Mycologia Iranica 11(2): 61– 72, 2024 Original Article DOI:10.22043/MI.2024.368044.1302

Phylogeny and taxonomy of *Penicillium* species associated with some foodstuffs in Mahabad, western Iran

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Abstract: Contamination of foodstuffs by mycotoxigenic fungi is a major threat to human health. During a study on mycotoxigenic fungi associated with raisins, dried fruits apricot, apple, and white mulberry, and a traditional food (Terkhêna), 50 *Penicillium* isolates were collected from Mahabad, west of Iran. These isolates showed various ISSR fingerprinting patterns generated by $(GTG)_5$ primer and grouped in 11 distinct clusters. A representative isolate from each cluster was selected for phylogeny and morphological studies. Phylogenetic analyses of *BenA* sequence data, resulted in the identification of five *Penicillium* species belong to two different sections *Chrysogena* (*P. chrysogenum* and *P. dipodomyus*) and *Fasciculata* (*P. crustosum*, *P. palitans* and *P. polonicum*). As far as we know, except *P. chrysogenum* and *P. polonicum* which have previously been reported from raisins, all other recognized species here are reported for the first time from all examined foodstuffs around the world. Moreover, *P. dipodomyus* is reported here as a new record for the mycobiota of Iran.

Keywords: *Aspergillaceae*, Fungal contamination, Mycotoxigenic fungi, Terkhêna.

INTRODUCTION

Dried fruits, as popular and nutrient-dense snacks, are viable and sustainable alternatives to fresh fruits which are always available throughout the year. Their intake may mitigate the incidence of cardiovascular disease, coronary heart disease, type 2 diabetes, obesity, and various other chronic conditions (Chang et al. 2016). These foodstuffs are rich in vital nutrients and bioactive compounds such as flavonoids, proanthocyanidins, phenolic acids, carotenoids, and phytoestrogens, and exhibit higher concentrations of these constituents than their fresh counterparts (Alasalvar et al. 2023). In the western region of Iran, Terkhêna (or Doowina) is a very popular fermented winter food produced from bulgur wheat soaked in buttermilk (or Doogh) supplemented with vegetables such as turnip or pennyroyal leaves which is usually stored for a long time as small dried pieces (Mohammadi and Ostovar 2023). Terkhêna with a higher amount of ash and protein than fat, is a rich source of minerals, vitamins, and free amino acids (Mohammadi and Ostovar 2023).

These foodstuffs are stored in warehouses usually for long periods and then fungal spoilage is a major threat affecting their quality and threatens human health due to mycotoxins contamination (Bhat et al. 2010, González-Curbelo and Kabak 2023). Dried fruits and foods are particularly vulnerable to fungal contamination, especially by species of *Penicillium*, *Aspergillus*, *Fusarium*, and *Alternaria*, well-known producers of deleterious mycotoxins. Identification of these mycotoxin-producing fungi is of paramount importance for ensuring food safety for consumers (Barboráková et al. 2023). Several *Penicillium* species are among the most common agents of postharvest spoilage, and found on a wide range of foodstuffs (Pitt and Hocking, 2022). Many of them, such as *P. expansum*, *P. chrysogenum*, and *P. crustosum*, are generally producing a number of potent mycotoxins (e.g. Penitrem A, Patulin, Citrinin, and Ochratoxin A) (Barkai-Golan 2008).

The genus *Penicillium*, meaning 'brush', was established by Link (1809), to encompass three species including *P. candidum*, *P. glaucum*, and *P. expansum* as the type species. Since then, about 1500 species names have been proposed (October 2024; https://www.indexfungorum.org) and the taxonomy of this genus has continuously developed (Houbraken and Samson 2011, Visagie et al. 2014, 2024). *Penicillium* is a well-recognized genus in *Aspergillaceae* found in diverse habitats, such as soil, indoor environments, and foods (Visagie et al. 2014). Recently, in an infrageneric classification 535 accepted species within the genus *Penicillium* have been placed in two subgenera (*Aspergilloides* and *Penicillium*), 32 sections and 101 series (Visagie et al. 2024).

In a study on phylogeny and taxonomy of mycotoxigenic fungi isolated from raisins, some dried fruits (apple, apricot, and white mulberry), and the traditional food Terkhêna, 415 fungal isolates belonging to four genera *Aspergillus*, *Penicillium*, *Rhizopus* (Ghaderi and Abdollahzadeh 2024) and

Submitted 19 Dec. 2024, accepted for publication 28 Dec. 2024
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Alternaria were collected. This study, focuses on molecular and morphological identification of *Penicillium* isolates.

MATERIALS AND METHODS

Sampling, fungal isolation and purification

Over 2021–2022, samples of raisins, dried fruits apple, apricot, and white mulberry, and the traditional food Terkhêna, were collected from various locations in Mahabad, western Iran. To isolate fungi, surface sterilized (3 min in 70% ethanol) small pieces of the samples were incubated on potato dextrose agar (PDA) and purified using the single-spore method on water agar (WA). Purified isolates were stored on PDA at 4–8 °C. Representative isolates of each species were deposited in the culture collection of the Iranian Research Institute of Plant Protection (IRAN, Tehran, Iran) (Table 1).

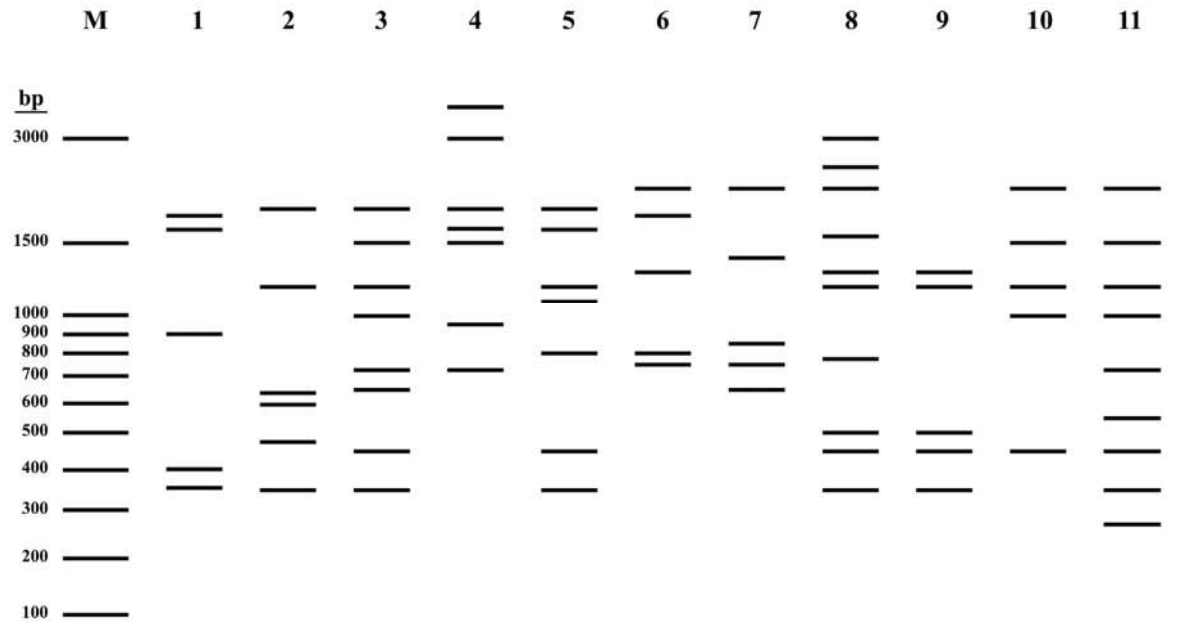

Fig. 1. DNA fingerprinting patterns of identified *Penicillium* species generated by primer $(GTG)_5$. 1: *P. chrysogenum* IRAN 5129C; 2: *P. crustosum* IRAN 5117C; 3–7: *P. dipodomyus* CJA OGhZ62, IRAN 5121C, CJA OGhT98, CJA OGhT58, CJA OGhZ48; 8, 9: *P. palitans* IRAN 5127C, CJA OGhK39; 10, 11: *P. polonicum* IRAN 5128C, CJA OGhD661. M: GeneRuler DNA Ladder Mix (100bp).

DNA extraction and sequencing

For DNA extraction, we followed the modified method of Raeder and Broda (1985), as described by Abdollahzadeh et al. (2009). To manage the costs of sequencing, the inter-simple sequence repeat (ISSR) technique was used as described by Alves et al. (2007) and isolates clustered at the species level based on DNA fingerprinting patterns generated by primer $(GTG)_5$. For PCR and electrophoresis conditions we followed Alves et al. (2007) and DNA banding profiles were visually analyzed and then a representative isolate from each cluster was subjected to sequencing, phylogenetic analyses and morphological examinations. For phylogenetic analyses, we selected *BenA* (β-tubulin) gene as DNA barcode and amplified using forward and reverse primers Bt2a (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′) (Glass and Donaldson 1995). PCR amplification was performed according to Visagie et al. (2014). PCR products were purified and sequenced by BGI (China) via BMG (Bio Magic Gene) Company (Karaj, Iran). Generated sequences were edited and extracted using BioEdit v. 7.0.0 (Hall, 2004) and submitted to GenBank (Table 1).

Phylogenetic analyses

The sequences generated in this study were analyzed using NCBI BLAST and aligned with the type or authentic strains of *Penicillium* species using MAFFT online service (Katoh et al. 2019). Phylogenetic analyses of the *BenA* alignment were performed using Maximum Likelihood (ML) and Bayesian Inference

Table 1. *Penicillium* isolates sequenced in this study.

Section	Species	Isolate No.[1]	Substrate	Coordinates (N, E)	GenBank accession numbers[2]
					BenA
Chrysogena	*P. chrysogenum*	IRAN 5129C	Apricot	36.904136, 45.754849	PQ790610
	P. dipodomyus	IRAN 5121C	Apricot	36.717915, 45.691892	PQ790609
		CJA OGhZ62	Apricot	36.930233, 45.747017	PQ811044
		CJA OGhT58	White Mulberry	36.673605, 46.033742	PQ811701
		CJA OGhZ48	Apricot	36.878823, 45.919165	PQ811703
		CJA OGhT98	White Mulberry	36.564531, 45.559975	PQ811702
Fasciculata	*P. crustosum*	IRAN 5117C	Terkhêna	36.692384, 45.958818	PQ790606
	P. palitans	IRAN 5127C	White Mulberry	36.809466, 45.742031	PQ790608
		CJA OGhK39	Raisin	36.820737, 45.807872	PQ811043
	P. polonicum	IRAN 5128C	Raisin	36.906752, 45.748396	PQ790607
		CJA OGhD661	Terkhêna	36.704849, 45.988875	PQ811042

[1] *IRAN* Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; *CJA* Personal cultures of Jafar Abdollahzadeh. [T] Ex-type. [2] *BenA* partial β-tubulin gene.

(BI) methods. Maximum Likelihood phylogenies were computed using the IQ-TREE v. 2.2.2.7 (Nguyen et al. 2015) and bootstrap values were calculated by ultrafast bootstrapping with 1000 replications using UFBoot (Hoang et al. 2018). The CIPRES Science Gateway web portal (Miller et al. 2012, www.phylo.org) was utilized to execute MrBayes v3.2.7a (Ronquist et al. 2012) as described by Bashiri et al. (2022). Phylogenetic trees were visualized using FigTree v. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree), and edited in Adobe Illustrator 2023 v. 27.1.0.189.

Macro-micromorphological observations

Representative isolates were incubated on malt extract agar (MEA) at 25 °C in the dark for 7 days. For media preparation and descriptions we followed Visagie et al. (2014). All species have been described based on growth rate, colony characteristics, and microscopic features. Fungal structures were mounted on a drop of lactic acid (60 %). Ethanol (70 %) was

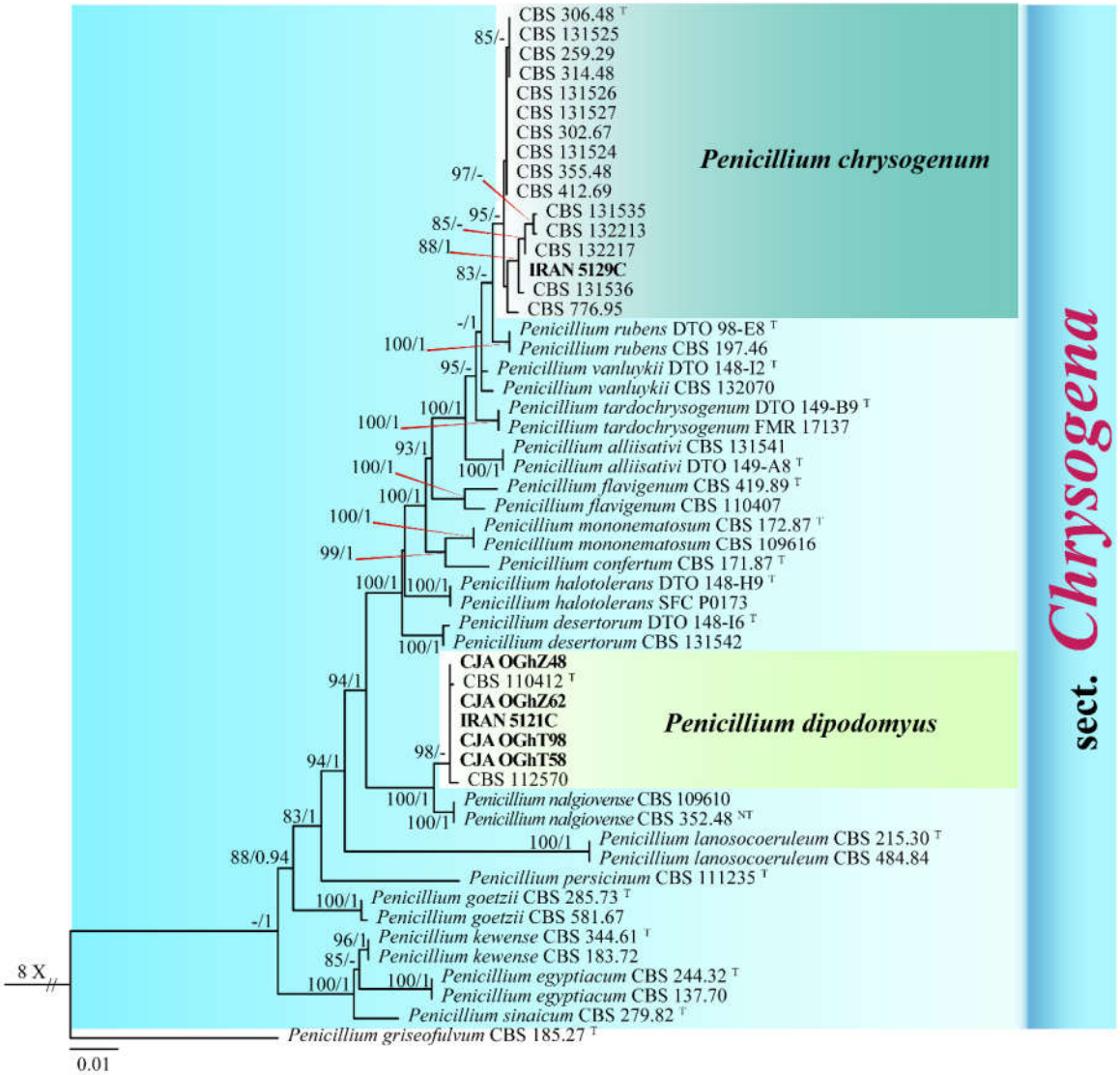

Fig. 2. Maximum likelihood phylogram of *Penicillium* sect. *Chrysogena* resulted based on *BenA* sequence data. ML/BI ultrafast bootstrap support (≥ 80%) and posterior probability (≥ 0.90) values are given at the nodes (ML-BS/PP). The phylogram was rooted to *Penicillium griseofulvum* CBS 185.27. The scale bar indicates the number of substitutions per site. Isolates sequenced in this study are shown in boldface.
[T] indicates ex-type strains. [NT] indicates neotype strains.

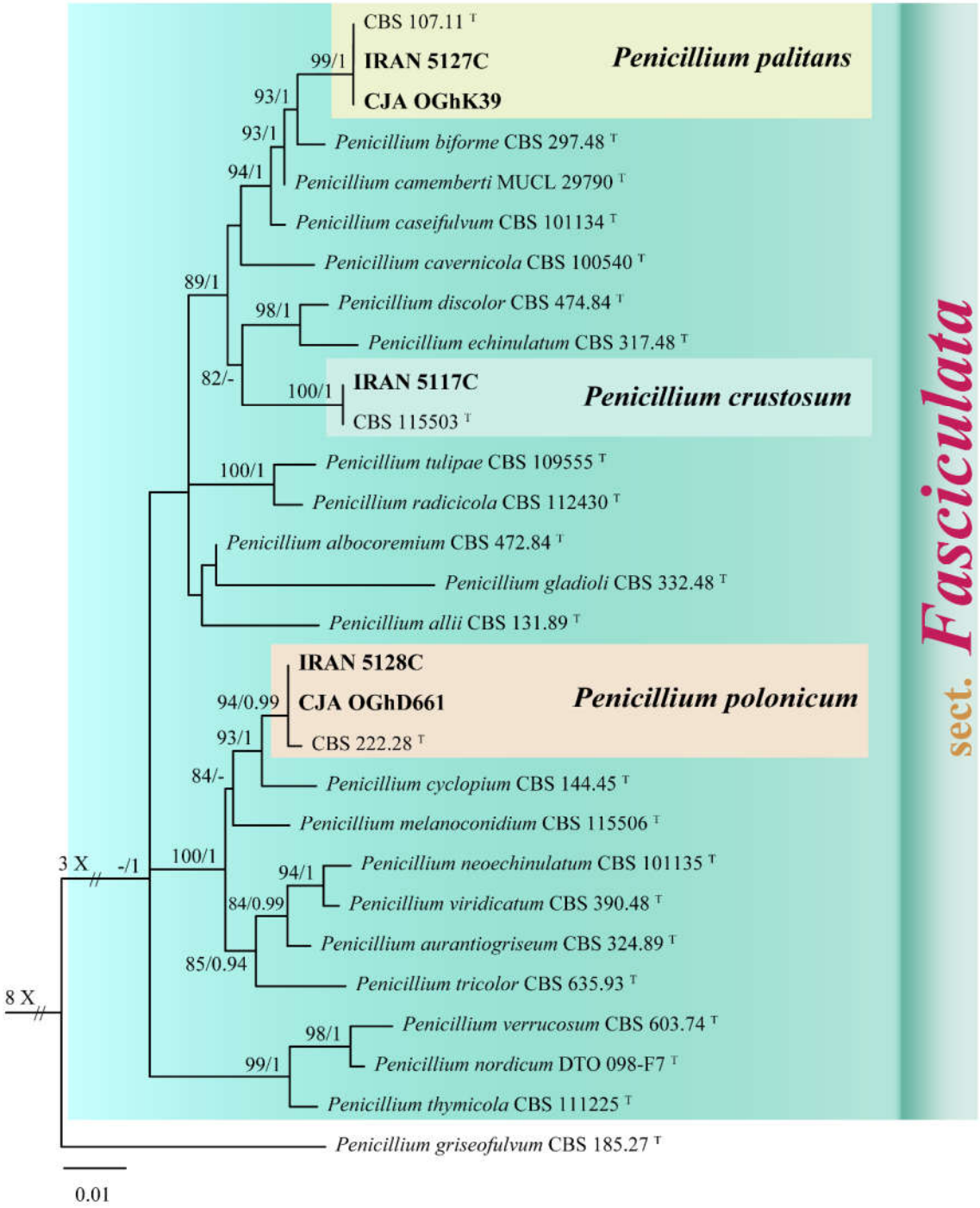

Fig. 3. Maximum likelihood phylogram of *Penicillium* sect. *Fasciculata* resulted based on *BenA* sequence data. ML/BI ultrafast bootstrap support (≥ 80%) and posterior probability (≥ 0.90) values are given at the nodes (ML-BS/PP). The phylogram was rooted to *Penicillium griseofulvum* CBS 185.27. The scale bar indicates the number of substitutions per site. Isolates sequenced in this study are shown in boldface. T indicates ex-type strains.

used to wash away excess conidia and to prevent air from being "trapped" when mounted in lactic acid. The fungal microscopic structures were documented using an Olympus BX51 microscope with differential interference contrast (DIC) illumination. Photomicrographs were obtained using an Olympus DP72 camera and a measurement module Cell Sens Entry v. 2.1. Dimensions of the fungal structures calculated based on at least 30 measurements, are presented as typical ranges with minimum and

maximum values in the brackets. For conidia, 50 measurements were computed, and the mean and standard deviation are also presented. Photoplates were prepared using Adobe Photoshop 2021 v. 22.5.8.

RESULTS AND DISCUSSION

Fungal isolation and clustering

A total of 50 fungal isolates with *Penicillium* morphology were obtained from the traditional food Terkhêna (n = 11 isolates), raisins (n = 7 isolates), and dried fruits apple (n = 8 isolates), apricot (n = 15 isolates) and white mulberry (n = 9 isolates). Isolates were grouped in 11 clusters based on DNA fingerprinting patterns (Fig. 1) and thus we selected 11 representative isolates for phylogenetic studies based on the partial sequence of *BenA* gene.

Phylogenetic analyses

Based on BLAST search of *BenA* generated sequences, our isolates were close to species in two sections *Chrysogena* (6 representative isolates) and *Fasciculata* (5 representative isolates). Two distinct datasets corresponding to two sections *Chrysogena* and *Fasciculata* were made and subjected to ML and BI phylogenetic analyses. After alignment, the dataset of *Chrysogena* section contained 467 characters including gaps. The RAxML analysis of the dataset with 427 distinct alignment patterns produced a best-scoring ML tree with lnL = -6171.6478 (Fig. 2). The bootstrap values (ML-BS) equal to or higher than 80 % were given on the nodes. The BI analysis generated 1552 trees from which 388 trees were discarded as burn-in. The consensus tree and posterior probability values (PP) were computed from the remaining 1164 trees. The average standard deviation of split frequencies was 0.009563 at the end of the run. Bayesian analysis resulted in a consensus tree with the same topology as the ML tree and posterior probability values (PP) equal or higher than 0.9 were mapped on the ML tree (Fig. 2). In this study, isolate IRAN 5129C placed in *P. chrysogenum* and five isolates IRAN 5121C, CJA OGhZ62, CJA OGhT58, CJA OGhZ48, and CJA OGhT98 were grouped with CBS 110412, the ex-type strain of *P. dipodomyus*.

The alignment of section *Fasciculata* dataset consisted of 456 characters including gaps. The RAxML analysis of the *Fasciculata* dataset with 143 distinct alignment patterns produced a best-scoring ML tree with lnL = -1596.217 (Fig. 3). The BI analysis resulted in 1422 trees, of which 354 were discarded as burn-in. The consensus tree and posterior probability values (PP) were calculated from the remaining 1068 trees. The average standard deviation of split frequencies was 0.009751 at the end of the run. The BI tree was mapped on the ML tree shown in Fig. 3 with bootstrap support (ML-BS ≥ 80 %) and posterior probability (PP ≥ 0.9) values given on the nodes. In both analyses isolates IRAN 5127C and CJA OGhK39 with no differences in *BenA* sequence with CBS 107.11, the type strain of *P. palitans,* were grouped in a well-supported clade (ML-BS/PP = 99/1). Isolates IRAN 5128C and CJA OGhD661 with one difference (substitution) with CBS 222.28, the type strain of *P. polonicum* constituted a well-supported clade (ML-BS/PP = 100/1). Isolates IRAN 5117C and CBS 115503, the type strain of *P. crustosum*, with no differences in *BenA* sequence data formed a well-supported clade in both ML and BI (ML-BS/PP = 100/1).

Taxonomy

Based on phylogenetic analyses, in this survey we identified five *Penicillium* species namely; *P. chrysogenum* and *P. dipodomyus* from section *Chrysogena* and *P. crustosum*, *P. palitans* and *P. polonicum* from section *Fasciculata* which are illustrated and discussed here in alphabetic order.

Penicillium chrysogenum Thom, U.S.D.A. Bur. Animal Industr. Bull. 118: 58 (1910)

Colony characters: MEA 25 °C, 7 d: diam 46 mm, radially sulcate, mycelium white, slightly floccose to velutinous with a feathery edge, light gray to greenish white, sporulation strong, exudates absent, soluble (diffusible) pigments absent, reverse radially sulcate and vivid yellow.

Micromorphology: Conidiophores bi-, ter-, and quarterverticillate. Stipes smooth-walled. Ramus cylindrical, (13–) 14.4–16.4 (–16.8) × (2.4–) 2.8–3.4 (–3.7) µm. Metulae cylindrical, (6.7–) 7–11.3 (–11.5) × (2.6–) 2.8–3.2 (–3.9) µm. Phialides cylindrical, (5.5–) 6–8 (–8.3) × (1.8–) 2–2.8 (–3) µm. Conidia smooth, globose to subglobose, (2–) 2.1–3 (–3.2) × (1.7–) 1.8–3 (–3.2) µm (av. ± S.D. = 2.4 ± 0.3 × 2.7 ± 0.3 µm) (Fig. 4).

Notes: Based on phylogeny of *BenA* sequence data, isolate IRAN 5129C obtained from dried fruit apricot was identified as *P. chrysogenum*. It was sequenced as a representative of a cluster containing two other isolates (on Terkhêna and dried fruit apple) with the same DNA banding profile generated by primer $(GTG)_5$. To date, as we know this species has only been isolated from raisins (Alghamdi et al. 2023), but it is reported here for the first time from Terkhêna and dried fruits apple and apricot around the world. To our knowledge, so far this species has not been reported from any kind of foodstuffs in Iran, but has been isolated from air (Naeimi et al. 2021), moldy bread (Delshad and Mostowfizadeh-Ghalamfarsa 2020) and Urmia lake water samples (Niknejad et al. 2013).

Penicillium crustosum Thom, The Penicillia: 399 (1930)

Colony characters: MEA 25 °C, 7 d: diam 39 mm, radially sulcate, mycelium white, velutinous, light

Fig. 4. *Penicillium chrysogenum* (IRAN 5129C). a. Colonies obverse (left) and reverse (right) on MEA at 25 °C after 7 d; b–e. Conidiophores; f. Conidia. Scale bars: b = 20 µm, c–f = 10 µm.

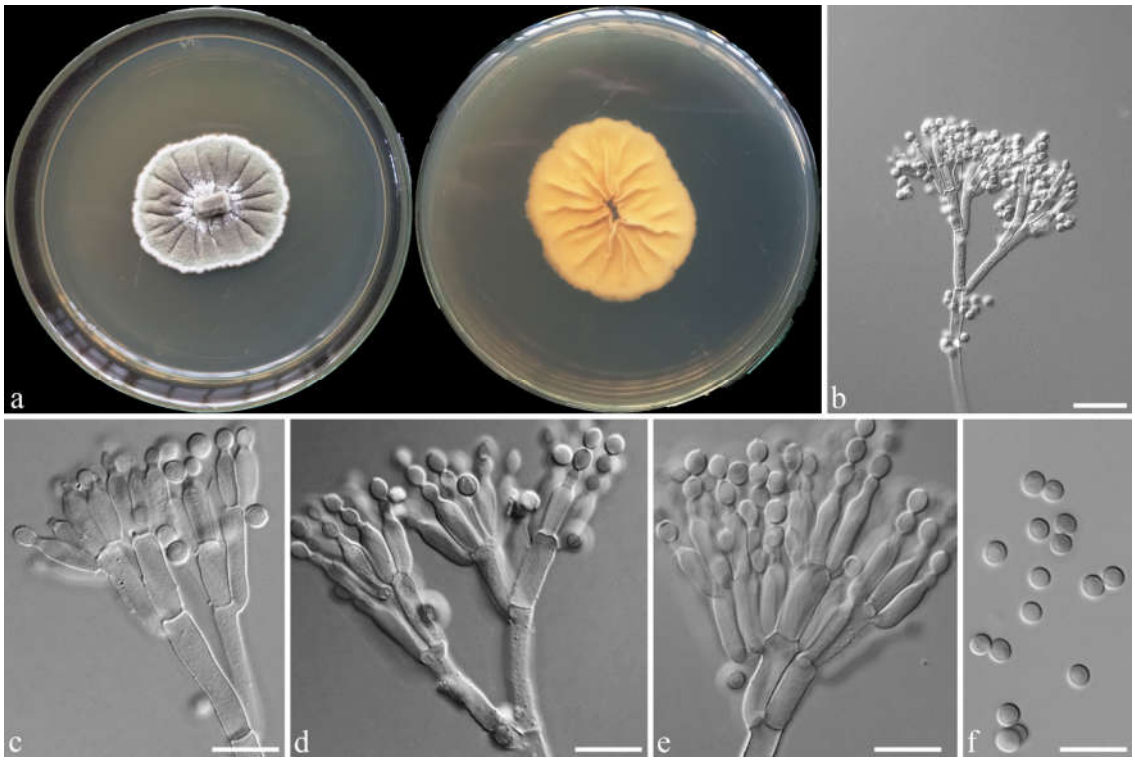

Fig. 5. *Penicillium crustosum* (IRAN 5117C). a. Colonies obverse (left) and reverse (right) on MEA at 25 °C after 7 d; b–e. Conidiophores; f. Conidia. Scale bars: b = 20 µm, c–f = 10 µm.

olive gray, sporulation strong, exudates absent, soluble (diffusible) pigments absent, reverse radially sulcate, light yellow.

Micromorphology: Conidiophores bi and terverticillate; Stipes smooth walled. Ramus cylindrical, (11.6–) 13.5–20.4 (–20.6) × (3.3–) 3.4–3.9 (–4.2) µm. Metulae cylindrical, (9.4–) 9.7–13.9 (–14.5) × (2.6–) 2.8–4.4 (–4.9) µm. Phialides cylindrical, (8.5–) 9–11.8 (–13) × (2.5–) 2.7–3.5 (–3.6) µm. Conidia smooth, globose to subglobose, (1.6–) 2.4–3.4 (–3.6) × (2.2–) 2.4–3.9 (–4) µm (av. ± S.D. = 2.9 ± 0.4 × 3.3 ± 0.4 µm) (Fig. 5).

Notes: Based on phylogeny of *BenA* sequence data, isolate IRAN 5117C obtained from Terkhêna was identified as *P. crustosum*. It was sequenced as a representative of a cluster containing another seven isolates with the same DNA banding profile generated by primer $(GTG)_5$. Here, we isolated *P. crustosum* from Terkhêna and dried fruits apple and apricot, which is the first report on these substrates around the world. As deduced from the literature, this species has not previously been reported from foodstuffs in Iran, but has been isolated from air (Naeimi et al. 2021) and fish samples (Ebrahimi Jafari et al. 2022).

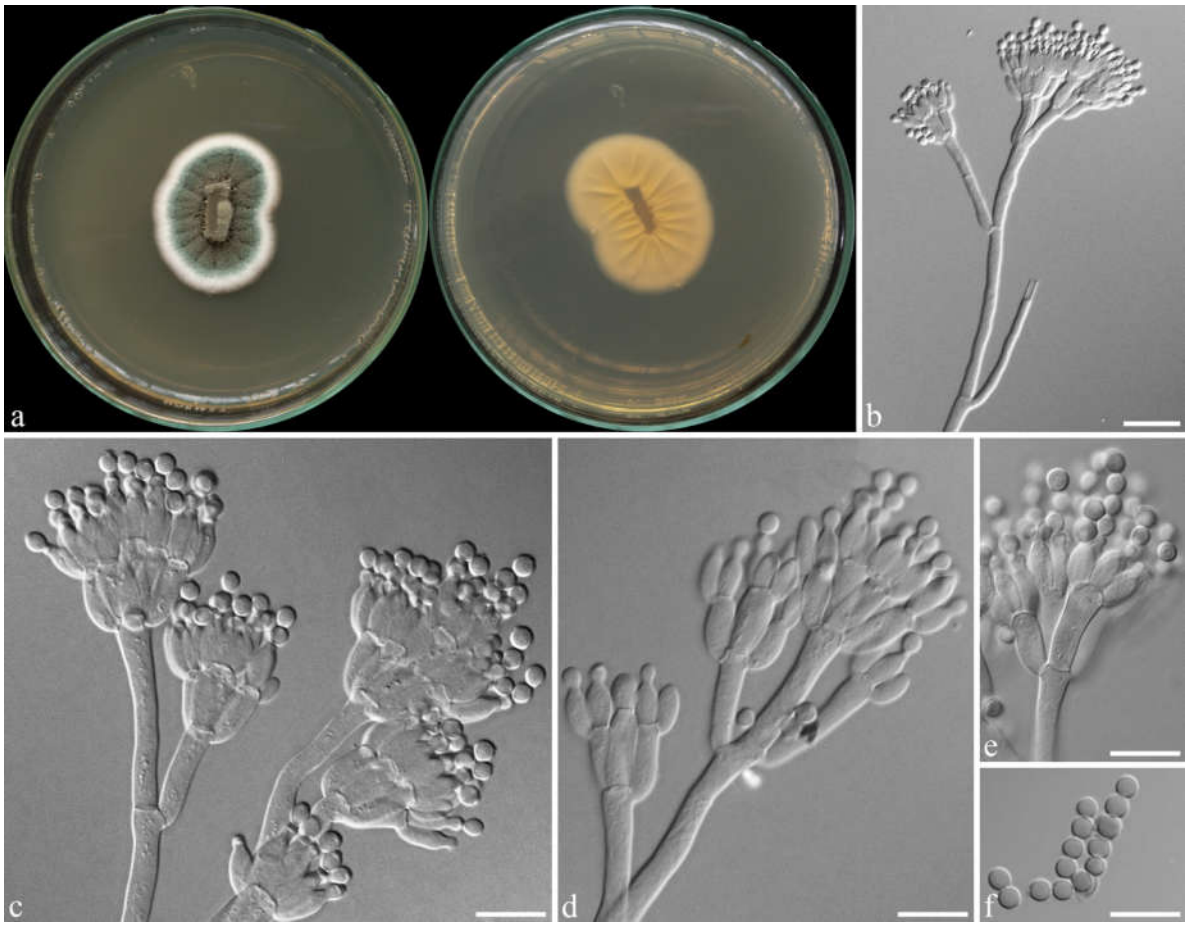

Fig. 6. *Penicillium dipodomyus* (IRAN 5121C). a. Colonies obverse (left) and reverse (right) on MEA at 25 °C after 7 d; b–e. Conidiophores; f. Conidia. Scale bars: b = 20 µm, c–f = 10 µm.

Penicillium dipodomyus (Frisvad, Filt. & Wicklow) Banke, Frisvad & S. Rosend., Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification: 271 (2000)

Colony characters: MEA 25 °C, 7 d: diam 35 mm, radially sulcate, mycelium white, velutinous with a feathery edge, grayish olive green to strong green, sporulation strong, exudates absent, soluble (diffusible) pigments absent, reverse radially sulcate, moderate yellow to light yellow.

Micromorphology: Conidiophore bi and terverticillate. Stipes smooth-walled. Ramus cylindrical, (12–) 13.5–20.4 (–25.6) × (3.5–) 3.7–4.2 (–4.4) µm. Metulae cylindrical, (6.6–) 7–10.8 (–12.8) × (2.9–) 3–4.3 (–4.8) µm. Phialides cylindrical, (4.6–) 5–8.5 (–9.1) × (2.1–) 2.3–4.7 (–3.8) µm. Conidia smooth, globose, (1.9–) 2.2–3.5 (–3.6) × (1.6–) 2.3–3.3 (–3.4) µm (av. ± S.D. = 2.9 ± 0.3 × 2.9 ± 0.3 µm) (Fig. 6).

Notes: Five isolates with different DNA banding profiles generated by $(GTG)_5$ selected as

representatives of five clusters (n = 22 isolates), placed in a single clade based on *BenA* sequence data, and characterized as *P. dipodomyus*. Here we isolated this species from Terkhêna, raisins and dried fruits apple, apricot, and white mulberry, which is reported on these substrates for the first time around the world. To date, there is no report of this species from Iran and thus it is a new record for Iran mycobiota.

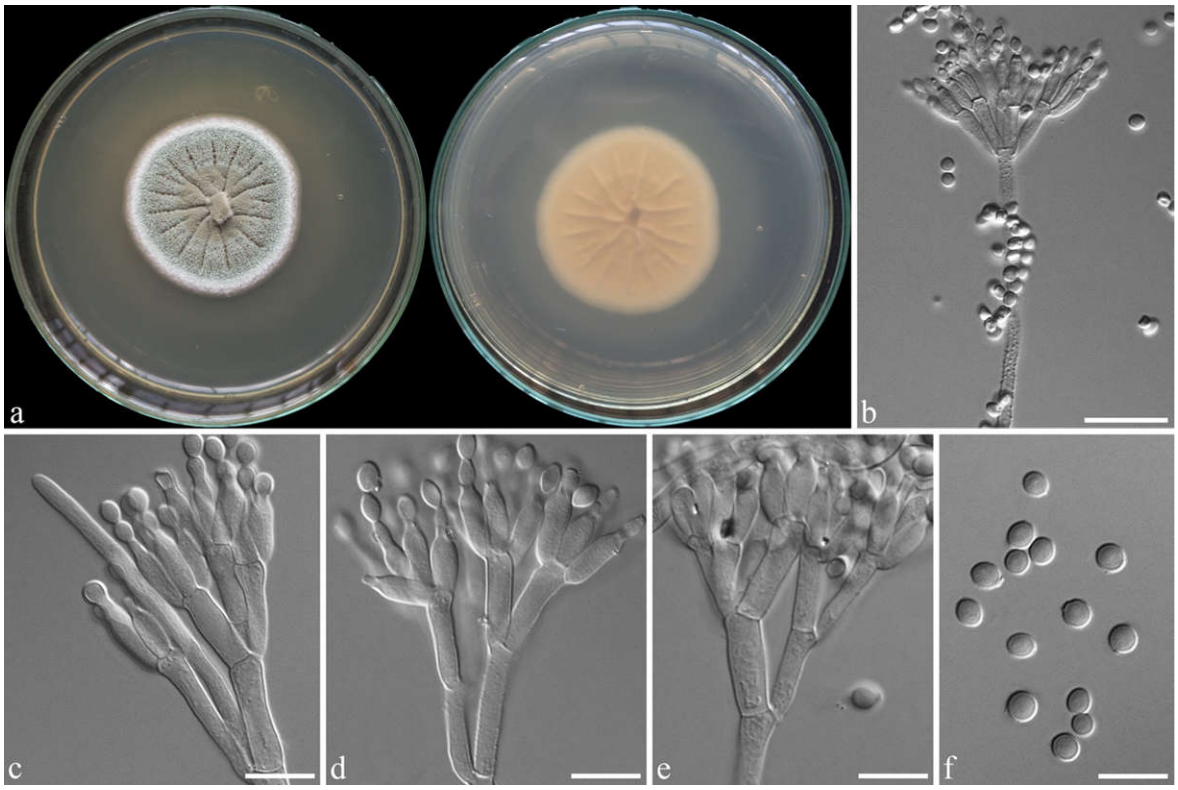

Fig. 7. *Penicillium palitans* (IRAN 5127C). a. Colonies obverse (left) and reverse (right) on MEA at 25 °C after 7 d; b–e. Conidiophores; f. Conidia. Scale bars: b = 20 µm, c–f = 10 µm.

Penicillium palitans Westling, Ark. Bot. 11 (1): 83 (1911)

Colony characters: MEA 25 °C, 7 d: diam 45 mm, radially sulcate, mycelium white, velutinous with a feathery edge, light grayish olive to pale green, sporulation strong, exudate droplets dark brown, soluble (diffusible) pigments absent, reverse radially sulcate, moderate orange yellow.

Micromorphology: Conidiophore terverticillate, rarely biverticillate. Stipes smooth-walled. Ramus cylindrical, (13.2–) 14.1–16.7 (–18.8) × (3–) 3.2–4 (–5.4) µm. Metulae cylindrical, (10–) 10.5–14.2 (–15.1) × (2.4–) 3–4 (–4.6) µm. Phialides cylindrical, (6.9–) 7.7–12 (–13.9) × (2.5–) 2.7–3.7 (–4) µm. Conidia smooth, ellipsoidal, (2.6–) 2.8–4.3 (–4.5) × (1.7–) 1.9–4.3 (–4.5) µm (av. ± S.D. = 3.1 ± 0.8 × 3.8 ± 0.5 µm) (Fig. 7).

Notes: Two representative isolates IRAN 5127C and CJA OGhK39, from two distinct clusters (n = 12 isolates) with different DNA banding profiles generated by $(GTG)_5$, with the same *BenA* sequence data placed in a single clade and characterized as *P. palitans*. We isolated this species from raisin and dried fruits apple, apricot and white mulberry, which is reported on these substrates for the first time around the world. As we know, *P. palitans* has not been reported from foodstuffs in Iran, but this species has previously been isolated from air (Naeimi et al. 2021) and bread samples (Abastabar et al. 2016).

Penicillium polonicum K.W. Zaleski, Bull. Int. Acad. Polon. Sci., Cl. Sci. Math., Sér. B., Sci. Nat. 1927: 445 (1927)

Colony characters: MEA 25 °C, 7 d: diam 45 mm, radially sulcate, mycelium white velutinous with a feathery edge, light greenish gray to pale green, sporulation strong, exudate droplets hyaline, soluble (diffusible) pigments absent, reverse radially sulcate, moderate orange yellow.

Micromorphology: Conidiophore terverticillate, rarely biverticillate. Stipes smooth walled. Ramus cylindrical (13.3–) 13.4–15.2 (–17.1) × (2.9–) 3.3–3.7 (–3.9) µm. Metulae cylindrical, (9.5–) 10.6–15.7 (–16.5) × (2.6–) 2.7–3.6 (–3.9) µm. Phialides cylindrical, (5.8–) 7.2–11.8 (–14.2) × (1.8–) 2–3.6 (–3.9) µm. Conidia smooth, globose to subglobose,

(2.2–) 2.4–3.8 (–4.3) × (1.7–) 2–3.6 (–4) µm (av. ± S.D. = 3.1 ± 0.4 × 2.7 ± 0.5 µm) (Fig. 8).

Notes: Two representative isolates IRAN 5128C and CJA OGhD661, from two distinct clusters (n = 5 isolates) with different DNA banding profiles generated by $(GTG)_5$, with the same *BenA* sequence data placed in a single clade and characterized as *P. polonicum*. Here, this species was isolated from Terkhêna, raisin and dried fruit apricot. So far, this species has only been isolated from raisin (Alghamdi et al. 2023), thus it is reported here for the first time from Terkhêna and dried fruit apricot around the world. As far as we know, *P. polonicum* has not been reported from foodstuffs in Iran. However, it has been isolated from air (Naeimi et al. 2021) and bread (Abastabar et al. 2016), and also as an endophyte in strawberry shoot and crown samples (Zargar et al. 2019).

Given the importance and popularity of the investigated foodstuffs, it is recommended to examine their mycotoxin contaminations in future studies.

Fig. 8. *Penicillium polonicum* (IRAN 5128C). a. Colonies obverse (left) and reverse (right) on MEA at 25 °C after 7 d; b–e. Conidiophores; f. Conidia. Scale bars: b = 20 µm, c–f = 10 µm.

ACKNOWLEDGMENTS

This study was financially supported by the University of Kurdistan. O. Ghaderi was financially supported by the ″Iranian Mycological Society″.

REFERENCES

Abastabar, M., Mirhendi, H., Hedayati, M.T., Shokohi, T., Rezaei-Matehkolaei, A., Mohammadi, R., et al. 2016. Genetic and morphological diversity of the genus *Penicillium* from Mazandaran and Tehran provinces, Iran. Jundishapur Journal of Microbiology 9: e28280.

Abdollahzadeh, J., Goltapeh, E.M., Javadi, A., Shams-Bakhsh, M., Zare, R. and Phillips, A.J.L. 2009. *Barriopsis iraniana* and *Phaeobotryon cupressi*: two new species of the *Botryosphaeriaceae* from trees in Iran. Persoonia 23: 1–8.

Alasalvar, C., Chang, S.K., Kris-Etherton, P.M., Sullivan, V.K., Petersen, K.S., Guasch-Ferré, M., et al. 2023. Dried fruits: bioactives, effects on gut microbiota, and possible health benefits—an update. Nutrients 15: 1611.

Alghamdi, R.G., Zabermawi, N.M., Altihani, F.A., Bokhari, F.M., Makki, R.M., Hassoubah., et al. 2023. Diversity and density of fungi isolated from dried fruits. Journal of Biochemical Technology 14: 45–55.

Alves, A., Phillips, A.J., Henriques, I. and Correia, A. 2007. Rapid differentiation of species of *Botryosphaeriaceae* by PCR fingerprinting. Research in Microbiology 158: 112–121.

Barboráková, Z., Jakabová, S., Maskova, Z., Mrvová, M., Uzsáková, V., Maková, J., et al. 2023. Toxin producing micromycetes of the genus *Penicillium* and *Aspergillus* on berries, grapes, and tomato

fruits in Slovak stores. Journal of Microbiology, Biotechnology and Food Sciences 13: e9927.

Barkai-Golan, R. 2008. *Penicillium* mycotoxins. In: Mycotoxins in fruits and vegetables. (R. Barkai-Golan and N. Paster, eds): 153–183. Academic Press, United States.

Bashiri, S., Abdollahzadeh, J. and Evidente, A. 2022. Diagnosing and pathogenicity of *Biscogniauxia* species, the causal agents of oak charcoal canker and decline in Zagros forests of Iran. Journal of Plant Pathology 104: 1011–1025.

Bhat, R., Rai, R.V. and Karim, A.A. 2010. Mycotoxins in food and feed: present status and future concerns. Comprehensive Reviews in Food Science and Food Safety 9: 57–81.

Chang, S.K., Alasalvar, C. and Shahidi, F. 2016. Review of dried fruits: Phytochemicals, antioxidant efficacies, and health benefits. Journal of Functional Foods 21: 113–132.

Delshad, D. and Mostowfizadeh-Ghalamfarsa, R. 2020. Identification of *Penicillium* species from citrus fruits and various bread types in Shiraz. Rostaniha 21: 65–78.

Ebrahimi Jafari, M., Bayat, M., Haghighi Khiabanian Asl, A. and Hashemi Hazaveh, S.J. 2022. Molecular identification, phylogenetic analysis and histopathological evaluation of gill fungal infection in some ornamental fish: First report and new species. Iranian Journal of Fisheries Sciences 21: 1316–1334.

Ghaderi, O. and Abdollahzadeh, J. (2024). 'Identification of *Rhizopus arrhizus* associated with some dried fruits and the traditional food Terkhêna in Mahabad, western Iran', Mycologia Iranica 11: 117–122.

Glass, N.L. and Donaldson, G.C. 1995. Development of premier sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Applied and Environmental Microbiology 61: 1323–1330.

González-Curbelo, M.Á. and Kabak, B. 2023. Occurrence of mycotoxins in dried fruits worldwide, with a focus on aflatoxins and ochratoxin A: a review. Toxins 15: 576.

Hall, T.A. 2004. BioEdit. Version 7.0.0. Department of Microbiology. United States, North Carolina State University.

Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q. and Vinh, L.S. 2018. UFBoot2: improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35: 518–522.

Houbraken, J. and Samson, R.A. 2011. Phylogeny of *Penicillium* and the segregation of *Trichocomaceae* into three families. Studies in Mycology 70: 1–51.

Katoh, K., Rozewicki, J. and Yamada, K.D. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20: 1160–1166.

Link, H.F. 1809. Observationes in ordines plantarum naturales. Dissertatio 1: 1–33.

Miller, M.A., Pfeiffer, W. and Schwartz, T. 2012. The CIPRES science gateway. Available online at: http://www.phylo.org/index.php/portal (Accessed August 2024).

Mohammadi, N. and Ostovar, N., 2023. Chemical composition, fatty acid composition, and volatile compounds of a traditional Kurdish fermented cereal food: Tarkhineh. Food Chemistry Advances 2: 100187.

Naeimi, B., Mohsenifard, I., Ansari, S., Sadeghzadeh, F., Khamisipour, G., Dobaradaran, S., et al. 2021. Phenotypic features and molecular study of airborne *Penicillium* species isolated in the northern part of the Persian Gulf, Bushehr, Iran. Current Medical Mycology 7: 22–28.

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A. and Minh, B.Q. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–274.

Niknejad, F., Moshfegh, M., Najafzadeh, M.J., Houbraken, J., Rezaei, S., Zarrini, G., et al. 2013. Halotolerant ability and α-amylase activity of some saltwater fungal isolates. Iranian Journal of Pharmaceutical Research 12: 113–119.

Pitt, J.I. and Hocking, A.D. 2022. Spoilage of stored, processed and preserved foods. In: Fungi and food spoilage. (J.I. Pitt and A.D. Hocking, eds): 537–568. Springer International Publishing, United States.

Raeder, U. and Broda, P. 1985. Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology 1: 17–20.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.

Visagie, C.M., Houbraken, J., Frisvad, J.C., Hong, S.B., Klaassen, C.H.W., Perrone, G., et al. 2014. Identification and nomenclature of the genus *Penicillium*. Studies in Mycology 78: 343–371.

Visagie, C.M., Yilmaz, N., Kocsubé, S., Frisvad, J.C., Hubka, V., Samson, R.A., et al. 2024. A review of recently introduced *Aspergillus*, *Penicillium*, *Talaromyces* and other *Eurotiales* species. Studies in Mycology 107: 1–66.

Zargar, D., Amini, J. and Abdollahzadeh, J. 2019. Biocontrol potential of endophytic *Penicillium* spp. against strawberry anthracnose. Biological Control of Pests and Plant Diseases 8: 47–58.

تبارشناسی و طبقه بندی گونه های *Penicillium* مرتبط با برخی مواد غذایی در شهرستان مهاباد (غرب ایران)

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چکیده: آلودگی مواد غذایی به قارچهای مولد زهرابه یک تهدید جدی برای سلامت انسان است. در یک مطالعه روی قارچهای مولد زهرابه مرتبط با کشمش، میوه های خشک زردآلو، سیب و توت سفید و غذای سنتی ترخینه، ۵۰ جدایه *Penicillium* از شهرستان مهاباد واقع در غرب ایران جمع آوری شد. براساس الگوهای اثرانگشت ISSR ایجاد شده با استفاده از آغازگر (GTG)$_5$ جدایه ها در ۱۱ گروه مجزا قرار گرفتند. از هر گروه یک جدایه به عنوان نماینده برای مطالعات تبارشناسی و ریخت‌شناسی انتخاب شد. براساس واکاوی های تبارشناسی توالی بخشی از ژن بتا-توبولین (*BenA*) جدایه‌ها به ۵ گونه شامل گونه‌های *P. chrysogenum* و *P. dipodomyus* از بخش *Chrysogena* و گونه‌های *P. crustosum*، *P. palitans* و *P. polonicum* از بخش *Fsciculata* تعلق داشتند. براساس اطلاعات موجود، غیر از دو گونه *P. chrysogenum* و *P. polonicum* که قبلا از کشمش گزارش شده اند، سایر گونه‌های شناسایی شده در این تحقیق برای اولین بار در دنیا از مواد غذایی بررسی شده گزارش می‌شوند. علاوه بر این، گونه *P. dipodomyus* به عنوان یک گزارش جدید برای مجموعه قارچهای ایران معرفی می شود.

کلمات کلیدی: آسپرژیلاسه، آلودگی قارچی، ترخینه، قارچهای مولد زهرابه

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تاریخ دریافت: ۲۹/۰۹/۱۴۰۳ تاریخ پذیرش: ۰۸/۱۰/۱۴۰۳