



## Novel endophytic species of *Talaromyces* sect. *Talaromyces* associated with saffron plant to the mycobiota of Iran

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**Abstract:** In an investigation of the biodiversity of endophytic fungal species associated with saffron plant in Iran's main saffron cultivation regions, five isolates belong to *Talaromyces* genus were obtained. The isolates identified as *Talaromyces versatilis*, *T. aurantiacus*, *T. pinophilus*, *T. funiculosus*, and *T. purpureogenus* by morphological and molecular criteria. To our knowledge, two species, including *Talaromyces versatilis* and *T. aurantiacus* are the first reports to the mycobiota of Iran.

**Keywords:**  $\beta$ -tubulin gene, morphology, phylogeny, sampling

### INTRODUCTION

Saffron is one of the oldest and globally most expensive plant spices known as an important medicinal plant cultivated since the past (Negbi et al. 1989). According to the latest statistics for saffron trade in 2019, Iran is the world's largest saffron producer, supplying 430 tons of the total 450 tons of saffron produced worldwide (<https://behinexir.com/global-saffron-market/>). Saffron stigma has become more popular due to its versatile biological and medicinal properties, such as aphrodisiac, antispas-

modic, expectorant, antidepressant, and stomachic activity (Abdullaev 1993, Richelson 1993, Wang et al. 2010). Medicinal plants are rich sources of endophytic fungi that lived within the tissues of host plants and have proven to be a wealthy pool of bioactive secondary metabolites; some of them are shared with the hosts (Yu et al. 2009, Kusari et al. 2009, Zhu et al. 2010, Kusari 2012).

The genus *Talaromyces* was introduced by Benjamin (1955) as a teleomorph genus related to the *Penicillium* species. Phylogenetic studies revealed that *Penicillium* was a polyphyletic and *Talaromyces* species, and *Penicillium* subgenus *Biverticillium* belonged to a clade distinct from *Penicillium sensu stricto* (Chen et al. 2016). Based on phylogenetic, phenotypic and extrolite data, and following the nomenclatural priority and single name nomenclature concepts, Samson et al. (2011) transferred the most accepted *Penicillium* subgenus *Biverticillium* species to *Talaromyces*. *Talaromyces* currently includes around 140 accepted species, grouped into seven sections, namely section Bacillispori, Helici, Islandici, Purpurei, Subinflati, *Talaromyces* and Trachyspermi. One of these sections is Sect. *Talaromyces*. Stolk and Samson (1972) introduced *Talaromyces* section *Talaromyces* as species that produce yellow ascomata, which can occasionally be white, creamish, pinkish or reddish, and have yellow ascospores. Conidiophores are usually from the biverticillate-symmetrical type, with some species that have reduced conidiophores with solitary phialides. Phialides are usually acerose, with a minor proportion of species having wider bases (Stolk & Samson 1972). Section *Talaromyces* species are commonly isolated from soil, indoor environments, humans with penicilliosis and food products (Yilmaz et al., 2014).

*Talaromyces atroroseum* and *T. minioluteus* have been reported from Iran (Ershad 2009; Khodaei et al. 2015). A large number of *Penicillium* species have been previously reported from Iran, though after revising *Talaromyces* genus by Samson et al. (2011) and separating it from the genus *Penicillium*, these species have not yet been reviewed.

*Talaromyces* species have a worldwide distribution isolated from a wide range of substrates, mainly in soil (Barbosa et al. 2018, Sun et al. 2020). *Talaromyces* include species that are medically and industrially important (Yilmaz et al., 2014). On the other hand, species of *Talaromyces* are good producers of anticancer, antibacterial, and antifungal compounds (Nicoletti et al. 2018). Several species also proved to be effective as biocontrol agents against soil-borne pathogens (Sun et al. 2020).

This study aimed to identify endophytic *Talaromyces* species isolated from saffron, collected from different saffron producer provinces in Iran. We provide morphological descriptions using macro- and micromorphological characters. Phenotypic characteristics, combined with partial  $\beta$ -*tubulin* sequences, were applied to identify isolates.

## MATERIALS AND METHODS

### Sample collection and isolation

Sampling was conducted from saffron farms in Khorasan Razavi, South Khorasan, North Khorasan, Kerman, Isfahan, Fars and Yazd provinces, Iran. Plant samples were collected from corms, leaves, and flowers without disease symptoms. Isolation of endophytic fungi was performed according to the protocol reported by Petrini (1986) with some modifications. First, healthy collected samples were washed under running water for 10 minutes. Afterward, superficial disinfection was performed for one minute in 75% ethanol, two minutes in dilute sodium hypochlorite solution (NaClO) (containing 1% active chlorine) and two minutes in 70% ethanol. Disinfected samples were washed in sterile distilled water thrice. After surface-disinfection, the samples were placed on WA, at 25°C in darkness condition. The living strains were deposited in the Culture Collection of Agricultural Biotechnology Research Institute of Iran (ABRRI).

### Morphological analysis

Morphological identification of *Talaromyces* species was performed using macroscopic and microscopic characteristics. Macroscopic characteristics such as colony diameter, texture, the color of conidia, mycelia, soluble pigments, and acid or possible base production were studied on MEA, OA, YES, CZ, CYA, and CYAS medium after one week of incubation at 25 °C in darkness. Moreover, microscopic characteristics, such as the number of branching points between stipe and phialides (i.e., solitary phialides to quaterverticillate), dimension, shape and texture of stipes, vesicles, metulae/branches (when present), phialides, conidia, cleistothecia, asci and ascospores (when present) on MEA medium, were measured after one week of incubation at 25 °C in darkness (Samson et al. 2019). All isolates were inoculated at the three-point method on all the media.

### DNA extraction and PCR amplification

DNA extraction was performed from one-week-old colonies grown on potato dextrose agar (PDA) at 25 °C in darkness, according to Zhong & Steffenson (2001). The  $\beta$ -*tubulin* gene region was partially amplified using primer pair BT2A/BT2b (5'-GGTAA CCAAATCGGTGCTGCTTTC/ACCCTCAGTGTGT GACCCTTGGC-3') (Glass & Donaldson 1995). Polymerase chain reaction (PCR) mixture was prepared in a total volume of 26  $\mu$ L containing 10  $\mu$ L Master Mix Red (CinnaGen, Iran), 1.5 mM MgCl<sub>2</sub>, 1  $\mu$ L of each primer (10 pmol) and 10–20 ng of genomic DNA. PCR condition for  $\beta$ -*tubulin* amplification was as follow: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 45 s, and extension at 72°C for 1 min; and final extension at 72°C for 10 min.

### DNA sequencing and phylogenetic analysis

The PCR products were purified using GeneAll Purification Kit (combo GP, 50p). The purified PCR products were sequenced in the forward direction by Cardiogenetics Research Center in Iran. The received sequences were edited by Pro Chromas Version 1.7.6 and Editseq version 5.01. The Basic Local Alignment Search Tool (BLAST) was used to compare the similarities of nucleotide sequences with GenBank database (<http://www.ncbi.nlm.nih.gov/blast/>). Sequences obtained from this study and the GenBank were aligned using MEGA 7 software and multiple alignments option (Muscle). Maximum likelihood analysis was performed. The best model for ML was selected based on the Akaike Information Criterion (AIC), which was calculated in MEGA. ML analysis was done by calculating an initial tree using BioNJ and the subsequent Heuristic search done with the Nearest-Neighbour- Interchange (NNI) option. The reliability for each group was evaluated by 1000 replications bootstrap. *Rasamsonia aegroticola* was selected as a suitable outgroup. The sequences used in this analysis were deposited in GenBank (Table 1).

## RESULTS AND DISCUSSION

### Morphological identification

Based on morphological characteristics, endophytic *Talaromyces* isolates obtained from leaf and corm of saffron in this study, belonged to the *Thalaromyces* section *Thalaromyces*. Overall, five *Talaromyces* isolates were obtained and according to morphological studies, the isolates were identified as *Talaromyces versatilis*, *T. aurantiacus*, *T. pinophilus*, *T. funiculosus* and *T. purpureogenus* species.

### Phylogenetic analysis

In this study, sequence data of the  $\beta$ -*tubulin* gene region was used to identify relationships within *Talaromyces* species. The  $\beta$ -*tubulin* gene generated 390- to 409-bp fragments and comprised 368 characters after alignment. The phylogenetic tree generated by  $\beta$ -*tubulin* sequences of species in sections *Thalaromyces*, *Purpurei*, *Trachyspermi*, *Bacillispori*

and *Islandici* from GenBank (Table. 1). The results are shown in Fig. 1, which demonstrate that five isolates examined in this study are belonged to five distinct species (Fig. 1). The isolate ABRIICC 10349 is close to *Talaromyces aurantiacus* and form a well-supported clade with 93% bootstrap. The isolate ABRIICC 10348, form a sister group with *T. versatilis* (94%). Additionally, isolates ABRIICC 10350, ABRIICC 10351 and ABRIICC 10352 are close to *T. pinophilus*, *T. funiculosus* and *T. purpureogenus* with high bootstrap support (100%), respectively.

Phylogenetic classification using  $\beta$ -*tubulin* genes showed that  $\beta$ -*tubulin* sequence had acceptable performance in species recognition and separating the *Thalaromyces* section species with a higher bootstrap value. As concluded in the investigation conducted by Visagie et al. (2014),  $\beta$ -*tubulin* could successfully identify *Talaromyces* species. They recommended  $\beta$ -*tubulin* as an identification marker for *Talaromyces* (Yilmaz et al. 2014). Except from the new species, all other isolates could be reliably identified using  $\beta$ -*tubulin* sequences (Barbosa et al. 2018).

## Taxonomy

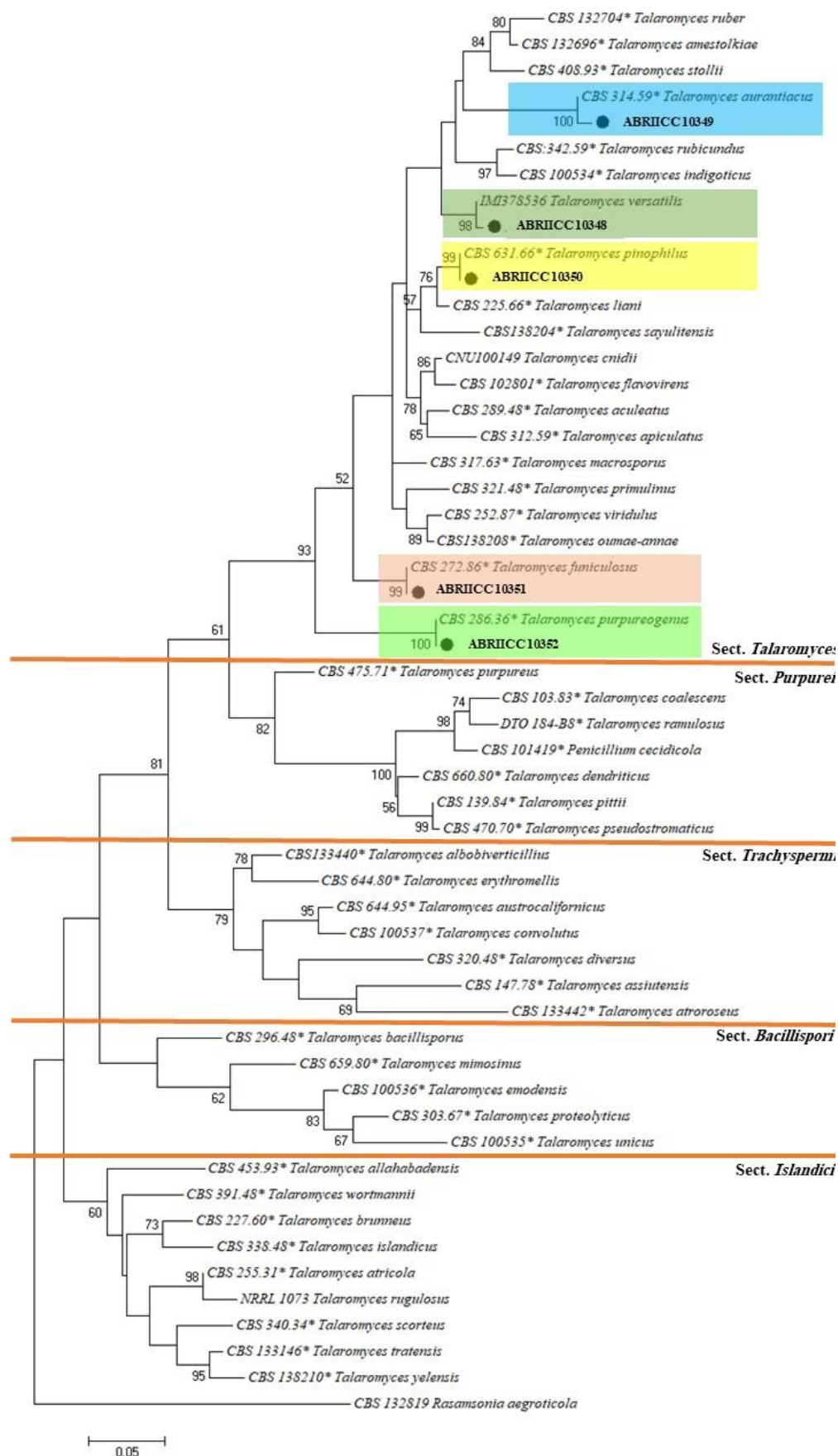
***Talaromyces aurantiacus* (J.H. Mill., Giddens & A.A. Foster) Samson, Yilmaz & Frisvad, Stud. Mycol. 71: 175. 2011.**

Basionym: *Penicillium aurantiacum* J.H. Mill., Giddens & A.A. Foster, Mycologia 49: 797. 1957.

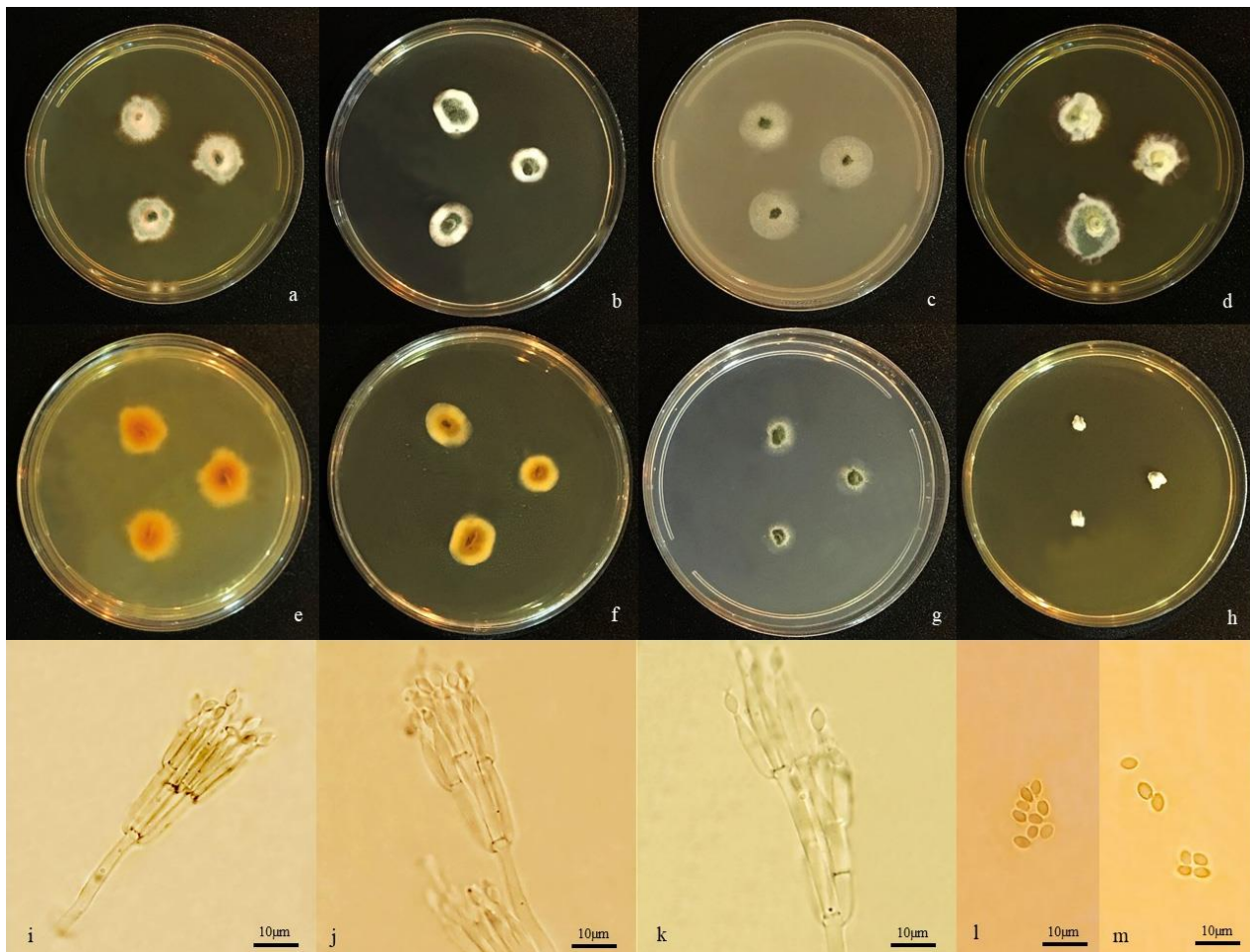
Colonies on MEA at 25°C, 19 mm growth; margins low, plane; mycelia white and light pink; texture floccose; reverse greyish orange. On OA at 25°C, 17 mm growth; colonies low, plane; margins low, plane; mycelia white; texture floccose; conidia en masse greyish green. On YES at 25°C, 21 mm growth; Colonies raised, slightly sulcate; margins low, plane; mycelia white; texture floccose; conidia en masse greyish green. On CZ at 25°C, 10 mm growth; margins low, plane; mycelia white; texture floccose; reverse greyish green. On CYA at 25°C, 15 mm growth; sterile white aerial mycelia, pale red appearance because of reverse coloring; margins low, plane; mycelia white; texture floccose and funiculose; reverse pastel red. On CYAS at 25°C, No growth (Fig. 2).

**Table 1.** *Talaromyces* strains used for phylogenetic analysis in this study.

Species	Basionym	Collection No.	Origin	Accession No. of $\beta$ - <i>tubulin</i>
<i>Talaromyces rubicundus</i>	<i>Penicillium rubicundum</i>	CBS:342.59	Soil (USA)	JX494309.1
<i>Paecilomyces aerugineus</i>	<i>Talaromyces aerugineus</i>	CBS 350.66	Debris (UK)	KJ865736.1
<i>T. aurantiacus</i>	<i>P. aurantiacum</i>	CBS 314.59	Soil (USA)	KF741917.1
<i>T. primulinus</i>	<i>P. primulinum</i>	CBS 321.48	USA	JX494305.1
<i>T. stollii</i>	<i>P. funiculosum</i>	CBS 408.93	Human (Netherlands)	JX315633.1
<i>T. ruber</i>	<i>P. rubrum</i>	CBS 132704	Aircraft fuel tank (UK)	JX315629.1
<i>T. amestolkiae</i>	<i>P. sp.</i>	CBS 132696	House dust (South Africa)	JX315623.1
<i>T. macrosporus</i>	<i>P. vermiculatum</i>	CBS 317.63	Apple juice (South Africa)	JX091382.1
<i>T. verruculosus</i>	<i>P. verruculosum</i>	CBS 388.48	Soil (USA)	KF741928.1
<i>T. viridulus</i>	<i>Geosmithia viridis</i>	CBS 252.87	Soil (Australia)	JX091385.1
<i>T. oumae-annae</i>	-----	CBS138208	House dust (South Africa)	KJ775203.1
<i>T. cnidii</i>	-----	CNU100149	medicinal crops (Korea)	KF183641.1
<i>T. flavovirens</i>	<i>P. aureocephalum</i>	CBS 102801	-----	JX091376.1
<i>T. aculeatus</i>	<i>P. aculeatum</i>	CBS 289.48	Textile (USA)	KF741929.1
<i>T. apiculatus</i>	<i>P. aculeatum</i> var. <i>apiculatum</i>	CBS 312.59	Soil (Japan)	KF741916.1
<i>T. liani</i>	<i>P. liani</i>	CBS 225.66	Soil (China)	JX091380.1
<i>T. sayulitensis</i>	-----	CBS138204	House dust (Mexico)	KJ775206.1
<i>T. indigoticus</i>	<i>P. indigoticum</i>	CBS 100534	Soil (Japan)	JX494308.1
<i>T. funiculosus</i>	<i>P. funiculosum</i>	CBS 272.86	Lagenaria vulgaris (India)	JX091383.1
<i>T. versatilis</i>	<i>P. versatilis</i>	IMI378536	Great Britain	KC992272.1
<i>T. pinophilus</i>	<i>P. pinophilum</i>	CBS 631.66	PVC (France)	JX091381.1
<i>T. purpureogenus</i>	<i>P. purpureogenum</i>	CBS 286.36	Japan	JX315639.1
<i>T. ramulosus</i>	<i>P. ramulosum</i>	DTO 184-B8	Soil, Malmesbury (South Africa)	FJ753290.1
<i>T. purpureus</i>	<i>P. purpureum</i>	CBS 475.71	Soil (France)	GU385739.1
<i>T. pseudostromaticus</i>	<i>P. pseudostromaticum</i>	CBS 470.70	Feather of <i>Hylocichla</i> (USA)	HQ156950.1
<i>T. pittii</i>	<i>P. pittii</i>	CBS 139.84	Clay soil under poplar trees (Spain)	KJ865728.1
<i>T. dendriticus</i>	<i>P. dendriticum</i>	CBS 660.80	Eucalyptus leaf litter (Australia)	JX091391.1
<i>T. coalescens</i>	<i>P. coalescens</i>	CBS 103.83	Soil under <i>Pinus</i> sp. (Spain)	JX091390.1
<i>T. cecidicola</i>	<i>P. cecidicola</i>	CBS 101419	Cynipid insect galls (USA)	FJ753295.1
<i>T. yelensis</i>	-----	CBS 138210	House dust (Micronesia)	KJ775210.1
<i>T. wortmannii</i>	<i>P. wortmannii</i>	CBS 391.48	Soil (Denmark)	KF984648.1
<i>T. tratensis</i>	-----	CBS 133146	Soil, Trat (Thailand)	KF984559.1
<i>T. scorteus</i>	<i>P. scorteum</i>	CBS 340.34	Military equipment (Japan)	KF984565.1
<i>T. rugulosus</i>	<i>P. rugulosum</i>	NRRL 1073	decaying twigs (France)	KF984575.1
<i>T. islandicus</i>	<i>P. islandicum</i>	CBS 338.48	South Africa	KF984655.1
<i>T. brunneus</i>	<i>P. brunneum</i>	CBS 227.60	Milled rice (Thailand)	KJ865722.1
<i>T. atricola</i>	<i>P. rugulosum</i>	CBS 255.31	-----	KF984566.1
<i>T. allahabadensis</i>	<i>P. allahabadense</i>	CBS 453.93	Cultivated soil (India)	KF984614.1
<i>T. purpurogenus</i>	<i>P. purpurogenus</i>	UTFC-S514	Corm of <i>Crocus sativus</i> (Kerman)	MW854300
<i>T. funiculosus</i>	<i>P. funiculosus</i>	UTFC-S236	Corm of <i>Crocus sativus</i> (Isfahan)	MW854301
<i>T. pinophilus</i>	<i>P. pinophilus</i>	UTFC-S429	Corm of <i>Crocus sativus</i> (South Khorasan)	MW854302
<i>T. aurantiacus</i>	-----	UTFC-S567	Leaf of <i>Crocus sativus</i> (Kerman)	MW854299
<i>T. versatilis</i>	-----	UTFC-S303	Corm of <i>Crocus sativus</i> (Yazd)	MW854298



**Fig. 1.** Maximum likelihood phylogram generated from partial  $\beta$ -tubulin sequence data, demonstrating phylogenetic relationships of *Talaromyces* species in *Talaromyces* section *Talaromyces*. The numbers above the branches represent branch support using 1000 bootstrap replications. *Rasamsonia aegroticola* JX273020.1 (CBS 132819) was used as an outgroup.



**Fig. 2.** Morphological characters of *Talaromyces aurantiacus*, isolate ABRIICC 1349. Colonies on a. MEA, b. CYA, c. OA, d. YES, e. MEA (reverse), f. CYA (reverse), g. CZ, h. CYAS, i–k. Conidiophores, l–m. Conidia.

Conidiophores biverticillate, consisting of a smooth-walled stipe,  $40\text{--}110 \times 2\text{--}3 \mu\text{m}$ , terminating in a whorl of two to five metulae. metulae  $8\text{--}15 \times 2.5\text{--}3 \mu\text{m}$ ; phialides acerose, two to six per metulae,  $10\text{--}17 \times 2\text{--}3 \mu\text{m}$ ; Conidia smooth, cylindrical to ellipsoidal,  $3\text{--}6 \times 1.5\text{--}2.5 \mu\text{m}$ . Ascomata not observed (Fig. 2).

*Specimen examined.* IRAN, Kerman Province, Dashtkhak region, on a leaf of *Crocus sativus* L., June 2018, H. Vardasbi, (ABRIICC 1349).

*Notes.* *Talaromyces aurantiacus* closely resembles *T. funiculosus*. *T. funiculosus* has strongly funiculate colonies on MEA and OA. This feature distinguishes *T. funiculosus* from *T. aurantiacus*.

Based on our knowledge, this is the first report of this species for the mycobiota of Iran.

***Talaromyces funiculosus* (Thom) Samson et al., Stud. Mycol. 71: 176. 2011.**

*Basionym:* *Penicillium funiculosum* Thom, Bull. Bur. Anim. Ind. U.S. Dep. Agric. 118: 69. 1910.

Colonies on MEA at  $25^\circ\text{C}$ , 24 mm growth; margins low, plane; mycelia white to light pink; texture funiculate; conidia en masse greyish green to dull green. On OA at  $25^\circ\text{C}$ , 16 mm growth; margins low,

plane; mycelia white; texture loosely funiculate to velvety. On YES at  $25^\circ\text{C}$ , 21 mm growth; colonies slightly raised at the center; margins low, plane; mycelia white; texture sterile aerial mycelia grow like funiculate; sporulation absents. On CZ at  $25^\circ\text{C}$ , 12 mm growth; margins low, sulcate; mycelium white; texture funiculate; conidia en masse greyish green to dull green. On CYA at  $25^\circ\text{C}$ , 16 mm growth; Colonies slightly raised at the center; margins low, plane; mycelia white; texture floccose; reverse light orange to greyish orange. On CYAS at  $25^\circ\text{C}$ , No growth (Fig. 3).

Conidiophores biverticillate, consisting of a smooth-walled stipe, terminating in a whorl of three to six metulae. metulae  $6.5\text{--}11.5 \times 2\text{--}4 \mu\text{m}$ ; phialides lanceolate, three to eight per metulae,  $7\text{--}11 \times 1.5\text{--}2.5 \mu\text{m}$ ; Conidia smooth, subglobose to ellipsoidal,  $2\text{--}3 \times 1\text{--}2 \mu\text{m}$ . Ascomata not observed (Fig. 3).

*Specimen examined.* IRAN, Isfahan Province, Natanz region, on corm of *Crocus sativus* L., June 2018, H. Vardasbi, (ABRIICC 1351).

*Notes.* *Talaromyces funiculosus* characteristically produces colonies that are strongly funiculate. It shows fast growth on general media at  $37^\circ\text{C}$ . These features distinguish *T. funiculosus* from other *Talaromyces* species.



**Fig. 3.** Morphological characters of *Talaromyces funiculosus*, isolate ABRIICC 1351. Colonies on a. MEA, b. CYA, c. OA, d. YES, e. MEA (reverse), f. CYA (reverse), g. CZ, h. CYAS, i–k. Conidiophores, l. Conidia.

*Talaromyces funiculosus*, have been previously reported from Iran as *Penicillium funiculosus* (Sadraei and Rahimizadeh 2016). This is the first study of this species, after revising *Talaromyces* genus by Samson et al. (2011). They classified *Talaromyces* as a new genus and distinguished it from *Penicillium*; accordingly, this is the first time that *T. funiculosus* species is reported from saffron in Iran.

***Talaromyces pinophilus* (Hedgc.) Samson et al., Stud. Mycol. 71: 176. 2011.**

Basionym: *Penicillium pinophilum* Hedgcock apud Thom, Bull. Bur. Anim. Ind. US Dept. Agric. 118: 37. 1910.

Colonies on MEA at 25°C, 21 mm growth; colonies slightly raised; margins low, plane; mycelia white and yellow; texture loosely funiculose to floccose especially in the center; reverse greyish orange. On OA at 25°C, 18 mm growth; margins low, plane; mycelia white and yellow; texture funiculose and floccose, especially in the center aerial mycelia; conidia en masse greyish yellow. On YES at 25°C, 22 mm growth; colonies slightly raised at the center; margins low, plane; mycelia white and yellow; texture floccose; conidia en masse greyish green; dark brown to brownish orange. On CZ at 25°C, 15 mm growth;

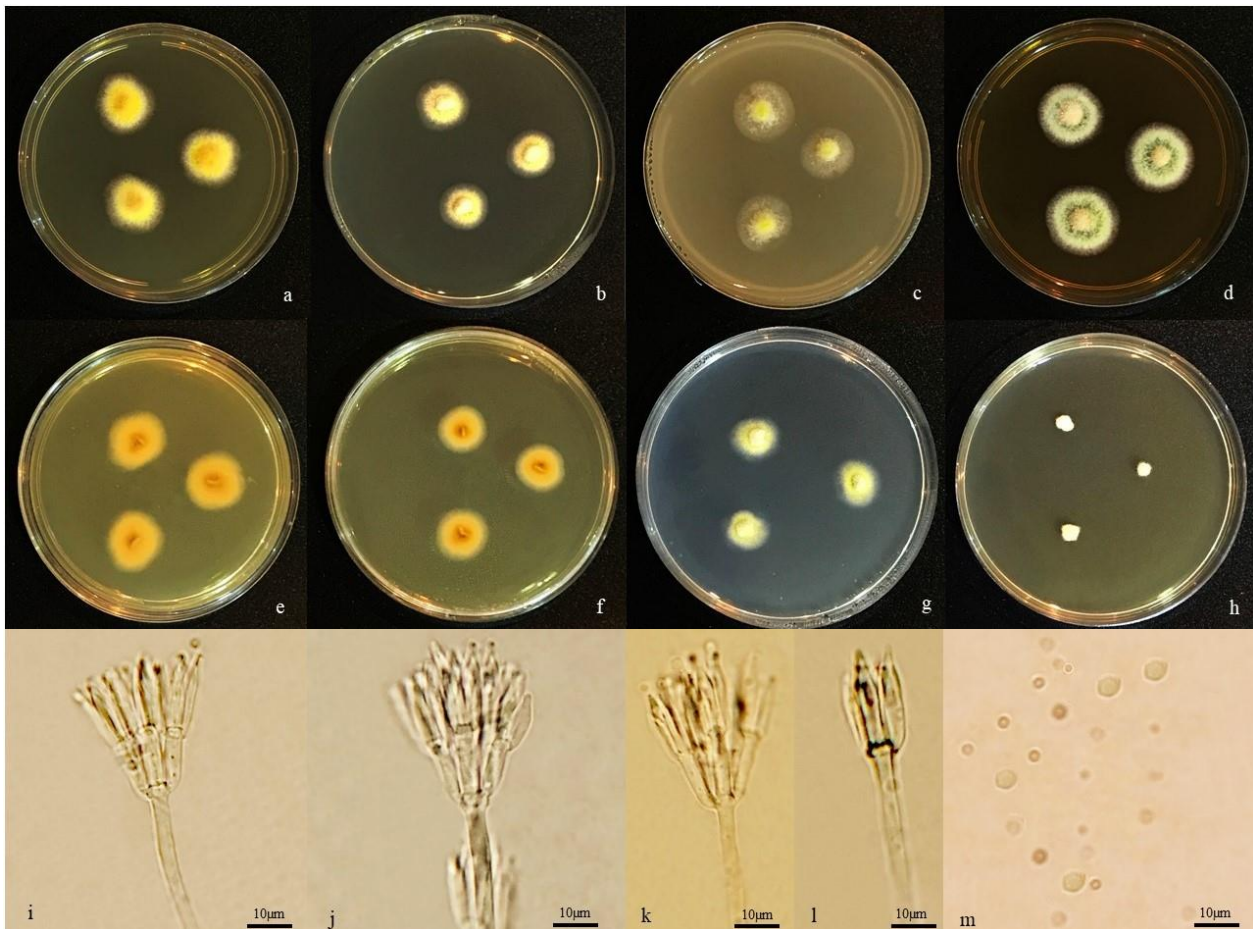
colonies raised at the center; margins low, plane; mycelia white and yellow; texture floccose; conidia en masse yellow. On CYA at 25°C, 16 mm growth; colonies raised at the center; margins low, plane; mycelia white and yellow; texture loosely funiculose and floccose especially in the center; reverse greyish orange to orange. On CYAS at 25°C, No growth (Fig. 4).

Conidiophores biverticillate, consisting of a smooth-walled stipe, terminating in a whorl of three to eight metulae rarely finely roughened with age. metulae 10–11 × 2.5–3 µm; phialides acerose, three to eight per metulae, 8.5–12 × 2–3 µm; Conidia with smooth to finely roughened walls, globose to subglobose, 2–3 × 2–3 µm. Ascomata not observed (Fig. 4).

*Specimen examined.* IRAN, South Khorasan Province, Sarayan region, on corm of *Crocus sativus* L., June 2018, H. Vardasbi, (ABRIICC 1350).

*Notes.* *Talaromyces pinophilus* closely resembles *T. stollii*, in terms of micro- and macromorphology. However, *T. stollii* grows slightly faster than *T. pinophilus* on CYA.

Based on investigations, *Talaromyces pinophilus*, have been previously reported from Iran as *Penicillium pinophilum* (Ershad 2009). This is the first study of this species, after revising *Talaromyces* genus by Samson et al. (2011).



**Fig. 4.** Morphological characters of *Talaromyces pinophilus*, isolate ABRIICC 1350. Colonies on a. MEA, b. CYA, c. OA, d. YES, e. MEA (reverse), f. CYA (reverse), g. CZ, h. CYAS, i–l. Conidiophores, m. Conidia.

***Talaromyces purpureogenus* (Stoll) Samson, Yilmaz, Frisvad & Seifert**

Basionym: *Penicillium purpureogenum* Stoll, Beitr. Charakt. *Penicillium*-Arten: 32. 1904.

Colonies on MEA at 25°C, 22 mm growth; margins low, plane, mycelium orange and white; texture floccose, with some velvety areas; reverse greyish orange. On OA at 25°C, 13 mm growth; low, margins low, plane; mycelium white and green; texture velvety and floccose; sporulation moderately dense to dense. On YES at 25°C, 17 mm growth; margins low, sulcate; mycelium white and orange; texture floccose; sporulation moderately dense; conidia en masse greyish green. On CZ at 25°C, 10 mm growth; margins low, sulcate; mycelium white; texture velvety; sporulation moderately dense; soluble pigment typically light red. On CYA at 25°C, 16 mm growth; margins very narrow, plane; mycelium white and red; texture floccose; sporulation moderately dense; conidia en masse dull green; reverse dark brown to reddish-brown. On CYAS at 25°C, No growth (Fig. 5).

Conidiophores strictly biverticillate, stipes smooth-walled, hyaline, 150–240 × 2.5–3 µm; Metulae and phialides 12–14 µm long. phialides

acerose, three to six per metula, Conidia ellipsoidal, sometimes subspherical, apiculate, irregularly roughened, 3–3.5 × 2–2.5 µm (Fig. 5).

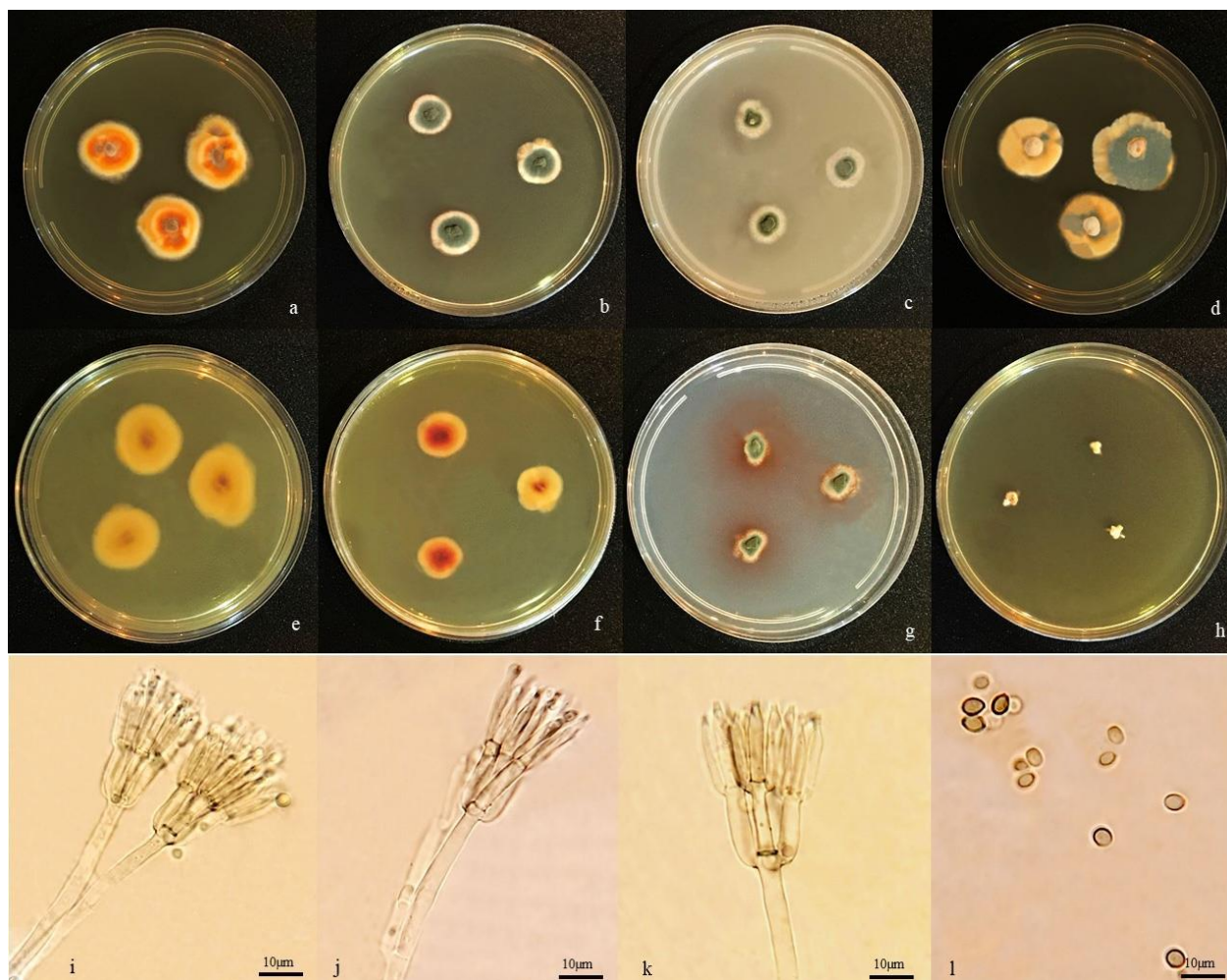
*Specimen examined.* IRAN, Kerman Province, Tarz region, on corm of *Crocus sativus* L., June 2018, H. Vardasbi, (ABRIICC 1352).

*Notes.* *Talaromyces purpureogenus* is not capable of growing at temperatures below 18°C, grows slower and produces a bright red diffusing pigment on CYA at 25°C. These characters distinguish *T. purpureogenus* from other *Talaromyces* species.

*Talaromyces purpureogenus* species is reported from saffron for the first time, in Iran. *Talaromyces purpureogenus*, have been previously reported from Iran as *Penicillium purpureogenum* (Sadraei and Rahimizadeh 2016). This is the first study of this species, after revising *Talaromyces* genus by Samson et al. (2011).

***Talaromyces versatilis* P.F. Cannon, Bridge & Buddie. 2013.**

Colonies on MEA at 25°C, 18 mm growth; margins low, plane; mycelia white; texture funiculose; sporulation moderately dense to dense; conidia en masse greyish green. On OA at 25°C, 19 mm growth;



**Fig. 5.** Morphological characters of *Talaromyces purpureogenus*, isolate ABRIICC 1352. Colonies on a. MEA, b. CYA, c. OA, d. YES, e. MEA (reverse), f. CYA (reverse), g. CZ, h. CYAS, i–k. Conidiophores, l. Conidia.

margins low, plane; mycelia white; texture floccose and velvety, especially in the center aerial mycelia. On YES at 25°C, 20 mm growth; margins low, plane; mycelia white; texture floccose; conidia en masse greyish green; reverse brownish orange. On CZ at 25°C, 17 mm growth; margins low, sulcate; mycelia white; texture floccose. On CYA at 25°C, 20 mm growth; margins low, plane; mycelia white; texture floccose; reverse greyish orange. On CYAS at 25°C, No growth (Fig. 6).

Conidiophores biverticillate; Stipes usually hyaline, smooth or rough-walled,  $35\text{--}100 \times 2\text{--}3 \mu\text{m}$ , terminating in a whorl of five to 11 metulae; metulae  $11\text{--}12 \times 2.5\text{--}3.5 \mu\text{m}$ ; phialides acerose, approximately equal length to metulae, three to five per metulae,  $11\text{--}12 \times 2.5\text{--}3.5 \mu\text{m}$ ; Conidia aseptate, green masse, usually globose to subglobose or ovoid,  $2.4\text{--}2.8 \times 2\text{--}2.4 \mu\text{m}$  (Fig. 6).

*Specimen examined.* IRAN, Yazd Province, Abarkouh region, on corm of *Crocus sativus* L., June 2017, H. Vardasbi, (ABRIICC 1348).

*Notes.* *Talaromyces versatilis* contains key enzymes involved in lignocellulose degradation, such as cellulases, which can hydrolyse pretreated

sugarcane bagasse to reach a hydrolysis yield up to 88 % (Maeda et al. 2013).

Based on investigations, *T. versatilis* has not been reported from Iran so far, and this is the first report of this species for the mycobiota of Iran.

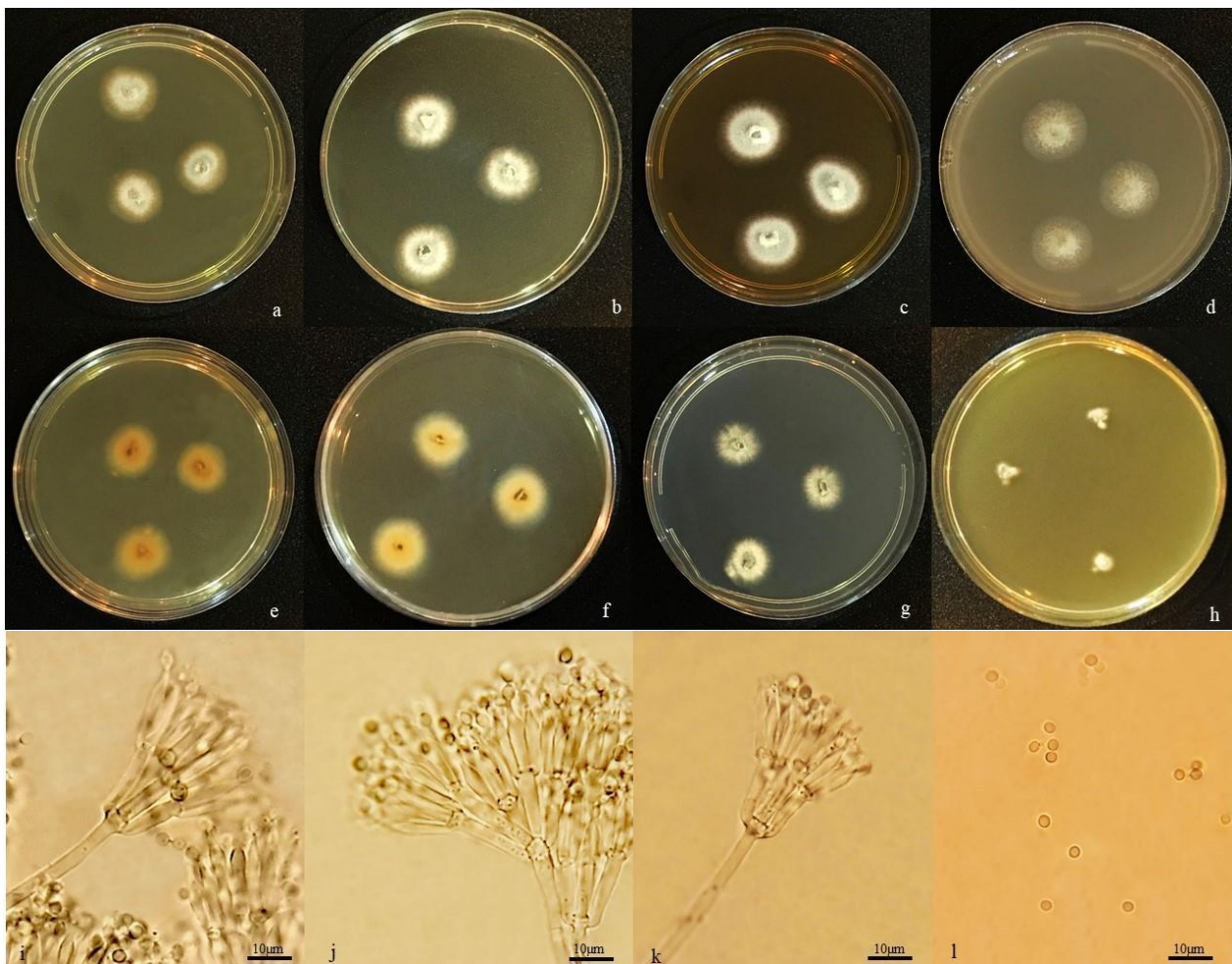
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**Fig. 6.** Morphological characters of *Talaromyces versatilis*, isolate ABRIICC 1348. Colonies on a. MEA, b. CYA, c. OA, d. YES, e. MEA (reverse), f. CYA (reverse), g. CZ, h. CYAS, i–k. Conidiophores, l. Conidia.

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## معرفی گونه‌های اندوفیت جدیدی از *Talaromyces* sect. *Talaromyces* همراه گیاه زعفران برای مایکوبیوتای ایران

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**چکیده:** در این پژوهش تنوع زیستی قارچ‌های اندوفیت همراه گیاه زعفران عمده‌ترین مناطق کشت زعفران در ایران، مورد بررسی قرار گرفت. پنج جدایه از جنس *Talaromyces* به دست آمد. این جدایه‌ها بر اساس ویژگی‌های ریخت‌شناسی و مولکولی به عنوان *Talaromyces versatilis*، *T. aurantiacus*، *T. pinophilus*، *T. funiculosus* و *T. purpureogenus* شناسایی شدند. طبق اطلاعات بدست آمده، دو گونه *Talaromyces versatilis* و *T. aurantiacus* اولین گزارش برای مایکوبیوتای ایران هستند.

**کلمات کلیدی:** ژن بتا-توبولین، ریخت‌شناسی، تبارشناسی، نمونه برداری