Mycologia Iranica 2025, Vol. 12 (No. 2): ??–??

Original Article

Morpho-Phylo taxonomy of three new *Microascus* species (*Microascaceae*, *Microascales*, *Ascomycota*) for Iran

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https://doi.org/10.22092/mi.2025.371343.1334

ABSTRACT

During a study on the biodiversity of ascomycetous micro-fungi from 2020 to 2024, several fungal isolates from healthy Cannabis seeds (*Cannabis sativa* L.) and healthy Camelthorn roots (*Alhagi maurorum* Medik), as endophytic fungi, and some of them from laboratory air, as airborne fungi, were recovered and characterized. Based on the morphological investigations, part of the identified fungi belonged to the genus *Microascus* (*Microascaceae*). Molecular phylogenetic analysis using the partial nucleotide sequence of the β-tubulin (*tub2*) gene and the resulting consensus phylogenetic tree revealed that the recovered isolates belong to three different *Microascus* species, including *M. cinereus* from healthy Camelthorn root, *M. croci* from laboratory air and *M. trigonosporus* from healthy Cannabis seed with high levels of sequence similarity with the relevant sequences from GenBank. In the resulting consensus phylogenetic tree, these three identified *Microascus* species were completely resolved from each other as well as from the other species with high bootstrap support and posterior probability, confirming the correct morphological identification and the effectiveness of the used genomic region. To the best of our knowledge, these three *Microascus* species are new to the funga of Iran. Additionally, the *Alhagi maurorum* and *Cannabis sativa* plants are reported here as new hosts (matrix nova) for *M. cinereus* and *M. trigonosporus*, respectively, in the world.

KEYWORDS

Biodiversity, β-tubulin, Fungi, Phylogeny, Taxonomy.

INTRODUCTION

Fungi exhibit substantial diversity in both morphological traits and nutritional modes, which facilitates the decomposition of organic matter and contributes to ecosystem stability and health. They also demonstrate adaptability to a broad spectrum of environmental conditions and habitats, with the capacity to parasitize or infect plants and animals, including mammals and insects (Anees-Hill et al. 2022). The family *Microascaceae* Luttr. ex Malloch (*Microascales*, *Sordariomycetes*, *Ascomycota*) was erected in 1951 by Luttrell to accommodate the genus *Microascus* Zukal. In recent decades, improvements in morphology and multi–locus phylogenetic analyses have enhanced our understanding of the taxonomy and species diversity within the family *Microascaceae*. Species in this family are primarily distinguished by their annellidic asexual morphs with dry, aseptate conidia, and their sexual morphs, which produce cleistothecial or perithecial, and carbonaceous ascomata that yield reniform, lunate, or triangular ascospores, sometimes having germ pores. Currently, around 290 species across 23 genera are recognized in the *Microascaceae* family. The majority of which are terrestrial saprobes or plant pathogens. The genus *Microascus* is characterized by ampulliform or globose perithecia possessing necks that are papillate or cylindrical in shape. The asci are unitunicate, ovate to globose in morphology, evanescent, and non-

Received: 08 Nov. 2025 Revised: 30 Nov. 2025 Accepted: 01 Dec. 2025 Published online: 06 Dec. 2025

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Published by Iranian Mycological Society (IrMS)—https://mij.areeo.ac.ir

pedicellate that irregularly disposed throughout the centrum. These asci are arranged in basipetal chains and contain 1–8 ascospores. Ascospores exhibit asymmetry and vary in shape, including triangular, lunate, or reniform configurations. Germ pores in the ascospores are concealed, and they are released in a mucus-bound, ribbon–like mass (cirrus) or a gelatinous ball at the apex of the ascomata. Species within the genus *Microascus* are commonly isolated from soil, plant debris, and indoor environments. Certain species exhibit pathogenicity toward animals, including mammals (particularly humans) and insects, with some documented as opportunistic human pathogens (Ustun et al. 2006, Sandoval-Denis et al. 2016, Wang et al. 2024). To date, the genus *Microascus* encompasses approximately seventy–eight accepted species that were listed in the Index Fungorum until November 2025 (https://www.indexfungorum.org).

Sequences of the genomic regions such as ITS, LSU, *tef1* and β-tubulin are widely used for distinguishing different species in the genus *Microascus* (Wei et al. 2024). Phylogenetic analyses of *Microascus* species have revealed that the sequences of the LSU, β–tubulin, and *tef1* genes are reliable molecular markers for robust species identification and delimitation. Sandoval-Denis et al. (2016) have investigated the phylogeny of the *Microascus*, *Scopulariopsis* Bainier and allied genera by integrating morphological, physiological, and multi–genic sequence analyses of ITS, LSU, *EF-1α*, and β-tubulin genomic regions. Based on the analyses and applying the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) criterion, they revealed that these two genera belong to two distinct evolutionary lineages.

According to the literature, only two studies have been carried out on the species identification in the genus *Microascus* in Iran, and only two *Microascus* species have been reported from Iran until now. For example, in a study performed in Iran, *M. cirrosus* Curzi was isolated and reported for the first time from Pistachio trees as a fungus associated with brown leaf spot disease (Mirzaee et al. 2010). Also, Karimzadeh et al. (2024) have reported *M. cirrosus* and *M. terreus* (Kamyschko) Jagielski, Sand.-Den. & Gené for the first time as endophytic fungi of *Capparis spinosa* L. from Iran. According to their report, *M. terreus* was introduced as a new species for Iran. Therefore, the primary objective of the present study was to survey the fungal isolates belonging to the genus *Microascus* in some habitats of Iran.

MATERIALS AND METHODS

Fungal strains

In this study, fungal strains were obtained from healthy seeds and plants, as well as from the laboratory air of the Plant Pathology Laboratory in the Department of Plant Protection, Faculty of Agriculture, College of Agriculture and Natural Resources, University of Tehran, Karaj, during 2020 to 2024. To identify the endophytic fungi of healthy Cannabis seeds (*Cannabis sativa* L.) and healthy Camelthorn roots (*Alhagi maurorum* Medik), healthy seed and plant samples were collected from South Khorasan and Golestan Provinces, respectively. The method proposed by Tan et al. (2012), with slight modifications, was used for surface sterilization of seeds and plant tissues and isolation of fungal endophytes. In the first stage, 70% ethanol was applied for two minutes; in the second stage, 1% sodium hypochlorite was applied for three minutes; and in the third stage, a final treatment with 70% ethanol was applied for two minutes to the seeds and plant pieces. Subsequently, the sterilized seed and tissue samples were rinsed three times with sterile distilled water for three minutes. Finally, seeds and plant tissues were dried between sterile filter papers (Götz et al. 2006). Seeds and Plant pieces were placed at regular intervals in Petri dishes containing water agar culture medium, and then incubated for one to two weeks at 25±2 °C in absolute darkness. Five fungal strains were also isolated from the air of the Plant Pathology laboratory (Department of Plant Protection, Faculty of Agriculture, College of Agriculture and Natural Resources, University of Tehran, Karaj) in Alborz Province. For spore trapping, 10 Petri dishes containing potato dextrose agar (PDA) culture medium with open lids were placed in the laboratory. After 10 days, the grown fungal colonies were investigated. Fungal growth from plant tissue was checked over two weeks, and the resulting fungal colonies were purified using single-spore and hyphal tip purification techniques. Pure fungal isolates were transferred to Petri dishes containing oat meal agar (OMA) culture medium and maintained at 25±2 °C under the alternating near–UV light (12 h light/12 h dark) until pure fungal colonies appeared. For long–term storage, fungal isolates were grown on sterile filter papers placed on PDA culture medium for seven to 10 days. Subsequently, colonized filter papers were taken from the surface of culture medium, and then were dried at room temperature for four to five days, and finally were stored at –20 °C for future use.

Morphological identification

The morphological characterization of the fungal isolates was performed based on the morphology of the colony, as well as the features of the fruiting bodies, such as ascomata, asci, ascospores, and conidial stage. Morphological studies of the obtained fungal isolates were performed on OMA culture medium, and the cultures were incubated under the near–ultraviolet (nUV) light (12 h light/12 h darkness) at 25 °C. After 14 to 35 days incubation of the pure fungal colonies, the fungal features were assessed by BH2 light microscope (Olympus, Japan) using the microscopic slide mounts prepared in lacto–phenol or lacto–phenol cotton blue solutions. Colony diameter of the fungal

Table 1. Partial β-tubulin gene sequences used for phylogenetic analyses. Newly generated sequences are in boldface. [T]: type material sequence.

Species	Strain	Host/Substrate	Country	GenBank accession no.
				tub2
Microascus alveolaris	CBS 150.64	*Allium cepa* L.	USA	KX924262
	CBS 139501[T]	Human, BAL fluid	USA	KX924263
M. atrogriseus	DTO 139-D7	Indoor	Germany	KX924267
	CBS 410.76	Burnt soil	Netherlands	KX924266
M. cinereus	**ABRIICC10455**	***Alhagi maurorum***	**Iran**	**PV090984**
	CBS 138709	Human	USA	KX924272
M. collaris	LC12599	Animal faeces	China	MK336121
	LC12598[T]	Plant debris	China	MK336120
M. croci	**ABRIICC10373**	**laboratory air**	**Iran**	**PV082623**
	HHAUF170524	Soil	China	MG595660
	CBS 158.44[T]	*Crocus* sp.	Netherlands	KX924287
M. expansus	CBS 138127[T]	Human sputum	USA	KX924294
	CBS 138127[T]	Human sputum	SPAIN	LM652624
M. gracilis	DTO 342-G8	Unknown	Netherlands	KX924303
	DTO 342-D6	Unknown	Netherlands	KX924302
M. hyalinus	CBS 134639	Goat dung	China	KX924306
	CBS 766.70[T]	Cow dung	USA	KX924305
M. levis	LC12495	Soil	China	MK336123
	LC12447	Animal faeces	China	MK336122
M. longicollis	PL 2302	Human	Czech Rep.	LT548282
	CBS 752.97	*Anacardium occidentale* L.	Brazil	KX924309
M. melanosporus	HHAUF170520	Soil	China	MG516737
	HHAUF170522	Soil	China	MG516738
M. micronesiensis	DTO 223-A5	Indoor	Micronesia	KX924341
	CBS 141523[T]	Indoor	Micronesia	KX924340
M. superficialis	LC12601	Animal faeces	China	MK336128
	LC12600	Animal faeces	China	MK336127
M. terreus	CBS 665.71	Soil	USA	KX924372
	CBS 807.73	Saline desert soil	Kuwait	KX924373
M. trautmannii	IHEM:28176	*Myotis daubentonii* Kuhl	Belgium	OU641439
	IHEM:27958	*Myotis daubentonii*	Belgium	OU641436
M. trigonosporus	**ABRIICC10370**	***Cannabis sativa***	**Iran**	**PV082622**
	CBS 366.65[T]	Unknown	India	KX924380
	DTO 220-I8	Indoor	Netherlands	KX924382
Kernia columnaris	CBS:159.66	Dung of hare	South Africa	MN982416

strains were usually measured after 14 days. Macro– and micro–morphological features of the different recovered isolates were measured according to Barron et al. (1961), Sandoval-Denis et al. (2016) and Sun et al. (2020). Photographs were taken with Sony camera mounted on a light microscope. The living cultures of the representative isolates were deposited in the Agricultural Microbial Collection of the Agricultural Biotechnology Research Institute of Iran, Karaj, Iran (ABRII).

Phylogenetic analyses

For molecular phylogenetic analyses, genomic DNA was extracted from the seven–day–old fungal mycelium of the representative isolates by the Zhong and Steffenson (2001) method with slight modifications. The partial fragment of the β-tubulin (*tub2*) gene was amplified by Bt2a (GGTAACCAAATCGGTGCTGCTTTC) and Bt2b (ACCCTCAGTGTAGTGACCCTTGGC) (Glass and Donaldson 1995). PCR amplification carried out in a final volume of 25 µl containing 10 µL of *Taq* DNA polymerase Mix Red–Mgcl2, 11 µL deionized water, 1 µL of each primer (10 pmol) and 2 µL template DNA. The PCR amplification of the partial sequence of the β-tubulin gene was done in a thermocycler with the following thermal cycling conditions. The initial denaturation at 94°C for 5 min, and then followed by 35 cycles each with denaturation at 94°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 30 s and a final extension at 72°C for 5 min. The PCR products were analysed by electrophoresis in 1.5% agarose gel using 1X Tris-Boric acid-EDTA (TBE) buffer. PCR products were sent to Codon Genetic Group (Tehran, Iran) for sequencing. The resulting sequences (560 bp for *tub2*) were separately subjected to BLAST search (Altschul et al. 1990) to find the nearest and relevant sequences in the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov; Sayers et al. 2025). Thirty-four β-tubulin (*tub2*) gene sequences of different *Microascus* species, most of which are type sequences, as well as *Kernia columnaris* (CBS 159.66; MN982416; *Microascaceae*) as an outgroup taxon, were selected for phylogenetic analyses (Table 1). Generated sequences in the present research, together with those retrieved from the GenBank (NCBI) were aligned using the MAFFT v. 7 online tools (https://mafft.cbrc.jp/alignment/server/index.html). Alignment was manually edited in BioEdit v.7.0.0. (Hall 2004). Phylogenetic analyses were carried out using the Maximum Likelihood (ML), Maximum Parsimony (MP), and Bayesian Inference (BI) methods. The maximum likelihood method was carried out through the CIPRES Science Gateway portal (https://www.phylo.org) (Miller et al. 2012). PAUP v. 4.0b10 software (Swofford, 2003) was used for MP analyses and MrBayes v. 3.2.6 was used for BI analyses (Ronquist and Huelsenbeck 2003).

The aligned sequences of the β-tubulin (*tub2*) gene (560 nt.) were subjected to the Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI). Of these, 333 characters were constant, and 227 characters were variable. Of the variable characters, 79 characters were parsimony–uninformative and 148 characters were parsimony–informative, and the gaps were treated as missing data. Maximum parsimony analysis of the remaining 148 parsimony–informative characters resulted in one most parsimonious tree (TL = 1428, CI = 0.689, RI = 0.808, HI= 0.311). IQ–TREE best tree (log-likelihood – 2683.990) was found after 20 iterations. The best evolutionary model that was selected by ModelFinder (Kalyaanamoorthy et al. 2017) in IQ–TREE was K3Pu+F+G4. The Bayesian analyses of the aligned partial sequences of β-tubulin (*tub2*) gene generated 1582 trees, from which 394 trees were discarded as burn-in (25%). The consensus tree and posterior probability values (PP) were calculated from the remaining 1188 trees. The average standard deviation of split frequencies was 0.009911 at the end of the run. *Kernia columnaris* isolate CBS 159.66 (MN982416) was used as an outgroup taxon to root the final tree. The resulting trees were analyzed with FigTree v1.4.3 (Cummings 2004), and annotated using Adobe Illustrator v2019 (Adobe Inc., USA). Bayesian inference posterior probabilities (pp) values and bootstrap (BS) percentages of the analyses were labelled at the nodes. Newly obtained sequences in the present study were deposited in GenBank (www.ncbi.nlm.nih.gov).

RESULTS

Fungal strains

The present study was conducted using the samples collected from the Golestan, South Khorasan, and Alborz Provinces of Iran. Airborne as well as the endophytic fungi associated with the healthy seeds and roots of Cannabis and Camelthorn plants were identified. A total of 110 fungal isolates were obtained, comprising five isolates from the air of the Plant Pathology laboratory, 75 isolates associated with the healthy roots of the Camelthorn plant (*Alhagi maurorum*), and 30 isolates associated with the healthy seeds of Cannabis (*Cannabis sativa*). The highest abundance of isolates was recorded from the Golestan Province, followed by Alborz and South Khorasan Provinces. (Fig. 1). The morphological investigation and grouping of the recovered fungal isolates indicated that they belong to three distinct species of the genus *Microascus*. The three recovered *Microascus* species were identified based on the morphological investigations as *M. cinereus* from healthy Camelthorn root, *M. croci* from laboratory air, and *M. trigonosporus* from healthy Cannabis seed.

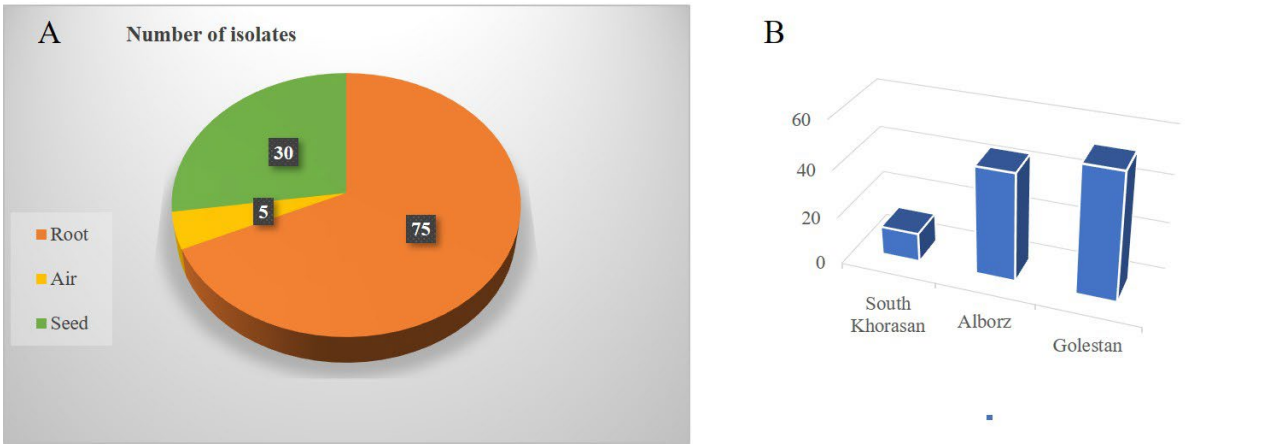

Fig. 1. Distribution of the *Microascus* isolates obtained from the healthy roots of Camelthorn and healthy seeds of Cannabis plants and from the air of the Plant Pathology laboratory (A); and in the sampled Provinces (B).

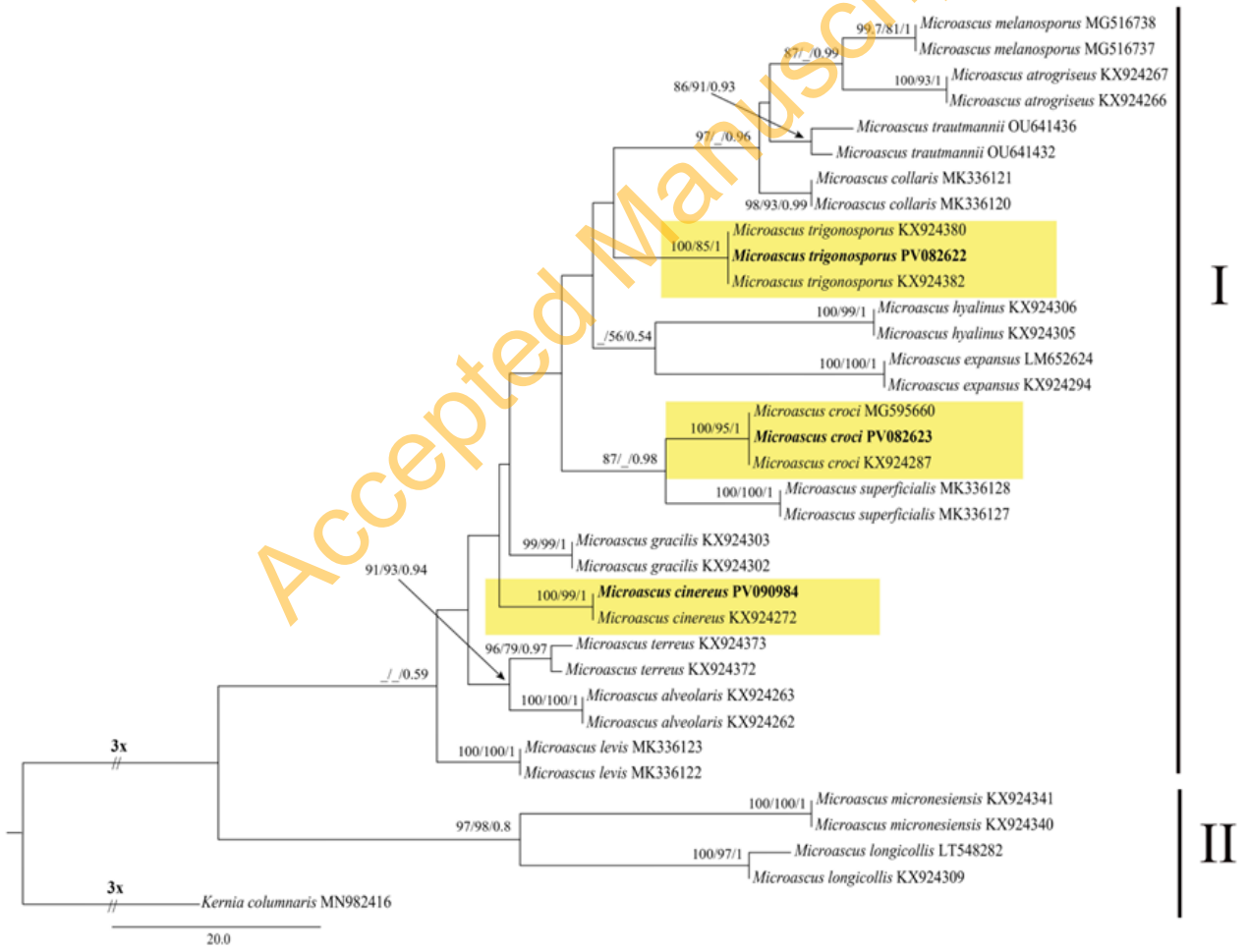

Fig. 2. Consensus phylogenetic tree of the 16 *Microascus* species constructed based on the partial nucleotide sequences of the β-tubulin (*tub2*) gene. Bootstrap support of Maximum Parsimony and Maximum Likelihood trees using the IQ–TREE and Bayesian Inference (BI) posterior probabilities are indicated at the nodes. The scale bar represents the expected number of changes per site. The tree is rooted to *Kernia columnaris* (CBS 159.66; MN982416). Strains of the present study are in boldface.

Phylogenetic analyses

In the reconstructed ML, MP and BI phylogenetic trees based on the partial nucleotide sequences of the β-tubulin (*tub2*) gene, 34 nucleotide sequences comprising 16 *Microascus* species were used. Our *Microascus* isolates (ABRIICC10370, ABRIICC10455, and ABRIICC10373) clustered with high bootstrap and posterior probability with the relevant *Microascus* species, indicating the ability of the used methods for phylogenetic analyses. In the resulting phylogenetic tree, two distinct clades appeared. Most of the *Microascus* species, including our three identified species, were grouped in the upper clade, namely Clade I, with high bootstrap support. In the Clade I the *M. cinereus* ABRIICC10455 (100/99/1), *M. croci* ABRIICC10373 (100/95/1) and *M. trigonosporus* ABRIICC10370 (100/85/1) were grouped with high bootstrap supports and posterior probabilities in MP, ML and BI trees with the relevant species that their sequences were retrieved from GenBank (NCBI) (Fig. 2). Based on the morphological and phylogenetic analyses, isolates ABRIICC10455 (GenBank accession no. PV090984), ABRIICC10373 (GenBank accession no. PV082623), and ABRIICC10370 (GenBank accession no. PV082622) were identified and characterized as *M. cinereus*, *Microascus croci* and *Microascus trigonosporus* respectively.

Taxonomy

Microascus cinereus Curzi, Boll. R. Staz. Patalog. Veget. Roma, N.S. 11: 60 (1931). Fig. 3

Colony on OMA reached 42 mm in diameter after 14 days at 25 °C under the alternating near–ultraviolet (nUV) light (12 h light/12 h dark). At first, the colony was velvety, pale, and becoming slightly olive-grey due to the production of ascomata. Perithecia black, carbonaceous, scattered or crowded, globose, textura angularis, 100–230 µm in diameter, papillate, glabrous, or with scattered hairs, hairs septate, and pigmented. Asci hyaline, sessile, mostly elliptical, sometimes globose, 12–16 × 8–11 µm ($\bar{x}$ = 14.5 × 10.6 µm, n = 20) in size, ascospores rounded at the ends, pale brown, 5–6 × 3–4 µm ($\bar{x}$ = 5.3 × 3.5 µm, n = 50) in size. Ascospores exuded as elongated cirrhi after twenty days. Conidiophores arising from the vegetative hyphae singly or in groups, broader at the center, tapering towards the extremities, usually 5.5–14 µm long ($\bar{x}$ = 12.4 µm, n = 50), sometimes almost obsolete, conidia borne in long chains, markedly truncate at the base, obtusely rounded at the tip, sometimes papillate, 3–4 × 2–3 µm ($\bar{x}$ = 3.5 × 2.6 µm, n = 50) in size, pale grayish-brown in color (Fig. 3). Morphological features of the investigated isolate (ABRIICC10455) were consistent with the description of *Microascus cinereus* provided by Barron et al. (1961).

Specimen examined: IRAN, Golestan Province, Aqqala County, N= 37°04'44.9" E= 54°27'50.0, recovered as endophytic fungus from healthy Camelthorn root (*Alhagi maurorum* Medik) Dec. 2024. S. Abdi Aghbolagh (ABRIICC10455), GenBank accession no. PV090984.

Notes: *Microascus cinereus* has been isolated from stored corn (Barron et al. 1961) and as brain abscess in a bone marrow transplant recipient (Baddley et al. 2000). *Microascus cinereus* was also isolated from *Cressa cretica* L., *Ammi majus* L., *Polypogon monspeliensis* (L.) Desf., *Prosopis farcta* (Banks & Sol.) J.F.Macbr. as an endophytic fungus (Al-Bedak et al. 2021). To the best of our knowledge, *Microascus cinereus* is a new species for the funga of Iran, as well as the Camelthorn plant is reported here as a new host (matrix nova) for this species in the world.

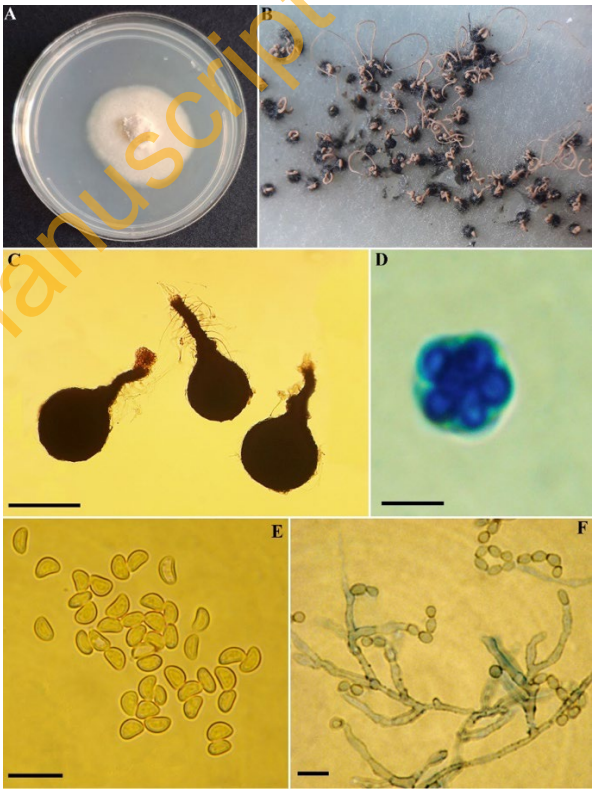

Fig. 3. *Microascus cinereus* (ABRIICC10455). A. Colony on OMA after 14 days of cultivation at 25 °C in alternating near–ultraviolet (nUV) light (12 h light/12 h dark) conditions, B–C. Ascomata after 20 days with elongated cirrhi, D. Ascus, E. Ascospores, F. Annellides and conidia. Scale bars: C = 200 µm; D = 10 µm; E =15 µm; F = 8 µm.

Microascus croci (J.F.H. Beyma) Sand.-Den., Gené & Guarro, in Sandoval–Denis, Gené, Sutton, Cano-Lira, de Hoog, Decock & Guarro, Persoonia 36: 17 (2015). Fig 4

Colony on OMA reached 42 mm in diameter after 14 days at 25 °C under the alternating near–ultraviolet (nUV) light (12 h light/12 h dark). Colony was grey to mouse-grey, flat, velvety to powdery, with an

irregular margin and olive grey at reverse. Vegetative hyphae septate, hyaline to pale brown, smooth- and thin-walled, 1.5–3 µm wide. Conidiophores absent. Annellides, solitary, lateral, measured 6–15 µm in size. Conidia, single or arranged in short chains, smooth, globose to broadly ellipsoid shaped, light brown in color, measured 3.5–4.5 × 3–4 µm ($\bar{x}$ = 4.1 × 3.5 µm, n = 50) in size. Sexual morph not observed (Fig. 4). Morphological features of the investigated isolate (ABRIICC10373) agree with the description of *Microascus croci* provided by Sandoval-Denis et al. (2016).

Specimen examined: IRAN, Alborz Province, Karaj County, N= 35°48'16.4" E= 50°59'48.6", recovered as airborne fungi from the air of the Plant Pathology laboratory of the University of Tehran. Dec. 2023. A. Atashi Khalilabad (ABRIICC10373), GenBank accession no. PV082623.

Note: *Microascus croci* has been isolated from *Crocus* sp. (Sandoval-Denis et al. 2016), air and nail (Jagielski et al. 2016). Also, this species was isolated from macroalgae *Ascoseira mirabilis* Skottsberg, *Adenocystis utricularis* (Bory) Skottsberg, *Phaeurus antarcticus* Skottsberg, and *Desmarestia anceps* Montage (Vega–Portalatino et al. 2023). To the best of our knowledge, *Microascus croci* is a new species for the funga of Iran.

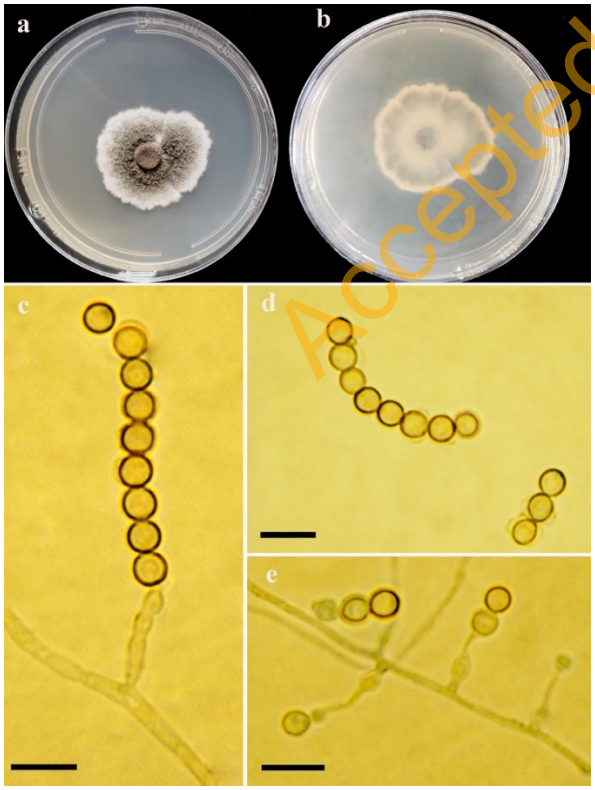

Fig. 4. *Microascus croci* (ABRIICC10373). A. Colony (obverse) on OMA, B. Colony (reverse) on OMA, after 14 days of cultivation at 25 °C under the alternating near–ultraviolet (nUV) light (12 h light/12 h dark) condition, and C–E. Annellides and conidia in chains. Scale bars: 10 µm.

Microascus trigonosporus C.W. Emmons & B.O. Dodge, Mycologia 23(5): 317 (1931). Fig 5

Colony on OMA grows slowly, floccose, white at the edge, reaching 38 mm in diameter after 14 days at 25 °C under the alternating near–ultraviolet (nUV) light (12 h light/12 h dark). The colony started to darken from the center, and then black granular ascomata subsequently formed. Perithecia black, globose to flask-shaped, carbonaceous, glabrous, textura angularis, 120–260 µm ($\bar{x}$ = 235 µm, n = 30) in diameter, neck up to 230 µm long, cylindrical. Asci hyaline, globose to ovoid, 5–8.5 µm in size, and sessile. Ascospores triangulate in planar view, concave on all three sides, 2.5–3.5 × 3–4.5 µm ($\bar{x}$ = 2.9 × 3.5 µm, n = 50) in size, rounded at the ends, and orange in mass. Asexual morph was not observed in the present study. Ascospores exuded as elongated cirrhi after twenty days (Fig. 5). Morphological features of the investigated isolate were similar to the description of *Microascus trigonosporus* provided by Barron et al. (1961) and Sandoval-Denis et al. (2016).

Specimen examined: IRAN, South Khorasan Province, Gazik District, E= 32°59'58.9 N= 60°13'36.1, recovered as endophytic fungus from healthy Cannabis seed (*Cannabis sativa* L.) Dec. 2020. A. Atashi Khalilabad (ABRIICC10370), GenBank accession no. PV082622.

Note: *Microascus trigonosporus* has been isolated from surface-sterilized cereal and legume seeds from Alabama, South Dakota, North Dakota, Michigan, Illinois, Minnesota, Iowa, Wisconsin, and Wyoming. *Microascus trigonosporus* has been listed as a component organism of the soil microflora. This species has also been isolated either from dermal lesions or cases of onychomycosis in humans (Barron et al. 1961). *Microascus trigonosporus* has been found in seeds of onion, sumac, sorghum, cereal, and legume (Huang et al. 1975). *Microascus trigonosporus* was also isolated from human lung transplant (Schoeppler et al. 2015). To the best of our knowledge, *Microascus trigonosporus* is new for the funga of Iran, as well as the Cannabis plant is reported here as new host (matrix nova) for this species in the world.

The living cultures of the representative strains of all three species identified in the present study were deposited in the Agricultural Microbial Collection of the Agricultural Biotechnology Research Institute of Iran, Karaj, Iran (ABRII) with accession numbers ABRIICC10455 for *Microascus cinereus*, ABRIICC10373 for *Microascus croci*, and ABRIICC10370 for *Microascus trigonosporus*.

DISCUSSION

In this study, three species of *Microascus* were

obtained from two different plant species (as endophytic fungi) and the Plant Pathology laboratory air (as airborne fungus) collected from South Khorasan,

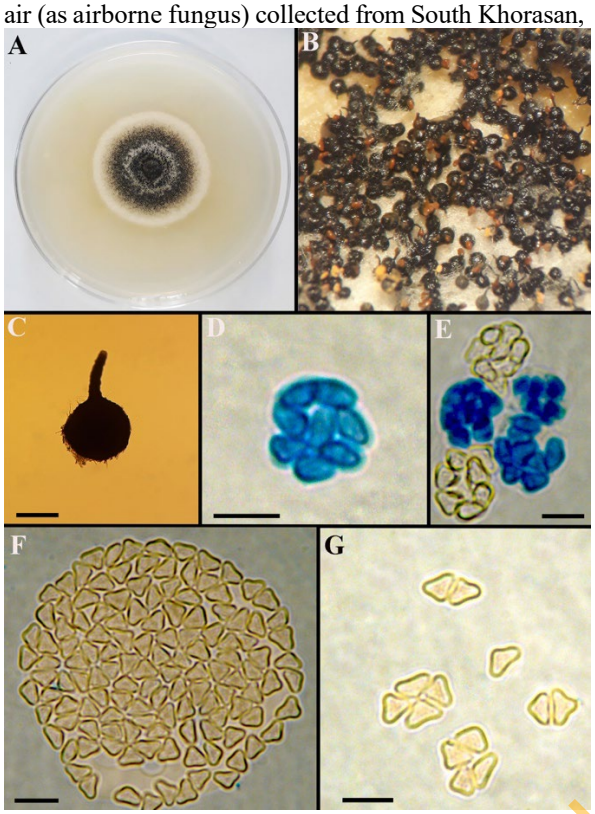

Fig. 5. *Microascus trigonosporus* (ABRIICC10370). A. Colony on OMA after 14 days of cultivation at 25 °C under the alternating near–ultraviolet (nUV) light (12 h light/12 h dark) condition, B-C. Ascomata after 20 days with orange cirrhi, D-E. Asci and ascospores, and F-G. Ascospores. Scale bars: C = 100 µm; D = G 5µm.

Golestan, and Alborz Provinces of Iran. After investigation of the morphological features and taking into account of molecular data, isolates were identified as *M. cinereus* from healthy Camelthorn root (*Alhagi maurorum*), *M. croci* from laboratory air, and *M. trigonosporus* from healthy Cannabis seed (*Cannabis sativa*). The genus *Microascus* was first established by Zukal (1885) and typified by *M. longirostris*. It is the largest genus of the *Microascaceae* family. Members of the genus *Microascus* are widespread, occurring in abundance in soil, dust and indoor air and in plant debris. Furthermore, some species are also recognized as important animal or human opportunistic pathogens (Zukal 1885, Barron et al. 1961, Brasch et al. 2019, Mhmoud et al. 2021). Based on the previous studies relating to the identification of various species within the genus *Microascus*, the nuclear DNA regions, including ITS, LSU, *tub2*, and *tef1* were used. Pursuant to our survey, the species of the genus *Microascus* were separated well by the partial nucleotide sequences of the β–tubulin (*tub2*) gene in the resulting consensus phylogenetic tree (Fig. 2), which is in consistent with relevant researches such as Sandoval-Denis et al. (2016), Sun et al. (2020), and Wei et al. (2024). According to the phylogenetic analysis, *M. croci* and *M. superficialis* clustered in the same subclade and received moderate bootstrap support. Based on the survey performed in 2021, *M. superficialis* has a sexual stage, whereas in the *M. croci* isolates, only the asexual morph is observed, confirming our findings about this species (Zhang et al. 2021). Morphologically, *Microascus alveolaris* resembles *M. trigonosporus*, with comparable triangular–shaped ascospores. *Microascus alveolaris* can be distinguished by its membranous and white colony, along with the smaller dimensions of its ascospores and narrower conidia. *Microascus gracilis* and *M. cinereus* are morphologically highly similar, which complicates their identification without considering the sexual morph. Morphologically, *M. gracilis* can be distinguished from *M. cinereus* by its lunate ascospores, the development of complex conidiophores, and the colony's morphology and color (Sandoval-Denis et al. 2016). Also, *Microascus terreus* and *M. trigonosporus* can be morphologically distinguished from each other by the bigger ascospores in *M. terreus* (Jagielski et al. 2016). Also, *Microascus cirrosus* is very similar to *M. cinereus*. However, *M. cirrosus* produces broadly reniform ascospores and bigger conidia, while *M. cinereus* produces broadly lunate or almost triangular ascospores and obovate to clavate conidia.

Based on the morphological and molecular investigations and to the best of our knowledge, these three *Microascus* species, including *M. cinereus* from healthy Camelthorn root (*Alhagi maurorum*), *M. croci* from air and *M. trigonosporus* from healthy Cannabis seed (*Cannabis sativa*) are new species for the funga of Iran and are reported here for the first time. Also, *Alhagi maurorum* and *Cannabis sativa* are reported here for the first time as new hosts (matrix nova) for *M. cinereus* and *M. trigonosporus,* respectively, in the world.

ACKNOWLEDGMENT

The present research was supported by the University of Tehran. The authors would like to express their appreciation to the University of Tehran.

AUTHOR CONTRIBUTION

Abbas Atashi Khalilabad Sampling, Methodology, Molecular analysis, writing manuscript draft; Sara Abdi Aghbolagh Sampling, writing manuscript draft; Ali Yaghoubi Hassanali-Deh Sampling, writing manuscript draft; Khalil-Berdi Fotouhifar Methodology, Supervising, and Writing the final draft of the manuscript.

DATA AVAILABILITY

All data are available in the manuscript except the

newly generated sequences were deposited in the GenBank (NCBI).

DECLARATION

In this manuscript, there is nothing to declare and the authors declare no conflicts of interest.

FUNDING

The authors declare that no financial support was received during this research.

ETHICS APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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آرایه‌بندی ریخت‌شناختی و تبارشناختی سه گونه جدید از جنس *Microascus* برای ایران (*Microascaceae*, *Microascales*, *Ascomycota*)

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چکیده

طی مطالعه‌ای در خصوص تنوع زیستی ریز–قارچ های آسکومیستی در ایران بین سال‌های ۱۳۹۹ تا ۱۴۰۳، چندین جدایه قارچی از بذور سالم گیاه شاهدانه (*Cannabis sativa* L.) و ریشه‌های سالم گیاه خارشتر (*Alhagi maurorum* Medik) به عنوان قارچ‌های اندوفیت، و برخی از آنها از هوای آزمایشگاه بیماری شناسی گیاهی، به عنوان قارچ‌های هوابُرد، جداسازی و شناسایی شدند. بر اساس بررسی ویژگی‌های ریخت شناختی، بخشی از قارچ‌های شناسایی شده به جنس *Microascus* (Microascaceae) تعلق داشتند. تجزیه و تحلیل‌های تبارزایی مولکولی با استفاده از بخشی از توالی نوکلئوتیدی ژن بتا–توبولین (*tub2*) و شجره توافقی تبارزایی حاصل، نشان دادند که جدایه های به دست آمده به سه گونه از جنس *Microascus* شامل گونه *M. cinereus* حاصل از ریشه سالم گیاه خارشتر، گونه *M. croci* حاصل از هوای آزمایشگاه و گونه *M. trigonosporus* حاصل از بذر سالم گیاه شاهدانه، تعلق دارند و توالی آنها با توالی های گونه‌های مرتبط از بانک ژن دارای تشابه بسیار زیادی است. در شجره توافقی تبارزایی حاصل، سه گونه شناسایی شده از جنس *Microascus* به طور کامل از یکدیگر و همچنین از سایر گونه‌های این جنس با حمایت اعتبارسنجی و احتمال خلفی زیاد جدا شدند، که تایید کننده شناسایی ریخت شناختی درست و همچنین نشان دهنده کارآمد بودن ناحیه ژنومی مورد استفاده می‌باشد. بر اساس اطلاعات ما، این سه گونه از جنس *Microascus* برای فونگای ایران جدید هستند و همچنین گیاهان *Alhagi maurorum* و *Cannabis sativa* به ترتیب برای گونه‌های قارچی *M. cinereus* و *M. trigonosporus* میزبان‌های گیاهی جدیدی (matrix nova) در دنیا گزارش می‌شوند.

کلمات کلیدی: بتا-توبولین، تبارزایی، تنوع زیستی، رده بندی، قارچ ها.

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تاریخ دریافت: ۱۴۰۴/۰۸/۱۷، تاریخ بازنگری: ۱۴۰۴/۰۹/۰۹، تاریخ پذیرش: ۱۴۰۴/۰۹/۱۰، تاریخ انتشار برخط: ۱۴۰۴/۰۹/۱۵