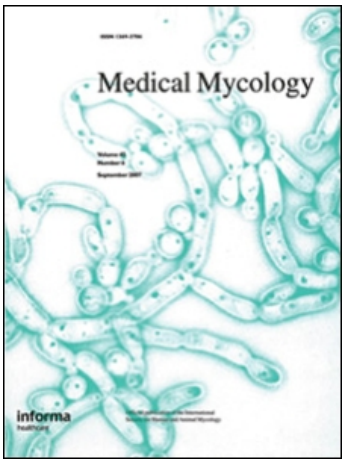

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Cladophialophora saturnica sp. nov., a new opportunistic species of Chaetothyriales revealed using molecular data

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Cladophialophora saturnica sp. nov., a new opportunistic species of *Chaetothyriales* revealed using molecular data

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While many members of the black yeasts genus *Cladophialophora* have been reported to cause diseases in humans, understanding of their natural niche is frequently lacking. Some species can be recovered from the natural environment by means of selective isolation techniques. The present study focuses on a *Cladophialophora* strain that caused an interdigital *tinea nigra*-like lesion in a HIV-positive Brazilian child. The fungal infection was successfully treated with oxiconazole. Similar strains had been recovered from the environment in Brazil, Uruguay and the Netherlands. The strains were characterized by sequencing the Internal Transcribed Spacer (ITS) regions and the small subunit (SSU) of the nuclear ribosomal RNA gene, as well as the elongation factor 1-alpha (EF1α) gene. Since no match with any known species was found, it is described as the new species, *Cladophialophora saturnica*.

Keywords black yeasts, *Cladophialophora*, cutaneous infection, taxonomy

Introduction

The fungal genus *Cladophialophora* currently contains seven species proven to be involved in human disease [1]. Among the most virulent species is *C. bantiana*, the main agent of a potentially fatal cerebral infection in transplant recipients and patients with leukemia, but also in immunocompetent individuals [2–4]. Another member of the genus, *C. carrionii,* is the etiologic agent of chromoblastomycosis, a common skin disease which is endemic in the arid climate zones of South America and Australia. This infection occurs primarily in immunocompetent individuals [5,6], and is supposed to result from the inoculation of plant debris contaminated with the fungus [7]. The remaining clinically relevant species of *Cladophialophora,* viz. *C. arxii, C. devriesii, C. emmonsii, C. boppii* and *C. modesta,* are very rarely reported as etiologic agents of disease.

Cladophialophora is an anamorph member of the *Chaetothyriales*, an ascomycete order that also comprises the black yeast genus *Exophiala* [1] and its filamentous relatives, *Fonsecaea* and *Phialophora*. The natural niche outside humans is unknown for most of these opportunists. In contrast to frequently expressed opinions, the fungi concerned are rarely isolated from dead plant material or rotten wood, and hardly ever from soil [8]. A selective isolation method is required to recover these fungi, e.g., the use of high temperature [9], a mouse vector [10,11], alkylbenzenes [12] or extraction via mineral oil [13,14]. At the phylogenetic base of the *Chaetothyriales* is located a group of plant-associated *Cladophialophora* species [15]. The present paper describes a species of *Cladophialophora,* based on

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isolate CBS 118724 that caused an interdigital infection in an immunocompromised child in Curitiba, Brazil.

Case report

The patient, a 4-year-old girl living in an institution since she was one-year-old was perinatally infected with HIV. The HIV diagnosis was made at the age of 8 months. Her immunodeficiency status was classified as A2 according to the 1994 revised categories by the Center for Disease Control and Prevention [16]. Her clinical category was characterized by mildly symptomatic features and moderate immunosuppression. Antiretroviral treatment was initiated at the age of 1 year and included zidovudine, lamivudine and ritonavir. She was treated at the hospital outpatient HIV clinic which she visited periodically for evaluation. At the moment of this report her weigh was normal for her age, with T- CD4 values of 1,142/mm^3 and T- CD8 of 1,116/mm^3. The number of HIV RNA viral copies was 11,000 copies/ml.

During physical evaluation, a non-symptomatic skin lesion was detected in the interdigital webs. The lesion was velvety, scaling, brownish black and sharply demarcated, non-macerated (Fig. 1) and clinically diagnosed as interdigital *tinea nigra*. To recover the etiologic agent, the area was soaked in 70% isopropanolol and scrapings from the skin were collected aseptically into a Petri dish using a sterile scalpel blade. Treatment with topical oxiconazole was initiated as pigmented hyphae were found during microscopical observation of the interdigital scrapings. Culture plates of Sabouraud's Dextrose Agar (SDA, Difco) containing chloramphenicol (50 µg/ml) and cycloheximide (500 µg/ml) were inoculated with portions of the scrapings and incubated at 25°C and 37°C.

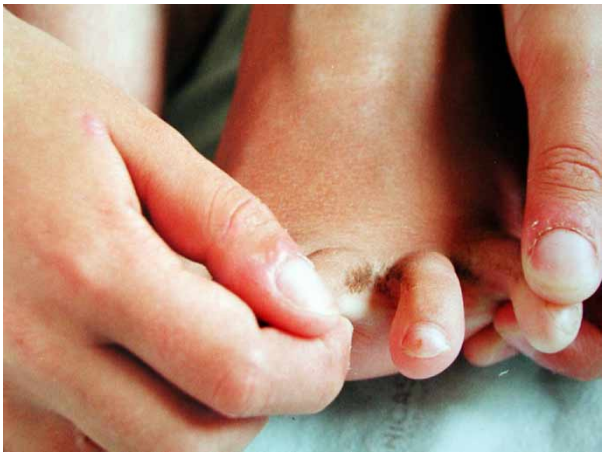

Fig. 1 A velvety, scaling, brownish black macular area in the interdigital webs.

A search for similar strains in the patient's environment was performed using the mineral oil technique. The melanized isolates obtained from this investigation, as well as four strains with molecular similarity found colonizing dead wood in Uruguay and developing in a biofilter in the Netherlands, were included in this study. Since no match of these strains was found with any described taxonomic entity, the fungus is reported here as a new species.

Materials and methods

Fungal strains and morphology

Strains studied are listed in Table 1. Stock cultures were maintained on slants of 2% malt extract agar (MEA) and oatmeal agar (OA) at 24°C. For morphological observation, potato dextrose agar (PDA) slide cultures were prepared and mounted in lactophenol. To evaluate the natural niche, 10 g samples of vegetable cover found in the patient's garden and from the surrounding area were added to 20 ml of sterile mineral oil in 100 ml of sterile saline, followed by the addition of antibiotics with subsequent vigorous shaking and incubation for 20 min at room temperature. Afterwards, the oil/saline interface was seeded on Mycosel agar plates and incubated at 30°C [13,14].

Physiology

Growth temperatures of strains CBS 114326, 102230, 109628, 109630 and 118724 were determined by incubating MEA culture plates in the dark for 2 weeks at temperatures ranging from 6–36°C at intervals of 3° as well as at 40°C [17].

DNA extraction

About 1 cm^2 mycelium segments from 20 to 30-day-old cultures were transferred to a 2 ml Eppendorf tube containing 300 µl CTAB (cetyltrimethylammonium bromide) buffer and about 80 mg of a silica mixture (silica gel H, Merck 7736, Darmstadt, Germany/ Kieselguhr Celite 545, Machery, Düren, Germany, 2:1, w/w). Cells were mechanically disrupted for approximately 1 min with a tight-fit sterile pestle. Subsequently, 200 µl CTAB buffer was added, the mixture was vortexed and incubated for 10 min at 65°C. After addition of 500 µl of chloroform, the solution was mixed and centrifuged for 5 min at 14,000 r.p.m. and the supernatant transferred to a new tube with 2 volumes of ice-cold 96% ethanol. DNA

Table 1 Isolation data of *Cladophialophora* strains examined

Name	CBS	Status	Other reference	GenBank ITS, EF1a	Source	Origin
Cladophialophora saturnica	109628		dH 12333; IHM 1727	EU103983, EU140601	Dead tree	Uruguay, Isla Grande del Queguay
Cladophialophora saturnica	109630		dH 12335; IHM 1733	—	Recently cut trunk, *Neotandia membranacea*	Uruguay, Isla Grande del Queguay
Cladophialophora saturnica	118724	T	157D; dH 12939	EU103984, EU140602	Interdigital toe lesion, child	Brazil, Paraná, Curitiba
Cladophialophora saturnica	102230		dH 11591, 4IIBPIRA	AY857508, EU140600	Litter, vegetable cover/soil	Brazil, Paraná, Curitiba
Cladophialophora saturnica	114326		ATCC 200384	AY857507, EU140603	Toluene biofilter	Netherlands, Wageningen
Cladophialophora devriesii	147.84	T	ATCC 56280; CDC 82-030890	EU103985, EU140595	Disseminated infection, male	USA, Grand Cayman Island
Cladophialophora devriesii	—		ISO 13F	—	Litter, vegetable cover/soil	Brazil, Paraná, Curitiba
Cladophialophora arxii	306.94	T	—	EU103986, EU140593	Tracheal abscess, male	Germany
Cladophialophora arxii	409.96		UAMH 5881; dH 15849	EU103987, EU140594	Male	—
Cladophialophora australiensis	112793	LT	—	EU035402, —	Sports drink	Australia
Cladophialophora chaetospira	114747		—	EU035403, —	*Phyllostachys bambusoides*	China
Cladophialophora chaetospira	115468		HKUCC 10147	EU035404, —	Bamboo	China
Cladophialophora minourae	987.96		IFM 4701; UAMH 5022	EU103988, EU140599	Rotting wood	Japan, Yachimata, Chiba
Cladophialophora minourae	556.83	T	ATCC 52853; IMI 298056	AY251087, EU140598	Decaying wood	Japan, Shiroi
Cladophialophora potulentorum	112222		CPC 1376; FRR 4946	EU035409, —	Sports drink	Australia
Cladophialophora potulentorum	114772		CPC 1375; FRR 4947	EU035410, —	Sports drink	Australia
Cladophialophora potulentorum	115144	LT	CPC 11048; FRR 3318	DQ008141, —	Apple juice	—
Fonsecaea monophora	289.93		dH 15691	AY366925, —	*Arctocephalus australis* (seabear)	Netherlands, Rotterdam, Blijdorp Zoo
Fonsecaea monophora	269.37	T	dH 12659	AY857511, —	—	
Fonsecaea monophora	102238		dH 11602, 1PLE	AY366927, —	Soil	Brazil
Fonsecaea monophora	102248		dH 11613	AY366926, —	Chromoblastomycosis, male	Brazil
Fonsecaea pedrosoi	271.37	T	ATCC 18658; IMI 134458; dH 15659	AY366914, —	Chromoblastomycosis, male	South America
Fonsecaea pedrosoi	272.37		dH 15661	AY366917, —	Chromoblastomycosis, male	—
Cladophialophora bantiana	173.52	T	CBS 100433	EU103989, EU140585	Brain abscess, male	USA
Cladophialophora bantiana	102586		dH 11331	EU103990, EU140586	Brain abscess, male	Brazil, Belo Horizonte
Cladophialophora bantiana	119719		Solna Lab. No. 1739/05; dH 14515	EU103991, EU140589	Skin graft, Tsunami victim	Thailand
Cladophialophora bantiana	678.79		CDC B-3658; NCMH 2249; NIH B-3839; UAMH 4992	EU103992, EU140592	Skin lesion, cat	USA
Cladophialophora bantiana	648.96		UAMH 3830	EU103993, EU140587	Liver, dog	Barbados
Cladophialophora bantiana	444.96		—	EU103994, EU140591	Disseminated infection, dog	South Africa, Pretoria, Onderstepoort
Cladophialophora bantiana	101158		ATCC 44223; CDC B-3426; dH 11313	AY857516, EU140588	Brain infection, human	Japan
Cladophialophora bantiana	101252		ATCC 58040; CDC B-3466; dH10749	AY857519, EU140590	Brain abscess, human	USA, Washington
Cladophialophora emmonsii	—		dH 13029 ; UTHSC 03-70	AY857518, EU140582	Brain	—
Cladophialophora emmonsii	640.96		CDC B-3634; NCMH 2248; UAMH 4991	EU103995, EU140584	Sub-cutaneous lesion, cat	—
Cladophialophora emmonsii	979.96	T	CDC B-3875; NCMH 2247; UAMH 4994a; dH16329	EU103996, EU140583	Sub-cutaneous lesion right fore-arm, human	USA, Virginia

Table 1 (*Continued*)

Name	CBS	Status	Other reference	GenBank ITS, EF1a	Source	Origin
Cladophialophora yegresii	114406		UNEFM SgSR1; dH 13275	EU137323, EU137263	*Stenocereus griseus* plant (*Cactaceae*)	Venezuela, Falcon State
Cladophialophora yegresii	114405	T	UNEFM SgSr3; dH 13276	EU137322, EU137262	*Stenocereus griseus* plant (*Cactaceae*)	Venezuela, Falcon State
Cladophialophora yegresii	114407		UNEFM SgSR1; dH 13274	EU137324, EU137264	*Stenocereus griseus* plant (*Cactaceae*)	Venezuela, Falcon State
Cladophialophora carrionii	114392		UNEFM 82267; dH 13261	EU137267, EU137211	Chromoblastomycosis leg lesion, female	Venezuela, Falcon State
Cladophialophora carrionii	114393		UNEFM 9801; dH 13262	EU137268, EU137212	Chromoblastomycosis hand lesion, male	Venezuela, Falcon State
Cladophialophora carrionii	114396		UNEFM 2001/1; dH 13265	EU137269, EU137213	Chromoblastomycosis arm lesion, male	Venezuela, Falcon State
Cladophialophora carrionii	114398		UNEFM 2003/1; dH 13267	EU137271, EU137215	Chromoblastomycosis arm lesion, female	Venezuela, Falcon State
Cladophialophora carrionii	260.83	T of *C. ajelloi*	CDC B-1352; FMC 282; ATCC44535	EU137292, EU 137234	Chromoblastomycosis skin lesion, male	Uganda
Cladophialophora carrionii	160.54	LT	ATCC 16264; CDC A-835; MUCL 40053; IFA 4808; dH 15445	AB109177/EU137266	Chromoblastomycosis, male	Australia
Cladophialophora boppii	126.86	T	FMC 292; dH 15357	EU103997, EU140596	Skin lesion, on limb,male	Brazil
Cladophialophora boppii	110029		det M-41/2001 56893; dH 12362	EU103998, EU140597	Scales of face, male	Netherlands, Dordrecht

Abbrevations used: ATCC = American Type Culture Collection, Manassas, U.S.A.; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DH = G.S. de Hoog private collection; IFM = Research Institute for Pathogenic Fungi, Chiba, Japan; IHM = Laboratory of Mycology, Faculty of Medicine, Montevideo Institute of Epidemiology and Hygiene, Montevideo, Uruguay; IMI = International Mycological Institute, London, U.K.; IWW = Rheinisch Westfählisches Institut für Wasserforschung, Mülheim an der Ruhr, Germany; GHP = G. Haase private collection; MUCL = Mycotheque de l'Université de Louvain, Louvain-la-Neuve, Belgium; NCMH = North Carolina Memorial Hospital, Chapel Hill, U.S.A.; RKI = Robert Koch Institute, Berlin, Germany; UAMH = Microfungus Herbarium and Collection, Edmonton, Canada; UTHSC = Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio, U.S.A.; UTMB = Medical Mycology Research Center, Galveston, U.S.A.; UNEFM = Universidade Nacional Experimental Francisco de Miranda, Coro, Falcon, Venezuela.
T = ex-type culture; LT = ex-lectotype culture; NT = ex-neotype culture.

was allowed to precipitate for 30 min at −20°C and then centrifuged for 5 min at 14,000 r.p.m. The pellets were washed with cold 70% ethanol, dried at room temperature, resuspended in 97.5 µl of TE-buffer with 2.5 µl of RNAse 20 U/ml^{-1}, and incubated for 5 min at 37°C, before storage at −20°C [18].

Sequencing and phylogenetic reconstruction

The ribosomal DNA Internal Transcribed Spacers (ITS) were amplified using primers V9G and LS266 and sequenced with the internal primers ITS1 and ITS4 [19]. The translation elongation factor 1 alpha (EF1α) was amplified and sequenced using EF1-728F and EF1-986R [20]. Amplicons were cleaned with GFX PCR DNA and gel band purification kit (GE Healthcare, UK). Sequencing was performed on an ABI 3730XL automatic sequencer. Sequences were edited using the SEQMAN package (DNAStar Inc., Madison, USA) and aligned using BioNumerics version 4.61 (Applied Maths, Kortrijk, Belgium). The phylogenies were reconstructed with MrAIC [7] using a Neighbor-Joining criterion. Bootstrap values of ITS and EF1α trees were calculated with the program Treefinder using a parsimony criterion and 1000 replicates [21]. Small Subunit (SSU) rDNA amplicons were generated with primers NS1 and NS24 and were sequenced with primers BF83, Oli1, Oli9, BF951, BF963, BF1438, Oli3 and BF1419 [22]. Nucleotide positions 96-1765 (with reference to *Saccharomyces cerevisiae*) of the SSU rDNA were aligned and a tree of *Chaetothyriales* was constructed with the package ARB developed by W. Ludwig [23], using a Neighbor-Joining criterion. For all analyses, the corrected Akaike Information Criterion (AICc) was used to select a substitution model with the program MrAIC [7]. The results of this selection are presented in Table 2.

Results

Cardinal growth temperatures showed that all cultures had their best development at 27°C (Fig. 2), although the range spanned from 9–36°C. No growth was observed at 40°C. Using the phylogenetic marker

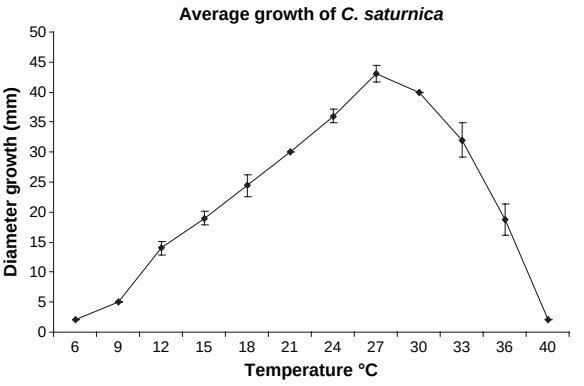

Fig. 2 Colony diameters at different temperatures ranging from 6°C to 40°C, measured after two weeks on 2% MEA were calculated for *C. saturnica* (CBS 102230, 109628, 118724, 114326, 109630).

nucSSU and a dataset representative of the *Chaetothyriales*, strain CBS 102230 was found to be a member of a Clade (1) primarily containing *Cladophialophora* and *Fonsecaea* species. Members of this clade are primarily known to cause diseases in humans, but some may be recovered from the environment, e.g., *C. minourae* (Fig. 3). Clade (2), the sister group of Clade (1), contained *Cladophialophora* and *Phialophora* species, most of which are known to be involved in or associated with human skin disorders. *Phialophora americana* is known as the anamorph of *Capronia semiimmersa*. Basal to these clades was a group (3) of environmental, mainly waterborne *Exophiala* species, including the teleomorph species *Capronia coronata*. The remaining members of *Chaetothyriales* were found at considerable phylogenetic distance, including *Cladophialophora modesta*. Of several recently described species of *Cladophialophora* [15], three were included in this study (*C. australiensis, C. chaetospira* and *C. potulentorum*). The others were not considered because they were only remotely related from our species of interest (CBS 118724).

For ITS sequences, MrAIC selected the HKY +G model. The base frequency of ITS was T = 0.2465, C = 0.2874, A = 0.2239, G = 0.2420, TC = 0.5339, AG = 0.4660. An ITS rDNA tree composed of alignable members of SSU clades (1) and (2) showed considerable

Table 2 Results from MrAIC using corrected Akaike information criterion (AICc)

Fragment/Gene	Model	df*	lnl*	AICc*	wAICc*
rDNA ITS	HKYG	78	−2617.4881	5415.8731	0.4995
EF1α	K2PG	55	−1460.1768	3065.5536	0.7023

*df = degrees of freedom; lnl = Log likelihood; AICc = corrected AIC; wAICc = weighted corrected AIC.

Fig. 3 Neighbor-joining tree based on positions 145–1640 of 115 SSU rRNA gene sequences genereated with the ARB package. *Cladophialophora modesta* CBS 985.96 was used as the out group.

differences between species (Fig. 4); regions with ambiguous alignments were removed from the analysis. The ex-type strains of *Cladophialophora arxii, C. devriesii, C. emmonsii* and *C. minourae* were found separated by 8.9, 10.4, 11.1 and 13.1% distance, respectively. Comparison in a local database on melanized fungi maintained at CBS showed four sequences identical to CBS 118724. These sequences were isolates from plant litter collected in the surroundings of the region where the patient lives in Brazil (CBS 102230), from a biofilter in the Netherlands (CBS 114326), and two from dead plant material in Uruguay (CBS 109628 and CBS 109630). The ex-type strain of *Cladophialophora devriesii* was the nearest neighbor at 6.5% ITS difference. Sequences of *C. modesta, C. hostae, C. humicola, C. scillae* and C. *sylvestris* were too distant for confident alignment and were therefore not included in the tree. The EF1α tree was built with substitution model K2P+G, the base frequency of which was; T = 0.2688, C = 0.2705, A = 0.2254, G = 0.2351, TC = 0.5394, AG = 0.4605. With EF1α (Fig. 5) the ex-type strains of *Cladophialophora arxii, C. devriesii, C. emmonsii* and *C. minourae* were found separated by 9.8, 10.4, 20.0 and 13.8% distance, respectively. The sequences could be aligned with confidence over almost their total lengths. Four strains had EF1α sequences nearly identical to CBS 118724 and were the same as the ones previously shown to be identical using ITS. The EF1α tree (Fig. 5) showed the same topology as the ITS tree. *Cladophialophora bantiana, C. carrionii* and *C. yegresii* formed a clade which

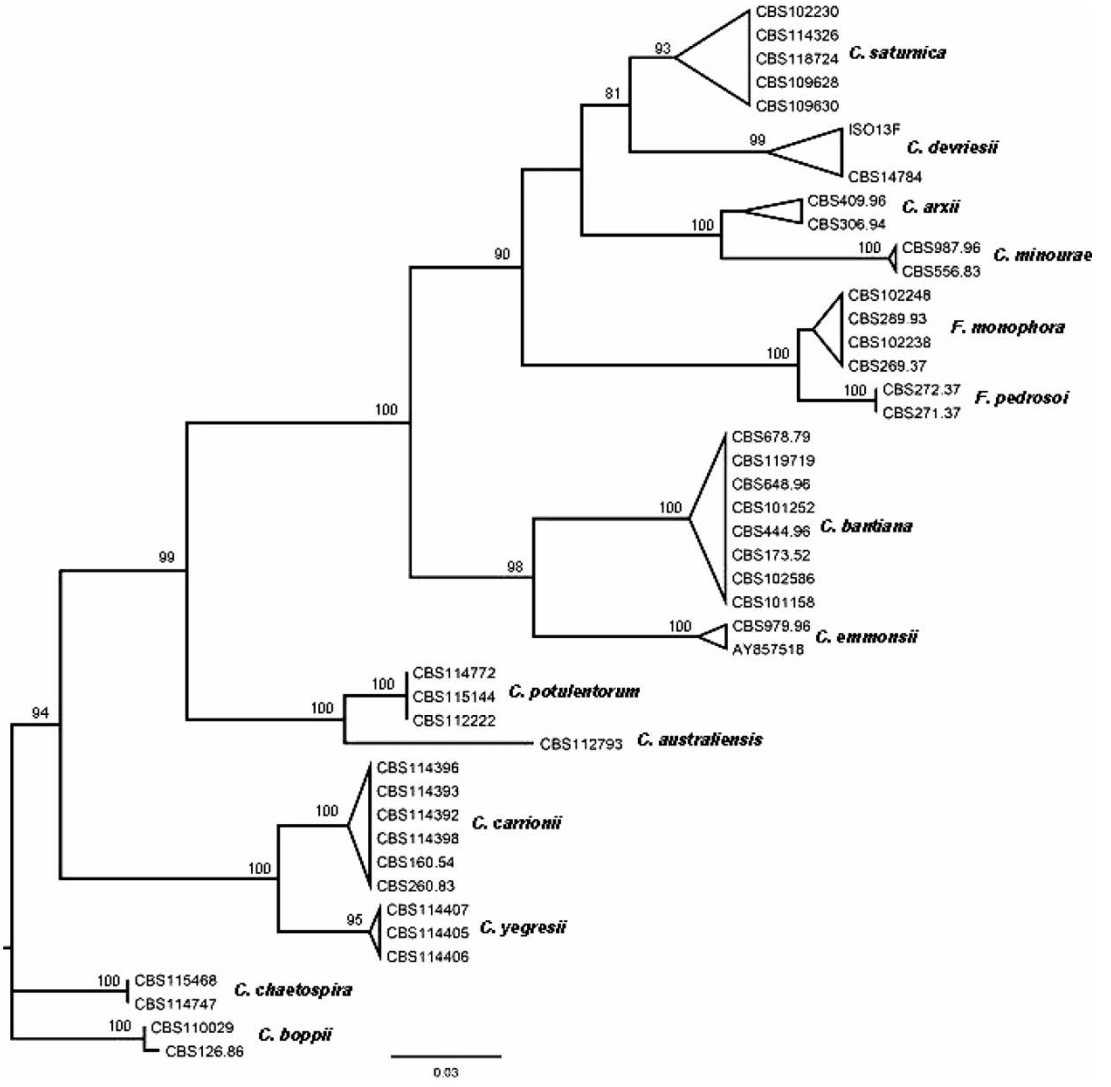

Fig. 4 Neighbor-joining tree of the *Cladophialophora* species based on rDNA ITS, generated using the HKY+G model. The model was calculated using ML in MrAIC software. Bootstrap set to 1000 replicates and cut-off = 80%. Cladophialophora boppii (CBS 126.86 and CBS 110029) were taken as out group.

was significantly different from CBS 118724. *Fonsecaea* species were found at a large phylogenetic distance. As the well-supported cluster including our strain of interest was located at a significant phylogenetic distance from the remaining taxa, we propose to recognize it as a new species with the following description:

Cladophialophora saturnica Badali, Carvalho, Vicente, Attili-Angelis, Kwiatkowski, Gerrits van den Ende & de Hoog, sp. nov. – Figs. 6 and 7. Mycobank MB491920.

Etym: named after Saturn-shaped conidia in face view.

Coloniae modice expandentes, siccae, velutinae, olivaceo-virides ad griseae, reversum olivaceo-nigrum. Cellulae gemmantes absentes. Hyphae septatae, dilute olivaceae. Conidiophora erecta, e denticulis prominentibus cellulas conidiogenas proferentia. Cellulae conidiogenae hyphis conidiophorisque paulo pallidiores, cylindricae, e denticulis hebetibus conidia limoniformia proferentes. Conidia unicellularia, olivacea, hilo paulo obscuriore, levia, catenas breves (2-3), acropetales, cohaerentes formantia. Ramoconidia nonnumquam massa conidiorum disrupta oriuntur. Chlamydosporae et phialides absentes. Teleomorphe ignota. Optime crescit 27°C, temperatura minima 9°C, maxima 36°C, 40°C haud crescit.

Typus vivus et exsiccatus CBS 118724 in CBS praeservatur.

Colonies (OA, 30°C), moderately expanding, dry, velvety, olivaceous green to gray; reverse olivaceous black. Budding cells absent. Hyphae septate, pale

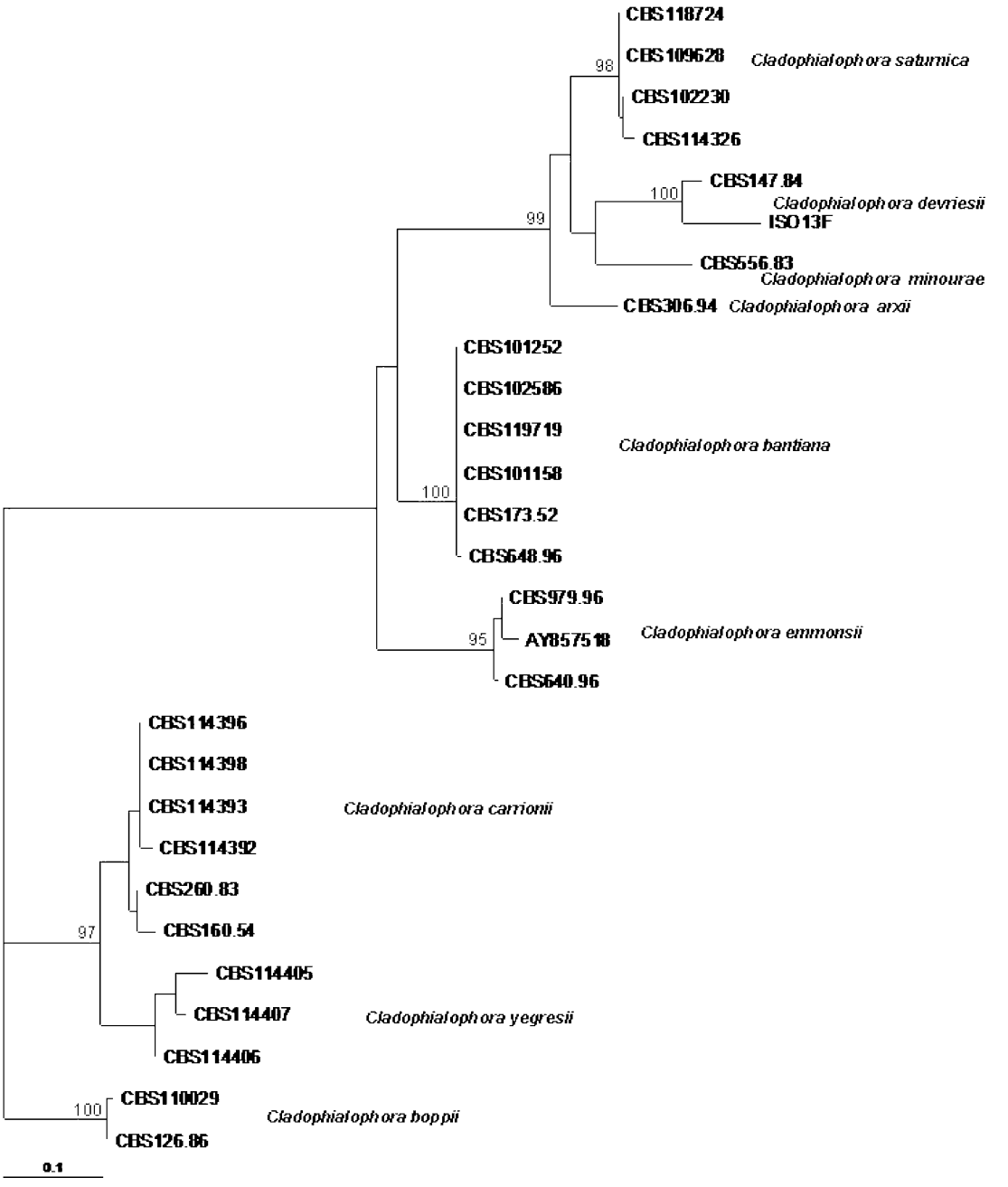

Fig. 5 Neighbor-joining tree of the *Cladophialophora* species based on EF1α, generated using the K2P+G model. The model was calculated using ML in MrAIC software. Bootstrap set to 1000 replicates and cut-off = 80%. Cladophialophora boppii (CBS 126.86 and CBS 110029) were taken as out group.

olivaceous. Conidiophores erect, with prominent denticles bearing the conidiogenous cells. Conidiogenous cells slightly lighter than the mycelia and conidiophore, cylindrical, branched with small, blunt denticles bearing lemon-shaped conidia. Conidia one-celled, olivaceous with pigmented scars, smooth-walled, arising in short, cohering, acropetal chains of 2-3; conidial scars pale pigmented. Ramoconidia occasionally produced by disarticulation of the conidial apparatus. Chlamydospores absent. Phialides absent. Teleomorph unknown. Optimal growth at 27°C, minimum and maximum 9 and 36°C, respectively. No growth at 40°C.

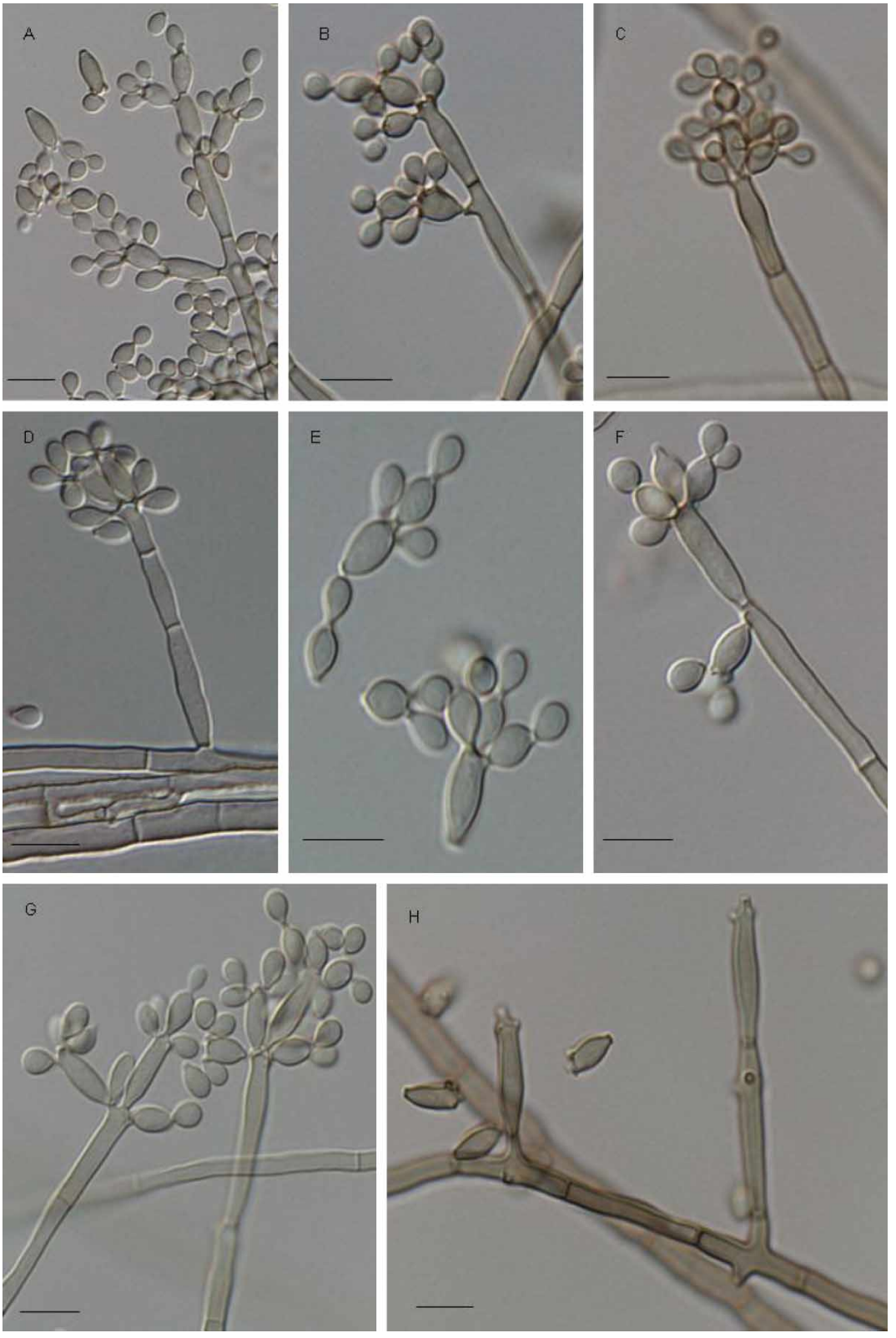

Fig. 6 Microscopic morphology of *Cladophialophora saturnica*. (A and B) CBS 102230, (C) CBS 109628, (D and E) CBS 114326, (F and G) CBS 118724, (H) CBS 109630. Scale bar = 10 μm.

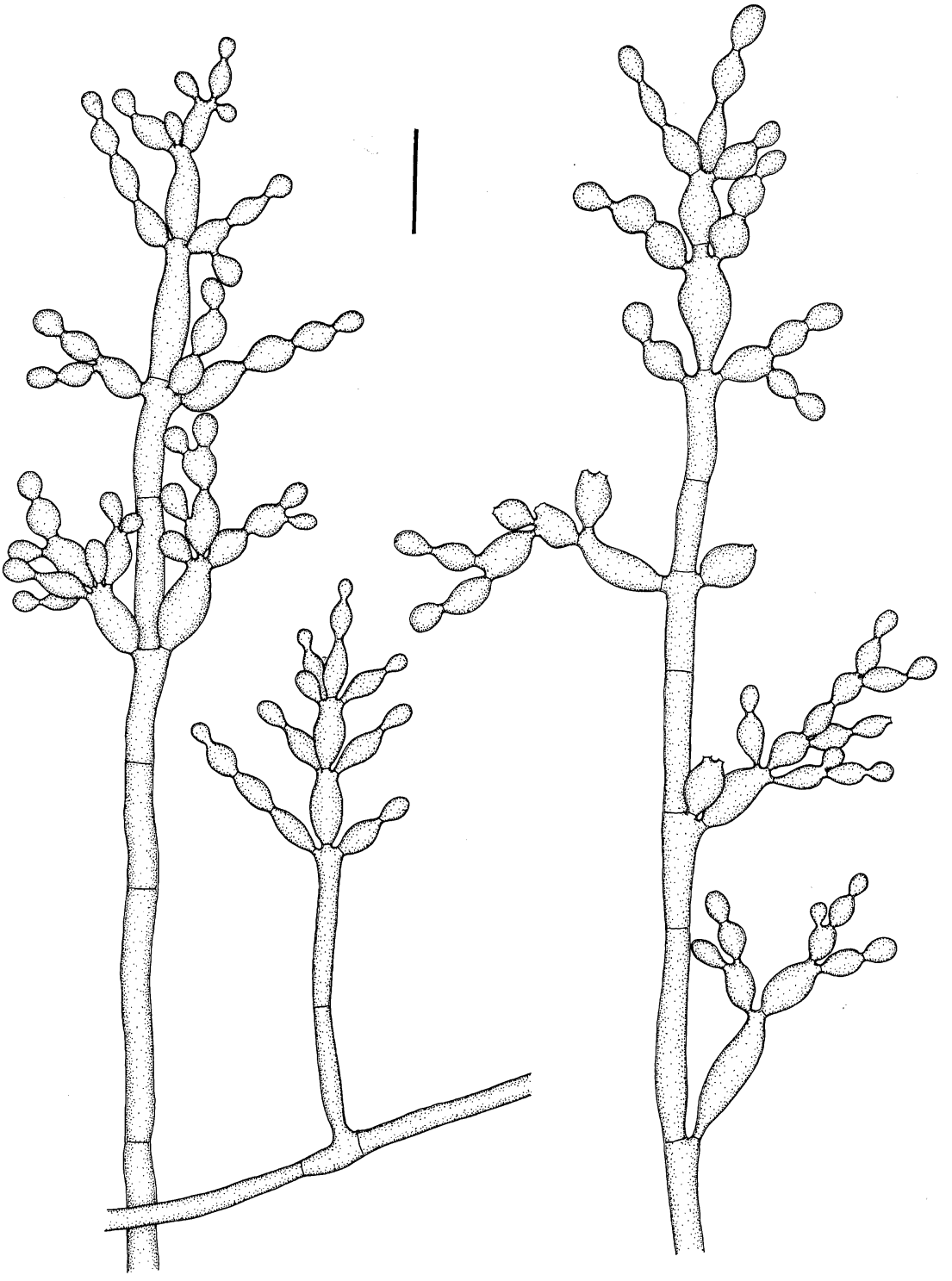

Fig. 7 Line drawing of microscopic morphology of *Cladophialophora saturnica*, strain CBS 118724. Conidiophores erect, with prominent denticles bearing the conidiogenous cells. Scale bar = 10 μm.

According to phylogenetic reconstruction, *C. saturnica* was the nearest neighbor with *C. devriesii* with a genetic distance of 6.5% and 10.4% for ITS and EF1α genes, respectively. The five isolates of *C. saturnica* formed a strongly supported monophyletic group (bootstrap = 93% in ITS and 98% in EF1 α). Although one of these five isolates caused disease in a Brazilian child with HIV infection, pathogenicity of these strains has to be evaluated with immunocompromised animal models.

Holotype: dried culture at CBS preserved as CBS H-19940; ex-type strain CBS 118724, isolated from interdigital, tinea nigra-like skin lesion of 4-year-old HIV-positive child, Curitiba, Brazil.

Discussion

This report describes a skin lesion that although appearing clinically insignificant was found to be caused by a member of the genus *Cladophialophora* which contains numerous species causing disease in immunocompetent and inmmunoincompetent individuals. This emphasizes the need for careful monitoring of patients with AIDS. The future patterns of emerging fungal opportunists in the twenty-first century will be influenced by immunocompromised hosts, permissive environmental conditions, and selective antifungal pressure [24]. Enhanced detection and identification of agents of infection in patient populations at risk are overdue.

The genus *Cladophialophora* is morphologically characterized by poorly or profusely branched chains of dry, rather strongly coherent, moderately melanized conidia. The conidial scars have pale pigmentation, in contrast to those of members of the saprobic genus *Cladosporium*, where pronounced, black conidial scars are present. Conidiophores are poorly differentiated, while those of *Cladosporium* species are mostly erect, significantly darker than the rest of the mycelium. Conidial chains of *Cladophialophora* species are coherent, while those of *Cladosporium* detach very easily.

Although species of *Cladophialophora* are predominantly involved in human disease [1], they might have their evolutionary origin in plant pathogens [7]. A recent study has investigated the phylogenetic placement of some plant-pathogenic species of *Cladophialophora* [15]. The results, although ambiguous, suggest that *C. hostae, C. scillae, C. sylvestris* and *C. humicola* are only distantly related from the clade including *C. saturnica*. *Cladophialophora*-like morphology is seen in several unrelated, environmental fungi, particularly in *Pseudocladosporium*/*Fusicladium* [15,25] and *Devriesia* [26]. These genera are assigned to different families within the *Dothideales* and the *Capnodiales*, two ascomycete orders for which species are only exceptionally encountered in a clinical setting. *Cladophialophora* species in the core of the *Chaetothyriales* share a marked clinical potential with numerous members of the family. All anamorph genera described to date, viz. *Cyphellophora, Exophiala, Fonsecaea, Rhinocladiella* and *Veronaea*, have been reported from infections in humans and warm- or cold-blooded vertebrates [1]. Within the order, the family *Herpotrichiellaceae* is based on characters of the teleomorph genus *Capronia*. Most anamorph genera are poorly supported in phylogenies using ribosomal markers [27]. Moreover, a similar morphology is observed in different subclades, and several clades are morphologically heterogeneous. In addition, *Cladophialophora* is polyphyletic, as for example, the causative agents of brain infection, *C. bantiana* and *C. modesta*, are clearly apart in SSU sequences (Fig. 3), and their ITS spacer regions are not even alignable. *Cladophialophora saturnica* is morphologically very similar to *C. devriesii* and *C. arxii*, which are comprise strains from the environment, as well as very rare agents of fatal dissemination in humans [28–30]. The numbers of strains available for study is as yet too small to be certain about genetic delineation of these species. With the development of selective isolation methods, the number of known species tends to increase in the group of the black yeasts. The use of these new isolation methods and other investigations are currently carried out with the aim to improve the knowledge of the ecology of these fungi. Despite a limited taxon sampling, the differences observed for all phylogenetic markers are considered sufficient to propose that the investigated environmental strain represents a new species of *Cladophialophora, C. saturnica*, with a clinical potential.

Species of *Cladophialophora* show a differential maximum growth at temperatures more or less coinciding with clinical predilection [1]. While species causing systemic infections are able to grow at 40°C, those that are involoved in chromoblastomycosis have a maximum growth at 37°C or in mildly cutaneous infections, such as *C. boppii,* are not able to grow at this these elevated temperatures. All strains of *C. saturnica* had an optimum growth around 27°C, and were still able to grow at 37°C, but not at 40°C (Fig. 2). This observation matches with the prevalent nature of this species as an environmental saprobe, with the potential to cause superficial infection in humans, similarly to other opportunistic species. Therefore, suggesting that potential pathogenesis in animal model should be evaluated for *C. saturnica.*

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