Biodiversity of *Aspergillus* species in soils of the National Park of Urmia Lake (NW Iran)

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Abstract

In the present study, nine species of *Aspergillus*, viz., *A. alliaceus*, *A. ochraceus*, *A. persii*, *A. leproris*, *A. parasiticus*, *A. fumigatiaffinis*, *A. sydowii*, *A. niger*, *A. tubingensis* and *Eurotium amstelodami* were obtained and identified from soils of the National Park of Urmia Lake, based on cultural and morphological characteristics. All of the species are newly recorded from hypersaline soils in Iran. Three species, namely, *A. persii*, *A. fumigatiaffinis* and *A. leproris* represent new records to the mycobiota of Iran. An identification key is also provided for *Aspergillus* species in soils of the National Park of Urmia Lake.

Keywords: *Aspergillus*, extreme environment, National Park of Urmia Lake, saline soils

تنوع زیستی گونه‌های *Aspergillus* در خاک‌های پارک ملی دریاچه ارومیه*

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خلاصه

در این مطالعه، تعداد نه گونه از جنس *Aspergillus* شامل: *A. alliaceus*، *A. ochraceous*، *A. persii*، *A. leproris*، *A. parasiticus*، *A. fumigatiaffinis*، *A. sydowii*، *A. niger*، *A. tubingensis* و *Eurotium amstelodami* که از نمونه‌های خاک از پارک ملی دریاچه ارومیه جمع‌آوری و جداسازی شده بودند، شناسایی شدند. شناسایی گونه‌ها براساس ترکیب صفات ریخت‌شناختی ماکروسکوپی و میکروسکوپی انجام گرفت. تمامی گونه‌ها برای نخستین بار از خاک‌های شور در ایران گزارش می‌شوند. از میان گونه‌های شناسایی شده، سه گونه *A. leproris*، *A. fumigatiaffinis* و *A. persii* به عنوان گونه‌های جدید برای میکوبیوتای ایران گزارش و توصیف می‌شوند. همچنین، کلید شناسایی برای گونه‌های *Aspergillus* جدا شده از خاک‌های پارک ملی دریاچه ارومیه ارایه شده است.

واژه‌های کلیدی: پارک ملی دریاچه ارومیه، خاک‌های شور، محیط‌های افراطی، *Aspergillus*

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Introduction

The genus *Aspergillus* was first described almost 300 years ago by Micheli in 1729 (Klich 2002, Hocking 2006). Since its description, it has become one of the best-known and most studied mold groups. Species of the this genus are important micro-organisms with respect to their positive impact as fermentation agents as well as their negative impact as degraders of agricultural products, their toxicity and pathogenicity (Klich 2002, Pitt & Samson 2007, Gregory *et al.* 1997, Suhail *et al.* 2007). Aspergilli are considered as common food spoilage fungi as well as major producers of toxic secondary metabolites on agricultural products. In addition, a large number of aspergilli are opportunistic pathogens of animals and humans (Krijgsheld *et al.* 2013, Varga *et al.* 2011).

Species of *Aspergillus* have a ubiquitous distribution, occurring on decaying vegetation, soil and dust worldwide (Dyer & O'Gorman 2012). They are most often found in terrestrial habitats and are commonly isolated from soil (Carroll & Wicklow 1992). The cosmopolitan distribution of aspergilli in diverse ranges of ecological niches is mainly attributed to their neutral reaction to abiotic growth conditions as they are not very selective in this respect. *Aspergillus* species have evolved a myriad of metabolites to adapt to the variety of niches they inhabit and they are not very selective with respect to abiotic growth conditions. For instance, they can grow over a wide range of temperature (6–55° C) and at relatively low humidity (Krijgsheld *et al.* 2013).

Studies on the occurrence of fungi in salterns have indicated that *Aspergillus* and *Penicillium* species are dominant among the mycobiota adapted to saline niches (Gunde-Cimerman *et al.* 2005) and tolerance for high salt concentrations is a characteristic, recognized for many *Aspergillus* species (Tresner & Hayes 1971).

In fact, *A. penicilloides* is among the well known xerophilic fungi (Krijgsheld *et al.* 2013). The most important halotolerant *Aspergillus* species are *A. niger*, *A. sydowii* and *Eurotium amstelodami* (Gunde-Cimerman *et al.* 2009); other species such as *A. flavus*, *A. tubingensis*, *A. candidus*, *A. fumigatus*, *A. melleus*, *A. niger*, *A. ochraceus*, *A. sydowii*, *A. terreus*, *A. sclerotiorum*, *A. versicolor* and *A. wentii* have been frequently isolated from the salterns (Gunde-Cimerman *et al.* 2005).

The National Park of Urmia Lake is a protected area with unique ecosystem, owing to variable ranges of salinity and pH in its soil. EC of soils in this region varies between 0.9–68 dS/m (Khara & Mayvan 2002, Samadi *et al.* 2013). This study was aimed to explore the biodiversity of *Aspergillus* species inhabiting hypersaline soils of the National Park of Urmia Lake in Iran.

Materials and Methods

- Description of the location and the sampling sites

Soil samples were collected at 10–15 cm depth from soils of the National Park of Urmia Lake islands and coastal areas of Urmia Lake, Iran, during 2011–12 (Fig. 1). Isolations were subsequently made by using soil dilution plate and Warcup soil plate methods on MEA (Malt Extract Agar), GPY (Glucose Peptone Yeast Extract Agar) and PDA (Potato Dextrose Agar) culture media containing 0–30% NaCl. Pure cultures were established using a single spore technique.

- Morphological analysis

For macro-morphological observations, isolates were cultivated on Czapek yeast (autolysate) agar (CYA), Czapek-Dox (Cz) agar, Yeast extract sucrose (YES) agar, oatmeal agar (OA) (medium compositions according to Samson *et al.* 2004) and MEA. The isolates were inoculated at three points on each plate of each medium and incubated at 25° C and 37° C in the dark for seven days. Colony growth characteristics were recorded on 7-day-old colonies. For microscopic observations, mounts were made in lactic acid from MEA colonies; a drop of alcohol was added to remove air bubbles and excess conidia. For micro-morphological examination light microscopy (Olympus BH2) was employed. Photographs were captured using a light Olympus-BX41 microscope with an Olympus digital camera system (DP 25) and software to analyze photographs. Cultures were deposited in culture collection Applied and Industrial Mycology department (DTO) at CBS Fungal Biodiversity Centre, The Netherlands.

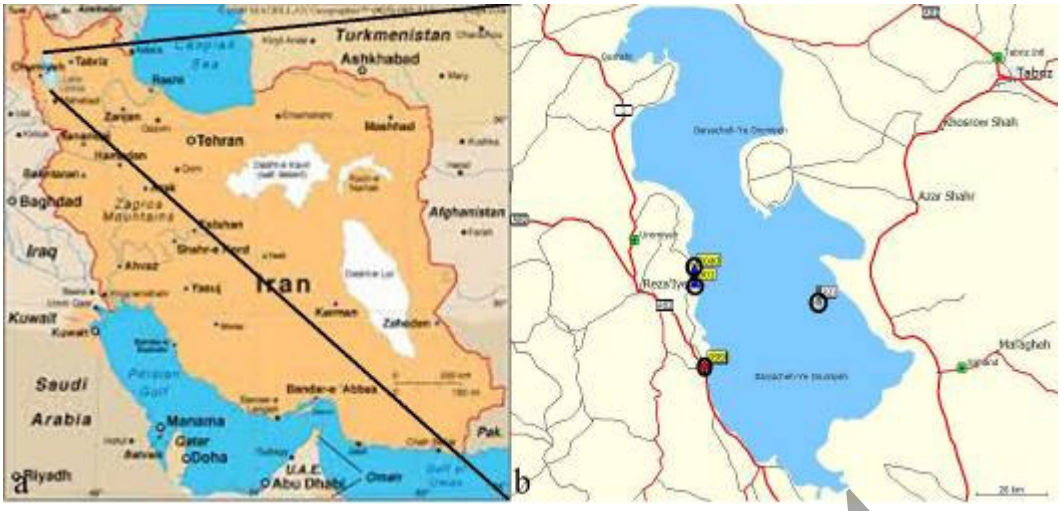

Fig. 1. Map of Iran (a) and the National Park of Urmia Lake (b), showing the sampling sites.

Table 1. List of *Aspergillus* species isolated from soil samples in the National Park of Urmia Lake in this study

DTO No.*	Name	Taxonomy	Locality
DTO 204-C5	*Eurotium amstelodami*	*Aspergillus* sect. *Aspergillus*	Kabodan Island, Iran
DTO 203-G9	*Aspergillus alliaceus*	*Aspergillus* sect. *Circumdati*	Kabodan Island, Iran
DTO 203-E2	*Aspergillus ochraceus*	*Aspergillus* sect. *Circumdati*	Mahabad road (seaside), Iran
DTO 203-B7	*Aspergillus persii*	*Aspergillus* sect. *Circumdati*	Jaddeh Darya (seaside), Iran
DTO 203-B8	*Aspergillus persii*	*Aspergillus* sect. *Circumdati*	Jaddeh Darya (seaside), Iran
DTO 203-B9	*Aspergillus persii*	*Aspergillus* sect. *Circumdati*	Jaddeh Darya (seaside), Iran
DTO 203-C1	*Aspergillus persii*	*Aspergillus* sect. *Circumdati*	Jaddeh Darya (seaside), Iran
DTO 203-D6	*Aspergillus persii*	*Aspergillus* sect. *Circumdati*	Mahabad road (seaside), Iran
DTO 203-H5	*Aspergillus persii*	*Aspergillus* sect. *Circumdati*	Kabodan Island, Iran
DTO 204-C3	*Aspergillus persii*	*Aspergillus* sect. *Circumdati*	Kabodan Island, Iran
DTO 203-D8	*Aspergillus alliaceus*	*Aspergillus* sect. *Flavi*	Aspear Island, Iran
DTO 203-D4	*Aspergillus leporis*	*Aspergillus* sect. *Flavi*	Aspear Island, Iran
DTO 203-D9	*Aspergillus leporis*	*Aspergillus* sect. *Flavi*	Aspear Island, Iran
DTO 203-E1	*Aspergillus leporis*	*Aspergillus* sect. *Flavi*	Aspear Island, Iran
DTO 203-C4	*Aspergillus parasiticus*	*Aspergillus* sect. *Flavi*	Aspear Island, Iran
DTO 203-H7	*Aspergillus parasiticus*	*Aspergillus* sect. *Flavi*	Kabodan Island, Iran
DTO 203-E3	*Aspergillus fumigatiaffinis*	*Aspergillus* sect. *Fumigati*	Aspear Island, Iran
DTO 203-E4	*Aspergillus fumigatiaffinis*	*Aspergillus* sect. *Fumigati*	Kabodan Island, Iran
DTO 203-H2	*Aspergillus fumigatiaffinis*	*Aspergillus* sect. *Fumigati*	Kabodan Island, Iran
DTO 204-F4	*Aspergillus fumigatiaffinis*	*Aspergillus* sect. *Fumigati*	Kabodan Island, Iran
DTO 204-E9	*Aspergillus fructus*	*Aspergillus* sect. *Nidulantes* (*Versicolores*)	Jaddeh Darya (seaside), Iran
DTO 204-C4	*Aspergillus sydowii*	*Aspergillus* sect. *Nidulantes* (*Versicolores*)	Kabodan Island, Iran
DTO 204-D3	*Aspergillus sydowii*	*Aspergillus* sect. *Nidulantes* (*Versicolores*)	Mahabad road (seaside), Iran
DTO 203-C5	*Aspergillus niger*	*Aspergillus* sect. *Nigri*	Aspear Island, Iran
DTO 203-H4	*Aspergillus niger*	*Aspergillus* sect. *Nigri*	Kabodan Island, Iran
DTO 203-H6	*Aspergillus niger*	*Aspergillus* sect. *Nigri*	Kabodan Island, Iran
DTO 203-H9	*Aspergillus tubingensis*	*Aspergillus* sect. *Nigri*	Kabodan Island, Iran

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Results and Discussion

In total, 27 isolates were obtained from the hypersaline soils of the National Park of Urmia Lake. Based on a combination of cultural and morphological characteristics nine *Aspergillus* species belonging to six sections, namely, *Aspergillus* (*Eurotium amstelodami*), *Circumdati* (*A. alliaceus*, *A. ochraceus* and *A. persii*), *Flavi* (*A. leproris* and *A. parasiticus*), *Fumigati* (*A. fumigatiaffinis*), *Versicolores* (*A. sydowii*) and *Nigri* (*A. niger* and *A. tubingensis*) were identified in the present study. All of the nine species are newly recorded from hypersaline soils in Iran. Three species, namely, *A. persii*, *A. fumigatiaffinis* and *A. leproris* represent new records to the mycobiota of Iran. Description and illustrations for the new species are presented.

1. ***Aspergillus fumigatiaffinis*** S.B. Hong, Frisvad & Samson, Mycologia 97 (6): 1326 (2006)

Macroscopic characteristics: Colonies attaining 32–43 mm diameter after seven days on MEA, floccose, plicate, mycelia white, sporulation limited, conidial mass greyish green (50), soluble pigment absent, exudates absent, reverse pale luteous (11), sclerotia absent (Fig. 2).

Microscopic characteristics: Stipes (3–)5–6(–7) µm in diameter, smooth-walled, aseptate, walls green-pigmented. Conidial heads columnar, vesicles (5–)7–8 (–10) × (5.5–)7–8(–10.5) µm, globose to subglobose, vesicles wall less than 1 µm, green. Conidium-bearing elements uniseriate, phialides (5–)6–7(–9) × 2–3 µm, cylindrical tapering to a distinct collulum, walls smooth, walls green–pigmented. Conidia 2–2.5 µm in diameter, globose to subglobose, smooth-walled, green (Fig. 2).

2. ***Aspergillus leporis*** States & M. Chr., Mycologia 58 (5): 738 (1966)

Macroscopic characteristics: Colonies attaining 42–68 mm diameter after seven days on MEA, floccose to velvety, plicate, colony depth about 2291 µm. Mycelia white, stromata absent, sporulating relatively dense, conidial mass citrine (13); reverse pale luteous (11), soluble pigment absent, exudates colorless, formed on sclerotia. Sclerotia developed on the surface of the colony after 6–8 days, at first appearing as white globular masses, later becoming black columnar masses with white apices, abundant (Fig. 3).

Microscopic characteristics: Mycelium smooth-walled, septate, (2–)3.5–5(–8) µm diameter, club–like mycelium frequently observed, (4–)6.5–8(–12) × (7–)10.5–13(–21) µm. Stipes (300–)381–478 (–880) × (5–) 8–9 (–14) µm, rough and thick-walled (1 µm), aseptate; foot cell asymmetric, stipe and foot cell uncolored to orange. Conidial heads radiate on CY20S, short columnar on CZ, YES, MEA and CYA, vesicles (6–)30–38(–60) × (8–)30–39(–62) µm, globose to subglobose, vesicles wall thickness less than 1 µm, uncolored. Conidium-bearing elements biseriate; the fertile part covering 3/4 upper part of the vesicle; metulae (6.5–)9–10(–13) × (2–)3.5–4.5 (–6) µm, cylindrical, walls smooth, uncolored; 2–5 phialides formed on each metulae. Phialides (7–)8–9 (–11.5) × 2–3 µm, cylindrical, with distinct collula, walls smooth, uncolored; conidia (2–)2.5–2.7(–3) × (2–)2.4–2.6(–3) µm in diameter, globose to subglobose, smooth-walled, yellow (Fig. 4).

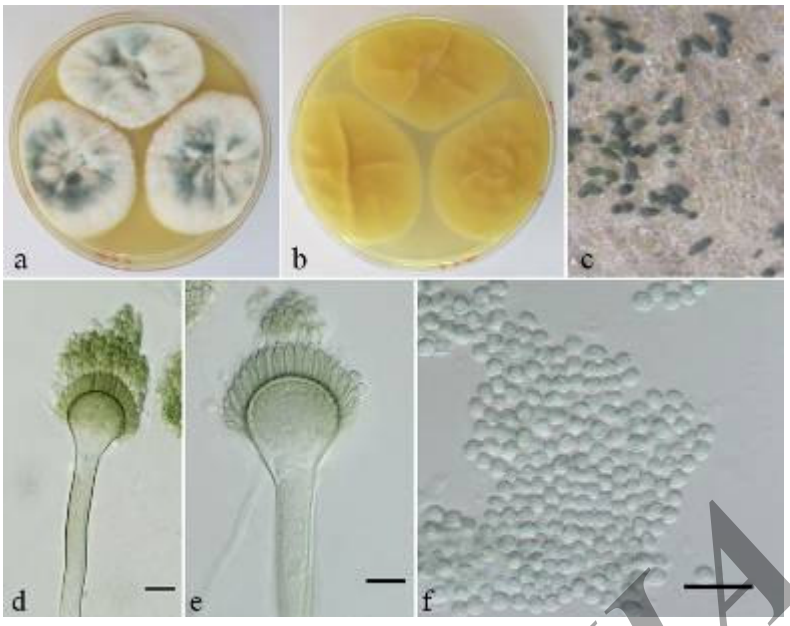

Fig. 2. *Aspergillus fumigatiaffinis*: a, b. Colony on MEA, c-d. Conidial head, e. Conidiophore, f. Conidia (Bar = 10 µm).

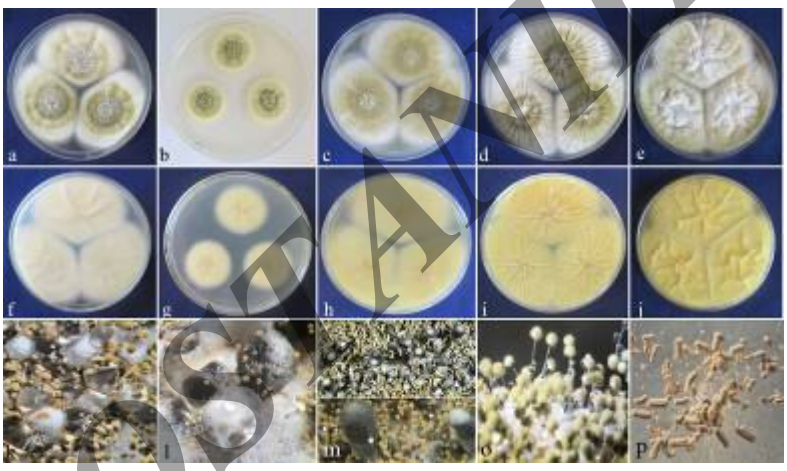

Fig. 3. *Aspergillus leporis*: Colonies after 7 d at 25° C. a, f. CYA, b, g. MEA, c, h. CZ, d, i. YES, e, j. CY20S, k. Exudates droplets, l. Globular sclerotia after 7 d m. Columnar sclerotia after 25 d on CY20S at 25° C, o. Conidial heads on CY20S, p. Conidial heads on YES.

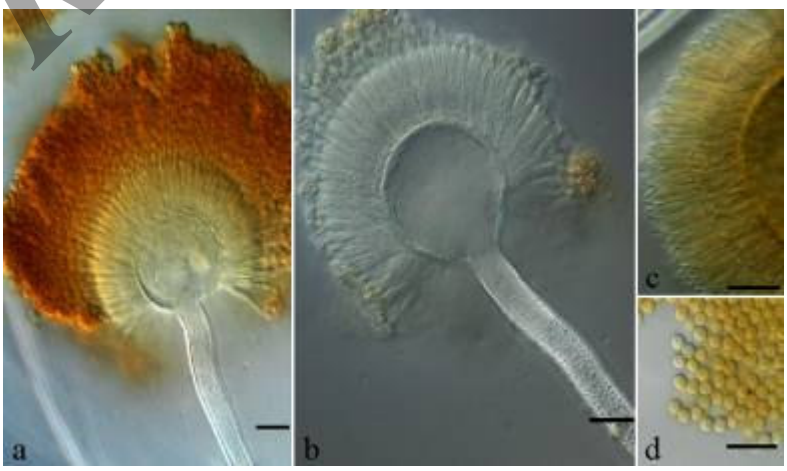

Fig. 4. *Aspergillus leporis*: a. Conidial heads, b. Conidiophores, c. Conidium-bearing elements, f. Conidia (Bar = 10 µm).

3. *Aspergillus persii* A.M. Corte & Zotti, Mycotaxon 83: 276 (2002)

Macroscopic characteristics: Colonies attaining 40–43 mm diameter after seven days on MEA, floccoseplan with few sulcate. Mycelia white, sporulating relatively sparse, conidial mass pure yellow (14), in reverse pale luteous (11), soluble pigment absent; sclerotia formed as white cottony tufts, turned to straw color (46) with age (after 3 weeks), uncolored exudates after two weeks produced on the surface of sclerotia (Fig. 5).

Microscopic characteristics: Stipes (450–)753–945 (–1300) × (5–)6–7(–9) µm, rough and thick-walled (1 µm), aseptate, yellow; foot cell in two forms: symmetric or asymmetric. Conidial heads firstly loosely radiate, after 12 days split into divergent segments, vesicles (14.5–)20–23(–32) × (22–)30–34(–42) µm, globose to subglobose, vesicle wall thickness 1 µm, yellow. Conidium-bearing elements biseriate or rarely uniseriate, the fertile part covering 3/4 upper part of the vesicle, metulae (6.5–)11–13(–18) × (3–)2–6(–7) µm, cylindrical, walls rough, yellow, Phialides (7–)8–9(–11) × 2–5 µm, cylindrical tapering to a distinct collulum, walls smooth, yellow. Conidia 2–3 µm in diameter, globose, smooth-walled, yellow (Fig. 6). Club-like enlargements 20–6 × 10–3.5 µm were formed on the mycelium.

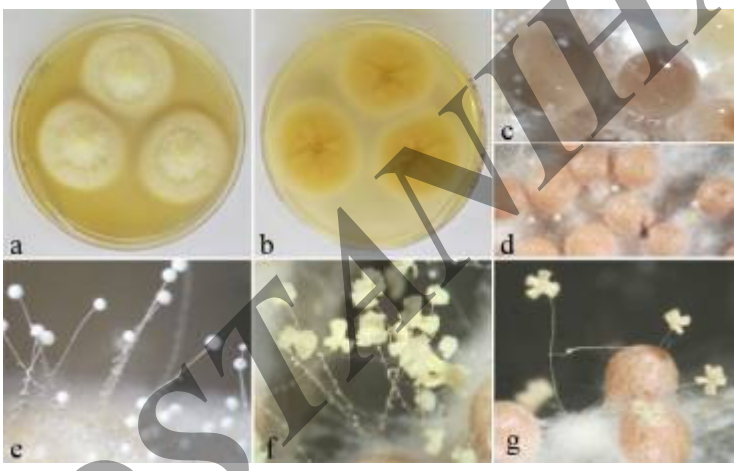

Fig. 5. *Aspergillus persii*: a, b. Colonies after 7 d at 25° C MEA, c. Exudates, d. Sclerotia, e, g. Conidia heads.

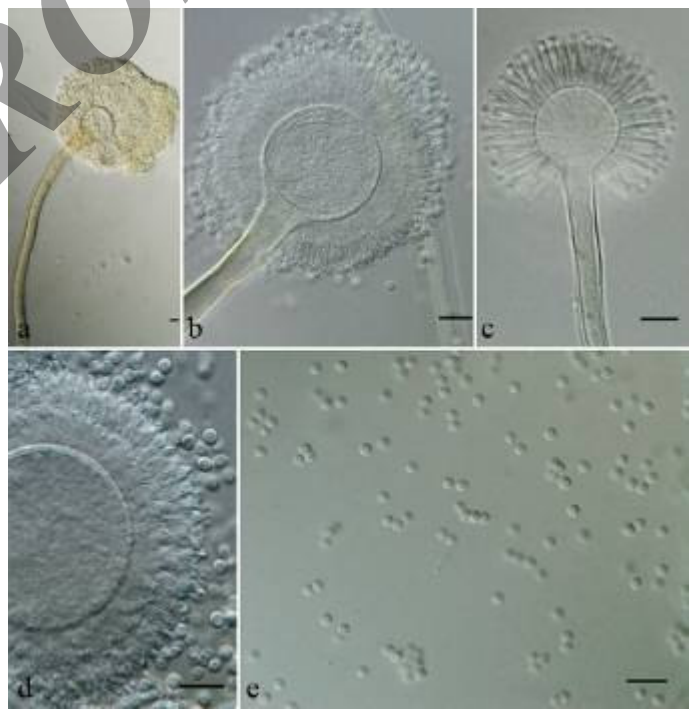

Fig. 6. *Aspergillus persii*: a-c. Conidiophores and aspergilla, d. Conidium-bearing elements, h. Conidia (Bar = 10 µm).

Identification key to the species of *Aspergillus* from soils of the National Park of Urmia Lake

Aspergilli uniseriate/biseriate ……. 1

Aspergilli biseriate ……... (subgenus ***Nidulants***) 2

1. Strictly uniseriate ……. 3

Uniseriate or biseriate ……. (subgenus ***Circumdati***) 4

2. Conidial head radiate, columnar with age, white to vinaceous buff; conidia smooth, thin-walled; cleistothecia absent; think-walled hyphal element or elongated hull cells present …….. section ***Flavipedes***

Conidial head radiate to loosely columnar, green to green and white, conidia mostly echinulate, sclerotia and globose to subglobose hulle cells some time present …… (section ***Versicolores***) 14

3. Conidial head clavate with spore masses splitting at maturity, in blue-green shades; vesicles strongly clavate …… subgenus ***Clavati***

Conidial head radiate to columnar, variable in color, vesicles globose to subclavate or turbinate ……. 5

4. Conidia black or white color …….. 8

Conidia not black or white color …… 9

5. Conidial head radiate to loosely to definitely columnar; osmophilic; cleistothecia present or absent …….. (subgenus ***Aspergillus***) 6

Conidial head radiate or compactly columnar, blue-green, fawn, not osmophilic, cleistothecia present or absent …… (subgenus ***Fumigati***) 7

6. Conidial head radiate, cleistothecia present, ascospore with convex surfaces rough …… ***A. amstelodami***

Conidial head loosely to definitely columnar, clistotheca absent ……. section ***Restricti***

7. Conidia grey green blue, colony white with center dull green …… ***A. fumigatiaffinis***

Conidia light orange to orange-gray …… section ***Cervini***

8. Conidia black or near black, conidiophore up to 3 mm in length …….. ***A. niger***

Conidia white or near white ……. section ***Candidia***

9. Conidial head yellow green, olive-brown or brown, conidiophore colorless, phialid uniseriate or biseriate, sclerotia red brown-purple brown or black at maturity …….. (section ***Flavi***) 10

Conidial head pale yellow, orange-yellow, buff or ochraceus, conidiophore pale yellow to brown, phialid biseriate, sclerotia varying in form and color ……. (section ***Circumdati***) 12

10. Conidial head radiate to loosely columnar, dark yellow green …….. 11

Conidial head tightly columnar and ecru-olive ……. ***A. leporis***

11. Conidial head radiate to very loosely columnar, conidia finely echinulate, phialid predominantly biseriate, sclerotia sometimes present …… ***A. flavus***

Conidial head radiate, conidia predominantly echinulate, phialide always uniseriate, sclerotia absent …….. ***A. parasiticus***

12. Conidial surface smooth and conidia globose to subglobose or ovoid, vesicle globose to subglobose or pyriform …… 13

Conidia surface smooth to finely roughed, conidia globose to broadly ellipsoid, vesicle globose to elongate …….... ***A. ochraceus***

13. Conidia globose, vesicle globose to subglobose …… ***A. persii***

Conidia subglobos to ovoid, vesicle globose to pyriform ……... ***A. alliaceus***

14. Conidial head variable in color, conidiophore hyaline or yellowish ……... ***A. versicolor***

Conidial head always blue-green when young, conidiophore colorless …… ***A. sydowii***

Discussion

The present study aimed to explore biodiversity of the genus *Aspergillus* in soils of the National Park of Urmia Lake. The National Park of Urmia Lake is a protected area with unique ecosystem, owing to variable ranges of salinity and pH in its soil. EC of soils in this region varies between 0.9–68 dS/m (Khara & Mayvan 2002, Samadi *et al*. 2013). Our results revealed a rich diversity of *Aspergillus* species in sampled areas. Contrary to earlier believes, recent studies have shown filamentous fungi as active inhabitants of saline environments such as solar salterns (Gunde-Cimerman *et al.* 2009). Saline environments encompass a heterogenous community of filamentous fungi including members of order *Wallemiales* (*Basidiomycota*), *Capnodiales*, *Dothideales* and *Eurotiales* (*Ascomycota*) (Gunde-Cimerman *et al.* 2009).

Eurotiales represent an important xerotolerant and halotolerant fungal group within *Ascomycota.* Members of this order have been isolated with high frequency from solar saltern, with *Aspergillus* and *Penicillium* as dominant groups (Gunde-Cimerman *et al.* 2005). *Aspergillus* is among the most abundant and widely distributed organisms on earth (Klich 2002). During this study, a rich diversity of *Aspergillus* species was observed in soils of the National Park of Urmia Lake. The *Aspergillus* isolates identified in this study belonged to four subgenera, namely, *Aspergillus*, *Fumigati*, *Nidulantes* and *Circumdati*.

The subgenus *Aspergillus* is characterized by radiate to loosely or definitely columnar conidial head, uniseriate aspergilli, grey green conidia and having a general xerophilic phenotype. Subgenus *Aspergillus* contains two sections *Restricti* and *Aspergillus* (Nagamani 2006, Klich, 2002). Species in section *Aspergillus* are usually characterized by having yellow cleistothecia, lenticular ascospores, uniseriate conidial heads in shades of green or blue, and often yellow-, orange- or red-encrusted hyphae (Hubka *et al.* 2013). *Aspergillus* section *Aspergillus* contains economically important, xerophilic fungi that are widely distributed in nature and the human environment and are known for their ability to grow on substrates with low water activity. *Eurotium* is the teleomorph genus associated with section *Aspergillus* (Hubka *et al.* 2013). Different species of the teleomorphic genus *Eurotium* have often been isolated from natural hypersaline water environments such as Dead Sea and hypersaline waters of salterns around the globe (Gunde-Cimerman 2009). *Eurotium amstelodami* was the only isolated species from this section in the soils of the National Park of Urmia Lake. *Eurotium amstelodami* is shown as one of the most important groups of halotolerant species (Gunde-Cimerman 2009). *Eurotium amstelodami* is the teleomorph state of *Aspergillus amestelodami* and it is characterized by abundant bright yellow cleistothecia, subglobose to globose, imparting a characteristic appearance to the colony; asci subglobose to globose, 8-spored; ascospores valves roughened with irregular ridges, conidia small (4.5–5 × 3.5–4 µm), echinulate (Domsch *et al.* 2007, Nagamani 2006).

Other subgenus with uniseriate aspergilli is subgenus *Fumigati* which is distinguished by uniseriate aspergilli, predominantly pyriform vesicles; grey green, blue green to orange conidia. Subgenus *Fumigati* contains two sections *Cervini*, with light orange to orange-grey conidia, and *Fumigati* (Klich 2002). *Aspergillus* section *Fumigati* includes species which are characterized by uniseriate aspergilli, columnar conidial heads in shades of green and flask-shaped vesicles. From section *Fumigati* anamorphic species *A. brevipes*, *A. duricaulis*, *A. fumigatus*, *A. lentulus*, *A. novofumigatus* and *A. fumigatiaffinis* have been reported from soil (Samson *et al.* 2007). In the present study, *A. fumigatiaffinis* was frequently isolated from soils of the National Park of Urmia Lake. *Aspergillus fumigatiaffinis* represents a new species to the mycobiota of Iran. *Aspergillus fumigatiaffinis* has been reported to occur on other substrates such as kangaroo rat and human. This species is pathogenic to humans. *A. fumigatiaffinis* can be distinguished from the other species in this section based on its comparatively small (sub) globose vesicles; minimum growth temperature at

10° C, and maximum growth temperature below 50° C (Samson *et al.* 2007).

Subgenus *Circumdati* was characterized by uniseriate or biseriate aspergilla and spherical to pyriform vesicles. Subgenus *Circumdati* contains seven sections, namely, *Wentii*, *Flavi*, *Nigri*, *Circumdati*, *Candidi*, *Cremei* and *Sparsi* (Klich 2002). During our study some isolates from four sections *Circumdati*, *Candidi*, *Flavi* and *Nigri* were obtained. *Aspergillus* subgenus *Circumdati* section *Circumdati* and its associated *Neopetromyces* teleomorph are known for production of several mycotoxins including ochratoxin A, penicillic acid, xanthomegnin, viomellein and vioxanthin. Section *Circumdati* contains species with yellow to ochre conidia, predominantly biseriate aspergilli and non-black sclerotia. Several species have been identified in this section using a polyphasic approach including morphological characters, extrolites profile and partial *β-tubulin* sequences (Frisvad 2004). From this section, *A. persii* was isolated this with high frequency from the soils of the National Park of Urmia Lake. This report is a new record for the mycobiota of Iran. *A. persii* has been reported as the causal agent of onychomycosis and nail infection in human (Zotti 2010).

From section *Flavi*; *Aspergillus leporis* and *A. paraiticus* were isolated from soils of the National Park of Urmia Lake. *Aspergillus leporis* can be distinguished from *A. parasiticus* using different morphological characteristics such as its ecru-olive and tightly columnar conidial heads vs green to dark dull yellow-green and loosely radiate conidial heads in *A. parasiticus*; thick-walled vesicles; smooth to finely roughened conidia vs prominently echinulate conidia in *A. parasiticus*; elongate sclerotia vs non-sclerotia in *A. parasiticus*. Also the morphological analysis by Christensen (1981) showed high similarity for *A. parasiticus* and *A. flavus*. *Aspergillus paraiticus* differs from *A. flavus* in its greener color, generally slower growth rate, formation of consistently short conidiophores with subglobose to clavate vesicles which never exceed 35 µm diameter, phialide always biseriate, globose, mostly 4.5 µm diameter, prominently echinulate and yellow conidia, sclerotinia absent (Christensen 1981).

We isolated two species, namely, *A. niger* and *A. tubingensis* from the section *Niger* in the soils of the National Park of Urmia Lake. Section *Niger* contains species with smooth-walled stipes and black or near black conidia (Klich 2002). Members of *Aspergillus* section *Nigri* are distributed worldwide, growing upon a wide variety of substrates (Abarca 2004). Although the main source of black aspergilli is soil, members of this section have been isolated from various substrates. *Aspergillus niger* occurs in soil depths ranging from 0–45 cm, but preferentially in the upper 15 cm and exhibit optimal growth at 3% NaCl (Domsch *et al.* 2007). Similar species, *A. tubingensis* is distinguished molecularly and morphologically but cannot be distinguished from *Aspergillus niger sensu lato* (Domsch *et al.* 2007, Samson *et al.* 2004). We used a set of species-specific primers developed by Khodei *et al*. for molecular identification of *A. tubingensis,* to confirm the identity of these isolates (unpublished data).

From the subgenus *Nidulantes* (section *Versicolores*); *A. sydowii* was isolated from the National Park of Urmia Lake. Section *versicolores* is characterized by conidial heads of radiate to loosely columnar, some shade of green or rarely green and white; uncolored to brown, smooth or roughened conidiophores; ovate to ellipsoidal vesicles, fertile over upper ½ to ¾ of the surface; biseriate phialides; globose to subglobose conidia, rarely elliptical, mostly echinulate; globose to subglobose sclerotia; hulle cells sometimes present (Nagamani 2006).

Previous studies have shown that, he most important halotolerant *Eurotiales* species include *Aspergillus sydowii*, *A. niger*, *Eurotium amstelodami* and *Penicillium chrysogenum* and species in *Emericella*. *Aspergillus versicolor* and *A. sydowii* have also been identified as part of fungal communities in hypersaline environments (Gunde-Cimerman 2009). Therefore, most of the isolated species from the National Park of Urmia Lake belong to xerophilic, halotolerant or halophilic fungi.

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