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New species of family *Saprolegniaceae* in Mazandaran, Iran

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Abstract: Oomycete species occupy many different environments and many ecological niches. The family *Saprolegniaceae* from *Oomycota* includes widely distributed water molds which usually behave as saprophytes on plants and animal debris. Members of some species may also be pathogenic for plants and fish. Oomycete species identification based on DNA is well established, but DNA barcoding with cytochrome c oxidase subunit I (*COXI*) and II (*COX II*) are a relatively new approach. In this study, 57 isolates were obtained from water samples in mazandaran province, Iran. After morphological identification by morphological keys, ITS, *COXI* , and *COX II* gene regions of 10 representatives of the isolates were sequenced and 3 genera and 6 species (*Newbya recurva, Achlya bisexualis, Dictyuchus monosporus, Saprolegnia ferax, S. bulbosa* , and *S. debaryana*) were identified through BLASTn in NCBI gene bank. The results described in this paper were indicated that except for ITS, *COXI* and *COXII* sequencing could also be valuable resources to *Saprolegniaceae* identification. Except for *S. ferax*, other described species were new reports for oomycete biota of Iran.

Keywords: *Oomycota*, water molds, cytochrome c oxidase, plant pathogens, ITS-rDNA, *Saprolegniaceae.*

INTRODUCTION

The fungi and oomycetes live in most ecological niches (Alexopoulos et al. 1996), especially oomycetes as water molds, live in fresh and salt waters (Emerson & Natvig 1981). Most saprophytic oomycetes decompose dead plant and animal debris, so they have an important role in the materials cycle in their habitat (Dick 1990). Some pathogenic species infect some rotifers, nematodes, mosquitos' larvae, crabs, fish eggs, and even fish (Alexopoulos et al. 1996).

The family *Saprolegniaceae* includes widely distributed water molds which usually behave as saprophytes on plants and animal debris, or are parasitic. This family is eucarpic, monoecious or dioecious organisms (Lecler et al. 2000, Johnson et al. 2002). The classic taxonomy of *Saprolegniaceae* is based on main morphological characteristics such as vegetative or sexual and asexual structures (Dick 1969, Seymour 1970, Willoughby 1978, Leclerc et al. 2000). Unfortunately, most isolates have some sexual or asexual reproduction problems with axenic media (Hatai et al. 1990, Diéguez-Uribeondo et al. 2007), some isolates need a long time to produce immature morphological structures (Leclerc et al. 2000).

Modes of zoospore discharge are the main feature for the identification of the *Saprolegniaceae* genera. In genus *Saprolegnia* spp., zoospores release slowly, but in *Achlya* spp. zoospores aggregate at the top of the zoosporangium and then release and swim (Markovskaja 2007).

Generally, molecular methods can be used for identification of *Saprolegnia* species (Beakes & Ford 1983, Molina et al. 1995). A large set of molecular evaluations of the Saprolegniaceae has been developed by Laclerc et al. (2000) on ITS and 28S regions of 40 species and 10 genera. Most Iranian studies on oomycetes focused on plant pathogens (Hatamian 2009, Nejadsattari 2000, Mousavi et al. 2009, Shahbazian et al. 2010, Nekuie Fard et al. 2011).Few taxonomic studies on *Oomycota*, especially *Saprolegniales* published in domestic journals and conferences. From the best examples of Iranian approaches to water molds are Masigol et al. (2018) and Bolboli & Mostowfizadeh-Ghalamfarsa (2019). We sought to evaluate the best possible identification methods as well as to identify species in these areas therefore we isolated and identified water mold of Mazandaran Province in the Caspian seasides as a rainy region with different rivers and many local small dams and dykes. This survey utilized morphological keys and molecular methods for isolate identification.

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MATERIALS AND METHODS

Sampling isolation, purification, and maintenance of isolate

All water samples were collected from Caspian Sea coasts, different rivers, streams, pools, and surface wells of various regions in Mazandaran in 2013-2014. The water samples were transferred to the lab and maintained at room temperature. Seeds of hemp, sesame, corn, and wheat were used as baits in 8 cm Petri plates to isolate water molds from collected waters. After the growth of mycelia around baits, were washed with distilled water, transferred into streptomycin (30 µg/ml) containing WA, then subjected to the hyphal tip method to purify isolates in PDA. The harvested isolates were maintained at 4°C for long-term storage and subcultured every 1-3 months to refresh cultures if it was necessary.

Morphological identification

The 5 mm agar disks of purified isolates were transferred to Petri plates containing sterile seeds in distilled water to produce sexual and asexual structures. Most morphological features surveyed according to the taxonomic keys, (Johnson et al. 2002 , Khulbe 2002). Microscopic studies like photography and drawings fulfilled by Nikon E600 microscope appointed to digital photography and drawing tube.

Molecular studies

Ten representatives of 57 identified isolates (Table 1) by morphological methods were subjected to DNA extraction (Petrisko et al. 2008) and amplified with ITS1, ITS4 (White et al. 1990), Oom*COXI*-Levup, and Fm85mod (Robideau et al. 2011) and coxR and coxF (Hudspeth et al. 2000) primers. Thermocycler program for amplification of the ITS region was: 94°C for 5 min of initial denaturation followed by 32 cycles of 94°C for 60 s, 58°C for 60 s, 72°C for 60 s, and a final extension at 72°C for 10 min. Thermocycler program for amplification of the cox1 and 2 regions was: 94°C for 5 min followed by 35 cycles of 94ºC for 30 s, 55°C for 30 s, 72°C for 30 s. A final extension step was made at 72°C for 10 min. After electrophoresis of PCR products on 2% agarose and staining with Ethidium bromide, photography of gels were fulfilled by Kodak gel logic 200. The PCR products were sequenced by Bioneer, South Korea. Generated sequences in this study, were edited manually and adjustments were made where necessary in the BioEdit v.7.0.5.2 (Hall 1999). The resulted sequences were subjected to NCBI BLASTn (https://blast.ncbi.nlm.nih.gov) to confirm their identities and comparison with the previously reported isolates available in the NCBI.

Obtained sequences in this study were deposited in the GenBank nucleotide database and accession numbers have been recorded. All sequences were aligned by using the CLUSTAL-W program (Kumar et al. 2016).

Table 1. Representative isolates from Mazandaran (Iran), used in this study and their GenBank accession numbers for ITS-rDNA and *COXI* and *COXII* regions.

Isolate	Collection locale	Time of sampling	Species	GenBank accession numbers		
				ITS	*COXI*	*COXII*
U11A	Sari	January, 2013	*A. conspicua*	KM272002	KM888097	KM888100
Sorec2A	Sari	December, 2012	*A. flagellata*	KM289007	KM888093	KM888101
Tajan2A	Sari	May, 2013	*A. bisexualis*	KM289008	KM888096	KM888102
Old	Sari	September, 2012	*N. recurva*	KF225572	KM888089	-
Beh8	Behshahr	December, 2012	*A. bisexualis*	KF225573	KM888091	KM888103
Daz2A	Sari	May, 2013	*D. monosporus*	KM289009	KM888095	KM888104
Dic1A	Amol	May, 2013	*D. monosporus*	KM289010	KM888094	KM888105
Ch1	Chalus	January, 2013	*S. bulbosa*	KF225574	KM888092	KM888099
Beh3	Behshahr	December, 2012	*S. ferax*	KF225575	KM361513	KM888106
K6	Kiasar	January, 2013	*S. ferax*	KF225576	KM888090	KM888098

RESULTS AND DISCUSSION

This study is one of the limited the molecular identification of the *Saprolegniaceae* members in Iran. A few study in Iran used the ITS region to research taxonomy information of *Saprolegniacea* (Masigol et al. 2018 and 2020).

A total of 450 water samples were collected from several regions in Mazandaran Province. One hundred fifty-seven water samples were included in water mold hyphae, and finally, based on morphological characteristics of 57 studied isolates and sequence data obtained from ITS-rDNA, *COXI* and *COXII* from representatives of the isolates (Table 1), six species belonging to *Saprolegniaceae* were identified. Based on the available literature, among the species, *Saprolegnia ferax* (Gruith.) Kütz.,was reported for the oomycete biota of Iran by Tajick-Ghanbary et al. (2008), but five other species, including *Newbya recurva* (Cornu) M. W. Dick & Mark A. Spencer, *Achlya bisexualis* Coker and A. Couch, *Dictyuchus monosporus* Leitgeb, *Saprolegnia bulbosa* Steciow, and *S. debaryana* Humphery were new reports for oomycete biota of Iran, which are described here.

Phylogenetic analysis

BLASTn alignment on NCBI revealed that the ITS and *COXI* sequences for representative isolates had the highest similarity with the our 10 representative isolates in accordance with morphological identification. Because there is not enough data support in the gene banks in the *COXI* and *COXII* regions, it was decided to draw a phylogenetic tree only for the ITS region (Fig. 1). Analysis of the ITS-rDNA region (Fig. 1) showed that 3 clades were distinguished from outgroups (*Albugo candida* HQ643111 and *Phytophthora infestans* CBS 120920). The identified isolates U11A and Sorec2A, Tajan2, Beh8, and Old clustered with *Achlya conspicua*, *A. flagellata*, *A. debaryana*, *A. bisexualis*, and *N. recurve* by 99% bootstrap support (Clade I). Results of previous studies have revealed that the species in this clade have nearly identical ITS sequences also, indicated the Achlya is not always a monophyletic unit, however, their identification should be based on the morphological characters. (Green & Dick 1972, Dick 1999, Riethmüller et al. 1999, Leclerc et al. 2000). Isolates Daz2A and Dic1A were well clustered with those of *D. monosporus* with 100% bootstrap support (Clade II). Also isolates Beh3, Ch1 and K8 were clustered with those of *S. ferax* and *S. bulbosa* with 100% bootstrap support (Clade III). According to the classical descriptions and illustrations in Johnson et al. (2002), it also appeared that *S. bulbosa* (Steciow et al. 2007) descriptions can fit into the description of *S. ferax* even with varying sizes of some particles. *S. ferax* and *S. bulbosa* and Ch1, Beh3 and K6 isolates can be members of a phylogenetic group with a name that is discussed in detail at the end of *S. bulbosa* description.

***Saprolegnia ferax* (Gruith.) Kütz**

This species first was reported in Iran by Tajik-Ghanbari et al. (2008), although in this report, the shape of the oogonium was described as only spherical but we reported spherical, obpyriform, napiform or obovate. The size of sporangia was reported 18-67 × 31-624 µm (Johnson et al. 2002), 15-35 × 180-350 µm (Khulbe 2001), 20-45 × 80-300 µm (Markovskaja et al. 2006), and 46.51 µm (average diameter) (Bolboli & Mostowfizadeh-Ghalamfarsa 2019) whereas, our report (15-28 × 30-540 µm) is almost equal with Johnson et al. (2002) and Khulbe (2001). Also, the size of oogonia was reported 60-80 µm (lowest 28 and highest 194) (Johnson et al. 2002) and 50-100 µm (Markovskaja et al. 2006), which is consistent with this report. Khulbe (2002) and Markovskaja et al. (2006) reported sporangia abundance, while Johnson et al. (2002) reported low sporangium number as the present report. Khulbe (2002) and Markovskaja et al. (2006) saw antheridia commonly. But, in this report they were seen occasionally as Johnson et al. (2002) reported. Khulbe (2001) cited the type of antheridium androgynous whereas, Johnson et al. (2002) and Markovskaja et al. (2006), such as present study, reported monoclinous or androgynous (Fig. 3, a5) and sometimes diclinous (Fig. 3, a4) but Bolboli & Mostowfizadeh-Ghalamfarsa et al. (2019) reported only monoclinous antheridia. Johnson et al. (2002) and Markovskaja et al. (2006), the same as present study, reported centric or subcentric oospores, and Khulbe (2001) only reported centric, but Bolboli & Mostowfizadeh-Ghalamfarsa et al. (2019) did not report that as one of the most important features of this species. K6 and Beh3 isolates, in ITS analysis, with accession numbers KF225576 and KF225575, respectively, *COXI* analysis with accession numbers KM888090 and KM361513 respectively and *COXII* with accession numbers KM888098 and KM888106 respectively, placed in *S. ferax* isolates.

***Saprolegnia bulbosa* Steciow 2007**

These isolates were monoecious, and mycelium was stout. The hyphae were more or less branched. Sporangia were mostly cylindrical (Fig. 3, b1), filiform or naviculate; 20-40 × 208-560 µm. Discharge and behavior were saprolegnoid. Gemmae were variable in shape, single (Fig. 3, b2), or catenulate. Oogonia were abundant, terminal, lateral (Fig. 3, b4 & b5), rarely intercalary; spherical and pyriform; 31-86 µm in diameter. The oogonial wall was pitted (Fig. 3, b3) and oogonial stalks were different and occasionally bent, curved or coiled. Oospores were subcentric (Fig. 2, b3) and rarely centric; 3-13 per oogonium, 14-26 µm in diameter; there were also unmatured oospores. Antheridial branches were not mostly persistent, monoclinous (Fig. 2, b5), androgynous (Fig. 2, b4), and sometimes diclinous. Steciow et al. (2007) named this species based on bulbous (swollen) basis in most of the oogonia, multiple hyphal swellings in the mycelium, and numerous bulbous antheridial branches. Also, these features were observed in the present survey, although not very distinct in the drawings. Sporangia size was 25-41 × 255-521 µm and the diameter of the oogonia is 97-45 µm, the diameter of the oospores is 15-35 µm and the number of oospores is 2-15, while only oogonium and oospores slightly were bigger than those reported by Steciow et al. (2007), it was consistent with the present research.

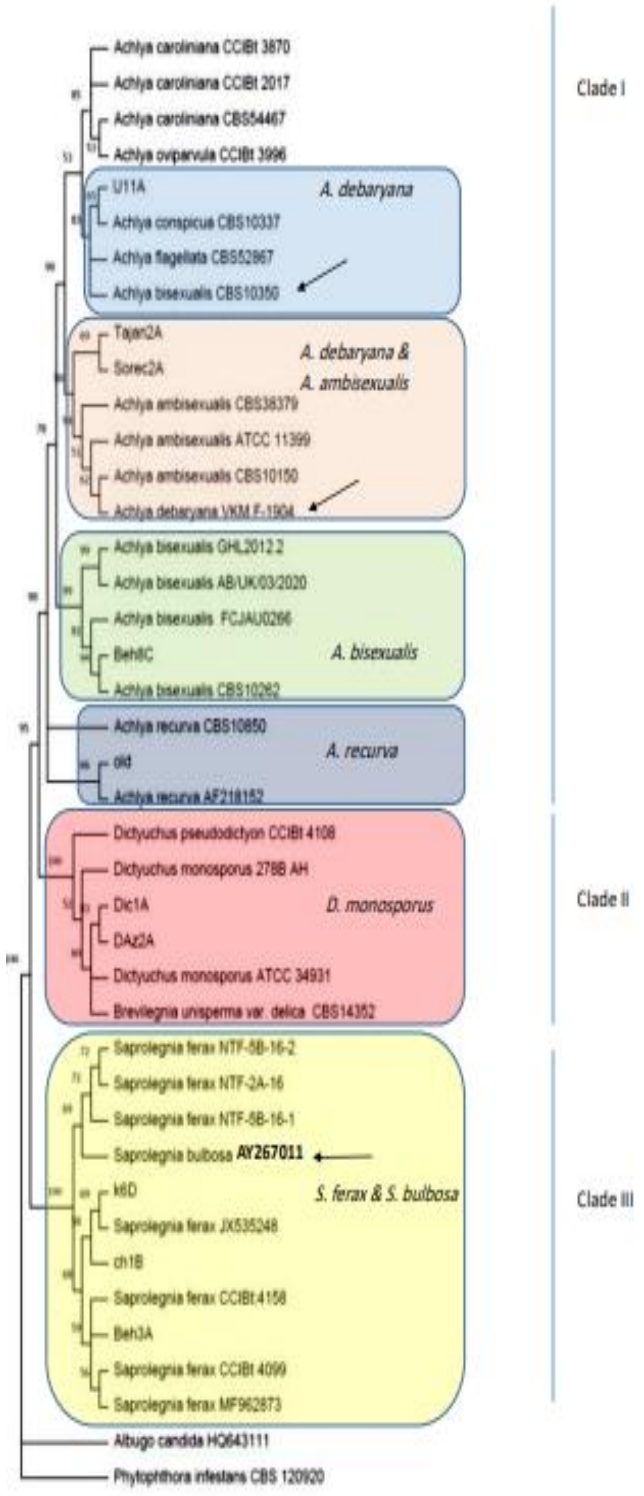

Fig. 1. Phylogenetic positions of isolates based on ITS rDNA region. It showed the Neighbor-Joining tree. All bootstrap values are indicated at 1000 repetitions (values smaller than 50% not shown).

A BLASTn search yielded in ITS the nearest identity with *S. ferax* (NTF-2A-16) and (99.58%) with *S. bulbosa* (AY267011). It seems that *S. bulbosa* (Steciow et al. 2007) descriptions can fit into the description of *S. ferax* species even with varying sizes according to the classical descriptions and illustrations in Johnson et al. (2002) book. Finally, it can be determined whether the isolate can be introduced as a new species, or should be named as *S. ferax* with help of molecular markers. In recent years, the same difference in conclusions has prompted researchers to introduce new examples to the world that may not be endorsed by other colleagues. We have described these two species separately based on the morphological differences. This is the first report of this species for Iran. This is the first report of this species for Iran.

***Dictyuchus monosporus* Leitgeb 1969**

These isolates were dioecious. Mycelia were slender to stout. hyphae were more or less branched (Fig. 3, a5). Sporangia were abundant, mostly elongate-cylindrical (Fig. 3, a1-3) to elongate-narrowly clavate and sometimes catenulate (Fig. 3, a4); 12-36 × 55-833 µm. Discharge and behavior were dictyucoid (Fig. 3, a7). Gemmae and sexual structures were absent. Johnson et al. (2002) reported that gemmae were not present or rarely seen and sporangia size was 10-40 × 60-780 µm which was consistent with the present report. However, catenulate sporangia were typically observed for the first time. In Khulbe's (2001) explanations, the sporangia size was 9–50 × 135–1000 µm, which was larger than the present studies's results, and Khulbe's (2001) reported gemmae and sexual organs had not been seen which is consistent with the present report. In ITS sequencing research, Daz2A and Dic1A isolates were registered with accession numbers KM289009 and KM289010, *COXI* accession numbers KM888095 and KM888094 and *COXII* accession numbers KM888104 and KM888105 that placed in the same isolates.

***Achlya bisexualis* Coker and A. Couch 1927**

These isolates were dioecious but capable of self-conjugation and some degree of interspecific compatibility. Hyphae were branched. Sporangia were fusiform and occasionally cylindrical; 20-40 × 85-410 µm. Discharge and behavior were Achlyoid (Fig. 3, b1 & b5). Gemmae were abundant and variable in shape, mostly single and terminal. The oogonia were lateral (Fig. 3, b2 & b7), sometimes terminal; spherical, obpyriform or oval; 55-127 µm in diameter. The oogonial wall was not pitted, and oogonial stalks were different. Oospores were eccentric; 8-12 per oogonium, 10-25 µm in diameter; antheridial branches arose from one thallus or in self-conjugating strains in diclinous (Fig. 3, b2 & b4) and abundantly branched; often were wrapped about the oogonium and its attendant hypha or stalks (Fig. 3, b2 & b4). Johnson et al. (2002) reported sporangia size is 25-45 × 110-400 µm, oogonia size is (35-) 60-75 (-130) µm and oospores were 22-26 µm in diameter and 5-10 number which oospores was slightly larger

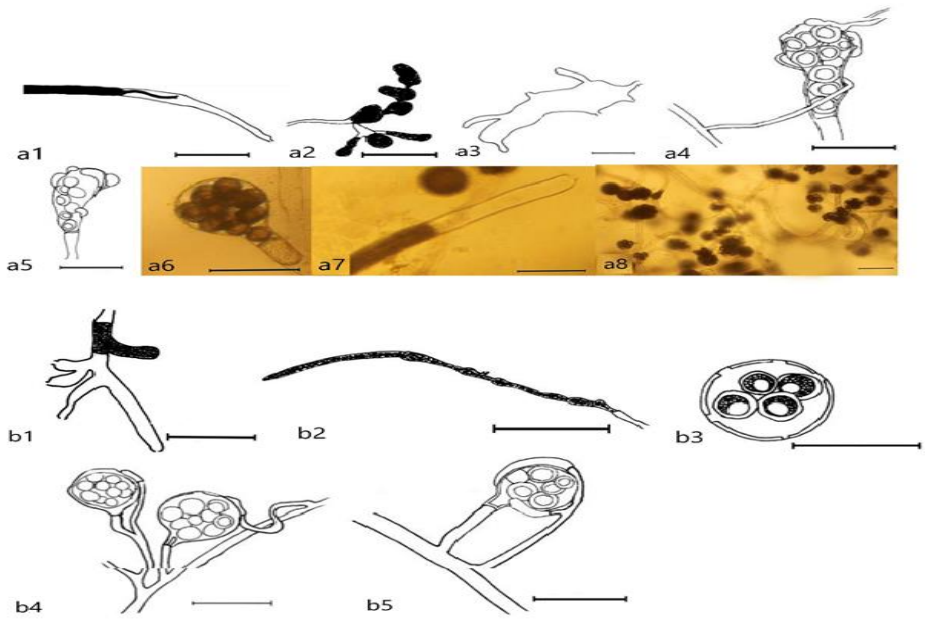

Fig. 2. a. *Saprolegnia ferax* a1. Internal proliferation in sporangium, a2. Single and catenulate gemmae, a3. Large sporangium with large exit tubes, a4. Oogonium and centric and sub-eccentric oospores and diclinous antheridium and a5. Oogonium with eccentric oospores and androgynous antheridium. a6. Oogonium with oospores, a7. Sporangium when spores discharged, and a8. Oogonia. b. *Saprolegnia bulbosa* b1. Sporangium, b2. Catenulate gemmae, b3. Oogonium and subeccentric oospores, b4. Oogonia and androgynous and monoclinous antheridia, and b5. Oogonium and monoclinous antheridium. — Scale bars a1, a2, b1 and b2 = 250 µm and Scale bars a3-8 and b3-6 = 70 µm.

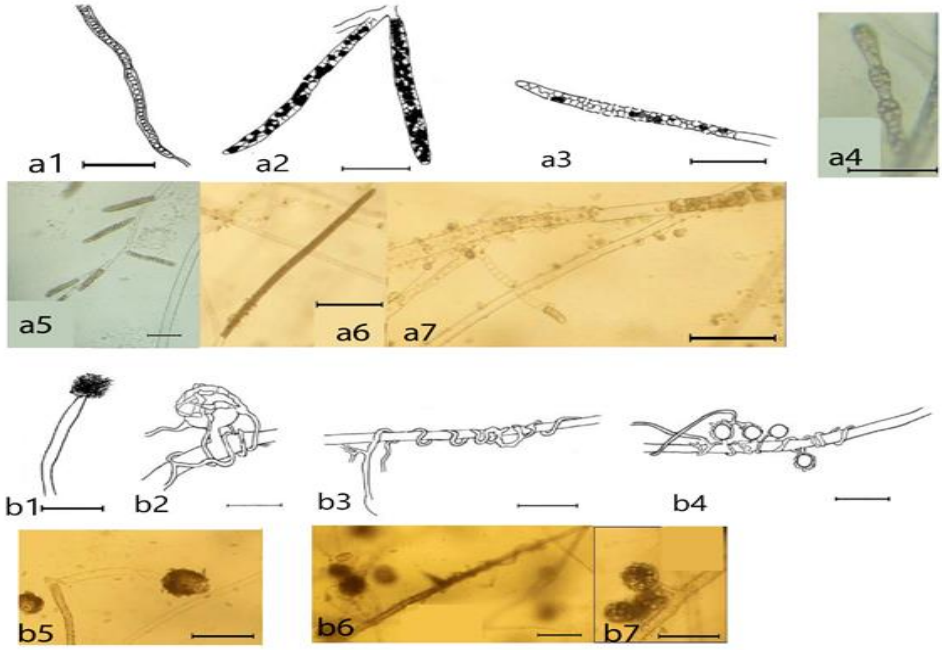

Fig. 3. a. *Dictyuchus monosporus* a1. Single-row sporangium, a2. Multi-row sporangia with empty and filled nets, a3. Multi-row sporangium with degraded portions of the wall. a4. Catenulate sporangia, a5. Sporangia, a6. Large sporangium, and a7. Sporangium with a network of cysts that some of the cysts are discharged and the zoospores emerging from the cysts. b. *Achlya bisexualis* b1. Sporangium, b2. Oogonium and diclinous antheridium, b3. Twisting hyphae around the mycelium, b4. Oogonium and diclinous antheridium and its attendant hyphae. b5. Sporangium and accumulation of cystic zoospores, b6. twisting hyphae around the mycelium, and b7. Oogonia and old antheridia. — Scale bars a1-7 = 100 µm, Scale bars b2 = 70 µm, and Scale bars b1,b3, b4, b5, b6 and b7 = 250 µm.

on average and their number was lower compared to the present report but overall the morphological identification of this species was consistent with Johnson et al. (2002).

It is interesting to note that the description of *Achlya bisexualis* is not found in Khulbe's (2001) identification key. A BLASTn search of Tajan2A isolate yielded the nearest identity (99.30%) with VKM F-1904 (*Achlya debaryana*) and (99.04%) ATCC 11399 (*Achlya ambisexualis*) and Beh8 isolate (100%) with AB/UK/03/2020 (*Achlya bisexualis*) respectively.

***Newbya recurva* (Cornu) M. W. Dick & Mark A. Spencer 2002**

These isolates were monoecious. Mycelium was stout and moderately branched. Appendages were formed on hyphae sometimes changed into antheridial branches (Fig. 4, a6). Sporangia were clavate, fusiform, or cylindrical; 15-28 × 30-540 µm. Discharge and behavior were Achlyoid (Fig. 4, a1). Gemmae were rare (Fig. 4, a2). The oogonia were lateral and rarely terminal (Fig. 4, a3-4 & a6-7) or intercalary (Fig. 4, a5); spherical or oval; 38-86 µm in diameter.

The oogonial wall was pitted and truncated (Fig. 4, a3-7). Oospores were eccentric (Fig. 4, a4); 1-9 per oogonium, 20-28 µm in diameter; Antheridial branches were androgynous (Fig. 4, a6-7), occasionally monoclinous (Fig. 4, a4) and rarely diclinous and abundantly branched (Fig. 4, a6). Johnson et al. (2002) reported sporangium was 8–81 ´ 81–820 µm, which was larger than the present report and the oogonia and oospores were (28-) 35-50 (-124) and (14-) 20-26 (-38) µm, respectively and oospore number was 4-8 and no gemmae was found that to correspond to this report. Khulbe (2002) reported sporangia as clavate but we found different types in our study and sporangia size 20–25 × 126–300 µm, which was smaller than the present report. Khulbe (2001) pointed to the presence of gemmae in his studies, but in the present report is rarely seen, as Johnson et al. (2002). Khulbe (2001) reported the oogonia were 25.8 – 64.8 µm in diameter, also a little smaller, and the oogonia were round.

Spencer et al. (2002) reported abundant oogonia, as much as (20-) 66-72 (-143) µm, 2-6 oospores, (11-) 22-28 (-46) in diameter, zoosporangium was formed rarely and their size was (149-) 250-465 (-741) × (12-) 16-40 (-53) and antheridium was monoclinous and rarely diclinous but based on our observations the average number of oospores was higher, the size of the oosporangia was smaller and androgynous type antheridium was observed. A BLASTn search of Old isolate yielded the nearest identity (98.80%) with AF218152 (*Newbya recurva*).

***Achlya debaryana* Humphery 1893**

These isolates were monoecious. Mycelium was stout and moderately branched (Fig. 4, b3). Sporangia were abundant and branched, clavate, fusiform or cylindrical and other shapes; 12-45 × 70-1100 µm. Discharge and behavior were Achlyoid (Fig. 4, b1-3). Gemmae were absent. Oogonia were lateral (Fig. 4, b4-5) and rarely terminal or intercalary; spherical or obpyriform; 45-120 µm in diameter. Oospores were eccentric (Fig. 4, b6); 3-17 per oogonium, 18-25 µm in diameter; Antheridial branches were abundantly branched (Fig. 4, b9), monoclinous (Fig. 4, b5) and occasionally diclinous (Fig. 4, b7) or androgynous (Fig. 4, b4). In Johnson et al. (2002) report sporangia were 10-53 × 78-1125 µm, oogonia were 45-70 µm and oospores were 20-28 µm and there were 2-14 oospores per oogonium, which is largely consistent with this report. They have rarely reported androgynous antheridial branches, whereas we observed this type of branching. Khulbe (2001) reported an abundance of gemmae, while we did not see any gemmae.

According to Khoulbe (2001) data, oogonium was 47-61.4 µm with 2 eccentric oospores, and with androgynous antheridium but according to our data the oogonium was larger with 3–17 eccentric oospores and antheridium (if present) was monoclinous and sometimes diclinous or androgynous.

The ITS sequences research revealed that the U11 isolate was 98.64% similar to *Achlya caroliniana* (CCIBt 2017), 99.04% to *Achlya oviparvula* (CCIBt 3996), 99.72% to *Achlya conspicua* (CBS10337) and 99.58% to *Achlya bisexualis* (CBS10350). The Sorek2A isolate was 98.47% similar to *Achlya debaryana* (VKM F-1904) and 98.33% similar to *Achlya caroliniana* (CCIBt 3870). Johnson et al. (2002) elaborated that both *A. conspicua* and *A. flagellate* are the same as *A. debaryana*, and all the morphological and molecular evidence supported this. Although Markovskaja (2004) opposed the species description and integration cited earlier reports of Johnson et al. (1956).

This report attempted to document both species in the form of *A. debaryana* for Iran, relying on the proper description and justification of the species (Fig. 1).

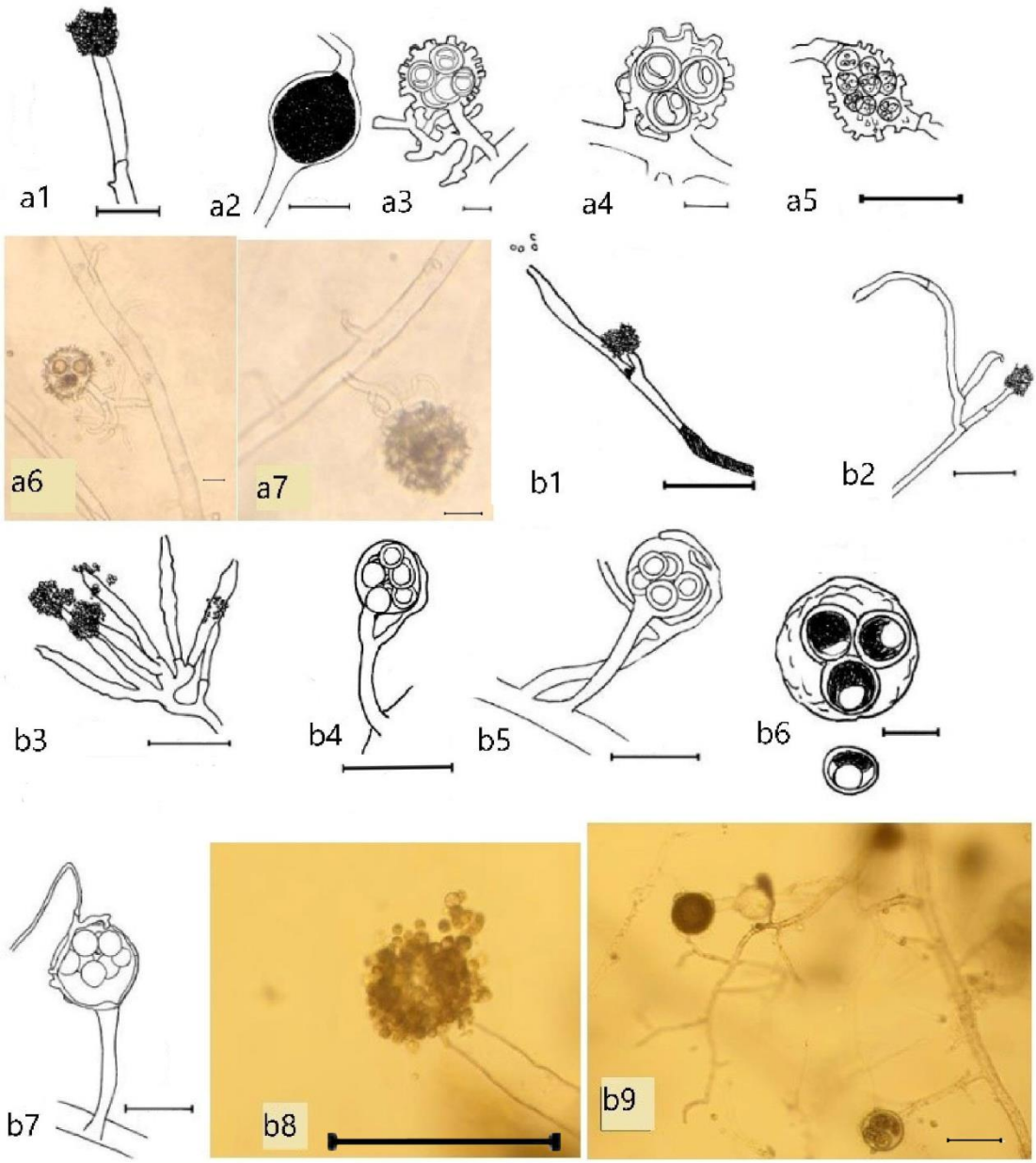

Fig. 4. a. *Newbya recurva* a1. Sporangium, a2. Single intercalary gemmae, a3. Oogonium and eccentric oospores and androgynous antheridium, a4. Oogonium and eccentric oospores and monoclinous antheridium, and a5. Intercalary oogonium and immature oospores. a6 and a7. Oogonium and oospores and androgynous antheridia. b. *Achlya debaryana* b1 & b2. Sporangium renewed basipetalous b3. Sporangium renewed cymosely, b4. Oogonium and androgynous antheridium, b5. Oogonium and monoclinous antheridium, b6. Oogonium and eccentric oospores, and b7. Oogonium and diclinous antheridium. b8. Sporangium and accumulation of cystic zoospores and b9. Oogonia with monoclinous antheridia and multiple hyphae branches. — Scale bars a1 and b1-3 = 250 µm, a2, a5, b4 and b9 = 100 µm, a3, a4, a6, a7 and b6 = 20 µm, b5 and b7 = 70 µm.

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جداسازی و شناسایی قارچ_های آب‌زی (خانواده *Saprolegniaceae*) استان مازندران

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چکیده: گونه های متعلق به اُمیست‌ها بسیاری از مناطق و آشیان های بوم‌شناختی را اشغال کرده‌اند. خانواده *Saprolegniaceae* متعلق به شاخه‌ی *Oomycota*، شامل گونه‌های زیادی از کپک های آب‌زی است که معمولا روی بقایای گیاهان و جانوران رفتار پوده‌رُستی دارند. اعضای برخی از این گونه ها همچنین می توانند بیمارگر گیاهان و ماهی ها باشند. استفاده از DNA برای شناسایی گونه های اُمیست‌ها به خوبی رایج شده است اما بارکد گذاری DNA با زیرواحد یک و دو سیتوکروم اکسیداز سی (*COXI* و *COX II*) روش نسبتا جدیدی است. در این بررسی پس از شناسایی ریخت‌شناختی با کلیدهای معتبر، نواحی ITS-rDNA، *COXI* و *COX II* ۱۰ نماینده از ۵۷ جدایه که نماینده سه جنس و شش گونه (*Achlya bisexualis*، *Dictyuchus monosporus* ، *Saprolegnia ferax*، *S. bulbosa* و *S. debaryana*، *Newbya recurva*) بودند توالی یابی شدند. نتایج به دست آمده در این بررسی این موضوع را تایید می کند که توالی یابی ناحیه‌های *COXI* و *COX II* و داده های ایجاد شده در کنار ناحیه ی ITS نیز می تواند یک منبع مناسب برای رده بندی اعضای خانواده ی *Saprolegniaceae* باشد. طبق این گزارش به غیر از گونه ی *S. ferax*، پنج گونه دیگر برای زیستگان اُمیستی ایران جدید هستند.

کلمات کلیدی: اُمیست‌ها، کپک های آب‌زی، سیتوکروم اکسیداز سی، بیمارگرهای گیاهی، *Saprolegniaceae*

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