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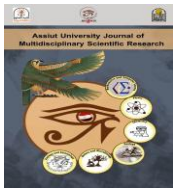

Biodiversity of *Chaetomium*-like genera in the Nile River, at Assiut, Egypt

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ABSTRACT

One of the most significant rivers in the world is the River Nile. It is the source of 96% of the country's renewable freshwater. The study of freshwater fungi is important because it reveals the hidden diversity and ecological significance of these organisms in aquatic ecosystems. It deepens our understanding of biodiversity, nutrient cycling, ecological interactions, and water quality, while also offering practical applications for environmental and biotechnological purposes. During surveys for freshwater ascomycetes in the Nile River as well as Ibrahimia and Fayama Canals at Assiut region, Egypt, ten species belonging to six genera of families: Chaetomiaceae, Podosporaceae, and Lasiosphaeriaceae, were isolated and identified. Among them, *Botryotrichum geniculatum, B. trichorobustum, Pseudorhypophila marina, Pseudothielavia terricola, Zopfiella indica,* and *Z. udagawae* were reported in the current study for the first time from Egypt. These species were isolated in pure cultures and identified based mainly on their morphological features. The identification of one species was confirmed genetically and identified as *Pseudothielavia terricola* AUMC 16038 (OQ581575). The treated species were described, and photographic illustrations were captured. The frequency, distribution, and hosts for each listed species were recorded. Seven out of ten fungal species have been recovered from *Phragmites australis*, which is considered in this study to be the ideal submerged plant for isolating freshwater

ascomycetous fungi. Continued research on freshwater fungi is of paramount importance, and it is crucial to prioritize conservation efforts to protect rare and endangered species.

INTRODUCTION

The largest phylum Ascomycota is a diverse group of fungi with different forms, from unicellular cells as in yeasts to complex fruiting bodies as in ascomata-forming ascomycetous fungi. One of the major Ascomycota classes is Sordariomycetes [1]. This class includes the family Chaetomiaceae, which contains species that are almost strictly saprophytic and are antagonistic against several plant pathogens [2]. In recent times, a lot of new genera and species have been discovered in this family, and other species have emerged without comprehensive descriptions or photographs. For example, the new genus *Batnamyces* is primarily identified based on its molecular phylogeny since scientists were unable to observe the distinctive structures of either the asexual or sexual morphs of the species within this genus [3]. In Egypt, there have been some new records reported in this family until now, such as *Chaetomium grande* for the Egyptian and African mycobiota [4]. For freshwater ecosystems, this group relies mostly on its incredible ability to adhere to available substrates and release enzymes to degrade complex organic contents, along with its ability to consume lignocellulose in the woody litter, softening the wood and liberating nutrients. So, they play an important role in nutrient and carbon cycling [5]. Consequently, this study aimed to isolate and identify fungi associated with dehydrated plant segments, deposited in some freshwater habitats (Nile River, Ibrahimia, and Fayama Canals) to attract some of these seldom-reported fungi.

MATERIALS AND METHODS

Description of the study sites

Three water bodies in Assiut Governorate, Egypt, were assigned for this investigation. Site 1 (the River Nile) has flowing, clear water, is devoid of vegetation, and is located far from any cultivated areas. Site 2 (Ibrahimia Canal) is a large irrigation canal with slow-flowing water and is found in sites cultivated with fruit trees and grasses. Site 3 (Fayama Canal) is a small canal in a village with standing water that is polluted with domestic waste and animal dung.

Collection of samples

Dehydrated stems of *Phragmites australis, Phoenix* sp., and *Ziziphus* sp. were deposited in the Nile River and two irrigation Canals (Ibrahimia and Fayama) at Assiut Governorate for a year as baiting substrates for freshwater fungi. Plant baits were retrieved. Samples were returned to the laboratory, washed with sterilized distilled water, cut into segments of ten to fifteen cm in length, and incubated in plastic boxes lined with sterilized moist tissue paper at room temperature. These humid chambers were moistened periodically with sterilized distilled water containing 0.5 g chloramphenicol/Liter according to [6].

Examination and preservation of obtained fungi

Samples were examined periodically for fungal fruiting structures during 3 months of incubation at room temperature. Fruiting bodies were picked from substrates using fine forceps under a stereomicroscope (Olympus SZ62), transferred to a sterilized glass slide with a sterilized drop of distilled water on it under a compound microscope, and degraded using sterilized fine forceps to release asci and ascospores. The Single Spore Isolation (SSI) technique was used to obtain a pure culture for each fungus. Cultures were isolated and preserved on potato dextrose agar media (Himedia). Woods with fungal species were dried at 60˚C for 24 h and deposited as herbarium materials. Photographs were taken using a digital camera (Toup Cam, model number LCMOS14000KPA). Methods used for the preparation of materials for light microscopy have been reported by [6]. Permanent slides and herbarium materials of the fungi recorded were deposited in the author's herbarium and can be examined on request. Identification of fungal species was mainly achieved morphologically, the following

sources of articles and keys were used to identify the documented fungi: [7, 8], the genus *Thielavia* by [9], and new cleistothecial Sordariaceae and a new family, Coniochaetaceae by [10]. The nomenclature of recorded species was unified through indexfungorum.org and mycobank.org.

DNA extraction, sequencing, and phylogenetic analysis

Pure culture from the fungal isolate was grown in yeast and malt extract with glucose (YMG) broth media (4 g yeast extract, 10 g glucose, 10 g malt extract in 1 litter distilled water) until sufficient mycelium had formed to allow DNA extraction. DNA extraction for polymerase chain reaction (PCR) was performed using the microbial DNA extraction kit (QIAGEN), Partial LSU ribosomal DNA was amplified using two primers LR0R (GTA CCC GCT GAA CTT AAG C) and LR7 (TAC TAC CAC CAA GAT C), PCR reactions, cycling parameters, and sequencing were carried out. The phylogenetic trees were constructed by CLC genomic 25 software using the sequences of relative fungal strains from the Gene bank database of the National Center for Biotechnology Information (NCBI).

Data analysis

The total number of fungal taxa was recorded and used to calculate the frequency of occurrence for a particular fungal species based on the following formula

$$\frac{\text{Collections number of a particular fungal taxa}}{\text{Number of samples examined}} \times 100$$

RESULTS

Ten fungal species belonging to six genera were isolated and their features at the macroscopic and microscopic levels on wood samples were used to identify them. (Table 1).

Table 1 List of fungi belonging to family Chaetomiaceae and other families collected from the Nile River, Ibrahimia, and Fayama Canals.

fungal species	family name	site	season	%F
Botryotrichum geniculatum	Chaetomiaceae	Fayama Canal	Winter and Spring	1.5
Botryotrichum trichorobustum	Chaetomiaceae	Nile River	Summer	0.1
Cladorrhinum hyalocarpum	Podosporaceae	Fayama Canal	Spring and Summer	0.7
Cladorrhinum leucotrichum	Podosporaceae	Ibrahimia Canal	Summer	0.2
Pseudorhypophila marina	Lasiosphaeriaceae	Nile River	Spring	0.3
Pseudothielavia terricola	Chaetomiaceae	Ibrahimia Canal	Summer	0.2
Stolonocarpus gigasporus	Chaetomiaceae	Ibrahimia Canal	Autumn	0.2
Zopfiella indica	Lasiosphaeriaceae	Fayama Canal	Winter	0.1
Zopfiella latipes	Lasiosphaeriaceae	Fayama Canal	Winter	3.5
Zopfiella udagawae	Lasiosphaeriaceae	Nile River	Spring	0.1

Botryotrichum geniculatum X. Wei Wang, P.J. Han & F.Y. Bai

Basionym: *Botryotrichum geniculatum* X. Wei Wang, P.J. Han & F.Y. Bai

Botryotrichum geniculatum was isolated for once in the world from soil under *Trollius chinensis*, in China by [7]. *Botryotrichum geniculatum* is a new record from Egypt. The fungus was isolated during winter and spring from the Fayama Canal and on *Phragmites australis* baits.

Macroscopic features: It is characterized morphologically by forming superficial ascomata, occasionally immersed, solitary, or clustered.

Microscopic features: Ascomata of the species exhibit a globose to subglobose shape and lack ostioles. They possess a dark olivaceous coloration attributed to the presence of ascomatal hairs. The ascomata themselves are spherical and have a diameter ranging from 300 to 400 µm. The ascomatal wall, which surrounds the ascomata, displays a dark brown to black hue. When the wall is young, it exhibits an intricate or epidermal texture, while in its mature state, it appears angular when observed from the surface. The entire surface of the ascoma is covered with ascomatal hairs, which resemble hyphae. These hairs are smooth or finely verrucose, slightly undulate in the upper part, and gradually taper and fade to a hyaline coloration towards the tips. In the lower part, the hairs exhibit a geniculate form and appear brown at the base. They measure 2 to 3.5 µm near the base and vary in length, with some reaching up to 400 µm. Moving on to the asci, they take on a clavate shape and consist of a spore-bearing part measuring 48 to 72 µm in length and 20 to 26 µm in width. These asci are accompanied by stalks that are 12 to 30 µm in length. Each ascus contains eight irregularly arranged, or biseriate, ascospores, which gradually disappear over time. Ascospores are composed of a single cell and have a smooth surface. When young, they appear hyaline, but as they mature, they darken in color and become a dark brown shade. Ascospores have an elongated, limoniform to broadly fusiform shape, occasionally displaying inequilateral characteristics. Their dimensions range from 16.5 to 25 µm in length and 11 to 14 µm in width. Notably, these spores feature an apical germ pore. Lastly, no information is available regarding the presence of an asexual morph for this species. (Fig. 1).

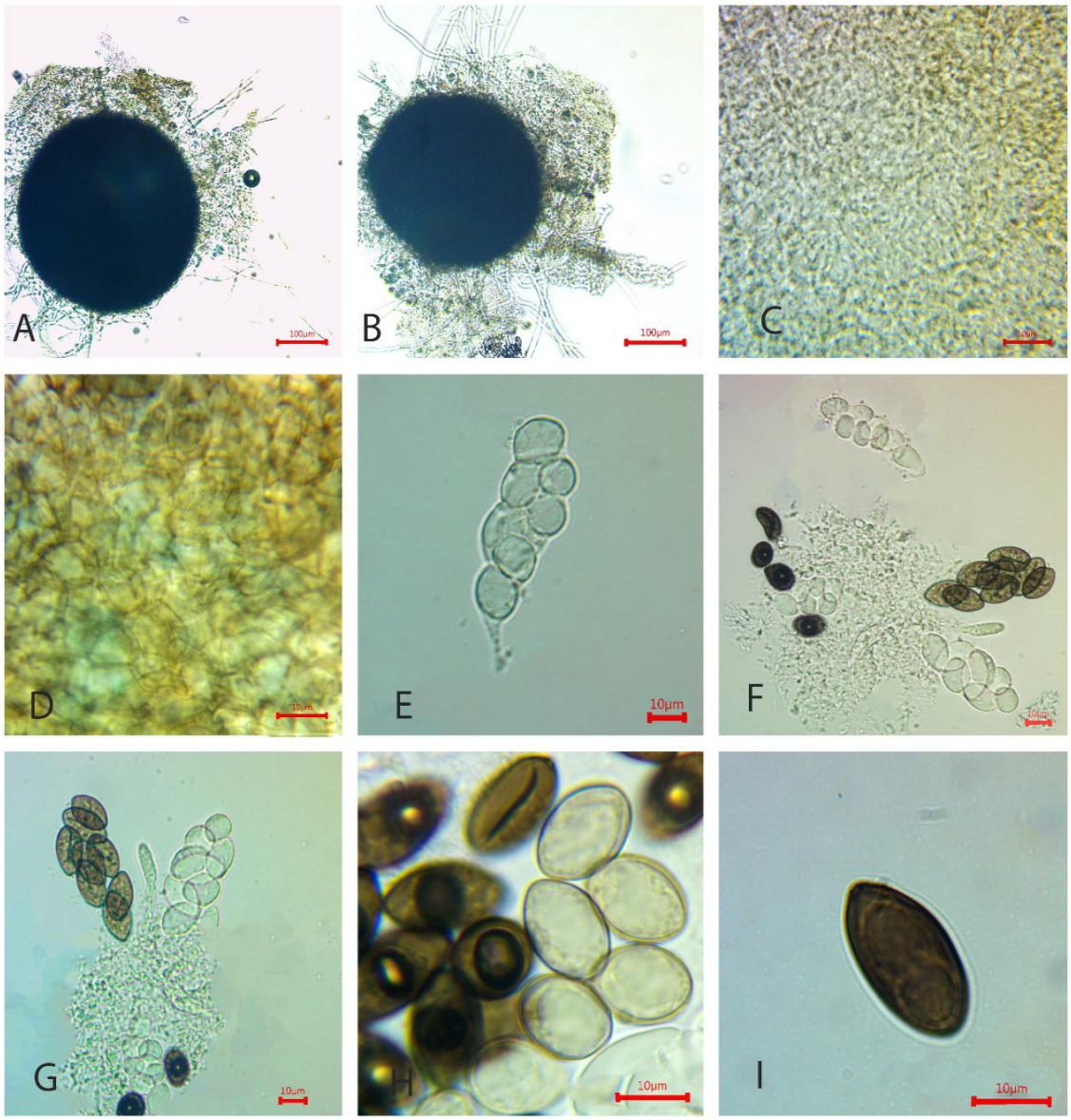

Fig. 1. *Botryotrichum geniculatum*. A–B. Mature ascomata. C–D. Structure of ascomatal wall in surface view. E–G. Asci. H–I. Ascospores. Scale bars: A–B = 100 µm; C–I = 10 µm.

Botryotrichum trichorobustum (Seth) X. Wei Wang & Houbraken

Basionym: *Chaetomidium trichorobustum* Seth

Synonym: *Thielavia trichorobusta* (Seth) Malloch and Cain

Botryotrichum trichorobustum was isolated from rabbit dung in Germany; Hamburg by [11], It is a new record from Egypt. The fungus was isolated during Summer from the Nile River, on *Phragmites australis* baits.

Macroscopic features: It is characterized morphologically by forming ascomata superficial, solitary.

Microscopic features: Ascomata is globose, non-ostiolate, dark brown to black, 250–300 µm diam., with long, yellow to pale brown ascomatal hairs. The ascomatal wall is dark brown, composed of epidermal texture in surface view. Asci are borne in a basal fascicle, clavate, spore-bearing part 65–80 × 10–19.5 µm, with stalk 15–20 µm long, containing eight uniseriate ascospores, ascospores broadly lemon-shaped, smooth, hyaline when young, then becoming dark olive-brown in color, 10.5–19 × 10.5–13.5 µm, with an apical germ pore. An asexual morph was not observed (Fig. 2).

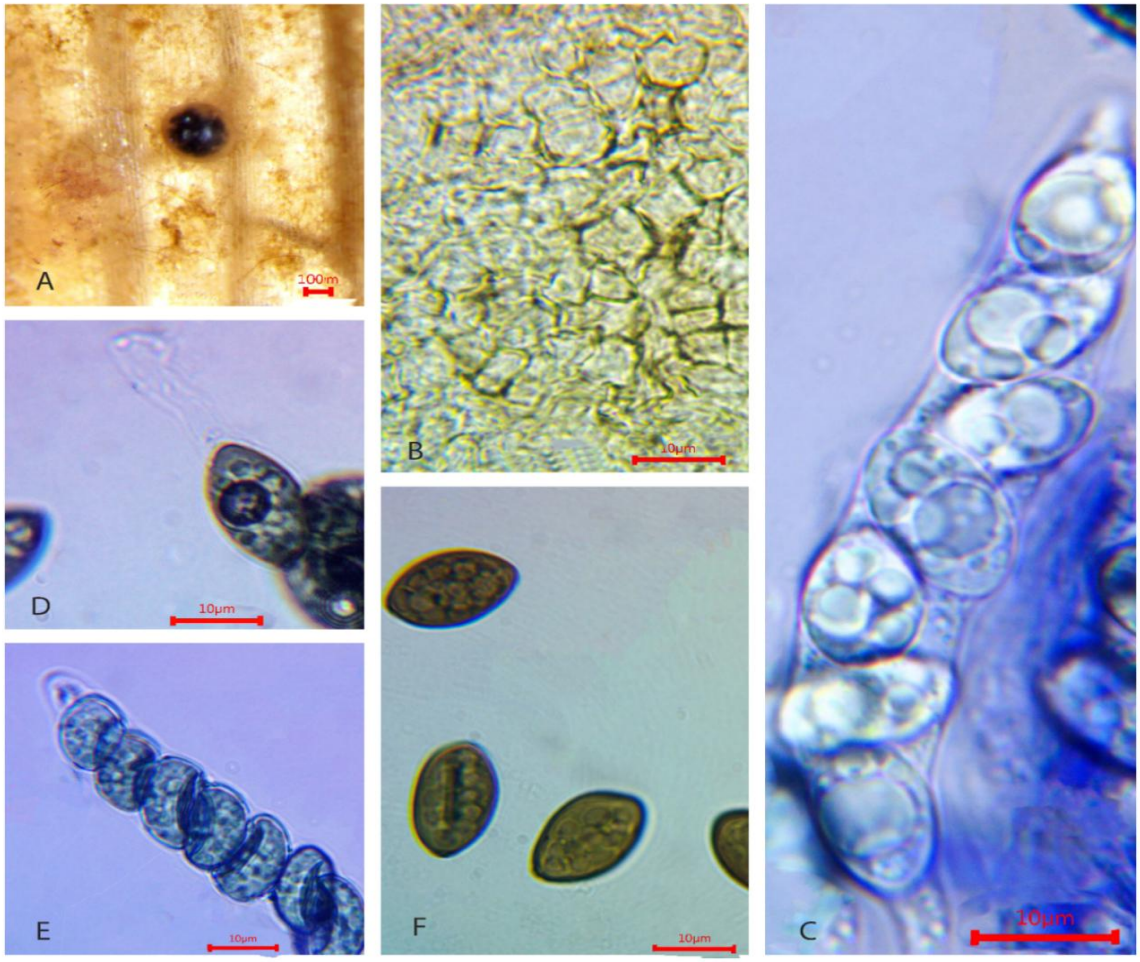

Fig. 2. *Botryotrichum trichorobustum*. A. Mature ascomata on *Phragmites australis* leaf. B. Structure of ascomatal wall in surface view. C–E. Immature asci. D. Ascus stalk. F. Ascospores. Scale bars: A = 100 μm; B–F = 10 μm.

Cladorrhinum hyalocarpum (Arx) X. Wei Wang & Houbraken
Basionym: *Thielavia hyalocarpa* Arx

Cladorrhinum hyalocarpum was isolated from soil in Netherlands; Flevoland, also from forest soil in Spain; Beseit. In this investigation, it was isolated during spring and summer seasons from the Fayama Canal, on *Phragmites australis* baits.

Macroscopic features: It is characterized morphologically by forming superficial or slightly submersed ascomata, occasionally immersed, solitary, or clustered.

Microscopic features: The ascomata, encompassing globose structures lacking an ostiole, exhibit a black hue with a globose form, boasting a diameter ranging from 150 to 400 μm. The ascomatal wall, displaying a pale brown coloration, assumes a semi-translucent state, thereby revealing a translucent quality and a composition marked by an epidermal or intricately textured surface when observed. Within this framework, the ascomatal hairs, akin to hyphae in appearance, possess a hyaline or sub-hyaline nature and measure approximately 2 to 4 μm in diameter near their base. The asci, cylindrical in shape, exhibit a spore-bearing region measuring 81.5 to 148 μm in length and 11.5 to 19.5 μm in width. These asci are accompanied by stalks spanning a length of 7 to 20.5 μm. Asci are 8-spored, deliquize early, uniseriate. Ascospores, consisting of a solitary cell, initially possess a hyaline appearance that transitions to a dark brown shade as they reach maturity. Exhibiting a smooth surface, these spores have a fusiform shape, measuring between 22.5 and 32 μm in length and 11.5 and 16 μm in width. Notably, an apical germ pore is present in these ascospores, facilitating germination. It is worth mentioning that no discernible asexual morphological characteristics were observed during the investigation. (Fig. 3).

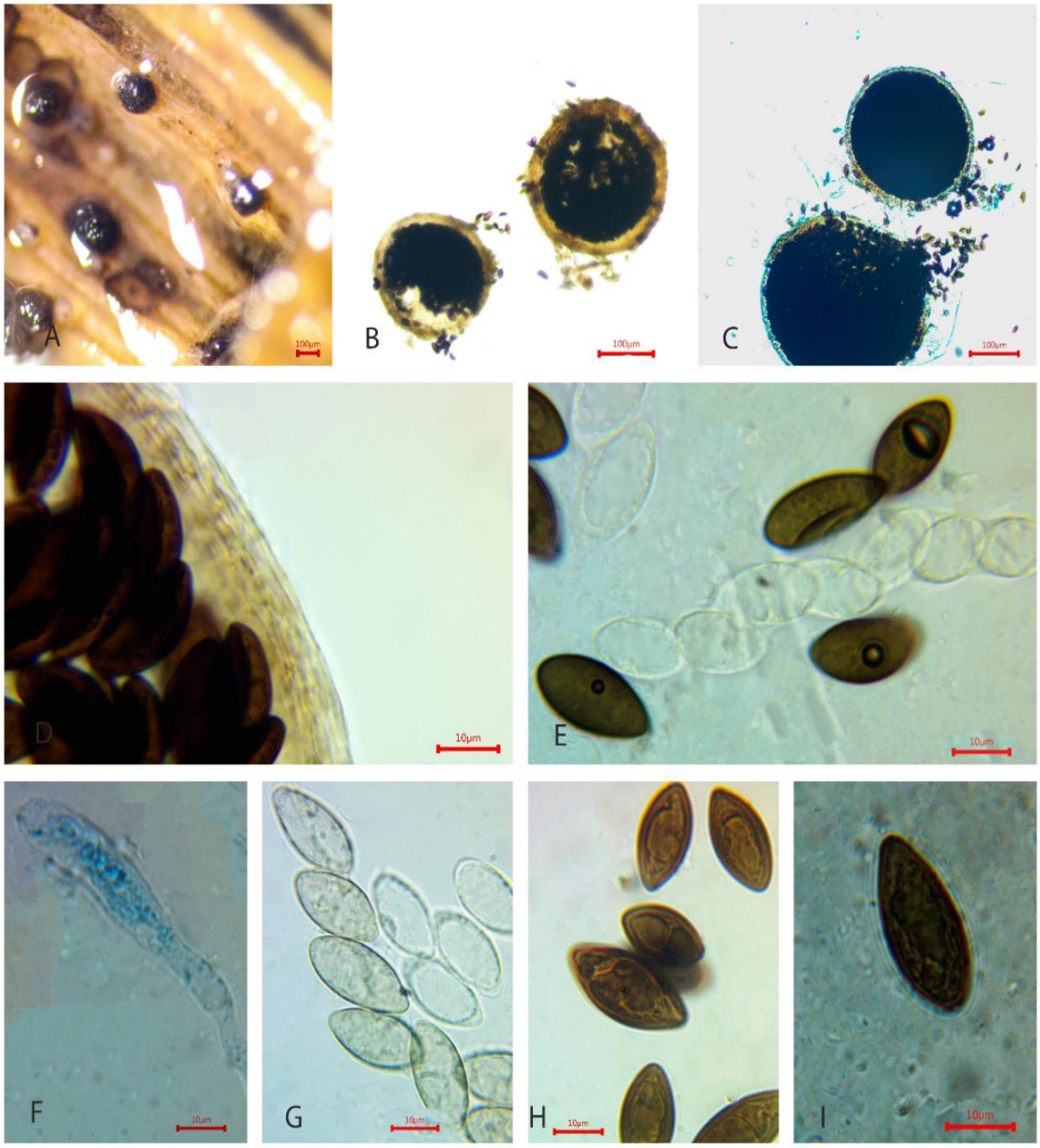

Fig. 3. *Cladorrhinum hyalocarpum*. A. Ascomata on *Phragmites australis* stem. B–C. Ascomata mounted in lactic acid. D. Structure of ascomatal wall in surface view. E–F. Asci. G–H. Ascospores. Scale bars A–C = 100 µm; D–I = 10 µm**.**

Cladorrhinum leucotrichum (Spegazzini) S.K. Huang & K.D. Hyde

Basionym: *Sordaria leucotricha* Spegazzini

Synonym: *Zopfiella leucotricha* (Speg.) Malloch and Cain

Synonym: *Podospora leucotricha* (Speg.) Niessl

Synonym: *Tripterospora leucotricha* (Speg.) N. Lundq

Cladorrhinum leucotrichum was isolated from the dung of deers in Italy by [12]. In this study, it was isolated during the summer season from the Ibrahimia Canal, on *Phragmites australis* baits.

Macroscopic features: It is characterized morphologically by ascomata superficial and solitary.

Microscopic features: Ascomata is globose to subglobose, 290–410 μm diam., non ostiolate, with hairs, uniformly distributed on the surface; Periderm 15–20 μm thick, membranaceous, pseudoparenchymatous, pale brown tissues. Asci 8-spored, biseriate, cylindrical, 70–130 × 15–25 μm, ascospores two-celled; upper cell 18–26 × 12–17 μm, limoniform, truncate at the base, smooth, thin-walled, dark brown, with a conspicuous apical germ pore, umbonate at the apex; lower cell 3–5 × 5–6 μm, cylindrical and hyaline. Anamorph was not observed (Fig. 4).

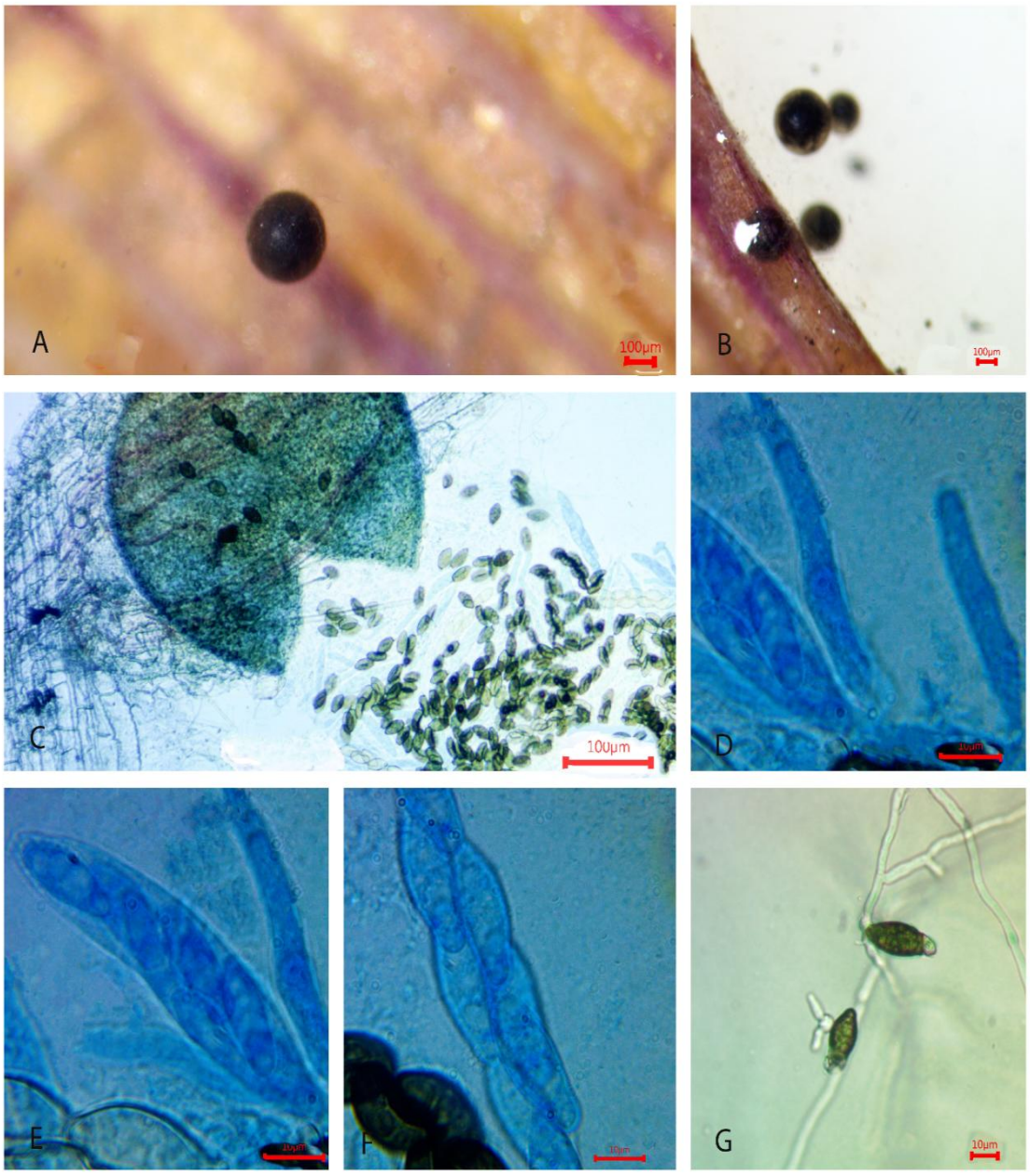

Fig. 4. *Cladorrhinum leucotrichum*. A–C. Ascomata on *Phragmites australis* stem. D–E. Immature asci. F. Ascus with ascospores. G. Germinating ascospores. Scale bars A–C = 100 µm, D–G = 10 µm.

Pseudorhypophila marina (Furuya & Udagawa) Y. Marín & Stchigel

Basionym: *Zopfiella marina* Furuya & Udagawa.

Synonym: *Zopfiella submersa* (Furuya & Udagawa) Guarro, Al-Saadoon, Gené & Abdullah.

Pseudorhypophila marina was initially identified from marine mud in China by [13], then recoded in Iraq on submerged wood by [14]. It is a new record from Egypt. The fungus was isolated during spring from the Nile River, on *Phoenix* sp. baits.

Macroscopic features: It is characterized morphologically by ascomata superficial and immersed, solitary and aggregated.

Microscopic features: The ascocarp, characterized by its globose to subglobose shape, possesses a diameter ranging from 235 to 400 µm and lacks an ostiole. The surface of the ascocarp is characterized by uniformly distributed hairs that are pale brown in color, unbranched, thin-walled, and have scarce septations. These hairs measure approximately 10 to 20 µm in length and 2.5 to 3.5 µm in width. The outer layer of the ascocarp, known as the periderm, has a thickness of 10 to 16 µm and exhibits a membranaceous and pseudoparenchymatous nature. It appears pale brown and consists of 8 to 13 layers of angular-textured cells, measuring 9 to 12 µm in diameter. Asci, containing eight spores each, display a cylindrical to clavate shape, with dimensions ranging from 90 to 120 µm in length and 14 to 20 µm in width. No paraphyses were observed in conjunction with the asci. Ascospores, consisting of two cells, have distinct characteristics. The upper cell, measuring 13.0 to 20.5 µm in length and 10 to 14 µm in width, adopts a limoniform shape with a truncated base. It possesses a smooth, thick wall and appears dark brown, featuring a conspicuous subapical germ pore. The lower cell, ranging in dimensions from 6 to 13 µm in length and 3 to 5 µm in width, is cylindrical in shape with a rounded end. It can vary in color from hyaline to slightly brown, occasionally appearing brown, and possesses a more or less thin wall. Notably, no anamorph was observed during the investigation. (Fig. 5).

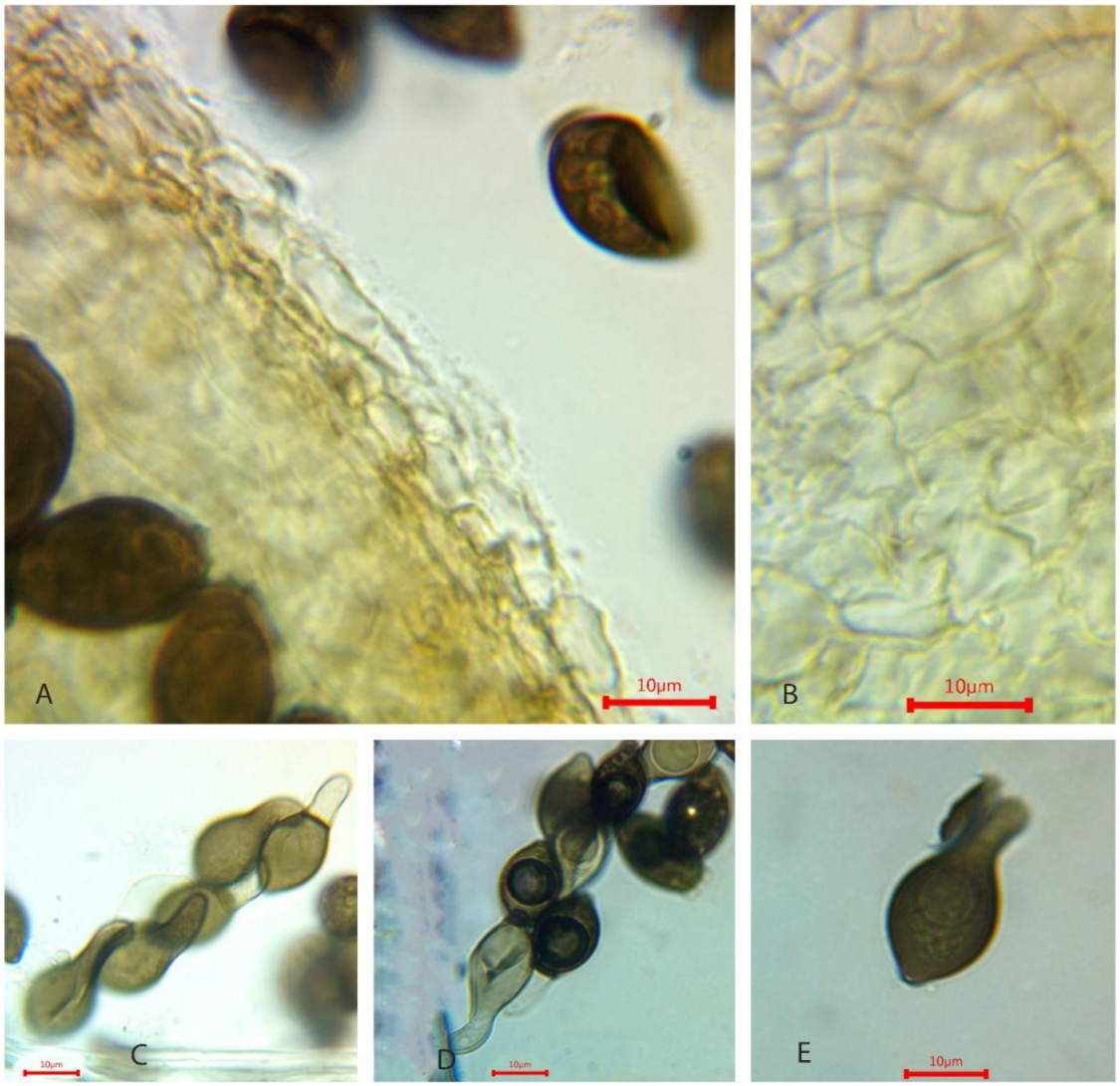

Fig. 5. *Pseudorhypophila marina*. A–B. Structure of ascomatal wall in surface view. C–D. Asci. E. Ascospore with subapical germ pore. Scale bars A–E = 10 µm.

Pseudothielavia terricola (J.C. Gilman & E.V. Abbott) X. Wei Wang & Houbraken Basionym: *Coniothyrium terricola* J.C. Gilman & E.V. Abbott

Synonym: *Thielavia terricola* (J.C. Gilman & E.V. Abbott) C.W. Emmons

Pseudothielavia terricola was originally isolated by [15] from the soil of Louisiana, United States, and was also recorded in both Israel and Congo. It is a new record from Egypt. The fungus was isolated during the summer season from the Ibrahimia Canal, on *Phragmites australis* baits.

Macroscopic features: It is characterized morphologically by forming ascomata superficial, solitary to aggregated.

Microscopic features: The ascomata lacks an ostiole and, when viewed under reflected light, appears a leaden black color when mature, owing to the presence of a dark ascomatal wall. These structures are spherical in shape, smooth, and have a diameter ranging from 85 to 200 µm. The ascomatal wall, on the other hand, is brown in color, and exhibits an epidermal texture when observed from the surface. The asci, which are subglobose to pyriform in shape, consist of a spore-bearing part measuring 18.5 to 28.5 µm in length and 15.5 to 22 µm in width. They contain eight irregularly arranged ascospores that eventually fade away. Ascospores themselves consist of a single cell and initially appear hyaline, gradually transitioning to an olivaceous brown hue as they mature. These spores are smooth, fusiform in shape, measuring 11 to 14.5 µm in length and 6 to 8 µm in width, and feature an apical germ pore. Notably, no asexual morphological features were observed. (Fig. 7).

According to the analysis of the Large Subunit (LSU) gene and phylogenetic tree illustrated in (Fig. 6), our strain is closely related to *Thielavia terricola* (currently: *Pseudothielavia terricola*) with similarity exceeding 99%.

Phylogenetic tree;

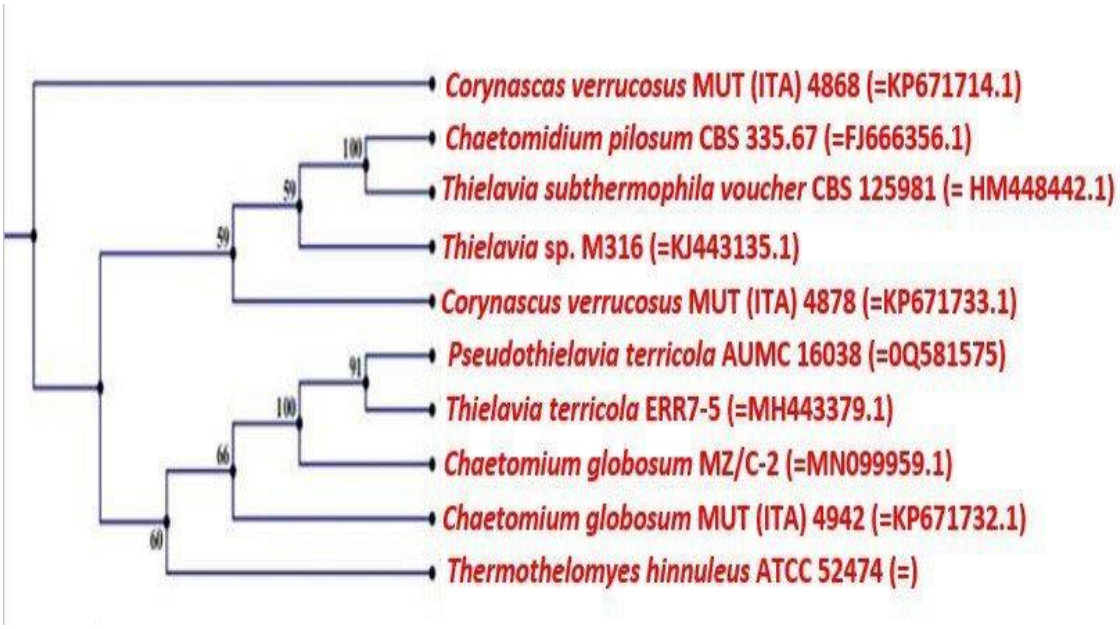

Fig. 6. Phylogenetic relationships of *Pseudothielavia terricola* and similar fungi, based on the nucleotide sequences of LSU rDNA.

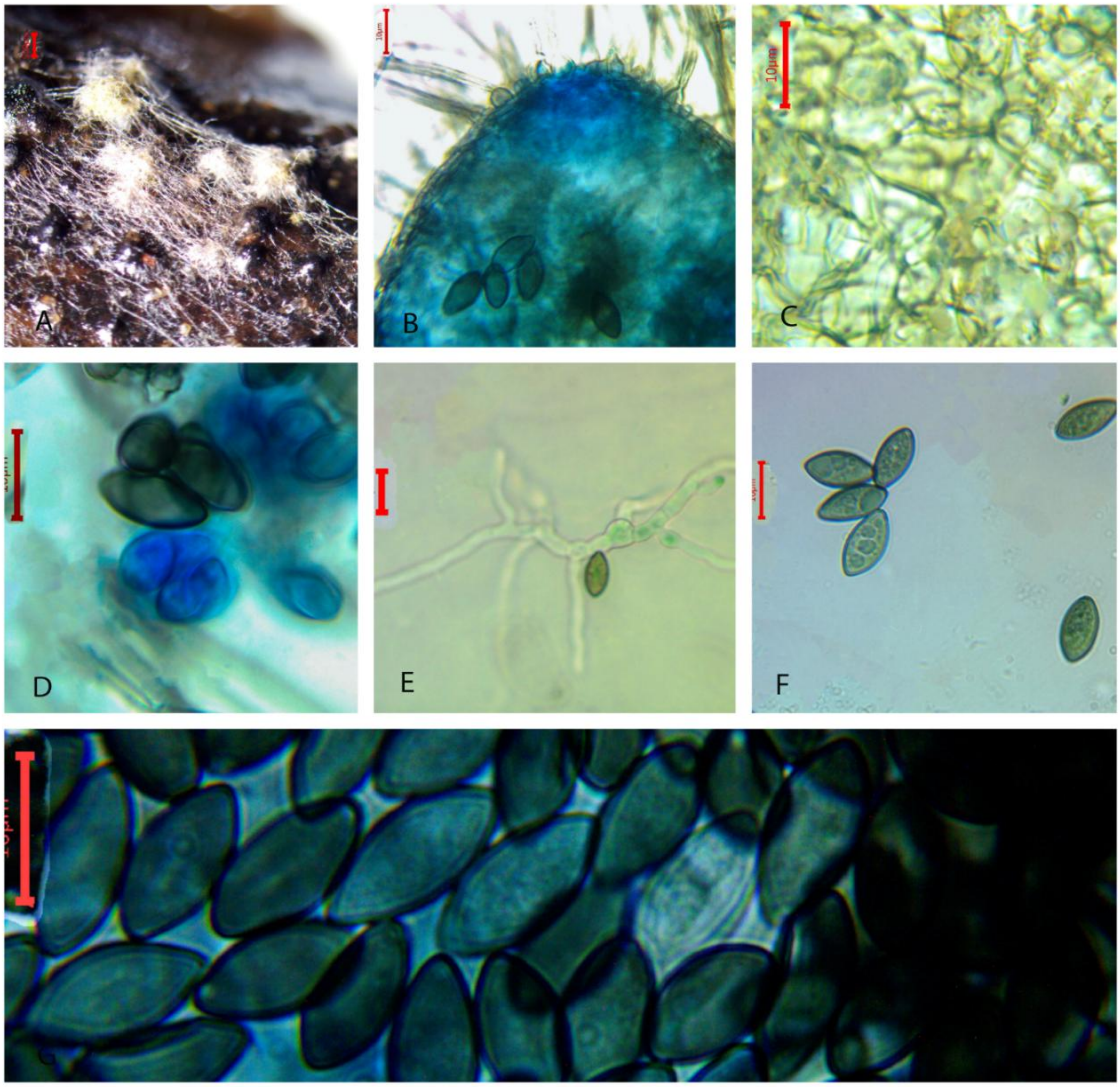

Fig. 7. *Pseudothielavia terricola.* A. Ascomata on *Phragmites australis* stem. B–C. Structure of ascomatal wall in surface view. D. Asci. E. Germinating ascospores. F–G. Mature ascospores in ascomata. Scale bars A = 200 μm; B–G = 10 μm.

Stolonocarpus gigasporus (Mustafa & Abdel-Azeem) X. Wei Wang & Houbraken

Basionym: *Thielavia gigaspora* Moustafa & Abdel-Azeem.

Stolonocarpus gigasporus was isolated from the dung of *Camelus dromedaries* in Egypt, El-Sheikh Zweid by [16]. In this study, it was recorded during autumn from the Ibrahimia Canal, on *Phragmites australis* baits.

Macroscopic features: It is characterized morphologically by forming superficial ascomata, solitary or clustered.

Microscopic features: The ascomata display a globose to subglobose shape, lack ostioles, and exhibit a fawn to olivaceous appearance when viewed under reflected light. This color is attributed to the presence of ascomatal hairs. The diameter of the ascomata ranges from 155 to 415 µm. The ascomatal wall, on the other hand, is brown in color and not translucent. It is composed of irregular, angular, or elongated cells, lending a unique structural composition to the ascomata. The ascomatal hairs resemble hyphae, exhibiting a flexuous nature. They are partly brown, septate, and measure approximately 1.5 to 3 µm in diameter near the base. Additionally, some of these hairs appear dark brown and have a slightly larger diameter, ranging from 3.5 to 5.5 µm near the base. The asci, which are arranged in fascicles, take on a cylindrical shape. They often exhibit geniculate or twisted forms. The spore-bearing portion of the asci measures 49 to 74 µm in length and 7.6 to 12.5 µm in width. These structures are accompanied by stalks, which are approximately 5.5 to 10 µm long. Each ascus contains eight uniseriate ascospores, although occasionally, a biseriate arrangement may be observed. These ascospores gradually vanish over time. The ascospores, consisting of a single cell, appear brown when mature. They possess a smooth surface and adopt an ellipsoidal shape with attenuated ends or a fusiform shape. The dimensions of the ascospores range from 23 to 29.5 µm in length and 12.9 to 16.1 µm in width. Notably, an apical germ pore is present in these spores, allowing for germination. During the investigation, no asexual morphological characteristics were observed in association with this species. (Fig. 8).

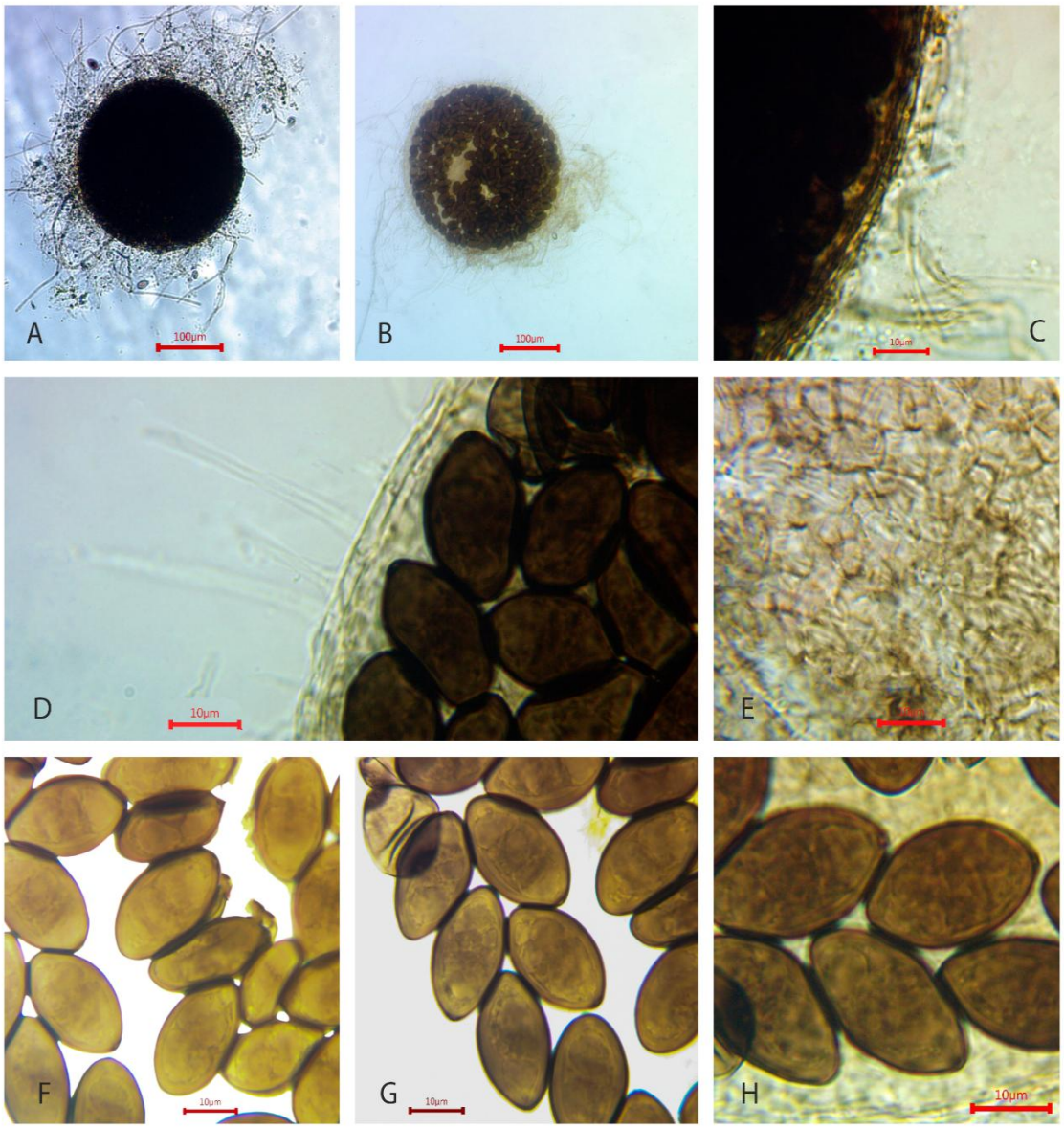

Fig. 8. *Stolonocarpus gigasporus*. A–B. Mature ascomata. C–E. Structure of ascomatal wall in surface view. F–H. Ascospores. Scale bars A–B = 100 µm; C–H = 10 µm.

Zopfiella indica Devadatha, Jeewon & V.V. Sarma

Basionym: *Zopfiella indica* Devadatha, Jeewon & V.V. Sarma

Zopfiella indica was isolated from India on mangrove wood bark. It is a new record from Egypt. The fungus was isolated during winter from the Fayama Canal and on *Ziziphus* sp. baits.

Macroscopic features: It is characterized morphologically by forming superficial and solitary ascomata.

Microscopic features: Ascomata is ostiolate, globose to subglobose, dark brown to black, 320–555 µm high, 220–405 µm diam. The peridium is 5–25 µm wide with an angular texture. Asci 115–200 × 15–45 µm, contain eight spores, unitunicate, cylindrical to clavate, evanescent, short pedicel, 20–75 µm × 2.5–7.5 µm, Ascospores biseriate, each consisting of a dark upper cell and a hyaline lower appendage; upper cell fusiform, hyaline at first turning olivaceous brown to dark brown at maturity (21.5–30 × 10–14) µm, lower appendage lash-like, hyaline, 8–55 × 3–8 µm, smooth-walled, mostly collapsing after maturation of ascospores, anamorph was not observed (Fig. 9).

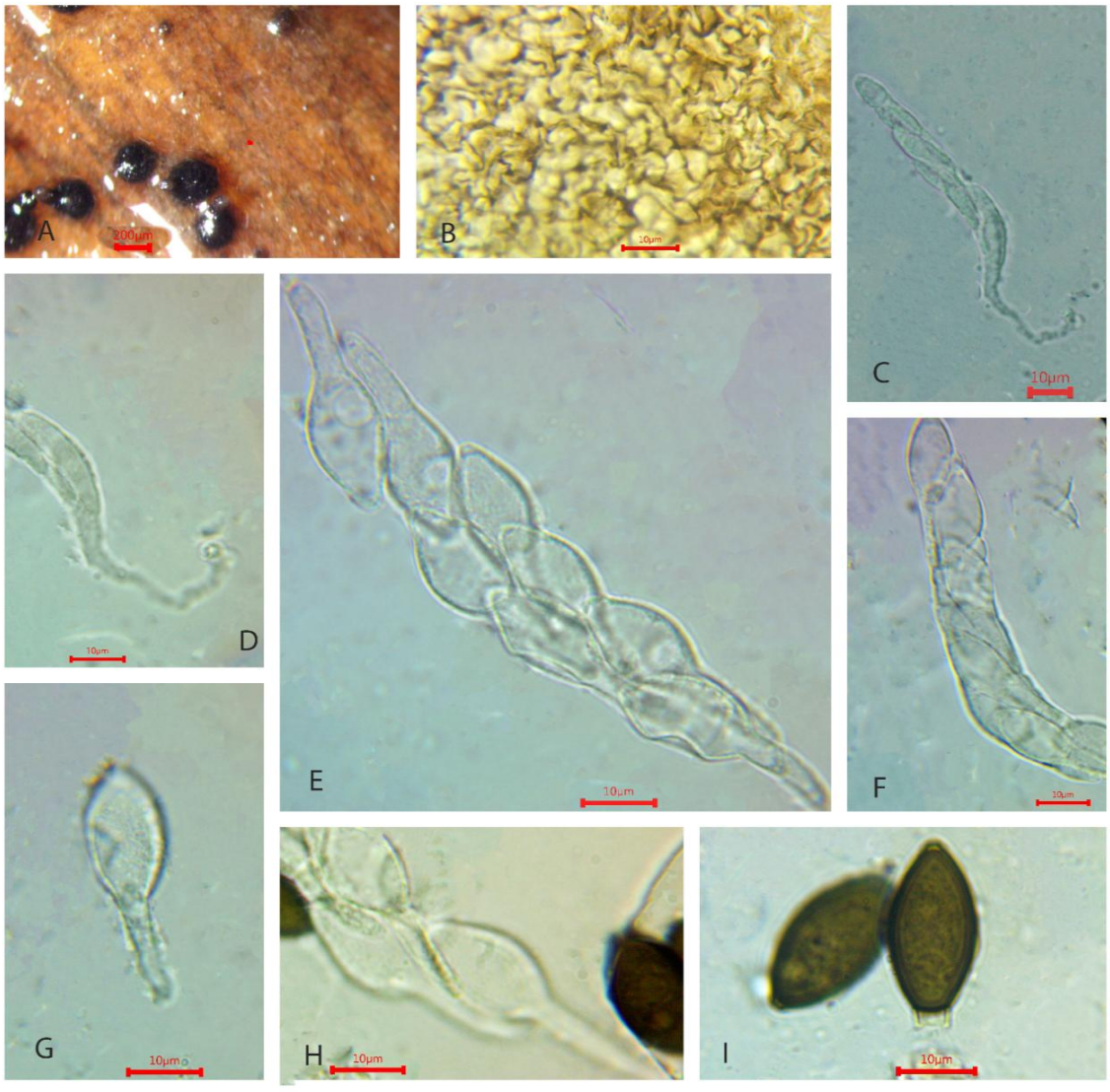

Fig. 9. *Zopfiella indica*. A. Ascomata on *Ziziphus* sp. Stem. B. Structure of ascomatal wall in surface view. C. Immature Ascus. D. Ascus stalk. E–F. Asci with ascospores. G–H. Immature ascospores. I. Mature Ascospores with collapsed pedicel. Scale bars A = 100 µm; B–I = 10 µm.

Zopfiella latipes (N. Lundqvist) Malloch & Cain

Basionym: *Tripterospora latipes* N. Lundqvist

Zopfiella latipes was initially isolated from the soil of Denmark, and then recorded on seagrass leaves, dead marsh grass, mangrove wood, and onion leaves from Hong Kong, India, Japan, Thailand, and Egypt. It is frequently recorded during all seasons autumn, winter, spring, and summer from the Nile River and Fayama Canal, on *Phragmites australis* baits.

Macroscopic features: It is characterized morphologically by forming superficial, solitary, or clustered ascomata.

Microscopic features: Ascomata is pale brown, blackish, globose, 0.5–1 mm diam, covered with yellow to pale brown, flexuous, and thick hairs, opening by dehiscence of the periderm. Periderm pseudoparenchymatous, angular texture, olivaceous brown. Paraphyses very evanescent, asci clavate, 8-spored, measure 100–110 × 15–25 µm. Ascospores are biseriate, with two cells: the upper cell hyaline when immature, olivaceous brown at maturity, 15–20.5 × 10–12 µm, truncated at the base; the lower pedicel is hyaline to pale yellow, conical in shape, 5–10 × 5–8 µm, with a subapical germ pore. Anamorph was not observed (Fig. 10).

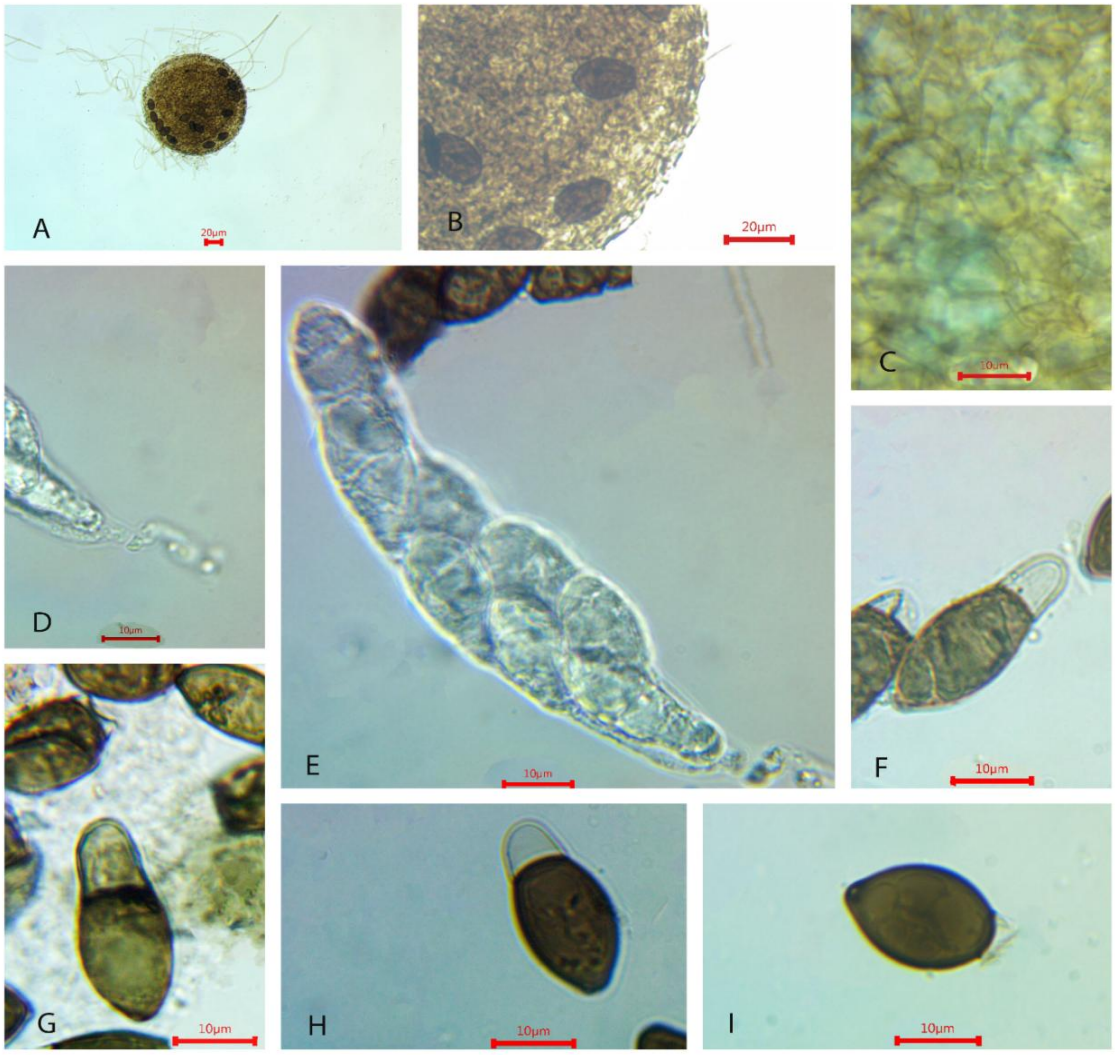

Fig. 10. *Zopfiella latipes*. A. Ascomata in lactic acid. B–C. Structure of ascomatal wall in surface view. D. Ascus stalk. E. Immature Ascus with ascospores. F–H. Ascospores. I. Ascospore with subapical germ pore. Scale bars A–B = 20 µm, C–I = 10 µm.

Zopfiella udagawae Guarro & Punsola

Guarro & Punsola identified *Zopfiella udagawae* in Spain on plant debris [17]. It isn't mentioned again in any other published papers. It is recorded in the current study as a new record for Egypt and the second record worldwide. The fungus was isolated during spring from the Nile River, on *Phoenix* sp. baits.

Macroscopic features: It is characterized morphologically by forming superficial to sub-immersed and solitary ascomata.

Microscopic features: The ascocarp exhibits a dark brown to black coloration and has a globose shape with a diameter ranging from 165 to 345 µm. It is cleistothecial and is adorned with pale brown, flexuous, and septate hairs that resemble hyphae. Peridium is relatively thick and initially semi-transparent, as it matures, it becomes dark brown and opaque. It is composed of irregular cells with thick walls. Asci 70–90 × 15–25 µm, are broadly clavate, have a rounded upper portion and feature a small, thickened ring at the apex. Notably, paraphyses are absent in this species. Each ascospore is arranged irregularly in two rows and consists of a dark upper cell and a hyaline lower appendage. The upper cell initially appears hyaline but later transforms into a dark brown color. It has a smooth and opaque surface, an irregular ellipsoidal shape, and measures 20-38 µm in length and 13-21 µm in width. The upper cell is pointed at the tip and truncated at the base, featuring a subapical circular germ pore measuring approximately 2 µm. In some cases, it may contain oil globules. On the other hand, the lower cell of the ascospore is hyaline, cylindrical, and elongated, and measures 15-50 µm in length and 5-8 µm in width. It can exhibit a straight or curved form and tends to collapse as it matures. No asexual morphological characteristics were observed in association with this species. (Fig. 11).

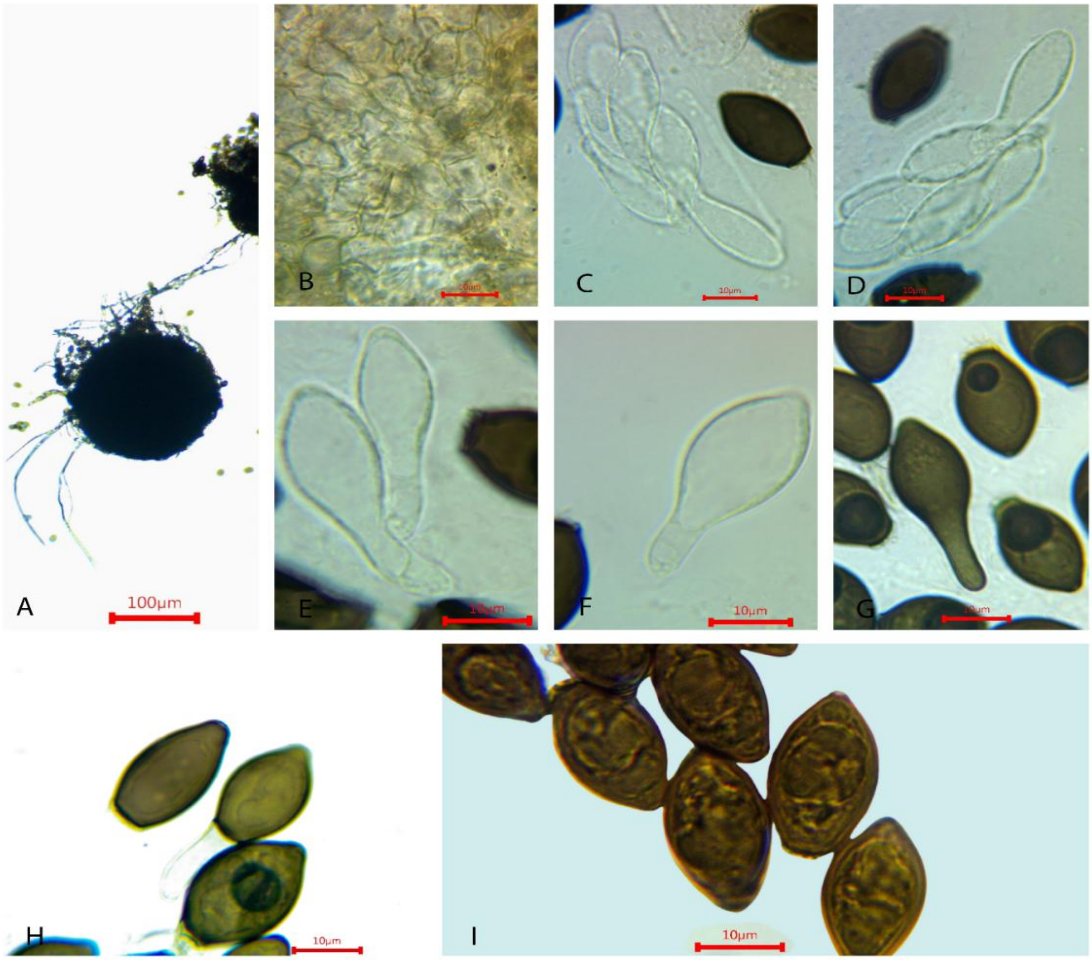

Fig. 11. *Zopfiella udagawae*. A. mature ascomata in lactic acid. B. Structure of ascomatal wall in surface view. C–D. Asci. E–G. Immature ascospores. H. Mature ascospores. I. Ascospores with collapsed pedicel. Scale bars A = 100 µm; B–I = 10 µm.

DISCUSSION

The Nile River is the life artery of Egypt [18]. Microorganisms, which serve as sensors for water contamination, have an impact on the Nile River's quality. This aquatic ecosystem is a home for a variety of fungal species, particularly those associated with aquatic Ascomycetes, and it is a promising source for several new taxa to science or at least new records for Egypt. Because the family Chaetomiaceae includes phenotypically diverse species, and their defined genera are highly polyphyletic and lack a clear

taxonomic overview, it was redefined using multigene phylogenetic analysis in combination with morphology [8].

Two species of *Botryotrichum* (*B. geniculatum* and *B. trichorobustum*) were identified from the Fayama Canal and the Nile River, respectively. *Botryotrichum*, *Chaetomium*, *Parathielavia*, and some other species related to the family Chaetomiaceae may be expanded to include other new species or a new combination. *B. geniculatum* was isolated as a species novel from the soil of China [8]. Both species are mainly characterized morphologically by producing globose, brown ascomata, a dark brown ascomatal wall, stalked asci, and clavate, each containing eight ascospores. Ascospores are one-celled, dark brown, and smooth. But the former has a shorter spore-bearing part, irregularly arranged or biseriate ascospores, and is larger in size (16.5– 25 × 11–14 μm). It was observed that *Pseudothielavia terricola* and *P. arxii* are phenotypically different, but phylogenetically they are closely related [8]. *Stolonocarpus gigasporus* is one of the *Thielavia*-like species in the family Chaetomiaceae.

Zopfiella latipes was recorded first in Egypt as a phylloplane fungus from onion leaves [19]. In the current study, three species of *Zopfiella* were treated, and two of them (*Z. indica*, and *Z. udagawae*) were recorded for the first time in Egypt. Genus *Zopfiella* is characterized phenotypically by ascomata being non-ostiolate or ostiolate, glabrous or adorned with hairs, asci being 4-or 8-spored, cylindrical or clavate, and peridia of different types [20].

Pseudorhypophila marina (formerly *Zopfiella marina*) is a new record from Egypt. *Zopfiella marina* was transferred by [20] to the new genus *Pseudorhypophila* on the basis of phylogenetically related studies. It is characterized by ascospores two-celled: the upper cell is limoniform, and the lower cell is hyaline to brown and cylindrical in shape.

The identification of *Pseudothielavia terricola* (formerly: *Thielavia terricola*) was confirmed through molecular analysis by the sequencing Large Subunit (LSU) gene. The resulting sequence was found to have high similarity with *Thielavia terricola* ERR7-5 (=MH443379.1) from the NCBI gene bank database.

CONCLUSION

The Nile River and its subsidiary canals (Ibrahimia & Fayama) are highly contaminated by Ascomycetous fungi, especially those related to the family Chaetomiaceae. Ten species belonging to five genera were treated here. Intensive studies on diverse substrates and ecosystems are needed to add new species or at least new fungal strains to be used in various aspects. Some members of Chaetomiaceae are difficult to be identified accurately based only on morphological features because most of them are morphologically similar; in addition, some strains are unable to be cultured in vitro on synthetic media. For these reasons, phylogenetic studies using multigene analysis are extremely required.

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