *Toxicocladosporium* species from cacti in Brazil, and description of *Neocladosporium* gen. nov.

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Abstract: Brazil harbours a unique ecosystem, the Caatinga, which belongs to the tropical dry forest biome. This region has an important diversity of organisms, and recently several new fungal species have been described from different hosts and substrates within it. During a survey of fungal endophyte diversity from cacti in this forest, we isolated cladosporium-like fungi that were subjected to morphological and multigene phylogenetic analyses including *actA*, ITS, LSU, *rpb2* and *tub2* gene sequences. Based on these analyses we identified two new species belonging to the genus *Toxicocladosporium*, described here as *T. cacti* and *T. immaculatum* spp. nov., isolated from *Pilosocereus gounellei* subsp. *gounellei* and *Melocactus zehntneri*, respectively. To improve the species recognition and assess species diversity in *Toxicocladosporium* we studied all ex-type strains of the genus, for which *actA*, *rpb2* and *tub2* barcodes were also generated. After phylogenetic reconstruction using five loci, we differentiated 13 species in the genus. *Toxicocladosporium velox* and *T. chlamydosporum* are synonymized based on their phylogenetic position and limited number of unique nucleotide differences. Six strains previously assigned to *T. leucadendri*, including the ex-type strain (CBS 131317) of that species, were found to belong to an undescribed genus here named as *Neocladosporium* gen. nov., with *N. leucadendri* comb. nov. as type species. Furthermore, this study proposes the *actA*, ITS, *rpb2* and *tub2* as main phylogenetic loci to recognise *Toxicocladosporium* species.

Key words:
Cladosporiaceae
Endophytic fungi
Multigene phylogeny
Taxonomy

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INTRODUCTION

The genus *Toxicocladosporium* (*Cladosporiaceae*, *Capnodiales*) was described by Crous *et al*. (2007) to accommodate cladosporium-like fungi having distinct "dark, thick-walled conidial and conidiophore septa, and lacking the typical coronate *Cladosporium* scar type". The type species of this genus, *T. irritans*, was isolated from mouldy paint in Suriname and named "*irritans*" because of the production of several volatile metabolites in culture, causing skin irritation when there is exposure to the fungus (Crous *et al*. 2007). After its introduction, several new species were described in the genus, which currently comprises 13 species reported from different host plants in studies from America (Suriname and USA), Africa (Madagascar and South Africa), Asia (China), and Oceania (Australia).

Similar to *Cladosporium*, *Toxicocladosporium* exhibits a widespread distribution and the capacity to colonise distinct substrates and plant families. Almost all species in this genus were described from plant species belonging to the families *Asteraceae*, *Cyperaceae*, *Myrtaceae*, *Pinaceae*, *Proteaceae*, *Rubiaceae*, and *Strelitziaceae* (Crous *et al*. 2009a, 2010a, b, 2011a, 2012a, b, 2013, 2014, Crous & Groenewald 2011), the exception being *T. irritans* and *T. hominis* described from mouldy paint (Crous *et al.* 2007) and a human bronchoalveolar lavage fluid specimen (Crous *et al.* 2016), respectively. In addition to species descriptions in this genus, few reports of isolation of *Toxicocladosporium* species, mainly *T. irritans*, have been published in different countries. For example, studying ancient laid-paper documents of the 17th century in Portugal, Mesquita *et al*. (2009) reported the isolation of *T. irritans*. Similar results were obtained in Italy by Piñar *et*

al. (2015) who used culture-independent molecular methods and scanning electron microscopy (SEM) to verify the fungi colonizing parchment manuscripts, and by Bonadonna *et al.* (2014), who reported *T. irritans* colonising tattoo inks. These reports may show similarities because the first isolation of *T. irritans* was associated with mouldy paint in Suriname (Crous *et al.* 2007). *Toxicocladosporium irritans* was also reported associated with patients having atopic dermatitis in Japan (Zhang *et al.* 2011), and it was isolated from human blood and a fingernail by Sandoval-Denis *et al*. (2015) in the USA. This species was also reported by Cruywagen *et al.* (2015) on baobab trees in southern Africa, and on equipment used in the International Space Station or Space Shuttle in Japan (Satoh *et al*. 2016).

There are also reports from other unusual substrates or hosts, including coffee scale insects in Vietnam (Nhạ *et al.* 2011), the vector of visceral leishmaniasis (*Lutzomyia longipalpis*) in Brazil (McCarthy *et al*. 2011), an unidentified sponge from Korea (Cho *et al*. 2016), patients with seborrheic dermatitis in Japan (Tanaka *et al.* 2014), outdoor dust samples in the USA (Barberán *et al*. 2015), and as a plant pathogen on African olive (*Olea europaea* subsp. *cuspidata*, *Oleaceae*) in Australia (Australian Government Department of Agriculture 2015). *Toxicocladosporium* and *Cladosporium* were also suggested as candidates for fungal structures found in the fossilized extinct aquatic angiosperm *Eorhiza arnoldii* in Canada (Klymiuk *et al.* 2013). These reports show that *Toxicocladosporium* host associations are not specific and may differ from *Cladosporium*, in which species tend to have confined host ranges, but with some exceptions (Bensch *et al*. 2012). *Toxicocladosporium chlamydosporum* and *T. rubrigenum* were, however, described from a single leaf spot of *Eucalyptus camaldulensis* (*Myrtaceae*) growing in Madagascar (Crous *et al.* 2009a). This example demonstrates that specimens from a single host and location can be colonized by genotypes representing different species (Bensch *et al*. 2012). *Toxicocladosporium* species may be recovered from inconspicuous substrates and extreme habitats, showing a lack of environmental preference and an ability to be associated with unusual materials and ecological conditions (McCarthy *et al*. 2011, Nhạ *et al*. 2011, Cho *et al*. 2016, Satoh *et al.* 2016).

Dematiaceous fungi isolated from different plant species in extreme environments generally live as endophytes (Redman *et al.* 2002, Suryanarayanan *et al*. 2011, Loro *et al*. 2012, Sun *et al.* 2012, Knapp *et al*. 2015). Although *Cladosporium* species are widely reported as endophytes (Bensch *et al.* 2012), the closely related genus *Toxicocladosporium* has not previously been reported as endophytic. All presently known associations of *Toxicocladosporium* species with plant material were as an epiphyte, saprobe, or phytopathogen, or with unusual substrates or hosts.

Plants living in dry environments are an important host for fungi with widespread distributions, and have always shown a great diversity of species (Fisher *et al*. 1994, Suryanarayanan *et al.* 2005, Khidir *et al.* 2010, Silva-Hughes *et al*. 2015, Fonseca-García *et al.* 2016). The Caatinga, one of the most important tropical dry forests in Brazil, harbours several cacti that prove to have a great diversity of endophytic fungi (Bezerra *et al.* 2012, 2013, Freire *et al*. 2015). Recently, Bezerra *et al*. (2017) described a new order in the class *Dothideomycetes* for endophytes isolated from the cactus *Tacinga inamoena* collected in the Caatinga.

We studied all ex-type strains of *Toxicocladosporium* species isolated from different substrates and hosts in order to report on the isolation and to describe those we recovered as endophytes from the cacti *Melocactus zehntneri* and *Pilosocereus gounellei* subsp. *gounellei* growing in the Caatinga. Using morphological characters and multigene phylogenetic analyses (*actA*, ITS, LSU, *rpb2* and *tub2*), the genus *Toxicocladosporium* and its respective species were re-evaluated. We aimed to determine the phylogenetic relationship of endophytes from cacti with species of *Toxicocladosporium*, provide an overview of hosts and substrates amongst *Toxicocladosporium* species, and propose new loci to assist with species differentiation in the genus.

MATERIALS AND METHODS

Endophytic fungi from cacti

Endophytic fungi were isolated as described by Bezerra *et al.* (2013) from the cacti *Melocactus zehntneri* and *Pilosocereus gounellei* subsp. *gounellei* growing in the Brazilian tropical dry forest (Caatinga), Catimbau National Park, Buíque municipality, Pernambuco state, Brazil (8º36'35'' S, 37º14'40'' W), and sustainable family farming plots, Itaíba municipality, Pernambuco state, Brazil (9º 08.895' S, 37º 12.069' W). The collections were authorized by the Ministério do Meio Ambiente (MMA)/Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio); permission number: 40331-1/ authentication code 87451826 issued on 4 November, 2013. In addition, 32 isolates selected on the basis of genetic and morphological relatedness with cacti endophytes, were obtained from the collection of the Westerdijk Fungal Biodiversity Institute (formerly CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands) and the CPC collection (collection of P.W. Crous, held at CBS) and included in the analyses (Table 1).

Morphology

Endophytes previously identified as belonging to *Toxicocladosporium* were cultured on malt extract agar (MEA), oatmeal agar (OA), potato dextrose agar (PDA), and synthetic nutrient deficient agar (SNA) (Crous *et al.* 2009c), and incubated at 22 °C under a natural day-night cycle. Macro- and micro-morphological features, and reproductive structures were visualized after 3 wk on MEA, OA, PDA, and/ or SNA culture media. Culture colours were evaluated using the charts of Rayner (1970). Slide preparations were mounted as described by Bensch *et al*. (2012) in clear lactic acid and/ or in Shear's solution. Endophytic strains are deposited in the culture collections of Micoteca URM Prof. Maria Auxiliadora Cavalcanti (Federal University of Pernambuco, Recife, Brazil – www.ufpe.br/micoteca, WCDM 604) and the CBS collection at Westerdijk Fungal Biodiversity Institute (under Material Transfer Agreement – MTA Nº 05/2015/Micoteca URM, issued on 14 April, 2015). Nomenclatural and taxonomic information were deposited in MycoBank (www.mycobank.org) (Crous *et al.* 2004).

DNA extraction, amplification (PCR) and sequencing

Genomic DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) according to the manufacturer's instructions. The primers LR0R and LR5 (Vilgalys & Hester 1990), ITS5 and ITS4 (White *et al.* 1990), ACT-512F and ACT-783R (Carbone & Kohn 1999), Bt2a and Bt2b or Bt10 (Glass & Donaldson 1995) and 5f2 and 7cr (O'Donnell *et al.* 2010) were used to amplify part of the nuclear ribosomal large subunit (LSU) of the rDNA, the ITS region (first and second ITS regions and intervening 5.8S nrDNA), the partial actin gene (*actA*), partial β-tubulin gene (*tub2*), and a fragment of the RNA polymerase second largest subunit gene (*rpb2*) respectively. Amplification and sequencing reactions, sequences analyses, and consensus sequences were performed as described by O'Donnell *et al*. (2010) and Bezerra *et al*. (2017). In addition, 136 DNA sequences representing 57 taxa were retrieved from GenBank and included in the phylogenetic analyses (Table 1).

Phylogenetic analyses

Following blast searches of the NCBI's GenBank nucleotide database for preliminary identifications, an initial backbone tree was constructed using ITS, LSU and *rpb2* sequences from *Cladosporiaceae* (Schubert *et al*. 2007a, b, Zalar *et al.* 2007, Crous *et al*. 2007, 2009b, 2011a, Bensch *et al*. 2010, 2012, 2015) and from the other six families in *Capnodiales* following Quaedvlieg *et al.* (2014) and Videira *et al.* (2016). *Parastagonospora nodorum* (CBS 110109) was used as outgroup. Firstly, the alignments for each locus were performed using the online MAFFT interface (Katoh & Standley 2013) followed by manual adjustments using MEGA v. 7 (Kumar *et al*. 2015). These alignments were used to infer preliminary phylogenetic relationships for *Toxicocladosporium* species in *Cladosporiaceae*.

A second, more inclusive analysis included *actA*, LSU, ITS, *rpb2* and *tub2* sequences derived from ex-type cultures of *Toxicocladosporium* species and endophytes isolated from cacti (Crous *et al.* 2007, 2009a, 2010a, b, 2011a, 2012a, b, 2013, 2014, 2016, Crous & Groenewald 2011). *Neocladosporium leucadendri* (CPC 18315 = CBS 131317), previously published as *Toxicocladosporium leucadendri*, was used as outgroup for that analysis.

Maximum Parsimony analyses (MP) were performed with PAUP v. 4.0b10 (Swofford 2003) and involved 1000 replicates of heuristic search with random addition of sequences. The tree bisection-reconnection option was used, with the branch swapping option set to "best-trees" only. Gaps were treated as missing data and all characters were unordered and given equal weight. The tree length (TL), consistency index (CI), Retention index (RI), and rescaled consistence index (RC) were calculated. Maximum parsimony bootstrap analyses (MP-BS) were performed using 1000 replicates. Maximum likelihood analyses (ML) were performed using RAxML-HPC2 v. 8.2.8 (Stamatakis 2014) on XSEDE in the CIPRES science gateway (http://www.phylo.org/). The robustness of the trees obtained was evaluated according to the level of bootstrap support (ML-BS), with the number of replicates determined automatically by the software. Bayesian analyses (BI) were performed using MrBayes v. 3.2.6 (Huelsenbeck & Ronquist 2001). The program was executed with four Markov chains in two simultaneous runs for 5 M generations with the stopval option on and saving trees every 1000 generations. The analyses were stopped when the two runs converged and the average standard deviation of split frequencies came below 0.01. The 50 % majority-rule consensus tree and the Bayesian posterior probabilities (BPP) were calculated after discarding the first 25 % of saved trees as "burn-in". The best fit evolutionary models were calculated independently for each gene data partition using MrModelTest v. 2.3 (Nylander 2004) following the Akaike information criterion and included in the analyses, in all cases selecting the GTR+I+G model. All resulting trees were plotted using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). All the analyses were first made independently for each locus and visually inspected for topological incongruences between nodes with significant statistical support before being combined into multigene datasets (Mason-Gamer & Kellogg 1996, Wiens 1998). The new sequences generated in this study were deposited in the NCBI's GenBank nucleotide database and the European Nucleotide Archive (Table 1) and the alignments and phylogenetic trees in TreeBASE (Study ID S20701).

RESULTS

In order to verify the relationship of *Toxicocladosporium* with other genera in the family *Cladosporiaceae*, we used ITS, LSU and *rpb2* sequences from representatives of 19 genera from seven families in *Capnodiales*. *Parastagonospora nodorum* (CBS 110109) was used as outgroup. The final combined alignment contained 68 isolates and 1750 characters (ITS: 427, LSU: 621 and *rpb2*: 702) of which 770 were parsimony-informative (ITS: 178, LSU: 161 and *rpb2*: 431), 117 were variable and parsimony-uninformative (ITS: 37, LSU: 59 and *rpb2*: 21), and 848 were constant (ITS: 427, LSU: 621 and *rpb2*: 702). Because of the high degree of sequence conservation, the LSU analysis alone was not able to resolve the generic limits in *Cladosporiaceae*, i.e. the *Toxicocladosporium* species did not form a monophyletic clade but were intermixed with species of *Cladosporium* (data not shown); thus, the combined ITS, LSU, and *rpb2* sequences were more informative when used in a combined alignment. Fig. 1 shows a RaxML tree and node support values obtained using MP, ML and BI analyses. Parsimony analysis resulted in 68 trees (TL = 4735; CI = 0.347; RI = 0.705; RC = 0.245). These analyses show that all *Toxicocladosporium* species cluster together in a clade (MP-BS 100 %, ML-BS 91 %, BPP 0.98) closely related to *Cladosporium*, with the exception of *T. leucadendri*. The ex-type strain of the latter species (CBS 131317) and five other isolates formed a distinct linage phylogenetically, close but unrelated to, the genera *Graphiopsis* and *Verrucocladosporium*, representing a different genus we describe here as *Neocladosporium*, with *N. leucadendri* as the type species.

The second alignment included ITS and LSU sequences from all the available ex-type strains of *Toxicocladosporium* species with *N. leucadendri* as outgroup. To further improve

Table 1. GenBank accession numbers and details of strains used in this study.

Species	Strain/isolate number[1]	Substrate/host (country)	GenBank accession numbers[2]				
			ITS	LSU	*actA*	*rpb2*	*tub2*
Acrodontium crateriforme	CBS 144.33^{T} (ex-type of *Chloridium crateriforme*)	*Tuberculina maxima* (The Netherlands)	FN666565	KX286952		KX288399	
A. luzulae	CBS 841.71^{T}	On leaf of *Carex* sp. (The Netherlands)	KX287273	KX286961		KX288410	
Cercospora beticola	CBS 116456	On *Beta vulgaris* (Italy)	DQ678091	DQ678091		KT216555	
C. capsici	CBS 118712	Unknown host, on calyx attached to fruit (Fiji)	GU214653	KF251800		KT216554	
Cladosporium allicinum	CBS 121624^{ET} = CPC 12211	On *Hordeum vulgare* (Belgium)	EF679350	KJ564335			
C. chalastosporoides	CBS 125985^{ET} = CPC 13864	On *Protea arborea* (South Africa)	HM148001	KJ564332		**LT799751**	
C. fusiforme	CBS 119414^{T}	Hypersaline water of Secovlje salterns (Slovenia)	DQ780388	KJ564333			
C. herbarum	CBS 121621^{ET} = CPC 12177	On *Hordeum vulgare* (The Netherlands)	EF679363	KJ564331		**LT799752**	
C. hillianum	CBS 125988^{T} = CPC 15459	On *Typha orientalis* (New Zealand)	HM148097	KJ564334			
C. iridis	CBS 138.40^{ET} (ex-epitype of *Scolicotrichum iridis*)	On *Iris* sp. (The Netherlands)	EF679370	EU167591		KT223022	
Dissoconium aciculare	CBS 204.89	On *Astragalus* sp. (Germany)	AY725520	GU214419		KX288435	
D. aciculare	CBS 342.82^{T}	On *Medicago lupulina* (Germany)	NR_119427	EU019266			
D. eucalypti	CBS 132084 = CPC 18969	On *Malus domestica* fruit (USA)	JQ622084	JQ622092			
D. proteae	CBS 122900^{T} = CPC 13853	On leaves of *Protea* sp. (Spain)	EU707897	EU707897			
Extremus adstrictus	CBS 118292^{T} = TRN96 (ex-type of *Devriesia adstricta*)	Rock sample (Spain)	NR_144954	KF310022			
E. antarcticus	CBS 136103^{T} = CCFEE 451 (ex-type of *Devriesia antarctica*)	Rock sample (Antarctica)	NR_138389	GU250360			
	CBS 136104 = CCFEE 5207	Rock sample (Antarctica)	KF309980	KF310021			
Graphiopsis chlorocephala	CBS 121522 = CPC 11383	On leaves of *Paeonia delavayi* (Germany)	EU009457	EU009457		**LT799753**	
	CBS 100405	On leaf and stem lesions on *Paeonia* sp. (New Zealand)	EU009456	EU009456		KT216520	
Mycodiella eucalypti	CBS 142098 = CPC 29458	On leaves of *Eucalyptus diversicolor* (Australia)	KY173420	KY173511		KY173586	
M. laricis-leptolepidis	MAFF 410081	On *Larix leptolepis* (Japan)	JX901767	JX901862			
M. sumatrensis	CBS 118499^{T} = CPC 11171 (ex-type of *Mycosphaerella sumatrensis*)	On *Eucalyptus* sp. (Indonesia)	KF901655	KF901994		**LT799754**	

Table 1. (Continued).

Species	Strain/isolate number[1]	Substrate/host (country)	GenBank accession numbers[2]				
			ITS	LSU	*actA*	*rpb2*	*tub2*
***Neocladosporium leucadendri* gen. sp. nov.**	CBS 131317^{T} = CPC 18315 (ex-type of *Toxicocladosporium leucadendri*)	On leaves of *Leucadendron* sp. (South Africa)	JQ044436	JQ044455	**LT821376**	**LT799755**	**KY706602**
	CPC 29090	On leaves of *Kunzea pauciflora* (Australia)	**LT799737**	**LT799744**		**LT799756**	
	CPC 29092	On leaves of *Hakea marginata* (Australia)	**LT799738**	**LT799745**		**LT799757**	
	CPC 29166	On leaves of *Hakea* sp. (Australia)	**LT799739**	**LT799746**		**LT799758**	
	CPC 29237	On leaves of *Banksia media* (Australia)	**LT799740**	**LT799747**		**LT799759**	
	CPC 29545	On leaves of *Petrophile* sp. (Australia)	**LT799741**	**LT799748**		**LT799760**	
Neodevriesia hilliana	CBS 123187^{T} = CPC 15382 (ex-type of *Devriesia hilliana*)	On leaves of *Macrozamia communis* (New Zealand)	NR_145098	GU214414		**LT799761**	
Neodevriesia sp.	CBS 118302 = TRN142	Rock sample (Spain)	AY559374	GU323975			
N. xanthorrhoeae	CBS 128219^{T} = CPC 17720 (ex-type of *Devriesia zantheria*)	On leaves of *Xanthorrhoea australis* (Australia)	NR_144962	HQ599606			
Parastagonospora nodorum	CBS 110109	On *Lolium perenne* (Denmark)	KF251177	EU754175			
Pseudocercospora eucalyptorum	CBS 132034 = CPC 13455	On *Eucalyptus* sp. (Portugal)	KF901690	KF902035		**LT799762**	
P. robusta	CBS 111175^{T} = CPC 1269	On *Eucalyptus robur* (Malaysia)	KF901678	KF902020		KX348075	
P. schizolobii	CBS 120029^{T} = CPC 12962 (ex-type of *Passalora schizolobii*)	On *Schizolobium parahybum* (Ecuador)	KF251322	KF251826			
Rachicladosporium cboliae	CBS 125424^{T} = CPC 14034	On twig debris (USA)	GU214650	GU214484		**LT799763**	
R. luculiae	CBS 121620^{T} = CPC 11407	On leaf of *Luculia* sp. (New Zealand)	EU040237	EU040237			
R. pini	CBS 129525^{T} = CPC 16770	On needles of *Pinus monophylla* (The Netherlands)	JF951145	JF951165		**LT799764**	
Ramichloridium apiculatum	CBS 400.76	Soil (Pakistan)	EU041794	EU041851		KX348077	
R. cucurbitae	CBS 132087^{T} = CPC 19423	On fruit of *Cucurbita maxima* (USA)	NR_120082	NG042613			
R. luteum	CBS 132088^{T} = CPC 18961	On fruit of *Malus domestica* (China)	NR_119684	JQ622099			
Ramularia endophylla	CBS 113265^{ET} (ex-epitype of *Sphaeria punctiformis*)	On dead leaves of *Quercus robur*	KF901725	KF902072		KP894673	
R. glennii	CBS 129441^{T}	From human bronchoalveolar lavage fluid (The Netherlands)	KJ504769	KJ504728		KJ504640	
Readeriella menaiensis	CBS 125003^{T} = CPC 14447	On leaves of *Eucalyptus oblonga* (Australia)	KX348084	KF901870		KX348084	
R. tasmanica	CBS 125002^{T} = CPC 13631	On leaves of *Eucalytus delegatensis* (Australia)	KF901761	KF902116		KX348086	

Table 1. (Continued).

Species	Strain/isolate number[1]	Substrate/host (country)	GenBank accession numbers[2]				
			ITS	LSU	*actA*	*rpb2*	*tub2*
Schizothyrium pomi	CBS 486.50	On *Polygonum sachalinense* (The Netherlands)	EF134948	KF902024			
	CBS 228.57	Unknown host (Italy)	EF134947	KF902007			
Teratosphaeria fibrillosa	CBS 121707^{ET} = CPC 13960	On leaves of *Protea* sp. (South Africa)	KF901728	KF902075		**LT799765**	
T. fimbriata	CBS 120736^{T} = CPC 13324 (ex-type of *Mycosphaerella fimbriata*)	On leaves of *Corymbia* sp. (Australia)	KF901577	KF901901		**LT799766**	
T. molleriana	CBS 118359 = CMW 11560	On *Eucalyptus globulus* (Australia)	KF901764	KF902120		KX348104	
Toxicocladosporium banksiae	CBS 128215^{T} = CPC 17280	On leaves of *Banksia emulata* (Australia)	HQ599598	HQ599599	**LT821371**	**LT799767**	**KY706597**
***T. cacti* sp. nov.**	URM 7489^{T} = CBS 141539	Endophyte from *Pilosocereus gounellei* subsp. *gounellei* (Brazil)	**KY752806**	**KY752819**	**LT821361**	**LT799768**	**KY706587**
	URM 7490 = CBS 141538	Endophyte from *Pilosocereus gounellei* subsp. *gounellei* (Brazil)	**KY752813**	**KY752826**	**LT821368**	**LT799769**	**KY706594**
	188 JB	Endophyte from *Pilosocereus gounellei* subsp. *gounellei* (Brazil)	**KY752803**	**KY752816**	**LT821358**	**LT799770**	**KY706584**
	191 JB	Endophyte from *Pilosocereus gounellei* subsp. *gounellei* (Brazil)	**KY752804**	**KY752817**	**LT821359**	**LT799771**	**KY706585**
	192 JB	Endophyte from *Pilosocereus gounellei* subsp. *gounellei* (Brazil)	**KY752805**	**KY752818**	**LT821360**	**LT799772**	**KY706586**
	195-2 JB	Endophyte from *Pilosocereus gounellei* subsp. *gounellei* (Brazil)	**KY752807**	**KY752820**	**LT821362**		**KY706588**
	225 JB	Endophyte from *Pilosocereus gounellei* subsp. *gounellei* (Brazil)	**KY752808**	**KY752821**	**LT821363**	**LT799773**	**KY706589**
	226 JB	Endophyte from *Pilosocereus gounellei* subsp. *gounellei* (Brazil)	**KY752809**	**KY752822**	**LT821364**	**LT799777**	**KY706590**
	231 JB	Endophyte from *Pilosocereus gounellei* subsp. *gounellei* (Brazil)	**KY752810**	**KY752823**	**LT821365**	**LT799774**	**KY706591**
	235 JB	Endophyte from *Pilosocereus gounellei* subsp. *gounellei* (Brazil)	**KY752811**	**KY752824**	**LT821366**	**LT799775**	**KY706592**
	236 JB	Endophyte from *Pilosocereus gounellei* subsp. *gounellei* (Brazil)	**KY752812**	**KY752825**	**LT821367**	**LT799776**	**KY706593**
	261-2 JB	Endophyte from *Pilosocereus gounellei* subsp. *gounellei* (Brazil)	**KY752814**	**KY752827**	**LT821369**		**KY706595**
T. chlamydosporum	CBS 124157^{T} = CPC 15709 (ex-type of *T. chlamydosporum*)	On leaf of *Eucalyptus camaldulensis* (Madagascar)	FJ790283	FJ790301	**LT821372**	**LT799778**	**KY706598**
	CBS 124159^{T} = CPC 15736 (ex-type of *T. velox*)	On leaf of *Eucalyptus camaldulensis* (Madagascar)	FJ790288	FJ790306	**LT821383**	**LT799779**	**KY706609**

Table 1. (Continued).

Species	Strain/isolate number[1]	Substrate/host (country)	GenBank accession numbers[2]				
			ITS	LSU	*actA*	*rpb2*	*tub2*
T. ficiniae	CBS 136406^{T} = CPC 21283	On leaves of *Ficinia indica* (South Africa)	KF777190	KF777241	**LT821373**	**LT799780**	**KY706599**
T. hominis	CBS 140694^{T} = FMR 13297	From human bronchoalveolar lavage fluid (USA)	LN834444	**KY752829**	**LT821374**	**LT799781**	**KY706600**
***T. immaculatum* sp. nov.**	URM 7491^{T} = CBS 141540	Endophyte from *Melocactus zehntneri* (Brazil)	**KY752815**	**KY752828**	**LT821370**	**LT799782**	**KY706596**
T. irritans	CBS 185.58^{T}	From mouldy paint (Suriname)	EU040243	EU040243	**LT821375**	**LT799783**	**KY706601**
T. pini	CBS 138005^{T} = CPC 23639	On needles of *Pinus* sp. (China)	KJ869160	KJ869217	**LT821377**	**LT799784**	**KY706603**
T. posoqueriae	CBS 133583^{T} = CPC 19305	On leaves of *Posoqueria latifolia* (Australia)	NR121555	KC005803	**LT821378**	**LT799785**	**KY706604**
T. protearum	CBS 126499^{T} = CPC 15254	On leaves of *Protea burchellii* (South Africa)	HQ599586	HQ599587	**LT821379**	**LT799786**	**KY706605**
T. pseudoveloxum	CBS 128775^{T} = CPC 18527	On leaf bracts of *Phaenocoma prolifera* (South Africa)	JF499847	JF499867	**LT821380**		**KY706606**
T. rubrigenum	CBS 124158^{T} = CPC 15735	On leaf of *Eucalyptus camaldulensis* (Madagascar)	FJ790287	FJ790305	**LT821381**	**LT799787**	**KY706607**
T. strelitziae	CBS 132535^{T} = CPC 19762	On leaves of *Strelitzia reginae* (South Africa)	NR111765	JX069858	**LT821382**	**LT799788**	**KY706608**
Undescribed species	CPC 29168	On *Cyperaceae* (Australia)	**LT799742**	**LT799749**		**LT799789**	
Undescribed species	CPC 29170	On *Cyperaceae* (Australia)	**LT799743**	**LT799750**		**LT799790**	
Uwebraunia australiensis	CBS 120729^{T} = CPC 13282	On *Eucalyptus platyphylla* (Australia)	KF442513	GQ852588		**LT799791**	
U. commune	CBS 110809^{T} = CPC 830	On *Eucalyptus nitens* (South Africa)	AY725536	KJ564336			
U. dekkeri	CPC 13264	On *Eucalyptus molucana* (Australia)	GQ852741	GQ852593		**LT799792**	
Verrucocladosporium dirinae	CBS 112794^{T}	On lichen *Dirina massiliensis* (United Kingdom)	EU040244	EU040244			

[1] CBS: Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands; CCFEE: Culture collection from extreme environments of the Dipartamento di Scienze Ambientali, University of Tuscia, Viterbo, Italy; CMW: Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI) of the University of Pretoria, Pretoria, South Africa; CPC: Culture collection of P.W. Crous, held at the Westerdijk Fungal Biodiversity Institute; FMR: Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, Reus, Spain; JB: Collection of J.D.P. Bezerra, housed at URM; TRN: C. Ruibal private collection, currently in MAF; URM: Micoteca URM Profa. Maria Auxiliadora Cavalcanti, Departamento de Micologia Prof. Chaves Batista, Universidade Federal de Pernambuco, Brazil. ^{T} ex-type strain, ^{ET} ex-epitype strain.

[2] ITS: first and second internal transcribed spacer regions and intervening 5.8S nrDNA; LSU: nuclear ribosomal large subunit of the rDNA; *actA*: actin gene; *rpb2*: RNA polymerase second largest subunit gene; *tub2*: β-tubulin gene. Names of the new taxa and sequences newly obtained in this study are shown in **bold**.

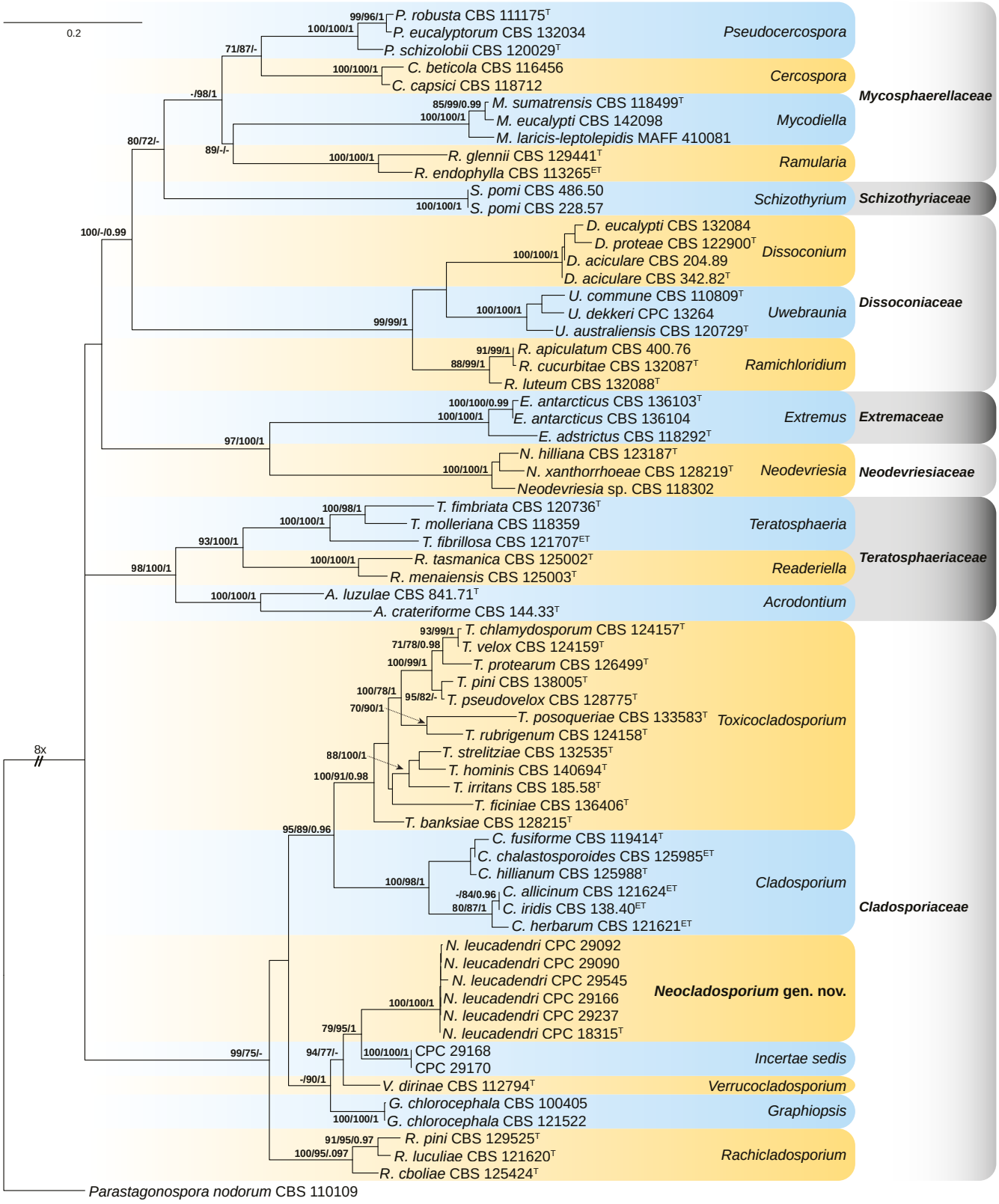

Fig. 1. Maximum likelihood (RaxML) tree obtained by phylogenetic analysis of the combined ITS and LSU rDNA and *rpb2* sequences of 67 taxa belonging to *Capnodiales*. The new genus, *Neocladosporium*, is shown in **bold**. Bootstrap support values from Maximum Parsimony (MP-BS) and Maximum Likelihood (ML-BS), and Bayesian posterior probabilities (BPP) above 70 % and 0.95, respectively, are indicated at the nodes (MP-BS/ML-BS/BPP). *Parastagonospora nodorum* (CBS 110109) was used as outgroup. T = ex-(holo-)type strain, ET = ex-epitype strain.

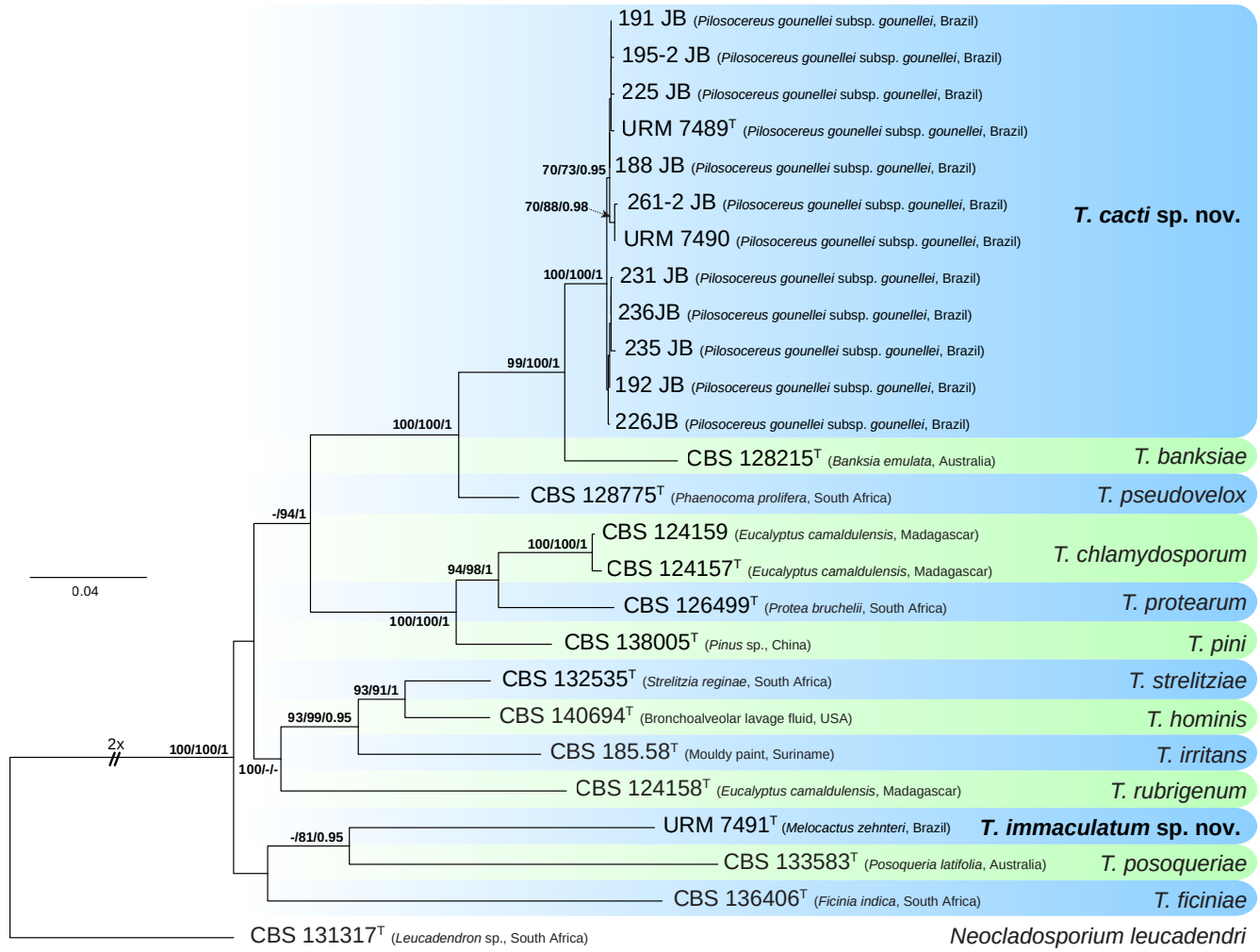

Fig. 2. Maximum likelihood (RaxML) tree obtained by phylogenetic analysis of the combined ITS rDNA, LSU rDNA, *actA*, *rpb2* and *tub2* datasets of the genus *Toxicocladosporium*. Newly introduced species are shown in **bold**. Bootstrap support values from Maximum Parsimony (MP-BS), Maximum Likelihood (ML-BS), and Bayesian posterior probabilities (BPP) above 70 % and 0.95, respectively, are indicated at the nodes (MP-BS/ML-BS/BPP). *Neocladosporium leucadandri* (CBS 131317) was used as outgroup. [T] ex-(holo-)type strain.

the species resolution, *actA*, *rpb2* and *tub2* sequences were also included in this analysis. This second phylogeny included sequences from 26 isolates (including the outgroup) and 2 562 characters (*actA*: 247, ITS: 396, LSU: 780, *rpb2*: 724 and *tub2*: 415) of which 482 were parsimony-informative (*actA*: 25, ITS: 22, LSU: 28, *rpb2*: 230 and *tub2*: 125), 215 were variable and parsimony-uninformative (*actA*: 35, ITS: 48, LSU: 28, *rpb2*: 68 and *tub2*: 36), and 1 820 were constant (*actA*: 123, ITS: 311, LSU: 726, *rpb2*: 420 and *tub2*: 240). The results of this analysis are shown in Fig. 2. Parsimony analysis resulted in a single tree showing the best score (TL = 1870; CI = 0.570; RI = 0.651; RC = 0.371). The endophytic isolates grouped in two linages; 12 isolates formed a fully-supported clade close to *T. banksiae* (CBS 128215) (MP-BS 100 %, ML-BS 100 %, BPP 1) while one isolate (URM 7491 = CBS 141540) formed a moderately supported monotypic linage closely related to but distinct from *T. ficiniae* (CBS 136406) and *T. posoqueriae* (CBS 133583) (MP-BS < 70 %, ML-BS 81 %, BPP 0.95). These two groups are described here as the new species *Toxicocladosporium cacti* (ex-type culture URM 7489 = CBS 141539) and *Toxicocladosporium immaculatum* (ex-type culture URM 7491 = CBS 141540), respectively.

In addition, the ex-type strains of *T. chlamydosporum* (CBS 124157) and *T. velox* (CBS 124159) always clustered together with high support values (MP-BS 100 %, ML-BS 100 %, BPP 1.00). From these phylogenetic results and based on the few nucleotide differences between the two species (*actA*: 1 nt and 1 gap, ITS: 5 nt, LSU: 1 nt, *rpb2*:0 nt and TUB: 0 nt) and given that both species show similar morphological and ecological features, we treat the name *T. velox* as a synonym of *T. chlamydosporum*.

LSU and ITS were informative loci to verify the relationship between genera and species groups. However, the *actA*, *rpb2* and *tub2* sequences were more informative to distinguish related species, especially in the case of *T. cacti*, which is closely related to *T. banksiae*.

Table 2. Morphological features of *Neocladosporium* and *Toxicocladosporium* species included in this paper. Newly described species names are shown in **bold**.

Species	Conidiophores in µm [number of septa]		Conidiogenous cells in µm	Ramoconidia in µm [number of septa]	Conidia in µm [number of septa]		References
	Macroconidiophores	Microconidiophores			intercalary	terminal	
N. leucadendri	50–150 × 3–5 [6–15]	-	8–20 × 4–6	25–45 × 3–5 [1–2]; secondary 15–20 × 3–4 [0–1]	9–11(–15) × (2.5–)3(–4)	(6–)7–8(–9) × (2.5–)3(–4)	Crous *et al.* (2011)
T. banksiae	50–130 × 3–4 [3–7]	10–40 × 2.5–4	6–20 × 2.5–3	(14–)17–25 × (2.5–)3–4 [0–1]	10–12(–20) × (2.5–)3–3.5 [0–1]	(7–)8–10(–11) × (2–)2.5–3 [0]	Crous *et al.* (2010)
T. cacti	to 130 × 2–3.5 [2–6]	21.5–34.5 × 2–5 [0–1]	13–16 × 2–3	10–14(–20.5) × 2–3 [0–1]; secondary 7–10(–14) × 2–3 [0–1]	6–9 × 2.5–3 {0–1]	5–6 × 2–2.5 [0]	This paper
T. chlamydosporum	20–60 × 3–5 [1–4]	to 15 × 5 [0–1]	10–25 × 3–4	(15–)16–17(–18) × (2.5–)3–4 [0–1]; secondary (9–)10–14(–16) × (2.5–)3–4 [0–1-]	(8–)9–11(–12) × 2.5–3(–3.5) [0–1]	6–10 × 2–2.5(–3) [0]	This paper and Crous *et al.* (2009)
T. ficiniae	10–40 × 3–5 [1–15]	-	5–15 × 2.5–4	15–35 × 3–4 [0]; secondary 12–20 × 2.5–3 [0–1]	(9–)10–11 × (2.5–)3	(7–)8–9 × (2.5–)3	Crous *et al.* (2013)
T. hominis	70–113 × 3–3.5	-	13–30 × 3–4	15–32 × 2–4 [0–2]; secondary 11–15 × 2.5–4 [0–1]	9–16 × 3–4 [0–1]	5.5–8 × 2.5–3.5	Crous *et al.* (2016)
T. immaculatum	to 100 × 2–3.5 [2–5]	12–25 × 2.5–3.5 [0–1]	10–14 × 2.5–3.5	14.5–22.5 × 2–4 [0–1]; secondary (7–)8–14(–18.5) × 2–3 [0–1]	11.5–13 × 2.5–3	8–10(–11) × 2–3	This paper
T. irritans	30–60 × 4–6 [2–7]	10–30 × 2.5–4 [0(1–2)]	7–12 × 3–4	7–15 × 3–5 [(0–)1(–3)]	-	(5–)6–8(–10) × (3–)4(–5) [0–1]	Crous *et al.* (2007)
T. pini	30–90 × 3–4 [2–8]	10–17 × 3–4	5–20 × 3–3.5	12–17 × 3(–3.5) [0–1]	12–14 × 3 [0–1]	8–10(–11) × 2.5(–3) [0–1]	Crous *et al.* (2014)
T. posoqueriae	50–200 × 4–7 [1–3]	-	10–20 × 4–7	5–15 × 4–5 [0]	-	(4–)6–7 × (3–)4	Crous *et al.* (2012)
T. protearum	30–80 × 3–4 [1–8]	-	10–20 × 2.5–3	15–20 × 2.5–3.5 [0–1]	-	(9–)11–13(–16) × (2–)2.5(–3) [0–1]	Crous *et al.* (2010)
T. pseudovelox	20–50 × 3–4 [2–5]	-	10–15 × 3–4	8–15 × 2.5–4 [0–1]	-	(6–)7–10(–11) × (2–)2.5(–3) [0]	Crous & Groenewald (2011)
T. rubrigenum	to 100 × 2–4 [1–8]	to 30 × 2–3 [0–1]	15–20 × 2.5–3	(13–)14–15(–16) × 2.5–3(–3.5); secondary (9–)10–12(–14) × 2.5–3(–3.5)	7–8(–9) × 2(–2.5)	(4–)6–7 × 2(–2.5)	Crous *et al.* (2009)
T. strelitziae	40–70 × 2–3.5 [2–5]	3–7 × 2.5–3.5	10–15 × 2.5–3.5	12–20 × 2–3.5 [0]; secondary 10–17 × 2–3.5 [0]	10–12 × 2–2.5	(5–)7–8(–9) × 2(–2.5)	Crous *et al.* (2012)

TAXONOMY

Our phylogenetic analyses revealed that the endophytic fungi from cactus species previously identified as *Toxicocladosporium* represent two new species in this genus. These newly proposed species are established based on phylogenetic analyses and morphological features. In addition, we introduce a new generic name, *Neocladosporium* to accommodate "*Toxicocladosporium*" *leucadendri*, which is not congeneric with *Toxicocladosporium*. In this section a bibliographic synopsis of the genus is compiled including key morphological features for identification, known host affiliations, substrates, and geographic distribution for all the currently accepted species of *Toxicocladosporium*. Table 2 summarises key morphological features of the *Neocladosporium* and *Toxicocladosporium* species included here.

Neocladosporium J.D.P. Bezerra, Sandoval-Denis, C.M. Souza-Motta & Crous, **gen. nov.**
MycoBank MB820266

Etymology: Named because of its similarity to the genus *Cladosporium*.

Diagnosis: Differs from *Toxicocladosporium* by its verruculose to warty ramoconidia, and from *Cladosporium* s. str. by its dark, thick-walled conidial and conidiophore septa, also lacking the typical coronate *Cladosporium* scar.

Type species: *Neocladosporium leucadendri* (Crous) J.D.P. Bezerra *et al.* 2017 (syn. *Toxicocladosporium leucadendri* Crous 2011).

Description: *Mycelium* consisting of pale brown, smooth, branched, septate hyphae. *Conidiophores* solitary, erect, unbranched or branched above, subcylindrical, straight to flexuous, apical septum becoming dark brown and thickened. *Conidiogenous cells* integrated, polyblastic, terminal and lateral, subcylindrical, smooth, brown; scars truncate, thickened and darkened. *Ramoconidia* medium brown, verruculose to warty, giving rise to branched chains of conidia, subcylindrical, polyblastic, brown, verruculose to warty, 0–1-septate, frequently forking close to apex; scars darkened, thickened. *Intercalary conidia* subcylindrical to fusoid-ellipsoidal, brown, smooth to somewhat warty. *Small terminal conidia* fusoid-ellipsoidal, brown, smooth; hila thickened and darkened.

Neocladosporium leucadendri (Crous) J.D.P. Bezerra, Sandoval-Denis, C.M. Souza-Motta & Crous, **comb. nov.**
MycoBank MB 820267
(Fig. 3)

Basionym: *Toxicocladosporium leucadendri* Crous, *Persoonia* **27**: 157 (2011).

Type: **South Africa**: *Western Cape Province*: Hermanus, Fernkloof Nature Reserve, on leaves of *Leucadendron* sp. (*Proteaceae*), 4 May 2010, *P.W. Crous* (CBS H-20774 – holotype; CPC 18315 = CBS 131317 – culture ex-type).

Description: Crous *et al.* (2011a).

Substrate and distribution: On leaves of *Leucadendron* sp. (*Proteaceae*) in the Western Cape province of South Africa (Crous *et al*. 2011a). On leaves of *Kunzea pauciflora* (*Myrtaceae*), and *Banksia media*, *Hakea* sp., and *Petrophile* sp. (*Proteaceae*) in Western Australia.

Other material examined: **Australia**: *Western Australia*: Albany, Fitzgerald River National Park, Point Ann, on leaves of *Banksia media* (*Proteaceae*), 21 Sep. 2015, *P.W. Crous* (CPC 29237); Denmark, Lights Beach, on leaves of *Hakea* sp. (*Proteaceae*), 19 Sep. 2015, *P.W. Crous* (CPC 29166); Wellstead, Cape Riche, on leaves of *Hakea marginata* (*Proteaceae*), 21 Sep. 2015, *P.W. Crous* (CPC 29092); ibid., on leaves of *Kunzea pauciflora* (*Myrtaceae*), 21 Sep. 2015, *P.W. Crous* (CPC 29090); Williams, Williams Nature Reserve, on leaves of *Petrophile* sp. (*Proteaceae*), 18 Sep. 2015, *P.W. Crous* (CPC 29545).

Notes: Crous *et al.* (2011a) published the strain CPC 18315 = CBS 131317 as *T. leucadendri* based on phylogenetic analyses using LSU and ITS sequences, and morphological characters. According to these authors, based on a combination of culture characteristics, conidiophore and conidial dimensions, it differs from known taxa, many of which also occur in the fynbos vegetation (Crous *et al.* 2011b). A megablast search of the NCBIs GenBank nucleotide sequence database using the ITS and LSU sequences of *N. leucadendri* retrieved as closest hits *Graphiopsis chlorocephala* and *Verrucocladosporium dirinae*, amongst others. In our phylogenetic analyses this strain appeared in a single lineage closely related to *Graphiopsis chlorocephala* and *Verrucocladosporium dirinae* as shown before by Crous *et al.* (2011a), but clearly separated from members of *Toxicocladosporium* (Fig. 1). Morphologically, *Neocladosporium leucadendri* is very similar to *Toxicocladosporium* species, but can be distinguished from it by size and ornamentation of ramoconidia (verruculose to warty) and ramoconidia frequently forking close to the apex. Sequences of ITS and LSU rDNA or *rpb2* are the best approach to separate *N. leucadendri* from *Toxicocladosporium* species and related genera. Also very similar to *Cladosporium s. str*., but differing in the size and ornamentation of the ramoconidia (verruculose to warty), which are frequently forking close to apex, dark, thick-walled conidial and conidiophore septa, and lacking the typical coronate *Cladosporium* scar type similar to *Toxicocladosporium* (David 1997, Crous *et al*. 2007); it differs from *Graphiopsis* which has morphological peculiarities on its conidiophores (cladosporioid and periconioid morphs), conidiogenous loci and hila (Schubert *et al*. 2007a, Braun *et al*. 2008); from *Rachicladosporium* which has an apical conidiophore rachis with inconspicuous to subconspicuous scars and unthickened, not darkened-refractive conidial hila (Crous *et al*. 2007); and from *Verrucocladosporium* which has mainly an unusual conidial and hyphal ornamentation (Crous *et al*. 2007). Because of our phylogenetic results and morphological observations, the new generic name *Neocladosporium*, is proposed to accommodate *N. leucadendri*.

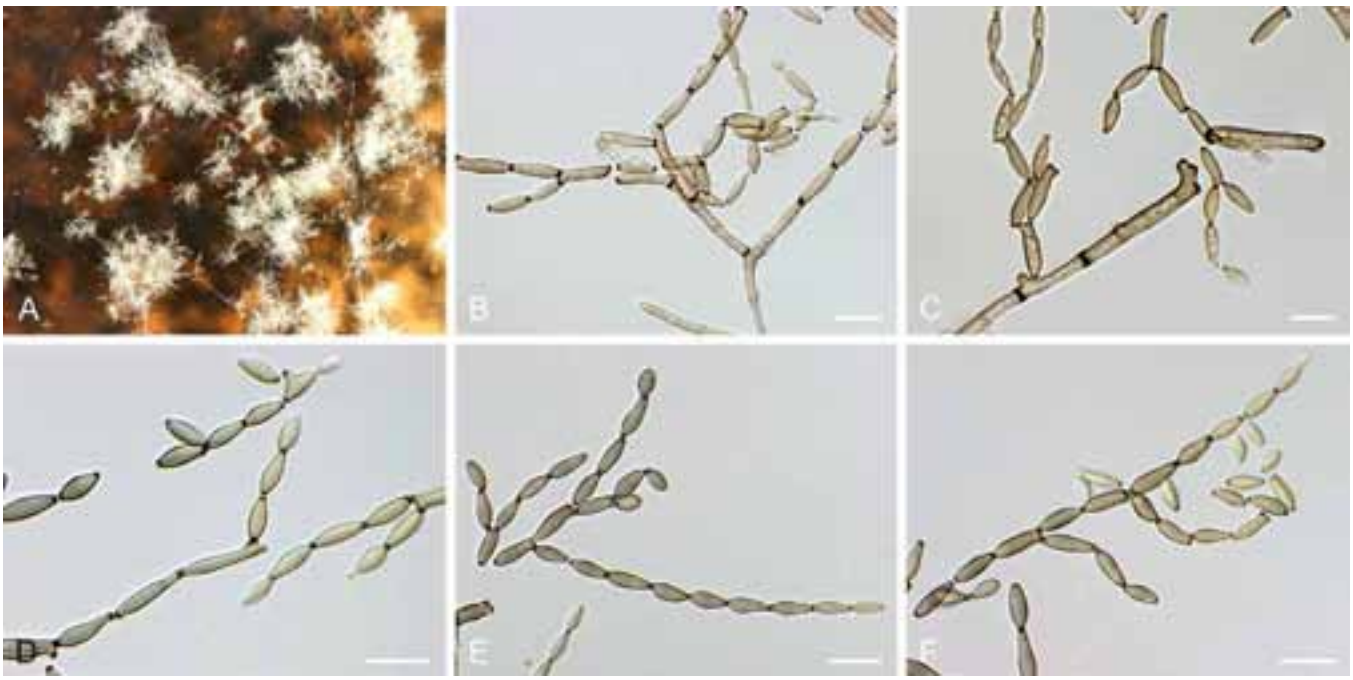

Fig. 3. *Neocladosporium leucadendri* (CBS 131317 – ex-type culture). **A.** Colony sporulating on MEA. **B–F.** Conidiophores giving rise to chains of conidia. Bars = 10 µm.

Toxicocladosporium Crous & U. Braun, *Stud. Mycol.* **58**: 39 (2007).

Type species: *Toxicocladosporium irritans* Crous & U. Braun 2007.

Notes: *Toxicocladosporium* was introduced by Crous *et al*. (2007) to accommodate cladosporium-like fungi having distinct dark, thick-walled conidial and conidiophore septa, and lacking the typical coronate *Cladosporium* scar type. After this original publication, several new species isolated from different substrates and hosts were introduced in this genus using morphological characters and phylogenetic analyses of ITS and LSU sequences (Crous *et al*. 2007, 2009a, 2010a, b, 2011a, 2012a, b, 2013, 2014, 2016, Crous & Groenewald 2011).

Toxicocladosporium banksiae Crous *et al*., *Persoonia* **25**: 147 (2010).

Type: **Australia**: *Queensland*: Noosa National Park, 26º34'14.0"S 153º4'21.6"E, on leaves of *Banksia* sp., 13 July 2009, *P.W. Crous et al.* (CBS H-20496 – holotype; CPC 17281, CPC 17280 = CBS 128215 – culture ex-type).

Description and illustration: Crous *et al.* (2010).

Substrate and distribution: On leaves of *Banksia* sp. (*Proteaceae*), Australia (Crous *et al*. 2010b).

Notes: According to Crous *et al*. (2010b), the ITS and LSU sequences of *T. banksiae* are close to those of *T. chlamydosporum* and *T. irritans*. The ITS sequences of *T. banksiae* also differ from those of *T. protearum*. Morphologically, *T. banksiae* differs from these three species in the size and shape of the intercalary and terminal conidia, ramoconidia, and presence or absence of chlamydospores. In our phylogeny using five different loci, this species is closely related to the new species *T. cacti* which differs in microconidiophore size [10–40 × 2.5–4 µm (aseptate) in *T. banksiae vs*. 21.5–34.5 × 2–5 µm (0–1-septate) in *T. cacti*], ramoconidia (14–25 × 2.5–4 µm *vs.* 10–20.5 × 2–3 µm), intercalary conidia (10–20 × 2.5–3.5 µm *vs.* 6–9 × 2.5–3 µm), terminal conidia (7–11 × 2–3 µm *vs.* 5–6 × 2–2.5 µm), and culture characteristics (colonies olivaceous grey reaching up to 7 mm diam in 2 wk in *T. banksiae vs.* colonies pale grey to grey, growing up to 30 mm diam in 3 wk and presence of a pale brown to brown exudate in *T. cacti*).

Toxicocladosporium cacti J.D.P. Bezerra, C.M. Souza-Motta & Crous, **sp. nov.**
MycoBank MB820264
(Fig. 4)

Etymology: Named after the nature of the host, a cactus, from which it was isolated.

Diagnosis: Differs from *T. banksiae* in its slightly smaller and less septate microconidiophores and conidia, and by its pale grey to grey colonies.

Type: **Brazil**: *Pernambuco*: Catimbau National Park, 8º36'35''S 37º14'40''W, as endophytic fungus from cactus *Pilosocereus gounellei* subsp. *gounellei*, Sep. 2013, *J.D.P. Bezerra* (URM 90068 – holotype; URM 7489 = CBS 141539 – culture ex-type).

Other material examined: **Brazil**: *Pernambuco*: Catimbau National Park, 8º36'35''S 37º14'40''W, as endophytic fungus from cactus *Pilosocereus gounellei* subsp. *gounellei*, Sep. 2013, *J.D.P. Bezerra*

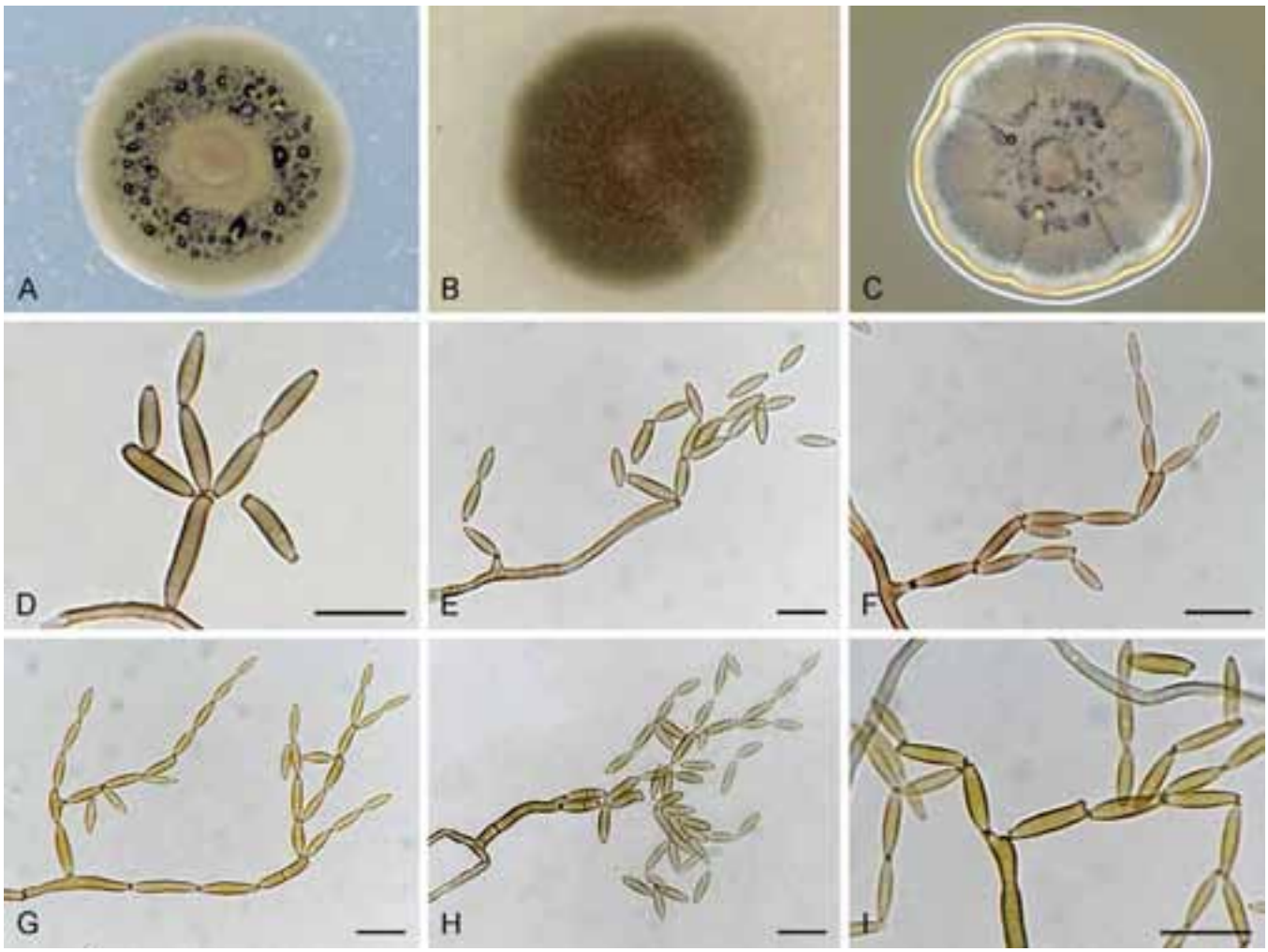

Fig. 4. *Toxicocladosporium cacti* (URM 7489 = CBS 141539 – ex-type culture). **A.** Colony sporulating on PDA. **B.** Colony sporulating on OA. **C.** Colony sporulating on MEA. **D–H.** Conidiophores and conidia. **I.** Ramoconidia and conidia. Bars = 10 µm.

(URM 7490 = CBS 141538, 188 JB, 191 JB, 192 JB, 195-2 JB, 225 JB, 231 JB, 235 JB, 236 JB, 226 JB, 261-2 JB).

Description: Mycelium consisting of branched, septate, smooth, brown, 2–2.5 µm wide hyphae; wall and septa becoming dark brown and thickened with age. *Conidiophores* dimorphic. *Macroconidiophores* solitary, arising from superficial mycelium, erect, brown, unbranched or branched above, finely verruculose, subcylindrical, straight to flexuous, up to 130 × 2–3.5 µm, 2–6-septate. *Microconidiophores* reduced to conidiogenous cells, rarely with one supporting cell, pale brown, smooth, erect, subcylindrical, 21.5–34.5 × 2–5 µm, 0–1-septate. *Conidiogenous cells* integrated, terminal or lateral, smooth, brown, 13–16 × 2–3 µm, proliferating sympodially with 1–2 apical loci; scars truncate, thickened and darkened, 1–1.5 µm wide. *Conidia* catenate in branched or unbranched chains, pale brown, thick-walled, septa dark and thick or inconspicuous, finely verruculose. *Primary ramoconidia* brown, finely verruculose, 0–1-septate, ellipsoidal to subcylindrical, 10–14(–20.5) × 2–3 µm; *secondary ramoconidia* brown, finely verruculose, 0–1-septate, ellipsoidal to subcylindrical, 7–10(–14) × 2–3 µm; scars darkened, thickened, 0.5–1 µm wide. *Intercalary conidia* subcylindrical to fusoid-ellipsoidal, 0–1-septate, brown, finely verruculose, 6–9 × 2.5–3 µm. *Small terminal conidia* fusoid-ellipsoidal, aseptate, brown, finely verruculose, 5–6 × 2–2.5 µm; hila thickened and darkened, 0.5–1 µm wide.

Culture characteristics (in a day-night cycle, 22 °C after 3 wk): *Colonies* on MEA are slightly folded and sulcate, velvety, pale grey to grey with a pale grey rim, reverse dark grey, reaching 30 mm diam; on OA flat to semi erumpent, spreading, with sparse to moderate aerial mycelium, smooth, surface and reverse pale grey to grey, to 29 mm; and on PDA surface and reverse olivaceous grey, to 25 mm. *Exudate* pale brown to brown observed on cultures growing on MEA and PDA.

Substrate and distribution: An endophytic fungus isolated from the cactus *Pilosocereus gounellei* subsp. *gounellei* (*Cactaceae*), Brazil.

Notes: *Toxicocladosporium cacti* is phylogenetically related to *T. banksiae* but differs morphologically from it in microconidiophore size and septation [21.5–34.5 × 2–5 µm (0–1-septate) *vs.* 10–40 × 2.5–4 µm], smaller ramoconidia (10–20.5 × 2–3 µm *vs.* 14–25 × 2.5–4 µm), intercalary conidia (6–9 × 2.5–3 µm *vs.* 10–20 × 2.5–3.5 µm), and small terminal conidia (5–6 × 2–2.5 µm *vs.* 7–11 × 2–3 µm).

Furthermore, the culture characteristics are different from those of *T. banksiae*, colonies pale grey to grey, growing to 30 mm diam in 3 wk with exudate pale brown to brown in *T. cacti vs.* colonies olivaceous grey reaching up to 7 mm diam after 2 wk in *T. banksiae*.

Toxicocladosporium chlamydosporum Crous & M.J. Wingf., *Persoonia* **22**: 90 (2009).

Synonym: *Toxicocladosporium velox* Crous & M.J. Wingf., *Persoonia* **22**: 92 (2009); as '*veloxum*'.

Types: **Madagascar**: Morondavo, on leaf of *Eucalyptus camaldulensis*, Aug. 2007, *M.J. Wingfield* (CBS H-20193 – holotype of *T. chlamydosporum*; CPC 15709 = CBS 124157 – culture ex-type); *ibid*. (CBS H-20196 – holotype of *T. velox*; CPC 15736 = CBS 124159 – culture ex-type).

Description: *Mycelium* consisting of branched, septate, smooth, brown, 2–3 µm wide hyphae, containing swollen, globose, dark brown chlamydospore-like cells to 12 µm diam. *Conidiophores* dimorphic. *Macroconidiophores* solitary, erect, arising from superficial mycelium, penicillate, subcylindrical, straight to once geniculate-sinuous, medium to dark brown, smooth to finely verruculose, 20–60 µm long, 3–5 µm wide at base, 1–4-septate, not swollen, and lacking rhizoids. *Microconidiophores* erect, subcylindrical, to 15 µm tall and 5 µm wide, 0–1-septate, medium brown. *Conidiogenous cells* terminal, integrated, subcylindrical, straight, medium brown, 10–25 × 3–4 µm, smooth to finely verruculose; loci terminal and lateral, flat tipped, thickened, darkened, at times subdenticulate, (0.5–)1–2 µm wide. *Conidia* in branched chains, brown, smooth to finely verruculose, ellipsoid to cylindrical-oblong. *Primary ramoconidia* rarely observed, 0–1-septate, fusoid-ellipsoidal to subcylindrical, (15–)16–17(–18) × (2.5–)3–4 µm. *Secondary ramoconidia* 0–1-septate, fusoid-ellipsoidal, (9–)10–14(–16) × (2.5–)3–4 µm. *Intercalary conidia* 0–1-septate, fusoid-ellipsoidal, (8–)9–11(–12) × 2.5–3(–3.5) µm. *Small terminal conidia* aseptate, fusoid-ellipsoidal, 6–10 × 2–2.5(–3) µm (conidia dark brown and verruculose on MEA) (based on Crous *et al.* 2009a).

Culture characteristics (in the dark, at 25 °C after 1 mo): *Colonies* on MEA erumpent, spreading, with sparse aerial mycelium; surface folded, irregular and sectored, with feathery margin, centre pale olivaceous grey to fuscous-black, outer region olivaceous grey to greyish sepia; reverse iron-grey to dark grey; reaching up to 25 mm diam. Black sclerotial bodies on MEA, consisting of an agglomeration of chlamydospore-like cells; they remain sterile, and eventually resemble hollow fruiting bodies, although they lack an ostiole or defined wall. On OA spreading, flat, with sparse aerial mycelium, and even catenulate margin; surface iron-grey with patches of pale olivaceous grey to smoke-grey; colonies reaching up to 30 mm diam (Crous *et al.* 2009a).

Substrate and distribution: On leaves of *Eucalyptus camaldulensis* (*Myrtaceae*), Madagascar (Crous *et al*. 2009a).

Notes: Crous *et al.* (2009a) described this species using ITS and LSU sequences, and morphological characters to differentiate it from *T. irritans*. *Toxicocladosporium chlamydosporum* differs from other species in the genus in the presence of larger ramoconidia, and longer, narrower intercalary conidia, and in that it forms chlamydospores and sclerotial bodies in culture. *Toxicocladosporium velox* was isolated from the same leaf spot (Crous *et al.* 2009a). Based on the limited nucleotide differences and their morphological similarity, we consider *T. velox* a synonym of *T. chlamydosporum*. A revised description is provided to enable *T. chlamydosporum* in its expanded circumscription to be distinguished from other species in the genus. This species is closely related to *T. protearum* which differs from it mainly in the size and degree of septation of its conidiophores [20–60 µm × 3–5 µm (1–4-septate) in *T. chlamydosporum vs.* 30–80 µm × 3–4 µm (1–8-septate) in *T. protearum*], ramoconidia (15–18 × 2.5–4 µm *vs.* 15–20 × 2.5–3.5 µm), and intercalary and terminal conidia (8–11 × 3–3.5 µm *vs.* 9–16 × 2–3 µm).

Toxicocladosporium ficiniae Crous & A.R. Wood, *Persoonia* **31**: 191 (2013).

Type: **South Africa**: *Western Cape Province*: Brackenfell, Cape Town, Bracken Nature Reserve, on leaves of *Ficinia indica* (*Cyperaceae*), 18 Aug. 2012, *A.R. Wood* (CBS H-21413 – holotype; CPC 21283, CPC 21282 = CBS 136406 – culture ex-type).

Description and illustration: Crous *et al.* (2013).

Substrate and distribution: On leaves of *Ficinia indica* (*Cyperaceae*), South Africa (Crous *et al.* 2013).

Notes: *Toxicocladosporium ficiniae* is phylogenetically related to *T. posoqueriae* which differs in conidiophore size and septation [10–40 × 3–5 µm (1–15-septate) *vs.* 50–200 × 4–7 µm (1–3-septate) in *T. posoqueriae*], and sizes of the conidiogenous cells (5–15 × 2.5–4 µm *vs.* 10–20 × 4–7 µm), primary ramoconidia (15–35 × 3–4 µm *vs.* 5–15 × 4–5 µm), and terminal conidia (7–9 × 2.5–3 µm *vs.* 4–7 × 3–4 µm).

Toxicocladosporium hominis Sandoval-Denis *et al.*, *Persoonia* **36**: 421 (2016).

Type: **USA**: *Florida*: Daytona Beach, from human bronchoalveolar lavage fluid, *D.A. Sutton* (FMR H-13297 – holotype; CBS H-22331 – isotype; FMR 13297 = UTHSCSA DI-13-172 = CBS 140694 – cultures ex-type).

Description and illustration: Crous *et al*. (2016).

Substrate and distribution: From human bronchoalveolar lavage fluid, USA (Crous *et al*. 2016).

Notes: *Toxicocladosporium hominis* is phylogenetically related and morphologically similar to *T. strelitziae* (Crous *et al.* 2012b), but differs from *T. strelitziae* in the production of larger conidiogenous cells (13–30 × 3–4 µm *vs.* 10–15 × 2.5–3.5 µm) and intercalary conidia (9–16 × 3–4 µm *vs.* 10–12 × 2–2.5 µm). In addition, the latter species has smooth to verruculose ramoconidia, secondary ramoconidia and intercalary conidia,

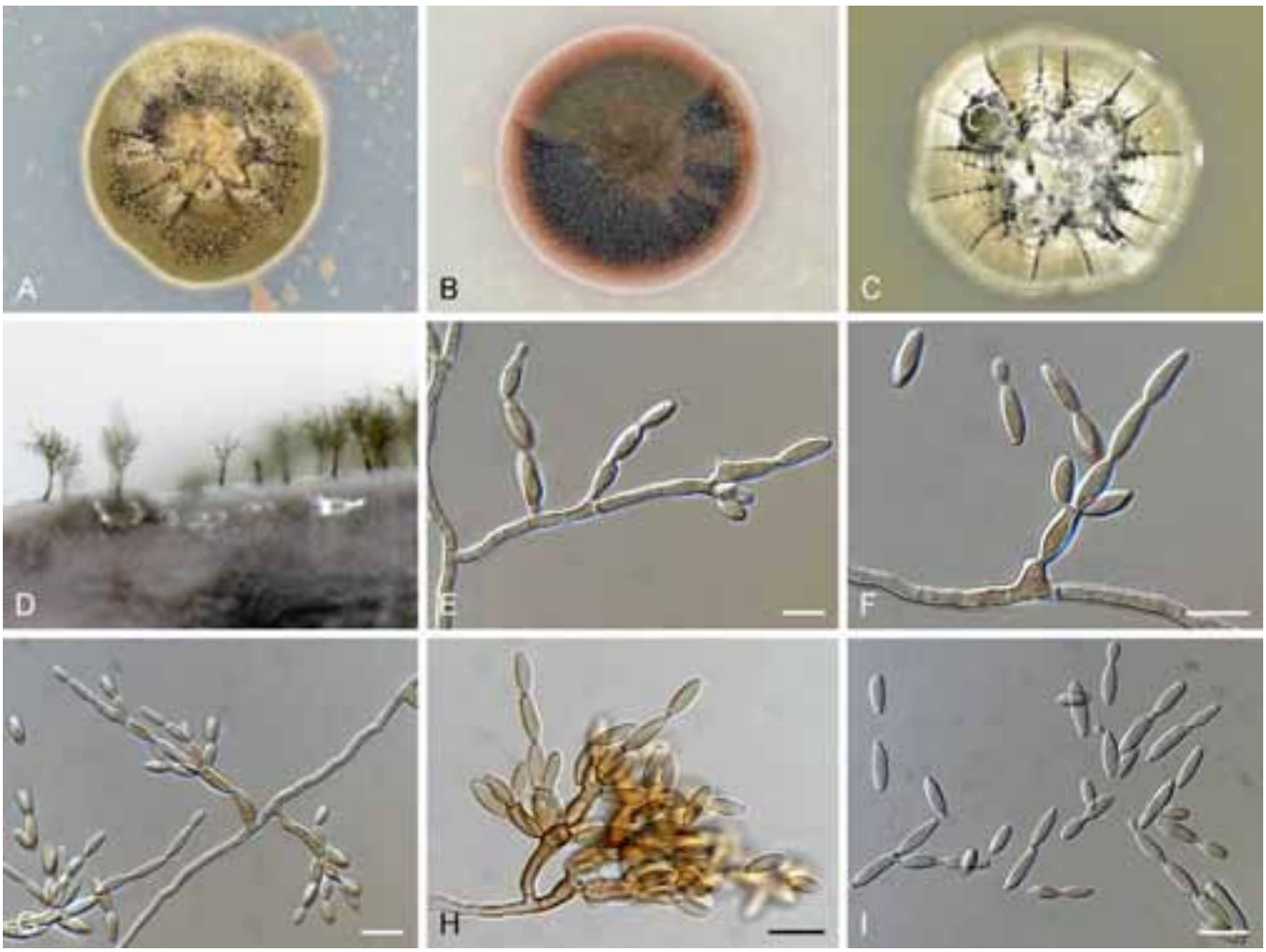

Fig. 5. *Toxicocladosporium immaculatum* (URM 7491 = CBS 141540 – ex-type culture). **A.** Colony sporulating on PDA. **B.** Colony sporulating on OA. **C.** Colony sporulating on MEA. **D.** Conidiophores. **E–G.** Conidiophores and conidia. **H.** Conidiophore and conidia after 1 mo on SNA at 22 ° C. **I.** Ramoconidia and conidia. Bars = 10 µm.

without constrictions in the medial portion or at the septum (Crous *et al*. 2016). Phylogenetically, this species is a distinct taxon closely related to *T. strelitziae* (Fig. 2).

Toxicocladosporium immaculatum J.D.P. Bezerra, C.M. Souza-Motta & Crous, **sp. nov.** MycoBank MB820265
(Fig. 5)

Etymology: Named after its pristine, well-developed, penicillate conidiophores.

Diagnosis: Differs from most *Toxicocladosporium* species by its red to dark red pigmented colonies when grown on OA. Different from *T. ficiniae* mainly by the larger and less septate conidiophores with shorter primary and secondary ramoconidia. Distinguished from *T. posoqueriae* by the slightly reduced conidiophores and conidia, and from *T. rubrigenum* by its less septate macroconidiophores, shorter microconidiophores and somewhat larger conidia.

Type: **Brazil**: *Pernambuco*: Itaíba, Curral Velho Farm, 9º 08.895 S 37º 12.069 W, as endophyte from cactus *Tacinga inamoena*, Sep. 2013, *J.D.P. Bezerra* (URM 90069 – holotype; URM 7491 = CBS 141540 – culture ex-type).

Description: *Mycelium* on SNA consisting of branched, septate, smooth to verruculose, pale brown, 2–3 µm wide hyphae. *Conidiophores* dimorphic, arising from superficial mycelium, erect to sinuous, brown, unbranched, finally verruculose, subcylindrical, straight to flexuous. *Macroconidiophores* up to 100 × 2–3.5 µm, 2–5-septate. *Microconidiophores* sometimes reduced to conidiogenous cells on hyphae, pale brown, smooth to finally verruculose, flexuous, subcylindrical, 12–25 × 2.5–3.5 µm, 0–1-septate. *Conidiogenous cells* integrated, polyblastic, terminal and lateral, smooth, becoming verruculose, brown, 10–14 × 2.5–3.5 µm; scars truncate, thickened and darkened, 1.5–2 µm wide. *Primary ramoconidia* medium brown, finely verruculose, 0–1-septate, subcylindrical, 14.5–22.5 × 2–4 µm. *Secondary ramoconidia* giving rise to branched chains of conidia, subcylindrical, polyblastic, brown, finely verruculose, 0–1-septate, (7–)8–14(–18.5) × 2–3 µm; scars darkened, thickened, 0.5–1 µm wide. *Intercalary conidia* subcylindrical to fusoid-ellipsoidal, brown, finely verruculose to verruculose, 11.5–13 × 2.5–3 µm. *Small terminal conidia* fusoid-ellipsoidal,

brown, finely verruculose, 8–10(–11) × 2–3 µm; hila thickened and darkened, 0.5–1 µm wide.

Culture characteristics (in a day-night cycle, at 22 °C after 3 wk): *Colonies* on MEA are folded and sulcate, velvety, pale grey to olive-yellowish with a very light grey rim, reverse dark brown, reaching 33 mm diam; on OA flat, spreading, with sparse to moderate aerial mycelium, smooth, surface olive, with a light grey rim, reverse dark brown, red to dark red pigmentation produced, growing up to 33 mm diam; and on PDA surface olivaceous to olivaceous yellowish, reverse dark green, with sparse to moderate aerial mycelium, reaching up to 33 mm diam. *Exudate* pale brown to brown on MEA and PDA.

Substrate and distribution: As an endophyte isolated from the cactus *Tacinga inamoena* (*Cactaceae*), Brazil.

Notes: *Toxicocladosporium immaculatum* is phylogenetically closely related to *T. ficiniae, T. posoqueriae* and *T. rubrigenum* (Fig. 2). It differs morphologically from *T. ficiniae* in conidiophore size and septation [up to 100 × 2–3.5 µm (2–5-septate) *vs.* 10–40 × 3–5 µm (1–15-septate) in *T. ficiniae*], conidiogenous cells (10–14 × 2.5–3.5 µm *vs.* 5–15 × 2.5–4 µm in *T. ficiniae*), ramoconidia size (primary 14.5–22.5 × 2–4 µm and secondary 7–18.5 × 2–3 µm *vs.* primary 15–35 × 3–4 µm and secondary 12–20 × 2.5–3 µm in *T. ficiniae*) and intercalary conidia (11.5–13 × 2.5–3 µm *vs.* 9–11 × 2.5–3 µm in *T. ficiniae*). It differs from *T. posoqueriae* in the size of the conidiophores [to 100 × 2–3.5 µm (2–5-septate) *vs.* 50–200 × 4–7 µm (1–3-septate) in *T. posoqueriae*], conidiogenous cells (10–14 × 2.5–3.5 µm *vs.* 10–20 × 4–7 µm in *T. posoqueriae*), ramoconidia (primary 14.5–22.5 × 2–4 µm and secondary 7–18.5 × 2–3 µm *vs.* 5–15 × 4–5 µm in *T. posoqueriae*) and terminal conidia (8–11 × 2–3 µm *vs.* 4–7 × 3–4 µm in *T. posoqueriae*). *Toxicocladosporium rubrigenum* differs in the size of the conidiophores [macroconidiophores to 100 × 2–3.5 µm (2–5-septate) *vs.* to 100 µm × 2–4 µm (1–8-septate) in *T. rubrigenum* and microconidiophores 12–25 × 2.5–3.5 µm *vs.* to 30 × 2–3 µm in *T. rubrigenum*], conidiogenous cells (10–14 × 2.5–3.5 µm *vs.* 15–20 × 2.5–3 µm in *T. rubrigenum*), ramoconidia (primary 14.5–22.5 × 2–4 µm and secondary 7–18.5 × 2–3 µm *vs.* primary 13–16 × 2.5–3.5 µm and secondary 9–14 × 2.5–3.5 µm in *T. rubrigenum*), intercalary conidia (11.5–13 × 2.5–3 µm *vs.* 7–9 × 2–2.5 µm in *T. rubrigenum*), and terminal conidia (8–11 × 2–3 µm *vs.* 4–7 × 2–2.5 µm in *T. rubrigenum*). Furthermore, *T. immaculatum* also differs from these species in colony colour, the presence of a red to dark red pigmentation in OA medium, and slower growth rates.

Toxicocladosporium irritans Crous & U. Braun, *Stud. Mycol.* **58**: 39 (2007).

Type: **Suriname:** *Paramaribo*: isolated from mouldy paint, Feb. 1958, *M.B. Schol-Schwarz* (CBS H-19892 – holotype; CBS 185.58 – culture ex-type).

Description and illustration: Crous *et al.* (2007).

Substrate and distribution: Isolated from mouldy paint, Suriname (Crous *et al.* 2007); ancient laid-paper documents, Portugal (Mesquita *et al.* 2009); associated with patients with atopic dermatitis, Japan (Zhang *et al.* 2011); colonizing tattoo inks, Italy (Bonadonna *et al.* 2014); on parchment manuscripts, Italy (Piñar *et al.* 2015); from human blood and finger nail, USA (Sandoval-Denis *et al.* 2015); on *Adansonia digitata*, South Africa (Cruywagen *et al.* 2015); and on equipment used in the International Space Station or Space Shuttle, Japan (Satoh *et al.* 2016).

Notes: Crous *et al.* (2007) described *Toxicocladosporium irritans* as producing volatile metabolites, which cause a skin rash within minutes of opening an inoculated dish for microscopic examination. Morphologically and phylogenetically it is very similar to *Cladosporium* s. str., and produces dimorphic conidiophores, which is also a feature commonly observed in that genus. It is distinct in having dark, thick-walled conidial and conidiophore septa, and lacking the typical coronate *Cladosporium* scar type (David 1997, Crous *et al.* 2007). In our phylogenetic analyses (Fig. 2), *T. irritans* forms a lineage related to *T. rubrigenum* and *T. hominis*. It differs from *T. rubrigenum* in the size and septation of the conidiophores [30–60 × 4–6 µm (2–7-septate) *vs.* to 100 µm × 2–4 µm (1–8-septate)], conidiogenous cells (7–12 × 3–4 µm *vs.* 15–20 × 2.5–3 µm), ramoconidia (7–15 × 3–5 µm *vs.* primary 13–16 × 2.5–3.5 µm and secondary 9–14 × 2.5–3.5 µm) and terminal conidia (5–10 × 3–5 µm *vs.* 4–7 × 2–2.5 µm). It differs from *T. hominis* in conidiophore size (70–113 × 3–3.5 µm), conidiogenous cells (13–30 × 3–4 µm), ramoconidia (primary 15–32 × 2–4 µm and secondary 11–15 × 2.5–4 µm), and intercalary conidia (9–16 × 3–4 µm).

Toxicocladosporium pini Crous & Y. Zhang ter, *Persoonia* **32**: 269 (2014).

Type: **China**: Beijing, Badaling, 40º20'45.1"N 116º00'48.3"E, on needles of *Pinus* sp. (*Pinaceae*), 1 Sept. 2013, *P.W. Crous & Y. Zhang* (CBS H-21719 – holotype; CPC 23639 = CBS 138005 – culture ex-type).

Description and illustration: Crous *et al.* (2014).

Substrate and distribution: On needles of *Pinus* sp. (*Pinaceae*), China (Crous *et al.* 2014).

Notes: According to Crous *et al*. (2014), *Toxicocladosporium pini* is morphologically similar to *T. pseudovelox* (ramoconidia 0–1-septate, broadly ellipsoid to subcylindrical, 8–15 × 2.5–4 µm; intercalary and terminal conidia ellipsoid, 6–11 × 2–3 µm) and *T. protearum* (ramoconidia 0–1-septate, subcylindrical, 15–20 × 2.5–3.5 µm; intercalary and terminal conidia subcylindrical to narrowly fusoid-ellipsoidal, 9–16 × 2–3 µm). Based on conidial dimensions, *T. pini* can be distinguished from *T. protearum*, but because of its morphological similarity to *T. pseudovelox* it can only be distinguished from that species by DNA data. Phylogenetically, this species is positioned as a distinct lineage between *T. protearum* and *T. strelitziae* (Fig. 2).

Toxicocladosporium posoqueriae Crous & R.G. Shivas, *Persoonia* **29**: 181 (2012).

Type: **Australia:** *Northern Territory*: Darwin, on leaves of *Posoqueria latifolia* (*Rubiaceae*), 12 Apr. 2011, *R.G. Shivas* (CBS H-21086 – holotype; CPC 19305 = CBS 133583 – culture ex-type).

Description and illustration: Crous *et al*. (2012b).

Substrate and distribution: On leaves of *Posoqueria latifolia* (*Rubiaceae*), Australia (Crous *et al*. 2012b).

Notes: According to Crous *et al*. (2012b), *Toxicocladosporium posoqueriae* differs from other members of the genus in that it has whorls of conidiogenous cells, resembling those of *Parapericoniella asterinae* (Heuchert *et al.* 2005, Bensch *et al.* 2012). This species is closely related to *T. ficiniae* which differs in conidiophore size and septation [50–200 × 4–7 µm (1–3-septate) *vs.* 10–40 × 3–5 µm (1–15-septate)], conidiogenous cells (10–20 × 4–7 µm *vs.* 5–15 × 2.5–4 µm), ramoconidia (5–15 × 4–5 µm *vs.* primary 15–35 × 3–4 µm and secondary 12–20 × 2.5–3 µm), intercalary conidia (9–11 × 2.5–3 µm) and terminal conidia (4–7 × 3–4 µm *vs.* 7–9 × 2.5–3 µm). It is also similar to the newly described *T. immaculatum* which differs from in the conidiophores [macroconidiophores to 100 × 2–3.5 µm (2–5-septate) and microconidiophores 12–25 × 2.5–3.5 µm], conidiogenous cells (10–14 × 2.5–3.5 µm), ramoconidia (primary 14.5–22.5 × 2–4 µm and secondary 7–18.5 × 2–3 µm), intercalary conidia (11.5–13 × 2.5–3 µm), and terminal conidia (8–11 × 2–3 µm).

Toxicocladosporium protearum Crous & Roets, *Persoonia* **25**: 135 (2010).

Type: **South Africa**: *Western Cape Province*: Stellenbosch, J.S. Marais Garden, on leaves of *Protea* sp., 22 Apr. 2008, *F. Roets* (CBS H-20490 – holotype; CPC 15254 = CBS 126499 – culture ex-type).

Description and illustration: Crous *et al*. (2010a).

Substrate and distribution: On leaves of *Protea* sp. (*Proteaceae*), South Africa (Crous *et al.* 2010a).

Notes: Blast analyses of the LSU and ITS sequences of *Toxicocladosporium protearum* showed that it is closely related to *T. chlamydosporum* and *T. irritans* (Crous *et al.* 2010a). Morphologically it differs from *T. chlamydosporum* which has smaller intercalary (8–11 × 3–3.5 µm) and terminal (6–10 × 2–3) conidia. Our phylogenetic analyses place *T. protearum* as a distinct lineage between *T. chlamydosporum* and *T. pini* which has larger macroconidiophores (30–90 × 3–4 µm), intercalary conidia (12–14 × 3 µm, 0–1-septate), and smaller terminal conidia (8–11 × 2.5–3 µm, 0–1-septate) (Crous *et al.* 2014).

Toxicocladosporium pseudovelox Crous, *Persoonia* **26**: 81 (2011); as '*pseudoveloxum*'.

Type: **South Africa**: *Western Cape Province*: Hermanus, Fernkloof Nature Reserve, 34º23'38"S 19º16'9.7"E, on leaf bracts of *Phaenocoma prolifera*, 2 May 2010, *K.L. Crous & P.W. Crous* (CBS H-20535 – holotype; CPC 18257 = CBS 128775 – culture ex-type).

Description and illustration: Crous & Groenewald (2011).

Substrate and distribution: On leaf bracts of *Phaenocoma prolifera* (*Asteraceae*), South Africa (Crous & Groenewald 2011).

Notes: Crous & Groenewald (2011) showed that *Toxicocladosporium pseudovelox* was similar to *T. chlamydosporum* and other *Toxicocladosporium* species, but has shorter ramoconidia (8–15 × 2.5–4 µm) than *T. chlamydosporum* (15–18 × 2.5–4 µm). *Toxicocladosporium pseudovelox* is closely related to *T. pini*, which has larger conidiophores [macroconidiophores 30–90 × 3–4 µm (2–8-septate) and microconidiophores 10–17 × 3–4 µm], conidiogenous cells (5–20 × 3–3.5 µm), and intercalary conidia (12–14 × 3 µm, 0–1-septate). *Toxicocladosporium pseudovelox* was placed in a basal position at a highly supported node, which clustered it with *T. pini*, *T. protearum*, and *T. chlamydosporum* (Fig. 2).

Toxicocladosporium rubrigenum Crous & M.J. Wingf., *Persoonia* **22**: 91 (2009).

Type: **Madagascar**: Morondavo, on leaf of *Eucalyptus camaldulensis*, Aug. 2007, *M.J. Wingfield* (CBS H-20195 – holotype; CPC 15735 = CBS 124158 – culture ex-type).

Description and illustration: Crous *et al.* (2009a).

Substrate and distribution: On leaf of *Eucalyptus camaldulensis* (*Myrtaceae*), Madagascar (Crous *et al.* 2009a).

Notes: This species differs from other *Toxicocladosporium* species in the production of densely branched penicillate conidiophores, and colonies that form a prominent red pigment on OA (Crous *et al.* 2009a). *Toxicocladosporium rubrigenum* is phylogenetically related to *T. irritans* and the new species *T. immaculatum* (Fig. 2). It differs from *T. irritans* in having longer and narrower conidiophores and conidiogenous cells (to 100 µm × 2–4 µm and 15–20 × 2.5–3 µm), as well as narrower ramoconidia (13–16 × 2.5–3.5 µm); and from *T. immaculatum* in the size of the conidiophores [macroconidiophores to 100 × 2–3.5 µm (2–5-septate) and microconidiophores 12–25 × 2.5–3.5 µm], conidiogenous cells (10–14 × 2.5–3.5 µm), ramoconidia (primary 14.5–22.5 × 2–4 µm and secondary 7–18.5 × 2–3 µm), intercalary conidia (11.5–13 × 2.5–3 µm), terminal conidia (8–11 × 2–3 µm), and culture characteristics (culture colour, pigmentation in the culture medium, production of exudates, and growth rates).

Toxicocladosporium strelitziae Crous, *Persoonia* **28**: 179 (2012).

Type: **South Africa**: *Mpumalanga Province*: Kruger Game Reserve, Satara Rest Camp, on leaves of *Strelitzia reginae* (*Strelitziaceae*), 11 July 2011, *P.W. Crous* (CBS H-20970 – holotype; CPC 19763, CPC 19762 = CBS 132535 – culture ex-type).

Description and illustration: Crous *et al.* (2012b).

Substrates and distribution: On leaves of *Strelitzia reginae* (*Strelitziaceae*), South Africa (Crous *et al*. 2012b).

Notes: In a previous phylogenetic analysis, *Toxicocladosporium strelitziae* was placed in close proximity to *T. pseudovelox* (Crous *et al.* 2012b), but in the present analysis is placed in a lineage distant from that species with *T. hominis* as the closest relative (Fig. 2). *Toxicocladosporium strelitziae* is distinct from *T. pseudovelox* in having longer, narrower conidiophores (40–70 × 2–3.5 µm *vs.* 20–50 × 3–4 µm in *T. pseudovelox*), and larger, aseptate ramoconidia (12–20 × 2–3.5 µm *vs.* 8–15 × 2.5–4 µm, 0–1-septate in *T. pseudovelox*), and from *T. hominis* which has larger conidiophores (40–70 × 2–3.5 µm in *T. strelitziae vs.* 70–113 × 3–3.5 µm in *T. hominis*), conidiogenous cells (10–15 × 2.5–3.5 µm in *T. strelitziae vs.* 13–30 × 3–4 µm), ramoconidia [primary 12–20 × 2–3.5 µm (aseptate) and secondary 10–17 × 2–3.5 µm (aseptate) in *T. strelitziae vs.* primary 15–32 × 2–4 µm (0–2-septate) and secondary 11–15 × 2.5–4 µm (0–1-septate) in *T. hominis*], and intercalary conidia [10–12 × 2–2.5 µm in *T. strelitziae vs.* 9–16 × 3–4 µm (0–1-septate) in *T. hominis*].

DISCUSSION

The generic name *Toxicocladosporium* was introduced by Crous *et al.* (2007) to accommodate fungi similar to *Cladosporium* species but with different conidiophore and conidium morphology and phylogeny. Following this description, several new species were reported mainly as epiphytic, saprobic or phytopathogenic fungi from all continents (Crous *et al.* 2009a, 2010a, b, 2011a, 2012a, b, 2013, 2014, 2016, Crous & Groenewald 2011). However, in contrast to *Cladosporium*, *Toxicocladosporium* species had not previously been reported as endophytic fungi (Bensch *et al.* 2012, Bezerra *et al.* 2012, 2013). The isolation of novel *Toxicocladosporium* species as endophytic fungi from cacti in a tropical dry forest (Caatinga) in Brazil is reported here for the first time, and illustrates the diversity of fungi present as endophytes in different hosts and ecosystems.

In this study we revisited all currently published species of *Toxicocladosporium* using morphology and phylogenetic analyses (including three new loci). Based on these data we proposed two new species and one new closely related genus. Using a multigene phylogeny to recognise taxa in *Dothideomycetes*, Schoch *et al*. (2006) showed that *Cladosporium* belongs to the family *Cladosporiaceae* (an older name for the previously published *Davidiellaceae*). Later, during the investigation of cladosporium-like taxa, Crous *et al.* (2007) studied several isolates and proposed different genera based on their morphology and phylogeny, using sequences of part of the LSU nrDNA. In their phylogenetic reconstruction, six new genera were proposed, including *Rachicladosporium*, *Toxicocladosporium,* and *Verrucocladosporium* as *incertae sedis*. Bensch *et al.* (2012) monographed the genus *Cladosporium* and showed that it belongs to the family *Cladosporiaceae* (*Capnodiales*, *Dothideomycetes*) along with other four genera, *Graphiopsis*, *Rachicladosporium*, *Toxicocladosporium*, and *Verrucocladosporium*. Using ITS, LSU and *rpb2* sequences from these genera, from all *Toxicocladosporium* species and from the other six families in *Capnodiales* we reconstructed the phylogenetic relationships of *Cladosporiaceae* and determined the phylogenetic position of each genus, including the newly described genus *Neocladosporium* (Fig. 1). Our results are similar to those of Bensch *et al.* (2012), who used LSU sequences to verify the relationship among these genera of the *Cladosporiaceae*.

Sandoval-Denis *et al.* (2015) studied clinical samples from the USA and reported the isolation of *Cladosporium* and *Toxicocladosporium* mainly obtained from respiratory specimens. These authors used phylogenetic analyses from all the available ITS and LSU sequences of *Toxicocladosporium* species except *T. leucadendri*, as well as morphological characters to identify two isolates as *T. irritans*, while a third isolate was unidentified, but phylogenetically positioned in a lineage between *T. rubrigenum* and *T. strelitziae*. In a subsequent paper the unidentified isolate was published as a new species, *T. hominis* (Crous *et al.* 2016). Sandoval-Denis *et al*. (2015) may not have included sequences from *T. leucadendri* in their analyses because this species appeared as a different genus, not belonging to *Toxicocladosporium s. str*. *Toxicocladosporium leucadendri* (CPC 18315 = CBS 131317) was published by Crous *et al*. (2011a) based on megablast searches in combination with culture characteristics, and conidiophore and conidial dimensions. Also, the phylogenetic analyses of the ITS and LSU sequences showed this strain in a single clade between *Graphiopsis chlorocephala* and *Verrucocladosporium dirinae*. The same result was observed in our phylogenetic analyses using the same loci, and also using *actA*, *rpb2* and *tub2* sequences. Based on these results, we introduced the new genus, *Neocladosporium*, with *N. leucadendri* as type species. Furthermore, based on the phylogenetic position and the small nucleotide differences between *T. chlamydosporum* and *T. velox*, we treat them as conspecific. These similarities can be also observed in the phylogenetic reconstruction published by Crous & Groenewald (2011), where *T. velox* and *T. chlamydosporum* are placed in the same clade with a high bootstrap support value. In addition, these authors used few morphological characters, such as the colour and size of conidia (darker brown and somewhat larger), absence and/or presence of chlamydospores and growth in culture to separate these species. These features are now combined in the revised circumscription of *T. chlamydosporum* presented here.

To improve the discrimination of species in the genus *Toxicocladosporium,* we generated *actA*, *rpb2* and *tub2* sequences from all the available ex-type strains as well as endophytic isolates generated in this study (Fig. 2). In our analyses using a combined matrix of ITS, LSU, *actA*, *rpb2* and *tub2* sequences, we recognise 13 species in this genus, including the two new species, *T. cacti* and *T. immaculatum*. As previously demonstrated in *Cladosporium* by different authors (Braun *et al.* 2003, Schubert *et al.* 2007b, Zalar *et al.* 2007, Bensch *et al*. 2010, 2012, 2015, Sandoval-Denis *et al.* 2016), ITS, and LSU sequences are less informative than *actA*, *rpb2* and *tub2* sequences to separate species in

Toxicocladosporium. In our analyses, rDNA sequences were very similar among some species, but are useful to separate genera (LSU) and species groups (ITS). After inclusion of *actA* sequences, the third most informative region after *rpb2* and *tub2*, respectively, the separation of species was improved. Sequences of *rpb2*, followed by *tub2* were the best loci to recognise species in our analyses. We therefore recommend these markers as barcoding targets for species recognition as well as for the description of new taxa in addition to ITS and *actA* sequences in this genus. The inclusion of *actA*, *rpb2* and *tub2* sequences in our analyses was crucial to facilitate the separation of *T. cacti* from *T. banksiae*, since rDNA sequences from endophytic isolates were closely related to *T. banksiae*, but could not unambiguously resolve both species. In contrast, the *actA, rpb2* or *tub2* loci consistently separate these two taxa with high statistical confidence (data not shown). A similar situation was observed in *Cladosporium* for which a combined phylogenetic analysis including ITS, translation elongation factor 1-alpha (*tef1*) and *actA* loci has been adopted in order to separate species within that genus, with ITS being the least informative locus (Bensch *et al.* 2012, 2015, Sandoval-Denis *et al.* 2016).

Our study shows that *Toxicocladosporium* species, as those of *Cladosporium* (Bensch *et al.* 2012, Bezerra *et al*. 2012, 2013), may be isolated as endophytic fungi from plants growing in tropical dry regions. This report also expands our knowledge about endophytes associated with cacti and highlights the mostly underestimated fungal diversity associated with this little-studied group of host plants, and as well as the importance of protecting them in their natural habitats.

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