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# New reports of endophytic fungi associated with cherry (*Prunus avium*) and sour cherry (*Prunus cerasus*) trees in Iran

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**Abstract**: Endophytic fungi constitute a remarkable multifarious group of microorganisms live within plants tissues without causing any obvious negative effect. Endophytic fungi have been found in every plant species examined to date. During an investigation on endophytic fungi, 123 fungal isolates were obtained from healthy twigs and leaves of cherry (*Prunus avium*) and sour cherry (*P. cerasus*) trees in Iran. The isolates identified based on sequence data of 18S rDNA (SSU) region, as well as morphological and cultural features. As a result, four species namely *Coniothyrium olivaceum*, *Collophora paarla*, *Sarocladium strictum* and *Quambalaria cyanescens* identified. All these identified species are new reports as endophytic fungi from *P. cerasus* and *P. avium* in the world. Among them, *Collophora paarla* and *Quambalaria cyanescens* are new taxa for the mycobiota of Iran.

**Key words:** Diversity, morphology, taxon, sequencing, phylogeny

## INTRODUCTION

Endophytic fungi are a group of fungi that colonize internal tissues of plants without causing any negative effects (Hirsh & Braun 1992). Endophytes may play many important and beneficial roles in the host plant. They also are rich source of novel bioactive compounds with huge potential for exploitation in a wide variety of medical, agricultural, and industrial areas (Tan & Ziu 2001). Endophytes have also recognized as potential sources of novel natural products for industrials, agricultural and pharmaceutical uses (Strobel & Daisy 2003).

Cherry (*Prunus avium* L.) and sour cherry (*P. cerasus* L.) from the family of *Rosaceae* are the most important stone fruit trees with valuable fruits worldwide (Nemati & Abdollahzadeh 2009). Stone fruit trees are a cultivated worldwide group of plants with a great economic importance which can be used as a model for endophyte studies (Pimenta et al. 2012). Fungal endophytes of fruit trees have investigated less frequently compare to other forest trees, and most of the researches have focused mainly on aerial parts, especially leaves, branches and fruits (Hortova & Novotny 2011). Haddadderafshi et al. (2011) isolated 150 endophytic fungal strains from 4500 cherry tissue segments. These isolates belonged to 25 different species of genera such as *Acremonium*, *Alternaria*, *Botryotinia*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Embellisia*, *Epicoccum*, *Fusarium*, *Glomerella*, *Macrophomina*, *Neonectria*, *Phoma*, *Phomopsis*, *Pyronema*, *Rhizoctonia*, *Rhizopycnis*, *Rosellinia* and *Xylaria*. Hortova & Novotny (2011) isolated some endophytic fungi from branches of sour cherry trees, however the isolates belonged to 15 fungal species. *Alternaria alternata* and *Aureobasidium pullulans* were the most frequent fungal species. Pimenta et al. (2012) obtained 163 fungal isolates and, finally 14 different species from plum (*Prunus domestica*) leaves, additionally they investigated their antagonistic activity against *Monilinia fructicola*.

The main goal of the present study was the identification and characterization of some endophytic fungi associated with cherry and sour cherry trees in different areas of Iran based on morphological and phylogenetic studies.

## MATERIALS AND METHODS

### Isolation of Endophytic Fungi

Fungal endophytes were isolated from healthy and living tissues of cherry (*P. avium*) and sour cherry (*P. cerasus*) trees that were collected from Western Azerbaijan, Eastern Azerbaijan, Ardabil, Isfahan, Ilam, Ghazvin, Hamedan, Razavi Khorasan, South Khorasan, North Khorasan and Kerman provinces of Iran during 2014–2015. Samples transferred to mycological laboratory of University of Tehran and stored at 4 °C for future use. The endophytic fungi were isolated using a method described by Refaei et al. (2011). Plant materials washed in running tap water for 10 min. After surface sterilization by immersion of plant tissues in 70% ethanol for 1 min, 2.5% sodium

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hypochlorite solution for 3 min, 70% ethanol for 30 s, and then rinsed with sterile water. The outer tissues of the plant materials removed with sterile scalpel and were cut in small pieces (0.5 $cm^2$) and then placed in Petri dishes containing 2% water agar (WA). Fungal isolates were purified on PDA culture medium using hyphal tip method, and then incubated at 24±1 °C until the pure fungal colonies were appeared. For long–term storage, fungal isolates were grown on sterile filter papers placed on PDA for 7–10 days. Subsequently, colonized filter papers were taken from the surface of culture medium, and were dried at room temperature for four or five days, and then stored at –20 °C for future use.

**Morphological examination**

The morphological identification of endophytic fungi were performed based on the morphology of fungal colony or hyphae, the characteristics of the fruiting bodies such as conidiomata, conidiogenous cells, conidiophores and conidia (Barnett & Hunter 1998; Chen et al. 2015; Damm et al. 2010; Giraldo et al. 2015; de Hoog & de Vries 1973). After 7–14 days incubation of the pure fungal colonies, the fungi assessed by light microscope using the microscopic slides that were prepared in lacto–phenol or lacto–phenol cotton blue solutions. Morphology of the studied characteristics such as colony color, conidia and conidiophore structures measured and recorded using a light microscope. Photographs were taken using BH2 Olympus microscope. Measurements made using macro– and micro–morphological features of different recovered isolates.

Morphological studies of *Coniothyrium olivaceum* was performed on oatmeal agar (OMA), malt extract agar (MEA) and potato dextrose agar (PDA), and the cultures were incubated under near–ultraviolet (nUV) light (12 h light/12 h darkness). Colony diameter measured after 14 days, micro–morphological features (conidiomata, conidiogenous cells and conidia) and measurements were performed according to Chen et al. (2015).

*Collophora paarla* isolates identified based on colony morphology on PDA and micro–morphological characteristics such as presence of conidiomata, microcyclic conidiation or endoconidia, size and shape of conidia and conidiophores (Damm et al. 2010, Gramaje et al. 2012). The cultures were incubated at 25 ºC in continuous dark condition. Colony diameter measured after 14 days.

Morphological characterization of *Sarocladium strictum* carried out from cultures grown on PDA. Culture incubated at 25 °C in constant dark condition. Colony diameter measured after 14 days. This species characterized based on colony morphology, conidiophores, phialides and conidia (Giraldo et al. 2015).

Morphological studies of *Quambalaria cyanescens* were done on PDA. The culture incubated in continuous dark condition at 25 °C. Colony diameter measured after 10 days and micro–morphological description provided based on measurements of conidiogenous cells, conidia and secondary conidia (de Hoog & de Vries 1973).

**Molecular examination**

After morphological identifications, one isolate of each morphotypes selected for molecular investigations. Genomic DNA of the isolates was extracted by the method of Zhong & Steffenson (2001) and partial sequence of 18S rDNA (SSU) locus was amplified and sequenced using the primer pairs NS1 and NS2 (White et al. 1990). PCR amplification carried out in a final volume of 25 µl containing 10 µL of *Taq* DNA Polymerase Mix Red–$Mgcl_2$, 11 µL deionized water, 0.2 pmol of each primer and 10–30 $ng.\mu L^{-1}$ template DNA. PCR amplification performed on Eppendorf Thermal Cycler (Mastercycler, ep gradient), with cycling conditions of 4 min at 95 °C for initial denaturation, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 50 s and a final extension at 72 °C for 10 min. PCR products were purified and directly sequenced in one direction using NS1 primer by Macrogen Company (Seoul, South Korea).

**Phylogenetic analyses**

DNA sequences were evaluated by Chromas 2.6 (Technelysium Pty Ltd., South Brisbane, Australia). Obtained nucleotide sequences compared with other fungal DNA sequences which deposited in GenBank (NCBI) database (www.ncbi.nlm.nih. gov/genbank/) using BLAST search tool. Also, the relevant sequences were obtained from NCBI for phylogenetic analyses, and evolutionary trees were generated using MEGA v. 7.0 (Kumar et al. 2016) with 1000 boot–strap replicates. Multiple sequence alignment performed with Muscle using MEGA v. 7.0 software and alignment manually corrected where it was necessary. Distance matrixes of the aligned sequences calculated by the Tamura–Nei model (Tamura & Nei 1993), and analyzed with the maximum likelihood algorithm (Felsenstein 1985). *Eremothecium gossypii* (AY046265) selected as an out–group taxon. The newly obtained nucleotide sequences of the 18S rDNA region in this study deposited in the GenBank using the Sequin software (NCBI, USA). Detailed information of the examined isolates in this study is provided in Table 1.

## RESULTS AND DISCUSSION

From total of 123 endophytic fungal isolates 116 and seven were from branches and leaves respectively which were obtained from *P. cerasus* and *P. avium*. In total, 58 strains including 56 from branches and two from leaves were isolated from *P. cerasus*. Furthermore, a total of 65 strains include; 60 from branches and five from leave were isolated from *P. avium*.

In this study, four species including *Coniothyrium olivaceum*, *Sarocladium strictum*, *Collophora paarla*

and *Quambalaria cyanescens* were identified and described based on both morphological criteria and molecular data. All four species are reported for the first time as endophytic fungi from *P. cerasus* and *P. avium* trees in the world. Furthermore, *Collophora paarla* and *Quambalaria cyanescens* are new taxa for the mycobiota of Iran.

**Taxonomy**

***Coniothyrium olivaceum*** Bonord., in Fuckel, Symbolae mycologicae: 377 (1869).

*Specimen examined*. IRAN, West Azerbaijan Prov., Khoi, recovered from branch of *P. cerasus*, Oct. 2015, (code of the isolates; AGA6, AGA7, AGA8, AGA9), recovered from branch of *P. avium*. October 2015, (code of the isolates; AG2, AG3, AG4, AG5, AG6), East Azerbaijan Prov., Marand, recovered from branch of *P. cerasus*, Oct. 2015, (code of the isolates; ASA1, ASA3, ASA8), recovered from branch of *P. avium*, Oct. 2015, (code of the isolates; AS1, AS2, AS3, AS4, AS10, AS12), Ardabil Prov., Sarein, recovered from branch of *P. cerasus*, Oct. 2015, (code of the isolates; ARA13, ARA14, ARA17, ARA18, ARA19), recovered from branch of *P. avium*, Oct. 2015, (code of the isolates; AR13, AR14, AR15, AR16, AR17, AR18, AR19), Kerman Prov., Rafsanjan, recovered from branch of *P. cerasus*, Oct. 2014, (code of the isolate; KEA1, KEA2, KEA3, KEA6, KEA7, KEA8, KEA10, KEA11, KEA12, KEA14, KEA15, KEA16, KEA17, KEA18, KEA19, KEA20, KEA31, KEA32, KEA33, KEA34, KEA35, KEA36), recovered from branch of *P. avium*, Oct. 2014, (code of the isolate; KE1, KE2, KE3, KE4, KE7, KE8, KE9, KE11, KE12, KE15, KE18, KE19, KE20, KE21, KE22), Sh. Abdollahi Aghdam. Code of selected isolate: UTFC–EP23 (UTFC is University of Tehran Fungal Culture Collection, at Mycology Laboratory, Department of Plant Protection, Faculty of Agricultural Science and Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.), isolated from West Azerbaijan Prov., Khoi, recovered from branch of *P. cerasus*, Oct. 2015.

Colonies on PDA reached to 53 mm in diameter after 14 days at 25 °C under Ultra Violet (UV) light (12 h light/12 h dark). Colonies on PDA and OMA respectively become green in center due to production of pycnidia. Colonies on MEA become brown in center after two weeks. Pycnidia reached to 0.5–2 mm in diameter, dark brown, immersed, spherical. Conidiogenous cells hyaline, smooth–walled, sub–cylindrical to ampulliform, 5–10(8.6) × 3–6(4.5) µm. Conidia 1–celled, pale brown, thick and smooth–walled, ellipsoidal to sub–cylindrical, 7–9(7.5) × 4–5(4.3) µm in diameter (Fig. 1). Morphological features of the investigated isolate were similar to description provided by Chen et al. (2015).

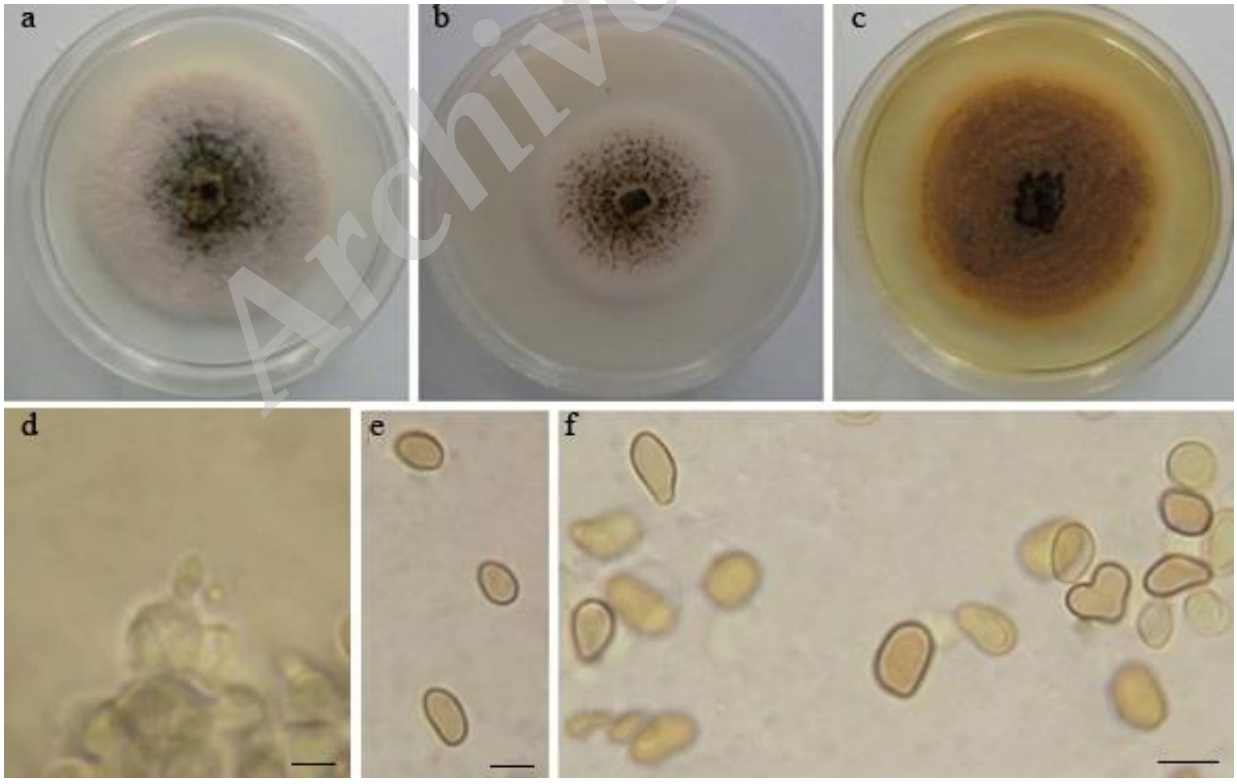


**Fig. 1.** *Coniothyrium olivaceum*, isolate UTFC–EP23. a. Colony on PDA; b. Colony on OA; c. Colony on MEA after 14 days; d. Conidiogenous cell; e., f. Conidia. — Scales bars = 10 µm.

***Collophora paarla*** Damm & Crous, in Damm, Fourie & Crous, Persoonia 24: 67 (2010).

*Specimen examined*. IRAN, Isfahan Prov., Khansar, recovered from branch of *P. avium*, May 2014, (code of the isolate; ES1–1, ES1–2, ES1–3), Ghazvin Prov., Boyin Zahra, recovered from branch of *P. avium*, July 2014, (code of the isolate; GA25, GA26), recovered from branch of *P. cerasus*, July 2014, (code of the isolate; GAA21), Sh. Abdollahi Aghdam. Code of selected isolate: UTFC–EP45, isolated from Isfahan Prov., Khansar, recovered from branch of *P. avium*, May 2014.

Conidiomata pseudopycnidial, solitary, subglobose, superficial, pale to dark brown (observed once on PDA and have not observed again). Conidiophores lining the inner conidiomatal cavity, hyaline, smooth–walled, filiform, branched, 10–45(27.5) × 2.5–4(2.9) µm in diameter. Conidiogenous cells enteroblastic, hyaline, mono–phialidic, 3–7(5.4) × 1–2.5(1.9) µm in diameter. Conidia of pseudopycnidia hyaline, aseptate, smooth–walled, cylindrical with obtuse ends, 2 × 1 µm in diameter.

Colonies on PDA reached to 32 mm in diameter after 14 days at 25°C in constant dark condition. Colonies slow growing, moist, cream, lacking aerial mycelium. Conidiophores, hyaline, branched, septate and filiform. Conidiophores mostly reduced to conidiogenous cells. Conidiogenous cells enteroblastic, intercalary, 1.5–4 (2.6) × 1–2(1.5) µm in diameter. Conidia hyaline, 1–celled, cylindrical, with both ends obtuse or with a papillate apex, smooth–walled, almost biguttulate, 5–10(7.8) × 1.5–3(2.1) µm in diameter. Endoconidia formed uniseriately within hyphae, hyaline, 1–celled, cylindrical, smooth–walled (Fig. 2). Morphological features of the investigated isolate were similar to description of *Collophora paarla* provided by Damm et al. (2010).

***Sarocladium strictum*** (W. Gams) Summerb., in Summerbell, Gueidan, Schroers, Hoog, Starink, Arocha Rosete, Guarro & Scott, Stud. Mycol. 68: 158 (2011).

*Specimen examined*. IRAN. West Azerbaijan Prov., Urmia, recovered from branch of *P. cerasus*, (code of the isolate; AGA1, AGA2, AGA3, AGA5), recovered from branch of *P. avium*, May 2014, (code of the isolate; AG9, AG10), Isfahan Prov., Khansar, recovered from branch of *P. cerasus*, May 2014, (code of the isolate; ESA17, ESA18), recovered from branch of *P. avium*, May 2014, (code of the isolate; ES1, ES2, ES5, ES6, ES10), recovered from leaf of *P. avium*, May 2014, (code of the isolate; ES14, ES15, ES18), Ilam Prov., Ilam, recovered from branch of *P. avium*, Sept. 2015, (code of the isolate; EL3, EL4, EL5, EL8), Razavi Khorasan Prov., Kashmar, recovered from branch of *P. avium*, Apr. 2014, (code of the isolate; KA3, KA6, KA8),

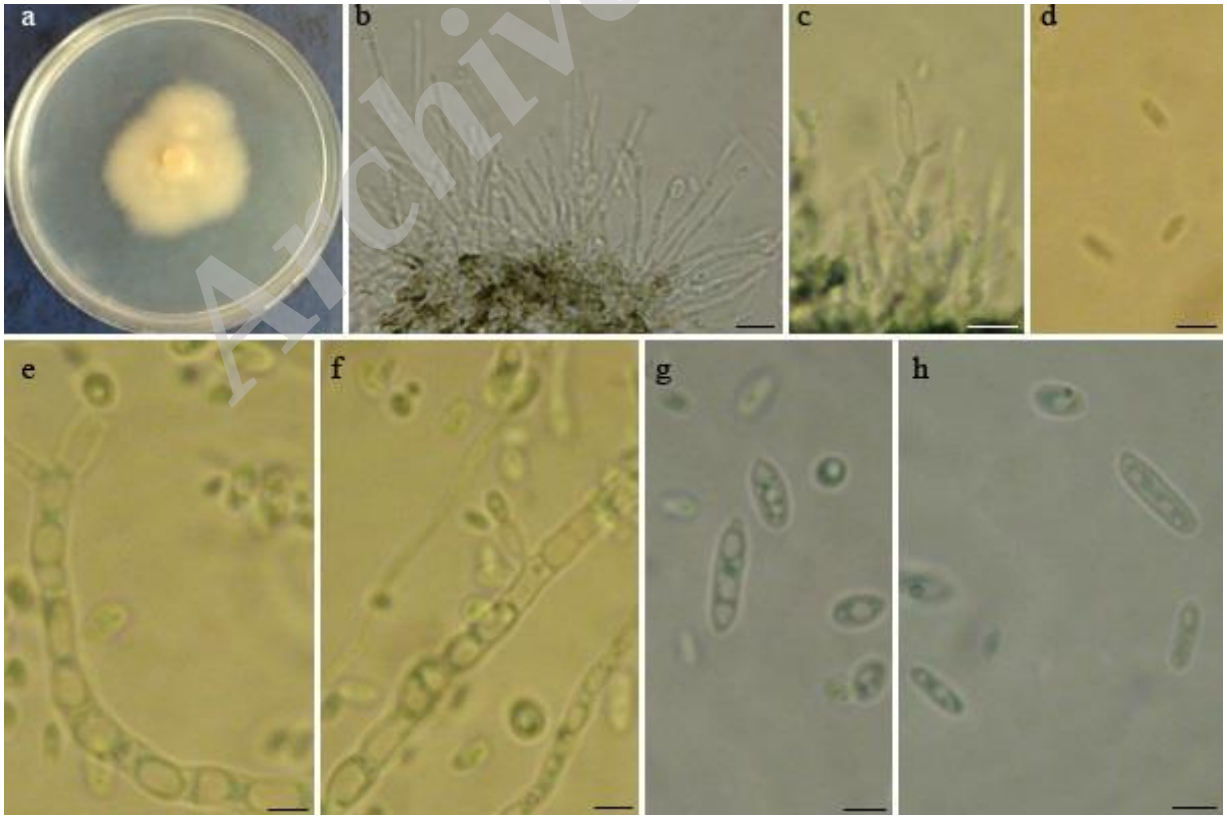


**Fig. 2.** *Collophora paarla*, isolate UTFC–EP45. a. Colony on PDA after 14 days; b. Conidiophora in pseodopycnidia; c. Conidiogenous cell in pseodopycnidia; d. Conidia in pseudopycnidia; e, f. Hyphae and endoconidia; g, h. Conidia. — Scales bars = 10 µm.

(code of the isolate; KA3, KA6, KA8), recovered from leaf of *P. avium*, Apr. 2014, (code of the isolate; KA9, KA26), South Khorasan Prov., Birjand, recovered from branch of *P. cerasus*, Sept. 2014, (code of the isolate; KJA1, KJA2, KJA3, KJA5, KJA5, KJA9), North Khorasan Prov., Esfarayen, recovered from branch of *P. cerasus*, May 2015, (code of the isolate; KSA8, KSA9, KSA11), recovered from leaf of *P. cerasus*, May 2015, (code of the isolate; KSA12, KSA13). Sh. Abdollahi Aghdam. Code of selected isolate: UTFC–EP36., isolated from West Azerbaijan Prov., Urmia, May 2014, recovered from branch of *P. cerasus*.

Colonies on PDA reached 41 mm in diameter in 14 days at 25°C in the continuous dark condition. Colonies were moist to smooth, pale orange to pink. Vegetative hyphae septate, hyaline, smooth–walled, hyphal coils formed abundantly. Phialides hyaline, slender, smooth–walled, arising from vegetative hyphae, 13–40(23.5) × 2–4(3.2) µm in diameter. Conidia grouped in slimy heads, 1–celled, hyaline, straight, cylindrical or ellipsoid, 4–10(6.7) × 1–3(2.2) µm in diameter (Fig. 3). Chlamydospores not observed. Morphological features of the investigated isolate were similar to description of *Sarocladium* provided by Gams (1971).

***Quambalaria cyanescens*** (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & R. Bauer, in de Beer, Begerow, Bauer, Pegg, Crous & Wingfield, Stud. Mycol. 55: 295 (2006).

*Specimen examined*. IRAN, Hamedan Prov., Malayer, recovered from branch of *P. avium*, June 2015, (code of the isolate; HA25, HA27, HA28, HA29, HA30, HA36, HA39, HA40), recovered from branch of *P. cerasus*, June 2015, (code of the isolate; HAA26, HAA27, HAA30, HAA31, HAA32, HAA34), Sh. Abdollahi Aghdam. Code of selected isolate: UTFC–EP47, isolated from Hamedan Prov., Malayer, June 2015, recovered from branch of *P. avium*.

Colonies on PDA reached to 15 mm in diameter after 10 days at 25°C in continuous dark condition. Colonies are restricted, farinose or velvety, snow–white, deep blue/violet pigment into agar. Hyphae are hyaline, smooth–walled, branched and sub–erect. Conidiogenous cells are undifferentiated, cylindrical and variable in size, with a cluster of small denticles apically, 5–25(19.6) × 1–1.5(1.3) µm in diameter. Conidia hyaline, smooth–walled, obovoid, 3–4(3.6) × 1.5–2(1.7) µm in diameter, larger conidia [4–7(5.8) × 2 µm] producing secondary conidia (Fig. 4). Morphological features of the investigated isolate were similar to description of *Quambalaria* provided by Smith & Batenburg–Van der Vegte (1985) and de Hoog & de Vrier (1973).

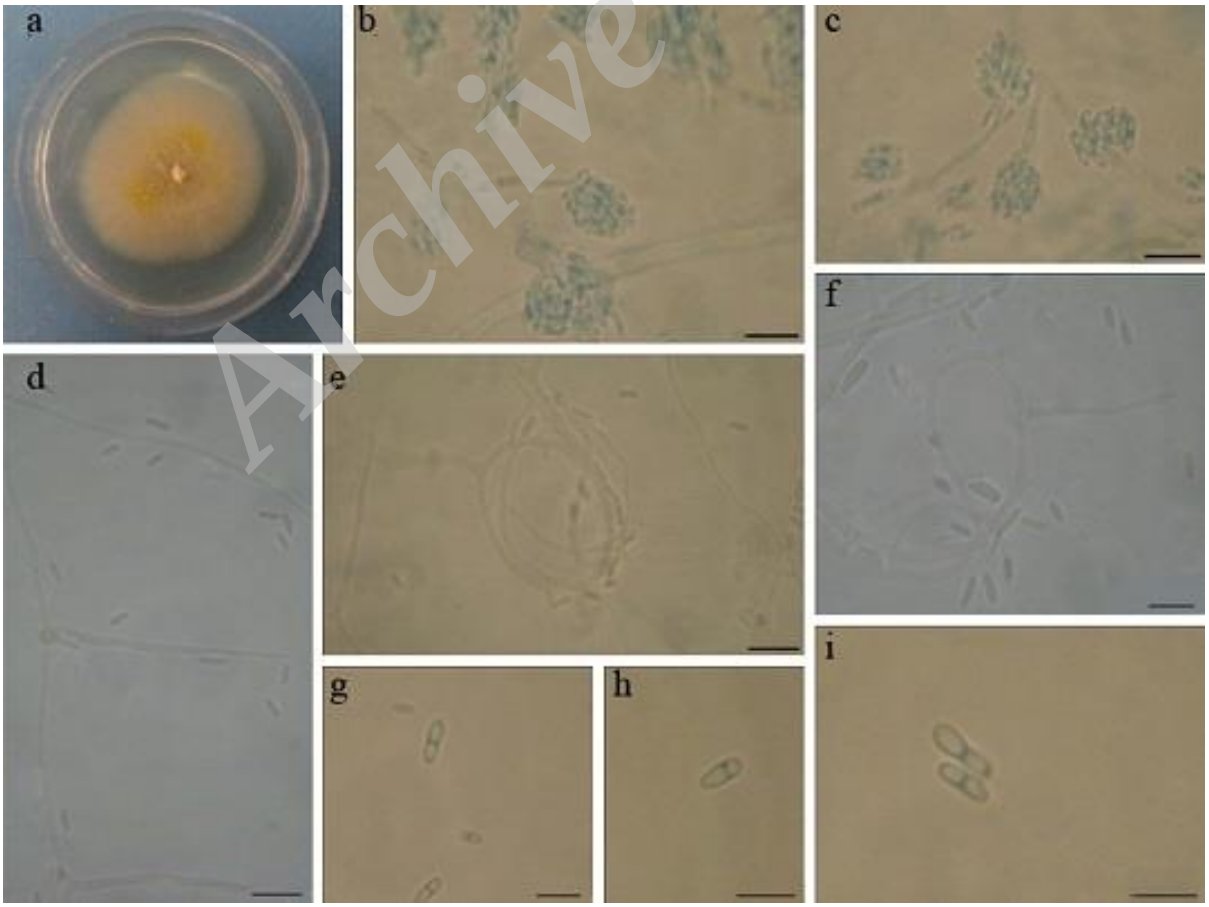


**Fig. 3**. *Sarocladium strictum*, isolate UTFC–EP36. a. Colony on PDA after 14 days; b, c. Slimy heads; d. Phialides; e, f. Hyphal coils; g–i. Conidia. — Scales bars = 10 µm.

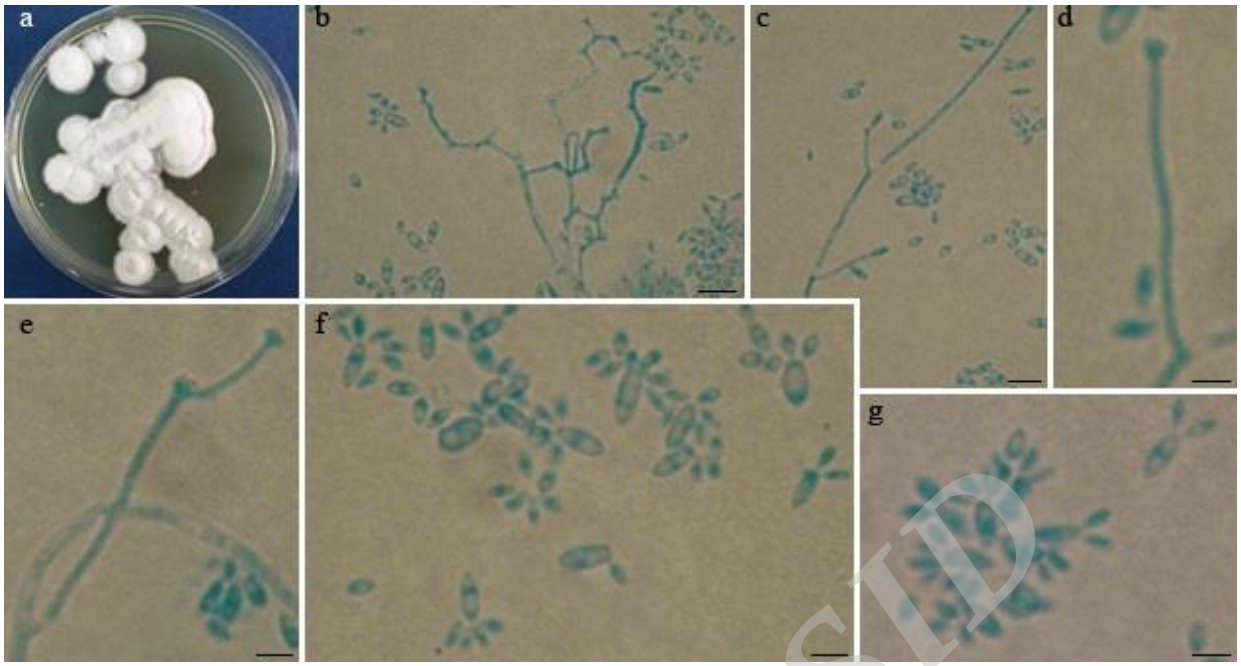


**Fig. 4**. *Quambalaria cyanescens*, isolate UTFC–EP47. a. Colony on PDA after 10 days; b–e. Conidiophores; f, g. Conidia. — Scales bars = 10 µm.

**Phylogenetic analysis**

The phylogenetic analyses performed using twenty–two 18S rDNA nucleotide sequences including our isolates and the others obtained from GenBank including the out–group (Table 1). DNA sequence analysis revealed that all investigated isolates are placed in four distinct clades, corresponding to four different fungal orders including *Pleosporales*, *Helotiales*, *Hypocreales* in

**Table 1.** Fungal strains used in the phylogenetic analysis.

| Fungal species | Isolate | Source (plant host) | Origin | NCBI accession no. | Reference |
|---|---|---|---|---|---|
| *Sarocladium strictum* | CBS 346.70 T | *Triticum aestivum* | Germany | HQ232211 | Summerbell et al. (2011) |
| | UTFC–EP36 | *Prunus cerasus* | Iran | MF000698 | |
| *S. bactrocephalum* | CBS 749.69 T | *Ustilago* sp. | Canada | HQ232180 | Summerbell et al. (2011) |
| | KF103 | | | KM096139 | Panzer et al. 2015 |
| *S. kiliense* | CBS 146.62 | | | HQ232197 | Summerbell et al. (2011) |
| | CBS 122.29 T | | | HQ232198 | Summerbell et al. (2011) |
| *Microsphaeropsis olivacea* | CBS 401.81 | | | AY642517 | Verkley et al. (2004) |
| | CBS 442.83 | | | AY642518 | Verkley et al. (2004) |
| | CBS 336.78 | | | AY642519 | Verkley et al. (2004) |
| | CBS 116669 | *Cytisus scoparius* | Netherlands | EU754071 | de Gruyter et al. (2009) |
| *Coniothyrium olivaceum* | UTFC–EP23 | *Prunus cerasus* | Iran | MF000696 | |
| *C. cereale* | CBS 122787 | | Germany | EU754052 | de Gruyter et al. (2009) |
| *Collophora paarla* | CBS:120877 T | *Prunus salicina* | South Africa | GQ154634 | Damm et al. (2010) |
| | UTFC–EP45 | *Prunus avium* | Iran | MF000694 | |
| | CBS:121443 | *Prunus salicina* | South Africa | GQ154633 | Damm et al. (2010) |
| | CBS:120878 | *Prunus salicina* | South Africa | GQ154632 | Damm et al. (2010) |
| *C. rubra* | CBS:120873 T | *Prunus persica* | South Africa | GQ154627 | Damm et al. (2010) |
| | CBS:121441 | *Prunus persica* var. *nucipersica* | South Africa | GQ154628 | Damm et al. (2010) |
| *C. capensis* | CBS:120879 T | *Prunus salicina* | South Africa | GQ154631 | Damm et al. (2010) |
| *C. africana* | CBS:120872 T | *Prunus salicina* | South Africa | GQ154630 | Damm et al. (2010) |
| *Quambalaria cyanescens* | CBS 876.73 | *Eucalyptus pauciflora* | Australia | KF706440 | Wang et al. (2014) |
| | UTFC–EP47 | *Prunus avium* | Iran | MF000697 | |
| | UM 1095 | Skin scraping | Malaysia | KT186108 | Kuan et al. (2015) |
| *Microstroma phylloplanum* | JCM 9035 | | | AB038131 | |
| | CBS8073 T | *Baksia collina* | Australia | AJ496258 | |
| *Sympodiomycopsis kandeliae* | CBS 11676 | | | KP322963 | Wang et al. (2015) |
| *S. paphiopedili* | IAM 13459 | | | D14006.1 | |
| | AFTOL–ID 1772 | | | DQ832239 | |
| *Eremothecium gossypii* | NRRL Y–1056 | | | AY046265 | Kurtzman & Robnett 2003 |

Basidiomycota. As shown in Fig. 5, all isolates from *P. avium* and *P. cerasus* hosts could be classified in four clades; *Pleosporales* (Clade I), *Helotiales* (Clade II), *Hypocreales* (Clade III), and *Microstromatales* (Clade IV).

*Coniothyrium* and *Microsphaeropsis* species grouped in the same cluster (Clade I) with 100% bootstrap support. Our examined isolate of *Coniothyrium olivaceum* (MF000696) was 100% identical to other isolates of this species in GenBank (AY642517) with 100% query coverage in BLAST search. According to Index Fungorum website, the current name of *M. olivacea* is *C. olivaceum*. The species belongs to order *Pleosporales*, taxonomically.

*Sarocladium* species grouped in the Clade II with 100% bootstrap support. A BLAST search showed that our examined isolate of *S. strictum* (MF000698) was 99% identical to other isolates of this species in GenBank (HQ232211) with 99% coverage. The species belongs to order *Hypocreales*taxonomically.

Different *Collophora* species were grouped in the same cluster (Clade III) with 87% bootstrap support. The partial 18S rDNA nucleotide sequence of our examined *Collophora paarla* isolate (MF000694) was 100% similar to other isolates of this species in GenBank (GQ154634) with 100% coverage in a BLAST search. The species belongs to order *Helotiales*, taxonomically.

The isolates in the Clade IV grouped with 100% bootstrap support. A BLAST search showed that partial 18S rDNA nucleotide sequence of our examined *Quambalaria cyanescens* isolate (MF000697) was 99% identical to other isolates of this species in GenBank (KT186108 and KF706440) with 100% coverage. The species is belonging to order *Microstromatales*, taxonomically.

The isolate UTFC–EP23 identified as *Coniothyrium olivaceum* based on morphology and the description provided by Chen et al. (2015) as well as based on molecular data. This species differs from other *Coniothyrium* species in characteristics of conidiogenous cells and conidial shape and size. This species has been reported from stem of *Hedera helix* in Austria, needles of *Pinus laricio* in France and dead twigs and pods of *Sarothamnus* sp. in Netherland (Chen et al. 2015). This species was reported as causal agent of brown spine rot in *Alhagi maurorum* in Iran (Razaghi and Zafari 2016). Petrini and Fisher (1988), isolated *M. olivacea* as endophytic fungi from xylem and stems of *Pinus sylvestris*. Hormazabal and Piontelli (2009), isolated this species as an endophytic fungi from Chilean gymnosperms. *Coniothyrium olivaceum* is reported for the first time as endophytic fungus from *P. cerasus* and *P. avium* trees in the world.

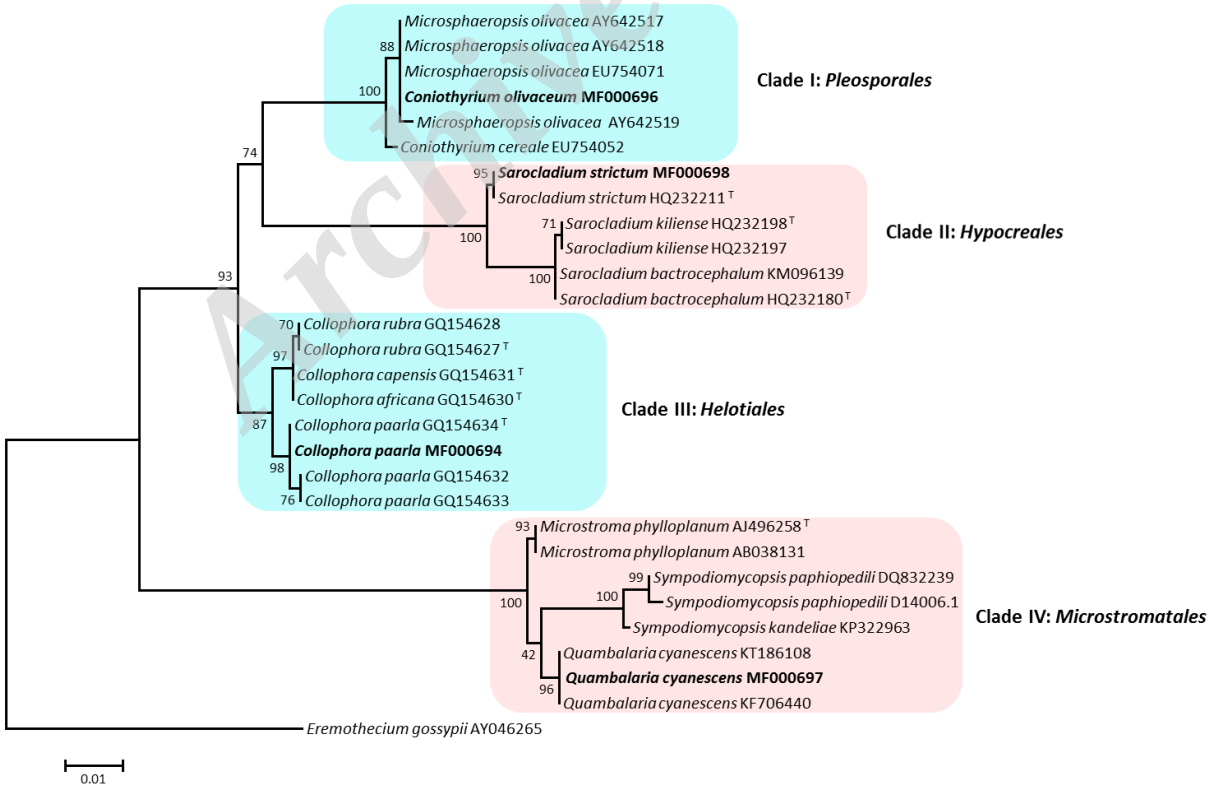


**Fig. 5**. A maximum likelihood tree inferred from 18S rDNA sequences of 29 isolates using MEGA v. 7.0 software. The numbers on the branches shows the bootstrap values of 1000 replicates. The length of branches is proportional to the number of base changes, indicated by the scale bar. *Eremothecium gossypii* (AY046265) was used as out–group.

The isolate UTFC–EP45 identified as *C. paarla* using morphological characteristics, according to the description provided by Damm et al. (2010). The genus has at least six species including *Collophora aceris*, *C. africana*, *C. capensis*, *C. hispanica*, *C. paarla* and *C. rubra* (Damm et al. 2010; Xie et al. 2013, Gramaje et al. 2012). *Collophora paarla* unlike the any other species of the *Collophora*, produceed endoconidia. Furthermore, conidiophores of this species in the conidiomata differ from other species. Unlike the five other species of the genus, *C. paarla* did not release any pigments into culture medium. *C. paarla* has been isolated from *Prunus salicina* in South Africa (Damm et al. 2010). Some *C. paarla* isolates have obtained from the dark brown necrosis symptoms in woods of *P. persica* (Damm et al. 2010). According to the morphological and molecular analysis, the fungus was identified as *C. paarla*. This is the first report of *Collophora paarla* as new taxon for the mycobiota of Iran. In addition, *C. paarla* is reported for the first time as endophytic fungus from *P. cerasus* and *P. avium* trees in the world.

Molecular data confirmed the morphological identification of UTFC–EP36 as *Sarocladium strictum*. Summerbell et al. (2011) have reported some *Sarocladium* species, such as *S. strictum*, segregated from *Acremonium*, based on phylogenetic analysis of SSU and LSU sequences. *Sarocldium strictum* has the morphological similarity and close relationship with *S. pseudostrictum*, but *S. strictum*, has a faster growth rate on PDA and larger phialides (Giraldo et al. 2015). *S. strictum* unlike *S. kiliense* does not formed unicellular chlamydospores (Perdomo et al. 2011). *Sarocldium strictum* has been reported from *Vitis sylvestris*, *Zea mays* and as fungi accompanying the scab symptoms in Iran (Ershad 2009; Ebrahimi and Fotouhifar, 2016). Species of *Sarocladium* have been reported as endophytes in grass species such as *Spinifex littoreus* and native Turkish grasses (Tunali et al. 2000; Yeh & Kirschner 2014**).** Here, *S. strictum* is reported for the first time as endophytic fungus from *P. cerasus* and *P. avium* trees in the world.

The isolate UTFC–EP47 was identified as *Quambalaria cyanescens* based on morphological features provided by Smith and Batenburg–Van der Vegte (1985), de Hoog and de Vrier (1973) and the molecular data. The fungus has six species including *Quambalaria coyrecup*, *Q. cyanescens*, *Q. eucalypti*, *Q. pitereka*, *Q. pusilla* and *Q. simpsonii* (Paap 2008; de Beer et al. 2006; Simpson 2000; Cheewangkoon et al. 2009). Our isolate of *Q. cyanescens* differs from other five *Quambalaria* species by the differences in colony color and conidial shape and size. *Quambalaria cyanescens* was isolated for the first time from human skin and was reported as *Sporothrix cyanescens* (Fan et al. 2014). This species is one of the rare clinical basidiomycetous pathogens and most reported species from the human in the 1990s (Fan et al. 2014). Almost all species of the genus *Quambalaria* are regarded as plant–pathogenic fungi, causing disease on species of Eucalyptus (de Beer et al. 2006). *Quambalaria cyanescens* generally regarded as a saprophyte that live or decay on different plant tissues (de Beer et al. 2006). Also, this species is associated with canker on *Eucalyptus pauciflora*, *Corymbia calophylla*, *C. ficifolia*, and *C. citriodora* in Australia (Pegg et al. 2008). *Quambalaria cyanescens* is new taxon for the mycobiota of Iran. Also, this species is reported for the first time as endophytic fungus from *P. cerasus* and *P. avium* trees in the world. It is well understood that endophytes might play important role in the growth and development of the host plant through providing of protection against various sources, and potential biological active natural products (Strobel et al. 2004). In this study, we investigated fungal endophytes associated with *P. avium* and *P. cerasus* using the morphological features and molecular data based only on the sequences of genomic SSU rDNA. This is the first study of fungal endophytes from these hosts in Iran. Some genera and species of endophytic fungi including *Coniothyrium olivaceum*, *Collophora paarla*, *Sarocladium strictum* and *Quambalaria cyanescens* were also isolated and identified in the present study.

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# گزارش جدیدی از قارچ های اندوفیت درختان آلبالو (*Prunus cerasus*) و گیلاس (*Prunus avium*) در ایران

**شیوا عبدالهی اقدم و خلیل بردی فتوحی فر ✉**
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**چکیده:** قارچ های اندوفیت گروهی از میکروارگانیسم‌های گوناگونی را شامل می‌شوند که بدون ایجاد علایم بیماری داخل بافت گیاهی زندگی می‌کنند. طی تحقیقی به منظور دستیابی به برخی قارچ‌های اندوفیت در ایران طی سال‌های ۱۳۹۳و ۱۳۹۴، ۱۲۳ جدایه های قارچی از درختان گیلاس (*Prunus avium*) و آلبالو (*Prunus cerasus*) در ایران جداسازی شدند. شناسایی جدایه‌ها بر اساس خصوصیات ریخت شناختی و رشدی و همچنین داده‌های مولکولی بر اساس داده های توالی ناحیه ژنومی 18S rDNA انجام شد. در این تحقیق چهار گونه شامل *Coniothyrium olivaceum*، *Collophora paarla*، *Sarocladium strictum* و *Quambalaria cyanescens* شناسایی شدند. تمامی گونه ها برای اولین بار به عنوان قارچ اندوفیت درختان آلبالو و گیلاس در دنیا گزارش می‌شوند. از بین گونه‌های شناسایی شده، گونه *Collophora paarla* و *Quambalaria cyanescens* برای اولین بار برای میکوبیوتای ایران معرفی می‌گردند.

**کلمات کلیدی:** تنوع، ریخت شناسی، آرایه، توالی یابی، فیلوژنی



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