SYMPOSIUM

*Chaetomium atrobrunneum* causing human eumycetoma: The first report

Najwa A. Mhmoud¹,², Antonella Santona³, Maura Fiamma³, Emmanuel Edwar Siddig¹, Massimo Deligios³, Sahar Mubarak Bakhiet¹,†, bob skeletalistro e³, Salvatore Rubino³, Ahmed Hassan Fahal¹*

¹ Mycetoma Research Centre, University of Khartoum, Khartoum, Sudan, ² Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan, ³ Department of Biomedical Sciences, University of Sassari, Sassari, Italy, ⁴ Institute for Endemic Diseases, University of Khartoum, Khartoum, Sudan

* ahfahal@hotmail.com, ahfahal@uofk.edu

**Author summary**

In this communication, a case of black grain eumycetoma produced by the fungus *C. atrobrunneum* is reported. The patient was initially misdiagnosed with *M. mycetomatis* eumycetoma based on the grains’ morphological and cytological features. However, further aerobic culture of the black grains generated a melanised fungus identified as *C. atrobrunneum* by conventional morphological methods and by internal transcribed spacer 2 (ITS2) ribosomal RNA gene sequencing. This is the first-ever report of *C. atrobrunneum* as a eumycetoma-causative organism of black grain eumycetoma. It is essential that the causative organism is identified to the species level, as this is important for proper patient management and to predict treatment outcome and prognosis.

**Overview**

Mycetoma is a chronic, progressive, granulomatous, subcutaneous inflammatory disease. It is caused by certain fungi and bacteria, and thus, it is classified as a eumycetoma and an actinomyctoma, respectively. More than 50 microorganisms were reported as mycetoma-causative organisms. *Madurella mycetomatis* is the most frequently reported eumycetoma-causative organism, with the highest worldwide disease burden. It generally affects children and young adults of low socioeconomic status, causing devastating deformities, disability, and high morbidity in late disease, and it has many severe effects on patients and communities. In this communication, we report on a 32-year-old patient from the White Nile state, Central Sudan. He presented with a painless subcutaneous swelling in the left foot of 7 years’ duration. The clinical diagnosis was *M. mycetomatis* eumycetoma, for which he underwent wide surgical excision. The black grains’ aerobic culture generated a melanised fungus identified as *Chaetomium atrobrunneum* by conventional morphological methods and by internal transcribed spacer 2 (ITS2) ribosomal RNA gene sequencing. The patient was started on empiric itraconazole, and fungal susceptibility was later confirmed by Sensititre YeastOne test (minimum inhibitory concentration [MIC] ≤ 0.06). No evidence of recurrence was observed after 1 year of treatment. Our study adds *C. atrobrunneum* from the Sordariales order and Chaetomiaceae family to the list of melanised fungi causing human black grain eumycetoma. Both phenotypic and genetic methods were fundamental to elucidate the eumycetoma associated with this unusual mould and to determine the appropriate therapy.
Introduction

Mycetoma is a chronic granulomatous inflammatory disease that is characterized by local swelling and draining sinuses that drain grains of different color and size depending on the causative agent; the disease is caused either by bacteria or fungus, with the latter being the most common causative agent of mycetoma reported in Sudan [1 – 3]. The two major factors for successful management of mycetoma patients are better identification of the causative agents and better prevention and treatment of infection [2, 4]. The identification of the causative agent is of value for proper treatment and identification of the drug of choice for patient treatment [5].

**Chaetomium** species are scattered worldwide, in animal dung, straw, paper, bird feathers, seeds, plant debris, and soil [6]. The genus, *Chaetomium*, encompasses more than 100 species, most of which grow best in a temperature ranging from 25–37˚C [7, 8]. This fungus is able to affect healthy and immunocompromised people; the most common pathogenic species was *Chaetomium globosum* [8 – 10]. Clinical features of infection have included those in association with onychomycosis [8, 9], keratitis [11, 12], sinusitis [13], lung empyema [14], pneumonia, and fatal disseminated cerebral mycosis [15]. However, *C. atrobrunneum* has not been previously reported to be associated with mycetoma infection.

Case report

The patient is a 32-year-old farmer from the White Nile, Central Sudan, who presented in 2010 to the Mycetoma Research Centre (MRC), Khartoum, Sudan, with a painless left foot swelling of 4 years’ duration. His condition started with a small, painless subcutaneous swelling on the heel of the left foot that gradually increased in size. In 2008, it was diagnosed as an abscess, for which he underwent surgical drainage under local anaesthesia at a district general hospital twice.

The patient had no medical comorbidities and had no family history of similar conditions. He had an ultrasound examination of the swelling, which showed a surgical scar and a single cavity that contained fluid collection and echogenic aggregated grains suggestive of residual mycetoma. He underwent fine needle aspiration for cytology, which showed black grains and inflammatory infiltrates in line with *M. mycetomatis* with type I and II tissue reactions. He was started on 400 mg of ketoconazole twice per day (BID), and he was on regular follow-up in the MRC for 18 months; he then dropped the follow-up and treatment.

In 2017, he was seen at El Andalous Health Centre, the White Nile, with the same left foot lesion, which had increased in size. On examination, he looked well and not pale. He was hemodynamically stable. The systemic examinations were unremarkable. Local examination showed a firm subcutaneous mass on the left heel, which was 4 × 4 cm, firm in consistency, and attached to the skin and had deep structures with multiple sinuses and discharge of black grains.

His liver function test showed serum bilirubin of 0.3 mg/dL, total protein of 8 g/dL, serum albumin of 5 g/dL, alkaline phosphatase of 98 U/L, aspartate aminotransferase (AST) of 15 U/L, and alanine aminotransferase (ALT) of 20 U/L. His renal function test showed normal blood urea of 21 mg/dL and serum creatinine of 0.51 mg/dL. His complete blood count examination showed leucocytosis, with a total white blood cell count of 12.0 × 10³, haemoglobin count of 12.1 g/dL, and platelet count of 397 × 10³. Lesion ultrasound examination findings were in line with eumycetoma. He underwent wide local excision of the mass under spinal anaesthesia with uneventful postoperative recovery. He was started on 200 mg BID of itraconazole and 5 mg daily of folic acid and received daily wound dressing.

The surgical biopsy and grains were persevered partly in normal saline for grain culture and partly in 10% formal saline for histopathological examination. A paraffin-processed tissue
block, which measured 6.5 × 3.8 × 1.5 cm, was prepared from the surgical biopsy (Fig 1). The tissue block was cut using a rotary microtome, and subsequently, 3–5 μm sections were obtained. The sections were stained with haematoxylin–eosin (HE) stain. Microscopical examination showed multiple black grains surrounded by granulation tissue. There were marked histiocytic and mixed inflammatory cellular infiltrates, in line with *M. mycetomatis* eumycetoma; however, a few differences were observed compared with *M. mycetomatis* eumycetoma (Table 1) [16]. There was an abundant extracellular matrix, which was yellow to brown in colour, and the fungus hyphae were seen at the periphery of the matrix as short filamentous structures, leaving an empty, cracking centre that contains only cement matrix infiltrated by neutrophils (Fig 2A and 2B).

![Image](https://doi.org/10.1371/journal.pntd.0007276.g001)

**Fig 1. Microphotograph showing the grossing appearance of the lesion that showed multiple black grains.**

https://doi.org/10.1371/journal.pntd.0007276.g001

| Eumycetoma features | *C. atrobrunneum* | *M. mycetomatis* [16] |
|---------------------|------------------|----------------------|
| Host immune reaction| Chronic granuloma | Chronic granuloma     |
| Cement matrix       | Abundant with yellow to brown colour | Abundant with brown colour |
| Fungus hyphae       | Periphery of the matrix with short filamentous structure | At the centre and periphery with long filament |

Table 1. The different characteristic features of *C. atrobrunneum* and *M. mycetomatis*.

https://doi.org/10.1371/journal.pntd.0007276.t001
The black grains were washed three times in saline solution and then cultivated on nonselective and selective media that included blood agar (BA), potato agar (PA), Sabouraud dextrose agar (SDA) with and without chloramphenicol (0.05 g/L), and gentamicin (0.1 g/L) at 37°C. Fungal growth appeared after 3 days in BA and 5 days on PA and SA. Microscopic examination of lactophenol cotton blue (LPCB) mount showed ascoma and ascospore cells resembling the typical Chaetomium spp. cells from the Chaetomiaceae family (Fig 2C and 2D).

DNA was extracted from the isolate cultured in Sabouraud liquid media (Oxoid) using YeaStar Genomic DNA Kit (Zymo Research, Tustin, California; United States); it was then amplified by PCR using specific ITS2 rRNA primers for fungi [17]. The specific amplicon was purified using DNA clean and concentrator (TM-5-Zymo Research, California, US) and were Sanger sequenced (BMR Genomics, Padova). Sequences were analysed using Geneious 11 software (http://www.geneious.com/), and the species was identified by BLAST database sequences comparison. A 570 bp sequence was obtained from the purified ITS2 amplicon, and it showed 99.6% identity with C. atrobrunneum (KX146507).

The patient was started empirically on 400 mg per day of itraconazole in two divided doses and 5 mg of folic acid once daily. Antifungal susceptibility testing was performed using the YeastOne (Sensititre; Thermo Scientific, Cleveland, Ohio, USA) system test as previously described for Aspergillus spp. [18]. For the in vitro susceptibility test, we used a commercial colorimetric microdilution assay; the isolate was first subcultured in SDA and incubated for 7 days. Fig 2. Microphotograph showing multiple black grains surrounded by granulation tissue with marked histiocytic and mixed inflammatory cellular infiltrates (HE 10×). (A) Grains of Chaetomium spp. showed abundant extracellular matrix, which was yellow to brown in colour, and the fungus hyphae are located at the periphery of the matrix with short filamentous structure. (B) The filamentous pattern of M. mycetomatis grains consists of brown septate and branched hyphae at the centre and periphery with long filament. (C, D) Microphotograph of LPCB mount showing ascoma and ascospore cells resembling the typical Chaetomium spp. cells (C) and conidia of M. mycetomatis (D). HE, haematoxylin–eosin; LPCB, lactophenol cotton blue.

https://doi.org/10.1371/journal.pntd.0007276.g002
days at 35˚C to obtain adequate sporulation. After that, we collected the conidia using a sterile cotton swab, suspended it in sterile normal saline with 10% Tween, and obtained the correct turbidity of 0.5 McFarland standard. Then, we added 100 μL of the suspension to 11 mL of YeastOne inoculum broth and incubated the plate at 35˚C for 48 hours. After 24 hours, we observed the positive control and the control in the SDA. For *Chaetomium* spp., we read the MIC as the lowest concentration with a blue colour. The interpretations of the MIC are based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint. *C. atrobrunneum* proved to be susceptible (S) to itraconazole, with MIC of S ≤ 0.06, (Table 2). The patient is on regular follow-up at the MRC, and he had no evidence of recurrence after 1 year of treatment.

The evolutionary history of the *C. atrobrunneum* strain isolated here was inferred using the neighbor-joining method [19], including ITS nucleotide sequences of *Chaetomium* and *Madurella* species (*n* = 66) of both clinical and environmental origins. The optimal tree had a branch length sum of 1.09302367. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches [20]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method [21] and are in units of the number of base substitutions per site. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 666 positions in the final dataset. Evolutionary analyses were conducted in Molecular Evolutionary Genetics Analysis (MEGA) X [22].

The phylogenetic tree showed *Madurella* inside the *Chaetomium* clade (Fig 3), with *C. atrobrunneum* BG9 closely related to the other *C. atrobrunneum* strains isolated from plants (MRDS7, MRDS8, and MRDS12) and from human (LVP-1), and *C. succineum* strains also isolated from plants. Only one *C. atrobrunneum* strain (WCH-CA001 ITS, human origin) clustered separately from the other *C. atrobrunneum*, with few *Chaetomium* spp.

### Discussion

Mycetoma is a badly neglected medical and health disease endemic in many tropical and subtropical countries around the world [23 – 25]. Certain environment factors such as rainfall, humidity, and temperature influence the geographical disease distribution [26 – 28]. One-third of all the reported mycetomas worldwide were from Sudan, where eumycetoma accounts for 70% of these cases and *M. mycetomatis* is the most frequently reported [29 – 31]. Mycetoma is believed to occur as a result of traumatic implantation of the causative organism into the subcutaneous tissue through minor trauma [26]. It then spreads to involve the skin, deep

| Antifungal agent | MIC |
|------------------|-----|
| Itraconazole     | S ≤ 0.06 |
| Posaconazol      | S ≤ 0.06 |
| Caspofungin      | S ≤ 0.06 |
| Mycafungin       | S ≤ 0.06 |
| Anidulafungin    | S ≤ 0.05 |
| Amphotericin     | R > 4 |
| 5-Fluorocitoxin  | R = 8 |
| Fluconazole      | R ≥ 256 |

Abbreviations: MIC, minimum inhibitory concentration; R, resistance; S, susceptible.

https://doi.org/10.1371/journal.pntd.0007276.t002
tissues, and bone, leading to massive destruction, deformities, and disabilities [32, 33]. If untreated, it can have a major impact on the affected patients, communities, and health system in endemic counties [34, 35].

In this communication, we reported and described the first case of black grain eumycetoma caused by C. atrobrunneum, a member of the Chaetomiaceae family and Sordariales order, which was initially clinically diagnosed as M. mycetomatis eumycetoma, the most frequent cause of eumycetoma worldwide and, in particular, in Sudan [36].

Fungi species causing eumycetoma belong to seven different orders (Table 3), with Sordariales, Pleorales, and Chaetothyriales able to produce black grains (Table 3).

Table 3. Eumycetoma-causing agents.

| Eumycetoma species       | Order              | Family            | Grain      | Ref  |
|--------------------------|--------------------|-------------------|------------|------|
| Exophiala jeanselmei     | Chaetothyriales    | Herpotrichiellaceae | Black      | [47] |
| Phaeoacremonium krajdenii| Diaporthales       | Togniniaceae      | White      | [48] |
| Phaeoacremonium parasiticum| Diaporthales     | Togniniaceae      | White      | [49] |
| Aspergillus flavus       | Eurotiales         | Aspergillaceae    | White      | [50] |
| Aspergillus hollandicus  | Eurotiales         | Aspergillaceae    | White      | [51] |
| Aspergillus nidulans     | Eurotiales         | Aspergillaceae    | White      | [52] |
| Acrocomium recifei       | Hypocreales        | Nectriaceae       | White      | [53] |
| Cylindrocarpon cyanescens| Hypocreales        | Nectriaceae       | White      | [54] |
| Cylindrocarpon destructans| Hypocreales        | Nectriaceae       | White      | [55] |
| Fusarium falciforme      | Hypocreales        | Nectriaceae       | White      | [56] |
| Fusarium solani          | Hypocreales        | Nectriaceae       | White      | [57] |
| Fusarium verticillioides | Hypocreales        | Nectriaceae       | White      | [56] |
| Acromonium kiliense      | Hypocreales        | Incertae sedis    | White      |      |
| Acromonium potronii      | Hypocreales        | Incertae sedis    | White      |      |
| Phialemonium obovatum    | Hypocreales        | Cephalothecaee    | White      | [57] |
| Scedosporium boydii      | Microascales       | Microascaleae     | White      | [58] |
| Microsporum canis        | Onygenales         | Arthrodermataceae | White      |      |
| Trichophyton sp.         | Onygenales         | Arthrodermataceae | White      |      |
| Neotestudina rosatii     | Pleosporeales      | Testudinaceae     | White/black| [28] |
| Bipolaris spicifera      | Pleosporeales      | Pleosporeae       | Black      |      |
| Curvularia geniculata    | Pleosporeales      | Pleosporeae       | Black      | [59] |
| Curvularia lunata        | Pleosporeales      | Pleosporeae       | Black      | [60] |
| Medicopsis romeroi       | Pleosporeales      | Neohendersoniaee | Black      | [61] |
| Falciformispora senegalensis| Pleosporeales      | Leptosphaericaee | Black      | [62] |
| Falciformispora tompkinsii| Pleosporeales      | Leptosphaericaee | Black      | [62] |
| Pseudochaetosphaerena larense| Pleosporeales      | Incertae sedis    | Black      | [62] |
| Corynespora cassiicola   | Pleosporeales      | Corynesporascaleae| Black      | [28] |
| Nigrograna mackinnonii   | Pleosporeales      | Nigrogranaee      | Black      | [28] |
| Madurella grisea         | Pleosporeales      | Chaetomiaceae     | Black      | [28] |
| Madurella fahalii        | Sordariales        | Chaetomiaceae     | Black      | [28] |
| M. mycetomatis          | Sordariales        | Chaetomiaceae     | Black      | [28] |
| Madurella pseudomycetomatis| Sordariales        | Chaetomiaceae     | Black      | [28] |
| Madurella tropicana      | Sordariales        | Chaetomiaceae     | Black      | [28] |
| C. atrobrunneum         | Sordariales        | Chaetomiaceae     | Black      | This study |
More than 100 Chaetomium species from the ascomycete Chaetomiaceae family were reported. Most of them can produce intricate fruiting bodies with characteristically shaped setae and ascospores, making them microscopically distinguishable from Madurella and other Sordariales species.

Chaetomium species commonly reside in soil enriched with animal dung or cellulosic materials and also in indoor environments [37]. Only a few cases of C. atrobrunneum human infections have been previously reported worldwide [38, 39]. This infection can cause minor disorders such as allergic reaction, onychomycosis, and sinusitis. In immunocompromised patients and bone marrow transplant recipients, it can cause serious and fatal infections such as empyema [40], pneumonia, and fatal disseminated cerebral disease [37].

C. atrobrunneum was rarely reported in eye infections. It was reported as a cause of keratitis in an adult male [41] and retinitis in a patient with Hodgkin lymphoma [42].

It was also reported in mixed infections, including cutaneous eyelid infection caused by C. atrobrunneum and Clavispora lusitaniae, [43] and in a fatal pneumonia caused by C. atrobrunneum and Aspergillus fumigatus [44].

The appropriate treatment for Chaetomium infections is unknown. Published in vitro susceptibility data for Chaetomium species have revealed resistance to flucytosine and fluconazole [45]. In the case reported here, C. atrobrunneum isolate was susceptible to itraconazole with a low MIC.

In the last 20 years, significant progress in fungal systematics and taxonomy has been achieved because of advancement in the next-generation sequencing technologies and bioinformatic tools [46].

In a recent phylogenetic study, the genus Madurella, comprising the species M. mycetomatis, M. pseudomycetomatis, M. fahalii, and M. tropicana, was found to cluster with C. atrobrunneum and other Chaetomium spp. within the Chaetomiaceae family. Here, we showed that M. mycetomatis and C. atrobrunneum are not only phylogenetically but also clinically related, causing human eumycetomas that are clinically indistinguishable and identifiable using both phenotypic and genetic methods.

In conclusion, we reported on the first human eumycetoma caused by C. atrobrunneum, adding another Sordariales species from Chaetomiaceae, such as M. mycetomatis, to the list of melanised fungi that cause human black grain mycetoma. This new case of eumycetoma confirmed Chaetomium spp.’s inclination to cause human infection, which needs to be explored.

Ethics statement
The study was approved by the Mycetoma Research Center Institutional Review Board (IRB) (5/2018). Written, informed consent to publish history, findings, and images for educational purposes was obtained from the patient.

Key learning points
- C. atrobrunneum is a rare cause of eumycetoma in Sudan.
- The histopathological discrimination between C. atrobrunneum grains and M. mycetomatis is frequently difficult and can be misleading.
- Molecular identification of mycetoma causative agent to the species level is mandatory.
References

1. Abbas M, Scolding PS, Yosif AA, EL Rahman RF, EL-Amin MO, Elbashir MK, et al. The disabling consequences of Mycetoma. PLoS Negl Trop Dis. 2018; 12(12):e0007019. https://doi.org/10.1371/journal.pntd.0007019 PMID: 30392253

2. Fahal AH, Suliman SH, Hay R. Mycetoma: The Spectrum of Clinical Presentation. Trop Med Infect Dis. 2018; 3(3):97.

3. Emmanuel P, Dumre SP, John S, Karbwang J, Hirayama K. Mycetoma: a clinical dilemma in resource limited settings. Ann Clin Microbiol Antimicrob. 2018; 17(1):35. https://doi.org/10.1186/s12941-018-0287-4 PMID: 30097030

4. Welsh O, Al-Abdely HM, Salinas-Carmona MC, Fahal AH. Mycetoma medical therapy. PLoS Negl Trop Dis. 2014; 8(10):e3218. https://doi.org/10.1371/journal.pntd.0003218 PMID: 25330342

5. Ahmed AA, van de Sande W, Fahal AH. Mycetoma laboratory diagnosis: Review article. PLoS Negl Trop Dis. 2017; 11(8):e0005638. https://doi.org/10.1371/journal.pntd.0005638 PMID: 28837657

6. Guarro J, Gene J, Stchigel AM. Developments in fungal taxonomy. Clin Microbiol Rev. 1999; 12(3):454–500. PMID: 10398676

7. Wang XW, Lombard L, Groenewald JZ, Li J, Videira SI, Samson RA, et al. Phylogenetic reassessment of the Chaetomium globosum species complex. Persoonia. 2015; 36:83–133. https://doi.org/10.3767/003158516X689657 PMID: 27616789

8. Kim DM, Lee MH, Suh MK, Ha KY, Kim H, Choi JS. Onychomycosis Caused by Chaetomium globosum. Ann Dermatol. 2017; 29(5):671–4. https://doi.org/10.5021/ad.2017.29.5.671 PMID: 28905835

9. Shih D, Lu G, Mei H, de Hoog GS, Zheng H, Liang G, et al. Onychomycosis due to Chaetomium globosum with yellowish black discoloration and periungual inflammation. Med Mycol Case Rep. 2016; 13:12–16. https://doi.org/10.1016/j.mmcr.2016.09.001 PMID: 27699147

10. Chowdhary A, Perfect J, de Hoog GS. Black Molds and Melanized Yeasts Pathogenic to Humans. Cold Spring Harb Perspect Med. 2015; 5(8):a019570.

11. Reddy M, Venugopal R, Prakash PY, Kamath YS. Corneal ulcer due to a rare coelomycetes fungus Chaetomium strumarium: Case report and global review of Chaetomium keratomycosis. Indian J Ophthalmol. 2017; 65(9):871–874. https://doi.org/10.4103/ijjo.uk.2016.17 PMID: 28905835

12. Kalamurthy J, Kalavathy CM, Nelson Jesudasan CA, Thomas PA. Keratitis due to Chaetomium sp. Case Rep Ophthalmo Med. 2012; 2011:696145.

13. Aru A, Munk-Nielsen L, Federzelli BH. The soil fungus Chaetomium in the human paranasal sinuses. Eur Arch Otorhinolaryngol. 1997; 254(7):350–352. PMID: 928672

14. Chowdhary A, Agarwal R, Meis JF. Filamentous Fungi in Respiratory Infections. What Lies Beyond Aspergillosis and Mucormycosis? PLoS Pathog. 2016; 12(4):e1005491. https://doi.org/10.1371/journal.ppat.1005491 PMID: 27124489

15. Abbott SP, Sigler L, McAleer R, McGough DA, Rinaldi MG, Mizell G. Fatal cerebral mycoses caused by the ascomycete Chaetomium strumarium. J Clin Microbiol. 1995; 33(10):2692–2698. PMID: 8567907

16. Siddig EE, Fahal AH. Histopathological Approach in Diagnosis of Mycetoma Causative Agents: A Mini Review. J Cytol Histol. 2017; 8:486.

17. Asemaninejad A, Weerasuriya N, Gloor GB, Lindo Z, Thom RG. New Primers for Discovering Fungal Diversity Using Nuclear Large Ribosomal DNA. PLoS ONE. 2016; 11(7):e0159043. https://doi.org/10.1371/journal.pone.0159043 PMID: 27391306

18. Melo E, Posteraro B, Vella A, De Carolis E, Torelli R, D’inizio T, et al. Susceptibility Testing of Common and Uncommon Aspergillus Species against Posaconazole and Other Mold-Active Antifungal Azoles Using the Sensititre Method. Antimicrob Agents Chemother. 2017; 61(6):e00168–17. https://doi.org/10.1128/AAC.00168-17 PMID: 28416358

19. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4(4):406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454 PMID: 3447015

20. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 1985; 39(4):783–791. https://doi.org/10.2307/15585646 PMID: 28561359

21. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci U S A. 2004; 101(30):11030–11035. https://doi.org/10.1073/pnas. 0404206101 PMID: 15258291

22. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol. 2018; 35(6):1547–1549. https://doi.org/10.1093/molbev/ may096 PMID: 29722887
23. Bakhiet SM, Fahal AH, Musa AM, Mohamed ESW, Omer RF, Ahmed ES, et al. A holistic approach to the mycetoma management. PLoS Negl Trop Dis. 2018; 12(5):e0006391. https://doi.org/10.1371/journal.pntd.0006391 PMID: 29746460

24. Fahal AH. Mycetoma thorn on the flesh. Trans R Soc Trop Med Hyg. 2004; 98(1):3–11. https://doi.org/10.1016/s0035-9203(03)00009-9 PMID: 14702833

25. Fahal AH, Hassan MA. Mycetoma. Br J Surg. 1992; 79(11):1138–1141. PMID: 1467883

26. Fahal AH, van de Sande WW. The Epidemiology of mycetoma. Current Fungal Infection Reports. 2012; 6(4):320–326.

27. Fahal AH. Mycetoma. In: Williams NS, Bulstrode CJK, O’Connell PR, editors. Bailey and Love’s Short Practice of Surgery. 26th ed. Oxford, UK: Oxford University Press; 2013; p 84–88.

28. van de Sande WW. Global burden of human mycetoma: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2013; 7:e2550. https://doi.org/10.1371/journal.pntd.0002550 PMID: 24244780

29. Fahal AH, Mahgoub ES, Hassan AME, Abdel-Rahman ME. Mycetoma in the Sudan: An Update from the Mycetoma Research Centre, University of Khartoum, Sudan. PLoS Negl Trop Dis. 2015; 9(3):e0003679. https://doi.org/10.1371/journal.pntd.0003679 PMID: 25816316

30. Ahmed AOA, van de Sande WW, Fahal A, Bakker-Woudenberg I, Verbrugh H, van Belkum A. Mycetoma caused by Madurella mycetomatis: a neglected infectious burden. Lancet Infect Dis. 2004; 4(9):566–574. https://doi.org/10.1016/S1473-3099(04)01131-4 PMID: 15336224.

31. Ahmed AOA, van de Sande WW, Fahal A, van Leeuwen W, Verbrugh H, van Belkum A. Management of mycetoma: major challenge in tropical mycoses with limited international recognition. Curr Opin Infect Dis. 2007; 20(2):146–151. https://doi.org/10.1097/QCO.0b013e32803d38fe PMID: 17496572

32. Suleiman SH, Wadaella ES, Fahal AH. The Surgical Treatment of Mycetoma. PLoS Negl Trop Dis. 2016; 10(6):e0004690. https://doi.org/10.1371/journal.pntd.0004690 PMID: 27363736

33. Fahal AH, Mahgoub ES, El Hassan AM, Jacoub AO, Hassan D. Head and Neck Mycetoma: The Mycetoma Research Centre Experience. PLoS Negl Trop Dis. 2015; 9(3):e0003587. https://doi.org/10.1371/journal.pntd.0003587 PMID: 25768090

34. Fahal AH. Mycetoma: A global medical and socio-economic dilemma. PLoS Negl Trop Dis. 2017; 11(4):e0005509. https://doi.org/10.1371/journal.pntd.0005509 PMID: 28426654

35. van de Sande WW, Fahal A, Ahmed SA, Serrano JA, Bonifaz A, Zijlstra E. Closing the mycetoma knowledge gap. Med Mycol. 2018; 56(suppl_1):153–164. https://doi.org/10.1093/mmy/myx061 PMID: 28992217

36. Samy AM, van de Sande WWJ, Fahal AH, Peterson AT. Mapping the Potential Risk of Mycetoma Infection in Sudan and South Sudan Using Ecological Niche Modeling. PLoS Negl Trop Dis. 2014; 8(10):e3250. https://doi.org/10.1371/journal.pntd.0003250 PMID: 25330098

37. Wang XW, Houbraken J, Groenewald JZ, Meijer M, Andersen B, Nielsen KF, et al. Diversity and taxonomy of Chaetomium and chaetomium-like fungi from indoor environments. Studies in Mycology. 2016; 84:145–224. https://doi.org/10.1016/j.simyco.2016.11.005 PMID: 28082757

38. Hoppin EC, McCoy EL, Rinaldi MG. Opportunistic mycotic infection caused by Chaetomium in a patient with acute leukemia. Cancer. 1983; 52:555–556. PMID: 6574805

39. Hubka V, Mencik K, Skorepova M, Lyskova P, Zalabska E. Phaeohyphomycosis and onychomycosis due to Chaetomium spp., including the first report of Chaetomium brassiliense in fungus from indoor environments. Studies in Mycology. 2016; 84:145–146. https://doi.org/10.1016/j.simyco.2016.11.005 PMID: 28082757

40. Balne PK, Nalamada S, Kodiganti M, Taneja M. Fungal keratitis caused by Chaetomium atrobrunneum. Cornea. 2012; 31(1):94–95. https://doi.org/10.1097/ICO.0b013e31821eedad PMID: 22045390

41. Al-Aidaroo s A, Bin-Hussain I, El Solh H, Kofide A, Thawadi S, Belgaumi A, et al. Invasive chaetomium infection in two immunocompromised pediatric patients. Pediatr Infect Dis J. 2007; 26(5):456–458. https://doi.org/10.1097/01.inf.0000259230.90103.ad PMID: 17468664

42. Zhang H, Ran Y, Li D, Liu Y, Xiang Y, Zhang R, Dai Y. Claviceps lusitaniae and Chaetomium atrobrunneum as rare agents of cutaneous infection. Mycopathologia. 2010; 169(5):373–380. https://doi.org/10.1007/s11046-009-9266-9 PMID: 20020214

43. Wang H, Liu Y, Chen SC, Long Y, Kong F, Xu YC. Chaetomium atrobrunneum and Aspergillus fumigatus in multiple tracheal aspirates: Copathogens or symbiosis. J Microbiol Immunol Infect. 2016; 49(2):281–285. https://doi.org/10.1016/j.jmii.2015.12.011 PMID: 26880338
45. Barron MA, Sutton DA, Veve R, Guaro J, Rinaldi M, Thompson E, et al. Invasive Mycotic Infections Caused by Chaetomium perlucidum, a New Agent of Cerebral Phaeohyphomycosis. J Clin Microbiol. 2003; 41(11):5302–5307. https://doi.org/10.1128/JCM.41.11.5302-5307.2003 PMID: 14605190

46. Zhang N, Luo J, Bhattacharya D. Advances in Fungal Phylogenomics and Their Impact on Fungal Systems. Adv Genet. 2017; 100:309–328. https://doi.org/10.1016/bs.adgen.2017.09.004 PMID: 29153403

47. Capoor MR, Khanna G, Nair D, Hasan A, Rajni, Deb M, Aggarwal P. Eumycetoma pedis due to Exophiala jeanselmei. Indian J Med Microbiol. 2007; 25:155–157. PMID: 17582190

48. Hemashettar BM, Siddaramappa B, Munjunathaswamy BS, Pangi AS, Pattan J, Andrade AT, et al. Phaeoacremonium krajdenii, a Cause of White Grain Eumycetoma. J Clin Microbiol. 2006; 44(12):4619–4622.

49. Colombier M-A, Alanio A, Denis B, Melica G, Garcia-Hermoso D, Levy B, et al. Dual Invasive Infection with Phaeoacremonium parasiticum and Paraconiothyrium cyclothyrioides in a Renal Transplant Recipient: Case Report and Comprehensive Review of the Literature of Phaeoacremonium Phaeohyphomycosis. J Clin Microbiol. 2015; 53(7):2084–2094. https://doi.org/10.1128/JCM.00295-15 PMID: 25903573

50. Ahmed SA, Abbas MA, Jouvion G, Al-Hatmi AM, de Hoog GS, Kolecka A, et al. Seventeen years of subcutaneous infection by Aspergillus flavus; eumycetoma confirmed by immunohistochemistry. Mycoses. 2015; 58(12):728–34. https://doi.org/10.1111/myc.12422 PMID: 26497138

51. Mhmoud NA, Ahmed SA, Fahal AH, de Hoog GS, Gerrits van den Ende AHG, van de Sande WWJ. Pleurostomophora ochracea, a Novel Agent of Human Eumycetoma with Yellow Grains. J Clin Microbiol. 2012; 50(9):2987–2994. https://doi.org/10.1128/JCM.01470-12 PMID: 22760037

52. Prasanna S, Grover N, Bhatt P, Sahni AK. A case of Aspergillus nidulans causing white granule mycetoma. Medical Journal, Armed Forces India. 2016; 72(1):88–90. https://doi.org/10.1016/j.mjafi.2014.11.003 PMID: 26900232

53. Koshi G, Padhye AA, Ajello L, Chandler FW. Acremonium recifei as an agent of mycetoma in India. Am J Trop Med Hyg. 1979; 28(4):692–6. PMID: 464189

54. Hemashettar BM, Siddaramappa B, Padhye AA, Sigler L, Chandler FW. White Grain Mycetoma Caused by a Cylindrocarpon sp. in India. J Clin Microbiol. 2000; 38(11):4288–4291. PMID: 11060115

55. Zoutman DE, Sigler L. Mycetoma of the foot caused by Phialemonium obovatum. J Clin Microbiol. 1986; 23(5):987–991. PMID: 3711290

56. Summerbell RC, Schroers H-J. Analysis of Phylogenetic Relationship of Cylindrocarpon lichenicola and Acremonium falciforme to the Fusarium solani Species Complex and a Review of Similarities in the Spectrum of Opportunistic Infections Caused by These Fungi. J Clin Microbiol. 2002; 40(8):2866–2875. https://doi.org/10.1128/JCM.40.8.2866-2875.2002 PMID: 12149344

57. Lomax LG, Cole JR, Padhye AA, Ajello L, Chandler FW, Smith BR. Osteolytic phaeohyphomycosis in a German shepherd dog caused by Phialemonium obovatum. J Clin Microbiol. 1986; 23(5):987–991. PMID: 3711290

58. Cortez KJ, Rolides E, Quiroz-Telles F, Meletiadis J, Antachopoulos C, Knudsen T, et al. Infections Caused by Scedosporium spp. Clin Microbiol Rev. 2008; 21(1):157–197. https://doi.org/10.1128/CMR.00039-07 PMID: 18202441

59. Shinde RS, Hanumantha S, Mantur BG, Parande MV. A Rare Case of Mycetoma Due to Curvularia. J Lab Physicians. 2015; 7(1):55–57. https://doi.org/10.4103/0974-2727.154799 PMID: 25949061

60. Elad D, Orgad U, Yakobsen B, Peri S, Golomb P, Trainin R, Tsur I, Shenkler S, Bor A. Eumycetoma caused by Curvularia lunata in a dog. Mycopathologia. 1991; 116(2):113–118. PMID: 1664052

61. Mathuram Thiyyagarajan U, Bagul A, Nicholson ML. A nodulo-cystic eumycetoma caused by Pyrenochaeta romeroi in a renal transplant recipient: A case report. J Med Case Rep. 2011; 5:460. https://doi.org/10.1186/1752-1947-5-460 PMID: 21917163

62. Machmachi H, Godineau N, Develoux M, Bretagne S, Bazeli A, Arnellam D, et al. Black grain mycetoma caused by Leptosphaeria tompkinsii. Med Mycol. 2011; 49(2):186–189. https://doi.org/10.3109/13693786.2010.524945 PMID: 21235319