



## Notes on some endophytic fungi isolated from *Quercus brantii* in Dena region of Kohgiluyeh and Boyer-Ahmad province, Iran

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**Abstract:** In this study, we report some endophytic fungi isolated from twigs of *Quercus brantii* in Dena region of Kohgiluyeh and Boyer-Ahmad province. The twigs were sampled from trees which had basidiocarps of the invasive basidiomycetous polypore fungus *Inonotus krawtzevii* on their trunk. Altogether, ca. 40 pure isolates were obtained. The ITS sequences were obtained for 16 isolates, and their phylogenetic relationships were analysed. Altogether, 13 taxa were identified based on morphological and molecular data. The identified species include: *Alternaria alternata*, *A. cf. arborescens*, *Kalmusia variispora*, *Purpureocillium lilacinum*, *Lentinus tigrinus*, and *Lopodostoma quercicola*. A number of isolates could be identified at generic level (*Coniochaeta*, *Cytospora*, *Diatrype*, *Pyronema*, *Ulocladium*), while the rest of isolates were identified only at family (Phaeosphaeriaceae), and order (Pleosporales) levels. The identified isolates belonged to ascomycetes, except one basidiomycetous fungus *L. tigrinus*. Brief descriptions and microscopic illustrations are given for some isolates.

**Key words:** Fungal endophytes of tree plants, morphology, Persian oak, PlutoF, rDNA ITS, Zagros

### INTRODUCTION

Dena is one of the protected areas in western Iran, and part of its range has been assigned as Dena

Biosphere Reserve in 2010. The area is located in the central Zagros region, in Kohgiluyeh and Boyer-Ahmad province, and has the typical vegetation in Zagros, consisting of open *Quercus* woodlands and angiosperm shrubs, with sporadic gymnosperm stands at higher elevation. The main oak species in the area is *Q. brantii* (Sagheb Talebi et al. 2014, Mozaffarian 2005), which has been subjected to a severe decline in the past years (e.g. Attarod et al. 2016, Hosseini 2011, Mirabolfathi 2013, Ghobad-Nejhad 2016). The possible link between endophytic fungi and oak decline has not been studied in Zagros.

Broadly, “endophytes are microorganisms that spend at least parts of their life cycle inside plants” (Hardoim et al. 2015), and they include fungi and bacteria. In contrast to some conventional definitions which restrict endophytes to a certain lifestyle within only living plant tissues (see Hardoim et al. 2015), this simple definition reflects multifaceted biology of endophytes substantiated by recent empirical studies (Peršoh 2015). It is corroborated that an endophytic species may change its mode of nutrition and function during its life cycle in a response to the host health conditions (Wrzosek et al. 2017, Szink et al. 2016), therefore the taxonomy of the fungus is not necessarily directly linked to its trophic habit. While the most recent count on magnitude of global fungal diversity (Hawksworth & Lücking 2017) estimates 2.2 to 3.8 million species of fungi to be present in the world, other studies approximate the number of endophytic fungi alone to be as high as 0.5–1.3 million species, reflecting their enormous diversity (Sieber 2007, Dreyfuss & Chapela 1994).

Fungal endophytes of native oak stands in the Zagros region have not been adequately studied (Ghobad-Nejhad et al. 2018 provided a short review on published records of fungal endophytes isolated from *Q. brantii*). To know more about the fungal endophytes of damaged oak trees, some fungal endophytes were isolated from twigs of *Q. brantii*. We focused on tree stands which were visibly attacked by the basidiomycetous polypore fungus *Inonotus krawtzevii* (Pilát) Pilát (oak’s *Inonotus*, see <http://www.mycolich.ir/species/3283>, accessed on 30 October 2017). Then, samples were taken from the tree stands with as

identical conditions as possible, as e.g. recommended by Torres et al. (2014) in endophyte studies. Moreover, we aimed to test if this polypore would be present endophytically inside oak twig tissue. The polypore has recently been reported from Iran, and has been shown to cause damage to Zagros oak stands (Ghobad-Nejhad 2016).

## MATERIALS AND METHODS

### Sampling and isolation

Twigs of *Quercus brantii* trees were collected in November 2016, in Dena National Park, Dashtak, in Kohgiluyeh and Boyer-Ahmad Province. Samples were taken from stands infected with visible basidiocarps of the polypore basidiomycete *Inonotus krawtzevii* (see Ghobad-Nejhad 2016 for a full description of this polypore). In the collecting area, five trees were randomly selected, and five twigs from each tree were sampled, and transported to the lab. Twigs were surface-sterilized following Unterseher (2011). From each twig, three segments were cut and put onto Petri dished with PDA medium kept at room temperature. Isolates were checked periodically for about one month and grown mycelia were moved to new dishes. Cultures are preserved at the Iranian Fungal Culture Collection (IRAN...C) at the Iranian Research Institute of Plant Protection, Tehran, Iran. Morphological examination of the cultured isolates and application of additional culture media for stimulating sporulation were done following Hajizadeh et al. (2015).

### Molecular study

Total DNA was extracted using DNA Extraction Mini Kit (YT9030, Taiwan). The ITS region (covering ITS1, 5.8S, ITS2, partial SSU/LSU) was amplified with Super PCR Master Mix (YT1553-5, Taiwan) mainly using the primer pair ITS1F/ITS4 (White et al. 1990; Gardes & Bruns 1993). The parameters for PCR reactions follow Ghobad-Nejhad & Langer (2017). The PCR product was purified and sequenced by Macrogen (Korea, Macrogen Inc.). The obtained DNA sequences were assembled using DNA Baser Sequence Assembler v4, and were submitted to GenBank. Their identification is reported in Table 1, with the accession numbers reported in Table 2.

The dataset for phylogenetic analyses was constructed using the newly obtained sequences together with additional relevant GenBank sequences retrieved from Blastn searches (Altschul et al. 1990), with 98–100 % identity and query coverage, and with particular notion to type strains or generic types, as much as possible. Doubtful and unpublished sequences and the sequences from environmental studies were avoided. To exclude the effect of flanking regions of 28S and 18S regions, the ITS region was partitioned into segments using ITSx (Bengtsson-Palme et al. 2013). Partitions were then blasted with massBlaster against the reference sequences at PlutoF (Abarenkov et al. 2010). The top-most matching sequences of

Species Hypothesis (see Abarenkov et al. 2010) were added to the final ITS dataset.

The sequences were aligned using MUSCLE 3.8 (Edgar 2004), and analyzed using MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003), with the same parameters as used by Ghobad-Nejhad (2016). The outgroup species was *Lentinus tigrinus* AF516518.

## RESULTS

In total, ca. 40 pure isolates were obtained in this study. After morphological studies, DNA was extracted from selected isolates and 16 ITS sequences were obtained (Table 1). Final dataset consisted of 56 taxa and 462 characters from which, 234 characters were informative, and 182 characters were constant.

The Bayesian phylogram resulted from the phylogenetic analysis (using SYM+I+G model of nucleotide evolution as suggested by MrModeltest 2.3 (Nylander 2004)) is shown in Fig. 1.

The identified isolates (except one) belonged to ascomycetes (Pezizomycotina), to the classes Dothideomycetes, Sordariomycetes, and Pezizomycetes, the orders Diaporthales, Hypocreales, Pezizales, Pleosporales, Sordariales, and Xylariales, and the families Coniochaetaceae, Diatrypaceae, Didymosphaeriaceae, Lopadostomataceae, Ophiocordycipitaceae, Phaeosphaeriaceae, Pleosporaceae, Pyronemataceae, and Valsaceae. There was only one basidiomycete fungus *Lentinus tigrinus* (Polyporales, Polyporaceae), while *I. krawtzevii* was not isolated. Several isolates remained as sterile mycelia, with no reproductive organs (Table 1) and these were studied using phylogenetic analysis based on careful taxon sampling as described above. The isolates identified at species level were as following: *Alternaria alternata*, A. cf. *arborescens*, *Kalmusia variispora*, *Lentinus tigrinus*, *Lopadostoma quercicola*, and *Purpureocillium lilacinum*. A number of isolates could be identified at generic level, and they belonged to the genera *Coniochaeta* (Sacc.) Cooke, *Cytospora* Ehrenb., *Diatrype* Fr., *Pyronema* Carus, and *Ulocladium* Preuss. The rest of isolates were identified only at family (Phaeosphaeriaceae), and order (Pleosporales) levels (Table 1). Brief description and microscopic illustrations are given for isolates as much as possible.

### Notes on some taxa

*Alternaria* cf. *arborescens* E.G. Simmons, Mycotaxon 70: 356 (1999). Fig. 2 c-g

The conidia with 1–3 longitudinal septa are much abundant in the isolate K3-4d1. Additional molecular examination would be required in the future to examine it at fine level.

Although the examined isolates of *Alternaria alternata* and A. cf. *arborescens* are morphologically distinct (Simmons 2007, Ellis 1971), their ITS sequences are highly similar and collapsed in polytomy.

**Table 1.** Taxa identified in this study. All strains were obtained from twigs of *Quercus brantii* collected in November 2016 in Dena National Park, Dashtak, in Kohgilouyeh and Boyer-Ahmad Province. Strains in bold are sterile. Classification is based on MycoBank.

Taxon	Classification (class, order, family)	Strain (DNA extraction no.)	IRAN accession numbers
<i>Alternaria alternata</i> (Fr.) Keissl.	Dothideomycetes, Pleosporales, Pleosporaceae	K3-4c (MG318)	IRAN 3013C
<i>A. cf. arborescens</i> E.G. Simmons	same as above	K3-4d1 (MG316)	IRAN 3041C
<i>Coniochaeta</i> sp. 1	Sordariomycetes, Sordariales, Coniochaetaceae	K3-5b (MG325), K3-4b	IRAN 3014C, IRAN 3015C
<i>Coniochaeta</i> sp. 2	same as above	<b>K4-2c</b> (MG323)	IRAN 3016C
<i>Cytospora</i> sp. 1	Sordariomycetes, Diaporthales, Valsaceae	<b>K1-2a</b> (MG322)	IRAN 3017C
<i>Cytospora</i> sp. 2	same as above	K5-1A (MG327)	IRAN 3042C
<i>Diatrype</i> sp.	Sordariomycetes, Xylariales, Diatrypaeceae	K4-3b (MG328)	IRAN 3043C
<i>Kalmusia variispora</i> (Verkley, Göker & Stielow) Ariyawansa & K.D. Hyde	Dothideomycetes, Pleosporales, Didymosphaeriaceae	<b>K1-a</b> (MG319), K3-3a (MG320)	IRAN 3018C, IRAN 3019C
<i>Lentinus tigrinus</i> (Bull.) Fr.	Agaricomycetes, Polyporales, Polyporaceae	<b>K4-2D</b> (MG331)	IRAN 3020C
<i>Lopadostoma quercicola</i> Jaklitsch, J. Fourn. & Voglmayr	Sordariomycetes, Xylariales, Lopadostomataceae	K5-4 (MG326)	IRAN 3044C
<i>Phaeosphaeriaceae</i> sp.	Dothideomycetes, Pleosporales	K4-3c (MG321)	IRAN 3021C
Pleosporales sp.	Dothideomycetes	<b>K2-1a</b> (MG322)	IRAN 3026C
<i>Purpureocillium lilacinum</i> (Thom)	Sordariomycetes, Hypocreales, Ophiocordycipitaceae	K1-5B (MG330)	IRAN 3022C
Luangsa-ard, Houbraken, Hywel-Jones & Samson			
<i>Pyronema</i> sp.	Pezizomycetes, Pezizales, Pyronemataceae	<b>K1-5</b> (MG314)	IRAN 3023C
<i>Ulocladium</i> sp.	Dothideomycetes, Pleosporales, Pleosporaceae	K3-4d3 (MG317), K3-4d2	IRAN 3024C, IRAN 3025C

(Fig. 1), corroborating the known idea that ITS is not the most appropriate marker for discriminating species of *Alternaria*. Future monographic studies on *Alternaria*-like taxa in Iran would apply that.

#### *Coniochaeta* sp. 1 Fig. 3

A phialosporic fungus. Small conidia are formed on denticles or branched conidiophores in heads. This anamorph resembles both *Lecythophora* Nannf. anamorph of *Coniochaeta* (Sacc.) Cooke and *Collophora* Damm & Crous.

We compared the ITS sequence of this isolate with sequences of type species of *Lecythophora* (current name *Coniochaeta*, typified with *C. ligniaria* (Grev.) Cooke) and *Collophora* (typified with *C. rubra* Damm & Crous). As seen in Fig. 1, sequence of the isolate K3-5b is nested in a polytomy composed of *Fimetariella rabenhorstii* (Niessl) N. Lundq. and *Coniochaeta* sp./*Coniochaeta taeniospora*, rooted with *C. ligniaria*, while *C. rubra* found a distant position. Even though we tried to select the reliable sequences, which cannot excluded the possibility of misidentification in these GenBank sequences. For now, we name the isolate K3-5b as *Coniochaeta* sp. 1 (older generic name) until more details become available.

#### *Coniochaeta* sp. 2

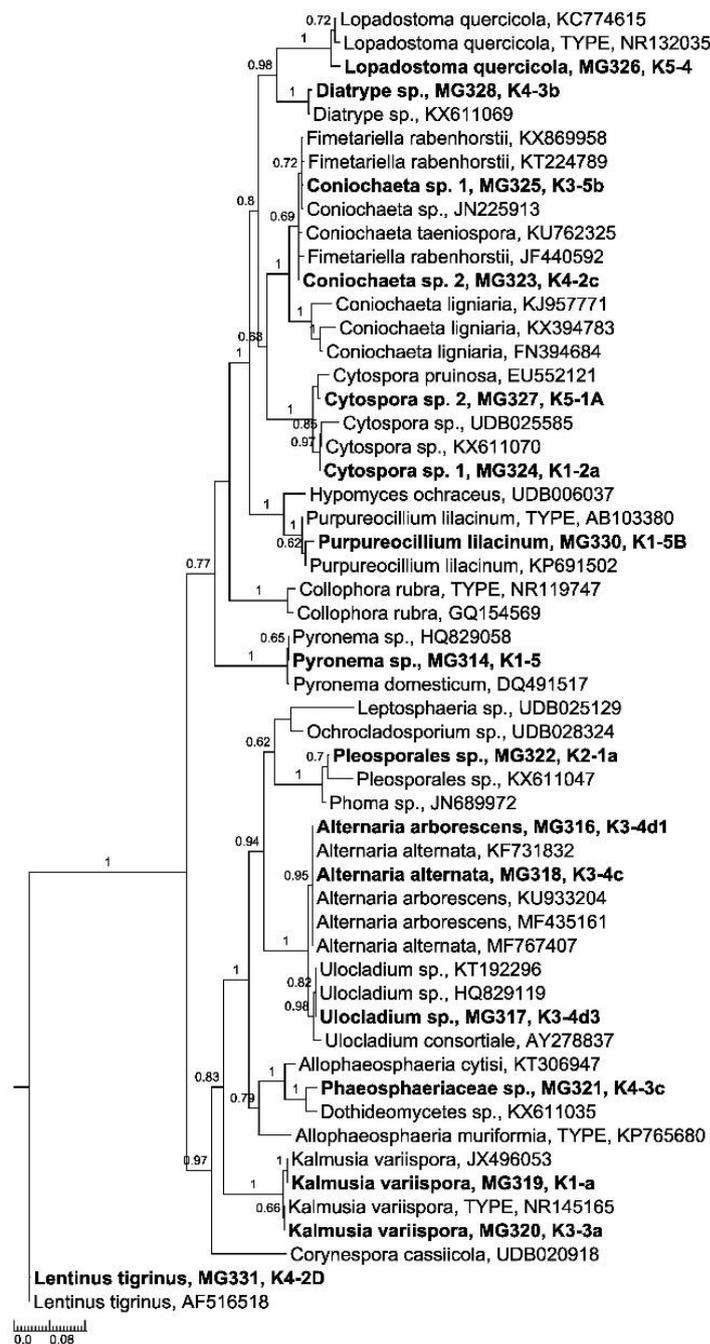
The studied isolate was sterile. Blast searches hit *Fimetariella rabenhorstii* and *Coniochaeta* sp. with high scores (99 % identity over 100 % query coverage). To infer its phylogenetic affiliation, authentic sequences of the generic type of *Fimetariella* N. Lundq. 1964 (i.e., *F. rabenhorstii*) and the type of *Coniochaeta* (i.e., *C. ligniaria*) were added to the dataset. As mentioned above, and shown in Fig. 1, *F. rabenhorstii* and *Coniochaeta* sp./*Coniochaeta taeniospora* do not appear as distinct clades, but their polytomy is outgrouped with *C. ligniaria*. For the time being, we regard the isolate K4-2c as an unidentified isolate of the genus *Coniochaeta*.

*Kalmusia variispora* (Verkley, Göker & Stielow) Ariyawansa & K.D. Hyde, Fungal Diversity 68: 85 (2014). Fig. 4

Colonies dark fuscus to rusty brown, more or less paler at center and at margins, producing long synnemata-like bodies on PDA. Conidiophores light brown, smooth, highly branched, moderately thick-walled, 3–4 µm wide, with numerous septa, producing light brown, smooth, globose to subglobose aleuroconidia or chlamydospore-like bodies in mid-segments or terminally, ca. 5.5–9.8 × 4–9.2 µm.

**Table 2.** List of fungal taxa used in the phylogenetic analysis. Accession numbers in bold were generated in this study. Accession numbers starting with UD were retrieved from UNITE database.

Taxon	Strain	ITS GenBank accession no.
<i>Alternaria alternata</i> (Fr.) Keissl.	BL7	KF731832
<i>A. alternate</i>	CF2.1B	MF767407
<i>A. alternate</i>	K3-4c	<b>MG208004</b>
<i>A. cf. arborescens</i> E.G. Simmons	K3-4d1	<b>MG208002</b>
<i>A. arborescens</i>	ECU113	MF435161
<i>A. arborescens</i>	A43	KU933204
<i>Allophaeosphaeria cytisi</i> Wanasinghe, Camporesi, E.B.G. Jones & K.D. Hyde	MFLUCC 15-0649	KT306947
<i>A. muriformia</i> Ariyaw., Camporesi & K.D. Hyde	MFLUCC 13-0349- (generic TYPE)	KP765680
<i>Collophora rubra</i> Damm & Crous	CBS120873 (TYPE material, generic TYPE)	NR_119747
<i>C. rubra</i>	STEU6416	GQ154569
<i>Coniochaeta ligniaria</i> (Grev.) Cooke	Fan & Guo 1302309 (generic TYPE)	KX394783
<i>C. ligniaria</i>	11G002	KJ957771
<i>C. ligniaria</i>	3836	FN394684
<i>Coniochaeta taeniospora</i> (Sacc.) Friebes, Jaklitsch & Voglmayr	LTA1	KU762325
<i>Coniochaeta</i> sp.	ICMP-18911	JN225913
<i>Coniochaeta</i> sp. 1	K3-5b	<b>MG208011</b>
<i>Coniochaeta</i> sp. 2	K4-2c	<b>MG208009</b>
<i>Corynespora cassiicola</i> (Berk. & M.A. Curtis) C.T. Wei	COAD1110	UDB020918, SH19758907.FU
<i>Cytospora pruinosa</i> (Fr.) Sacc.	CBS119207	EU552121
<i>Cytospora</i> sp.	TU116803	UDB025585, SH20085907.FU
<i>Cytospora</i> sp.	C2	KX611070
<i>Cytospora</i> sp. 1	K1-2a	<b>MG208010</b>
<i>Cytospora</i> sp. 2	K5-1A	<b>MG208013</b>
<i>Diatrype</i> sp.	C1	KX611069
<i>Diatrype</i> sp.	K4-3b	<b>MG208014</b>
<i>Dothideomycetes</i> sp.	A48	KX611035
<i>Fimetariella rabenhorstii</i> (Niessl) N. Lundq.	ARSL-051214-10 (generic TYPE)	KX869958
<i>F. rabenhorstii</i>	VL284	JF440592
<i>F. rabenhorstii</i>	S11	KT224789
<i>Hypomyces ochraceus</i> (Pers.) Tul. & C. Tul.	JF02178	UDB006037, SH19885307.FU
<i>Kalmusia variispora</i> (Verkley, Göker & Stielow) Ariyawansa & K.D. Hyde	K1-a	<b>MG208005</b>
<i>K. variispora</i>	K3-3a	<b>MG208006</b>
<i>K. variispora</i>	CBS121517 (TYPE material)	NR145165
<i>K. variispora</i>	CBS197.82	JX496053
<i>Lentinus tigrinus</i> (Bull.) Fr.	LE(BIN)0861-SBI-5	AF516518
<i>L. tigrinus</i>	K4-2D	<b>MG208016</b>
<i>Leptosphaeria</i> sp.	TU116318	UDB025129, SH19212707.FU
<i>Lopadostoma quercicola</i> Jaklitsch, J. Fourn. & Voglmayr	LG9	KC774615
<i>L. quercicola</i>	CBS134633 (TYPE material)	NR_132035
<i>L. quercicola</i>	K5-4	<b>MG208012</b>
<i>Ochrocladosporium</i> sp.	TU124352	UDB028324
Phaeosphaeriaceae sp.	K4-3c	<b>MG208007</b>
<i>Phoma</i> sp.	R193	JN689972
Pleosporales sp.	A75	KX611047
Pleosporales sp.	K2-1a	<b>MG208008</b>
<i>Purpureocillium lilacinum</i> (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson	44736-03-02-01	KP691502
<i>P. lilacinum</i>	K1-5B	<b>MG208015</b>
<i>Pyronema domesticum</i> (Sowerby) Sacc.	AFTOL ID949	DQ491517
<i>Pyronema</i> sp.	CID007	HQ829058
<i>Pyronema</i> sp.	K1-5	<b>MG208001</b>
<i>Ulocladium consortiale</i> (Thüm.) E.G. Simmons	bmp3151001	AY278837
<i>Ulocladium</i> sp.	K3-4d3	<b>MG208003</b>
<i>Ulocladium</i> sp.	50a1-1	KT192296
<i>Ulocladium</i> sp.	CID225	HQ829119

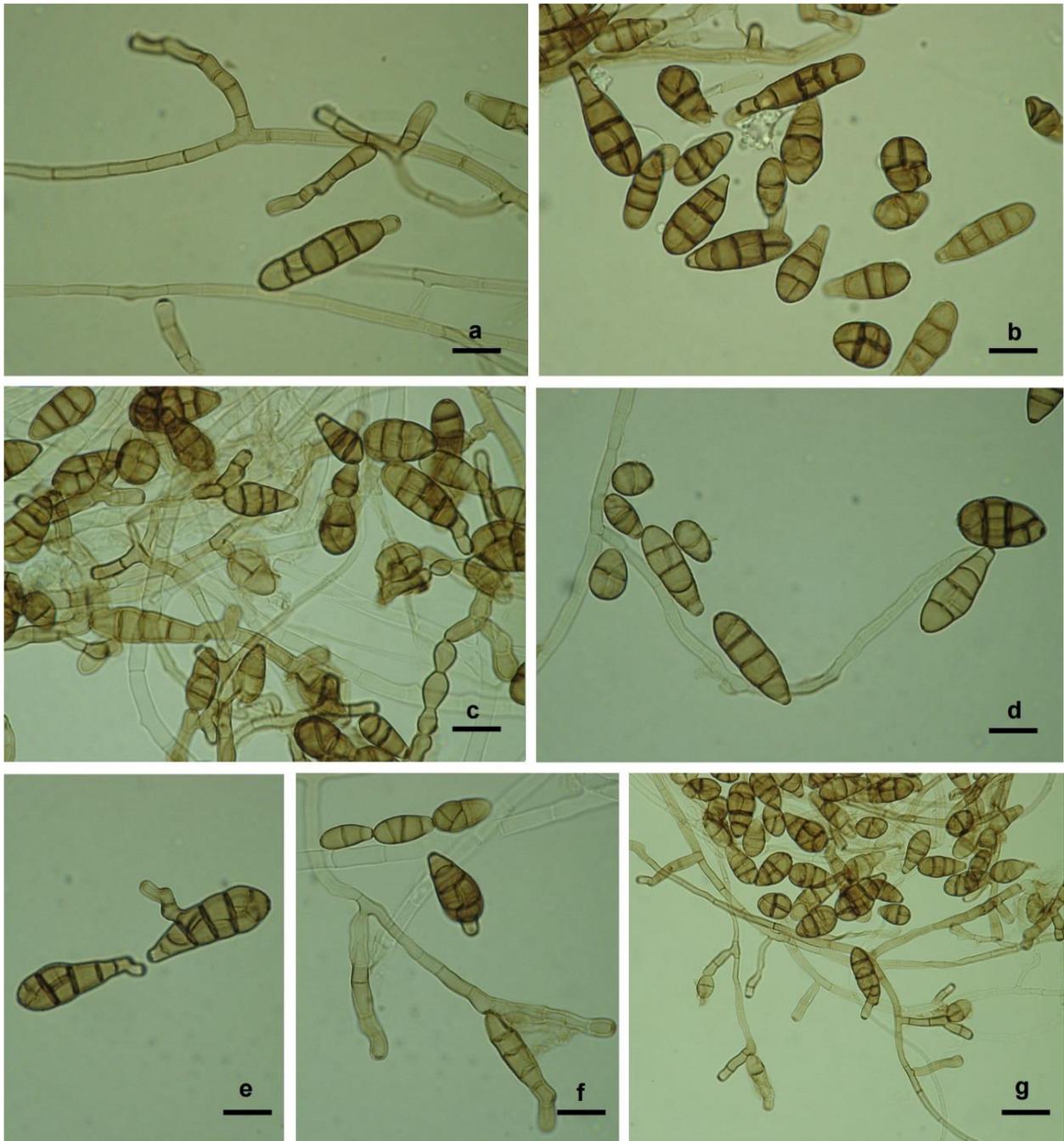


**Fig. 1.** Bayesian phylogram representing the phylogenetic relationships of the ITS sequences of fungal endophytes isolated from *Quercus brantii* in this study (bold terminals). Numbers above branches are Bayesian posterior probabilities. The tree is rooted with *Lentinus tigrinus*.

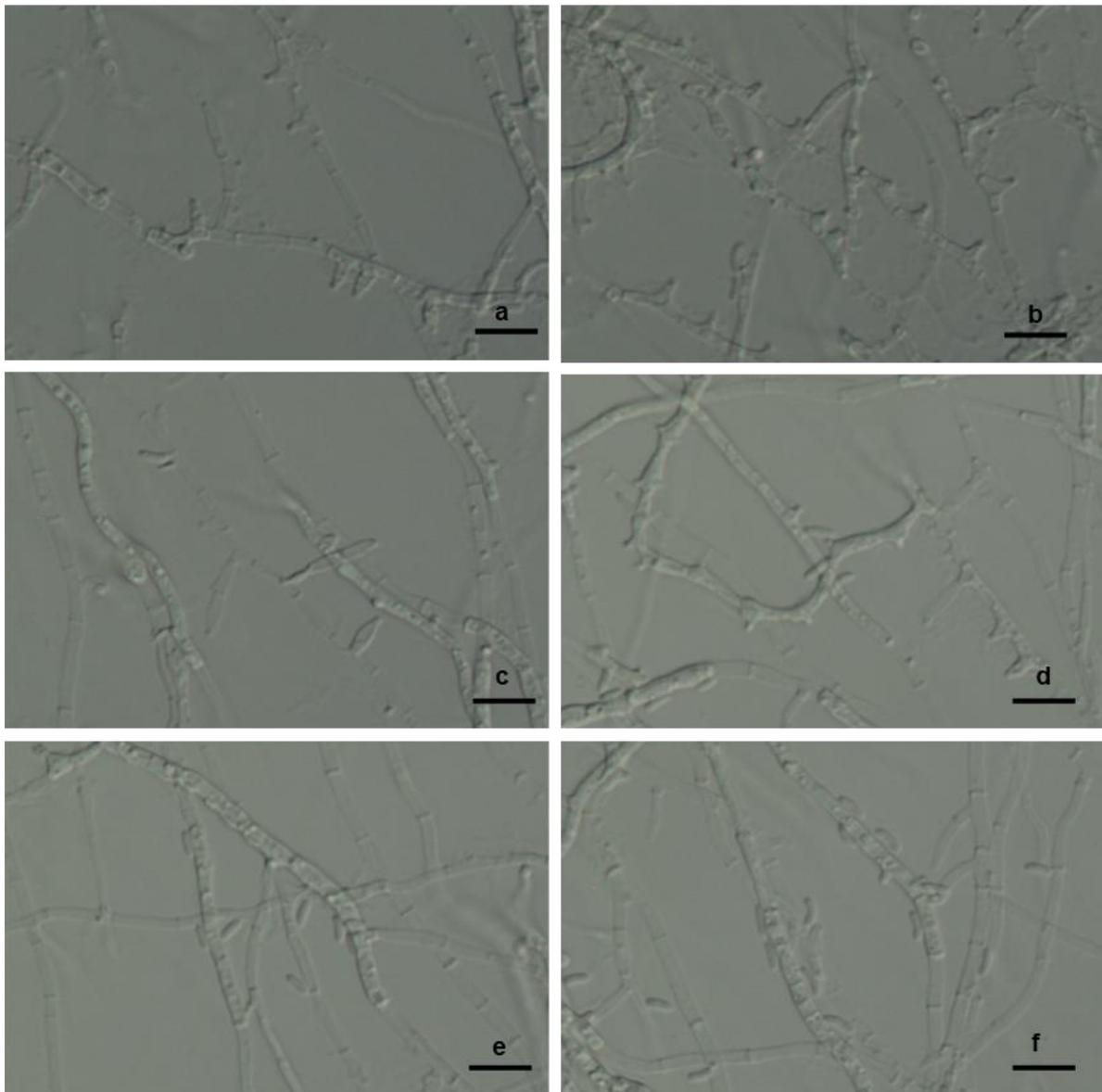
The ITS sequence of this isolate matches very well with authentic *Kalmusia variispora* sequences in GenBank, including the ex-type sequence. *Dendrothyrium variisporum* Verkley, Göker & Stielow, a pycnidial fungus, has been synonymized under *Kalmusia variispora* (Ariyawansa et al. 2014, Verkley et al. 2014). However, our isolate produces long synnemata-like bodies on PDA, producing aleuroconidia or swellings on repeatedly branched, dendroid conidiophores (Fig. 4) that are absent in both *Dendrothyrium* Verkley, Göker & Stielow and *Kalmusia* Niessl. Therefore, sequencing an additional

gene region like  $\beta$ -*tubulin* would be needed to decide if this isolate can be introduced as a novel anamorph for *K. variispora*. Abdollahi Aghdam & Fotouhifar (2017) recently reported *D. variisporum* as new to Iran, and their identification was based on ITS Blast (although no accession number was given in their study).

The sterile isolate K1-a also seems to be conspecific with K3-3a, and their sequences match each other with 100 % identity over 98 % query coverage.



**Fig. 2.** *Alternaria alternaria* (a, b) and *A. cf. arborescens* (c-g). — Scale bars a-f = 10 μm, g = 20 μm.



**Fig. 3.** *Coniochaete* sp. 1. a–d. Conidiogenous cells; e, f. Conidia. — Scale bars = 10  $\mu$ m.

### **Phaeosphaeriaceae sp.**

No morphological data are available for this isolate. However, in Blast searches, it exactly matches the isolate A48 (945 bp) studied by Hagh Doust et al. (2017), also isolated from *Q. brantii* in Iran, and shows very lower match with other accessions. Similar to the isolate by Hagh Doust et al. (2017), the amplified ITS region for this isolate is 911 bp long, containing a long SSU portion (432 bp). Separate blasting of ITS segments in GenBank retrieved members of Phaeosphaeriaceae. Blasting the ITS region after removing SSU and LSU fragments retrieved *Allophaeosphaeria cytisi* KT306947 with 92 % identity over 99 % query coverage.

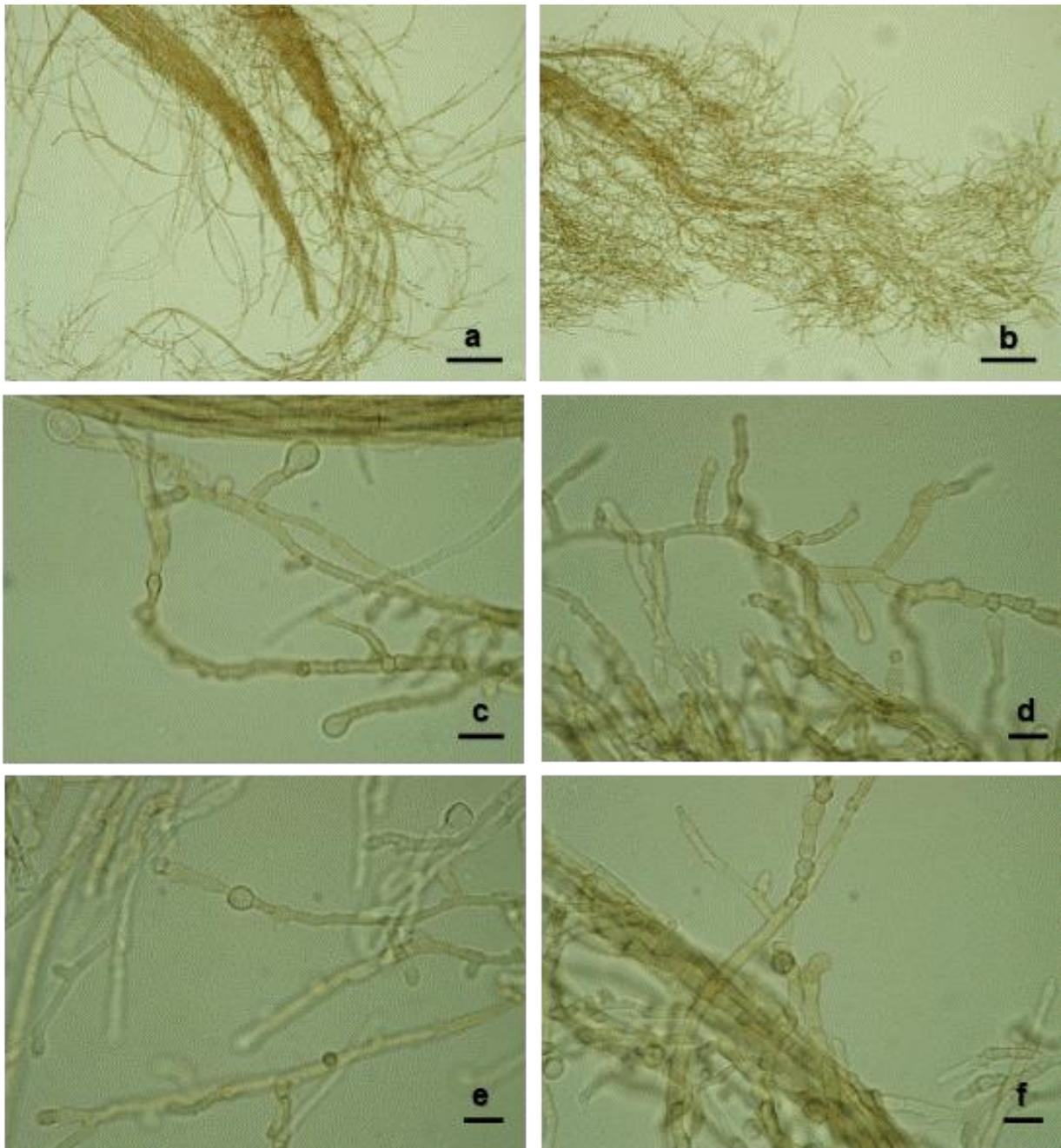
*Allophaeosphaeria* Ariyaw., Camporesi & K.D. Hyde is a small genus recently established by Liu et al. (2015). The genus is typified with *A. muriformia*

Ariyaw., Camporesi & K.D. Hyde and currently contains 4 species.

From the phylogenetic analysis (Fig. 1), we believe that our isolate K4-3c is a member of the family Phaeosphaeriaceae (Pleosporales, Dothideomycetes), and may be closely related to the genus *Allophaeosphaeria*.

### **Pleosporales sp.**

The isolate K2-1a remained essentially sterile on culture media that we examined. However, its ITS Blast hits the isolate A75 studied by Hagh Doust et al. (2017), also isolated from *Q. brantii* in Iran as an endophyte, with 99 % identity over 100 % query coverage. Hits to other accessions retrieved very low coverage. The ITS sequence for this isolate contains a 460 bp long SSU fragment, and very short (only 14 bp) ITS2.



**Fig. 4.** *Kalmusia variispora*. a, b. Synnemata; c–f. Aleuroconidia or chlamydospore-like swellings on dendroid hyphae. — Scale bars a, b = 60  $\mu$ m, c–f = 10  $\mu$ m.

Partitioned blasting retrieved hits to different families in Pleosporales such as Didymellaceae, Fenestellaceae, and Leptosphaeriaceae. Blast against UNITE hit *Leptosphaeria* sp. but only with low score. Therefore, it can be established that the isolate K2-1a is a member of Pleosporales, yet further taxonomic rank assignments would require more detailed studies.

***Purpureocillium lilacinum*** (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson, FEMS Microbiology Letters 321: 144 (2011). Fig. 5 = *Paecilomyces lilacinus* (Thom) Samson

Colonies uniform, pinkish to purplish white with floccose upper surface, relatively rapidly growing on PDA. Conidiophores thick-walled, 2.5–3.5  $\mu$ m wide, bearing short, divergent phialids. Conidia fusiform to subglobose, ca. 2–3  $\times$  1.8–2.8  $\mu$ m, with smooth, thin, pale pink walls, formed in divergent chains. A full description of the species is available in the study by Luangsa-ard et al. (2011).

It is noteworthy that *P. lilacinum* is basically known as a saprotrophic fungus residing in soil, but also as an emerging human pathogen in immune-compromised individuals (Saghrouni et al. 2013), and has also been reported as insect pathogen in Iran

(Ghazavi et al. 2005). Here, it is reported as endophyte of *Q. brantii* for the first time.

*Ulocladium* sp. Fig. 6

Colonies yellowish-brown, more or less felty. Conidiophores light brown, moderately branched at right angles, curved or geniculate at terminal segments, thin- to thick-walled, smooth, 3.5–4.5  $\mu\text{m}$  wide, frequently septate, more pigmented at terminal

branches and conidiogenous cells. Conidia dark to golden brown, apically formed on short conidiogenous cells with 1–2 scars, subglobose to widely ellipsoid, walls slightly verrucose; with 1–2 (3) transverse and 1–4 longitudinal septa, (11.8) 12–16 (16.5)  $\times$  9–12 (12.5)  $\mu\text{m}$ .

References used for morphological examination include: Ellis (1971, 1976), Wang et al. (2010), and Runa et al. (2009). Additional molecular examination is required to sufficiently name this species.

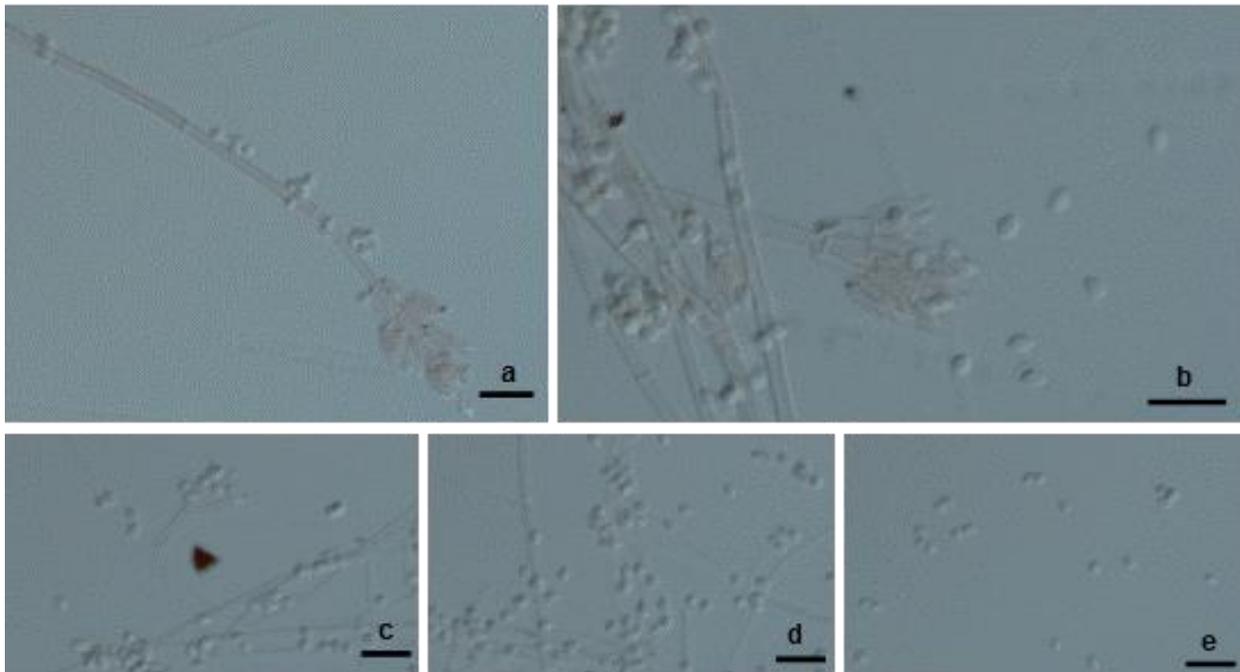


Fig. 5. *Purpureocillium lilacinum*. a–c. Conidiophores; d, e. Conidia. — Scale bars = 10  $\mu\text{m}$ .

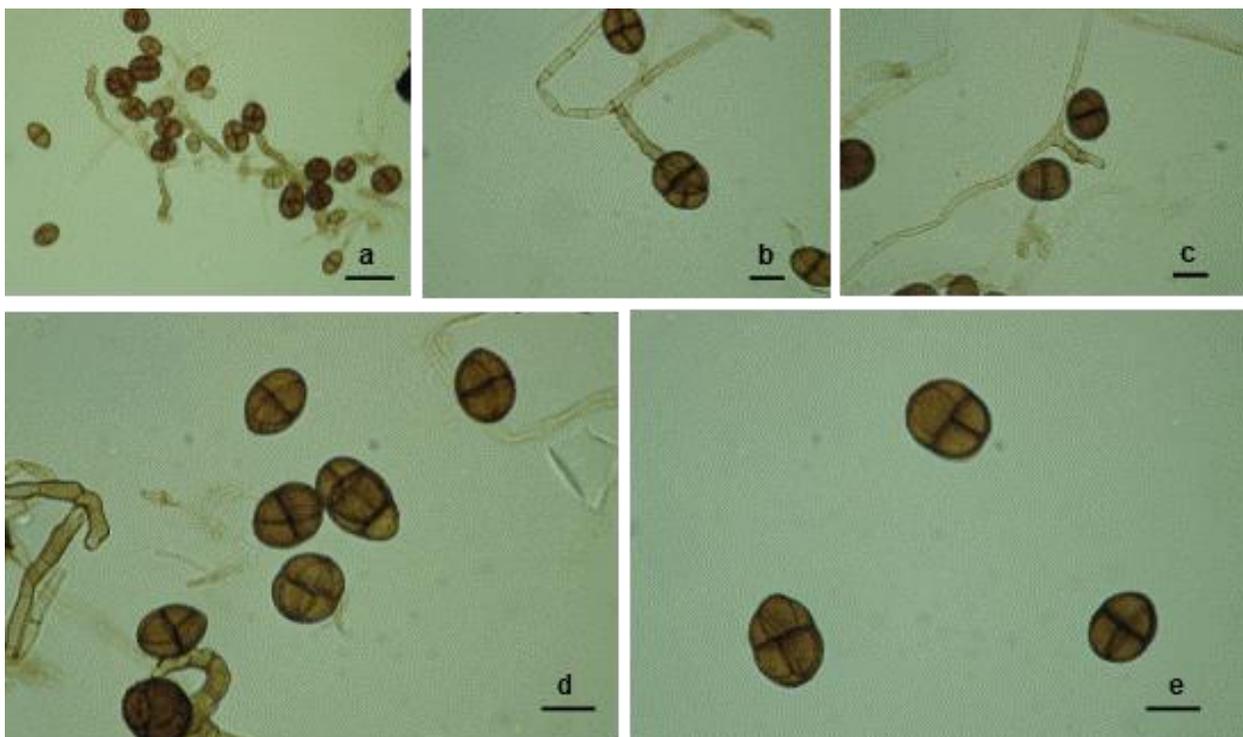


Fig. 6. *Ulocladium* sp. a–c. Conidiophores; d, e. Conidia. — Scale bars a = 20  $\mu\text{m}$ , b, c = 10  $\mu\text{m}$ .

## DISCUSSION

Here, we reported on some fungal endophytes isolates from twigs of *Q. brantii*. The isolated taxa belonged to four classes, 7 orders, and 10 fungal families. It seems that fungal endophytes of *Q. brantii* may be taxonomically and phylogenetically diverse, deserving more studies on Zagros oak mycobiome.

Despite some attempts, several isolates remained sterile in culture media. In the recent study by Ashkezari & Fotouhifar (2017) on fungal endophytes of *Taxus baccata* in Iran, they noted that 67.2 % of isolates remained sterile (mycelia sterilia), while they removed these isolates from further examinations. In the article review by Sun & Guo (2012), they summarized that up to 54 % of endophytic fungi obtained in culture-based techniques do not sporulate, despite application of various spore-stimulating culture conditions. It is evident that molecular techniques are essential to assign sterile isolates to taxonomic ranks.

In this study, all except one of our isolates were ascomycetous. The isolated basidiomycetous fungus (*L. tigrinus*) is primarily a saprotrophic wood-decay fungus, inhabiting decaying wood of various angiosperm trees. Parfitt et al. (2010) believed that wood-decay fungi are only rarely detected in culture-based endophytic studies "presumably because propagules have not received the appropriate germination cues". According to Rungjindamai et al. (2008) and Pinruan et al. (2010), the majority of isolated basidiomycetous endophytes (including *Lentinus* in their study) seem to be white-rot saprotrophic species, and their (transient) endophytic lifestyle may be a strategy contributing to the subsequent host decay in later stages (Parfitt et al. 2010, Boddy & Rayner 1983).

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## برخی قارچ‌های درون‌زی جدا شده از درختان *Quercus brantii* در منطقه دنا، استان کهگیلویه و بویراحمد

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**چکیده:** در این تحقیق، تعدادی قارچ درون‌زی (اندوفیت) جدا شده از شاخه‌های درختان *Quercus brantii* در منطقه دنا، استان کهگیلویه و بویراحمد، گزارش می‌شود. شاخه‌ها از درختان آلوده به قارچ پلی‌پور *Inonotus krawtzevii* نمونه‌گیری شدند. در مجموع، حدود ۴۰ جدایه خالص بدست آمد. همچنین توالی ناحیه ITS نیز برای ۱۶ جدایه تهیه شد و قرابت جدایه‌ها با آنالیز فیلوژنتیکی بررسی گردید و نهایتاً ۱۳ تاکسون در سطوح مختلف تاکسونومیکی شناسایی شد. گونه‌های شناسایی شده عبارتند از: *Alternaria lilacinum*، تعدادی جدایه فقط در سطح جنس (*Ulocladium*, *Pyronema*, *Diatrype*, *Cytospora*, *Coniochaeta*) و جدایه‌های دیگر تنها در سطح تیره (*Phaeosphaeriaceae*) یا راسته (*Pleosporales*) قابل شناسایی بودند. به جز گونه بازیدیومیستی *L. tigrinus* سایر جدایه‌ها متعلق به آسکومیست‌ها بودند. تصاویر میکروسکوپی تعدادی از جدایه‌ها ارائه شده است.

**کلمات کلیدی:** قارچ‌های اندوفیت درختان، مورفولوژی، بلوط ایرانی، PlutoF، ناحیه DNA ITS، زاگرس