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

Article info: Submitted: 16 March 2017; Accepted: 24 April 2017; Published: 1 May 2017.

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) and a human bronchoalveolar lavage fluid specimen (Crous et al. 2016), respectively. In addition to species descriptions in this genus, few reports of 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

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. iritans* colonising tattoo inks. These reports may show similarities because the first isolation of *T. iritans* was associated with mouldy paint in Suriname (Crous et al. 2007). *Toxicocladosporium iritans* 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 (Nha 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 seborrhoeic 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, Nha 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-Garcia 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, Biuei municipality, Pernambuco state, Brazil (8°36′35″ S, 37°12′06″ W), and sustainable family farming plots, Itaíba municipality, Pernambuco state, Brazil (9°′ 08.895″ S, 37° 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 LRS (Vilgalys & Hester 1990), ITS5 and ITS4 (White et al. 1990), ACT-512F and ACT-738R (Carbone & Kohn 1999), B2a and B2b or B10 (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, 2012, 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 (Swoford 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 consistency 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 lineage 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 | Substrate/host (country) | GenBank accession numbers |
|--------------------------|-----------------------|--------------------------|--------------------------|
| **Acrodontium crateriforme** | CBS 144.33T (ex-type of *Chloridium crateriforme*) | Tuberculina maxima (The Netherlands) | FN666565 KX286952 KX288399 |
| A. luzulae               | CBS 841.71T           | On leaf of Carax 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 121624ET = CPC 12211 | On *Hordeum vulgare* (Belgium) | EF679350 KJ564335 LT799751 |
| C. chalastosporoides    | CBS 125985ET = CPC 13864 | On *Protea arborea* (South Africa) | HM148001 KJ564332 LT799751 |
| C. fusiforme            | CBS 119414T           | Hypersaline water of Secovlje saltm (Slovenia) | DQ780388 KJ564333 LTS99752 |
| C. herbarum             | CBS 121621ET = CPC 12177 | On *Hordeum vulgare* (The Netherlands) | EF679363 KJ564333 LT799752 |
| C. hillianum            | CBS 125986 = CPC 15459 | On Typha orientalis (New Zealand) | HM148097 KJ564334 |
| C. iridis               | CBS 138.40ET (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 342.82T           | On *Medicago lupulina* (Germany) | NR_119427 EU019266 |
| D. eucalypti            | CBS 132084 = CPC 18969 | On *Malus domestica* fruit (USA) | JQ622084 JQ622092 |
| D. proteae              | CBS 122900ET = CPC 13853 | On leaves of *Protea* sp. (Spain) | EU707897 EU707897 |
| Extremus adstrictus     | CBS 118292ET = TRN96 (ex-type of *Devriesia adstricta*) | Rock sample (Spain) | NR_144954 KF310022 LT799753 |
| E. antarcticus          | CBS 136103ET = CCFEE 451 (ex-type of *Devriesia antarctica*) | Rock sample (Antarctica) | NR_138389 GU250360 |
| **Graphiopsis chlorocephala** | CBS 136104 = CCFEE 5207 | Rock sample (Antarctica) | KF309980 KF310021 LT799753 |
| M. eucalypti            | CBS 121522 = CPC 11383 | On leaves of *Paeonia delavayi* (Germany) | EU009457 EU009457 LT799753 |
| MAFF 410081             | CBS 100405            | On leaf and stem lesions on *Paeonia* sp. (New Zealand) | EU009456 EU009456 KT216520 |
| Mycosphaerella sumatrensis | CBS 118499T = CPC 11171 (ex-type of *Mycosphaerella sumatrensis*) | On *Eucalyptus* sp. (Indonesia) | KF901655 KF901994 LT799754 |

| Species                  | Strain/isolate number | Substrate/host (country) | GenBank accession numbers |
|--------------------------|-----------------------|--------------------------|--------------------------|
| **Graphiopsis chlorocephala** | CBS 136104 = CCFEE 5207 | Rock sample (Antarctica) | KF309980 KF310021 LT799753 |
| M. eucalypti            | CBS 121522 = CPC 11383 | On leaves of *Paeonia delavayi* (Germany) | EU009457 EU009457 LT799753 |
| MAFF 410081             | CBS 100405            | On leaf and stem lesions on *Paeonia* sp. (New Zealand) | EU009456 EU009456 KT216520 |
| Mycosphaerella sumatrensis | CBS 118499T = CPC 11171 (ex-type of *Mycosphaerella sumatrensis*) | On *Eucalyptus* sp. (Indonesia) | KF901655 KF901994 LT799754 |
| Species                        | Strain/isolate number¹ | Substrate/host (country)                  | GenBank accession numbers² |
|-------------------------------|------------------------|------------------------------------------|---------------------------|
| **Toxicocladosporium**        |                        |                                          |                           |
| *leucadendri* gen. sp. nov.   | CBS 131317⁷ = CPC 18315 (ex-type of *Toxicocladosporium leucadendri*) | On leaves of Leucadendron sp. (South Africa) | JQ044436 JQ044455 LT821376 LT799755 KY706602 |
| Neocladosporium               | CBS 29090              | On leaves of Kunzea pauciflora (Australia) | LT799737 LT799744          |
| Neocladosporium               | CBS 29092              | On leaves of Hakea marginata (Australia)  | LT799738 LT799745          |
| Neocladosporium               | CBS 29166              | On leaves of Hakea sp. (Australia)        | LT799739 LT799746          |
| Neocladosporium               | CBS 29237              | On leaves of Banksia media (Australia)     | LT799740 LT799747          |
| Neocladosporium               | CBS 29545              | On leaves of Petrophile sp. (Australia)    | LT799741 LT799748          |
| Neodevriesia hilliana         | CBS 123187¹ = 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)                       | NR_144962 HQ599606        |
| *N. xanthorrhoeae*            | CBS 128219¹ = CPC 17720 (ex-type of *Devriesia xanthorrhoea*) | 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         |
| *P. robusta*                  | CBS 111175¹ = CPC 1269 | On Eucalyptus robur (Malaysia)            | KF901678 KF902020         |
| *P. schizolobii*              | CBS 120029⁷ = CPC 12962 (ex-type of *Passalora schizolobii*) | On Schizolobium parahybum (Ecuador)       | KF251322 KF251826         |
| *Rachicladosporium cbiolae*   | CBS 125424⁷ = CPC 14034 | On twig debris (USA)                      | GU214650 GU214484 LT799763 |
| *R. luciae*                   | CBS 121620⁴ = CPC 11407 | On leaf of Lucilia sp. (New Zealand)       | EU040237 EU040237         |
| *R. pini*                     | CBS 129525⁷ = CPC 16770 | On needles of Pinus monophylla (The Netherlands) | JF951145 JF951165 LT799764 |
| *Ramichloridium apiculatum*   | CBS 400.76             | Soil (Pakistan)                           | EU041794 EU041851         |
| *R. cucurbitae*               | CBS 132087⁷ = CPC 19423 | On fruit of Cucurbita maxima (USA)         | NR_120082 NG042613        |
| *R. luteum*                   | CBS 132088⁷ = CPC 18961 | On fruit of Malus domestica (China)        | NR_119684 JQ622099        |
| *Ramularia endophylla*        | CBS 113265⁷ (ex-epitype of *Sphaeria punctiformis*) | On dead leaves of Quercus robur     | KF901725 KF902072 KF894673 |
| *R. gefenii*                  | CBS 129441¹            | From human bronchoalveolar lavage fluid (The Netherlands) | KJ504769 KJ504728 KJ504640 |
| *Readeriella menaiensis*      | CBS 125003⁵ = CPC 14447 | On leaves of Eucalyptus oblonga (Australia) | KX348084 KF901870 KX348084 |
| *R. tasmanica*                | CBS 125002² = CPC 13631 | On leaves of Eucalyptus delegatensis (Australia) | KF901761 KF902116 KX348086 |
| Species                     | Strain/isolate number\(^1\) | Substrate/host (country)                                                                 | GenBank accession numbers\(^2\) |
|-----------------------------|------------------------------|------------------------------------------------------------------------------------------|--------------------------------|
| *Schizothyrium pomi*        | CBS 486.50                   | On Polygonum sachalinense (The Netherlands)                                                | EF134948 KF902024 |
|                             | CBS 228.57                   | Unknown host (Italy)                                                                     | EF134947 KF902007 |
| *Teratosphaeria fibrillosa* | CBS 121707\(^2\) = CPC 13960 | On leaves of Protea sp. (South Africa)                                                    | KF901728 KF902075 LT799765 |
| *T. fimbriata*              | CBS 120736\(^2\) = CPC 13324 | 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\(^5\) = CPC 17280 | On leaves of Banksia emulata (Australia)                                                  | HQ599598 HQ599599 LT821371 LT799767 KY706597 |
| *T. cacti* sp. nov.         | URM 7489\(^7\) = 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\(^7\) = CPC 15709 | On leaf of Eucalyptus camaldulensis (Madagascar)                                           | FJ790283 FJ790301 LT821372 LT799778 KY706598 |
|                             | CBS 124159\(^7\) = CPC 15736 | 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⁾ |
|-------------------------|--------------------------|----------------------------------------------------------------------------------------|------------------------------|
| T. ficiniae             | CBS 136406⁽²⁾ = CPC 21283 | On leaves of Ficinia indica (South Africa)                                               | KF777190 KF777241 LT821373 LT799780 KY706599 |
| T. hominis              | CBS 140694⁽³⁾ = FMR 13297 | From human bronchoalveolar lavage fluid (USA)                                            | LN834444 KY752829 LT821374 LT799781 KY706600 |
| T. immaculatum sp. nov. | URM 7491⁽¹⁾ = CBS 141540  | Endophyte from Melocactus zehntneri (Brazil)                                            | KY752815 KY752828 LT821370 LT799782 KY706596 |
| T. irritans             | CBS 185.58⁽⁴⁾            | From mouldy paint (Suriname)                                                             | EU040243 EU040243 LT821375 LT799783 KY706601 |
| T. pini                 | CBS 138005⁽⁵⁾ = CPC 23639 | On needles of Pinus sp. (China)                                                          | KJ869160 KJ869217 LT821377 LT799784 KY706603 |
| T. posoqueriae          | CBS 133583⁽³⁾ = CPC 19305 | On leaves of Posoqueria latifolia (Australia)                                          | NR121555 KC005803 LT821378 LT799785 KY706604 |
| T. proteanum            | CBS 126499⁽⁶⁾ = CPC 15254 | On leaves of Protea burchellii (South Africa)                                           | HQ599586 HQ599587 LT821379 LT799786 KY706605 |
| T. pseudoveloxum        | CBS 128775⁽⁷⁾ = CPC 18527 | On leaf bracts of Phaenocoma prolifera (South Africa)                                  | JF499847 JF499867 LT821380 KY706606 |
| T. rubrigenum           | CBS 124158⁽⁷⁾ = CPC 15735 | On leaf of Eucalyptus carreadulensis (Madagascar)                                       | FJ790027 FJ790035 LT821381 LT799787 KY706607 |
| T. strelitziae          | CBS 132535⁽⁸⁾ = CPC 19762 | On leaves of Strelitzia reginae (South Africa)                                          | NR111765 JX069858 LT821382 LT799788 KY706608 |
| Undescribed species     | CPC 29 168               | On Cyperaceae (Australia)                                                               | LT799742 LT799749 LT799789 |
| Undescribed species     | CPC 29 170               | On Cyperaceae (Australia)                                                               | LT799743 LT799750 LT799790 |
| Uwebraunia australiensis| CBS 120729⁽⁹⁾ = CPC 13282 | On Eucalyptus platypylla (Australia)                                                   | KF442513 GQ852588 LT799791 |
| U. commune              | CBS 110809⁽⁹⁾ = CPC 830  | On Eucalyptus nitens (South Africa)                                                    | AY725536 KJ564336 |
| U. dekkeri              | CPC 13264                | On Eucalyptus molucana (Australia)                                                     | GQ852741 GQ852593 LT799792 |
| Verrucocladosporium dirinae | CBS 112794⁽¹⁾          | On lichen Dirina massiliensis (United Kingdom)                                         | EU040244 EU040244 |

1 CBS: Westerdijk Fungal Biodiversity Institute, Uppsalaalaan 8, 3554 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: Facultad de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, Reus, Spain; JB: Collection of J.D.P. Bezerra, housed at URN; TRN: C. Rubal private collection, currently in MAF; URM: Micoloca URM Profa. Maria Auxiliadora Cavalcanti, Departamento de Micologia Prof. Chaves Batista, Universidade Federal de Pernambuco, Brazil.⁽¹⁾ ex-type strain,⁽²⁾ ex-epitype strain.

2 ITS: first and second internal transcribed spacer regions and intervening 5.8S rDNA; 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-LS) 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. * = ex-(holo-)type strain, ** = ex-epitype strain.
New Toxicocladosporium species from cacti in Brazil

The results of this analysis are shown in Fig. 2. 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/BPP). A fully-supported clade is indicated with a bold line. The species resolution, actA, rpb2, and tub2 datasets 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: 48, LSU: 28, rpb2: 125, and tub2: 230). 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 lineages: 12 isolates formed a fully-supported clade close to T. banksiae (CBS 128215) (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. banksiae as a synonym of T. chlamydosporum

The species resolution, actA, rpb2, and tub2 datasets 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: 48, LSU: 28, rpb2: 125, and tub2: 230). 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 lineages: 12 isolates formed a fully-supported clade close to T. banksiae (CBS 128215) (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. banksiae as a synonym of T. chlamydosporum.

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.

The results of this analysis are shown in Fig. 2. 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/BPP). A fully-supported clade is indicated with a bold line. The species resolution, actA, rpb2, and tub2 datasets 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: 48, 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 lineages: 12 isolates formed a fully-supported clade close to T. banksiae (CBS 128215) (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. banksiae as a synonym of T. chlamydosporum.

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         |
|------------------------|----------------------------------------|---------------------------|--------------------------------------|---------------------------------|---------------------|
| **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) [0–1] | 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] | 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] | 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] | 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 | 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] | 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    | This paper |
| **T. irritans**         | 30–60 × 4–6 [2–7]                      | 10–30 × 2.5–4             | 7–12 × 3–4                           | 7–15 × 3–5 [0–1] [1–3]           | -                   | 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] | Crous et al. (2014) |
| **T. posoqueriae**      | 50–200 × 4–7 [1–3]                     | -                         | 10–20 × 4–7                          | 5–15 × 4–5 [0]                  | -                   | 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]            | -                   | Crous et al. (2010) |
| **T. pseudovelox**      | 20–50 × 3–4 [2–5]                      | -                         | 10–15 × 3–4                          | 8–15 × 2.5–4 [0–1]              | -                   | 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) | Crous et al. (2009) |
| **T. streltziae**       | 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 | 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

Etyymology: 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 29168); 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 NCBI’s 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 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. leuca-
dendri.
**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, 26°34’14.0”S 153°4’21.6”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, 8°36’35”S 37°14’40”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, 8°36’35”S 37°14’40”W, as endophytic fungus from cactus *Pilosocereus gounellei* subsp. *gounellei*, Sep. 2013, J.D.P. Bezerra.
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(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).

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![Fig. 4.](image_url) "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.
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-oblung. *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 × 2.5–3 μm (conidia dark brown and verruculose on MEA) (based on Crous et al. 2009a).

*Cultivar 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 sclerotic 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 sclerotal 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 conidiофores [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 × 3–5 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 conidiofere 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 conidigenous 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 conidigenous 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,
New Toxicocladosporium species from cacti in Brazil

**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: *Pemambuco*: Italba, Curral Velho Farm, 9° 08.895 S 37° 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 red dark 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 × 2.5–2 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. 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 × 2.25 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, 40°20′45.1″N 116°00′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. strelitziæ* (Fig. 2).
**Toxicocladosporium species from cacti in Brazil**

**Type:** Australia: Northern Territory: Darwin, on leaves of Posoqueria latifolia (Rubiaceae), 12 Apr. 2011, R.G. Shivash (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) 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 protearum was placed in a basal position at a highly supported node, which clustered with T. pini, T. protearum, and T. chlamydosporum. (Fig. 2).

**Toxicocladosporium pseudovelox** Crous, Persoonia 26: 81 (2011); as ‘pseudooveloxum’.

**Type:** South Africa: Western Cape Province: Hermanus, Fernkloof Nature Reserve, 34°23′38″S 19°16′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 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 larger 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), and terminal conidia (8–11 × 2–3 μm).

**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).

**Substrate and distribution:** On leaves of Posoqueria latifolia (Rubiaceae), South Africa (Crous & Groenewald 2011).

**Notes:** This species is closely related to 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).
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, asperate 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), conidigenous 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 Verrucocladosprium 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 Verrucocladosprium. Using ITS, LSU and rpb2 sequences from these genera, from all Toxicocladosporium species and from the other six families in Capnodiales we re-constructed 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 loci has been adopted in order to separate species within that genus, 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.

ACKNOWLEDGEMENTS

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Process 203132/2014-9), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Fundação de Amparo à Ciência e Tecnologia de Pernambuco (FACEPE) of Brazil for financial support and scholarships. We also thank Konstanze Bensch and David L. Hawksworth for the valuable comments and suggestions to improve the manuscript. We extend our thanks to the Universidade Federal de Pernambuco and to the MycoBank: an online initiative to launch mycology into the 21st century. MycoBank, the teleomorph of Cladosporium s. str. Mycological Progress 2: 3–18. Braun U, Crous PW, Schubert K (2008) Taxonomic revision of the genus Cladosporium s. lat. 8. Reinroduction of Graphiopsis (=Dichocladosporium) with further reassessments of cladosporoid hyphomycetes. Mycota 103: 207–216.

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