Curvularia ahvazensis and *C. rouhanii* spp. nov. from Iran

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Abstract

Three isolates of *Curvularia* were obtained from diseased *Eucalyptus* sp., *Syngonium vellozianum* and *Zinnia elegans*. Phylogenetic trees inferred from combined dataset of the internal transcribed spacer ribosomal DNA (ITS), partial glyceraldehyde-3-phosphate dehydrogenase (GPDH), and partial translation elongation factor 1- α (EF1 α) along with morphological characterization, showed they are two novel species. *Curvularia ahvazensis* sp. nov. and *C. rouhanii* sp. nov. are described and illustrated and shown to be morphologically distinct from previously known species of *Curvularia*.

Key words – Two new species – Common zinnia – *Eucalyptus* – syngonium – multi-locus phylogenetic analysis

Introduction

Helminthosporium-like fungi associated with *Cochliobolus*, *Helminthosporium*, *Bipolaris* and *Curvularia*, contains some of the most highly damaging plant pathogens (Webster & Webber 2007). These genera produce dark coloured multi-septate conidia and belong to the family Pleosporaceae, class Dothideomycetes (Hibbett 2007, Wijayawardene et al. 2017, 2018). *Curvularia sensu lato* (Boedijn 1933) is generally characterized by curved and euseptate phragmoconidia with an unevenly swollen central cell, usually less strongly pigmented end cells, and a protuberant hilum in some species; with blastic-sympodial and tretic conidiogenesis. Recently, *Curvularia* species have been subjected to multigene phylogenetic analyses in combination with morphology (Manamgoda et al. 2012a, b, 2014, 2015, Tan et al. 2014, 2018, Tomaso-Peterson et al. 2016, Marin-Felix et al. 2017a, b, Heidari et al. 2018, Liang et al. 2018). As a result of this, several *Bipolaris* species are now accepted in the genus *Curvularia* (Manamgoda et al. 2012a, b, 2014, 2015, Tan et al. 2014, 2018, Tomaso-Peterson et al. 2016). The partial regions of ITS (internal nuclear ribosomal transcribed spacer region), GPDH (glyceraldehyde-3-phosphate dehydrogenase) and EF1 α (translation elongation factor 1-a) were used exclusively or in combination for the species delimitation of *Curvularia* (Manamgoda et al. 2012a, b, 2014, 2015, Tan et al. 2014, 2018, Tomaso-Peterson et al. 2016, Marin-Felix et al. 2017a, b, Heidari et al. 2018, Liang et al. 2018). Including the most recent new species descriptions (Marin-Felix et al. 2017a, b, Heidari et al. 2018, Tan et al. 2018, Liang et al. 2018), *Curvularia sensu stricto* includes 92 validated species with very wide morphological variation (<http://www.mycobank.org>).

In the present study, two new species of *Curvularia* isolated in Iran are described and illustrated through a polyphasic approach. A multi-locus phylogeny (ITS, GPDH and EF1 α), combined with morphological analysis, was used to delimit them from previously described species.

Material and Methods

Isolates and purification

During 2015, symptomatic plants of *Eucalyptus* sp. (with stem canker and die-back), *Zinnia elegans* (with wilting and root rot) and *Syngonium vellozianum* (with leaf blight) were sampled from Ahvaz and Behbahan in the southwest of Iran. Small tissue pieces (0.3–1 cm) were cut from the interface between healthy and diseased tissue and surface-disinfected for 2–4 min in a solution containing 2 % (v/v) sodium hypochlorite. They were then rinsed three times in sterile distilled water (2 min) and air-dried in a microbiological hood (Alexopoulos & Beneke 1962). Plant pieces were then plated on potato dextrose agar (PDA) supplemented with streptomycin (30 mg/L) and incubated at 28 °C for 5–10 days. Following incubation, three isolates having distoseptate phragmoconidia were obtained and shown to be two new species of *Curvularia*. The type specimens (dried cultures) of the new species are deposited in Herbarium Ministerii Iranici Agriculturae, Iranian Research Institute of Plant Protection, Tehran, Iran. Ex-type living cultures are deposited in IRAN (Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran), CBS (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands) and SCUA (Collection of Fungal Cultures, Department of Plant Protection, Shahid Chamran University of Ahvaz, Iran).

Growth characterization and morphological observations

Growth rates and colony characteristics were determined on PDA (Merck) and corn meal agar (CMA, Sigma Aldrich) at three different temperatures (28, 35 and 40 °C) in a 12-h fluorescent light-dark regime. The colony diameter was measured daily for at least eight days. Sporulation structures (including conidia, conidiophores and conidiogenous cells) were examined and measured using 40x and 100x objective lens of a Leitz Wetzlar (SM-LUX) Basic Biological light microscope. Slide cultures were prepared according to the protocol described by Beneke & Rogers (1996). Measurements are reported as a maximum and minimum range in association with 95% confidence limits and standard deviation. Images were prepared and recorded with an OLYMPUS BX51 microscope fitted with an OLYMPUS DP12 digital camera.

Biomass preparation, DNA extraction and amplification

Mycelial biomass was recovered from cultures grown in Erlenmeyer flasks containing potato-dextrose-broth medium (PDB) and passed through filter papers. The biomass was freeze-dried (Freeze-Dryer, Alpha 1–2LD Plus, Christ) and powdered in a mortar containing liquid nitrogen. Genomic DNA was extracted from approximately 100 mg of freeze-dried mycelium using an organic method described by Raeder & Broda (1985), with some modification (Ahmadpour et al. 2017). A part of nuclear DNA, containing the ITS 1 & 2, 5.8S and LSU-D1/D2 regions, was amplified by PCR using the primer combinations ITS1 and NL4 (White et al. 1990, O'Donnell 1993). A fragment of the GPDH gene was amplified using the primer pair gpd1 and gpd2 (Berbee et al. 1999). A partial region of EF1 α was amplified using the primer pair EF1-983 and EF1-2218R (Schoch et al. 2009). Each polymerase chain reaction (PCR) was performed in a 50 μ l reaction volume which contained 5 μ l 10 $\times$ PCR buffer, 1.6 μ l of each primer (10 μ M), 6 μ l MgCl₂ (25 mM), 1 μ l template DNA, and 0.25 μ l Taq DNA polymerase (5 units/ μ l, GenetBio, South Korea). Amplicons were produced in an automated thermal cycler (MJ MiniTM Gradient Thermal Cycler) with an initial melting step at 95 °C for 3 min, followed by 35 cycles of 30 s denaturation at 94 °C, 40 s primers annealing at 54 °C (ITS), 56 °C (GPDH) or 62 °C (EF1 α), 1 min extension at 72 °C,

and a 10 min extension at 72 °C. PCR products of the expected size were purified by GF-1 AmbiClean Kit (Vivantis, Malaysia) according to the manufacturer's instructions.

Sequencing, DNA analysis and phylogeny

Amplicons were sequenced in both directions using the original PCR primers by Macrogen Company (Humanizing Genomics, Macrogen, South Korea). The obtained sequences were edited and assembled using BioEdit Sequence Alignment Editor Version 7.0.9.0 (Hall 1999) and DNA Baser Sequence Assembler v4 programs (2013, Heracle BioSoft, www.DnaBaser.com), respectively. The generated sequences were deposited in GenBank (Table 1). To study the phylogeny of *Curvularia* including the isolates under study and many of the known species, two combined matrix, containing ITS and GPDH (two-locus) and ITS, GPDH and EF1 α (three-locus) sequences, were constructed. *Bipolaris drechsleri* was selected as outgroup taxon. Sequences were aligned with Clustal W in MEGA version 6 (Tamura et al. 2013) and manually refined in BioEdit 7.0.9.0 (Hall 1999). Maximum likelihood (ML) analyses of the combined datasets was performed with MEGA version 6, using best-fitting nucleotide substitution model, as suggested by ML model test, subtree-pruning-regrafting (SPR) algorithm and following options: gaps (insertion/deletions) were included in analysis, bootstrap (BP) analyses were done with 1000 replicates, initial trees for ML were made by NJ/BioNJ algorithm and branch swap filter was set very strong. Statistical support of nodes of the phylogenetic trees is indicated as ML bootstrap values (BS) = 50–100%. Two combined matrix used for phylogenetic analysis were deposited in the Treebase (<http://purl.org/phylo/treebase/phyloids/study/TB2:S22741>).

Results

Sequences and phylogenetic analysis

The final *Curvularia* alignment to construct phylogenetic trees included 121 strains (Table 1), representing many of the known *Curvularia* species, two new species described in the present study and the outgroup taxon. The combined matrix consisted of 1652 characters including alignment gaps (ITS: 468, GPDH: 501, and EF1 α : 683). The number of conserved sites was 1172 (ITS: 327, GPDH: 294, and EF1 α : 551). The number of variable and parsimony uninformative sites was 74 (ITS: 26, GPDH: 20, and EF1 α : 28) and 386 sites were variable and parsimony informative (ITS: 101, GPDH: 181, and EF1 α : 104). The best models of evolution calculated with MEGA 6 were the TN93 (Tamura-Nei) and GTR (General Time Reversible) with Invariant Site and Gamma Distribution (G + I) used for ML analysis of two- and three- locus datasets, respectively. In both two- and three- locus phylogenetic trees inferred from the combined dataset of ITS-GPDH and ITS-GPDH-EF1 α (Fig. 1), both strains (CBS 144674 and CBS 144675) of *C. rouhanii* cluster together with BS 73% and 93% support, respectively. This species also associated with two other taxa, *C. buchloes* and *C. spicifera*, in a larger clade with BS 94% support (Fig. 1). In three-locus phylogenetic tree, *C. ahvazensis* clustered with *C. hawaiiensis* into a distinct clade with BS 92% support. Two species, *C. ahvazensis* and *C. hawaiiensis*, could also be clearly distinguished based on morphology. The phylogenetic tree based on ITS-GPDH dataset (not shown) was constructed to show delimitation of some *Curvularia* species for which their EF1 α sequences were not available.

Taxonomy

Curvularia ahvazensis M. Mehrabi-Koushki & G. Babaahmadi, sp. nov.

Fig. 2

MycoBank: MB823459

Typification – IRAN, KHUZESTAN PROVINCE: Ahvaz. On rotten roots of common zinnia (*Zinnia elegans*), December 2015, G. Babaahmadi and M. Mehrabi-Koushki (holotype: IRAN 16942F, ex-type cultures: CBS 144673 = IRAN 3136C = SCUA-1bi).

Etymology – Name refers to the city Ahvaz, where the fungus was collected.

Morphology on PDA – *Hyphae* hyaline to pale brown, branched, septate, 2.5–5 µm diam. *Conidiophores* arising singly, septate, generally equal width in basal and median parts, upper part wider, straight or flexuous, sometimes geniculate at apex, mostly un-branched, sometimes bifurcated, cell walls thicker than those of vegetative hyphae, brown, darker towards apex, (7.5–)12–137.5(–187) × 2–4 µm, 95% confidence limits = 37.3–53.8 × 2.7–3.1 µm, ($\bar{x} \pm SD = 45.5 \pm 36.9 \times 2.9 \pm 0.7$ µm, n = 45). *Conidiogenous cells* integrated or discrete, smooth or with verruculose nodes, terminal or intercalary, proliferating sympodially, with circular and slightly thickened scars, pale brown to brown, subcylindrical to slightly swollen. *Conidia* smooth-walled, straight or very-slightly curved, ellipsoid, oblong or cylindrical, tapering towards rounded ends, pale brown to brown, 2–6-distoseptate, end cells not paler, (10–)13.5–27(–35) × (2.5–)5–7.8 µm, 95% confidence limits = 20.6–22.1 × 6.4–6.7 µm, ($\bar{x} \pm SD = 21.4 \pm 3.7 \times 6.5 \pm 0.7$ µm, n = 50). *Hila* slightly protruding, darkened, thickened, 1.5–3 µm diam. *Chlamydospore* and *sexual morph* not observed.

Table 1 Strains used in this study and their GenBank accession numbers. The new species are indicated in bold.

Species	Strain no.*	Source	Origin	GenBank Accession numbers		
				ITS	GPDH	EF1α
<i>B. drechsleri</i>	MUS0028	<i>Microstegium vimineum</i>	USA	KF500532	KF500535	KM093761
<i>C. aerea</i>	BRIP 61232b	<i>Oryza sativa</i>	Australia	KU552200	KU552162	KU552155
<i>C. affinis</i>	CBS 154.34	Unknown	Indonesia	KJ909780	KM230401	KM196566
<i>C. ahvazensis</i>	CBS 144673; IRAN 3136C; SCUA-1bi	<i>Zinnia elegans</i>	Iran	KX139029	MG428693	MG428686
<i>C. akaii</i>	CBS 317.86	<i>Themada triandra</i>	Japan	KJ909782	KM230402	KM196569
<i>C. akaiiensis</i>	BRIP 16080	–	–	KJ415539	KJ415407	KJ415453
<i>C. alcornii</i>	MFLUCC 100703	<i>Zea</i>	Thailand	JX256420	JX276433	JX266589
<i>C. americana</i>	UTHSC 08-3414	<i>Homo sapiens</i>	USA	HE861833	HF565488	-
<i>C. asianensis</i>	MFLUCC 100711	<i>Panicum</i> sp.	Thailand	JX256424	JX276436	JX266593
<i>C. australiensis</i>	BRIP 12044	<i>Oryza sativa</i>	–	KJ415540	KJ415406	KJ415452
<i>C. australiensis</i>	CBS 172.57	<i>Oryza sativa</i>	Vietnam	JN601026	JN601036	JN601003
<i>C. australis</i>	BRIP 12247a	<i>Eragrostis cilianensis</i>	Australia	KC424609	KC747759	KC503954
<i>C. australis</i>	BRIP 12521	<i>Sporobolus carolii</i>	–	KJ415541	KJ415405	KJ415451
<i>C. bannonii</i>	BRIP 16732	<i>Jacquemontia tamnifolia</i>	USA	KJ415542	KJ415404	KJ415450
<i>C. beasleyi</i>	BRIP 10972	<i>Chloris gayana</i>	Australia	MH414892	MH433638	MH433654
<i>C. beasleyi</i>	BRIP 16732	<i>Leersia hexandra</i>	Australia	MH414893	MH433639	MH433655
<i>C. beerburumensis</i>	BRIP 12942	<i>Eragrostis bahiensis</i>	Australia	MH414894	MH433634	MH433657
<i>C. beerburumensis</i>	BRIP 12555	<i>Eragrostis sororia</i>	Australia	MH414895	MH433640	MH433656
<i>C. boeremae</i>	IMI 164633	<i>Portulaca oleracea</i>	India	MH414911	MH433641	-
<i>C. borrieriae</i>	AR5176r	<i>Sorghum bicolor</i>	South Africa	KP400637	KP419986	KP735690

Table 1 Continued.

Species	Strain no.*	Source	Origin	GenBank Accession numbers		
				ITS	GPDH	EF1 α
<i>C. borrieriae</i>	MFLUCC 11–0422	Unknown grass	Thailand	KP400638	KP419987	KM196571
<i>C. bothriochloae</i>	BRIP 12522	<i>Bothriochloa</i>	Australia	KJ415543	KJ415403	KJ415449
<i>C. brachyspora</i>	CBS 186.50	Soil	India	KJ922372	KM061784	KM230405
<i>C. buchloes</i>	CBS 246.49	<i>Buchloe dactyloides</i>	USA	KJ909765	KM061789	KM196588
<i>C. caricapapayae</i>	CBS 135941	<i>Carica papaya</i>	India	HG778984	HG779146	–
<i>C. chlamydospora</i>	UTHSC 072764	Toe nail	USA	HG779021	HG779151	–
<i>C. clavata</i>	BRIP:61680	<i>Oryza</i> sp.	Australia	KU552205	KU552167	KU552159
<i>C. coatesiae</i>	BRIP 24261	<i>Litchi chinensis</i>	Australia	MH414897	MH433636	MH433659
<i>C. coatesiae</i>	BRIP 24170	Air	Australia	MH414896	MH433635	MH433658
<i>C. coicis</i>	CBS 192.29	<i>Coix lacryma</i>	Japan	JN192373	JN600962	JN601006
<i>C. colbranii</i>	BRIP 13066	<i>Crinum zeylanicum</i>	Australia	MH414898	MH433642	MH433660
<i>C. crustacea</i>	BRIP 13524	<i>Sporobolus</i> sp.	Indonesia	KJ415544	KJ415402	KJ415448
<i>C. dactyloctenicola</i>	CPC 28810	<i>Dactyloctenium aegyptium</i>	Thailand	MF490815	MF490837	MF490858
<i>C. dactyloctenii</i>	BRIP 12846	<i>Dactyloctenium radulans</i>	Australia	KJ415545	KJ415401	KJ415447
<i>C. ellisii</i>	CBS 193.62	Air	Pakistan	JN192375	JN600963	JN601007
<i>C. eragrosticola</i>	BRIP 12538	<i>Eragrostis pilosa</i>	Australia	MH414899	MH433643	MH433661
<i>C. eragrostidis</i>	CBS 189.48	–	–	HG778986	HG779154	–
<i>C. geniculata</i>	CBS 187.50	Unknown seed	Indonesia	KJ909781	KM083609	KM230410
<i>C. gladioli</i>	ICMP 6160	<i>Gladiolus</i> sp.	New Zealand	JX256426	JX276438	JX266595
<i>C. graminicola</i>	BRIP 23186a	–	Australia	JN192376	JN600964	JN601008
<i>C. harveyi</i>	BRIP 57412	<i>Triticum aestivum</i>	Australia	KJ415546	KJ415400	KJ415446
<i>C. hawaiiensis</i>	BRIP 11987	<i>Oryza sativa</i>	USA	KJ415547	KJ415399	KJ415445
<i>C. heteropogonicola</i>	BRIP 14579	<i>Heteropogon contortus</i>	India	KJ415548	KJ415398	KJ415444
<i>C. heteropogonis</i>	CBS 284.91	<i>Heteropogon contortus</i>	Australia	JN192379	JN600969	JN601013
<i>C. hominis</i>	AR 5118	<i>Lolium perene</i>	USA	KP400639	KP419988	KM196580
<i>C. hominis</i>	MFLUCC 120191	Unknown grass	Thailand	KP400640	KP419989	KM196581
<i>C. homomorpha</i>	CBS 156.60	–	–	JN192380	JN600970	JN601014
<i>C. inaequalis</i>	CBS 102.42	Sand dune soil	France	KJ922375	KM061787	KM196574
<i>C. inaequalis</i>	DAOM 20022	<i>Pisum sativum</i>	Canada	KJ922374	KM061786	KM196575
<i>C. intermedia</i>	B19	<i>Polygonatum sibiricum</i>	China	MF370184	MF370185	MF370186
<i>C. ischaemi</i>	CBS 630.82	<i>Ischaemum indicum</i>	Fiji	JX256428	JX276440	–
<i>C. kenpeggii</i>	BRIP 14530	<i>Triticum aestivum</i>	Australia	MH414900	MH433644	MH433662
<i>C. lamingtonensis</i>	BRIP 12259	<i>Microlaena stipoides</i>	Australia	MH414901	MH433645	MH433663
<i>C. lunata</i>	CBS 730.96	lung biopsy	USA	JX256429	JX276441	JX266596
<i>C. malina</i>	CBS 131274	<i>Zoysia</i> grass	USA	JF812154	KP153179	KR493095
<i>C. malina</i>	FLS–119	<i>Bermuda</i> grass	USA	KR493070	KR493083	KR493093

Table 1 Continued.

Species	Strain no.*	Source	Origin	GenBank Accession numbers		
				ITS	GPDH	EF1 α
<i>C. meibaldsii</i>	BRIP 12900	<i>Cynodon tranvaalensis</i>	Australia	MH414902	MH433647	MH433664
<i>C. meibaldsii</i>	BRIP 13983	<i>Cynodon dactylon</i>	Australia	MH414903	MH433646	MH433665
<i>C. microspora</i>	GUCC 6272	<i>Hippeastrum striatum</i>	China	MF139088	MF139097	MF139115
<i>C. microspora</i>	GUCC 6273	<i>Hippeastrum striatum</i>	China	MF139089	MF139098	MF139116
<i>C. miyakei</i>	CBS197.29	<i>Eragrostis pilosa</i>	Japan	KJ909770	KM083611	KM196568
<i>C. mosaddeghii</i>	IRAN 3131C	<i>Syzygium cumini</i>	Iran	MG846737	MH392155	MH392152
<i>C. mosaddeghii</i>	IRAN 3123C	<i>Vigna unguiculata</i>	Iran	MG971270	MG975597	MH392151
<i>C. muehlenbeckiae</i>	MUS 0031	<i>Sorghum</i> sp.	USA	KP400647	KP419996	KM196578
<i>C. muehlenbeckiae</i>	AR 5180	<i>Sorghum</i> sp.	USA	KP400649	KP419998	-
<i>C. neergaardii</i>	BRIP 12919	<i>Oryza sativa</i>	Ghana	KJ415550	KJ415397	KJ415443
<i>C. neergaardii</i>	DAOM 228085	Desert soil	Chile	KJ909784	KM083615	KM196593
<i>C. neoindica</i>	IMI 129790	<i>Brassica nigra</i>	India	MH414910	MH433649	MH433667
<i>C. nicotiae</i>	BRIP 11983	—	—	KJ415551	KJ415396	KJ415442
<i>C. nodosa</i>	CPC 28801	<i>Urochloa reptans</i>	Thailand	MF490817	MF490839	MF490860
<i>C. nodosa</i>	CPC 28812	<i>Chloris barbata</i>	Thailand	MF490818	MF490840	MF490861
<i>C. nodulosa</i>	CBS 160.58	—	—	JN601033	JN600975	JN601019
<i>C. oryzae</i>	CBS 169 53	<i>Oryza sativa</i>	Vietnam	KP400650	KP645344	KM196590
<i>C. ovariicola</i>	BRIP 15882	—	—	JN601031	JN600971	JN601020
<i>C. pallescens</i>	CBS 156.35	Air	Java	KJ922380	KM083606	KM196570
<i>C. papendorffii</i>	BRIP 57608	<i>Acacia karroo</i>	—	KJ415552	KJ415395	KJ415441
<i>C. papendorffii</i>	CBS308.67	<i>Acacia karroo</i>	South Africa	KJ909774	KM083617	KM196594
<i>C. perotidis</i>	CBS 350.90	<i>Perotis rara</i>	Cape York	JN192385	JN601021	JN601021
<i>C. petersonii</i>	BRIP 14642	<i>Dactyloctenium aegyptium</i>	Australia	MH414905	MH433650	MH433668
<i>C. pisi</i>	CBS 190.48	<i>Pisum sativum</i>	Canada	KY905678	KY905690	KY905697
<i>C. platzii</i>	BRIP 27703b	<i>Cenchrus clandestinum</i>	Australia	MH414906	MH433651	MH433669
<i>C. portulacae</i>	BRIP 14837	Soil	—	KJ415554	KJ415392	KJ415439
<i>C. portulacae</i>	CBS 239.48	<i>Portulaca oleracea</i>	USA	KJ909775	KM083616	KM230404
<i>C. prasadii</i>	CBS 143.64	<i>Jasminum sambac</i>	India	KJ922373	KM061785	KM230408
<i>C. protuberata</i>	5876	<i>Fragaria</i> sp.	—	KT012665	KT012626	KT012587
<i>C. protuberata</i>	CBS 376.65	<i>Deschampsia flexuosa</i>	UK	KJ922376	KM083605	KM196576
<i>C. pseudobrachyspora</i>	CPC 28808	<i>Eleusine indica</i>	Thailand	MF490819	MF490841	MF490862
<i>C. pseudolunata</i>	UTHSC 092092	Nasal sinus	USA	HE861842	HF565459	—
<i>C. pseudorobusta</i>	UTHSC 083458	Nasal sinus	USA	HE861838	HF565476	—
<i>C. ravenelii</i>	BRIP 13165	<i>Sporobolus fertilis</i>	Australia	JN192386	JN600978	JN601024
<i>C. reesii</i>	BRIP 4358	Air	Australia	MH414907	MH433637	MH433670

Table 1 Continued.

Species	Strain no.*	Source	Origin	GenBank Accession numbers		
				ITS	GPDH	EF1 α
<i>C. richardiae</i>	BRIP 4371	<i>Richardia brasiliensis</i>	Australia	KJ415555	KJ415391	KJ415438
<i>C. robusta</i>	CBS624 68	<i>Dichanthium annulatum</i>	USA	KJ909783	KM083613	KM196577
<i>C. rouhanii</i>	CBS 144674; SCUA-2bi	<i>Syngonium vellozianum</i>	Iran	KX139030,	MG428694	MG428687
<i>C. rouhanii</i>	CBS 144675; IRAN 3138C; SCUA-4bi	<i>Eucalyptus</i> sp.	Iran	KX139032	MG428696	MG428689
<i>C. ryleyi</i>	BRIP 12554	<i>Sporobolus creber</i>	–	KJ415556	KJ415390	KJ415437
<i>C. ryleyi</i>	CBS349.90	<i>Sporobolus creber</i>	Australia	KJ909766	KM083612	KM196567
<i>C. senegalensis</i>	CBS 149.71	–	–	HG779001	HG779128	–
<i>C. soli</i>	CBS 222.96	Soil	Papua New Guinea	KY905679	KY905691	KY905698
<i>C. sorghina</i>	BRIP 15900	<i>Sorghum bicolor</i>	Australia	KJ415558	KJ415388	KJ415435
<i>C. spicifera</i>	CBS 274.52	Soil	Spain	JN192387	JN600979	JN601023
<i>C. sporobolicola</i>	BRIP 23040b	<i>Sporobolus australasicus</i>	Australia	MH414908	MH433652	MH433671
<i>C. subpapendorffii</i>	CBS656.74	Desert soil	Egypt	KJ909777	KM061791	KM196585
<i>C. trifolii</i>	AR5169	<i>Sorghum bicolor</i>	South Africa	KP400656	KP645345	KP735694
<i>C. tripogonis</i>	BRIP 12375	Unknown	Australia	JN192388	JN600980	JN601025
<i>C. tropicalis</i>	BRIP 14834	<i>Coffea arabica</i>	India	KJ415559	KJ415387	KJ415434
<i>C. tsudae</i>	BRIP 10970a	<i>Chloris gayana</i>	Australia	KC424605	KC747755	KC503940
<i>C. tsudae</i>	MAFF 236750	<i>Chloris gayana</i>	Japan	KP400651	KM061790	KM230409
<i>C. tuberculata</i>	CBS 14663	<i>Zea mays</i>	India	JX256433	JX276445	JX266599
<i>C. uncinata</i>	CBS 221.52	<i>Oryza sativa</i>	Vietnam	HG779024	HG779134	–
<i>C. variabilis</i>	CPC 28813	<i>Digitaria ciliaris</i>	Thailand	MF490820	MF490842	MF490863
<i>C. variabilis</i>	CPC 28815	<i>Chloris barbata</i>	Thailand	NR154866	MF490844	MF490865
<i>C. verruculosa</i>	CBS150 63	<i>Punica granatum</i>	India	KP400652	KP645346	KP735695
<i>C. verruculosa</i>	MFLUCC 100690	<i>Oryza sativa</i>	Thailand	JX256437	JX276448	JX266602
<i>C. warraberensis</i>	BRIP 14817	<i>Dactyloctenium aegyptium</i>	Australia	MH414909	MH433653	MH433672
<i>Curvularia</i> sp.	AR 5117	<i>Lolium perenne</i>	USA	KP400655	KP645349	KP735698
<i>Curvularia</i> sp.	BRIP 17439	<i>Trianthema portulacastrum</i>	Australia	AF081449	AF081406	MH445455
<i>Curvularia</i> sp.	MFLUCC 100709	<i>Oryza sativa</i>	Thailand	JX256442	JX276453	–
<i>Curvularia</i> sp.	MFLUCC 100739	<i>Oryza sativa</i>	Thailand	JX256443	JX276454	JX266603
<i>Curvularia</i> sp.	MFLUCC 120177	Unknown grass	Thailand	KP400654	KP645348	KP735697

Table 1 Continued.

Species	Strain no.*	Source	Origin	GenBank Accession numbers		
				ITS	GPDH	EF1 α
<i>Curvularia</i> sp.	UTHSC 08809	Human	USA	HE861826	HF565477	–

*BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at Westerdijk Fungal Biodiversity Institute; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; ICMP: International Collection of Micro-organisms from Plants, Landcare Research, Private Bag 92170, Auckland, New Zealand; IMI: International Mycological Institute, Kew, UK; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan, MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; UTHSC: Fungus Testing Laboratory, Department of Pathology at the University of Texas Health Science Center, San Antonio, Texas, USA; SCUA: the Collection of Fungal Cultures, Department of Plant Protection, Shahid Chamran University of Ahvaz, Iran.

Cultural characters – Colonies on PDA attaining 60–65 mm diam. after 8 d incubation at 28±0.5 °C, 48–52 mm diam. at 35±0.5 °C and 9–9.4 mm diam. at 40±0.5 °C, circular with entire margin, greenish grey (green jade) with colorless edge, paler with age, aerial mycelium sparse to abundant, floccose with age; reverse greenish black, paler towards the edge of the colony, with age becoming darker. Colonies on CMA, 80–84 mm diam. after 8 d incubation at 28±0.5 °C, looser than on PDA, circular with entire margin, blackish green with paler edge, paler with age, aerial mycelium sparse to abundant, with age slightly floccose; reverse greenish black, paler and greener towards the edge of the colony, with age becoming darker.

Habitat – common zinnia.

Distribution – Iran (Ahvaz).

Notes – In the phylogenetic analysis of combined ITS-GPDH-EF1 α dataset (Fig. 1), *Curvularia ahvazensis* is phylogenetically related to *C. dactyloctenii* (Alcorn) Y.P. Tan & R.G. Shivas (Alcorn 1982, Tan et al. 2014, 2018), *C. hawaiiensis* (Bugnic.) Manamgoda, L. Cai & K.D. Hyde (Manamgoda et al. 2012), and *C. nodosa* Y. Marín, Cheew. & Crous (Marin-Felix et al. 2017b). The length of *C. ahvazensis* conidia (13.5–27 μ m) is similar to those of *C. nodosa* (14.5–25 μ m), while the conidia of *C. dactyloctenii* (32–55 μ m long) and *C. hawaiiensis* (12–37 μ m long) are longer. The conidia of *C. ahvazensis* (5–7.8 μ m wide) and *C. dactyloctenii* (6–8 μ m wide) are narrower than those of *C. hawaiiensis* (8–10 μ m wide) and *C. nodosa* (7–9.5 μ m wide). Conidiophores of *C. ahvazensis* are 2–4 μ m wide, while in *C. hawaiiensis* and *C. nodosa* they are 2–6 μ m and 3–5.5 μ m wide, respectively.

Curvularia rouhanii M. Mehrabi-Koushki & G. Babaahmadi, sp. nov.

Fig. 3

MycoBank: MB823474

Typification – IRAN, KHUZESTAN PROVINCE: Ahvaz. On blighted leaves of syngonium (*Syngonium vellozianum*), Oct 2015, *G. Babaahmadi* and *M. Mehrabi-Koushki* (holotype: IRAN 17006F, ex-type cultures: CBS 144674 = SCUA-2bi-2).

Etymology – In reference to the significant contribution of Prof. Hamid Rouhani to develop biological control of plant diseases in Iran.

Morphology on PDA – *Hyphae* hyaline to pale brown, poorly branched, septate, occasionally with pear-shaped swollen cells, 2–6 μ m diam. *Conidiophores* arising singly, septate, generally equal wide in median or upper parts, base thinner with a narrowed basal cell, straight or flexuous, apically geniculate, un-branched, cell walls thicker than those of vegetative hyphae, brown, paler towards apex, (17.5–)25–155(–230) $\times$ 2.5–5(5.5) μ m, 95% confidence limits = 63.9–83.2 $\times$ 3.8–4.1 μ m, ($\bar{x}$ $\pm$ SD = 73.6 $\pm$ 47.6 $\times$ 4 $\pm$ 0.8 μ m, n = 50). *Conidiogenous cells* integrated or discrete, smooth or with verruculose nodes, terminal or intercalary, proliferating sympodially, with circular and thickened scars, brown to dark brown, subcylindrical to swollen. *Conidia* smooth-walled, straight or very slightly curved, ellipsoid to cylindrical, tapering towards rounded ends, pale brown

Figure 1 – Phylogenetic tree generated from a ML analysis based on a concatenated alignment of ITS, GPDH and EF1 α sequences of *Curvularia* strains representing most previously known species and new taxa. The tree was rooted with *Bipolaris drechsleri* MUS0028. Bootstrap values greater than 50% (expressed as percentages of 1000 replications) are shown at the nodes.

to brown, (2–)4-distoseptate, end cells not paler, 15–30.5 $\times$ (5.3–)6.5–10 μ m, 95% confidence limits = 21.6–23.2 $\times$ 8.3–8.7 μ m, ($\bar{x} \pm SD = 22.4 \pm 4 \times 8.5 \pm 1 \mu$ m, $n = 50$). *Hila* mostly inconspicuous sometime very slightly protruding, darkened, thickened, 2–3 μ m diam. *Chlamydospores* unicellular or multicellular (pseudosclerotoid), smooth, dark brown to black. *Swollen cells* hyaline to pale brown, intercalary, mainly in clusters, variable in size and shape. Sexual morph not observed.

Cultural characters – Colonies on PDA attaining 50–55 mm diam. after 8 d incubation at 28 $\pm$ 0.5 $^{\circ}$ C, 84–88 mm diam. at 35 $\pm$ 0.5 $^{\circ}$ C and 16–18 mm diam. at 40 $\pm$ 0.5 $^{\circ}$ C, circular with slightly undulate margin, greenish brown with colorless edge, paler with age, aerial mycelium abundant, highly floccose with age; reverse greenish black, blacker with age. Colonies on CMA, 82–86 mm diam. after 8 d of inoculation at 28 $\pm$ 0.5 $^{\circ}$ C, looser than on PDA, circular with entire margin, pale brown with lighter edge, paler with age, aerial mycelium sparse, slightly floccose with age; reverse pale brown with lighter edge.

Additional cultures examined – IRAN, KHUZESTAN PROVINCE: Behbahan. From *Eucalyptus* sp., Oct 2015, G. Babaahmadi and M. Mehrabi-Koushki (CBS 144675 = IRAN 3138C).

Habitat – *Syngonium* and *Eucalyptus*.

Distribution – Iran (Ahvaz and Behbahan).

Notes – Phylogenetic analyses indicate that this species is closely related to *C. buchloes* (Lefebvre & A.G. Johnson) Manamgoda, Rossman & K.D Hyde and *C. spicifera* (Bainier) Boedijn (Fig. 1), but is morphologically distinct. *Curvularia rouhanii* can be easily distinguished from *C. buchloes* (Manamgoda et al. 2015) by the production of shorter conidia with fewer septa [(2–)4-distoseptate and 15–30.5 μ m long in *C. rouhanii* vs. 3–8-distoseptate and (34–)40–69(–71) μ m long in *C. buchloes*]. Furthermore, the conidia of *C. buchloes* are more curved than those of *C. rouhanii* (Manamgoda et al. 2015). The conidia of *C. rouhanii* differ from *C. spicifera* (Bainier 1908) in having more septa, and shorter and narrower conidia (*C. spicifera* are predominantly 3-distoseptate and 30–36 $\times$ 11–14 μ m).

Figure 2 – *Curvularia ahvazensis* (CBS 144673). a–b Colony on PDA (top and reverse). c–d Colony on CMA (top and reverse). e–g Conidiophores, conidiogenous cells and conidia.

Figure 3 – *Curvularia rouhanii* (CBS 144674). a–b Colony on PDA (top and reverse). c–d Colony on CMA (top and reverse). e–f Conidiophores, conidiogenous cells and conidia. g Swollen cells and multicellular chlamydospores.

Discussion

The combination of phylogenetic analysis with morphological characterization showed that both *Curvularia ahvazensis* and *C. rouhanii*, are novel species. In both two- and three-locus-based phylogenetic trees, the combined datasets separated both new taxa from previously known species of *Curvularia*. In addition to their phylogenetic delimitation, morphological differences, including conidial size, shape and septation, can distinguish them from their closest relatives among the *Curvularia* species. Phylogenetic trees obtained from maximum likelihood analysis in this study yielded the same overall topology as that presented in previous surveys (Manamgoda et al. 2012a, b, 2014, 2015, Tan et al. 2014, 2018, Tomaso-Peterson et al. 2016, Marin-Felix et al. 2017a, b, Heidari et al. 2018, Liang et al. 2018).

Curvularia species are commonly isolated from soil and living or dead parts of plants (Manamgoda et al. 2012a, b, 2014, 2015, Tan et al. 2014, 2018, Ariyawansa et al. 2015, Tomaso-Peterson et al. 2016, Marin-Felix et al. 2017a, b, Wijayawardene et al. 2017, 2018, Heidari et al. 2018, Liang et al. 2018). Some species have been also found in human tissues (Manamgoda et al. 2012a, da Cunha et al. 2013, Madrid et al. 2014). *Curvularia ahvazensis* was isolated from rotten roots of *Zinnia elegans* while *C. rouhanii* was isolated from blighted leaves of *Syngonium vellozianum* and *Eucalyptus*. *Zinnia elegans* was previously reported to be host of *C. hawaiiensis* (Manamgoda et al. 2015). *Eucalyptus* was known to be host of *C. eragrostidis*, *C. hawaiiensis*, *C. pallescens* and *C. tuberculata* (Manamgoda et al. 2011, 2015). This is the first report of a *Curvularia* species obtained from *Syngonium vellozianum*. Several species of *Curvularia* have been previously reported on herbaceous and woody plants from different areas of Iran, including *C. affinis*, *C. australiensis*, *C. clavata*, *C. hawaiiensis*, *C. inaequalis*, *C. lunata*, *C. mosaddeghii*, *C. pallescens*, *C. papendorffii*, *C. prasadii*, *C. senegalensis*, *C. trifolii*, *C. tuberculata* and *C. verruculosa* (Ershad 2009, Ahmadpour et al. 2013, Heidari et al. 2018, <https://www.ncbi.nlm.nih.gov>).

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