Phaeohyphomycosis and onychomycosis due to Chaetomium spp., including the first report of Chaetomium brasiliense infection

VIT HUBKA*, KAREL MENCL†, MAGDALENA SKOREPOVA‡, PAVLINA LYSKOVA§ & EVA ZALABSKA†

*Department of Botany, Charles University in Prague, Czech Republic, †Department of Clinical Microbiology, Pardubice Regional Hospital Inc., Czech Republic, ‡Dermatological Clinic, Charles University in Prague, First Faculty of Medicine, Czech Republic, and §Regional Institute of Public Health, Prague, Czech Republic

Chaetomium species have been rarely described as aetiological agents of invasive and dermatomycotic infections in humans. The majority of cases have been reported within the last two decades. Treatment failed in most of these cases. In this paper we present two cases in which Chaetomium spp. can be clearly identified as an aetiological agent in pathological conditions. In the first report, we describe a new aetiological agent, Chaetomium brasiliense, which was implicated in a case of otitis externa in a patient with spinocellular carcinoma basis cranii. The patient had been repeatedly treated for relapsing otitis externa and had previously undergone surgery several times for otitis media. The fungal aetiology was confirmed by repeated positive culture and histologic studies. The second case involved onychomycosis with strikingly brown nail discoloration due to Chaetomium globosum in an otherwise healthy patient. The nail lesion was successfully cured by oral terbinafine. The determination of both species was supported by sequencing of rDNA regions. The morphological aspect of Chaetomium spp. identification is also discussed. In vitro antifungal susceptibility tests demonstrated that both isolates were susceptible to terbinafine andazole derivates except fluconazole. Amphotericin B was effective only against the C. brasiliense strain. We review the literature to summarize clinical presentations, histologic findings, and treatment strategies.

Keywords otitis externa, nail discoloration, antifungal agents, disc diffusion method, cancer patient

Introduction

The increasing incidence of dermatomycotic infections by non-dermatophyte filamentous fungi (NDFF) across several decades is indisputable. However, the incidence of NDFF dermatomycotic infections, particularly onychomycosis, is highly variable and depends upon laboratory methodologies used. The conventional criteria for the diagnosis of NDFF onychomycosis [1] and other dermatomycotic infections were modified and recently re-evaluated [2–5]. Reliable and simplified criteria for the identification of NDFF dermatomycotic infections are an indispensable foundation for the diagnosis and treatment of this problematic clinical entity. It is important to note that only a reduced number of antifungal drugs effective against dermatophytes have action spectra that include NDFF [6].

The term phaeohyphomycosis refers to a heterogenous group of mycotic infections that are caused by a group of fungi containing melanin pigments in their cell wall. They are called phaeoid fungi (rather than dematiaceous) which also includes members of the genus Chaetomium. While there is a variety of definitions, the one based upon histopathological finding is the one most frequently accepted in medical mycology. Employing this definition, phaeohyphomycoses encompass cutaneous, subcutaneous and systemic infections in which the aetiological agent develops...
in the host tissue in the form of dark-walled phaeoid mycelial elements [7,8]. Chaetomium is a member of the ascomycete family Chaetomiaceae (order Sordariales) and encompasses more than 100 species. Most of the species colonize cellulose-rich substrata, such as plant debris, herbivore dung, paper or textiles [9,10]. Many species produce mycotoxins of medical and agricultural interest [11,12]. Chaetomium spp. are generally referred to as soil saprophyles and are also commonly isolated from the soil by techniques for isolation of keratinophilic fungi [13,14]. Chaetomium globosum was also detected in the air of special-care units of a hospital [15] and as a contaminant of fluid for peritoneal dialysis [16].

Recently, some species of Chaetomium have been reported to be of medical interest as aetiological agents of systemic and dermatomycotic infections [17]. No conidial anamorphs are known in medically important Chaetomium species with the exception of C. strumarium, which produces small conidia on solitary phialides [18]. Other species propagate strictly by ascospores. Infections due to a fungus without an anamorphic state are exceptional in medical mycology.

In this paper, we report on otitis externa caused by C. brasiliense and onychomycosis caused by C. globosum. Case report 1 presents the first instance of infection due to C. brasiliense. Case report 2 represents the second case of onychomycosis due to Chaetomium in Europe in addition to a 2007 Spanish case [19]. Susceptibility to common antifungal agents was characterized in the case isolates and some other clinical strains of Chaetomium to approximately assess the antifungal susceptibility pattern. The literature was reviewed to summarize clinico-histopathological presentation and treatment of nail and skin infections due to Chaetomium spp. Morphology, as well as sequence data analysis were used in the identification of the isolates. Recommendations and important features for identification of Chaetomium spp. are also summarized in this paper.

Case report 1

A 61-year-old woman was admitted to an otorhinolaryngological (ORL) ward in May 2008 with a 2-week history of marked left-sided hearing loss. The patient had had surgery four times for otitis media in her personal history, the last in 1970. She had suffered a head injury with otalgia and infraauricular oedema in 2002 and had previously experienced repeated inflammation in the left auditory canal.

The patient underwent many neurological and ORL examinations in 2007. Hypacusis and hypesthesia anterior to the left tragus and mandible while reclining to the right with an open mouth were diagnosed in August 2007. The frontal wall of the left auditory canal was damaged with numerous granulations and sanguineum species. Progressive, persistent neuralgia of the trigeminal nerve combined with hypesthesia of the lower lip was followed by incomplete facial paresis in December 2007. Additional symptoms were neurogenic cervicalgia and tingling on the left side of the tongue. CT (August 2007) and MRI (June 2007) examinations revealed no clear signs of a tumor. The patient was treated with ciprofloxacin drops for a supposed relapse of bacterial otitis externa (4 drops twice per day for 10 days; this corresponds to 0.6 mg of active substance per day). After 2 weeks of treatment, the patient complained about ringing in her ear and developed fluid in the left ear. Repeated CT examination (May 2008) revealed multiple inflammatory changes in the tympanic cavity and basis cranii. Diagnostic paracentesis of the membra tympani was performed (June 2008), with several tissue samples recovered for histological and microbiological examination. An invasive, moderately differentiated spinocellular carcinoma was confirmed by histological examination and abundant fungal growth was noted in cultures. The recovery of the fungus led us to perform further examinations of the previously obtained tissue samples (hypertrophic granulation tissue from the external auditory canal and Eustachian tube). Brown walled fungal elements were revealed in the tissue sections. Using Grocott methenamine silver stain (GMS) the fungal structures were highlighted, occasional thick-walled mycotic hyphae and thick-walled spherical mycotic cells were observed (Fig. 1).

Mycotic otitis externa was verified by repeated sampling. The obverse of colonies on Sabouraud glucose agar (SGA) was initially white due to abundant aerial mycelia and later turned grey (Supplementary Fig. 1 – online only), while the reverse was black. The primordia of fruiting bodies with numerous ascomatal hairs developed within 2 weeks but failed to mature over several weeks of incubation. The fungus grew rapidly at 37°C. Further studies were conducted on corn meal agar (CMA) and on potato carrot agar

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Fig. 1 Histopathology taken from a nodular lesion from the external auditory canal. Occasional thick-walled, mycotic hyphae and thick-walled spherical bodies were discovered using Grocott silver staining.
The fungus grew on these media at 25°C and the isolate was identified as Chaetomium brasiliense (Fig. 2, Supplementary Figs. 2–6 – online only) according to Arx et al. [10].

Despite these findings, the patient was not subjected to antifungal therapy. Surgical therapy was not indicated due to the advanced stage of the cancer. The patient underwent radiotherapy (70 Gy/33 fractions/7 weeks) and chemotherapy (cis-dichlorodiammineplatinum [II]; 40 mg 4 times in the period of 6 weeks) with no evident improvement. Analgesic therapy was prescribed and the patient was under long-term dispensarization. Serum biochemical and hematological analysis and peripheral blood count remained in the reference limits until the advanced stage of the disease. The patient did not suffer from any other immunosuppressive or chronic disease and died a year later (September 2009) of a bacterial respiratory infection and respiratory failure secondary to advanced cancer.

Case report 2

A 48-year-old male presented with a severe nail infection in December 2006. He first noticed a possible infection in the nail plate of the first digit of the left foot a year earlier following a traumatic haematoma acquired during a basketball game. Later, it spread from the distal margin of the nail proximally with the nail plate of the left second digit becoming affected. The nails were discoloured, dystrophic and thickened by hyperkeratosis (Fig. 3A).

The patient had previously been treated for flexural eczema with topical corticosteroids. At the time, the eczema manifested intermittently on the hands and was successfully treated by local corticosteroids application. The patient was otherwise healthy and was not regularly taking any other medications.

The direct microscopic examination of the affected nails in 20% KOH mounts revealed aggregates of swollen, thick-walled brown cells and hyphae (Fig. 3B). Nail clippings were inoculated onto SGA slants with and without chloramphenicol (Bio-Rad) and incubated at 27°C. Extensive growth of a non-dermatophyte hyphomycete in pure culture was observed after 2 weeks of incubation and the same fungus was recovered during a subsequent visit. Colonies on SGA were initially white and cottony but became dark olive green with age. Microscopic examination of the colonies showed subglobose perithecia with numerous ascomatal hairs and lemon-shaped ascospores. Growth at 37°C was restricted. Further studies were conducted as described in the first case. The isolate was identified based on its morphology (Fig. 4, Supplementary Figs. 1, 6–8 – online only) as Chaetomium globosum according to Arx et al. [10].

![Fig. 2 Chaetomium brasiliense CCF 3878 – case 1. Perithecium with spirally coiled ascomatal hairs (A), scale bar, 50 μm; cylindrical ascus (B), scale bar, 10 μm; ovate ascospores (C) with a single germ pore, scale bar, 10 μm.](image-url)
A daily oral dose of terbinafine (250 mg) was administered for 3 months. After 8 months (November 2007), the nail plates showed healthy growth and only slight hyperkeratosis persisted in the nail plate of the left big toe. KOH preparations and cultures were negative.

Materials and methods

Preservation of strains

Cultures of the case isolates were entered into the Culture Collection of Fungi (CCF), Department of Botany, Charles Fig. 3 Onychomycosis due to *Chaetomium globosum* – case 2. Thickened, dystrophic and hyperpigmented toenails (A). Direct microscopic examination of the nail sample in 20% KOH revealed brown, thick-walled fungal elements (B).

Fig. 4 *Chaetomium globosum* CCF 4060 – case 2. Perithecium with undulate ascomatal hairs (A), scale bar, 100 μm; clavate ascus (B), scale bar, 10 μm; limoniform biapiculate ascospores (C), scale bar, 10 μm.
University in Prague, and were designated as stated in Table 1.

**Antifungal susceptibility testing**

The *in vitro* antifungal drug susceptibility of the *Chaetomium* isolates was investigated by the disc diffusion method. Seven clinical isolates of *Chaetomium* belonging to three species (*C. brasiliense*, *C. globosum*, and *C. funicola*) were chosen for susceptibility testing (Supplementary Table S1 – online only).

**Inoculum preparation**

The isolates were grown on PCA for 3 weeks at 25°C. The inoculum was prepared by transferring the perithecia via a sterile swap into 15 ml sterile tubes containing 5 ml of sterile distilled water. The suspension was vigorously vortexed for 15 sec. Heavy hyphal fragments and perithecial wall fragments were removed by passing the suspension through a sterile nylon filter (pore size 20 μm). A homogenous suspension, consisting mainly of ascospores, was adjusted spectrophotometrically at 530 nm by adding sterile water to obtain optical densities that ranged from 0.092–0.110 (78–81% transmittance).

**Plate inoculations**

Suspensions were applied to the surfaces of 90 mm RPMI 1640 agar plates (TRIOS) by dipping a sterile swab applicator into the ascospore suspension and streaking it across the surface of the plates in three directions. The plates were allowed to dry at ambient temperature for 20 min.

**Disc diffusion method**

The paper discs (ITEST) of amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, miconazole, ketoconazole, voriconazole and terbinafine were applied to the surface of the inoculated plates, which were then incubated at 25°C and read after 48 and 72 h. Zone diameters were measured to the nearest whole millimetre at the point where there was a prominent reduction in growth (80%). Zone diameter categories were assigned as susceptible, intermediate (susceptible dose-dependent) and resistant according to the manufacturer’s instructions (Supplementary Table S2 – online only).

**DNA analysis**

Genomic DNA was isolated from 7-day-old cultures using a Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc.). Amplification of the rDNA ITS (ITS1-5.8S-ITS2) region was performed using the primers ITS5 and ITS4S. A partial sequence of LSU rDNA was amplified with the primer set NL1 and LR6. PCR amplification was performed using the same cycling conditions for both rDNA regions and followed the procedures described in Kolarik et al. [21]. PCR product purification and sequencing was performed into the ascospore suspension and streaking it across the surface of the plates in three directions. The plates were allowed to dry at ambient temperature for 20 min.

Table 1  Reported cases of dermatomycotic *Chaetomium* infections.

<table>
<thead>
<tr>
<th>Patient/Reference</th>
<th>Species</th>
<th>Clinical features</th>
<th>Location</th>
<th>Age/ Sex of patient</th>
<th>Predisposing factor</th>
<th>Treatment outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td><em>C. brasiliense</em></td>
<td>Otitis externa</td>
<td>Czech Republic</td>
<td>61/F</td>
<td>Injury of head, surgeries, tumor</td>
<td>No antymycotic therapy</td>
</tr>
<tr>
<td>(CCF‡ 3878)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 2</td>
<td><em>C. globosum</em></td>
<td>Onychomycosis (toenails)*</td>
<td>Czech Republic</td>
<td>48/M</td>
<td>Trauma, eczema</td>
<td>Cured</td>
</tr>
<tr>
<td>(CCF 4060)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[19]</td>
<td><em>C. globosum</em></td>
<td>Onychomycosis (toenails)*</td>
<td>Spain</td>
<td>23/M</td>
<td>Football player</td>
<td>Cured</td>
</tr>
<tr>
<td>[30]</td>
<td><em>C. globosum</em></td>
<td>Onychomycosis (toenails)*</td>
<td>USA</td>
<td>83/F</td>
<td>Age</td>
<td>NR</td>
</tr>
<tr>
<td>[26]</td>
<td><em>C. globosum</em></td>
<td>Onychomycosis (fingernails, toenail)#</td>
<td>Japan</td>
<td>57/M</td>
<td>Intensive traveling</td>
<td>Improvement</td>
</tr>
<tr>
<td>[27]</td>
<td><em>C. globosum</em></td>
<td>Onychomycosis (fingernails)#</td>
<td>Brasil</td>
<td>62/F</td>
<td>Trauma</td>
<td>Failed</td>
</tr>
<tr>
<td>[28]</td>
<td><em>C. globosum</em></td>
<td>Onychomycosis (fingernails)#</td>
<td>India</td>
<td>26/M</td>
<td>Trauma</td>
<td>NR</td>
</tr>
<tr>
<td>[32]</td>
<td><em>C. globosum</em></td>
<td>Onychomycosis (fingernails)#</td>
<td>India</td>
<td>25/M</td>
<td>Acid burn</td>
<td>NR</td>
</tr>
<tr>
<td>[59]</td>
<td><em>C. globosum</em></td>
<td>Subcutaneous phaeohyphomycosis#</td>
<td>China</td>
<td>13/M</td>
<td>Farmer</td>
<td>NR</td>
</tr>
<tr>
<td>[29]</td>
<td><em>C. globosum</em></td>
<td>Subcutaneous phaeohyphomycosis#</td>
<td>China</td>
<td>14/M</td>
<td>Immunodeficiency, cardiomyopathy</td>
<td>Failed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[27]</td>
<td><em>C. globosum</em></td>
<td>Superficial phaeohyphomycosis#</td>
<td>Brasil</td>
<td>64/F</td>
<td>Trauma</td>
<td>Cured</td>
</tr>
<tr>
<td>[25]</td>
<td><em>C. globosum</em></td>
<td>Superficial phaeohyphomycosis#</td>
<td>Italy</td>
<td>46/M</td>
<td>N/A</td>
<td>Cured</td>
</tr>
<tr>
<td>[60]</td>
<td><em>C. faricola</em></td>
<td>Superficial phaeohyphomycosis#</td>
<td>Germany</td>
<td>71/M</td>
<td>Age</td>
<td>NR</td>
</tr>
<tr>
<td>[58]</td>
<td><em>C. faricola</em></td>
<td>Chromoblastomycosis</td>
<td>Panama</td>
<td>83/M</td>
<td>Age, farmer</td>
<td>Failed</td>
</tr>
<tr>
<td>[61]</td>
<td><em>C. marorum</em></td>
<td>Subcutaneous phaeohyphomycosis#</td>
<td>China</td>
<td>25/F</td>
<td>Farmer</td>
<td>NR</td>
</tr>
<tr>
<td>[31]</td>
<td>Chaetomium sp.</td>
<td>Onychomycosis (toenails)*</td>
<td>Spain</td>
<td>11/F</td>
<td>Eczema</td>
<td>Cured</td>
</tr>
<tr>
<td>[47]</td>
<td>Chaetomium sp.</td>
<td>Superficial phaeohyphomycosis#</td>
<td>Brazil</td>
<td>8/F</td>
<td>N/A</td>
<td>NR</td>
</tr>
</tbody>
</table>

# Brown-colored hyphae were observed in direct microscopy.
* Hyaline, septate hyphae were observed in direct microscopy.
‡ CCF, the isolated are maintained in the Culture Collection of Fungi (CCF), Department of Botany, Charles University in Prague; N/A, not reported.

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at Macrogen Inc. (Seoul, South Korea). Using a BLAST similarity search, obtained sequences were compared with sequences listed on the GenBank server. Sequences were deposited into EMBL database under accession numbers FR718872-3 and FR823008-9.

Results

Disc diffusion method

Drug susceptibility test results for both case isolates are summarized in Table 2 and the results for all seven isolates are included in Supplementary Table S1 – online only. For all antifungal agents, except for amphotericin B, there were no significant differences in the inhibition zone diameters when read after 48 h or 72 h, i.e., the values remained in the same category. For amphotericin B, the diameter of the inhibition zone was markedly reduced after 72 h. C. funicola strains, although susceptible after 48 h, were scored as resistant after 72 h (Supplementary Table S1 – online only).

All the Chaetomium strains tested showed resistance by the disk diffusion method to 5-fluorocytosine and fluconazole. Conversely, all strains were susceptible to itraconazole, voriconazole, miconazole and ketoconazole. The C. globosum strains were resistant to amphotericin B and intermediate susceptible to terbinafine in contrast to the strains of C. funicola and C. brasiliense that were susceptible.

Molecular data analysis

The morphological identification of the C. brasiliense strain CCF 3878 was supported by a partial LSU sequence that was 100% similar to the C. brasiliense strain sequenced by Untereiner et al. [22]. Sequence of the ITS region showed only a 92–93% similarity to some related Chaetomium spp. and to some other genera of ascomycetes. No sequence of the ITS region of C. brasiliense had previously been deposited into GenBank.

The ITS, as well as partial LSU sequence of the C. globosum strain CCF 4060 was 100% similar to C. globosum isolates sequenced by Sugiyama et al. [23] and to some other C. globosum isolates deposited in the internationally recognized culture collections. The LSU region was 99% (535/537 bp) similar to C. globosum isolate MUCL 39889 sequenced by Untereiner et al. [22]. In addition, the ITS sequence showed 100% similarity to ‘C. brasiliense’ strain NRRL 22999 (GU183109) and the LSU sequence was 100% similar to ‘C. murorum’ strain UOA 9860 (GQ376100).

Discussion

Chaetomium spp. are not considered to be keratinolytic, although they are frequently isolated from soil via the hair baiting technique [13,14]. In the natural process of keratinized substrates degradation, Chaetomium spp. may contribute significantly at a certain stage of succession, and participate in the degradation of complex substances in a natural scenario [14,24]. This suggests that Chaetomium spp. have the potential to degrade damaged nails and skin. Age-related nail and skin changes, including reduced function, are well known. Phaeohyphomycoses are generally believed to occur as a result of traumatic implantation of fungal material from contaminated plants or soil, other cases have been reported in elderly, as well as immunodeficient patients [8]. Similarly, Chaetomium spp. typically cause nail and skin infections secondary to some transient insult or secondary to some underlying problem, although a few cases without evident primary insult have been reported [25,26]. These infections are characterized by chronicity and difficulty in treatment. The infection progresses slowly and patients usually present several years after its initiation [19,27,28].

Nail and skin infections incited by Chaetomium spp. occur predominantly in posttraumatic immunocompetent patients, with only one case reported in a patient with evident immunodeficiency [29] (Table 1). Clinical presentations of cutaneous infections include superficial phaeohyphomycosis, deep subcutaneous phaeohyphomycosis, or chromoblastomycosis (Table 1). One case of erythematous epilation in a dog due to C. globosum has been described [23]. Nail infections manifest as onychomycosis with strikingly brown to black [27,28,30], or yellow-brown [19,26,31,32] discoloration. Toenails, as well as fingernails may be affected and the lesions may spread to the surrounding skin [27]. Similarly, the intensive brown to brown-black nail discoloration may occur in nail lesions caused by some other filamentous fungi such as Trichophyton rubrum var. nigricans, Scytalidium dimidiatum, Alternaria alternata, Microascus desmosporus [33], Curvularia lunata [34], Exophiala dermatitidis [35], Fusarium oxysporum [36], Aspergillus spp. [37], Onychochola canadensis [38,39] and Scopularopsis brumptii [40]. In differential diagnosis it may be important to exclude melanonychia associated with subungual melanoma in some instances [39].
**Table 3** The morphological features differentiating clinically relevant *Chaetomium* spp. recovered from nail and skin infections.

<table>
<thead>
<tr>
<th>Species name</th>
<th>Ascus</th>
<th>Ascospores: shape; size (μm)</th>
<th>Colonies (CMA, 25°C)</th>
<th>Ascomatal hairs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. brasiliense</em></td>
<td>Cylindrical&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Ovate; 7–8.5 × 6–7 × 5–6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Grey; black reverse</td>
<td>Spirally coiled&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. funicola</em></td>
<td>Clavate&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Ovate, slightly apiculate; 6–7.5 × 4–4.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>White to pale grey; yellow reverse</td>
<td>Partly long unbranched, partly shorter repeatedly dichotomously branched&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. globosum</em></td>
<td>Clavate&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Limoniform, biapiculate; 9–12 × 8–10 × 6–8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Olivaceous brown; often with colored exudate</td>
<td>Undulate&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. muroorum</em></td>
<td>Clavate</td>
<td>Ellipsoidal to broadly fusiform; 13–17 × 7–9</td>
<td>Grey to olivaceous brown</td>
<td>Flexuous, often recurved at the apex</td>
</tr>
</tbody>
</table>

<sup>a</sup>According to Arx et al. [10].
<sup>b</sup>Fig. 2, Suppl. Figs 4 & 5; <sup>c</sup>Suppl. Fig. 10; <sup>d</sup>Fig. 4, Suppl. Fig. 8; <sup>e</sup>Fig. 6, Suppl. Figs. 2 & 3; <sup>f</sup>Suppl. Fig. 9; <sup>g</sup>Fig. 4, Suppl. Fig. 7.

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Infections due to Chaetomium species

Among other typical morphological features (Table 3), cylindrical asci (Fig. 1, Supplementary Figs. 4 & 5) that are present in a limited number of Chaetomium species. C. murorum can also be easily differentiated from C. globosum by morphology (Table 3).

Only three Chaetomium spp. have been shown to cause dermatomycotic infections in humans (Table 1). We have updated this observation by identifying a fourth species, C. brasiliense, which was implicated in a case of otitis externa in a cancer patient. Mycotic aetiology was confirmed by repeatedly positive recovery of the same fungus and by histology. A possible connection between chronic inflammation of the external auditory canal and development of cancer is questionable. Mycotic aetiology was not investigated in this patient despite the recurrence of her otitis externa. It is a known fact that inflammation is a critical component in tumor progression. Many types of cancer arise from sites of infection, chronic irritation and inflammation [48]. We are not able to establish the beginning of fungal infection. The fungal pathogen could be present for several months or even for several years. Chaetomium spp. produce many classes of bioactive secondary metabolites with various biological properties such as immunomodulatory, cytotoxic, and anti-tumor effects that may, together with autoaggressive character of chronic inflammation, participate in cancerogenesis [11,12,49,50]. According to 2006 data, C. brasiliense has never been isolated in the Czech Republic [51].

In addition to the two presented case reports, we found that Chaetomium spp. are relatively frequently isolated from dermatomycotic specimens. Fourteen isolates were recovered from specimens in the last 4 years (a study on incidence and spectrum of NDFF is a part of the first author’s MA thesis and is currently in preparation). This collection included one C. brasiliense, three C. funicola, and ten C. globosum isolates. All isolates are listed in Supplementary Table S3 – online only. Some of the isolates were tested for susceptibility to some antifungal agents (Supplementary Table S1 – online only). In only the two cases presented here was the non-dermatophyte aetiology diagnosed based on repeated examination. In other suspicious cases, the aetiological significance of isolated strains was unclear as repeated sampling was not performed. Some direct microscopic examinations were positive for hyaline, irregular, septate hyphae. In other observations, septate, brown-coloured hyphae or thick-walled brown cells were found. This finding is in agreement with previous reports that variously described either hyaline, septate hyphae or brown-coloured fungal elements (Table 1). Microscopic observation of the infectious fungal elements in samples can possibly demonstrate great variability. The mycelium produced by Chaetomium spp. is hyaline, only the fruiting bodies are dark pigmented. The placement of the genus Chaetomium in the dematiaceous hyphomycetes is controversial and epistemologically incorrect. The ability to produce melamins is widespread in fungi and some fungal pathogens traditionally not considered phaeoid have the ability to synthesize melanin in vivo during infection. This may lead to confusions in the diagnosis [52]. This is the reason why we prefer the definition of phaeohyphomycosis based on histological findings rather than on phenotypic trait of the aetiological agent being dark-pigmented under laboratory conditions. Enhanced melamins synthesis is generally associated with stress [53] and its production is linked to virulence in pathogenic fungi [54].

Therapy results for Chaetomium infections are equivocal. Despite the use of several drugs, either alone or in combination, treatment failed in the majority of cases of systemic infections. Treatment results of the cases of invasive Chaetomium infections were summarized by Al-Aidaroos et al. [41], while those of skin and nail infections are reviewed in Table 1. There are only a few cases of dermatomycotic infections that include information on therapy and only in some was the treatment successful. There have been just three cases of onychomycosis due to Chaetomium which describe effective therapy. A case of onychomycosis was partially cured by oral itraconazole [26] and another two cases were completely cured by topical and oral terbinafine [19] or oral itraconazole and topical miconazole [31]. Two cases of superficial skin lesions were successfully treated with topical oxiconazole [27] or oral terbinafine in combination with topical toconazole [25]. A skin lesion in a dog was cured by systemic and topical ketoconazole [15]. We chose oral terbinafine for treatment of our cases. The nail lesions were nearly cured after 8 months. Only two cases of disseminated infection were cured after the use of amphotericin B [44,55], in most of the other cases the patients died before Chaetomium was identified.

Previously published data on antifungal susceptibility obtained by the modified broth microdilution reference procedure of the Clinical and Laboratory Standards Institute (CLSI) for filamentous fungi are available for some Chaetomium spp. and for some antifungal agents [43,56]. No criteria for susceptibility testing of species that disseminate only via ascospores have been established. Consequently, the results study should be considered to be approximate. In some other studies, only one or two strains of C. globosum [23,44,26,28,29] or C. perlucidum [42] were tested with markedly different results. C. brasiliense has never been tested. The disc diffusion method that we chose shows only a low percentage of major, very major and minor errors (shifts between susceptible and intermediately susceptible or between intermediately susceptible and resistant) in filamentous fungi and is not as dependent on incubation time as other methods based on diffusion gradient [57].

Chaetomium isolates showed resistance to 5-fluorocytosine and fluconazole (Supplementary Table S1 – online only). This is in agreement with previous reports [23,26,43,44].
for some Chaetomium spp. We expanded this finding to C. brasiliense. The described resistance is probably typical for the entire genus Chaetomium and is reported frequently in filamentous fungi. Fluconazole therapy was shown to be ineffective in vivo as well [58]. Conversely, all the Chaetomium isolates tested appeared to be susceptible to azole derivatives excepting fluconazole. Inter-species differences in susceptibility to amphoteracin B and terbinafine were found. C. globosum strains were resistant to amphoteracin B and intermediately susceptible to terbinafine in contrast with the strains of C. funicola and C. brasiliense that were susceptible (Supplementary Table S1 – online only). The strain of C. brasiliense was susceptible to the highest number of antifungal agents tested. Based on limited in vivo data, oral terbinafine and oral itraconazole seem to be effective in the treatment of dermatomycotic infections due to Chaetomium spp.

Conclusions

In conclusion, nail and skin infections due to Chaetomium spp. occur predominantly in immunocompetent patients, typically presenting with secondary to traumatic implantation, or in elderly people. The infection may manifest as phaeohyphomycosis, chromoblastomycosis or onychomycosis with brown discoloration. The infection progresses slowly and patients usually present several years after it first appears. For the first time ever, we report a case of infection caused by C. brasiliense, involving otitis externa in a cancer patient that manifested histologically as phaeohyphomycosis. The second report deals with a case of onychomycosis due to C. globosum which included such typical signs of nail infection due to Chaetomium, as history of nail trauma and brown discoloration of the affected nail. Therapy strategy is not established due to the small number of cases. The infection has been most effectively eradicated when treatment included terbinafine or itraconazole. Chaetomium species have a specific antifungal susceptibility pattern that includes primary resistance to 5-fluorocytosine and fluconazole. Important interspecies differences in antifungal susceptibility were observed particularly with amphoteracin B.

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Note

“The EMBL accession numbers for the ITS region and LSU rDNA of Chaetomium brasiliense and C. globosum are FR718872-3 and FR823008-9.

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Supplementary material available online
Supplementary Figs 1–10.
Supplementary Tables 1–3.

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