MAYARA LUISA ROCHA FREITAS

NEW SPECIES AND NEW RECORDS OF CONIDIAL FUNGI FROM SUBMERGED DECAYED LEAVES IN BRAZIL

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de *Magister Scientiae*.

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APROVADA: 16 de julho de 2018.

Maria Catarina Megumi Kasuya

André Wilson Campos Rosado

Olinto Liparini Pereira
(Orientador)

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BIOGRAFIA

MAYARA LUISA ROCHA FREITAS, filha de Luis Carlos de Freitas e Joelma Eleotério Rocha Freitas, nasceu em Timóteo, Minas Gerais, no dia 10 de julho de 1990.

Em 2010 ingressou no curso de graduação em Ciências Biológicas na Universidade Federal de Viçosa. Participou do Programa Ciências Sem Fronteiras em 2014 na University of the West of England, Inglaterra, Reino Unido. Graduou-se em julho de 2016.

Em agosto de 2016, iniciou o mestrado no Programa de Pós-Graduação em Microbiologia Agrícola na Universidade Federal de Viçosa, concentrando seus estudos na área de micologia (taxonomia e filogenia molecular de fungos conidiais).

SUMÁRIO

ABSTRACT

FREITAS, Mayara Luisa Rocha, M.Sc., Universidade Federal de Viçosa, July, 2018. **New species and new records of conidial fungi from submerged decayed leaves in Brazil**. Adviser: Olinto Liparini Pereira

Fungi are recognized as cosmopolitan and carry out various ecological functions in the ecosystem, including the decomposition of organic matter. They recycle the litter present in the soil, contributing to mineralization of nutrients. In aquatic environments, this saprophytic role contributes to increase the palatability of plant material used by organisms of other trophic levels. Several representatives of the Fungi kingdom are present in the aquatic environments and, among these, the conidial fungi. In the Atlantic Forest biome, studies on fungi in the aquatic environment are still scarce. The aim of this work was to carry out a taxonomic and phylogenetic study of the conidial fungi species associated with decomposed plant substrates submerged in water bodies of three forest fragments located in the Serra do Brigadeiro, Minas Gerais. The collected samples were taken to the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas of Universidade Federal de Viçosa, where the isolations and identification of associated fungi were made. The genus *Cladosporium* was the most abundant fungal group. By means of the morphology of vegetative and reproductive structures of this fungus, the growth of in vitro cultures and phylogenetic analyzes, it was concluded that all the isolates belong to the *Cladosporium cladosporioides* complex, two species reported for the first time in Brazil and a new species be proposed. This study contributes to increasing the knowledge of the diversity of conidial fungi in aquatic environments in the Brazilian Atlantic Forest and emphasizes the importance of exploring new habitats in mycological researches.

RESUMO

FREITAS, Mayara Luisa Rocha, M.Sc., Universidade Federal de Viçosa, julho de 2018. **Nova espécie e novos relatos de fungos conidiais em serapilheira submersa no Brasil**. Orientador: Olinto Liparini Pereira

Os fungos são reconhecidamente cosmopolitas e desenvolvem diversas funções ecológicas no ecossistema, entre elas a decomposição da matéria orgânica. Eles reciclam a serapilheira presente no solo, contribuindo para mineralização de nutrientes. Nos ambientes aquáticos, esse papel saprofítico, contribui para aumentar a palatabilidade do material vegetal utilizado por organismos de outros níveis tróficos. Diversos representantes do reino Fungi estão presentes nos ambientes aquáticos e, dentre esses, os fungos conidiais. No bioma da Mata Atlântica os estudos sobre fungos no ambiente aquático ainda são escassos. Assim, o presente trabalho teve como objetivo realizar um estudo taxonômico e filogenético das espécies de fungos conidiais associadas a substratos vegetais em decomposição submersos em corpos d'água de três fragmentos florestais localizados na Serra do Brigadeiro, Minas Gerais. As amostras coletadas foram levadas ao Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas da Universidade Federal de Viçosa onde foram feitos os isolamentos e identificação dos fungos associados. O gênero *Cladosporium* foi o grupo de fungos mais abundante. Por meio da morfologia das estruturas vegetativas e reprodutivas desse fungo, do crescimento das culturas *in vitro* e de análises filogenéticas, concluiu-se que todos os isolados pertencem ao complexo *Cladosporium cladosporioides*, sendo dois deles relatados pela primeira vez no Brasil e uma espécie nova a ser proposta. Este estudo contribui para ampliar o conhecimento da diversidade de fungos conidiais em ambientes aquáticos na Mata Atlântica Brasileira e ressalta a importância de se explorar novos habitats em pesquisas micológicas.

INTRODUÇÃO GERAL

Os hifomicetes aquáticos pertencem ao reino Fungi (Hibbett *et al*. 2007), geralmente apresentam conídios tetraradiados ou sigmóides (Dang *et al*. 2007) e a maioria das espécies descritas pertencem ao filo Ascomycota, enquanto pouquíssimas pertencem ao filo Basidiomycota (Shearer *et al.* 2007). Eles também são conhecidos como hifomicetos de água doce ou fungos Ingoldianos (Bärlocher 1992) e foram observados pela primeira vez em um pequeno riacho na cidade de Leicester, Reino Unido. Ingold (1942) observou a presença de esporos na superfície da espuma formada pelo fluxo contínuo de água no local. Posteriormente foram encontrados fungos que estavam crescendo e esporulando em folhas da serapilheira com esporos semelhantes aos anteriormente encontrados, no leito do rio (Ingold 1942) certificando, assim, a origem daqueles esporos na superfície aquática.

Acredita-se que com o resultado da intensa colonização desses fungos na serapilheira submersa ocorra um aumento da atividade exoenzimatica local, contribuindo para degradação das folhas, tornando-as palatáveis para invertebrados aquáticos que vivem nesses ambientes (Bärlocher 1985, 2005, Raviraja *et al*. 1998). Sendo assim, esses fungos são importantes como intermediários de fluxo de energia entre a matéria orgânica e os invertebrados presente nos rios (Bärlocher 1985).

A biodiversidade desses fungos é intensamente estudada nas regiões temperadas, no entanto, mais estudos sobre sua caracterização e diversidade nas regiões tropicais são necessários (Wong *et al*. 1998, Duarte *et al*. 2016). Pequenos riachos e lagos encontrados em florestas tropicais têm se mostrado como potenciais habitats para fungos conidiais que ainda não foram descritos na literatura (Castañeda *et al*. 2006). Atualmente, no Brasil, os estudos têm se concentrado principalmente nos estados da Bahia e em São Paulo (Barbosa

et al. 2008, Cruz & Gusmão 2009, Monteiro & Gusmão 2014, Costa & Gusmão 2017, Fiuza *et al*. 2017). No entanto, ainda faltam informações básicas sobre a diversidade taxonômica desse grupo nas regiões de Mata Atlântica do estado de Minas Gerais, o que implica em desvendar a diversidade dos fungos conidiais nesse bioma e seu papel no ecossistema.

Sendo assim, o objetivo do presente trabalho foi explorar a diversidade dos fungos conidiais na mesorregião da Zona da Mata Mineira. O estudo foi baseado em análises filogenéticas e morfológicas do principal grupo de fungos encontrados, o gênero *Cladosporium*.

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Artigo

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New species and new records of conidial fungi from submerged decayed leaves in Brazil

New species and new records of conidial fungi from submerged decayed leaves in Brazil

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Abstract

Aquatic hyphomycetes are conidial fungi, whose sexual phases is in the Ascomycota or Basidiomycota phyla. We surveyed *Cladosporium* spp. associated with submerged leaf litter, from three localities belonging to Atlantic Forest biome in the State of Minas Gerais, Brazil. The species *Cladosporium angulosum* and *Cladosporium anthropophilum* are reported for the first time in Brazil, and a new species for the genus will be proposed. A multilocus DNA sequence typing approach employing ITS, Actin and Translation elongation 1-α factor region/genes, associated with morphological and cultural analysis, were used to identify these species.

Introduction

The genus *Cladosporium* was initially described by Persoon (1794) as *Dematium herbarum* Pers., which was later established by Link (1816) as *Cladosporium herbarum* (Pers.) (Heuchert *et al*. 2005; Schubert 2005; Crous *et al*. 2007b). Since then, several authors accepted this genus and many dematiaceous hyphomycetes were incorrectly classified as *Cladosporium*, due to the morphological similarity between the groups, as formation of catenulate conidia. Nevertheless, nowadays the genus *Cladosporium* is considered to have its exclusive morphological characteristic, a type of scar and conidial hila, which is characterized by a central convex part (dome), surrounded by a raised periclinal rim, namely "coronate", which distinguished it from other similar genera (David 1997; Braun *et al*. 2003).

Cladosporium is a cosmopolitan genus and commonly isolated from various substrates (Bensch *et al*., 2012; Crous *et al*., 2007a; Seifert *et al*., 2011). Species of this dematiaceous fungi exhibit different lifestyles. Some species may inhabit outdoor and indoor environments and others are able to live in extreme environments (Bensch *et al*., 2012, Grum-Grzhimaylo *et al*., 2016, Bensch *et al*., 2018). They perform symbiotic relationships with plants, such as potential use as biological control agent (Abdel-Baky & Abdel-Salam 2003, Torres *et al.* 2017). Some species of *Cladosporium* are known to be important plant pathogens, which may occur on stems, leaves and fruits on different hosts (Bensch *et al*. 2012).

Cladosporium has holoblastic conidiogenesis with amero- or phragmoconidia formed in acropetal chains (Bensch *et al*. 2012). Morphologically, it is characterized by the ramoconidia, secondary ramoconida, intercalary conidia and small terminal conidia (Schubert *et al*. 2007). Recent researches have presented polyphasic approaches carried out to delimitate the three species complexes, *Cladosporium herbaum* (Schubert *et al*.

2007), *Cladosporium sphaerospermum* (Dugan *et al*. 2008) and *Cladosporium cladosporioides* (Bensch *et al*. 2010). Among the approaches is the study of conidia morphology with use of scanning electron microscopy techniques and the phylogenetic revisions of the genus.

Small streams and lakes found in tropical forests have been shown as potential habitats for conidial fungi that have not yet been described in the literature (Castañeda *et al.* 2006). Castañeda *et al* (2005) described for the first time in Mexico the fungus *Idriella cagnizarii* collected in the native tropical forest Los Tuxtlas. This fungus was found in decomposing plant material that was submerged in water. The new species, *Acumispora verruculosa* and *Pleurophragmium aquaticum*, were also found in decomposing leaves in streams of the same Mexican rainforest (Abarca *et al*. 2007). Similarly, *Brevicatenospora enteroproliferata* and *Beltraniopsis aquatica* were described from submerged litter collected in Cuban tropical forest (Castañeda *et al*. 2006). However, studies with this group of fungi are still scarce, requiring a deeper study of the diversity of this group.

In Brazil, studies with conidial fungi on litter have been concentrated in the states of São Paulo and Bahia, where new species have been described. (Barbosa *et al.* 2008, Cruz & Gusmão 2009, Monteiro & Gusmão 2014, Costa & Gusmão 2017, Fiuza et al. 2017). However, studies in the Atlantic Forest of the state of Minas Gerais is rare or none existent. Thereby, this study aimed to carry out a taxonomic survey of aquatic hyphomycetes in Zona da Mata Mineira, Brazil, and provide descriptions, and illustrations, including geographical distribution for new records and new species.

Materials and methods

Sample collection

Submerged decayed leaves in water was collected in fragments of Atlantic Forest in the Zona da Mata Mineira, in months on September and November of 2017. The samples were collected in streams at Tombo da Cachoeira (20°44'24" S, 42°37'53" W) in the municipality of Canaã -MG, at Cachoeira do Milita (20°45'25" S, 42°35'53" W) and Parque Estadual Serra do Brigadeiro (PESB), both located in the municipality of Araponga - MG. At the PESB, the samples were collected on Trilha do Encontro (20°45'14" S, 42°28'44" W), Trilha do Moinho do Zeca (20°43'14" S, 42°28'44" W), near Trilha da Pedra do Pato (20°43'55" S, 42°28'44" W) and in the small river next to the Casa de Hóspedes (20 ° 42'59 "S, 42 ° 28'51" W).

All plant material collected were packed in kraft paper bags according to Leão-Ferreira *et al*. (2013) and sent to the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas, Universidade Federal de Viçosa. The decayed leaves was submitted to the washing under in running tap water (Harleyand & Waid 1955), as adapted by Castañeda-Ruiz *et al*. (2006). Further, they were air-dried and kept in moist chamber at room temperature. During 30 days, the reproductive structures of conidial fungi were observed, collected using a fine needle and mounted between a microscope slide and coverslip, in lactoglycerol. Direct isolations were performed using growing medium of Potato (4 g/L) Dextrose (20 g/L) Agar (15g/L) and Carrot (30 g/L) Corn (30 g/L) Agar (20 g/L). The isolates were identified and stored at 25° C with constant light. The monosporic cultures were obtained according to procedures established by Pinho *et al.* 2016.

Daily, the isolates were analysed and the fungal cultures were stored in 1.5mL microtubes containing 10% glycerol solution, 0.5% saline solution and in anhydrous silica gel (Mota *et al*. 2003, Gonçalves *et al*. 2016). The isolates were deposited in the culture collection “Coleção Octávio Almeida Drummond” (COAD) of the Universidade

Federal de Viçosa (Viçosa, Brazil). Additionally, the material to be proposed of holotype of a new species was deposited (metabolically inactive culture) in the herbarium of Universidade Federal de Viçosa (VIC).

DNA extraction, PCR and sequencing

Monosporic cultures of *Cladosporium* spp. were grown on Potato Dextrose Agar at 25 °C with constant light for five days. The mycelium was scraped with a sterile toothpick and transferred to 1.5 mL microtube. Genomic DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) according to Pinho *et al*. (2013). Part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer, the 5.8S rRNA gene, the second internal transcribed spacer and the 5' end of the 28S rRNA gene; translation elongation factor 1-alpha (TEF1-α), and actin (ACT) were amplified using the respective primers ITS5 (White *et al.* 1990)/LR5 (Vilgalys & Hester 1990), EF1-728F (Carbone & Kohn, 1999)/EF2 (O'Donnell *et al*. 1998) and ACT-512F/ACT-783R (Carbone & Kohn 1999). The PCR reaction of each isolate was performed with 12.5 µl of Dream Taq TM PCR Master Mix 2× (MBI Fermentas, Vilnius, Lithuania); 1 µl of each forward and reverse primer at 10 µM (Invitrogen, Carlsbad, CA); 1 µl of dimethyl sulfoxide (DMSO, Sigma–Aldrich, St. Louis, MO); 7.5 µl of nuclease-free water and 2 µl of genomic DNA (50 ng/µL). The cycling conditions of genomic regions comprised an initial denaturation at 95 ºC for 2 min, followed by 35 cycles of denaturation at 94 ºC for 30 s, elongation at 72 ºC for 1 min, and a final elongation step at 72 ºC for 10 min. The anneling temperature of the primers ACT-512F/ACT-783R, ITS5/LR5, EF1-728F/ EF2 were respectively 61 °C, 50 °C and 48 °C for 30 s. The PCR products were analysed by electrophoresis on 2% agarose gels stained with GelRed™ (Biotium Inc., Hayward, CA) in a 1× TAE buffer and

visualized under UV light to check for the amplification size. The PCR product was purified using 5 µl of the amplicons and 2 µl the ExoProStar 1-Step PCR. The final product of this purification was diluted with 15 µl of nuclease-free water. The sequencing was performed by Macrogen Inc., Korea (http://www.macrogen.com).

Sequence alignment and phylogenetic analyses

The nucleotide sequences were edited and checked manually to obtain the consensus sequences using BioEdit software (Hall 2013). The consensus sequences were compared to the GenBank database (http://www.ncbi.nlm.nih.gov) using the BLAST program. Sequences of *Cladosporium* spp. were downloaded in the FASTA format (Table 1) and aligned using the multiple sequence alignment program MUSCLE® (Edgar 2004), which is executed by MEGA v. 6 software (Tamura *et al*. 2013).

Phylogenetic analyses were done by Bayesian inference (BI) employing a Markov Chain Monte Carlo method. The methods were used for each gene/locus separately, as well as the combined dataset (ITS/LSU, TEF1-α, and ACT). The best nucleotide substitution model was determined for each gene using MrMODELTEST 2.3 (Posada & Crandall 1998) and selected according to the Akaike Information Criterion (AIC). Phylogenetic analysis was carried out in the CIPRES portal (Miller *et al.* 2010) using MrBayes v.3.2.3 (Ronquist *et al.* 2012). Four MCMC chains were run simultaneously, from random trees up to 10 000 000 generations. Trees were sampled every 1 000 generations, burning 25% of all trees obtained. Posterior probabilities (Rannala & Yang 1996) were determined in the most consensus tree among the 15 000 remaining trees. Trees were visualized in FigTree v. 1.4.3 (Rambaut 2016) and exported to graphics programs.

Morphological studies

A representative isolate from each identified species in the phylogenetic analysis was used for morphological characterization. Mycelium was cultivated on PDA and Malt Extract Agar (2g/L) for 14 days at 25 °C, under near- ultraviolet light. The colonies were measured and evaluated according to the charts of Rayner (1970). Microscopic preparations were made by slide culture technique for measurements of vegetative and reproductive structures (Riddell 1950). The fungi were grown on Synthetic Nutrient-Poor Agar (SNA) plug placed directly between a sterile microscope slide and coverslip. The slide was kept in a moist chamber inside a Petri dish in the dark at 25 °C for seven days. The plug was removed, and the coverslip was mounted in a drop of lactoglycerol on another slide. A second preparation was made with the fungal growth on the original slide using the same mounting fluid (Dhingra & Sinclair 1995). Photographic images were taken using differential interference contrast (DIC) illumination at Olympus BX53 microscope equipped with a digital camera, Olympus Q-Color5™. Measurements (n = 30) of the relevant morphological characteristics were made using the Olympus cellSens software.

Results

A total of 63 fungal isolates were obtained from the decayed leaves in small watercourses. Diversity genus of fungi were found colonizing the litter submerged in water. Some isolates were characterized morphologically; they are *Clonostachys* sp., *Codinaea* sp., *Cylindrocladium* sp., *Chalara* sp. and *Periconia* sp. Others are preserved for future identification. The group with the greatest representability among the isolates

was *Cladosporium* spp. Thus, we chose to characterize, morphologically and phylogenetically, those genera found in the submerged decayed leaves.

Amplicons of 530-999, 170-186 and 198-228 base pairs (pb) were generated for ITS/LSU, ACT, and TEF1-α respectively. Sequencing was successfully performed for 10 isolates (COAD 2487, 2488, 2491, 2492, 2494, 2496, 2497, 2498, 2499 and 2500) and they will be deposited in GenBank. An initial Bayesian inference analysis containing ITS and LSU combined sequences data from the three *Cladosporium* complexes (*C. cladosporioides*, *C. herbarum* and *C. sphaerospermum*), showed that our 10 isolates belong to the *C. cladosporioides* complex. It was then performed a second combined analysis with this species complex.

The combined alignment of 126 taxa (including *C. herbarum* as outgroup) of tree loci was represented by a total of 944 characters. The phylogenetic analyses (Figure 1) revealed that two isolates corresponded to cosmopolitan *Cladosporium cladosporioides* (COAD 2491 and 2492), three isolates clustered together with *Cladosporium angulosum* (COAD 2498, 2499, 2500) and three others grouped together with *Cladosporium anthropophilum* (COAD 2488, 2496 and 2497).

Two isolates were clustered into a clade representing one putative new species. *Cladosporium* sp. COAD 2487 and *Cladosporium* sp. COAD 2494 showed to be phylogenetically related to *C. anthropophilum*. However, both were separated from *C. anthropophilum*, forming a new group, with high support value (1) indicating to be a possible new species within the genus *Cladosporium*.

Taxonomy

All isolates have a typical *Cladosporium* morphology such as holoblastic conidiogenesis with different shapes of conidia formed in acropetal chains, presence de

ramoconidia (sometimes), secondary ramoconidia, intercalary conidia and terminal conidia. The main morphological characteristics of each *Cladosporium* species is available in Table 2. Based on phylogenetic analyses, morphological and cultural characteristics a description of a new putative species is proposed below:

Cladosporium sp. VIC 44468 (to be proposed as new species) M.L.R. Freitas & O.L. Pereira (Figure 2)

MycoBank: MB826908

GenBank: - (ITS), - (ACT), - (TEF1-α)

Systematic position: Ascomycota, Pezizomycotina, Dothideomycetes, Dothideomycetidae, Capnodiales, Cladosporiaceae

Type:—BRAZIL. Minas Gerais: Canaã, 626- 642 m, 20° 45' 25" S, 42° 35' 53" W, on submerged litter in streams, 7 September 2017 VIC 44468 (to be proposed as holotype), culture COAD 2487 (to be proposed as ex-type).

Description: *Mycelium* sparsely formed, 1.5–2 µm wide, pluriseptate, medium to dark brown, sometimes subhyaline, smooth or asperulate. *Conidiophores* solitary, cylindrical, 44–225 × 2–3 µm, arising terminal and laterally from hyphae, erect or somewhat flexuous, dark brown, septum conspicuous and walls thickened. *Conidiogenous cells* integrated, mainly terminal, cylindrical, sometimes geniculate-sinuous, proliferation sympodial, 7.5–42.5 × 2–3.5 µm, conidiogenous loci (1– 3) thickened and darkened. *Ramoconidia* cylindrical, 8–17.5 × 2.5–4µm, 0–1 septa, pale brown, truncate base, somewhat thickened. *Secondary ramoconidia* oblong, oblong-ellipsoid, 5–12.5 × 2–3.5 µm, 2–3 distal hila, narrowed base, pale brown. *Conidia intercalary* numerous,

catenulate, acrogenous, branching in all directions, ellipsoidal, aseptate, medium to dark brown, 3.5–6 × 2–3.5 µm, hila protuberant. C*onidia terminal*, subglobose to ellipsoidal, 2.5–4.5 × 2–3 µm, aseptate, medium to dark brown, hila protuberant.

Culture characteristics: Colonies on PDA attaining 36 mm diam, dark green to olive green, velvety, furrowed; reverse olivaceous grey. On MEA attaining 57 mm diam, dark green to olive green, pale olivaceous grey central mycelium; velvety; reverse olivaceous grey.

Additional specimens examined.—BRAZIL. Minas Gerais: Canaã, 626- 642 m, 20° 45' 25" S, 42° 35' 53" W, on submerged litter in streams, 7 September 2017 (culture COAD 2494).

Discussion

Cladosporium have been isolated from different environments and substrates (Crous *et al*. 2007a, Bensch *et al*. 2012, 2015, Sandoval-Denis *et al*. 2016), nevertheless there are no descriptions of species of the genus *Cladosporium* isolated from submerged litter in water.

In addition to morphological and cultural characterization, the use of molecular tools is essential for the correct identification of *Cladosporium* species (Sandoval-Denis *et al*. 2016). Some phylogenetic studies have already proposed the use of internal spacers of the rDNA genes (ITS), actin (ACT) and translation elongation factor 1-α (TEF1-α) to elucidate species diversity within the genus (Bench *et al*. 2010, 2015, 2018), therefore the choice of these regions for the present study.

In total four species were identified in this study, all species belonging to the *C. cladosporioides* complex. *Cladosporium cladosporioides*, a species that has a

cosmopolitan distribution, *Cladosporium angulosum*, *Cladosporium anthropophilum* and *Cladosporium* sp. VIC 44468 to be proposed as a new species. In Brazil, there are still few studies dedicated to this group of conidial fungi, and only three species are originally from Brazil, *Cladosporium maracuja,* from leaves of *Passiflora* sp. (Viégas 1946); *Cladosporium rugulovarians*, isolated from leaf sheaths of unidentified *Poaceae* (Bensch *et al*. 2015); and *Cladosporium langeronii* isolated from man ulcero-nodular mycosis of hand and arm (Zalar *et al*. 2007).

Cladosporium angulosum (Sandoval-Denis *et al*. 2016) was isolated from bronchoalveolar fluid of humans. Previously, some isolates were erroneously identified as *C. perangustum* (Bensch *et al.*, 2010, 2012, 2015), but besides the phylogenetic distance between the species, a conspicuous morphological characteristic of *C. angulosum* are the conidiophores usually branched forming a 90º angle. In this work our the isolates were phylogenetically grouped with type CSB 140692. However, COAD 2498, 2499 and 2500 were isolated from decomposing plant material. Therefore, *C. angulosum* besides being originally as a clinical species may present another life style, such as saprophytic fungus of plant materials.

Cladosporium anthropophilum is probably saprobic fungi, but due to the number of isolates found in human and animal body fluids, they can also represent a clinically relevant fungus (Sandoval-Denis *et al*. 2016). This species has as morphological characteristics longer conidiophores (up to 438 µm) and conidia terminal oval to ellipsoidal. The isolates obtained in our study do not present ramoconidia, only secondary ramoconidia.

In spite of the new taxon introduced here, the collection of isolates also meant that the concept of hosts and distributions of some species could be expanded. There are no reports of *Cladosporium* spp. isolated from submerged decayed leaves in tropical forests.

It is the first time that *C. angulosum* (Figure 3) and *C. anthropophilum* (Figure 4) are reported in Brazil.

In addition, considering the size of our country, with abundance of rivers, streams and waterfalls, the number of aquatic conidial fungi described in Brazil is small. More efforts are needed to the knowledge of the ecology and taxonomy of this group.

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CONCLUSÕES GERAIS

- Esta é a primeira ocorrência de *Cladosporium angulosum* e *Cladosporium anthropophilum* no Brasil e primeira ocorrência em serapilheira;
- *Cladosporium* sp. VIC 44468 é uma nova espécie dentro do complexo *C. cladosporioides*, a ser proposta segundo o Código de Nomenclatura para Algas, Fungos e Plantas;
- O presente trabalho corrobora estudos sobre a diversidade de espécies de *Cladosporium* nas regiões tropicais. No entanto, outros levantamentos são necessários para aumentar o conhecimento sobre a diversidade e ecologia desse importante gênero.

ANEXOS

TABLE 1. GenBank accession numbers of *Cladosporium* species, *C. cladosporioides* complex, used in the phylogenetic analyses. The samples obtained in this study are highlighted in bold.

Species	Sample	Host/ Substrate	GenBank Access Number		
			ITS	TEF1-α	ACT
C. acalyphae	CBS 125982	*Acalypha autralis*	HM147994	HM148235	HM148481
C. alboflavescens	CBS 140690	Bronchoalveolar lavage fluid	LN834420	LN834516	LN834604
C. angulosum	CBS 140692	Bronchoalveolar lavage fluid	LN834425	LN834521	LN834609
C. angulosum	CPC 11516	*Acacia mangium*	HM148127	HM 148371	HM148616
C. angulosum	**COAD 2498**	Leaf litter	-	-	-
C. angulosum	**COAD 2499**	Leaf litter	-	-	-
C. angulosum	**COAD 2500**	Leaf litter	-	-	-
C. angustisporum	CBS 125983	*Alloxylon wickhamii*	HM147995	HM148236	HM148482
C. angustisporum	DTO 127-E6	Air sample, bakery	KP701935	KP701812	KP702057
C. angustiterminale	CBS 140480	*Banksia grandis*	KT6000379	KT6000476	KT6000575
C. anthropophilum	CBS 140685	Bronchoalveolar lavage fluid	LN834437	LN834533	LN834621
C. anthropophilum	CBS 117483	-	HM148007	HM148248	HM148494
C. anthropophilum	**COAD 2488**	Leaf litter	-	-	-
C. anthropophilum	**COAD 2496**	Leaf litter	-	-	-
C. anthropophilum	**COAD 2497**	Leaf litter	-	-	-
C. asperulatum	CBS 126340	*Protea susannae*	HM147998	HM148239	HM148485
C. asperulatum	CBS 126339	*Eucalyptus* leaf litter	HM147997	HM148238	HM148484
C. australiense	CBS 125984	*Eucalyptus moluccana*	HM147999	HM148240	HM148486
C. austroafricanum	CBS 140481	Leaf litter	KT600381	KT600478	KT600577
C. chalastosporoides	CBS 125985	Fruiting bodies of *Teratosphaeria proteaearboreae*	HM148001	HM148242	HM148488
C. chasmanthicola	CBS 142612	Leaf spots on *Chasmanthe aethiopica*	KY646221	KY646224	KY646227
C. chubutense	CBS 124457	*Pinus ponderosa*	FJ936158	FJ936161	FJ936165
C. cladosporioides	CBS 112388	Air, indoor environment	HM148003	HM148244	HM148491
C. cladosporioides	CBS 113738	Grape bud	HM148004	HM148245	HM148492
C. cladosporioides	CPC 15626	Wild plant	KT600387	KT600484	KT600582
C. cladosporioides	**COAD 2491**	Leaf litter	-	-	-
C. cladosporioides	**COAD 2492**	Leaf litter	-	-	-
C. colocasiae	CBS 119542	*Colocasia esculenta*	HM148066	HM148309	HM148554
C. colocasiae	CBS 386.64	*Colocasia esculenta*	HM148067	HM148310	HM148555
C. colombiae	CBS 274.80B	*Cortaderia* sp.	FJ936159	FJ936163	FJ936166

C. crousii	CBS 140686	Bronchoalveolar lavage fluid	LN834431	LN834527	LN834615
C. cucumerinum	CBS 171.52	*Cucumis sativus*	HM148072	HM148316	HM148561
C. cucumerinum	CBS 172.54	*Cucumis sativus*	HM148073	HM148317	HM148562
C. delicatulum	CBS 126344	*Tilia cordata*, leaves	HM148081	HM148325	HM148570
C. delicatulum	CPC 15612	*Juglans regia*	KT600389	KT600486	KT600584
C. europaeum	CPC 14238	*Sambucus nigra*, fruit	HM148055	HM148297	HM148542
C. europaeum	CPC 14296	Indoor building material, school	HM148056	HM148298	HM148543
C. exasperatum	CBS 125986	*Eucalyptus tintinnans*	HM148090	HM148334	HM148579
C. exile	CBS 125987	Chasmothecia of *Phyllactinia*	HM148091	HM148335	HM148580
C. flabelliforme	CBS 126345	*Melaleuca cajuputi*	HM148092	HM148336	HM148581
C. flabelliforme	UTHSC-DI-13267	Human, sputum	LN834361	LN834457	LN834545
C. flavovirens	CBS 140462	Human, toenails	LN834440	LN834536	LN834624
C. funiculosum	CBS 122129	Leaf of *Vigna umbellata*	HM148094	HM148338	HM148582
C. funiculosum	CBS 122128	Ficus carica	HM148093	HM148337	HM148582
C. gamsianum	CBS 125989	*Strelitzia* sp.	HM148085	HM148339	HM148584
C. gamsianum	CPC 15617	*Glycine max*, seeds	KT600392	KT600489	KT600587
C. globisporum	CBS 81296	Meat stamp	HM148096	HM148340	HM148585
C. globisporum	CPC 19124	Indoor environment, window	MF472985	MF473413	MF473834
C. grevilleae	CBS 114271	*Grevillea* sp., leaves	JF770450	JF770472	JF770473
C. herbarum	CBS 121621	*Hordeum vulgare*	EF679363	EF679440	EF679516
C. hillianum	CBS 125988	*Typha orientalis*, leaf mold	HM148097	HM148341	HM148586
C. hillianum	CPC 15458	*Typha orientalis*, leaf mold	HM148098	HM148342	HM148587
C. inversicolor	CBS 401.80	*Triticum aestivum*, leaf	HM148101	HM148345	HM148590
C. inversicolor	CBS 14365	Leaf of *Tilia* sp.	HM148100	HM148344	HM148589
C. inversicolor	CBS 139573	Indoor air, archive	KP701874	KP701751	KP701997
C. ipereniae	CBS 140483	*Puya* sp.	KT600394	KT600491	KT600589
C. ipereniae	CPC 16855	*Arctostaphylos pallida*	KT600395	KT600492	KT600590
C. iranicum	CBS 126346	Leaf of *Citrus sinensis*	HM148110	HM148354	HM148599
C. kenpeggii	CBS 142613	Leaves of *Passiflora edulis*	KY646222	KY646225	KY646228
C. licheniphilum	CBS 125990	*P. orbicularis* and *Physcia* sp. on *Acer platanoides*	HM148111	HM148355	HM148600
C. longicatenatum	CBS 140485	Unknown plant	KT600403	KT600500	KT600598
C. lycoperdinum	CBS 574.78C	*Aureobasidium caulivorum*	HM148115	HM148359	HM148604
C. lycoperdinum	CBS 126347	From galls of *Apiosporina*	HM148112	HM148356	HM148601

		morbosa on *Prunus* sp.			
C. montecillanum	CBS 140486	Pine needles	KT600406	KT600504	KT600602
C. montecillanum	CPC 17804	Pine needles	KT600408	KT600506	KT600604
C. myrtacearum	CBS 126350	*Corymbia foelsheana*	HM148117	HM148361	HM148606
C. myrtacearum	CBS 126349	*Eucalyptus placita*	HM148116	HM148360	HM148605
C. needhamense	CBS 143359	Indoor air sample, office	MF473142	MF473570	MF473991
C. neerlandicum	CBS 143360	Swab sample, achive	KP701887	KP701764	KP702010
C. neopsychrotolerans	CGMCC 3.18031	*Saussurea involucrata*, rhizosphere soil	KX938383	KX938400	KX938366
C. neopsychrotolerans	CGMCC 3.18032	*Saussurea involucrata*, rhizosphere soil	KX938384	KX938401	KX938367
C. oxysporum	CBS 125991	Soil, near the terracotta army	HM148118	HM148362	HM148607
C. oxysporum	CBS 126351	Indoor air	HM148119	HM148363	HM148608
C. paracladosporioides	CBS 171154	-	HM148120	HM148364	HM148609
C. parapenidielloides	CBS 140487	*Eucalyptus* sp.	KT600410	KT600508	KT600606
C. perangustum	CBS 125996	*Cussonia* sp.	HM148121	HM148365	HM148610
C. perangustum	CPC 13730	*Protea caffra*	HM148140	HM148384	HM148629
C. phaenocomae	CBS 128769	*Phaenocoma prolifera*	JF499837	JF499875	JF499881
C. phyllactiniicola	CBS 126992	Chasmothecia of *Phyllactinia guttata* on leaves of *Corylus avellana*	HM148150	HM148394	HM148639
C. phyllactiniicola	CBS 126353	Chasmothecia of *Phyllactinia guttata* on leaves of *Corylus avellana*	HM148153	HM148397	HM148642
C. phyllophilum	CBS 125992	*Taphrina* sp. on *Prunus cerasus*	HM148154	HM148398	HM148643
C. phyllophilum	CPC 13873	On *Teratosphaeria proteae-arboreae* on *Protea arborea*	HM148155	HM148399	HM148644
C. pini-ponderosae	CBS 124456	*Pinus ponderosa*	FJ936160	FJ936164	FJ936167
C. pseudochalastosporoides	CBS 140490	Pine needles	KT600415	KT600513	KT600611
C. pseudocladosporioides	CBS 125993	Outside air	HM148158	HM148402	HM148647
C. pseudocladosporioides	CPC 12850	Pruned wood	HM148169	HM148413	HM148658
C. pseudocladosporioides	CPC 14992	*Eucalyptus* sp.	HM148192	HM148436	HM148681
C. pseudocladosporioides	CPC 13992	Coffee tree	HM148174	HM148418	HM148663
C. pseudocladosporioides	CPC 13529	*Sagittaria graminea*	HM148172	HM148416	HM148661
C. puris	**COAD 2487**	Leaf litter	-	-	-
C. puris	**COAD 2494**	Leaf litter	-	-	-
C. rectoides	CBS 125994	*Vitis flexuosa*	HM148193	HM148438	HM148683
C. rectoides	CBS 126357	*Plectranthus* sp.	HM148194	HM148439	HM148684
C. rugulovarians	CBS 140495	Unidentified *Poaceae*, leaf sheaths	KT600459	KT600558	KT600656

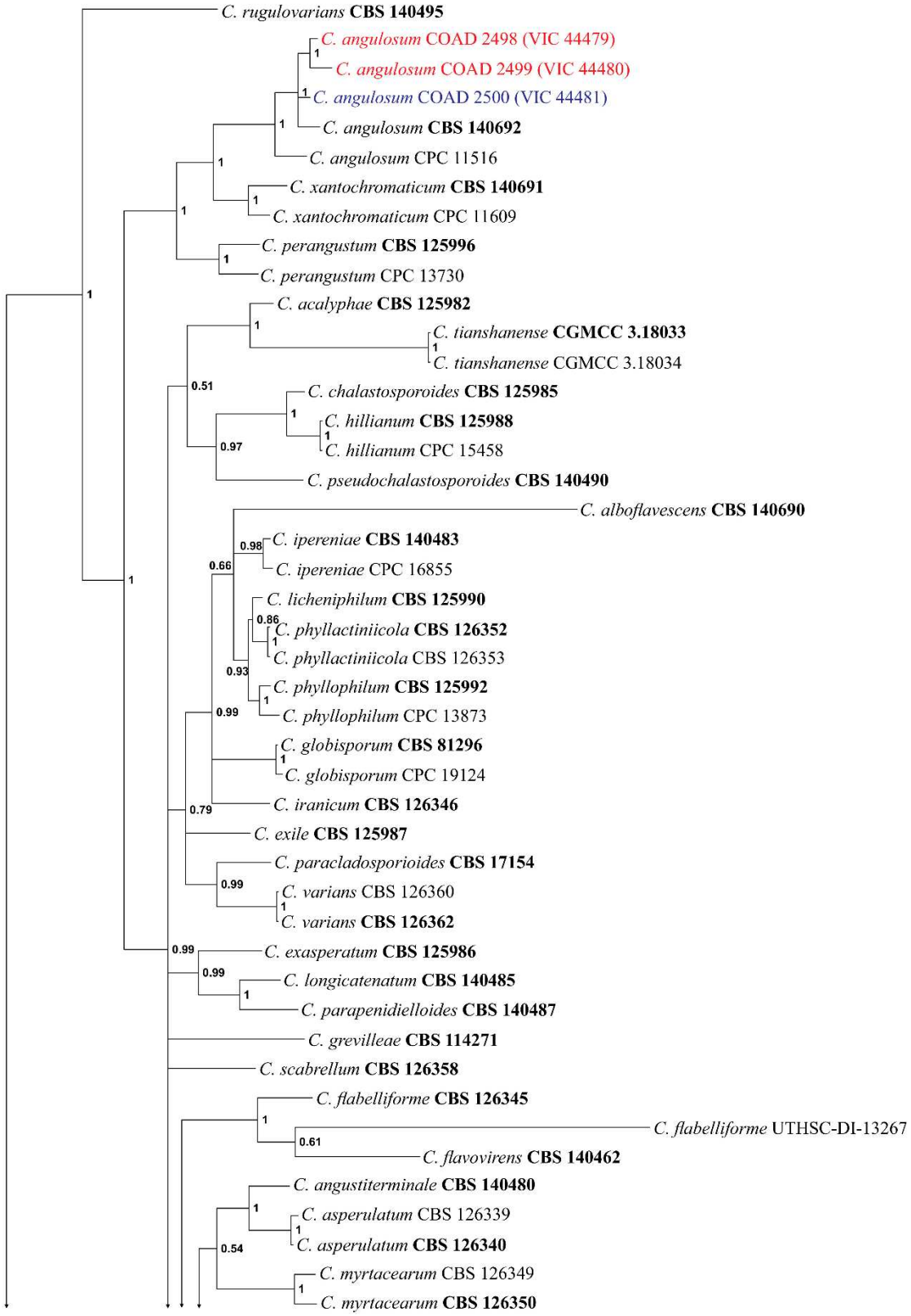
C. scabrellum	CBS 126358	*Ruscus hypoglossum*	HM148195	HM148440	HM148685
C. silenes	CBS 109082	*Silene maritima*	EF679354	EF679429	EF679506
C. sinuatum	CGMCC 3.18086	Soil	KX938385	KX938402	KX938368
C. sinuatum	CGMCC 3.18087	Soil	KX938386	KX938403	KX938369
C. subuliforme	CBS 126500	*Chamaedorea metallica*	HM148196	HM148441	HM148686
C. subuliforme	CPC 15833	*Citrus* sp.	KT600453	KT600552	KT600650
C. tenuissimum	CBS 117.79	Fruit	HM148200	HM148445	HM148690
C. tenuissimum	CBS 125995	*Lagerstroemia* sp.	HM148197	HM148442	HM148687
C. tenuissimum	CPC 10538	*Musa* sp.	HM148202	HM148447	HM148692
C. tenuissimum	CPC 10882	*Gnaphalium affine*	HM148204	HM148449	HM148694
C. tianshanense	CGMCC 3.18033	*Saussurea involucrata*, rhizosphere soil	KX938381	KX938398	KX938364
C. tianshanense	CGMCC 3.18034	*Saussurea involucrata*, rhizosphere soil	KX938382	KX938399	KX938365
C. uredinicola	CPC 5380	Hyperparasite on *Cronartium fusiforme*	AY251071	HM148467	HM148712
C. uwebraunianum	DTO 072-D8	Indoor air, archive	MF473306	MF473729	MF474156
C. uwebraunianum	CBS 139572	Indoor air, archive	KP701873	KP701750	KP701996
C. varians	CBS 126362	*Catalpa bungei*	HM148224	HM148470	HM148715
C. varians	CBS 126360	*Ulmus* sp.	HM148222	HM148468	HM148713
C. verrucocladosporioides	CBS 126363	*Rhus chinensis*	HM148226	HM148472	HM148717
C. vicinum	CBS 143366	Indoor air semple	MF473311	MF473734	MF474161
C. vicinum	CPC 13867	*Leptosphaeria* sp.	HM148059	HM148301	HM148546
C. vignae	CBS 121.25	*Vigna unguiculata*, living stems	HM148227	HM148473	HM148718
C. welwitschiicola	CBS 142614	Dead leaf of *Welwitschia mirabilis*	KY646223	KY646226	KY646229
C. westerdijkiae	CBS 113746	Bing cherry fruits	HM148061	HM148303	HM148548
C. westerdijkiae	CPC 10150	Fatoua vilosa	HM148062	HM148304	HM148549
C. westerdijkiae	CPC 14284	Triticum sp., grain	HM148065	HM148307	HM148552
C. xantochromaticum	CBS 140691	Human, BAL	LN834415	LN834511	LN834599
C. xantochromaticum	CPC 11609	*Musa* sp.	EF679356	EF679431	EF679508
C. xylophilum	CBS 125997	*Picea abies*, dead wood	HM148230	HM148476	HM148721
C. xylophilum	CBS 113749	Bing cherry fruits	HM148228	HM148474	HM148719

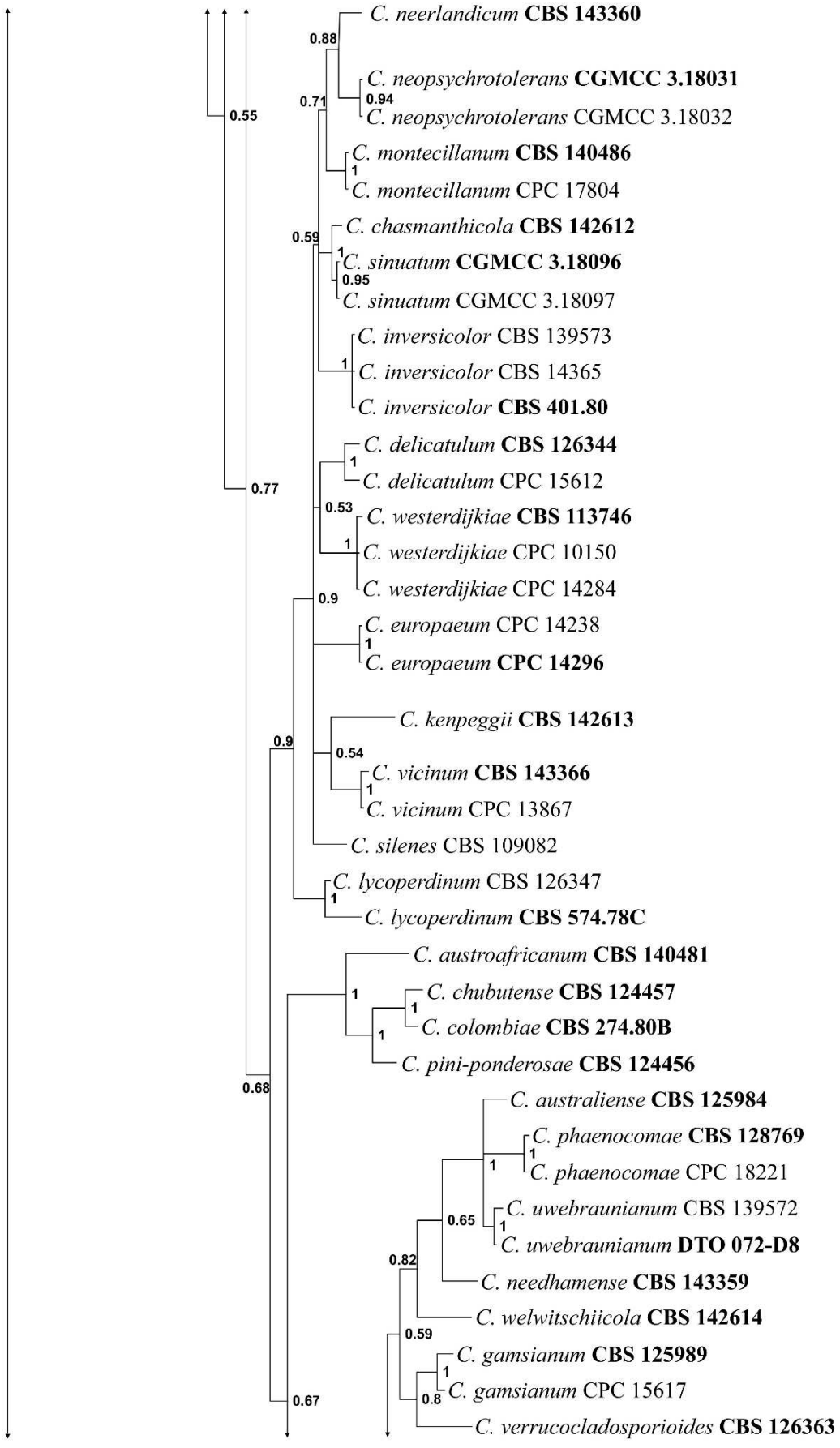
TABLE 2. Main morphological characteristics of *Cladosporium* species. Measures in µm.

Species	Hypha	Conidiophore	Conidiogenous cells	Ramoconidia	Secondary ramoconidia	Conidia intercalary	Conidia terminal	Reference
C. angulosum	1.5–3	up to 150 × 3–4	8–46 × 2–3.5	24.5–46 × 2–3.5	8–17 × 2.5–3	3.5–4.5 × 2–2.5	4–6 × 2–3	Sandoval-Denis *et al*. 2016
C. angulosum (COAD 2500)	2–3	15–60 × 2–4	9–36.5 × 3–4	*	5–24 × 2.5–3	3–6 × 2–3	3–3.5 × 2–2.5	This paper
C. anthropophilum	2–3	up to 550 × 2–5	15–54 × 3–5	20–42 × 2–5	7–38 × 2–5	4.5–11 × 2–3	3.5–9 × 2–3	Sandoval-Denis *et al*. 2016
C. anthropophilum (COAD 2497)	2–3	30.5–438 × 2–5	13–87.5 × 2–4.5	*	5–14 × 2.5–3.5	4.5–6.5 × 2.5–4	3–4 × 2–3	This paper
C. cladosporioides	2– 4	40–300 × 3–4	16–38	15–50 × 3–5	10–33 × 2.5–3	5–12 × 2.5–4	3–6 × 2–2.5	Bensch *et al*. 2010
C. cladosporioides (COAD 2492)	2– 4	28–184 × 2.5–4	7–58 × 2.5–4	17.5–20.5 × 3–3.5	7.5–15 × 2.5–4	4–6 × 2–4	3–6 × 2–3	This paper
Cladosporium sp. VIC 44468 (to be propoded as new species)	1.5–2	44–225 × 2–3	7.5–42.5 × 2–3.5	8–17.5 × 2.5–4	5–12.5 × 2–3.5	3.5–6 × 2–3.5	2.5–4.5 × 2–3	This paper

* Not observed

FIGURE 1. Multilocus phylogenetic tree inferred from Bayesian analysis based on the combined ITS, TEF1-α, and ACT sequences. Bayesian posterior probabilities are indicated next to the nodes. Culture numbers with *type* status are printed in **bold** face. The species in this study are highlighted in the colours in red (Canaã), blue (Araponga) and green (Parque Estadual Serra do Brigadeiro). The tree was rooted with *C. herbarum* CBS 121621




C. neerlandicum CBS 143360
C. neopsychrotolerans CGMCC 3.18031
C. neopsychrotolerans CGMCC 3.18032
C. montecillanum CBS 140486
C. montecillanum CPC 17804
C. chasmanthicola CBS 142612
C. sinuatum CGMCC 3.18096
C. sinuatum CGMCC 3.18097
C. inversicolor CBS 139573
C. inversicolor CBS 14365
C. inversicolor CBS 401.80
C. delicatulum CBS 126344
C. delicatulum CPC 15612
C. westerdijkiae CBS 113746
C. westerdijkiae CPC 10150
C. westerdijkiae CPC 14284
C. europaeum CPC 14238
C. europaeum CPC 14296
C. kenpeggii CBS 142613
C. vicinum CBS 143366
C. vicinum CPC 13867
C. silenes CBS 109082
C. lycoperdinum CBS 126347
C. lycoperdinum CBS 574.78C
C. austroafricanum CBS 140481
C. chubutense CBS 124457
C. colombiae CBS 274.80B
C. pini-ponderosae CBS 124456
C. australiense CBS 125984
C. phaenocomae CBS 128769
C. phaenocomae CPC 18221
C. uwebraunianum CBS 139572
C. uwebraunianum DTO 072-D8
C. needhamense CBS 143359
C. welwitschiicola CBS 142614
C. gamsianum CBS 125989
C. gamsianum CPC 15617
C. verrucocladosporioides CBS 126363

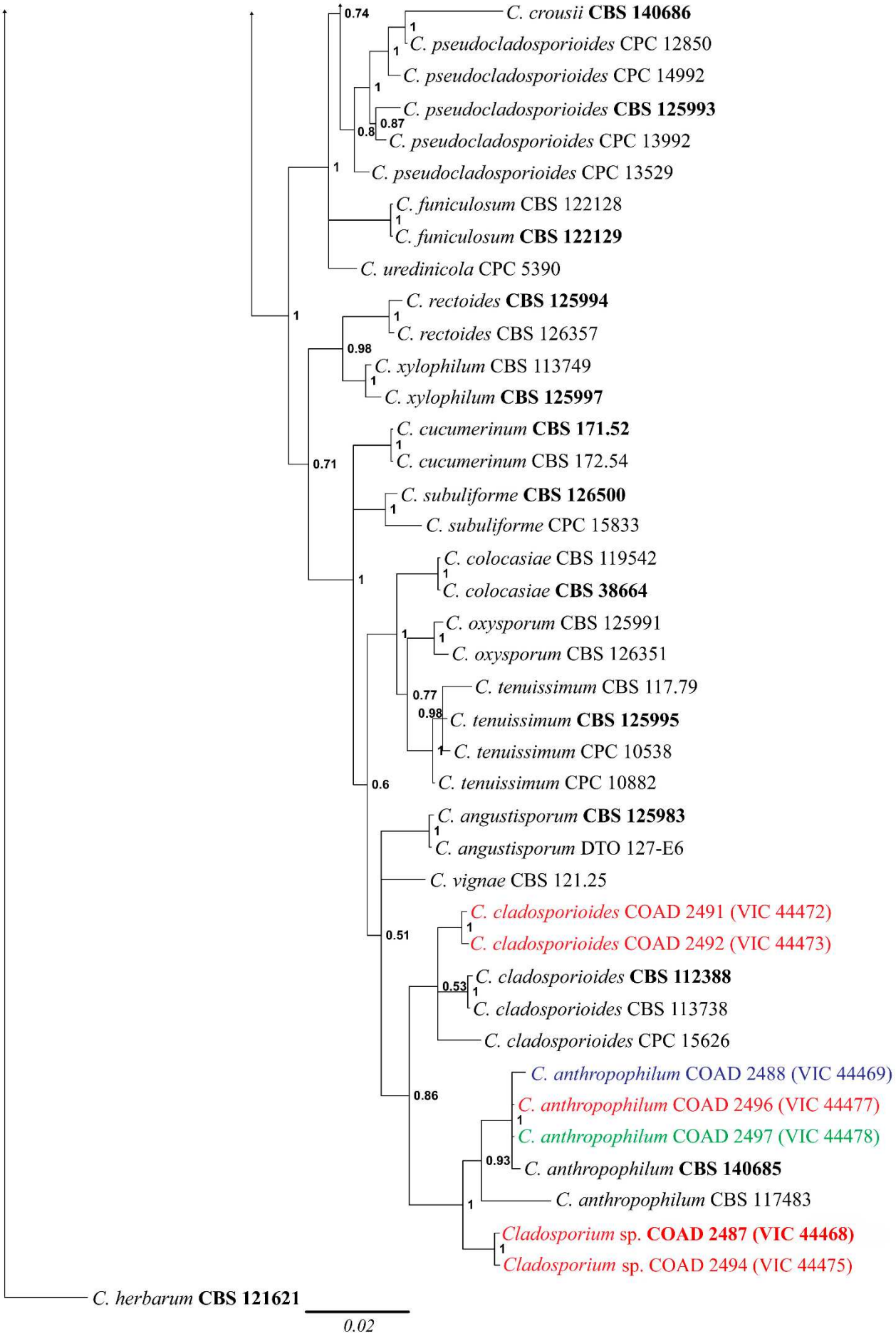

C. crousii CBS 140686
C. pseudocladosporioides CPC 12850
C. pseudocladosporioides CPC 14992
C. pseudocladosporioides CBS 125993
C. pseudocladosporioides CPC 13992
C. pseudocladosporioides CPC 13529
C. funiculosum CBS 122128
C. funiculosum CBS 122129
C. uredinicola CPC 5390
C. rectoides CBS 125994
C. rectoides CBS 126357
C. xylophilum CBS 113749
C. xylophilum CBS 125997
C. cucumerinum CBS 171.52
C. cucumerinum CBS 172.54
C. subuliforme CBS 126500
C. subuliforme CPC 15833
C. colocasiae CBS 119542
C. colocasiae CBS 38664
C. oxysporum CBS 125991
C. oxysporum CBS 126351
C. tenuissimum CBS 117.79
C. tenuissimum CBS 125995
C. tenuissimum CPC 10538
C. tenuissimum CPC 10882
C. angustisporum CBS 125983
C. angustisporum DTO 127-E6
C. vignae CBS 121.25
C. cladosporioides COAD 2491 (VIC 44472)
C. cladosporioides COAD 2492 (VIC 44473)
C. cladosporioides CBS 112388
C. cladosporioides CBS 113738
C. cladosporioides CPC 15626
C. anthropophilum COAD 2488 (VIC 44469)
C. anthropophilum COAD 2496 (VIC 44477)
C. anthropophilum COAD 2497 (VIC 44478)
C. anthropophilum CBS 140685
C. anthropophilum CBS 117483
Cladosporium sp. COAD 2487 (VIC 44468)
Cladosporium sp. COAD 2494 (VIC 44475)
C. herbarum CBS 121621
0.02

FIGURE 2. *Cladosporium* VIC 44468 (to be proposed as new species). **A.** Cannã-MG. **B.** Leaf litter submerse in watercourse. **C –D.** Colonies on MEA and PDA, respectively. **E.** Overview of reproductive structures of the fungus. **F.** Ramoconidia and conidia formed in acropetal chains. **G.** Secondary ramoconidia. **H.** Conidia. Scale bars = 10µm.

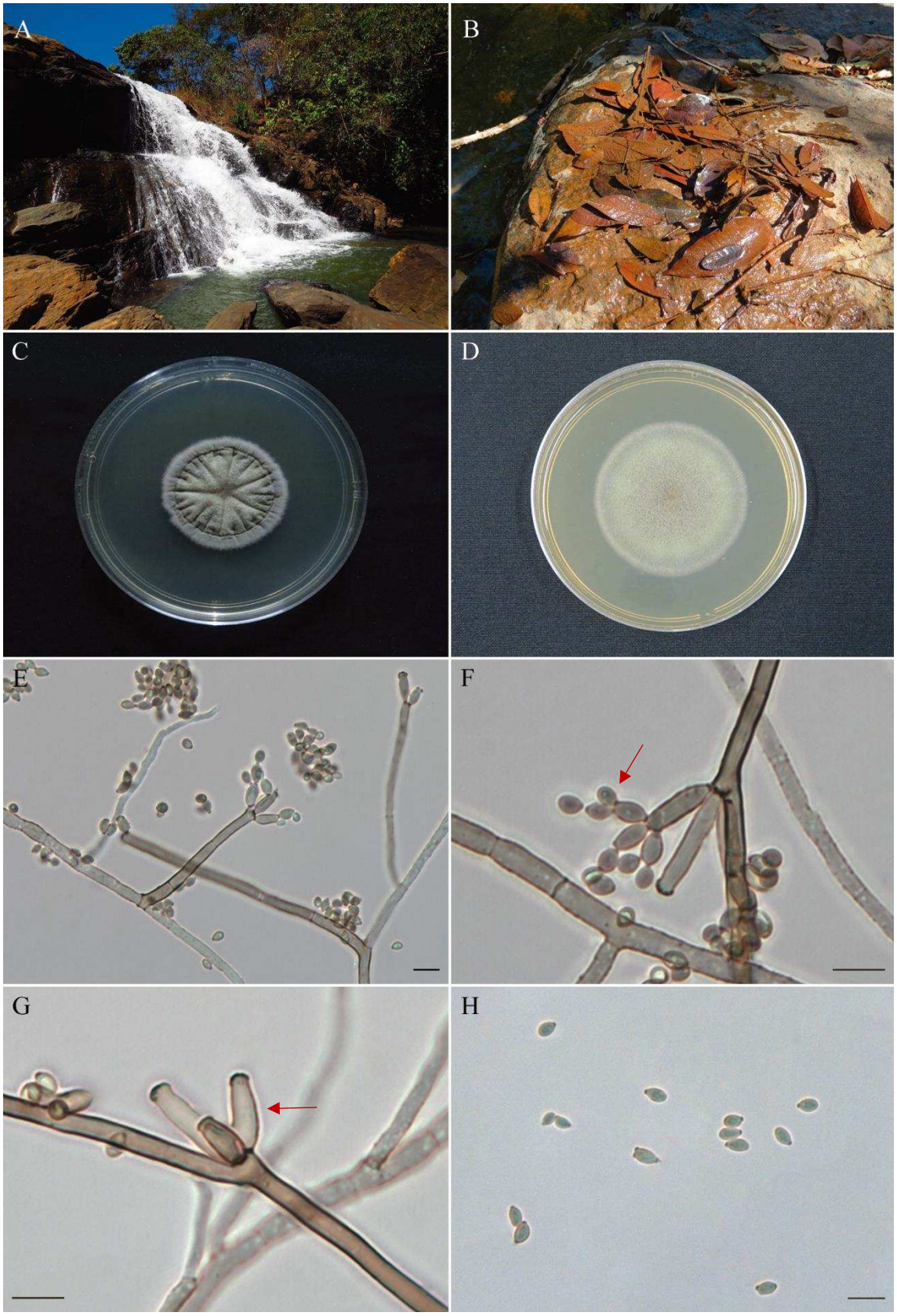


FIGURE 3. *Cladosporium angulosum* COAD 2500. **A.** Araponga-MG. **B-C.** Colonies on MEA and PDA, respectively **D.** Overview of reproductive structures of the fungus. **E.** Conidiophores branched forming a 90º angle. **F-G.** Secondary ramoconidia and conidia formed in acropetal chains. Scale bars = 10µm.

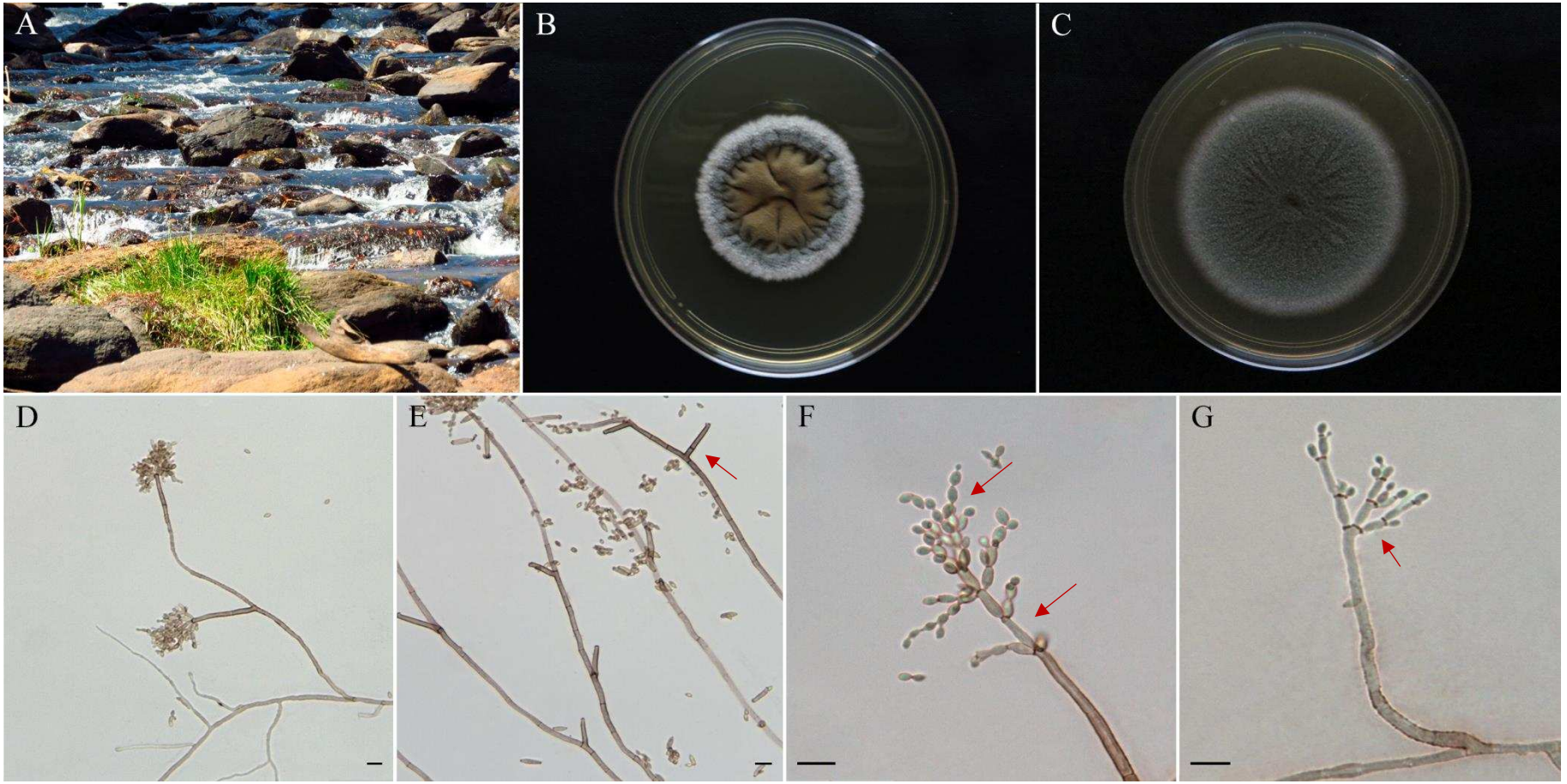


FIGURE 4. *Cladosporium anthropophilum* COAD 2497. **A.** Trilha do Encontro on PESB. **B-C.** Colonies on MEA and PDA, respectively **D.** Conidiogenous cell and secondary ramoconidia. **E.** Long and erect conidiophores. **F.** Conidiophores and chains of conidia. Scale bars = 10µm.