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

M. Arzanlou¹ · R. Samadi¹ · J. C. Frisvad² · J. Houbraken³ · Y. Ghosta⁴

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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 139561T) 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 139558T) 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 4410T), 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

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

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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). 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 BX41) was employed. Photographs were captured using a 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
| Species name         | Sectional classification | Collection numbers | Substrate and location         | BenA | CaM | RPB2 | ITS |
|----------------------|--------------------------|--------------------|--------------------------------|------|-----|------|-----|
| A. alabamensis       | Terrei                   | CBS 125693T = UAB20T = DTO 045-CS5T | Human; wound; Alabama, USA     | KP987049 | EU147583 | KP987018 | KP987071 |
| A. allahabadii       | Terrei                   | CBS 164.63T = NRRL 4539T = ATCC 15055T = IMI 139273T | Garden soil, pH 7.6; Allahabad, India | EF669531 | EF669559 | EF669643 | EF669601 |
| A. ambigua           | Terrei                   | CBS 117.58T = NRRL 4737T = ATCC 16827T = IMI 139274T | Savannah soil; Terrini, Somalia | EF669534 | EF669564 | EF669648 | EF669606 |
| A. ardalensis        | Flavipedes              | CFF 4031T = NRRL 62824T = CBS 134372T | Soil; near Cueva de Dona Trinidad, Ardales, Andalucia, Spain | HG916683 | HG916725 | HG916704 | FR733808 |
| A. aureoterreus      | Terrei                   | CBS 503.65T = NRRL 1923T = ATCC 16793T = IMI 82431T | Soil; Texas, USA              | EF669524 | EF669538 | EF669624 | EF669580 |
| A. brevijanus        | Jani                     | CBS 111.46T = NRRL 1935T = ATCC 16828T = IMI 16066T = NRRL 302T = ATCC 24487T | Soil; Alameda, Mexico        | EU014078 | EF669540 | EF669624 | EF669582 |
| A. candidus          | Candidi                  | CBS 566.65NT = NRRL 303NT = ATCC 1002NT = IMI 16264NT = IMI 91889NT | Unknown substrate and location | EU014089 | EF669550 | EF669634 | EF669692 |
| A. capensis          | Flavipedes              | CBS 138188T = DTO 179-E6T | House dust; Cape Town, South Africa | KJ775072 | KJ775279 | KP987020 | KJ775550 |
| A. carneas           | Terrei                   | CBS 494.65T = NRRL 527T = ATCC 16798T = IMI 135818T | Air; Washington DC, USA       | EF669529 | EF669569 | EF669653 | EF669611 |
| A. citrinoterreus    | Terrei                   | GM 228 = CBS 138921T | Human sputum; Madrid, Spain    | LN680657 | LN680685 | n/a | KP175260 |
| A. flavipes          | Flavipes                 | NRRL 302T = ATCC 24487T = IMI 171885T | Received by Charles Thom in 1922 from Da Fonseca as Bainier’s culture of *Sterigmatocystus flavipes*. | EU014085 | EF669549 | EF669633 | EF669591 |
| A. floccosus         | Terrei                   | CBS 116.37T = IBT 10846T = IBT 22556T = DTO 067-BrT | Dead beetle, Uruguay. Received as Blochwitz’s strain of *A. archiflavipes*. | FP987053 | FP987070 | FP987019 | FP987083 |
| A. hortai            | Terrei                   | CBS 1242T = IBT 26384T = DTO 051-D6T | Ear of man; Brazil            | FJ491714 | FP987066 | FP987021 | FP987086 |
| A. iizukae           | Flavipedes              | CBS 541.69T = NRRL 3750T = IMI 141552T | 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, Aspear Island; Urmia, Iran | KP987044 | KP987059 | KP987033 | KP987076 |
| A. iranicus          | Terrei                   | CCTU 756T = DTO 203-D7T = CBS 139561T = IBT 32596T | Soil, Panama                  | EU014076 | EF669536 | EF669620 | EF669578 |
| A. janus             | Jani                     | CBS 118.45T = NRRL 1787T = IMI 16065T = NCTC 6970T | Natural truffle soil; near Aups, Province, France | EU014079 | EF669575 | EF669659 | EF669617 |
| A. luppii            | Flavipes                 | NRRL 6326T = CBS 653.74T = CFF 4545T | Savannah soil; Somalia         | EF669515 | EF669655 | EF669649 | EF669607 |
| A. microcysticus     | Terrei                   | CBS 120.58T = NRRL 4749T = ATCC 16826T = IMI 139275T | House dust; Yela of Kosrae Island, Micronesia | KJ775085 | KP987067 | KP987023 | KJ775548 |
| A. microcysticus     | Flavipes                 | CBS 138183T = DTO 267-D5T | House dust; Yela of Kosrae Island, Micronesia | KP987047 | KP987062 | KP987036 | KP987079 |
| A. microcysticus     | Flavipes                 | DTO 247-H3 | House dust; Mexico             | KP987048 | KP987063 | KP987037 | KP987080 |
| A. microcysticus     | Flavipes                 | DTO 266-D3 | House dust; Mexico             | KP987047 | KP987062 | KP987036 | KP987080 |
| A. microcysticus     | Flavipes                 | NRRL 295 = ATCC 16814 = CBS 585.65 = IMI 135422 = CFF 4554 = FRR 0295 | Dairy products; Minnesota, USA | EU014081 | EF669546 | EF669630 | EF669588 |
| A. microcysticus     | Flavipes                 | NRRL 4263 = CFF 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 134923 = CCF 4555 = DTO 31-6-C6¹         | Soil, Haiti, Type of *Aspergillus frequens*             | EU014082 | EF669560 | EF66944  | EF669602  |
| A. mostiensis               | Flavipedes               | CCF 4410¹ = NRRL 62819¹ = CBS 134395¹ = DTO 31-6-C6¹ = IMI 137213 = IBT 32594      | Soil near Movie cave; Dobrogea, Mangalia, Romania       | HG916697 | HG916740 | HG916718 | KP987089  |
| A. mostiensis               | Flavipedes               | CCTU 749 = DTO 203-C9 = CBS 139559 = IMI 137213 = IBT 32594                        | Soil, Kabodan Island; Urmia, Iran                       | KP987043 | KP987058 | KP987032 | KP987075  |
| A. micronesiensis           | Flavipedes               | CCTU 788 = DTO 203-H3 = CBS 139562                                               | Soil, Kabodan Island; Urmia, Iran                       | KP987046 | KP987061 | KP987035 | KP987078  |
| A. mostiensis               | Flavipedes               | NRRL 4610 = IMI 350352 = CCF 4551                                                | Soil; Fons Parisien, Haiti                             | EU014080 | EF669562 | EF669646 | EF669604  |
| A. neoafricanus             | Terrei                   | CBS 130.55¹ = RNR 2399¹ = ATCC 16792¹ = IMI 61457¹ = MUCL 31316¹ = NRRL A-3175¹ | Cellulose material buried in forest soil, Pak Thong Chai, Thailand       | EU014084 | EF669572 | EF669656 | EF669614  |
| A. neoflavipes              | Flavipedes               | CBS 260.73¹ = NNRL 5504¹ = ATCC 24484¹ = IMI 171883¹ = CCF 4552¹                    | Cellulose material buried in forest soil, Pak Thong Chai, Thailand       | EF669532 | EF669574 | EF669658 | EF669616  |
| A. neovires                 | Terrei                   | CBS 261.73¹ = NNRL 5299¹ = ATCC 24482¹ = IMI 171878¹                              | Cellulose material buried in forest soil, Pak Thong Chai, Thailand       | EF669508 | EF669570 | KP987024 | KP987060  |
| A. niveus                   | Terrei                   | CBS 115.27¹ = NRRL 5505¹                                                         | Unknown source and location                              | EF669528 | EF669573 | EF669657 | EF669615  |
| A. polyporicola             | Flavipedes               | NRRL 32683¹ = CCF 4553¹                                                          | Basididia of *Earlicula scabrosa* (Polyporales); Alien Wet Forest, Hilo, Hawaii, USA | EU014088 | EF669553 | EF669637 | EF669595  |
| A. pseudoterreus            | Terrei                   | CBS 123890¹ = NRRL 401¹                                                          | Soil, Argentina                                         | EF669523 | EF669556 | EF669640 | EF669598  |
| A. spekeius                 | Flavipedes               | CCF 4425¹ = CBS 13437¹ = NRRL 62826¹                                             | Cave sediment; Nerja Cave, Andalusia, Spain             | HG916698 | HG916741 | HG916719 | HG919005  |
| A. micronesiensis           | Flavipedes               | IMI 357699 = DTO 305-B6 = IBT 23707                                              | Soil; West Bengal, India. Type of *A. sunderbani*        | KP987052 | KP987069 | KP987026 | KP987084  |
| A. templicina                | Flavipedes               | CBS 138180 = DTO 267-H4                                                           | House dust, Thailand                                    | KJ775087 | KP987064 | KP987038 | KP987081  |
| A. templicola               | Flavipedes               | CBS 138181¹ = DTO 270-C6¹                                                        | Dust from church; Mexico                                | KJ775092 | KP987017 | KP775545 | KP987060  |
| A. templicola               | Flavipedes               | CCF 4698 = NRRL 62825                                                            | Soil near Movie cave; Mangalia, Romania. Type of *Aspergillus mangaliensis* | HG916695 | HG916738 | HG916716 | HG919002  |
| A. templicola               | Flavipedes               | CCF 869 = NRRL 62823                                                             | Industrial material; China                              | HG916696 | HG916739 | HG916717 | HG915903  |
| A. terreus                  | Terrei                   | CCF 601.65¹ = NRRL 255¹ = ATCC 1007¹ = IMI 017294¹ = NRRL 543¹ = ATCC 1012¹ = IMI 137213 = IBT 32597 | Soil; Connecticut, USA                                  | EF669519 | EF669544 | EF669628 | EF669586  |
| A. umiensis                 | Flavipedes               | CCTU 734 = DTO 203-B = CBS 139557 = IBT 32597                                    | Soil, Jade Darya (seaside); Urmia, Iran                 | KP987039 | KP987055 | KP987029 | KP987072  |
| A. umiensis                 | Flavipedes               | CCTU 742¹ = DTO 203-C = CBS 139558¹ = IBT 32593¹                                 | Soil, Jade Darya (seaside); Urmia, Iran                 | KP987042 | KP987056 | KP987030 | KP987073  |
| A. umiensis                 | Flavipedes               | CCTU 743 = DTO 203-C = 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 stop rule option, stopping the analysis at an average standard deviation of split frequencies of 0.01. Aspergillus candidus NNRL 3037 (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 Flavipeses, Terrei and Jani including the outgroup (A. candidus NNRL 3037) 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 two well-supported groups, representing three sections in *Aspergillus: Flavipeses, Terrei and Jani*. Twelve species are currently accepted in section *Flavipeses*, including the novel species *A. urmiensis*. The type strains of *A. templicola* (CBS 138181T) and *A. mangaliensis* (CCF 4698T) form a well-supported clade, together with two other strains (CBS 138180 and CCF 869). Similarly, *A. micronesiensis* (CBS 138183T) and *A. frequentis* (NRRL 4578T) fall into the same clade. Five isolates obtained in our study belong to section *Flavipeses*. 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 4410T) and are identified accordingly.

The isolates CBS 139560 and CBS 139561T, 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, gregatin, 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 MB8117473. 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, gregatin, 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.
Aspergillus species belonging to RP2 and a combined dataset of sequences showing the relationship of species belonging to Aspergillus sections Flavipes, 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** Best-scoring Maximum Likelihood trees based on *BenA*, *CaM*, RP2 and a combined dataset of sequences showing the relationship of species belonging to *Aspergillus* sections *Flavipes*, *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.
**RPB2**

- A. templicola CBS 138181\(^7\)
- A. templicola CBS 138180
- A. templicola CCF 4698 (T of A. mangaliensis)
- A. capensis CBS 138188\(^1\)
- A. iizukaæ NRRL 3750\(^1\)
  - A. flavipes NRRL 302\(^1\)
  - A. flavipes DTO 309-I5
  - A. flavipes DTO 303-I4 (T of A. archilavipes)
  - A. ardaliensis CCF 4031\(^1\)
- A. urmiensis sp. nov. CBS 139557\(^1\)
- A. urmiensis sp. nov. CBS 139558
- A. urmiensis sp. nov. CBS 139766

**Combined**

- A. urmiensis sp. nov. CBS 139766
- A. urmiensis sp. nov. CBS 139558
- A. urmiensis sp. nov. CBS 139766

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 velvet, 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, spatulate, 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, uncolored. 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
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. Samadi: 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, CYA 37 °C 21–23; CZ 37 °C 16–20; MEA 37 °C 17–19; YES 37 °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.
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 undulate 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 undulate 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 with lightly umbonate in centre; reverse luteous (12) (Fig. 3). CZ 25 °C, 7d: mycelium ochreous (44); soluble pigment slightly produced, luteous; exudate absent; reverse orange (7) (Fig. 3). No ascomata, ascospores or Hülle 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 collumell. 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. speleaeus by larger colony diameters (16–20 vs. 0–17 mm) on CYA incubated at 37 °C. This new species can be differentiated from A. ardalenis based on the diameter of the vesicles (A. ardalenis, 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. microniosiensis and A. izukae 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 (e.g. 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. ardalenis, A. flavipes, A. frequens, A. izukae, A. luppii, A. mangaliensis, A. movilensis, A. neoflavipes, A. polyporicola and A. speleaeus) (Hubka et al. 2015). Concurrently, another three additions to this section were made (A. templicola, A. capensis and A. microniosiensis) (Visagie et al. 2014). The type strains of A. frequens (NRRL 45785) and A. microniosiensis (CBS 1381832) shared identical BenA, CaM and RPB2 sequences. Based on these data, A. frequens (Hubka et al. 2015) is reduced here to synonymy with A. microniosiensis (Visagie et al. 2014). Furthermore, the invalidly described species Aspergillus sunderbanii (Arts 40.3, 40.4, 40.5) is also a synonym of A. microniosiensis. The type strain of A. mangaliensis CCF 46984 is phylogenetically close, but not identical, to the type of A. templicola (CBS 1381812) (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 44103) 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. aureotterreus*, *A. carneus*, *A. citrinoterreus*, *A. floccosus*, *A. hortai*, *A. microcysticus*, *A. neoaficanus*, *A. neoidicus*, *A. niveus*, *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. neoidicus*. Besides the unique phylogenetic position, the *A. iranicus* isolates can also be differentiated from *A. carneus*, *A. niveus*, *A. allahabadii* and *A. neoidicus* 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.

Extralite profile analyses revealed that *A. iranicus* isolates produce citrinin, gregatinins, and a terrequinone in common, and isolate CBS 139560 additionally produces an extralite tentatively identified as asperamide. The hepatotoxic extralite citrinin is also known from several other species in this section, namely, *A. alabamensis*, *A. allahabadii*, *A. carneus*, *A. floccosus*, *A. hortai*, *A. neoidicus*, *A. niveus* and *A. pseudoterreus* (Samson et al. 2011). A diverse array of metabolites, including acetylaranotin, asperphenamate, aspochalamins, aspulvinones, astellixin, asterriquinone, aszonalenis, atrovenetins, butyrolactones, citreosocopumars, citreovirdins, citrinis, decaturins, fulvic acid, geodins, gregatins, mevinolins, serantrypine, 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 gregatin 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. umiensis* 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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References

Ainsworth GC (1976) Introduction to the history of mycology. Cambridge University Press, Cambridge

Asem A., Eimanifar A., Djamal M., De los Rios P. and Wink M. (2014) Biodiversity of the Hypersaline Urmia Lake National Park (NW Iran). Diversity 6:102–132
