



Application of the consolidated species concept to *Cercospora* spp. from Iran

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Abstract The genus *Cercospora* includes many important plant pathogenic fungi associated with leaf spot diseases on a wide range of hosts. The mainland of Iran covers various climatic regions with a great biodiversity of vascular plants, and a correspondingly high diversity of cercosporoid fungi. However, most of the cercosporoid species found to date have been identified on the basis of morphological characteristics and there are no cultures that support these identifications. In this study the Consolidated Species Concept was applied to differentiate *Cercospora* species collected from Iran. A total of 161 *Cercospora* isolates recovered from 74 host species in northern Iran were studied by molecular phylogenetic analysis. Our results revealed a rich diversity of *Cercospora* species in northern Iran. Twenty species were identified based on sequence data of five genomic loci (ITS, TEF1- α , actin, calmodulin and histone H3), host, cultural and morphological data. Six novel species, viz. *C. convolvulicola*, *C. conyzae-canadensis*, *C. cylindracea*, *C. iranica*, *C. pseudochenopodii* and *C. sorghicola*, are introduced. The most common taxon was *Cercospora* cf. *flagellaris*, which remains an unresolved species complex with a wide host range. New hosts were recorded for previously known *Cercospora* species, including *C. apii*, *C. armoraciae*, *C. beticola*, *C. cf. richardicola*, *C. rumicis*, *Cercospora* sp. G and *C. zebrina*.

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INTRODUCTION

Species of *Cercospora* (*Mycosphaerellaceae*) are often associated with leaf spots, but also cause necrotic lesions on flowers, fruits, bracts, seeds and pedicels of many cultivated and native plants in a range of climates worldwide (Goodwin et al. 2001, Crous & Braun 2003, Agrios 2005). The genus includes many important pathogens of agricultural crops, including cereals, vegetables, ornamentals, forest trees and grasses. Examples are *C. beticola* on sugar beet (Weiland & Koch 2004), *C. zonata* on faba beans (Kimber 2011), *C. zea-maydis* and *C. zeina* on maize (Crous et al. 2006) and *C. carotae* on carrots (Kushalappa et al. 1989). Some species are considered potential biocontrol agents of weeds, including *C. caricis* on *Cyperus rotundus* and *C. rodmanii* on water hyacinth (Morris & Crous 1994, Charudattan 2001, Inglis et al. 2001, Tessmann et al. 2001, Praveena & Naseema 2004).

The genus *Cercospora* was established by Fresenius (in Fuckel 1863), and *C. penicillata* was later designated as the type species of the genus (Crous & Braun 2003). Since the description of *Cercospora*, the taxonomy of this genus together with the description of individual species has proven highly problematic. Morphological traits in *Cercospora* are generally conserved and specific morphological characters (including conidial shape and size, the presence or absence of external mycelium and conidiophore morphology), have often been used to describe

and identify *Cercospora* species, despite their limitations. The paucity of useful morphological characters and high level of intraspecific variation has meant species definition in this genus being largely dependent on host plant association, i.e., a species of *Cercospora* was described as new when found on a different host species (Chupp 1954, Ellis 1971). The classification of *Cercospora* species is clouded by a history of taxonomic recombinations and name changes. While the description of new species from different hosts has increased the number of species on the one hand (Pollack 1987), the synonymy of names has decreased the species number on the other (Crous & Braun 2003). A significant problem for the taxonomy of *Cercospora* is the degree of host-specificity of the various species. Host data for *Cercospora* spp. is not well known, and should be avoided as the primary criterion for identification purposes. Extensive host inoculation experiments have shown that identification of *Cercospora* spp. by host specificity alone is error prone, because many species are not restricted to a single host. For example, several taxa including *C. apii*, *C. beticola*, *C. canescens* and *C. zebrina*, occur on different unrelated plant families and have broad host ranges (Crous & Braun 2003, Lartey et al. 2005, Bakhshi et al. 2012b, Groenewald et al. 2013).

During the course of monographic studies on *Cercospora*, Crous & Braun (2003) proposed that only genetically and morphologically distinguishable taxa should be treated as separate species. They recognised 659 names in the genus *Cercospora*, with a further 281 names referred to *C. apii* s.lat. Based on molecular data and morphological examinations, Crous & Braun (2003) concluded that *C. apii*-like fungi form a morphologically uniform, complicated assemblage of taxa in which the process of speciation has not concluded. They introduced the concept of 'compound species' consisting of morphologically indistinguishable species with different races (host range) for a complex of plurivorous taxa, which were morphologically indistinguishable.

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Cercospora was often linked to the sexual genus *Mycosphaerella* according to phylogenetic analyses based on nrDNA sequence loci, especially ITS and later 28S nrDNA (Stewart et al. 1999, Crous et al. 2000). Contrary to an earlier indication that many diverse asexual genera were linked to *Mycosphaerella* (Arzanlou et al. 2007, 2008), it was later shown that *Mycosphaerella* was polyphyletic (Crous et al. 2007). Subsequently, *Mycosphaerella* was split into numerous genera, correlating with different asexual morphs (Crous et al. 2009a, b, Quaedvlieg et al. 2013, Verkley et al. 2013). In this regard, *Mycosphaerella* s.str. is now restricted to taxa that form *Ramularia* asexual morphs (Verkley et al. 2004, Groenewald et al. 2013). Following a proposal accepted by the International Code of Nomenclature for Algae, Fungi and Plants (ICN) (Hawksworth 2011, Norvell 2011), the asexual name *Ramularia* (1833) was chosen over the younger, confused sexual name *Mycosphaerella* (1884) (Crous et al. 2009a, b, Hyde et al. 2013, Kirk et al. 2013). The genus *Cercospora* is now considered a holomorphic genus in its own right (Groenewald et al. 2013), with some species exhibiting the ability to form mycosphaerella-like sexual morphs (Corlett 1991, Crous et al. 2004b).

In recent years, multi-gene DNA sequence datasets have proven useful for *Cercospora* species identification (Crous et al. 2004c, Groenewald et al. 2005, 2006, 2010, Montenegro-Calderón et al. 2011). The most inclusive study to date was that of Groenewald et al. (2013), who compared 360 *Cercospora* isolates, isolated from 161 host species from 39 countries. One important outcome of this study was that several species originally referred to *C. apii* s.lat. based on morphology (Crous & Braun 2003), were separated as distinct phylogenetic species. This also led to the conclusion that morphology alone frequently provides an insufficient basis for species discrimination in the genus *Cercospora*. Furthermore, multilocus DNA sequence typing integrated with ecology, morphology and cultural characteristics, referred to as the Consolidated Species Concept (Quaedvlieg et al. 2014), proved the most effective method for the recognition of *Cercospora* spp. (Groenewald et al. 2010, 2013).

The mainland of Iran covers various climatic regions with a great biodiversity of vascular plants, and corresponding diversity of cercosporoid fungi. However, most of the species to date have been identified and described on the basis of morphological characteristics sensu Chupp (1954), with no attempt to derive cultures or molecular data. In order to further an understanding of this group of fungi in Iran, we initially assembled a checklist (Bakhshi et al. 2012a). Our primary aim was to describe *Cercospora* spp. from the north and northwest of Iran based on freshly collected specimens, derived cultures, and DNA sequence data. To achieve this aim, we sequenced the ITS locus (including ITS1, 5.8S nrDNA gene and ITS2), together with parts of four protein coding genes, viz. translation elongation factor 1-alpha (TEF1- α), actin (ACT), calmodulin (CAL) and histone H3 (HIS), and compared these data to publically available sequence data.

MATERIAL AND METHODS

Specimens and isolates

Leaf samples colonised with *Cercospora* spp. were collected in the field from different provinces, including Guilan, Mazandaran, Ardabil, Zanjan, West and East Azerbaijan and taken to the laboratory. Leaves were examined directly under a Nikon SMZ 1500 stereo-microscope to observe sporulation. Conidia were scraped from a single leaf spot, and single conidial colonies were established on 2 % malt extract agar (MEA; Fluka, Hamburg, Germany) (Bakhshi et al. 2011). Dried specimens were maintained in the Fungal Herbarium of the Iranian Research

Institute of Plant Protection (IRAN). Axenic cultures were deposited in the Culture Collection of Tabriz University (CCTU) and the Centraalbureau voor Schimmelcultures (CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands). A complete list of the isolates used in this study is presented in Table 1.

DNA extraction, amplification and sequencing

Isolates were grown on MEA for 10 d at 25 °C in the dark, and genomic DNA was extracted using the protocol of Möller et al. (1992). The DNA samples were subsequently diluted 50–100 times in preparation for further DNA amplification reactions. Five loci were sequenced for each isolate. The primers V9G (de Hoog & Gerrits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify part of the nuclear rRNA operon (ITS) spanning the 3' end of 18S rRNA gene, the first internal transcribed spacer, the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene. Part of the actin gene (ACT) was amplified using the primer set ACT-512F and ACT-783R (Carbone & Kohn 1999), whereas the primer set CylH3F and CylH3R (Crous et al. 2004c) was used to amplify part of the histone H3 gene (HIS). Primers employed for the amplification of translation elongation factor 1-alpha (TEF1- α) included EF1-728F and EF1-986R (Carbone & Kohn 1999) or EF-2 (O'Donnell et al. 1998), while the primer set CAL-228F and CAL-737R (Carbone & Kohn 1999) or CAL-2Rd (Groenewald et al. 2013) was used to amplify part of the calmodulin gene (CAL). The PCRs were performed in a total volume of 12.5 μ L. The ITS, HIS, TEF1- α and ACT mixtures contained 5–10 ng genomic DNA, 1X PCR buffer (Bioline, London, UK), 2 mM MgCl₂ (Bioline), 40 μ M of each dNTP, 0.7 μ L DMSO, 0.2 μ M of each primer and 0.5 Unit GoTaq® Flexi DNA polymerase (Promega, Madison, USA). The CAL PCR mixture differed from the original mix by containing 2.5 mM MgCl₂ and 10–20 ng genomic DNA. The PCR conditions for ITS, HIS, TEF1- α and ACT consisted of an initial denaturation step of 5 min at 95 °C followed by 40 cycles of 30 s at 95 °C, 30 s at 52 °C and 45 s at 72 °C, then 5 min at 72 °C and PCR conditions for CAL consisted of an initial denaturation step of 5 min at 95 °C followed by 40 cycles of 30 s at 95 °C, 40 s at 58 °C/55 °C and 50 s at 72 °C using respectively CAL-737R or CAL-2Rd as reverse primer and final elongation step of 5 min at 72 °C. Following PCR amplification, amplicons were visualized on a 1.2 % agarose gel stained with GelRed™ (Biotium, Hayward, CA, USA) and viewed under ultra-violet light and sizes of amplicons were determined against a HyperLadder™ I molecular marker (Bioline). The ABI Prism BigDye® Terminator Cycle sequencing reaction kit v. 3.1 (Applied Biosystems™, Foster City, CA, USA) was used for sequencing of PCR products in both directions using the same primers pairs used for amplification, following the manufacturer's instructions. Sequencing products were purified through a 96-well multiscreen HV plate (Millipore) containing Sephadex G-50 (Sigma Aldrich, St. Louis, MO) as outlined by the manufacturer and analysed with an ABI Prism 3730XL Automated DNA analyzer (Life Technologies Europe BV, Applied Biosystems™, Bleiswijk, The Netherlands) according to manufacturer's recommendation.

Phylogenetic analyses

The raw trace files were edited using MEGA v. 5 (Tamura et al. 2011) and a consensus sequence was generated manually for each set of trace files from a given reaction. The generated sequences were compared with other fungal DNA sequences from NCBI's GenBank sequence database using BLAST; sequences with high similarity were added to the alignments. Sequences of *Ramularia endophylla* (isolate CBS 113265) were used as the outgroup based on availability and phylogenetic relationship with *Cercospora*. A basic alignment of the obtained sequences

Table 1 Collection details and GenBank accession numbers of isolates included in this study.

Species	Culture accession number(s) ¹	Host	Host Family	Origin	Collector	ITS	TEF1- α	ACT	CAL	HIS
<i>Cercospora althaeina</i>	CCTU 1001	<i>Althaea rosea</i>	Malvaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886392	KJ886231	KJ885909	KJ885748	KJ886070
	CCTU 1026	<i>Althaea rosea</i>	Malvaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886393	KJ886232	KJ885910	KJ885749	KJ886071
	CCTU 1028	<i>Althaea rosea</i>	Malvaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886394	KJ886233	KJ885911	KJ885750	KJ886072
	CCTU 1071	<i>Malva sylvestris</i>	Malvaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886395	KJ886234	KJ885912	KJ885751	KJ886073
	CCTU 1152	<i>Althaea rosea</i>	Malvaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886396	KJ886235	KJ885913	KJ885752	KJ886074
	CCTU 1194	<i>Malva sylvestris</i>	Malvaceae	Iran, East Azerbaijan, Kaleibar	M. Arzanlou	KJ886397	KJ886236	KJ885914	KJ885753	KJ886075
	CCTU 1222	<i>Malva sylvestris</i>	Malvaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886398	KJ886237	KJ885915	KJ885754	KJ886076
	CCTU 1249	<i>Malva sylvestris</i>	Malvaceae	Iran, East Azerbaijan, Kaleibar	M. Arzanlou	KJ886399	KJ886238	KJ885916	KJ885755	KJ886077
	CCTU 1041; CPC 24910	<i>Plantago lanceolata</i>	Plantaginaceae	Iran, Guilan, Chaboksar	M. Bakhshi	KJ886400	KJ886239	KJ885917	KJ885756	KJ886078
	CCTU 1047	<i>Plantago lanceolata</i>	Plantaginaceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886401	KJ886240	KJ885918	KJ885757	KJ886079
	CCTU 1082; CBS 138728	<i>Plantago lanceolata</i>	Plantaginaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886402	KJ886241	KJ885919	KJ885758	KJ886080
	CCTU 1095	<i>Plantago lanceolata</i>	Plantaginaceae	Iran, East Azerbaijan, Horand	M. Bakhshi	KJ886403	KJ886242	KJ885920	KJ885759	KJ886081
	CCTU 1179	<i>Plantago lanceolata</i>	Plantaginaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886404	KJ886243	KJ885921	KJ885760	KJ886082
	CCTU 1063	<i>Ecballium elaterium</i> *	Cucurbitaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886405	KJ886244	KJ885922	KJ885761	KJ886083
	CCTU 1217	<i>Ecballium elaterium</i>	Cucurbitaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886406	KJ886245	KJ885923	KJ885762	KJ886084
CCTU 1134	<i>Heliotropium europaeum</i>	Boraginaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886407	KJ886246	KJ885924	KJ885763	KJ886085	
CCTU 1200; CBS 138581	<i>Heliotropium europaeum</i>	Boraginaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886408	KJ886247	KJ885925	KJ885764	KJ886086	
CCTU 1061	<i>Cynanchum acutum</i> *	Apocynaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886409	KJ886248	KJ885926	KJ885765	KJ886087	
CCTU 1069	<i>Cynanchum acutum</i>	Apocynaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886410	KJ886249	KJ885927	KJ885766	KJ886088	
CCTU 1086; CBS 136037	<i>Cynanchum acutum</i>	Apocynaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886411	KJ886250	KJ885928	KJ885767	KJ886089	
CCTU 1215	<i>Cynanchum acutum</i>	Apocynaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886412	KJ886251	KJ885929	KJ885768	KJ886090	
CCTU 1219; CBS 136155	<i>Cynanchum acutum</i>	Apocynaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886413	KJ886252	KJ885930	KJ885769	KJ886091	
CCTU 1013	?	?	Iran, East Azerbaijan, Mianeh	M. Torbati	KJ886414	KJ886253	KJ885931	KJ885770	KJ886092	
CCTU 1022; CBS 136028	?	?	Iran, East Azerbaijan, Mianeh	M. Torbati	KJ886415	KJ886254	KJ885932	KJ885771	KJ886093	
CCTU 1040; CBS 136131	<i>Tanacetum balsamita</i> *	Asteraceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886416	KJ886255	KJ885933	KJ885772	KJ886094	
CCTU 1107	?	?	Iran, Zanjan, Tarom	M. Bakhshi	KJ886417	KJ886256	KJ885934	KJ885773	KJ886095	
CCTU 1117; CBS 136132	<i>Cardaria draba</i>	Brassicaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886418	KJ886257	KJ885935	KJ885774	KJ886096	
CCTU 1234	<i>Cardaria draba</i>	Brassicaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886419	KJ886258	KJ885936	KJ885775	KJ886097	
CCTU 1127; CBS 136133	<i>Capparis spinosa</i> *	Capparidaceae	Iran, Khuzestan, Ahvaz	E. Mohammadian	KJ886420	KJ886259	KJ885937	KJ885776	KJ886098	
CCTU 1127.2	<i>Capparis spinosa</i>	Capparidaceae	Iran, Khuzestan, Ahvaz	E. Mohammadian	KJ886421	KJ886260	KJ885938	KJ885777	KJ886099	
CCTU 1190; CBS 136134	<i>Coronilla varia</i>	Fabaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886422	KJ886261	KJ885939	KJ885778	KJ886100	
CCTU 1035	<i>Malva sylvestris</i>	Malvaceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886423	KJ886262	KJ885940	KJ885779	KJ886101	
CCTU 1057	<i>Chenopodium</i> sp.	Chenopodiaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886424	KJ886263	KJ885941	KJ885780	KJ886102	
CCTU 1065	<i>Chenopodium</i> sp.	Chenopodiaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886425	KJ886264	KJ885942	KJ885781	KJ886103	
CCTU 1074; CPC 24909	<i>Malva neglecta</i>	Malvaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886426	KJ886265	KJ885943	KJ885782	KJ886104	
CCTU 1087	<i>Chenopodium</i> sp.	Chenopodiaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886427	KJ886266	KJ885944	KJ885783	KJ886105	
CCTU 1088; CBS 138582	<i>Sonchus asper</i> *	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886428	KJ886267	KJ885945	KJ885784	KJ886106	
CCTU 1089; CPC 24911	<i>Plantago lanceolata</i> *	Plantaginaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886429	KJ886268	KJ885946	KJ885785	KJ886107	
CCTU 1108	<i>Plantago lanceolata</i>	Plantaginaceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886430	KJ886269	KJ885947	KJ885786	KJ886108	
CCTU 1109	<i>Malva sylvestris</i>	Malvaceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886431	KJ886270	KJ885948	KJ885787	KJ886109	
CCTU 1135	<i>Beta vulgaris</i>	Chenopodiaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886432	KJ886271	KJ885949	KJ885788	KJ886110	
CCTU 1199; CBS 136128	<i>Rumex crispus</i> *	Polygonaceae	Iran, Mazandaran, Ramsar	M. Bakhshi	KJ886433	KJ886272	KJ885950	KJ885789	KJ886111	
CCTU 1201	<i>Malva neglecta</i>	Malvaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886434	KJ886273	KJ885951	KJ885790	KJ886112	
CCTU 1205; CBS 136127	<i>Sesamum indicum</i> *	Pedaliaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886435	KJ886274	KJ885952	KJ885791	KJ886113	
CCTU 1208	<i>Sonchus</i> sp.*	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886436	KJ886275	KJ885953	KJ885792	KJ886114	
CCTU 1033	<i>Chenopodium album</i>	Chenopodiaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886437	KJ886276	KJ885954	KJ885793	KJ886115	
CCTU 1060	<i>Chenopodium album</i>	Chenopodiaceae	Iran, Guilan, Bandar-e Anzali	M. Bakhshi	KJ886438	KJ886277	KJ885955	KJ885794	KJ886116	
CCTU 1157	<i>Chenopodium album</i>	Chenopodiaceae	Iran, Guilan, Langroud	M. Bakhshi	KJ886439	KJ886278	KJ885956	KJ885795	KJ886117	
CCTU 1163	<i>Chenopodium album</i>	Chenopodiaceae	Iran, Guilan, Lahijan	M. Bakhshi	KJ886440	KJ886279	KJ885957	KJ885796	KJ886118	
CCTU 1083; CBS 136126 (ex-type)	<i>Convolvulus arvensis</i>	Convolvulaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886441	KJ886280	KJ885958	KJ885797	KJ886119	
CCTU 1083.2	<i>Convolvulus arvensis</i>	Convolvulaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886442	KJ886281	KJ885959	KJ885798	KJ886120	
CCTU 1008	<i>Conyza canadensis</i>	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886443	KJ886282	KJ885960	KJ885799	KJ886121	
CCTU 1105	<i>Conyza canadensis</i>	Asteraceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886444	KJ886283	KJ885961	KJ885800	KJ886122	
CCTU 1119; CBS 135978 (ex-type)	<i>Conyza canadensis</i>	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886445	KJ886284	KJ885962	KJ885801	KJ886123	

Table 1 (cont.)

Species	Culture accession number(s) ¹	Host	Host Family	Origin	Collector	GenBank accession numbers ²				
						ITS	TEF1- α	ACT	CAL	HIS
<i>Cercospora cylindracea</i>	CCTU 1016	<i>Cichorium intybus</i>	Asteraceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886446	KJ886285	KJ885963	KJ885802	KJ886124
	CCTU 1044; CBS 136021	<i>Lactuca serriola</i>	Asteraceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886447	KJ886286	KJ885964	KJ885803	KJ886125
	CCTU 1049	<i>Lactuca serriola</i>	Asteraceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886448	KJ886287	KJ885965	KJ885804	KJ886126
	CCTU 1081; CBS 138580 (ex-type)	<i>Lactuca serriola</i>	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886449	KJ886288	KJ885966	KJ885805	KJ886127
	CCTU 1114	<i>Cichorium intybus</i>	Asteraceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886450	KJ886289	KJ885967	KJ885806	KJ886128
	CCTU 1183	<i>Lactuca serriola</i>	Asteraceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886451	KJ886290	KJ885968	KJ885807	KJ886129
	CCTU 1189	<i>Lactuca serriola</i>	Asteraceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886452	KJ886291	KJ885969	KJ885808	KJ886130
	CCTU 1207	<i>Lactuca serriola</i>	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886453	KJ886292	KJ885970	KJ885809	KJ886131
	CCTU 1209	<i>Xanthium strumarium</i> *	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886454	KJ886293	KJ885971	KJ885810	KJ886132
	CCTU 1006; CBS 136030	<i>Impatiens balsamina</i> *	Balsaminaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886455	KJ886294	KJ885972	KJ885811	KJ886133
	CCTU 1007; CBS 136031	<i>Hydrangea</i> sp.	Hydrangeaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886456	KJ886295	KJ885973	KJ885812	KJ886134
	CCTU 1010; CBS 136032	<i>Pelargonium hortorum</i> *	Geraniaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886457	KJ886296	KJ885974	KJ885813	KJ886135
	CCTU 1021; CBS 136033	<i>Amaranthus retroflexus</i>	Amaranthaceae	Iran, Guilan, Fuman	M. Bakhshi	KJ886458	KJ886297	KJ885975	KJ885814	KJ886136
	CCTU 1027; CBS 136034	<i>Lepidium sativum</i> *	Brassicaceae	Iran, Guilan, Chamkhaleh	M. Bakhshi	KJ886459	KJ886298	KJ885976	KJ885815	KJ886137
	CCTU 1029; CBS 136035	<i>Cucurbita maxima</i> *	Cucurbitaceae	Iran, Guilan, Rudsar	M. Bakhshi	KJ886460	KJ886299	KJ885977	KJ885816	KJ886138
	CCTU 1031; CBS 136036	<i>Urtica dioica</i> *	Urticaceae	Iran, Guilan, Sowme'eh Sara	M. Bakhshi	KJ886461	KJ886300	KJ885978	KJ885817	KJ886139
	CCTU 1048; CBS 136029	<i>Xanthium strumarium</i>	Asteraceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886462	KJ886301	KJ885979	KJ885818	KJ886140
CCTU 1055	<i>Hibiscus trionum</i> *	Malvaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886463	KJ886302	KJ885980	KJ885819	KJ886141	
CCTU 1059; CBS 136136	<i>Ecballium elaterium</i> *	Cucurbitaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886464	KJ886303	KJ885981	KJ885820	KJ886142	
CCTU 1064	<i>Amaranthus retroflexus</i>	Amaranthaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886465	KJ886304	KJ885982	KJ885821	KJ886143	
CCTU 1068	<i>Xanthium spinosum</i> *	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886466	KJ886305	KJ885983	KJ885822	KJ886144	
CCTU 1070; CBS 136137	<i>Gossypium herbaceum</i> *	Malvaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886467	KJ886306	KJ885984	KJ885823	KJ886145	
CCTU 1072	<i>Amaranthus biitoides</i>	Amaranthaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886468	KJ886307	KJ885985	KJ885824	KJ886146	
CCTU 1075	<i>Raphanus sativus</i> *	Brassicaceae	Iran, Guilan, Sowme'eh Sara	M. Bakhshi	KJ886469	KJ886308	KJ885986	KJ885825	KJ886147	
CCTU 1084; CBS 136156	<i>Amaranthus</i> sp.	Amaranthaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886470	KJ886309	KJ885987	KJ885826	KJ886148	
CCTU 1085	<i>Xanthium strumarium</i>	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886471	KJ886310	KJ885988	KJ885827	KJ886149	
CCTU 1093	<i>Buxus microphylla</i> *	Buxaceae	Iran, Mazandaran, Abbas abad	M. Bakhshi	KJ886472	KJ886311	KJ885989	KJ885828	KJ886150	
CCTU 1115; CBS 136139	<i>Cercis siliquastrum</i> *	Caesalpiniaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886473	KJ886312	KJ885990	KJ885829	KJ886151	
CCTU 1118; CBS 136140	<i>Populus deltoides</i>	Salicaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886474	KJ886313	KJ885991	KJ885830	KJ886152	
CCTU 1120	<i>Raphanus sativus</i>	Brassicaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886475	KJ886314	KJ885992	KJ885831	KJ886153	
CCTU 1128; CBS 136141	<i>Phaseolus vulgaris</i> *	Fabaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886476	KJ886315	KJ885993	KJ885832	KJ886154	
CCTU 1130; CBS 136142	<i>Olea europaea</i> *	Oleaceae	Iran, Zanjan, Tarom	M. Torbati	KJ886477	KJ886316	KJ885994	KJ885833	KJ886155	
CCTU 1136	<i>Cucurbita pepo</i> *	Cucurbitaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886478	KJ886317	KJ885995	KJ885834	KJ886156	
CCTU 1138	<i>Phaseolus vulgaris</i>	Fabaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886479	KJ886318	KJ885996	KJ885835	KJ886157	
CCTU 1139	<i>Phaseolus vulgaris</i>	Fabaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886480	KJ886319	KJ885997	KJ885836	KJ886158	
CCTU 1140; CBS 136143	<i>Calendula officinalis</i> *	Asteraceae	Iran, Guilan, Astara	M. Bakhshi	KJ886481	KJ886320	KJ885998	KJ885837	KJ886159	
CCTU 1141; CBS 136144	<i>Tagetes patula</i> *	Fabaceae	Iran, Guilan, Rudsar	M. Bakhshi	KJ886482	KJ886321	KJ885999	KJ885838	KJ886160	
CCTU 1142	<i>Phaseolus vulgaris</i>	Fabaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886483	KJ886322	KJ886000	KJ885839	KJ886161	
CCTU 1143; CBS 136145	<i>Datura stramonium</i> *	Solanaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886484	KJ886323	KJ886001	KJ885840	KJ886162	
CCTU 1145	<i>Cucurbita</i> sp.*	Cucurbitaceae	Iran, Guilan, Fuman	M. Bakhshi	KJ886485	KJ886324	KJ886002	KJ885841	KJ886163	
CCTU 1147	<i>Urtica dioica</i>	Urticaceae	Iran, Guilan, Masal	M. Bakhshi	KJ886486	KJ886325	KJ886003	KJ885842	KJ886164	
CCTU 1149; CBS 136146	<i>Leucantherum superbum</i> *	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886487	KJ886326	KJ886004	KJ885843	KJ886165	
CCTU 1150	<i>Buxus microphylla</i>	Buxaceae	Iran, Guilan, Fuman	M. Bakhshi	KJ886488	KJ886327	KJ886005	KJ885844	KJ886166	
CCTU 1154; CBS 136147	<i>Abutilon theophrasti</i> *	Malvaceae	Iran, Guilan, Rasht	M. Bakhshi	KJ886489	KJ886328	KJ886006	KJ885845	KJ886167	
CCTU 1155; 11	<i>Phaseolus vulgaris</i>	Fabaceae	Iran, Guilan, Fuman	M. Bakhshi	KJ886490	KJ886329	KJ886007	KJ885846	KJ886168	
CCTU 1156	<i>Xanthium strumarium</i>	Asteraceae	Iran, Guilan, Rasht	M. Bakhshi	KJ886491	KJ886330	KJ886008	KJ885847	KJ886169	
CCTU 1158	<i>Xanthium strumarium</i>	Asteraceae	Iran, Guilan, Langroud	M. Bakhshi	KJ886492	KJ886331	KJ886009	KJ885848	KJ886170	
CCTU 1159; CBS 136148	<i>Arachis hypogaea</i> *	Fabaceae	Iran, Guilan, Lahijan	M. Bakhshi	KJ886493	KJ886332	KJ886010	KJ885849	KJ886171	
CCTU 1160; CBS 136149	<i>Vicia faba</i> *	Fabaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886494	KJ886333	KJ886011	KJ885850	KJ886172	
CCTU 1161	<i>Phaseolus vulgaris</i>	Fabaceae	Iran, Guilan, Lahijan	M. Bakhshi	KJ886495	KJ886334	KJ886012	KJ885851	KJ886173	
CCTU 1162	<i>Citrullus lanatus</i>	Cucurbitaceae	Iran, Guilan, Lahijan	M. Bakhshi	KJ886496	KJ886335	KJ886013	KJ885852	KJ886174	
CCTU 1164	<i>Phaseolus vulgaris</i>	Fabaceae	Iran, Guilan, Lahijan	M. Bakhshi	KJ886497	KJ886336	KJ886014	KJ885853	KJ886175	
CCTU 1167; CBS 136150	<i>Anubias</i> sp.*	Araceae	Iran, Guilan, Kiashtahr	M. Bakhshi	KJ886498	KJ886337	KJ886015	KJ885854	KJ886176	

CCTU 1168	<i>Phaseolus vulgaris</i>	Fabaceae	Iran, Guilan, Kiasahr	M. Bakhshi	KJ886499	KJ886338	KJ886016	KJ885855	KJ886177
CCTU 1171	<i>Raphanus sativus</i>	Brassicaceae	Iran, Guilan, Kiasahr	M. Bakhshi	KJ886500	KJ886339	KJ886017	KJ885856	KJ886178
CCTU 1172	?	?	Iran, Guilan, Kiasahr	M. Bakhshi	KJ886501	KJ886340	KJ886018	KJ885857	KJ886179
CCTU 1175	<i>Phaseolus vulgaris</i>	Fabaceae	Iran, Guilan, Sowme'eh Sara	M. Bakhshi	KJ886502	KJ886341	KJ886019	KJ885858	KJ886180
CCTU 1195	<i>Datura stramonium</i>	Solanaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886503	KJ886342	KJ886020	KJ885859	KJ886181
CCTU 1198; CBS 136151	<i>Acer velutinum*</i>	Aceraceae	Iran, Mazandaran, Ramsar	M. Bakhshi	KJ886504	KJ886343	KJ886021	KJ885860	KJ886182
CCTU 1204	<i>Abutilon theophrasti</i>	Malvaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886505	KJ886344	KJ886022	KJ885861	KJ886183
CCTU 1209; CBS 136152	<i>Glycine max*</i>	Fabaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886506	KJ886345	KJ886023	KJ885862	KJ886184
CCTU 1210	<i>Glycine max</i>	Fabaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886507	KJ886346	KJ886024	KJ885863	KJ886185
CCTU 1211	<i>Glycine max</i>	Fabaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886508	KJ886347	KJ886025	KJ885864	KJ886186
CCTU 1212; CBS 136153	<i>Silybum marianum*</i>	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886509	KJ886348	KJ886026	KJ885865	KJ886187
CCTU 1216	<i>Ecballium elaterium</i>	Cucurbitaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886510	KJ886349	KJ886027	KJ885866	KJ886188
CCTU 1218	<i>Hibiscus trionum</i>	Malvaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886511	KJ886350	KJ886028	KJ885867	KJ886189
CCTU 1223; CBS 136154	<i>Eclipta prostrata*</i>	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886512	KJ886351	KJ886029	KJ885868	KJ886190
CCTU 1137; CBS 136124 (ex-type)	<i>Vicia faba</i>	Fabaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886513	KJ886352	KJ886030	KJ885869	KJ886191
CCTU 1137.2	<i>Vicia faba</i>	Fabaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886514	KJ886353	KJ886031	KJ885870	KJ886192
CCTU 1196; CBS 136123	<i>Hydrangea</i> sp.	Hydrangeaceae	Iran, Mazandaran, Ramsar	M. Bakhshi	KJ886515	KJ886354	KJ886032	KJ885871	KJ886193
CCTU 1038; CBS 136022 (ex-type)	<i>Chenopodium</i> sp.	Chenopodiaceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886516	KJ886355	KJ886033	KJ885872	KJ886194
CCTU 1045	<i>Chenopodium</i> sp.	Chenopodiaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886517	KJ886356	KJ886034	KJ885873	KJ886195
CCTU 1176	<i>Chenopodium album</i>	Chenopodiaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886518	KJ886357	KJ886035	KJ885874	KJ886196
CCTU 1004	<i>Bidens tripartita*</i>	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886519	KJ886358	KJ886036	KJ885875	KJ886197
CCTU 1121	<i>Urtica dioica*</i>	Urticaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886520	KJ886359	KJ886037	KJ885876	KJ886198
CCTU 1123	<i>Rumex crispus</i>	Polygonaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886521	KJ886360	KJ886038	KJ885877	KJ886199
CCTU 1129	<i>Rumex crispus</i>	Polygonaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886522	KJ886361	KJ886039	KJ885878	KJ886200
CCTU 1043; CBS 136038	<i>Solanum nigrum</i>	Solanaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886523	KJ886362	KJ886040	KJ885879	KJ886201
CCTU 1050	<i>Solanum nigrum</i>	Solanaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886524	KJ886363	KJ886041	KJ885880	KJ886202
CCTU 1173; CBS 136448 (ex-type)	<i>Sorghum halepense</i>	Poaceae	Iran, Guilan, Kiasahr	M. Bakhshi	KJ886525	KJ886364	KJ886042	KJ885881	KJ886203
CCTU 1173.2	<i>Sorghum halepense</i>	Poaceae	Iran, Guilan, Kiasahr	M. Bakhshi	KJ886526	KJ886365	KJ886043	KJ885882	KJ886204
CCTU 1002	<i>Celosia cristata</i>	Amaranthaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886527	KJ886366	KJ886044	KJ885883	KJ886205
CCTU 1015; CBS 136024	<i>Plantago major</i>	Plantaginaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886528	KJ886367	KJ886045	KJ885884	KJ886206
CCTU 1020; CBS 136023	<i>Sorghum halepense</i>	Poaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886529	KJ886368	KJ886046	KJ885885	KJ886207
CCTU 1030; CBS 136026	<i>Bidens tripartita</i>	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886530	KJ886369	KJ886047	KJ885886	KJ886208
CCTU 1046	<i>Plantago major</i>	Plantaginaceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886531	KJ886370	KJ886048	KJ885887	KJ886209
CCTU 1053; CBS 136027	<i>Cichorium intybus</i>	Asteraceae	Iran, Guilan, Sowme'eh Sara	M. Bakhshi	KJ886532	KJ886371	KJ886049	KJ885888	KJ886210
CCTU 1054	<i>Amaranthus</i> sp.	Amaranthaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886533	KJ886372	KJ886050	KJ885889	KJ886211
CCTU 1058	?	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886534	KJ886373	KJ886051	KJ885890	KJ886212
CCTU 1079; CBS 136025	<i>Amaranthus retroflexus</i>	Amaranthaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886535	KJ886374	KJ886052	KJ885891	KJ886213
CCTU 1090	<i>Abutilon theophrasti</i>	Malvaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886536	KJ886375	KJ886053	KJ885892	KJ886214
CCTU 1116	<i>Plantago major</i>	Plantaginaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886537	KJ886376	KJ886054	KJ885893	KJ886215
CCTU 1122	<i>Amaranthus</i> sp.	Amaranthaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886538	KJ886377	KJ886055	KJ885894	KJ886216
CCTU 1144; CBS 136130	<i>Cucurbita maxima</i>	Cucurbitaceae	Iran, Guilan, Masal	M. Bakhshi	KJ886539	KJ886378	KJ886056	KJ885895	KJ886217
CCTU 1197	<i>Bidens tripartita</i>	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886540	KJ886379	KJ886057	KJ885896	KJ886218
CCTU 1148; CBS 136125	<i>Coreopsis</i> sp.	Asteraceae	Iran, Guilan, Rasht	M. Bakhshi	KJ886541	KJ886380	KJ886058	KJ885897	KJ886219
CCTU 1148.2	<i>Coreopsis</i> sp.	Asteraceae	Iran, Guilan, Rasht	M. Bakhshi	KJ886542	KJ886381	KJ886059	KJ885898	KJ886220
CCTU 1025	<i>Viola</i> sp.	Violaceae	Iran, Mazandaran, Nowshahr	M. Bakhshi	KJ886543	KJ886382	KJ886060	KJ885899	KJ886221
CCTU 1012; CBS 136129	<i>Medicago</i> sp.	Fabaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886544	KJ886383	KJ886061	KJ885900	KJ886222
CCTU 1039	<i>Alhagi camelorum*</i>	Fabaceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886545	KJ886384	KJ886062	KJ885901	KJ886223
CCTU 1110	<i>Medicago sativa</i>	Fabaceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886546	KJ886385	KJ886063	KJ885902	KJ886224
CCTU 1180	<i>Medicago sativa</i>	Fabaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886547	KJ886386	KJ886064	KJ885903	KJ886225
CCTU 1181	<i>Trifolium repens</i>	Fabaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886548	KJ886387	KJ886065	KJ885904	KJ886226
CCTU 1185	<i>Vicia</i> sp.*	Fabaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886549	KJ886388	KJ886066	KJ885905	KJ886227
CCTU 1225	<i>Medicago sativa</i>	Fabaceae	Iran, East Azerbaijan, Marand	M. Bakhshi	KJ886550	KJ886389	KJ886067	KJ885906	KJ886228
CCTU 1239; CBS 135977	<i>Vitis vinifera*</i>	Vitaceae	Iran, East Azerbaijan, Kaleibar	M. Arzanlou	KJ886551	KJ886390	KJ886068	KJ885907	KJ886229
CCTU 1003	<i>Zinnia elegans</i>	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886552	KJ886391	KJ886069	KJ885908	KJ886230
CBS 113265	Leaf litter	—	The Netherlands, Utrecht	G.J.M. Verkley	KF251329	KF253276	KF253634	KF253981	KP207603

¹ CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CCTU: Culture Collection of Tabriz University, Tabriz, Iran.

² ITS: internal transcribed spacers and intervening 5.8S rDNA; TEF1- α : translation elongation factor 1- α ; ACT: actin; CAL: calmodulin; HIS: histone H3

* new host records.

in this study together with the sequence data from GenBank and the outgroup sequences was first done using MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/index.html>) (Katoh et al. 2002); and when considered necessary, manual adjustments were made by eye in MEGA v. 5 (Tamura et al. 2011).

Phylogenetic analyses were based on Bayesian inference (BI). For this purpose, the best nucleotide substitution model for each partition was determined using MrModeltest v. 2.2 (Nylander 2004). Based on the results of MrModeltest, a phylogenetic reconstruction under optimal criteria per partition was performed for the aligned combined dataset and phylogenetic trees were generated using MrBayes v. 3.2.1. (Ronquist & Huelsenbeck 2003). A Markov Chain Monte Carlo (MCMC) algorithm of four chains was started in parallel from a random tree topology with the heating parameter set at 0.15 and lasted until the average standard deviation of split frequencies came below 0.01. Trees were saved every 1 000 generations, the first 25 % of saved trees were discarded as the 'burn-in' phase and posterior probabilities (PP) determined from the remaining trees.

The resulting phylogenetic tree was printed with Geneious v. 5.6.7 (Drummond et al. 2012). Newly generated sequences in this study were deposited in NCBI's GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov>; Table 1) and alignments and phylogenetic trees in TreeBASE (www.treebase.org). The GenBank accession numbers for the strains obtained from NCBI can be found in Groenewald et al. (2013).

Taxonomy

All taxonomic descriptions are based on structures from herbarium material. Diseased leaf tissues were observed under a stereo-microscope and relevant morphological structures (stromata, conidiophores and conidia) were picked up from lesions with a sterile inoculation needle and mounted on glass slides in clear lactic acid. Thirty measurements were made at $\times 1\ 000$ magnification using a Nikon Eclipse 80i light microscope for each microscopic structure, and 95 % confidence intervals were derived for the measurements with extreme values given in parentheses. High-resolution photographic images of microscopic fungal structures were captured with a Nikon digital sight DS-f1 high definition colour camera mounted on the Nikon Eclipse 80i light microscope. Adobe Photoshop CS3 was used for the final editing of acquired images and photographic preparations. Colony colours on MEA were determined after 1 mo at 25 °C in the dark in duplicate. The mycological colour charts of Rayner (1970) were used to define colours of the fungal colonies. Nomenclatural novelties and descriptions were deposited in MycoBank (www.Mycobank.org; Crous et al. 2004a). The naming system for tentatively applied names used by Groenewald et al. (2013) is continued in this manuscript to simplify comparison between the studies.

RESULTS

DNA sequencing and phylogenetic analysis

A total of 161 *Cercospora* isolates corresponding to 74 host species and 28 host families were collected for DNA sequence analysis from the north and north-western parts of Iran. Approximately 700, 300, 200, 450 and 400 bp were obtained for ITS, TEF1- α , ACT, CAL and HIS loci, respectively.

The resulting concatenated alignment contains 294 ingroup taxa (including 133 taxa from NCBI, and 161 taxa from this study) with a total of 1 634 characters (including alignment gaps). *Ramularia endophylla* (isolate CBS 113265) was used as the outgroup taxon. Four sets of four Ns were used in the alignment to separate adjacent loci and were excluded from the phylogenetic analyses. The gene boundaries were 1–474

bp for ITS, 479–802 bp for TEF1- α , 807–1 006 bp for ACT, 1 011–1 268 bp for CAL and 1 273–1 634 bp for HIS. Based on the results of MrModeltest, a GTR+G model with a gamma-distributed rate variation for ITS, ACT and CAL, and HKY+G with gamma-distributed rates for TEF1- α were suggested while HIS required HKY+I+G with inverse gamma distributed. All partitions had dirichlet base frequencies. From this alignment 1 618 characters were used for the Bayesian analysis; these contained 625 unique site patterns (54, 211, 112, 124 and 124 for ITS, TEF1- α , ACT, CAL and HIS, respectively). The Bayesian analysis lasted 28 720 000 generations and delineated a total of 57 442 trees. After discarding the first 25 % of sampled trees (the first 7 180 000 generations) for burn-in, the consensus trees and posterior probabilities were calculated from the remaining 43 082 trees.

All genes were also assessed individually using Bayesian analysis under the above-mentioned substitution models, for each data partition (data not shown). The ITS region had limited resolution for almost all species in *Cercospora*, and was only able to distinguish *C. chenopodii*, *C. solani* and *C. sorghicola* from the other species examined. Based on the TEF1- α region, we were able to distinguish seven of the 20 species including *C. chenopodii*, *C. conyzae-canadensis*, *C. rumicis*, *C. solani*, *C. sorghicola*, *C. violae* and *C. cf. zinniae*, whereas nine of the 20 species including *C. althaeina*, *C. chenopodii*, *C. conyzae-canadensis*, *C. cylindracea*, *C. pseudochenopodii*, *C. solani*, *C. sorghicola*, *C. violae* and *C. cf. zinniae* were distinguished in the ACT phylogeny. Based on the CAL region, we were able to differentiate eight of the 20 lineages, i.e. *C. armoraciae*, *C. beticola*, *C. conyzae-canadensis*, *C. solani*, *C. sorghicola*, *Cercospora* sp. T, *C. violae* and *C. cf. zinniae*. Based on the HIS region, we distinguished 10 of the 20 lineages, including *C. chenopodii*, *C. conyzae-canadensis*, *C. cylindracea*, *C. pseudochenopodii*, *C. rumicis*, *C. solani*, *C. sorghicola*, *Cercospora* sp. G sensu Groenewald et al. (2013), *C. violae* and *C. zebrina*.

Based on results of the multigene concatenated BI phylogenies, the posterior probability (PP) support for the grouping of most species ranged from 1 to 0.97 as found by Groenewald et al. (2013). However support for deeper nodes were often lower, indicating that the branching may be uncertain for the root of these species. As previously stated by Groenewald et al. (2013), no single locus was found which could reliably distinguish all species and, occurrences of the same sequence(s) shared between multiple species in one locus, were observed.

Taxonomy

The Consolidated Species Concept was employed in this study to distinguish species, revealing a rich diversity among the *Cercospora* species studied. Twenty lineages of *Cercospora* from Iran were resolved based on the clustering and support in the Bayesian tree obtained from the combined ITS, TEF1- α , ACT, CAL and HIS alignment (Fig. 1). Data are alphabetically summarised in Table 1. Eight species including *C. althaeina*, *C. apii* (species complex), *C. armoraciae* (species complex), *C. beticola*, *C. chenopodii*, *C. rumicis*, *C. violae* and *C. zebrina* were assigned to existing species names. Three more species including *C. cf. flagellaris* (species complex), *C. cf. richardii-cola* and *C. cf. zinniae* were morphologically similar to existing species, but as explained by Groenewald et al. (2013), these names could not be applied in cases where the ex-type strain was unavailable. In these cases, species were indicated with 'cf.' in the species name.

In addition, several new hosts were recognised for the previously known *Cercospora* species including *C. apii*, *C. armoraciae*,

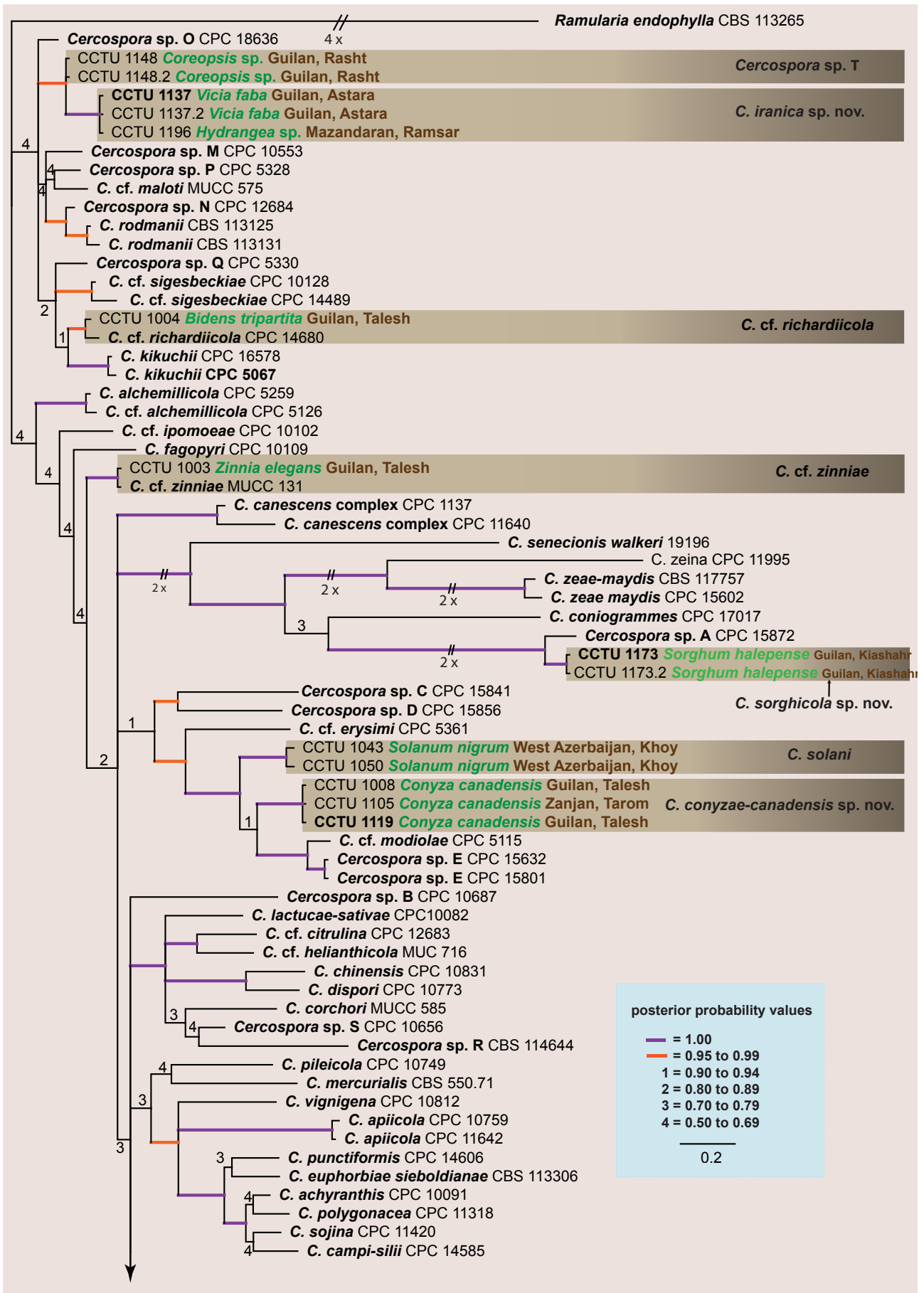


Fig. 1 (Part 1) Consensus phylogram (50 % majority rule) of 43 082 trees resulting from a Bayesian analysis of the combined 5-gene sequence alignment using MrBayes v. 3.2.1. Bayesian posterior probabilities are indicated with colour-coded branches and numbers (see legend) and the scale bar indicates 0.2 expected changes per site. Lineages from Iran are indicated in coloured blocks and species names in black text. Hosts and provinces of origin are indicated in green and brown text, respectively. The tree was rooted to *Ramularia endophylla* (isolate CBS 113265).

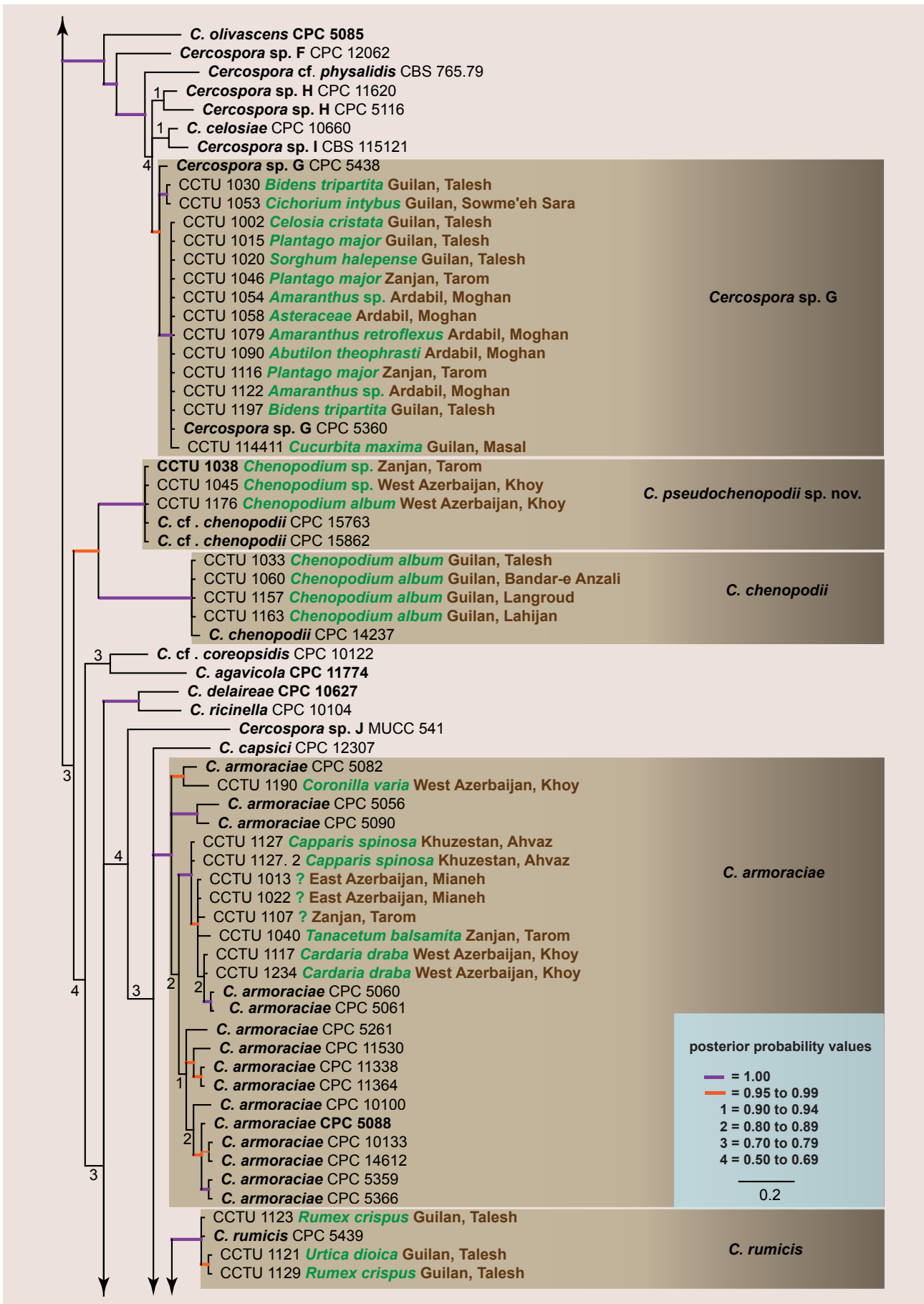


Fig. 1 (cont.) (Part 2)

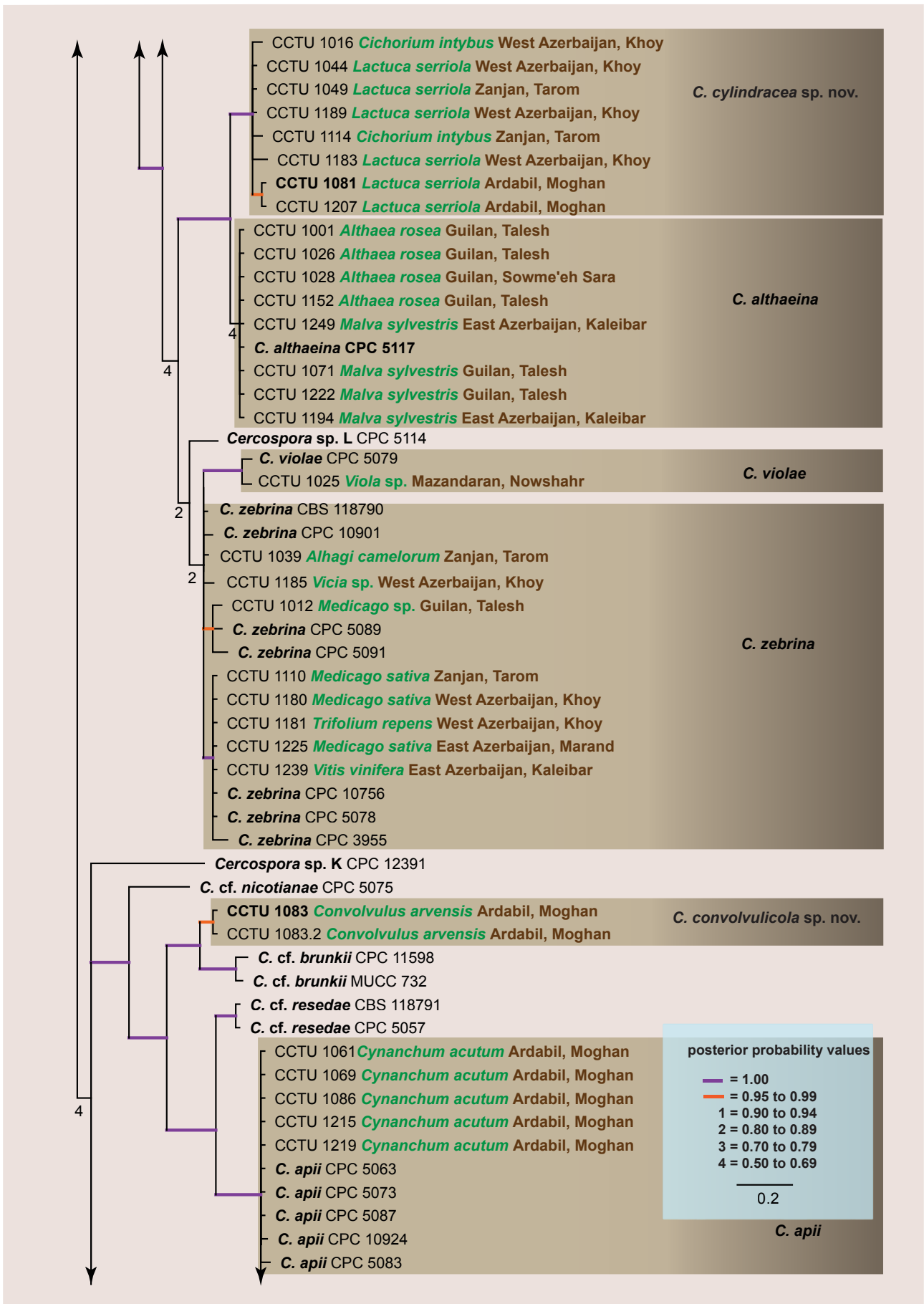


Fig. 1 (cont.) (Part 3)

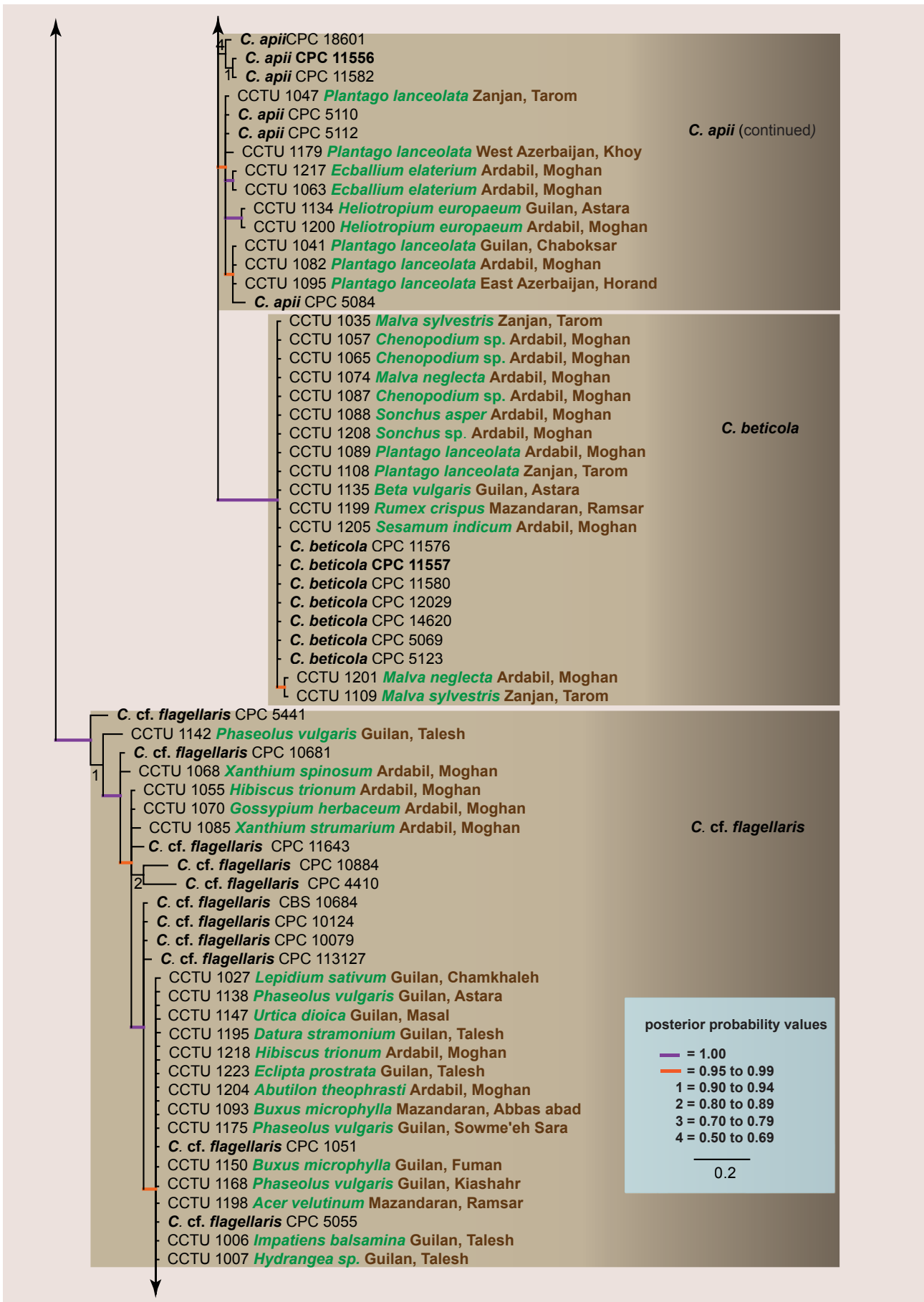


Fig. 1 (cont.) (Part 4)

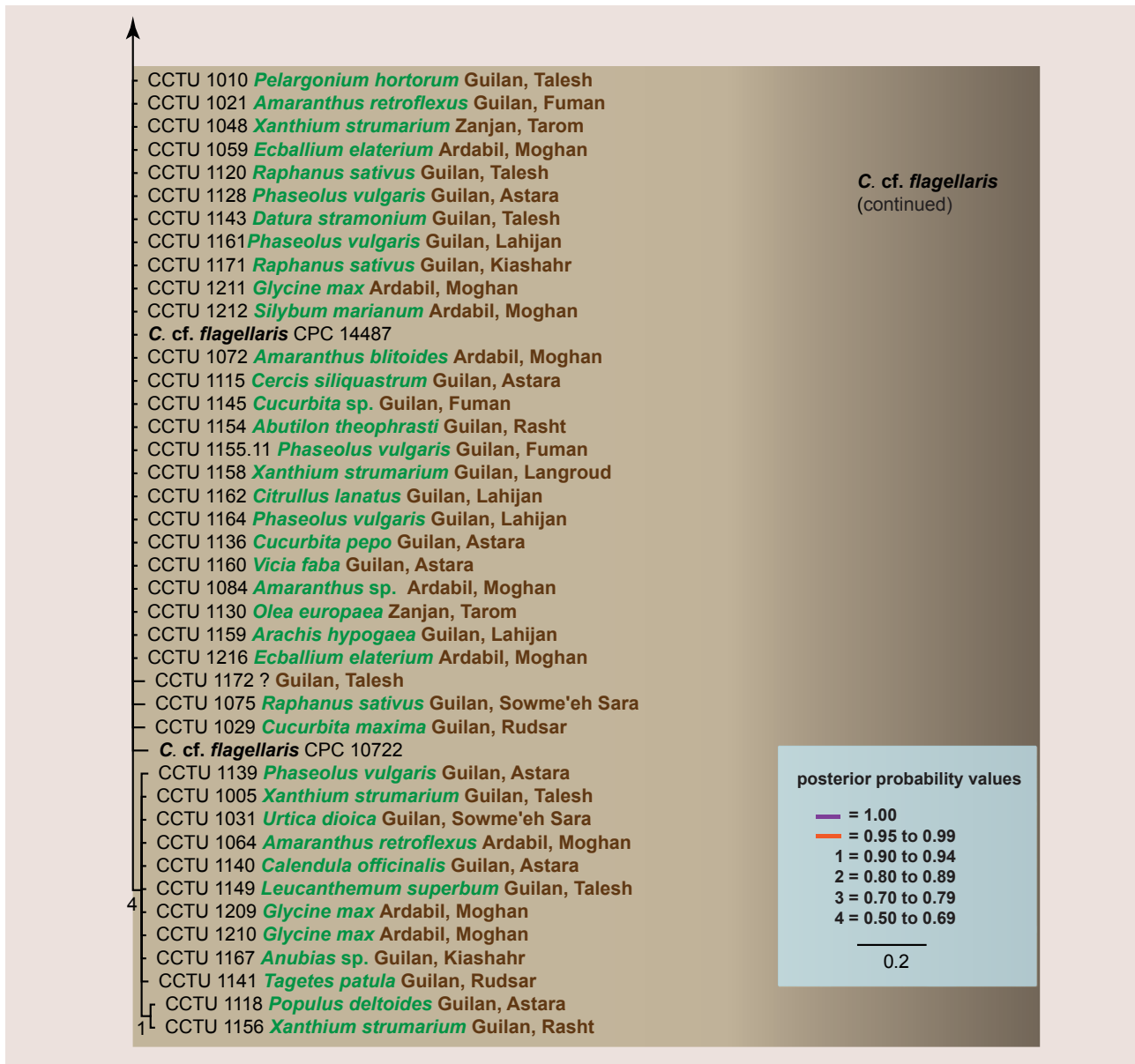


Fig. 1 (cont.) (Part 5)

C. beticola, *C. cf. richardiicola*, *C. rumicis*, *Cercospora* sp. G and *C. zebrina*. Novel host records are shown with an asterisk in Table 1. *Cercospora* cf. *flagellaris* and *Cercospora* sp. G sensu Groenewald et al. (2013), two species with wide host ranges (infecting 18 and six host families respectively in this study), were common species in the sampled areas, and sometimes these two species infected the same host at the same time.

Furthermore, eight additional lineages were recognised in this study. Identification of these lineages required careful morphological comparison and consideration of host-fungus relationships, as well as knowledge of the relevant scientific literature (Crous & Braun 2003) and databases (Systematic Mycology and Microbiology Laboratory (SMML), <http://nt.ars-grin.gov/fungalDATABASES/fungushost/fungushost.cfm>).

Cercospora chenopodii Fresen., Beitr. Mykol.: 92. 1863 — Fig. 2

Additional synonyms in Groenewald et al. (2013)

Description in planta — *Leaf spots* amphigenous, distinct, circular to subcircular, 2–6 mm diam, pale brown with black dots (stroma with conidiophores), definite margin, surrounded

by a dark pink border. *Mycelium* internal. *Caespituli* amphigenous, brown. *Conidiophores* aggregated in dense fascicles (5–35), arising from the upper cells of a moderately developed brown stroma, up to 70 µm wide; conidiophores medium brown, becoming pale brown towards the apex, 2–8-septate, straight to variously curved, unbranched, (40–)62–72(–90) × 4–6 µm, width of conidiogenous cells immediately behind the fertile region is often narrower. *Conidiogenous cells* intercalary and terminal, unbranched, pale brown, smooth, proliferating sympodially, 20–50 × 4–6 µm, mostly mono-local, sometimes multi-local; loci thickened, darkened, protuberant, refractive, apical or lateral, 2–3.5 µm diam. *Conidia* solitary, smooth, subcylindrical, straight to slightly curved, hyaline, distinctly (0–)2–4(–5)-septate, apex obtuse, base obconically truncate, sometimes constricted at the septa, (20–)27–32(–40) × 5–6(–7) µm; hila thickened, darkened, refractive, 2–4 µm diam.

Specimens examined. IRAN, Guilan Province, Talesh, on leaves of *Chenopodium album* (*Chenopodiaceae*), Sept. 2011, M. Bakhshi, CCTU 1033; Guilan Province, Bandar-e Anzali, on leaves of *C. album* (*Chenopodiaceae*), June 2012, M. Bakhshi, CCTU 1060; Guilan Province, Langroud, on leaves of *C. album* (*Chenopodiaceae*), Aug. 2012, M. Bakhshi, CCTU 1157; Guilan Province, Lahijan, on leaves of *C. album* (*Chenopodiaceae*), Aug. 2012, M. Bakhshi, CCTU 1163.

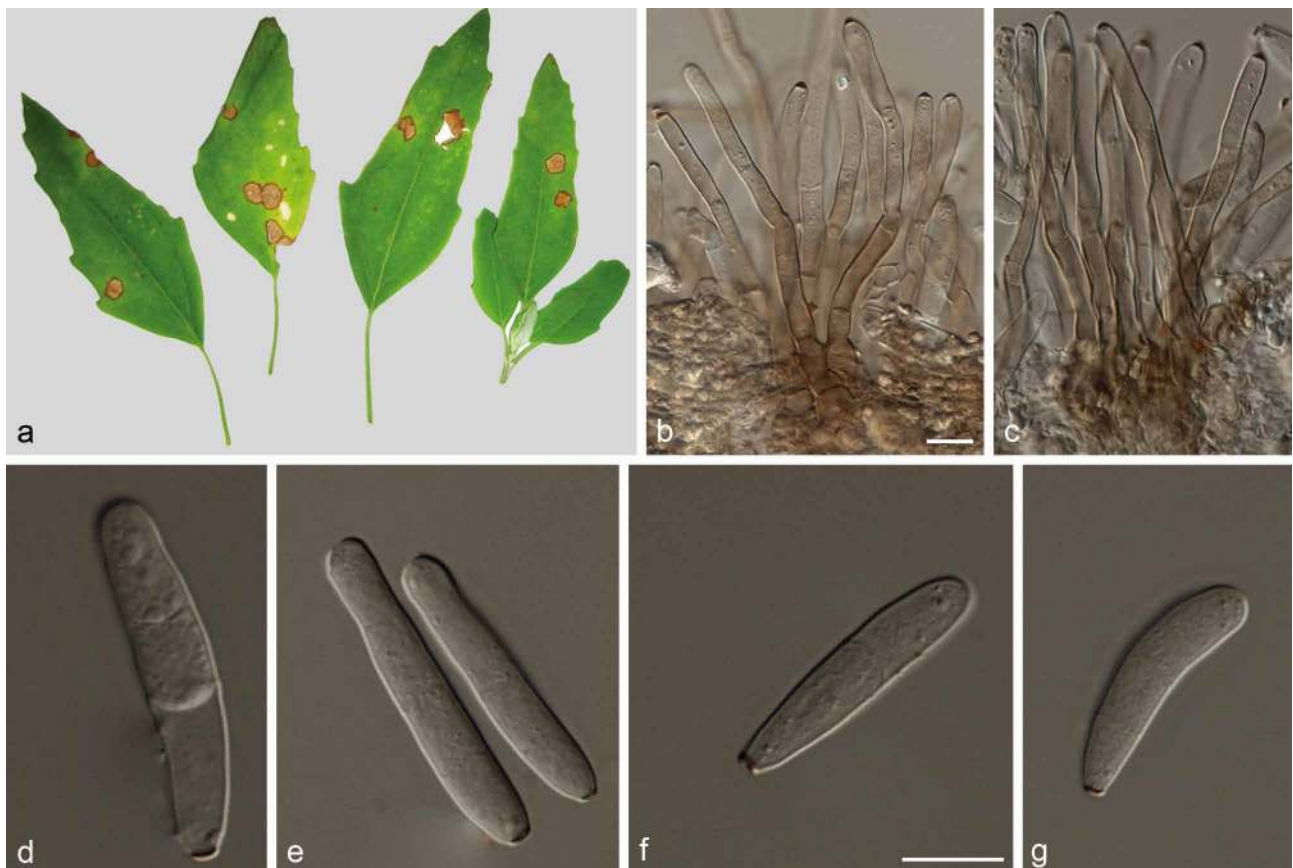


Fig. 2 *Cercospora chenopodii* (CCTU 1033). a. Leaf spots; b. c. fasciculate conidiophores; d–g. conidia. — Scale bars = 10 μ m.

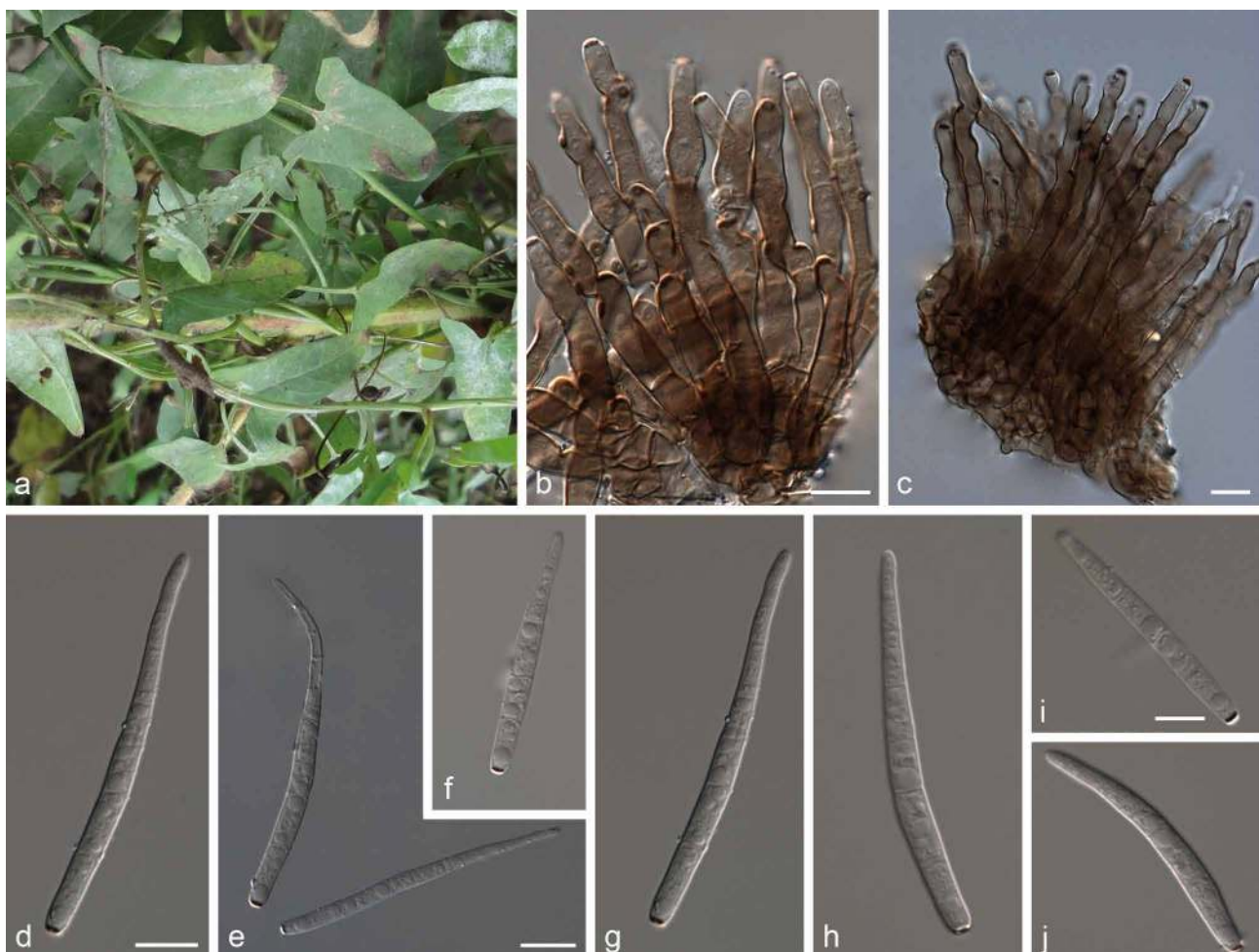


Fig. 3 *Cercospora convulvicolae* (CBS 136126). a. Leaf spots; b. c. fasciculate conidiophores; d–j. conidia. — Scale bars = 10 μ m.

Cercospora convolvulicola M. Bakhshi, Arzanlou, Babai-ahari, Crous & U. Braun, *sp. nov.* — MycoBank MB809116; Fig. 3

Etymology. Named after the host genus on which it was collected, *Convolvulus*.

Description in planta — *Leaf spots* circular to subcircular, 2–8 mm, grey-brown to brown, not surrounded by margin of different colour. *Mycelium* internal. *Caespituli* amphigenous, brown. *Conidiophores* straight or sinuously geniculate, in dense fascicles, arising from the upper cells of a well-developed, intraepidermal and substomatal, brown stroma, up to 40 µm diam; conidiophores pale brown to brown, simple, rarely branched, moderately thick-walled, irregular in width, attenuated at the upper portion, often constricted at septa and proliferating point, 35–50(–70) × (3–)4–6 µm, 2–5-septate. *Conidiogenous cells* intercalary and terminal, proliferating sympodially, 10–20 × 3–5.5 µm, multi-local; loci distinctly thickened, apical, lateral or formed on the shoulders caused by geniculation, sometimes circumspersed, protuberant, 1.5–2.5 µm. *Conidia* solitary, hyaline, subcylindrical to obclavate, straight or slightly curved, truncate to somewhat obconically truncate at the base, subacute or sub-obtusely rounded at the apex, 35–50(–65) × (2.5–)3.5–4.5 µm, 3–8-septate, guttulate; hila thickened, darkened, refractive, 1.5–2.5 µm diam.

Cultural characteristics — Colonies on MEA reaching 55 mm diam after 20 d at 25 °C in the dark; flat with smooth, even margins and moderate aerial mycelium; surface olivaceous-grey, reverse dark iron-grey.

Specimens examined. IRAN, Ardabil Province, Moghan, on *Convolvulus arvensis* (*Convolvulaceae*), Oct. 2011, M. Bakhshi (holotype IRAN 16454 F, culture ex-type CCTU 1083 = CBS 136126); Moghan, on *C. arvensis* (*Convolvulaceae*), Oct. 2011, M. Bakhshi, CCTU 1083.2.

Notes — Based on individual gene trees, the two isolates representing this species are never supported in their own clade; in the TEF1- α and ACT phylogenies, they are intermixed with *C. cf. flagellaris* and *C. cf. brunckii*; in the CAL phylogeny with *C. apii* and *C. cf. brunckii*, and in the HIS phylogeny with *C. rodmanii*, *C. cf. zinniae* and *Cercospora* spp. N, P and Q sensu Groenewald et al. (2013). Shared alleles are the likely cause for the separate position of *C. convolvulicola* in the combined phylogeny (Fig. 1, part 3). *Cercospora convolvulicola* is sister to *C. cf. brunckii* and appears to be specific to *Convolvulus arvensis*. The only species known from *Convolvulus arvensis*, is *C. ipomoea*. *Cercospora cf. ipomoea* (tentative name for *C. ipomoea*) has a different phylogenetic position. *Cercospora convolvulicola* differs morphologically from *C. ipomoea*, by having dense conidiophores and shorter, guttulate, subcylindrical to obclavate conidia (Fig. 3).

Cercospora conyzae-canadensis M. Bakhshi, Arzanlou, Babai-ahari, Crous & U. Braun, *sp. nov.* — MycoBank MB809117; Fig. 4

Etymology. Named after the host plant from which it was collected, *Conyza canadensis*.

Description in planta — *Leaf spots* amphigenous, circular, 1–4 mm diam, grey to pale brown with dark brown margins. *Mycelium* internal. *Caespituli* amphigenous, brown. *Conidiophores* aggregated in loose fascicles (3–15), arising from a weakly developed, intraepidermal and substomatal, dark brown stroma, up to 30 µm diam; conidiophores brown to dark brown, 2–6-septate, straight to geniculate-sinuous due to sympodial proliferation, simple, thick-walled, uniform in width, often constricted at the proliferating point, (57–)97–112(–140) × 4.5–5.5 µm. *Conidiogenous cells* intercalary and terminal, pale brown to brown, proliferating sympodially, 20–40 × 4–5.5 µm, multi-local;



Fig. 4 *Cercospora conyzae-canadensis* (CBS 135978). a. Leaf spots; b. c. fasciculate conidiophores; d–h. conidia. — Scale bars = 10 µm.

loci distinctly thickened, darkened and somewhat refractive, apical or formed on shoulders caused by sympodial proliferation, 2–3.5 µm diam. *Conidia* solitary, filiform to obclavate-cylindrical, straight to slightly curved, hyaline, (32–)60–94(–170) × 3.5–5.5 µm, (3–)7–12(–17)-septate, with subobtusely rounded apices and truncate to obconically truncate bases; hila thickened, darkened, refractive, 1.5–2.5 µm diam.

Cultural characteristics — Colonies on MEA reaching 24 mm diam after 20 d at 25 °C in the dark; erumpent with smooth, irregular margins and sparse aerial mycelium; dark olivaceous-green on the surface, dark blue-green underneath.

Specimens examined. IRAN, Guilan Province, Talesh, on *Conyza canadensis* (Asteraceae), Nov. 2012, *M. Bakhshi* (holotype IRAN 16455 F, culture ex-type CCTU 1119 = CBS 135978); Talesh, on *C. canadensis* (Asteraceae), Aug. 2011, *M. Bakhshi*, CCTU 1008; Zanjan Province, Tarom, on *C. canadensis* (Asteraceae), Aug. 2012, *M. Bakhshi*, CCTU 1105.

Notes — *Cercospora conyzae-canadensis* must be regarded as a new species, based on its distinct phylogenetic position. In the individual gene trees (ACT, TEF1- α , CAL and HIS), it is distinguished from all other species. In the combined tree (Fig. 1, part 1), it is a sister taxon to the clade including *C. cf. modiolae* and *Cercospora* sp. E sensu Groenewald et al. (2013). Three species of *Cercospora*, including *C. bidentis*, *C. erigeronicola* and *C. nilghirensis*, have been reported from *Conyza*. *Cercospora conyzae-canadensis* is morphologically distinguished from those species by its moderately developed stroma, loose fascicles and dark brown conidiophores. *Cercospora erigeronicola* is distinct in having shorter and narrower, 0–3-septate conidia, 15–45 × 2–3.5 µm. *Cercospora conyzae-canadensis* is morphologically close to *C. nilghirensis* in conidial shape and size. However *C. nilghirensis*, described from India on *Conyza ambigua*, lacks stromata and has numerous longer conidiophores that are densely fasciculate. *Cercospora conyzae-canadensis* appears to be specific to *Conyza canadensis*.

Cercospora cylindracea M. Bakhshi, Arzanlou, Babai-ahari, Crous & U. Braun, *sp. nov.* — MycoBank MB809118; Fig. 5

Etymology. Name derived from the cylindrical conidia.

Description in planta — *Leaf spots* distinct, circular to sub-circular, sometimes angular, pale brown, with broad brown margin, sometimes appearing as an eye spot, 1–7 mm diam. *Mycelium* internal. *Caespituli* amphigenous, brown. *Conidiophores* in divergent fascicles (4–25), arising from the upper cells of a moderately to well-developed, intraepidermal and substomatal, brown stroma, up to 30 µm diam; conidiophores pale brown to brown, thick-walled, 1–6-septate, straight, sinuous to distinctly geniculate, flexuous, (35–)55–65(–90) × 4–5.5 µm, irregular in wide, conically narrowed at the apex. *Conidiogenous cells* terminal or intercalary, unbranched, pale brown, smooth, proliferating sympodially, 15–30 × 3.5–5 µm, multi-local; loci thickened, darkened, refractive, protuberant, apical, lateral or circumsperised, 1.5–2.5 µm diam. *Conidia* solitary, subcylindrical to cylindrical, straight to mildly curved, hyaline, distinctly 1–10-septate, obtuse at the apex, subtruncate at the base, (30–)45–60(–90) × 3.5–5.5 µm; hila thickened, darkened, refractive, 1.5–2.5 µm diam.

Cultural characteristics — Colonies on MEA reaching 62 mm diam after 20 d at 25 °C in the dark; erumpent, folded, with smooth, even margins and sparse to moderate aerial mycelium; surface olivaceous-grey, reverse dark olivaceous-grey.

Specimens examined. IRAN, Ardabil Province, Moghan, on *Lactuca serriola* (Asteraceae), Sept. 2011, *M. Bakhshi* (holotype IRAN 16468 F, culture ex-type CCTU 1081 = CBS 138580); Moghan, on *L. serriola* (Asteraceae), Oct. 2012, *M. Bakhshi*, CCTU 1207; West Azerbaijan Province, Khoy, on *Cichorium intybus* (Asteraceae), June 2011, *M. Arzanlou*, CCTU 1016; Khoy, on *L. serriola* (Asteraceae), Sept. 2011, *M. Arzanlou*, CCTU 1044 = CBS 136021; Khoy, on *L. serriola* (Asteraceae), Sept. 2011, *M. Arzanlou*, CCTU 1049; Khoy, on *L. serriola* (Asteraceae), Sept. 2012, *M. Arzanlou*, CCTU 1183; Khoy, on



Fig. 5 *Cercospora cylindracea* (CBS 138580). a. Leaf spots on *Cichorium intybus*; b. leaf spots on *Lactuca serriola*; c–e. fasciculate conidiophores; f–k. conidia. — Scale bars = 10 µm.

L. serriola (Asteraceae), Sept. 2012, M. Arzanlou, CCTU 1189; Zanjan Province, Tarom, on *C. intybus* (Asteraceae), Oct. 2011, M. Bakhshi, CCTU 1114.

Notes — *Cercospora cylindracea* clusters as a sister taxon to the *C. althaeina* clade in the combined tree (Fig. 1, part 3). The host range of *C. cylindracea* is limited to *Lactuca serriola* and *Cichorium intybus* (both in the Asteraceae). *Cercospora cylindracea* is distinguished from *C. althaeina* in the HIS and ACT phylogenies but not in the TEF1- α phylogeny. In the CAL phylogeny, isolates are intermixed with those of *C. zebrina*, *Cercospora* sp. L sensu Groenewald et al. (2013) and *C. althaeina*. Three species of *Cercospora* including *C. apii*, *C. lactuca-sativae* and *C. cichorii*, are known from *Lactuca serriola* and *Cichorium intybus*. *Cercospora cylindracea* is separated in the combined gene tree from *C. apii* and *C. lactuca-sativae* as circumscribed in Groenewald et al. (2013) who studied Japanese material on *Lactuca sativa*. *Cercospora cylindracea* differs from *C. cichorii* and *C. lactuca-sativae* by its cylindrical to subcylindrical conidia. Furthermore, the conidiogenous loci in *C. lactuca-sativae* are broader, 2.5–3.5 μm , than in *C. cylindracea*.

Cercospora iranica M. Bakhshi, Arzanlou, Babai-ahari, Crous & U. Braun, sp. nov. — MycoBank MB809119; Fig. 6

Etymology. Named after Iran, the country of the type location.

Description in planta — *Leaf spots* amphigenous, circular, 1–7 mm, first appearing as red-brown spots, later centre becoming grey with red-brown borders on upper and lower surface. *Mycelium* internal. *Caespituli* amphigenous, brown. *Conidiophores* aggregated in moderately dense fascicles (8–20), arising from a well-developed, erumpent, dark brown stroma, up to 40 μm diam; conidiophores brown, becoming pale brown towards the apex, 2–6-septate, straight to geniculate-sinuuous due to sympodial proliferation, simple, uniform in width, sometimes con-

stricted at the proliferating point, (30–)62–71(–90) \times 4–5.5(–6) μm . *Conidiogenous cells* intercalary and terminal, pale brown to brown, proliferating sympodially, 15–35 \times 4–5 μm , multi-local; loci distinctly thickened, darkened and somewhat refractive, apical, lateral or formed on shoulders caused by geniculation, 2–3.5 μm diam. *Conidia* solitary, obclavate when smaller, longer ones filiform to acicular, straight to slightly curved, hyaline, (27–)52–67(–95) \times 2–4 μm , (3–)7–10(–14)-septate, with subobtusely rounded apices and truncate or long obconically truncate bases; hila thickened, darkened, refractive, 1.5–2 μm diam.

Cultural characteristics — Colonies on MEA reaching 60 mm diam after 20 d at 25 °C in the dark; erumpent with smooth, even margins and moderate aerial mycelium; surface pale grey-olivaceous in centre, vinaceous-grey in outer region, reverse iron-grey in centre, dark pink-grey in outer region.

Specimens examined. IRAN, Guilan Province, Astara, on leaves of *Vicia faba* (Fabaceae), June 2012, M. Bakhshi (holotype IRAN 16466 F, culture ex-type CCTU 1137 = CBS 136124); Astara, on leaves of *V. faba* (Fabaceae), June 2012, M. Bakhshi, CCTU 1137.2; Mazandaran Province, Ramsar, on leaves of *Hydrangea* sp. (Hydrangeaceae), Sept. 2012, M. Bakhshi, CCTU 1196 = CBS 136123.

Notes — In the TEF1- α , HIS and ACT phylogeny, isolates of *C. iranica* and *Cercospora* sp. T cluster together in a distinct well-supported clade. In the CAL phylogeny, *C. iranica* forms a distinct clade, whereas *Cercospora* sp. T cannot be distinguished from *Cercospora* spp. M, O, P and Q (sensu Groenewald et al. 2013), nor from *C. alchemillicola* and *C. cf. sigesbeckiae*. The different CAL sequences explain the basal position of *Cercospora* sp. T to the *C. iranica* clade in the combined phylogeny (Fig. 1, part 1). *Cercospora zonata*, the causal agent of *Cercospora* leaf spot of faba beans (Kimber 2011) is morphologically quite distinct from *C. iranica* in having much broader (3–6 μm) obclavate-cylindrical conidia with short

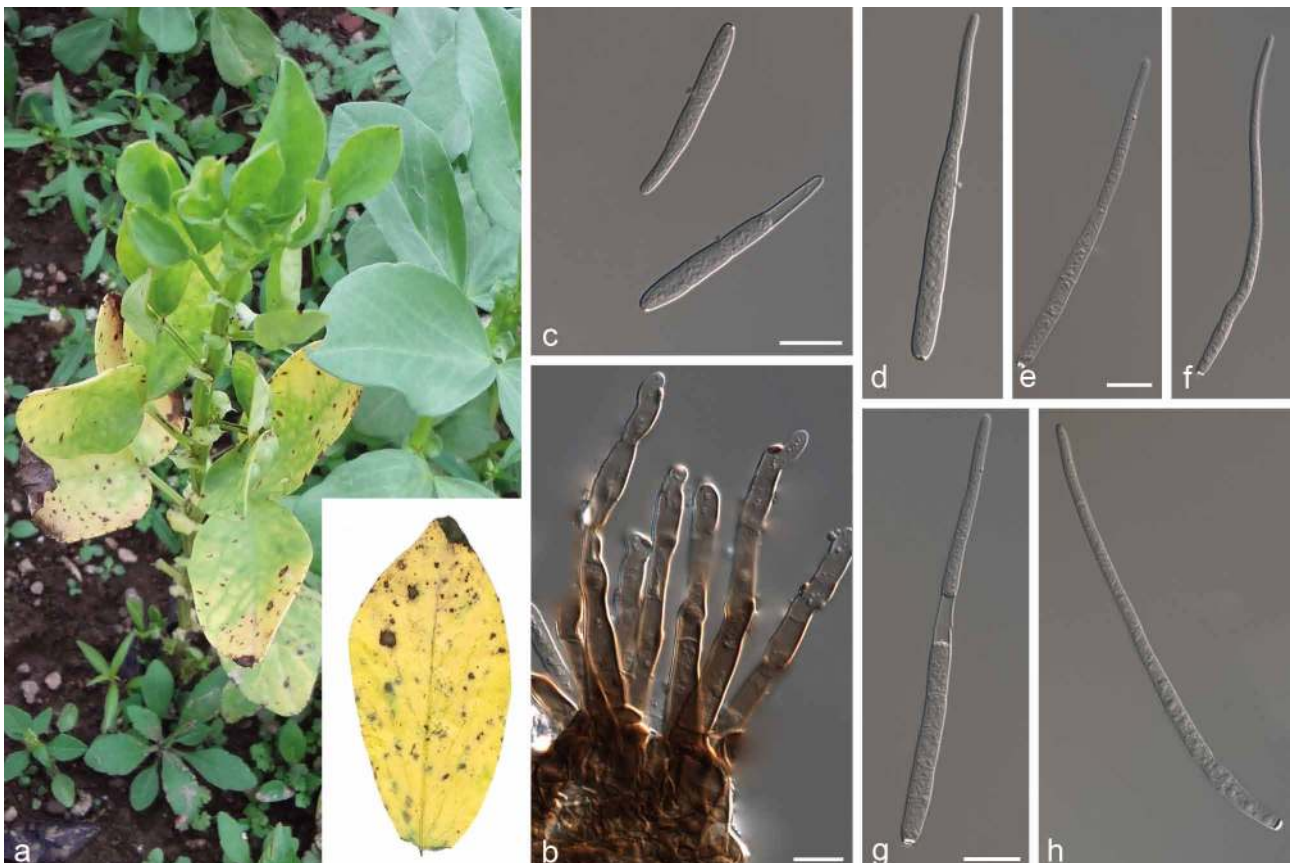


Fig. 6 *Cercospora iranica* (CBS 136124). a. Leaf spots; b. fasciculate conidiophores; c–h. conidia. — Scale bars = 10 μm .

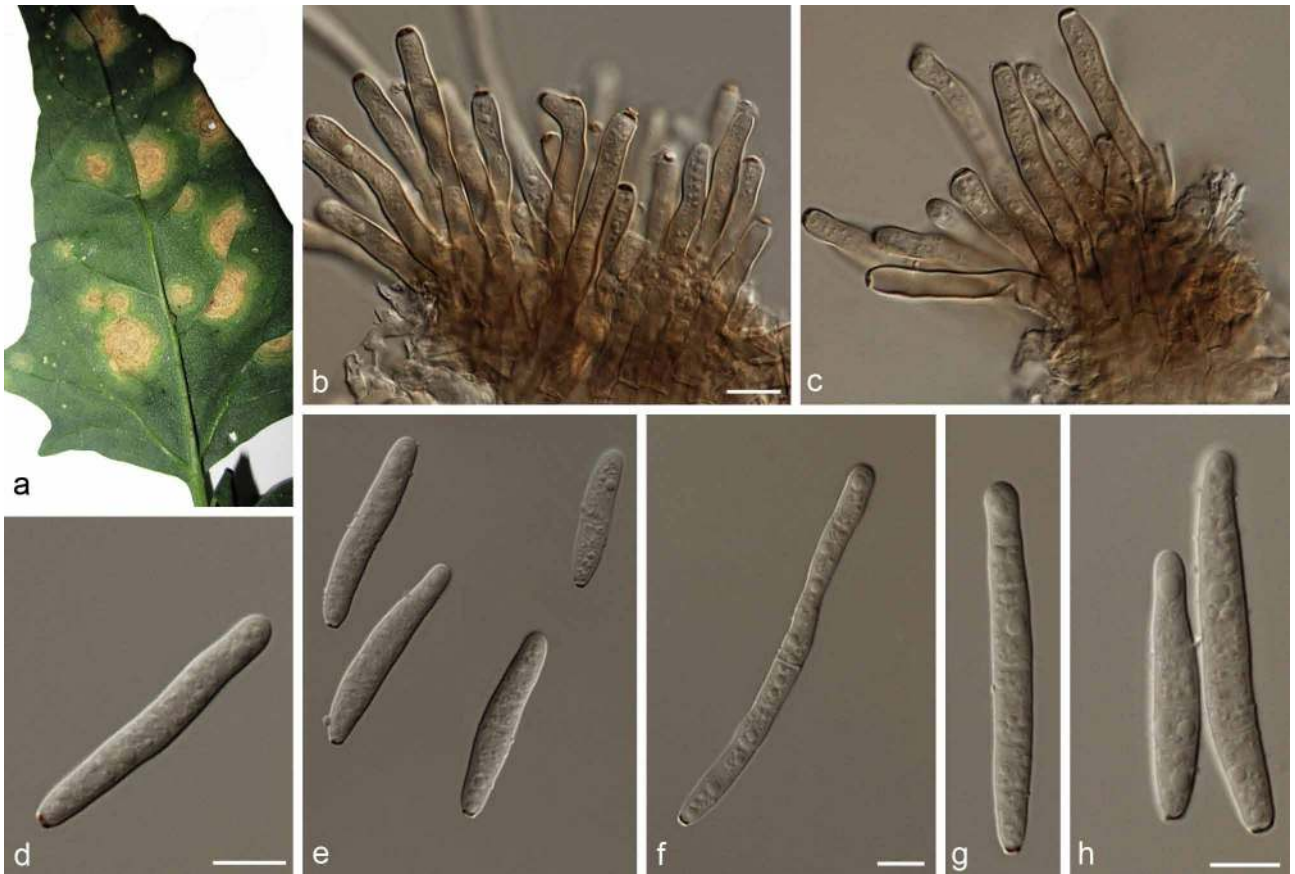


Fig. 7 *Cercospora pseudochenopodii* (CBS 136022). a. Leaf spots; b. c. fasciculate conidiophores; d–h. conidia. — Scale bars = 10 μ m.



Fig. 8 *Cercospora solani* (CBS 136038). a. Leaf spots; b. c. fasciculate conidiophores; d–h. conidia. — Scale bars = 10 μ m.

obconical base and larger hila, 2–2.5 µm wide (various collections examined, including topotype material of *C. zonata*: on *Vicia faba*, Portugal, May 1884, F. Moller, Rabenh., Fung. Eur. Exs. 3294, B, HAL). Caespituli that arise from a well-developed, erumpent stroma on the leaf surface is a unique morphological character of this species on *Vicia faba*.

Cercospora pseudochenopodii M. Bakhshi, Arzanlou, Babai-ahari & Crous, *sp. nov.* — MycoBank MB809120; Fig. 7

Etymology. Named after its superficial resemblance to *Cercospora chenopodii*.

Description in planta — *Leaf spots* amphigenous, circular to irregular, 5–12 mm diam, pale brown, with concentric rings on adaxial and abaxial surface (stroma with conidiophores), indefinite margin, not surrounded by a border of different colour. *Mycelium* internal. *Caespituli* amphigenous, brown. *Conidiophores* aggregated in dense fascicles (8–40), emerging through stomatal openings or erumpent through the cuticle, arising from the upper cells of a moderately developed brown stroma, up to 60 µm wide; conidiophores pale brown to brown, 2–5-septate, thick-walled, mainly straight, sometimes geniculate in upper part, unbranched, almost uniform in width, (32–)39–45(–60) × (3.5–)4.5–5(–6.5) µm. *Conidiogenous cells* terminal, unbranched, pale brown, smooth, tapering to flat-tipped apical loci, proliferating sympodially, 10–30 × 3.5–6.5 µm, mostly mono-local, sometimes multi-local; loci apical or formed on shoulders caused by geniculation, thickened, darkened, protuberant, somewhat refractive, 2–4 µm diam. *Conidia* solitary, guttulate, cylindrical to subcylindrical, straight to slightly curved, hyaline, (0–)2–4(–5)-septate, apex obtuse, base obconically truncate, (25–)37–44.5(–70) × (4–)5–5.5(–7) µm; hila thickened, darkened, refractive, 2–4 µm diam.

Cultural characteristics — Colonies on MEA reaching 24 mm diam after 20 d at 25 °C; smooth to folded, erumpent with even margins and moderate aerial mycelium; surface smoke-grey in centre, olivaceous-grey in outer region; reverse olivaceous-grey.

Specimens examined. IRAN, Zanjan Province, Tarom on leaves of *Chenopodium* sp. (*Chenopodiaceae*), 26 Sept. 2011, M. Bakhshi (holotype IRAN 16467 F, culture ex-type CCTU 1038 = CBS 136022); West Azerbaijan Province, Khoy, on *Chenopodium* sp. (*Chenopodiaceae*), 20 Sept. 2011, M. Arzanlou, CCTU 1045; Khoy, on leaves of *C. album* (*Chenopodiaceae*), 1 Sept. 2012, M. Arzanlou, CCTU 1176.

Notes — Groenewald et al. (2013) regarded this species as a cryptic taxon, *C. cf. chenopodii*, since they did not have sufficient isolates of *C. chenopodii* for comparison. In the present study, we have included additional collections of both species. Based on robust phylogenetic differences, *C. pseudo-chenopodii* must be regarded as a distinct species. There are slight differences in morphology and symptoms between *C. chenopodii* and *C. pseudochenopodii*, i.e., leaf spots with concentric rings without definite margins; conidia slightly longer and narrower (Fig. 7), which refer only to the collections examined. *Cercospora chenopodii* is widespread and represented by numerous collections. The two species are, however, indistinguishable, and can only be differentiated by DNA sequence analyses. *Cercospora pseudochenopodii* has distinct ACT and HIS phylogenies, but based on CAL sequence data, it cannot be differentiated from *C. chenopodii*. In the ITS and TEF1-α phylogeny, *C. pseudochenopodii* is intermixed with some other species, but it is distinct from *C. chenopodii*. In the combined tree (Fig. 1, part 2), it sits in a well-supported clade sister to *C. chenopodii*.

Cercospora solani Thüm., Hedwigia 19: 135. 1880 and Contr. Fl. Mycol. Lusat. II: 15. 1880 — Fig. 8

Description in planta — *Leaf spots* amphigenous, subcircular to irregular, 8–27 mm diam, with grey to black dots (stroma with conidiophores) and dark grey margins. *Mycelium* internal. *Caespituli* amphigenous, brown. *Conidiophores* aggregated in moderately dense fascicles (6–20), arising from a well-developed, intraepidermal and substomatal, brown stromata, 10–55 µm diam; conidiophores pale brown to brown, 2–6-septate, straight to geniculate-sinuuous due to sympodial proliferation, simple, rarely branched, almost uniform in width, often constricted at the proliferating point, (45–)64–75(–100) × 4–5 µm. *Conidiogenous cells* intercalary and terminal, pale brown to brown, tapering to flat-tipped apical loci, proliferating sympodially, 20–35 × 4–5 µm, multi-local; loci distinctly thickened, darkened and somewhat refractive, apical or formed on shoulders caused by geniculation, 2–3.5 µm diam. *Conidia* solitary, subcylindrical or somewhat narrowed towards the tip, straight to slightly curved, hyaline, thin-walled, (26–)48–59(–92) × (3.5–)4.5–5.5 µm, distinctly (2–)3–7(–8)-septate, with subobtusely rounded apices and truncate bases; hila distinctly thickened, darkened, refractive, 1.5–2.5 µm diam.

Cultural characteristics — Colonies on MEA slow growing, reaching 15 mm diam after 20 d at 25 °C in the dark; erumpent with smooth, even margins and sparse aerial mycelium; grey-olivaceous on the surface, reverse iron-grey.

Specimens examined. IRAN, West Azerbaijan Province, Khoy, on leaves of *Solanum nigrum* (*Solanaceae*), Sept. 2011, M. Arzanlou, CCTU 1043 = CBS 136038; Khoy, on leaves of *S. nigrum* (*Solanaceae*), Sept. 2011, M. Arzanlou, CCTU 1050.

Notes — *Cercospora solani* is supported in all of the individual gene trees. In the combined tree, it is a sister taxon to the clade including *C. conyzae-canadensis*, *C. cf. modiolae* and *Cercospora* sp. E sensu Groenewald et al. (2013) (Fig. 1, part 1). Ten species of *Cercospora* have been reported from *Solanum*, including *C. apii*, *C. canescens*, *C. lanugiflori*, *C. physalidis*, *C. puyana*, *C. sciadophila*, *C. solanacea*, *C. solani*, *C. solanigena* and *C. solani-nigri*. *Cercospora solani* is phylogenetically distinct from *C. apii*, *C. canescens* and *C. physalidis*. Among the other candidate species, the status of *C. lanugiflori*, *C. sciadophila* and *C. solanigena* are uncertain, as their type collections are lacking (Crous & Braun 2003); symptoms of *C. puyana* are different, and *C. solanacea* has been reduced to synonymy with *Pseudocercospora trichophila* var. *punctata* (Braun & Urteaga 2013). *Cercospora solani-nigri* is also a *Pseudocercospora* and heterotypic synonym of *P. atromarginalis* (type material examined by U. Braun: on *Solanum nigrum*, India, Poona, 18 Dec. 1957, P.P. Chiddarwar, BPI 441404). The description of *C. solani* in Chupp (1954) is misleading. It is unclear on which collections Chupp's (1954) description was based. The name *C. solani* has often been confusingly applied. However, type material of *C. solani* has been examined by U. Braun (on *Solanum nigrum*, Portugal, Coimbra, Jan. 1879, F. Moller, Thüm., Mycoth. Univ. 2070, HAL) and was shown to be a true *Cercospora* s.str. characterised by cylindrical to subacicular (somewhat apically attenuated) conidia. The type of *C. solani* agrees well with the present material from Iran.

Cercospora sorghicola M. Bakhshi, Arzanlou, Babai-ahari, Crous & U. Braun, *sp. nov.* — MycoBank MB809121; Fig. 9

Etymology. Derived from the host genus, *Sorghum*.

Description in planta — *Leaf spots* amphigenous, initially dark purple spots that enlarge over time into linear-oblong lesions with dark purple centre and dark red-purple margins, 5–35 mm long. *Mycelium* internal. *Caespituli* amphigenous, brown.

Ceroidiophores aggregated in loose or dense fascicles (5–40), arising from the upper cells of a well-developed, intraepidermal and substomatal, brown stroma, up to 50 µm diam; conidiophores pale brown to brown, paler towards the apex, simple, unbranched, 1–8-septate, straight or flexuous caused by sympodial proliferation, almost uniform in width, sometimes conical at the apex, (45–)70–80(–100) × 4–5.5 µm. *Conidiogenous cells*

terminal or intercalary, unbranched, pale brown, smooth, proliferating sympodially, 20–40 × 3.5–5.5 µm, multi-local; loci thickened, darkened, refractive, protuberant, apical, lateral, 2–4 µm diam. *Conidia* solitary, smooth, acicular, cylindro-obclavate to obclavate, straight or curved, successively tapering towards the apex, hyaline, (3–)8–13(–17)-septate, apex subacute to subobtuse, base truncate to obconically truncate, (21–)80–100

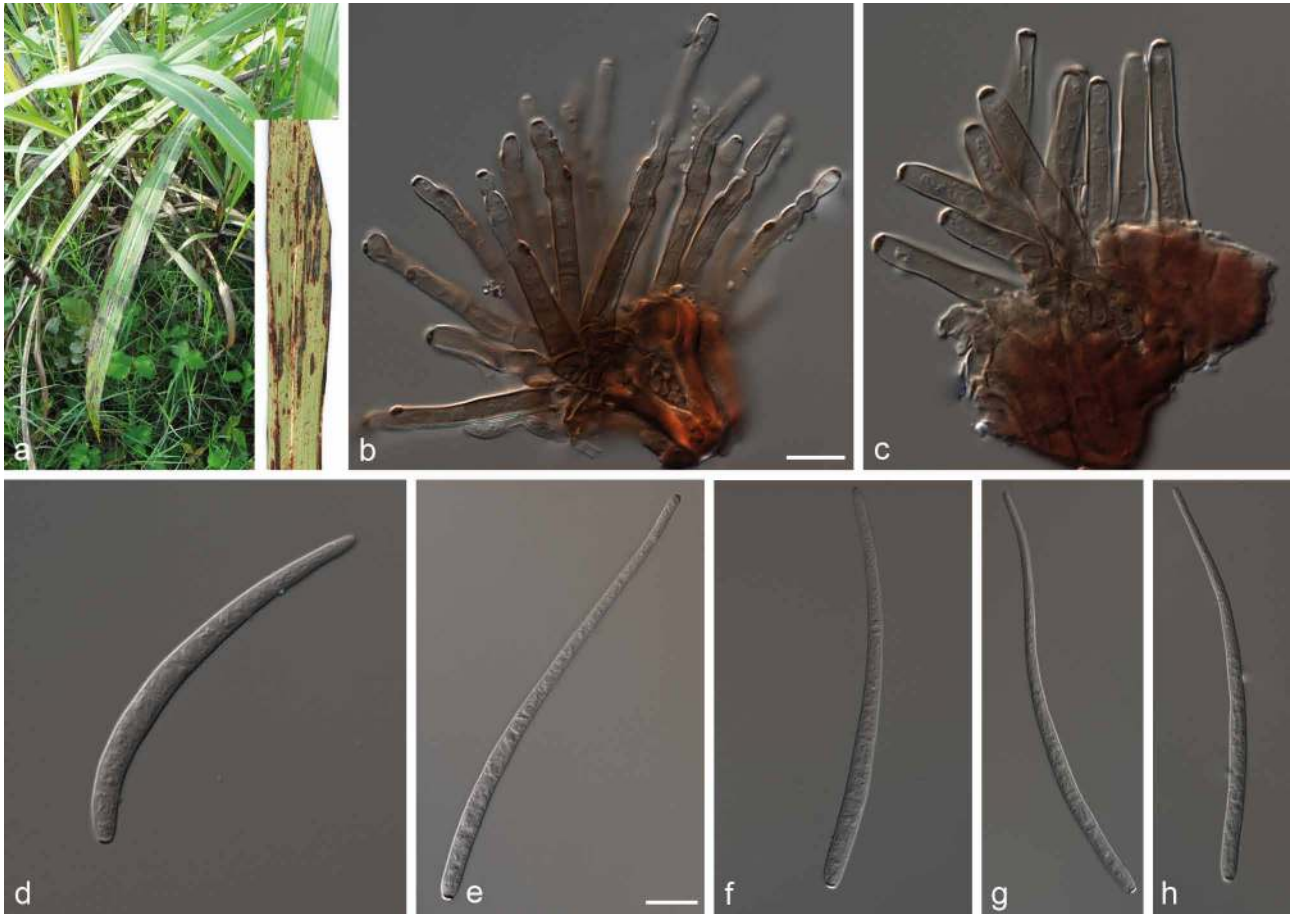


Fig. 9 *Cercospora sorghicola* (CBS 136448). a. Leaf spots; b. c. fasciculate conidiophores; d–h. conidia. — Scale bars = 10 µm.

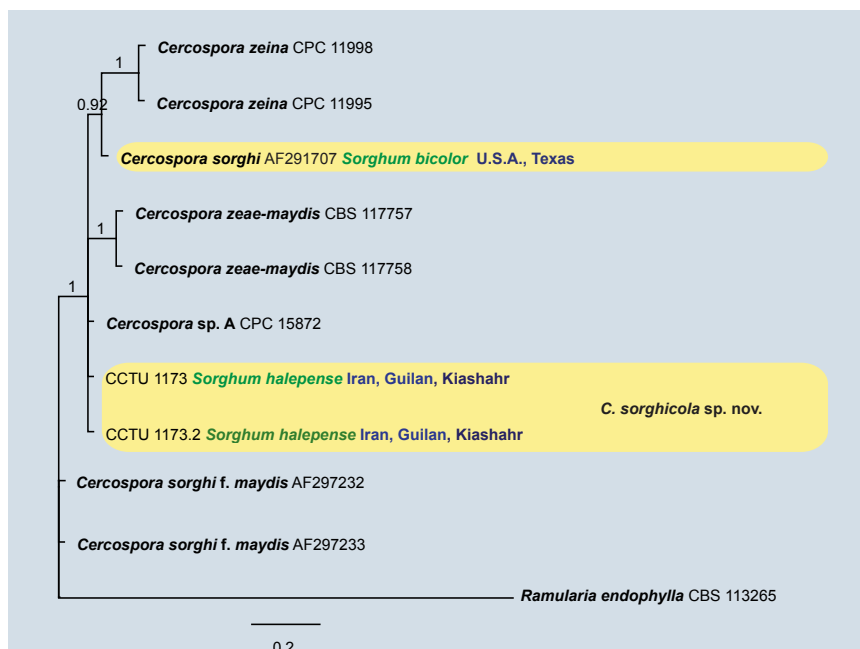


Fig. 10 Consensus phylogram (50% majority rule) of 346 trees resulting from a Bayesian analysis of the ITS sequence alignment using MrBayes v. 3.2.1. The tree was rooted to *Ramularia endophylla* (strain CBS 113265).



Fig. 11 *Cercospora* sp. T (CBS 136125). a. b. Leaf spots; c. intraepidermal caespituli; d. substomatal caespituli; e. fasciculate conidiophores; f–h. conidia. — Scale bars = 10 μ m.

(–150) \times 3–4(–5) μ m; hila distinctly thickened, darkened, refractive, 1.5–2.5 μ m diam.

Cultural characteristics — Colonies on MEA reaching 45 mm diam after 20 d at 25 °C in the dark; flat with smooth, even margins and moderate aerial mycelium; surface olivaceous-green, reverse dark olivaceous-green.

Specimens examined. IRAN, Guilan Province, Kiashahr, on *Sorghum halepense* (Poaceae), Aug. 2012, M. Bakhshi (holotype IRAN 16457 F, culture ex-type CCTU 1173 = CBS 136448); Kiashahr, on *S. halepense* (Poaceae), Aug. 2012, M. Bakhshi, CCTU 1173.2.

Notes — In the individual gene trees (TEF1- α , ACT, CAL and HIS phylogeny), *C. sorghicola* always resides in a well-supported clade including *C. sorghicola* and *Cercospora* sp. A sensu Groenewald et al. (2013). In the combined tree (Fig. 1, part 1), it forms a distinct clade from *Cercospora* sp. A and these two species are sister taxa. The variation between these two species is based on one nucleotide change in ITS (one insertion in *Cercospora* sp. A), three nucleotides in TEF1- α (three transitions), two nucleotides in CAL (two transversions), two nucleotides in ACT (one transition and one transversion) and four nucleotide changes in HIS (one transversion and three transitions).

Because sequences for the TEF1- α , ACT, CAL and HIS loci were not available in NCBI for *C. sorghi*, which has been reported from *Sorghum* spp., a separate tree that included *C. sorghicola*, *Cercospora* sp. A, *C. sorghi* (GenBank AF291707) and other closely related species was generated using only ITS sequences. In this tree *C. sorghicola* and *C. sorghi* reside in different lineages (Fig. 10). Two nucleotide changes at ITS (one

transition and one insertion) explain the different position of the isolates used in the current study and *C. sorghi*. *Cercospora sorghicola* is also morphologically different from *C. sorghi* by its longer, wider and multi-septate conidia.

***Cercospora* sp. T** — Fig. 11

Description in planta — *Leaf spots* amphigenous, subcircular to irregular, 5–12 mm diam, grey-brown with indefinite margins. *Mycelium* internal. *Caespituli* amphigenous, brown. *Conidiophores* aggregated in loose fascicles (2–8), arising from a weakly developed, intraepidermal and substomatal, dark brown stroma, up to 25 μ m diam; conidiophores brown to dark brown, 5–14-septate, straight to geniculate-sinuous due to sympodial proliferation, simple, unbranched, thick-walled, uniform in width, (95–)152–175(–215) \times 3.5–5 μ m. *Conidiogenous cells* intercalary and terminal, proliferating sympodially, multi-local; loci thickened, darkened, protuberant, apical or formed on shoulders caused by geniculation, 1.5–3 μ m diam. *Conidia* solitary, hyaline, filiform to acicular, straight to slightly curved, with truncate base and acute to subobtus apices, (72–)93–115(–180) \times (2–)3–4 μ m, (7–)10–14(–20)-septate.

Cultural characteristics — Colonies on MEA reaching 65 mm diam after 20 d at 25 °C in the dark; smooth, flat, with even margins and moderate aerial mycelium; surface smoke-grey; reverse iron-grey.

Specimens examined. IRAN, Guilan Province, Rasht, on leaves of *Coreopsis* sp. (Asteraceae), June 2012, M. Bakhshi, CCTU 1148 = CBS 136125; Rasht, on leaves of *Coreopsis* sp. (Asteraceae), June 2012, M. Bakhshi, CCTU 1148.2.

Notes — For phylogeny, see the notes under *C. iranica*. Two species of *Cercospora*, including *C. bidentis* and *C. coreopsidis*, have been reported from *Coreopsis* spp. *Cercospora* sp. T is morphologically distinct from *C. bidentis* by lacking or having small stroma, loose fascicles and dark brown conidiophores. According to its independent phylogenetic position (Fig. 1, part 1), *Cercospora* sp. T probably represents a host-specific species. Furthermore, *Cercospora* sp. T and *C. cf. coreopsidis* (tentative name for an examined and sequenced Korean sample of *C. coreopsidis* sensu Shin & Kim 2001, see Groenewald et al. 2013), which are both host-specific to *Coreopsis* spp., are phylogenetically distinct. We presently do not have phylogenetic data from North American material on *Coreopsis*, which would fix the application of the name *C. coreopsidis*. The relationship between *Cercospora* sp. T on *Coreopsis* from Iran and *C. cf. coreopsidis* on *Coreopsis* from Korea needs resolution.

DISCUSSION

This study provides a broad framework for the genus *Cercospora* in Iran. These fungi are very common and widespread

in different climates and regions of this country. Until now, 33 species of *Cercospora* s.str. have been recorded from Iran (Bakhshi et al. 2012a, Hesami et al. 2012, Pirnia et al. 2012). The identification of these taxa has mostly relied on host association and morphological characteristics sensu Chupp (1954). Unfortunately, there are few living cultures available for molecular study. In the present paper, multilocus sequence typing (MLST) was employed for the first time to discriminate among Iranian *Cercospora* species, which are described according to their DNA phylogeny, ecology, morphological and cultural characteristics, by employing the Consolidated Species Concept as outlined by Quaedvlieg et al. (2014).

Phylogenetic performance of the five loci (ITS, TEF1- α , ACT, CAL and HIS) employed for phylogenetic inference in this study was previously reported by Groenewald et al. (2010, 2013). Our study indicated that the ITS region has limited resolution when used for species comparison in *Cercospora*, especially with regard to *C. apii* s.lat. (Goodwin et al. 2001, Pretorius et al. 2003, Groenewald et al. 2010, 2013). The other loci screened in this study had different levels of success in resolving species boundaries. The TEF1- α region was able to distinguish

Table 2 Host-fungus index for the Iranian *Cercospora* spp. examined in this study.

Host Family	Host species	Species	Host Family	Host species	Species
Aceraceae	<i>Acer velutinum</i>	<i>C. cf. flagellaris</i>	Cucurbitaceae	<i>Cucurbita maxima</i>	<i>C. cf. flagellaris</i> <i>Cercospora</i> sp. G
Amaranthaceae	<i>Amaranthus blitoides</i> <i>Amaranthus retroflexus</i>	<i>C. cf. flagellaris</i> <i>Cercospora</i> sp. G		<i>Cucurbita pepo</i> <i>Cucurbita</i> sp.	<i>C. cf. flagellaris</i> <i>C. cf. flagellaris</i>
	<i>Amaranthus</i> spp.	<i>Cercospora</i> sp. G		<i>Citrullus lanatus</i> <i>Ecballium elaterium</i>	<i>C. cf. flagellaris</i> <i>C. apii</i> <i>C. cf. flagellaris</i>
	<i>Celosia cristata</i>	<i>Cercospora</i> sp. G	Fabaceae	<i>Arachis hypogaea</i> <i>Alhagi camelorum</i> <i>Coronilla varia</i> <i>Glycine max</i> <i>Medicago sativa</i> <i>Medicago</i> sp. <i>Phaseolus vulgaris</i> <i>Trifolium repens</i> <i>Vicia faba</i>	<i>C. cf. flagellaris</i> <i>C. zebrina</i> <i>C. armoraciae</i> <i>C. cf. flagellaris</i> <i>C. zebrina</i> <i>C. zebrina</i> <i>C. cf. flagellaris</i> <i>C. zebrina</i> <i>C. cf. flagellaris</i> <i>C. iranica</i> <i>C. zebrina</i>
Apocynaceae	<i>Cynanchum acutum</i>	<i>C. apii</i>		<i>Vicia</i> sp.	<i>C. cf. flagellaris</i> <i>C. zebrina</i>
Araceae	<i>Anubias</i> sp.	<i>C. cf. flagellaris</i>	Geraniaceae	<i>Pelargonium hortorum</i>	<i>C. cf. flagellaris</i>
Asteraceae	<i>Bidens tripartita</i>	<i>C. cf. richardiicola</i> <i>Cercospora</i> sp. G	Hydrangeaceae	<i>Hydrangea</i> sp.	<i>C. cf. flagellaris</i> <i>C. iranica</i>
	<i>Calendula officinalis</i> <i>Cichorium intybus</i>	<i>C. cf. flagellaris</i> <i>Cercospora</i> sp. G	Malvaceae	<i>Abutilon theophrasti</i>	<i>C. cf. flagellaris</i> <i>Cercospora</i> sp. G
	<i>Conyza canadensis</i> <i>Coreopsis</i> sp.	<i>C. cylindracea</i> <i>C. conyzae-canadensis</i> <i>Cercospora</i> sp. T		<i>Althaea rosea</i> <i>Gossypium herbaceum</i> <i>Hibiscus trionum</i> <i>Malva sylvestris</i> <i>Malva neglecta</i>	<i>C. althaeina</i> <i>C. cf. flagellaris</i> <i>C. cf. flagellaris</i> <i>C. althaeina</i> <i>C. beticola</i> <i>C. beticola</i>
	<i>Eclipta prostrata</i> <i>Lactuca serriola</i> <i>Leucanthemum superbum</i> <i>Silybum marianum</i> <i>Sonchus asper</i> <i>Sonchus</i> sp. <i>Tagetes patula</i> <i>Tanacetum balsamita</i> <i>Xanthium spinosum</i> <i>Xanthium strumarium</i> <i>Zinnia elegans</i>	<i>C. cf. flagellaris</i> <i>C. cylindracea</i> <i>C. cf. flagellaris</i> <i>C. cf. flagellaris</i> <i>C. beticola</i> <i>C. beticola</i> <i>C. cf. flagellaris</i> <i>C. armoraciae</i> <i>C. cf. flagellaris</i> <i>C. cf. flagellaris</i> <i>C. cf. zinniae</i>	Oleaceae	<i>Olea europaea</i>	<i>C. cf. flagellaris</i>
Balsaminaceae	<i>Impatiens balsamina</i>	<i>C. cf. flagellaris</i>	Pedaliaceae	<i>Sesamum indicum</i>	<i>C. beticola</i>
Boraginaceae	<i>Heliotropium europaeum</i>	<i>C. apii</i>	Plantaginaceae	<i>Plantago lanceolata</i> <i>Plantago major</i>	<i>C. apii</i> <i>C. beticola</i> <i>Cercospora</i> sp. G
Brassicaceae	<i>Cardaria draba</i> <i>Lepidium sativum</i> <i>Raphanus sativus</i>	<i>C. armoraciae</i> <i>C. cf. flagellaris</i> <i>C. cf. flagellaris</i>	Poaceae	<i>Sorghum halepense</i>	<i>Cercospora</i> sp. G <i>C. sorghicola</i>
Buxaceae	<i>Buxus microphylla</i>	<i>C. cf. flagellaris</i>	Polygonaceae	<i>Rumex crispus</i>	<i>C. beticola</i> <i>C. rumicis</i>
Caesalpinaceae	<i>Cercis siliquastrum</i>	<i>C. cf. flagellaris</i>	Salicaceae	<i>Populus deltoides</i>	<i>C. cf. flagellaris</i>
Capparidaceae	<i>Capparis spinosa</i>	<i>C. armoraciae</i>	Solanaceae	<i>Datura stramonium</i> <i>Solanum nigrum</i>	<i>C. cf. flagellaris</i> <i>C. solani</i>
Chenopodiaceae	<i>Beta vulgaris</i> <i>Chenopodium album</i>	<i>C. beticola</i> <i>C. chenopodii</i> <i>C. pseudochenopodii</i>	Urticaceae	<i>Urtica dioica</i>	<i>C. cf. flagellaris</i> <i>C. rumicis</i>
	<i>Chenopodium</i> spp.	<i>C. beticola</i> <i>C. pseudochenopodii</i>	Violaceae	<i>Viola</i> sp.	<i>C. violae</i>
Convolvulaceae	<i>Convolvulus arvensis</i>	<i>C. convolvulicola</i>	Vitaceae	<i>Vitis vinifera</i>	<i>C. zebrina</i>

only 35 % of 20 lineages, whereas the actin region had 45 % clade recovery. Although the CAL region only distinguished 40 % of the species, it remains essential to distinguish several species: *C. apii* from *C. beticola*, *C. cf. flagellaris* from *C. convolvulicola*, and *C. iranica* from *Cercospora* sp. T. The HIS region was slightly more effective and discriminated half of the detected species. These data show the importance of all five loci in combined analysis for *Cercospora* taxonomy and are congruent with previous studies of Groenewald et al. (2010, 2013). Despite this, the sequences of these five loci are still too conserved in *Cercospora*, and there is still need to find the best barcoding locus (loci) for *Cercospora*.

In the present study 20 species of *Cercospora* were identified from northern Iran based on a combination of sequence data, host-fungus relation and morphological characters. Results obtained in this study show that 60 isolates of *Cercospora* obtained from 18 host families in different groups of plants viz. agricultural crops, ornamentals, forest trees and weeds grouped within the *C. cf. flagellaris* species complex. This complex was previously treated by Groenewald et al. (2013) from nine host families (in total encompassing 23 host families). *Cercospora cf. flagellaris* is morphologically similar to *C. flagellaris* (= *C. apii* s.lat.), but names could not be applied with confidence, and isolates from the original host and country (*Phytolaca decandra*, USA) need to be included to confirm the true identity of this species. We suspect that this species could split into several species once more DNA loci are screened and pathogenicity tests are conducted.

In recent years several groups have attempted to study *Cercospora* spp. from Iran based solely on morphological characters and host range (Hesami et al. 2012). According to our data, most of these records are unreliable or incorrect, and probably reside in the *C. cf. flagellaris* clade (Hesami et al. 2012, Pirnia et al. 2012). On the other hand, many earlier records were identified as *C. apii*, which according to results of our study, has a much narrower host range than generally recognised.

Although the isolates investigated during this study represent the largest collection of *Cercospora* species so far subjected to DNA sequence analysis from Iran, there are still some issues that need to be resolved. The identity of two groups of species remains questionable. Names based on American or European type specimens should not be assumed for the identification of identical diseases on the same hosts in Asia, Africa or South America and vice versa (Crous et al. 2013, Groenewald et al. 2013). This was the case for the first group of the species with questionable identity in our study, which were indicated with 'cf.' in the species name, e.g. *C. cf. richardiicola*, *C. cf. flagellaris* and *C. cf. zinniae*. In the case of the second species group, the clade contains isolates from multiple hosts, e.g. in *Cercospora* sp. G, isolates from six host families (*Amaranthaceae*, *Asteraceae*, *Cucurbitaceae*, *Malvaceae*, *Plantaginaceae*, *Poaceae*) were found. To resolve these taxonomic problems, additional species described by Chupp (1954) and Crous & Braun (2003), which are not currently known from their DNA must be epitypified, thus allowing DNA sequence-based analyses to stabilize the names used in different phylogenetic lineages. Furthermore, in future studies of *Cercospora*, additional loci must be included in the analyses to obtain better resolution of the species.

The data presented here confirm that some *Cercospora* species are host-specific, e.g. *C. chenopodii* and *C. pseudochenopodii* on *Chenopodium* spp., *C. violae* on *Viola* spp., *C. cf. zinniae* on *Zinnia elegans*, *C. conyzae-canadensis* on *Conyza canadensis*, *C. convolvulicola* on *Convolvulus arvensis*, *C. solani* on *Solanum nigrum* and *C. sorghicola* on *Sorghum halepense*; some species are restricted to one host family e.g. *C. althaeina* on *Malvaceae*, *C. cylindracea* on *Asteraceae*; whereas others have

wide host ranges, e.g. *C. apii*, *C. armoraciae*, *C. beticola*, *C. cf. flagellaris* and *Cercospora* sp. G. However, it is not acceptable to recognise the host range of a species without confirmatory pathogenicity tests. For example, it still remains to be seen whether isolates from different hosts with similar morphology to *C. cf. flagellaris*, have the ability to cross-infect hosts (Table 2).

The present study was initiated to resolve the taxonomy of the genus *Cercospora* in Iran by employing the Consolidated Species Concept. Our results indicate a rich diversity of this genus in the north and north-west of Iran. Future studies will be directed towards resolving the taxonomy of the genus *Cercospora* in other parts of Iran, and also the taxonomy of other cercospora-like pathogens of agricultural crops of major economic importance.

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