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Some species of fungi associated with declined Persian oak trees in Ilam province with emphasis on new records to mycobiota of Iran

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Abstract

Zagros vegetation zone is one of the most important forest regions in Iran, which consists of a diverse group of arboreal species, especially oaks (*Quercus* spp.). Ilam province located in west of Iran and in Zagros vegetation zone which has 641000 ha of oak forests that its dominant species is Persian oak (*Q. brantii*). Oak trees decline is a complicated phenomenon that may result from different kinds of agents such as fungi. In order to study on fungi associated with oak trees decline, different parts of symptomatic Persian oak trees were sampled in different regions of Ilam province during the summer and autumn of 2014–15. Fungal species were identified according to either morphological or molecular characteristics obtained from ITS of ribosomal DNA. Eleven species of eight fungal genera were identified that all of them are reported for the first time as Persian oak-associated species. Also three species including *Immersidiscosia eucalypti*, *Petriella sordida*, and *Neocamarosporium obiones* are reported and fully described here as new records to mycobiota of Iran.

Keywords: Morphology, phylogeny, *Quercus* spp., ribosomal DNA, Zagros vegetation zone

برخی قارچ‌های همراه با درختان بلوط ایرانی با علایم زوال در استان ایلام با تاکید بر آرایه‌های جدید برای میکوبیوتای ایران*

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خلاصه

ناحیه رویشی زاگرس به عنوان یکی از مهم‌ترین مناطق جنگلی ایران، در بردارنده گونه‌های درختی مختلفی به ویژه گونه‌های بلوط (*Quercus* spp.) می‌باشد. استان ایلام که در حوزه رویشی زاگرس قرار می‌گیرد دارای جنگل‌های وسیعی بوده که گونه غالب جنگل‌های این ناحیه را بلوط ایرانی (*Q. brantii*) تشکیل می‌دهد. زوال بلوط پدیده‌ای است که می‌تواند در اثر عوامل مختلفی از جمله قارچ‌ها ایجاد شود. به این ترتیب در این مطالعه، به منظور جمع‌آوری و بررسی عوامل قارچی همراه با علایم زوال درختان بلوط در استان ایلام، نمونه‌برداری از مناطق مختلف جنگلی استان ایلام و از اندام‌های مختلف درختان بلوط طی تابستان و پاییز سال‌های ۱۳۹۳ و ۱۳۹۴ انجام پذیرفت. شناسایی گونه‌های قارچی براساس خصوصیات ریخت‌شناختی و اطلاعات توالی حاصل از نواحی ITS از DNA ریبوزومی صورت پذیرفت. به این ترتیب، تعداد ۱۱ گونه قارچی از هشت جنس مختلف قارچی شناسایی شد. گزارش وجود گونه‌های *Immersidiscosia eucalypti*، *Petriella sordida* و *Neocamarosporium obiones* برای فلور قارچی ایران جدید می‌باشد. علاوه‌براین، تمامی گونه‌های قارچی شناسایی شده در این مطالعه، برای نخستین بار از درختان بلوط ایرانی جداسازی و گزارش می‌شوند.

واژه‌های کلیدی: دی.ان.ای ریبوزومی، ریخت‌شناسی، فیلوژنی، مناطق جنگلی، ناحیه رویشی زاگرس

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Introduction

Zagros forest zone is one of the most important forest area in Zagros Mountains located in western parts of Iran and contains about 5.2 billion hectares of Iran's forests area (Jazirehi & Ebrahimi Rostaghi 2013). This ecologically important area is host for a diverse group of plants such as trees and shrubs (Sagheb Talebi *et al*. 2014). Oaks (*Quercus* spp.) are the most dominant tree species present in this area (Sagheb Talebi *et al*. 2014). Among *Quercus* spp., Persian oak (*Quercus brantii*) is more widespread and frequent than other species (Jazirehi & Ebrahimi Rostaghi *l.c*.). Ilam province covers about 641000 ha (10%) of forest regions in Zagros forest zone (Ahmadi *et al*. 2014).

The decline is one of the most important diseases of oak trees around the world especially in Iran that resulted in destruction of oak trees in Zagros forest ecosystems. Oak decline is a complicated phenomenon that is generally caused by a diverse group of biotic and abiotic stresses (Akilli *et al*. 2013). Undoubtedly, biotic stresses play an important role on appearing decline symptoms on their host plants (Akilli *et al*. l.c.). One of the most major groups of organisms affecting the oaks is those fungi, which can basically take into consideration in any oak decline projects.

A brief overview on the literature reveals that, the high numbers of studies have tried to determine the diversity of fungal communities associated with oak decline symptoms worldwide (Kowalski 1996, Bruhn *et al*. 2000, Thomas *et al*. 2002, Ragazzi *et al*. 2003, Kelley *et al*. 2009, Henriques *et al*. 2012, Mirabolfathy 2013, Linaldeddu *et al*. 2014). For instance, the association of fungal species including *Botryosphaeria dothidea*, *Diplodia corticola*, *D. seriata*, and *Neofusicoccum parvum* with evergreen oak (*Q. ilex*), decline has been reported by Linaldeddu *et al*. (2014). Luque *et al*. (2000) have isolated *Biscogniauxia mediterranea*, *Botryosphaeria stevensii*, *Ophiostoma quercus*, *Phomopsis* sp., *Graphium* sp., and *Dendrophoma myriadea* from leaves and stems of cork oak (*Q. suber*). Several species such as *Apiognomonia quercina*, *Colpoma quercinum*, *Diplodia mutila*, and *Phomopsis quercina* have been isolated from *Quercus* spp. in Italy (Ragazzi *et al*. 2003). In investigations on oak trees with decline symptoms in Zagros forests by Mirabolfathi *et al*. (2013) and Mirabolfathi (2013), two species, *Biscogniauxia mediterranea*, and *Obolarina persica* have reported. In addition, several species *viz*. *Cladosporium tenellum*, *Paecilomyces formosus*, *Petriella guttulata*, *Preussia australis*, and *Sordaria sibutii* have also been reported as endophytes of oak trees in Zagros forests (Hajizadeh *et al*. 2015).

There is limited information concerning the fungal communities along with oak trees with decline symptoms in the Zagros forest region. The aims of this study was: a) to collect the fungal isolates accompanying the oak trees with decline symptoms, and b) to identify recovered fungal isolates based on morphology and DNA sequence data, and reconstruct their phylogenetic relationships.

Materials and Methods

- Sampling and isolation of fungi

In different forest regions of Ilam province (western Iran) including Tang-e Dalab, Chagha Sabz, Mehran, Eyvan (Dareh Deraz), and Malek Shahi during the summer and autumn of 2014–15; several symptomatic samples were collected from different parts (stems, leaves and roots) of Persian oak trees, placed in separate paper bags enclosed by sample information (plant tissues, geographic location and date of sampling). In mycological lab, samples were washed under running tap water to remove dusts or other surface contaminations and were subjected to air dry at room temperature for 2–3 h. Plant tissues were cut into small pieces (about 1–2 cm) and woody samples (branches and roots) were disinfested with 1% sodium hypochlorite for 1 min and ethanol 70% for 30 seconds, and then rinsed twice with sterile water. Leaf samples were surface sterilized using ethanol 70% for 1 min and rinsed twice with the sterile water. In order to isolate of fungi, samples were further cut into smaller segments (about 5

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mm) at sterile conditions and paced on 2% Water Agar (2% WA) as well as Potato Dextrose Agar (PDA) and then incubated at 25 °C for 5–7 days. Fungi purification was done by transferring single spore and/or single hyphal tip grown on 2% Water Agar (2% WA) onto Potato Dextrose Agar. Purified isolates were stored on PDA slants at 4 °C for future studies.

- Morphological diagnosis

Morphological species identification was conducted on six general culture media, including Potato Dextrose Agar (PDA), Oat Meal Agar (OA), Potato Carrot Agar (PCA), Carnation Leaf Agar (CLA), Rice Straw Agar (RSA), and 2% Water Agar supplemented with oak tissues (2% WA + sterile host material including leaves and branches). Macro- and micro-morphological characters such as colonies tissue, color and diameter, sexual and asexual states characteristics were recorded and compared with the literature (Barron *et al*. 1961, Tanaka *et al*. 2011, Gruyter *et al*. 2013, Woudenberg *et al*. 2013, Verkley *et al*. 2014). Micro-measurements and micrographs were done by using lactophenol and lactophenol-cotton blue slide mounts with Nikon (E600) light microscope. All identified species have been deposited at the Microbial of Agriculture Biotechnology Research Institute of Iran Culture Collections (ABRIICC).

- DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted according to the protocol described by Zhong & Steffenson (2001). The PCR amplifications were done with the primer pairs NS1/NS4 for SSU and ITS1/ITS4 for ITS-rDNA (White *et al*. 1990), and LROR/LR5 for LSU (Rehner & Samuels, 1995). The PCR was performed in a final volume of 25 µl containing 17.85 µl deionized water, 2.5 µl PCR buffer 10X (Sinagene, Iran), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.75 U of Taq DNA polymerase (Sinagene, Iran), 0.2 pmol of each primer and 10–30 ng/µl DNA template. Conditions for each genomic region consisted of an initial denaturation of 4 min at 94 °C followed by 35 cycles denaturation of 50 s at 94 °C, annealing of 50 s at 57 °C and elongation of 50 s at 72 °C, with a final elongation step of 10 min at 72 °C. The PCR products were visualized on a 1% agarose gel to validate the presence and size of amplicons. The PCR products were purified and sequenced by Macrogen Corporation (South Korea).

- Alignment and phylogenetic analysis

Newly generated sequences were observed and edited in BioEdit Ver. 7.2.5 (Hall 1999) and were subjected to BLAST search tool in GenBank nucleotide database. Required sequences for phylogenetic analysis were retrieved from GenBank (Table 1) and multiple sequence alignments were generated using Clustal X software (Thompson *et al.* 1997). Phylogenetic estimates were evaluated using the Maximum Parsimony Analyses (MP) in MEGA 6.0 (Tamura *et al.* 2013). MP analyses were done by using heuristic searches with 1000 random sequence additions and branch swapping with Tree-Bisection-Reconnection (TBR) algorithm, gaps treated as missing data and the reliability of resultant trees was determined by bootstrap values in 1000 replicates (Felsenstein 1985). All sequences in present study were submitted to GenBank nucleotide database and accession numbers have been recorded.

Results and Discussion

Ninety-eight isolates of fungi were obtained from twigs, trunck, leaf and root. Finaly, eleven species belong to eight genera including *Alternaria atra*, *A. infectoria*, *A. consortialis*, *A. molorum*, *Chaetomium globosum*, *Epicoccum nigrum*, *Immersidiscosia eucalypti*, *Kalmusia variispora*, *Petriella sordida*, *Neocamarosporium obiones*, and *Sordaria fimicola* were identified by morphological characteristics and molecular data. *I. eucalypti*, *P. sordida*, and *N. obiones* are reported as new records to micobiota of Iran.

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- Phylogenetic analyses

Ninety-eight isolates were morphologically characterized, and were placed into 11 species belonging to eight genera *viz*. *Alternaria*, *Epicoccum*, *Kalmusia*, *Neocamarosporium*, *Petriella*, *Immersidiscosia*, *Sordaria*, and *Cheatomium*. The PCR amplification of ITS-rDNAproduced 500–570 bp DNA fragments. Multiple alignment of ITS-rDNA sequences of 11 isolates of this study together with the ITS-rDNA sequences of 25 species downloaded from GenBank and *Paecilomyces divaricatus* as the outgroup taxon (Table 1), yielded a 556-characters (nucleotides + gaps) dataset, of which 225, 298 and 276 characters were constant, parsimony uninformative and parsimony informative, respectively. MP analysis of ITS-rDNA confirmed identification of all morphologically identified species (Fig. 1). Phylogenetic analysis of ITS-rDNA revealed all identified species are well-clustered in two highly supported clades belonging to *Dothidiomycetes* and *Sordariomycetes* (Fig. 1).

Toxonomy

Immersidiscosia eucalypti (Pat.) Kaz. Tanaka, Okane & Hosoya, in Tanaka, Endo, Hirayama, Okane, Hosoya & Sato, Persoonia, Mol. Phyl. Evol. Fungi 26: 94 (2011). (Fig. 2)

Colonies on OA yellow to pale orange, with white arachnoid mycelia, 27 mm in diam. after 10 days (Fig. 2a), on PDA 37 mm after seven days; pycnidia developed slightly on 2% WA + oak tissues and RSA media after three weeks, immerged globose to ovoid and commonly unilocular; conidiophores cylindrical, branched and up to 40 µm in length (Fig. 2b); conidiogenous cells holoblastic, hyaline, cylindrical and 1.5–2 × 4–18 µm in size (Fig. 2c-d); conidia cylindrical, hyaline, with a filiform appendage at the both ends, with three transverse septa and 2.5–3 × 14–21 µm in size; apical and basal cells of conidia 2.5–3 µm in length and the assemblage length of two median cells 2.5–3 × 14–21 µm; lengths of apical and basal appendages 7.5–15 µm and 9–15 µm, respectively (Fig. 2e-f).

Note: The genus *Immersidiscosia* is monotypic with only one species, i.e. *I. eucalypti* (http://www.indexfungorum.org), which was first described from dead leaves of *Laurus nobilis*. (Tanaka *et al.* 2011). Although, *Quercus myrsinifolia*, *Eucalyptus* sp., and *Ardisia japonica* are the additional hosts for this species (Tanaka *et al*. 2011). In the present study, this species was isolated from Persian oak leaves with blight symptoms. The closest genus to the *Immersidiscosia* is *Discosia* that can be well distinguished from each other by ITS-rDNA (Fig. 1, clade H). In this study, the GenBank blast searches of LSU region sequences for two isolates; 17RA1 (KY825092) and 17BR1 (KY825093) shows high similarity to *I. eucaliypti* (AB593723) too, (823/823 bp (100%) identity and 819/821 bp (99%) identity, respectively).

Specimens examined: Isolates 17RA1 (ABRIICC 10035) and 17BR1 (ABRIICC 10036), isolated from Persian oak leaves, Ilam province, Tang-e Dalab, N33 42.212 E46 22.739, Sept. 2014 and Oct. 2015, A. Alidadi and S. Karami.

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Table 1. ITS1-rDNA sequences used in phylogenetic analysis

Taxon	Strain	GenBank No.	Reference
Alternaria atra	**91RF2**	**KY751367**	**This study**
A. atra	UAMH 7840	AY625072	Meklin *et al*. 2004
A. consortialis	**45SP**	**KY751366**	**This study**
A. consortialis	CBS 104.31	KC584247	Vu *et al*. 2019
A. infectoria	**18SA**	**KY751365**	**This study**
A. infectoria	CBS 112250	FJ214897	Andersen *et al*. 2009
A. malorum	**112SA**	**KY751368**	**This study**
A. malorum	CBS 900.87	FJ214860	Crous *et al*. 2009
Ascochyta pisi	CBS 122750	KT389477	Chen *et al*. 2015
A. rabiei	CBS 237.37	KT389479	Chen *et al*. 2015
Chaetomium elatum	C29	HM365236	Asgari & Zare 2011
C. globosum	**57SA**	**KY783412**	**This study**
C. globosum	C63	HM365254	Asgari & Zare 2011
C. globosum	CBS 161.52	KM655335	Vu *et al*. 2019
Discosia pseudoartocreas	CPC 21117	KF777161	Crous *et al*. 2013
D. pseudoartocreas	346Jb14	KU516455	Crous *et al*. 2013
Epicoccum nigrum	**30SA1**	**KY783413**	**This study**
E. nigrum	CBS 505.85	FJ426997	Aveskamp *et al*. 2009
E. nigrum	Zbf-S21	KX065012	Li *et al*. 2016
Immersidiscosia eucalypti	**17RA1**	**KY783415**	**This study**
I. eucalypti	KT2191	AB594791	Tanaka *et al*. 2011
I. eucalypti	KT2115	AB594793	Tanaka *et al*. 2011
Kalmusia italica	MFLUCC 13-0066	KP325440	Thambugala *et al*. 2015
K. variispora	**95SA2**	**KY783414**	**This study**
K. variispora	CBS 197.82	JX496053	Verkley *et al*. 2014
K. sarothamni	CBS 116474	KF796676	Zhang *et al*. 2014
Paecilomyces divaricatus	CBS 284.48	MH856344	Vu *et al*. 2019
Petriella sordida	**90SA3**	**KY783417**	**This study**
P. sordida	CBS 297.58	AY882359	Rainer *et al*. 2006
P. setifera	CBS 745.69	AY882350	Vu *et al*. 2019
Neocamarosporium betae	CBS 523.66	FJ426981	Aveskamp *et al*. 2009
N. obiones	**NBR1**	**KY783416**	**This study**
N. obiones	CBS 432.77	GU230752	De Gruyter *et al*. 2012
N. obiones	CBS 786.68	MH859227	De Gruyter *et al*. 2012
Sordaria fimicola	**96SA**	**KY783418**	**This study**
S. fimicola	CBS 508.50	AY681188	Vu *et al*. 2019
S. sibutii	CBS 768.96	AY681180	Vu *et al*. 2019

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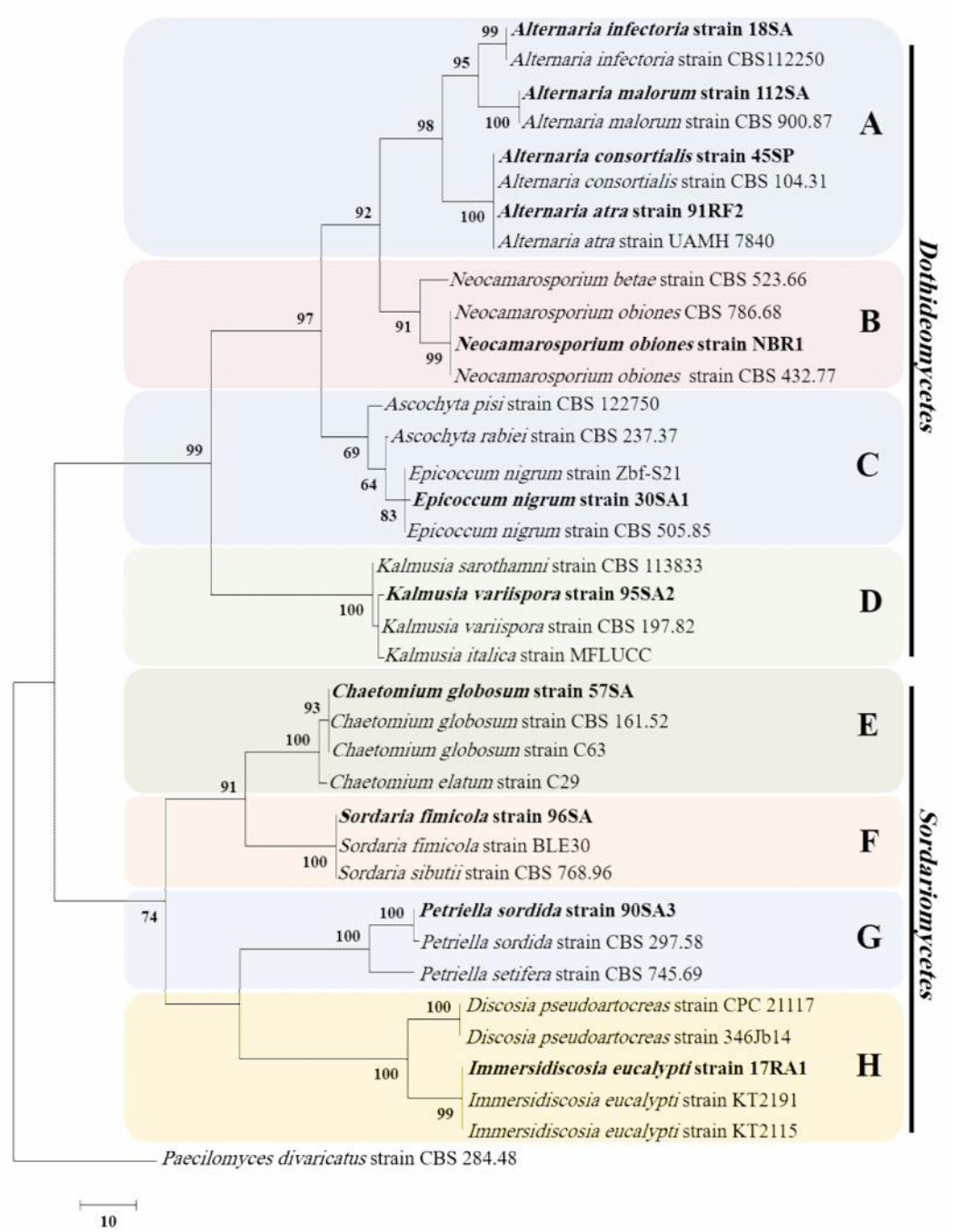

Fig. 1. One of the most parsimonious tree inferred from the ITS rDNA sequences of 31 taxa belong to *Dothideomycetes* and *Sordariomycetes*. The numbers in front of the nodes show the bootstrap values from 1000 replicates. The ITS sequence of *Paecilomyces divaricatus* was used as out group.

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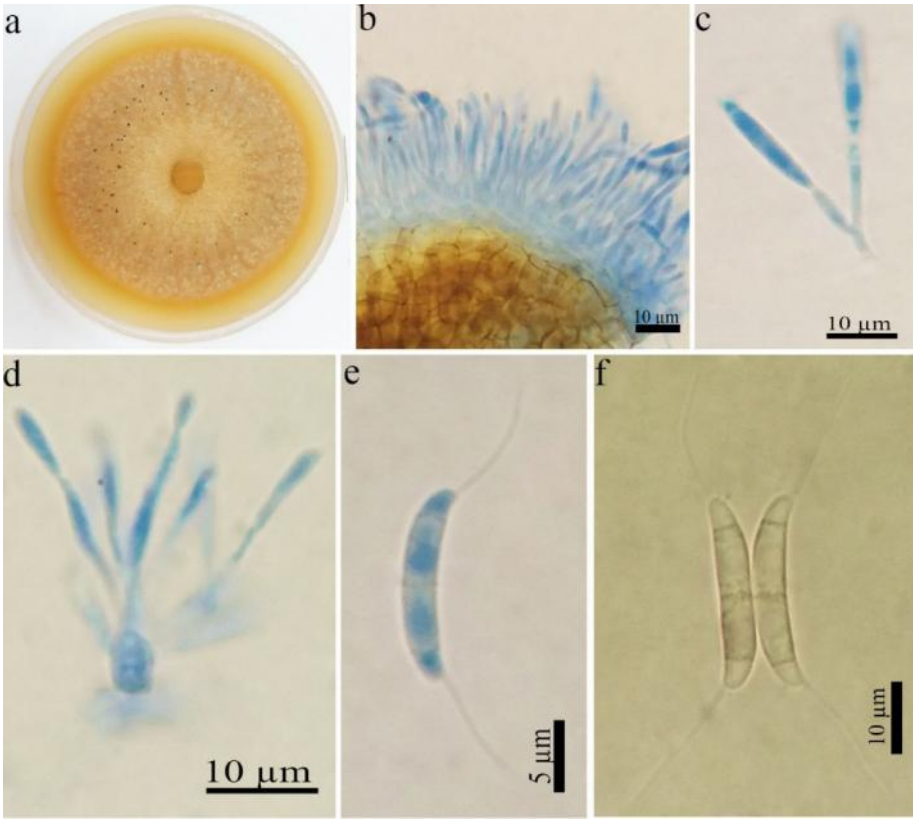

Fig. 2. *Immersidiscosia eucalypti*: a. Colony on OA, b. Conidiomata and conidiophores c-d. Conidiogenous cells, e-f. Conidia.

Kalmusia variispora (Verkley, Göker & Stielow) Ariyawansa & K.D. Hyde, in Ariyawansa, Tanaka, Thambugala, Phookamsak, Tian, Campo, Fungal Diversity 68: 85 (2014). (Fig. 3)

Colonies on PDA cottony, olive green at center, white at margin and 30 mm in diam. after 10 days (Fig. 3a); on OA olive green with white and loose aerial arachnoid hyphae (Fig. 3b). Pycnidia developed on OA three weeks after incubation at 25 °C under 12/12 h nUV photoperiod, mostly spherical, dark brown to black, thick-walled with angular tissues and a distinct ostiole, commonly observed in cross-section as multi-locular and/or sometimes with merged locules and seen as uni-locular (Fig. 2d). Conidiophores simple or branched with integrated conidiogenus cell (Fig. 3e-f). Conidiogenous cells phialidic, cylindrical to oblong at the apex and 4–15 × 2–4 µm in size. Conidia varied in shape, spherical to ovoid, hyaline at beginning then turning to olive brown to pale brown, aseptate and 2.5–3 × 1–1.5 µm in size (Fig. 3g-h), usually exudate out from pycnidia in a black droplet (Fig. 3c).

Note: Verkley *et al*. (2014) have first reported this species on *Erica carnea*, and *Vitis vinifera.* In present study, this species is reported for the first time on Persian oak. This species was originally known as *Dendrothyrium variisporum*. In a multi-gene based study by Ariawansa *et al*. (2014), *D. variisporum* and *D. longisporum* were well clustered with *K. ebuli* in a same clade. ITS-rDNA sequences of the members of this genus are nearly identical and in this study *K. variisporum*, and *K. italica* were grouped in the same sub-clade (Fig. 1, clade d). Ariyawansa *et al*. (2014) separated these species from each other based on analyses of concatenated ITS-rDNA with LSU, SSU and β-tubulin gene sequences. In this study, the LSU regions were amplified and sequenced. The blast searches of the LSU region sequences of this species in GenBank

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(KY825094) revealed 100% identity (873/873 bp) to *K. variiposa* (CBS 121517) as well.

Specimen examined: Isolate 95SA2 (ABRIICC 10037), isolated from Persian oak branches, Ilam province, Chagha Sabz, N33 35.669 E46 26.411, Oct. 2015, A. Alidadi and S. Karami.

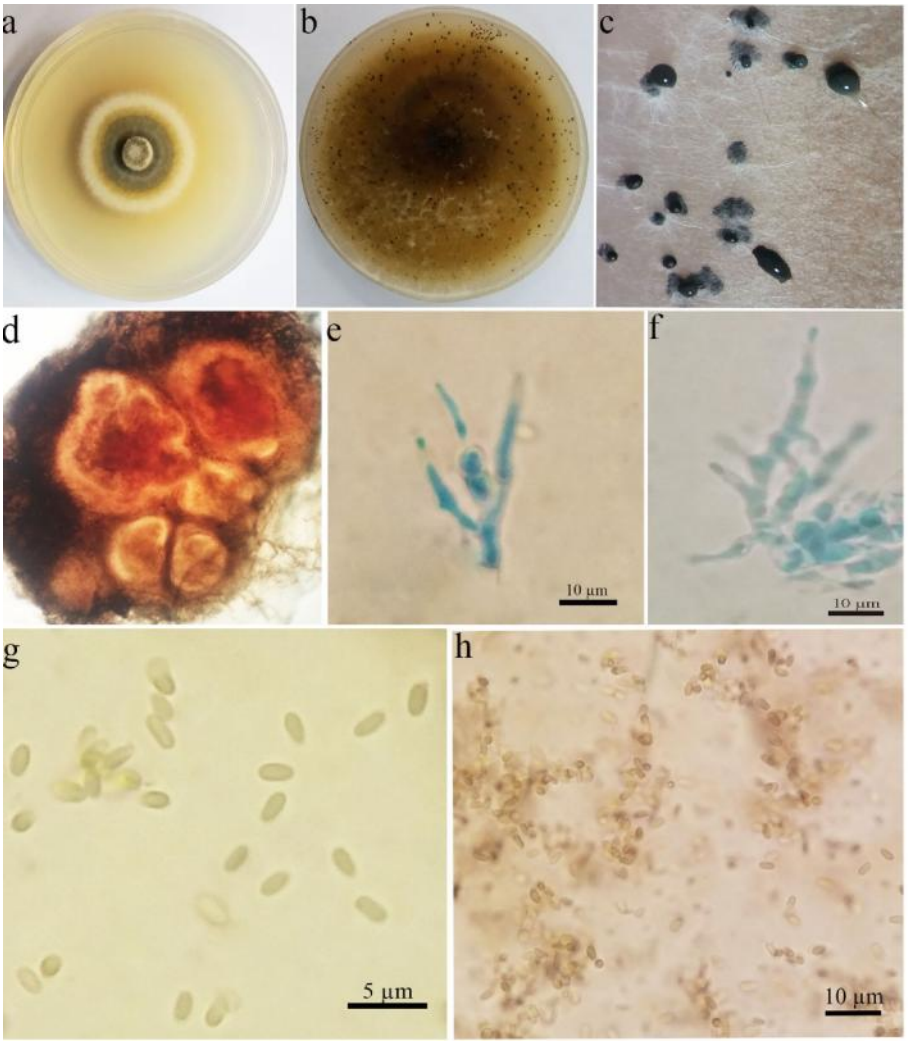

Fig. 3. *Kalmusia variispora*: a. Colony on PDA, b. Colony on OA, c. Pycnidia formed on OA, d. Cross-section of pycnidia, e-f. Conidiogenous cell, g-h. Conidia.

Petriella sordida (Zukal) G.L. Barron & J.C. Gilman, Can. J. Bot. 39: 839 (1961). (Fig. 4)

Colonies on PDA white, flat, slow growing and reaching 40 mm in 30 days (Fig. 4a); on OA 30 mm and on CMA 22 mm. This species apparently forms two different kinds of conidial forms; *Graphium* and *Sporotrichum* state. The *Sporotrichum* state develops on PDA two days after incubation, and includes hyaline and spherical to ovoid conidia with 4–7 µm in size, produced on hyaline, simple and narrow conidiophores which resulted terminally from vegetative hyphae (Fig. 4g). The *Graphium*-like conidial state develops on OA, 7–10 days after incubation as straight and pedicellate synnema, dark brown in color and up to 0.5–0.95 mm in length (Fig. 4h). Conidia one-celled, kidney shape or cylindrical and 2.5–4 × 6–10 µm in size (Fig. 4i). Ascomata were produced on CLA about 14 days after incubation (Fig. 4b). Perithecia black, globuse to subglobuse at the base, covered with brown, septate hairs and with short perithecial neck covered with short hairs. These hairs are

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curly at the base of perithecia (Fig. 4d). Asci clavate, spherical to ovoid, reddish brown with a lot of oil droplets and 4.5–5.5 × 9–11 µm in size (Fig. 4e-f).

Note: The most morphologically closest species to *Petriella sordida* is *P. guttulata*. However, these species are differentiated from each other by oblong ellipsoid conidia with truncate at the base, ascomata without peritecial neck, and spherical, asymmetric and smaller ascospores in *P. guttulata* (Barron *et al.* 1961). This species is reported here for the first time as an endophytic species from trunks of Persian oak trees with decline symptoms and is also new record to mycobiota of Iran.

Specimen examined: Isolate 90SA3 (ABRIICC 10038), isolated from trunks of Persian oak trees, Ilam province, Chagha Sabz, N33 35.737 E46 26.523, Sept. 2014 and Oct. 2015, A. Alidadi and S. Karami.

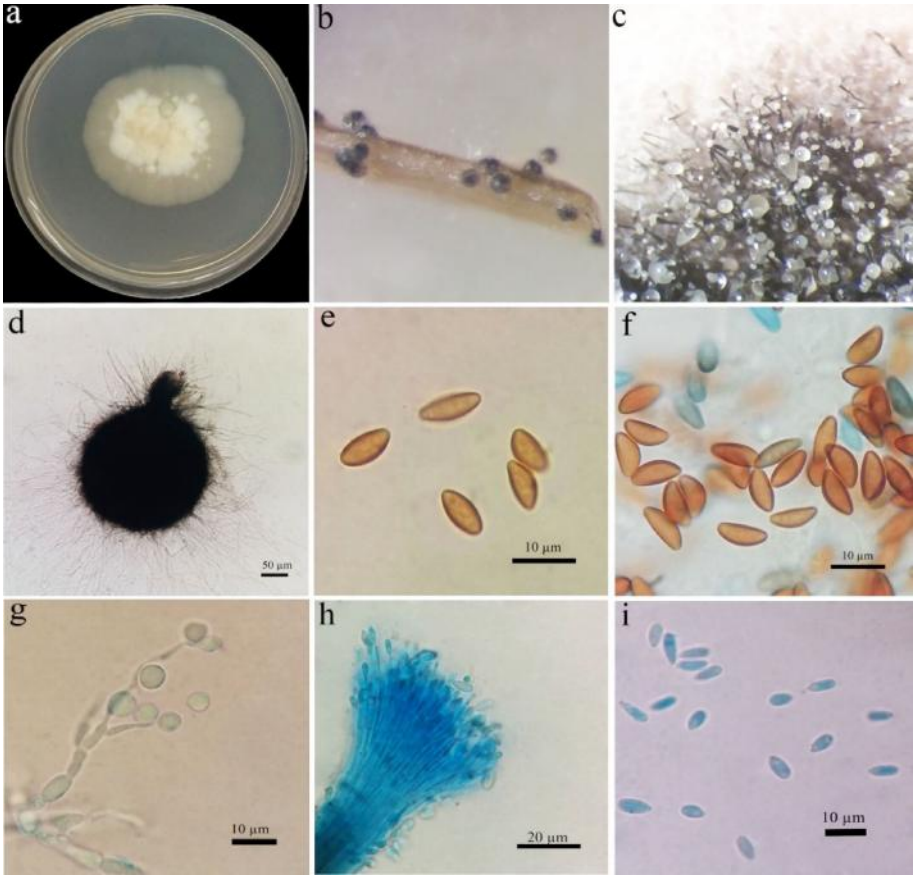

Fig. 4. *Petriella sordida*: a. Colony on PDA, b. Perithecia on CLA, c. Synnema formed on OA, d. Perithecia, e-f. Ascospores, g. Conidiophores and conidia of *Sporotrichum* state, h-i. Synnema and conidia of *Graphiam* state.

Neocamarosporium obiones (Jaap) Wanas. & K.D. Hyde, in Wanasinghe, Hyde, Crous, Wijayawardene, Jeewon, Jones, Bhat, Phillips, Groenewald, Dayarathne, Phukhamsakda, Thambugala, Bulgakov, Camporesi, Gafforov, Mortimer & Karunarathna, Stud. Mycol. 87: 249 (2017) (Fig. 5).

Colonies on MEA cottony, white to gray and 40 mm after 10 days under darkness at 25 °C and (Fig. 5b). Pycnidia developed on PDA under 12/12 h photoperiod nUV at 25 °C seven days after incubation (Fig. 5c-d), ovoid in shape with distinct ostiole covered with mycelial hairs and 250–260 µm in size (Fig. 5e). Pycnidial wall consisted of an outer layer with darkened and thickened pseudoparenchyma cells and a hyaline inner layer including hyaline conidiophores. Conidiphores small, hyaline, produced from pycnidial inner layer, pyriform with a short neck and rarely long, aseptate and 5–10 × 3–4 µm in size (Fig. 5f-g). Conidia single, yellow to pale brown, with 1–2

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transverse septa, ellipsoid to ovoid and 10–13(–15) × 4–5(–6) in size (Fig. 5h-i). Chlamydospores were also produce on vegetative hyphae.

Note: This species was formerly placed in *Ascochyta* (*Didymellaceae*) and known as *A. obiones* that was first described on *Halimione portulacoides* (Dickinson & Morgan 1966). As a result of a phylogenetic study by De Gruyter *et al*. (2013), this species was clustered with *A. hyalospora*, *A. caulina*, and *Phoma betea* in *Pleosporaceae* and was named as *Pleospora halimiones*. The subsequent studies on *Pleosporineae* (*Dothideomycetes*) based on multi-gene phylogenetic analysis showed that the *P. halimiones* clustered with species of *Neocamarosporium* genus in the *Neocamarosporiaceae* family. Thus, the *P. halimiones* is synonymized under *Neocamarosporium obiones* (Wanasinghe *et al.* 2017).

We identified this species according to morphological characteristics as well as phylogenetic analysis based on ITS-rDNA sequences (Fig. 1, clade b-c). In addition, the blast searches of newly generated LSU (KY950252) and SSU (KY950253) sequence were revealed a high identity to *N. obiones* (CBS 432.77) (100% identity). This species is reported for the first time from Persian oak leaves and is also new records to mycobiota of Iran.

Specimen examined: Isolate NBR1 (ABRIICC 10039), isolated from Persian oak leaves, Ilam province, Mehran, N33 32.463 E46 14.996, Sept. 2014, A. Alidadi and S. Karami.

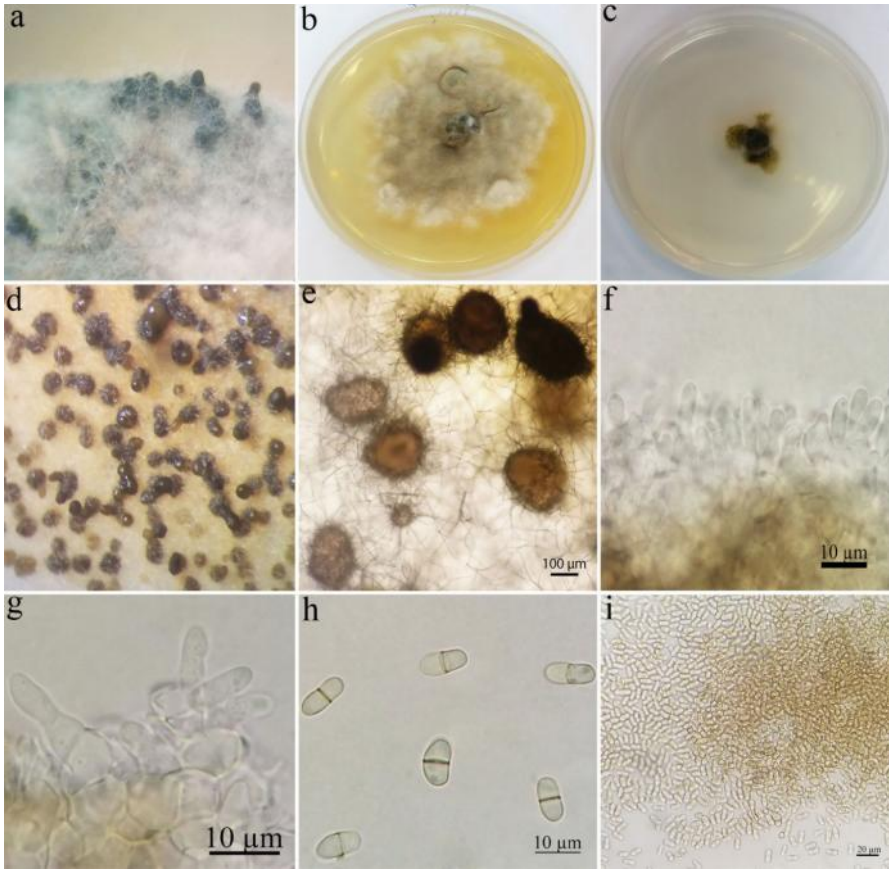

Fig. 5. *Neocamarosporium obiones*: a. Pycnidia on oak leaf surface, b. Colony on MEA, c-d. Pycnidia on PDA surface after seven days, e. Pycnidia with mycelial hairs, f-g. Conidiophores, h-i. Conidia.

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Alternaria atra (Preuss) Woudenb. & Crous, Stud. Mycol. 75(1): 204 (2013). (Fig. 6a-b)

The main morphological characteristics of this species are the production of conidia with golden yellow, pale brown to brown, with warty outer conidial wall, spherical to ellipsoidal shape and with cruciform conidial septa; 1–2 transverse septa and 1–2 longitudinal septa (Fig. 6a-b).

Note: This species was formerly known as *Ulocladium atrum* (Simmons 1998). In a revision by Woudenberg *et al*. (2013), all Alternarioid fungi were kept under a single generic name as *Alternaria* and resulted in the synonymy of genus *Ulocladium* as section *Ulocladioides* within genus *Alternaria*. In the present study, *A. atra* and *A. consortialis* well clustered with the *Alternaria* species in section *Ulocladioides* (100% bootstrap values; Fig. 1, clade a), showing that, the ITS-rDNA sequences as a universal barcode for fungi, has less phylogenetic information within this genus and needed some more genomic loci to accurate species delineation in the genus (Woudenberg *et al*. 2013). This species has frequently been reported from Iran on different substrates (Ershad 2009, Hergholi *et al*. 2015).

Specimen examined: Isolate 91RF2 (ABRIICC 10033), isolated from Persian oak branches, Ilam province, Chagha Sabz, N33 35.723 E46 26.518, Sept. 2014, A. Alidadi and S. Karami.

Alternaria consortialis (Thüm.) J.W. Groves & S. Hughes [as 'consortiale'], in Hughes, Can. J. Bot. 31: 636 (1953). (Fig. 6c, e)

The most important morphological characteristics of this species are the conidia often are produced singly or in short chains (2–3 spores), usually with smooth and rarely verrucous wall surface, 1–5 transverse septa and 1–3 longitudinal septa (Fig. 6d-e).

Note: This species was formerly placed in genus *Ulocladium* as *U. consortiale* (Woudenberg *et al*. 2013). It has transferred to the genus *Alternaria* by the mutli-gene phylogeny, and currently known as *A. consortialis* (Woudenberg *et al*. 2013). This species was first reported as an endophytic fungus of peach and apricot trees in Iran (Hashemlou *et al*. 2015).

Specimen examined: Isolate 45SA (ABRIICC 10032), isolated from Persian oak roots, Ilam province, Chagha Sabz, N33 35.775 E46 26.497, Sept. 2014, A. Alidadi and S. Karami.

Alternaria infectoria E.G. Simmons, Mycotaxon 25(1): 298 (1986). (Fig. 6f-h)

Colonies on PCA were olive at center and white at margin, sporulation at dense clumps of conidia in short to moderate conidial chains in which the first-formed conidia are robust and large (Fig. 6f-h).

Note: *Alternaria infectoria* was first reported by Ghosta *et al*. (2003) from wheat seeds in Iran. In the last revision of the genus *Alternaria*, 27 sections were determined that *A. infectoria* is placed in section *Infectoriae* as the type species (Lawrence *et al*. 2016). In this study, *A. infectoria* was isolated from trunks and branches of Persian oak trees.

Specimen examined: Isolate 18SA (ABRIICC 10030), isolated from trunks of Persian oak trees, Ilam province, Tang-e Dalab, N33 42.188 E46 22.684, Oct. 2015, A. Alidadi and S. Karami.

Alternaria malorum (Ruehle) U. Braun, Crous & Dugan, in Braun, Crous, Dugan & de Hoog, Mycol. Progr. 2(1): 5 (2003). (Fig. 6i-l)

Sporulation abundant from both agar surface and aerial mycelia in long and branched to unbranched spore chains. Conidiophores were short, straight to somewhat curved. Conidia were cylindrical, pale olive, one-celled with smooth outer wall. Ramoconidia were rectangular with 1–3 even darkened and thickened transverse septa (Fig. 6j-l).

Note: This species has been recognized as *Chalastospora gossypii* (Crous *et al*. 2009), and currently is placed in section *Chalastospora* within genus *Alternaria* as *A. malourum* (Woudenberg *et al*. 2013). It has been reported from Iran on barley (Asgari *et al*. 2004).

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However, Hergholi *et al*. (2015) have also reported *A. malorum* as endophyte of *Vitis vinifera*.

Specimen examined: Isolate 112SA (ABRIICC 10028), isolated from Persian oak roots, Ilam province, Tang-e Dalab, N33 51.0220 E46 11.938, Oct. 2015, A. Alidadi and S. Karami.

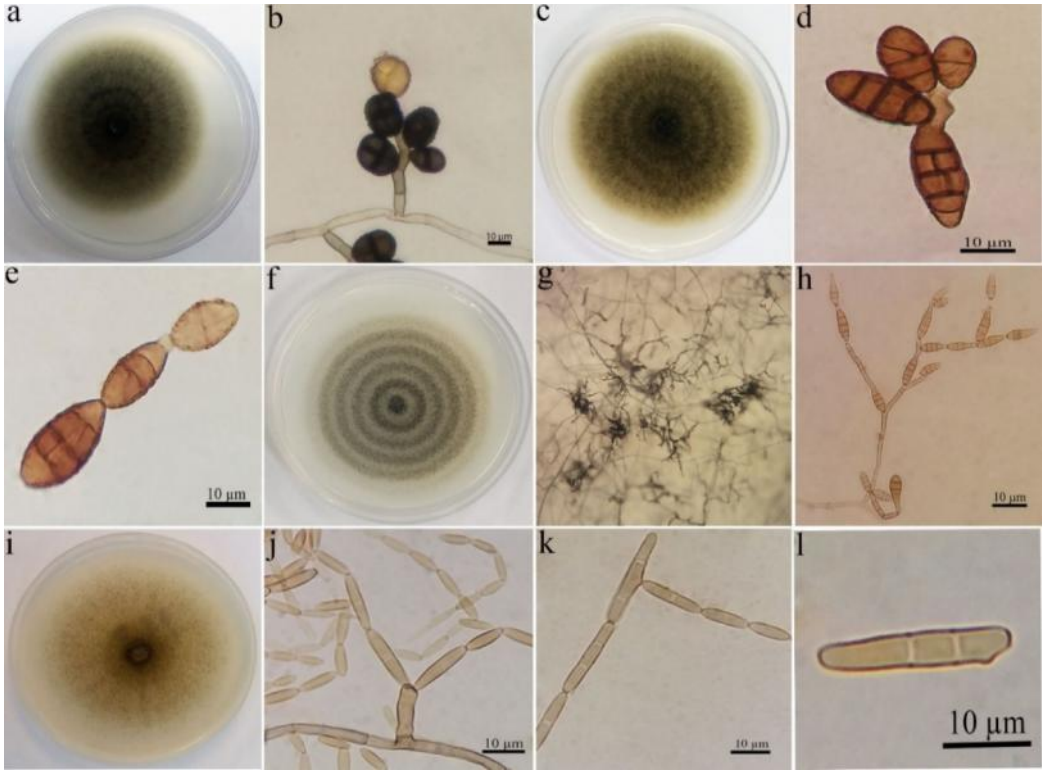

Fig. 6. a-b. *Alternaria atra* (a. Colony on PCA, b. Conidiophores and conidia), c-e. *Alternaria consortialis* (c. Colony on PCA, d. Secondary coniciophores, e. e. Short chains of conidia,, f-h. *Alternaria infectoria* (f. Colony on PCA, g. Sporulation on PCA surface, h. Branching chains conidia), i-l. *Alternaria malorum* (i. Colony on PCA, j-k. Branching chains, l. Ramoconidia).

Chaetomium globosum Kunze, in Kunze & Schmidt, Mykologische Hefte (Leipzig) 1: 16 (1817). (Fig. 7a-c)

Colonies on MEA cottony, yellowish brown at center and white at margin. Perithecia brown, spherical to ellipsoidal and covered with long and covered with long and curly hairs at the upper half of ascomata. Asci hyaline, clavate, with long pedicel and consisting eight lime shape, one-celled and dark brown to brown ascospores (Fig. 7a-c).

Note: This species has been reported from different hosts including cotton, maize and soybean in Iran (Ershad 2009). Based on the literature, this species has only been reported from three oak species *viz. Quercus germana*, *Q. robur*, and *Q. sartorii* (Heredia 1993, Mulenko 2008), and here is reported on Persian oak.

Specimen examined: Isolate 57SA (ABRIICC 10029), isolated from Persian oak branches, Ilam province, Dareh Draz, N33 46.153 E46 22.205, Sept. 2014, A. Alidadi and S. Karami.

Epicoccum nigrum Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin 8: 32 (1816). (Fig. 7d-f).

Colonies on PDA smooth, olive green at center and white at margin. Sporodochia pulvinate and appeared as a black conidial mass on OA. Conidia spherical to pyriform, dark brown with irregular trans- and longitudinal septa (Fig. 7d-f).

Note: *Epiccocum nigrum* is a ubiquitous fungal species and were frequently isolated from a wide variety of

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substrates such as soils, plants, air, animals, foods, textiles etc. It is commonly known as a potential saprobe associating with plant debris decay; however, it can also see as secondary plant pathogen (Ellis 1971, Domsch *et al*. 2007). In Iran, *E. nigrum* has been isolated from peach, apricot and almond trees (Dokhanchi *et al*. 2014), yew trees (Jam Ashkezari *et al*. 2013), grape vine (Hergholi *et al*. 2015), and here is reported on Persian oak.

Specimen examined: Isolate 30SA1 (ABRIICC 10034), isolated from trunks of Persian oak trees, Ilam province, Tang-e Dalab, N33 41.830 E46 24.014, Sept. 2014, A. Alidadi and S. Karami.

Sordaria fimicola (Roberge ex Desm.) Ces. & De Not., Comm. Soc. Crittog. Ital. 1(fasc. 4): 226 (1863). (Fig. 7g-h)

Colonies on PDA white at beginning then subsequently turning to dark brown. Perithecia formed abundantly on PDA. Asci hyaline and 8-spored. Ascospores ellipsoidal, pale yellow when are young and brown to dark brown at maturity, and surrounded by a hyaline gelatinous sheath with a germinating pore at the apex (Fig. 7g-h).

Note: *Sordaria fimicola* is phylogenetically close to *S. sibutii* based on the ITS-rDNA sequences (Fig. 1, clade e). However, *S. sibutii* is differentiated from *S. fimicola* due to its ascospores shape, dimensions and outer wall smoothness as well as lack of hyaline gelatinous sheath surrounding the ascospores (Huhndorf *et al*. 2004, Cai *et al*. 2006). *Sordaria fimicola* has been described as endophyte of *Q. ilex* in Spain (Collado *et al*. 1996). *Sordaria sibutii*, the closest species to *S. fimicola*, has been previously reported on *Q. brantii* in Iran (Hajizadeh *et al*. 2015). However, this study is the first report of *S. fimicola* on branches of Persian oak trees with decline symptoms in Iran.

Specimen examined: Isolate 96SA (ABRIICC 10040), isolated from Persian oak branches, Ilam province, Chagha Sabz, N33 35.694 E46 26.449, Oct. 2015, A. Alidadi and S. Karami.

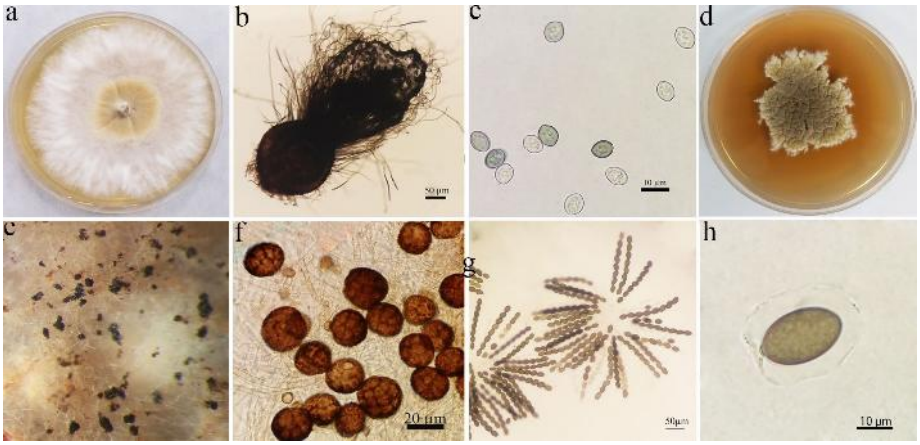

Fig. 7. a-c. *Chaetomium globosum* (a. Colony on MEA, b. Ascomata, c. Ascospors), d-f. *Epicoccum nigrum* (d. Colony on PDA, e. Sporodochium, f. Conidia), g-h. *Sordaria fimicola* (g. Asci, h. Ascospores with gelatin sheath).

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