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ORIGINAL ARTICLE

Two novel *Aspergillus* species from hypersaline soils of The National Park of Lake Urmia, Iran

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Abstract Two novel *Aspergillus* species, one belonging to the section *Terrei* and the other to section *Flavipedes*, were isolated from hypersaline soils of The National Park of Lake Urmia (Iran) and are here described as *Aspergillus iranicus* and *Aspergillus urmiensis*. A polyphasic taxonomic approach comprising extrolite profiles, phenotypic characters and molecular data (beta-tubulin, calmodulin and ribosomal polymerase II second largest subunit gene sequences) was applied to determine their novel taxonomic status. *Aspergillus iranicus* (CBS 139561^T) is phylogenetically related to *A. carneus*, *A. niveus*, *A. allahabadii* and *A. neoindicus*, and it can be differentiated from those species by a unique extrolite pattern (citrinin, gregatins, and a terrequinone) and its conidial colour. *Aspergillus urmiensis* (CBS 139558^T) shares a most recent common ancestor with *A. templicola*. The former species produces globose vesicles, and those of *A. templicola* are predominantly elongate. The *Aspergillus urmiensis* isolates produce several uncharacterized extrolites. Two other strains obtained during this study reside in a clade, together with the type strain of *A. movilensis* (CCF 4410^T), and are identified accordingly. Based on the phylogenetic data presented in this study, *A. frequens* is reduced to synonymy with *A. micronesiensis* and *A. mangaliensis* is considered to be a synonym of *A. templicola*.

Keywords *Aspergillus* section *Terrei* · *Aspergillus* section *Flavipedes* · Extrolite profile · Extreme environment · Gregatins

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Introduction

The genus *Aspergillus* was described almost 300 years ago in 1729 by Micheli (Ainsworth 1976; Pitt and Hocking 1997). Since the description of the genus, it became one of the best-known and most studied fungi. *Aspergillus* species are important microorganisms and can have positive and negative impacts on man. They are used in food fermentations (e.g. *A. oryzae*, *A. sojae*, *A. luchuensis*) and for the production of drugs and enzymes (e.g. *A. terreus*, *A. niger*). Their negative impacts include degradation of agricultural products and spoilage of food and feed, production of mycotoxins and infection of animals and humans (Klich 2002a, b; Krijgsheld et al. 2013; Gregory and Thomas 1997; Suhail et al. 2007).

Species of *Aspergillus* have a ubiquitous distribution and occur on decaying vegetation, soil and dust worldwide (Dyer and O'Gorman 2012). They are found in terrestrial habitats and are commonly isolated from soil (Carroll and Wicklow 1992). The cosmopolitan distribution of *Aspergillus* in diverse ranges of ecological niches is mainly attributed to their neutral reaction to abiotic growth conditions as they are not very selective in this respect (Krijgsheld et al. 2013). Studies on the occurrence of fungi in salterns have indicated that *Aspergillus* and *Penicillium* species are among the

predominant genera in these environments (Cantrell et al. 2011). The ability to tolerate high salt concentrations is a characteristic recognized for many species of *Aspergillus* (Tresner and Hayes 1971).

Changes in the International Code of Nomenclature for algae, fungi and plants have led to discussions whether to split *Aspergillus* into multiple genera or to keep it as one genus (Samson et al. 2014; Pitt and Taylor 2014). If the proposal of Samson is followed, then the genus comprises more than 340 accepted species. Based on a combination of multilocus sequence data and morphological traits, four subgenera and 23 sections are recognized in *Aspergillus* (Houbraken et al. 2014; Jurjević et al. 2015). During the survey of *Aspergillus* species in soil, several isolates belonging to the sections *Flavipedes* and *Terrei* were obtained. These sections are phylogenetically related and belong to subgenus *Circumdati* (Houbraken and Samson 2011; Jurjević et al. 2015). The taxonomy of these sections has been studied in detail; however, there is confusion in section *Flavipedes* because two studies describing new, similar species were published online around the same time (Hubka et al. 2015; Visagie et al. 2014).

Lake Urmia, located in the northwest of Iran between the provinces East and West Azerbaijan, is the largest lake in the Middle East and the second saltiest lake in the world after the Dead Sea. The National Park of Urmia Lake is a protected area and comprises two ecosystems (water and land). The salinity of the lake ranges between 120 g/L and more than 300 g/L and the lake is surrounded by marsh lands (Asem et al. 2014). Its land ecosystem consists of 102 islands, covering an area of 7816 ha (Asem et al. 2014). During the investigation of the biodiversity of *Aspergillus* species inhabiting hypersaline soils of this National Park, we discovered strains belonging to the sections *Terrei* and *Flavipedes*, which did not fit into any of the described species of *Aspergillus*. We used a polyphasic taxonomic approach to fully characterize these novel species. The macro- and micromorphology of the isolates were examined and extrolite patterns determined. For phylogenetic analysis, partial sequences of the β-tubulin (*BenA*), calmodulin (*CaM*) and ribosomal polymerase II second largest subunit (*RPB2*) genes were analyzed.

Materials and methods

Isolates

Soil samples were collected at 10–15 cm depth from two islands (Aspear and Kabodan) and the coastal areas of Lake Urmia, Iran, during 2011 and 2012. Isolations were carried out using the soil dilution plate and Warcup soil plate method (Warcup 1950) on malt extract agar (MEA), glucose peptone yeast extract agar (GPY) and potato dextrose agar (PDA) culture media containing NaCl concentrations varying from 0 to 30 %. Single spore isolations were made to obtain pure cultures. Dried cultures of the types are preserved at the fungarium of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands. The living strains (Table 1) were deposited in the Culture Collection of Tabriz University (CCTU), CBS-KNAW and the internal culture collection of the Applied and Industrial Mycology group (DTO) of CBS-KNAW.

Morphological analysis

For macro-morphological observations, isolates were cultivated on Czapek yeast autolysate agar (CYA), Czapek agar (CZA), yeast extract sucrose agar (YES), oatmeal agar (OA) (medium compositions according to Samson and Frisvad 2004) and malt extract agar (2 % MEA; Merck, Germany). The isolates were inoculated at three points on each plate of each medium and incubated at 25 °C and 37 °C in the dark. Colony growth characteristics were recorded after 7 days of incubation. Colour names and numeric codes used in the description refer to Klich (2002b). For microscopic observations, mounts were made in lactic acid from colonies grown on MEA; a drop of alcohol was added to remove air bubbles and excess conidia. For micro-morphological examination, light microscopy (Olympus BH2) was employed. Photographs were captured using a Olympus-BX41 light microscope with an Olympus digital camera system (DP 25).

Extrolite analysis

Cultures were grown for 7 days on CYA and YES agar prior extrolite extraction. Three agar plugs were extracted per agar medium as described before (Houbraken et al. 2012; Nielsen et al. 2011). The extracts were analysed using UHPLC-DAD and compounds were identified against an internal database of UV spectra and literature. Standards were available for the extrolites reported in Nielsen et al. (2011).

Phylogenetic analyses

Strains were grown for 3–10 days on MEA prior to DNA extraction. Genomic DNA was extracted using the Ultraclean™ Microbial DNA isolation kit (MoBio, Solana Beach, USA). After DNA extraction, parts of the *BenA*, *CaM*, internal transcribed spacers (ITS) and *RPB2* regions were amplified, sequenced and annotated (Houbraken et al. 2012; Houbraken and Samson 2011). The newly generated sequences were supplemented with sequence data from GenBank. After compilation of the sequence data sets, all datasets were aligned using the MAFFT multiple sequence alignment software v.7.221 (Katoh and Standley 2013). The best model for the maximum likelihood analysis was selected based on the Akaike Information Criterion (AIC), which was

Table 1 List of strains and the GenBank accession numbers used in the phylogenetic analyses in this study

Species name	Sectional classification	Collection numbers	Substrate and location	*BenA*	*CaM*	*RPB2*	ITS
A. alabamensis	*Terrei*	CBS 125693^{T} = UAB20^{T} = DTO 045-C5^{T}	Human, wound; Alabama, USA	KP987049	EU147583	KP987018	KP987071
A. allahabadii	*Terrei*	CBS 164.63^{T} = NRRL 4539^{T} = ATCC 15055^{T} = IMI 139273^{T}	Garden soil, pH 7.6; Allahabad, India	EF669531	EF669559	EF669643	EF669601
A. ambiguus	*Terrei*	CBS 117.58^{T} = NRRL 4737^{T} = ATCC 16827^{T} = IMI 139274^{T}	Savannah soil; Terrini, Somalia	EF669534	EF669564	EF669648	EF669606
A. ardalensis	*Flavipedes*	CCF 4031^{T} = NRRL 62824^{T} = CBS 134372^{T}	Soil; near Cueva de Doña Trinidad, Ardales, Andalucia, Spain	HG916683	HG916725	HG916704	FR733808
A. aureoterreus	*Terrei*	CBS 503.65^{T} = NRRL 1923^{T} = ATCC 16793^{T} = IMI 82431^{T}	Soil; Texas, USA	EF669524	EF669538	EF669622	EF669580
A. brevijanus	*Jani*	CBS 111.46^{T} = NRRL 1935^{T} = ATCC 16828^{T} = CBS 119.45^{T} = IMI 16066^{T}	Soil; Alameda, Mexico	EU014078	EF669540	EF669624	EF669582
A. candidus	*Candidi*	CBS 566.65^{NT} = NRRL 303^{NT} = ATCC 1002^{NT} = IMI 16264^{NT} = IMI 91889^{NT}	Unknown substrate and location	EU014089	EF669550	EF669634	EF669592
A. capensis	*Flavipedes*	CBS 138188^{T} = DTO 179-E6^{T}	House dust; Cape Town, South Africa	KJ775072	KJ775279	KP987020	KJ775550
A. carneus	*Terrei*	CBS 494.65^{T} = NRRL 527^{T} = ATCC 16798^{T} = IMI 135818^{T}	Air; Washington DC, USA	EF669529	EF669569	EF669653	EF669611
A. citrinoterreus	*Terrei*	GM 228^{T} = CBS 138921^{T}	Human sputum; Madrid, Spain	LN680657	LN680685	n/a	KP175260
A. flavipes	*Flavipedes*	NRRL 302^{T} = ATCC 24487^{T} = IMI 171885^{T}	Received by Charles Thom in 1922 from Da Fonseca as Bainier's culture of *Sterigmatocystus flavipes.*	EU014085	EF669549	EF669633	EF669591
A. flavipes	*Flavipedes*	NRRL 4852 = DTO 303-I4 = CCF 4836 = IMI 345934	Dead beetle, Uruguay. Received as Blochwitz's strain of *A. archiflavipes.*	KP987053	KP987070	KP987019	KP987083
A. floccosus	*Terrei*	CBS 116.37^{T} = IBT 10846^{T} = IBT 22556^{T} = DTO 067-B7^{T}	Waste cloth; Wuchang, China	FJ491714	KP987066	KP987021	KP987086
A. hortai	*Terrei*	CBS 124230^{T} = NRRL 274^{T} = ATCC 10070^{T} = IBT 26384^{T} = DTO 051-D6^{T}	Ear of man; Brazil	FJ491706	KP987054	KP987022	KP987087
A. iizukae	*Flavipedes*	CBS 541.69^{T} = NRRL 3750^{T} = IMI 141552^{T}	Soil from stratigraphic drilling core; Fujioka, Gymna Prefecture, Japan	EU014086	EF669555	EF669639	EF669597
A. iranicus	*Terrei*	CCTU 750 = DTO 203-D1 = CBS 139560 = IBT 32595	Soil, Jade Darya (seaside); Urmia, Iran	KP987044	KP987059	KP987033	KP987076
A. iranicus	*Terrei*	CCTU 756^{T} = DTO 203-D7^{T} = CBS 139561^{T} = IBT 32596^{T}	Soil, Aspear Island; Urmia, Iran	KP987045	KP987060	KP987034	KP987077
A. janus	*Jani*	CBS 118.45^{T} = NRRL 1787^{T} = IMI 16065^{T} = NCTC 6970^{T}	Soil, Panama	EU014076	EF669536	EF669620	EF669578
A. luppii	*Flavipedes*	NRRL 6326^{T} = CBS 653.74^{T} = CCF 4545^{T}	Natural truffle soil; near Aups, Province, France	EU014079	EF669575	EF669659	EF669617
A. microcysticus	*Terrei*	CBS 120.58^{T} = NRRL 4749^{T} = ATCC 16826^{T} = IMI 139275^{T}	Savannah soil; Somalia	EF669515	EF669565	EF669649	EF669607
A. micronesiensis	*Flavipedes*	CBS 138183^{T} = DTO 267-D5^{T}	House dust; Yela of Kosrae Island, Micronesia	KJ775085	KP987067	KP987023	KJ775548
A. micronesiensis	*Flavipedes*	CCF 4005	Hospital, indoor air; Králové, Czech Republic	HG916685	HG916727	HG916706	FR727135
A. micronesiensis	*Flavipedes*	DTO 247-H3	House dust; Mexico	KP987047	KP987062	KP987036	KP987079
A. micronesiensis	*Flavipedes*	DTO 266-D3	House dust; Mexico	KP987048	KP987063	KP987037	KP987080
A. micronesiensis	*Flavipedes*	NRRL 295 = ATCC 16814 = CBS 585.65 = IMI 135422 = CCF 4554 = FRR 0295	Dairy products; Minnesota, USA	EU014081	EF669546	EF669630	EF669588
A. micronesiensis	*Flavipedes*	NRRL 4263 = CCF 4556	Soil; Dehradun New Forest, India	EU014083	EF669558	EF669642	EF669600

Table 1 (continued)

Species name	Sectional classification	Collection numbers	Substrate and location	*BenA*	*CaM*	*RPB2*	ITS
A. micronesiensis	*Flavipedes*	NRRL 4578 = ATCC 16805 = CBS 586.65 = IMI 135423 = CCF 4555	Soil; Haiti. Type of *Aspergillus frequens*	EU014082	EF669560	EF669644	EF669602
A. movilensis	*Flavipedes*	CCF 4410^T = NRRL 62819^T = CBS 134395^T = DTO 316-C6^T	Soil near Movile cave; Dobrogea, Mangalia, Romania	HG916697	HG916740	HG916718	KP987089
A. movilensis	*Flavipedes*	CCTU 749 = DTO 203-C9 = CBS 139559 = IBT 32594	Soil, Kabodan Island; Urmia, Iran	KP987043	KP987058	KP987032	KP987075
A. movilensis	*Flavipedes*	CCTU 788 = DTO 203-H3 = CBS 139562	Soil, Kabodan Island; Urmia, Iran	KP987046	KP987061	KP987035	KP987078
A. movilensis	*Flavipedes*	NRRL 4610 = IMI 350352 = CCF 4551	Soil; Fons Parisien, Haiti	EU014080	EF669562	EF669646	EF669604
A. neoafricanus	*Terrei*	CBS 130.55^T = NRRL 2399^T = ATCC 16792^T = IMI 61457^T = MUCL 31316^T = NRRL A-3175^T	Soil; Tafo, Ghana	EF669516	EF669543	EF669627	EF669585
A. neoflavipes	*Flavipedes*	CBS 260.73^T = NNRL 5504^T = ATCC 24484^T = IMI 171883^T = CCF 4552^T	Cellulose material buried in forest soil; Pak Thong Chai, Thailand	EU014084	EF669572	EF669656	EF669614
A. neoindicus	*Terrei*	CBS 444.75^T = NRRL 6134^T = IMI 334935^T	Soil; Maharashtra, India	EF669532	EF669574	EF669658	EF669616
A. neoniveus	*Flavipedes*	CBS 261.73^T = NRRL 5299^T = ATCC 24482^T = IMI 171878^T	Forest soil; near Pak Thong Chai, Thailand	EU014098	EF669570	KP987024	EF669612
A. niveus	*Terrei*	CBS 115.27^T = NRRL 5505^T	Unknown source and location	EF669528	EF669573	EF669657	EF669615
A. polyporicola	*Flavipedes*	NRRL 32683^T = CCF 4553^T	Basidioma of *Earliella scabrosa* (Polyporales); Alien Wet Forest, Hilo, Hawaii, USA	EU014088	EF669553	EF669637	EF669595
A. pseudoterreus	*Terrei*	CBS 123890^T = NRRL 4017^T	Soil, Argentina	EF669523	EF669556	EF669640	EF669598
A. spelaeus	*Flavipedes*	CCF 4425^T = CBS 134371^T = NRRL 62826^T	Cave sediment; Nerja Cave, Andalusia, Spain	HG916698	HG916741	HG916719	HG915905
A. micronesiensis	*Flavipedes*	IMI 357699 = DTO 305-B6 = IBT 23707	Soil; West Bengal, India. Type of *A. sunderbanii.*	KP987052	KP987069	KP987026	KP987084
A. templicola	*Flavipedes*	CBS 138180 = DTO 267-H4	House dust; Thailand	KJ775087	KP987064	KP987038	KP987081
A. templicola	*Flavipedes*	CBS 138181^T = DTO 270-C6^T	Dust from church; Mexico	KJ775092	KJ775394	KP987017	KJ775545
A. templicola	*Flavipedes*	CCF 4698 = NRRL 62825	Soil near Movile cave; Mangalia, Romania. Type of *Aspergillus mangaliensis*	HG916695	HG916738	HG916716	HG915902
A. templicola	*Flavipedes*	CCF 869 = NRRL 62823	Industrial material; China	HG916696	HG916739	HG916717	HG915903
A. terreus	*Terrei*	CBS 601.65^T = NRRL 255^T = ATCC 10071^T = ATCC 1012^T = IMI 017294ii^T = NRRL 543^T	Soil; Connecticut, USA	EF669519	EF669544	EF669628	EF669586
A. urmiensis	*Flavipedes*	CCTU 734 = DTO 203-B3 = CBS 139557 = IBT 32597	Soil, Jade Darya (seaside); Urmia, Iran	KP987039	KP987055	KP987029	KP987072
A. urmiensis	*Flavipedes*	CCTU 742^T = DTO 203-C2^T = CBS 139558^T = IBT 32593^T	Soil, Jade Darya (seaside); Urmia, Iran	KP987041	KP987056	KP987030	KP987073
A. urmiensis	*Flavipedes*	CCTU 743 = DTO 203-C3 = CBS 139766 = IBT 32598	Soil, Jade Darya (seaside); Urmia, Iran	KP987042	KP987057	KP987031	KP987074

Acronyms of culture collections in alphabetic order: *ATCC* American Type Culture Collection, Manassas, Virginia; *CBS* Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; *CCF* Culture Collection of Fungi at the Department of Botany of Charles University in Prague; *CCTU* Culture Collection of Tabriz University; *DTO* Internal collection of Dept. Applied and Industrial Mycology housed at CBS; *FRR* Food Fungal Culture Collection, North Ride, Australia; *IMI* CABI's collection of fungi and bacteria, Egham, UK; *NRRL*, Agricultural Research Service Culture Collection, Peoria, Illinois

calculated in MEGA6. All positions containing gaps and missing data were eliminated. A ML analysis was performed on the individual and combined datasets. The individual datasets were analysed in MEGA v.6.0.6 (Tamura et al. 2013) and the combined multilocus alignment in RAxML (randomised accelerated maximum likelihood, v.7.0.4) software (Stamatakis et al. 2008). In the RAxML analysis, each dataset was treated as a separate partition. The statistical support was evaluated by 1000 bootstrap replicates. A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v.3.1.1 (Ronquist and Huelsenbeck 2003). Models of nucleotide substitution for each gene were included for each partition. The Bayesian analysis was performed with two sets of four chains (one cold and three heated) and the stoprule option, stopping the analysis at an average standard deviation of split frequencies of 0.01. *Aspergillus candidus* NRRL 303^T (sect. *Candidi*) was used as an outgroup. Newly obtained sequences were deposited in GenBank, see Table 1.

Results

DNA phylogeny and identification

A total of 48 isolates belonging to sections *Flavipedes*, *Terrei* and *Jani* including the outgroup (*A. candidus* NRRL 303^T) were included in the multigene analysis (gene boundaries of *BenA*: 1–554; *CaM*: 555–1160; ITS: 1161–1706; *RPB2*: 1707–2679). 2679 characters including gaps were processed, 1198 distinct alignment patterns were present and the proportion of gaps in the alignment was 7.28 %. For Bayesian analysis, a HKY+G+I model was selected for *BenA*, and a GTR+G+I model for the *CaM* and *RPB2* dataset. The posterior probability values correlated well with the bootstrap supports from the ML analysis (Fig. 1).

The results of the combined analysis is shown in Fig. 1 and demonstrates that the isolates can be divided into three well-supported groups, representing three sections in *Aspergillus*: *Flavipedes*, *Terrei* and *Jani.* Twelve species are currently accepted in section *Flavipedes*, including the novel species *A. urmiensis*. The type strains of *A. templicola* (CBS 138181^T) and *A. mangaliensis* (CCF 4698^T) form a well-supported clade, together with two other strains (CBS 138180 and CCF 869). Similarly, *A. micronesiensis* (CBS 138183^T) and *A. frequens* (NRRL 4578^T) fall into the same clade. Five isolates obtained in our study belong to section *Flavipedes*. Isolates CBS 139558, CBS 139766 and CBS 139557 form a well-supported clade in all analyses. This group of isolates is described here as *Aspergillus urmiensis*. Based on the combined analysis, this new species is a sister species of *A. templicola*. Two other isolates from Iranian soil (CBS 139559 and CBS 139562) reside in a clade together with the type strain of *A. movilensis* (CCF 4410^T) and are identified accordingly.

The isolates CBS 139560 and CBS 139561^T, described as a new species *A. iranicus* in this study, have identical *BenA*, *CaM*, ITS and *RPB2* sequences. These isolates cluster together in all analyses and never with any of the other accepted species in section *Terrei*. The exact phylogenetic position of these isolates is unresolved in the *CaM* and *RPB2* analysis, but the *BenA* and combined analyses show that these strains are basal to *A. carneus*, *A. niveus*, *A. allahabadii* and *A. neoindicus*.

Extrolites analysis

The extrolites profiles of the strains isolated during this study were determined. Both *A. iranicus* strains produced citrinin, gregatins, and a terrequinone and CBS 139560 produced an additional compound tentatively identified as asperamide. The *A. urmiensis* isolates had similar extrolite profiles; however, none of the detected compounds could be identified and remain uncharacterized. The two *A. movilensis* strains isolated in this study produced asperphenamate, aspochalasins, a butyrolactone and other unique extrolites. The phylogenetically closely related strain NRRL 4610 (= IBT 30185), which was identified as *A. movilensis* by Hubka et al. (2015), produced asperphenamate, a butyrolactone and a cyclic peptide resembling psychrophilin. This extrolite profile is similar to those of the two *A. movilensis* strains from this study.

Taxonomy

Aspergillus iranicus Arzanlou, Houbraken & Samadi, **sp. nov**. Mycobank MB817473. Figure 2.

Etymology: in reference to the ex-type strain, which was isolated from hypersaline soil in Iran.

Diagnosis: Phylogenetically basal to *A. carneus*, *A. niveus*, *A. allahabadii* and *A. neoindicus*. Good growth on CYA at 37 °C (34–38 mm), radiate conidial heads, accessory conidia produced. Unique extrolite profile: citrinin, gregatins, terrequinone X (maybe terrequinone A).

Typus: Iran, Urmia, Aspear Island, soil, 2012, isolated by U. Ghosta and R. Samad (holotype CBS H-22338, culture ex-type CCTU 756 = CBS 139561 = IBT 32596 = DTO 203-D7).

Additional material examined: Iran, Jade Darya (seaside), Urmia, soil, 2011, isolated by U. Ghosta and R. Samad, CCTU 750 = CBS 139560 = IBT 32595 = DTO 203-D1.

ITS barcode: KP987077 (alternative markers: *BenA* = KP987045; *CaM* = KP987060; *RPB2* = KP987034).

Colony diameter (mm): 7 days, 25 °C, CYA 28–32; CZ 24–28; MEA 30–34; YES 23–27; 7 days, 37 °C, CYA37 °C 34–38; CZ37 °C 37–39; MEA37 °C 36–40; YES37 °C 36–40.

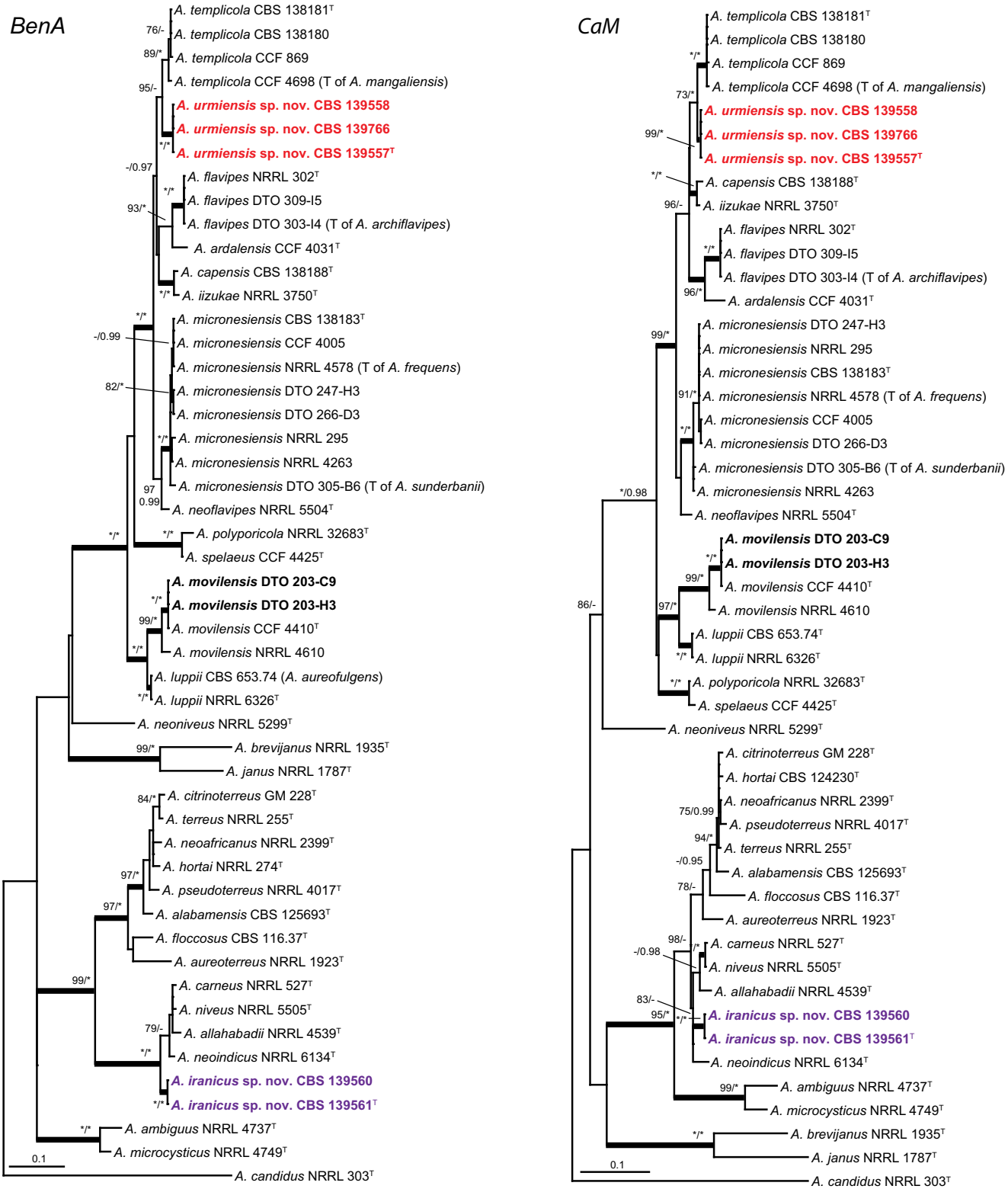

Fig. 1 Best-scoring Maximum Likelihood trees based on *BenA*, *CaM*, *RPB2* and a combined dataset of sequences showing the relationship of species belonging to *Aspergillus* sections *Flavipedes*, *Jani* and *Terrei*. The strains in *bold* were isolated in this study. The bootstrap percentages of the Maximum Likelihood (ML) analysis are presented at the nodes together with the posterior probability (pp) values (ML/pp). Bootstrap values below 70 % and less than 0.95 pp are omitted or indicated as a hyphen, whereas *asterisks* indicate full support (100 % bootstrap or 1.00 pp). The branches with more than 95 % bootstrap support and 1.00 in the Bayesian analysis are *thickened*

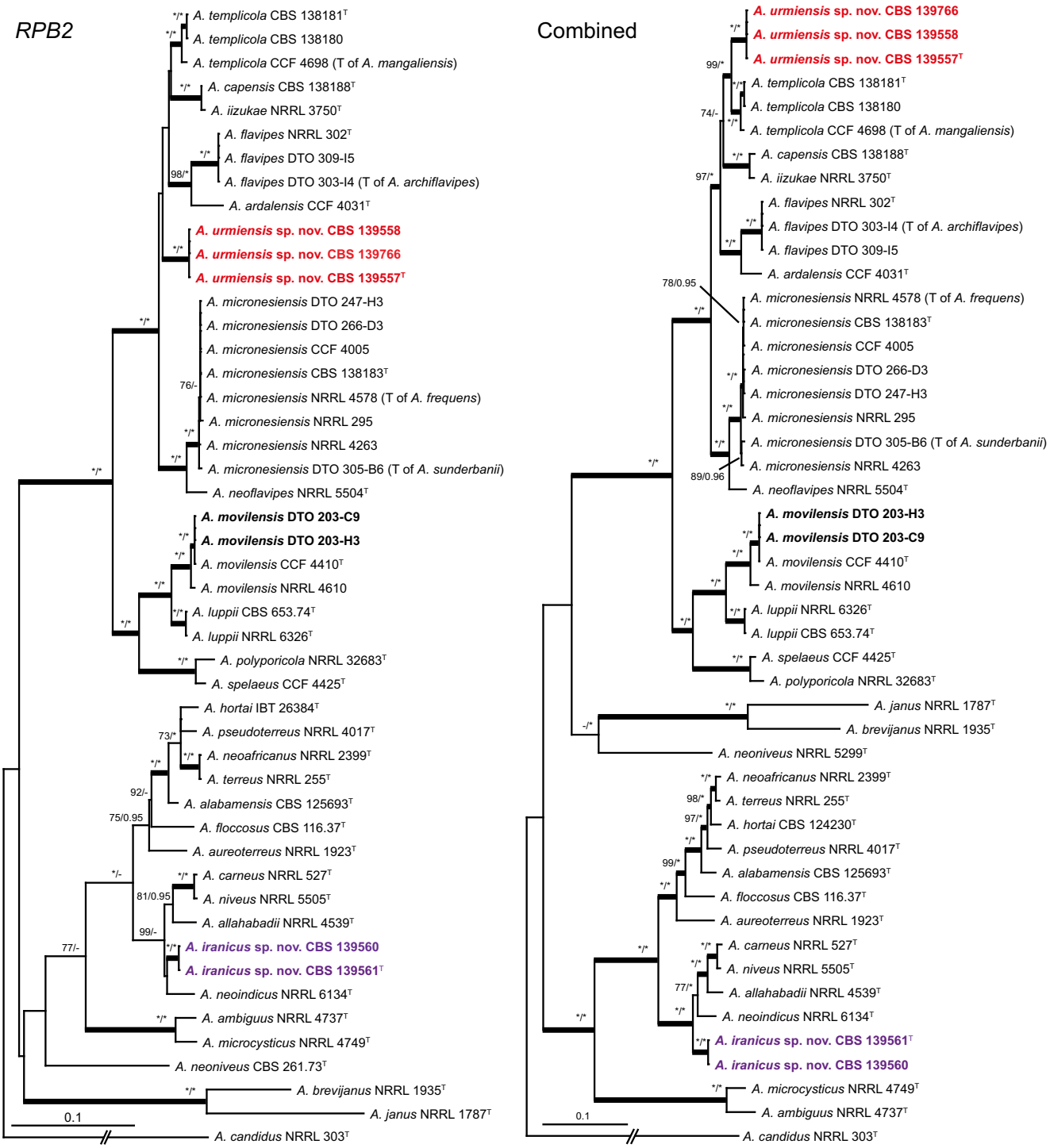

Fig. 1 (continued)

Colony characters: CYA 25 °C, 7 days: mycelium white; sclerotia absent; sporulation dense; conidial mass white, colour of the colony changed to peach (4) after 3 weeks; soluble pigment absent; colonies felt, centrally velutinous, sulcate; reverse honey (64) and sulfur yellow (15) in the deeper parts of the sulcations. YES 25 °C, 7 days: mycelium white; sclerotia absent; sporulation moderate; conidial mass white; soluble pigment absent; exudate sparse, amber (47); colony texture velvety, floccose in centre; sulcate; reverse pale luteous (11) to luteous (12) (Fig. 2). CZ 25 7 days: mycelium white at margin to greenish yellow in the centre (16); sclerotia absent; sporulation moderate in centre, conidial mass white; colonies felt, centrally floccose, sulcate; soluble pigment absent; greenish yellow (16) exudate produced after 14 days;

reverse citrine (13). MEA 25 °C, 7 days: mycelium white; sclerotia absent; sporulation dense; conidial mass white; soluble pigment absent; exudate absent; colonies velutinous to lightly floccose; sulcate; reverse pale luteous (11) (Fig. 2).

Micromorphology: Stipes (375–) 550–625 (−800) × (2.5–) 4–5 (−7) μm, smooth, aseptate to occasionally septate, walls pale yellow pigmented, thick-walled (1 μm). Foot cell in two forms: symmetric and asymmetric. Conidial heads radiate on MEA, YES, CZ and radiate to loosely columnar on CYA. Vesicles (14.5–) 20–23 (−32) × (7–) 11–13 (−16) μm, spathulate, wall thickness less than 1 μm, uncoloured. Conidiophores biseriate; the fertile part covering 1/3 to 1/4 of the upper part of the vesicle, occasionally small conidiophores with diminutive heads present. Metulae (5–) 6–7 (−8) × (2–) 3 (−4) μm, cylindrical, walls smooth, uncolorued. Phialides, 1–3 on each metula, (5–) 6–7 (−9) × 2–3 μm, cylindrical tapering to a distinct collulum. Conidia 2–2.5 × 1.8–2.5 μm in diameter, globose to subglobose, smooth-walled, hyaline (Fig. 2). Accessory conidia abundant, sessile or on the short, hyaline, micronematous conidiophores bearing conidia, globose, subglobose, elliptical, clavate, commonly truncate (4–) 5–6 (−7) μm (Fig. 2).

Notes: *Aspergillus iranicus* is phylogenetically related to *A. carneus*, *A. niveus*, *A. allahabadii* and *A. neoindicus*; however, it can be differentiated from these species by a combination of cultural and micro-morphological characteristics. *Aspergillus neoindicus* produces yellow-green mycelial tufts and the mycelium of *A. iranicus* is white. Furthermore, the conidial colour en masse of *A. iranicus* is in shades of yellow and this feature is not shared with *A. niveus* (white) and

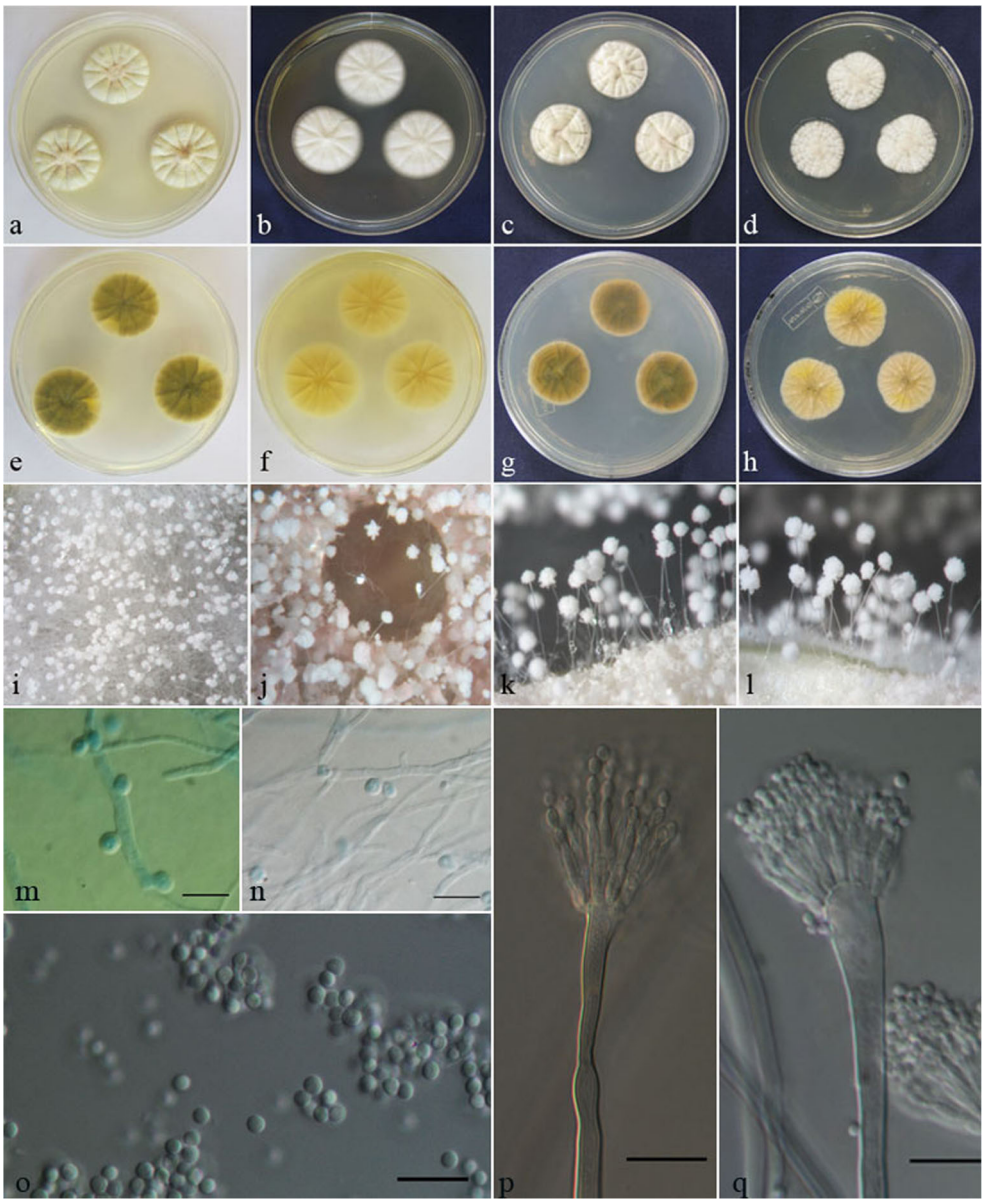

Fig. 2 *Aspergillus iranicus* CCTU 756. Colonies after 7 days at 25 °C: **a**, **e** CYA; **b**, **f** MEA; **c**, **g** CZ; **d**, **h** YES. **i** Details of colony on MEA; **j** exudate; (**k**, **l**) conidial heads; **m**, **n** accessory conidia; (**o**) conidia; **p**, **q** Conidiophores. *Scale bars* 10 μm

A. carneus (vinaceous fawn). *Aspergillus iranicus* produces accessory conidia and those were also reported in *A. terreus*, *A. carneus*, *A. niveus* and *A. alabamensis*.

Aspergillus urmiensis Arzanlou, Houbraken & Samadi, **sp. nov**. Mycobank MB817474. Figure 3.

Etymology: in reference to the ex-type strain, which was isolated from soil in Urmia, West Azerbaijan province, Iran.

Diagnosis: Conidial colour on CYA, MEA and YES ochreous, good growth on CYA incubated at 37 °C (16–20 mm), vesicles subglobose to globose measuring (17–) 20–23 (−30) × (16–) 19–22 (−30) μm.

Typus: Iran, Urmia, Jade Darya (seaside), soil, 2011, isolated by U. Ghosta and R. Samadi (holotype CBS H-22671, culture ex-type CCTU 742 = CBS 139558 = IBT 32593 = DTO 203-C2).

Additional material examined: Iran, soil, 2011, isolated by U. Ghosta and R. Samad: CCTU 734 = CBS 139557 = DTO 203-B3; CCTU 743 = CBS 139766 = IBT 32598 = DTO 203-C3.

ITS barcode: KP987073 (alternative markers: *BenA* = KP987041; *CaM* = KP987056; *RPB2* = KP987030).

Colony diameter (mm): 7 days, 25 °C, CYA 28–32; CZ 20–24; MEA 23–27; YES 21–24; 7 days, 37 °C, CYA37 °C 21–23; CZ37 °C 16–20; MEA37 °C 17–19; YES37 °C 18–20.

Colony characters: CYA 25 °C, 7 days: mycelium white; sporulation strong; conidial mass ochreous (44) sclerotia absent; soluble pigment luteous (7); exudate after 21 days produced; umber-coloured (9); colony texture floccose in centre to felt in margin; sulcate with low umbonate in centre; reverse sienna (8) and one umber (9) line present in middle of colony.

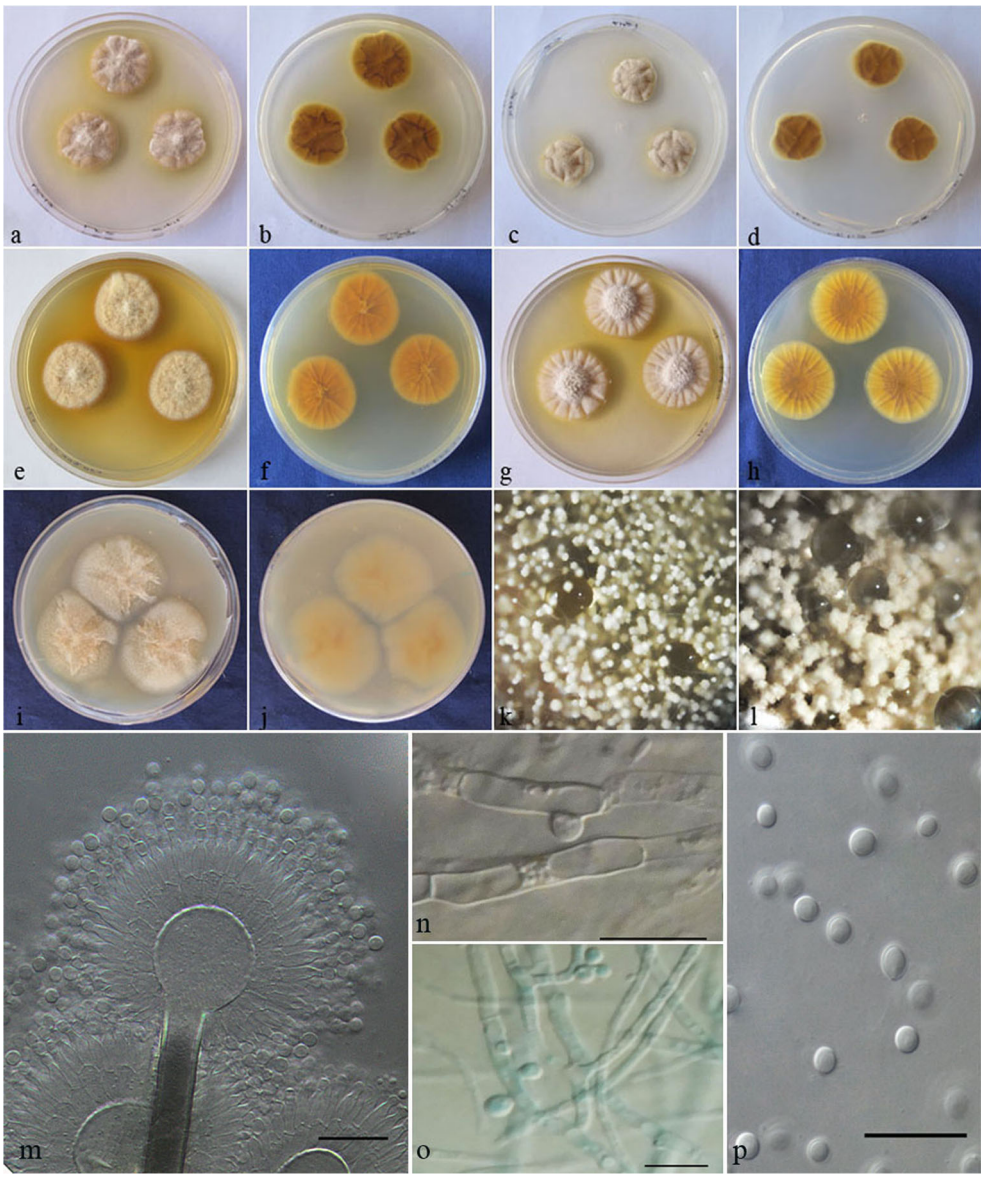

Fig. 3 *Aspergillus urmiensis* CCTU 742. Colonies after 7 days at 25 °C: **a**, **b** CYA; **c**, **d** CZ; **e**, **f** MEA; **g**, **h** YES. Colonies after 14 days at 25 °C: **i**, **j** OA. Conidial heads (**k**) CYA; **l** MEA. **m** Conidiophores; **n**, **o** accessory conidia; **p** conidia. *Scale bars*10 μm

YES 25 °C, 7 days: submerged mycelium at the margin of colony ochreous (44); white aerial mycelium appeared after 28 days; sporulation strong; conidial mass ochreous (44); sclerotia absent; soluble pigment luteous (12); exudate absent; colony texture lanose in centre to felt in margin; sulcate with umbonate in centre; reverse luteous (12) in centre pale luteous (11) in margin of colony. CZ 25 °C, 7d: mycelium white; sporulation strong, conidial mass ochreous (44); sclerotia absent; colony texture lanose; sulcate with lightly umbonate in centre; soluble pigment slightly produced, luteous; exudate absent; reverse orange (7) (Fig. 3). MEA 25 °C, 7 days: mycelium ochreous (44); sporulation strong; conidial mass ochreous (44); sclerotia absent; orange (7) uncoloured exudate after 14 days frequently produced; colony texture lanose; sulcate; reverse luteous (12) (Fig. 3).

Micromorphology: Stipes mostly hyaline close to the vesicle and light brown to brown closer to the base, (350–) 700–850 (−1330) × (5–) 8–10 (−12) μm, smooth-walled, wall thickening 1 μm, aseptate, rarely with one septum, foot cell symmetric or asymmetric, amber (47). Conidial heads radiate; vesicles (17–) 20–23 (−30) × (16–) 19–22 (−30) μm, subglobose to globose, wall thickness less than 0.8 μm, uncoloured. Conidiophores biseriate or uniseriate; Metulae (4–) 5–6 (−7.5) × (1.5–) 2–3 (−4) μm, wedge-shaped, walls smooth, uncoloured, covering 4/5 of the upper part of the vesicle. Phialides 2–5 on each metula, (2–) 5–7 (−8) × (1–) 1.5–2 (−3) μm, cylindrical, with distinct collulum. Conidia 2–3 μm, globose, smooth-walled, hyaline (Fig. 3). Accessory conidia present in relatively small numbers, sessile or on the short, hyaline, micronematous conidiophores bearing conidia, globose, subglobose, clavate, commonly truncate (4–) 5–6 (−7) μm (Fig. 3). No ascomata, ascospores or Hülle cells observed.

Notes: *Aspergillus urmiensis* is phylogenetically most closely related to *A. templicola*. The former species produces globose vesicles, and those of *A. templicola* are predominantly elongate. *Aspergillus urmiensis* can be differentiated from *A. luppii*, *A. movilensis*, *A. polyporicola* and *A. spelaeus* by larger colony diameters (16–20 vs. 0–17 mm) on CYA incubated at 37 °C. This new species can be differentiated from *A. ardalensis* based on the diameter of the vesicles (*A. ardalensis*, 18.5 μm; *A. urmiensis*, 22 μm). *A. neoflavipes* produces bright yellow colonies on CYA and MEA, and has a sexual state; both features are not observed in *A. urmiensis*. *A. micronesiensis* and *A. iizukae* generally produce Hülle cells and these structures were not detected in *A. urmiensis* (Hubka et al. 2015; Visagie et al. 2014).

Discussion

The National Park of Urmia has a unique ecosystem which consists of a range of regular to extreme environmental conditions (Asem et al. 2014). During a survey on the biodiversity of *Aspergillus* species inhabiting hypersaline soils of the Urmia Lake basin, we discovered strains belonging to the sections *Terrei* and *Flavipedes*, and some that could not be reliably identified to any described *Aspergillus* species. Species in section *Terrei* and *Flavipedes* are phenotypically related and the taxonomy of these sections based on morphology is troublesome. In the past, species currently classified in section *Terrei* were placed in the section *Flavipedes* due to overlaps in cultural and morphological characteristics (Raper and Fennell 1965; Samson 1979; Hubka et al. 2015). DNA sequencing and phylogenetic analysis has provided a reproducible and robust tool for species classification and identification in fungi including Aspergilli (Hong et al. 2005; Peterson 2008; Schoch et al. 2012; Samson et al. 2014). Sequence data from different genomic regions (eg. *BenA*, *CaM*, ITS, large ribosomal subunit (LSU) and *RPB2*) have been employed to delineate sections and species boundaries in *Aspergillus* (e.g. Peterson 2000, 2008; Varga et al. 2005; Hubka et al. 2015). The taxonomy of section *Flavipedes* was recently revised and 10 species were accepted (seven described as new) (*A. ardalensis*, *A. flavipedes*, *A. frequens*, *A. iizukae*, *A. luppii*, *A. mangaliensis*, *A. movilensis*, *A. neoflavipedes*, *A. polyporicola* and *A. spelaeus*) (Hubka et al. 2015). Concurrently, another three additions to this section were made (*A. templicola*, *A. capensis* and *A. micronesiensis*) (Visagie et al. 2014). The type strains of *A. frequens* (NRRL 4578^T) and *A. micronesiensis* (CBS 138183^T) shared identical *BenA, CaM* and *RPB2* sequences. Based on these data, *A. frequens* (Hubka et al. 2015) is reduced here to synonymy with *A. micronesiensis* (Visagie et al. 2014). Furthermore, the invalidly described species *Aspergillus sunderbanii* (Arts 40.3, 40.4, 40.5) is also a synonym of *A. micronesiensis*. The type strain of *A. mangaliensis* CCF 4698^T is phylogenetically close, but not identical, to the type of *A. templicola* (CBS 138181^T) (similarity *BenA* 98.8 %; *CaM* 98.7 %; *RPB2* 99.1 %). Based on gene concordance, they could represent two separate species; however, due to the high similarity in the investigated gene regions, we treat both as conspecific. Analysing additional strains in future will generate more insight on the status of these species.

In this study, different isolates from section *Flavipedes* were isolated from soils with different amounts of salinity up to 70 dS/m of the seaside and islands of the National Park of Lake Urmia. Two isolates (CBS 139559 and CBS 13562) reside in a clade together with the type strain of *A. movilensis* (CCF 4410^T) and are accordingly identified as such. The isolates CBS 139558, CBS 139766 and CBS 139557 formed a well-supported clade in both single-gene and combined phylogenetic analyses. This group of isolates is described here as a new species named *Aspergillus urmiensis*. *A. urmiensis* is phylogenetically closely related to *A. templicola* and can be differentiated from other species

belonging to section *Flavipedes* by a combination of cultural and morphological characters such as growth rate on CYA incubated at 37 °C, conidial colour, the shape and diameter of the vesicles and the presence or absence of Hülle cells and/or ascomata. This species also produces accessory conidia, a feature shared with all other members of section *Flavipedes* (Hubka et al. 2015).

Two strains (CBS 139560 and CBS 139561) isolated during this study belong to section *Terrei* and form a lineage distinct from the other accepted species. Currently, 15 species are accepted in this section: *A. alabamensis*, *A. allahabadii*, *A. ambiguous*, *A. aureoterreus*, *A. carneus*, *A. citrinoterreus*, *A. floccosus*, *A. hortai*, *A. microcysticus*, *A. neoafricanus*, *A. neoindicus*, *A. neoniveus, A. niveus, A. pseudoterreus* and *A. terreus* (Peterson 2008; Balajee et al. 2009; Samson et al. 2011; Guinea et al. 2015). We describe the new species *A. iranicus* in this section based on the concordance between the *BenA*, *CaM* and *RPB2* genes and the unique phylogenetic position of the isolates in section *Terrei* in the combined analyses. Both isolates have identical *BenA*, *CaM*, ITS and *RPB2* sequences. Analysis of the *CaM* and *RPB2* data sets could not resolve the exact phylogenetic position of these isolates, but the *BenA* and combined analysis show that the strains are basal to *A. carneus*, *A. niveus*, *A. allahabadii* and *A. neoindicus*. Besides the unique phylogenetic position, the *A. iranicus* isolates can also be differentiated from *A. carneus*, *A. niveus*, *A. allahabadii* and *A. neoindicus* by a combination of cultural and micro-morphological characteristics (see "Taxonomy").

Members of the section *Flavipedes* are known from different types of soil, especially in subtropical and tropical soils. Many species in this section are adapted to reduced water activity conditions and are able to grow in natural dry habitats. For example, *A. flavipes* isolates tolerate relatively high concentrations of osmotically active solutes in media, being able to grow on media with 40 % (w/v) sucrose and 25 % (w/v) NaCl (Tresner and Hayes 1971; Moustafa and AL-Musallam 1975) and were isolated from natural habitats with high NaCl concentration such as salterns (Moustafa 1975; Butinar et al. 2011; Cantrell et al. 2011), brackish water (Pawar and Thirumalachar 1966) or coastal sand of the Dead Sea (Grishkhan et al. 2003). The most well-known species from section *Terrei* is *A. terreus*, a cosmopolitan species known from desert and grassland soils, compost heaps, and also as contaminants on stored corn, barley and peanuts (Kozakiewicz 1989). This and other species such as *A. alabamensis*, *A. citrinoterreus* and *A. hortai* are also clinically significant (Balajee et al. 2009). In the present study, *A. iranicus* is described as new species in this section from hypersaline soils of the Urmia Lake basin. There is no report available on the tolerance of species in section *Terrei* to high concentrations of osmotically active solutes in media.

Extrolite profile analyses revealed that *A. iranicus* isolates produce citrinin, gregatins, and a terrequinone in common, and isolate CBS 139560 additionally produces an extrolite tentatively identified as asperamide. The hepatotoxic extrolite citrinin is also known from several other species in this section, namely, *A. alabamensis*, *A. allahabadii*, *A. carneus*, *A. floccosus*, *A. hortai*, *A. neoindicus*, *A. niveus* and *A. pseudoterreus* (Samson et al. 2011). A diverse array of metabolites, including acetylaranotin, asperphenamate, aspochalamins, aspulvinones, asteltoxin, asterric acid, asterriquinones, aszonalenins, atrovenetins, butyrolactones, citreoisocoumarins, citreoviridins, citrinins, decaturins, fulvic acid, geodins, gregatins, mevinolins, serantrypinone, terreic acid (only the precursor 3,6-dihydroxytoluquinone found), terreins, terrequinones, terretonins and territrems, are known from section *Terrei* species (Samson et al. 2011). Two additional metabolites namely gregatins and a compound tentatively identified as asperamide were found in *A. iranicus* and these compounds are new for the section *Terrei*. Members of the section *Flavipedes* are rich producers of bioactive secondary metabolites, some of which possess biotechnological and pharmacological significance (Hubka et al. 2015). *Aspergillus flavipes* is well studied with respect to extrolite production. A wide array of bioactive compounds is reported to be produced by this species and was listed by Hubka et al. (2015). *Aspergillus movilensis* CBS 139559 produces asperphenamate, aspochalasins, a butyrolactone and other unique extrolites. Strain NRRL 4610 (=IBT 30185) identified as *A. movilensis* by Hubka et al. (2015) produces asperphenamate, a butyrolactone and a cyclic peptide resembling psychrophilin, and this pattern of extrolites is very similar to that of CBS 139559. The *A. urmiensis* isolates (CBS 139558, CBS 139766 and CBS 139557) have similar extrolite profiles and produce several uncharacterised extrolites. These extrolites did not match with any of the known secondary metabolites in *Aspergillus* and might represent novel bioactive compounds. These compounds need structure elucidation and can be further evaluated on their pharmacological and biotechnological significance.

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