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Diversity of Lignicolous Freshwater Fungi from Sohag Governorate, Egypt, with the Description of a New Record: *Minutisphaera aspera*

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Abstract: This study investigated the diversity of lignicolous freshwater fungi on submerged decaying wood from four plant species: *Salix alba*, *Ziziphus spina-christi*, *Phoenix dactylifera*, and *Phragmites australis*. Random sampling and baiting techniques were employed in the Nile River and irrigation canals at Sohag Governorate, Egypt, between February 2021 and September 2022. These fungi play critical roles in wood decomposition and nutrient cycling in freshwater ecosystems. A total of 126 fungal taxa were identified, comprising 48 sexual ascomycetes, 73 asexual ascomycetes, and five Basidiomycetes from 4,448 fungal collections recorded from 1,410 submerged samples. Among the 126 taxa recorded, 17 represent new records for Egypt, including four species that are new to science. Asexual taxa predominated in the fungal community, with a ratio of asexual ascomycetes taxa to sexual ascomycetes of 1.5/1.0. The fungi were mainly distributed within the classes Sordariomycetes (52%) and Dothideomycetes (44%). The number of fungi recorded from Sohag Nile River, EL-Maragha Nile River and EL-Maragha irrigation canal were 98, 97, and 77, respectively. *Dictyocheirospora heptaspora* was the only species common to all three sites. Fifty-five species were consistently recorded across the three studied sites. One hundred and two taxa were identified from baited samples, while 99 were identified from randomly collected samples. Seventy-four fungi were consistently recorded across both sampling methods, while 28 and 24 fungi were unique to baited and randomly collected samples, respectively. The most common genera included *Dictyocheirospora* with 286 records, *Halobyssothecium* (248), *Pseudohalonectria* (240), *Ophioceras* (230) and *Achaetomium* (205). *Jahnula* was the most speciose genus comprising five species. Frequent species included *Dictyocheirospora heptaspora* (20%), *Pseudohalonectria lignicola* (17%), *Ophioceras commune* (16%), *Zopfiella latipes* (14%), *Limnoperdon incarnatum* (13%), and *Hapalosphaeria deformans* (12%). *Minutisphaera aspera* was reported for the first time in Egypt during this study. Phylogenetic analyses of ITS rDNA sequence data, along with morphological characteristics, were used to identify the new collection.
Keywords: Fungal diversity, Fungal ecology, Lignicolous fungi, wood baits, Nile River.

1. Introduction

Freshwater fungi are organisms that complete whole or part of their life cycle, rely on free freshwater or on submerged substrates found in lentic and lotic ecosystems [1, 2]. They can be found in various freshwater environments, such as rivers, streams, ponds, lakes, swamps, and artificial habitats including pools, reservoirs, drainage ditches, water-cooling towers, water pipes, street gutters, wastewater treatment, and sewage systems [1, 3-7].

Freshwater fungi are classified into thirteen phyla, with documentation of 3,870 species. The majority belong to Ascomycota, which includes 2,968 species across 1,018 genera. This is followed by Chytridiomycota, which has 333 species in 97 genera, and Basidiomycota, which comprises 218 species in 100 genera [8-11]. Lignicolous freshwater fungi grow on decaying wood submerged in various freshwater environments [5, 10]. They play a key role in decomposition of the submerged woody debris in aquatic habitats by breaking down lignocelluloses and releasing essential nutrients [5]. Sordariomycetes and Dothideomycetes are the most speciose classes of lignicolous freshwater fungi [12, 13] with few species in Eurotiomycetes [14, 15] and Orbiliomycetes [16].

Studies of freshwater fungi colonizing decaying submerged plant debris in Egypt have significantly advanced in the last two decades. Three new genera and twenty-two new species have been described as new to science from freshwater environments in Egypt [17-28].

Sohag Governorate is located in Upper Egypt, between latitudes 26°15′00″ to 26°45′00″N and longitudes 31°15′00″ to 32°00′00″E and covering a total area of 11,022 km^2. The Nile River and its associated irrigation canals constitute the primary source of surface water in Sohag Governorate. The Nile River is generally regarded as the longest river in the world, extending over 6,800 km through northeastern Africa. The riparian vegetation of the riverbanks in Sohag Governorate primarily includes *Arundo donax*, *Phragmites australis*, *Vachellia nilotica*, *Eucalyptus* spp., *Salix* spp., *Ziziphus spina-christi,* and various other dwarf shrubs [29].

This research was designed to explore the diversity of freshwater fungi on the submerged decaying wood of four plant species (*Salix alba*, *Ziziphus spina-christi*, *Phoenix dactylifera* and *Phragmites australis*) using random sampling and baiting techniques from the Nile River and irrigation canals at Sohag Governorate, Egypt.

2. Materials and methods

2.1. Sample collection, morphological study and isolation

A total of 1,410 submerged decaying wood samples were collected from the following plant species: *Phoenix dactylifera* (Arecaceae), *Phragmites australis* (Poaceae), *Salix alba* (Salicaceae), *Ziziphus spina-christi* (Rhamnaceae) and unidentified dicot wood. The samples were obtained using baiting techniques (690 samples) and random sampling (720 samples). The test blocks were 14 x 2 cm and were sourced from the selected plant wood. Sets of ten pieces of the same type were tightly bound with rope and then attached to a larger base timber. A total of 100 wood samples from each plant were baited at three sites: the Nile River (26°33'55.8"N 31°42'21.8"E) at Sohag city; the Nile River (26°44'49.2"N 31°34'23.7"E) at EL-Maragha; and the irrigation canal (26°39'16.1"N 31°37'43.1"E) at Nagaa Taie, EL-Maragha. The samples were submerged at these three sites at Sohag Governorate, Egypt, for 18 months, from February 2021 to September 2022, and were retrieved every three months.

A total of 720 samples of *Phoenix dactylifera*, *Phragmites australis*, and unidentified dicot wood were collected randomly from the same sites every three months during the same period. Collected samples were placed in clean plastic bags and brought to the laboratory, where they were incubated in plastic boxes lined with sterilized moist tissue paper at room temperature. The examination of samples, and pure cultures of the fungi was obtained following the methods described previously [30, 31]. Fruiting structures were sectioned using a Leica CM 1100 Cryostat (Leica Biosystems, Nussloch, Germany). Micrographs were obtained with an Olympus BX51 microscope equipped with Toup Tek XCAM1080PHA (Toup Tek, Zhejiang, China) digital imaging system. Pure cultures and herbarium specimens are preserved at the Sohag University Microbial Culture Collection, Egypt (SUMCC).

2.2. DNA extraction, sequencing, and phylogenetic analyses

Cultures grown on peptone and yeast with glucose (PYG) broth [30] were used for genomic DNA extraction using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). PCR amplification and sequencing of the ITS regions were conducted using the primer pairs ITS1 and ITS4 [32], following the methods outlined by Abdel-Wahab et al. [33] by Solgent Co. Ltd (South Korea). Sequencher 4.2.2 (Gene Codes Corporation) was employed to assemble the sequences, and ClustalX [34] was used to align them with pertinent ones that were obtained from GenBank. Phylogenetic analyses were performed based on maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI). The ML analysis was conducted by RAxMLGUI v. 2.0.13 [35], the BI analysis was conducted in MrBayes v. 3.1.2 [36], and the MP analysis by PAUP* 4 [37] with details as described by Bakhit and Abdel-Wahab [38, 39]. The obtained sequence was deposited in the NCBI GenBank.

2.3. Data analysis

The following data were calculated for each of the study sites and sample types.

Percentage occurrence of each fungus =

$$\frac{\text{Number of collections of the fungus} \times 100}{\text{Number of samples collected}}$$

Number of fungi per sample =

$$\frac{\text{Total number of fungal collections}}{\text{Number of samples collected}}$$

Diversity indices (Shannon-Weiner and Menhinick's) and similarity indices (Jaccard and Sørenson) were calculated for the communities as described in Abdel-Aziz [25].

2.4. Statistical analysis

Fungal diversity indices and similarity indices were estimated for the communities using PAleontological STatistics (PAST), Version 4.17c, USA [40] and The Multi-Variate Statistical Package (MVSP) software [41].

3. Results and Discussion

A total of 4,448 fungal collections were recorded from 1,410 decaying submerged wood samples of the following plant species: *Salix alba*, *Ziziphus spina-christi*, *Phoenix dactylifera*, *Phragmites australis*, and unidentified dicot wood. One hundred and twenty-six fungal taxa were identified during this study, comprising 48 sexual ascomycetes, 73 asexual ascomycetes, and five Basidiomycetes (Table 1). Of the 126 taxa recorded, 17 represent new records for Egypt, including four species that are new to science, of which a new species was described in a previous article, namely *Murispora purpurea* [28]. The taxonomic placements of ten species were confirmed by molecular data.

Studies of lignicolous freshwater fungi are mainly concentrated in tropical, subtropical, and temperate regions [42], with their diversity in tropical regions being significantly higher than that in temperate regions [5, 43]. Freshwater fungi have been relatively well-studied in Asia, particularly in China and Thailand [42, 44-47]. Zhang et al. [48] documented 173 species belonging to 112 genera collected from freshwater habitats in Thailand. Hu et al. [49] reported 782 species of freshwater fungi in China. Xu et al. [47] introduced 42 new species, five new genera, and three new families from submerged decaying wood in China.

Several studies have been carried out to document lignicolous freshwater fungi from the Nile River and irrigation canals at Sohag Governorate. Abdel-Aziz [50] reported 52 fungal species, including 37 sexual ascomycetes, 12 asexual taxa, and 3 Basidiomycetes, from 400 samples collected from the Nile River at Sohag Governorates. Abdel-Aziz [51] documented 62 fungal species, comprising 42 sexual ascomycetes, and 20 asexual taxa, from 135 wood samples collected from the Nile River at Sohag. Abdel-Aziz [52] identified 13 fungal species, including 9 sexual ascomycetes, and 4 asexual taxa, from 100 samples collected from the Nile River at Sohag Governorate. Abdel-Aziz [23] recorded 99 fungal taxa, including 42 sexual ascomycetes, 55 asexual taxa and 2 Basidiomycetes from the Nile River at Sohag. Species richness in this study which included 126 species was higher than that recorded in previous studies from the Nile River at Sohag [23, 50-52] but lower than that reported by Bakhit [53], who identified 198 fungal taxa (110 ascomycetes, 81 asexual taxa, and 7 Basidiomycetes) from the Nile River and Nile Delta

irrigation canals at eight governorates. Greater fungal diversity is observed when samples are collected periodically over a longer time period because of the seasonal occurrence of certain taxa [54]. Hyde et al. [5] stated that factors such as riparian vegetation, disturbances including pollution, stream drying, and study methods, can affect the diversity of lignicolous freshwater fungi. Additionally, water chemistry and geographic location also impact fungal colonization.

Asexual taxa dominated the fungal community in the present study, with a ratio of asexual ascomycetes taxa / sexual ascomycetes was 1.5/1.0. This is also in agreement with previous studies from freshwater habitats [23, 51, 54-58]. In contrast, sexual ascomycetes were found to dominate the freshwater fungal community in the Nile River and Delta region of Egypt [50, 52, 53, 59, 60]. The reasons for the variation in the dominance of asexual fungi over sexual ascomycetes remain unclear [23]. Ho et al. [56] suggested that environmental parameters may influence the reproductive modes of fungi.

A list of recorded species, along with their frequencies of occurrence, is provided in Table (1). Fungi recorded are classified as very frequent (above 20%), frequent (10–20%), common (5–10%) and infrequent (below 5%). The frequencies of occurrence for all taxa ranged from 0.1% to 20%. Frequent species included *Dictyocheirospora heptaspora* (20%), *Pseudohalonectria lignicola* (17%), *Ophioceras commune* (16%), *Zopfiella latipes* (14%), *Limnoperdon incarnatum* (13%), and *Hapalosphaeria deformans* (12%) (Fig. 1. A), in this respect, the genera *Dictyocheirospora*, *Pseudohalonectria*, and *Ophioceras* are cosmopolitan in distribution and largely reported from freshwater habitats in the USA and Asia [11].

The mean number of species per genus (S/G) was 1.2. The most common genera included *Dictyocheirospora* represented by 286 records, *Halobyssothecium* (248); *Pseudohalonectria* (240); *Ophioceras* (230); *Achaetomium* (205); *Zopfiella* (202); *Limnoperdon* (187); *Hapalosphaeria* (173); *Dictyosporella* (136); *Atractium* and *Murispora* (121); *Lolia* (113), and *Natantispora* (102). Among the reported genera, 16 are represented by two or more species: *Jahnula* (5 species), *Graphium*, *Xylomyces*, *Savoryella*, and *Halobyssothecium* (3 species each); and two species belong to each of the following genera: *Achaetomium*, *Minutisphaera*, *Robillarda*, *Canalisporium*, *Aniptodera*, *Dictyosporium*, *Dictyosporella*, *Gliocladiopsis*, *Pleurotheciella*, *Pseudorobillarda*, and *Verticillium*. Some genera, such as *Jahnula*, *Minutisphaera, Halobyssothecium*, *Pseudohalonectria*, *Hapalosphaeria*, and *Natantispora* are saprobes exclusively on aquatic habitats. Six species of *Jahnula* (*J. aquatica*, *J. bipileata*, *J. dianchia*, *J. granulosa*, *J. poonythii* and *J. sangamonensis*) have been recorded formerly from freshwater habitats in Egypt [23, 29, 51, 60]. *Canalisporium* species are common on submerged decaying wood in freshwater habitats [61-63]. Five species of this genus have been recorded previously from freshwater environments at Sohag, Governorate [53, 63].

The dominance diversity curve (Fig. 1. B) illustrates that many fungal taxa were recorded as infrequent species, while a small number were classified as frequent in the present study. A total of one hundred and eleven fungi were identified as infrequent species and are listed in table (1). Most species reported from freshwater habitats are often collected only once or have a low frequency of occurrence [23, 64, 65].

The total number of fungal records was 4,448, documented from 1,410 samples. The number of fungi per sample ranged from 3.0 (in the Nile River at Sohag) to 3.4 (in the Nile River at El-Maragha), as shown in table (2). The average number of fungi per sample in this study was 3.15, which is considered higher than that recorded in previous studies conducted at Sohag, Egypt [23, 50-52]. The average number of fungi per sample in other regions of the world ranged from 1.5 to 3.8 [56, 57, 66-68].

Ascomycota predominates over other fungal groups in freshwater habitats [11, 23, 69]. Within Ascomycota, which includes 2,968 species and 1,018 genera, the class Sordariomycetes is the most abundant, comprising 823 species and 298 genera, followed by the class Dothideomycetes with 677 species and 229 genera [11]. In the current study, Ascomycetes and their asexual morphs comprised the majority of the fungal community, with only five basidiomycete species recorded. The fungi were mainly distributed in Sordariomycetes (52%) and Dothideomycetes (44%). The dominance of Sordariomycetes and Dothideomycetes in freshwater environments may be explained by their abilities to produce superficial to immersed ascomata with a gelatinous centrum, active ascospore dispersal, and ascospore equipped with elaborate appendages or gelatinous sheaths [70].

Discomycetes was represented by only two taxa during the present study namely, *Ascobolus behnitziensis* and *Orbilia caudata*. Few Discomycetes species have been reported from wood from tropical streams, while Shearer [3] listed 112 species from temperate regions. Nine species belonging to 5 genera of discomycetes were identified from freshwater habitats in Nile Delta region localities [53, 60]. Abdel-Aziz [23] identified only one species of discomycete, *Orbillia* sp., however, the occurrence of discomycetes on wood submerged in tropical and subtropical rivers is rare [5].

Basidiomycota was represented by five species during the present study (Table 1). *Limnoperdon incarnatum* and *Flammulina* sp., accounted for 13% and 3.6% of the total frequency of occurrence, respectively. Basidiomycetes have rarely been encountered on decaying plant materials in freshwater habitats [66, 71, 72]. Abdel-Aziz [23] collected two species, *Limnoperdon incarnatum* and *Ceratorhiza* sp. from freshwater environments at Sohag Governorate. Calabon et al. [11] updated the list of freshwater Basidiomycetes, reporting a total of 218 species across100 genera.

A total of 17 fungal species identified in the present study have not been previously recorded in Egypt including four species are new to science based on molecular and morphological studies (*Murispora purpurea*, *Paoayensis nilotica*, *Scytalidium sohagensis* and *Sohagmyces aquatica*). The other 13 newly recorded taxa are *Coleodictyospora muriformis*, *Flammulina* sp., *Gliocladiopsis aquaticus*, *Graphium penicillioides*, *Halobyssothecium phragmitis*, *Lindgomyces apiculatus*, *Longipedicellata aquatica*, *Mariannaea elegans*, *Massariosphaeria roumeguerei*,

Minutisphaera aspera, *Pleurotheciella saprophytica*, *Xylomyces giganteus*, and *Xylomyces rhizophorae.*

Although numerous studies have examined freshwater fungi colonizing wood and herbaceous stems from the Nile River and there is little overlap in the species identified across these studies. Abdel-Aziz [50] reported 52 fungal taxa; only eight species were also found in the present study. Abdel-Aziz [51] documented 62 fungal taxa, with twelve species overlapping with the present study. Abdel-Aziz [23] recorded a total of 99 fungal species, from 400 samples. Of the 99 taxa identified in that study, 17 species were also found in the present study. A total of 198 freshwater fungal species were identified from the Nile River and irrigation canals in the Nile Delta region, Egypt [53, 60]. Of these 198 taxa, 28 species were also recorded in the present study.

Ninety-eight, 97, and 77 fungal species were recorded from the Sohag Nile River, EL-Maragha Nile River, and EL-Maragha irrigation canal, respectively. The diversity of fungi found in the Nile River samples was higher than that reported in the irrigation canal samples (Table 2). The lowest values of the Shannon (3.89) and Menhinick (2.08) diversity indices were observed in the El-Maragha irrigation canal samples (Table 2). Similarity indices ranged from 0.77 to 0.83 for Sørenson and from 0.526 to 0.652 for Jaccard indicating a high similarity between the mycota of the studied sites. Fifty–five species were consistently recorded across all three sites, and twenty-two fungal species overlapped between the two Nile River sites (Fig. 1. C). The fungi were mainly distributed within the classes Sordariomycetes and Dothideomycetes at the three studied sites (Fig. 1. E). Fungal communities in freshwater environments become increasingly diverse with greater geographic distance, difference in structure and diversity of riparian vegetation, and variation in water quality.

One hundred and two taxa were identified from baited samples, while 99 taxa were identified from randomly collected samples at the three studied sites. Our results revealed that baited samples contained a higher diversity of freshwater fungi than randomly collected samples. The average number of fungi per baited sample (3.75) was higher than that observed in randomly collected samples (2.62). Frequencies of occurrence of taxa in baited samples ranged from 0.3% to 32.8%, while it was ranged from 0.3% to 15.8% in naturally occurring wood. Seventy-four fungi (58.7%) were consistently recorded across both sample types, while 28 and 24 fungi were unique to baited and randomly collected samples, respectively (Fig. 1. D). Sordariomycetes and Dothideomycetes predominate over other fungal classes in the two methods samples (Fig. 1. F). Jaccard and Sørenson's similarity indices were 0.59 and 0.76, respectively, indicating a high similarity between the mycota obtained by the two methods. Greater species diversity has been reported for submerged test blocks as compared to naturally occurring wood in many formerly studies [56, 73]. Conversely, Abdel-Aziz [50] observed that randomly submerged decaying wood samples harbor a higher diversity of fungal species than submerged test blocks of *Casuarina, Eucalyptus*, and *Salix* baits in the Nile River at Sohag Governorate. Wood baits in freshwater are colonized by a distinct group of fungi [73, 74].

3.1. Fungal Diversity at Different Sites

3.1.1. Fungal Diversity at the Sohag Nile River

Ninety-eight fungal species were collected, comprising 38 sexual ascomycetes, 57 asexual taxa, and 3 Basidiomycetes identified from 1,458 fungal collections derived from 480 samples collected from the Nile River at Sohag City. The frequencies of occurrence of all taxa ranged from 0.2% to 27%. The number of fungi per sample was 3.0., with *Pseudohalonectria lignicola* (27%) and *Dictyocheirospora heptaspora* (22%) being the very frequent fungi. Frequent fungi included *Murispora purpurea* (16%), *Ophioceras commune* (15%), *Halobyssothecium unicellular*e (11%), and *Atractium stilbaster* (17%) (Table 1).

3.1.2. Fungal Diversity at the EL-Maragha Nile River

Ninety-seven fungi (40 sexual ascomycetes, 53 asexual taxa, and four Basidiomycetes) were identified from 1,617 fungal collections that recorded from 480 samples collected from the Nile River in EL-Maragha. The frequencies of occurrences of all taxa ranged from 0.2% to 26%. The average number of fungi per sample (3.4) was the highest among the studied sites. *Dictyocheirospora heptaspora*, with a frequency of 26%, was the only very frequent species. Other frequently observed species included *Hapalosphaeria deformans* (17%), *Ophioceras commune* (16%), *Massariosphaeria roumeguerei* (13%), *Zopfiella latipes* (12%), *Dictyosporella aquatica* (11%), *Lolia aquatica* (10%), and *Achaetomium globosum* (12%).

3.1.3. Fungal Diversity at the EL-Maragha Irrigation Canal

Seventy-seven fungi (34 sexual ascomycetes, 40 asexual taxa, and 3 Basidiomycetes) were collected, comprising a total of 1,691 fungal collections derived from 450 samples collected from an irrigation canal in EL-Maragha (Table 1). Species richness was lower than that recorded at the other two sites. The frequencies of occurrence for all taxa ranged from 0.2% to 38%. The average number of fungi per sample was 3.1. The most frequent species were *Pseudohalonectria lignicola* (38%), *Dictyocheirospora heptaspora* (35%), *Ophioceras commune* (26%), *Limnoperdon incarnatum* (25%), and *Zopfiella latipes* (22%). Other frequently occurring species included *Achaetomium globosum* (13%), *Acrogenospora sphaerocephala*, and *Hapalosphaeria deformans* (12%), *Natantispora retorquens*, *Halobyssothecium unicellulare* (11%), and *Sammeyersia grandispora* (10%).

3.2. Diversity of Fungi from Baited and Randomly Collected Samples

3.2.1 Diversity of Fungi on Baits

One hundred and two fungal species (39 sexual ascomycetes, 59 asexual taxa, and four Basidiomycetes) were collected with a total of 2,585 fungal collections from 690 baited samples (Table1). The average number of fungi per wood sample was 3.75. Frequencies of occurrence of all taxa ranged from 0.3% to 32.8 %, with *Dictyocheirospora heptaspora* (32.8%), *Pseudohalonectria lignicola* (32.3%), *Limnoperdon incarnatum* (22.9%), and *Ophioceras commune* (21.7 %) were very frequent species. Frequent species included *Atractium stilbaster* (16.7%), *Achaetomium globosum* (14.9%), *Zopfiella latipes* (12.8%),

Jahnula granulosa (11.9%), *Hapalosphaeria deformans* (11.7%), and *Halobyssothecium unicellulare* (11.4%) (Table 1). The ratio of asexual taxa to sexual taxa was 1.5/1.00.

3.2.2 Diversity of Fungi on Randomly Collected Samples

Ninety-nine species, including 42 sexual ascomycetes, 52 asexual taxa, and five Basidiomycetes, were identified from 1,884 fungal collections recorded from 720 samples collected randomly from the studied sites (Table 1). The number of fungi per wood sample was 2.62 which is lower than the number reported from baits. The frequencies of occurrence for all taxa ranged from 0.3% to 15.8%. The most frequent species were *Zopfiella latipes* (15.8%), *Hapalosphaeria deformans* (13.1%), *Murispora purpurea* (11.9%), and *Dictyosporella aquatica* (10.3%). The ratio of asexual ascomycetes to sexual ascomycetes was 1.23/1.00 which is lower than that recorded on baits.

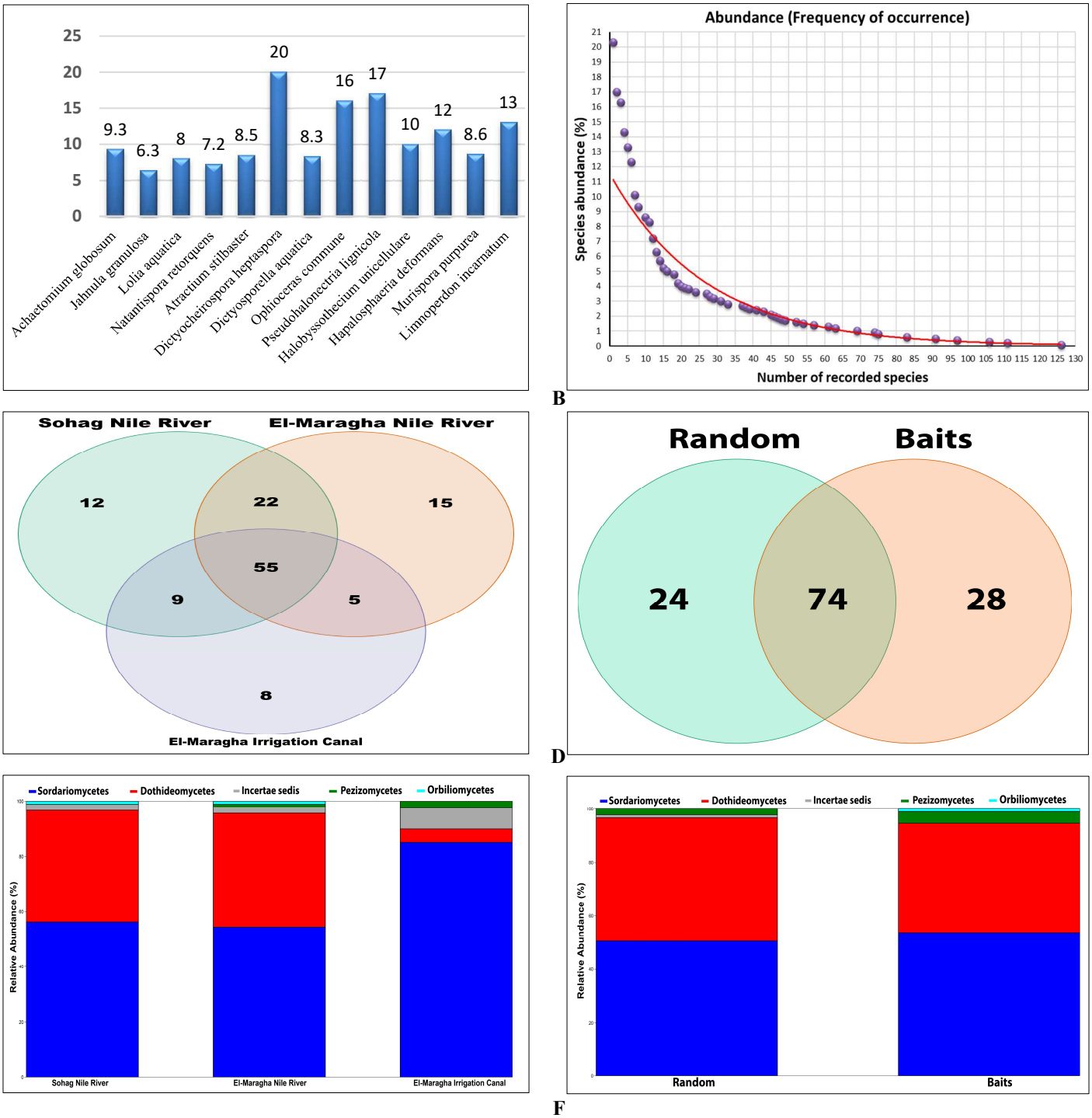

Fig. 1: A. Frequencies of occurrence (%) of the most frequent species. **B**. Dominance- diversity curve for all species collected from the studied sites. **C**. Number of shared and specific species according to the sites. **D**. Number of shared and specific species according to the sampling techniques. **E**. Relative abundance of classes across different sites. **F**. Relative abundance of classes in the two sampling techniques.

Table 1: Frequency of occurrence of fungal species collected at each of the three sites along Sohag Governorate.

Species	Collection Sites			Total	Collection Method
	SNR	ENR	EIC		
	%	%	%	%	
Sexual ascomycetes					
Achaetomium globosum J.N. Rai & J.P. Tewari	5	10	13	9.3	R, B
Achaetomium indicum Kulshr, Raych. & A.Z.M. Khan	2.7	8	4.7	5.2	R, B
Aliquandostipite separans (Abdel-Wahab & El-Shar.) J. Campb., Raja, A. Ferrer, Sivichai & Shearer	0.2	-	1.8	0.6	R, B
Aniptodera aquadulcis (S.Y. Hsieh, H.S. Chang & E.B.G. Jones) J. Campb., J.L. Anderson & Shearer	-	0.8	-	0.3	B
Aniptodera chesapeakensis Shearer & MA Mill.	0.2	0.6	4	1.6	R, B
Annulatascus aquaticus W.H. Ho, K.D. Hyde & Hodgkiss	6.3	1	4.4	3.9	B
Anthostomella sp.	-	0.4	-	0.1	R
Ascobolus behnitziensis Kirschst.	-	2.3	3.8	2	R
Astrosphaeriella exorrhiza Boise	1.3	0.4	-	0.6	R, B
Cladorrhinum leucotrichum (Speg.) S.K. Huang & K.D. Hyde	0.8	6.9	1.1	3	R, B
Fusoidigranularius nilensis (Abdel-Wahab & Abdel-Aziz) W. Dong, H. Zhang & K.D. Hyde	2.9	-	-	1	B
Halobyssothecium voraginesporum (Abdel-Wahab, Bahkali & Jones) M.S. Calabon, K.D. Hyde & E.B.G. Jones	5.2	4.2	2.4	4	R, B
Herpotrichia sp.	-	0.8	-	0.3	B
[&] *Hongkongmyces* sp.	0.8	0.6	2.4	1.3	R, B
Jahnula aquatica (Kirschst.) Kirschst.	0.4	-	-	0.1	R
Jahnula bipileata Raja & Shearer	0.2	-	-	0.1	R
Jahnula dianchia S.K. Huang & K.D. Hyde	1.9	0.6	0.9	1.1	R, B
Jahnula granulosa KD Hyde & SW Wong	7.1	7.5	4.2	6.3	R, B
Jahanula sp.	-	-	0.2	0.1	B
Leptosphaeria sp.	-	0.4	-	0.1	R
Lindgomyces apiculatus K. Hiray. & Kaz. Tanaka	3.5	1	0.7	1.8	R
Lolia aquatica Abdel-Aziz & Abdel-Wahab	3.3	10	3.1	5.7	R, B
Longipedicellata aquatica W. Dong, H. Zhang & K.D. Hyde	-	1.5	0.2	0.6	R, B
Lophodermium sp.	-	-	2.9	0.9	R
Massarina carolinensis Kohlm, Volkm.-Kohlm. & O.E. Erikss	-	1.7	-	0.6	R
Massariosphaeria roumeguerei (Sacc.) Leuchtm	1	13	0.4	5	R, B
Microascus cinereus C.A. Fuentes & F.A. Wo	0.6	0.2	-	0.3	R, B
[&] *Minutisphaera aspera* Raja, Oberlies, Shearer & A.N. Mill	1.3	5.8	-	2.4	R, B
Minutisphaera fimbriatispora Shearer, AN Mill. & A Ferrer.	0.8	5	5.8	3.8	R, B
Naïs aquatica KD Hyde	3.8	0.8	1.1	1.9	R, B
Natantispora retorquens (Shearer & JL Crane) J. Campb, JL.	3.8	6.9	11	7.2	R, B
Nectria sp.	0.6	-	0.9	0.5	R, B
Neohelicascus egyptiacus (Abdel-Wahab & Abdel-Aziz) W. Dong, K.D. Hyde & H. Zhang	2.9	0.8	0.4	1.4	R, B
Neojahnula australiensis (K.D. Hyde) W. Dong, H. Zhang & K.D. Hyde	0.8	0.2	-	0.4	B
Ophioceras commune Shearer, J.L. Crane & W. Chen	15	18	16	16	R, B
Orbilia caudata Starbäck	0.6	0.8	-	0.5	B
[&] *Paoayensis nilotica* sp. nov.	0.6	1.5	5.1	2.3	R, B
Pseudohalonectria lignicola Minoura & T. Muroi	27	4.6	20	17	R, B
Plenodomus agnitus (Desm.) Gruyter, Aveskamp & Verkley	5.4	1.7	0.9	2.7	R, B
Podospora inquinata Udagawa & S. Ueda	3.1	6	5.1	4.8	R, B
Porosphaerellopsis sp.	1.3	1.9	6.7	3.2	R, B

Table 1: Continued.

Species	Collection Sites			Total	Collection Method
	SNR	ENR	EIC		
	%	%	%	%	
Rivulicola cygnea Raja & Shearer	0.6	0.2	-	0.3	R
Sammeyersia grandispora (Meyers) S.Y. Guo, E.B.G. Jones & K.L. Pang	0.4	-	8	2.7	R, B
Savoryella aquatica K.D. Hyde	0.4	0.4	0.7	0.5	R, B
Savoryella lignicola E.B.G. Jones & R.A. Eaton	0.8	4	2.9	2.6	R, B
Trichoderma sp.	1.3	1.5	0.9	1.2	R, B
Westerdykella dispersa (Clum) Cejp & Milko	-	0.4	0.9	0.4	R, B
Zopfiella latipes (N. Lundq.) Malloch & Cain.	9.2	12	22	14	R, B
Asexual ascomycetes					
Acremonium masseei (Sacc.) W. Gams	0.6	-	-	0.2	B
Acrogenospora sphaerocephala (Berk. & Broome) M.B. Ellis	1.5	0.8	11	4.2	B
Acrocalymma medicaginis Alcorn & J.A.G. Irwin	-	-	0.9	0.3	B
Atractium stilbaster Link	14	6	6	8.6	R, B
Bartalinia sp.	0.2	-	-	0.1	B
Brachiosphaera tropicalis Nawawi	1.3	0.4	2.2	1.3	R, B
& *Brocchiosphaera microspora* (R.F. Castañeda & W.B. Kendr.) K. Yamag., Chuaseehar. & Nakagiri	0.4	4.2	-	1.6	B
Canalisporium caribense (Hol.-Jech. & Mercado) Nawawi & Kuthub	0.2	0.2	0.4	0.3	R, B
Canalisporium jinghongense L. Cai, K.D. Hyde & McKenzie	4.4	0.8	1.8	2.3	B
Chaetopsina aquatica M.S. Bakhit & A.E. Abdel-Aziz	0.2	2.5	1.8	1.5	R, B
Chaetosphaeronema clematidis Phukhams., Ertz, Gerstmans & K.D. Hyde	-	0.2	-	0.1	R
& *Chloridium gonytrichii* (F.A. Fernández & Huhndorf) Réblová & Seifert	0.8	2.7	0.2	1.3	R, B
Codinaea paniculata Réblová & J. Fourn	-	0.6	1.1	0.6	R
Coleodictyospora muriformis W. Dong, Doilom & K.D. Hyde	0.6	0.2	-	0.3	R
Colletogloeum sp.	-	0.4	-	0.1	B
& *Corynespora* sp.	4.6	8.8	0.9	4.8	R, B
Creosphaeria sp.	1.7	0.8	-	0.9	R, B
Clavariana aquatica Nawawi	0.4	-	2	0.8	R, B
Clonostachys rogersoniana Schroers	-	1.5	-	0.5	B
Cucurbitinus constrictus (I. Schmidt) L.L. Liu & Z.Y. Liu	3.5	2.7	2.7	3	R, B
Dictyoarthrinium sacchari (JA Stev.) Damon	0.4	-	-	0.1	R
Dictyocheirospora heptaspora (Garov.) M.J. D'souza, Boonmee & K.D. Hyde	22	26	13	20	R, B
Dictyosporella aquatica Abdel-Aziz	7.5	11	6.7	8.3	R, B
Dictyosporella sp.	2.1	1.9	-	1.3	R, B
Dictyosporium aquaticum Abdel-Aziz	2.5	3.5	-	2.1	R, B
Dictyosporium elegans Corda	-	1.5	-	0.5	B
Diplocladiella taurina Cazau, Aramb. & Cabello	1	-	0.7	0.6	R, B
Gliocladiopsis aquatica Y.Z. Lu, R.H. Perera & K.D. Hyde	0.6	4	-	1.6	R, B
Gliocladiopsis sp.	-	0.6	-	0.2	R
Graphium laricis K. Jacobs, Kirisits & M.J. Wingf.	-	-	0.2	0.1	R
Graphium sp.	2.7	5	2.2	3.3	R, B
Graphium penicillioides corda	-	2.5	-	0.9	R, B
Halobyssothecium phragmitis M.S. Calabon, E.B.G. Jones, S. Tibell & K.D. Hyde	5.6	4	0.9	3.5	R, B
Halobyssothecium unicellulare (Abdel-Aziz) M.S. Calabon, K.D. Hyde & E.B.G. Jones	11	7.5	11	10	R, B
Hapalosphaeria deformans (Syd & P. Syd.) Syd.	8.5	17	11	12	R, B
Koorchalomella salmonispora Abdel-Aziz & Abdel-Wahab	-	1.9	-	0.6	R

Table 1: Continued.

Species	Collection sites			Total	Collection method
	SNR	ENR	EIC		
	%	%	%	%	
Lasiodiplodia theobromae (Pat.) Griffon & Maubl	2.7	0.6	7.6	3.5	R, B
[&]*Mariannaea elegans* G. Arnaud	0.4	1	-	0.5	B
Monodictys sp.	3.3	2.1	1.8	2.4	R, B
[&]*Murispora purpurea* Bakhit, A.E. Abdel-Aziz & Abdel-Aziz	16	4.4	5.1	8.6	R, B
Myrothecium sp.	2.5	-	-	0.9	B
Parafuscosporella nilotica Abdel-Aziz	0.6	4.2	3.8	2.8	R, B
Pestalotia sp.	0.2	-	-	0.1	R
Periconia prolifica Anastasiou	0.6	-	-	0.2	R
Phaeoisaria clematidis (Fuckel) S. Hughes	-	-	0.2	0.1	R
Phoma sp.	2.3	1	1.8	1.7	R, B
Pleurotheciella nilotica Abdel-Aziz & Abdel-Wahab	-	0.8	0.9	0.6	R, B
Pleurotheciella saprophytica Z.L. Luo, H.Y. Su & K.D. Hyde	0.2	0.6	-	0.3	R
Pseudorobillarda sp.1	2.1	0.8	0	1	R, B
Pseudorobillarda sp.2	2.9	0.8	0.4	1.4	R, B
Robillarda sp.	1.9	0.6	-	0.9	R
Robillarda sohagensis Abdel-Wahab & Abul-Ezz & Bakhit	0.2	0.4	-	0.2	R, B
Savoryella nypae (K.D. Hyde & Goh) S.N. Zhang, K.D. Hyde & J.K. Liu	1.7	1.3	-	1	R, B
[&] *Scytalidium sohagensis* sp. nov.	-	1	-	0.4	B
Septoria sp.	-	0.8	-	0.3	B
[&] *Sohagmyces aquatica* sp. nov	0.2	-	4.4	1.5	R, B
Stagonospora vitensis Unamuno	0.2	1.3	-	0.5	B
Synnematous sp.	0.4	-	-	0.1	R
Taeniolella phialosperma Ts. Watan.	-	-	0.2	0.1	B
Tetraploa aristata Berk. & broome	0.2	-	-	0.1	R
Torula herbarum (Pers). Link	0.8	2.7	4.7	2.7	R, B
Trichocladium englandense KD Hyde and Goh	5.8	1.5	1.1	2.8	R, B
Unknown coelomycetes sp.1	2.7	3.3	0.7	2.3	R, B
Unknown coelomycetes sp.2	2.7	7.1	1.3	3.8	R, B
Unknown hyphomycetes sp.1	1.3	-	-	0.4	B
Verticillium sp.1	1	-	0.4	0.5	B
Verticillium sp.2	0.4	2.9	4.9	2.7	B
Volucrispora sp.	-	-	1.1	0.4	B
*Volutella ciliata (*Alb. & Schwein.) Fr.	1.7	1.9	-	1.2	R, B
Xylaria arbuscula Sacc.	0.6	2.3	-	1	B
Xylomyces aquaticus (Dudka) K.D. Hyde & Goh	4	4.4	2	3.5	R, B
Xylomyces giganteus Goh, W.H. Ho, K.D. Hyde & C.K.M. Tsui	0.2	-	0.4	0.2	B
Xylomyces rhizophorae Kohlm. & Volkm. Kohlm.	1	-	2.2	1.1	R, B
Basidiomycetes					
Ceratorhiza sp.	4.2	1	2.2	2.5	R, B
Flammulina sp.	7.1	3.5	-	3.6	R, B
Limnoperdon incarnatum G.A. Escobar	6.9	13	20	13	R, B
Unknown Basidiomycetes sp.1	-	4.2	-	1.4	R, B
Unknown Basidiomycetes sp.2	-	-	1.1	0.4	R

Abbreviations: SNR= Sohag Nile River, ENR= EL-Maragha Nile River, EIC= EL-Maragha irrigation canal, R=Random, B=Baits, [&] supported by molecular data.

Table 2: Fungal diversity at the three studied sites

	Collection sites			Total
	SNR	ENR	EIC	
Total number of fungal collections	1458	1617	1373	4448
Total number of samples collected	480	480	450	1410
Number of fungi per wood sample	3.0	3.4	3.1	3.2
Total number of fungal taxa	98	97	77	126
Number of sexual ascomycetes	38	40	34	48
Number of asexual ascomycetes	57	53	40	73
Number of Basidiomycetes	3	4	3	5
Shannon - Winner diversity index	4.07	4.14	3.89	4.32
Menhinick diversity index	2.57	2.41	2.08	1.89

The taxonomical position of *Minutisphaera aspera* (SUMCC 22012) was confirmed based on morphological and molecular studies in this article.

Minutisphaera aspera Raja, Oberlies, Shearer & A.N. Mill., Mycologia 107(4): 854 (2015). Figure (3)

Index Fungorum number: 811063

GenBank numbers – ITS: PX069565.

Phylogenetic analysis

The ITS dataset consisted of 23 strains, including 15 belonging to the genus *Minutisphaera*, 6 representatives from genera within Aliquandostipitaceae, and 2 taxa from *Myrmaecium* in Valsariaceae, which were used as outgroup. The MP dataset consisted of 500 characters including: 240 constant, 16 variable parsimony uninformative and 244 parsimony-informative characters. MP analyses resulted in a single most parsimonious tree with a tree length of 552 steps, a consistency index (CI) of 0.7409, a retention index (RI) of 0.8595 and a rescaled consistency index (RC) of 0.6369. A best scoring RAxML tree with a final likelihood value of -2995.211660 is presented in Fig. N. The matrix contained 265 distinct patterns with 9.78% undetermined characters or gaps. Estimated base frequencies were as follows: A= 0.215790, C=0.266376, G= 0.308889, T= 0.208945; substitution rates, AC=0.866592, AG=3.235018, AT=2.163212, CG= 0.881394, CT=11.016855, GT=1.0; and gamma distribution shape parameter $\alpha = 1.776931$. The topologies obtained from the three analyses were closely similar. In the phylogenetic analysis, our new strain (SUMCC 22012) clustered together with *Minutisphaera aspera* (G427-1a, G427-1b) strains, forming a sister group to *M. japonica* (JCM 18560, JCM 18561, JCM 18562) based on the ITS dataset (Fig. 2).

Taxonomy

Saprobic on submerged decaying wood of *Salix alba*. **Sexual morph: Ascomata** 83–166 µm high, 103–152 µm diam. (mean= 109 × 121 µm, n= 8), apothecioid, globose to subglobose, dark brown to black, scattered, superficial. **Peridium** 9–17.5 µm wide, comprising two layers; the outer layers composed of irregular, dark brown cells of *textura angularis*; the inner layer is composed of hyaline, subglobose to flattened cells of *textura angularis*. **Hamathecium** 1.5–2.0 µm wide, septate, hyaline, cellular pseudoparaphyses. **Asci** 47–84 × 22–41 µm (mean= 67.5 × 31.3 µm, n = 16), 8-spored, bitunicate, fissitunicate, subglobose to clavate, sessile to short pedicellate, apically rounded, without an ocular chamber. **Ascospores** 26–35 × 10–16 µm (mean = 30.7 × 12.2 µm, n= 36), biseriate to multiseriate, fusiform to ellipsoidal, one-septate with the septum median to sub median. The septum is hyaline and thin in immature ascospores and then becoming thicker and darker in mature ascospores. The upper cell is longer and wider than the lower cell. Ascospores are hyaline when young and multiguttulate becoming dark brown and rough-walled with age. They are surrounded by hyaline sheath in immature ascospores; the sheath becomes condensed and dark forming a verruculose ascospore wall covering in older ascospores. **Asexual morph:** Undetermined.

Materials examined: Egypt, Sohag Governorate, EL-Maragha City, the Nile River (26°44'49.2"N 31°34'23.7"E), on submerged decaying wood test blocks of *Salix alba* (Salicaceae), January. 2022, coll. A. F. Mahmoud, SUMCC H-22012; Sohag Governorate, Sohag City, the Nile River (26°33'55.8"N 31°42'21.8"E), on decaying stem of *Phragmites australis* (Poaceae), March. 2022, coll. A. F. Mahmoud, SUMCC H-22013.

Known distribution: USA [75], China [76], Egypt (This study).

Notes:

The genus *Minutisphaera* Shearer, A.N. Mill. & A. Ferrer was established by Ferrer et al. [77] to accommodate *M. fimbriatispora*, which was described from submerged wood in freshwater habitats in the USA. Raja et al. [75, 78] introduced three new species, i.e. *M. aspera*, *M. japonica*, and *M. parafimbriatispora* from Japan and the USA. The genus is characterized by small, erumpent to superficial, dark-pigmented ascomata, and 1–2-septate, clavate to broadly fusiform, hyaline to brown ascospores, with or without a gelatinous sheath [75, 77, 78]. Additionally, two new species *M. aquaticum* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su and *M. thailandensis* R.J. Xu, Boonmee & K.D. Hyde, were discovered from Thailand from freshwater habitats [79, 80]. Currently, the genus comprises six species that are saprobes in freshwater habitats. Raja et al. [78] introduced the order Minutisphaerales to accommodate the *Minutisphaera* species. Minutisphaerales is a small order with only two genera: *Acrogenospora* and *Minutisphaera* in two families [81].

Minutisphaera aspera was described from submerged decorticated wood, USA [78]. Since then, it has only been found once in freshwater stream in Yuanjiang River Basin, China [76].

This species is characterized by having ascospores that are hyaline when young becoming dark brown and rough-walled with age and surrounded by dark sheath form a verruculose ascospore wall covering [78]. Our new collection was identified as *M. aspera* supported by morphological and phylogenetic analyses. Morphologically, our collection has smaller ascomata (83–166 µm high, 103–152 µm diam. vs 235–480 µm diam., in the original description) and slightly wider ascospore (10–16 µm vs. 9–14 µm). In all other aspects it is morphologically similar to the holotype [78]. This is the first report of *M. aspera* in Egypt and Africa. *Minutisphaera aspera* produces four secondary metabolites including dipeptides and aromatic polyketides [78].

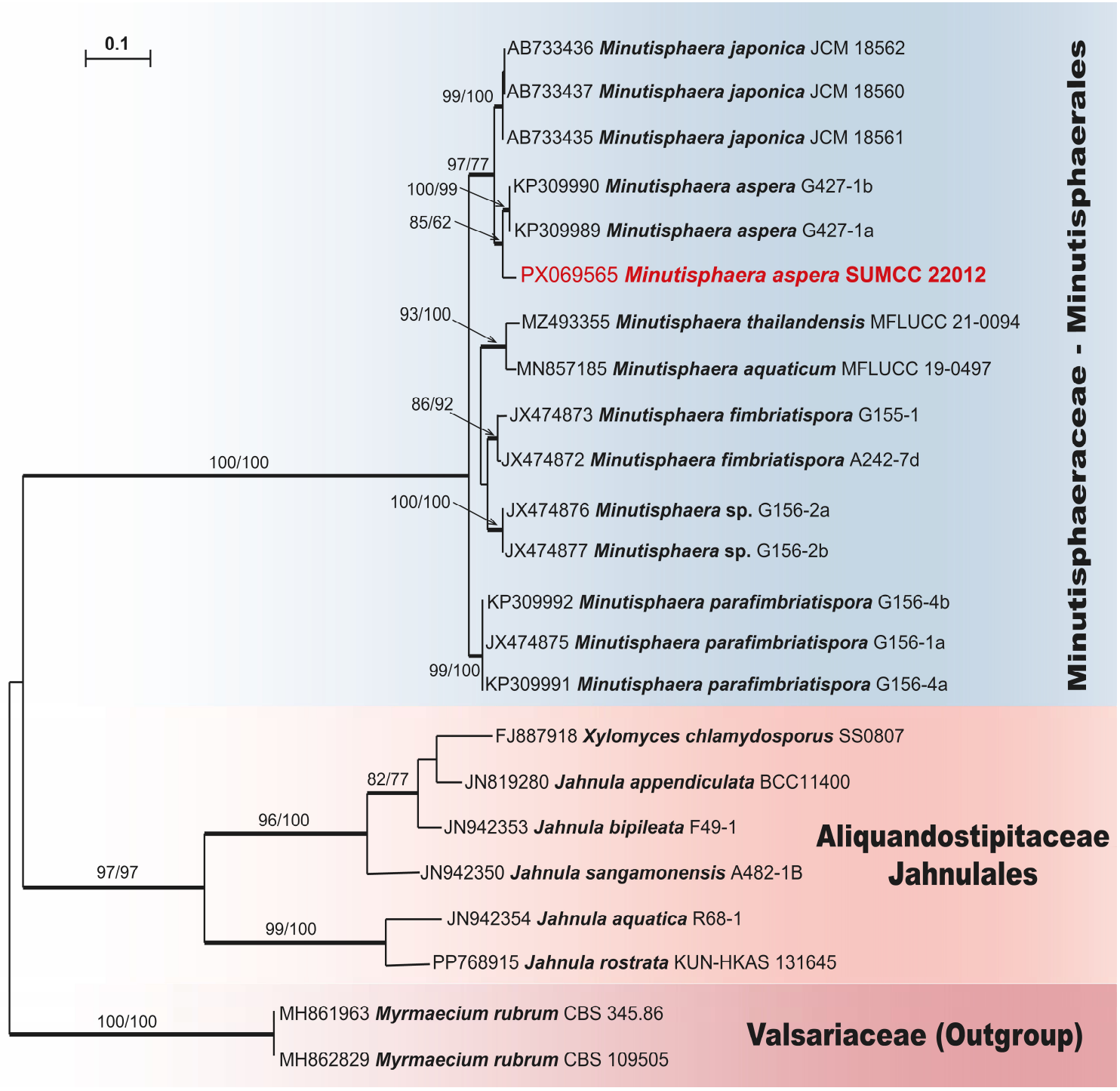

Fig. 2. RAxML phylogenetic tree of ITS sequence dataset for *Minutisphaera aspera* (SUMCC 22012) with other species of *Minutisphaera* and their related species. Bootstrap support on the nodes represents ML and MP ≥ 70 % and Bayesian pp ≥ 0.95. The tree is rooted to *Myrmaecium rbrum* (CBS 345.86, CBS 109505). Newly generated sequences are indicated in red.

Fig. 3. *Minutisphaera aspera* (SUMCC H-22012). **A**. Ascomata on natural substrate. **B**, **C**. Sections of ascoma. **D**. Peridium. E. Squash mounts of ascoma. **F–I** Asci. J. Pseudoparaphyses. **K–M.** Ascospores. Scale bars: **B–F** = 20 µm, **G–M** = 10 µm.

CRediT authorship contribution statement:

Conceptualization, Faten A. Abdel-Aziz (FAA), and Mahmoud S. Bakhit (MSB); methodology, Asmaa F. Mahmoud (AFM), MSB; software, AFM, MSB; Data curation FAA, MSB, AFM; validation, FAA, MSB, AFM; Writing – original draft, MSB, AFM; Writing – review & editing FAA, MSB; Supervision, FAA, MSB; Project administration FAA.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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