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Ascotricha funiculosa a new species for the funga of Iran

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Travertine stone (Abbas Abad type) is one of the widely used stones in Iran. The mines of this stone, one of the most luxurious travertine stones in the world with a white-background color, are located in Mahalat, although its production is mainly carried out in Isfahan province. In our visits of various building stone sites in Hafiz town, Shiraz, Fars province in 2021, loose and rotten stones with black streaks were observed from which the rocks are cracked, causing a severe damage to stonework's industry (Fig. 1a). To isolate fungi, small pieces (5 × 5 mm) of stone samples were surface disinfested with 0.5% sodium hypochlorite for 1 min, washed three times with sterilized distillate water and plated on potato dextrose agar (PDA; Merck, Darmstadt, Germany). Purification of isolates was done by hyphal tip technique. Among the isolated fungi, one isolate that was recovered from Imperator work stone in Hafiz town, Shiraz (Fars province) was identified as *Ascotricha funiculosa* (Guarro & Calvo) D.W. Li & G.H. Zhao, based on morphological features (Guarro and Calvo 1983) and sequences data. Macroscopic and microscopic features of the fungus are given here.

Colonies were slow-growing, attaining a diameter of 17 mm in seven days on PDA (Merck, Darmstadt, Germany), first dull white, then becoming grayish with a white edge (Fig. 1b). The colony on the reverse side of the agar plate was blackish (Fig. 1c). Conidiophores were borne mostly as short vertical branches from individual hyphae, commonly 70-130 × 2.5–4.5 µm, composed of a differentiated supporting hyphae bearing sterile swollen cells hyaline and groups of conidiogenous cells. Stipe was smooth, simple or once branched, septate, hyaline when young, becoming olive-brown at maturity, swollen cells hyalin, sterile, thin-walled, 6-8 × 4-5 µm, rounded above (Fig. 1d-h). Conidiogenous cells were lateral and terminal, sympodial, developing conidia on denticles (Fig. 1g-h). Conidia were smooth, subglobose to ellipsoidal, unicellular, hyaline when young, becoming light brown at maturity, with a minute scar at the base, and 4–6 × 3.5–4.5 µm ($\bar{x}$ = 5 × 3.5 µm, n = 100) (Fig. 1i).

For confirmation of morphological identification, DNA of representative isolate was extracted using a commercial kit (Zagros Bioidea Co., Razi University Incubator, Kermanshah, Iran). PCR was performed using primers ITS1 (CCGTAGGTGAACCTGCGC) and ITS4 (TCCTCCGCTTATTGATATGC) for the internal transcribed spacer region (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA (rDNA) (White et al. 1990), and Bt2a (GGTAACCAAATCGGTGCTGC TTTC) and Bt2b (ACCCTCAGTGTAGTGACCC TTGGC) for a part of the *β-tubulin* gene (Glass & Donaldson 1995). The PCR product was submitted for sequencing to a capillary sequencing machine (Pishgam Biotech Co., Tehran, Iran). The sequence generated in this study was deposited in GenBank under accession number OK324153 (ITS) and OK337389 (*β-tubulin* gene).

BLAST analysis revealed a high nucleotide identity (99% for ITS and 98.7% for *β-tubulin* gene) with the ITS region and *β-tubulin* gene of *Ascotricha funiculosa* isolate CBS 323.86 (KU684134 and KU683762) that was recently reported from North American (Cheng et al. 2015). Two datasets, including individual aligned sequences of ITS, and the combined ITS and *β-tubulin* datasets (ITS- *β-tubulin*), were used to phylogenetic analysis of *Ascotricha*. Phylogenetic analyses were performed using maximum likelihood (ML) method in the MEGA Ver. X program (Kumar *et al*. 2018). Phylogenetic analyses based on ITS (Fig. 2), and combined ITS and *β-tubulin* gene sequences (Fig. 3) of our isolates and 22 selected isolates of *Ascotricha* (Table 1) showed that our isolates are closely related to *A. funiculosa* (Figs. 3, 4).

The isolates formed a well-supported clade with the reliable reference strains of *A. funiculosa* (Figs. 2, 3), placed separately from the other species of *Ascotricha*. The result of the phylogenetic analysis was in accordance with the molecular identification based on DNA sequences in BLAST search, thus resolving the morphological identification.

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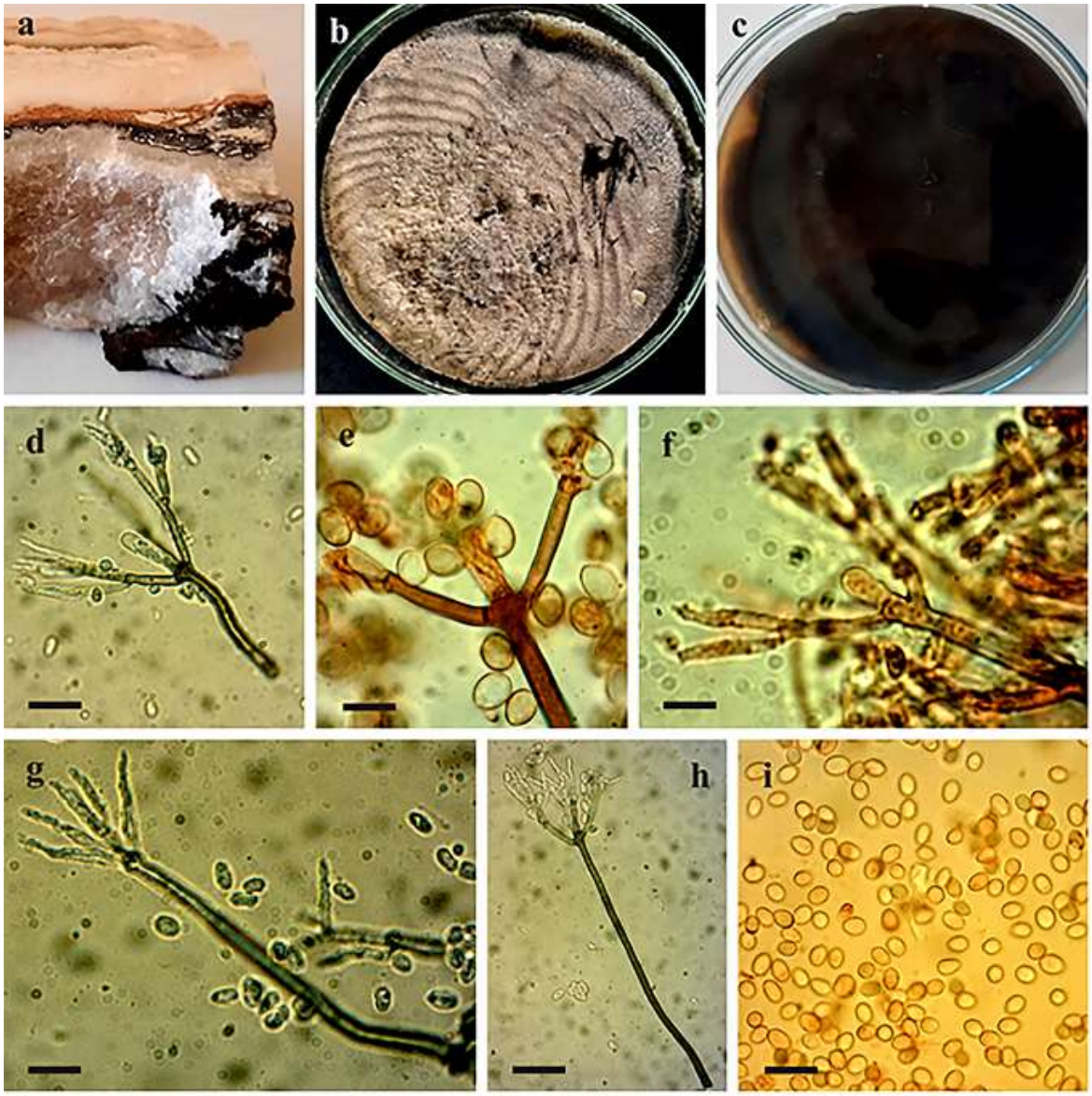

Fig. 1. Travertine stone colonized by *Ascotricha funiculosa* (a). Colony on PDA after 21 days (b), Reverse side of colony (c), Conidiophores and conidiogenous cells (d-h), conidia (i). Bars: d, h = 10 µm, e, f, g, i= 5 µm.

In Iran, *Ascotricha chartarum* has been reported from Abbas abad travertine stone (Jamali 2021). Information about of *A. funiculosa* is rare and this species previously has been reported from Spain. *A. funiculosa* differs from other species in the morphology of its conidiogenous cells (Guarro & Calvo 1983) and in lacking a sexual stage. In this study also, sexual stage was not seen in culture media. *Ascotricha funiculosa* is new to the Iranian funga, and is reported for the first time from building stone in the world.

A subculture of this fungus is preserved at the Iranian Fungal Culture Collection of the Iranian Research Institute of Plant Protection (Tehran, Iran) under accession number IRAN 4558C. The genus *Ascotricha* (Ascomycetes, Xylariaceae) was erected by Berkeley in 1838 to accommodate the single species *A. chartarum* (Hawksworth 1971). So far, 29, and 30 *Ascotricha* species are recorded in the MycoBank and Index Fungorum, respectively.

Many researchers have reported *Ascotricha* species in the China, Germany, India, Italy, New Zealand, North America, Portugal, and Spain, (Stchigel & Guarro 1998; Udagawa & Uchiyama 1999; Li & Yang 2004; U'Ren et al. 2016; Vu et al. 2019) but no such studies have been carried out in Iran.

Key words: Stone deterioration, Xylariaceae, *Dicyma*, β-tubulin gene, Iran

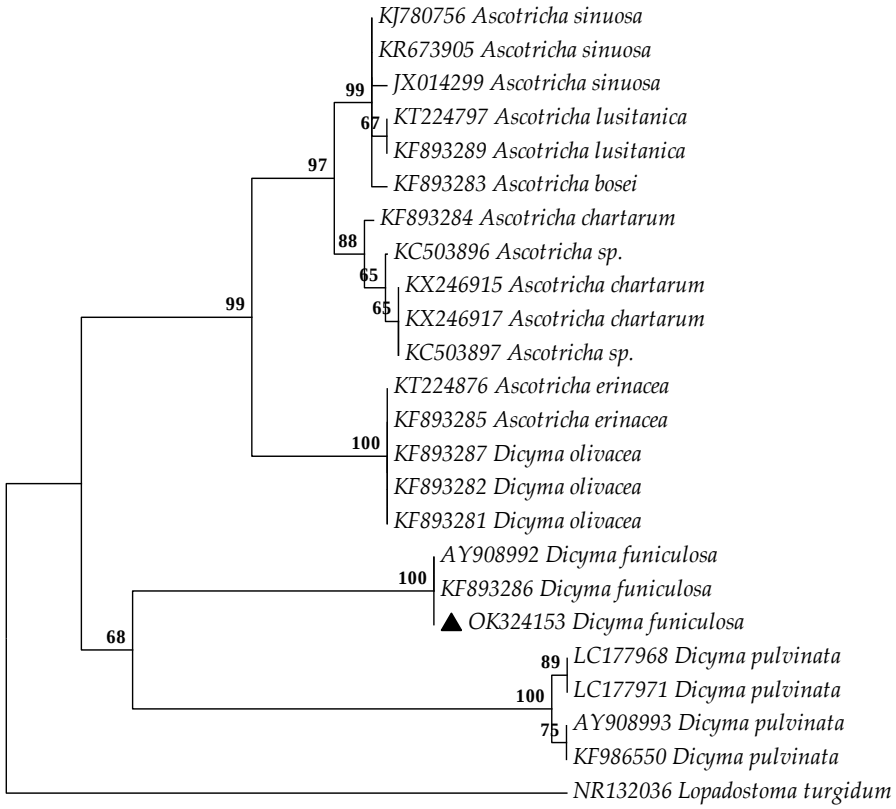

Fig. 2. Phylogenetic tree generated from a Maximum liklihood inference based on ITS dataset. Maximum likelihood bootstrap are indicated at the nodes. Black triangle refer to Iranian isolate.

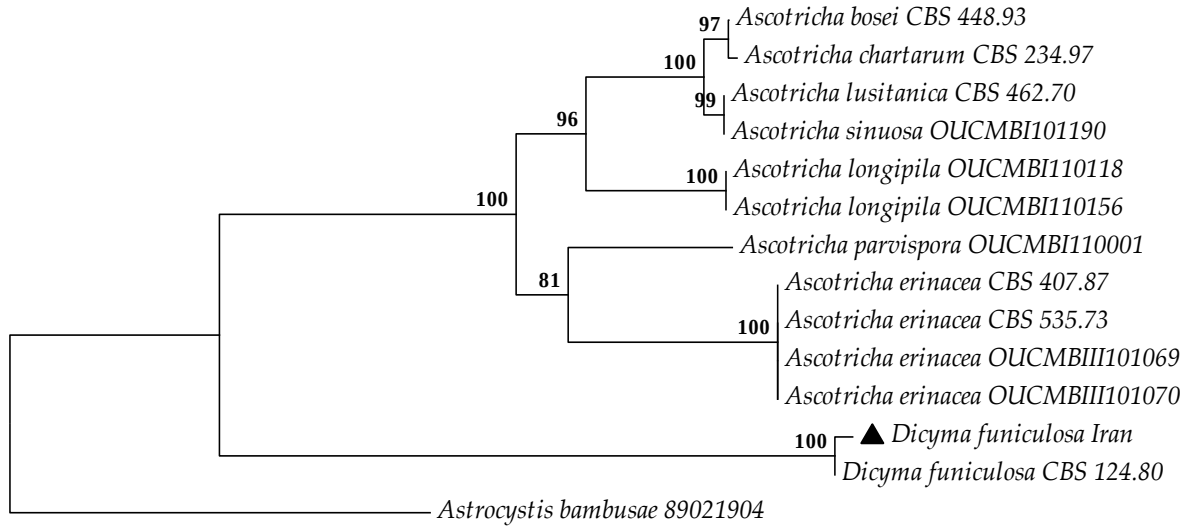

Fig. 3. Phylogenetic tree generated from a Maximum liklihood inference based on combined ITS and *β-tubulin* datasets. Maximum likelihood bootstrap are indicated at the nodes. Black triangle refer to Iranian isolate.

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Table 1. Overview of all taxa and corresponding DNA sequences selected for the molecular phylogeny

Species	Isolate No.	GenBank Accession No.		Authors
		ITS	β-tubulin	
Ascotricha bosei	CBS 448.93 (T)	KF893283	KF893270	Cheng et al. 2015
A. chartarum	CBS 234.97	KF893284	KF893271	Cheng et al. 2015
A. chartarum	B4C	KX246915	-	Okpalanozie et al. 2016
A. chartarum	B4F	KX246917	-	Okpalanozie et al. 2016
A.erinacea	CBS 407.87	KF893287	KF893274	Cheng et al. 2015
A.erinacea	CBS 535.73	KF893285	KF893272	Cheng et al. 2015
A.erinacea	OUCMBIII $_{10}$ 1069	KF893281	KF893268	Cheng et al. 2015
A.erinacea	OUCMBIII $_{10}$ 1070	KF893282	KF893269	Cheng et al. 2015
A.erinacea	S98	KT224876	-	Li and Zhao 2018
A. longipila	OUCMBI$_{11}$0118 (T)	KC503896	KF893265	Cheng et al. 2015
A. longipila	OUCMBI$_{11}$0156 (IT)	KC503897	KF893264	Cheng et al. 2015
A. lusitanica	CBS 462.70 (IT)	KF893289	KF893275	Cheng et al. 2015
A. lusitanica	S19	KT224797	-	Li and Zhao 2018
A. parvispora	OUCMBI$_{11}$0001 (T)	JX014298	KF893267	Cheng et al. 2015
A. sinuosa	OUCMBI$_{10}$1190 (T)	JX014299	KF893266	Cheng et al. 2015
A. sinuosa	A1S5	KJ780756	-	Li and Zhao 2018
A. sinuosa	F8	KR673905	-	Li and Zhao 2018
Dicyma funiculosa	CBS 124.80 (T)	KF893286	KF893273	Cheng et al. 2015
D. funiculosa	CBS323.86	AY908992	-	Uren et al. 2016
D. funiculosa	-	**OK324153**	**OK337389**	**This study**
D. pulvinata	414-3	LC177968	-	Li and Zhao 2018
D. pulvinata	KACC44502	LC177971	-	Li and Zhao 2018
D. pulvinata	CBS194.56	AY908993	-	Uren et al. 2016
Dicyma pulvinata	LCC11	KF986550	-	Li and Zhao 2018

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