Mycologia Iranica 2025, Vol. 12 (No. 1): 21–42 JIrMS

Original Article

Biodiversity of *Aspergillus* species in some dried fruits and the traditional food Terkhêna, with description of *Aspergillus mahabadiensis* sp. nov. from Mahabad, western Iran

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https://doi.org/10.22092/mi.2025.368273.1305

ABSTRACT

Dried fruits and the traditional food 'Terkhêna' are popular foods since ancient times in the western part of Iran. Microbiological food spoilage and toxin production by fungi during storage are serious threats to human health. In this research, we studied 317 *Aspergillus* isolates obtained from Terkhêna, raisins and three dried fruits apple, apricot and white mulberry. Based on morphological and molecular data (sequences of *CaM* gene) our isolates are assigned to *Aspergillus* species in subgenus *Circumdati*, namely *A. luchuensis*, *A. niger* and *A. tubingensis* in section *Nigri*, *A. flavus*/*A. oryzae* complex in section *Flavi*, and *A. terreus* and a new species candidate in section *Terrei*. Multigene phylogeny based on *CaM*, *BenA* and *RPB2* sequence data and morphological examinations confirmed the recognition of one novel species in section *Terrei* that is here formally described as *Aspergillus mahabadiensis* sp. nov. Two species *Aspergillus niger* and *A. tubingensis* were the most frequent species. Some fungus-substrate associations are here reported for the first time.

KEYWORDS

DNA barcoding, Food safety, Mycotoxigenic fungi, Phylogeny, Taxonomy.

INTRODUCTION

Dried fruits, as nutrient-rich healthful snack foods, have been popular in Iran. Historically, they constitute the major part of the Mediterranean diet dates back to the millennial generations in Mesopotamia (incl. western Iran) northern part of the Fertile Crescent. Considering their roles in human health due to their bioactive compounds, micronutrients, fiber content and antioxidant properties daily intake of dried fruits is recommended by the World Health Organization (Alasalvar et al. 2023). In western parts of Iran, raisins and dried fruits such as apple, apricot and white mulberry are the most common traditional snacks. In this part of the country, a special type of traditional dried food known as 'Terkhêna' is also very popular cold season food which is made of bulgur wheat, turnip (*Brassica rapa* L.) or pennyroyal (*Mentha pulegium* L.) leaves and buttermilk (Mohammadi and Ostovar 2023). Terkhêna is stored dry, and commonly consumed to prevent or treat cold, rhinitis and similar diseases. Since these foodstuffs are processed traditionally and stored in the warehouse for a long time, fungal spoilage and hazards related to their mycotoxins are major concerns for human health (Bhat et al. 2010, Wu et al. 2018, González-Curbelo et al. 2023).

Among the various food spoilage fungi, *Aspergillus* members are ubiquitous and well-known mycotoxigenic species. The genus *Aspergillus*, introduced by Micheli (1729), is a diverse, widespread, and economically significant fungus, which seriously affects food and

Received: 10 Jan. 2025 Revised: 10 Feb. 2025 Accepted: 15 Feb. 2025 Published online: 22 Feb. 2025

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

human health. *Aspergillus* species occur in diverse environments and are found on various substrates at a wide temperature range, as well as under low humidity conditions (Bhat et al. 2010, Krijgsheld et al. 2013). Members of *Aspergillus* are considered by researchers in various fields of biotechnology and industrial mycology (metabolites production, medicines, fermentation and food processing), food and indoor mycology (food spoilage and mycotoxins production) and medical mycology (human and animal fungal diseases) (Samson et al. 2014).

Taxonomy of *Aspergillus* has traditionally relied on morphological features, but in recent decades, affected by chemotaxonomic and phylogenetic species concepts and following a polyphasic systematic approach, researchers have used extrolite profiles and DNA sequence data together with phenotypic characters (Samson et al. 2014). The first monographs on *Aspergillus* were published by Thom and Raper (1945) and Raper and Fennell (1965). Following Raper and Fennell (1965) who divided the genus *Aspergillus* into 18 groups, infrageneric classification in *Aspergillus* based on phenotypic data was further developed by Gams et al. (1985) and subgenera and sections were introduced under the genus level, which is supported by current phylogenetic analyses. The taxonomic history has been discussed by Samson et al. (2014) and Houbraken et al. (2020) which updated the taxonomy of *Aspergillus* in a polyphasic approach. Recent multigene phylogenetic studies often supported by phenotypic, physiologic and/or extrolite data, have listed and classified 453 *Aspergillus* species in six subgenera, 28 sections, and 76 series (Houbraken et al. 2020, Visagie et al. 2024). Among these, *Circumdati* with 11 sections (e.g. *Flavi*, *Nigri* and *Terrei*) is the second species-rich subgenus after *Nidulantes* (Houbraken et al. 2020, Visagie et al. 2024).

Members of section *Flavi* are of special interest to researchers in terms of food safety, due to the production of some hazardous mycotoxins such as aflatoxins and ochratoxins (Frisvad et al. 2019). They can exist in the form of sclerotia, conidia and mycelium, and can be found in soil, indoor environments and a wide range of foods (Varga et al. 2011). Section *Flavi*, containing 35 species, resided in subgen. *Circumdati* and classified into eight series including ser. *Flavi*. In terms of food safety, *Aspergillus flavus*/*A. oryzae* complex is of great importance in ser. *Flavi* (Houbraken et al. 2020).

Aspergillus species in section *Nigri* are commonly regarded as safe, and considered in biotechnology and industrial mycology for producing fungal natural products such as enzymes and organic acids (Andersen et al. 2011, Yang et al. 2017) and food fermentation (Hong et al. 2014). However, some species in this section cause food spoilage (Samson et al. 2019) or produce mycotoxins on a wide variety of foods and feeds (Frisvad et al. 2018), and some members are known as minor pathogens that can cause various mycoses in animal and human (Nargesi et al. 2022). In a phylogenetic revision on *Aspergillus* and allied genera, section *Nigri* with 29 species placed in subgen. *Circumdati* and divided into five series including *Carbonarii*, *Heteromorphi*, *Homomorphi*, *Japonici* and *Nigri* (Houbraken et al. 2020). Recently, Bian et al. (2022) in an extensive phylogenomic study reduced the number of species in series *Nigri* from 10 species recognized by Houbraken et al. (2020) to six species, i.e. *A. brasiliensis*, *A. eucalypticola*, *A. luchuensis*, *A. niger*, *A. tubingensis* and *A. vadensis*.

Aspergillus section *Terrei* was established based on *A. terreus*, the most well-known and widespread species with great significance in fermentation, food and medicine industries due to the ability to produce a wide range of secondary metabolites such as enzymes, acids, mycotoxins and some drugs, antitumor and antiviral compounds (Samson et al. 2011). Section *Terrei* species are classified into three series *Terrei*, *Nivei* and *Ambigui* (Houbraken et al. 2020). Series *Terrei* and *Nivei* are distinguished based on conidia en masse which are in shades of brown in *Terrei* and in shades of yellow, vinaceous fawn or white in *Nivei*. The species in series *Ambigui* grow slower than the members of series *Terrei* and *Nivei* (Houbraken et al., 2020). Thus far, 22 species have been accepted in this section, with most of them being isolated from soil, clinical samples, and a limited number of plant products such as wheat flour, barley, peanuts and corn (Samson et al. 2011, Barros Correia et al. 2020, Houbraken et al. 2020, Wang and Zhuang 2022, Cañete-Gibas et al. 2023). Of these, *A. terreus* is one of the well-known species that causes invasive aspergillosis with higher mortality rates (Lass-Flörl et al. 2021).

During an extensive study on taxonomy and phylogeny of mycotoxigenic fungi associated with raisins, three dried fruits (apple, apricot, white mulberry) and a traditional food known as 'Terkhêna' in Mahabad, located in western Iran, we studied a large collection of 415 fungal isolates belonging to four genera *Alternaria*, *Aspergillus*, *Penicillium* and *Rhizopus*. Of these, *Aspergillus* with 317 isolates (76.4%) was the most prevalent genus followed by *Penicillium* with 50 isolates (12%) belonging to five species (Ghaderi and Abdollahzadeh 2024a), *Rhizopus* with 33 isolates (8%) identified as *R. arrhizus* (Ghaderi and Abdollahzadeh 2024b), and *Alternaria* with 15 isolates (3.6%). In this paper, we focus on the phylogeny and taxonomy of *Aspergillus* species and introduce a novel species in section *Terrei*, named here *Aspergillus mahabadiensis* sp. nov., isolated from samples of dried apricot and the traditional food Terkhêna.

MATERIALS AND METHODS

Sampling and Isolation

During 2021–2022, samples of raisins, dried fruits including apple, apricot and white mulberry, and the traditional food Terkhêna were collected from various locations in Mahabad, Iran. Fungi were isolated by direct plating of surface sterilized (3 min in 70% ethanol) small pieces of the samples on potato dextrose agar (PDA). Pure fungal isolates were obtained using the hyphal tip or single spore techniques on Water

Agar (WA 1.5%) and stored on PDA at 4–8 °C. Representative isolates were deposited in the culture collection of the Iranian Research Institute of Plant Protection (IRAN, Tehran, Iran) and the CBS collection of the Westerdijk Institute, Utrecht, The Netherlands.

DNA extraction, PCR amplification and sequencing

Fungal isolates were grown in potato dextrose broth (PDB) for 7–12 days at 20–25 °C. The modified Raeder and Broda (1985) method was followed for DNA extraction from dried mycelium as described by Abdollahzadeh et al. (2009). To manage the costs of DNA sequencing and time for morphological studies, we used the inter-simple sequence repeat (ISSR) technique to cluster fungal isolates based on DNA fingerprinting patterns generated with $(GTG)_5$ primer (Meyer et al. 1991, Alves et al. 2007). PCR and electrophoresis conditions were adjusted following Alves et al. (2007). DNA fingerprinting profiles were visually analyzed, and a representative isolate from each cluster was selected for further studies. A part of the calmodulin (*CaM*) gene of representative isolates was amplified and sequenced using primer pairs CF1/CF4 (Peterson et al. 2005). For comprehensive phylogenetic analysis of the potential new species, ITS region (ITS1-5.8S-ITS2, using primer pairs ITS5/ITS4 (White et al. 1990), a part of beta-tubulin (*BenA*, using primer pairs Bt2a/Bt2b (Glass and Donaldson 1995) and RNA polymerase II second largest subunit (*RPB2*, using primer pairs 5F2) (Sung et al. 2007) or 5F/7cR (Liu et al. 1999) were amplified and sequenced. The PCR conditions were as follows: an initial denaturation step of 5 min at 95°C followed by 35 cycles of 30 s at 95°C, 30 s at 55°C (ITS, *CaM* and *BenA*)/52°C (*RPB2*), 60 s at 72°C, with a final extension of 7 min at 72°C. Generated sequences in this survey were submitted to GenBank (Table 1).

Phylogenetic analyses

The generated sequences of *CaM* locus were subjected to BLAST analysis in GenBank. Based on the BLAST analysis, our sequences were supplemented with the sequences of the type specimens or authentic specimens of existing species and aligned using online MAFFT v. 7 (Katoh et al. 2019) and edited manually in BioEdit v.

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

Section	Species	Isolate No.[1]	Substrate	Coordinates (DD)	GenBank accession numbers[2]			
					ITS	*CaM*	*BenA*	*RPB2*
Flavi	*A. flavus/A. oryzae*	IRAN 5119C	Terkhêna	36.649795, 45.739374	N.S.	PQ344956	N.S.	N.S.
Nigri	*A. luchuensis*	IRAN 5120C	White Mulberries	36.863849, 45.755258	N.S.	PQ344960	N.S.	N.S.
	A. niger	IRAN 5126C	White Mulberries	36.877358, 45.778903	N.S.	PQ344963	N.S.	N.S.
		CJA OGhS9	Apples	36.764079, 45.725067	N.S.	PQ344965	N.S.	N.S.
		CJA OGhK108	Raisins	36.611758, 45.827911	N.S.	PQ344964	N.S.	N.S.
	A. tubingensis	IRAN 5122C	Raisins	36.762110, 45.636150	N.S.	PQ344958	N.S.	N.S.
		CJA OGhS40	Apples	36.820715, 45.807501	N.S.	PQ344961	N.S.	N.S.
		CJA OGhK106	Raisins	36.617319, 45.518825	N.S.	PQ344959	N.S.	N.S.
		CJA OGhK4	Raisins	36.753143, 45.720660	N.S.	PQ344957	N.S.	N.S.
Terrei	*A. mahabadiensis*	IRAN 4982C	Apricots	36.762386, 45.734450	PQ845699	PP544439	PP544443	PP544441
		IRAN 4983C [T]	Terkhêna	36.753150, 45.721956	PQ846961	PP544440	PP544444	PP544442
		DTO 519-H3	Apricots	36.497720, 45.566984	N.S.	PQ587367	PQ588709	PQ963942
		DTO 519-H4	Apricots	36.901956, 45.765277	N.S.	PQ587368	PQ588714	PQ963943
		DTO 519-H5	Apricots	36.815705, 45.736087	N.S.	PQ587369	PQ588710	PQ963944
		DTO 519-H6	Terkhêna	36.416470, 45.517483	N.S.	PQ587370	PQ588713	PQ963945
	A. terreus	IRAN 5116C	White Mulberries	36.627992, 45.706870	N.S.	PQ344962	N.S.	N.S.

[1] *IRAN* Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran; *CJA* Personal cultures of Jafar Abdollahzadeh. *DTO* internal culture collection of the Department of Applied and Industrial Mycology of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. [T] Ex-type. [2] *CaM* partial calmodulin gene; *BenA* partial β-tubulin gene; *RPB2* partial RNA polymerase II second largest subunit gene; ITS internal transcribed spacers of rDNA; N.S. Not Sequenced.

7.0.5 (Hall, 2004), if needed. The same procedure was followed for *BenA* and *RPB2* sequences of the new species located in section *Terrei*. In section *Terrei*, both single and multigene phylogenies were conducted. Thus, for multigene phylogenetic analyses, single-locus alignments were concatenated with Mesquite 3.70 (Maddison and Maddison 2023). All single and combined alignments were analyzed by Maximum

Likelihood (ML) and Bayesian Inference (BI). In BI analyses, the general time reversible (GTR + G + I) model was used for all single and multigene phylogenies. The BI was executed through the online CIPRES Science Gateway (Miller et al. 2012) using MrBayes v. 3.2.7a (Ronquist et al. 2012), as described by Bashiri et al. (2022). The ML analysis was executed in IQ-TREE v. 2.2.2.7 (Nguyen et al. 2015) For ML analyses the most suitable model was determined for each locus using Modelfinder (Kalyaanamoorthy et al. 2017) and ultrafast bootstrapping executed with 1000 replications using UFBoot (Hoang et al. 2018), both integrated into IQ-TREE. The phylogenetic trees obtained from the analyses were visualized in FigTree v. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree) and edited in Adobe Illustrator 2023 v. 27.1.0.189. Alignments and trees were deposited in TreeBASE (www.treebase.org, S31425) and taxonomic novelties in MycoBank (www. MycoBank.org, Crous et al. 2004).

Macro and micromorphological observations

The macromorphological characteristics were observed on three-point inoculated agar media malt extract agar (MEA), Czapek yeast extract autolysate agar (CYA), yeast extract sucrose agar (YES), oatmeal agar (OA) and Czapek's agar (CZ). Culture media were prepared as described by Samson et al. (2014). All inoculated media were incubated at 25 °C for 7 d, with additional CYA plates incubated at 27 °C, 30 °C, 33 °C and 37 °C. Culture characteristics including colony diameter, texture, degree of sporulation, obverse and reverse colony color (according to Rayner, 1970), production of soluble pigments, sclerotia or ascomata and exudates were recorded after 7 d of incubation. For microscopic observations, mounts were prepared in lactic acid (60%) and excess conidia were removed using ethanol (70%). The shape, color and dimension of the fungal structure were documented using an Olympus BX51 microscope equipped with an Olympus DP72 camera and a measurement module Cell Sens Entry v. 2.1. Dimensions of fungal structures were estimated based on at least 30 microscopic measurements and presented as the range of measurements with extremes in brackets followed by mean ± standard deviation. The recorded images were used to prepare photoplates in Adobe Photoshop 2021 v. 22.5.8.

RESULTS

Sampling, fungal isolation and DNA sequencing

In an extensive phylogenetic study on mycotoxigenic fungi associated with some foodstuffs in Mahabad, western Iran, we obtained 415 fungal isolates from 519 samples of raisins (n = 119), dried fruits including apple (n = 100), apricot (n = 100) and white mulberry (n = 100), and a traditional food ‘Terkhêna’ (n = 100). A total of 317 out of 415 isolates (76.4%) belonged to *Aspergillus*, including 81 from raisins, 64 from dried apple, 64 from dried apricot, 52 from dried white mulberry and 56 from Terkhêna. Based on DNA fingerprinting profiles generated by $(GTG)_5$ primer, all 317 *Aspergillus* isolates were placed in 11 clusters (Fig. 1), and thus 11 representative isolates were selected for phylogenetic analyses.

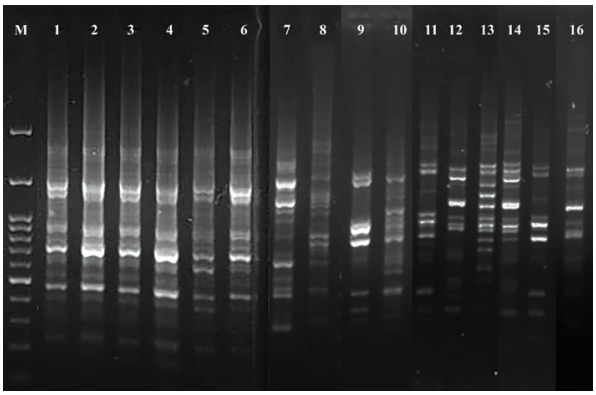

Fig. 1. DNA fingerprinting patterns of sequenced isolates generated by primer $(GTG)_5$. Lanes 1–6: *A. mahabadiensis* sp. nov.; 7: *A. falvus/A. oryzae* (IRAN 5119C); 8: *A. terreus* (IRAN 5116C); 9–11: *A. niger* (CJA OGhK108, CJA OGhS9, IRAN 5126C); 13: *A. luchuensis* (IRAN 5120C); 12, 14–16: *A. tubingensis* (CJA OGhK4, CJA OGhK106, IRAN 5122C, CJA OGhS40). M: GeneRuler DNA Ladder Mix (100bp).

Phylogeny

BLAST search of *CaM* sequences revealed that our isolates are placed in sections *Flavi*, *Nigri* and *Terrei*. Based on phylogenetic analyses of *CaM* sequence data our isolates placed in *A. flavus*/*A. oryzae* in section *Flavi* (Fig. 2), *A. luchuensis*, *A. niger* and *A. tubingensis* in section *Nigri* (Fig. 3), and *A. terreus* together with a potential new species in section *Terrei* (Fig. 4). Identified species with their distribution and number of isolates obtained from each substrate are shown in Fig. 5.

Aspergillus oryzae is known as non-aflatoxigenic domesticated form of *A. falvus* even though some *A. flavus* strains have lost their aflatoxin production ability (Yu et al. 2004). Based on phylogenetic analyses of *BenA*, *CaM* and *RPB2* sequence data, the two species *A. flavus* and *A. oryzae* are conspecific, but due to the importance of aflatoxins production in food safety researchers still know *A. oryzae* as a legitimate species and differentiate these two species based on the aflatoxin production ability, origin of the strains and morphology (Frisvad et al. 2019). Most of the researchers have identified isolates from fermented foods as *A. oryzae* and placed isolates from other substrates even with no aflatoxin production ability in *A. flavus*. In this study, the toxigenic properties of the isolates were not considered and based on origin and morphology some isolates showed an intermediate status. For example, IRAN 5119C morphologically

belongs to *A. flavus* by smaller and rough conidia (3.2–4 µm vs. 4.5–8 µm, rough vs. smooth to rough), degree of sporulation (strong vs. weak) and color of conidia *en masse* (yellow to green vs. green to brown), while as an isolate obtained from Terkhêna, a fermented traditional food, is an *A. oryzae* isolate. Thus, with no emphasis on a particular species our isolates are considered as members of *A. flavus*/*A. oryzae* complex.

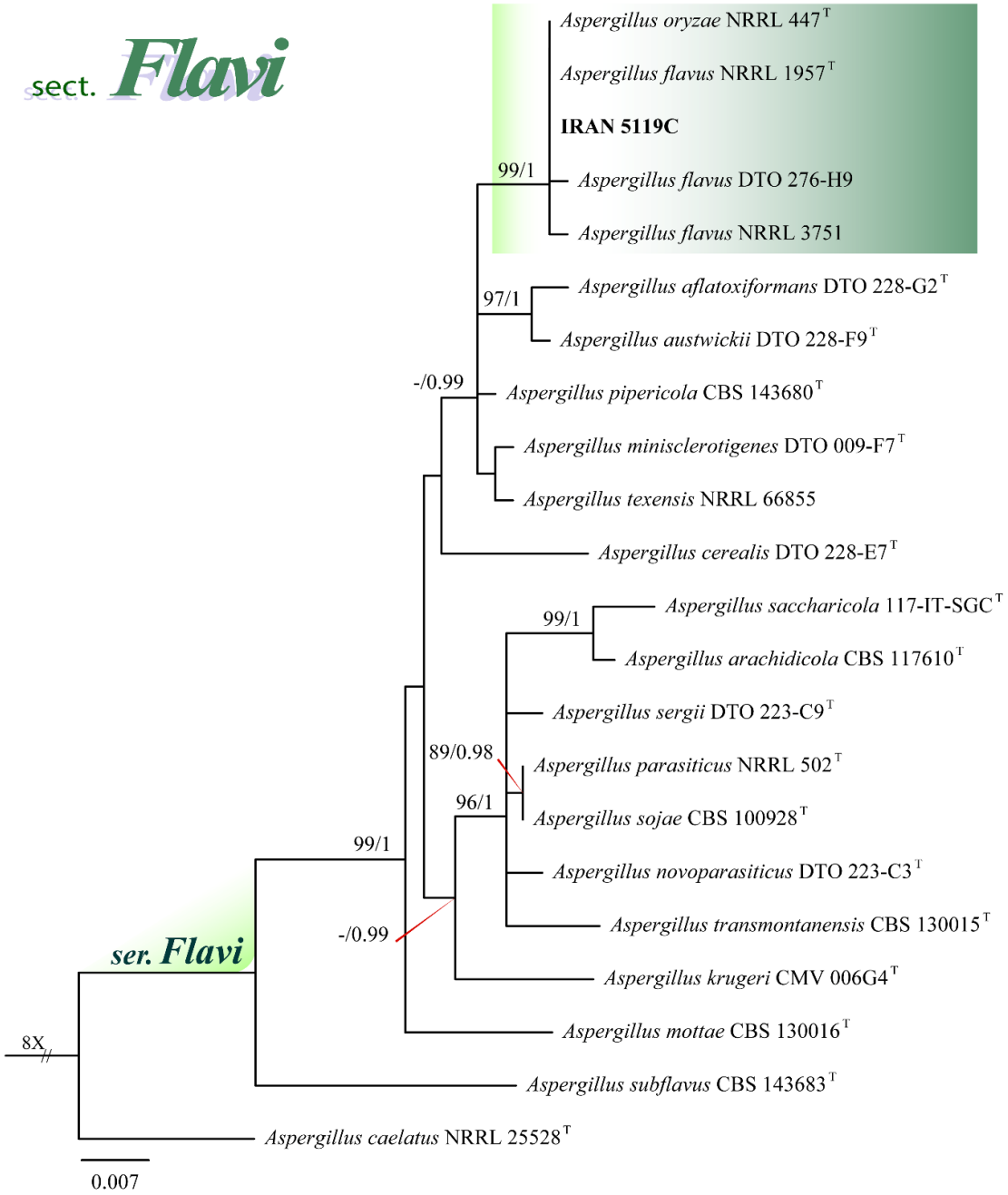

Fig. 2. Maximum likelihood phylogram of *Aspergillus* section *Flavi* (series *Flavi*) based on *CaM* sequence data. ML/BI ultrafast bootstrap support (≥80%) and posterior probability values (≥0.95) are shown at the nodes. The phylogenetic tree was rooted with *Aspergillus caelatus* NRRL 25528. Strain sequenced in this study is in boldface. T = Ex-type strain.

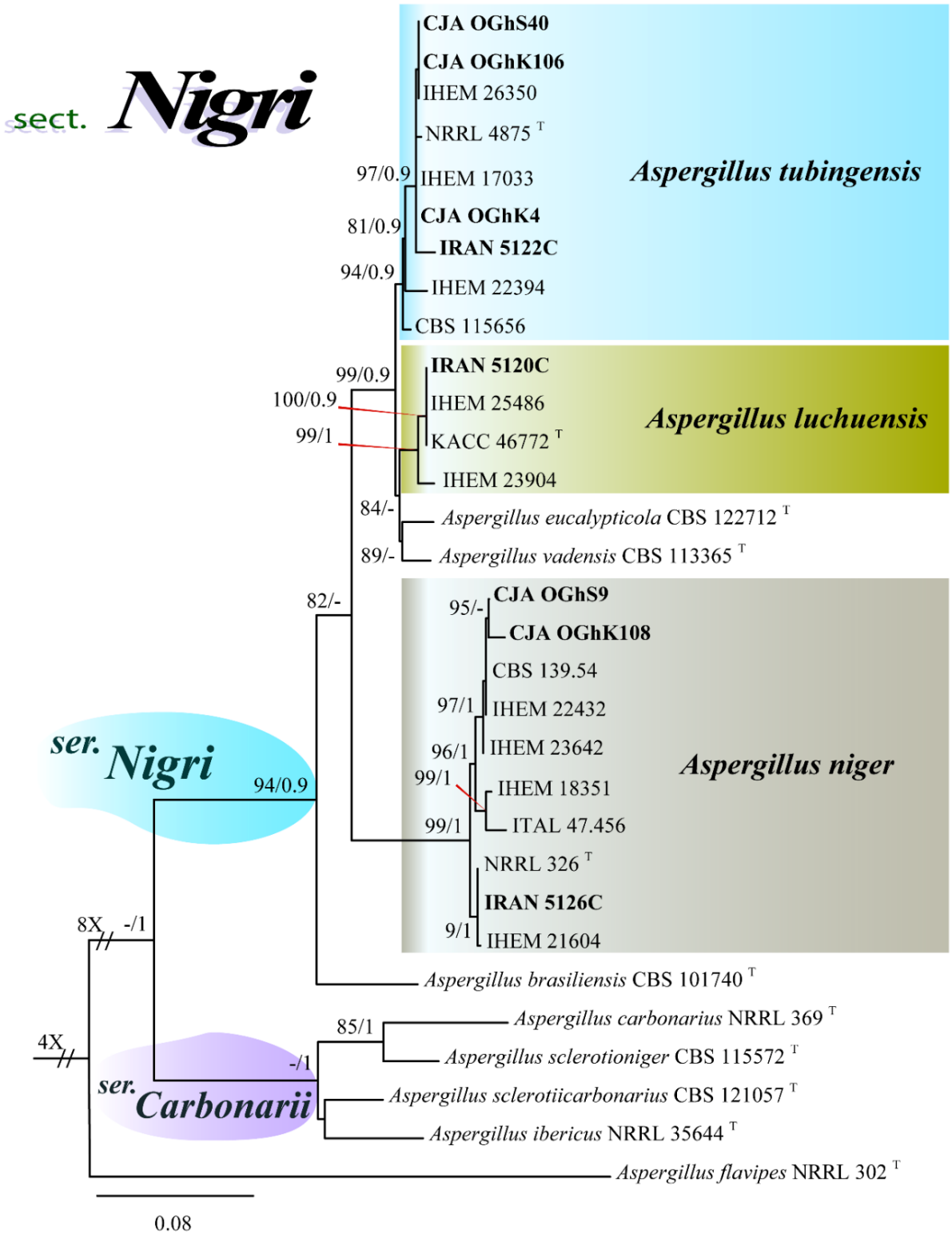

Fig. 3. Maximum likelihood phylogram of *Aspergillus* section *Nigri* (series *Nigri* and *Carbonarii*) based on *CaM* sequence data. ML/BI ultrafast bootstrap support (≥80%) and posterior probability values (≥0.95) are shown at the nodes. The phylogenetic tree was rooted with *Aspergillus flavipes* NRRL 302. Strains sequenced in this study are in boldface. ^{T} = Ex-type strain.

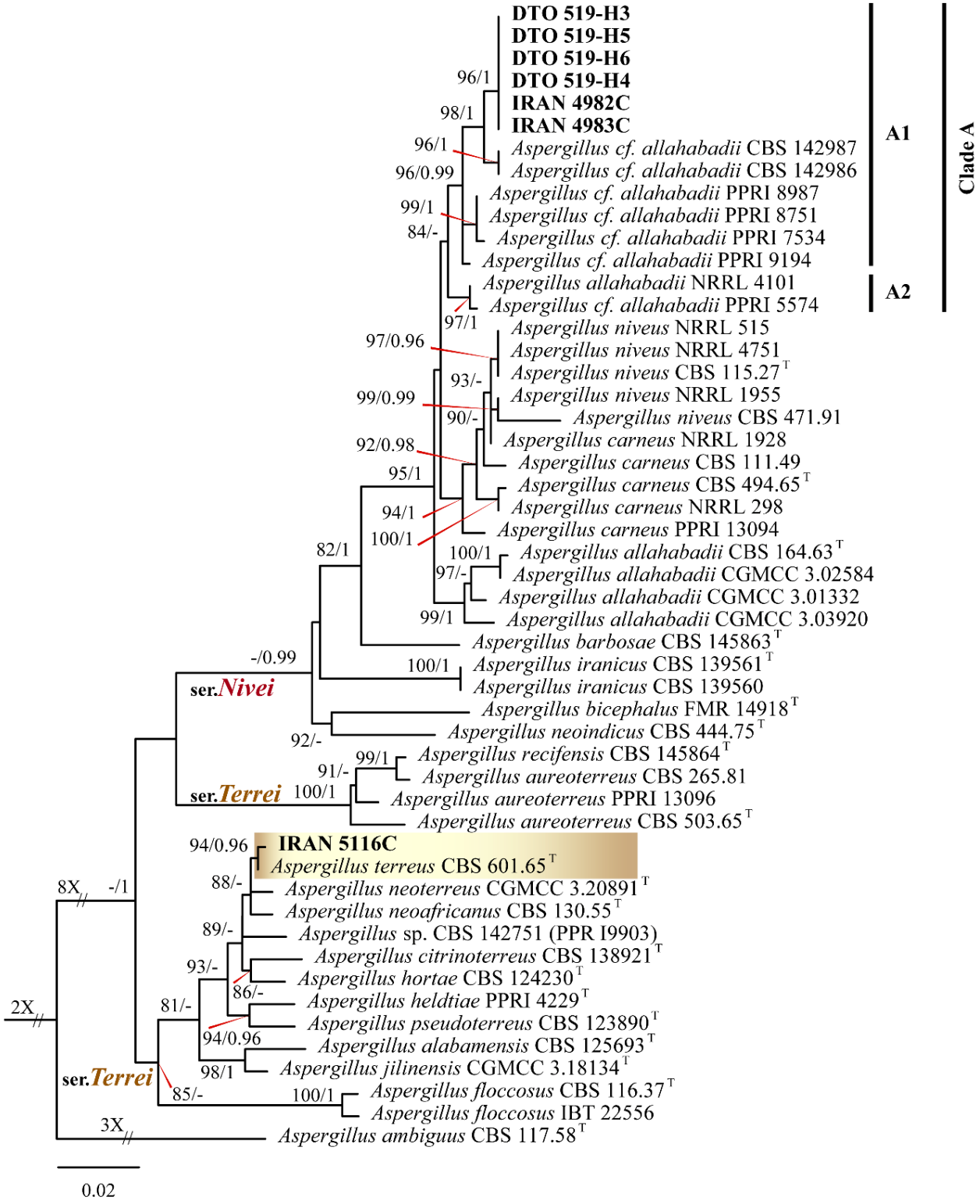

Fig. 4. Maximum likelihood phylogram of *Aspergillus* section *Terrei* (series *Nivei* and *Terrei*) based on *CaM* sequence data. ML/BI ultrafast bootstrap support (≥80%) and posterior probability values (≥0.95) are shown at the nodes. The phylogenetic tree was rooted with *Aspergillus ambiguus* CBS 117.58. Strains sequenced in this study are in boldface. ^{T} = Ex-type strain.

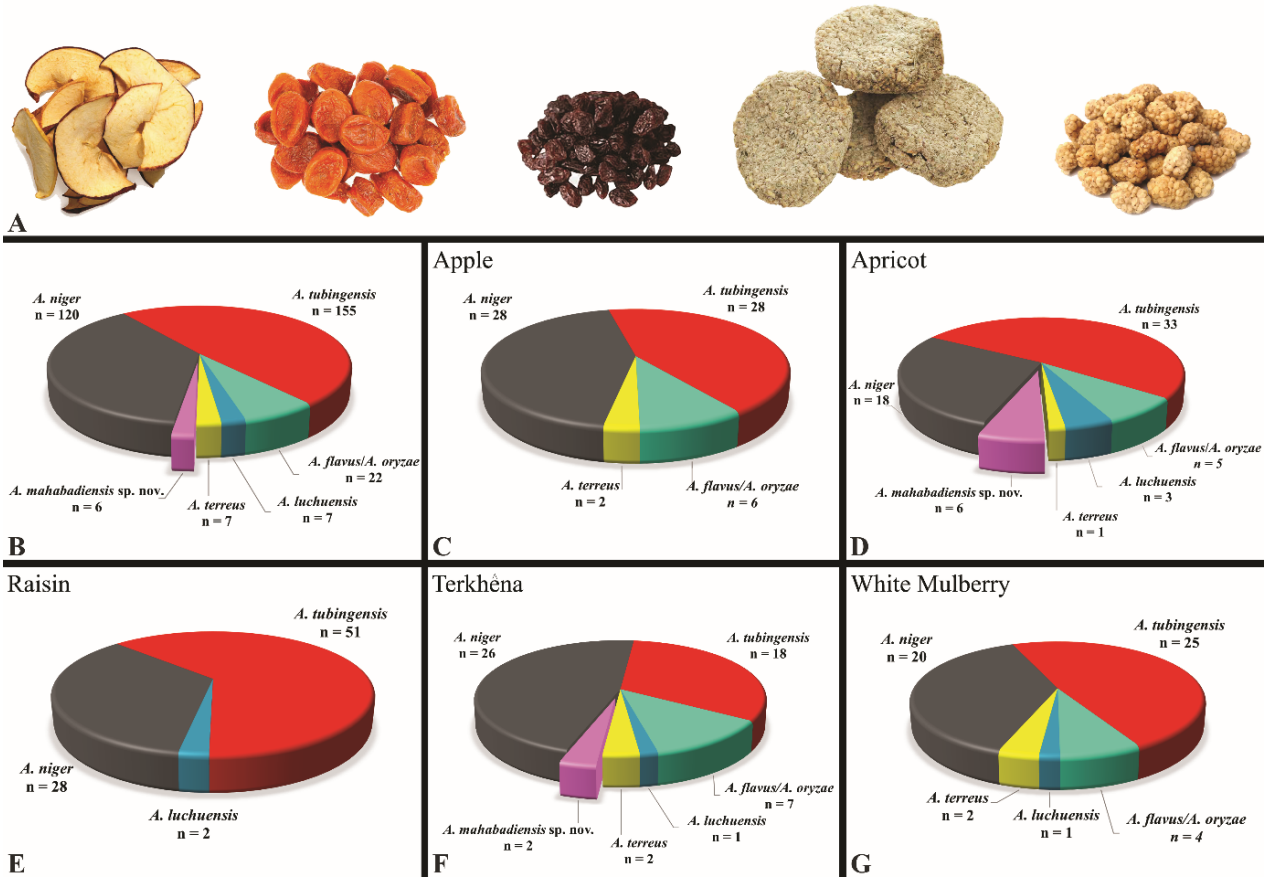

Fig. 5. Substrates examined: left to right apple, apricot, raisins, Terkhêna and white mulberry (A), identified *Aspergillus* species (B), and their distribution on each substrate (C–G).

To confirm the potential new species based on representative isolate IRAN 4983C in section *Terrei*, all other five isolates with the same DNA fingerprinting profile were selected for sequencing in a part of *CaM* and more DNA barcodes (ITS, *BenA*, *RPB2*) necessary for *Aspergillus* phylogeny. In ML analyses, the nucleotide substitution model TPM2+F+I was used for ITS, K2P + I for *BenA*, TNe + I for *CaM* and TIM2e + G4 for both *RPB2* and the combined phylogeny. In BI analyses, the general time reversible (GTR + G + I) model was used for all single and multigene phylogenies.

Since ITS sequence data is insufficient for discrimination of all *Aspergillus* species, both ML and BI analyses resulted in an unresolved tree and our isolates were not correctly identified based on ITS phylogeny (Fig. 6). In both single-gene phylogenies *CaM* (Fig. 4) and *BenA* (Fig. 7) our isolates together with seven isolates previously identified as *A.* cf. *allahabadii* and *A. allahabadii* NRRL 4101 (Visagie and Houbraken 2020, Visagie et al. 2024) were placed in a large clade (A) representing a potential new species separate and distant from *A. allahabadii*. This clade is highly supported in ML (*BenA*: 94%; *CaM*: 84%), but posterior probability is lacking in BI analyses (*BenA*: 0.8; *CaM*: 0.84). In *CaM* phylogeny, more resolution was observed and two strongly supported subclades (A1: BS = 96%, PP = 0.99 and A2: BS = 97%, PP = 1) were recognized within clade A in both ML and BI analyses. In *RPB2* phylogeny, A1 divided to three separate clades (A1-1, A1-2, A1-3) representing three potential new species, and A2 constituted a distinct clade representing a new potential species as a sister group with CBS 164.63, ex-type strain of *A. allahabadii* (Fig. 8). Some incongruences were observed between single-gene phylogenies regarding discrimination of the two series *Terrei* and *Nivei* (Fig. 4), position of *A. barbosae* (Figs 7, 8) and the degree of resolution in clades containing *A. careus*, *A. niveus*, *A. cf. allahabadii* isolates from South Africa and our isolates from Iran (Figs 4, 7, 8). However, our isolates together with *A. cf. allahabadii* isolates were obviously distinguished from the CBS 164.63 ex-type strain of *A. allahabadii* in all three single-gene phylogenies. Thus, we combined all three alignments and provided a multigene dataset (*CaM*: 50, *BenA*: 52, *RPB2*: 37 sequences). The concatenated alignment consisting of 2095 characters (*CaM*: 558, *BenA*: 527, *RPB2*: 1010), including alignment gaps, was analyzed by Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analysis of the dataset with 733 distinct alignment patterns and 456 parsimony-informative characters, yielded a best-

scoring ML tree (lnL = -9953.326). The BI phylogenetic tree was mapped on the ML tree presented in Fig. 9 with ML/BI bootstrap support and posterior probability values at the nodes.

Multigene phylogenetic analyses resulted in a fully resolved tree in both ML and BI analyses. All isolates of *A. allahabadii*, *A. cf. allahabadii* and our isolates were placed in a large unsupported clade containing *A. allahabadii* and four potential new species (A1-1, A1-2, A1-3, A2). In accordance with *RPB2* phylogeny, subclade A1 was split in three clades (A1-1, A1-2, A1-3) representing three new species and subclade A2 was placed in a clade as sister group with *A. allahabadii*. The degree of nucleotide variation in all three loci within and between four potential new species and *A. allahabadii* is summarized in Table 2. In all single and multigene phylogenies, our isolates were grouped close to isolates CBS 142986 and CBS 142987 and appeared as a candidate for a new species in a subclade with poor (Fig. 4) or strong (Fig. 7) statistical supports or in a well-supported distinct clade (Figs 8, 9). The small degree of nucleotide variation (*BenA*: one substitution; *CaM*: four substitutions; *RPB2*: one substitution) in this clade was interpreted as infraspecies variation. Thus, we recognized this clade as a new species distinguished from all described species in series *Nivei* and named as *A. mahabadiensis* sp. nov., which is described here.

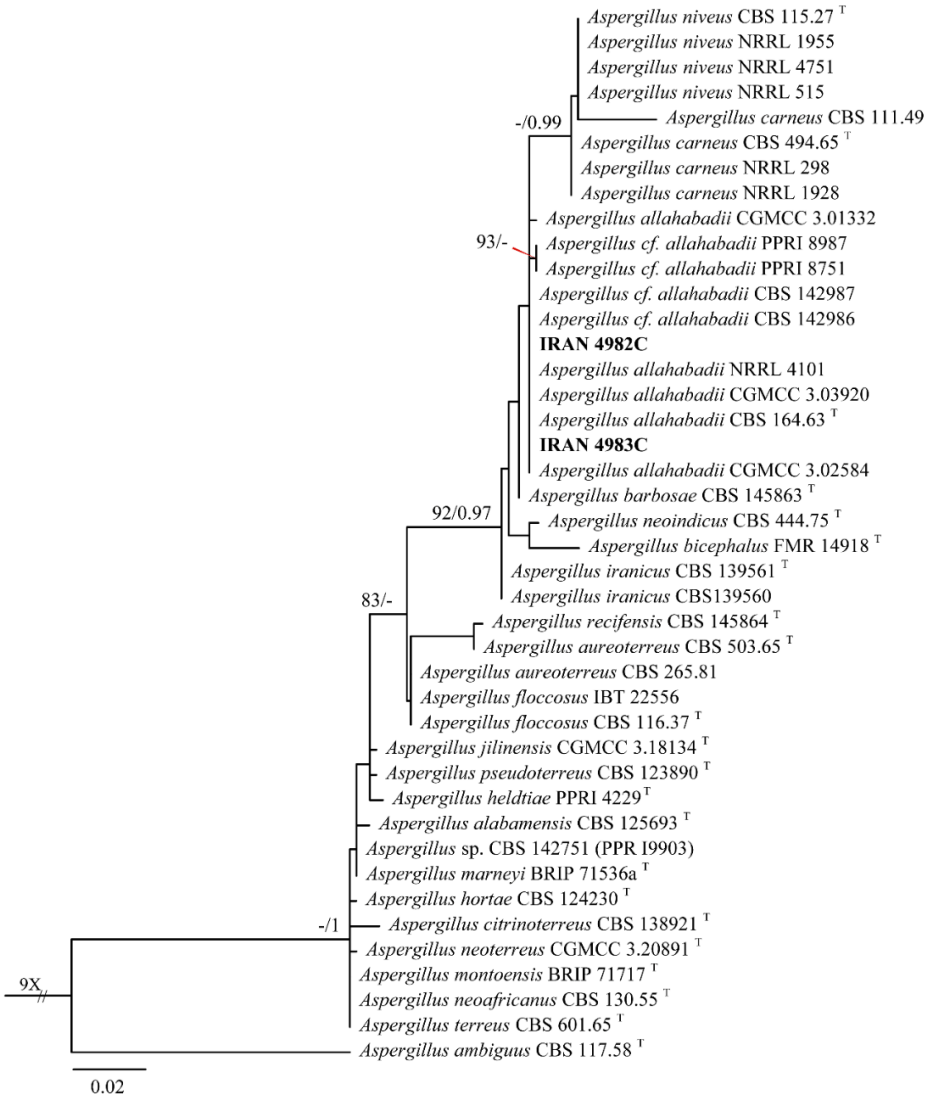

Fig. 6. Maximum likelihood phylogram of *Aspergillus* section *Terrei* (series *Nivei* and *Terrei*) based on ITS sequence data. ML/BI ultrafast bootstrap support (≥80%) and posterior probability values (≥0.95) are shown at the nodes. The phylogenetic tree was rooted with *Aspergillus ambiguus* CBS 117.58. Strains sequenced in this study are in boldface. ^{T} = Ex-type strain.

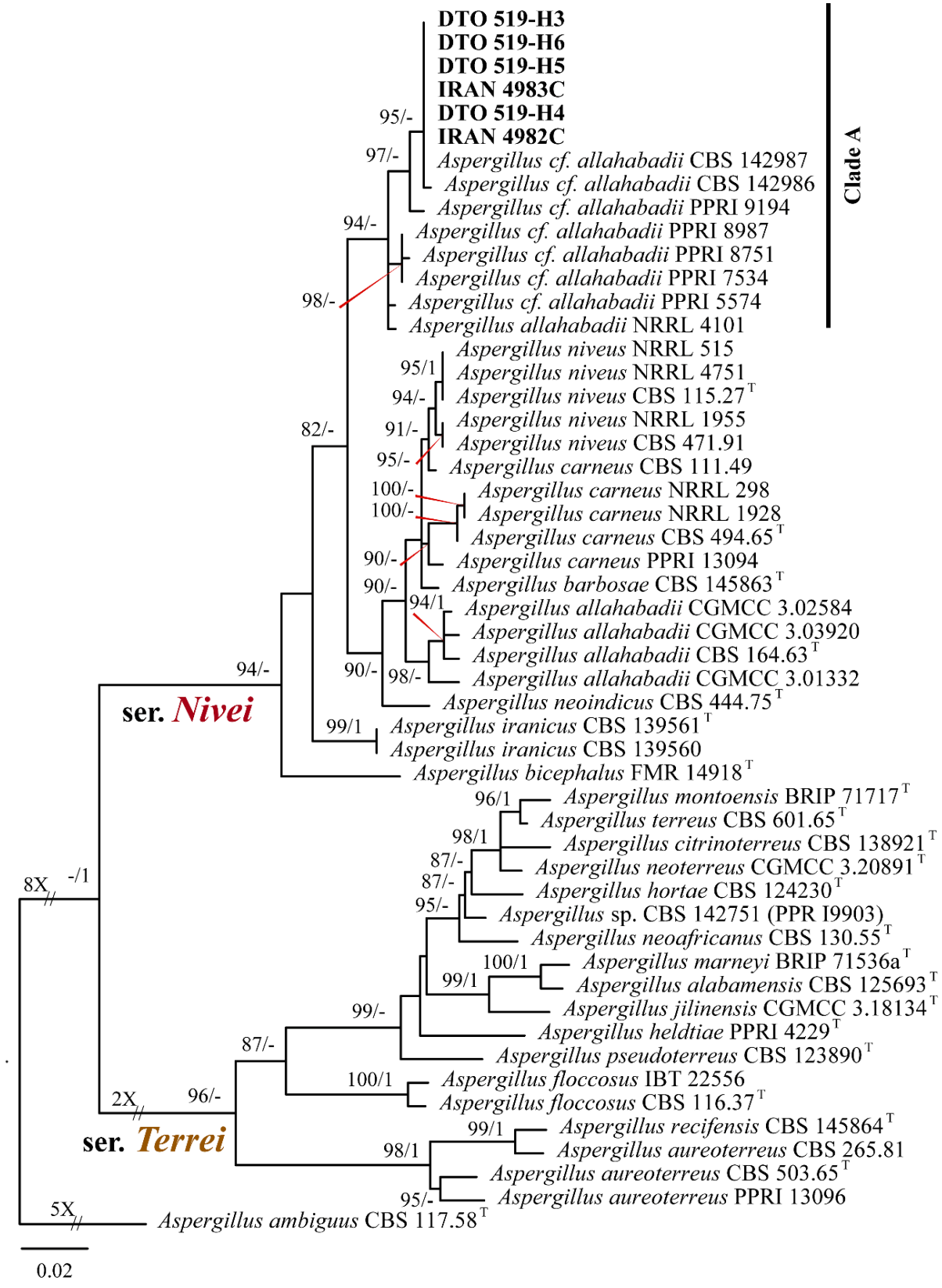

Fig. 7. Maximum likelihood phylogram of *Aspergillus* section *Terrei* (series *Nivei* and *Terrei*) based on *BenA* sequence data. ML/BI ultrafast bootstrap support (≥80%) and posterior probability values (≥0.95) are shown at the nodes. The phylogenetic tree was rooted with *Aspergillus ambiguus* CBS 117.58. Strains sequenced in this study are in boldface. ^{T} = Ex-type strain.

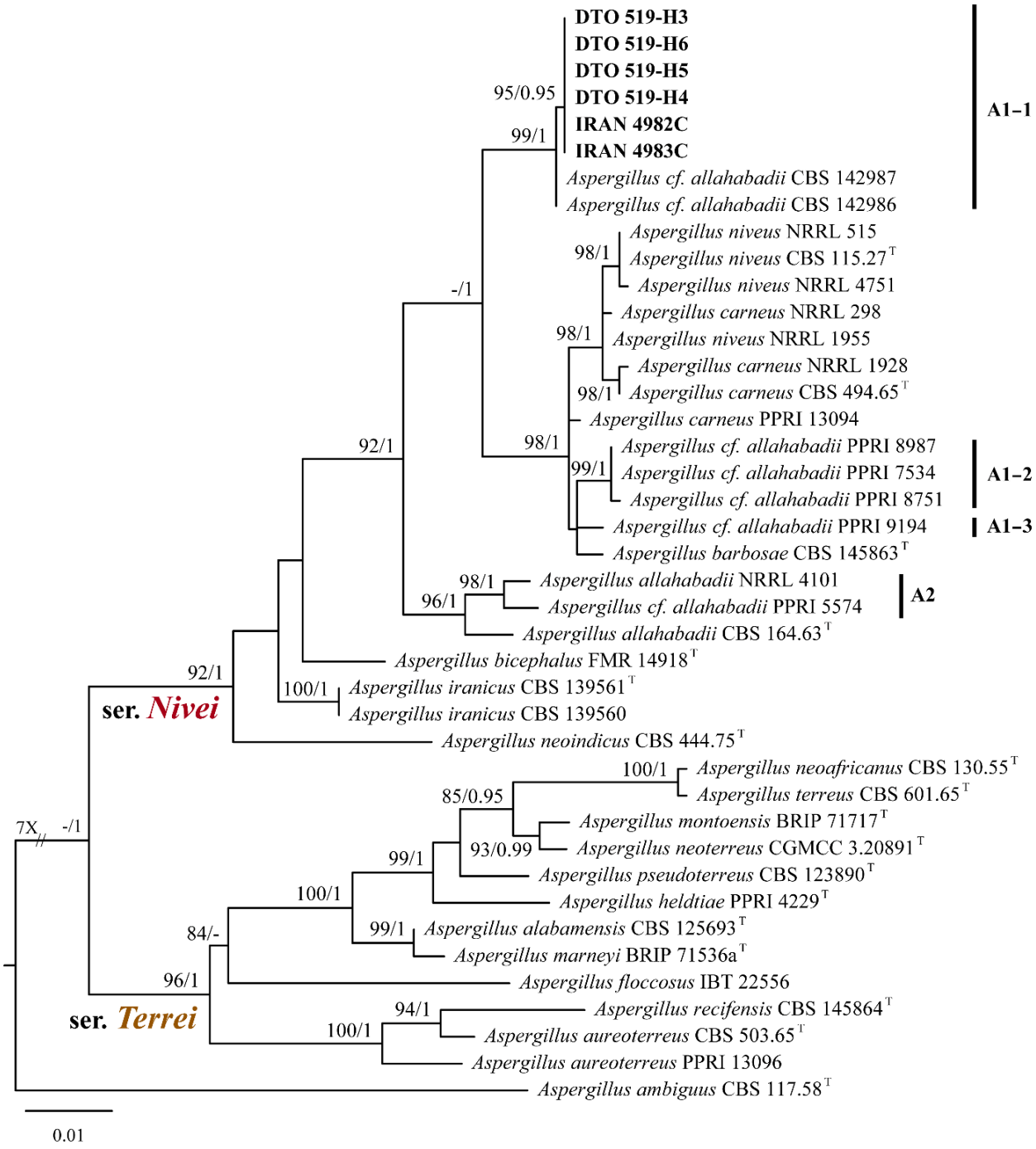

Fig. 8. Maximum likelihood phylogram of *Aspergillus* section *Terrei* (series *Nivei* and *Terrei*) based on *RPB2* sequence data. ML/BI ultrafast bootstrap support (≥80%) and posterior probability values (≥0.95) are shown at the nodes. The phylogenetic tree was rooted with *Aspergillus ambiguus* CBS 117.58. Strains sequenced in this study are in boldface. ^{T} = Ex-type strain.

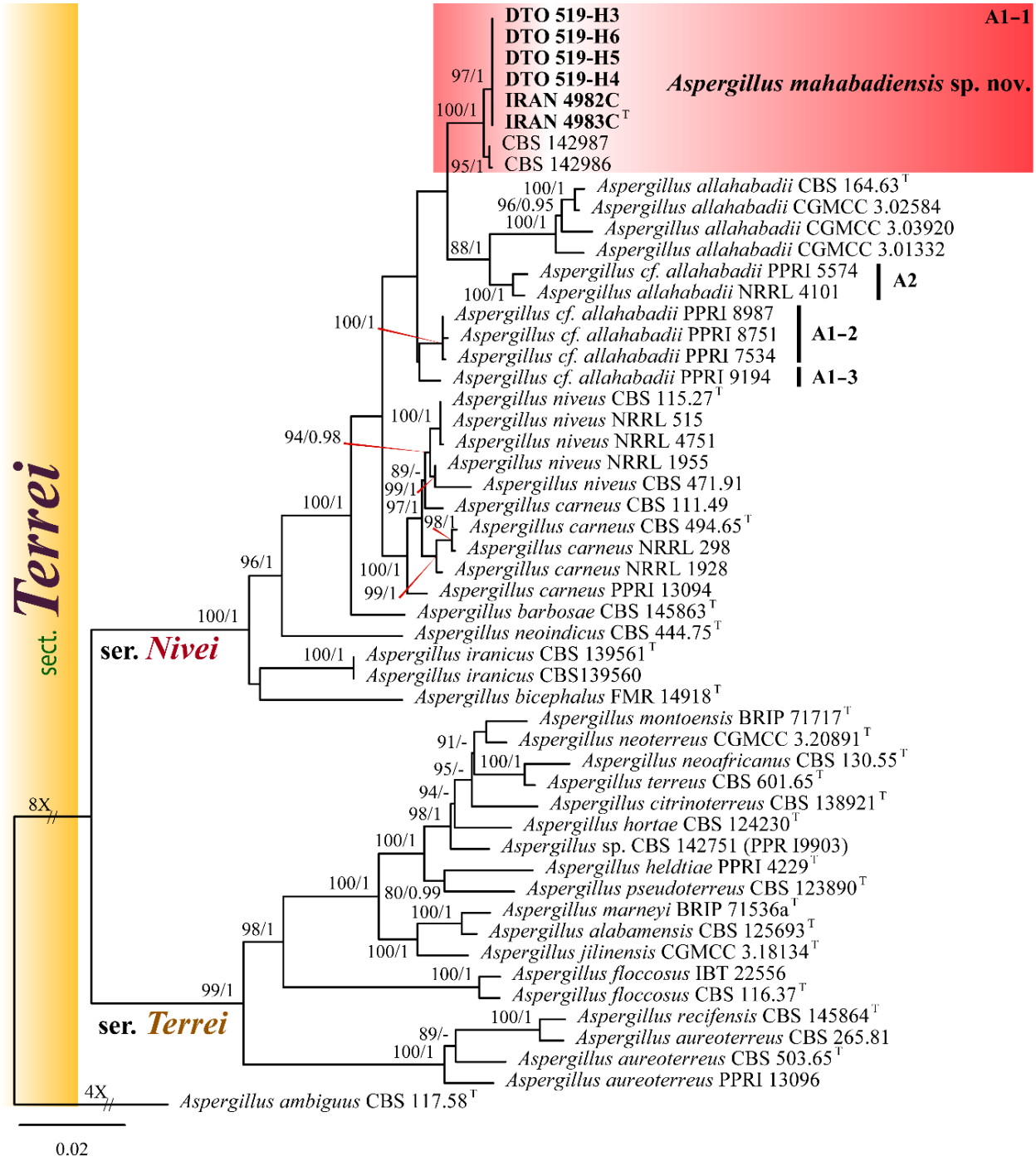

Fig. 9. Maximum likelihood phylogram of *Aspergillus* section *Terrei* (series *Nivei* and *Terrei*) based on concatenated dataset of *BenA*, *CaM* and *RPB2* sequences data. ML/BI ultrafast bootstrap support (≥80%) and posterior probability values (≥0.95) are shown at the nodes. The phylogenetic tree was rooted with *Aspergillus ambiguus* CBS 117.58. Strains sequenced in this study are in boldface. ^{T} = Ex-type strain.

Table 2. The degree of nucleotide variation in three different loci (*BenA*, *CaM*, *RPB2*) within and between recognized new species, *A. allahadadii* and closely related clades.

Species/clade	*A. mahabadiensis* (n = 8) *	A1-2 (n = 3)	A1-3 (n = 1)	A2 (n = 2)	*A. allahabadii* (n = 4)
A. mahabadiensis (A1-1)	1, 4, 1	8–9, 7–8, 23–24	4–5, 6, 22–23	6–8, 11, 28–29	22–23, 19, 24–26
A1-2		1, 1, 1	8–9, 3–4, 6–7	3–4, 8–9, 31–35	20–21, 14–19, 30–31
A1-3				7, 7, 37–38	23, 15–17, 34
A2				2, 2, 7	19–20, 11–16, 13–14
A. allahabadii					4–8, 4–12, ?

* n: number of isolates in each species/clade; ? *RPB2* sequence data is available only for the ex-type isolate.

Taxonomy

Based on phylogenetic analyses of DNA sequence data, we identified *A. flavus*/*A. oryzae* (sect. *Flavi*), *A. luchuensis*, *A. niger*, *A. tubingensis* (sect. *Nigri*) and *A. terreus* (sect. *Terrei*) (Fig. 10) as known *Aspergillus* species together with a new species from section *Terrei* (ser. *Nivei*) named as *A. mahabadiensis* sp. nov., which is described and illustrated here.

Fig. 10. Colonies on MEA and micromorphology of identified *Aspergillus* species. (A–D) *A. niger* IRAN 5126C, (E–H) *A. tubingensis* IRAN 5122C, (I–L) *A. luchuensis* IRAN 5120C, (M–P) *A. terreus* IRAN 5116C, and (Q–T) *A. flavus*/*A. oryzae* IRAN 5119C. Scale Bars: B, F, J, N, R = 20 µm; C, D, G, H, K, L, O, P, S, T =10 µm.

Aspergillus mahabadiensis **Abdollahz. & O. Ghaderi, sp. nov.; Fig. 11.**

MycoBank: MB856047

Holotype: Iran, Western Azarbaijan Province, Mahabad, 36°45'11.3"N, 45°43'19.0"E, from Terkhêna a traditional food, October 04, 2021, O. Ghaderi (IRAN 18461F, living culture IRAN 4983C = CBS 151400).

Etymology: The specific epithet refers to Mahabad, a city located in the west of Iran from which the ex-type strain IRAN 4983C was isolated.

In: *Aspergillus* subgen. *Circumdati* sect. *Terrei* ser. *Nivei*.

Diagnosis: The species is phylogenetically distinct from other members of ser. *Nivei*. Phenotypically, it can be differentiated from all closely related species, i.e., *A. allahabadii*, *A. barbosae*, *A. carneus* and *A. niveus*, by faster growth rate on CZ at 25 °C (32–33 mm/1W), larger conidia (2.8–3 µm) and the presence of diminutive forms.

DNA barcodes: ITS = PQ846961; *BenA* = PP544444; *CaM* = PP544440; *RPB2* = PP544442.

Colony diam, *7 d (in mm)*: CYA 25 °C 40–41 mm; CYA 30 °C 44–46 mm; CYA 37 °C 52–53 mm; MEA 25 °C 26–30 mm; YES 25 °C 55–56 mm; CZ 25 °C 32–33 mm; OA 25 °C 24–25 mm.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, radially sulcate; margins entire, regular; mycelia white; texture floccose; sporulation moderately dense; conidia *en masse* rosy buff (61) to rosy vinaceous (58); sclerotia absent; soluble pigments absent; exudates clear or hyaline droplets; reverse pale luteous (11) to ochreous (44). YES 25 °C, 7 d: Colonies moderately deep, randomly sulcate; margins entire, regular; mycelia white; texture floccose; sporulation dense; conidia *en masse* in shades of white to pale vinaceous (85); sclerotia absent; soluble pigment absent; exudate absent; reverse pale luteous (11) to sienna (8) or bay (6). MEA 25 °C, 7 d: Colonies moderately deep, radially sulcate; margins entire, regular; mycelia white; texture floccose; sporulation moderately sparse; conidia *en masse* in shades of white; sclerotia absent; soluble pigments absent; exudates absent; reverse white or hyaline. CZ 25 °C, 7 d: Colonies moderately deep, plane, slightly concentric at the center; margins entire, regular; mycelia white; texture floccose; sporulation dense; conidia *en masse* in shades of white to pale vinaceous (85); sclerotia absent; soluble pigments absent; exudates clear to buff (45); reverse pale luteous (11) to sienna (8) or bay (6). OA 25 °C, 7 d: Colonies moderately deep, plane, concentric; margins entire, regular; mycelia white; texture floccose; sporulation dense; conidia *en masse* in shades of white to pale vinaceous (85) or yellow (as small spots) at the center and white at the margin; sclerotia absent; soluble pigments absent; exudates absent; reverse white or hyaline.

Micromorphology: Conidial heads hyaline, radiate, biseriate. Conidiophores with smooth stipes, hyaline, aseptate, 164–301 × 2.3–7.6 µm, foot cell asymmetric. Vesicles hyaline, subglobose to subclavate, 6.2–18.9 µm wide, fertile over the upper half to two-thirds, rarely proliferant. Metulae hyaline, oblong to wedge-shaped, 4.4–8.2 × 1.5–3.1 µm. Phialides hyaline, flask-shaped, 3–5 on each metulae, 4.9–8.1 × 1.8–2.5 µm. Conidia hyaline, globose, smooth, (2.4–)2.8–3(–3.2) µm (2.9 ± 0.2, n = 50). Diminutive forms (small uniseriate or biseriate vesicles appearing penicillate, sessile metulae bearing phialides, phialides directly on hyphae) were observed. Accessory conidia hyaline, ovoid to ellipsoid, base truncate, 3.4–4.9 × 2.6–4.1 µm.

Additional specimens examined: IRAN, West Azarbaijan Province, Mahabad (36°45'44.6"N, 45°44'04.0"E), from dried apricot, 23 October 2021, O. Ghaderi, IRAN 4982C = CBS 151399; Miriseh village (36°29'51.8"N, 45°34'01.1"E), from dried apricot, 03 October 2021, O. Ghaderi, CJA OGhZ17 = DTO519-H3 = CBS 152878; Laj village (36°54'07.0"N, 45°45'55.0"E), from dried apricot, 23 October 2021, O. Ghaderi, CJA OGhZ61 = DTO519-H4 = CBS 152879; Yusef Kandi village (36°48'56.5"N, 45°44'09.9"E), from dried apricot, 25 October 2021, O. Ghaderi, CJA OGhZ75 = DTO519-H5 = CBS 152880; Jandaran village (36°24'59.3"N, 45°31'02.9"E), from Terkhêna a traditional food, 03 October 2021, O. Ghaderi, CJA OGhD15 = DTO519-H6 = CBS 152878.

Notes: Phylogenetic analyses revealed that *Aspergillus mahabadiensis* is a distinct species placed in ser. *Nivei* close to *A. allahabadii*, *A. barbosae*, *A. carneus* and *A. niveus* and some potential new species. Morphological examinations indicated that it is possible to separate *A. mahabadiensis* from closely related species. We have listed some important colony and morphological characters in *Aspergillus* taxonomy to differentiate *A. mahabadiensis* from closely related species (Table 3).

DISCUSSION

People living in the Zagros region located in the west of Iran, traditionally process and store some foods needed throughout the year. Raisins and dried fruits such as apple, apricot and white mulberry together with Terkhêna, a popular dried traditional food, are the most commonly consumed foods. Food seldom seen as an ecosystem, possibly because it is not considered as a “natural” system. The association between foodstuffs and fungi has existed for thousands of years, considered an important ecosystem (Pitt and Hocking 2009). Foods and dried fruits are subjected to fungal contaminations during process and storage (Wehner and Rabie 1970, Tournas et al. 2015, Snyder and Worobo 2018, Alghamdi et al. 2023). Fungi affect directly their quality and marketability, and on the other hand due to mycotoxin production indirectly threaten human health over time (Drusch and Ragab 2003, Trucksess and Scott 2008, Jard et al. 2011, Hartwig et al. 2020, Nikolchina

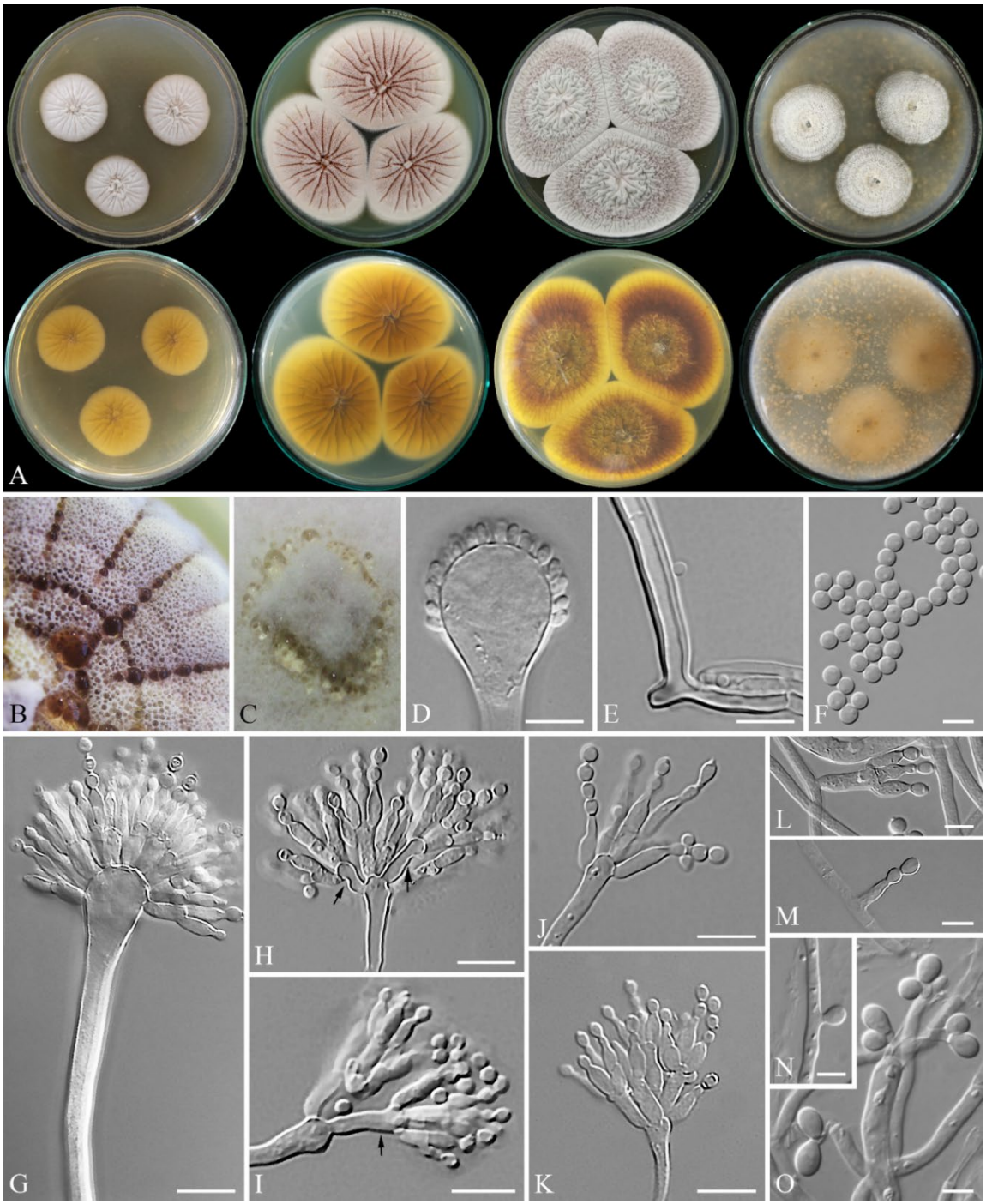

Fig. 11. *Aspergillus mahabadiensis* IRAN 4983C: (A) 7 d old colonies at 25 °C: top row left to right, obverse MEA, CYA, YES, and OA; bottom row left to right, reverse MEA, CYA, YES, and OA, (B) exudates on CYA, (C) exudates on CZ, (D) immature conidial head, (E) asymmetric foot cell, (F) conidia, (G) common biseriate conidial head and conidiophore, (H–I) proliferant vesicles (black arrows), (J, K) diminutive penicillate conidiophores, (L, M) reduced forms: (L) sessile metulae bearing phialides, (M) phialide directly on hyphae, (N, O) accessory conidia. Scale bars: D, E, G–K = 10 µm; F, L–O = 5 µm.

Table 3. Phenotypical features to distinguish *A. mahabadiensis* from closely related species in ser. *Nivei*.

Characters	*A. allahabadii*	*A. barbosae*	*A. carneus*	*A. mahabadiensis*	*A. niveus*
Conidia *en masse*	White to glaucous blue	Gray to greenish	Pale vinaceous fawn to vinaceous fawn	White to pale vinaceous/rosy buff to rosy vinaceous	White
Conidial head	Mostly biseriate/sometimes uniseriate	Biseriate	Biseriate	Biseriate	Biseriate
Foot cell	Asymmetric	Symmetric/asymmetric	Symmetric/asymmetric	Asymmetric	Symmetric/asymmetric
Stipe (µm)	250–360 (–1500) × 3.5–6	170–540 × 2.5–3	250–400 (–1000) × 3.5–6	164–302 × 2.3–7.6	Up to 600 (–1000)
Vesicle width (µm)	5.6–13	8–10	5.5–9 (–10)	(6.2–)9–13(–18.9)	8–15
Vesicle shape	Ovoid/hemispherical	Pyriform	Hemispherical	Subglobose to subclavate	Hemispherical
Phialide dimension (µm)	4.5–6.8 × 1.8	4.5–6 × 1.5–2.5	5–5.5 × 1.8–2	4.8–8 × 1.8–2.5	5–7 × 2–2.5
Metulae dimension (µm)	4.5–5.6 × 2.4	4–6 × 1.5–2	5.5–6.0 × 2.0–2.5	4.4–8 × 1.5–3.1	5–8 × 2.5–3
Conidial dimension (µm)	2–3	2–2.5 × 1.5–3	2.4–2.8	2.8–3	2–2.5
Conidial shape	Globose to subglobose	Subglobose	Globose to subglobose	Globose	Globose
Proliferating structures	Observed (frequently, phialides)	Observed (vesicles)	-	Observed (vesicles)	Observed
Diminutive forms	Not observed	Not observed	Not observed	Observed	Not observed
Accessory conidia	Not observed	Not observed	Observed	Observed	Observed
Colony diam./CYA/25 °C	35–37/2W	28–30/1W	–	40–41/1W	–
Colony diam./YES/25 °C	37–50/1W	32–34/1W	–	55–56/1W	–
Colony diam./MEA/25 °C	25–37/1W	22–25/1W	–	26–30/1W	–
Colony diam./OA/25 °C	32–37/1W	20–21/1W	–	24–25/1W	–
Colony diam./CZ/25 °C	40–50/2–3W	25–26/1W	40–50/2W	32–33/1W	25–30/2W
Colony diam./CYA 30 °C	–	42–47/1W	–	44–46/1W	–
Colony diam./CYA 37 °C	50–51/1W	42–44/1W	–	52–53/1W	–
References	Mehrotra and Agnihotri 1962, Zhang et al. 2018	Barrosa Correia et al. 2020	Raper and Fennell 1965	This study	Raper and Fennell 1965

and Rodrigues 2021, Naeem et al. 2022). A broad spectrum of fungi encompassing the most important genera *Aspergillus*, *Fusarium* and *Penicillium* are found in association with food spoilage and mycotoxin contaminations during processing and storage (Reddy et al. 2010, Houbraken and Samson 2017, Schaarschmidt and Fauhl-Hassek 2018). *Aspergillus* is a species-rich fungal genus encompassing the most important mycotoxigenic species, which are of significance in agriculture and food production and consequently for human, livestock and poultry health (Bhat et al. 2010, Jard et al. 2011, Kumar et al. 2016, Taniwaki et al. 2018, Ráduly et al. 2020). The precise identification of food spoilage fungi is a fundamental step to developing prevention and intervention strategies to minimize food waste and safeguard food quality (Filtenborg et al. 1996, Houbraken and Samson 2017).

As deduced from the literature, among the agricultural commodities few studies have considered mycotoxigenic fungi associated with dried fruits. Likewise, few studies, mostly on raisins, have considered fungi associated with dried fruits in Iran (Sedaghati et al. 2011, Ghezel Sefloo et al. 2012, Khodaei et al. 2014, Mirabolfathy et al. 2014, Sarabi et al. 2015, Amirmijani et al. 2018, Mehraban et al. 2019).

In an extensive study, we focused on the identification of mycotoxigenic fungi associated with raisins, some dried fruits including apple, apricot and white mulberry and Terkhêna a traditional food common and popular in Zagros region of Iran. A large fungal collection morphologically belonging to four genera *Alternaria*, *Aspergillus*, *Penicillium* (Ghaderi and Abdollahzadeh 2024a) and *Rhizopus* (Ghaderi and Abdollahzadeh 2024b) were isolated. A majority of the fungal isolates (76.4%) belonged to the genus *Aspergillus*. Using ISSR technique, our isolates were grouped into 11 clusters and thus we significantly reduced the number of isolates from 317 to 11 for phylogenetic and micromorphological studies. The isolates selected based on ISSR fingerprinting profile were placed in six *Aspergillus* species which is due to the fact that even though some researchers applied the DNA fingerprinting patterns for discrimination at interspecies level (Alves et al. 2007, Abdollahzadeh and Zolfaghari 2014), actually these techniques (e.g. ISSR, RAPD, rep-PCR, RFLP and SSR) are used for genotyping of fungal species in population genetic studies to calculate the genetic variation at infraspecies level and thus they can detect the degree of diversity more than the interspecies level. In this study, four selected isolates as representatives of four different DNA banding profiles were placed in *A. flavus/A. oryzae*, *A. luchuensis*, *A. mahabadiensis*, *A. terreus*) which indicates the ability of this technique to discriminate at the species level. In addition, the placement of seven isolates with different DNA fingerprinting profiles in two *Aspergillus* species (*A. tubingensis* and *A. niger*) reflects the genetic diversity within these two species and indicates that screening of our isolates using ISSR technique most likely didn't lead to underestimation at the species level and loss of any species. Sequencing of large collections is not affordable for all researchers around the world and phenotypic characters are not reliable thus it is recommended to examine the efficiency of DNA fingerprinting techniques in the discrimination of fungal species in more fungal groups especially in the important fungi such as *Aspergillus* as an interesting fungus for researchers in various scientific fields.

In a polyphasic approach based on morphology, DNA fingerprinting profile and sequence data, we identified *A. flavus*/*A. oryzae*, *A. luchuensis*, *A. niger*, *A. terreus*, *A. tubingensis*, and *A. mahabadiensis* sp. nov. We isolated *A. mahabadiensis* sp. nov., only from dried apricot and Terkhêna. It is distinguished from close species including *A. barbosae*, *A. carneus*, *A. niveus* and *A. allahabadii* in all single and multigene phylogenetic analyses of the main DNA barcodes *CaM*, *BenA* and *RPB2*. Moreover, *A. mahabadiensis* can be distinguished from the close species based on a combination of micromorphological features, colony characters and growth rates on different culture media. Among these, the presence of diminutive forms, conidial dimension, and growth rate on CZ at 25 °C are the most useful and effective characters in species recognition. Moreover, color of conidia *en masse* can differentiate *A. mahabadiensis* (pale vinaceous/rosy buff to rosy vinaceous) from *A. allahabadii* (White to glaucous blue), *A. barbosae* (Gray to greenish), *A. niveus* (White), but it is somehow similar with *A. carneus* (Pale vinaceous fawn to vinaceous fawn). Growth rates on CYA at 25 °C and 37 °C and on YES at 25 °C, which are available only for *A. allahabadii* and *A. barbosae*, are discriminative and faster in *A. mahabadiensis*. Accessory conidia produced by *A. mahabadiensis* have been reported for *A. carneus* and *A. niveus* but not observed in *A. allahabadii* and *A. barbosae*.

Based on a multigene phylogeny, Visagie and Houbraken (2020) detected a large degree of variation within *A. allahabadii* and recognized some clades with potential as new species in series *Nivei* but they didn't introduce them due to the small number of isolates and geographic origin. In our study, one of those clades consisted of isolates CBS 142986 and CBS 142987 recognized as conspecific with our isolates identified as *A. mahabadiensis* sp. nov., and the other clades are close to this new species. *Aspergillus mahabadiensis* sp. nov. is obviously differentiated from these potential new species based on *CaM* and specifically *RPB2* and multigene phylogenies.

In our survey, *A. tubingensis* (n = 155) and *A. niger* (n = 120) with 275 isolates out of 317 *Aspergillus* isolates were determined as the two most frequent species, both isolated from all examined foodstuffs. We didn’t isolate *A. flavus*/*A. oryzae* and *A. terreus* from raisins and *A. luchuensis* from dried apple. We found all

identified *Aspergillus* species on dried apricot and Terkhêna, and this is the first research on fungi associated with Terkhêna. So far as we know, some identified species here are reported for the first time on dried apple (*A. terreus*, *A. tubingensis*), apricot (*A. luchuensis*, *A. tubingensis*) and white mulberry (*A. luchuensis*, *A. terreus*, *A. tubingensis*) around the globe. Based on our knowledge, *A. luchuensis* is reported for the first time on foods in Iran and no information is available in the country on *Aspergillus* species on dried apple, apricot and white mulberry. Considering the consequences of mycotoxigenic fungi for human health, metabolite profiling and detection of mycotoxins especially for *A. mahabadiensis* sp. nov., in culture and substrates is significant to investigate in future studies.

ACKNOWLEDGMENT

Mahshid Saedi for amplification and sequencing of DTO isolates in Westerdijk Institute, Utrecht, The Netherlands.

AUTHOR CONTRIBUTION

Formal analysis, Investigation, Software, Microscopy, Photography, TreeBASE and GenBank submissions, Writing – original draft, OGh; Conceptualization, Funding acquisition, Resources, Methodology, Supervision, Validation, Writing – review & editing, JA.

DATA AVAILABILITY

All data are available in online repositories. The names of the repository/repositories and accession number(s) can be found in the article. Requests for more data and materials should be addressed to Jafar Abdollahzadeh.

DECLARATION

The authors declare that there is no conflict of interest.

FUNDING

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

ETHICS APPROVAL

Not applicable.

REFERENCES

Abdollahzadeh J, Goltapeh, EM, Javadi A, Shams-Bakhsh M, Zare R, … et al. 2009. *Barriopsis iraniana* and *Phaeobotryon cupressi*: two new species of the *Botryosphaeriaceae* from trees in Iran. Persoonia-Molecular Phylogeny and Evolution of Fungi. 23(1): 1-8. https://doi.org/10.3767/ 003158509X467552.

Abdollahzadeh J, Zolfaghari S. 2014. Efficiency of rep-PCR fingerprinting as a useful technique for molecular typing of plant pathogenic fungal species: *Botryosphaeriaceae* species as a case study. FEMS Microbiology Letters. 361(2): 144-157. https://doi.org/10.1111/1574-6968.12624.

Alasalvar C, Chang SK, Kris-Etherton PM, Sullivan VK, Petersen KS, … et al. 2023. Dried fruits: Bioactives, effects on gut microbiota, and possible health benefits—An update. Nutrients. 15(7): 1611. https://doi.org/10.3390/nu15071611.

Alghamdi RG, Zabermawi NM, Altihani FA, Bokhari FM, Makki RM, … et al. 2023. Diversity and density of fungi isolated from dried fruits. Journal of Biochemical Technology. 14(4): 45-55. https://doi.org/10.51847/WpwifxHnGg.

Alves A, Phillips AJ, Henriques I, Correia A. 2007. Rapid differentiation of species of *Botryosphaeriaceae* by PCR fingerprinting. Research in Microbiology. 158(2): 112-121. https://doi.org/10.1016/j.resmic.2006.10.003.

Amirmijani A, Sadeghian M, Karimi B. 2018. Fungal contamination associated with some dried fruits in Iran. Novel Research in Microbiology Journal. 2(6): 105-113. https://doi: 10.21608/NRMJ.2018.22703.

Andersen MR, Salazar, MP, Schaap PJ, van de Vondervoort PJ, Culley D, … et al. 2011. Comparative genomics of citric-acid-producing *Aspergillus niger* ATCC 1015 versus enzyme-producing CBS 513.88. Genome Research. 21(6): 885-897. https://doi:10.1101/gr.112169.110.

Barros Correia AC, Barbosa RN, Frisvad JC, Houbraken J, Souza-Motta, CM. 2020. The polyphasic re-identification of a Brazilian *Aspergillus* section *Terrei* collection led to the discovery of two new species. Mycological Progress 19: 885-903. https://doi.org/10.1007/s11557-020-01605-4.

Bashiri S, Abdollahzadeh J, 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(3): 1011-1025. https://doi.org/ 10.1007/ s42161-022-01124-z.

Bhat R, Rai RV, Karim AA. 2010. Mycotoxins in food and feed: present status and future concerns. Comprehensive Reviews in Food Science and Food Safety. (1): 57-81. https://doi.org/10.1111/j.1541-4337.2009.00094.x.

Bian C, Kusuya Y, Sklenář F, D'hooge E, Yaguchi T, … et al. 2022. Reducing the number of accepted species in *Aspergillus* series *Nigri*. Studies in Mycology. 102(1): 95-132. https://doi.org/10.3114/sim. 2022.102.03.

Cañete-Gibas CF, Patterson HP, Sanders CJ, Mele J, Fan H, … et al. 2023. Species distribution and antifungal susceptibilities of *Aspergillus* Section *Terrei* isolates in clinical samples from the United States and description of *Aspergillus pseudoalabamensis* sp. nov. Pathogens. 12(4): 579. https://doi.org/10.3390/pathogens12040579.

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G. 2004. MycoBank: an online initiative to launch

mycology into the 21st century. Studies in Mycology. 50: 19-22.

Drusch S, Ragab W. 2003. Mycotoxins in fruits, fruit juices, and dried fruits. Journal of Food Protection. 66(8): 1514-1527. https://doi.org/10. 4315/ 0362-028X-66.8.1514.

Filtenborg O, Frisvad JC, Thrane U. 1996. Moulds in food spoilage. International Journal of Food Microbiology. 33(1): 85-102. https://doi.org/10. 1016/ 0168-1605(96)01153-1.

Frisvad JC, Hubka V, Ezekiel CN, Hong SB, Nováková A, … et al. 2019. Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins, ochratoxins and other mycotoxins. Studies in Mycology. 93(1): 1-63. https://doi.org/ 10. 1016/j.simyco.2018.06.001.

Frisvad JC, Møller LL, Larsen TO, Kumar R, Arnau J. 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*. Applied Microbiology and Biotechnology. 102: 9481-9515. https://doi.org/10.1007/s00253-018-9354-1.

Gams W, Christensen M, Onions AH, Samson RA. 1985. Infrageneric taxa of *Aspergillus*. In: Advances in *Penicillium* and *Aspergillus* systematics. (Samson RA and Pitt JI, eds): 55-62. Springer, United States.

Ghaderi O, Abdollahzadeh J. 2024a. Phylogeny and taxonomy of *Penicillium* species associated with some foodstuffs in Mahabad, western Iran. Mycologia Iranica. 11(2): 61-72. DOI:10.22043/ MI.2024.368044.1302.

Ghaderi O, Abdollahzadeh J. 2024b. Identification of *Rhizopus arrhizus* associated with some dried fruits and the traditional food Terkhêna in Mahabad, western Iran. Mycologia Iranica. 11(1): 117-122. DOI: 10.22043/mi.2024.367535.1291.

Ghezel Sefloo N, Pirnia M, Karimi-Shahri M. 2011. Identification of some black *Aspergillus* from various raisins in Khorasan-e-Razavi Province. Acta Horticulturae. 963: 189-197. https://doi.org/10.17660/ActaHortic.2012.963.30.

Glass NL, Donaldson GC. 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. https://doi.org/ 10.1128/ aem.61.4.1323-1330.1995.

González-Curbelo MÁ, Kabak B. 2023. Occurrence of mycotoxins in dried fruits worldwide, with a focus on aflatoxins and ochratoxin A: A review. Toxins. 15(9): 576. https://doi.org/10.3390/ toxins15090576.

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

Hartwig N, Ferreira CFJ, Colazzo CC, Kupski L, Badiale-Furlong E. 2019. Dry fruit as source of fungal contaminants or functional compounds?. Food Science and Technology. 40(1): 76-83. https://doi.org/10.1590/fst.32118.

Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS. 2018. UFBoot2: improving the ultrafast bootstrap approximation. Molecular Biology and Evolution. 35(2): 518-522. https://doi:10.1093/ molbev/ msx281.

Hong SB, Yamada O, Samson RA. 2014. Taxonomic re-evaluation of black koji molds. Applied Microbiology and Biotechnology. 98: 555-561. https://doi.org/10.1007/s00253-013-5332-9.

Houbraken J, Samson RA. 2017. Current taxonomy and identification of foodborne fungi. Current Opinion in Food Science. 17: 84-88. https://doi.org /10.1016/j.cofs.2017.10.010.

Houbraken J, Kocsubé S, Visagie CM, Yilmaz N, Wang XC, … et al. 2020. Classification of *Aspergillus*, *Penicillium*, *Talaromyces* and related genera (*Eurotiales*): An overview of families, genera, subgenera, sections, series and species. Studies in Mycology. 95: 5-169. https://doi.org/10.1016/j.simyco.2020.05.002.

Jard G, Liboz T, Mathieu F, Guyonvarc'h A, Lebrihi A. 2011. Review of mycotoxin reduction in food and feed: From prevention in the field to detoxification by adsorption or transformation. Food Additives and Contaminants. 28(11): 1590-1609.https://doi.org/10.1080/19440049.2011.595 377.

Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics. 20(4): 1160-1166. https://doi.org/10.1093/bib/bbx108.

Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods. 14: 587-589. https://doi.org/10.1038/nmeth.4285.

Khodaei A, Arzanlou M, Babaiahari A, Darvishi F. 2014. Identification of black Aspergilli species on grape and raisin in Southern regions of East and West Azerbaijan provinces. Journal of Applied Research in Plant Protection. 3(1): 49-64.

Krijgsheld P, Bleichrodt RV, Van Veluw GJ, Wang F, Müller WH, … et al. 2013. Development in *Aspergillus*. Studies in Mycology 74(1): 1-29. https://doi.org/10.3114/sim0006.

Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG. 2017. Aflatoxins: A global concern for food safety, human health and their management. Frontiers in Microbiology. 7: 2170. https://doi.org/10.3389/fmicb.2016.02170.

Lass-Flörl C, Dietl AM, Kontoyiannis DP, Brock M. 2021. *Aspergillus terreus* species complex. Clinical Microbiology Reviews. 34(4): e00311-20. https://doi.org/10.1128/CMR.00311-20.

Liu YJ, Whelen S, Hall BD. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Molecular Biology

and Evolution. 16(12): 1799-808. https://doi.org/10.1093/oxfordjournals.molbev.a026092.

Maddison WP, Maddison DR. 2023. Mesquite: a modular system for evolutionary analysis (Version 3.70) [Computer software]. http://www.mesquiteproject.org.

Mehraban A, Nasr R, Eslami M, Amrollahi H. 2019. Molecular and morphological identification of fungi isolated from sour cherry, plum and apple fruit leather in Iran. Gene Reports. 17: 100500. https://doi.org/10.1016/j.genrep.2019.100500

Mehrotra BS, Agnihotri VP. 1962. Two new species of *Aspergillus* from India. Mycologia. 54(4): 400-406. https://doi.org/10.1080/00275514.1962.12025015

Meyer W, Koch A, Niemann C, Beyermann B, Epplen JT, … et al. 1991. Differentiation of species and strains among filamentous fungi by DNA fingerprinting. Current Genetics. 19: 239-242. https://doi.org/10.1007/BF00336493.

Micheli PA. 1729. Nova plantarvm genera ivxta Tovrnefortii methodvm disposita. 1st ed. Florence, Italy.

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

Mirabolfathy M, Karami-Osboo R, Hosseinian L. 2014. Ochratoxin A and ochratoxin-producing isolates of *Aspergillus* section *Nigri* in raisin in Iran. Iranian Journal of Plant Pathology. 50: 1-16. https://doi.org/10.3390/ijerph19063404.

Mohammadi N, Ostovar N. 2023. Chemical composition, fatty acid composition, and volatile compounds of a traditional Kurdish fermented cereal food: Tarkhineh. Food Chemistry Advances. 2: 100187. https://doi.org/10.1016/j.focha.2023.100187.

Naeem I, Ismail A, Rehman AU, Ismail Z, Saima S, … et al. 2022. Prevalence of aflatoxins in selected dry fruits, impact of storage conditions on contamination levels and associated health risks on Pakistani consumers. International Journal of Environmental Research and Public Health. 19(6): 3404. https://doi.org/10.3390/ijerph19063404.

Nargesi S, Jafarzadeh J, Najafzadeh MJ, Nouripour-Sisakht S, Haghani I, … et al. 2022. Molecular identification and antifungal susceptibility of clinically relevant and cryptic species of *Aspergillus* sections *Flavi* and *Nigri*. Journal of Medical Microbiology. 71(4): 001480. https://doi.org/ 10.1099/jmm.0.001480.

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution. 32(1): 268-274. https://doi.org/10.1093/molbev/msu300.

Nikolchina I, Rodrigues P. 2021. A preliminary study on mycobiota and ochratoxin a contamination in commercial palm dates (*Phoenix dactylifera*). Mycotoxin Research. 37(3): 215-220. https://doi.org/10.1007/s12550-021-00432-0.

Peterson SW, Vega FE, Posada F, Nagai C. 2005. *Penicillium coffeae*, a new endophytic species isolated from a coffee plant and its phylogenetic relationship to *P. fellutanum*, *P. thiersii* and *P. brocae* based on parsimony analysis of multilocus DNA sequences. Mycologia. 97: 659-666. http://doi.org/10.1080/15572536.2006.11832796.

Pitt JI, Hocking AD. 2009. Fungi and food spoilage. 3rd ed. Springer, United States.

Ráduly Z, Szabó L, Madar A, Pócsi I, Csernoch L. 2019. Toxicological and medical aspects of *Aspergillus*-derived Mycotoxins entering the feed and food chain. Frontiers in Microbiology. 10: 2908. https://doi.org/10.3389/fmicb.2019.02908.

Raeder U, Broda P. 1985. Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology. 1(1): 17-20. https://doi.org/10.1111/j.1472-765X.1985.tb01479.x.

Raper KB, Fennell DI. 1965. The genus *Aspergillus*. 1st ed. Williams and Wilkins, United States.

Rayner RW. 1970. A mycological colour chart. Commonwealth Mycological Institute, United Kingdom.

Reddy KRN, Salleh B, Saad B, Abbas HK, Abel CA, … et al. 2010. An overview of mycotoxin contamination in foods and its implications for human health. Toxin Reviews. 29(1): 3-26. https://doi.org/10.3109/15569541003598553.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, … et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology. 61(3): 539-542. https://doi.org/10.1093/sysbio/sys029.

Samson RA, Houbraken J, Thrane U, Frisvad JC, Andersen B. 2019. Food and indoor fungi. 2nd ed. Westerdijk Fungal Biodiversity Institute, Netherlands.

Samson RA, Peterson SW, Frisvad JC, Varga J. 2011. New species in *Aspergillus* section *Terrei*. Studies in Mycology. 69(1): 39-55. https://doi.org/ 10.3114/ sim.2011.69.0.

Samson RA, Visagie CM, Houbraken J, Hong SB, Hubka V, … et al. 2014. Phylogeny, identification and nomenclature of the genus *Aspergillus*. Studies in Mycology. 78(1): 141-173. https://doi.org/10.1016/j.simyco.2014.07.004.

Sarabi MS, Shahidi F, Bahrami AR, Mortazavi SA, Nassiry MR, … et al. 2015. Isolation and identification of fungi from raisins varieties in Khorasane Razavi based on morphological and molecular properties. Iranian Food Science and Technology Research Journal. 11(1): 13-21. https://doi.org/10.22067/ifstrj.v11i1.43948.

Schaarschmidt S, Fauhl-Hassek C. 2018. The fate of mycotoxins during the processing of wheat for

human consumption. Comprehensive Reviews in Food Science and Food Safety. 17(3): 556-593. https://doi.org/10.1111/1541-4337.12338.

Sedaghati E, Rahmani R, Khodaygan P, Nadi M. 2011. Morphological identification of *Aspergillus* species isolated from fresh grape and raisin in Rafsanjan markets. Acta Horticulturae. 963: 51-53. https://doi.org/10.17660/ActaHortic.2012.963.7

Snyder AB, Worobo RW. 2018. Fungal spoilage in food processing. Journal of Food Protection. 81(6): 1035-1040. https://doi.org/10.4315/0362-028X.JFP-18-031.

Sung G-H, Sung J-M, Hywel-Jones NL, Spatafora JW. 2007. A multigene phylogeny of *Clavicipitaceae* (Ascomycota, Fungi): identification of localized incongruence using a combinational bootstrap approach. Molecular Phylogenetics and Evolution. 44: 1204-1223. https://doi.org/10.1016/j.ympev.2007.03.011.

Taniwaki MH, Pitt JI, Magan N. 2018. *Aspergillus* species and mycotoxins: occurrence and importance in major food commodities. Current Opinion in Food Science. 23: 38-43. https://doi.org/10.1016/j.cofs.2018.05.008.

Thom C, Raper KB. 1945. A manual of the Aspergilli. 1st ed. The Williams and Wilkins Company, United States.

Tournas VH, Niazi NS, Kohn JS. 2015. Fungal presence in selected tree nuts and dried fruits. Microbiology Insights. 8: MBI-S24308. https://doi.org/10.4137/MBI.S24308.

Trucksess MW, Scott PM. 2008. Mycotoxins in botanicals and dried fruits: a review. Food Additives and Contaminants. 25(2): 181-192. https://doi.org/10.1080/02652030701567459.

Varga J, Frisvad JC, Samson R. 2011. Two new aflatoxin producing species, and an overview of *Aspergillus* section *Flavi*. Studies in Mycology. 69(1): 57-80. https://doi.org/10.3114/ sim. 2011. 69.05.

Visagie CM, Houbraken J. 2020. Updating the taxonomy of *Aspergillus* in South Africa. Studies in Mycology. 95(1): 253-292. https://doi.org/10.1016/j.simyco.2020.02.003.

Visagie CM, Yilmaz N, Kocsubé S, Frisvad JC, Hubka V, … et al. 2024. A review of recently introduced *Aspergillus*, *Penicillium*, *Talaromyces* and other *Eurotiales* species. Studies in Mycology. 107: 1-66. https://doi.org/10.3114/sim.2024.107.01.

Wang XC, Zhuang WY. 2022. New species of *Aspergillus* (*Aspergillaceae*) from tropical islands of China. Journal of Fungi. 8(3): 225. https://doi.org/10.3390/jof8030225.

Wehner FC, Rabie CJ. 1970. The micro-organisms in nuts and dried fruits. Phytophylactica. 2(3): 165-170.https://hdl.handle.net/10520/AJA03701263_445.

White TJ, Bruns T, Lee SJWT, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications. (M.A. Innis, D.H. Garfield, J.J. Sninsky and T.J. White, eds): 315-322. Academic Press, United States. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1.

Wu Q, Xie L, Xu H. 2018. Determination of toxigenic fungi and aflatoxins in nuts and dried fruits using imaging and spectroscopic techniques. Food Chemistry. 252: 228-242. https://doi.org/10.1016/j.foodchem.2018.01.076.

Yang L, Lübeck M, Lübeck PS. 2017. *Aspergillus* as a versatile cell factory for organic acid production. Fungal Biology Reviews. 31(1): 33-49. https://doi.org/10.1016/j.fbr.2016.11.001.

Yu J, Chang P, Ehrlich KC, Cary JW, Bhatnagar D, … et al. 2004. Clustered pathway genes in aflatoxin biosynthesis. Applied and Environmental Microbiology. 70: 1253-1262. https://doi.org/10.1128/AEM.70.3.1253.

Zhang YS, Ding GA, Sun BD, Zhou YG, Zhao GZ, … et al. 2018. Phylogeny of *Aspergillus* section *Terrei* with two new records from the China General Microbiological Culture Collection Centre. Phytotaxa. 382(3): 275-87. https://doi.org/10.11646/phytotaxa.382.3.4.

تنوع زیستی گونه‌های آسپرژیلوس در برخی میوه‌های خشک و غذای سنتی ترخینه با توصیف گونه جدید *Aspergillus mahabadiensis* sp. nov. از شهرستان مهاباد (غرب ایران)

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چکیده

میوه‌های خشک و غذای سنتی "ترخینه" از زمان‌های قدیم جزو غذاهای محبوب و مورد علاقه مردم در مناطق غرب ایران هستند. فساد میکروبی مواد غذایی و تولید سموم توسط قارچها در طول دوره نگهداری، تهدیدات جدی برای سلامت انسان محسوب می‌شوند. در این تحقیق، تعداد ۳۱۷ جدایه *Aspergillus* به‌دست آمده از کشمش، میوه‌های خشک سیب، زردآلو و توت سفید و غذای سنتی ترخینه مورد مطالعه قرار گرفتند. براساس داده‌های ریختی و مولکولی (توالی ژن *CaM*)، جدایه‌ها به گونه‌های *Aspergillus* در زیرجنس *Circumdati* از جمله *A. luchuensis*، *A. niger* و *A. tubingensis* در بخش *Nigri*، *A. flavus/A. oryzae* در بخش *Flavi* و *A. terreus* و یک کاندید گونه جدید در بخش *Terrei* تعلق داشتند. فیلوژنی چندژنی بر اساس داده‌های توالی ژن‌های *CaM*، *BenA* و *RPB2* و بررسی‌های ریختی، شناسایی یک گونه جدید در بخش *Terrei* را تأیید کرد که تحت عنوان *Aspergillus mahabadiensis* sp. nov. توصیف و معرفی می‌شود. گونه‌های *A. niger* و *A. tubingensis* بیشترین فراوانی را به خود اختصاص دادند. برخی از گونه‌های *Aspergillus* برای اولین بار از مواد غذایی مطالعه شده در این تحقیق گزارش می‌شوند.

کلمات کلیدی

خط شناسه DNA، ایمنی غذایی، قارچ‌های مولد سموم قارچی، تبارزایی، تاکسونومی.

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تاریخ دریافت: ۱۴۰۳/۱۰/۲۱، تاریخ بازنگری: ۱۴۰۳/۱۱/۲۲، تاریخ پذیرش: ۱۴۰۳/۱۱/۲۷، تاریخ انتشار برخط: ۱۴۰۳/۱۲/۴