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

H. BADALI*†‡, V. O. CARVALHO§, V. VICENTE+, D. ATTILI-ANGELIS^, I. B. KWIAKTOWSKI+, A. H. G. GERRITS VAN DEN ENDE* & G. S. DE HOOG*†

*Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, †Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran, §Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands, ‡Division of Pediatric Dermatology, Department of Pediatrics, Federal University of Paraná, Curitiba, Paraná, Brazil, +Division of Basic Pathology, Department of Pathology, Federal University of Paraná, Curitiba, Paraná, Brazil, and ^UNESP Department of Biochemistry and Microbiology, Institute of Biosciences, Rio Claro, SP, Brazil.

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
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 weight was normal for her age, with T-CD4 values of 1,142/mm³ and T-CD8 of 1,116/mm³. 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% isopropanol 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 microscopic 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.

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°C as well as at 40°C [17].

DNA extraction

About 1 cm² 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>
<thead>
<tr>
<th>Name</th>
<th>CBS</th>
<th>Status</th>
<th>Other reference</th>
<th>GenBank ITS, EF1α</th>
<th>Source</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladophialophora saturnica</td>
<td>109628</td>
<td>dH 12333; IHM 1727</td>
<td>EU103983, EU140601</td>
<td>Dead tree</td>
<td>Uruguay, Isla Grande del Queguay</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora saturnica</td>
<td>109630</td>
<td>dH 12335; IHM 1733</td>
<td>—</td>
<td>Recently cut trunk, Neotania membranacea</td>
<td>Uruguay, Isla Grande del Queguay</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora saturnica</td>
<td>118724</td>
<td>T 157D; dH 12939</td>
<td>EU103984, EU140602</td>
<td>Interdigital toe lesion, child</td>
<td>Brazil, Paraná, Curitiba</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora saturnica</td>
<td>102230</td>
<td>dH 11591; 41BPIRA</td>
<td>AY857508, EU140600</td>
<td>Litter, vegetable cover/soil</td>
<td>Brazil, Paraná, Curitiba</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora saturnica</td>
<td>114326</td>
<td>ATCC 200384</td>
<td>AY857507, EU140603</td>
<td>Tobacco biofilter</td>
<td>Netherlands, Wageningen</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora saturnica</td>
<td>118724</td>
<td>T ATCC 56280; CDC 82-030890</td>
<td>EU103985, EU140595</td>
<td>Disseminated infection, male</td>
<td>USA, Grand Cayman Island</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora devriesii</td>
<td>147.84</td>
<td>T ATCC 56280; CDC 82-030890</td>
<td>EU103985, EU140595</td>
<td>Litter, vegetable cover/soil</td>
<td>Brazil, Paraná, Curitiba</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora arxii</td>
<td>306.94</td>
<td>T</td>
<td>EU103986, EU140593</td>
<td>Tracheal abscess, male</td>
<td>Germany</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora arxii</td>
<td>409.96</td>
<td>UAMH 5881; dH 15849</td>
<td>EU103987, EU140594</td>
<td>Male</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora arxii</td>
<td>112793</td>
<td>LT</td>
<td>EU035402, —</td>
<td>Sports drink</td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora arxii</td>
<td>114747</td>
<td></td>
<td>EU035403, —</td>
<td>Phyllostachys bambusoides</td>
<td>China</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora arxii</td>
<td>115468</td>
<td>HKUCC 10147</td>
<td>EU035404, —</td>
<td>Bamboo</td>
<td>China</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora minorae</td>
<td>987.96</td>
<td>IFM 4701; UAMH 5022</td>
<td>EU103988, EU140599</td>
<td>Rotting wood</td>
<td>Japan, Yachimata, Chiba</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora minorae</td>
<td>556.83</td>
<td>ATCC 52853; IMI 298056</td>
<td>EU103987, EU140598</td>
<td>Decaying wood</td>
<td>Japan, Shiroi</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora potulentorum</td>
<td>111222</td>
<td>CPC 1376; FRR 4946</td>
<td>EU035409, —</td>
<td>Sports drink</td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora potulentorum</td>
<td>114772</td>
<td>CPC 1375; FRR 4947</td>
<td>EU035410, —</td>
<td>Sports drink</td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora potulentorum</td>
<td>115144</td>
<td>CPC 11048; FRR 3318</td>
<td>DQ008141, —</td>
<td>Apple juice</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Fonsecaea monophora</td>
<td>289.93</td>
<td></td>
<td>EU366925, — Arctocephalus australis</td>
<td>Subcutaneous lesion, cat</td>
<td>Netherlands, Rotterdam, Blijdorp Zoo</td>
<td></td>
</tr>
<tr>
<td>Fonsecaea monophora</td>
<td>269.37</td>
<td>T</td>
<td>EU366923, —</td>
<td>Chromoblastomycosis, male</td>
<td>Brazil</td>
<td></td>
</tr>
<tr>
<td>Fonsecaea monophora</td>
<td>102238</td>
<td>dH 11602, 1PLE</td>
<td>EU366926, —</td>
<td>Chromoblastomycosis, male</td>
<td>Brazil</td>
<td></td>
</tr>
<tr>
<td>Fonsecaea monophora</td>
<td>102248</td>
<td>dH 11613</td>
<td>EU366914, — Chromoblastomycosis, male</td>
<td>South America</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Fonsecaea pedrosi</td>
<td>271.37</td>
<td>ATCC 18658; IMI 134458; dH 15659</td>
<td>EU366917, — Chromoblastomycosis, male</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Fonsecaea pedrosi</td>
<td>272.37</td>
<td></td>
<td>EU366927, —</td>
<td>Chromoblastomycosis, male</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora bantiana</td>
<td>173.52</td>
<td>CBS 100433</td>
<td>EU103989, EU140585</td>
<td>Brain abscess, male</td>
<td>USA</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora bantiana</td>
<td>102586</td>
<td>dH 11331</td>
<td>EU103990, EU140586</td>
<td>Brain abscess, male</td>
<td>Brazil, Belo Horizonte</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora bantiana</td>
<td>119719</td>
<td>Solna Lab. No. 1739/05; dH 14515</td>
<td>EU103991, EU140589</td>
<td>Skin graft, Tsunami victim</td>
<td>Thailand</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora bantiana</td>
<td>678.79</td>
<td>CPC B-3658; NCMM 2249; NIH B-3839; UAMH 4992</td>
<td>EU103992, EU140592</td>
<td>Skin lesion, cat</td>
<td>USA</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora bantiana</td>
<td>648.96</td>
<td>UAMH 3830</td>
<td>EU103993, EU140587</td>
<td>Liver, dog</td>
<td>Barbados</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora bantiana</td>
<td>444.96</td>
<td></td>
<td>EU103994, EU140591</td>
<td>Disseminated infection, dog</td>
<td>South Africa, Pretoria, Ondersteypoort</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora bantiana</td>
<td>101158</td>
<td>ATCC 44223; CDC B-3426; dH 11313</td>
<td>EU857516, EU140588</td>
<td>Brain infection, dog</td>
<td>Japan</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora bantiana</td>
<td>101252</td>
<td>ATCC 58040; CDC B-3466; dH10749</td>
<td>EU857519, EU140590</td>
<td>Brain abscess, human</td>
<td>USA, Washington</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora emmonsii</td>
<td>102586</td>
<td>dH 13029; UTHSC 03-70</td>
<td>EU857518, EU140582</td>
<td>Brain</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora emmonsii</td>
<td>640.96</td>
<td>CDC B-3634; NCMM 2248; UAMH 4991</td>
<td>EU103995, EU140584</td>
<td>Sub-cutaneous lesion, cat</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Cladophialophora emmonsii</td>
<td>979.96</td>
<td>CDC B-3875; NCMM 2247; UAMH 4994a; dH16329</td>
<td>EU103996, EU140583</td>
<td>Sub-cutaneous lesion right forearm, human</td>
<td>USA, Virginia</td>
<td></td>
</tr>
</tbody>
</table>

Cladophialophora saturnica sp. nov., a new opportunistic species
<table>
<thead>
<tr>
<th>Name</th>
<th>CBS</th>
<th>Status</th>
<th>Other reference</th>
<th>GenBank ITS, EF1a</th>
<th>Source</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladophialophora yegresii</td>
<td>114406</td>
<td></td>
<td>UNEFM SpSR1; dH 13275</td>
<td>EU137323, EU137263</td>
<td><em>Stenocereus griseus</em> plant (Cactaceae)</td>
<td>Venezuela, Falcon State</td>
</tr>
<tr>
<td>Cladophialophora yegresii</td>
<td>114405 T</td>
<td></td>
<td>UNEFM SpSr3; dH 13276</td>
<td>EU137322, EU137262</td>
<td><em>Stenocereus griseus</em> plant (Cactaceae)</td>
<td>Venezuela, Falcon State</td>
</tr>
<tr>
<td>Cladophialophora yegresii</td>
<td>114407</td>
<td></td>
<td>UNEFM SpSR1; dH 13274</td>
<td>EU137324, EU137264</td>
<td><em>Stenocereus griseus</em> plant (Cactaceae)</td>
<td>Venezuela, Falcon State</td>
</tr>
<tr>
<td>Cladophialophora carrionii</td>
<td>114392</td>
<td></td>
<td>UNEFM 82267; dH 13261</td>
<td>EU137267, EU137211</td>
<td>Chromoblastomycosis leg lesion, female</td>
<td>Venezuela, Falcon State</td>
</tr>
<tr>
<td>Cladophialophora carrionii</td>
<td>114393</td>
<td></td>
<td>UNEFM 9801; dH 13262</td>
<td>EU137268, EU137212</td>
<td>Chromoblastomycosis hand lesion, male</td>
<td>Venezuela, Falcon State</td>
</tr>
<tr>
<td>Cladophialophora carrionii</td>
<td>114396</td>
<td></td>
<td>UNEFM 2001/1; dH 13265</td>
<td>EU137269, EU137213</td>
<td>Chromoblastomycosis arm lesion, male</td>
<td>Venezuela, Falcon State</td>
</tr>
<tr>
<td>Cladophialophora carrionii</td>
<td>114398</td>
<td></td>
<td>UNEFM 2003/1; dH 13267</td>
<td>EU137271, EU137215</td>
<td>Chromoblastomycosis arm lesion, female</td>
<td>Venezuela, Falcon State</td>
</tr>
<tr>
<td>Cladophialophora carrionii</td>
<td>260.83 T of C. ajelloi</td>
<td></td>
<td>CDC B-1352; FMC 282; ATCC4435</td>
<td>EU137292, EU137234</td>
<td>Chromoblastomycosis skin lesion, male</td>
<td>Uganda</td>
</tr>
<tr>
<td>Cladophialophora carrionii</td>
<td>160.54 LT</td>
<td></td>
<td>ATCC 16264; CDC A-835; MUCL 40053; IFA 4808; dH 15445</td>
<td>AB109177/EU137266</td>
<td>Chromoblastomycosis, male</td>
<td>Australia</td>
</tr>
<tr>
<td>Cladophialophora boppii</td>
<td>126.86 T</td>
<td></td>
<td>FMC 292; dH 15357</td>
<td>EU103997, EU140596</td>
<td>Skin lesion, on limb, male</td>
<td>Brazil</td>
</tr>
<tr>
<td>Cladophialophora boppii</td>
<td>110029</td>
<td></td>
<td>det M-41/2001 56893; dH 12362</td>
<td>EU103998, EU140597</td>
<td>Scales of face, male</td>
<td>Netherlands, Dordrecht</td>
</tr>
</tbody>
</table>

Abbreviations 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 Westfälisches Institut für Wasserforschung, Mülheim an der Ruhr, Germany; GHP = G. Haase private collection; MUCL = Mycothèque 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.; 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, 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 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 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

![Fig. 2](image-url) 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).

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

<table>
<thead>
<tr>
<th>Fragment/Gene</th>
<th>Model</th>
<th>df*</th>
<th>lnL*</th>
<th>AICc*</th>
<th>wAICc*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rDNA ITS</td>
<td>HKYG</td>
<td>78</td>
<td>−2617.4881</td>
<td>5415.8731</td>
<td>0.4995</td>
</tr>
<tr>
<td>EF1α</td>
<td>K2PG</td>
<td>55</td>
<td>−1460.1768</td>
<td>3065.5536</td>
<td>0.7023</td>
</tr>
</tbody>
</table>

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

© 2009 ISHAM, Medical Mycology 47, 55–66
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.

**Fig. 3** Neighbor-joining tree based on positions 145–1640 of 115 SSU rRNA gene sequences generated with the ARB package. *Cladophialophora modesta* CBS 985.96 was used as the out group.
Cladophialophora saturnica sp. nov., a new opportunistic species

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.


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 Cladophialaphora 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%. Cladophialaphora boppii (CBS 126.86 and CBS 110029) were taken as out group.
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 EF1α). 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 immunoincompetent 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 involved in chromoblastomycosis have a maximum growth at 37°C or in mildly cutaneous infections, such as C. boppii, are not able to grow at 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.
Acknowledgements

The work of Hamid Badali was financially supported by the Ministry of Health and Education of Iran and the School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran. The authors thank Cécile Gueidan for critical reading of the manuscript. Katia Cruz and Kasper Luijsterburg are acknowledged for help in building up part of the sequence database.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References


This paper was first published online on iFirst on 6 August 2008.