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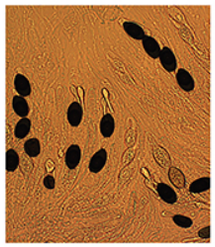

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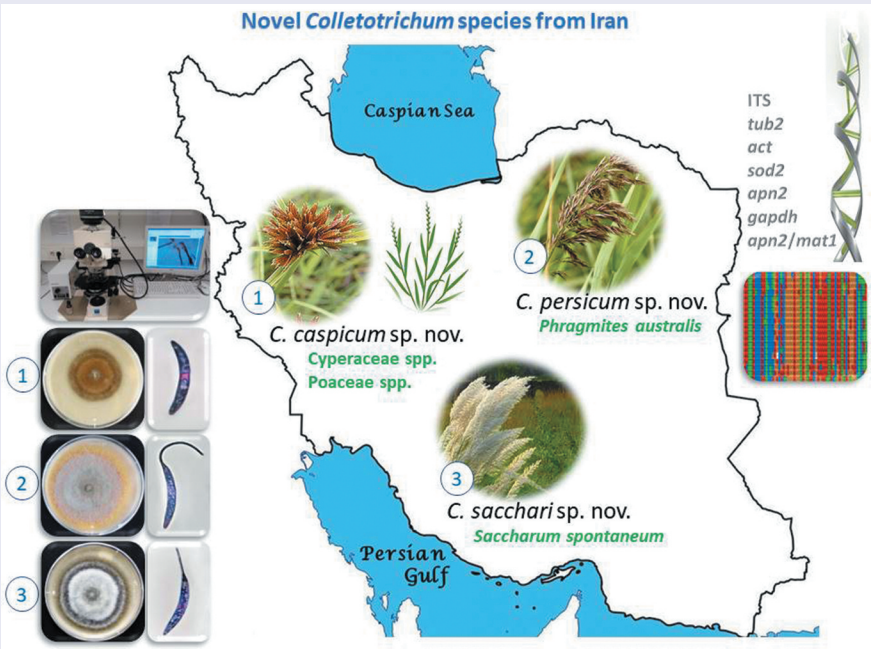
New species of *Colletotrichum* from wild Poaceae and Cyperaceae plants in Iran

A. Alizadeh [a], M. Javan-Nikkhah [b], R. Nourmohammadi Nazarian [a], F. Liu [c], R. Zare [d], K. B. Fotouhifar [b], E. H. Stukenbrock [e], and U. Damm [f]

[a]Department of Plant Protection, Azarbaijan Shahid Madani University, Tabriz 5375171379, Iran; [b]Department of Plant Protection, Faculty of Agricultural Science and Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj 77871-31587, Iran; [c]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No. 3 1st Beichen West Road, Chaoyang District, 100101, Beijing, China; [d]Department of Botany, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), P.O. Box 19395-1454, Tehran, Iran; [e]Environmental Genomics, Botanical Institute, Christian-Albrechts University of Kiel, Germany and Max Planck Institute for Evolutionary Biology, Plön, Germany; [f]Department of Botany, Senckenberg Museum of Natural History Görlitz, PF 300 154, 02806 Görlitz, Germany

ABSTRACT

Twenty-two *Colletotrichum* strains were isolated from anthracnose symptoms or leaf spots on leaves of various wild Poaceae and Cyperaceae plants collected in three provinces of Iran and tentatively identified as belonging to the Graminicola species complex based on morphology. All strains were studied via a polyphasic approach combining colony characteristics, morphology and phylogeny inferred from multi-locus sequences, including the nuc rDNA ITS1-5.8S-ITS2 (ITS), partial sequences of the β-tubulin (*tub2*), actin (*act*), manganese superoxide dismutase 2 (*sod2*), DNA lyase 2 (*apn2*) genes, a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), and the intergenic spacer between the *apn2* gene and the *mat1* idiomorph (*apn2/mat1*). Six species were distinguished, including three new species, namely *C. caspicum, C. persicum*, and *C. sacchari*, and three previously described species, *C. cereale, C. nicholsonii* and *C. sublineola*. Comprehensive morphological descriptions and illustrations are provided for all species. Furthermore, this study provided new insights into the distribution and host range of known species.



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INTRODUCTION

Colletotrichum species are mainly known as pathogens of major importance causing anthracnose diseases of a wide range of plant families in tropical, subtropical, and temperate climates, but also of crown and stem rots, red rots, ripe rot, damping-off of blossoms, seedling blights and brown blotch diseases (Alizadeh et al. 2015;

CONTACT U. Damm ulrike.damm@senckenberg.de

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Arzanlou et al. 2015; Cannon et al. 2012; Lenné 2002; Marin-Felix et al. 2017). Some species are responsible for significant yield losses of economically important fruits such as strawberry, citrus, and banana, as well as many other crops including coffee, cereals, legumes, vegetables, forage plants and ornamentals (Atghia et al. 2015; Backman et al. 1982; Cannon et al. 2012; Liu et al. 2015; Phoulivong et al. 2010; Prihastuti et al. 2009; Smith and Black 1990; Yan et al. 2015).

Accurate identification of plant pathogenic fungi is a key step to study species diversity and epidemiology and therefore of uttermost importance for the development of effective control strategies for plant diseases (Alizadeh et al. 2015; Cai et al. 2009). Traditionally, the host-plant association has been used as the basis for identification and defining species in the genus *Colletotrichum*. Accordingly, several hundred species were described assuming that *Colletotrichum* species were specific to their host plants (Cannon et al. 2012). Von Arx (1957) treated many of these species as synonyms and reduced the number of *Colletotrichum* species to 11 based on morphological characters with little or no attention to host specificity, which represented a drastic move in *Colletotrichum* systematics. Consequently, *Colletotrichum* species have been identified and delimited on morphological characters for many years (Simmonds 1965; Sutton 1992; Than et al. 2008a, 2008b; Thaung 2008). Delineation of *Colletotrichum* species based on morphology alone however is nearly impossible due to the lack of reliable morphological characters (Cai et al. 2009; Cannon et al. 2012; Hyde et al. 2010, 2009b).

In recent years, multi-locus sequence analyses combined with geographical, ecological, pathological and morphological data have been used for species differentiation within the genus *Colletotrichum* (Cai et al. 2009; Marin-Felix et al. 2017). This strategy led to significant progress in the systematics of *Colletotrichum* and revealed about 250 species within the genus, most of them belonging to large species complexes, namely the Acutatum, Agaves, Boninense, Dematium, Destructivum, Dracaenophilum, Gigasporum, Gloeosporioides, Graminicola, Magnum, Orbiculare, Orchidearum, Spaethianum and Truncatum species complexes (Bhunjun et al. 2021; Crouch et al. 2009c; Damm et al. 2009, 2012a, 2012b, 2013, 2014, 2019; Liu et al. 2013, 2014; Weir et al. 2012).

Colletotrichum species associated with more than 40 genera of Poaceae as well as with *Bletilla ochracea* (Orchidaceae) belong to the Graminicola species complex (Crouch 2014; Crouch et al. 2009a, 2009c, 2009d; Crouch and Tomaso-Peterson 2012; Moriwaki and Tsukiboshi 2009; Tao et al. 2013). Seven species from Poaceae hosts and the orchid *Bletilla ochracea* that are closely related to *C. caudatum*, form falcate conidia with a filiform appendage, except for one species, *C. ochraceae*, and are either regarded as the Caudatum subclade of the Graminicola species complex (Bhunjun et al. 2021; Crouch 2014; Tao et al. 2013; Zhang et al. 2020) or as Caudatum species complex (Jayawardena et al. 2016; Marin-Felix et al. 2017).

Within a survey, *Colletotrichum* species were isolated from wild plants with anthracnose symptoms or leaf spots collected in Northern Iran; some of them were previously reported (Alizadeh et al. 2015). Iran is a country with a high biodiversity that includes more than 8000 recorded plant species (Owfi 2020), of which many are considered as endemic or native plants (Noroozi et al. 2016). Wild Poaceae and Cyperaceae (Poales) species are regarded as the most important weeds in Iran (Keshavarzi 2021). However, the knowledge of *Colletotrichum* species associated with these plants in Iran is very low, and there is no report of a *Colletotrichum* species from any Poaceae or Cyperaceae in Iran (Farr and Rossman 2021), except for a recent report of *C. karsti* on *Cyperus* sp. in Iran originating from the same survey as this study (Alizadeh et al. 2015). *Colletotrichum karsti* forms straight conidia and belongs to the *C. boninense* species complex (Damm et al. 2012b). However, the majority of strains from the two plant families formed curved conidia and were closely related to the Graminicola species complex based on preliminary blastn searches on NCBI GenBank.

Therefore, the aim of this study is to characterize the *Colletotrichum* species from wild Poaceae and Cyperaceae plants in Northern Iran, based on a combination of morphology, culture characteristics, and multigene analyses.

MATERIALS AND METHODS

Sample collection and fungal isolation.—Samples were collected from wild Poaceae and Cyperaceae plants with anthracnose symptoms or leaf spots in the Alborz, Golestan and Guilan provinces of Iran during 2011–2013. Leaf samples (approximately 1 cm diam) were washed in tap water, surface-disinfected in 2% sodium hypochlorite solution for 1 min, rinsed in sterile distilled water and moist incubated in glass petri dishes on autoclaved paper towels soaked with sterile tap water. Petri dishes were kept at 20–25°C in the dark. Conidial masses or mycelia indicative of *Colletotrichum* were transferred to water agar (WA, 2%) plates supplemented with chloramphenicol (50 mg/L). Single spore or single hyphae isolates were obtained on potato dextrose agar (PDA, Merck, Darmstadt, Germany) (Goh 1999) and deposited

in the Mycology Laboratory of the College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran (UTFC) and the Iranian Fungal Culture Collection (IRAN) at the Iranian Research Institute of Plant Protection, Tehran, Iran. The ex-type strains of new species and some additional strains were deposited in the culture collections of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands (CBS) and the Senckenberg Museum of Natural History Görlitz, Germany (GLMC), as well as the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany (DSMZ). Type specimens were deposited in the herbarium of the Iranian Research Institute of Plant Protection (IRAN) (TABLE 1).

Morphological analysis.—Both cultural and microscopic features were studied on synthetic nutrient agar (SNA, Nirenberg 1976), and on oatmeal agar (OA, Crous et al. 2019) according to Alizadeh et al. (2015). Cultures were inoculated with 5-mm diam plugs from 4–7 d old cultures. To enhance sporulation, isolates were transferred to SNA amended with autoclaved filter paper and double-autoclaved stems of *Anthriscus sylvestris*. SNA and OA cultures were incubated at 25°C under near-UV light with a 12-hours photoperiod for 10 d. Measurements and photomicrographs of fungal structures (conidia, conidiophores, conidiomata, setae and appressoria) were made according to Damm et al. (2007). Appressoria were observed on the reverse side of SNA plates. Conidia were taken from acervuli. Microscopic preparations were made in clear lactic acid or methylene blue, with at least 40 measurements per structure and observed with a Nikon SMZ1000 dissecting microscope (DM) or with an Olympus BX53 microscope with differential interference contrast illumination (DIC). Colony characters and pigment production on SNA and OA were documented after 10 d. Growth rates were measured after seven and ten days. The descriptions provided were based on ex-type strains; data of strains with distinct morphological differences were added.

Phylogenetic analysis.—Fungal isolates on PDA were incubated at 25°C for 7–10 d. Genomic DNA was extracted using a standard phenol-chloroform extraction protocol (Sambrook and Russel 2001). The 5.8S nuclear ribosomal RNA gene with the two flanking internal transcribed spacers (ITS), a partial sequence of the manganese superoxide dismutase gene (*sod2*), the 3′ end of the DNA lyase gene (*apn2*), the 5′ end of the DNA lyase gene and mating type protein genes-like gene (*apn2*/*mat1*), partial sequences of the beta-tubulin (*tub2*) and actin (*act*) gens and a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) were amplified using the primer pairs ITS-1 + ITS-4 (White et al. 1990), SOD625F/R, Apn1W1F/R and mat1M72F/R (Crouch et al. 2009c), GDF1 + GDR1 (Guerber et al. 2003), *Act*-512F + *Act*-783R (Carbone and Kohn 1999) and T1 (O'Donnell and Cigelnik 1997) + Bt-2b (Glass and Donaldson 1995), respectively, following the protocols explained in the respective references, except for *tub2, act* and *gapdh*. The PCR for these three loci was performed as following: an initial step of 5 min at 98°C, 35 cycles of 10 s at 98°C, 20 s at 65°C and 20 s at 72°C, followed by 10 min at 72°C. PCR was performed in a TProfessional Thermocycler (Biometra, Germany) in a total volume of 25 µL. The PCR mixture contained 1 µL genomic DNA, 0.2 µM of each primer, 1 × HF Phusion PCR buffer (Thermo Scientific, Germany), 2 mM MgCl2, 20 µM of each dNTP, 0.75µ DMSO and 0.25 U Phusion High-Fidelity polymerase (Thermo Scientific, Germany). The PCR products were purified by the Wizard Genomic DNA Purification Kit (Promega, USA) and sequenced by Macrogen (Amsterdam, the Netherlands) with the amplifying primers.

The DNA sequences were used to obtain consensus sequences using BioNumerics 7.6.3 (Applied Math, St-Marthens-Lathem, Belgium); alignments were assembled and manually adjusted using BioNumerics and Sequence Alignment Editor 2.0a11 (Rambaut 2002). The sequences of the examined Iranian *Colletotrichum* isolates were compared with other fungal DNA sequences using the blast tools of the NCBI GenBank database (www.ncbi.nlm.nih.gov/genbank/) and the EPPO-Q-bank Fungi database (https://qbank.eppo.int/fungi/). Sequence data from ex-type and reference strains of known *Colletotrichum* species of the Graminicola species complex including its Caudatum subclade and of the outgroup *C. gloeosporioides* strain CBS 112999 (Cannon et al. 2012; Crouch 2014; Crouch et al. 2009a, 2006, 2009c; Crouch and Tomaso-Peterson 2012; Crouch et al. 2009d; Damm et al. 2012b; Moriwaki and Tsukiboshi 2009; O'Connell et al. 2012; Prihastuti et al. 2010; Rojas et al. 2010; Tao et al. 2013; Weir et al. 2012; Zhang et al. 2020) were obtained from NCBI GenBank (TABLE 1).

Maximum parsimony analyses were performed on multi-locus alignments 1 (ITS, *sod2, apn2, apn2/mat1*) following the studies of Crouch et al. (2009c) and Crouch (2014), and 2 (ITS, *tub2, act, gapdh*) following Tao et al. (2013) as well as for each locus separately with PAUP (Phylogenetic Analysis Using Parsimony) 4.0b10 (Swofford 2003) using the heuristic search option with 100 random sequence additions and tree bisection and

Table 1. Strains of *Colletotrichum* spp. studied, with collection details and GenBank accession numbers.

Species	Isolate[1]	Host	Host family	Country	GenBank number[2]						
					ITS	*sod2*	*apn2*	*apn2/mat1*	*tub2*	*act*	*gapdh*
C. alcornii	IMI 176619*	*Imperata cylindrica*	Poaceae	Australia	JX076858	EU554187	EU364987	FJ377901	–	–	–
C. axonopodis	IMI 279189*	*Axonopus fissifolius*	Poaceae	Australia	MN521699	–	EU364993	FJ377907	**MW740339**	**MW822638**	**MW740213**
C. baltimorense	BPI892771 = SD11*	*Sorghastrum nutans*	Poaceae	USA	JX076866	JX076886	JX076927	JX076905	–	–	–
C. baltimorense	BPI892765 = SD2	*Sorghastrum nutans*	Poaceae	USA	JX076867	JX076887	–	JX076906	–	–	–
C. baltimorense	BPI892768 = SD7	*Sorghastrum nutans*	Poaceae	USA	–	JX076890	JX076930	JX076909	–	–	–
C. baltimorense	BPI892769 = SD6	*Sorghastrum nutans*	Poaceae	USA	JX076869	JX076889	JX076929	JX076908	–	–	–
C. baltimorense	BPI892766 = SD3	*Sorghastrum nutans*	Poaceae	USA	JX076868	JX076888	JX076928	JX076907	–	–	–
C. baltimorense	SD9	*Sorghastrum nutans*	Poaceae	USA	JX076870	JX076891	JX076931	JX076910	–	–	–
C. caspicum	IRAN 4290C = UTFC 359	*Cynodon dactilon*	Poaceae	Iran	**MW741438**	**MW822609**	**MW822632**	**MW915575**	**MW740341**	**–**	**–**
C. caspicum	IRAN 3708C = UTFC 364	*Cyperus* sp.	Cyperaceae	Iran	**MW741440**	**MW822611**	**MW822634**	**MW915577**	**MW740343**	**–**	**–**
C. caspicum	IRAN 3709C = UTFC 365*	*Cyperus* sp.	Cyperaceae	Iran	**MW741443**	**MW822612**	**MW822635**	**MW915578**	**MW740344**	**–**	**–**
C. caspicum	IRAN 4292C = UTFC 373	*Cyperus* sp.	Cyperaceae	Iran	**MW741441**	**MW822613**	**MW822636**	**MW915579**	**MW740345**	**–**	**–**
C. caspicum	LC7364	Poaceae sp.	Poaceae	China	**MW741437**	**MW822608**	**–**	**–**	**MW740340**	**MW822639**	**–**
C. caspicum	IRAN 4291C = UTFC 362	Poaceae sp.	Poaceae	Iran	**MW741439**	**MW822610**	**MW822633**	**MW915576**	**MW740342**	**–**	**–**
C. caspicum	IRAN 4293C = UTFC 376	Poaceae sp.	Poaceae	Iran	**MW741442**	**MW822614**	**MW822637**	**MW915580**	**MW740346**	**–**	**–**
C. caudasporum	CGMCC 3.15106*	*Bletilla ochracea*	Orchidaceae	China	JX625162	–	–	–	JX625190	KC843526	KC843512
C. caudatum	BPI423339* (lectotype)	*Sorghastrum nutans*	Poaceae	USA	–	JX076915	–	–	–	–	–
C. caudatum	CBS 131602	*Sorghastrum nutans*	Poaceae	USA	JX076860	JX076878	JX076932	JX076893	MW740347	MW822640	MW740214
C. cereale	CBS 305.69	*Brachypodium sylvaticum*	Poaceae	Germany	EU554109	EU554213	EU365016	FJ377930	**MW740349**	**MW822642**	**MW740216**
C. cereale	CBS 129663 = KS-20BIG	*Bromus inermis*	Poaceae	USA	JQ005774	DQ133277	EU365064	FJ377977	**MW740348**	JQ005837	**MW740215**
C. cereale	IRAN 3717C = UTFC 383	*Lolium* sp.	Poaceae	Iran	–	**MW822621**	**MW822631**	**–**	**MW740352**	–	–
C. cereale	IRAN 3705C = UTFC 360	*Saccharum spontaneum*	Poaceae	Iran	**MW741436**	**MW822619**	**MW822629**	**MW915573**	**MW740350**	–	–
C. cereale	IRAN 3706C = UTFC 361	*Saccharum spontaneum*	Poaceae	Iran	**MW741467**	**MW822620**	**MW822630**	**MW915574**	**MW740351**	–	–
C. duyunense	CGMCC 3.15105*	*Bletilla ochracea*	Orchidaceae	China	JX625160	–	–	–	JX625187	KC843530	KC843515
C. echinochloae	MAFF 511473*	*Echinochloa utilis*	Poaceae	Japan	AB439811	AB440153	–	–	–	–	–
C. eleusines	MAFF 511155*	*Eleusines indica*	Poaceae	Japan	JX519218	EU554234	EU365038	–	JX519243	JX519234	**MW740217**
C. endophytum	CGMCC 3.15107	*Bletilla ochracea*	Orchidaceae	China	HM751814	–	–	–	JX625196	KC843533	KC843520
C. endophytum	CGMCC 3.15108*	*Bletilla ochracea*	Orchidaceae	China	JX625177	**MW822615**	–	–	JX625206	KC843532	KC843521
C. eremochloae	CBS 129661 = C05*	*Eremochloae ophiuroides*	Poaceae	USA	JX519220	JQ478449	–	JQ478476/JQ478462	JX519245	JX51923	**MW740218**
C. eremochloae	CBS 129664 = C01	*Eremochloae ophiuroides*	Poaceae	USA	JQ478446	JQ478448	–	JQ478475/JQ478461	**MW740355**	**MW822645**	**MW740219**
C. falcatum	CBS 127945 = LC885 = CGMCC 3.14187*	*Saccharum officinarum*	Poaceae	Indonesia	JQ005772	–	HM569770	HM569769	JQ005856	JQ005835	**MW740220**
C. falcatum	MAFF 306170	*Saccharum officinarum*	Poaceae	Japan	EU554111	EU554214	EU365018	FJ377932	–	–	–
C. falcatum	MAFF 306299	*Saccharum officinarum*	Poaceae	Thailand	EU554112	EU554215	EU365019	FJ377933	–	–	–
C. gloeosporioides	CBS 112999*	*Citrus sinensis*	Rutaceae	Italy	JQ005152	JX010365	GU994416	JQ899278	JQ005587	JQ005500	JQ005239
C. graminicola	CBS 130836 = M1001 = CgM2	*Zea mays*	Poaceae	USA	JQ005767	XM_008091695	EU365081	FJ377994	JQ005851	JQ005831	**MW740221**
C. graminicola	CBS 130839	*Zea mays*	Poaceae	Brazil	JQ005768	–	EU365082	FJ377995	JQ005852	JQ005832	**MW740222**
C. hainanense	CBS 14590 = XJ 9–1*	*Axonopus compressus*	Poaceae	China	KY242705	KY242711	KY242714	KY242708	–	–	**–**
C. hainanense	CBS 145901 = YF 10–2	*Axonopus compressus*	Poaceae	China	KY242706	KY242712	KY242715	KY242709	–	–	**–**
C. hanaui	MAFF 305404*	*Digitaria ciliaris*	Poaceae	Japan	JX519217	EU554205	EU365008	FJ377922	JX519242	–	**MW740223**
C. hanaui	MAFF 511014	*Digitaria ciliaris*	Poaceae	Japan	EU554124	EU554227	EU365031	FJ377944	–	–	–
C. jacksonii	MAFF 511152	*Echinochloa esculenta*	Poaceae	Japan	EU554130	EU554233	EU365037	FJ377950	–	–	–
C. jacksonii	MAFF 305460*	*Echinochloa esculenta*	Poaceae	Japan	JX519216	EU554212	–	FJ377929	JX519241	JX519233	**MW740224**
C. miscanthi	CGMCC 3.15116	*Bletilla ochracea*	Orchidaceae	China	HM751812	–	–	–	JX625189	KC843531	KC843519
C. miscanthi	MAFF 510857*	*Miscanthus sinensis*	Poaceae	Japan	JX519221	EU554224	EU365028	–	JX519246	JX519237	**MW740225**
C. navitas	CBS 125086 = 9038–158a*	*Panicum virgatum*	Poaceae	USA	JQ005769	GQ919073	–	GQ919069/GQ919071	JQ005853	JQ005832	**MW740209**
C. navitas	9032d	*Panicum virgatum*	Poaceae	USA	GQ919068	GQ919074	–	GQ919070/GQ919072	–	–	–
C. nicholsonii	MAFF 305391	*Paspalum dilatatum*	Poaceae	Japan	EU554099	#	EU365006	FJ377920	–	–	–

(*Continued*)

Table 1. (Continued).

Species	Isolate[1]	Host	Host family	Country	GenBank number[2]						
					ITS	*sod2*	*apn2*	*apn2/mat1*	*tub2*	*act*	*gapdh*
C. nicholsonii	MAFF 305428	*Paspalum dilatatum*	Poaceae	Japan	EU554103	EU554207	EU365010	FJ377924	–	–	–
C. nicholsonii	MAFF 510916	*Paspalum dilatatum*	Poaceae	Japan	EU554122	EU554225	EU365029	FJ377942	–	–	–
C. nicholsonii	MAFF 511115*	*Paspalum dilatatum*	Poaceae	Japan	JQ005770	EU554229	EU365033	FJ377946	JQ005854	JQ005833	**MW740226**
C. nicholsonii	IRAN 4294C = UTFC 366	*Paspalum dilatatum*	Poaceae	Iran	–	**MW822616**	**MW822625**	**MW915571**	**MW740364**	–	–
C. nicholsonii	IRAN 3710C = UTFC 368	*Paspalum dilatatum*	Poaceae	Iran	–	**MW822617**	**–**	**–**	**–**	–	–
C. nicholsonii	IRAN 3715C = UTFC 379	*Paspalum dilatatum*	Poaceae	Iran	–	**MW822618**	**–**	**–**	**–**	–	–
C. ochraceae	CGMCC 3.15102	*Bletilla ochracea*	Orchidaceae	China	JX625166	–	–	–	JX625194	KC843528	KC843514
C. ochraceae	CGMCC 3.15103	*Bletilla ochracea*	Orchidaceae	China	JX625167	–	–	–	JX625195	KC843529	KC843516
C. ochraceae	CGMCC 3.15104*	*Bletilla ochracea*	Orchidaceae	China	JX625156	–	–	–	JX625183	KC843527	KC843513
C. paspali	MAFF 305403*	*Paspalum notatum*	Poaceae	Japan	JX519219	EU554204	EU365007	FJ377921	JX519244	JX519235	**MW740210**
C. paspali	MAFF 511000	*Paspalum notatum*	Poaceae	Japan	EU554123	EU554226	EU365030	FJ377943	–	–	–
C. persicum	IRAN 3711C = UTFC 369	*Phragmites australis*	Poaceae	Iran	**MW741432**	**MW822603**	**MW822623**	**MW915569**	**MW740366**	**MW822654**	**MW740227**
C. persicum	IRAN 3712C = UTFC 370*	*Phragmites australis*	Poaceae	Iran	**MW741433**	**MW822604**	**MW822624**	**MW915570**	**MW740367**	**MW822655**	**MW740228**
C. sacchari	IRAN 3707C = UTFC 363*	*Saccharum spontaneum*	Poaceae	Iran	**MW741431**	**MW822602**	**MW822622**	**MW915568**	**MW740368**	**MW822656**	**MW740229**
C. somersetense	CBS 131599 = JAC 11–11 = BPI892770*	*Sorghastrum nutans*	Poaceae	USA	JX076862	JX076880	JX076918	JX076895	**MW740374**	**MW822658**	**MW740230**
C. somersetense	CBS 131601 = JAC 11–13 = BPI892764	*Sorghastrum nutans*	Poaceae	USA	JX076863	JX076881	JX076919	JX076896	**MW740375**	**MW822659**	**MW740231**
C. somersetense	JAC 11–10	*Sorghastrum nutans*	Poaceae	USA	JX076861	JX076879	JX076917	JX076894	–	–	–
C. somersetense	JAC 11–14	*Sorghastrum nutans*	Poaceae	USA	JX076864	–	JX076920	JX076897	–	–	–
C. somersetense	JAC 11–15	*Sorghastrum nutans*	Poaceae	USA	JX076865	–	JX076921	JX076898	–	–	–
C. sublineola	IRAN 4297C = UTFC 381	*Saccharum spontaneum*	Poaceae	Iran	**MW741435**	–	**MW822628**	–	**MW740373**	–	–
C. sublineola	CBS 131301*	*Sorghum bicolor*	Poaceae	Burkina Faso	JQ005771	DQ132051	EU365121	FJ378029	JQ005855	JQ005834	**MW740232**
C. sublineola	MAFF 305361	*Sorghum bicolor*	Poaceae	Japan	AB057439	EU554198	EU365001	FJ377915	–	**–**	–
C. sublineola	MAFF 510020	*Sorghum bicolor*	Poaceae	Japan	#	EU554219	EU365023	FJ377937	–	–	–
C. sublineola	IRAN 3714C = UTFC 372	*Sorghum helepense*	Poaceae	Iran	–	**MW822605**	–	–	**MW740370**	–	–
C. sublineola	IRAN 4295C = UTFC 378	*Sorghum helepense*	Poaceae	Iran	–	**MW822606**	**MW822626**	–	**MW740371**	–	–
C. sublineola	IRAN 4296C = UTFC 380	*Sorghum helepense*	Poaceae	Iran	**MW741434**	**MW822607**	**MW822627**	**MW915572**	**MW740372**	–	–
C. zoysiae	MAFF 238573*	*Zoysia tenuifolia*	Poaceae	Japan	JX076871	–	JX076922	JX076899	–	–	–
C. zoysiae	MAFF 238574	*Zoysia tenuifolia*	Poaceae	Japan	JX076872	JX076882	JX076923	JX076900	–	–	–
C. zoysiae	MAFF 238575	*Zoysia tenuifolia*	Poaceae	Japan	JX076873	JX076883	–	JX076901	–	–	–
C. zoysiae	MAFF 238576	*Zoysia tenuifolia*	Poaceae	Japan	JX076874	JX076884	JX076924	JX076902	–	–	–
C. zoysiae	MAFF 238577	Zoysia tenuifolia	Poaceae	Japan	JX076875	JX076885	JX076925	JX076903	–	–	–

[1]BPI: U.S. National Fungus Collections, USA; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; IRAN: Iranian Fungal Culture Collection at the Iranian Research Institute of Plant Protection, Tehran, Iran; LC: Working collection of Lei Cai, housed at the Chinese Academy of Sciences, Institute of Microbiology, State Key Laboratory of Mycology, Beijing, China; MAFF: MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; UTFC: Culture collection of the University of Tehran, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

[2]ITS: internal transcribed spacers and intervening 5.8S nrDNA; *sod2*: partial sequence of the manganese superoxide dismutase gene; *apn2*: the 3′ end of the DNA lyase gene; *apn2/mat1*: the 5′ end of the DNA lyase gene and mating type protein genes-like gene, *tub2*: partial beta-tubulin gene; *act*: partial actin gene; *gapdh*: partial glyceraldehyde-3-phosphate dehydrogenase gene. Sequences generated in this study are emphasised in bold face.

#sequence downloaded from NIAS GenBank (https://www.gene.affrc.go.jp/databases-micro_search_en.php). *ex-holotype, ex-epitype or ex-neotype culture or lectotype.

reconstruction (TBR) as the branch-swapping algorithm. The individual gene trees were assessed for clade conflicts between phylogenies. Alignment gaps were treated as missing and all characters were unordered and of equal weight. No more than 10 trees of score (length) greater than or equal to 10 were saved in each replicate. Tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for the resulting trees. The robustness of the trees obtained was evaluated by 100 000 bootstrap replications using the fast-stepwise addition algorithm (Hillis and Bull 1993). A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) for the combined sequence datasets. Models of nucleotide substitution for each gene determined by MrModeltest 2.3 (Nylander 2004) were included for each gene partition. The analyses of two MCMC chains were run from random trees for 1000 000 generations and sampled every 100 generations. The likelihood score of the two runs were 3400 and 3000 for the first analysis and 1100 and 1000 for the second analysis and therefore, the first 3200 and 1050 (the averages of both) trees, respectively, were discarded as the burn-in phases of the analyses and posterior probabilities determined from the remaining trees. Sequences derived in this study have been lodged in GenBank, the alignment and trees in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S28088), and taxonomic novelties in MycoBank.

RESULTS

In the analyses of multi-locus alignment 1 (gene boundaries of ITS: 1–572, *sod2*: 583–1 328, *apn2*: 1339–2182, *apn2*/*mat1*: 2193–3779), 78 strains from Poaceae and Cyperaceae as well as the outgroup, and 3779 characters including the alignment gaps were processed, of which 1379 characters were parsimony-informative, 509 parsimony-uninformative and 1891 constant. After a heuristic search using PAUP, 430 equally most parsimonious trees were retained (length = 3747 steps, CI = 0.670, RI = 0.880, RC = 0.590, HI = 0.330), of which one is shown in FIG. 1. The topology of these trees was similar, which was verified for a large selection of trees (not shown). They differed not only in the position of strains within species and of branch lengths, but also in the position of some of the species. Within the Caudatum subclade, especially the position of single-strain species and of the two *C. caudatum* sequences differed. For the Bayesian analysis, a GTR+I+G model was selected for ITS, a HKY+I+G model for *sod2* and a GTR+G model for *apn2* and *apn2*/*mat1*, and incorporated in the analysis. The consensus tree obtained from Bayesian analysis confirmed the tree topology obtained with parsimony (not shown). Most of the Bayesian posterior probability values agreed with bootstrap support values.

The three strains UTFC 363, UTFC 369 and UTFC 370 belong to a clade with a bootstrap support of 79% and a Bayesian posterior probability value of 1 representing the Caudatum clade of the Graminicola species complex. Within this clade, there are few supported clades; strains UTFC 369 and UTFC 370 form one of them (91/0.93), while strain UTFC 363 forms a single-strain clade. Most of the remaining species of the Graminicola species complex were represented by well supported clades. Each three strains collected in Iran cluster within *C. cereale* (99/1) and *C. nicholsonii* (87/1). Further four strains group within the *C. sublineola*/*eremochloae* clade (88/1). Seven strains from Poaceae and Cyperaceae from Iran and one strain from China form a well-supported clade basal to the other species of the Graminicola species complex (99/1). In some of the 430 most parsimonious trees, however, this clade was not basal, but clustered with the *C. sublineola*/*eremochloae* clade.

In the analyses of multi-locus alignment 2 (gene boundaries of ITS: 1–572, *tub2*: 583–1324, *act*: 1335–1632, *gapdh*: 1643–1973), 46 strains from Poaceae and Cyperaceae as well as the outgroup and 1973 characters including the alignment gaps were processed, of which 512 characters were parsimony-informative, 193 parsimony-uninformative and 1268 constant. After a heuristic search using PAUP, 711 equally most parsimonious trees were retained (length = 1 367 steps, CI = 0.699, RI = 0.851, RC = 0.594, HI = 0.301), of which one is shown in FIG. 2. The topology of these trees was similar, which was verified for a large selection of trees (not shown). They differed mainly in the position of strains within the species and branch lengths of clades. In some trees, *C. duyunense* was basal to the remaining species of the Caudatum subclade, and in some trees, the *C. eremochloae* clade was integrated in the *C. sublineola* clade, but separate in the others. For the Bayesian analysis, a GTR+I+G model was selected for ITS, a HKY+G model for *tub2*, a GTR+I model for *gapdh*, and a HKY+I model for *act*, and incorporated in the analysis. The consensus tree obtained from Bayesian analysis confirmed the tree topology obtained with parsimony (not shown). Most of the Bayesian posterior probability values agreed with bootstrap support values.

Strains UTFC 363, UTFC 369 and UTFC 370 belong to a long-branched clade with a bootstrap support of

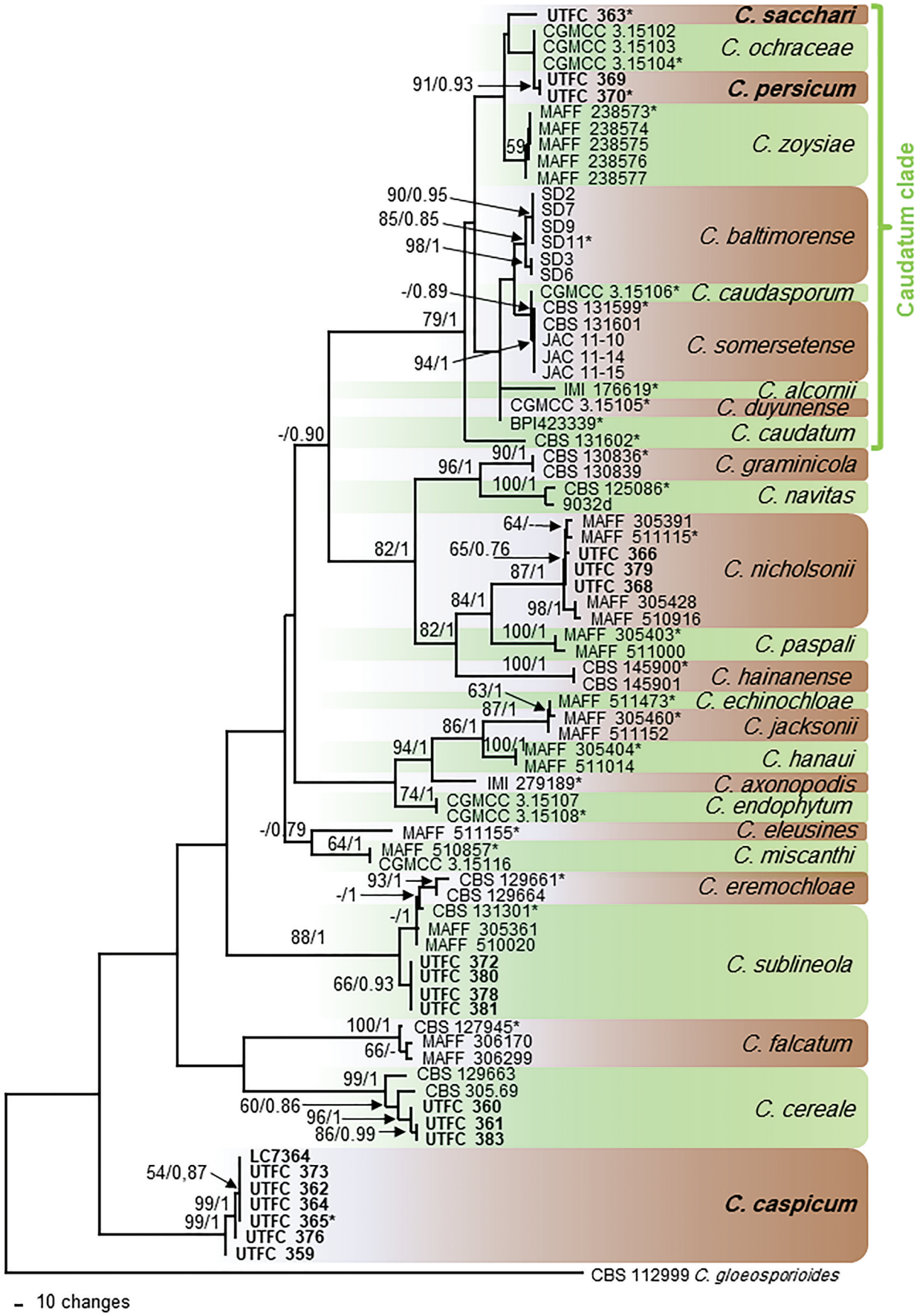

Figure 1. The first of 430 equally most parsimonious trees obtained from a heuristic search of the combined ITS, *sod2, apn2*, and *apn2/mat1* sequence alignment of the Graminicola species complex. Bootstrap support values above 50 % and Bayesian posterior probability values above 0.70 are shown at the nodes. *Colletotrichum gloeosporioides* strain CBS 112999 is used as outgroup. Numbers of types or ex-type strains are emphasized with an asterisk. The Caudatum subclade is indicated with a green bracket.

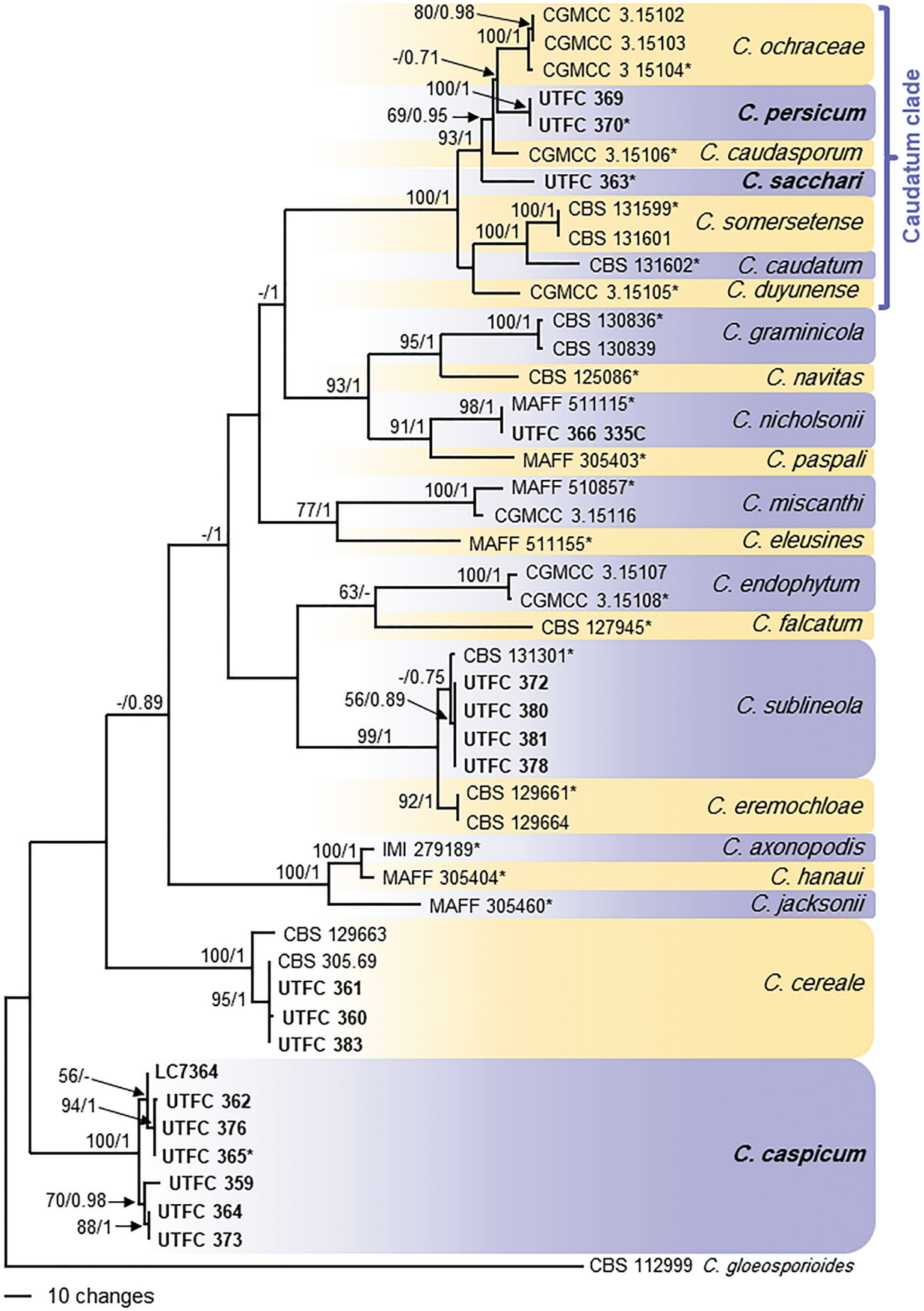

Figure 2. The first of 711 equally most parsimonious trees obtained from a heuristic search of the combined ITS, *tub2, act*, and *gapdh* sequence alignment of the Graminicola species complex. Bootstrap support values above 50 % and Bayesian posterior probability values above 0.70 are shown at the nodes. *Colletotrichum gloeosporioides* strain CBS 112999 is used as outgroup. Numbers of types or ex-type strains are emphasized with an asterisk. The Caudatum subclade is indicated with a blue bracket.

100% and a Bayesian posterior probability value of 1, representing the Caudatum clade of the Graminicola species complex. Strains UTFC 369 and UTFC 370 form a well-supported clade (100/1), while strain UTFC 363 forms a single-strain clade on a long branch. The clade formed by strains UTFC 369 and UTFC 370 was well supported in all single-locus phylogenies, except for ITS (not shown). Both clades are sister clades of *C. ochraceae* and *C. caudasporum*. Most of the other species of the Graminicola species complex were represented by well supported clades. Seven strains from Poaceae and Cyperaceae from Iran and one strain from China form a well-supported clade basal to the other species of the Graminicola species complex (100/1). Strains UTFC 360, UTFC 361 and UTFC 363 cluster with *C. cereale* and strain UTFC 366 with *C. nicholsonii* with high support values (100/1 and 98/1, respectively); four further strains group with *C. sublineola* with low support (-/0.75).

The topologies of the single-locus phylogenies (not shown) did not show any conflicts, which allowed us to combine them in multi-locus alignments 1 and 2, respectively; they also agreed with the topologies of the respective multi-locus phylogenies. The placement of the strains isolated in this study was the same in the single-locus phylogenies as in the multi-locus phylogenies, and the clades formed by unidentified strains were supported in all of the single-locus phylogenies, except for the clade formed by strains UTFC 369 and UTFC 370 that was not supported in the ITS phylogeny; the branch of these two strains and that of UTFC 363 was very short.

TAXONOMY

Based on the DNA sequence data and morphology, the 22 isolates (TABLE 1) from wild Poaceae and Cyperaceae plants in Iran belong to six *Colletotrichum* species, including *C. cereale, C. nicholsonii* and *C. sublineola* and three species that are new to science. All species studied in culture are characterized below.

Colletotrichum caspicum Alizadeh, Damm, F. Liu, Jav.-Nikkh. & Stukenbr. sp. nov. FIG. 3
MycoBank: MB839808

Typification: IRAN. GUILAN: Rasht, from leaves of *Cyperus* sp., Aug 2012, *A. Alizadeh* (**holotype** IRAN 17632F). Ex-holotype culture IRAN 3709C = UTFC 365 = CBS 148570 = DSM 113396 = GLMC 2641. GenBank: ITS = MW741443; *sod2* = MW822612; *apn2* = MW822635; *apn2/mat1* = MW915578; *tub2* = MW740344.

Diagnosis: Conidia on SNA measure 14–25 × 3–4.5 µm and on *Anthriscus* stem 14–28 × 2.5–4.5 µm, appressoria on SNA 5–21 × 4–9 µm (all strains included). The species differs from all other *Colletotrichum* species by its unique DNA sequences (ITS, *sod2, apn2, apn2/mat1, tub2, act*) and from its closest relative *C. cereale* also by its slower growth and lower sporulation.

Etymology: Referring to the Caspian Sea, near which this fungus was first collected.

On Anthriscus *stem:* Conidiomata scarce, not observed in strains UTFC 359, UTFC 362 and UTFC 364, basal cells not observed, setae and conidiophores mostly formed directly on hyphae. Setae medium to dark brown, 3–5-septate, 58–103 µm long, smooth-walled, tapering to an acute apex, base cylindrical, conical or inflated, 4.5–7.5 µm diam, no setae observed in UTFC 359. Conidiophores rarely observed, hyaline to pale brown, simple or septate and branched, up to 60 µm long. Conidiogenous cells hyaline to very pale brown, smooth-walled, cylindrical, 5–25 × 3–4 µm, opening 1–1.5 µm, collarette and periclinal thickening not observed. Conidia enteroblastic, aseptate, hyaline, smooth-walled, falcate, almost uniformly curved, apex ± acute, base truncate, 14–25 × 3–4.5 µm, mean ± SD = 20.5 ± 2.6 × 3.7 ± 0.3 µm, L/W ratio = 5.6. Conidia of strain UTFC 376 larger and narrower, measuring 21–28 × 2.5–4 µm, mean ± SD = 25 ± 1.7 × 3.3 ± 0.28 µm, L/W ratio = 7.6. UTFC 359 not sporulating.

On SNA: Hyphae hyaline, smooth-walled, septate, branched, 1–6 µm diam. Conidiomata rarely observed, conidiophores and setae mostly formed directly on hyphae. Setae only observed in UTFC 376, medium to dark brown, smooth-walled, 2–4-septate, straight, 67–130 µm long, tapering to an acute apex, base cylindrical to conical, sometimes inflated, 5–9.5 µm diam. Conidiophores rarely observed, hyaline to pale brown, simple or septate and branched, up to 60 µm long. Conidiogenous cells hyaline to very pale brown, smooth-walled, cylindrical, 5–27 × 3–4 µm, opening 1–1.5 µm, collarette and periclinal thickening not observed. Conidia enteroblastic, aseptate, hyaline, smooth-walled, falcate, almost uniformly curved, apex ± acute, base truncate, 14.5–25 × 3–4.5 µm, mean ± SD = 21 ± 2.5 × 3.9 ± 0.3 µm, L/W ratio = 5.2. UTFC 359 not sporulating. Hyphal appressoria solitary, aseptate, smooth-walled, pale to medium brown, globose, subglobose to ellipsoidal, sometimes clavate or irregulare shaped, entire edge, more or less lobed, 5–9 × 4–6 µm, mean ± SD = 7.5 ± 1 × 5 ± 0.6 µm, L/W ratio = 1.5. Appressoria of strain UTFC 376 larger, measuring 6–21 × 4–9 µm, mean ± SD = 11.3 ± 3.9 × 6.4 ± 1.6 µm, L/W ratio = 1.8.

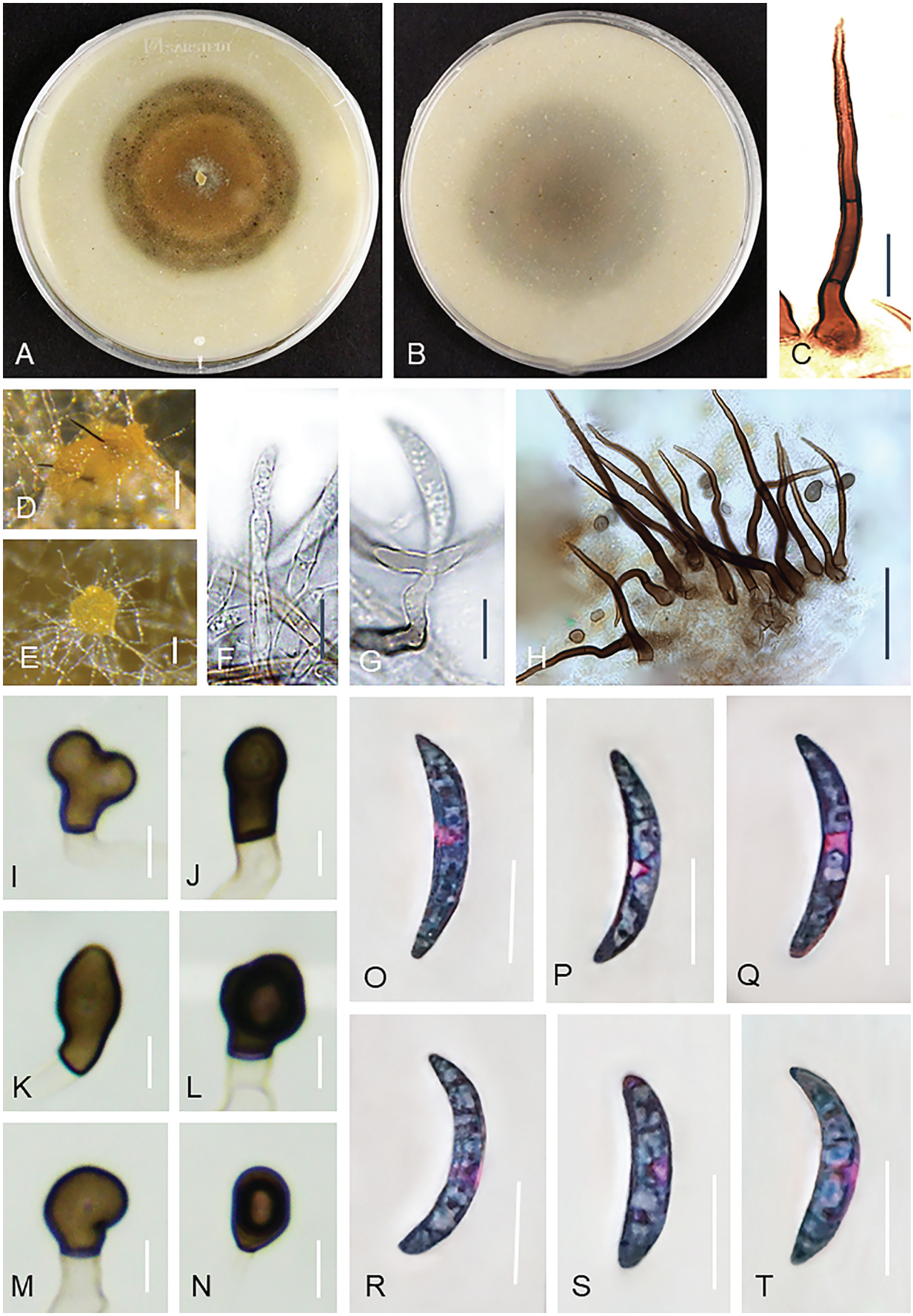

Figure 3. *Colletotrichum caspicum* (A–C, E, G, I–T from ex-holotype strain UTFC 365. D from strain UTFC 376. F, H from strain UTFC 373). A–B. Colony on OA after 7 d, A. upper and B. reverse side. C, H. Setae. D–E. Acervuli. F–G. Conidiophores. I–N. Appressoria. O–T. Conidia. C–H, O–T. from *Anthriscus* stem. I–N. from SNA. D, E. DM. C, F–T. DIC. Scale bars: D, E = 50 µm, C, F–T = 10 µm.

Culture characteristics: Colonies on OA flat and effuse with entire margin, ocher brown, with a grayish brown margin, reverse pale brown, 57–60 mm diam in 7 d. Colony color very different in strain UTFC 359, yellow, ocher brown to grayish brown at the center, reverse pale yellow to honey, yellowish gray in the center. Colonies on SNA flat with entire margin, hyaline to pale honey, reverse same colors, 55–60 mm diam in 7 d (>85 mm diam in 10 d). Conidia in mass buff to orange.

Additional specimens examined: IRAN. GUILAN: Rasht, from leaves of *Cyperus* sp., Aug 2012, *A. Alizadeh*, culture IRAN 3708C = UTFC 364; IRAN. GOLESTAN: Gorgan, Tooskestan, from leaves of a Poaceae sp., Aug 2011, *A. Alizadeh*, culture IRAN 4291C = UTFC 362; IRAN. ALBORZ: Karaj, from leaves of *Cynodon dactilon*, Jun 2012, *A. Alizadeh*, culture IRAN 4290C = UTFC 359 = CBS 148569 = DSM 113395 = GLMC 2642; IRAN. GUILAN: Lahijan, from leaves of a Poaceae sp., Oct 2013, *A. Alizadeh*, culture IRAN 4293C = UTFC 376; IRAN. GOLESTAN: Bandar Torkaman, from leaves of *Cyperus* sp., Oct 2013, *A. Alizadeh*, culture IRAN 4292C = UTFC 373.

Notes: Colletotrichum caspicum was isolated from Poaceae and Cyperaceae species, which indicates its wide host range. While several species were described from different Poaceae, no *Colletotrichum* species was previously described from *Cyperus* species (Cyperaceae). However, a few species were reported from this host genus (Farr and Rossman 2021), including *C. fructicola* on *Cyperus microiria* in Japan (Hirayama et al. 2018), *C. karsti* on *Cyperus* sp. in Iran (Alizadeh et al. 2015), and *C. truncatum* and its synonym *C. dematium* f. *truncatum* on *Cyperus rotundus* in Brazil and in the United States, respectively (Damm et al. 2009; Roy 1982), only the latter forms curved conidia, and all belong to other species complexes than *C. caspicum*.

Colonies of *C. caspicum* differ from those of its closest relative *C. cereale* that is faster growing and more strongly sporulating than *C. caspicum*; the orange conidial masses of *C. cereale* are visible in the center of the OA colony (FIGS. 3A, 4A).

Colletotrichum caspicum is not closely related to any other *Colletotrichum* species and can be identified with sequences of all loci available. In blastn searches on NCBI GenBank, the ITS sequence of the ex-type strain, UTFC 365, matched with 99% identity (1 and 2 nucleotides difference, respectively) with *Colletotrichum* sp. 2 strain C_3_1 from leaves of *Carex secalina* (Cyperaceae) in Poland (MG978338, Górzyńska et al. 2019) and *Colletotrichum* sp. strain MBD_1013 from a leaf of *Dalea purpurea* (Fabaceae) in the USA (MK595495, M. B. DeMers and G. May, unpubl. data), while the closest ex-type strain was with 97% identity (17 nc. diff.) that of *C. verruculosum*, IMI 45525 (GU227806), belonging to the Spaethianum species complex (Damm et al. 2009). Closest matches with the *act* sequence of *C. caspicum* strain LC7364 were with 93% identity (17 nc. diff.) *C. cereale* strains CBS 129663 (JQ005837, O'Connell et al. 2012) and CGMCC 3.15110 (KC843534, Tao et al. 2013). Closest matches with the *tub2* sequences of *C. caspicum* were with 89% identity several *C. tofieldiae* strains and with 88% identity *C. graminicola* strain STE-U 5298 (AY376587, Lubbe et al. 2004); closest ex-type strain was with 89% identity that of *C. hanaui*. Closest matches with the *sod2, apn2* and *apn2/mat1* sequences of *C. caspicum* were with ≤ 90% and ≤ 82% identity, respectively, those of several species of the Graminicola species complex.

Colletotrichum cereale Manns, Ohio Agric. Exp. Stn. Bull. 203: 207 (1909). FIG. 4

On Anthriscus stem: Conidiomata acervular, abundant. Setae abundant, medium to dark brown, 2–4-septate, straight, 120–270 µm long, smooth-walled, tip rounded to somewhat acute, base cylindrical to conical, sometimes inflated, 4–8 µm diam. Conidiophores hyaline to pale brown, simple, septate, up to 80 µm long. Conidiogenous cells hyaline to pale brown, smooth-walled, clavate or cylindrical, 5–18 × 3–6 µm, opening 1.5–2 µm, collarette 1–1.5 µm long, periclinal thickening visible. Conidia enteroblastic, aseptate, hyaline, smooth-walled, falcate, central part often with nearly parallel walls, more strongly curved toward the acute apex, base truncate, 22.5–28.5 × 4–4.5 µm, mean ± SD = 24.7 ± 1.4 × 4.5 ± 0.3 µm, L/W ratio = 5.4.

On SNA: Hyphae septate, branched, hyaline, 2–5.5 µm diam. Conidiomata acervular, abundant on agar surface. Setae abundant, medium to dark brown, smooth-walled, 2–4-septate, straight, 85–252 µm long, tip round or somewhat acute, base cylindrical to conical, sometimes inflated, 4–8 µm diam. Conidiogenous cells hyaline to pale brown, smooth-walled, clavate or cylindrical, 6–25 × 3–6 µm, opening 1.5–2 µm, collarette 1–1.5 µm long, periclinal thickening visible. Conidia enteroblastic, aseptate, hyaline, smooth-walled, falcate, central part often with nearly parallel walls, more strongly curved toward the acute ends, base truncate, 22–27 × 3.5–4.5 µm, mean ± SD = 23.4 ± 1.3 × 4 ± 0.4 µm, L/W ratio = 5.8. Hyphal appressoria solitary, aseptate, smooth-walled, medium to very dark brown, ellipsoidal to clavate, sometimes irregularly shaped, entire edge, more or less lobed, 7.5–13.5 × 6–10.5 µm, mean ± SD = 12.5 ± 1.9 × 9.5 ± 1.6 µm, L/W ratio = 1.7.

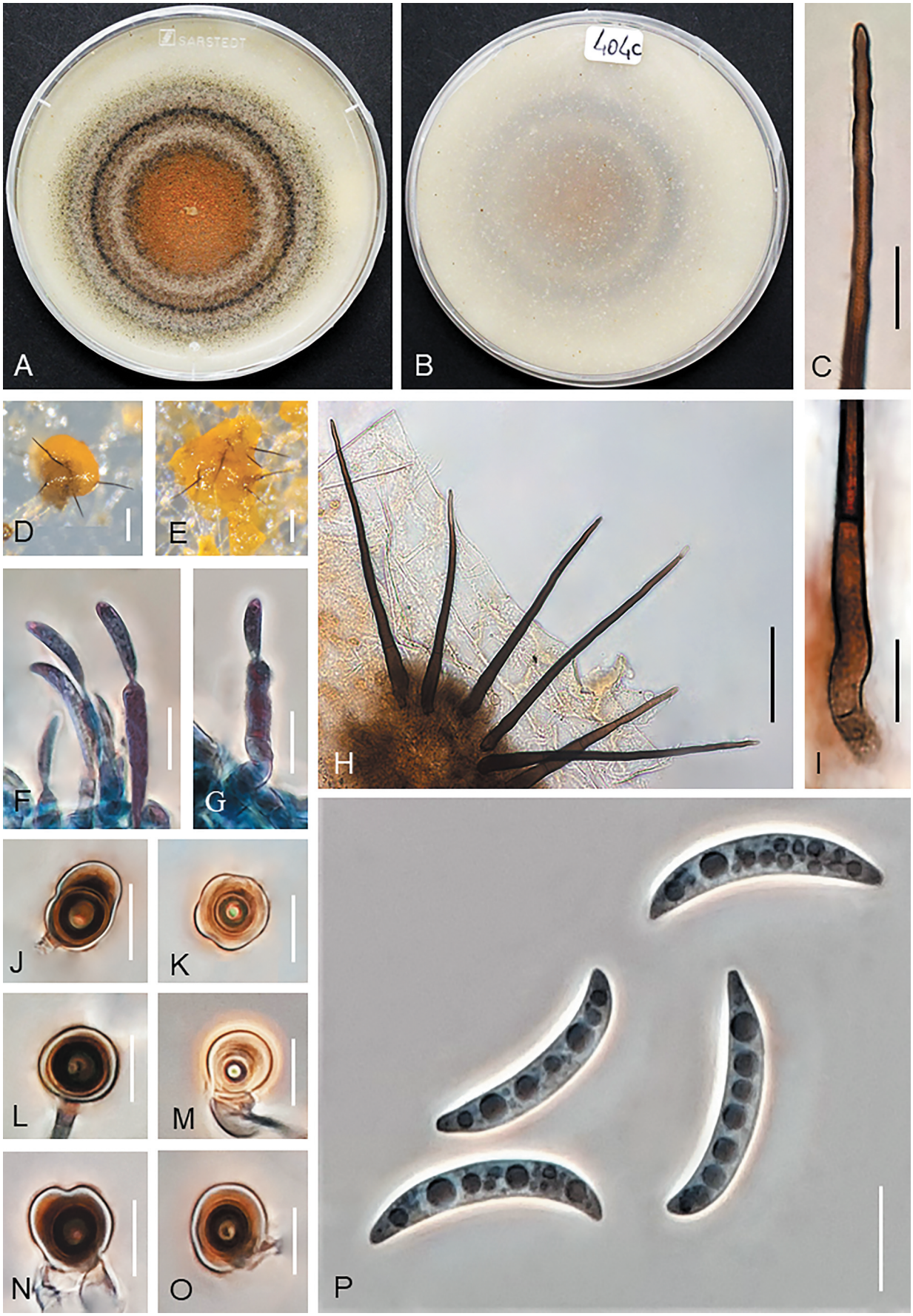

Figure 4. *Colletotrichum cereale* (from strain UTFC 383). A–B. Colony on OA after 7 d, A. upper and B. reverse side. C. Tip of a seta. D, E, H. Acervuli with setae. F, G. Conidiophores. I. Base of a seta. J–O. Appressoria. P. Conidia. C, F–I, P. from *Anthriscus* stem. D–E, J–O. from SNA. D, E. DM. C, F–P. DIC. Scale bars: D, E = 50 µm, C, F–*P* = 10 µm.

Culture characteristics: Colonies on OA flat with entire margin, orange at center due to abundant sporulation, smoky gray, pale to dark brown with darker brown spots toward the margin, partly covered by fluffy, white to smoky gray aerial mycelia, reverse buff, pale gray to cinnamon, 68–70 mm diam in 7 days at 25°C. Colonies on SNA flat with entire margin, hyaline, filter paper, *Anthriscus* stem and agar medium covered with white to pale olivaceous gray aerial mycelium, reverse hyaline to buff, 67–70 mm diam in 7 d (>90 mm diam in 10 d). Conidia in mass orange.

Specimens examined: IRAN. GOLESTAN: Gorgan, from leaves of *Saccharum spontaneum*, Jun 2013, *A. Alizadeh*, culture IRAN 3705C = UTFC 360 = CBS 148572 = DSM113398 = GLMC 2643; IRAN. GOLESTAN: Gorgan, from leaves of *Saccharum spontaneum*, Jul 2012, *A. Alizadeh*, living culture IRAN 3706C = UTFC 361; IRAN. GUILAN: Lahijan, from leaves of *Lolium* sp., Oct 2013, *A. Alizadeh*, living culture IRAN 3717C = UTFC 383 = CBS 148571 = DSM 113397 = GLMC 2644.

Notes: Colletotrichum cereale is known as a species with a worldwide distribution, and both as a plant pathogen of cool-season grasses (C3) of the subfamily *Pooideae* (Crouch et al. 2009d) and an endophyte of *Bletilla ochracea* (Orchidaceae) (Tao et al. 2013). Crouch et al. (2006) regarded the species as a distinct taxon with highly specialized populations corresponding to ecosystem and/or host plant. The strains examined in this study have similar conidia and appressoria shapes and dimensions as *C. cereale* strain CBS 129663 (Crouch et al. 2006). All loci included in this study separate *C. cereale* distinctly from all other species.

Colletotrichum nicholsonii J.A. Crouch, B.B. Clarke, J.F. White et B.I. Hillman. FIG. 5

On Anthriscus stem: Conidiomata acervular, abundant. Setae abundant, medium to dark brown, 2–4-septate, straight, up to 170 µm long, smooth-walled, tapering to an acute to rounded apex, base cylindrical, sometimes bent or inflated, 6–8 µm diam. Conidiophores pale to medium brown, branched, septate, up to 70 µm long. Conidiogenous cells pale to medium brown, smooth-walled, clavate or cylindrical, up to 25 µm long, opening 1.5–2 µm, collarette 1–1.5 µm long, periclinal thickening visible. Conidia enteroblastic, aseptate, hyaline, smooth-walled, falcate, strongly bent in the upper third toward an acute apex, base truncate 18.5–23.5 × 4.5–5.5 µm, mean ± SD = 21.5 ± 1 × 5 ± 0.3 µm, L/W ratio = 4.4.

On SNA: Hyphae hyaline, smooth-walled, septate, branched, 1.5–6 µm diam. Conidiomata acervular, abundant, especially on agar surface and on filter paper. Setae abundant, medium to dark brown, smooth-walled, 2–4-septate, straight, up to 260 µm long, tip round or somewhat acute, base cylindrical to conical, sometimes inflated, 2.5–5 µm diam. Conidiophores pale to medium brown, branched, septate, up to 80 µm long. Conidiogenous cells pale to medium brown, smooth-walled, clavate to cylindrical, up to 30 µm long, opening 1.5–2 µm, collarette and periclinal thickening not visible. Conidia enteroblastic, aseptate, hyaline, smooth-walled, falcate, strongly bent in the upper third toward an acute apex, base truncate, 14.5–24.5 × 4.5–5 µm, mean ± SD = 18.6 ± 1.1 × 4.9 ± 0.2 µm, L/W ratio = 4.4. Hyphal appressoria solitary, aseptate, smooth-walled, medium to dark brown, globose, oblong, ovoid, obovoid or clavate, edges entire or more or less lobed, 10.5–18 × 5–12 µm, mean ± SD = 13.5 ± 1.3 × 10 ± 1 µm, L/W ratio = 1.4.

Culture characteristics: Colonies on OA flat with entire margin, creamy white to gray, becoming dark gray with age in the center, reverse medium to dark gray, 69–71 mm diam in 7 d. Colonies on SNA flat with entire margin, hyaline, filter paper, *Anthriscus* stem and agar medium partly covered with white to pale olivaceous gray aerial mycelium, reverse hyaline to pale gray, 67–73 mm diam in 7 d (>90 diam mm in 10 d). Conidia in mass white to pale gray.

Specimens examined: IRAN. GUILAN: Rasht, from leaves of *Paspalum dilatatum*, Aug 2013, *A. Alizadeh*, culture IRAN 4294C = UTFC 366; IRAN GUILAN: Rasht, from leaves of *Paspalum dilatatum*, Oct 2013, *A. Alizadeh*, culture IRAN 3710C = UTFC 368; IRAN. GUILAN: Lahijan, from leaves of *Paspalum dilatatum*, Oct 2013, *A. Alizadeh*, culture IRAN 3715C = UTFC 379.

Notes: Colletotrichum nicholsonii was isolated from leaves of *Paspalum dilatatum* in this study. The colony characteristics, setae, conidial shape and dimension agree with the holotype of C. *nicholsonii* (Crouch et al. 2009c). In the phylogram, our three strains confidently clustered with the ex-type strain of *C. nicholsonii* (MAFF 511115) and strain MAFF 305428.

Colletotrichum persicum Alizadeh, Damm, Jav.-Nikkh & Stukenbr. sp. nov. FIG. 6

MycoBank: MB839810

Typification: IRAN. GUILAN: Kuchesfahan, from leaves of *Phragmites australis*, Oct 2013, *A. Alizadeh* (**holotype** IRAN 17633F). Ex-holotype culture IRAN 3712C = UTFC 370 = CBS 148574 = DSM 113400 = GLMC 2645. GenBank: ITS = MW741433; *sod2* = MW822604; *apn2* = MW822624; *apn2/mat1* = MW915570; *tub2* = MW740367; *act* = MW822655; *gapdh* = MW740228.

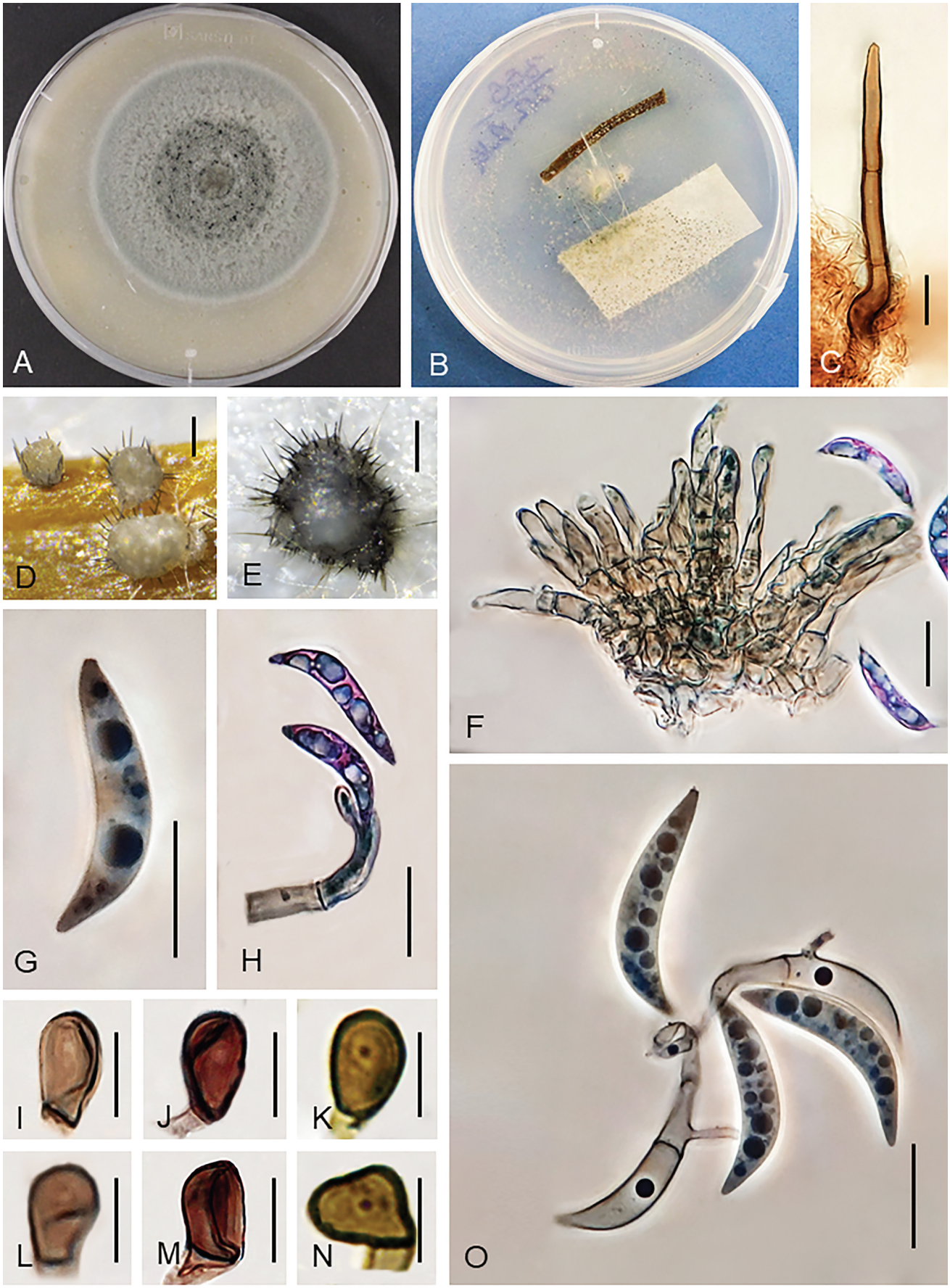

Figure 5. *Colletotrichum nicholsonii* (from strain UTFC 366). A. Colony on OA after 7 d. B. Colony on SNA amended with a double autoclaved stem of *Anthriscus sylvestris* and autoclaved filter paper. C. Seta. D, E. Acervuli. F, H. Conidiophores. G, O. Conidia. I–N. Appressoria. C, D. from *Anthriscus* stem. E. from autoclaved filter paper. F–O. from SNA. D–E. DM. C, F–O. DIC. Scale bars: D–E = 50 µm, C, F–O = 10 µm.

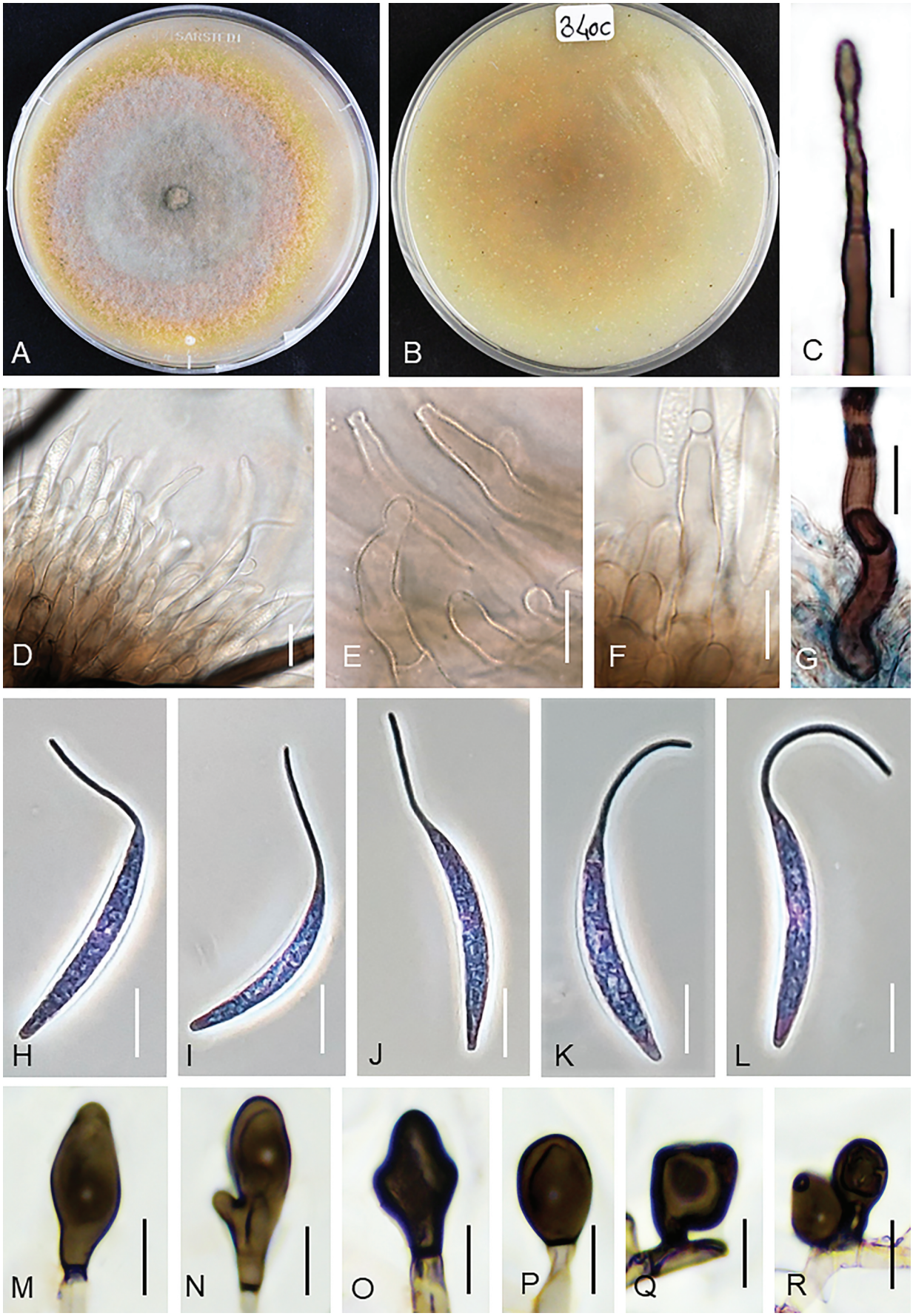

Figure 6. *Colletotrichum persicum* (from ex-holotype strain UTFC 370). A–B. Colony on OA after 7 d, A. upper and B. reverse side. C. Tip of a seta. D–F. Conidiophores. G. Basis of a seta. H–L. Conidia. M–R. Appressoria. C–L. from *Anthriscus* stem. M–R. from SNA. C–R. DIC. Scale bars: C–R = 10 µm.

Diagnosis: Conidia on *Anthriscus* stem measure 26–35.5 × 3–5.5 µm (excluding appendages) and appressoria on SNA 10–23 × 5–13 µm. The species differs from all other *Colletotrichum* species by its unique DNA sequences (as far as currently comparable) and by the existence or the length, respectively, of conidial appendages, which exceeds those of all other species (11–20 µm long, mean = 15.2 µm). Value, site and direction of their curvature are very variable in contrast to the uniform appendages of *C. sacchari*.

Etymology: Referring to the ancient name of Iran, the country from which this fungus was first collected.

On Anthriscus *stem:* Conidiomata acervular, conidiophores and setae formed on a basal cushion of pale brown angular cells. Setae abundant, medium to dark brown, 3–5-septate, 75–198 µm long, smooth-walled, tapering toward a round or slightly acute, base cylindrical to conical, sometimes inflated, 4–7 µm diam. Conidiophores, hyaline, pale to medium brown, branched, septate, up to 70 µm long. Conidiogenous cells pale to medium brown, smooth-walled, clavate, cylindrical, opening 1.5–2 µm, collarette 1–1.5 µm long, periclinal thickening visible. Conidia enteroblastic, aseptate, hyaline, smooth-walled, falcate, apex prolonged into a filiform appendage, 26–35.5 × 3–5.5 µm, mean ± SD = 30.4 ± 2.09 × 4.3 ± 0.6 µm, L/W ratio = 7.1 (excluding appendage), conidial appendage 11–20 µm long, mean ± SD = 15.2 ± 2.3 µm.

On SNA: Hyphae septate, branched, hyaline to pale brown, 1–6 µm diam. Not sporulating. Setae not observed. Hyphal appressoria solitary, aseptate, smooth-walled, pale to dark brown, globose, subglobose to ellipsoidal, oblong to clavate, edges entire or more or less lobed, 10–23 × 5–13 µm, mean ± SD = 16 ± 4.1 × 9 ± 1.9 µm, L/W ratio = 1.8.

Culture characteristics: Colonies on OA flat with entire margin, surface partly covered with white to pale mouse gray cottony aerial mycelium, luteous at the margin, a yellow pigment diffusing into the agar medium, reverse luteous, 58–60 mm diam in 7 d. Colonies on SNA flat with entire, sometimes fimbriate or irregular margin, filter paper, *Anthriscus* stem and agar medium covered with fluffy, olivaceous gray aerial mycelium; reverse olivaceous gray, dark olivaceous gray in the center, 60–62 mm diam in 7 d (>90 diam mm in 10 d). Conidia in mass yellow.

Additional specimen examined: IRAN. GUILAN: Kuchesfahan, from leaves of *Phragmites australis*, Oct 2013, *A. Alizadeh*, culture IRAN 3711C = UTFC 369 = CBS 148573 = DSM 113399 = GLMC 2646.

Notes: No *Colletotrichum* species was previously described from *Phragmites*, but Grove (1916) described a variety, namely *C. lineola* var. *phragmitis*, from culms of *Phragmites* in Moreton, Cheshire, UK. The spores of this fungus were oblong-fusoid, measuring 15–25 × 3–4 µm; that means, they are not only shorter than those of *C. persicum*, but also have a different shape. The only report of a *Colletotrichum* species from *Phragmites* based on molecular data is that of *C. destructivum* (Destructivum species complex) on *Phragmites* sp. in the USA (Damm et al. 2014), while the study of Wong and Hyde (2001) includes unidentified *Colletotrichum* species on *Phragmites australis* in Hong Kong.

Colletotrichum persicum can be identified with sequences of all loci available. In a blastn search on NCBI GenBank, the ITS sequence of the ex-type strain UTFC 370 was 99% identical (2 nucleotides difference) with those of *C. caudatum* isolates MAFF 305700 and I-99 (AB042304 and AB042305, Moriwaki et al. 2002), closest ex-type strain was with 3 nucleotides difference that of *C. caudasporum*. Closest matches with the *gapdh* sequence of *C. persicum* were with 97% identity (8 nc. diff.) *C. ochraceae* strains CGMCC 3.15102 and CGMCC 3.15104 (ex-type); closest matches with the *act* and *tub2* sequences with 98% (6 nc. diff.) and 99% (5 nc. diff.), respectively, the *C. caudasporum* strain. The *sod2* sequence was 97% identical (12 nc. diff.) with those of all *C. zoysiae* strains (no *sod2* sequence of the ex-type strain available). Closest matches with the *apn2* sequences of *C. persicum* were those of *C. caudatum* isolate MAFF 305700 (JX076926, Crouch 2014) with 99% identity (9 nc. diff.); the closest ex-type strain that of *C. zoysiae* with 98% identity. Closest matches with the *apn2*/*mat1* sequence of *C. persicum* were those of *C. baltimorense* isolates SD3 and SD6 with 97% identity (27 nc. diff.); the closest ex-type strains that of *C. somersetense* with 96% identity.

Like the species from *Saccharum spontaneum* that is described below, *C. persicum* belongs to the Caudatum subclade of the Graminicola species complex that is characterized by the formation of curved conidia with a filiform appendage (Crouch 2014). However, *C. ochraceae*, the closest neighbor of *C. persicum*, is an exception as it forms conidia without appendages (Tao et al. 2013). Therefore, the two species can easily be distinguished from each other by the existence of a filiform appendage. With an average of 15.2 µm (11–20 µm) the conidial appendages of *C. persicum* are larger than those of all other species in this clade. In contrast to *C. duyunensis*, in which setae are lacking, *C. persicum* forms abundant setae.

Colletotrichum sacchari Alizadeh, Damm, Jav.-Nikkh & Stukenbr. sp. nov. FIG. 7
MycoBank: MB839811

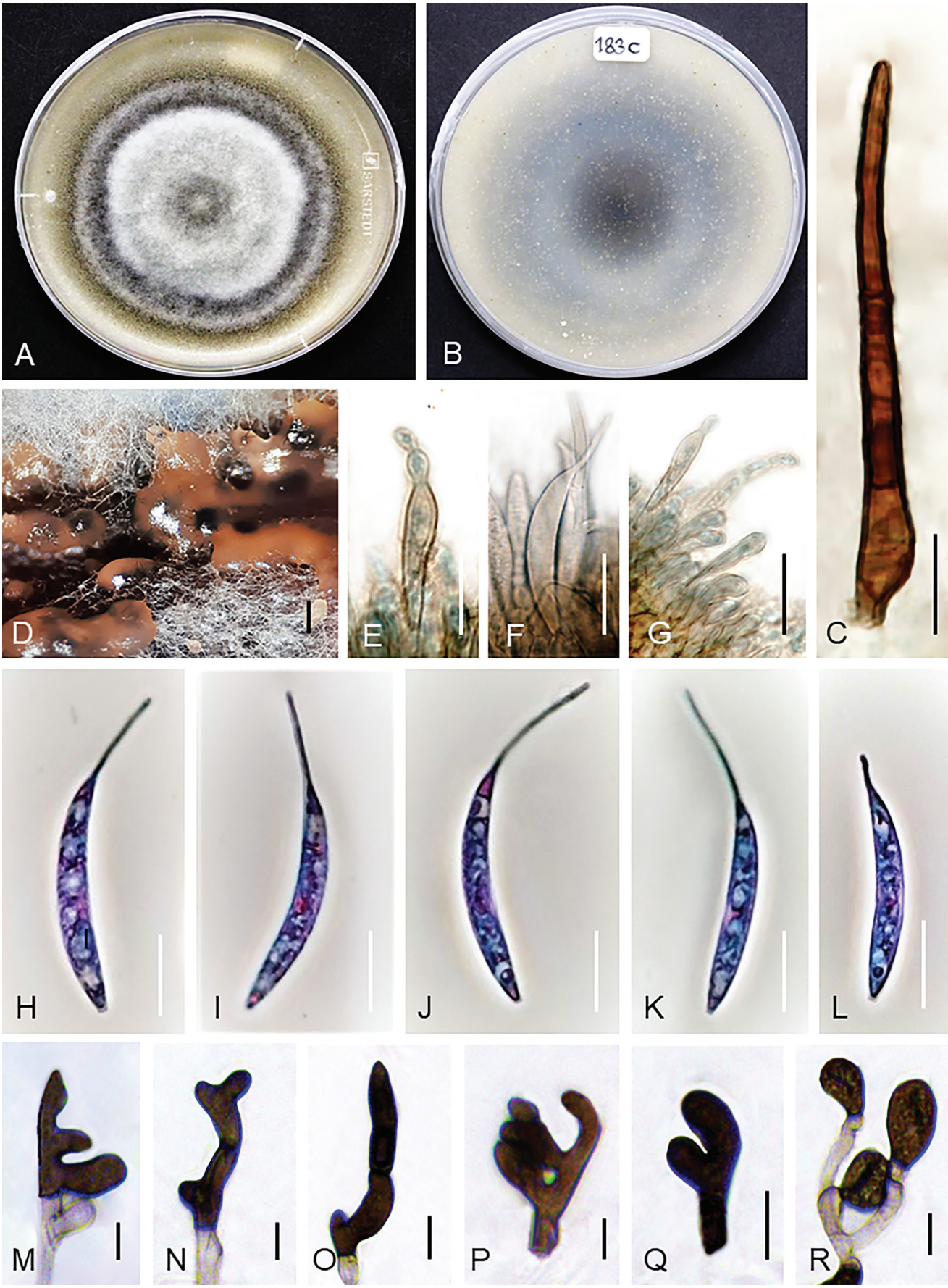

Figure 7. *Colletotrichum sacchari* (from ex-holotype strain UTFC 363). A–B. Colony on OA after 7 d, A. upper and B. reverse side. C. Seta. D. Acervuli. E–G. Conidiophores. H–L. Conidia. M–R. Appressoria. C–L. from *Anthriscus* stem. M–R. from SNA. D. DM. C, E–R. DIC. Scale bars: D = 50 µm, C, E–R = 10 µm.

Typification: IRAN. Golestan: Galugah, from leaves of *Saccharum spontaneum*, Sep 2012, *A. Alizadeh & A. Pordel* (**holotype** IRAN 17630F). Ex-holotype culture IRAN 3707C = UTFC 363 = CBS 148575 = DSM 113401 = GLMC 2647. GenBank: ITS = MW741431; *sod2* = MW822602; *apn2* = MW822622; *apn2/mat1* = MW915568; *tub2* = MW740368; *act* = MW822656; *gapdh* = MW740229.

Diagnosis: Conidia on *Anthriscus* stem measure 23–31.5 × 4.5–6 µm (excluding appendages), conidial appendages 11–16.5 µm and appressoria on SNA 11.5–50 × 6–19.5 µm. The species differs from all other *Colletotrichum* species by its unique DNA sequences (as far as currently comparable) and the often deeply lobed to branched and septate appressoria. Conidial appendages are uniform regarding value, site and direction of their curvature in contrast to the very variable appendages of *C. persicum*, while they are lacking in *C. ochraceae*.

Etymology: Referring to the host, *Saccharum*.

On Anthriscus stem: Conidiomata acervular, forming cushions of pale brown angular cells, from which setae and conidiophores are produced. Setae abundant, medium to dark brown, 2–4-septate, however septation hardly visible, straight, sometimes bent, 121–226 µm long, smooth-walled, tapering toward a round or slightly acute apex, base cylindrical to conical, sometimes inflated, 4–7.5 µm diam. Conidiophores pale to medium brown, simple, septate, up to 70 µm long. Conidiogenous cells pale to medium brown, smooth-walled, clavate or cylindrical, opening 1.5–2 µm, collarette and periclinal thickening not observed. Conidia enteroblastic, aseptate, hyaline, smooth-walled, falcate, apex prolonged to a filiform appendage, 23–31.5 × 4.5–6 µm, mean ± SD = 27.5 ± 1.8 × 4.7 ± 0.2 µm, L/W ratio = 5.8 (excluding appendage), conidial appendage 11–16.5 µm long, mean ± SD = 13.5 ± 1.6 µm.

On SNA: Hyphae hyaline to pale brown, smooth-walled, septate, branched, 1–6 µm diam. Not sporulating. Setae not observed. Hyphal appressoria smooth-walled, medium to dark brown, either aseptate, globose, subglobose, ellipsoidal to clavate with entire margin or more complex, irregularly shaped, elongate cylindrical, deeply lobed to branched and septate, 11.5–50 × 6–19.5 µm, mean ± SD = 18.8 ± 8.3 × 10.5 ± 2.8 µm, L/W ratio = 1.8.

Culture characteristics: Colonies on OA flat with entire margin, almost entirely covered with whitish to smoky gray, cottony aerial mycelium, olivaceous at the margin, reverse pale gray, pale blue or pale violet, dark gray at the center, 69–70 mm diam in 7 d. Colonies on SNA flat with entire margin, hyaline; filter paper, *Anthriscus* stem and agar medium covered with whitish, buff to smoky gray aerial mycelium, reverse hyaline with black spots mainly under the filter paper, 70–72 mm diam in 7 d (>90 diam mm in 10 d). Conidia in mass orange.

Notes: Colletotrichum sacchari is not closely related to *C. falcatum* that was also described from *Saccharum*, however from *S. officinarum* in Indonesia. The ex-type strains of *C. sacchari* and *C. falcatum* belong to different clades in the phylogenies of this study (FIGS. 1, 2). In contrast to *C. falcatum, C. sacchari* belongs to the Caudatum subclade of the Graminicola species complex that is characterized by the formation of curved conidia with appendages (Crouch 2014). Von Arx (1957) synonymized *Colletotrichum* species with falcate conidia from Poaceae, including *C. falcatum*, with *C. graminicola*, however considered the existence of formae speciales on different hosts. After several pathogenicity tests on sorghum, sugar cane and rye with a strain from maize failed, Messiaen et al. (1959) implemented this by naming some formae speciales of *C. graminicola*, including *C. graminicola* f. sp. *sacchari*, apparently referring to *C. falcatum* however, lacking any definite connection to it.

There is only one report of a *Colletotrichum* species from *S. spontaneum* listed in the USDA fungal database (Farr and Rossman 2021), that of *C. falcatum* on *S. spontaneum* in India. However, there are several reports from *S. officinarum*, including: *C. crassipes* in Malaysia (Liu 1977), *C. exoticum* in India (Mathur 1979), *C. falcatum* in many countries (Sutton 1980), *C. gloeosporioides* in India (Viswanathan et al. 2003), *C. graminicola* in several countries mostly located in south America (Farr and Stevenson 1963), *C. graminicola* f. sp. *sacchari* in Brazil (Mendes et al. 1998), *C. graminicola* (as *C. graminicolum*) in Pakistan (Ahmad et al. 1997) and *Colletotrichum* sp. in India (Alvarez 1976; Mathur 1979). Most of these reports are very old and there is no DNA data to support the identification of the reported fungi. Moreover, some of the reported species have not yet been revised in modern terms. However, none of these fungi produce conidia with a filiform appendage that is the prominent feature of the members of the Caudatum clade, including *C. sacchari* and it is therefore unlikely that any of these reports refers to *C. sacchari*. In this study, we also isolated *C. cereale* and *C. sublineola* from *S. spontaneum* in Iran.

Colletotrichum sacchari can be identified with sequences of all loci studied here. In a blastn search on NCBI GenBank, the ITS sequence of the ex-type strain UTFC 363 was 99% identical (3 nc. diff.) with that of *C. ochraceae* strains CGMCC 3.15102 and CGMCC 3.15103 and *C. zoysiae* strains MAFF 238573 (ex-type),

MAFF 238574 and MAFF 238575 that are included in this study. Closest matches with the *gapdh* sequence of *C. sacchari* were with 97% identity (8 nc. diff.), *C. ochraceae* strains CGMCC 3.15102 and CGMCC 3.15104 (ex-type); closest match with the *act* and *tub2* sequences was with 98% (5 nc. diff.) *C. caudasporum* strain CGMCC 3.15106. The *sod2* sequence was 98% identical (11 nc. diff.) with those of *C. zoysiae*, including its ex-type and 98% identical (4 nc. diff.) with that of the lectotype of *C. caudatum*, BPI423339; the latter sequence is however very short. Closest matches with the *apn2* and *apn2/mat1* sequences of *C. sacchari* were those of *C. caudatum* isolate MAFF 305700 (JX076926, Crouch 2014) with 99% and 97% identity (8 and 34 nc. diff.), respectively; the closest ex-type strains those of *C. zoysiae* and *C. somersetense* with 98% and 96% identity, respectively.

Colletotrichum sacchari differs morphologically from *C. persicum* that is described above in terms of conidial dimension, shape and size of appressoria, size and conidial appendages. The L/W ratio of conidia of *C. sacchari* is lower than that of *C. persicum* (5.8 vs. 7.1). Moreover, the conidial appendages of *C. sacchari* are very uniform, like an extension of the conidium; the direction of their curvature is usually toward the dorsal side or longitudinal axis of the conidia. In contrast, the conidial appendages of *C. persicum* are very variable regarding value, site and direction of the curvature that is sometimes toward the dorsal and sometimes toward the ventral side of the conidium. They vary from nearly straight to strongly curved, are sometimes bent just at its origin at the conidium and sometimes near its tip, just as they would be flexible. The conidial appendages of *C. sacchari* are longer than those of *C. alcornii*, that measure 2.0–6.0 µm. *C. sacchari* apparently differs from *C. duyunensis* by setae formation (abundant setae in *C. sacchari* vs. absence of setae in *C. duyunensis*). *Colletotrichum sacchari* can easily be distinguished from all others species in the caudatum clade by shape and size of appressoria (FIG. 7).

Colletotrichum sublineola Henn. ex Sacc. & Trotter, Syll. Fung. (Abellini) 22: 1206 (1913). FIG. 8.

On Anthriscus stem: Conidiomata acervular, abundant. Setae abundant, medium to dark brown, 2–4-septate, straight, up to 150 µm long, smooth-walled, tapering to an ± acute apex, base cylindrical, sometimes bent or inflated, 6–7.5 µm diam. Conidiophores hyaline or hyaline to pale brown, branched, septate, up to 50 µm long. Conidiogenous cells hyaline or hyaline to pale brown, smooth-walled, clavate to cylindrical, opening 1.5–2 µm, collarette and periclinal thickening not visible. Conidia enteroblastic, aseptate, hyaline, smooth-walled, falcate, slightly more strongly curved toward the acute apexes than the truncate bases, 23–27.5 × 4–4.5 µm, mean ± SD = 25.2 ± 1.2 × 4.2 ± 0.2 µm, L/W ratio = 6.

On SNA: Hyphae septate, branched, hyaline, 1.5–6 µm, Conidiomata acervular, abundant. Setae abundant, smooth-walled, pale to medium brown, 2–4-septate, straight, up to 140 µm long, tapering toward a round or slightly acute apex, base cylindrical to conical, sometimes inflated, 2.5–7.5 µm diam. Conidiophores pale to medium brown, branched, septate, up to 50 µm long. Conidiogenous cells pale to medium brown, smooth-walled, clavate to cylindrical, opening 1.5–2 µm, collarette and periclinal thickening not visible. Conidia enteroblastic, aseptate, hyaline, smooth-walled, falcate, slightly more strongly curved toward the acute apexes than the truncate bases, 20–29.5 × 3.5–5 µm, mean ± SD = 23 ± 2.1 × 3.5 ± 0.2 µm, L/W ratio = 6.5. Hyphal appressoria solitary, aseptate, smooth-walled, pale to medium brown, globose, oblong, ovoid, obovoid to clavate, edges entire or more or less lobed, 10–21.5 × 10–17 µm, mean ± SD = 16.5 ± 1.3 × 14.8 ± 1.4 µm, L/W ratio = 1.4.

Culture characteristics: Colonies on OA flat with entire margin, pale olivaceous gray to greenish gray, partly covered with gray, felty to wooly aerial mycelium, and in the center with orange conidial masses, yellow to luteous pigment diffusing into the agar medium, reverse pale olivaceous gray to luteous, 73–75 mm diam in 7 d. Colonies on SNA flat with entire margin, hyaline, filter paper, *Anthriscus* stem and agar medium covered with white to pale olivaceous gray aerial mycelium; reverse hyaline, 69–70 mm diam in 7 d (>90 diam mm in 10 d). Conidia in mass orange.

Specimens examined: IRAN. GUILAN: Rasht, from leaves of *Sorghum halepense*, Oct 2013, *A. Alizadeh*, culture IRAN 4296C = UTFC 380; IRAN. GUILAN: Rasht, from leaves of *Saccharum spontaneum*, Oct 2013, *A. Alizadeh*, culture IRAN 4297C = UTFC 381 = CBS 148576 = DSM 133402= GLMC 2648; IRAN. GUILAN: Rasht, from leaves of *Sorghum halepense*, Oct 2013, *A. Alizadeh*, culture IRAN 4295C = UTFC 378; IRAN. GUILAN: Rasht, from leaves of *Sorghum halepense*, Oct 2013, *A. Alizadeh*, culture IRAN 3714C = UTFC 372.

Notes: Colletotrichum sublineola was previously only reported from *Sorghum* species, including *S. bicolor* (United States, Brazil, Burkina Faso, Japan, South Africa, Sudan, Togo, Zambia), *S. halepense* (United States), *Sorghum* sp. (Togo, United States) and *S. vulgare* var. *technicum* (United States) (Farr and Rossman 2021). Moreover, this species was so far only reported from *S. halepense* (Johnsongrass) in the United States (Alabama, Florida, Georgia, Indiana, Kentucky,

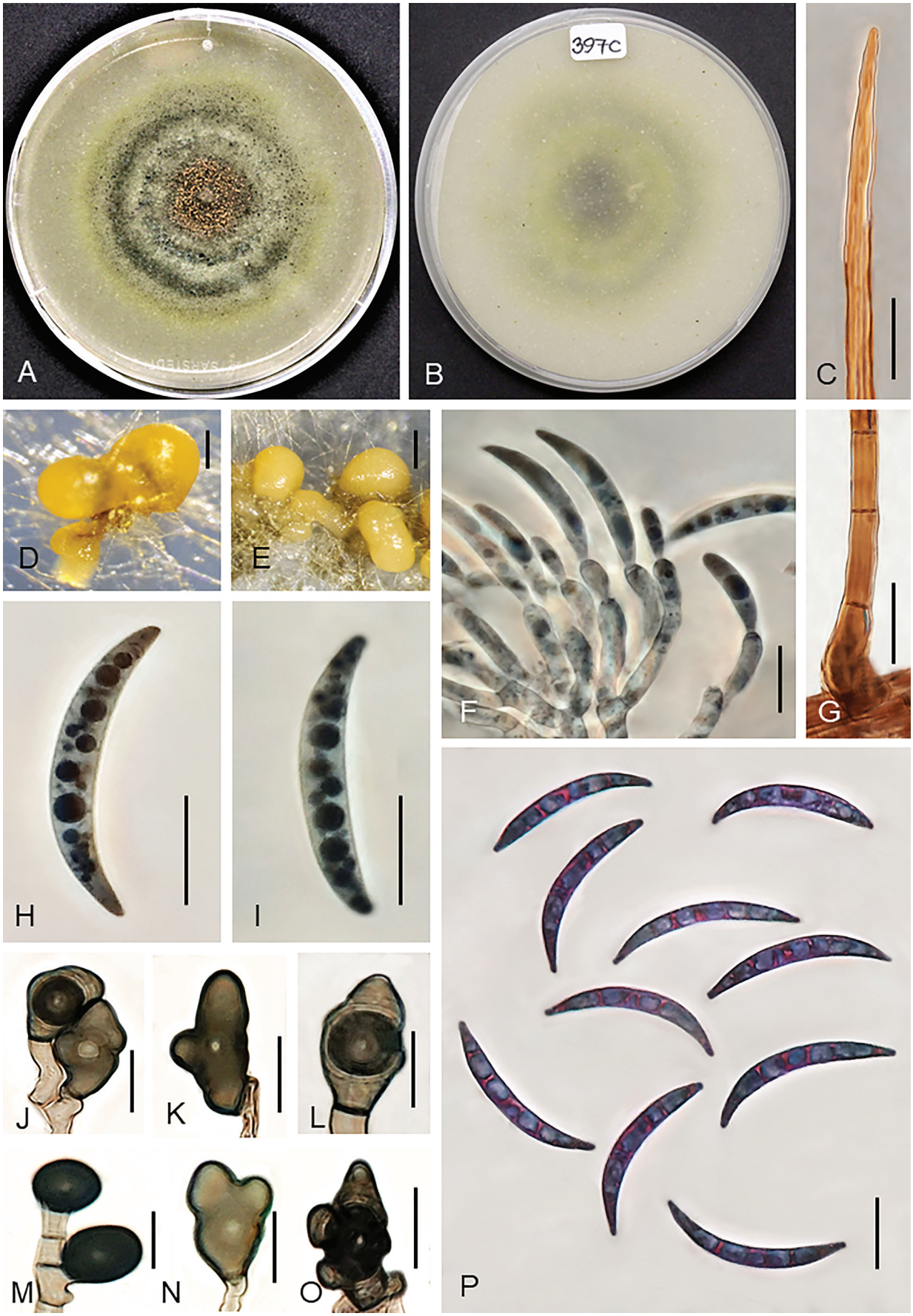

Figure 8. *Colletotrichum sublineola* (from strain UTFC 381). A–B. Colony on OA after 7 d, A. upper and B. reverse side. C. Tip of a seta. D, E. Acervuli. F. Conidiophores. G. Base of a seta. H, I, P. Conidia. J–O. Appressoria. C, G–I. from *Anthriscus* stem. D–F, J–P. from SNA. D, E. DM. C, F–P. DIC. Scale bars: D–E = 50 µm, C, F–*P* = 10 µm.

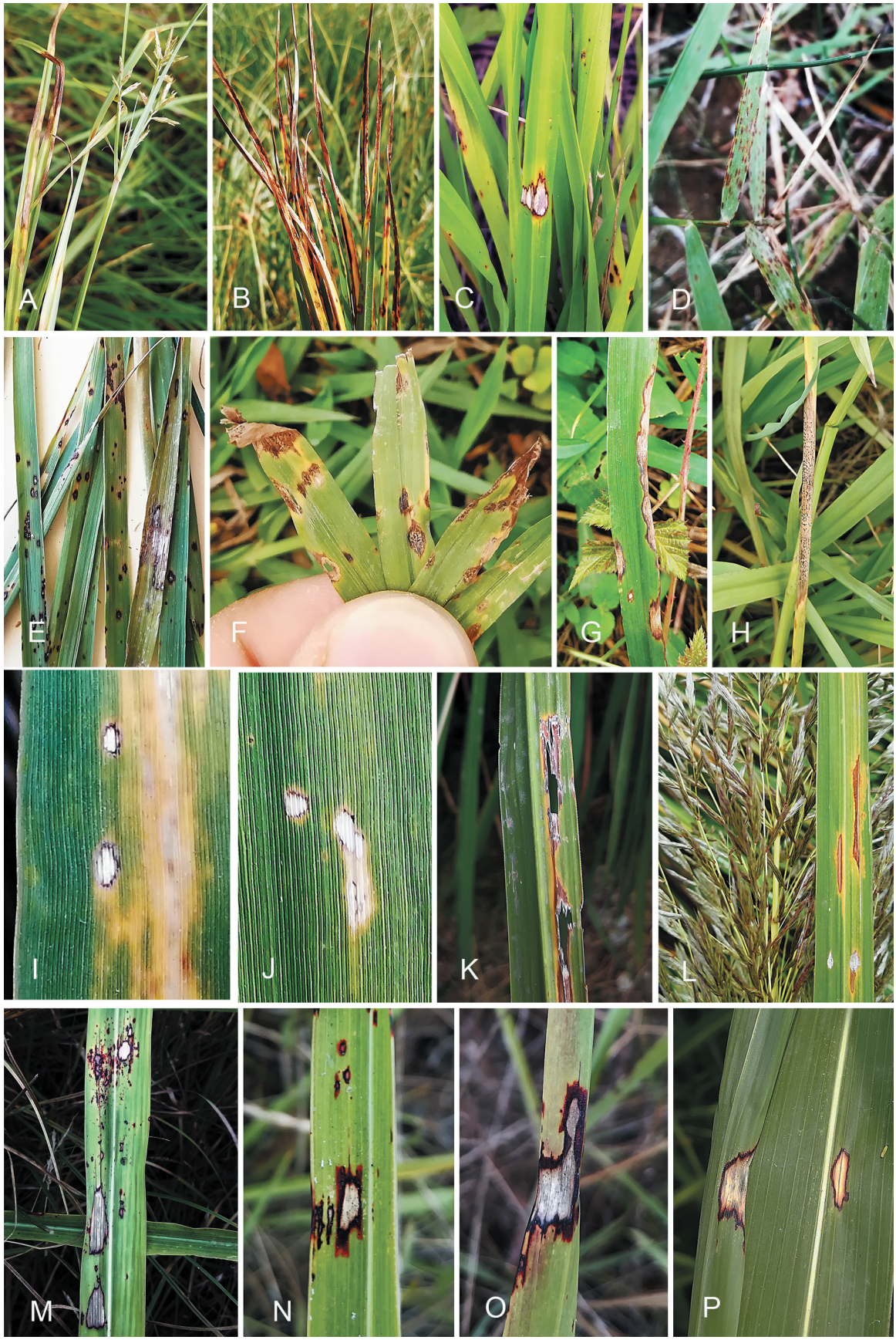

Figure 9. Wild Poaceae and Cyperaceae plants with symptoms of anthracnose or leaf spots from which *Colletotrichum* species were isolated in this study. A–B. *Cyperus* sp. (*C. caspicum*). C. Poaceae sp. (*C. caspicum*). D. *Cynodon dactylon* (*C. caspicum*). E. *Lolium* sp. (*C. cereale*). F–H. *Paspalum dilatatum* (*C. nicholsonii*). I–J. *Phragmites australis* (*C. persicum*). K–L. *Saccharum spontaneum* (*C. sacchari*). M. Anthracnose symptoms on *Sorghum halepense* (*C. sublineola*). N–P. *Sorghum halepense* (*C. sublineola*).

North Carolina, Texas). Considering that, this species is identified from *Sorghum halepense* and also from *Saccharum spontaneum* in this study, presumably still little is known about its geographical distribution and host range. Xavier et al. (2018) showed that *C. sublineola* populations obtained from *Sorghum bicolor* and *S. halepense* from the southeastern United States are genetically distinct, although both are capable of infecting different cultivars of *Sorghum bicolor*.

DISCUSSION

In this study, six *Colletotrichum* species were differentiated that were associated with anthracnose symptoms on leaves or leaf spot diseases of wild Poaceae and Cyperaceae plants in Iran (FIG. 9), including three known species, *C. cereale, C. nicholsonii* and *C. sublineola*, while three species, namely *C. caspicum, C. persicum* and *C. sacchari* were newly described using a polyphasic approach combining multilocus phylogeny, colony characteristics, morphology, collection and host data.

In simultaneous studies, different sets of loci had previously been used for discriminating species among the different species complexes in the genus *Colletotrichum*. For example, Crouch et al. (2009c) and Crouch (2014) used ITS, *sod2, apn2* and *apn2/mat1* to study the Graminicola species complex and its Caudatum subclade. In contrast, Tao et al. (2013) applied ITS, *gapdh, act* and *tub2* for studying endophytic *Colletotrichum* species from *Bletilla ochracea*, including species of the Graminicola species complex. As a result, individual species were characterized with different loci, and ITS is the only locus available for all species of the Graminicola complex described in these studies and therefore, the only locus that can be compared with all species. However, ITS sequences are known to have a resolution that is too low to resolve all species of the genus *Colletotrichum* (Crouch et al. 2009b; Damm et al. 2012a). Moreover, as only few morphological data of previously described *Colletotrichum* species from Poaceae are available for comparison, taxonomic studies of species in the Graminicola complex are challenging, and both identification and discovering of species are impeded. Some of the previously described species, especially those in the Caudatum subclade as well as *C. echinochloae* and *C. jacksonii* (Crouch et al. 2009c; Moriwaki and Tsukiboshi 2009), might be synonyms, which however, cannot be proven with certainty on this basis. We tried to solve this problem for the strains obtained from Poaceae and Cyperaceae collected in Iran in this study by calculating phylogenies from different data sets based either on the combinations of loci used by Crouch et al. (2009c), Crouch (2014) or those used by Tao et al. (2013) and the inclusion of as many strains and taxa as possible in each of them, which was however a compromise. Missing data resulted in low or lacking support values, especially in the tree based on ITS, *sod2, apn2* and *apn2/mat1* sequences (FIG. 1). Therefore, the data sets, especially those of the ex-type strains, need to be completed in further studies and the suitability of the individual loci for species delimitation within the Graminicola complex evaluated.

The results of this study are very valuable for understanding the host-pathogen relationships in the Graminicola complex. Crouch et al. (2009d) detected two distinct lineages within the Graminicola complex corresponding to the C3 and C4 photosynthetic pathways of their Poaceae hosts. The C4 lineage comprises several *Colletotrichum* species (including those later referred to as Caudatum subclade) that inhabit warm-season (C4) grasses, for example maize and sorghum. These species are apparently very host specific; they are exclusively associated with single host genera or species. In contrast, the C3 lineage, only consists of *C. cereale*, that has a wide host-range and had only been isolated from cool-season (C3) grasses. Later, Tao et al. (2013) isolated six species belonging to the Graminicola complex, both from the C3 and the C4 lineage, for the first time from a plant host other than Poaceae (*Bletilla ochracea*, Orchidaceae). These species were however isolated as endophytes of this orchid, while they are pathogens of Poaceae hosts. In this study, *C. cereale* (C3 lineage) was isolated from both cool-season (C3, *Lolium* sp.) and warm-season (C4, *Saccharum spontaneum*) grasses with anthracnose symptoms or leaf spots. Moreover, the new species *C. persicum* was described in the C4 lineage from *Phragmites australis*, which is a very adaptable grass with characteristics both of the C3 and C4 pathways (Milke et al. 2020). These are further exceptions of the splitting of the Graminicola complex in a C3 and a C4 lineage. Moreover, the distant location of the *C. cereale* clade (C3 lineage) cannot be entirely confirmed with the phylogenies in this paper.

To our best of knowledge, this study comprises the first reports of *C. cereale, C. nicholsonii* and *C. sublineola* for the mycobiota of Iran. *Saccharum spontaneum* (C4) was identified as a common host of both *C. sublineola* and *C. cereale*. Moreover, after the differentiation of the strains from *Eremochloae* from *C. sublineola* and their recognition as a separate species, *C. eremochloae* by Crouch and Tomaso-Peterson (2012), *C. sublineola* was regarded as only occurring on *Sorghum* species (Crouch and Tomaso-Peterson 2012; Xavier et al. 2018). In this study, *C. sublineola* was isolated not only from *Sorghum halepense* but also from *Saccharum spontaneum* that represents a new report of a host genus for a species of the C4 lineage of the Graminicola complex

that was previously regarded as being restricted to one host genus or species each.

The newly described species *C. caspicum* is not restricted to Poaceae. The strains originated from at least two hosts belonging to different plant families, Poaceae and Cyperaceae, and in two countries in Asia, Iran and China. Results of blast searches based on ITS sequences indicate an even wider host spectrum and a further distribution of this species that might include Europe (Poland) and North America (USA) (Górzyńska et al. 2019; M.B. DeMers and G. May, unpubl. data). However, these results still need to be confirmed based on sequencing of further loci.

The systematic position of *C. caspicum* in the two phylogenies in this study is basal to all other species of the Graminicola complex, which also means it belongs neither to the C3 nor the C4 lineage. Blastn searches on NCBI GenBank indicate a close relationship of this species both to the Spaethianum and the Graminicola species complex. However, based on preliminary analyses, including the ex-type strains of this complex, *C. caspicum* does not belong to the Spaethianum complex, and the species was tentatively assigned to the Graminicola complex. However, individual species of most of the species in the Graminicola complex are not closely related; backbone support is missing or low (Marin-Felix et al. 2017; Bhunjun et al. 2021; this study). Therefore, the systematic position of all species of the Graminicola complex needs to be revised with a more complete sequence data set that would allow a better analysis of all *Colletotrichum* species combined.

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ORCID

A. Alizadeh http://orcid.org/0000-0002-7094-2795
M. Javan-Nikkhah http://orcid.org/0000-0001-9533-0157
R. Nourmohammadi Nazarian http://orcid.org/0000-0002-5122-9986
R. Zare http://orcid.org/0000-0002-5931-4560
K. B. Fotouhifar http://orcid.org/0000-0002-4790-6171
E. H. Stukenbrock http://orcid.org/0000-0001-8590-3345
U. Damm http://orcid.org/0000-0002-2252-5196

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