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A preliminary report of aquatic hyphomycetes isolated from Anzali lagoon (Gilan province, North of Iran)

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Abstract

Aquatic hyphomycetes equipped with a rich array of extracellular enzymes are involved in bioremediation of anthropogenic pollutants, plastics, aromatic contaminants and petroleum hydrocarbons. The current study contributes to the knowledge of Iranian aquatic hyphomycetes. Sampling was conducted in Anzali lagoon, Gilan province, Iran during 2017. Among 55 fungal isolates obtained during this study, eight species including *Arthrobotrys oligosporus*, *Ceratorhiza hydrophila*, *Fusarium* cf. *ensiforme*, *F. incarnatum* species complex, *Myrmecridium schulzeri*, *Paecilomyces variotii*, *Sarocladium subulatum*, and *Volutella citrinella* were identified based on their morphological characteristics. Furthermore, three species assigned to the genera *Arthrobotrys*, *Fusarium* and *Sarocladium* remained unidentified. Molecular studies using mainly ITS, LSU and SSU rDNA and in some cases β-tubulin (*tub2*) and translation elongation factor 1-*a* (*tef1*) determined the phylogenetic position of the isolates among closely related species. The occurrence of members of *Fusarium*, *Sarocladium*, and *Ceratorhiza*, known as rice disease agents, in freshwater ecosystems is interesting. This is the first report of aquatic hyphomycetes communities in Anzali lagoon, Iran. In this study, *Sarocladium subulatum*, *Myrmecridium schulzeri*, and *Volutella citrinella* are reported for the first time for Iran.

Keywords: Anamorphic fungi, biodiversity, environmental pollutants, freshwater fungi, mitosporic fungi

گزارش مقدماتی از هیفومیست‌های آبزی تالاب انزلی (استان گیلان)*

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خلاصه

هیفومیست‌های آبزی تولیدکننده تعدادی زیادی آنزیم‌های خارج سلولی هستند که در تجزیه زیستی آلودگی‌های ناشی از فعالیت‌های انسانی، پلاستیک، ترکیبات آروماتیک و هیدروکربن‌های نفتی نقش دارند. مطالعه حاضر با هدف افزایش دانش ما از هیفومیست‌های آبزی در ایران صورت گرفت. نمونه‌برداری طی سال ۱۳۹۶ از تالاب انزلی در استان گیلان انجام گرفت. از میان ۵۵ جدایه قارچی که در این مطالعه به دست آمد، تعداد هشت گونه شامل *Arthrobotrys oligosporus*، *Ceratorhiza hydrophila*، *Fusarium* cf. *ensiforme*، *F. incarnatum* species complex، *Myrmecridium schulzeri*، *Paecilomyces variotii*، *Sarocladium subulatum* و *Volutella citrinella* برمبنای خصوصیات ریخت‌شناختی شناسایی شدند. علاوه براین، سه گونه متعلق به جنس‌های *Arthrobotrys*، *Fusarium* و *Sarocladium* ناشناخته باقی ماندند. شناسایی مولکولی اغلب گونه‌ها با استفاده از سه ناحیه ژنی شامل فاصله ترانویسی‌شده داخلی (ITS)، زیرواحد کوچک (SSU) و بزرگ (LSU) دی اِن اِی ریبوزومی (rDNA)، همچنین در مواردی از سازه امتداد ترجمه یک آلفا (*tef1*) و بتاتوبولین (*tub2*) موقعیت جدایه‌ها را در میان گونه‌های نزدیک نشان داد. حضور جنس‌هایی نظیر *Fusarium*، *Sarocladium* و *Ceratorhiza* در زیست‌بوم‌های آب شیرین که به عنوان عوامل بیماری‌زای برنج مطرح هستند، جالب توجه است. این نخستین گزارش از جمعیت‌های هیفومیستی در تالاب انزلی است. در این تحقیق، *Sarocladium subulatum*، *Myrmecridium schulzeri* و *Volutella citrinella* برای نخستین بار از ایران گزارش می‌شوند.

واژه‌های کلیدی: آلودگی‌های محیطی، تنوع زیستی، قارچ‌های آب‌های شیرین، قارچ‌های میتوسپوریک، قارچ‌های آنامورفیک

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Introduction

"Aquatic hyphomycetes", also known as waterborne hyphomycetes and Ingoldian fungi (Descals & Moralejo 2001), were named by Ingold (1942) to refer an ecologically defined, polyphyletic group of "true fungi". They are defined as a group of fungi mainly comprised of meiosporic and mitosporic Ascomycota (Webster 1992, Shearer *et al.* 2007). Four ecological descriptions of aquatic hyphomycetes including indwellers (constant activity), migrants (periodic or sporadic activity), versatiles (sporadic activity) and transients (those exhibiting no activity) have been proposed (Park 1972). Freshwater hyphomycetes have a worldwide distribution in lotic (rivers, streams, creeks, brooks) and lentic (lakes, ponds, swamps, pools) habitats, water droplets or dew on the leaves, rain waters, canopies and stem flow (Udaiyan & Hosagoudar 1991, Sridhar *et al.* 2006). The number of known and unknown species in different geographical areas reaches to 1500 and 165, respectively (Shearer *et al.* 2007) compared with the global fungal diversity ranging from 0.5 to 10 million species (Blackwell 2011).

The ecology and taxonomy of this pre-mentioned unexplored diversity are getting more attention due to realization of their contributions in ecosystem functions and services (Wurzbacher *et al.* 2010, Grossart & Rojas-Jimenez 2016, Grossart *et al.* 2019). For instance, aquatic hyphomycetes equipped with a rich array of extracellular enzymes are involved in bioremediation of anthropogenic pollutants, plastics, aromatic contaminants, petroleum hydrocarbons, etc. (Simister *et al.* 2015). In addition, the degradation of autochthonous (internal) and allochthonous (external) organic compounds by hyphomycetous fungi, as well as their incorporation into the aquatic food webs have consequences for freshwater ecosystems' health (Grinhut *et al.* 2011, Kisand *et al.* 2013).

Subphylum *Pezizomycotina* which makes up the majority of *Ascomycota* with more than 30,000 species is an ecologically diverse group. A small percentage of *Pezizomycotina* also contains aquatic taxa (Spatafora *et al*. 2006). In this context, the presence of some genera in aquatic habitats is more interesting due to their ecological relevance and economic importance. For example, *Fusarium* species which are considered as one of the major pathogens associated with rice diseases (Aoki *et al.* 2014, Bigirimana *et al*. 2015, Gao *et al*. 2015), have been reported from different freshwater ecosystems (Booth 1971, Révay & Gönczöl 1990, Casas & Descals 1997, Pascoal *et al*. 2005). Other examples are *Arthrobotrys* species that have an important role as ideal agents for controlling parasitic nematodes of plants and animals (Yang *et al*. 2007). They are broadly distributed in aquatic ecosystems (Yang *et al.* 2011).

In Iran, the majority of researches have focused on aquatic oomycetes causing significant infection of both dead and living fish and eggs (Jalilpoor *et al.* 2006, Ghasemi Pirbalouti *et al.* 2009, Ghiasi *et al*. 2010, Fadaeifard *et al*. 2011, Nekuiefard *et al.* 2011, Khosravi *et al*. 2012, Masigol *et al.* 2018). Apart from these studies, only one investigation has reported *Alatospora acuminata* Ingold, *Anguilospora* sp., *Clavariopsis aquatica* de Wildeman, *Lunulospora curvula* Ingold, *Tetracladium marchalianum* de Wildeman, *Trichocladium angelicum* Roldan & Honrubia, *Tricladium* sp., *Triscelosphorus monosporus* Ingold, and *Vargamyces aquaticus* (Dudka) Toth from Zayande-Roud River (Zare-Maivan & Ghaderian 1993).

For better understanding of diversity of the Iranian aquatic hyphomycetes, we have sampled Anzali lagoon (North of Iran) during 2017, in particular because of its ecological importance in the region. Among 25 freshwater ecosystems designated as wetlands of international importance from Iran by the "Ramsar Convention", Anzali wetland is one of the most important and biggest habitats, but studies of its fungal community are until today largely ignored. The objectives of this study were (i) to isolate hyphomycetous fungi from decaying leaves and twigs on the water surface, (ii) to identify them morphologically, and (iii) to determine their phylogenetic placement using

a combination of molecular markers including ITS, LSU, SSU of rDNA, *tef1* and *tub2*.

Materials and Methods

- Sampling and isolation

Samples of decaying leaves and twigs on the water surface were collected from Anzali lagoon (Guilan province, North of Iran) (37° 28' 16" N, 49° 27' 44" E) and brought to the laboratory in separate sterile polyethylene bags. Plant materials were moist-incubated in Petri dishes and examined every three days under a stereomicroscope to detect fruiting bodies for four weeks (Descals 1997). Fungal isolates and pure cultures were obtained by transferring fruiting bodies and mycelia to Malt Extract Agar (MEA) consisting of 30 g malt extract, 5 g mycological peptone and 15 g agar/liter (Gallowey & Burgess 1952) using the hyphal-tip technique. Representatives of fungal species were deposited at the Culture Collection of the Iranian Research Institute of Plant Protection (IRAN C), Tehran, Iran.

- Morphological identification

For each isolate, 30 measurements per replicate were taken for the required determination of morphological characters. All measurements and observations were made using an Olympus BH-2 microscope (Olympus Optical, Tokyo, Japan) equipped with AM4023-Digital Microscope 1.3 MPixel 72.5 30-USB 2.0 (Dino-Lite, Taiwan). Potato Dextrose Agar [PDA: composed of extract of potatoes (200 g), 20 g glucose, and 20 g agar/liter], Carnation Leaf Agar [CLA: composed of carnation leaves (5 g) and 20 g agar/liter], Synthetic Nutrient Agar [SNA: composed of KNO_3 (one g), KH_2PO_4 (one g), $MgSO_4.7H_2O$ (0.5 g), KCL (0.5 g), glucose (0.2 g), sucrose (0.2 g), and 20 g agar/liter], Oat Meal Agar [OA: 60 g oat meal and 12.5 g agar/liter], and Malt Extract Agar [MEA: malt extract (30 g), mycological peptone (5 g) and 15 g agar/liter] were synthesized according to the instructions (Nelson *et al*. 1983, Downes & Ito 2001, Murray *et al.* 2003).

Morphological identification was performed based on following literature: for *Arthrobotrys* isolate Haard (1968) and Can (1985), for *Ceratorhiza* isolate Cedeno *et al*. (1997), Xu *et al.* (2010) and Amirmijani *et al.* (2012), for *Fusarium* isolates Gerlach & Nirenberg (1982), Leslie & Summerell (2008), Nalim *et al.* (2011) and Chehri *et al*. (2015), for *Myrmecridium* isolate Arzanlou *et al.* (2007) and Peintner *et al*. (2016), for *Paecilomyces* isolate Samson (1974), Samson *et al.* (2009) and Nguyen *et al*. (2016), for *Sarocladium* isolate Giraldo *et al.* (2015) and Liu *et al*. (2017), and for *Volutella* isolate Seifert (1985) and Gräfenhan *et al.* (2011).

- DNA isolation, PCR amplification and Sequencing

DNA extraction was conducted using a slightly modified protocol of Montero-Pau *et al*. (2008). Briefly, the clot of mycelia of isolates growing in the malt extract broth were transferred to the 1.5 mL tubes containing 100 µL of alkaline lysis buffer (25 mM NaOH, 0.2 mM disodium EDTA, pH 8.0) and centrifuged for 30 min. at 9000 rpm. The tubes were incubated at 95 °C for 30 min. and then cooled on ice for five min. Finally, 100 µL of neutralizing solution (40 mM Tris-HCl, pH 5.0) were added to the tubes. The final solution was vortexed and kept at -20 °C. Partial sequences of the five loci *viz.* Large Subunit (LSU), Internal Transcribed Spacer (ITS), and Small Subunit (SSU) of rDNA, β -tubulin (*tub2*) and translation elongation factor 1-*a* (*tef1*) were amplified in a Flexibler PCR Thermocycler (Analytik Jena AG, Germany) using the primers (Table 1).

Table 1. Primers used in this study

Target DNA	Primer	Primer sequence 5'–3'	Reference
LSU rDNA	LR0R LR5	ACCCGCTGAACTTAAGC- TCCTGAGGGAAACTTCG	Vilgalys & Hester (1990)
ITS rDNA	ITS1 ITS4	TCCGTAGTGAACCTGCGG TCCTCCGCTTATTGATATGC	White *et al*. (1990)
SSU rDNA	SSU817 SSU1536	TTAGCATGGAATAATRRAATAGGA ATTGCAATGCYCTATCCCCA	Borneman & Hartin (2000)
tub2	Btub2Fd Btub4Rd	GTBCACCTYCARACCGGYCARTG CCRGAYTGRCCRAARACRAAGTTGTC	Woudenberg *et al*. (2009)
tef1	EF1-983F EF11567R	GCYCCYGGHCAYCGTGAYTTYAT ACHGTRCCRATACCACCRATCTT	Rehner & Buckley (2005)

For the LSU, ITS, and SSU rDNA, initial denaturation at 95 °C for 3 min. was followed by 40 cycles at the following conditions: 30 s at 95 °C, 45 s at 58 °C and 2 min. at 72 °C. A final extension at 72 °C for 8 min. completed the PCR. For the *tub2* and *tef1* primers, touch-down PCR was performed where an initial denaturation at 95 °C for 5 min. followed by 10 cycles of 45 s at 95 °C, 45 s starting at 68 °C and dropping by one °C per cycle until reached at 58 °C temperature with one min. extension at 72 °C. The initial ten cycles were followed by 35 cycles of 45 s at 95 °C, 45 s at 58 °C, and one min. at 72 °C. A final extension at 72 °C for 5 min. completed the PCR. The resulting fragments were sequenced using the PCR primers and the ABI Prism BigDye® Terminator Cycle Sequencing Reaction Kit Ver. 3.1 (Applied BiosystemsTM, Foster City, CA, USA), following the protocol of the manufacture.

The resulting sequences were edited using BioEdit Ver. 7.0.5 software (Hall 1999) and submitted to GenBank (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov). Accession numbers are shown in table 2.

- BLASTN search and Phylogenetic analysis

In addition to morphological identification, the taxonomic assignment of the isolates was performed by comparing sequences against the NCBI's GenBank sequence database using the BLASTN search method. We selected and downloaded sequences of some authentic isolates (preferably type sequences) for performing phylogenetic analyses. Since ITS and LSU sequences were obtained for all studied species, therefore, we combined these two regions to illustrate a general view for the phylogenetic relationship of all species.

The obtained sequences from GenBank together with the novel generated sequences during this study, were aligned with MAFFT Ver. 7 online interface using default settings (http://mafft. cbrc.jp/alignment/server/) (Katoh & Standley 2013) for each locus and whenever necessary, manually improved in MEGA Ver. 7 (Kumar *et al*. 2016). The LSU+ITS alignments were concatenated with Mesquite Ver. 2.75 (Maddison & Maddison 2011).

The best nucleotide substitution model was selected independently for each locus using MrModeltest Ver. 2.3 (Nylander 2004). A Bayesian phylogenetic reconstruction was performed with MrBayes Ver. 3.2.6 (Ronquist *et al*. 2012) based on the results of MrModeltest. The heating parameter was set at 0.15 and burn-in was set to 25%, and trees were saved for 1000 generations each. Posterior probabilities were determined by Markov Chain Monte Carlo (MCMC) analysis in MrBayes Ver. 3.2.6. Four simultaneous Markov chains were run for 10000000 generations and trees were sampled every 100th generation, until the average standard deviation of split frequencies reached to a value of 0.01 (stopval=0.01). Finally, the resulting phylogenetic tree was printed with Geneious Ver. 8.1.8 (Kearse *et al*. 2012).

Table 2. List of fungal isolates used in phylogenetic analyses and species identification

Taxa	Strain	GenBak Accession No.				
		LSU	ITS	SSU	*tub2*	*tef1*
Arthrobotrys arthrobotryoides	CBS 119.54	MH868798	MH857262			
A. conoides	YMF 1.00009	_	MF948387			
A. dianchiensis	1.00571	_	MH179720			
A. flagrans	CBS 583.91	AY261132	KT215213			
A. javanica	CBS 534.63 (TYPE)	_	NR 159640			
A. oligospora	CBS 111.37	MH867347	MH855843			
A. oligospora	TWF 1000	_	MN014021			
A. oligosporus	**IRAN 3331C**	**MH367082**	**MH367064**	**MK400439**	**_**	**_**
A. paucispora	ATCC 96704	_	EF445991			
A. pyriformis	YMF1.00020	_	MF948391			
A. scaphoides	CBS 226.52 (TYPE)	_	NR145361			
A. sinensis	YMF 1.00025	_	MF948394			
Arthrobotrys sp.	YMF 1.00564	DQ517532	_			
***Arthrobotrys* sp.**	**IRAN 3650C**	**MH367076**	**MH367058**	**MN461249**	**_**	**_**
A. superba	CBS 109.52	MH868471	_			
A. vermicola	1.03505	_	MH179790			
Aspergillus aculeatinus	LrBF24	_	MG543742			
A. aculeatus	KKU-CT2	_	LC102114			
A. japonicus	CBS 114.51	_	FJ629335			
A. violaceofuscus	CBS 102.23	MH866243	_			
Byssochlamys fulva	CBS 146.48 (TYPE)	NG063990	_			
B. nivea	CBS 100.11 (TYPE)	NG058631	_			
B. spectabilis	CBS 101075 (TYPE)	_	NR130679			
B. spectabilis	CBS 339.51	MH868409	_			
B. spectabilis	NR23	_	MH270551			
Ceratorhiz ahydrophila	**IRAN 3325C**	**MH367071**	**MH367053**	**_**	**_**	**MK400707**
Fusarium andiyazi	82-422	_	JQ363717			
F. andiyazi	CBS 134430	_	KC954400			
F. andiyazi	CBS 134430	_	KC954400			
F. begoniae	CBS 452.97 (TYPE)	MH874266	NR 111864			
F. begoniae	GSO4	_	MK616409			
F. dlaminii	CBS 738.97	_	MH862668			
F. ensiforme	CPC 27191	_	LT746248			
F.* cf. *ensiforme	**IRAN 3326C**	**MH367072**	**MH367054**	**MK400435**	**_**	**MK400704**

Table 2 (contd)

F. fujikuroi	H 24	KX375774	_			
F. incarnatum	CBS 130318	MH877331	MH865896			
F. incarnatum species complex	**IRAN 3330C**	**MH367080**	**MH367062**	**MK400438**	**_**	**_**
F. oxysporum f. sp. *elaeidis*	CBS 219.49	MH868036	MH856500			
F. pseudensiforme	CBS 125729	MH875116	MH863652			
F. pseudensiforme	S 1834 GJS02952	_	JF433037			
F. solani	CPC 27192	_	LT746269			
F. solani	ZB11060970		KX783368			
F. solani	ZB11263610		KX783355			
***Fusarium* sp.**	**IRAN 3332C**	**MH367083**	**MH367065**	**MN461252**		**MN475874**
Myrmecridium phragmitis	CBS 131311 (TYPE)	JQ044444	JQ044425			
M. schulzeri	CBS 100.54	EU041826	EU041769			
M. schulzeri	CBS 642.76	EU041834	EU041777			
M. schulzeri	**IRAN 3647C**	**MH367078**	**MH367060**	**MK400437**	**_**	**MK400706**
M. schulzeri	NRRL 62975	_	KM056332			
M. spartii	CPC 24953	KR611902	KR611884			
Peacilomyces variotii	**IRAN 3648C**	**MH367079**	**MH367061**	**MK400428**	**MK410642**	**_**
Sarocladium kiliense	CBS 122.29 (TYPE)	MH866490	_			
***Sarocladium* sp.**	**IRAN 3649C**	**MH367070**	**MH367052**	**MN461247**	**_**	**_**
S. strictum	CBS 376.70	MH871474	MH859725			
S. subulatum	CBS 217.35	MH867162	MH855652			
S. subulatum	**IRAN 3324C**	**MH367075**	**MH367057**	**MK400436**	**MK400692**	**MK400705**
S. subulatum	MUCL 9939 (TYPE)	HG965075	HG965031			
Sclerotium hydrophilum	CBS 201.27	MH866422	FJ231396			
S. hydrophilum	VC 228	_	KT362098			
Stilbella aciculosa	CBS 154.22	MH866237	MH854730			
S. aciculosa	DAOM 201.73	MH872363	MH860661			
Volutella aeria	LC 6216	KU746753	KU746707			
V. ciliata	DAOM 226718	_	HQ897802			
V. citrinella	DAOM 226716	HQ843770	_			
V. citrinella	DAOM 226720	HQ843771	HQ897821			
V. citrinella	**IRAN 3327C**	**MH367073**	**MH367055**	**MK400429**	**_**	**MK410643**
V. consors	CBS 122767	KM231632	KM231767			

Accession numbers of the studied isolates and the new generated sequences are highlighted in bold.
***** ITS and LSU sequences were obtained for all studied species (both regions were used for phylogenetic analysis).

Results

- Sequencing and phylogenetic analysis

Sequencing of rDNA ITS, LSU, and SSU were successful for all species, except for SSU in *Ceratorhiza hydrophila* isolate. Sequence of *tub2* and *tef1* loci obtained for three and seven taxa, respectively (Table 2). Hence, ITS and LSU sequences were concatenated for the final alignment, consisting of 1608 characters (including the alignment gaps), representing 66 taxa including 55 taxa from NCBI and 11 taxa from the present study. *Sclerotium hydrophilum* Sacc. (= *Ceratorhiza hydrophila*, isolates CBS 128854 and VC 2282) were used as outgroup taxa. The gene boundaries were 1–894 bp and 895–1608 bp for LSU, and ITS, respectively.

Based on the results of MrModeltest, the Bayesian analysis performed with the GTR+I+G substitution model, with inverse gamma rates and with dirichlet base frequencies for both LSU and ITS. The alignment contained a total of 850 unique site patterns (366 for LSU and 484 for ITS). The Bayesian analysis lasted 400000 generations and saved a total of 802 trees. After discarding the first 25% of sampled trees (200) for burn-in, the consensus trees and posterior probabilities (PP) were calculated from the remaining 602 trees (Fig. 1). After comparing the obtained sequences against reliable sequences in GenBank and in the constructed phylogenetic tree, most of our taxa were closely affiliated with accessible sequences and clustered in highly supported clades containing reliable sequences. These results were used for further consideration for precise species description.

- Taxonomy

During the present investigation, 55 fungal isolates were obtained from Anzali lagoon in 2017 from plant debris floating on the water surface. They belonged to seven genera including *Arthrobotrys*, *Ceratorhiza*, *Fusarium*, *Myrmecridium*, *Paecilomyces*, *Sarocladium*, and *Volutella.* Some isolates remained morphologically unknown since no morphological structures were produced/available. Among all isolates, eight fungal taxa including *Arthrobotrys oligosporus*, *Ceratorhiza hydrophila*, *Fusarium* cf. *ensiforme*, *F. incarnatum* complex species, *Myrmecridium schulzeri*, *Paecilomyces variotii*, *Sarocladium subulatum*, and *Volutella citrinella* were identified using a combination of morphological and molecular characteristics. Three species *viz. M. schulzeri*, *S. subulatum*, and *V. citrinella* are hereby reported for the first time from Iran. Furthermore, three species belonging to *Arthrobotrys*, *Fusarium*, and *Sarocladium* remained unidentified. All taxa, except for *Ceratorhiza hydrophila*, are herewith described in details. Furthermore, some notes are also provided for the species if necessary.

Arthrobotrys oligosporus Fresen., Beitr. Mykol. 1: 18 (1850), Fig. 2a–d

Colonies on PDA at 25 °C attaining 70–73 mm diam. in 14 days, white to cream, aerial mycelium extensive, margins regular, moderate sporulation; reverse cream. Mycelia: 1.25–3.75 µm wide, hyaline, septate and branched. Conidiophores arising singly from mycelium, unbranched, 150–412 × 3.75–7.5 µm. Conidiophore tip and nodular areas: swollen, 5–12.5 µm, bearing 5–25 conidia on wart like sterigmata in a tight capitate head. Conidia: 25–30 × 12.5–15 µm, two-celled, the distal cell usually 1.5–2 times longer than the proximal cells.

Material examined: Iran: Guilan prov., Anzali lagoon, on merged rotten leaves, 10 Aug. 2017, F. Rezakhani (IRAN 3331C); GenBank: SSU = MK400439, ITS = MH367064, LSU = MH367082.

Notes: This species is distinguished by its proliferating unbranched erect conidiophores with inflated conidiogenous heads, bearing up to 10 conidia on narrow, obovoid to pyriform two-cell conidia with unequal cell size. After running BLAST search, several ITS sequences with 97–100% similarity to our sequences (such as MN014021, isolate TWF 1000; MH855843, isolate CBS 111.37), were all assigned to *Arthrobotrys oligosporus*. However, none of them represented the type material.

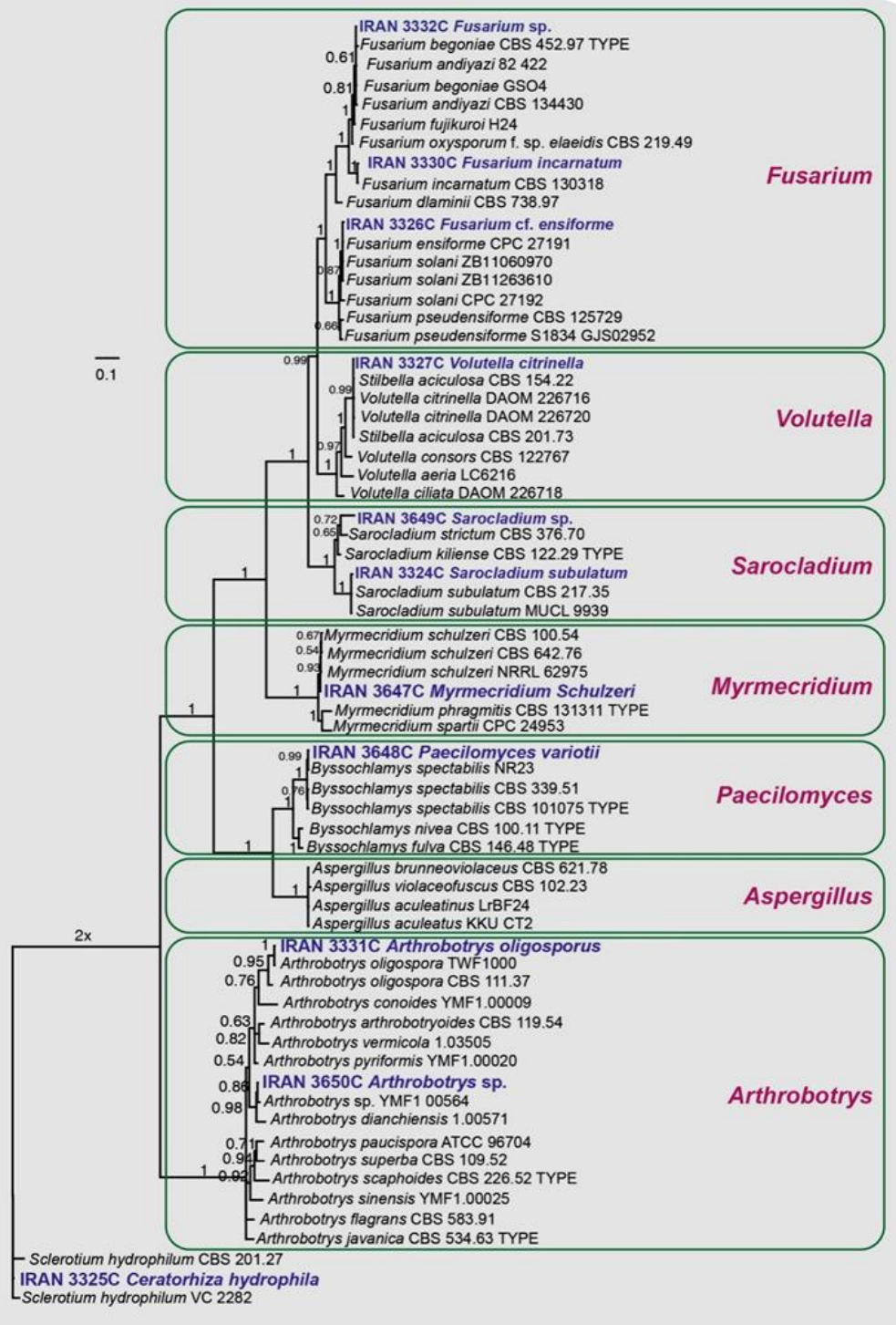

Fig. 1. Consensus phylogram (50% majority rule) of 602 trees resulting from a Bayesian analysis of the combined LSU and ITS sequence alignment using MrBayes Ver. 3.2.6. The scale bar indicates 0.1 expected changes per site. The tree was rooted to *Sclerotium hydrophilum* (isolates CBS 128854 and VC 2282).

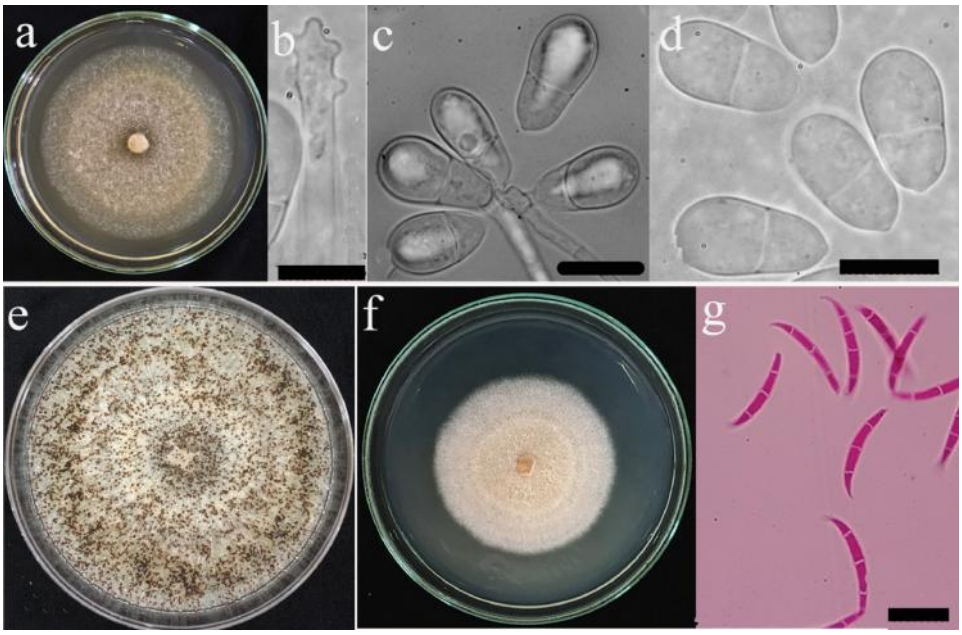

Fig. 2. *Arthrobotrys oligosporus*: a. Colony on PDA after 14 days at 25 °C, b. Denticles on conidiogenous cell, c. Conidia on conidiophore, d. Conidia, e. *Ceratorhiza hydrophila*: Colony on PDA after seven days at 25 °C, f-g: *Fusarium incarnatum* species complex (Bars = 20 µm).

Arthrobotrys oligosporus is a well-known and most widespread soil inhabiting nematode capturing fungus. This fungus has been recorded in a variety of environment. This is the first report of this fungus from an aquatic habitat in Iran. This species has been reported from freshwater ecosystems such as Dianchi and Pand lakes in China as well as Vestfold Hills, East Antarctica (Andrassy & Gibson 2007, Zhang *et al*. 2013). It belongs to the nematode-trapping hyphomycetes (family: *Orbiliaceae*) which contains the largest group of predaceous fungi (Yang *et al.* 2011).

Ceratorhiza hydrophila (Sacc.) Z.H. Xu, T.C. Harr., M.L. Gleason & Batzer, Mycologia 102(2): 340 (2010), Fig. 2e

Ceratorhiza hydrophila (syn.: *Sclerotium hydrophilum* Sacc.) is a well-known fungus described and illustrated from rice fields in Guilan province (Janipour *et al*. 2009, Amirmijani *et al.* 2012). This fungus is well characterized with white colonies and round, regular, small sclerotia (281–576 µm) which is formed on the surface of the colony. Carascal *et al*. (2017) isolated this species from submerged woods from Taal Lake, Philippines and showed its potential for butachlor biodegradation. *Ceratorhiza hydrophila* has been also proposed as a source of laccase enzyme in the treatment of textile dyes such as indigo in freshwater ecosystems (Campos *et al*. 2001).

Material examined: Iran: Guilan prov., Anzali lagoon, on merged rotten leaves, 10 Aug. 2017, F. Rezakhani (IRAN 3325C); GenBank: ITS = MH367053, LSU = MH367071, *tef1* = MK400707.

Fusarium* cf. *ensiforme Wollenw. & Reinking, Phytopathology 15(3): 169 (1925), Fig. 3a–c

Colonies on PDA at 25 °C attaining 29–30 mm diam. in four days, pink-white, after a month turning into apricot; reverse amber. Phialides: 25–107.5 × 1.25–3.75 µm. White sporodochia produced on carnation leaves agar. Macroconidia: arising from sporodochia, apical cells curved, foot cells well-developed, 1–4-septate, 30–50 × 3–5 µm. Microconidia: oval, elongate, 0–3-septate, 8–24 × 2–5 µm.

Material examined: Iran: Guilan prov., Anzali lagoon, on merged rotten leaves, 15 Sept. 2017, F. Rezakhani

(IRAN 3326C); GenBank: SSU = MK400435, ITS = MH367054, LSU = MH367072, *tef1* = MK400704.

Notes: Morphologically this fungus belongs to *Fusarium solani* species complex. However, several species have been segregated recently from *F. solani* which are morphologically very close to each other. Our ITS BLAST search of the isolate revealed a high sequence similarity (99.4–99.6%) to several *F. solani* (such as KX783368, KX783355) as well as two *F. ensiforme* sequences (LT746248, LT746247 provided by Sandoval-Denis *et al.* 2018). However, it was clearly different from the epitype ITS sequences of *F. solani sensu stricto* provided by Schroers *et al.* (2016). *Tef1* blast search just found one sequence with more than 99% similarity assigned to *Nectria haematococca* (= *F. solani sensu lat*., XM003053163). Unfortunately, *tef1* sequence available for *F. ensiforme* (by Sandoval-Denis *et al*. 2018) is not from the same gene region and they are not comparable. Hence, we introduce this fungus as *F.* cf. *ensiforme.*

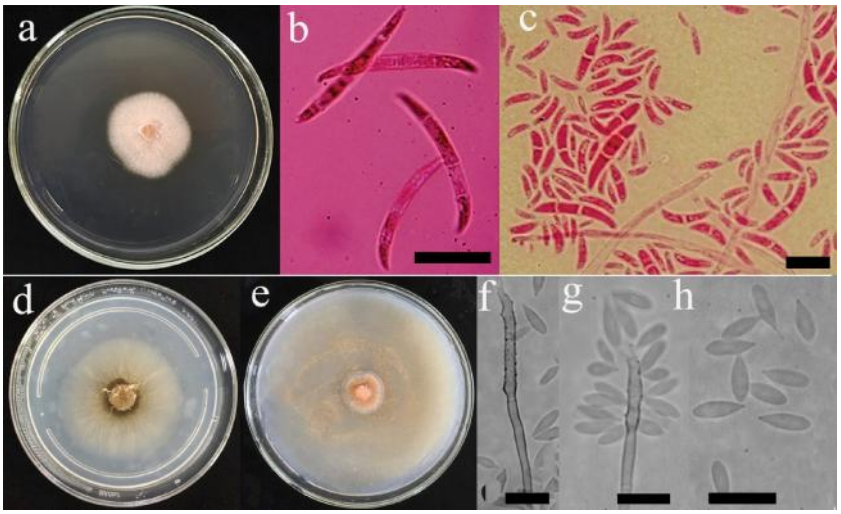

Fig. 3. *Fusarium* cf. *ensiforme*: a. Colony on PDA after 4 days at 25 °C, b. Macroconidia, c. Microconidia and macroconidia (stained with acid fuchsin); *Myrmecridium schulzeri*: d. Colony on OA after 14 days at 25 °C, e. Colony on SNA after 14 days at 25 °C, f. Rachis with scattered and pimple-shaped denticles, g. Macronematous conidiophore, h. Conidia (Bars: b, c = 20 µm; f-h = 10 µm).

Fusarium incarnatum (Desm.) Sacc., species complex, in Berkeley, Grevillea 3 (no. 27): 98 (1875) Fig. 2f, g

Colonies on PDA at 25 °C attaining 51–53 mm diam. in four days, yellow-salmon, aerial mycelium abundant; reverse ochreous. Phialides: mostly monophialidic, sometimes forming polyphialides, 7.5–32.5 × 1.25–2.5 µm. Orange sporodochia produced on carnation leaves agar. Macroconidia: arising from sporodochia, abundant, slender, apical cells curved and tapering to a point, basal cells foot-shaped, 3–5-septate, 22–38 × 2–4 µm. Microconidia: pyriform, 0–1-septate, 8.5–16 × 2–3 µm. Mesoconidia: fusiform, 2–3-septate, 18–25 × 2–4 µm. Chlamydospores: formed on the hyphae both singly and in chains, globose to subglobose, 7.5–20 × 7.5–10 µm.

Material examined: Iran: Guilan prov., Anzali lagoon, on merged rotten leaves, 15 Sept. 2017, F. Rezakhani (IRAN 3330C); GenBank: SSU = MK400438, ITS = MH367062, LSU = MH367080.

Notes: The taxonomic status of *Fusarium incarnatum* species complex is very complicated (O'Donnell *et al.* 2012). Therefore, identification of related species is limited due to high morphological similarities. ITS and LSU sequences of our isolates showed very high similarity (99–100%) to several species belonging to this complex. Unfortunately, we failed to get sequences of

other gene regions for this fungus. Therefore, we assigned it to *Fusarium incarnatum* species complex.

This is the first report of *F*. cf. *ensiforme* and *F. incarnatum* species complex from aquatic habitats. Nowadays, not only their presence in aquatic habitat but also leakage of their highly toxic mycotoxins from runoff of Fusarium-infested agricultural fields is getting more attention due to considerable economic and health risks (Butcheli *et al*. 2008). Members of *Fusarium* have been also detected in water distribution systems of several hospitals in the world (Sautor *et al*. 2012). Interestingly, it has been shown that, certain species such as *F. dimerum* Penz. and *F. oxysporum sensu* Smith & Swingle are adapting to a specific aquatic environment and gaining some properties which are not present in soil-borne *Fusarium* species (Steinberg *et al.* 2015).

Myrmecridium schulzeri (Sacc.) Arzanlou, W. Gams & Crous, in Arzanlou, Groenewald, Gams, Braun, Shin & Crous, Stud. Mycol. 58: 84 (2007), Fig. 3d-h

Colonies on OA at 25 °C reaching 20 mm diam. in 14 days, orange at the center and white in margin; reverse salmon; on SNA colonies reaching 40 mm diam. at 25 °C in 14 days, dark green at the center and white in margin; reverse the same. Conidiophores: unbranched, straight, thick-walled, septate, up to 187.5 µm long, 2.5–3.75 µm wide, with 2–5 additional septa. Conidiogenous cells: cylindrical, variable in length, subhyaline to pale brown, forming a straight rachis with scattered, pimple-shaped denticles less than one µm long, pigmented. Conidia: solitary, subhyaline, thin-walled, obovoid, 5–10 × 2–4 µm; hilum pigmented.

Material examined: Iran: Guilan prov., Anzali lagoon, on merged rotten leaves, 6 Jul. 2017, F. Rezakhani (IRAN 3647C); GenBank: SSU = MK400437, ITS = MH367060, LSU = MH367078, *tef1* = MK400706.

Notes: Morphology of this fungus fully agrees with the description available in Hughes (1951) for *Acrotheca acuta* Grove. Recently, this species has been transferred to the genus *Myrmecridium* Arzanlou, W. Gams & Crous (Arzanlou *et al.* 2007). According to the writer's BLAST search, the ITS sequence showed 99.79% (Identity: 483/484) similarity to accession numbers EU041777 and EU041769. This is the first report of this species from an aquatic habitat in the world. The genus *Myrmecridium* is an uncommon soil saprophyte with a widespread distribution. In addition, this is the first report of this taxon from Iran.

Paecilomyces variotii Bainier [as 'Varioti'], Bull. Soc. mycol. Fr. 23(1): 27 (1907), Fig. 4

Colonies on MEA fast growing, covering the Petri dish (8 cm) within seven days at 25 and 30 °C, but with slightly higher growth rate at 37 °C, green-yellow, powdery; reverse green-white. Conidiophores: mostly short and irregularly branched. Phialides: consisting of a cylindrical basal portion, tapering abruptly into a long cylindrical neck, 13–20 × 1.5–5.0 µm. Conidia: ellipsoidal, single or in chains, hyaline, one-celled, 4–7 × 2–4 µm.

Material examined: Iran: Guilan prov., Anzali lagoon, on merged rotten leaves, 6 Jul. 2017, F. Rezakhani (IRAN 3648C); GenBank: SSU = MK400428, ITS = MH367061, LSU = MH367079, *tub2* = MK410642.

Notes: By conducting a polyphasic approach, Samson *et al.* (2009) showed that, the type species of *Byssochlamys* and its related anamorphic genus *Paecilomyces s. str*. are congeneric. They accepted nine species in the *Byssochlamys*/*Paecilomyces* complex and provided a key for these species. According to the key, our isolate was identified as *Byssochlamys spectabilis* (anamorph = *Paecilomyces variotii*). However, we were not able to observe any chlamydospore. Although, ITS and LSU sequences from our isolate were more than 99% similar to several sequences belonging to *Byssochlamys spectabilis* available in GenBank, we found a slightly higher difference against the sequence of type materials (NR130679, isolate CBS 101075). According to recent rules of fungal nomenclature, Rossman *et al*. (2016) recommended the use of *Paecilomyces* over *Byssochlamys.*

Note: *Paecilomyces variotii* is a commonly occurring species in air and food particularly associated with many types of human infections (Houbraken *et al.* 2010). The isolation of this species from Anzali lagoon is important since direct discharging of hospital waste water containing human infections has been considered as one of the main environmental problems by several authors (Jafari 2009, Ziarati *et al.* 2015, Noorhosseini *et al.* 2017).

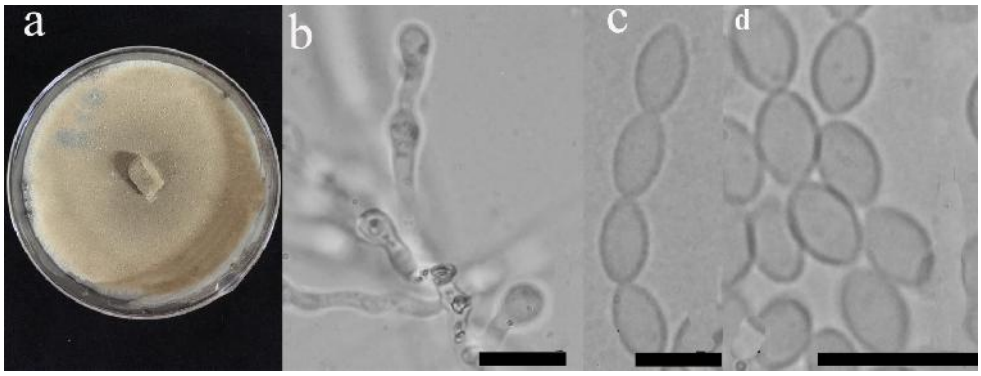

Fig. 4. *Paecilomyces variotii*: a. Colony on MEA after 7 days at 25 °C, b. Branched conidiophore and club-shaped phialides, c. Conidia in chain, d. Conidia (Bars = 10 µm).

Sarocladium subulatum A. Giraldo, Gené & Guarro, in Giraldo, Gené, Sutton, Madrid, de Hoog, Cano, Decock, Crous & Guarro, Persoonia 34: 20 (2014), Fig. 5

Colonies on OA at 25 °C attaining 20–22 mm diam. in 14 days, yellowish-white, powdery, flat, margin diffuse; reverse dark. On PDA colonies reaching 16–17 mm diam. in 14 days at 25 °C, yellowish-white, margin lobulated; reverse honey. Vegetative hyphae: septate, hyaline, smooth, 1.5–2.5 µm wide. Conidiophores: erect, simple, hyaline, smooth. Phialides: arising directly from vegetative hyphae, straight or slightly flexuous, subulate, 17.5–35 µm long, 2–2.5 µm wide at the base, adelophialides sometimes present on OA. Conidia: unicellular, fusiform, 4–9 × 1–2 µm, hyaline, thin- and smooth-walled, sometimes arranged in chains with shorter length. Chlamydospores and sexual morphs were not observed.

Material examined: Iran: Guilan prov., Anzali lagoon, on merged rotten leaves, 6 Jul. 2018, F. Rezakhani (IRAN 3324C); GenBank: SSU = MK400436, ITS = MH367057, LSU = MH367075, *tub2* = MK400692, *tef1* = MK400705.

Notes: In this study, *Sarocladium subulatum* is reported for the first time from Iran and is closely related to *S. bacillisporum* and *S. terricola*, based both on morphological and molecular characterization. However, it can be distinguished by its slower growth rate on OA and PDA at 25 °C and conidial size (Giraldo *et al*. 2015). ITS and LSU sequences from our species showed two substitution against type strain provided by Giraldo *et al.* (2015) (accession numbers HG965031 and HG965075 for ITS and LSU, respectively). There was no available *tef1* sequence for *S. subulatum* in GenBank. However, *tef1* sequence of *S. terricola* (accession number MG993030) showed 99% (435/441 base pair) similarity to our fungus.

It seems that, *Sarocladium* is an ecologically diverse genus. For instance, *S. mycophilum* Helfer found on *Cortinarius subsertipes* Romagn., and *S. zeae* are the only mycoparasit and endophyte species, respectively (Helfer 1991). In addition, *S. kiliense* (Grütz) Summerb. and *S. strictum* (W. Gams) Summerb. are clinically important (Giraldo *et al.* 2015). Therefore, it is not wondering that, some species might be found in aquatic ecosystems like our isolate.

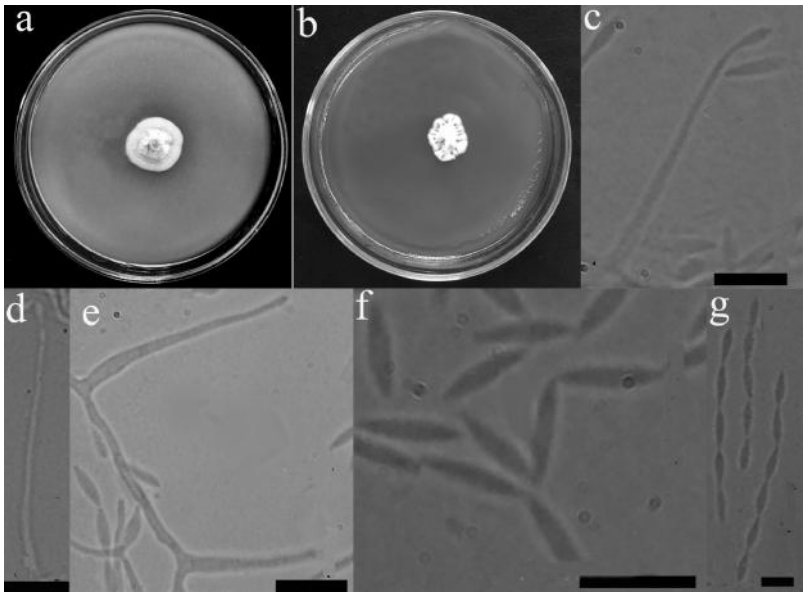

Fig. 5. *Sarocladium subulatum*: a. Colony on OA after 14 days at 25 °C, b. Colony on PDA after 14 days at 25 °C, c-e. Phialides arising directly from hyphae, f. Conidia, g. Conidia in chain (Bars = 10 µm).

Volutella citrinella (Cooke & Massee) Seifert, in Gräfenhan, Schroers, Nirenberg & Seifert, Stud. Mycol. 68: 110 (2011), Fig. 6

Colonies on PDA at 25 °C attaining 20–23 mm diam. in seven days, olivaceous at first then turning into white, conidial mass was produced on surface. Synnemata: lightly colored, 300–1450 µm tall and 25–50 µm wide. Marginal hyphae: verrucose near capitulum. Conidiogenous cells: phialidic, 13–25 × 1.5–2 µm. Conidia: ellipsoidal to oblong-ellipsoidal, 3–5 × 1–2 µm, conidial mass yellow-white, no aerial mycelium or chlamydospore were produced on PDA.

Material examined: Iran: Guilan prov., Anzali lagoon, on merged rotten leaves, 10 Aug. 2017, F. Rezakhani (IRAN 3327C); GenBank: SSU = MK400429, ITS = MH367055, LSU = MH367073, *tef1* = MK410643.

Notes: *Volutella s. str*. includes perithecial, sporodochial and synnematal species, with some of them formerly included in *Stilbella* (Gräfenhan *et al*. 2011). Gräfenhan *et al*. (2011) accepted three species in the genus. *Volutella citrinella* is differentiated from two other species in having synnematous conidiomata. In the phylogenetic tree, it clustered with *V. citrinella* (strain DAOM: 226720, ITS and LSU accession numbers: HQ897821 and HQ843771, respectively) with high sequence similarity: 99% (520/522) and 100% for ITS and LSU gene regions, respectively. These sequences have been provided by Gräfenhan *et al.* (2011). This is the first report of this species from Iran.

Volutella can be isolated from diverse habitats in soil as facultative plant pathogens, saprophytes, and decomposers on plant debris (Chilton 1954, Šafránková 2007).

Arthrobotrys sp.

Colonies on PDA at 25 °C attaining 23–28 mm diam. in seven days, aerial mycelium extensive, margins regular, milky; reverse dark moccasin. Mycelium: 1.25–2.5 µm wide, hyline and septate. Conidiophores: arising singly from mycelium, 37.5–350 × 2–3 µm. Conidiophore tip and nodular areas: swollen, 3.75–10 µm, bearing 5–25 conidia on wart like sterigmata in a tight capitate head. Conidia: 4–7 septa, 62.5–125 × 10–12.5 µm.

Material examined: Iran: Guilan prov., Anzali lagoon, on merged rotten leaves, 10 Aug. 2017, F. Rezakhani (IRAN 3650C); GenBank: SSU = MN461247, ITS = MH367058, LSU = MH367076.

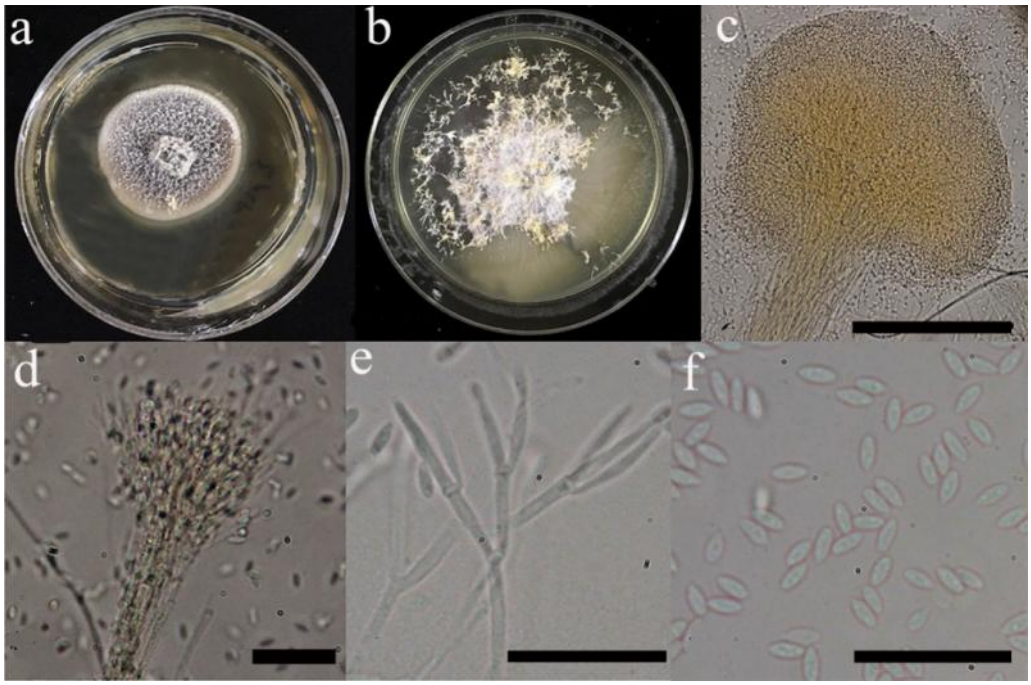

Fig. 6. *Volutella citrinella*: a. Colony on MEA after 20 d at 25 °C, b. Colony on PDA after seven days at 25 °C, c. Synnema, d, e. Conidiophores and phialides, e. Conidia (Bars: c = 40 µm; d-f = 10 µm).

Notes: The closest sequence in GenBank for ITS belongs to *Arthrobotrys dianchiensis* **(**isolate 1.00571, accession number MH179720) with 99% similarity (503/509 base pair). We found one unverified sequence from *tef1* of *Arthrobotrys dianchiensis* (isolate 1.00571, accession number MH179573) very close related to our fungus, just with one substitution along ca. 360 bp. No LSU sequence is available for *Arthrobotrys dianchiensis* in GenBank. However, the LSU sequence from *Arthrobotrys arthrobotryoides* (MH868798) showed a 99% (856/869) similarity to our fungus.

Fusarium sp.

Colonies on PDA at 25 °C reaching 45–48 mm diam. after four days, pale pink at the center and margin as well; reverse creamy. Phialides: mostly monophialidic and branched, 23.5–62.5 × 1.25–2.5 µm. Sporodochia rarely produced on CLA. Macroconidia: arising from sporodochia, rare, slender, apical cells curved and tapering to a point, basal cells foot-shaped, 1–3-septate, 25–40 × 2–4 µm. Microconidia: pyriform, 0–1-septate, 7–15 × 1–3 µm. Chlamydospores weren't formed.

Material examined: Iran: Guilan prov., Anzali lagoon, on merged rotten leaves, 10 Aug. 2017, F. Rezakhani (IRAN 3332C); GenBank: ITS = MH367065, LSU = MH367083, SSU = MN461252, *tef1* = MN475874.

Notes: Phylogenetically, this fungus clustered together with sequences belonging to the *Gibberella fujikuroi* species complex (Fig. 1). The ITS sequence showed more than 99% base similarity to some species belonging to this complex. The closest *tef1* sequence to this fungus belongs to *Fusarium dlaminii* (strain NRRL 13164, accession number KU171721) with 98.8% sequence similarity (428/433). However, none of these sequences belong to any type materials and therefore, we introduce this fungus as an unknown species.

Sarocladium sp.

Colonies on OA at 25 °C attaining 26–28 mm diam. in 14 days, orange-yellow, powdery, flat, margin diffuse; reverse orange. On PDA colonies reaching 16–17 mm diam. in 14 days at 25 °C, orange at center, margin orange; reverse honey. Vegetative hyphae: septate, hyaline, smooth, 1–1.5 µm wide. Conidiophores: erect, simple and rarely branched. Phialides: present,

12.5–37.5 µm long, 1–2 µm wide at the base, adelophialides absent on OA. Conidia: unicellular, cylindrical, 4–6 × 1–2 µm, hyaline to pale, arranged in clumps at the tip of the phialides. Chlamydospores and sexual morph were not observed.

Material examined: Iran: Guilan prov., Anzali lagoon, on merged rotten leaves, 10 Aug. 2017, F. Rezakhani (IRAN 3649C); GenBank: SSU = MN461247, ITS = MH367052, LSU = MH367070, *tef1* = MN475877, *tub2* = MN475873.

Notes: This fungus is closely related to *S. strictum* and *S. kiliense*. ITS and LSU sequence comparison showed a 98.6% (9515/523 base pair) and 98% (564/576) similarity to *Sarocladium strictum*, and *S. kiliense* (the closest sequences available in GenBank), respectively.

Discussion

Many *Fusarium* and *Sarocladium* species have been reported from rice fields as the causal agent of sheath and root rot diseases (Desjardins *et al*. 2000, Naeimi *et al*. 2003, Ebadi *et al*. 2014). This is also true for *Ceratorhiza* that can cause aggregate sheath spot disease of rice (Cintas & Webster 2001, Ayyadurai *et al*. 2005, Saravanakumar *et al*. 2009). Interestingly, we observed the full circulation of water from the lagoon to surrounding rice fields and vice versa (Fig. 7).

Although, it is not possible to determine the original niche of these species, it may imply aquatic hyphomycetes communities could be affected by their surroundings and have a double life in terrestrial and aquatic environments (Chauvet *et al*. 2016, Selosse *et al*. 2008). Thus, we suggest conducting pathogenicity tests of these aquatic isolates and comparing them with their terrestrial counterparts to investigate whether they are (morphologically, genetically and physiologically) the same ones as previously found in the rice fields.

In this study, we isolated and described eight aquatic hyphomycetes species from Anzali lagoon (Guilan province, North of Iran). This is the first report of the fungal community of freshwater ecosystems in Iran. It is worth mentioning that, we were biased by our specific isolation method. So, further investigation using different isolation methods combining with high throughput molecular toolboxes will help us to reveal the unknown fungal diversity in aquatic ecosystems. Although giving a precise description of known taxa and discovering new ones are crucial from a fungal taxonomy point of view, it should not distract us from exploring their ecological roles. For example, Masigol *et al*. (2019) investigated the ability of the isolates used in this study to evaluate their enzymatic activities including lignino-, cellulo-, hemicellulo-, pectino- and chinitolytic properties. Nearly, all isolates showed at least two major enzymatic activities. This calls scholars, mycologists in particular, to consider aquatic fungi from both ecological and taxonomical points of view.

Fig. 7. Circulation of water from the field to the lagoon and vice versa (Anzali lagoon, North of Iran).

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