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Curvularia mosaddeghii sp. nov., a novel species from the family Pleosporaceae

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Abstract

The new species *C. mosaddeghii* sp. nov. isolated from plants of *Syzygium cumini* and *Vigna unguiculata* is described and illustrated. Three-locus DNA sequence based phylogeny, in combination with morphology of the asexual morph, were used to characterize this species. Phylogenetic analysis used combined sequences of internal transcribed spacer regions 1 & 2 and 5.8S nrDNA (ITS), partial glyceraldehyde-3-phosphate dehydrogenase (*GPDH*) and part of the translation elongation factor 1-α (*EF1α*). In the phylogenetic trees, both isolates of *C. mosaddeghii* clustered together as a monophyletic clade with strong support, distinct from other previously known species of *Curvularia*. Morphologically, this species is distinguished from closely related species by having narrower conidia and hila.

Key words – Ahvaz – Jambolan – Cowpea – Mycoflora – New species

Introduction

Curvularia belongs to Pleosporaceae and is widely distributed in soil water and plants and infects humans and animals (Sivanesan 1987, Manamgoda et al. 2011, 2012 a, b, da Cunha et al. 2013, Rangaswamy et al. 2013, Verma et al. 2013, Hyde et al. 2014, Ariyawansa et al. 2015, Wijayawardene et al. 2017, 2018). *Curvularia* species are mainly saprobes often found in an association with hosts and non-host living organisms in tropical regions (Manamgoda et al. 2011, 2012a, b, 2014, 2015, Scott & Carter 2014, Tan et al. 2014), some are also opportunistic pathogens throughout the world (Manamgoda et al. 2011, Madrid et al. 2014). Some species are endophytes in various plant species (Tadych et al. 2012, Gautam et al. 2013, Jena & Tayung 2013).

Curvularia was established by Boedijn in 1933, with the description of *C. lunata* (Wakker) Boedijn (Boedijn 1933) as the type species. Taxonomic classification of helminthosporium-like fungi based on morphological characteristics is insufficient and artificial, and species identification can only be elucidated using molecular studies (Manamgoda et al. 2012a, b, 2014, 2015, Madrid et al. 2014, Tan et al. 2014, Tomaso-Peterson et al. 2016, Marin-Felix et al. 2017a, b). In order to resolve phylogenetic relationships and improve the systematics of *Curvularia* and *Bipolaris* species, the nucleotide sequences of ITS (internal nuclear ribosomal transcribed spacer region), *GPDH* (glyceraldehyde-3-phosphate dehydrogenase) and *EF1α* (translation elongation factor 1-a) were used exclusively or in combination for species delimitation (Manamgoda et al. 2012a, b, 2014, 2015, Madrid et al. 2014, Tan et al. 2014, Tomaso-Peterson et al. 2016, Marin-Felix et al. 2017a, b). According to the most recent phylogenetic analysis of *Bipolaris*- and *Curvularia*-like

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taxa (Manamgoda et al. 2015, Tomaso-Peterson et al. 2016, Marin-Felix et al. 2017a, b), *Curvularia* was emended as a monophyletic genus to accommodate 75 currently validated and five unknown species.

In the present study, a new species of *Curvularia* isolated from Iran is described through polyphasic approaches. A multi-locus phylogeny (ITS, *GPDH* and *EF1α*), combined with the morphological analysis of the asexual morph, was used to separate these unknown species from previously described species.

Materials & Methods

Isolates and typification

Two *Curvularia* isolates identified in this study were recovered from leaf spot of jambolan (*Syzygium cumini*) and healthy roots of cowpea (*Vigna unguiculata*) growing in Ahvaz in the southwest of Iran. The type specimens (dried cultures) are deposited in Herbarium Ministerii Iranici Agriculturae, Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN 16938F). The ex-type living culture and other isolate under survey are deposited in the Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN 3131C and IRAN 3123C). In addition, they are kept in Collection of Fungal Cultures, Department of Plant Protection, Shahid Chamran University of Ahvaz, Iran (SCUA-Ahv-Syz-P and SCUA-Ahw-Vig-1Rb) (Table 1).

Morphological and physiological study

The morphological characteristics of asexual morph were examined using 3-day to 14-day-old cultures grown on potato dextrose agar (PDA, Merck) at 28 °C in a 12-h fluorescent light-and-dark. These characteristics (including those of the mycelia, conidiophore, and conidia) were studied using the method of Riddle and Briggs (1950). The colour was determined as described in Methuen handbook of color (Kornerup & Wanscher 1967). The slide cultures were prepared according to the protocol described by Beneke & Rogers (1996). Sixty measurements for each character were made with the 40× and 100× objective lens of a Leitz wetzlar (SM-LUX) Basic Biological Light Microscope. The photomicrographs were recorded with an OLYMPUS BX51 microscope fixed with an OLYMPUS DP12 digital camera. The isolates were preliminarily identified based on morphology documented in literature review (Manamgoda et al. 2012a, b, 2014, 2015, Madrid et al. 2014, Tan et al. 2014, Tomaso-Peterson et al. 2016, Marin-Felix et al. 2017a, b). The rate of mycelial growth was determined at 28 °C.

Fungal biomass preparation

Single spore cultures of each *Curvularia* isolates were inoculated into Erlenmeyer flasks containing potato-dextrose-broth medium. The flasks were incubated at 180 rpm for 10–15 days at 28 ºC to achieve the maximum growth of fungi. The mycelial biomass was collected and washed by passing through filter papers using sterile distilled water. The mycelia were freeze-dried (Freeze-Dryer, Alpha 1–2LD Plus, Christ) and powdered in the mortar containing liquid nitrogen. The mycelia powders was collected into 10 ml tubes and stored in the -20 ºC freezer until consumed.

DNA extraction and amplification

Fungal DNA was isolated from 100 mg of freeze-dried mycelia using a Phenol:chloroform based protocol as previously described by Raeder & Broda (1985), with some modification (Ahmadpour et al. 2017). Mycelial lysates was extracted two times by Phenol:chloroform:isoamyl alcohol, followed by extracting with chloroform:isoamyl alcohol. DNA samples were analyzed using a spectrophotometer (eppendorf BioPhotometer plus) and loading on the gel. Partial regions of nrDNA (internal transcribed spacer regions 1 & 2, 5.8S, LSU-D1/D2), *GPDH* and *EF1α* were amplified with a thermal cycler (MJ Mini^{TM} Gradient Thermal Cycler) using primers ITS1 and NL4 (White et al. 1990), gpd1 and gpd2 (Berbee et al. 1999) and EF1983 and EF12218R, respectively

Table 1 Strains used in this study and their GenBank accession numbers. The sequences of newly described species are indicated in bold.

Species name	isolate name or strain no.*	Source or host	origin	GenBank Accession numbers		
				ITS	*GPDH*	*EF1α*
B. drechsleri	MUS0028	*Microstegium vimineum*	USA	KF500532	KF500535	KM093761
C. aeria	CBS 294.61	Air	Brazil	HE861850	HF565450	–
C. affinis	CBS 154.34	Unknown	Indonesia	KJ909780	KM230401	KM196566
C. affinis	CBS 185.49	–	–	HG778982	HG779127	–
C. akaii	CBS 317.86	*Themada triandra*	Japan	KJ909782	KM230402	KM196569
C. akaiiensis	BRIP 16080	–	–	KJ415539	KJ415407	KJ415453
C. alcornii	MFLUCC 100703	*Zea*	Thailand	JX256420	JX276433	JX266589
C. americana	UTHSC 072649	Toe tissue	USA	HE861834	HF565486	–
C. americana	UTHSC 08278	Peritoneal dialysis	USA	HE861832	HF565487	–
C. asianensis	MFLUCC 100685	*Saccharum* sp.	Thailand	JX256425	JX276437	JX266594
C. asianensis	MFLUCC 100711	*Panicum* sp.	Thailand	JX256424	JX276436	JX266593
C. australiensis	BRIP 12044	*Oryza sativa*	–	KJ415540	KJ415406	KJ415452
C. australiensis	CBS 172.57	*Oryza sativa*	Vietnam	JN601026	JN601036	JN601003
C. australis	BRIP 12247a	*Eragrostis cilianensis*	Australia	KC424609	KC747759	KC503954
C. australis	BRIP 12521	*Sporobolus carolii*	–	KJ415541	KJ415405	KJ415451
C. bannonii	BRIP 16732	*Jacquemontia tamnifolia*	USA	KJ415542	KJ415404	KJ415450
C. bannonii	DAOM196762	Tent wall	USA	KP400634	KP419983	KP735688
C. borreriae	AR5176r	*Sorghum bicolor*	South Africa	KP400637	KP419986	KP735690
C. borreriae	MFLUCC 11–0422	Unknown grass	Thailand	KP400638	KP419987	KM196571
C. bothriochloae	BRIP 12522	*Bothriochloa*	Australia	KJ415543	KJ415403	KJ415449
C. brachyspora	CBS 186.50	Soil	India	KJ922372	KM061784	KM230405
C. brachyspora	ZW020185	–	–	HM053667	HM053655	–
C. buchloes	CBS 246.49	*Buchloe dactyloides*	USA	KJ909765	KM061789	KM196588
C. caricapapayae	CBS 135941	*Carica papaya*	India	HG778984	HG779146	–
C. chlamydospora	UTHSC 072764	Toe nail	USA	HG779021	HG779151	–
C. clavata	BRIP:61680	*Oryza* sp.	Australia	KU552205	KU552167	KU552159
C. coicis	CBS 192.29	*Coix lacryma*	Japan	JN192373	JN600962	JN601006
C. crustacea	BRIP 13524	*Sporobolus* sp.	Indonesia	KJ415544	KJ415402	KJ415448
C. dactyloctenicola	CPC 28810	*Dactyloctenium aegyptium*	Thailand	MF490815	MF490837	MF490858
C. dactyloctenii	BRIP 12846	*Dactyloctenium radulans*	Australia	KJ415545	KJ415401	KJ415447
C. ellisii	CBS 193.62	Air	Pakistan	JN192375	JN600963	JN601007
C. ellisii	IMI 75862	Air	Pakistan	KJ922379	KM061792	–
C. eragrostidis	CBS 189.48	–	–	HG778986	HG779154	–
C. geniculata	CBS 187.50	Unknown seed	Indonesia	KJ909781	KM083609	KM230410
C. gladioli	CBS 210.79	–	–	HG778987	HG779123	–
C. gladioli	ICMP 6160	*Gladiolus* sp.	New Zealand	JX256426	JX276438	JX266595
C. graminicola	BRIP 23186a	–	Australia	JN192376	JN600964	JN601008
C. harveyi	BRIP 57412	*Triticum aestivum*	Australia	KJ415546	KJ415400	KJ415446
C. hawaiiensis	BRIP 11987	*Oryza sativa*	USA	KJ415547	KJ415399	KJ415445
C. heteropogonicola	BRIP 14579	*Heteropogon contortus*	India	KJ415548	KJ415398	KJ415444
C. heteropogonis	CBS 284.91	*Heteropogon contortus*	Australia	JN192379	JN600969	JN601013
C. heteropogonis	CBS 511.91	–	–	HF934918	HG779122	–

Table 1 Continued.

Species name	isolate name or strain no.*	Source or host	origin	GenBank Accession numbers		
				ITS	*GPDH*	*EF1α*
C. hominis	AR 5118	*Lolium perene*	USA	KP400639	KP419988	KM196580
C. hominis	MFLUCC 120191	Unknown grass	Thailand	KP400640	KP419989	KM196581
C. homomorpha	CBS 156.60	–	–	JN192380	JN600970	JN601014
C. inaequalis	CBS 102.42	Sand dune soil	France	KJ922375	KM061787	KM196574
C. inaequalis	DAOM 20022	*Pisum sativum*	Canada	KJ922374	KM061786	KM196575
C. intermedius	CBS 334.64	–	–	HG778991	HG779155	–
C. intermedius	UTHSC 09–3240	–	–	HE861855	HF565469	–
C. ischaemi	CBS 630.82	–	–	JX256428	JX276440	–
C. ischaemi	ICMP 6172	*Ischaemum indicum*	New Zealand	JX256428	JX276440	–
C. lunata	CBS 730.96	Lung biopsy	USA	JX256429	JX276441	JX266596
C. lunata	CBS 157.34	Unknown substrate	Indonesia	JX256430	JX276442	JX266597
C. malina	CBS 131274	zoysiagrass	USA	JF812154	KP153179	KR493095
C. malina	FLS–119	*Bermuda grass*	USA	KR493070	KR493083	KR493093
C. miyakei	CBS197.29	*Eragrostis pilosa*	Japan	KJ909770	KM083611	KM196568
C. mosaddeghii	**IRAN 3131C; SCUA-Ahv-Syz-P**	***Syzygium cumini***	**Iran**	**MG846737**	**MH392155**	**MH392152**
C. mosaddeghii	**IRAN 3123C; SCUA-Ahw-Vig-1Rb**	***Vigna unguiculata***	**Iran**	**MG971270**	**MG975597**	**MH392151**
C. muehlenbeckiae	AR5180	*Sorghum bicolor*	Japan	KP400649	KP419998	KM196579
C. muehlenbeckiae	MUS 0031	*Sorghum* sp.	USA	KP400647	KP419996	KM196578
C. neergaardii	BRIP 12919	*Oryza sativa*	Ghana	KJ415550	KJ415397	KJ415443
C. neergaardii	DAOM 228085	desert soil	Chile	KJ909784	KM083615	KM196593
C. neoindica	BRIP 17439	*Trianthema portulacastrum*	Australia	AF081449	AF081406	–
C. nicotiae	BRIP 11983	–	–	KJ415551	KJ415396	KJ415442
C. nicotiae	CBS 655.74	Desert soil	Algeria	KJ909772	KM083614	–
C. nisikadoi	CBS 192.29	–	–	AF081447	AF081410	–
C. nodosa	CPC 28801	*Urochloa reptans*	Thailand	MF490817	MF490839	MF490860
C. nodosa	CPC 28812	*Chloris barbata*	Thailand	MF490818	MF490840	MF490861
C. nodulosa	CBS 160.58	–	–	JN601033	JN600975	JN601019
C. oryzae	CBS 169 53	*Oryza sativa*	Vietnam	KP400650	KP645344	KM196590
C. ovariicola	BRIP 15882	–	–	JN601031	JN600971	JN601020
C. ovariicola	CBS 286.91	–	–	HG778994	HG779145	–
C. pallescens	CBS 156.35	Air	Java	KJ922380	KM083606	KM196570
C. papendorfii	BRIP 57608	*Acacia karroo*	–	KJ415552	KJ415395	KJ415441
C. papendorfii	CBS308.67	*Acacia karroo*	South Africa	KJ909774	KM083617	KM196594
C. perotidis	CBS 350.90	*Perotis rara*	Cape York	JN192385	JN601021	–
C. pisi	CBS 190.48	*Pisum sativum*	Canada	KY905678	KY905690	KY905697
C. portulacae	BRIP 14837	*Soil*	–	KJ415554	KJ415392	KJ415439
C. portulacae	CBS 239.48	*Portulaca oleracea*	USA	KJ909775	KM083616	KM230404
C. prasadii	CBS 143.64	*Jasminum sambac*	India	KJ922373	KM061785	KM230408
C. prasadii	CBS 144.64	–	–	HG778997	HG779149	–
C. protuberata	5876	*Fragaria* sp.	–	KT012665	KT012626	KT012587
C. protuberata	CBS 376.65	*Deschampsia flexuosa*	UK	KJ922376	KM083605	KM196576

Table 1 Continued.

Species name	isolate name or strain no.*	Source or host	origin	GenBank Accession numbers		
				ITS	*GPDH*	*EF1α*
C. pseudobrachyspora	CPC 28808	*Eleusine indica*	Thailand	MF490819	MF490841	MF490862
C. pseudolunata	UTHSC 092092	Nasal sinus	USA	HE861842	HF565459	–
C. pseudorobusta	UTHSC 083458	Nasal sinus	USA	HE861838	HF565476	–
C. ravenelii	BRIP 13165	*Sporobolus fertilis*	Australia	JN192386	JN600978	JN601024
C. ravenelii	CBS 127709	–	–	HG778999	HG779109	–
C. richardiae	BRIP 4371	*Richardia brasiliensis*	Australia	KJ415555	KJ415391	KJ415438
C. robusta	CBS624 68	*Dichanthium annulatum*	USA	KJ909783	KM083613	KM196577
C. ryleyi	BRIP 12554	*Sporobolus creber*	–	KJ415556	KJ415390	KJ415437
C. ryleyi	CBS349.90	*Sporobolus creber*	Yetman	KJ909766	KM083612	KM196567
C. senegalensis	CBS 149.71	–	–	HG779001	HG779128	–
C. senegalensis	ZM020571	–	–	JN006787	HQ394208	–
C. soli	CBS 222.96	*soil*	Papua New Guinea	KY905679	KY905691	KY905698
C. sorghina	BRIP 15900	*Sorghum bicolor*	Australia	KJ415558	KJ415388	KJ415435
C. spicifera	CBS 274.52	Soil	Spain	JN192387	JN600979	JN601023
C. subpapendorfii	CBS656.74	Desert soil	Egypt	KJ909777	KM061791	KM196585
C. trifolii	AR5169	*Sorghum bicolor*	South Africa	KP400656	KP645345	KP735694
C. tripogonis	BRIP 12375	Unknown	Australia	JN192388	JN600980	JN601025
C. tropicalis	BRIP 14834	*Coffea arabica*	India	KJ415559	KJ415387	KJ415434
C. tsudae	BRIP 10970a	*Chloris gayana*	Australia	KC424605	KC747755	KC503940
C. tsudae	MAFF 236750	*Chloris gayana*	Japan	KP400651	KM061790	KM230409
C. tuberculata	CBS 14663	*Zea mays*	India	JX256433	JX276445	JX266599
C. uncinata	CBS 221.52	*Oryza sativa*	Vietnam	HG779024	HG779134	–
C. variabilis	CPC 28813	*Digitaria ciliaris*	Thailand	MF490820	MF490842	MF490863
C. variabilis	CPC 28815	*Chloris barbata*	Thailand	NR154866	MF490844	MF490865
C. verruculosa	CBS150 63	*Punica granatum*	India	KP400652	KP645346	KP735695
C. verruculosa	MFLUCC 100690	*Oryza sativa*	Thailand	JX256437	JX276448	JX266602
Curvularia sp.	AR5117	*Lolium perene*	USA	KP400655	KP645349	KP735698
Curvularia sp.	MFLUCC 100709	*Oryza sativa*	Thailand	JX256442	JX276453	–
Curvularia sp.	MFLUCC 100739	*Oryza sativa*	Thailand	JX256443	JX276454	JX266603
Curvularia sp.	MFLUCC 120177	Unknown grass	Thailand	KP400654	KP645348	KP735697
Curvularia 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; 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; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; SCUA: the Collection of Fungal Cultures, Department of Plant Protection, Shahid Chamran University of Ahvaz, Iran

(Schoch et al. 2009). Each polymerase chain reaction (PCR) contained 5 µl of 10x prime Taq Reaction Buffer (GenBio, South Korea), 3 mM $MgCl_2$, 0.4 mM dNTPs (mix), 0.4 µM of each primer, 0.6 µL of Prime Taq DNA Polymerase (5 units/µl, GenetBio), and 5 ng/µL template DNA adjusted with purified water (Mili-Q Water) to a final volume of 50 µl. Amplification reactions started with an initial denaturation step at 95 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 54 °C (ITS), 56 °C (*GPDH*) or 62 °C (*EF1α*) for 40 s and 72 °C for 1 min. A final elongation step at 72 °C for 10 min was set to complete the amplification.

Purification and sequencing

The PCR products were size-fractionated by gel electrophoresis in 1% agarose (Hispan Agar) run in 1.0× Tris-acetic acid-EDTA (TAE) buffer stained with commercial safe stain (SinaClon, Iran) and then photographed under UV light. Amplified PCR products of the expected size were excised and purified by GF-1 AmbiClean Kit (Vivantis, Malaysia) according to the manufacturer's instructions. The purified amplicons were read in both directions using original PCR primers by Macrogen Company (Humanizing Genomics, Macrogen, South Korea). BioEdit Sequence Alignment Editor Version 7.0.9.0 (Hall 1999) was used to edit raw ABI chromatograms, after which the forward and reverse sequences were assembled using DNA Baser Sequence Assembler v4 programs (2013, Heracle BioSoft, www.DnaBaser.com). The generated sequences were deposited in GenBank (Table 1).

Phylogenetic analyses

The nucleotide sequences of ITS, *GPDH* and *EF1α* regions were compared to those of reference in GenBank using maximum likelihood (ML) algorithm in MEGA version 6 (Tamura et al. 2013). The combined datasets of ITS-*GPDH* (two-locus) and ITS-*GPDH*-*EF1α* (three-locus) used for phylogenetic analysis contained the sequence of the isolates under study and reference strains from previously known species of *Curvularia*, as well as outgroup taxon (Table 1). These reference sequences (Table 1) were mostly from Manamgoda et al. (2015), Madrid et al. (2014), Tan et al. (2014), Marin-Felix et al. (2017a, b). The species of *Bipolaris drechsleri* was used as outgroup taxon to root phylogenetic trees. Single and multiple sequence alignments were generated with Clustal W in MEGA version 6 (Tamura et al. 2013) using these parameters: pairwise alignment (gap opening 15, gap extension 6.6) and multiple alignment (gap opening 15, gap extension 6.6, transition weight 0.5, delay divergent sequences 30%). Alignments were checked visually and edited manually where necessary. The phylograms were constructed with best-fitting nucleotide substitution model, as suggested by ML model test using MEGA version 6 (Tamura et al. 2013), Subtree-Pruning-Regrafting (SPR) algorithm and following options: 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. Two combined matrix used for phylogenetic analysis were deposited in the Treebase database (http://purl.org/phylo/treebase/phylows/study/TB2: S22797).

Results

Sequences analysis, BLASTn algorithm and phylogeny

PCR fragments of approx. 500 bp were successfully amplified for ITS region using primer pair ITS1 and ITS4 and approx. 1100 bp for *EF1α* were obtained using the EF1-983 and EF1-2218R primers pair. Amplicons of approx. 700 bp was successfully produced for *GPDH* using primers gpd1 and gpd2. The sequences of ITS, *GPDH* and *EF1α* belonging to the isolates under study were submitted to GenBank (Table 1). The isolates of the new described and previously known species of *Curvularia* shared 74% sequence identity in the ITS region (468 bp) attributed to 36 SNPs and 82 bp insertion/deletion, 60% sequence identity in the *GPDH* region (507 bp) attributed to 86 SNPs and 111 bp insertion/deletion, and 77% sequence identity in the *EF1α* region (677 bp) attributed to 125 SNPs.

In the BLASTn analysis of the ITS sequence, the closest related species to *Curvularia mosaddeghii* were *C. hominis* (HG779011) and *C. muehlenbeckiae* (HG779002) with 98% sequence identity. In the analysis of the *GPDH*, the species with the highest sequence identity to new species were *Curvularia hominis* (HG779106), *Curvularia muehlenbeckiae* (HG779108) and *Curvularia pisi* (KY905690) with 96%, 95% and 95% similarity, respectively. The *EF1α* sequences of the new species showed maximum identity to different species, including *Curvularia akaiiensis*, *C. australiensis*, *C. buchloes*, *C. chiangmaiensis*, *C. hawaiiensis*, *C. pisi*, *C. sorghina*, *C. subpapendorfii*, *C. tropicalis* and *C. variabilis*.

The combined ITS-*GPDH* and ITS-*GPDH-EF1α* datasets consisted of 228 and 258 sequences from 114 and 86 taxa including the outgroup taxon, respectively (Table 1). The best model of evolution for phylogenetic analysis was the Tamura-Nei with Invariant Site and Gamma Distribution (TN93 + G + I). ITS-*GPDH* tree was constructed to show delimitation of new species to some *Curvullaria* species which their *EF1α* sequences were not available and excluded in three-locus based tree (not shown, supplemented figure). In both two- and three-locus based trees (Fig. 1), the isolates of *Curvularia mosaddeghii* formed a monophyletic clade with strong bootstrap 99% support, distinct from the other previously known species of *Curvularia*. Phylogenetic analyses based on ITS-*GPDH* and ITS-*GPDH-EF1α* dataset showed that the closest relatives of new species are *C. hominis*, *C. muehlenbeckiae* and *C. pisi* with 73% and 93% BS support, respectively (Fig. 1).

Taxonomy

Curvularia mosaddeghii M. Mehrabi-Koushki & R. Farokhinejad, sp. nov., Fig. 2

MycoBank: MB 825506; Facesoffungi number: FoF04768

Etymology – In reference to Prof. S. Saeed Mosaddegh who significantly contributed to the progress of plant protection science in Khuzestan Province, Iran.

Typification – IRAN, KHUZESTAN PROVINCE: Ahvaz. From jambolan leaf spot (*Syzygium cumini*), October 2017, *K. Heidari and R. Farokhinejad* (holotype: IRAN 16938F), ex-type cultures: IRAN 3131C = SCUA-Ahv-Syz-P).

Morphology on PDA – *Hyphae* sub-hyaline to brown, branched, septate, thin and smooth-walled, 3–5.25(–7) μm diam. *Conidiophores* arising singly, septate, generally equal wide in basal parts, tapering in median and upper parts, straight or flexuous, geniculate in apex, frequently unbranched, cells walls thicker than those of the vegetative hyphae, pale brown to brown, 30–161(–227) × (2.6–)3–5.1 μm, 95% confidence limits = 77.7–104.8 × 3.6–4 μm, ($\bar{x}$ ± SD = 91 ± 45 × 3.8 ± 0.6 μm, n = 60). *Conidiogenous cells* mostly integrated, smooth or with verruculose nodes, terminal or intercalary, proliferating sympodially, with circular and thickened scars, brown, cylindrical to swollen. *Conidia* with bipolar-germination, (1–)4-celled, smooth-walled or slightly verruculose, asymmetrically swollen and curved at the third cell from base, rarely symmetric swelling and straight, pale to dark brown, end cells paler and thin-walled than central cells, 11.1–26.3(–32) × 7.1–10(–11.1) μm, 95% confidence limits = 19.9–22.5 × 8.6–9.2 μm, ($\bar{x}$ ± SD = 21.2 ± 4.3 × 8.9 ± 1 μm, n = 60). *Hilum* very slightly protruding, darkened, thickened, 1.5–2.3 μm diam. *Chlamydospore* and *sexual morph* not observed.

Cultural characters on PDA – Colonies growing 55–60 mm diam after 8 d incubation at 28±0.5 °C, circular with filiform margin, dark green to greenish black, aerial mycelium sparse to moderate, floccose with age; reverse greyish green to brownish black.

Habitats – jambolan and cowpea

Distribution – Iran (Ahvaz).

Additional cultures examined – IRAN, KHUZESTAN PROVINCE: Ahvaz. Endophyte in cowpea root (*Vigna unguiculata*), Sep-2016, *S. Janbozorgi and M. Mehrabi-Koushki* (IRAN 3123C = SCUA-Ahw-Vig-1Rb).

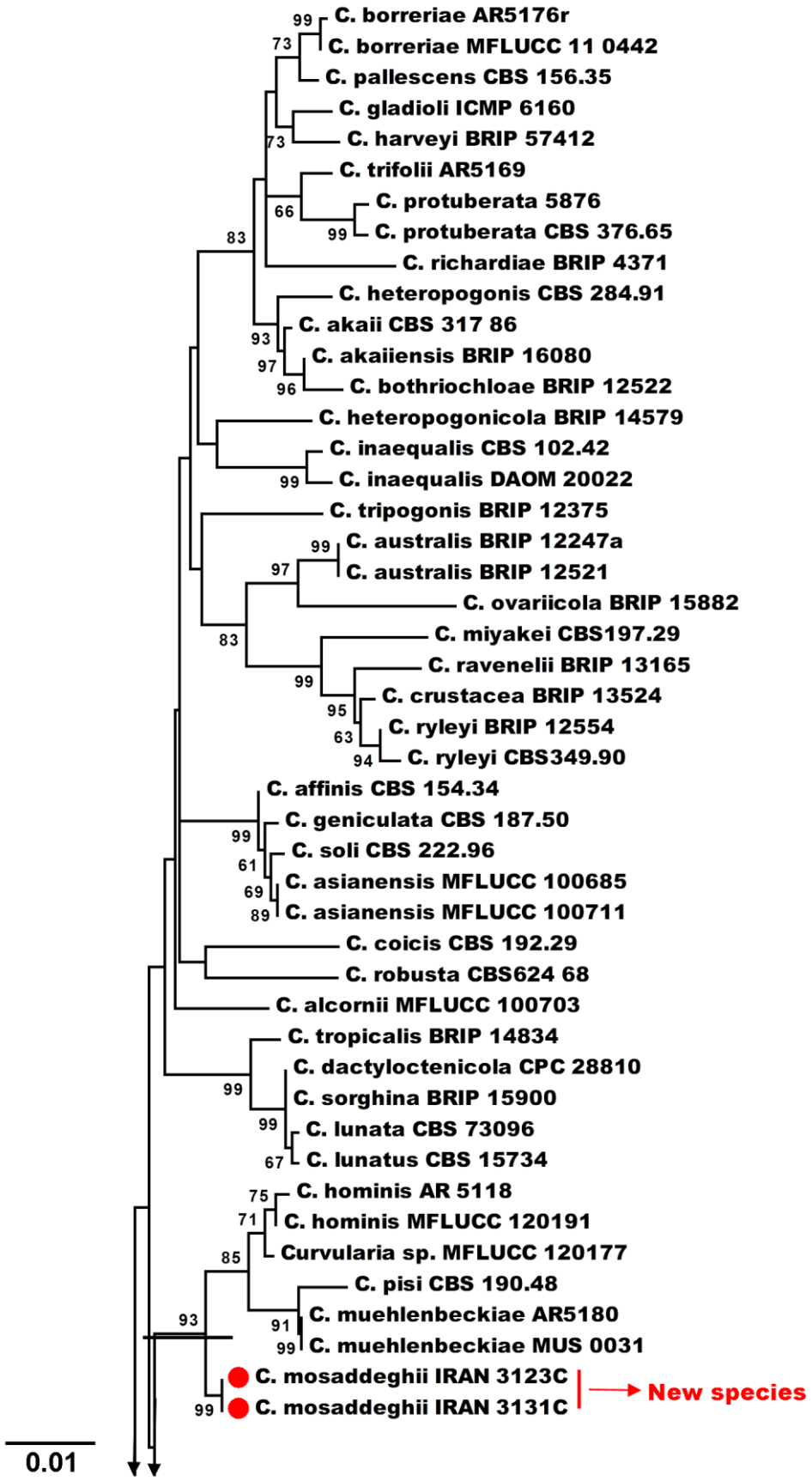

Figure 1 – Phylogenetic tree generated from a ML analysis based on a concatenated alignment of ITS, GPDH and EF1α sequences of 85 *Curvullaria* strains representing most previously known species and new taxon. Bootstrap values greater than 50% (expressed as percentages of 1000 replications) are shown at the nodes.

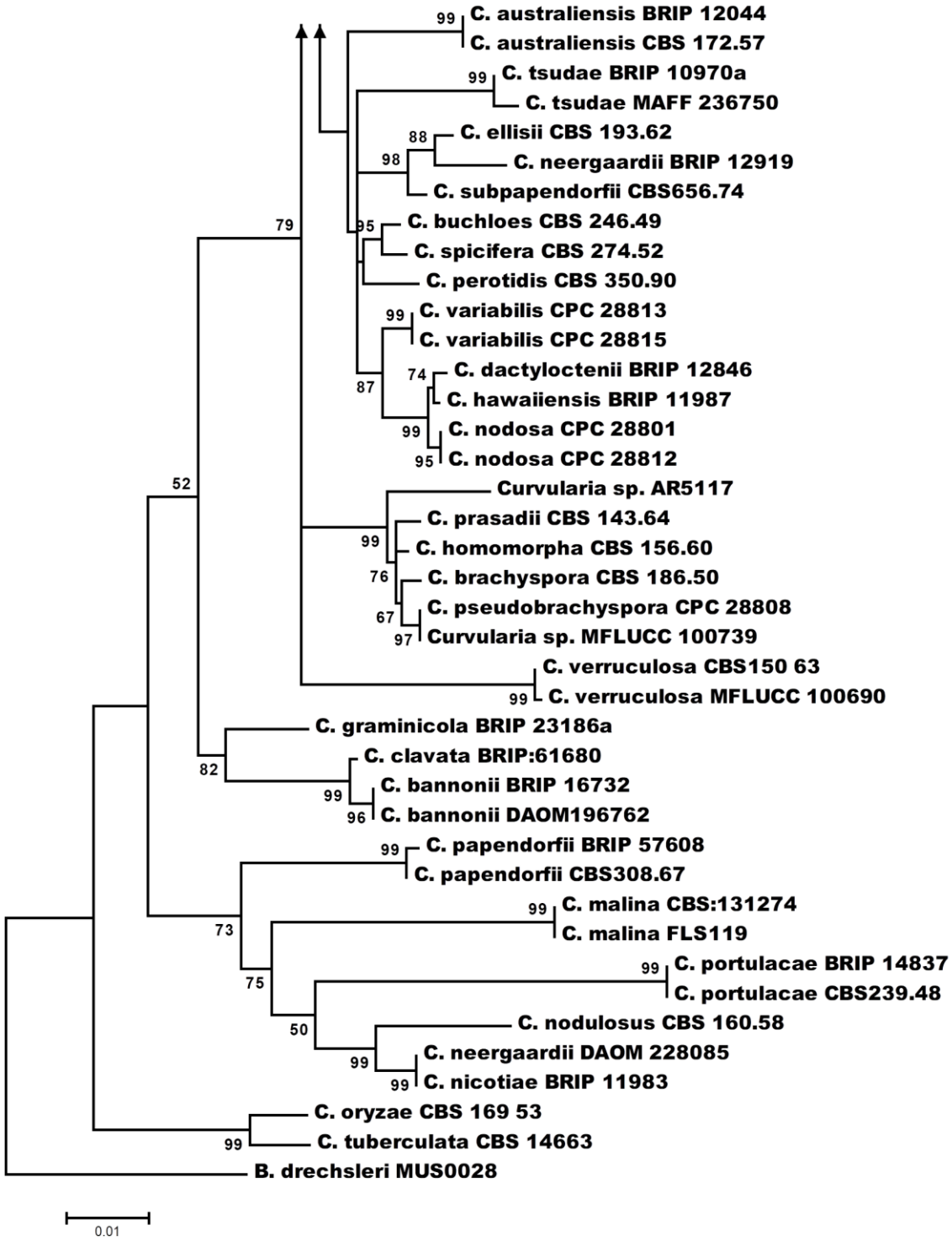

Figure 1 – Continued.

Notes – *Curvularia mosaddeghii* is slightly different morphologically from closely related species of *Curvularia,* i.e. *C. hominis* Da Cunha, Madrid, Gené & Cano (Madrid et al. 2014), *C. muehlenbeckiae* Madrid, Da Cunha, Gené, Guarro & Crous (Madrid et al. 2014) and *C. pisi* Y. Marin & Crous (Marin-Felix et al. 2017a). *C. mosaddeghii* can be easily distinguished from three other species by the production of less wide conidia (7.1–10 μm wide vs. 7–14, 8.5–12 and 9–15.5 μm wide in *C. hominis*, *C. muehlenbeckiae* and *C. pisi*, respectively). The conidia in *C. mosaddeghii*, *C. muehlenbeckiae* and *C. pisi* differ from *C. hominis* in being less septate

(predominantly 3-distoseptate vs. 3-4-distoseptate). In addition, hila in *C. mosaddeghii* are narrower than the three other species.

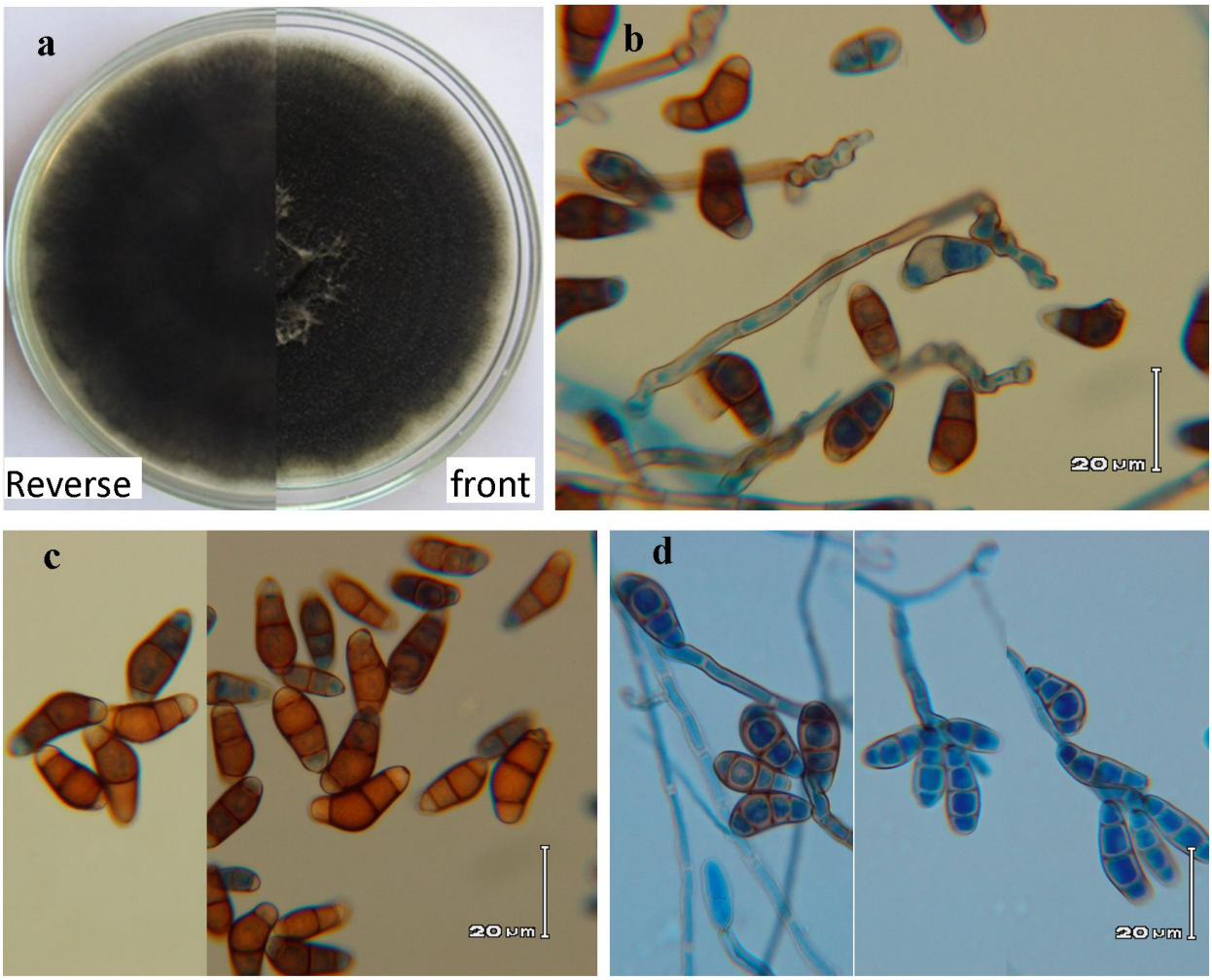

Figure 2 – *Curvularia mosaddeghii* (IRAN 3131C). a Colony on PDA (front and reverse). b–d Conidiophores and conidia.

Discussion

In our study, a new species of *curvularia* is described, which are ascribed to the new species of *Curvularia mosaddeghii*. Multi-locus phylogenetic analysis and morphology showed that *C. mosaddeghii* isolates are quite distinct from the other previously described *Curvularia* species, such as *C. hominis*, *C. muehlenbeckiae* and *C. pisi*. Our phylogenetic analyzes included all the *Curvularia* taxa used in the studies of Manamgoda et al. (2015), Madrid et al. (2014), Tan et al. (2014), Tomaso-Peterson et al. (2016) and Marin-Felix et al. (2017a, b). In three-locus based phylogenetic tree, the combined dataset (inferred from ITS, *GPDH* and *EF1α* gene sequences) was sufficient to delimit most closely related species in genus *Curvularia*. Here, the *GPDH* sequence showed the highest degree of polymorphism among the genomic regions used.

Curvularia mosaddeghii were isolated from jambolan leaf spot and cowpea healthy root, none of its near relatives, including *C. hominis*, *C. muehlenbeckiae* and *C. pisi*, have yet been reported from these hosts. *Curvularia hominis* and *C. muehlenbeckiae* have been isolated from human tissues and *Muehlenbeckia* sp. leaf in USA, respectively (Madrid et al. 2014). *C. pisi* was reported from the seeds of *Pisum sativum* in Canada (Marin-Felix et al. 2017a). *Curvularia mosaddeghii* is known to be as endophyte within *Vigna unguiculata*, which is also host for three other species of *Curvularia*, *C. eragrostidis* (Henn.) J.A. Mey., *C. lunata* (Wakker) Boedijn and *C. verruculosa* Tandon & Bilgrami (Ahmad et al., 1993, Manamgoda et a., 2011, Mogle and Maske

2012, Abdulwehab et al. 2015). However, these species are morphologically and phylogenetically different to *C. mosaddeghii*. Morphologically, these species differ from *C. mosaddeghii* in having wider conidia.

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References

Abdulwehab SA, El-Nagerabi SAF, Elshafie AE. 2015 – Leguminicolous fungi associated with some seeds of Sudanese legumes. Biodiversitas 16, 269-280.

Ahmad I, Iftikhar S, Bhutta AR. 1993 – Seed-borne microorganisms in Pakistan, Check List 1991. Pakistan Agricultural Research council. Islamabad. 32 pp.

Ahmadpour SA, Mehrabi-Koushki M, Farokhinejad R. 2017 – *Neodidymelliopsis farokhinejadii*, a new fungal species from dead branches of trees in Iran. Sydowia 69,171–182

Ariyawansa HA, Thambugala K, Manamgoda DS, Jayawardena R et al. 2015 - Towards a natural classification and backbone tree for Pleosporaceae. Fungal Diversity 71, 85–139.

Beneke ES, Rogers AL. 1996 – Medical mycology and human mycoses. Star Publishing Company, Belmont, 239 pp.

Berbee M, Pirseyedi M, Hubbard S. 1999 – *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 91, 964–977.

Boedijn KB. 1933 – Ueber einige phragmosporen Dematiazeen. Bulletin du Jardin botanique de Buitenzorg 13, 120–134.

da Cunha KC, Sutton DA, Fothergill AW, Gene J, Cano J, Madrid H, de Hoog S, Crous PW, Guarroa J. 2013 – In vitro antifungal susceptibility and molecular identity of 99 clinical isolates of the opportunistic fungal genus *Curvularia*. Diagnostic Microbiology and Infectious Disease 76, 168–174.

Gautam AK, Kant M, Thakur Y. 2013 – Isolation of endophytic fungi from *Cannabis sativa* and study their antifungal potential. Archive of Phytopathology and Plant Protection 46, 627–635.

Hall TA. 1999 – BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.

Hyde KD, Nilsson RH, Alias SA, Ariyawansa HA et al. 2014 – One stop shop: backbones trees for important phytopathogenic genera: I. Fungal Diversity 67, 21–125.

Jena SK, Tayung K. 2013 – Endophytic fungal communities associated with two ethno-medicinal plants of Similipal Biosphere Reserve, India and their antimicrobial prospective. Journal of Applied Pharmaceutical Science 3, 7.

Kornerup A,Wanscher JH. 1967 – Methuen handbook of colour. Methuen & Co. Ltd, London

Madrid H, da Cunha KC, Gene J, Dijksterhuis J, Cano J, Sutton DA, Guarro J, Crous PW. 2014 – Novel *Curvularia* species from clinical specimens. Persoonia 33, 48–60.

Manamgoda DS, Cai L, Bahkali AH, Chukeatirote E, Hyde KD. 2011 – *Cochliobolus*: an overview and current status of species. Fungal Diversity 51, 3–42.

Manamgoda DS, Cai L, McKenzie EH, Crous PW, Madrid H, Chukeatirote E, Shivas RG, Tan YP, Hyde KD. 2012a – A phylogenetic and taxonomic re-evaluation of the *Bipolaris-Cochliobolus-Curvularia* complex. Fungal Diversity 56, 131–44.

Manamgoda DS, Cai L, McKenzie EHC, Chukeatirote E, Hyde KD. 2012b – Two new *Curvularia* species from northern Thailand. Sydowia 64, 255–266.

Manamgoda DS, Rossman AY, Castlebury LA, Chukeatirote E, Hyde KD. 2015 – taxonomic and phylogenetic re-appraisal of the genus *Curvularia* (Pleosporaceae): human and plant pathogens. Phytotaxa 212, 175–198.

Manamgoda DS, Rossman AY, Castlebury LA, Crous PW, Madrid H, Chukeatirote E, Hyde KD. 2014 – The genus *Bipolaris*. Studies in Mycology 79, 221–288.
Marin-Felix Y, Groenewald JZ, Cai L, Chen Q, Marincowitz S et al. 2017a – Genera of phytopathogenic fungi: GOPHY 1. Studies in Mycology 86, 99–216.
Marin-Felix Y, Senwanna C, Cheewangkoon R, Crous PW. 2017b – New species and records of *Bipolaris* and *Curvularia* from Thailand. Mycosphere 8, 1556–1574.
Mogle UP, Maske SR. 2012 – Efficacy of bioagents and fungicides on seed mycoflora, germination and vigour index of cowpea. Science Research Reporter 2, 321–326.
Rangaswamy BE, Francis F, Prakash KK, Manjunath NS. 2013 – Variability in airborne bacterial and fungal population in the tertiary health care centre. Aerobiologia 29, 473–479.
Raeder U, Broda P. 1985 – Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology 1, 17–20.
Riddle OC, Briggs FN. 1950 – Inheritance of resistance to scald in barley. Hilgardia 20, 19–27.
Schoch C, Crous PW, Groenewald J, Boehm E, Burgess TI, De Gruyter J, De Hoog G, Dixon L, Grube M, Gueidan C. 2009 – A class-wide phylogenetic assessment of *Dothideomycetes*. Studies in Mycology 64, 1–15.
Scott EM, Carter RT. 2014 – Canine keratomycosis in 11 dogs: A case series (2000–2011). Journal of the American Animal Hospital Association 50, 112–118.
Sivanesan A. 1987 – Graminicolous species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and their teleomorphs. Mycological Papers 158, 1–261.
Tadych M, Bergen M, Johnson JC, Polashock J, Vorsa N. 2012 – Endophytic and pathogenic fungi of developing cranberry ovaries from flower to mature fruit: diversity and succession. Fungal Diversity 54, 101–116.
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013 – MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30, 2725-2729.
Tan YP, Madrid H, Crous PW, Shivas RG. 2014 – Johnalcornia gen. et. comb. nov., and nine new combinations in *Curvularia* based on molecular phylogenetic analysis. Australasian Plant Pathology 43, 589–603.
Tomaso-Peterson M, Jo YK, Vines PL, Hoffmann FG. 2016 – *Curvularia malina* sp. nov. incites a new disease of warm-season turfgrasses in the southeastern United States. Mycologia 108, 915–924.
Verma P, Singh S, Singh R. 2013 – Seven species of *Curvularia* isolated from three lakes of Bhopal. Advances in Life Science and Technology 8, 13–15.
White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA,Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322.
Wijayawardene NN, Hyde KD, Rajeshkumar KC, Hawksworth DL et al. 2017 – Notes for genera: Ascomycota. Fungal Diversity 86, 1–594.
Wijayawardene NN, Hyde KD, Lumbsch T, Liu JK et al. 2018 – Outline of Ascomycota – 2017. Fungal Diversity 88, 167–263.