

Fungal Communities on Decaying Submerged Leaves in Freshwater Habitats in Sohag Governorate, Egypt

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Abstract: Randomly collected decaying leaves and senescent, dried leaf litter of *Eucalyptus rostrata*, *Ficus nitida*, *Phoenix dactylifera*, and *Phragmites australis* submerged for one year in the River Nile, small and large irrigation canals at Sohag, Egypt, were investigated for freshwater fungi. Thirty-six fungal species belonging to 33 genera were identified from 982 fungal collections recorded from 864 samples. The most common genera were *Halobyssothecium* represented by 221 records, *Aspergillus* (190), and *Cylindrocladiella* (102). Of the 36 taxa recorded, five are new to science, of which *Robillarda sohagensis* was previously described. Multivariate clustering analysis based on similarity between the 96 fungal communities has produced 13 groups. The type of collected samples; random or baited and the host are the important factors that influenced the fungal communities. *Halobyssothecium unicellulare* was collected from 59 communities followed by *Aspergillus flavus* (48) and *Cylindrocladiella pseudohawaiiensis* (32). The highest numbers of species and records were reported from randomly collected leaves of *Phoenix dactylifera* and *Phragmites australis* from both the River Nile and the small irrigation canal. *Bartalinia robillardoides* was reported for the first time from freshwater habitats in Egypt during the present study. A description and illustration of the Egyptian collection are provided. Phylogenetic analyses of the LSU rDNA sequence confirmed its identity.

Keywords: Foliicolous fungi, fungal diversity, fungal ecology, leaf baits, molecular phylogenetics, multivariate cluster analysis, River Nile

1. Introduction

Freshwater fungi colonize submerged or partially submerged plant debris such as leaves, woods, and twigs [1]. Shearer et al. [2] divided the freshwater fungi into five groups: Chytridiomycota, Ascomycota, Anamorphic fungi, Basidiomycota, and the non-fungal Saprolegniales of the Oomycetes. Calabon et al. [3] updated the classification of freshwater fungi under thirteen phyla. They documented 3870 fungal species known from freshwater environments belonging to 1,361 genera, 386 families, 145 orders, and 45 classes.

Streams and rivers often depend on plant litter as a source of carbon, nutrients, and energy that drive ecosystem processes [4]. Most aquatic fungi can colonize and grow on a wide range of substrates [5-7]. Leaf decomposition in freshwater ecosystems includes leaching, microbial degradation, and physical and biotic fragmentation [8,9].

Filamentous freshwater fungi colonizing wood and herbaceous stems were fairly studied [2,3,10], while filamentous fungi colonizing decaying leaves in freshwater habitats are seldom studied. Abdel-Raheem [11] recorded 39 species of aquatic hyphomycetes from submerged decaying leaves in the River Nile, Egypt. Abdel-Aziz [12] recorded 64 species of freshwater fungi (40 ascomycetes, 20 asexual fungi, and 4 basidiomycetes) from decaying submerged herbaceous and woody samples collected from both River Nile and irrigation canals in Upper Egypt. Pang et al. [13] introduced a novel order Jahnulales with a new ascomycete species *Patescospora*

separans Abdel-Wahab & El-Sharouny on decaying submerged woody sample from freshwater habitat in Egypt. Abdel-Aziz [14] continued her work on freshwater fungi and reported 116 species from three different freshwater sites namely: High Dam Lake, River Nile, and irrigation canals from Aswan to Cairo governorates.

Abdel-Raheem [15] investigated the impact of four distinct aquatic techniques; lead mapping, random leaf sampling, millipore filtration, and spores in foam on the diversity of aquatic hyphomycetes in the River Nile. El-Sharouny [16] reported 74 species of freshwater fungi (40 ascomycetes, 34 asexual fungi) from decaying submerged samples of *Phragmites australis* from the River Nile and irrigation canals in Aswan and Qena governorates. Abdel-Aziz [17] identified 56 species of freshwater fungi (33 ascomycetes, 19 asexual fungi, and 4 basidiomycetes) from the River Nile and irrigation canals in Aswan, Qena and Sohag governorates. One hundred and ninety-eight species of freshwater fungi (110 ascomycetes, 81 asexual fungi, and 7 basidiomycetes) were recorded from decaying submerged woody samples collected from the River Nile and irrigation canals in the River Nile delta region [18,19]. Abdel-Aziz [20] recorded 99 species of freshwater fungi (42 ascomycetes, 55 asexual fungi, and 2 basidiomycetes) from the River Nile in Sohag governorate.

Different taxonomical studies of freshwater fungi colonizing decaying submerged plant debris were carried out in Egypt [21-35].

This research was designed to study freshwater fungi on the

submerged leaves of four tree species (*Eucalyptus rostrata*, *Ficus nitida*, *Phoenix dactylifera*, and *Phragmites australis*) using two methods: baits and random samples in the River Nile and irrigation canals in Sohag governorate, Egypt in the period from December 2015 to December 2016.

2. Materials and method

2.1. Sample collection, morphological study and isolation

A total of 432 senescent and dried leaf litter baits of four tree species; *Eucalyptus rostrata* (Myrtaceae), *Ficus nitida* (Moraceae), *Phoenix dactylifera* (Arecaceae), and *Phragmites australis* (Poaceae) were submerged in the River Nile and irrigation canals in Sohag governorate, Egypt for one year from December 2015 to December 2016. The leaves of the four tree species were baited in plastic mesh bags at three sites: River Nile (26°33'53"N, 31°42'19"E) in Sohag city; large irrigation canal (26°37'22.4"N, 31°44'18.1"E) and small irrigation canal (26°37'25.4"N, 31°44'05.6"E) in Sawama-sharq, Akhmim. Leaf packs were constructed by placing leaves from each tree species in twelve plastic mesh bags and tied using ropes and baited at each site. Samples were retrieved monthly. Also, the same leaves number (432) of the four tree species were collected randomly from the same sites monthly for one year.

Collected samples were returned to the laboratory and incubated in sterile Petri dishes lined with sterile, wet filter paper at room temperature and were sprayed with sterile distilled water from time to time to avoid dryness. Samples were examined periodically over 3 months of incubation for the presence of fungal sporulating structures using an Olympus SZ61 stereomicroscope (Olympus, Tokyo, Japan). Fruiting structures were sectioned by Leica CM 1100 Cryostat (Leica Biosystems, Nussloch, Germany). Micrographs were obtained with an Olympus BX51 differential interference contrast light microscope equipped with Olympus DP12 digital imaging system. The pure cultures of the fungi were obtained according to the methods described previously [34] and were preserved in cryotube containing 10% glycerol at -80°C. Pure cultures, permanent slides and herbarium materials were deposited at Sohag University microbial culture collection, Egypt (SUMCC).

2.2. Data analysis

Similarity between fungal communities of baited and randomly collected samples from the studied three sites for the four tree species and during the different seasons was compared using multivariate cluster analyses, PAST software version 4.09 [36].

2.3. DNA extraction, sequencing, and phylogenetic analysis

Fungal mycelium for DNA extraction was collected from pure fungal cultures grown in GPY broth [31] and genomic DNA was extracted using the Microbial DNA Extraction Kit

(MOBIO; Mo Bio Laboratories, Carlsbad, CA, USA). Partial LSU ribosomal DNA was amplified using primers LROR and LR7 [37]. PCR reactions, cycling parameters and sequencing were carried out as described by Abdel-Wahab et al. [38]. Sequences were assembled using Sequencher 4.2.2 and aligned with pertinent ones retrieved from GenBank using ClustalX [39]. Phylogenetic analyses were carried out using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses. Aligned sequences were analyzed using PAUP* 4 [40] and MEGAX [41]. Posterior probabilities (BYPP) were obtained in MrBayes version 3.1.2 [42]. Details of the phylogenetics methods used are described [43]. Obtained sequence of our isolate was deposited in NCBI GenBank.

3. Results and discussion

A total of 982 fungal collections were recorded from 864 decaying submerged leaves of the four tree species: *Eucalyptus rostrata*, *Ficus nitida*, *Phoenix dactylifera*, and *Phragmites australis*. Ninety-six fungal communities were observed during this study. The number of species, number of records, and the most common fungi in each community are listed in table (1). Thirty-six fungal species belonging to 33 genera were identified (Table 2). Of the 36 taxa recorded, five are new to science, of which a new species was described in a previous article namely *Robillarda sohagensis* Abdel-Wahab, Abul-Ezz & Bakhit [34].

Studies of freshwater fungi are mainly focused on lignicolous freshwater fungi but fungi on other hosts are poorly reported [10]. In freshwater environments, of 3870 fungal species have been recorded [3], only 418 species were reported from submerged decaying leaves. Species richness recorded during this study (36 species) was lower than that recorded from decaying submerged wood from freshwater habitats in Egypt [18,20] and other countries [44-47]. However, many studies carried out on leaf litter from several countries have reported smaller numbers of species than the present study [48]. Asexual taxa dominated the fungal community with a ratio of sexual ascomycetes to asexual ascomycetes taxa was 0.48:1. This finding is common in both submerged decaying wood and leaves. The occurrences of few sexual ascomycetes on leaf litter might be due to some features of wood, such as persistence over time or nutrient content [47,49].

The most common genera in this study were *Halobyssothecium* represented by 221 records, *Aspergillus* (190), and *Cylindrocladiella* (102) (Table 3). *Halobyssothecium unicellulare* (Abdel-Aziz) M.S. Calabon, KD. Hyde & EBG. Jones, *Aspergillus flavus* Link, *Cylindrocladiella pseudohawaiiensis* L. Lombard & Crous, *Pyrenochaetopsis terricola* KY. Wang, YL. Jiang & Y. Chen, *Volutella ciliata* Volutella ciliata (Alb. & Schwein.) Fr. and *Lolia aquatica* Abdel-Aziz & Abdel-Wahab were the most common species. *Pyrenochaetopsis terricola* was reported for the first time from aquatic habitats during the current study.

Table 1: The Ninety-six studied fungal communities during the present study.

	Type	No. of species	No. of record	The most common species		Type	No. of species	No. of record	The most common species
1	S – R – PA – WIN	8	18	<i>Volutella ciliata</i>	49	L – B – PA – WIN	4	13	<i>Halobyssothecium unicellulare</i>
2	S – R – PA – SPR	7	13	<i>Phaeosphaeria oryzae</i>	50	L – B – PA – SPR	2	2	<i>H. unicellulare</i> and Unknown Coelomycete sp. 2
3	S – R – PA – SUM	4	11	<i>Ceratorhiza</i> sp.	51	L – B – PA – SUM	0	0	–
4	S – R – PA – AUT	7	18	<i>Lolia aquatica</i>	52	L – B – PA – AUT	1	6	–
5	S – R – PD – WIN	6	23	<i>Aspergillus flavus</i>	53	L – B – PD – WIN	4	20	Unknown Coelomycete sp. 2
6	S – R – PD – SPR	13	30	<i>Halobyssothecium unicellulare</i>	54	L – B – PA – SPR	4	16	Unknown Coelomycete sp. 2
7	S – R – PD – SUM	5	22	<i>Halobyssothecium voragine sporum</i>	55	L – B – PA – SUM	3	15	Unknown Coelomycete sp. 2
8	S – R – PD – AUT	7	26	<i>Halobyssothecium unicellulare</i>	56	L – B – PA – AUT	2	4	<i>Halobyssothecium unicellulare</i>
9	S – R – FN – WIN	6	10	<i>A. flavus</i> and <i>V. ciliata</i>	57	L – B – FN – WIN	2	13	<i>Halobyssothecium unicellulare</i>
10	S – R – FN – SPR	1	1	<i>Lolia aquatica</i>	58	L – B – FN – SPR	2	4	<i>Aspergillus flavus</i> and <i>H. unicellulare</i>
11	S – R – FN – SUM	3	4	<i>Cylindrocladiella pseudohawaiiensis</i>	59	L – B – FN – SUM	1	1	<i>Aspergillus flavus</i>
12	S – R – FN – AUT	6	15	<i>A. flavus</i> , <i>P. terricola</i> and <i>V. ciliata</i>	60	L – B – FN – AUT	2	5	<i>Aspergillus flavus</i>
13	S – R – ER – WIN	8	20	<i>Cylindrocladiella pseudohawaiiensis</i>	61	L – B – ER – WIN	3	9	<i>Aspergillus flavus</i>
14	S – R – ER – SPR	3	10	<i>Cylindrocladiella pseudohawaiiensis</i>	62	L – B – ER – SPR	1	3	<i>Aspergillus flavus</i>
15	S – R – ER – SUM	0	0	–	63	L – B – ER – SUM	0	0	–
16	S – R – ER – AUT	3	9	<i>Cylindrocladiella pseudohawaiiensis</i>	64	L – B – ER – AUT	2	3	<i>Graphium</i> sp.
17	S – B – PA – WIN	4	16	<i>Aspergillus flavus</i>	65	N – R – PA – WIN	10	28	<i>Lolia aquatica</i> and <i>Myrothecium atroviride</i>
18	S – B – PA – SPR	0	0	–	66	N – R – PA – SPR	5	12	Unknown Coelomycete sp. 2
19	S – B – PA – SUM	0	0	–	67	N – R – PA – SUM	6	17	<i>Halobyssothecium unicellulare</i>
20	S – B – PA – AUT	3	11	<i>Achaetomium</i> sp.	68	N – R – PA – AUT	8	20	<i>Halobyssothecium unicellulare</i>
21	S – B – PD – WIN	4	20	<i>A. flavus</i> and Unknown Coelomycete sp. 2	69	N – R – PD – WIN	13	27	<i>Aspergillus flavus</i>
22	S – B – PD – SPR	3	14	Unknown Coelomycete sp. 2	70	N – R – PD – SPR	7	13	<i>H. voragine sporum</i> , <i>H. unicellulare</i> and <i>Torula herbarum</i>
23	S – B – PD – SUM	1	1	Unknown Coelomycete sp. 2	71	N – R – PD – SUM	2	9	<i>Aspergillus flavus</i>
24	S – B – PD – AUT	2	3	Unknown Coelomycete sp. 2	72	N – R – PD – AUT	4	14	<i>Halobyssothecium unicellulare</i>
25	S – B – FN – WIN	2	12	<i>Aspergillus flavus</i> and <i>H. unicellulare</i>	73	N – R – FN – WIN	7	25	<i>Cylindrocladiella pseudohawaiiensis</i>
26	S – B – FN – SPR	2	5	<i>Aspergillus flavus</i>	74	N – R – FN – SPR	3	9	<i>Halobyssothecium unicellulare</i>
27	S – B – FN – SUM	0	0	–	75	N – R – FN – SUM	3	5	<i>Aspergillus niger</i>
28	S – B – FN – AUT	1	1	Unknown Coelomycete sp. 2	76	N – R – FN – AUT	7	16	<i>Cylindrocladiella pseudohawaiiensis</i>
29	S – B – ER – WIN	3	9	<i>A. flavus</i>	77	N – R – ER – WIN	7	23	<i>Aspergillus flavus</i>
30	S – B – ER – SPR	0	0	–	78	N – R – ER – SPR	2	7	<i>Cylindrocladiella pseudohawaiiensis</i>
31	S – B – ER – SUM	0	0	–	79	N – R – ER – SUM	5	5	<i>A. flavus</i> , <i>H. unicellulare</i> and <i>P. terricola</i>
32	S – B – ER – AUT	1	1	<i>Achaetomium</i> sp.	80	N – R – ER – AUT	6	16	<i>Cylindrocladiella pseudohawaiiensis</i>
33	L – R – PA – WIN	8	11	<i>M. atroviride</i> and <i>V. ciliata</i>	81	N – B – PD – WIN	5	9	<i>Halobyssothecium unicellulare</i>
34	L – R – PA – SPR	6	13	<i>Ceratorhiza</i> sp. and <i>H. unicellulare</i>	82	N – B – PD – SPR	0	0	–
35	L – R – PA – SUM	5	8	<i>Ph. oryzae</i> and <i>H. unicellulare</i>	83	N – B – PD – SUM	0	0	–
36	L – R – PA – AUT	8	16	<i>Halobyssothecium unicellulare</i>	84	N – B – PA – AUT	5	14	<i>Halobyssothecium unicellulare</i>
37	L – R – PD – WIN	8	23	<i>Halobyssothecium unicellulare</i>	85	N – B – PD – WIN	6	19	<i>Halobyssothecium unicellulare</i>
38	L – R – PD – SPR	6	27	<i>Halobyssothecium unicellulare</i>	86	N – B – PD – SPR	3	12	<i>Halobyssothecium unicellulare</i>
39	L – R – PD – SUM	6	22	Unknown ascomycete sp.	87	N – B – PD – SUM	4	10	<i>Aspergillus flavus</i> and <i>H. unicellulare</i>
40	L – R – PD – AUT	9	25	<i>Halobyssothecium unicellulare</i>	88	N – B – PD – AUT	4	11	<i>Aspergillus flavus</i> and <i>H. unicellulare</i>
41	L – R – FN – WIN	3	6	<i>Aspergillus flavus</i>	89	N – B – FN – WIN	3	15	<i>Halobyssothecium unicellulare</i>
42	L – R – FN – SPR	2	2	<i>C. pseudohawaiiensis</i> and <i>M. atroviride</i>	90	N – B – FN – SPR	0	0	–
43	L – R – FN – SUM	2	2	<i>Aspergillus flavus</i> and <i>A. niger</i>	91	N – B – FN – SUM	0	0	–
44	L – R – FN – AUT	2	4	<i>Cylindrocladiella pseudohawaiiensis</i>	92	N – B – FN – AUT	2	5	<i>Halobyssothecium unicellulare</i>
45	L – R – ER – WIN	5	8	<i>Pyrenochaetopsis terricola</i>	93	N – B – ER – WIN	4	7	<i>Halobyssothecium unicellulare</i>
46	L – R – ER – SPR	5	12	<i>Cylindrocladiella pseudohawaiiensis</i>	94	N – B – ER – SPR	1	1	<i>Aspergillus flavus</i>
47	L – R – ER – SUM	2	2	<i>Microthyrium</i> sp. and <i>C. pseudohawaiiensis</i>	95	N – B – ER – SUM	1	3	<i>Aspergillus flavus</i>
48	L – R – ER – AUT	6	12	<i>Cylindrocladiella pseudohawaiiensis</i>	96	N – B – ER – AUT	0	0	–

Abbreviations: S = Small irrigation canal; L = Large irrigation canal; N = River Nile; R = Random; B = Baits; ER = *Eucalyptus rostrata*; FN = *Ficus nitida*; PD = *Phoenix dactylifera* and PA = *Phragmites australis*; WIN = winter; SPR = spring; SUM = summer; AUT = autumn.

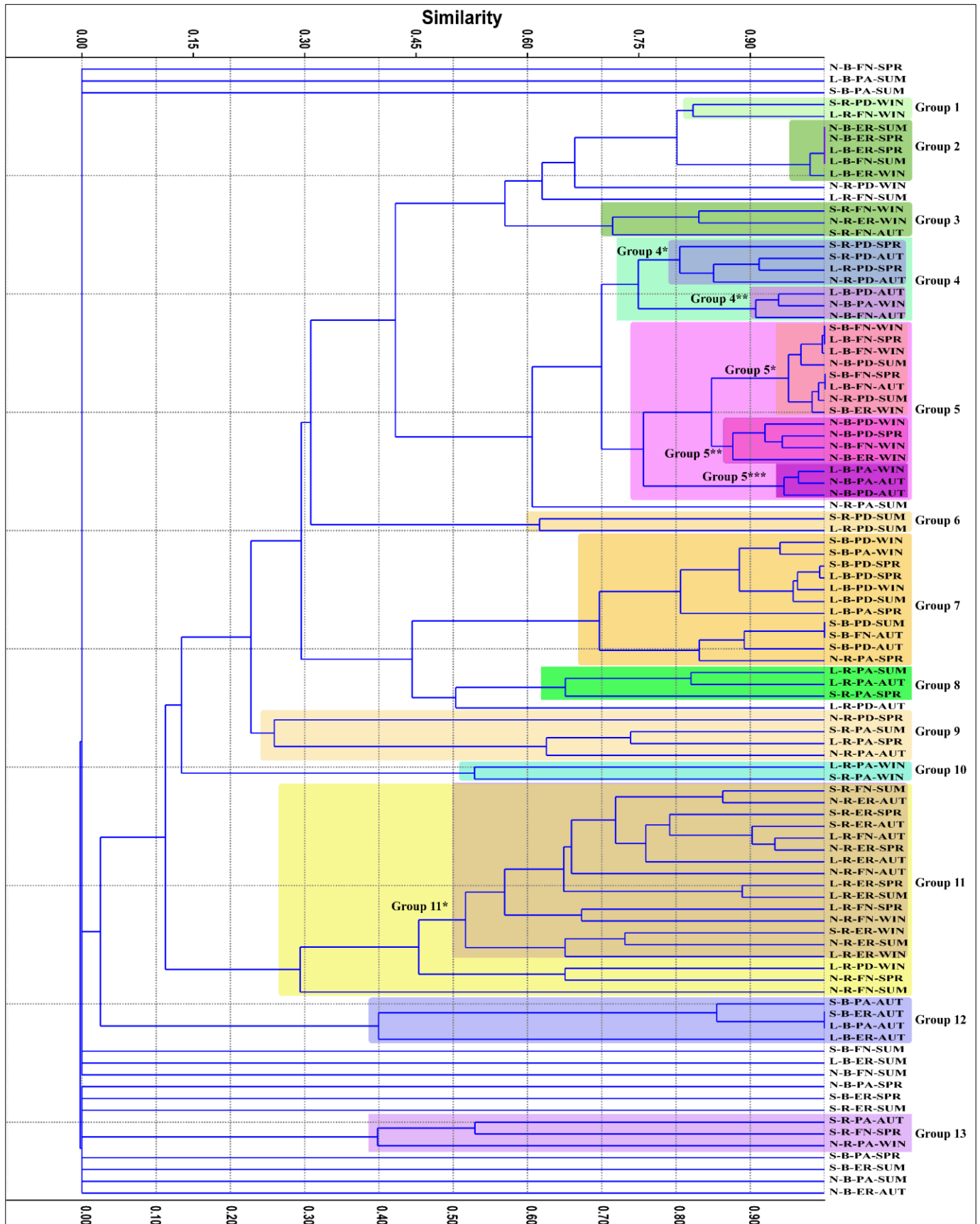


Figure 1: Dendrogram generated from multivariate cluster analyses based on the similarity between the ninety-six studied fungal communities in Sohag, Egypt using PAST4.05.

Table 2: Fungi recorded during the present study.

No	Recorded Fungi	No. of P.C.	Mean of N.
1	<i>Achaetomium</i> sp.	14	0.33
2	<i>Acremonium</i> sp.	11	0.20
3	<i>Alternaria alternata</i>	7	0.20
4	<i>Bartalinia robillardoides</i>	8	0.20
5	<i>Bisifusarium phragmites</i> sp. nov.	1	0.01
6	<i>Aspergillus flavus</i>	48	1.76
7	<i>Aspergillus niger</i>	10	0.22
8	<i>Ceratorhiza</i> sp.	4	0.14
9	<i>Cylindrocladiella pseudohawaiiensis</i>	32	1.06
10	<i>Cylindrocladium quinqueseptatum</i>	2	0.06
11	<i>Dictyosporium aquaticum</i>	1	0.01
12	<i>Dictyosporium musae</i>	4	0.04
13	<i>Drechslera biseptata</i>	6	0.14
14	<i>Graphium</i> sp.	1	0.02
15	<i>Halobyssothecium voragineporum</i>	4	0.11
16	<i>Halobyssothecium unicellulare</i>	59	2.22
17	<i>Koorchalomella salmonispora</i>	2	0.05
18	<i>Limnoperdon incarnatum</i>	4	0.04
19	<i>Lolia aquatica</i>	16	0.33
20	<i>Lophodermium</i> sp.	1	0.01
21	<i>Lulworthia medusa</i>	1	0.01
22	<i>Massariosphaeria</i> sp.	1	0.01
23	<i>Microthyrium</i> sp.	5	0.07
24	<i>Minutisphaera fimbriatispora</i>	1	0.01
25	<i>Myrothecium atroviride</i>	9	0.20
26	<i>Neopyrenochaeta foliicola</i> sp. nov.	2	0.08
27	<i>Phaeosphaeria oryzae</i>	9	0.20
28	<i>Phomatospora</i> sp.	1	0.01
29	<i>Pyrenochaetopsis terricola</i>	18	0.36
30	<i>Robillarda sohagensis</i>	3	0.04
31	<i>Torula herbarum</i>	5	0.09
32	Unknown ascomycete sp.	17	0.42
33	Unknown Coelomycete sp. 1	7	0.21
34	Unknown Coelomycete sp. 2	29	0.92
35	Unknown Coelomycete sp. 3	1	0.02
36	<i>Volutella ciliata</i>	18	0.42

No. of P.C. = Number of positive communities; Mean of N. = Mean of number of records

Numerous freshwater ascomycetes genera, such as *Aniptodera*, *Annulatascus*, *Halosarphaea*, *Jahnula*, *Kirchsteiniotelia*, *Massarina*, *Nais*, and *Natantispora* were frequently recorded in submerged decaying wood [18,20,45,47] but not recorded during the present study. Previous genera were recorded from wood and herbaceous stem samples and decaying leaves might not be suitable for their growth and reproduction as they degrade at a much rapid rate. Abdel-Aziz [20] recorded 99 species from submerged decaying wood, date palm rachis, and *Phragmites australis* culm samples; collected randomly from the River Nile at Sohag Governorate. *Ceratorhiza* sp.,

Table 3: Genera recorded during the present study.

Genus	No. of collections	%
<i>Achaetomium</i>	32	3.3
<i>Acremonium</i>	19	1.9
<i>Alternaria</i>	19	1.9
<i>Aspergillus</i>	190	19.3
<i>Bartalinia</i>	19	1.9
<i>Bisifusarium</i>	1	0.1
<i>Ceratorhiza</i>	13	1.3
<i>Cylindrocladiella</i>	102	10.4
<i>Cylindrocladium</i>	6	0.6
<i>Dictyosporium</i>	5	0.5
<i>Drechslera</i>	13	1.3
<i>Graphium</i>	2	0.2
<i>Halobyssothecium</i>	221	22.5
<i>Koorchalomella</i>	5	0.5
<i>Limnoperdon</i>	4	0.4
<i>Lolia</i>	32	3.3
<i>Lophodermium</i>	1	0.1
<i>Lulworthia</i>	1	0.1
<i>Massariosphaeria</i>	1	0.1
<i>Microthyrium</i>	7	0.7
<i>Minutisphaera</i>	1	0.1
<i>Myrothecium</i>	19	1.9
<i>Neopyrenochaeta</i>	11	1.1
<i>Phaeosphaeria</i>	19	1.9
<i>Phomatospora</i>	1	0.1
<i>Pyrenochaetopsis</i>	35	3.6
<i>Robillarda</i>	4	0.4
<i>Torula</i>	9	0.9
Unknown Ascomycete sp.	40	4.1
Unknown Coelomycete sp. 1	20	2.0
Unknown Coelomycete sp. 2	88	9.0
Unknown Coelomycete sp. 3	2	0.2
<i>Volutella</i>	40	4.1

Lolia aquatica and *Limnoperdon* sp., were the dominant fungi. *Dictyosporium*, *Monodictys*-like, *Aniptodera*, *Lolia*, *Podospora*, and *Zopfiella* were the speciose genera. Abdel-Raheem [15] reported that *Triscelophorus monosporus*, *Anguilospora longissima*, *Flagellospora curvula*, and *Tetracladium marchalianum* were the dominant hyphomycetes species in the River Nile (Upper Egypt). However, these fungi are generally studied by different methods [15]. *Bisifusarium*, *Lophodermium*, *Lulworthia*, *Minutisphaera*, and *Phomatospora* were represented by only one record in this study. About 65% of the ascomycetes reported from freshwater habitats have been reported only once [2]. *Halobyssothecium unicellulare* was collected in 59 communities followed by *Aspergillus flavus* in 48 and *Cylindrocladiella pseudohawaiiensis* in 32 (Table 2).

This study showed that randomly collected samples have higher freshwater fungi than those of baited samples. The highest number of species and records was reported in randomly collected leaves of *Phoenix dactylifera* and *Phragmites australis* from both the River Nile and the small irrigation canal (Table 1). Most of the baited samples of *Phragmites australis* and *Eucalyptus rostrata* during summer and spring were not colonized by any fungi. Multivariate clustering analysis based on similarity between the 96 fungal communities has produced 13 groups (Figure 1). These groups are briefly explained in the following paragraphs:

Group 1. These 2 fungal communities were reported on randomly collected samples of *Ficus nitida* and *Phoenix dactylifera* collected from small irrigation canal and large irrigation canal during winter season. The most common fungus in these communities was *Aspergillus flavus*.

Group 2. These 5 fungal communities were reported on baited samples of *Eucalyptus rostrata* and *Ficus nitida* collected from a large irrigation canal and River Nile. The most common fungus in these communities was *Aspergillus flavus*.

Group 3. These 3 fungal communities were reported on randomly collected samples of *Eucalyptus rostrata* and *Ficus nitida* collected from the small irrigation canal and the River Nile during the winter and autumn seasons. The most common species in these groups were *Aspergillus flavus* and *Volutella ciliata*.

Group 4. These 7 fungal communities were reported on random samples of *Phoenix dactylifera* collected from the three studied sites during different seasons. The most common species in these communities was *Halobyssothecium unicellulare*.

Group 4*. Four fungal communities were reported on randomly collected samples of *Phoenix dactylifera* that were collected from the three studied sites during the spring and autumn seasons. **Group 4**.** Three fungal communities were reported on baited samples of *Ficus nitida*, *Phoenix dactylifera*, and *Phragmites australis* that were collected from the large irrigation canal and the River Nile during winter and autumn seasons.

Group 5. These 14 fungal communities were reported on baited samples of different types of habitats that were collected from the three studied sites during different seasons. The most common species in these communities were *Aspergillus flavus* and *Halobyssothecium unicellulare*.

Group 5*. Seven fungal communities were reported on baited samples of different types of habitats collected from the three studied sites during different seasons. **Group 5**.** Four fungal communities were reported on baited samples of different types of habitats collected from the River Nile during the winter and spring seasons. **Group 5***.** Three fungal communities were reported on baited samples of *Phoenix dactylifera* and *Phragmites australis* collected from the large irrigation canal and the River Nile during the winter and autumn seasons.

Group 6. These 2 fungal communities were reported on randomly collected samples of *Phoenix dactylifera* collected from small irrigation canal and large irrigation canal during summer season. The most common species in these communities was unknown ascomycete sp.

Group 7. These 10 fungal communities were reported on baited

samples of different tree species collected from the small and the large irrigation canals during the different seasons. The most common species in these communities were *Aspergillus flavus* and unknown coelomycete sp. 2.

Group 8. These 4 fungal communities were reported on randomly collected samples of *Phragmites australis* collected from the small and the large irrigation canals during the different seasons. *Phaeosphaeria oryzae* and *Halobyssothecium unicellulare* were the most common species in these communities.

Group 9. These 4 fungal communities were reported on randomly collected samples of *Phragmites australis* and *Phoenix dactylifera* collected from the three studied sites during the different seasons. The most common species in these communities were *Ceratorhiza* sp. and *Halobyssothecium unicellulare*.

Group 10. These 2 fungal communities were reported on randomly collected samples of *Phragmites australis* collected from the small irrigation canal and the large irrigation canal during the winter season. *Volutella ciliata* was the most common species in these communities.

Group 11. These 18 fungal communities were reported on randomly collected samples of different tree species collected from the three studied sites during the different seasons. The most common species in these communities were *Aspergillus niger*, *Cylindrocycladia pseudohawaiiensis*, *Halobyssothecium unicellulare*, and *Pyrenochaetopsis terricola*. **Group 11*.** These 15 fungal communities were reported on randomly collected samples of *Eucalyptus rostrata* and *Ficus nitida* collected from the three studied sites during different seasons. The most common species in these communities were *Aspergillus niger* and *Halobyssothecium unicellulare*.

Group 12. These 4 fungal communities were reported on baited samples of *Eucalyptus rostrata* and *Phragmites australis* collected from the three studied sites during the autumn season. The most common species in these communities were *Achaetomium* sp., *Graphium* sp., and *Halobyssothecium unicellulare*.

Group 13. These 3 fungal communities were reported on randomly collected samples of *Phragmites australis* and *Ficus nitida* collected from the small irrigation canal and the River Nile during different seasons. The most common species in these communities was *Lolia aquatica*.

Our results reveal thirteen communities of freshwater foliicolous fungi showing different distributions and responses over the samples type, and host, seasons, and sites of collection. The type of collected samples; random or baited and the host are the important factors that influence the fungal communities. The other two factors include sites and seasonality that have less influence on fungal communities. Previous studies showed that the hosts are important factors that influence the fungal communities [44,50,51] and that some fungi might be host-specific [52].

The taxonomical position of *Bartalinia robillardoides* Tassi (SUMCC 16009) was confirmed based on morphological and molecular studies in the article.

Bartalinia robillardoides Tassi, Bulletin Labor. Orto Bot. de R. Univ. Siena 3: 145 (1900) Figure 3.

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Phylogenetic analysis

The LSU rDNA dataset consisted of 32 taxa, of which 15 belong to the genus *Bartalinia*, and 17 representatives of other genera in Sporocadaceae. The maximum parsimony dataset consisted of 750 characters of which 15 characters are excluded, 692

characters are constant, 18 are variable parsimony-uninformative, and 40 parsimony-informative characters. Maximum parsimony analyses resulted in 7 most parsimonious trees with a tree length of 71 steps, a consistency index of 0.7887, a retention index of 0.9102, and a rescaled consistency index of 0.7179. Maximum likelihood analysis yielded one tree (-ln likelihood = 1586.24). The Bayesian phylogenetic tree is shown in Figure 2. Our phylogenetic analysis placed the new isolate of *Bartalinia robillardoides* (SUMCC 16009) within *Bartalinia* and clusters with other strains of *B. robillardoides*.

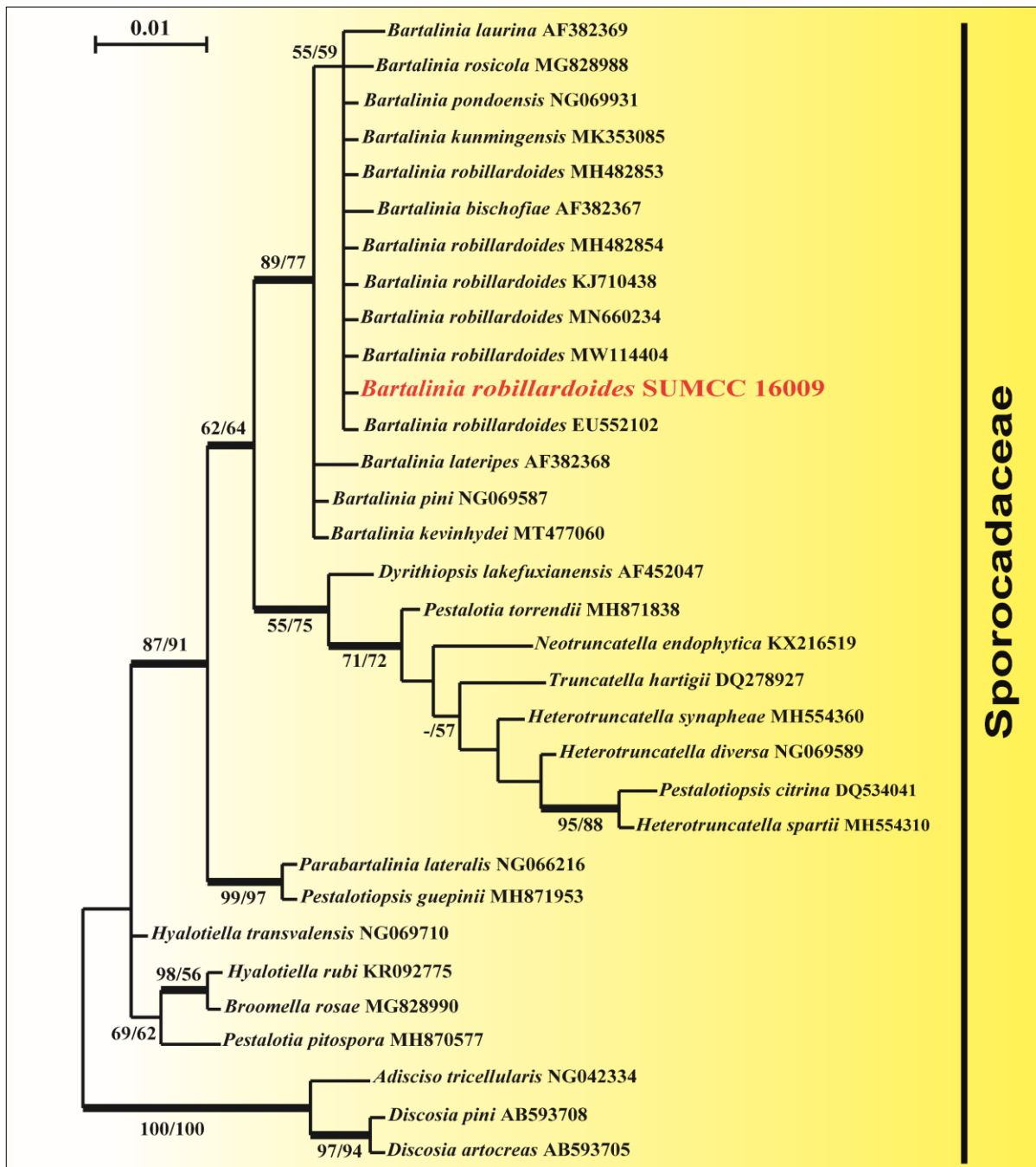


Figure 2. Phylogram generated from Bayesian analysis based on LSU sequence data of *Bartalinia robillardoides* (SUMCC 16009) along with other species of *Bartalinia* and representatives of Sporocadaceae. Bootstrap support on the nodes represents ML and MP $\geq 50\%$. Branches receiving Bayesian PP $\geq 95\%$ are in bold. The sequence of our new isolate is in red.

Taxonomy

Description

Foliicolous. **Sexual morph:** Undetermined. **Asexual morph** Coelomycetous. **Conidiomata** pycnidial, globose to subglobose, scattered to gregarious, erumpent, then superficial, unilocular, glabrous, 95–175 µm high, 142.5–237.5 µm diam. (\bar{x} = 136.3 × 185.6 µm, n = 19), dark brown to black, ostiolate; ostiole papillate. **Conidiomata walls** 18–32 µm thick, comprising several cell layers of light brown to brown *textura*

angularis, the inner layer is thin-walled, hyaline to pale brown, flattened cells. **Conidiophores** absent. **Conidiogenous cells** ampulliform, colorless, thin-walled, smooth, 2.5–5 × 1.5–2.5 (\bar{x} = 3.4 × 1.8 µm, n = 12). **Conidia** subcylindrical to fusiform, hyaline, 4-septate, 19–28 × 3–5 (\bar{x} = 24.4 × 3.7 µm, n = 38), smooth, constricted at the septa, bearing appendages; apical appendage branches almost three, attenuated, flexuous, divergent, 15–16 (\bar{x} = 15.5 µm long, n = 22); basal appendage single, filiform, excentric, 4–8 (\bar{x} = 5.8 µm long, n = 25).

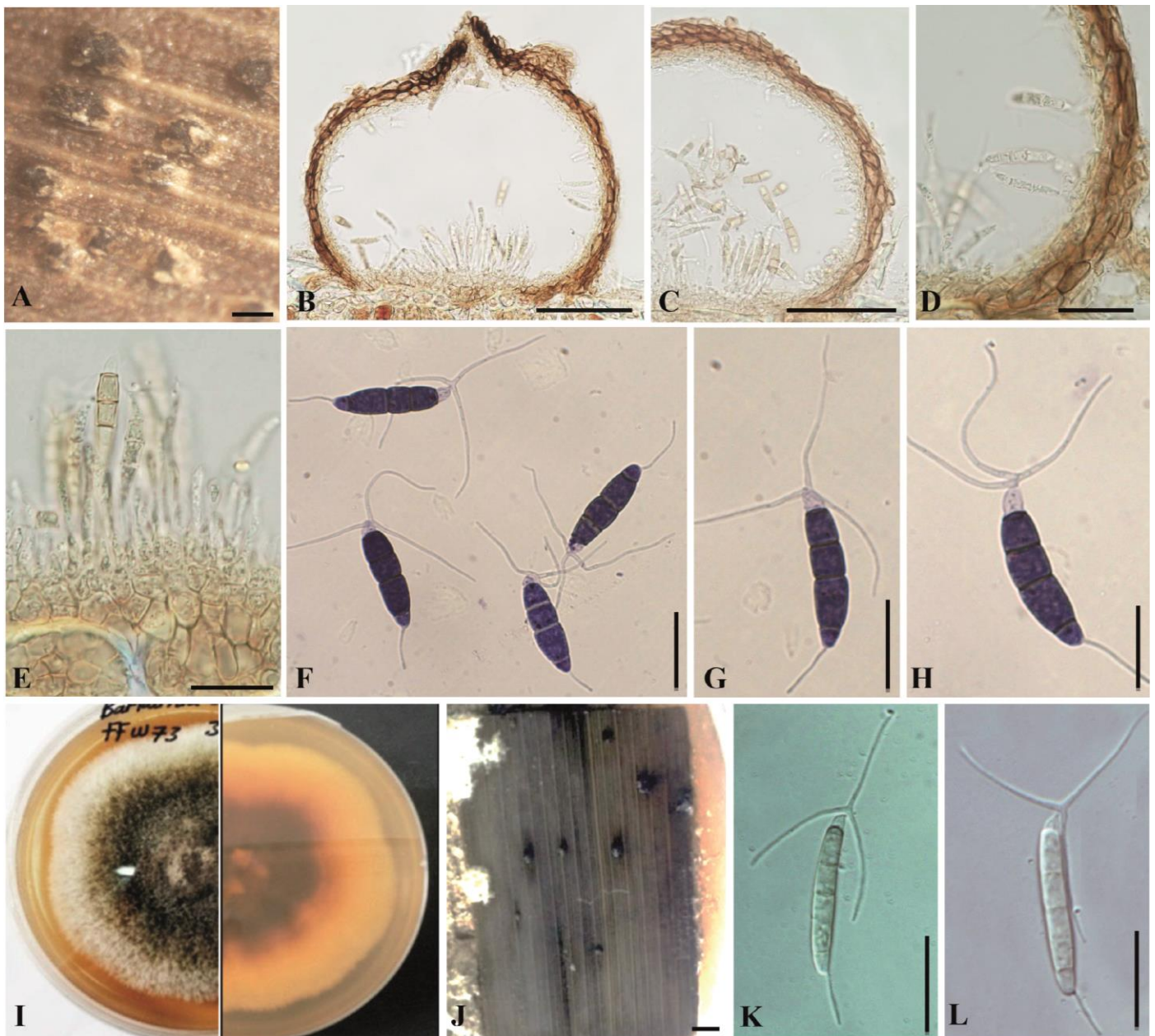


Figure 3. *Bartalinia robillardoides* (SUMCC 16009). **A.** Pycnidia on *phragmite* leaves. **B, C.** Vertical sections through pycnidia. **D.** Magnified part of the conidiomatal wall. **E.** Conidiogenous cells attached to conidia. **F - H.** Conidia (in toluidine blue). **I.** Colony on PDA (Upper and reverse view). **J.** Pycnidia sporulating on *Phragmites* leaf from culture. **K, L.** Conidia. **J - L** Photos from culture. Bars: **A, J** = 100, **B, C** = 50 µm; **D, E** = 20 µm; **F - H** and **K, L** = 10 µm.

Culture characteristics: Colonies on PDA reaching 87 mm diam., after 3 weeks at 22 °C, dense growth, spreading, circular with regular edges, from above olivaceous in the center grayish in the outer, from below reddish brown in the center, dull yellow in the outer. Conidiomata formed on sterilized *Phragmites* leaves incubated with the pure culture of the fungus were scattered, brownish-black to black, superficial to erumpent, conidia 16–23 × 2.5–3.8 μm (\bar{x} = 18.25 × 2.9 μm, n=26), cylindrical, hyaline to pale brown, slightly constricted at the septa.

Material Examined. Egypt, Sohag City, Irrigation canal (26°37'22.4"N, 31°44'18.1"E), decaying submerged leaves of *Phoenix dactylifera*, 22 January 2016, coll. Samar R. Abo Al-Ezz. The culture is deposited in Sohag University microbial culture collection, in Egypt (SUMCC 16009).

Habitat or host plant. On decaying submerged leaves of *Ficus nitida* and *Phoenix dactylifera* in the River Nile and irrigation canals.

Known Distribution: Australia, China, Egypt, India, Italy, Myanmar, Netherlands, South Africa, Ukraine and Venezuela.

GenBank accession numbers – LSU: OR294207.

Notes

The genus *Bartalinia* was established by Tassi [53] to accommodate *B. robillardoides* Tassi which was described from decaying leaves of *Callistemon speciosus* in Italy. Morgan-Jones et al. [54] and Sutton [55] accepted nine species in the genus and emphasized that this genus needed taxonomic revision. *Bartalinia* was regarded as a synonym of *Seimatosporium* by von Arx [56]. Nag Raj [57] retained the genus because of differences in their conidial appendages. Molecular studies by Crous et al. [58] and Liu et al. [59] confirmed that.

Nag Raj [57] accepted only six species including the type species and transferred three species to other genera. Senanayake et al [60] reported that *Bartalinia* includes 19 names. Wanasinghe et al. [61] described *Bartalinia rosicola* Wanas., Camporesi, EBG Jones & KD Hyde on dead aerial spines of *Rosa canina* in Italy. *Bartalinia pini* F. Liu, L. Cai & Crous was described from Uganda and USA [62]. Another five new species; *B. kunmingensis* Thiyag., Wanas., Phookamsak & KD Hyde, *B. kevinhydei* Doilom, Tibpromma & DJ Bhat, *B. bidenticola* Htet, Mapook & KD Hyde, *B. caryotae* Senan., Kular. & KD Hyde and *B. adonidiae* Konta & KD Hyde were described from China and Thailand [63-66].

Presently, twenty-six species are recognized in the genus. They have been recorded on a wide range of hosts in terrestrial and freshwater habitats. The genus was accommodated in Bartaliniaceae by Senanayake et al. [67] based on morpho-molecular studies. Later, Jaklitsch et al. [68] treated Bartaliniaceae as a synonym under Sporocadaceae. *Bartalinia robillardoides* were reported from freshwater habitat on decaying submerged leaves in the river Vinalopó (Spain) [69] and from freshwater samples in Korea [70]. It was described for the first time from freshwater habits in Egypt during the present investigation. Phylogenetic analysis based on LSU rDNA placed our new isolate (SUMCC 16009) in *Bartalinia* clade with other strains of *Bartalinia robillardoides* (CBS 122686, MFLU 18-2593, CBS 122705). Morphologically, our isolation was similar to *B. robillardoides*, as described by Morgan-Jones et al. [54]

and Crous et al. [58]. This species has been reported to produce taxol, an anticancer drug [71].

CRedit authorship contribution statement:

Mahmoud S. Bakhit: Conceptualization, Investigation, Data curation, Validation Writing – original draft, Writing – review & editing, Project administration. Samar R. Abul-Ezz: Investigation, Methodology, Data curation. Mohamed A. Abdel-Wahab: Conceptualization, Supervision, Writing – review & editing, Project administration.

Data availability

The corresponding author can provide data supporting the findings of this study upon request.

Declaration of competing interest

The authors declare that they do not have any competing financial interests or personal relationships that could have appeared to influence the findings reported in this paper.

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