



<https://doi.org/10.11646/phytotaxa.343.3.9>

## New record of *Chaetomium grande* Asgari & Zare (Chaetomiaceae) for the Egyptian and African mycobiota

AHMED M. ABDEL-AZEEM<sup>1</sup>, ROBERT A. BLANCHETTE<sup>2</sup> & BENJAMIN W. HELD<sup>2</sup>

<sup>1</sup>Department of Botany, Faculty of Science, University of Suez Canal, Ismailia 41522, Egypt.

E-mail: [ahmed\\_abdelazeem@science.suez.edu.eg](mailto:ahmed_abdelazeem@science.suez.edu.eg)

<sup>2</sup>Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, US. E-mail: [robertb@umn.edu](mailto:robertb@umn.edu), [bheld@umn.edu](mailto:bheld@umn.edu)

### Abstract

The first record of *Chaetomium grande* (Ascomycota, Chaetomiaceae) for the Egyptian and African fungi is reported here. The species was found during an extensive taxonomic and ecological revision of the genus *Chaetomium* supported by Science and Development Technology Fund in Egypt. *Ch. grande* identified phenotypically and was subjected to sequencing for confirmation. The internal transcribed spacer (ITS) 1–5.8 s – ITS2 rDNA sequences obtained were compared with those deposited in the GenBank Database and registered with accession number MF787599 in the NCBI Database. We provide an updated full description and illustration of the species.

**Key words:** Ascomycota, Endobionts, Saint Katherine Protectorate, Sinai, STDF, Taxonomy

### Introduction

*Chaetomium* is a large genus of the ascomycetous family Chaetomiaceae that was established by Gustav Kunze (1817) based on *Ch. globosum* as its type species. Since the establishment of the genus, more than 300 species have been described, many of which were synonymized/excluded and only 163 species were accepted (Doveri 2013). By scanning of available sources of information concerning genus *Chaetomium* in Egypt, it was possible to determine 53 species and one variety isolated/reported from different substrates (Moustafa & Abdel-Azeem 2005) without any record of *Chaetomium grande* until now. Here we report *Ch. grande* hosted *Verbascum sinaiticum* which constitutes the first record of this species in Egypt and Africa.

### Materials & Methods

#### Study area, sampling and isolation of endobiont fungi

One hundred samples of four plant species namely: *Artemisia herba-alba* Asso; *Chiliadenus montanus* (Vahl) Brullo; *Origanum syriacum* L.; and *Verbascum sinaiticum* Benth were collected from twenty localities in four wadis namely: Wadi Itlah (28°58'72.3" N, 33°92'01.7" E), Wadi Tala (28°34'02.3" N, 33°55'55.8" E), Wadi El-Arbaein (28°54'54" N, 33°55'36" E) and Gebel Ahmar (28°52'83" N, 33°61'83" E) in Saint Katherine Protectorate, South Sinai, Egypt.

A total of 400 plates were used for the isolation of endobiont mycobiota (100 plates/plant). Pieces of stem and leaves (5mm<sup>2</sup>, four pieces in each plate) were surface sterilized and cut. The sections were washed three times in running water, immersed in 70% ethanol for 1–5min, dipped in 5% NaOCl for 3–5min, according to the plant thickness, and then dipped in 70% ethanol for 0.5min (Abdel-Azeem & Salem 2012), before being plated on appropriate isolation media. For primary isolation, Czapek's yeast extract agar (CYA), Oat Meal Agar (OA), and Potato Dextrose Agar (PDA) media supplemented with Rose bengal (1/1500), chloramphenicol (50 ppm) were used. Samples were collected under permission of the Saint Katherine Protectorate for scientific purposes and no endangered species were involved in the study.

### Phenotypic identification

Identification of recovered *Chaetomium* isolates was conducted up to the species level based on phenotypic characteristics using the relevant identification keys for *Chaetomium* (Asgari & Zare 2011, Doveri 2013). The names of the authors of fungal taxa were abbreviated according to Kirk and Ansell (1992). All name corrections, authorities, and taxonomic assignments of recorded species in the present study were checked against the Index Fungorum database (2017). All isolated taxa are maintained in the Fungarium of Suez Canal University (FSCU) at Arab Society for Fungal Conservation (ASFC) in Botany Department, Faculty of Science, University of Suez Canal, Ismailia, Egypt.

### Molecular identification and phylogenetic analysis

DNA was extracted from fungal culture using an adapted chloroform procedure (Arenz & Blanchette 2011). The internal transcribed spacer (ITS) region of ribosomal DNA was targeted for PCR amplification with the primers ITS1 and ITS4 for large subunit amplification (White *et al.* 1990). PCR amplifications were done using Amplitaq Gold PCR Master-mix (Applied Biosystems, Foster City, CA) and 1 ml of template DNA using the following parameters: 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, and a final extension step of 5 min at 72 °C. PCR amplicons were visualized on a 1 % agarose gel using SYBR green 1 (Life Technologies, Grand Island, NY,US) prestain and a Dark Reader DR45 transilluminator (Clare Chemical Research, Denver, CO, US). Primers used for PCR were used for sequencing reactions on automated DNA sequencer (Model 3100; Perkin-Elmer Inc/Applied Biosystems—Bioneer, South Korea), according to the manufacturer's protocol. Consensus sequences were assembled using Geneious 9.0 (Kearse *et al.* 2012) and compared to those in GenBank using BLASTn for identification. The sequences of *Ch. grande* were compared with reference ITS sequences from the GenBank database at the National Center for Biotechnology Information (NCBI), using the basic local alignment search tool (BLAST). Evolutionary distance matrices based on the neighbor-joining algorithm (with max sequence difference of 0.75) were calculated using Kimura's two-parameter model (Kimura 1980). Tree topology was inferred by the neighbor-joining method in the program MEGA7 (Kumar *et al.* 2016), with bootstrap values based on 1,000 replications.

### Light and scanning electron microscopy examination

Fruiting bodies of *Ch. grande* was mounted in deionized water according to Abdel-Azeem (1998) to examine their appendages, ascospores and texture by light microscope. For scanning electron microscopy fungus was grown in Petri dishes on the appropriate media to obtain typical and good sporulation. Small pieces of agar (6–8 × 5 mm) with the fungus (or, when fresh, parts of the natural substrate) were cut from the colonies, put into stubs using double-sided sticky tape, stubs and fixed in unbuffered aqueous 2% OsO<sub>4</sub> overnight. The samples were then transferred to a critical point drying apparatus (Balzer) and dried in CO<sub>2</sub>. The specimens were coated with gold in a sputter coater (Polaron) and examined and photographed with a Hitachi S3500 scanning electron microscope.

## Results

Five species of *Chaetomium* were recovered from four medicinal plants during this extensive survey namely: *Ch. bostrychodes* Zopf., *Ch. globosum* Knuze, *Ch. grande* Asgari & Zare, *Ch. piluliferum* J. Daniels and *Ch. senegalense* L.M. Ames. All taxa were previously reported in Egypt except *Ch. grande*.

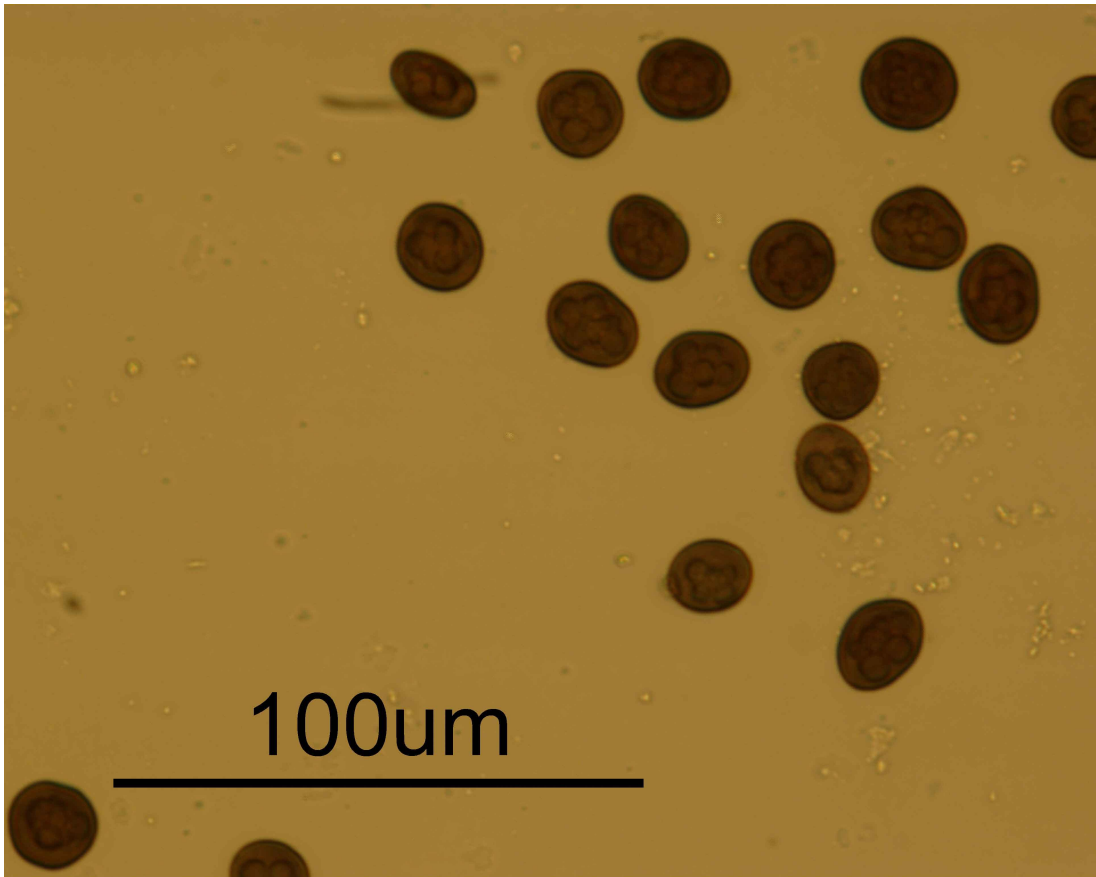
### Taxonomic treatment

*Chaetomium grande* Asgari & Zare, Mycologia 103(4): 874 (2011)

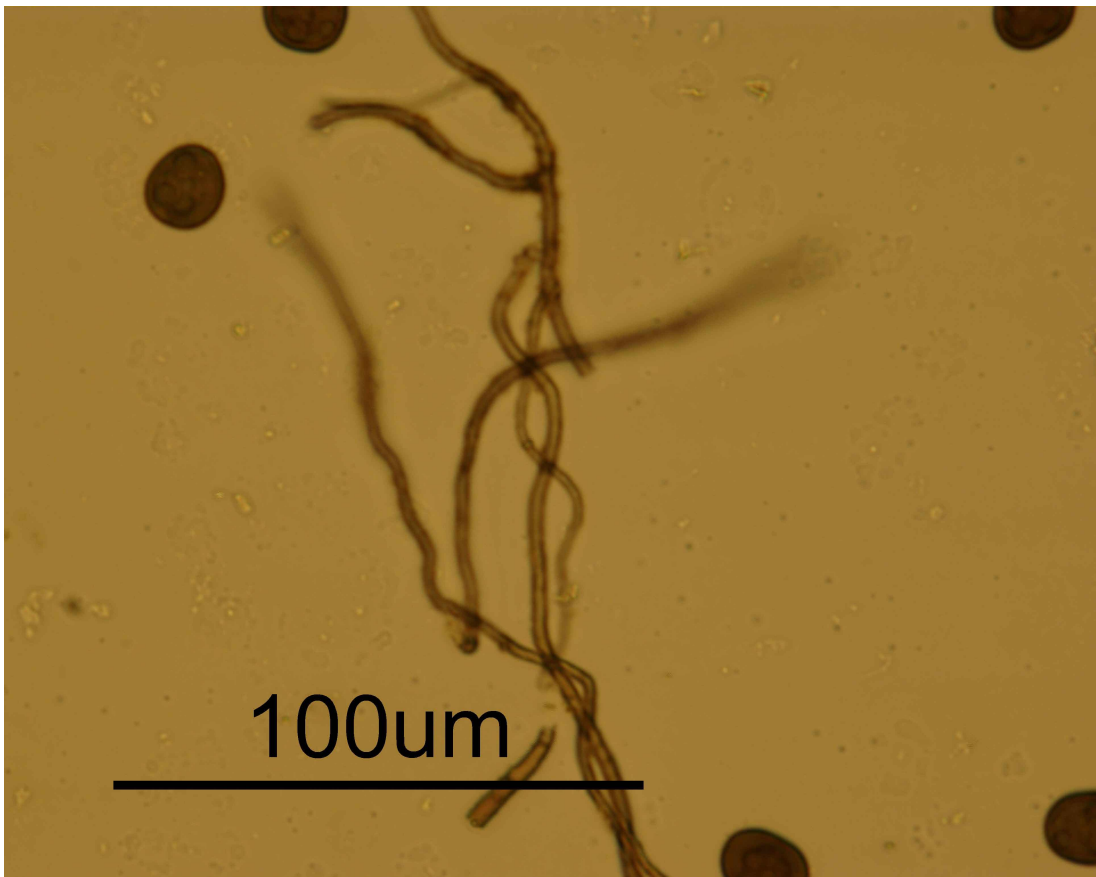
MF787599 (FIG. 1 A–E)

**Specimens Examined:**—Egypt, isolated from *Verbascum sinaiticum*, Saint Katherine Protectorate, South Sinai, Egypt, 11 May 2013, 27 August 2013, 30 November 2014, 28 December 2015, leg. Ahmed M. Abdel-Azeem, det. Ahmed .M. Abdel-Azeem (SCUF-155, 189, 209, 255).

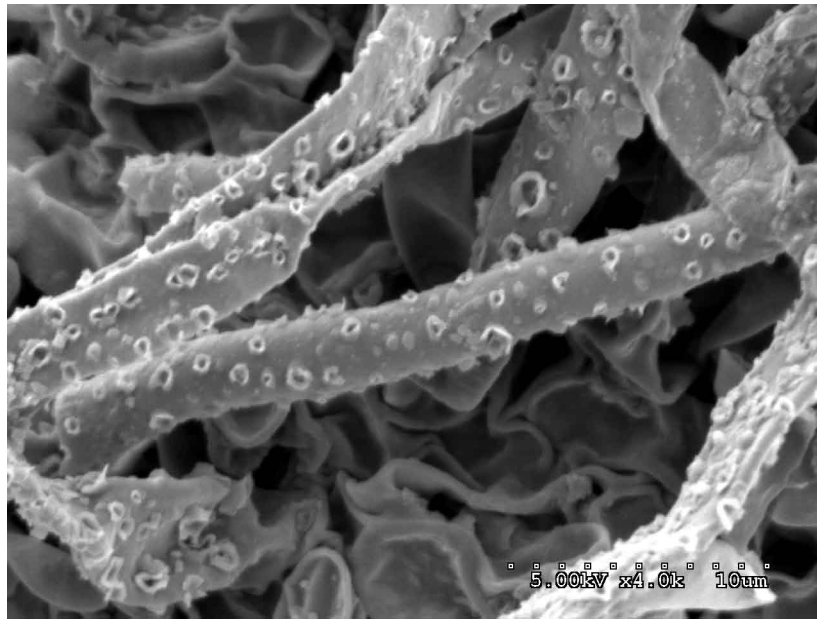
Colonies 33–(47)–52 mm diam on OMA in 7 d at 28 C, dirty white; reverse olivaceous white. Ascومات maturing within 14d, olivaceous gray or yellowish green in reflected light, globose, subglobose to ovoid, 200–400 µm diam, with narrowly rounded base and broad ostiole. Peridium brown or dark brown, of textura intricata. Ascومات hairs dark brown, partly long (up to 900 µm), sinous to undulate, with narrowly pointed tip, irregularly branched, verrucose (ornaments cupulate at maturity), 2.5–5 µm wide at the base, 2–3 µm in the middle part. Asci clavate, eight-spored,



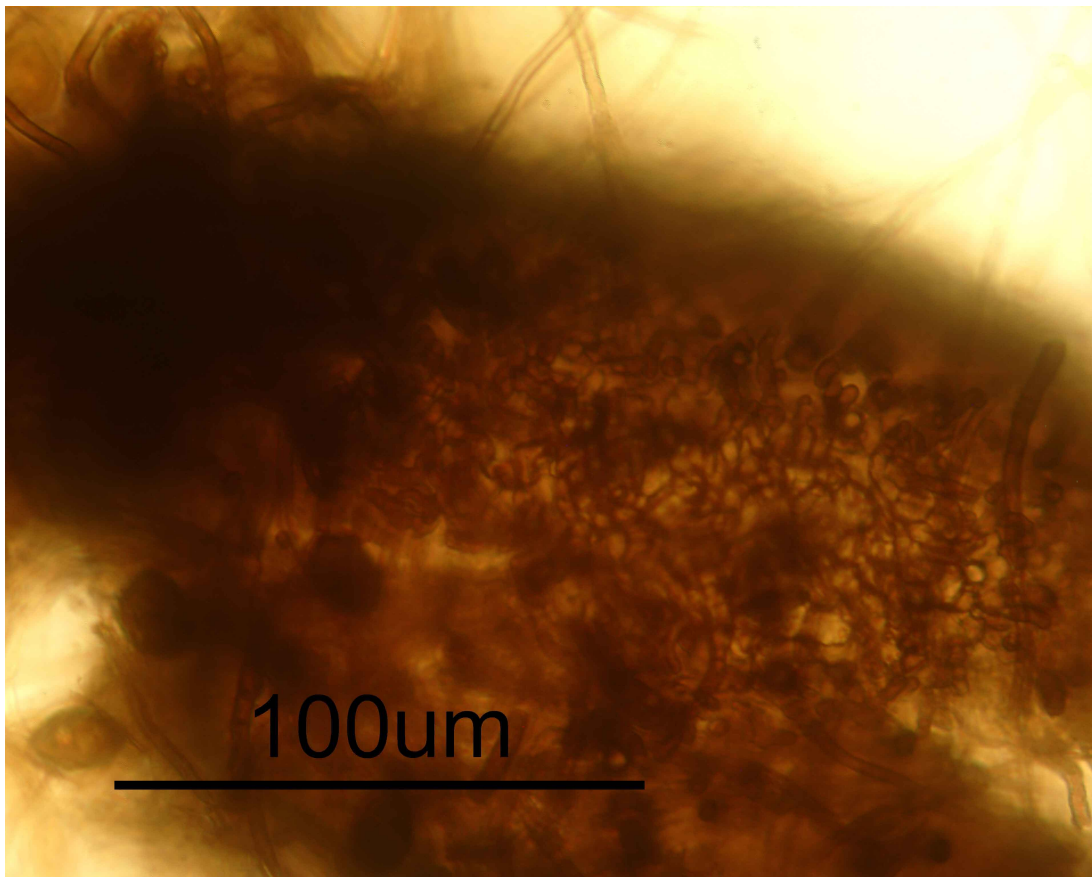
**FIGURE 1 A:** Mature ascospores.



**FIGURE 1 B:** Branched ascomatal hair.



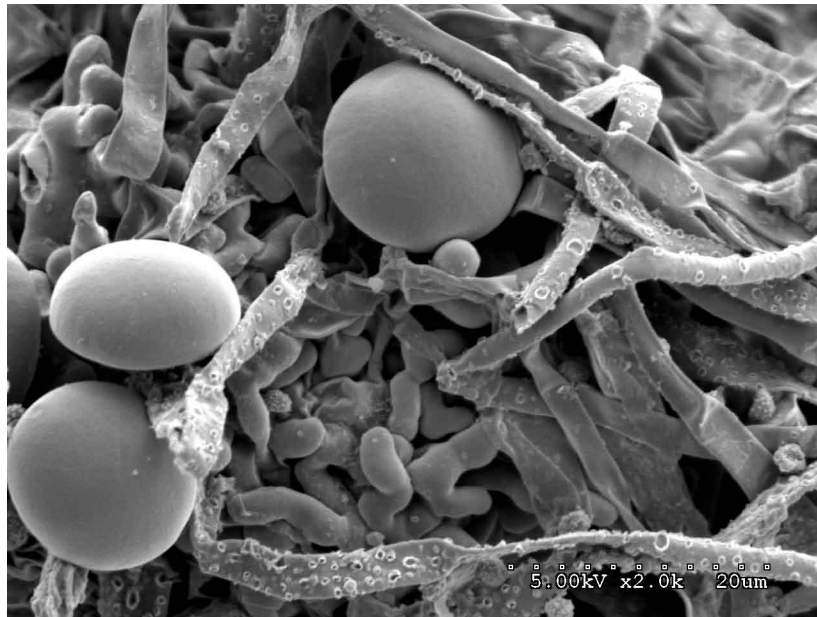
**FIGURE 1 C:** Ascumatal hairs showing cupulate ornaments by SEM.



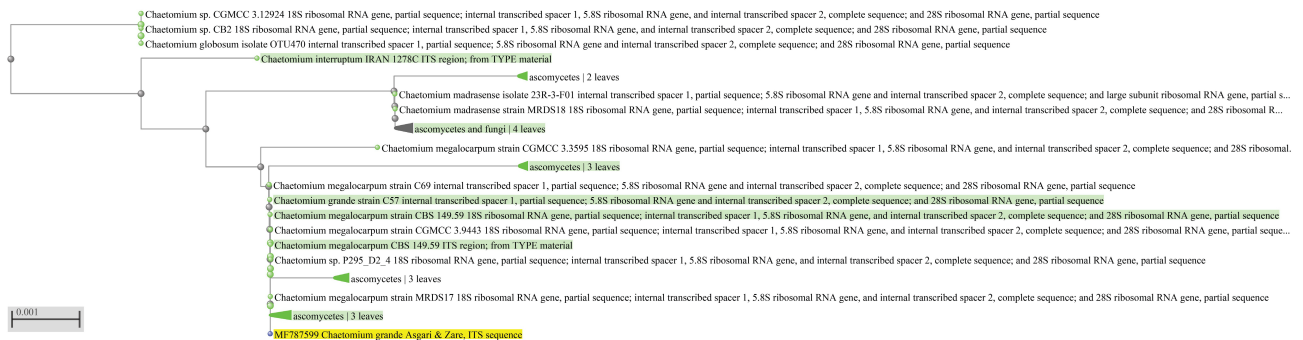
**FIGURE 1 D:** *Textura intricata* mounted by deionized water under light microscope.

short stalked, 55–95 (–100) × 25–40 μm. Ascospores dark brown, thick-walled, broadly ellipsoidal or spherical, 16–20 × 14.5–18 × 12–14 μm, with two rounded, sometimes slightly protuberant, polar germ pores; immature ascospores containing several small guttules, mature ascospores with a single large guttule (FIG. 1). Anamorph not recorded. Isolate MF787599 was subjected to molecular identification based on sequence analysis of the ITS regions. A search of the GenBank database via BLAST revealed that the ITS region sequence of MF787599 exhibited high similarity (100%) with *Ch. grande*. This relationship was also evident from the phylogenetic tree constructed using ITS region sequences. The isolate MF787599 clustered together with *Ch. megalocarpum* isolates, thus confirming their closest relationship at the species level (FIG. 2).





**FIGURE 1 F:** Mature ascospores and textura intricata by SEM.



**FIGURE 2:** ITS sequence tree of *Ch. grande* (MF787599) made through NCBI blast based on neighbour joining method with maximum sequence difference of 0.75.

**Conservation status:**—Conservation of fungi in Egypt and worldwide remains very low, and more education on fungal conservation is urgently needed. Although the species distribution is restricted only in South Sinai, Egypt, and in this state, data deficient (DD) is the conservation status of this fungal species according to the IUCN criteria (2010).

### Acknowledgements

This work was supported by the Science and Development Technology Fund in Egypt (STF project No. 12295). The authors would like to thank Ms. Fatma M. Salem for her invaluable support during laboratory work and we would also like to thank the two anonymous reviewers for the valuable suggestions.

### References

Abdel-Azeem, A. (1998) *Cytogenetical and Biological Studies on the fungus Chaetomiopsis*. M.Sc. Thesis, Suez Canal University, Ismailia, Egypt, 240 pp.

Abdel-Azeem, A. & Salem, F.M. (2012) Biodiversity of laccase producing fungi in Egypt. *Mycosphere* 3 (5): 900–920. <https://doi.org/10.5943/mycosphere/3/6/4>

Arenz, B. & Blanchette, R. (2011) Distribution and abundance of soil fungi in Antarctica at sites on the Peninsula, Ross Sea Region and

- McMurdo Dry Valleys. *Soil Biol. Biochem.* 43: 308–315.  
<https://doi.org/10.1016/j.soilbio.2010.10.016>
- Asgari, B. & Zare, R. (2011) The genus *Chaetomium* in Iran, a phylogenetic study including six new species. *Mycologia* 103: 863–882.  
<https://doi.org/10.3852/10-349>
- Doveri, F. (2013) An additional update on the genus *Chaetomium* with descriptions of two coprophilous species, new to Italy. *Mycosphere* 4: 820–846.  
<https://doi.org/10.5943/mycosphere/4/4/17>
- Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular Evolution* 16: 111–120.  
<https://doi.org/10.1007/BF01731581>
- Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.  
<https://doi.org/10.1093/molbev/msw054>
- Kunze, G. & Schmidt, J.K. (1817) *Mykologische Hefte* 1 (1). Leipzig, 16 pp.
- Index Fungorum database. Available from: <http://www.indexfungorum.org/names/Names.asp> (accessed 7 December 2017)
- IUCN. (2008) *Guidelines for Using the IUCN Red List Categories and Criteria*. Version 8.1. Available from: <http://intranet.iucn.org/webfiles/doc/SSC/RedList/RedListGuidelines.pdf> (accessed 7 December 2017)
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.  
<https://doi.org/10.1093/bioinformatics/bts199>
- Kirk, P.M. & Ansell, A.E. (1992) *Authors of Fungal Names*. Kew: CAB International.
- Moustafa, A.F. & Abdel-Azeem, A.M. (2005) The genus *Chaetomium* in Egypt. *El-Minia Science Bulletin* 16: 235–256.
- White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR Protocols: a guide to methods and applications*. Academic Press, San Diego, California, pp. 315–322.  
<http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1>