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Notes on *Dictyuchus* species (Stramenopila, Oomycetes) from Anzali lagoon, Iran

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Abstract: Nine *Dictyuchus* isolates were obtained from floating and decaying twigs and leaves in Anzali lagoon, Anzali County, Guilan province, Iran. They are interestingly distinguished from current *Dictyuchus* species by the presence of dictyoid and achlyoid type of zoospore discharge, abundant fusiform and spherical gemma, and the absence of any sexual apparatus. In addition, phylogenetic analyses of the internal transcribed spacer (ITS) region of the nuclear rDNA and cytochrome c oxidase subunit I (*cox1*) gene sequences using Maximum likelihood method indicate its novelty. We preferred not to introduce the isolates as new species due to low number of and sometimes unreliable sequences and lack of type species and suggested to verify *Dictyuchus sterilis* which has been excluded before. We discussed about taxonomy of the genus in details and provided a revised key to the species. In addition, it was shown that sterility *in vitro* might be common in this genus.

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INTRODUCTION

Oomycetes form a group of fungus-like microorganisms, which are present in marine, freshwater and terrestrial environments (Dick 2001, Beaks et al. 2012). They belong to the *Stramenopiles* lineage within the *Stramenopiles-Alveolata-Rhizaria* (SAR) super group (Kirk et al. 2008, Burki 2014). Of the nine orders within the Oomycetes, *Peronosporales*, *Pythiales*, and *Saprolegniales* are well-studied due to their impact on agriculture and natural ecosystems (Judelson 2012). While *Peronosporales* are predominantly freshwater saprophytes of plant and animal debris, *Pythiales* are primarily known as soil-born saprotrophs and necrotrophic pathogens (Cooke et al. 2000; Riethmuller et al. 2002; Levesque & de Cock 2004; Beakes & Sekimoto 2009). The order *Saprolegniales* is divided into three well-supported families, *Saprolegniaceae*, *Achlyaceae* and *Verrucalvaceae* (Beaks et al. 2014).

Of the 22 genera within the *Saprolegniales*, *Achlya*, *Saprolegnia*, and *Aphanomyces* seem to be widely distributed throughout the world (Johnson et al. 2002). Since the late 19th century, mycologists have focused on their identification and phylogenetic relationships because some genera can cause severe disease in fish and amphibians (Urban et al. 2015; Rezinciuc et al. 2016; Marano et al. 2017). According to all available identification keys, the zoospore release mechanism and the form and shape of sexual reproductive organs are important features to delineate different *Saprolegniales* genera (Johnson 1956; Scott 1961; Dick 1969; Seymour 1970; Johnson et al. 2002). However, the lack of type species and sexual structures *in vitro* and unstable morphological features have forced mycologists to look for alternative tools such as molecular markers (Sandoval-Sierra et al. 2014). Recently, cytochrome c oxidase subunit I (*cox1*) and internal transcribed

spacer (ITS) of rDNA have been accepted as DNA barcodes for identification of oomycetes (Robideau et al. 2011).

The genus *Dictyuchus* Leitgeb belongs to the family *Achlyaceae* (Beakes et al. 2014). The distinctive characteristic of the genus *Dictyuchus* is the dictyucoid discharge mode (Leitgeb 1868; Steciow et al. 2014), meaning releasing of spores individually from cysts in an intact sporangium, leaving a network of cyst walls (Johnson et al. 2002). Currently, there are seventeen records of *Dictyuchus* species given in the Index Fungorum community resource (Index Fungorum, 2019), but according to the newest literature, *D. monosporus* and *D. pseudodictyon* are only valid species (Johnson et al. 2002). The rest are considered as imperfectly known species or excluded from the genus or transferred to other genera. Although most of the difficulties regarding *Dictyuchus* species delineation stem from its ambiguous sexual behaviour (Humphrey 1890, 1893; Kaufman 1915; Coker 1923), they are still mainly separated based on their dioecious or monoecious behaviour (Johnson et al. 2002), meaning the ability to produce antheridia and oogonia on one single (dioecious) or different (monoecious) hyphae (Johnson et al. 2002). Despite the fact that there are more than 190,000 partial nuclear and mitochondrial sequences assigned to *Saprolegniales* in the GenBank database (GenBank, NCBI, USA; [Online] http://www.ncbi.nlm.nih.gov/), *Dictyuchus* is among the least-studied genera.

During a survey of aquatic oomycetes in twelve different freshwater ecosystems located in northern Iran and north-eastern Germany, only nine out of 150 isolates were identified morphologically as *Dictyuchus* sp. using their zoospore discharge character. More detailed investigations revealed morphological and morphometric differences among the isolates compared to two other known *Dictyuchus* species. Phylogenetic analyses of mitochondrial (cytochrome c oxidase I (*cox1*)) and nuclear rDNA sequences (ITS) were also conducted to investigate the position of this species in the genus *Dictyuchus*.

MATERIALS AND METHODS

Sampling and isolation

Dictyuchus isolates were isolated by the methods described earlier by Coker (1923), Sparrow (1960) and Seymour (1970) over several months in 2017 (Table 1). Samples of decaying leaves of the dominant local vegetation (*Typha* spp. L.) collected from Anzali lagoon (37° 28′ 16″ N, 49° 27′ 44″ E) were moved to the mycology laboratory of the University of Guilan, Iran, in separate sterile polyethylene bags. Briefly, the samples were cut into several approximately equal pieces, and after being washed with distilled water, were placed in sterilized plates containing 10 ml sterile distilled water with 10 sterilized hemp (*Cannabis sativa* L.) seed halves at 20-25°C (Middleton 1943). After three to five days, a piece of mycelia from colonized hemp seed halves was transferred to a fresh CMA-PARP medium (CMA-PARP; 40 g/L ground corn meal, 0.5 g/L ampicillin, 0.01 rifampicin, 0.2 g/L delvocid and 0.1 g/L pentachloronitrobenzene (PCNB), 15 g/L agar) (Kannwischer and Mitchell 1981). This step was repeated three to five times to achieve bacterial free (axenic) colonies. Then, a single hypha was transferred to cornmeal agar (CMA; 40 g/L ground corn meal, 15 g/L agar) (Seymour & Fuller 1987). The hyphal-tip technique was conducted three to five times to obtain a pure culture in CMA. The specimens of this new species were then deposited in the Fungal Herbarium of the Iranian Research Institute of Plant Protection (IRAN).

Characterization of morphological features

For each strain, thirty measurements per replicate were taken of the following characters: breadth of mycelia, length and breadth of sporangia, gemma diamater, and colony and cyst diameter. 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).

Although temperature and nutrition has been considered effective on initiation and maturation of oomycetes, there was no report on suitable chemically defined medium and temperature for inducing oospore formation by *Dictyuchus* species. Thus, in order to investigate sexual behavior, several treatments, were used. The nutrition treatments included (1) reciprocal culturing of all isolates with one another and *Trichoderma* sp. (Brasier et al. 1978) on CMA, (2) hemp seed agar (HSA; 60 g/L ground hemp seeds, 15 g/L agar) (Hendrix 1964), (3) soybean agar (SA; 100 g/L ground soybean seeds, 15 g/L agar) (Savage et al. 1968), (4) rape seed extract agar (REA; 100g/L ground rape seeds, 15 g/L) (Satour 1967), (5) carrot juice agar (CJA; 250 g/L boiled carrot extract, 20 g/L agar) (Ershad 1971), (6) mPmTG (2, 0.4, 0.4 and 12 g/L glucose, tryptone, peptonized milk and agar, respectively) (Moreau and Moreau 1936b), and (7) immersing colonized CMA in glycerin (4%) (Moreau and Moreau 1936a) and (8) culturing the isolates in Petri dishes containing 10 boiled hemp seeds in distilled lake water and distilled water (50/50). The temperature treatments also included culturing the isolates in 5, 10, 15, 20 and 25 °C in Petri dishes containing 10 boiled hemp seeds in distilled lake water and distilled water (50/50).

DNA extraction and PCR

DNA extraction was conducted based on protocol of Montero-Pau et al. (2008). Briefly, 100 µL of alkaline lysis buffer (25 mM NaOH, 0.2 mM disodium EDTA, pH 8.0) was aliquoted into 1.5 mL tubes. Malt extract broth (MEB; 17 g/L malt extract) (Galloway & Burgess 1962) was used for isolates' growth. Mycelial mass was then transferred to the

tube and centrifuged for 30 minutes at 9000 rpm. The tube was incubated at 95°C for 30 minutes and then cooled on ice for five minutes. Finally, 100 µL of neutralizing solution (40 mM Tris-HCl, pH 5.0) was added to the tubes. The final solution was vortexed for 5 minutes and kept at -20°C. Nuclear ITS-rDNA region as well as mitochondrial *cox1* was amplified in a Flexible PCR Thermocycler (Analytikjena, Germany) using ITS1/ITS4 (White et al. 1990) and OomCoxI-Levup/OomCoxI-Levlo (Robideau et al. 2011) primers. Thermocycler program for amplification of the ITS region was: 94°C for 2 min of initial denaturation followed by 32 cycles of 94°C for 15 s, 53°C for 15 s, 72°C for 30 s, and a final extension at 72°C for 5 min. Thermocycler program for amplification of the *cox1* region was: 95°C for 2 min followed by 35 cycles of 95ºC for 1 min, 55°C for 1 min, 72°C for 1 min. A final extension step was made at 72°C for 10 min. The resulting sequences were edited by using the Bioedit software (Hall 1999) and submitted to GenBank (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov) database (Table 2).

Phylogenetic analysis

The ITS and *cox1* sequences of the isolates together with sequences of the representative genera of *Saprolegniaceae*, retrieved from GenBank, were used to performed phylogenetic analysis. The sequences were aligned using MAFFT (Katoh & Standley 2013), refined manually using BioEdit (Hall et al. 2011), and analyzed with MEGA7 using maximum likelihood method (Kumar et al. 2016).

RESULTS

In total, nine *Dictyuchus* sp. isolates were obtained from three different regions in Anzali lagoon, Iran, and studied morphologically, morphometrically, and phylogenetically. The genus *Dictyuchus* is located within the clade recognized by the presence of eccentric oospores.

Table 1. *Dictyuchus* sp. isolates from Anzali lagoon (Anzali County, Iran) used in this study and their GenBank accession numbers for ITS-rDNA and *cox1* regions.

Isolate	Isolation Date	Coordinates	GenBank accession numbers	
			ITS	*cox1*
B952-1AA	January, 2017	37° 25' 44.0" N, 49° 27' 29.5" E	MH253594	-
F962-2	March, 2017	37° 27' 55.6" N, 49° 28' 08.4" E	MH253589	MK396251
S961-4	May, 2017	37° 27' 55.6" N, 49° 28' 08.4" E	MH253592	-
B952-1A	August, 2017	37° 26' 20.1" N, 49° 27' 18.8" E	MK400430	MK396250
D952-5B	September, 2017	37° 25' 44.0" N, 49° 27' 29.5" E	MH253595	-
E952-6	October, 2017	37° 26' 20.1" N, 49° 27' 18.8" E	MH253585	-
O963-5	November, 2017	37° 27' 55.6" N, 49° 28' 08.4" E	MH253588	-
M963-11A	December, 2017	37° 25' 44.0" N, 49° 27' 29.5" E	MK400432	-
O962-8	December, 2017	37° 26' 20.1" N, 49° 27' 18.8" E	MK400431	MK396252

Table 2. Comparison of morphological characters of *Dictyuchus* sp. isolates used in this study, *Dictyuchus monosporus* and *D. pseudodictyon.*

Morphological features		*Dictyuchus* isolates sp.	*D. monosporus*	*D. pseudodictyon*
Discharge mode		Dictyucoid or achlyoid,	Dictyucoid, sometimes aplanoid	Dictyucoid, rarely achlyoid
Gemma				
	shape	Spherical or fusiform, occasionally irregular shape in agar medium, when spherical, single or catenulate, lateral, when fusiform, mostly intercalary	Absent or very rare, when present, obpyriform to short-cylindrical	Cylindrical, fusiform, or obpyriform, rarely doliform; terminal or intercalary, single or catenulate
	size	77.01-176.11 µm	NA*	NA
Sexual reproduction		Sterile	Dioecious	Monoecious
Sporangia				
	shape	Filiform, branched, small to very long, breaking of from hyphae regardless of spore matureness	Elongate-cylindrical to elongate-narrowly clavate; straight, curved, or slightly irregular; unbranched or branched	Fusiform or clavate, occasionally cylindrical; straight, occasionally curved or bent, rarely branched
	renewal	Sympodial fashion	Sympodially or in a cymose fashion	Sympodially or in a cymose fashion
	size	80.45-391.84×11.78-34.49 µm	60-780×10-40 µm	70-603×10-44 µm
Cyst diameter		5.94-10.94 µm	10-17 µm	9-15 µm
Hyphae				
	Width	13.47-43.15 µm	NA	NA

* NA: Not assigned.

According to our morphological and morphometric studies, the isolates are separated from their allied species mainly based on its dictyoid and achlyoid spore discharge mode, abundant fusiform and spherical gemma, abundance presence of branched sporangia, and absence of sexual reproduction (Fig. 1, Table 2) (Leclerc et al. 2000; Johnson et al. 2002). The size of sporangia and cyst were also different from their closely related species (Fig. 2, Table 3).

Phylogenetic analyses

The phylogenetic analysis of the ITS sequences (698 bp) of different genera belonging to the family *Saprolegniaceae* (*Saprolegniales*, *Oomycota*), inferred from maximum likelihood method was consistent with the morphology-based taxonomy. According to the ITS derived phylogeny, all the isolates analyzed in this study were associated with *Dictyuchus* spp. (Fig. 3). The phylogentic analysis of the *cox1* sequences (413 bp) also led to robust separation of our isolates from *Brevilegnia* spp. (Fig. 4). ITS and *cox1* analyses involved 10 and 6 nucleotide sequences, respectively. However, as it was described before, there were clear different morphological and morphometric characters that raised the question about the novelty of the isolates. The matrix and the resulting tree can be viewed on TreeBASE (accession 23950; http://purl. org/phylo/ treebase/phylows/study/TB2:S23950).

***Dictyuchus* sp. (Fig. 1)**

Mycelium is dense; main hyphae are slender, sparingly branched, hyaline to dark, with 13.47–43.15 µm (average 29.99 µm) width. The arrangement of mycelia becomes irregular and segmented in old cultures. Sporangia are abundant, mostly branched and fusiform, occasionally curved, renewed sympodially, 80.45–391.84 length×11.78–34.49 width µm (average 210.75 µm), mainly detaching from hyphae regardless of the time the spores become mature, and very long in old cultures.

Zoospores rarely form in small sporangia, usually monoplanetic, spores' discharge behave as dictyoid, in which cysts leaves a network of wall material and sporangium wall may or may not disintegrate in part or entirely, and achlyoid, in which spores clusters (66.25–169.28 µm diam. (average 117.43 µm) do not usually persist at the exit orifice, and may fall away from the sporangium or just collapse. Cysts are 5.94–10.94 µm in diameter. (average 8.82 µm), gemmae is mostly present, spherical and fusiform, when spherical always terminal (77.01–176.11 µm) (average 122.25 µm in diam.), when fusiform

Table 3. Morphometric features (µm) of *Dictyuchus* sp. isolates isolated from Anzali lagoon, Iran.

	Isolates								
	B952-1AA	**F962-2**	**S961-4**	**E952-6**	**O963-5**	**M963-11A**	**O962-8**	**B952-1A**	**B952-3B**
Sporangia									
Length mean	239.05	170.92	210.51	213.56	233.39	195.45	189.01	212.25	232.65
Length range	135.29–391.84	94.9–241.66	118.01–310.1	103.36–360.66	124.61–351.23	80.45–287.78	104.04–294.92	161.07–257.46	113.15–312.58
Breadth mean	25.69	23.06	26.92	18.31	20.75	25.84	20.67	32.61	25.90
Breadth range	14.84–34.67	19.02–31.04	15.62–38.18	11.78–25.68	14.63–27.59	19.66–33.88	10.68–30.56	25.93–48.78	10.06–34.49
Cyst									
Mean	8.02	8.86	8.85	9.73	9.01	9.0	8.35	9.16	8.37
Diam. range	5.94–9.39	6.34–10.71	7–10.1	7.98–10.71	7.21–10.52	7.73–10.94	7.0–9.34	6.9–10.51	6.02–9.61
Hyphae									
Mean	38.27	25.67	28.17	37.60	29.65	31.78	31.73	22.25	24.84
Diam. range	15.43–75.33	13.47–50.04	12.44–48.07	13.47–67.22	12.68–59.41	15.96–64.96	14.18–88.16	11.92–39.01	12.2–43.15
Gemma									
Mean	127.34	-	125.57	-	111.2	124.87	116.83	127.72	118.56
Diam. range	96.93–171.75	-	77.01–164.07	-	78.09–134.7	89.95–164.07	80.82–154.6	96.08–176.11	87.94–149.1
Spore clumps									
Mean	116.53	125.54	122.35	112.98	120.25	124.66	127.56	125.25	123.45
Diam. range	62.25–165.32	63.15–157.43	69.28–150.55	62.75–164.89	70.21–144.39	65.45–136.32	66.24–142.52	65.92–169.28	67.28–116.36
Average growth rate (mm d^{-1}) on CMA at (°C)									
5	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0
15	7	7	6	5.5	6	6.5	7	7.5	7
20	11	11	9	9	10	9.5	9	10.5	11
25	17	15	17	15.5	15.5	15	17	16.5	17
30	25	24	24	24	24	25	23	24	25
35	25	25	24	25	24	23	22	25	24
40	0	0	0	0	0	0	0	0	0

mainly intercalary, sometimes catenulate (overabundant in HSA medium) (number of continuous gemma 2–5), and sometimes has irregular shapes in agar media. No specific pattern was observed for any of isolates on CMA. Sexual apparatuses are not produced in any treatments examined.

Specimen examined. IRAN, Guilan Province, Anzali County, Anzali lagoon, 37°25'11.0"N 49°26'24.4"E, on decaying leaves, 20 Jul. 2017, H. Masigol (GenBank Acc. No: ITS – MH253594, MH253589, MH253592, MK400430, MH253595, MH253585, MH253588, MK400431 and MK400432, *cox1* – MK396251 and MK396250).

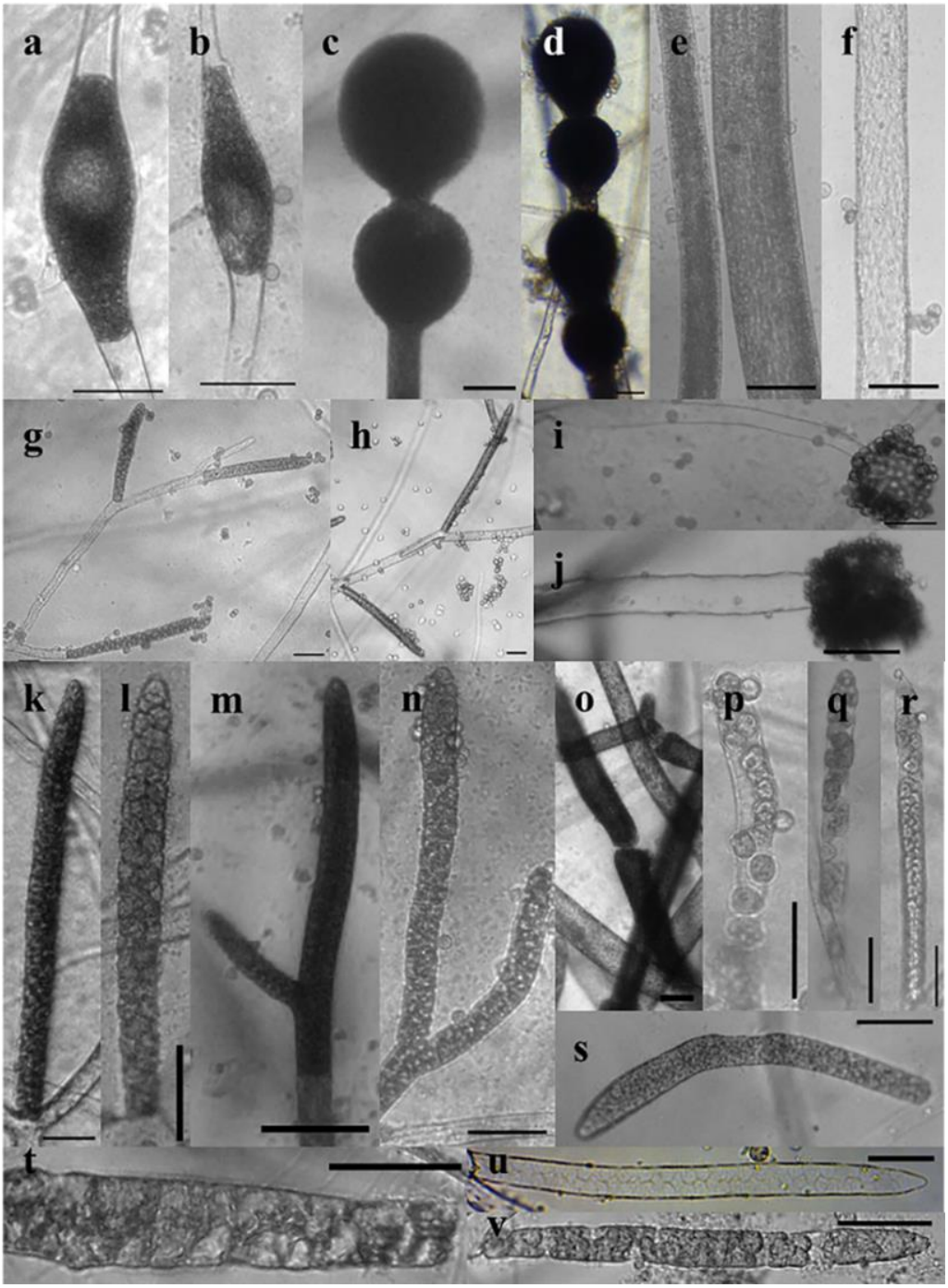

Fig. 1. Morphological structures of *Dictyuchus* sp. isolates: **a–b**. fusiform, intercalary gemma; **c–d.** spherical, terminal, catenulate gemma; **e–f.** dark (e) and hyaline (f) slender hyphae; **g–h.** sympodial renewal of fusiform sporangia, usually with only two successions; **i–j.** achlyoid discharge mode of zoospores four days after inoculation in water culture; **k.** immature fusiform sporangia; **l.** mature sporangia before dictyoid discharge; **m–n.** immature (m) and mature branched sporangia; **o.** segmentation of hyphae in one month water culture; **p–r, t-v.** dictyoid discharge mode of zoospores 10 days after inoculation in water culture in different stage of disintegration; (s) curved and detached immature sporangia. — Scale bars = 50 μm.

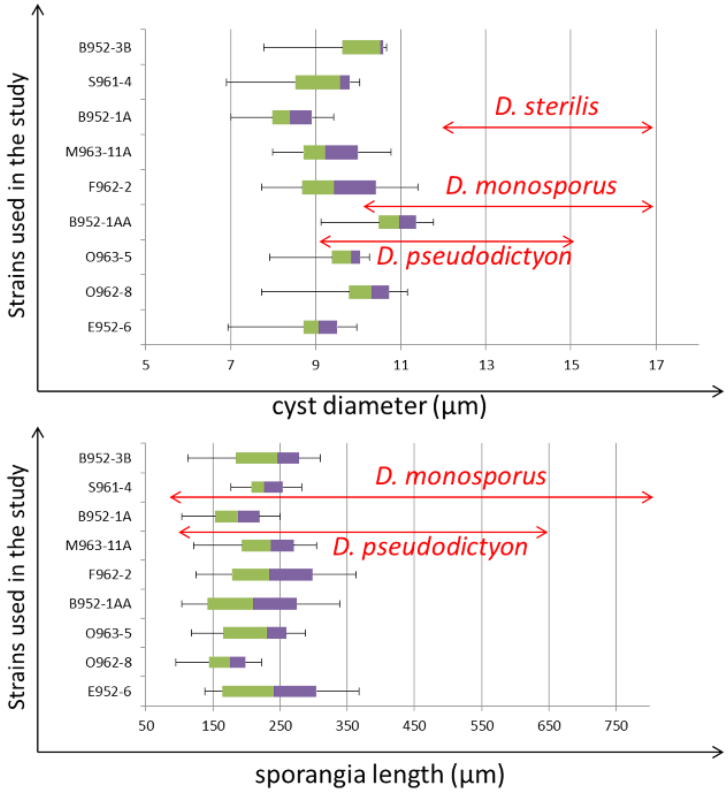

Fig. 2. Boxplots representation of cyst diameter (µm) range (up) and sporangia length (µm) (bottom) of *Dictyuchus* sp. isolates inferred from 30 measurements of each character. The measurements have been compared to *Dictyuchus monosporus*, *D. pseudodictyon* and *D. sterilis* (when available).

Fig. 3. Molecular phylogenetic tree of the *Dictyuchus* isolates (*Saprolegniales*, *Oomycota*). The analysis was performed on alignment of the ITS1-5.8S-ITS2 region of nuclea rDNA (610 bp) using the maximum likelihood method. Numbers next to the branches shows the bootstraps values ≥ 50%. HQ643117 *Aphanomyces euteiches* was considered as outgroup.

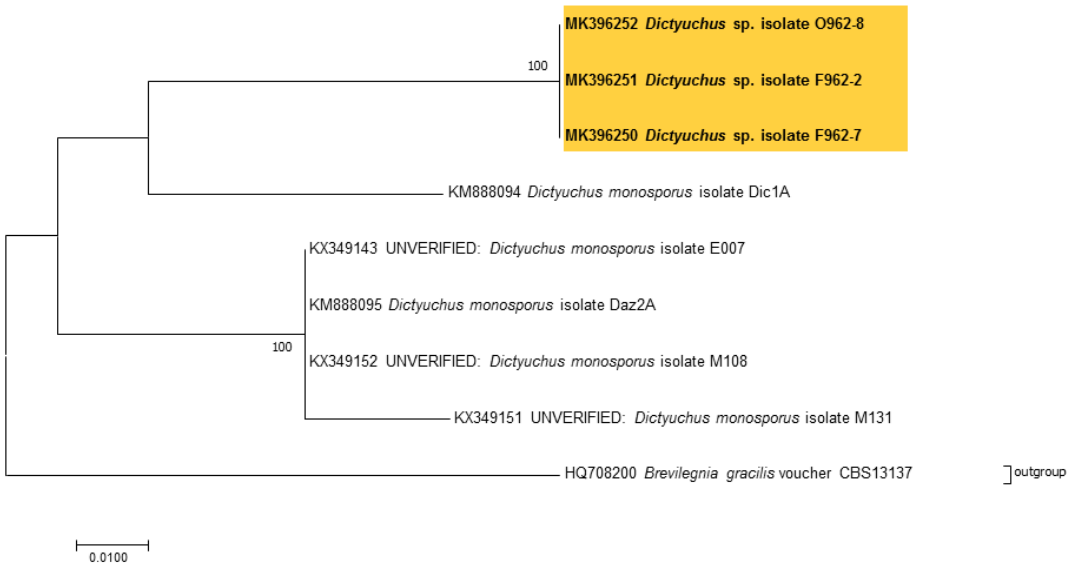

Fig. 4. Molecular phylogenetic tree of the *Dictyuchus* isolates (*Saprolegniales*, *Oomycota*). The analysis was performed on alignment of the mitochondrial *cox1* region (529 bp) using the maximum likelihood method. Numbers next to the branches shows the bootstraps values ≥ 50%. HQ708200 *Brevilegnia gracilis* was considered as outgroup.

Key to *Dictyuchus* species

1. Antheridia or oogonia or both present……...……...……...……..... 2
1.* Absence of antheridia and oogonia ……...……... 3
2. Dioecious, Anth. or oog. branches, or both, cross-induced……...……..... *D. monosporus*
2.* Monoecious, Antheridia or oogonia self-induced……...……. *D. pseudodictyon*
3. Gemma present, spherical (terminal) or fusiform (intercalary), achlyoid or dictyoid discharge mode, mainly branched sporangia, cyst diam. 6-11 µm……...… *Dictyuchus* sp.
3. Gemma absent, dictyoid discharge mode, cyst diam. 12-17 µm……...……..…… *D. sterilis* (?)

DISCUSSION

The number of submitted GenBank sequences of *Dictyuchus* taxa and its closest genus, *Brevilegnia*, are scares. In addition, in some cases, we failed to verify authenticity of *Dictyuchus* sp. submitted sequences. For instance, Abdulhag & Shahzad (1998) reported *Brevilegnia* sp. from Pakistan without presenting any morphological and morphometric features in their paper and then submitted it as *Dictyuchus monosporus* in GenBank (KP663638 and KP663638). We also tracked down the accession numbers KM289010, KM289009, KM888094 and KM888095 which had been reported from approximately the same geographical location as we isolated the examined isolates but neither morphological and morphometric features nor live cultures were available. Although these mentioned ambiguous isolates are clustered with our isolates, our isolates are clearly separated from *D. monosporus* according to its most valid and recent description (Johnsoon et al. 2002) (Table 2). We investigated the available literature on *Dictyuchus* isolates and its closely related genera from different origins. In most cases neither morphological and morphometric nor molecular data set has presented (El-Hissy et al., 2000, Czeczuga et al. 2003, Kiziewicz and Kurzątkowska 2004, Kiziewicz 2005, Kiziewicz and Nalepa, 2008, Mousavi et al. 2009). They mostly rather focused on seasonal distribution and correlation with physico-chemical features of the ecosystems.

Dictyuchus sterilis was proposed as a new and sterile species by Coker (1923) mainly due to its asexual nature. Johnson et al. (2002) showed a skeptical attitude toward Coker description validity, emphasizing that there is no way to be sure that Coker's isolates were strictly and permanently asexual or can they be alleged to be mating isolates of *D. monosporus*. This species is not also mentioned by Sparrow (1943, 1960) and Dick (2001). However,

two arguments were raised by Coker (1923) at the first place to justify his nomenclature; (1) very positive sterility of *D. sterilis* over a series of ten years of cultures in various media, representing over a hundred findings, although no further explanations were presented about these various media and (2) isolation and characterization of the same sterile isolates by Coker himself (63 times from Feb., 1912, to Dec., 1913) and several other authors from different regions (Coker, 1923). Interestingly, Rattan et al. (1978) also reported 36 isolated of *Dictyuchus* from Iraq, failed to produce oogonia and remained sterile.

Morphological features including the abundant presence of fusiform and spherical gemma, achlyoid zoospore discharge mode, branched shape and sympodial branching of sporangia, detaching of sporangia before the maturity of spores, and cyst diameter (Figure 2) can separate our isolates from *D. sterilis*. However, both fail to produce any sexual organs. This might imply sterility is, as Coker has stated before, a permanent feature and is not limited to *D. sterilis*. This feature has been observed before in other oomycetes genera such as *Achlya*, *Saprolegnia* and *Pythium* (Coker 1923; Seymour 1970; Johnson et al. 2002). On the other hand, our isolates were separated phylogenetically from *D. sterilis* AF036545 (Figure 3) which highlights our speculations about the novelty of our isolates. Nevertheless, due to lack of reliable sequences from type material of *Dictyuchus* species, we prefer not to introduce our isolates as a new species and adjourn it to further investigations. In particular, isolation of more geographically distributed *Dictyuchus* sp. isolates would be helpful to fill the current knowledge gap, both morphologically and phylogenetically.

We also examined the possibility of using morphometric features as a source of taxonomic value as it has been recently proposed by Sandoval-Sierra and Diéguez-Uribeondo (2015), leading to the separation of *Saprolegnia aenigmatica* Sand.-Sierra & Diég.-Urib., and *S. racemosa* Sand.-Sierra & Diég.-Urib. (Sandoval-Sierra & Diéguez-Uribeondo 2015). The lack of morphological and morphometric data about *Dictyuchus* species, compared to other oomycetes, is more noticeable, yet the analyses on our isolates indicated that this species could be distinguished using sporangia length and cyst diameter range (Figure 2). In this view, having more isolates of the described species could prevent ambiguous descriptions due to the variability of measurements.

Currently, the number of sequences of *Dictyuchus* spp. available in GenBank is very low. Although introducing new *Dictyuchus* spp. sequences to GenBank is helpful for exploring its unknown diversity, rendering this data set to a source of incorrectly named isolates, miss-assigned species names, as it has happened for *Achlya* and *Saprolegnia* species, must be absolutely avoided. So far, no clear agreement has been reached toward *Dictyuchus* species delineation. Only two species has been accepted by Johnson et al. (2002), yet 15 records which are accepted by Index Fungorum have irregularly been accepted or missed in old textbooks (Coker 1923; Fitzpatrick 1930; Sparrow 1943). A solution proposed by Sandoval-Sierra et al. (2014) for *Saprolegnia* species, called the definition of the molecular operational taxonomic units (MOTUs) approach avoiding possible miss-assignment of *Saprolegnia* species.

All in all, to better understanding of the difficulty in *Dictyuchus* classification and related genera, we recommend retrieving of more isolates of this genus to unravel its "true" genetic diversity. Moreover, no type material sequence data currently available for *Dictyuchus* species which is crucial to constructing molecular based taxonomy and species identification. By doing so, it is not implausible to witness qualitative and quantitative improvements in species descriptions of *Dictyuchus* species over the next decades, as it has been the case for *Phytophthora*, *Pythium*, etc. (Levesque 2011). In this context, preventing imprecise morphological identification at the species level without presenting morphological and morphometric data is a matter of importance. Meanwhile, one should prevent the unjustifiable preference of available molecular toolboxes over classic identification, as it has happened for other *Saprolegniales.*

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ملاحظاتی در مورد جنس *Dictyuchus* (Stramenopila, Oomycetes) جداشده از تالاب انزلی، ایران

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چکیده: نه استرین متعلق به جنس *Dictyuchus* از بقایای گیاهی شناور در تالاب انزلی جداسازی گردید. این جدایه‌ها با داشتن ویژگی‌های رهاسازی زئوسپور به شکل dictyoid و achlyoid، فراوانی ژمای کشیده و کروی و فقدان هرگونه اندام جنسی از گونه-های فعلی جنس *Dictyuchus* متمایز می‌شوند. علاوه بر این، واکاوی‌های فیلوژنتیکی نواحی ژنومی ITS-rDNA هسته ای و *cox1* میتوکندریایی با روش maximum likelihood حاکی از جدید بودن این جدایه ها است. ما ترجیح می‌دهیم به علت مشکلات فعلی، جدایه‌ها را به عنوان گونه جدید معرفی نکنیم. ما همچنین پیشنهاد می‌دهیم گونه *Dictyuchus sterilis* که پیش از این بی اعتبار گردیده بود مورد بررسی قرار گیرد. ما در مورد تاکسونومی این جنس بحث کردیم و یک کلید اصلاح‌شده را پیشنهاد دادیم. علاوه بر این، نشان داده شد که عقیم بودن در شرایط آزمایشگاهی ممکن است در این جنس معمول باشد.

واژه های کلیدی: تنوع زیستی، اکوسیستم آب شیرین، اومیست ها، ساپرولگنیالز، ناباروری

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