

Novel primers improve species delimitation in *Cercospora*

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Abstract: The genus *Cercospora* includes many important plant pathogens that are commonly associated with leaf spot diseases on a wide range of cultivated and wild plant species. Due to the lack of useful morphological features and high levels of intraspecific variation, host plant association has long been a decisive criterion for species delimitation in *Cercospora*. Because several taxa have broader host ranges, reliance on host data in *Cercospora* taxonomy has proven problematic. Recent studies have revealed multi-gene DNA sequence data to be highly informative for species identification in *Cercospora*, especially when used in a concatenated alignment. In spite of this approach, however, several species complexes remained unresolved as no single gene proved informative enough to act as DNA barcoding locus for the genus. Therefore, the aims of the present study were firstly to improve species delimitation in the genus *Cercospora* by testing additional genes and primers on a broad set of species, and secondly to find the best DNA barcoding gene(s) for species delimitation. Novel primers were developed for *tub2* and *rpb2* to supplement previously published primers for these loci. To this end, 145 *Cercospora* isolates from the Iranian mycobiota together with 25 additional reference isolates preserved in the Westerdijk Fungal Biodiversity Institute were subjected to an eight-gene (ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, *rpb2* and *gapdh*) analysis. Results from this study provided new insights into DNA barcoding in *Cercospora*, and revealed *gapdh* to be a promising gene for species delimitation when supplemented with *cmdA*, *tef1* and *tub2*. The robust eight-gene phylogeny revealed several novel clades within the existing *Cercospora* species complexes, such as *C. apii*, *C. armoraciae*, *C. beticola*, *C. cf. flagellaris* and *Cercospora* sp. G. The *C. apii* s. lat. isolates are distributed over three clades, namely *C. apii* s. str., *C. plantaginis* and *C. uwebrauniana* sp. nov. The *C. armoraciae* s. lat. isolates are distributed over two clades, *C. armoraciae* s. str. and *C. bizzoeriana*. The *C. beticola* s. lat. isolates are distributed over two clades, namely *C. beticola* s. str. and *C. gamsiana*, which is newly described.

Key words:

Bar codes
biodiversity
Cercospora apii complex
host specificity
multi-gene phylogeny
new taxa

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INTRODUCTION

Fungi belonging to the genus *Cercospora* (*Mycosphaerellaceae*, *Capnodiales*) are common etiological agents of leaf spots, but some also cause necrotic lesions on flowers, fruits, bracts, seeds and pedicels of many woody and herbaceous plants in a range of climates worldwide (Ellis 1976, Crous & Braun 2003, Agrios 2005, Groenewald *et al.* 2013, Bakhshi *et al.* 2015a).

Cercospora is a species-rich genus of cercosporoid fungi that was established by Fresenius (1863) for passalora-like species with pluriseptate conidia. During the course of the next 100 years, the concept of *Cercospora* had been continuously widened (Saccardo 1880, Solheim 1930) and all kinds of superficially similar species, with or without conspicuous

conidiogenous loci, with hyaline or pigmented conidia, formed singly or in chains, were assigned to this genus (Braun *et al.* 2013). In 1954, the genus was monographed by Chupp (1954), who treated 1419 *Cercospora* species while applying this broad generic concept. He also stated that species of *Cercospora* were generally host-specific and used this argument as the basis of formulating the concept that each plant host genus or family would have its own *Cercospora* species. The number of *Cercospora* species increased rapidly to more than 3000, which led Pollack (1987) to publish her annotated list of *Cercospora* names. Since the introduction of the genus, several attempts to split *Cercospora* s. lat. into smaller generic units have been made by applying a combination of characters such as conidiomatal structure, mycelium, conidiophores, conidiogenous cells, and conidia

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(e.g. Deighton 1973, 1979, 1983, Ellis 1971, 1976, Braun 1995, 1998). Crous & Braun (2003) published an annotated list of the names published in *Cercospora* and *Passalora* and used the structure of conidiogenous loci and hila as well as the absence or presence of pigmentation in conidiophores and conidia in their revision. They recognised 659 names in *Cercospora*, with a further 281 species names reduced to synonymy with *C. apii* s. lat., since they were morphologically not or barely distinguishable from *C. apii* s. str. Braun et al. (2013, 2014, 2015a, b, 2016) published a series of papers in a stepwise approach at plant family level in order to update the monograph of *Cercospora* and allied genera.

Scientific advances in DNA sequencing and supplementary software to store, share and compare the emerging molecular data have revolutionised the procedures underpinning the discovery and identification of fungal taxa, including the cercosporoid fungi (Crous & Groenewald 2005, Groenewald et al. 2013, Bakhshi et al. 2015a, Nguanhom et al. 2015, Guatimosim et al. 2017). Numerous molecular studies of *Cercospora* species have been conducted based on ITS nrDNA data as well as multi-gene approaches (Stewart et al. 1999, Crous et al. 2000, 2004b, 2009a, b, Goodwin et al. 2001, Tessmann et al. 2001, Pretorius et al. 2003, Groenewald et al. 2005, 2006, 2013, Montenegro-Calderón et al. 2011, Bakhshi et al. 2012b, 2015a, Nguanhom et al. 2015, Soares et al. 2015, Albu et al. 2016, Guatimosim et al. 2017, Guillin et al. 2017). A comprehensive and detailed molecular examination of *Cercospora* s. str. based on a multi-locus DNA sequence dataset of five genomic loci including the ITS (ITS1, 5.8S nrRNA gene and ITS2), together with parts of four protein coding genes, viz. translation elongation factor 1-alpha (*tef1*), actin (*actA*), calmodulin (*cmdA*) and histone H3 (*his3*) was conducted by Groenewald et al. (2013). The main conclusion of this study was that *C. apii* s. lat. could not be confirmed as a plurivorous monophyletic species, and that several lineages originally referred to *C. apii* s. lat., or considered close to this complex based on morphology (Crous & Braun 2003), were separated as distinct phylogenetic species. Hence, speciation within *Cercospora* s. str. is more complicated than formerly assumed, and far from being resolved. To date, multi-locus DNA sequence analyses combined with ecology, morphology and cultural characteristics, referred to as the Consolidated Species Concept (Quaedvlieg et al. 2014), proved the most effective method for the delimitation of *Cercospora* species (Groenewald et al. 2010, 2013).

At a higher taxonomic level, among the genera of cercosporoid fungi, the monophyly of *Cercospora* s. str. has until recently been tested based on phylogenetic association of taxa with the type species of *Cercospora*, *C. apii* (Groenewald et al. 2013, Bakhshi et al. 2015a, Braun & Crous 2016). Bakhshi et al. (2015b) recovered some cercospora-like isolates from *Ammi majus*, and in their subsequent multi-gene phylogenetic study (28S nrDNA, ITS, *actA*, *tef1* and *his3*), elucidated these isolates to represent a new genus, *Neocercospora*, clustering in a clade in *Mycosphaerellaceae* apart from *Cercospora* s. str., suggesting that cercospora-like morphologies are not necessarily part of a single monophyletic genus. This finding led to the conclusion that identification and descriptions of new cercospora-like taxa should be avoided without support

of molecular sequence data, not only at species but also at generic level.

Species of *Cercospora* are known to be widely distributed, occurring on a broad range of plant hosts in many climate zones of Iran (Bakhshi et al. 2012, Hesami et al. 2012, Pirnia et al. 2012), where the biodiversity of the genus has recently received much attention (Bakhshi et al. 2015a, b). The most inclusive study was that of Bakhshi et al. (2015a), who compared 161 *Cercospora* isolates, recovered from 74 host species from Iran based on DNA sequence data of five genomic loci (ITS, *tef1*, *actA*, *cmdA* and *his3*), host, cultural, and morphological data, revealing a rich species diversity. However, the problem concerning species delimitation in *Cercospora* due to the high level of conservation among DNA sequences of commonly used loci, (i.e. ITS, *tef1*, *actA*, *cmdA*, and *his3*), could not be resolved. Furthermore, cryptic clades in several species complexes remained unresolved in the five-gene phylogenetic tree, for example *C. apii*, *C. armoraciae*, *C. cf. flagellaris*, and *Cercospora* sp. G (Groenewald et al. 2013, Bakhshi et al. 2015a). Therefore, the aim of the present study was to assess three additional potential candidate gene regions including the partial β -tubulin (*tub2*) gene, part of the second largest subunit of RNA-polymerase II (*rpb2*) gene, and part of the glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene, in order to firstly generate an eight-gene DNA dataset to resolve cryptic taxa within these species complexes, and secondly to identify the best barcoding gene(s) for species resolution in *Cercospora*.

MATERIAL AND METHODS

Specimens and isolates

A total of 170 strains, including 145 previously identified as *Cercospora* species in Bakhshi et al. (2015a), as well as 25 other related strains formerly identified by Groenewald et al. (2013), were studied. Isolates used in this study (Table 1) are maintained in the collection of the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands, the working collection of Pedro Crous (CPC; housed at CBS), the culture collection of the Iranian Research Institute of Plant Protection (IRAN C), Tehran, Iran, and the culture collection of Tabriz University (CCTU), Tabriz, Iran. Type material of the new species recognized is preserved in the Fungal Herbarium of the Iranian Research Institute of Plant Protection (IRAN F).

DNA extraction and PCR amplification

DNA samples comprised those previously extracted by Bakhshi et al. (2015a) and Groenewald et al. (2013). Three additional partial nuclear genes were targeted for PCR amplification and sequencing, namely, glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), RNA polymerase II second largest subunit (*rpb2*), and β -tubulin (*tub2*), using corresponding primer sets (Table 2). PCR amplifications were performed in a total volume of 12.5 μ L on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). The *gapdh* PCR mixture consisted of 5–10 ng genomic DNA, 1 \times PCR buffer (Bioline, London), 2 mM MgCl₂ (Bioline), 50 μ M of each dNTP, 0.5 μ L BSA (10 mg/ml) (Promega, Madison, WI), 0.28 μ M of each primer and 0.5 units GoTaq® Flexi DNA

Table 1. Collection details and GenBank accession numbers of isolates included in this study. Ex-type isolates and newly generated sequences are highlighted in **bold**.

Species	Culture accession number (s) ¹	Host	Host Family	Origion	Collector	GenBank accession numbers ²							
						ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>
<i>Cercospora althaeina</i>	CCTU 1028	<i>Althaea rosea</i>	<i>Malvaceae</i>	Iran, Guilan, Sowme'eh Sara	M. Bakhshi	KJ886394	KJ886233	KJ885911	KJ885750	KJ886072	MH496336	MH511833	MH496166
	CCTU 1001	<i>Althaea rosea</i>	<i>Malvaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886392	KJ886231	KJ885909	KJ885748	KJ886070	MH496337	MH511834	MH496167
	CCTU 1026	<i>Althaea rosea</i>	<i>Malvaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886393	KJ886232	KJ885910	KJ885749	KJ886071	MH496338	MH511835	MH496168
	CCTU 1152	<i>Althaea rosea</i>	<i>Malvaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886396	KJ886235	KJ885913	KJ885752	KJ886074	MH496339	MH511836	MH496169
	CBS 248.67; CPC 5117 (TYPE)	<i>Althaea rosea</i>	<i>Malvaceae</i>	Romania, Fundulea	O. Constantinescu	JX143530	JX143284	JX143038	JX142792	JX142546	MH496340	–	MH496170
	CCTU 1194; IRAN 2674C	<i>Malva sylvestris</i>	<i>Malvaceae</i>	Iran, East Azerbaijan, Kaleybar	M. Arzanlou	KJ886397	KJ886236	KJ885914	KJ885753	KJ886075	MH496341	MH511837	MH496171
	CCTU 1071	<i>Malva sylvestris</i>	<i>Malvaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886395	KJ886234	KJ885912	KJ885751	KJ886073	MH496342	MH511838	MH496172
<i>Cercospora apii</i>	CBS 116455; CPC 11556 (TYPE)	<i>Apium graveolens</i>	<i>Apiaceae</i>	Germany, Heilbron	K. Schrameyer	AY840519	AY840486	AY840450	AY840417	AY840384	MH496343	–	MH496173
	CBS 536.71; CPC 5087	<i>Apium graveolens</i>	<i>Apiaceae</i>	Romania, Bucuresti	O. Constantinescu	AY752133	AY752166	AY752194	AY752225	AY752256	MH496344	MH511839	MH496174
	CCTU 1069	<i>Cynanchum acutum</i>	<i>Apocynaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886410	KJ886249	KJ885927	KJ885766	KJ886088	MH496345	MH511840	MH496175
	CCTU 1086; CBS 136037; IRAN 2655C	<i>Cynanchum acutum</i>	<i>Apocynaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886411	KJ886250	KJ885928	KJ885767	KJ886089	MH496346	MH511841	MH496176
	CCTU 1215	<i>Cynanchum acutum</i>	<i>Apocynaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886412	KJ886251	KJ885929	KJ885768	KJ886090	MH496347	MH511842	MH496177
	CCTU 1219; CBS 136155	<i>Cynanchum acutum</i>	<i>Apocynaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886413	KJ886252	KJ885930	KJ885769	KJ886091	MH496348	MH511843	MH496178
	CPC 5112	<i>Molucella laevis</i>	<i>Lamiaceae</i>	New zealand, Auckland	C.F. Hill	DQ233321	DQ233347	DQ233373	DQ233399	DQ233425	MH496349	MH511844	MH496179
	CBS 110813; CPC 5110; 01-3	<i>Molucella laevis</i>	<i>Lamiaceae</i>	U.S.A., California	S.T. Koike	AY156918	DQ233345	DQ233371	DQ233397	DQ233423	MH496350	MH511845	MH496180
<i>Cercospora armoraciae</i>	CBS 250.67; CPC 5088 (TYPE)	<i>Armoracia rusticana</i> (= <i>A. lapathifolia</i>)	<i>Brassicaceae</i>	Romania, Fundulea	O. Constantinescu	JX143545	JX143299	JX143053	JX142807	JX142561	MH496351	–	MH496181
<i>Cercospora beticola</i>	CPC 12028	<i>Beta vulgaris</i>	<i>Chenopodiaceae</i>	Egypt	M. Hasem	DQ233336	DQ233362	DQ233388	DQ233414	DQ233437	MH496352	MH511846	MH496182
	CPC 12029	<i>Beta vulgaris</i>	<i>Chenopodiaceae</i>	Egypt	M. Hasem	DQ233337	DQ233363	DQ233389	DQ233415	DQ233438	MH496353	MH511847	MH496183
	CCTU 1135	<i>Beta vulgaris</i>	<i>Chenopodiaceae</i>	Iran, Guilan, Astara	M. Bakhshi	KJ886432	KJ886271	KJ885949	KJ885788	KJ886110	MH496354	MH511848	MH496184
	CBS 116456; CPC 11557 (TYPE)	<i>Beta vulgaris</i>	<i>Chenopodiaceae</i>	Italy, Ravenna	V. Rossi	AY840527	AY840494	AY840458	AY840425	AY840392	MH496355	KT216555	MH496185
	CCTU 1057; IRAN 2651C	<i>Chenopodium</i> sp.	<i>Chenopodiaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886424	KJ886263	KJ885941	KJ885780	KJ886102	MH496356	MH511849	MH496186
	CCTU 1065	<i>Chenopodium</i> sp.	<i>Chenopodiaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886425	KJ886264	KJ885942	KJ885781	KJ886103	MH496357	MH511850	MH496187
	CCTU 1087	<i>Chenopodium</i> sp.	<i>Chenopodiaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886427	KJ886266	KJ885944	KJ885783	KJ886105	MH496358	MH511851	MH496188
	CCTU 1089; CPC 24911	<i>Plantago lanceolata</i>	<i>Plantaginaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886429	KJ886268	KJ885946	KJ885785	KJ886107	MH496359	MH511852	MH496189
	CCTU 1108	<i>Plantago lanceolata</i>	<i>Plantaginaceae</i>	Iran, Zanjan, Tarom	M. Bakhshi	KJ886430	KJ886269	KJ885947	KJ885786	KJ886108	MH496360	MH511853	MH496190

Table 1. (Continued).

Species	Culture accession number (s) ¹	Host	Host Family	Origion	Collector	GenBank accession numbers ²							
						ITS	tef1	actA	cmdA	his3	tub2	rpb2	gapdh
	CCTU 1088; CBS 138582	<i>Sonchus asper</i>	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886428	KJ886267	KJ885945	KJ885784	KJ886106	MH496361	MH511854	MH496191
<i>Cercospora bizzozeriana</i>	CCTU 1013	?	?	Iran, East Azerbaijan, Mianeh	M. Torbati	KJ886414	KJ886253	KJ885931	KJ885770	KJ886092	MH496362	MH511855	MH496192
	CCTU 1022; CBS 136028	?	?	Iran, East Azerbaijan, Mianeh	M. Torbati	KJ886415	KJ886254	KJ885932	KJ885771	KJ886093	MH496363	MH511856	MH496193
	CCTU 1127; CBS 136133	<i>Capparis spinosa</i>	Capparidaceae	Iran, Khuzestan, Ahvaz	E. Mohammadian	KJ886420	KJ886259	KJ885937	KJ885776	KJ886098	MH496364	MH511857	MH496194
	CCTU 1117; CBS 136132	<i>Cardaria draba</i>	Brassicaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886418	KJ886257	KJ885935	KJ885774	KJ886096	MH496365	MH511858	MH496195
	CCTU 1234	<i>Cardaria draba</i>	Brassicaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886419	KJ886258	KJ885936	KJ885775	KJ886097	MH496366	MH511859	MH496196
	CCTU 1107	?	?	Iran, Zanjan, Tarom	M. Bakhshi	KJ886417	KJ886256	KJ885934	KJ885773	KJ886095	MH496367	MH511860	MH496197
	CBS 258.67; CPC 5061 (TYPE)	<i>Cardaria draba</i>	Brassicaceae	Romania, Fundulea	O. Constantinescu	JX143546	JX143300	JX143054	JX142808	JX142562	MH496368	–	MH496198
	CBS 540.71; IMI 161110; CPC 5060	<i>Cardaria draba</i>	Brassicaceae	Romania, Hagieni	O. Constantinescu	JX143548	JX143302	JX143056	JX142810	JX142564	MH496369	–	MH496199
	CCTU 1040; CBS 136131	<i>Tanacetum balsamita</i>	Asteraceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886416	KJ886255	KJ885933	KJ885772	KJ886094	MH496370	MH511861	MH496200
<i>Cercospora chenopodii</i>	CCTU 1060; IRAN 2652C	<i>Chenopodium album</i>	Chenopodiaceae	Iran, Guilan, Bandar-e Anzali	M. Bakhshi	KJ886438	KJ886277	KJ885955	KJ885794	KJ886116	MH496371	MH511862	MH496201
	CCTU 1163	<i>Chenopodium album</i>	Chenopodiaceae	Iran, Guilan, Lahijan	M. Bakhshi	KJ886440	KJ886279	KJ885957	KJ885796	KJ886118	MH496372	MH511863	MH496202
	CCTU 1033	<i>Chenopodium album</i>	Chenopodiaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886437	KJ886276	KJ885954	KJ885793	KJ886115	MH496373	MH511864	MH496203
<i>Cercospora convolvulicola</i>	CCTU 1083; CBS 136126 (TYPE)	<i>Convolvulus arvensis</i>	Convolvulaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886441	KJ886280	KJ885958	KJ885797	KJ886119	MH496374	MH511865	MH496204
	CCTU 1083.2	<i>Convolvulus arvensis</i>	Convolvulaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886442	KJ886281	KJ885959	KJ885798	KJ886120	MH496375	MH511866	MH496205
<i>Cercospora conyzae-canadensis</i>	CCTU 1008	<i>Conyza canadensis</i>	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886443	KJ886282	KJ885960	KJ885799	KJ886121	MH496376	MH511867	MH496206
	CCTU 1119; CBS 135978 (TYPE)	<i>Conyza canadensis</i>	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886445	KJ886284	KJ885962	KJ885801	KJ886123	MH496377	MH511868	MH496207
	CCTU 1105; IRAN 2657C	<i>Conyza canadensis</i>	Asteraceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886444	KJ886283	KJ885961	KJ885800	KJ886122	MH496378	MH511869	MH496208
<i>Cercospora cylindracea</i>	CCTU 1016	<i>Cichorium intybus</i>	Asteraceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886446	KJ886285	KJ885963	KJ885802	KJ886124	MH496379	MH511870	MH496209
	CCTU 1114	<i>Cichorium intybus</i>	Asteraceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886450	KJ886289	KJ885967	KJ885806	KJ886128	MH496380	MH511871	MH496210
	CCTU 1081; CBS 138580; IRAN 2654C (TYPE)	<i>Lactuca serriola</i>	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886449	KJ886288	KJ885966	KJ885805	KJ886127	MH496381	MH511872	MH496211
	CCTU 1207	<i>Lactuca serriola</i>	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886453	KJ886292	KJ885970	KJ885809	KJ886131	MH496382	MH511873	MH496212
	CCTU 1044; CBS 136021	<i>Lactuca serriola</i>	Asteraceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886447	KJ886286	KJ885964	KJ885803	KJ886125	MH496383	MH511874	MH496213
	CCTU 1183	<i>Lactuca serriola</i>	Asteraceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886451	KJ886290	KJ885968	KJ885807	KJ886129	MH496384	MH511875	MH496214
	CCTU 1189	<i>Lactuca serriola</i>	Asteraceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886452	KJ886291	KJ885969	KJ885808	KJ886130	MH496385	MH511876	MH496215

Table 1. (Continued).

Species	Culture accession number (s) ¹	Host	Host Family	Origion	Collector	GenBank accession numbers ²							
						ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>
<i>Cercospora</i> cf. <i>flagellaris</i> clade 1	CCTU 1049	<i>Lactuca serriola</i>	Asteraceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886448	KJ886287	KJ885965	KJ885804	KJ886126	MH496386	MH511877	MH496216
	CPC 5441	<i>Amaranthus</i> sp.	Amaranthaceae	Fiji	C.F. Hill	JX143611	JX143370	JX143124	JX142878	JX142632	MH496387	MH511878	MH496217
	CCTU 1159; CBS 136148	<i>Arachis hypogaea</i>	Fabaceae	Iran, Guilan, Lahijan	M. Bakhshi	KJ886493	KJ886332	KJ886010	KJ885849	KJ886171	MH496388	MH511879	MH496218
	CCTU 1162; IRAN 2670C	<i>Citrullus lanatus</i>	Cucurbitaceae	Iran, Guilan, Lahijan	M. Bakhshi	KJ886496	KJ886335	KJ886013	KJ885852	KJ886174	MH496389	MH511880	MH496219
	CBS 132653; CPC 10884	<i>Dysphania ambrosioides</i> (= <i>Chenopodium ambrosioides</i>)	Chenopodiaceae	South Korea, Jeju	H.D. Shin	JX143603	JX143361	JX143115	JX142869	JX142623	MH496390	MH511881	MH496220
	CCTU 1007; CBS 136031	<i>Hydrangea</i> sp.	Hydrangeaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886456	KJ886295	KJ885973	KJ885812	KJ886134	MH496391	MH511882	MH496221
	CCTU 1027; CBS 136034	<i>Lepidium sativum</i>	Brassicaceae	Iran, Guilan, Chamkhaleh	M. Bakhshi	KJ886459	KJ886298	KJ885976	KJ885815	KJ886137	MH496392	MH511883	MH496222
	CCTU 1128; CBS 136141; IRAN 2661C	<i>Phaseolus vulgaris</i>	Fabaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886476	KJ886315	KJ885993	KJ885832	KJ886154	MH496393	MH511884	MH496223
	CCTU 1168; IRAN 2715C	<i>Phaseolus vulgaris</i>	Fabaceae	Iran, Guilan, Kiashahr	M. Bakhshi	KJ886499	KJ886338	KJ886016	KJ885855	KJ886177	MH496394	MH511885	MH496224
	CPC 1051	<i>Populus deltoides</i>	Salicaceae	South Africa	P.W. Crous	AY260069	JX143367	JX143121	JX142875	JX142629	MH496395	MH511886	MH496225
CCTU 1171	<i>Raphanus sativus</i>	Brassicaceae	Iran, Guilan, Kiashahr	M. Bakhshi	KJ886500	KJ886339	KJ886017	KJ885856	KJ886178	MH496396	MH511887	MH496226	
CCTU 1120	<i>Raphanus sativus</i>	Brassicaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886475	KJ886314	KJ885992	KJ885831	KJ886153	MH496397	MH511888	MH496227	
CCTU 1031; CBS 136036; IRAN 2648C	<i>Urtica dioica</i>	Urticaceae	Iran, Guilan, Sowme'eh Sara	M. Bakhshi	KJ886461	KJ886300	KJ885978	KJ885817	KJ886139	MH496398	MH511889	MH496228	
<i>Cercospora</i> cf. <i>flagellaris</i> clade 2	CCTU 1204	<i>Abutilon theophrasti</i>	Malvaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886505	KJ886344	KJ886022	KJ885861	KJ886183	MH496399	MH511890	MH496229
	CCTU 1198; CBS 136151	<i>Acer velutinum</i>	Aceraceae	Iran, Mazandaran, Ramsar	M. Bakhshi	KJ886504	KJ886343	KJ886021	KJ885860	KJ886182	MH496400	MH511891	MH496230
	CBS 132667; CPC 11643	<i>Celosia argentea</i> var. <i>cristata</i> (= <i>C. cristata</i>)	Amaranthaceae	South Korea, Hoengseong	H.D. Shin	JX143604	JX143362	JX143116	JX142870	JX142624	MH496401	MH511892	MH496231
	CCTU 1115; CBS 136139; IRAN 2659C	<i>Cercis siliquastrum</i>	Caesalpinaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886473	KJ886312	KJ885990	KJ885829	KJ886151	MH496402	MH511893	MH496232
	CCTU 1195	<i>Datura stramonium</i>	Solanaceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886503	KJ886342	KJ886020	KJ885859	KJ886181	MH496403	MH511894	MH496233
	CCTU 1059; CBS 136136	<i>Ecballium elaterium</i>	Cucurbitaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886464	KJ886303	KJ885981	KJ885820	KJ886142	MH496404	MH511895	MH496234
	CCTU 1216; IRAN 2717C	<i>Ecballium elaterium</i>	Cucurbitaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886510	KJ886349	KJ886027	KJ885866	KJ886188	MH496405	MH511896	MH496235
	CCTU 1223; CBS 136154; IRAN 2683C	<i>Eclipta prostrata</i>	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886512	KJ886351	KJ886029	KJ885868	KJ886190	MH496406	MH511897	MH496236
	CCTU 1068	<i>Xanthium spinosum</i>	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886466	KJ886305	KJ885983	KJ885822	KJ886144	MH496407	MH511898	MH496237
	CCTU 1085	<i>Xanthium strumarium</i>	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886471	KJ886310	KJ885988	KJ885827	KJ886149	MH496408	MH511899	MH496238

Table 1. (Continued).

Species	Culture accession number (s) ¹	Host	Host Family	Origion	Collector	GenBank accession numbers ²							
						ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>
<i>Cercospora</i> cf. <i>flagellaris</i> clade 3	CCTU 1172	<i>Oenothera biennis</i>	<i>Onagraceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886501	KJ886340	KJ886018	KJ885857	KJ886179	MH496409	MH511900	MH496239
	CCTU 1154; CBS 136147	<i>Abutilon theophrasti</i>	<i>Malvaceae</i>	Iran, Guilan, Rasht	M. Bakhshi	KJ886489	KJ886328	KJ886006	KJ885845	KJ886167	MH496410	MH511901	MH496240
	CCTU 1072; IRAN 2653C	<i>Amaranthus blitoides</i>	<i>Amaranthaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886468	KJ886307	KJ885985	KJ885824	KJ886146	MH496411	MH511902	MH496241
	CCTU 1064	<i>Amaranthus retroflexus</i>	<i>Amaranthaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886465	KJ886304	KJ885982	KJ885821	KJ886143	MH496412	MH511903	MH496242
	CCTU 1021; CBS 136033	<i>Amaranthus retroflexus</i>	<i>Amaranthaceae</i>	Iran, Guilan, Fuman	M. Bakhshi	KJ886458	KJ886297	KJ885975	KJ885814	KJ886136	MH496413	MH511904	MH496243
	CCTU 1084; CBS 136156	<i>Amaranthus</i> sp.	<i>Amaranthaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886470	KJ886309	KJ885987	KJ885826	KJ886148	MH496414	MH511905	MH496244
	CCTU 1167; CBS 136150	<i>Anubias</i> sp.	<i>Araceae</i>	Iran, Guilan, Kiashahr	M. Bakhshi	KJ886498	KJ886337	KJ886015	KJ885854	KJ886176	MH496415	MH511906	MH496245
	CBS 143.51; CPC 5055	<i>Bromus</i> sp.	<i>Poaceae</i>	—	M.D. Whitehead	JX143607	JX143365	JX143119	JX142873	JX142627	MH496416	MH511907	MH496246
	CCTU 1150	<i>Buxus microphylla</i>	<i>Buxaceae</i>	Iran, Guilan, Fuman	M. Bakhshi	KJ886488	KJ886327	KJ886005	KJ885844	KJ886166	MH496417	MH511908	MH496247
	CCTU 1140; CBS 136143; IRAN 2666C	<i>Calendula officinalis</i>	<i>Asteraceae</i>	Iran, Guilan, Astar	M. Bakhshi	KJ886481	KJ886320	KJ885998	KJ885837	KJ886159	MH496418	MH511909	MH496248
	CBS 115482; A207 Bs+; CPC 4410	<i>Citrus</i> sp.	<i>Rutaceae</i>	South Africa, Messina	M.C. Pretorius	AY260070	DQ835095	DQ835114	DQ835141	DQ835168	MH496419	MH511910	MH496249
	CCTU 1029; CBS 136035; IRAN 2647C	<i>Cucurbita maxima</i>	<i>Cucurbitaceae</i>	Iran, Guilan, Rudsar	M. Bakhshi	KJ886460	KJ886299	KJ885977	KJ885816	KJ886138	MH496420	MH511911	MH496250
	CCTU 1136	<i>Cucurbita pepo</i>	<i>Cucurbitaceae</i>	Iran, Guilan, Astar	M. Bakhshi	KJ886478	KJ886317	KJ885995	KJ885834	KJ886156	MH496421	MH511912	MH496251
	CCTU 1143; CBS 136145	<i>Datura stramonium</i>	<i>Solanaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886484	KJ886323	KJ886001	KJ885840	KJ886162	MH496422	MH511913	MH496252
	CCTU 1209; CBS 136152	<i>Glycine max</i>	<i>Fabaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886506	KJ886345	KJ886023	KJ885862	KJ886184	MH496423	MH511914	MH496253
	CCTU 1210; IRAN 2679C	<i>Glycine max</i>	<i>Fabaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886507	KJ886346	KJ886024	KJ885863	KJ886185	MH496424	MH511915	MH496254
	CCTU 1211	<i>Glycine max</i>	<i>Fabaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886508	KJ886347	KJ886025	KJ885864	KJ886186	MH496425	MH511916	MH496255
	CCTU 1218; IRAN 2682C	<i>Hibiscus trionum</i>	<i>Malvaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886511	KJ886350	KJ886028	KJ885867	KJ886189	MH496426	MH511917	MH496256
	CCTU 1006; CBS 136030	<i>Impatiens balsamina</i>	<i>Balsaminaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886455	KJ886294	KJ885972	KJ885811	KJ886133	MH496427	MH511918	MH496257
	CCTU 1130; CBS 136142	<i>Olea europaea</i>	<i>Oleaceae</i>	Iran, Zanjan, Tarom	M. Torbati	KJ886477	KJ886316	KJ885994	KJ885833	KJ886155	MH496428	MH511919	MH496258
	CCTU 1010; CBS 136032	<i>Pelargonium hortorum</i>	<i>Geraniaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886457	KJ886296	KJ885974	KJ885813	KJ886135	MH496429	MH511920	MH496259
	CCTU 1138; IRAN 2664C	<i>Phaseolus vulgaris</i>	<i>Fabaceae</i>	Iran, Guilan, Astar	M. Bakhshi	KJ886479	KJ886318	KJ885996	KJ885835	KJ886157	MH496430	MH511921	MH496260
	CCTU 1139; IRAN 2665C	<i>Phaseolus vulgaris</i>	<i>Fabaceae</i>	Iran, Guilan, Astar	M. Bakhshi	KJ886480	KJ886319	KJ885997	KJ885836	KJ886158	MH496431	MH511922	MH496261
	CCTU 1155.11	<i>Phaseolus vulgaris</i>	<i>Fabaceae</i>	Iran, Guilan, Fuman	M. Bakhshi	KJ886490	KJ886329	KJ886007	KJ885846	KJ886168	MH496432	MH511923	MH496262
	CCTU 1161; IRAN 2669C	<i>Phaseolus vulgaris</i>	<i>Fabaceae</i>	Iran, Guilan, Lahijan	M. Bakhshi	KJ886495	KJ886334	KJ886012	KJ885851	KJ886173	MH496433	MH511924	MH496263
	CCTU 1175; IRAN 2673C	<i>Phaseolus vulgaris</i>	<i>Fabaceae</i>	Iran, Guilan, Sowme'eh Sara	M. Bakhshi	KJ886502	KJ886341	KJ886019	KJ885858	KJ886180	MH496434	MH511925	MH496264
	CCTU 1142; IRAN 2667C	<i>Phaseolus vulgaris</i>	<i>Fabaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886483	KJ886322	KJ886000	KJ885839	KJ886161	MH496435	MH511926	MH496265

Table 1. (Continued).

Species	Culture accession number (s) ¹	Host	Host Family	Origion	Collector	GenBank accession numbers ²							
						ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>
	CCTU 1118; CBS 136140; IRAN 2660C	<i>Populus deltoides</i>	Salicaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886474	KJ886313	KJ885991	KJ885830	KJ886152	MH496436	MH511927	MH496266
	CCTU 1075	<i>Raphanus sativus</i>	Brassicaceae	Iran, Guilan, Sowme'eh Sara	M. Bakhshi	KJ886469	KJ886308	KJ885986	KJ885825	KJ886147	MH496437	MH511928	MH496267
	CCTU 1212; CBS 136153; IRAN 2680C	<i>Silybum marianum</i>	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886509	KJ886348	KJ886026	KJ885865	KJ886187	MH496438	MH511929	MH496268
	CCTU 1141; CBS 136144	<i>Tagetes patula</i>	Asteraceae	Iran, Guilan, Rudsar	M. Bakhshi	KJ886482	KJ886321	KJ885999	KJ885838	KJ886160	MH496439	MH511930	MH496269
	CCTU 1147	<i>Urtica dioica</i>	Urticaceae	Iran, Guilan, Masal	M. Bakhshi	KJ886486	KJ886325	KJ886003	KJ885842	KJ886164	MH496440	MH511931	MH496270
	CCTU 1160; CBS 136149	<i>Vicia faba</i>	Fabaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886494	KJ886333	KJ886011	KJ885850	KJ886172	MH496441	MH511932	MH496271
	CCTU 1158; IRAN 2668C	<i>Xanthium strumarium</i>	Asteraceae	Iran, Guilan, Langarud	M. Bakhshi	KJ886492	KJ886331	KJ886009	KJ885848	KJ886170	MH496442	MH511933	MH496272
	CCTU 1156	<i>Xanthium strumarium</i>	Asteraceae	Iran, Guilan, Rasht	M. Bakhshi	KJ886491	KJ886330	KJ886008	KJ885847	KJ886169	MH496443	MH511934	MH496273
	CCTU 1005; IRAN 2644C	<i>Xanthium strumarium</i>	Asteraceae	Iran, Guilan, Talesh	M. Bakhshi	KJ886454	KJ886293	KJ885971	KJ885810	KJ886132	MH496444	MH511935	MH496274
	CCTU 1048; CBS 136029	<i>Xanthium strumarium</i>	Asteraceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886462	KJ886301	KJ885979	KJ885818	KJ886140	MH496445	MH511936	MH496275
<i>Cercospora gamsiana</i>	CBS 144962; CCTU 1074; CPC 24909 (TYPE)	<i>Malva neglecta</i>	Malvaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886426	KJ886265	KJ885943	KJ885782	KJ886104	MH496446	MH511937	MH496276
	CCTU 1035	<i>Malva sylvestris</i>	Malvaceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886423	KJ886262	KJ885940	KJ885779	KJ886101	MH496447	MH511938	MH496277
	CCTU 1109	<i>Malva sylvestris</i>	Malvaceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886431	KJ886270	KJ885948	KJ885787	KJ886109	MH496448	MH511939	MH496278
	CCTU 1199; CBS 136128; IRAN 2675C	<i>Rumex crispus</i>	Polygonaceae	Iran, Mazandaran, Ramsar	M. Bakhshi	KJ886433	KJ886272	KJ885950	KJ885789	KJ886111	MH496449	MH511940	MH496279
	CCTU 1205; CBS 136127; IRAN 2677C	<i>Sesamum indicum</i>	Pedaliaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886435	KJ886274	KJ885952	KJ885791	KJ886113	MH496450	MH511941	MH496280
	CCTU 1208; IRAN 2678C	<i>Sonchus</i> sp.	Asteraceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886436	KJ886275	KJ885953	KJ885792	KJ886114	MH496451	MH511942	MH496281
<i>Cercospora</i> cf. <i>gossypii</i>	CCTU 1070; CBS 136137	<i>Gossypium herbaceum</i>	Malvaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886467	KJ886306	KJ885984	KJ885823	KJ886145	MH496452	MH511943	MH496282
	CCTU 1055; IRAN 2650C	<i>Hibiscus trionum</i>	Malvaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886463	KJ886302	KJ885980	KJ885819	KJ886141	MH496453	MH511944	MH496283
<i>Cercospora iranica</i>	CCTU 1196; CBS 136123	<i>Hydrangea</i> sp.	Hydrangeaceae	Iran, Mazandaran, Ramsar	M. Bakhshi	KJ886515	KJ886354	KJ886032	KJ885871	KJ886193	MH496454	MH511945	MH496284
	CCTU 1137; CBS 136124 (TYPE)	<i>Vicia faba</i>	Fabaceae	Iran, Guilan, Astara	M. Bakhshi	KJ886513	KJ886352	KJ886030	KJ885869	KJ886191	MH496455	MH511946	MH496285
<i>Cercospora plantaginis</i>	CCTU 1082; CBS 138728	<i>Plantago lanceolata</i>	Plantaginaceae	Iran, Ardabil, Moghan	M. Bakhshi	KJ886402	KJ886241	KJ885919	KJ885758	KJ886080	MH496456	MH511947	MH496286
	CCTU 1095	<i>Plantago lanceolata</i>	Plantaginaceae	Iran, East Azerbaijan, Horand	M. Bakhshi	KJ886403	KJ886242	KJ885920	KJ885759	KJ886081	MH496457	MH511948	MH496287
	CCTU 1041; CPC 24910	<i>Plantago lanceolata</i>	Plantaginaceae	Iran, Guilan, Chaboksar	M. Bakhshi	KJ886400	KJ886239	KJ885917	KJ885756	KJ886078	MH496458	MH511949	MH496288
	CCTU 1179; IRAN 2716C	<i>Plantago lanceolata</i>	Plantaginaceae	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886404	KJ886243	KJ885921	KJ885760	KJ886082	MH496459	MH511950	MH496289
	CCTU 1047	<i>Plantago lanceolata</i>	Plantaginaceae	Iran, Zanjan, Tarom	M. Bakhshi	KJ886401	KJ886240	KJ885918	KJ885757	KJ886079	MH496460	MH511951	MH496290

Table 1. (Continued).

Species	Culture accession number (s) ¹	Host	Host Family	Origion	Collector	GenBank accession numbers ²							
						ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>
	CBS 252.67; CPC 5084 (TYPE)	<i>Plantago lanceolata</i>	<i>Plantaginaceae</i>	Romania, Domnesti	O. Constantinescu	DQ233318	DQ233342	DQ233368	DQ233394	DQ233420	MH496461	_	MH496291
<i>Cercospora pseudochenopodii</i>	CCTU 1176	<i>Chenopodium album</i>	<i>Chenopodiaceae</i>	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886518	KJ886357	KJ886035	KJ885874	KJ886196	MH496462	MH511952	MH496292
	CCTU 1045	<i>Chenopodium</i> sp.	<i>Chenopodiaceae</i>	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886517	KJ886356	KJ886034	KJ885873	KJ886195	MH496463	MH511953	MH496293
	CCTU 1038; CBS 136022; IRAN 2649C (TYPE)	<i>Chenopodium</i> sp.	<i>Chenopodiaceae</i>	Iran, Zanjan, Tarom	M. Bakhshi	KJ886516	KJ886355	KJ886033	KJ885872	KJ886194	MH496464	MH511954	MH496294
<i>Cercospora</i> cf. <i>richardiicola</i>	CCTU 1004	<i>Bidens tripartita</i>	<i>Asteraceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886519	KJ886358	KJ886036	KJ885875	KJ886197	MH496465	MH511955	MH496295
<i>Cercospora rumicis</i>	CCTU 1123	<i>Rumex crispus</i>	<i>Polygonaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886521	KJ886360	KJ886038	KJ885877	KJ886199	MH496466	MH511956	MH496296
	CCTU 1129; IRAN 2662C	<i>Rumex crispus</i>	<i>Polygonaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886522	KJ886361	KJ886039	KJ885878	KJ886200	MH496467	MH511957	MH496297
	CCTU 1121	<i>Urtica dioica</i>	<i>Urticaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886520	KJ886359	KJ886037	KJ885876	KJ886198	MH496468	MH511958	MH496298
<i>Cercospora solani</i>	CCTU 1043; CBS 136038	<i>Solanum nigrum</i>	<i>Solanaceae</i>	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886523	KJ886362	KJ886040	KJ885879	KJ886201	MH496469	MH511959	MH496299
	CCTU 1050	<i>Solanum nigrum</i>	<i>Solanaceae</i>	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886524	KJ886363	KJ886041	KJ885880	KJ886202	MH496470	MH511960	MH496300
<i>Cercospora sorghicola</i>	CCTU 1173; CBS 136448; IRAN 2672C (TYPE)	<i>Sorghum halepense</i>	<i>Poaceae</i>	Iran, Guilan, Kiashahr	M. Bakhshi	KJ886525	KJ886364	KJ886042	KJ885881	KJ886203	MH496471	MH511961	MH496301
<i>Cercospora</i> sp. G clade 1	CCTU 1197	<i>Bidens tripartita</i>	<i>Asteraceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886540	KJ886379	KJ886057	KJ885896	KJ886218	MH496472	MH511962	MH496302
	CCTU 1015; CBS 136024; IRAN 2645C	<i>Plantago major</i>	<i>Plantaginaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886528	KJ886367	KJ886045	KJ885884	KJ886206	MH496473	MH511963	MH496303
	CPC 5438	<i>Salvia viscosa</i>	<i>Lamiaceae</i>	New Zealand, Manurewa	C.F. Hill	JX143682	JX143442	JX143196	JX142950	JX142704	MH496474	_	MH496304
<i>Cercospora</i> sp. G clade 2	CCTU 1058	<i>Helminthotheca echiooides</i>	<i>Asteraceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886534	KJ886373	KJ886051	KJ885890	KJ886212	MH496475	MH511964	MH496305
	CCTU 1090	<i>Abutilon theophrasti</i>	<i>Malvaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886536	KJ886375	KJ886053	KJ885892	KJ886214	MH496476	MH511965	MH496306
	CCTU 1079; CBS 136025	<i>Amaranthus retroflexus</i>	<i>Amaranthaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886535	KJ886374	KJ886052	KJ885891	KJ886213	MH496477	MH511966	MH496307
	CCTU 1054	<i>Amaranthus</i> sp.	<i>Amaranthaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886533	KJ886372	KJ886050	KJ885889	KJ886211	MH496478	MH511967	MH496308
	CCTU 1122	<i>Amaranthus</i> sp.	<i>Amaranthaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886538	KJ886377	KJ886055	KJ885894	KJ886216	MH496479	MH511968	MH496309
	CBS 115518; CPC 5360	<i>Bidens frondosa</i>	<i>Asteraceae</i>	New Zealand, Kopuku	C.F. Hill	JX143681	JX143441	JX143195	JX142949	JX142703	MH496480	_	MH496310
	CCTU 1030; CBS 136026	<i>Bidens tripartita</i>	<i>Asteraceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886530	KJ886369	KJ886047	KJ885886	KJ886208	MH496481	MH511969	MH496311
	CCTU 1002	<i>Celosia cristata</i>	<i>Amaranthaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886527	KJ886366	KJ886044	KJ885883	KJ886205	MH496482	MH511970	MH496312
	CCTU 1053; CBS 136027	<i>Cichorium intybus</i>	<i>Asteraceae</i>	Iran, Guilan, Sowme'eh Sara	M. Bakhshi	KJ886532	KJ886371	KJ886049	KJ885888	KJ886210	MH496483	MH511971	MH496313
	CCTU 1144; CBS 136130	<i>Cucurbita maxima</i>	<i>Cucurbitaceae</i>	Iran, Guilan, Masal	M. Bakhshi	KJ886539	KJ886378	KJ886056	KJ885895	KJ886217	MH496484	MH511972	MH496314

Table 1. (Continued).

Species	Culture accession number (s) ¹	Host	Host Family	Origion	Collector	GenBank accession numbers ²							
						ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>
	CCTU 1046	<i>Plantago major</i>	<i>Plantaginaceae</i>	Iran, Zanjan, Tarom	M. Bakhshi	KJ886531	KJ886370	KJ886048	KJ885887	KJ886209	MH496485	MH511973	MH496315
	CCTU 1116	<i>Plantago major</i>	<i>Plantaginaceae</i>	Iran, Zanjan, Tarom	M. Bakhshi	KJ886537	KJ886376	KJ886054	KJ885893	KJ886215	MH496486	MH511974	MH496316
	CCTU 1020; CBS 136023	<i>Sorghum halepense</i>	<i>Poaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886529	KJ886368	KJ886046	KJ885885	KJ886207	MH496487	MH511975	MH496317
<i>Cercospora</i> sp. T	CCTU 1148; CBS 136125	<i>Coreopsis</i> sp.	<i>Asteraceae</i>	Iran, Guilan, Rasht	M. Bakhshi	KJ886541	KJ886380	KJ886058	KJ885897	KJ886219	MH496488	MH511976	MH496318
<i>Cercospora uwebrauniana</i>	CCTU 1200; CBS 138581 (TYPE)	<i>Heliotropium europaeum</i>	<i>Boraginaceae</i>	Iran, Ardabil, Moghan	M. Bakhshi	KJ886408	KJ886247	KJ885925	KJ885764	KJ886086	MH496489	MH511977	MH496319
	CCTU 1134	<i>Heliotropium europaeum</i>	<i>Boraginaceae</i>	Iran, Guilan, Astara	M. Bakhshi	KJ886407	KJ886246	KJ885924	KJ885763	KJ886085	MH496490	MH511978	MH496320
<i>Cercospora violae</i>	CCTU 1025; IRAN 2646C	<i>Viola</i> sp.	<i>Violaceae</i>	Iran, Mazandaran, Nowshahr	M. Bakhshi	KJ886543	KJ886382	KJ886060	KJ885899	KJ886221	MH496491	MH511979	MH496321
	CBS 251.67; CPC 5079 (TYPE)	<i>Viola tricolor</i>	<i>Violaceae</i>	Romania, Cazanele Dunarii	O. Constantinescu	JX143737	JX143496	JX143250	JX143004	JX142758	MH496492	—	MH496322
<i>Cercospora zebrina</i>	CCTU 1039	<i>Alhagi camelorum</i>	<i>Fabaceae</i>	Iran, Zanjan, Tarom	M. Bakhshi	KJ886545	KJ886384	KJ886062	KJ885901	KJ886223	MH496493	MH511980	MH496323
	CBS 108.22; CPC 5091	<i>Medicago arabica</i> (= <i>M. maculata</i>)	<i>Fabaceae</i>	—	E.F. Hopkins	JX143744	JX143503	JX143257	JX143011	JX142765	MH496494	—	MH496324
	CCTU 1225	<i>Medicago sativa</i>	<i>Fabaceae</i>	Iran, East Azerbaijan, Marand	M. Bakhshi	KJ886550	KJ886389	KJ886067	KJ885906	KJ886228	MH496495	MH511981	MH496325
	CCTU 1180	<i>Medicago sativa</i>	<i>Fabaceae</i>	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886547	KJ886386	KJ886064	KJ885903	KJ886225	MH496496	MH511982	MH496326
	CCTU 1110; IRAN 2658C	<i>Medicago sativa</i>	<i>Fabaceae</i>	Iran, Zanjan, Tarom	M. Bakhshi	KJ886546	KJ886385	KJ886063	KJ885902	KJ886224	MH496497	MH511983	MH496327
	CCTU 1012; CBS 136129	<i>Medicago</i> sp.	<i>Fabaceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886544	KJ886383	KJ886061	KJ885900	KJ886222	MH496498	MH511984	MH496328
	CCTU 1181	<i>Trifolium repens</i>	<i>Fabaceae</i>	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886548	KJ886387	KJ886065	KJ885904	KJ886226	MH496499	MH511985	MH496329
	CBS 113070; CPC 5367	<i>Trifolium repens</i>	<i>Fabaceae</i>	New Zealand, Blockhouse Bay	C.F. Hill	JX143745	JX143507	JX143261	JX143015	JX142769	MH496500	—	MH496330
	CBS 118790; IMI 262766; WA 2030; WAC 7973	<i>Trifolium subterraneum</i>	<i>Fabaceae</i>	Australia	M.J. Barbeti	JX143748	JX143510	JX143264	JX143018	JX142772	MH496501	—	MH496331
	CBS 129.39; CPC 5078	<i>Trifolium subterraneum</i>	<i>Fabaceae</i>	U.S.A., Wisconsin—	—	JX143750	JX143512	JX143266	JX143020	JX142774	MH496502	—	MH496332
	CCTU 1185	<i>Vicia</i> sp.	<i>Fabaceae</i>	Iran, West Azerbaijan, Khoy	M. Arzanlou	KJ886549	KJ886388	KJ886066	KJ885905	KJ886227	MH496503	MH511986	MH496333

Table 1. (Continued).

Species	Culture accession number (s) ¹	Host	Host Family	Origion	Collector	GenBank accession numbers ²							
						ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>
<i>Cercospora</i> cf. <i>zinniae</i>	CCTU 1239; CBS 135977	<i>Vitis vinifera</i>	<i>Vitaceae</i>	Iran, East Azerbaijan, Kaleybar	M. Arzanlou	KJ886551	KJ886390	KJ886068	KJ885907	KJ886229	MH496504	MH511987	MH496334
	CCTU 1003	<i>Zinnia elegans</i>	<i>Asteraceae</i>	Iran, Guilan, Talesh	M. Bakhshi	KJ886552	KJ886391	KJ886069	KJ885908	KJ886230	MH496505	MH511988	MH496335

¹ CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CCTU: Culture Collection of Tabriz University, Tabriz, Iran; CPC: Culture collection of Pedro Crous, housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakenham Lane, U.K.; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran; WAC: Department of Agriculture Western Australia Plant Pathogen Collection, Perth, Australia.

² ITS: internal transcribed spacers and intervening 5.8S nrDNA; *tef1*: partial translation elongation factor 1-alpha gene, *actA*: partial actin gene, *cmdA*: partial calmodulin gene, *his3*: partial histone H3 gene, *tub2*: partial beta-tubulin gene, *rpb2*: partial RNA polymerase II gene, *gapdh*: partial glyceraldehyde-3-phosphate dehydrogenase gene.

Table 2. Primer combinations used during this study for amplification and sequencing.

Locus	Primer	Primer sequence 5' to 3'	Annealing temperature (°C)	Orientation	Reference
Beta-tubulin (<i>tub2</i>)	T1	AAC ATG CGT GAG ATT GTA AGT	48	Forward	O'Donnell & Cigelnik 1997
	β-Sandy-R	GCR CGN GGV ACR TAC TTG TT	48	Reverse	Stukenbrock <i>et al.</i> 2012
	BT-1F	GTC CWC ACC GCC CCT GAT	56	Forward	This study
	BT-1R	CTT GTT RCC RGA AGC CTR TGS	56	Reverse	This study
RNA polymerase II second largest subunit (<i>rpb2</i>)	fRPB2-5F	GAY GAY MGW GAT CAY TTY GG	47	Forward	Liu <i>et al.</i> 1999
	fRPB2-414R	ACM ANN CCC CAR TGN GWR TTR TG	47	Reverse	Quaedvlieg <i>et al.</i> 2011
	fRPB2-7cF	ATG GGY AAR CAA GCY ATG GG	49	Forward	Liu <i>et al.</i> 1999
	fRPB2-11aR	GCR TGG ATC TTR TCR TCS ACC	49	Reverse	Liu <i>et al.</i> 1999
	RPB2-C5F	TGG GGA GAY CAR AAR AAA GC	60→58→56	Forward	This study
	RPB2-C8R	ACG GAA TCT TCC TGG TTG TA	60→58→56	Reverse	This study
Glyceraldehyde-3-phosphate dehydrogenase (<i>gapdh</i>)	Gpd1-LM	ATT GGC CGC ATC GTC TTC CGC AA	60→58→53	Forward	Myllys <i>et al.</i> 2002
	Gpd2-LM	CCC ACT CGT TGT CGT ACC A	60→58→53	Reverse	Myllys <i>et al.</i> 2002

Table 3. Phylogenetic data and the substitution models used in the phylogenetic analysis, per locus. Abbreviations of loci follow Table 1.

Locus	ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>
Number of characters	470	291	187	248	358	415	1229	869
Unique site patterns	16	75	48	66	63	105	259	231
Substitution model used	SYM-gamma	K80-gamma	K80-gamma	K80-gamma	HKY-gamma	GTR-gamma	GTR-gamma	GTR-I-gamma
Number of generations (n)	2 405 000							
Total number of trees (n)	4 812							
Sampled trees (n)	3 610							

polymerase (Promega). The *tub2* PCR mixture contained 5–10 ng genomic DNA, 1 × PCR buffer, 2 mM MgCl₂, 40 μM of each dNTP, 0 μL / 0.5 μL BSA, 0.25 μM of each primer and 0.5 units GoTaq® Flexi DNA polymerase using respectively the BT-1F/BT-1R (this study) or T1 (O'Donnell & Cigelnik 1997)/β-Sandy-R (Stukenbrock *et al.* 2012) primer sets. The *rpb2* gene was amplified in three parts with three primer sets. Part three was only amplified in some selected species in order to design a new reverse primer for amplification of part two. The *rpb2* PCR mixtures using the fRPB2-5F (Liu *et al.* 1999)/fRPB2-414R (Quaedvlieg *et al.* 2011) primer set consisted of 5–10 ng genomic DNA, 1 × PCR buffer, 2 mM MgCl₂, 40 μM of each dNTP, 0.5 μL BSA, 0.2 μM of each primer and 0.5 units GoTaq® Flexi DNA polymerase. The PCR mixtures using RPB2-C5F/RPB2-C8R (this study) and fRPB2-7cF/fRPB2-11aR primer sets (Liu *et al.* 1999) were the same as *gapdh*.

To obtain the partial *tub2* and *rpb2* (using the fRPB2-5F/fRPB2-414R and fRPB2-7cF/fRPB2-11aR primer sets) sequences, PCR amplification conditions were set as follows: an initial denaturation temperature of 94 °C for 3 min, followed by 40 (*tub2*) or 45 (*rpb2*) cycles of denaturation temperature of 94 °C for 30 s, primer annealing at the temperature stipulated in Table 2 for 30 s, primer extension at 72 °C for 45 s and a final extension step at 72 °C for 5 min.

A touchdown PCR protocol was used to amplify the partial *gapdh* (using the Gpd1-LM/Gpd2-LM primer set (Myllys *et al.* 2002)) and *rpb2* (using the RPB2-C5F/RPB2-C8R primer set) sequences: initial denaturation (94 °C, 5 min), five amplification cycles (94 °C, 45 s; 60 °C, 45 s; 72 °C, 90 s), five amplification cycles (94 °C, 45 s; 58 °C, 45 s; 72 °C, 90 s), 30 amplification cycles (94 °C, 45 s; 53 °C (*gapdh*) or 56 °C (*rpb2*), 45 s; 72 °C, 90 s) and a final extension step (72 °C, 5 min). PCR products were visualised by electrophoresis using a 1.2 % agarose gel, stained with GelRed™ (Biotium, Hayward, CA) and viewed under ultra-violet light. Size estimates were made using a HyperLadder™ I molecular marker (Biolone).

Sequencing and phylogenetic analyses

The resulting PCR fragments were sequenced in both directions using the same primers used for amplification (Table 2) and the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems, Foster City, CA), following the manufacturer's instructions. DNA sequencing amplicons were purified through Sephadex G-50 Superfine columns (SigmaAldrich, St Louis, MO) in 96-well MultiScreen HV plates (Millipore, Billerica, MA) as outlined by the manufacturer and analysed with an ABI Prism 3730xl Automated DNA analyser (Life Technologies Europe BV, Applied Biosystems™, Bleiswijk, The Netherlands).

The raw DNA sequences of *tub2*, *gapdh* and *rpb2* were edited using MEGA v. 6 (Tamura *et al.* 2013) and forward and reverse sequences for each isolate were assembled manually to generate consensus sequences. Two parts of the *rpb2* gene (part amplified with the fRPB2-5F/fRPB2-414R primer set + part amplified with the RPB2-C5F/RPB2-C8R primer set) were compiled manually using MEGA v. 6. The assembled consensus sequences were initially aligned with MEGA v. 6 and optimised with the multiple sequence

alignment online interface of MAFFT using default settings (<http://mafft.cbrc.jp/alignment/server/>) (Kato & Standley 2013), and adjusted manually where necessary. In addition, sequences of the same isolates corresponding to the ITS locus (including ITS1, 5.8S, ITS2), together with parts of four protein coding genes, *viz.* translation elongation factor 1-alpha (*tef1*), actin (*actA*), calmodulin (*cmdA*) and histone H3 (*his3*), were retrieved from the NCBI's GenBank nucleotide database and included in the analyses, after separate alignment as described above. Sequences of *Cercospora sorghicola* (CBS 136448 = IRAN 2672C) were used as outgroup. Evolutionary models for phylogenetic analyses were selected independently for each locus using MrModeltest v. 2.3 (Nylander 2004) under the Akaike Information Criterion (AIC) (Table 3). The individual alignments of the different loci were subsequently concatenated with Mesquite v. 2.75 (Maddison & Maddison 2011) prior to being subjected to a combined multi-gene analysis. Given the different sizes of the data partitions, they could not be properly used in statistical tests for (in) congruency. Phylogenetic reconstruction was performed using Bayesian inference (BI) Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.2.2 (Ronquist *et al.* 2012). Two simultaneous MCMC analyses, each consisting of four Markov chains, were run from random trees until the average standard deviation of split frequencies reached a value of 0.01, with trees saved every 100 generations and the heating parameter was set to 0.15. Burn-in phase was set to 25 % and the posterior probabilities (Rannala & Yang 1996) were calculated from the remaining trees. The resulting phylogenetic tree was generated with Geneious v. 5.6.7 (Drummond *et al.* 2012).

All new sequences generated in this study were deposited in NCBI's GenBank nucleotide database (www.ncbi.nlm.nih.gov; Table 1) and the alignment and phylogenetic trees in TreeBASE S22944 (www.TreeBASE.org).

Morphology

Morphological descriptions are based on structures from dried material. Diseased leaf tissues were viewed under a Nikon® SMZ1500 stereo-microscope and taxonomically informative morphological structures (stromata, conidiophores and conidia) were picked up from lesions with a sterile dissecting needle and mounted on glass slides in clear lactic acid. Structures were examined under a Nikon Eclipse 80i light microscope, and photographed using a mounted Nikon digital sight DS-f1 high definition colour camera.

Thirty measurements were made at ×1000 for each microscopic structure, and 95 % confidence intervals were derived for the measurements with extreme values given in parentheses. Colony macro-morphology on MEA was determined after 1 mo at 25 °C in the dark in duplicate and colony colour was described using the mycological colour charts of Rayner (1970). Nomenclatural novelties and descriptions were deposited in MycoBank (www.mycobank.org; Crous *et al.* 2004). The naming system for tentatively applied names used by Groenewald *et al.* (2013) and Bakhshi *et al.* (2015a) is continued in this manuscript to simplify comparison between the studies.

Identification of the best-performing DNA barcode

The dataset of the eight loci, ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, *rpb2* and *gapdh*, was individually tested for two factors: Kimura-2-parameter (K2P) values (barcode gap) and molecular phylogenetic resolution (clade recovery). Inter- and intraspecific distances of eight loci were calculated for each single-locus sequence data alignment, using MEGA v. 6.0 with the Kimura-2-parameter distance values using the pairwise deletion model. Microsoft Excel 2010 was subsequently used to sort these distance values into distribution bins (from distance 0–0.1 with intervals of 0.01 between bins) and the frequency of entries for each individual bin was then plotted against the Kimura-2-parameter distance of each bin.

In addition, Bayesian analyses using the corresponding nucleotide substitution models (Table 3) were applied to each data partition to check the stability and robustness of each species clade (clade recovery) under the different loci (data not shown, trees deposited in TreeBASE S22944) (Table 4). The clade recovery and Kimura-2-parameter values for each locus were calculated after applying the consolidated species concept to the results of eight-gene phylogenetic tree.

Allele group designation

The isolates in each of the *Cercospora* species complexes, including *C. apii*, *C. armoraciae*, *C. beticola*, *C. cf. flagellaris*, and *Cercospora* sp. G, were compared using the individual alignments of the eight single loci in MEGA v. 6. Allele groups

Table 4. Summary of clade support (Bayesian posterior probabilities (PP) values) for each species and locus or combination of loci. Green cells represent the PP values of species which are supported as distinct species, purple cells represent the PP values of species which are indistinct from one other species; while white cells represent species which cannot be distinguished from several other species for the given locus or combination of loci. The K2P inter-/intraspecies variation ratio as well as the number of species in the three different coloured categories are indicated per locus below the table. Abbreviations of loci follow Table 1.

Locus/Loci	ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>	All 8 loci
<i>C. althaeina</i>		1	0.91		0.79	1	0.97	0.7	1
<i>C. apii</i>								1	1
<i>C. armoraciae</i>		0.73		1	0.98	1	?	1	1
<i>C. beticola</i>				1				1	1
<i>C. bizzoeriana</i>		0.96		1	0.98	1	1	1	1
<i>C. chenopodii</i>	0.99	1	1	0.95	1	1	1	1	1
<i>C. convolvulicola</i>			1	0.99				1	1
<i>C. conyzae-canadensis</i>		0.96	1	0.92	1	1	1	0.57	1
<i>C. cylindracea</i>		1	0.9		0.99	0.98	0.97	0.75	1
<i>C. cf. flagellaris</i> clade 1								1	1
<i>C. cf. flagellaris</i> clade 2								1	1
<i>C. cf. flagellaris</i> clade 3								1	1
<i>C. gamsiana</i>				1				1	1
<i>C. cf. gossypii</i>								1	1
<i>C. iranica</i>		1	0.94	1	1	0.98	1	0.91	1
<i>C. plantaginis</i>								1	1
<i>C. pseudochenopodii</i>		1	1	0.95	1	0.99	1	1	1
<i>C. cf. richardiicola</i>		1	1	1	1	1	1	1	1
<i>C. rumicis</i>		0.99			1		1	1	1
<i>C. solani</i>	1	0.99	0.93	0.83	1	1	1	1	1
<i>C. sorghicola</i>	1	1	1	1	1	1	1	1	1
<i>Cercospora</i> sp. G clade 1		0.98		1	0.86	1	1	1	1
<i>Cercospora</i> sp. G clade 2		0.98		1	0.86	1	1	1	1
<i>Cercospora</i> sp. T		1	0.94	1	1	1	1	0.91	1
<i>C. uwebrauniana</i>		1			0.76	0.99		1	1
<i>C. violae</i>		0.97	1	0.93	0.94		1	1	1
<i>C. zebrina</i>		0.73	0.87		0.84		1	0.75	1
<i>C. cf. zinniae</i>		1	1	1	1	1	1	1	1
K2P inter-/intraspecies variation ratio	4	127	15	76	13	71	74	44	
Number of distinct species	3	11	12	9	13	12	9	17	
Number of two indistinct species	0	8	2	8	6	4	8	11	
Number of unresolved species	25	9	14	11	9	12	10	0	

were established for each locus based on sequence identity, i.e. each sequence with one or more nucleotide difference from the other sequence was regarded as a different allele.

RESULTS

DNA amplification and phylogenetic analysis

New primers were designed for *rpb2* and *tub2* in this study (Table 2) and proved to be effective for the selected *Cercospora* species. Approximately 400, 1000, and 1200 bp were obtained for *tub2*, *gapdh* and *rpb2* loci, respectively. The final concatenated eight-locus alignment contained 169 ingroup taxa and a total of 4 099 characters including alignment gaps were processed. The gene boundaries were 1–470 bp for ITS, 475–765 bp for *tef1*, 770–956 bp for *actA*, 961–1 208 bp for *cmdA*, 1 213–1 570 bp for *his3*, 1 575–1 989 bp for *tub2*, 1 994–3 222 bp for *rpb2*, and 3 227–4 099 bp for *gapdh*. For the total alignment, 28 characters which were artificially introduced as spacers to separate the loci, were excluded from the phylogenetic analyses. The alignment contained 863 unique site patterns (Table 3).

The Bayesian analysis lasted 2 405 000 generations and generated 4 812 trees from which the first 1 202 trees (25 %), representing the burn-in phase of the analyses, were discarded, and the remaining trees (3 610) were used for calculating posterior probabilities (PP) values in the phylogenetic tree (50 % majority rule consensus tree) (Fig. 1).

TAXONOMY

Species delimitation in the genus *Cercospora* in this study follows the Consolidated Species Concept accepted in recent revisions of the taxonomy of cercosporoid fungi (e.g. Groenewald *et al.* 2013, Crous *et al.* 2013, Bakhshi *et al.* 2015a, Videira *et al.* 2017). Twenty-eight lineages of *Cercospora* were resolved based on the clustering and support in the Bayesian tree obtained from the combined ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, *rpb2*, and *gapdh* alignment (Fig. 1, Table 4). Of these, 15 species including *C. althaeina*, *C. chenopodii*, *C. convolvulicola*, *C. conyzae-canadensis*, *C. cylindracea*, *C. iranica*, *C. pseudochenopodii*, *C. cf. richardiicola*, *C. rumicis*, *C. solani*, *C. sorghicola*, *Cercospora* sp. T, *C. violae*, *C. zebрина*, and *C. cf. zinnia*, were the same as those also accepted before in the five-gene phylogenetic tree (ITS, *tef1*, *actA*, *cmdA*, and *his3*) (Bakhshi *et al.* 2015a). However, the eight-gene phylogenetic tree separated strains previously recognised as *C. apii*, *C. armoraciae*, *C. beticola*, *C. cf. flagellaris*, and *Cercospora* sp. G, based on five-gene phylogenetic tree (Groenewald *et al.* 2013, Bakhshi *et al.* 2015a) into at least three, two, two, four and two well-supported clades respectively (Fig. 1). Some of these clades are supported by the host range or morphological characters of the isolates and are therefore described as new below.

Cercospora apii complex

The 16 isolates previously recognised as *C. apii* based on five-gene phylogenetic tree (Groenewald *et al.* 2013, Bakhshi *et al.* 2015a) are assigned here to three lineages based on

the eight-gene phylogenetic tree, host association, and morphology, including *C. apii* s. str., *C. uwebrauniana* sp. nov., and *C. plantaginis* (Fig. 1, part 2). The results of allele group designation for the isolates in this complex detected one, four, two, two, four, three, four and two allele groups for the ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, *rpb2*, and *gapdh* sequences, respectively (Table 5).

***Cercospora apii* Fresen., *Beitr. Mykol.* 3: 91 (1863).**
Sensu Groenewald *et al.*, *Phytopathology* 95: 954 (2005).
(Fig. 2)

Type: Germany: Oestrich, on *Apium graveolens* (Apiaceae), *Fuckel*, *Fungi rhen.* 117, in HAL (**lectotype** designated by Groenewald *et al.* 2005); Heilbronn, Landwirtschaftsamt, on *A. graveolens*, 10 Aug. 2004, K. Schrameyer (CBS 116455 = CPC 11556 – **epitype** designated by Groenewald *et al.* 2005).

Description: Leaf spots amphigenous, distinct, circular to subcircular, 1–9 mm diam, white-grey in centre, surrounded by a dark purple-brown border. *Mycelium* internal. *Caespituli* amphigenous, brown. *Conidiophores* aggregated in moderately dense fascicles (4–15), arising from the upper cells of a well-developed brown stroma, to 50 µm wide; conidiophores brown, becoming pale brown towards the apex, 1–6-septate, straight to variously curved, unbranched, uniform in width, (45–)80–95(–125) × 4–5.5 µm. *Conidiogenous cells* integrated, lateral or terminal, unbranched, brown, smooth, proliferating sympodially, 20–40 × 3.5–5 µm, multi-local; loci thickened, darkened, refractive, apical or lateral, 2–3.5 µm diam. *Conidia* solitary, smooth, obclavate-cylindrical to acicular, straight to slightly curved, hyaline, distinctly 3–9(–15)-septate, apex subacute or subobtusely rounded, base subtruncate to obconically truncate, (30–)65–80(–115) × 3–5 µm; hila thickened, darkened, refractive, 2–3.5 µm diam.

Note: This clade includes the ex-epitype strain of *C. apii* (isolate CBS 116455 = CPC 11556), therefore we fixed the application of *C. apii* s. str. to this clade.

Specimens examined: Germany: Heilbronn, Landwirtschaftsamt, on *A. graveolens*, K. Schrameyer (CBS 116455 = CPC 11556 – ex-epitype culture). – **Iran:** Ardabil Province: Moghan, on leaves of *Cynanchum acutum* (Apocynaceae), Oct. 2011, M. Bakhshi (IRAN 17016F, IRAN 17017F, CCTU 1069, CCTU 1086 = IRAN 2655C = CBS 136037); Moghan, on leaves of *C. acutum*, Oct. 2012, M. Bakhshi (IRAN 17018F, IRAN 17019F, CCTU 1215, CCTU 1219 = CBS 136155). – **New Zealand:** Auckland, on *M. laevis*, C.F. Hill (CPC 5112). – **Romania:** Bucuresti, on *A. graveolens*, 2 Oct. 1969, O. Constantinescu (CBS 536.71 = CPC 5087). – **USA:** California: on *Moluccella laevis* (Lamiaceae), S.T. Koike (CBS 110813 = CPC 5110).

***Cercospora plantaginis* Sacc., *Michelia* 1: 267 (1878).**
(Fig. 3)

Type: Italy: Selva, on *Plantago lanceolata* (Plantaginaceae), Sep. 1873, P.A. Saccardo (PAD, s.n. – holotype, according to Art. 9.1, Note 1). – **Romania:** Domnesti, on *P. lanceolata*, 3 Aug. 1965, O. Constantinescu (CBS 252.67 – **epitype**

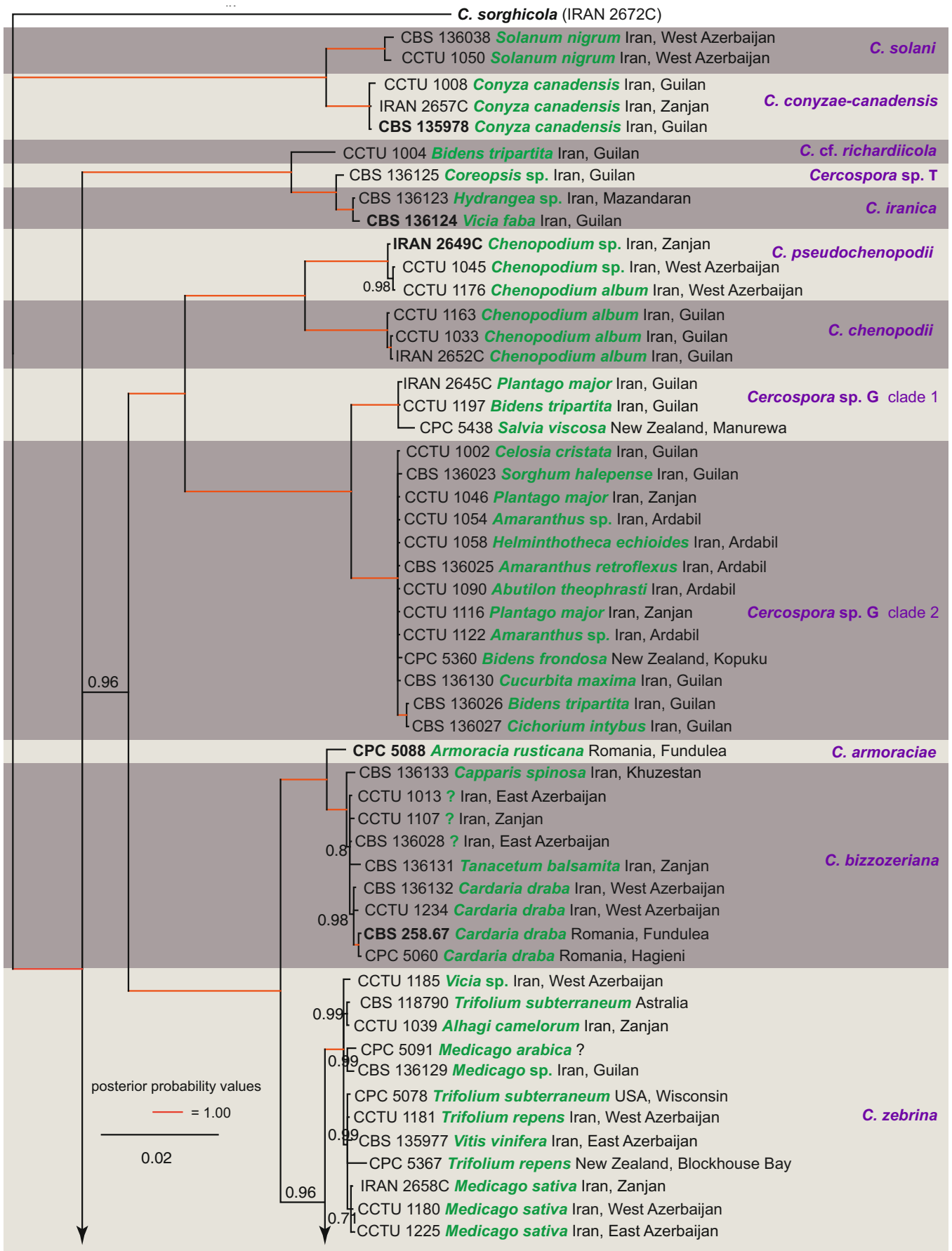


Fig. 1. Consensus phylogram (50 % majority rule) of 3 610 trees resulting from a Bayesian analysis of the combined eight-gene sequence alignment using MrBayes v. 3.2.2. The scale bar indicates 0.02 expected changes per site. Hosts and country of origin are indicated in green and black text, respectively. The tree was rooted to *Cercospora sorghicola* (isolate CBS 136448 = IRAN 2672C).

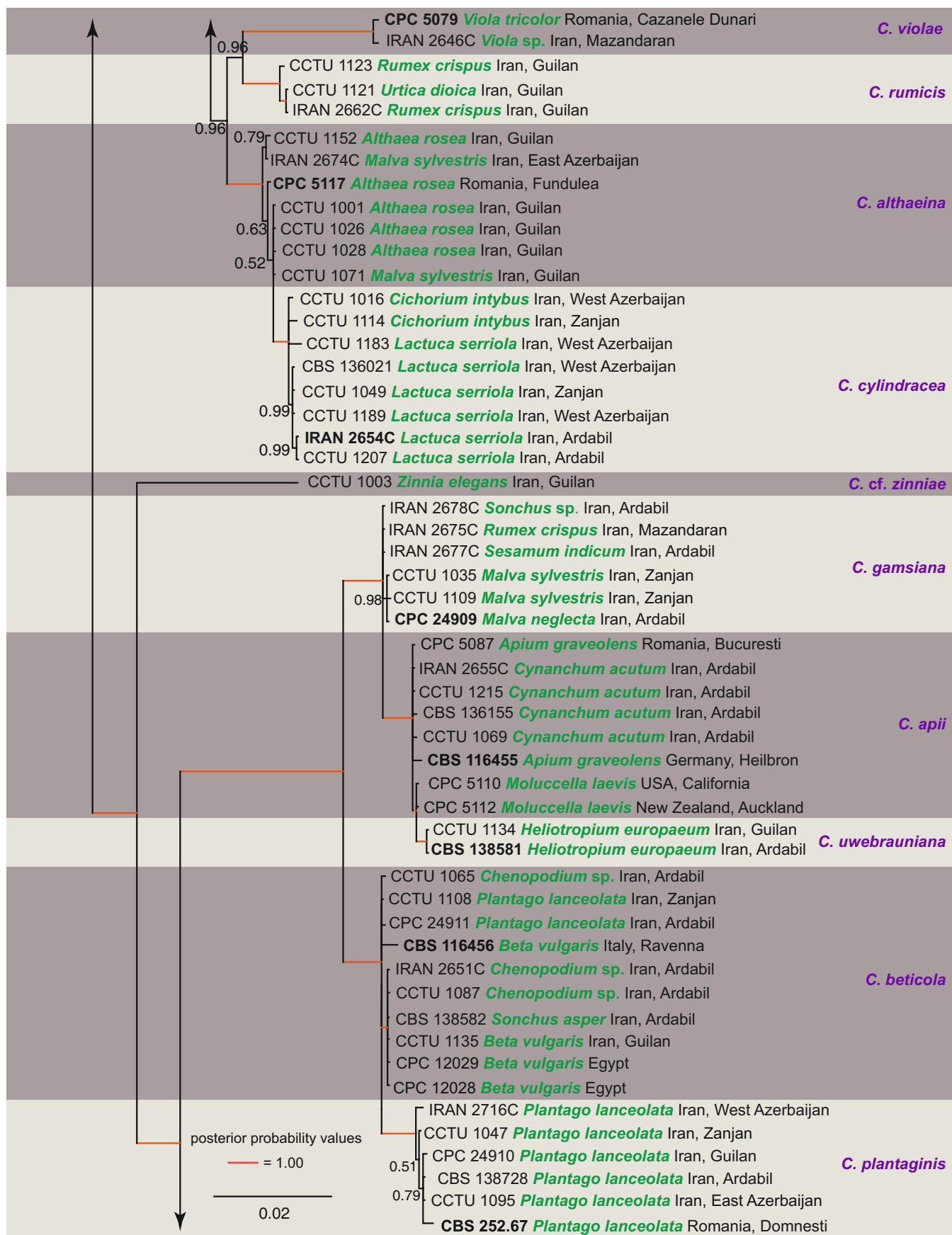


Fig. 1. (Continued).

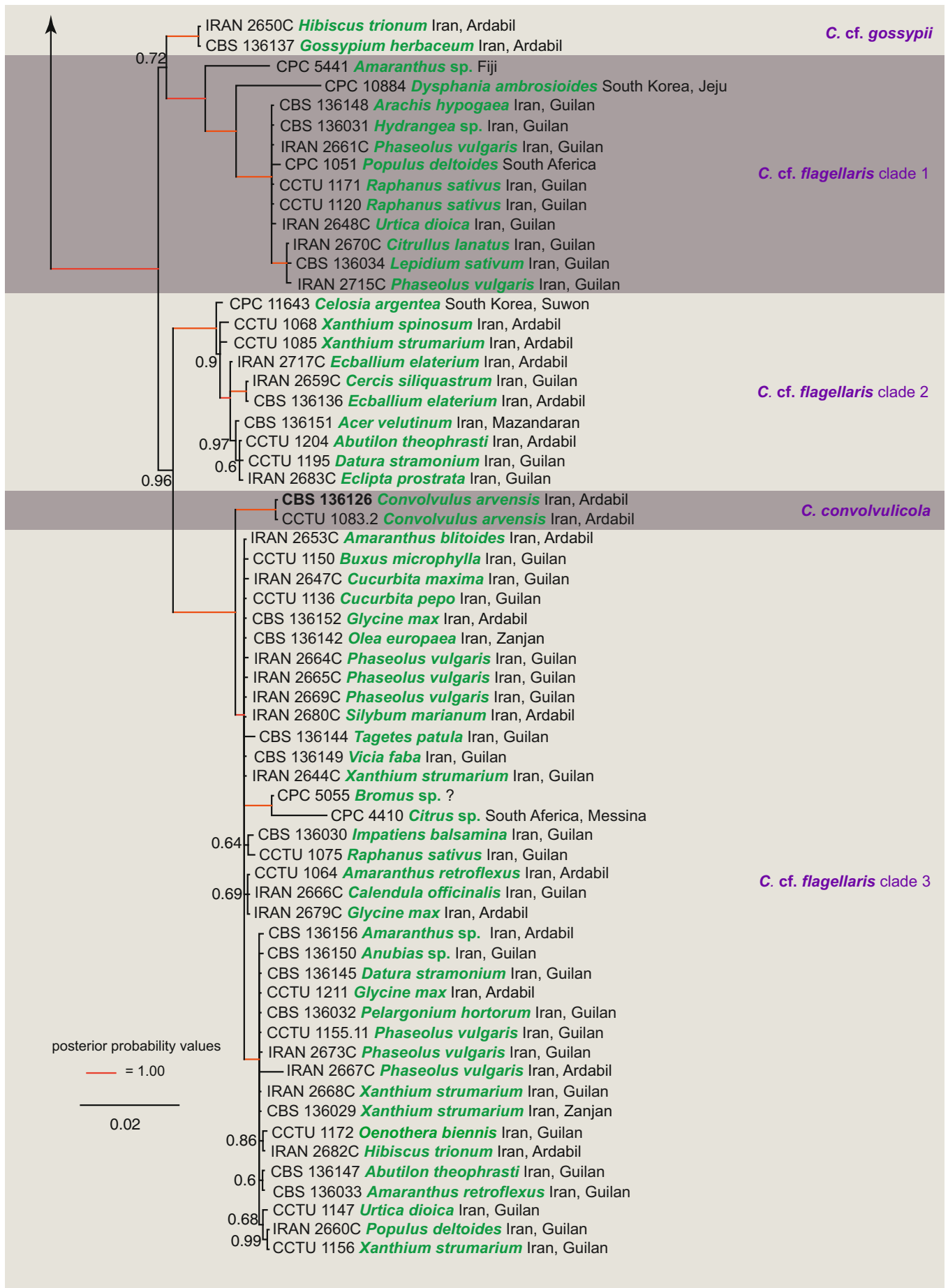


Fig. 1. (Continued).

Table 5. Results from allele group designation per locus for *Cercospora apii* s. lat. isolates in this study. Abbreviations of loci and collection accession numbers follow Table 1.

Species	Culture accession number	Host	Allele group per locus							
			ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>
<i>C. apii</i> s. str.	CCTU 1069	<i>Cynanchum acutum</i>	I	II	I	I	II	I	IV	I
	CCTU 1086; CBS 136037; IRAN 2655C	<i>Cynanchum acutum</i>	I	II	I	I	II	I	I	I
	CCTU 1215	<i>Cynanchum acutum</i>	I	II	I	I	II	I	I	I
	CCTU 1219; CBS 136155	<i>Cynanchum acutum</i>	I	II	I	I	II	I	II	I
	CBS 536.71; CPC 5087	<i>Apium graveolens</i>	I	II	I	I	II	I	I	I
	CBS 116455; CPC 11556 (TYPE)	<i>Apium graveolens</i>	I	I	I	I	I	I	–	I
	CBS 110813; CPC 5110	<i>Molucella laevis</i>	I	II	I	II	II	I	I	I
	CPC 5112	<i>Molucella laevis</i>	I	II	I	II	II	I	I	I
	<i>C. plantaginis</i>	CCTU 1041; CPC 24910	<i>Plantago lanceolata</i>	I	II	I	II	III	I	I
CCTU 1047		<i>Plantago lanceolata</i>	I	II	I	II	II	II	I	II
CCTU 1082; CBS 138728		<i>Plantago lanceolata</i>	I	II	I	II	III	II	I	II
CCTU 1095		<i>Plantago lanceolata</i>	I	II	I	II	III	II	I	II
CCTU 1179		<i>Plantago lanceolata</i>	I	II	II	II	II	I	III	II
CBS 252.67; CPC 5084 (TYPE)		<i>Plantago lanceolata</i>	I	III	II	II	III	II	–	II
<i>C. uwebrauniana</i>	CCTU 1134	<i>Heliotropium europaeum</i>	I	IV	I	II	IV	III	I	I
	CCTU 1200; CBS 138581 (TYPE)	<i>Heliotropium europaeum</i>	I	IV	I	II	IV	III	I	I

**Fig. 2.** *Cercospora apii* (CBS 136037). **A.** Leaf spots. **B–C.** Fasciculate conidiophores. **D–H.** Conidia. Bars = 10 μ m.



Fig. 3. *Cercospora plantaginis* (CPC 24910). A. Leaf spots. B–C. Fasciculate conidiophores. D–J. Conidia. Bars = 10 μ m.

designated here, MBT 383093, preserved as a metabolically inactive culture).

Description: Leaf spots amphigenous, circular to subcircular, 1–4 mm diam, white to grey with distinct raised brown borders. Mycelium internal. Caespituli amphigenous, brown. Conidiophores aggregated in loose fascicles, arising from a moderately developed, intraepidermal and substomatal, dark brown stroma, to 30 μ m diam; conidiophores brown at the base, becoming paler towards the apex, 2–10-septate, straight to geniculate-sinuuous due to sympodial proliferation, simple, uniform in width, somewhat constricted at the proliferating point, (45–)60–85 \times 4–5 μ m. Conidiogenous cells integrated, terminal or lateral, pale brown to brown, proliferating sympodially, 8–25 \times 3.5–5 μ m, multi-local; loci distinctly thickened, darkened and somewhat refractive, apical or formed on shoulders caused by sympodial proliferation, 2–3 μ m diam. Conidia solitary, subcylindrical, filiform to acicular, straight to mildly curved, hyaline, (40–)60–70(–105) \times 2–3.5 μ m, (4–)8–13(–17)-septate, with subobtuse to subacute apices and truncate bases; hila thickened, darkened, refractive, 1.5–2.5 μ m diam.

Notes: Based on the results of the eight-gene phylogenetic tree, all isolates obtained from *P. lanceolata* from five different provinces in Iran together with a European isolate from this host plant, previously recognised as *C. apii* based on a five-gene phylogenetic tree (Groenewald et al. 2013, Bakhshi et al. 2015a), cluster separately from the other isolates in this clade (Fig. 1, part 2). Three species of *Cercospora*, including *C. apii*, *C. pantoleuca* and *C. plantaginis*, have been reported from *Plantago* (Crous & Braun 2003, <https://nt.ars-grin.gov/fungal-databases/>). This species is morphologically close to *C. plantaginis* described from Italy on *P. lanceolata* (Chupp 1954). Since one European isolate from *P. lanceolata* in Romania (CBS 252.67 = CPC 5084) also resides in this clade, we designate an epitype here for this species, and fix the application of the name *C. plantaginis* to this clade.

Additional specimens examined: Iran: Guilan Province: Chaboksar, on *P. lanceolata*, Jul. 2012, M. Bakhshi (IRAN 17076F, CCTU 1041 = CPC 24910). Zanjan Province: Tarom, Pasar, on *P. lanceolata*, Sep. 2011, M. Bakhshi (IRAN 17078F, CCTU 1047). Ardabil Province: Moghan, on *P. lanceolata*, Sep. 2011, M. Bakhshi (CCTU 1082 = CBS 138728). East Azerbaijan Province: Arasbaran, Horand, on *P.*

lanceolata, Oct. 2011, *M. Bakhshi* (CCTU 1095). West Azerbaijan Province: Khoy, Firouragh, on *P. lanceolata*, Sep. 2012, *M. Arzanlou* (IRAN 17077F, CCTU 1179 = IRAN 2716C).

***Cercospora uwebrauniana* M. Bakhshi & Crous, sp. nov.**

MycoBank MB827521
(Fig. 4)

Etymology: Named in honour of Uwe Braun, who has published extensively on the genus *Cercospora*, and also provided a modern treatment for allied genera of *Mycosphaerellaceae*.

Diagnosis: Differs from *C. taurica* in the cylindrical conidia with truncate or subtruncate bases and somewhat shorter and wider conidia, (23–)38–48(–70) × 4.5–8 µm vs 40–110 × (2.5–)4–6(–7) µm in *C. taurica*.

Type: Iran: Ardabil Province: Moghan, on *Heliotropium europaeum* (*Boraginaceae*), Oct. 2012, *M. Bakhshi* (IRAN 16864F – holotype; CCTU 1200 = CBS 138581 – ex-type culture).

Description: Leaf spots distinct, circular to irregular, 3–10 mm, grey-brown to dark brown, surrounded by brown margin. Mycelium internal. Caespituli amphigenous, brown. Conidiophores in moderately dense fascicles, arising from the upper cells of a moderately developed, intraepidermal and substomatal, brown stroma, to 40 µm wide; conidiophores straight to slightly geniculate, pale brown to brown, unbranched, regular in width, (60–)115–145(–230) × 3.5–5.5 µm, 2–9-septate. Conidiogenous cells integrated, terminal, brown, proliferating sympodially, 15–35 × 3.5–5.5 µm, mostly mono-local, sometimes multi-local; loci distinctly thickened, darkened, refractive, apical or formed on the shoulders caused by geniculation, 2–3.5 µm. Conidia solitary, hyaline, subcylindrical to cylindrical, straight or slightly curved, truncate to subtruncate at the base, obtuse to rounded at the apex, (23–)38–48(–70) × 4.5–8 µm, (0–)3–4(–9)-septate; hila thickened, darkened, refractive, 1.5–3 µm diam.

Notes: Two isolates, obtained from *H. europaeum* in different provinces in Iran, clustered in a small clade within *C. apii* s. str. (Fig. 1, part 2). This independent clade is supported by *tef1*, *his3* and *tub2* from *C. apii* s. str. Morphologically, these two strains are completely distinct from their most closely related species in the phylogenetic tree, namely *C. apii* (conidia acicular, subacute or subobtusely rounded at the apex, (30–)65–80(–115) × 3–5 µm), *C. beticola* (conidia subacute to acute apex, (40–)90–140(–300) × 2–5 µm), *C. gamsiana* (conidia subobtusely at the apex, (27–)49–62(–100) × 2–4 µm) and *C. plantaginis* (conidia subobtusely to subacute apices, (40–)60–70(–105) × 2–3.5 µm), by the obtuse to rounded apex, wider and shorter conidia ((23–)38–48(–70) × 4.5–8 µm), and are regarded as a separate species, appearing to be confined to *H. europaeum*.

Presently, three species of *Cercospora* have been described from *Heliotropium*, *C. apii*, *C. heliotropiicola*, and *C. taurica* (Crous & Braun 2003, <https://nt.ars-grin.gov/fungaldatabases/>). *Cercospora uwebrauniana* differs from *C. taurica*

in the cylindrical conidia with truncate or subtruncate bases and somewhat shorter and wider conidia, (23–)38–48(–70) × 4.5–8 µm vs 40–110 × (2.5–)4–6(–7) µm in *C. taurica* (Braun 2002). In addition, *C. taurica* has obclavate-cylindrical conidia with obconically truncate bases and rather wider conidiophores, 4–9 µm diam (Braun 2002). *Cercospora heliotropiicola* is morphologically quite distinct from *C. uwebrauniana* in having acicular or subulate, much thinner (2–3 µm wide) and longer (to 300 µm long) conidia with subobtusely or acute apex (Pons & Sutton 1996).

Additional specimen examined: Iran: Guilan Province: Astara, on *H. europaeum*, Jun. 2012, *M. Bakhshi* (IRAN 17096F, CCTU 1134).

***Cercospora armoraciae* complex**

The 10 isolates previously recognised as *C. armoraciae* based on a five-gene phylogenetic tree (Groenewald *et al.* 2013, Bakhshi *et al.* 2015a) are assigned to two lineages here, based on the eight-gene phylogenetic tree, including *C. armoraciae* s. str. and *C. bizzozeriana* (Fig. 1, part 1). The results of allele group designation for the isolates in this complex revealed one, three, one, two, seven, three, three and two allele groups for the ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, *rpb2* and *gapdh* sequences, respectively (Table 6).

***Cercospora armoraciae* Sacc., *Nuovo Giorn. Bot. Ital.* 8: 188 (1876).**

Note: This clade includes the ex-type culture of *C. armoraciae* (CBS 250.67).

***Cercospora bizzozeriana* Sacc. & Berl., *Malpighia* 2: 248 (1888).
(Fig. 5)**

Type: Italy: Padova, on *Lepidium latifolium* (*Brassicaceae*), (Berlese, *Malpighia* 1: tab. XIV, fig. 23, 1887 – lectotype, designated here, MBT 383343); Romania: Fundulea, on *Cardaria draba*, isol. by O. Constantinescu [deposited in the CBS culture collection in 1967] (CBS 258.67 – **epitype designated here**, MBT 383154, preserved as a metabolically inactive culture).

Notes: Type material of *C. bizzozeriana* is not preserved in Saccardo's herbarium (see Gola 1930). Therefore, the original illustration published by Saccardo & Berlese (in Berlese 1888) is designated as lectotype (according to Art. 9.3 and 9.4). Berlese's article "Fungi veneti novi vel critici" was split into several parts published in *Malpighia* 1 (1887) and 2 (1888). The description of *C. bizzozeriana* was published in vol 2, but with reference to tab. XIV, fig. 23 already issued in vol. 1.

Description: Leaf spots amphigenous, circular, 1–5 mm, white to white-grey with grey to black dots (stroma with conidiophores) and definite brown border. Mycelium internal. Caespituli amphigenous, brown. Conidiophores aggregated in dense fascicles, arising from a well-developed, brown stroma, to 75 µm diam; conidiophores brown, 1–5-septate, straight to geniculate-sinuous due to sympodial proliferation, simple, sometimes branched, uniform in width, sometimes

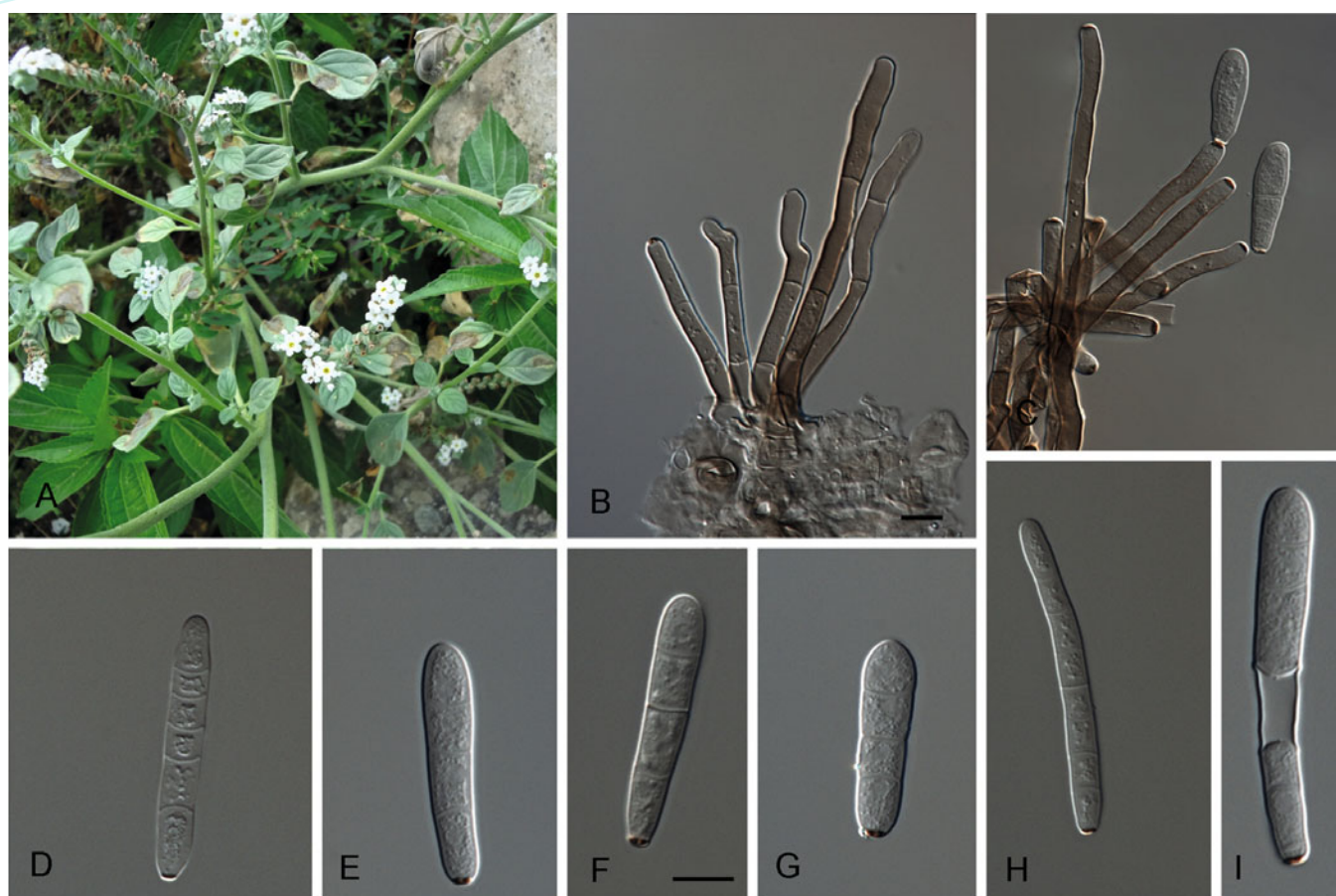


Fig. 4. *Cercospora uwebrauniana* (CBS 138581). A. Leaf spots. B–C. Fasciculate conidiophores. D–I. Conidia. Bars = 10 μ m.

constricted at the proliferating point, (30–)50–60(–80) \times 4–7 μ m. *Conidiogenous cells* integrated, terminal or lateral, pale brown to brown, proliferating sympodially, 10–25 \times 3–6 μ m, multi-local; loci distinctly thickened, darkened and somewhat refractive, apical, lateral or formed on shoulders caused by geniculation, 1.5–3 μ m diam. *Conidia* solitary, obclavate-cylindrical, straight to slightly curved, hyaline, (20–)60–80(–125) \times 3–6 μ m, 2–10-septate, with obtuse apices and subtruncate or obconically truncate bases; hila thickened, darkened, refractive, 1.5–3 μ m diam.

Notes: Isolates obtained from different host species including *Tanacetum balsamita*, *Capparis spinosa* and *Cardaria draba* clustered in a clade distinct from the ex-type isolate of *C. armoraciae*, and are regarded as a separate taxon. In addition, five isolates obtained from *Car. draba* (three from Iran and two from Romania) all cluster in this clade. Until now, three species of *Cercospora* are known from these host species, including *C. bizzozeriana*, *C. chrysanthemi* and *C. capparis* (Crous & Braun 2003, <https://nt.ars-grin.gov/fungal-databases/>). *Cercospora chrysanthemi* is in the *C. apii*

Table 6. Results from allele group designation per locus for *Cercospora armoraciae* s. lat. isolates in this study. Abbreviations of loci and collection accession numbers follow Table 1.

Species	Culture accession number	Host	Allele group per locus							
			ITS	tef1	actA	cmdA	his3	tub2	rpb2	gapdh
<i>C. armoraciae</i> s. str.	CBS 250.67; CPC 5088 (TYPE)	<i>Armoracia rusticana</i> (= <i>A. lapathifolia</i>)	I	I	I	I	I	I	–	II
<i>C. bizzozeriana</i>	CCTU 1013	?	I	II	I	I	III	I	I	I
	CCTU 1022; CBS 136028	?	I	II	I	I	III	I	I	I
	CCTU 1040; CBS 136131	<i>Tanacetum balsamita</i>	I	III	I	II	VI	I	II	I
	CCTU 1107	?	I	II	I	I	VII	I	I	I
	CCTU 1117; CBS 136132	<i>Cardaria draba</i>	I	II	I	I	V	I	I	I
	CCTU 1234	<i>Cardaria draba</i>	I	II	I	I	V	III	I	I
	CCTU 1127; CBS 136133	<i>Capparis spinosa</i>	I	II	I	I	IV	II	III	I
	CBS 540.71; CPC 5060	<i>Cardaria draba</i>	I	II	I	I	II	I	–	I
	CBS 258.67; CPC 5061 (TYPE)	<i>Cardaria draba</i>	I	II	I	I	II	I	–	I



Fig. 5. *Cercospora bizzozeriana* (CBS 136132). A–B. Leaf spots. C. Fasciculate conidiophores. D–J. Conidia. Bars = 10 μ m.

s. lat. complex (Crous & Braun 2003). *Cercospora capparis* differs from this species by the narrower (4–5.5 μ m diam) conidiophores and 3–5 μ m diam conidia (Chupp 1954). The species is morphologically close to *C. bizzozeriana* which was described from Italy on *Car. draba* (Chupp 1954). Since two European isolates from *Car. draba* in Romania also reside in this clade, we designate an epitype here (ex-epitype culture CBS 258.67 = CPC 5061) for this species, and fix the application of *C. bizzozeriana* to this clade.

Additional specimens examined: **Iran:** West Azerbaijan Province: Khoy, Firouragh, on leaves of *Car. draba*, Nov. 2011, M. Arzanlou (CCTU 1117 = CBS 136132); Khoy, Firouragh, on leaves of *Car. draba*, Oct. 2012, M. Arzanlou (IRAN 17027F, CCTU 1234). **Zanjan Province:** Tarom, Haroun Abad, on leaves of *Tanacetum balsamita* (Asteraceae), Sep. 2011, M. Bakhshi (IRAN 17029F, CCTU 1040 = CBS 136131); Tarom, Mamalan, Oct. 2011, M. Bakhshi (IRAN 17028F, CCTU 1107); Mianeh, Oct. 2012, M. Torbati (IRAN 17025F, IRAN 17026F, CCTU 1013, CCTU 1022 = CBS 136028). **Khuzestan Province:** Ahvaz, on leaves of *Capparis spinosa* (Capparidaceae), Dec. 2011, E. Mohammadian (CCTU 1127 = CBS 136133). – **Romania:** Hagieni, on *Car. draba*, O. Constantinescu (CBS 540.71 = IMI 161110 = CPC 5060).

Cercospora beticola complex

The 16 isolates previously recognised as *C. beticola* based on a five-gene phylogenetic analysis (Groenewald *et al.*

2013, Bakhshi *et al.* 2015a), are assigned to two lineages based on the eight-gene phylogenetic analysis (Fig. 1, part 2). One, one, one, one, one, two, three and four allele groups were distinguished for the ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, *rpb2* and *gapdh* sequences, respectively (Table 7).

***Cercospora beticola* Sacc., Nuovo Giorn. Bot. Ital. 8: 189 (1876).**

Sensu Groenewald *et al.*, *Phytopathology* 95: 954 (2005).

(Fig. 6)

Type: Italy: Vittorio (Treviso), on *Beta vulgaris* (Chenopodiaceae), Sep. 1897, P.A. Saccardo, Fungi ital. no. 197 (PAD – neotype designated by Groenewald *et al.* 2005); Ravenna, on *B. vulgaris*, 10 Jul. 2003, V. Rossi (CBS 116456 = CPC 11557 – epitype designated by Groenewald *et al.* 2005).

Description: Leaf spots amphigenous, distinct, circular to subcircular, 1–7 mm diam, white-grey, with grey dots (stroma with conidiophores), surrounded by distinct brown border. Mycelium internal. Caespituli amphigenous, brown. Conidiophores aggregated in loose to dense fascicles, emerging through stomatal openings or erumpent through the cuticle, arising from the upper cells of a moderately to well-developed brown stroma, to 110 μ m diam; conidiophores brown, becoming paler towards apex, 2–8-septate, thick-

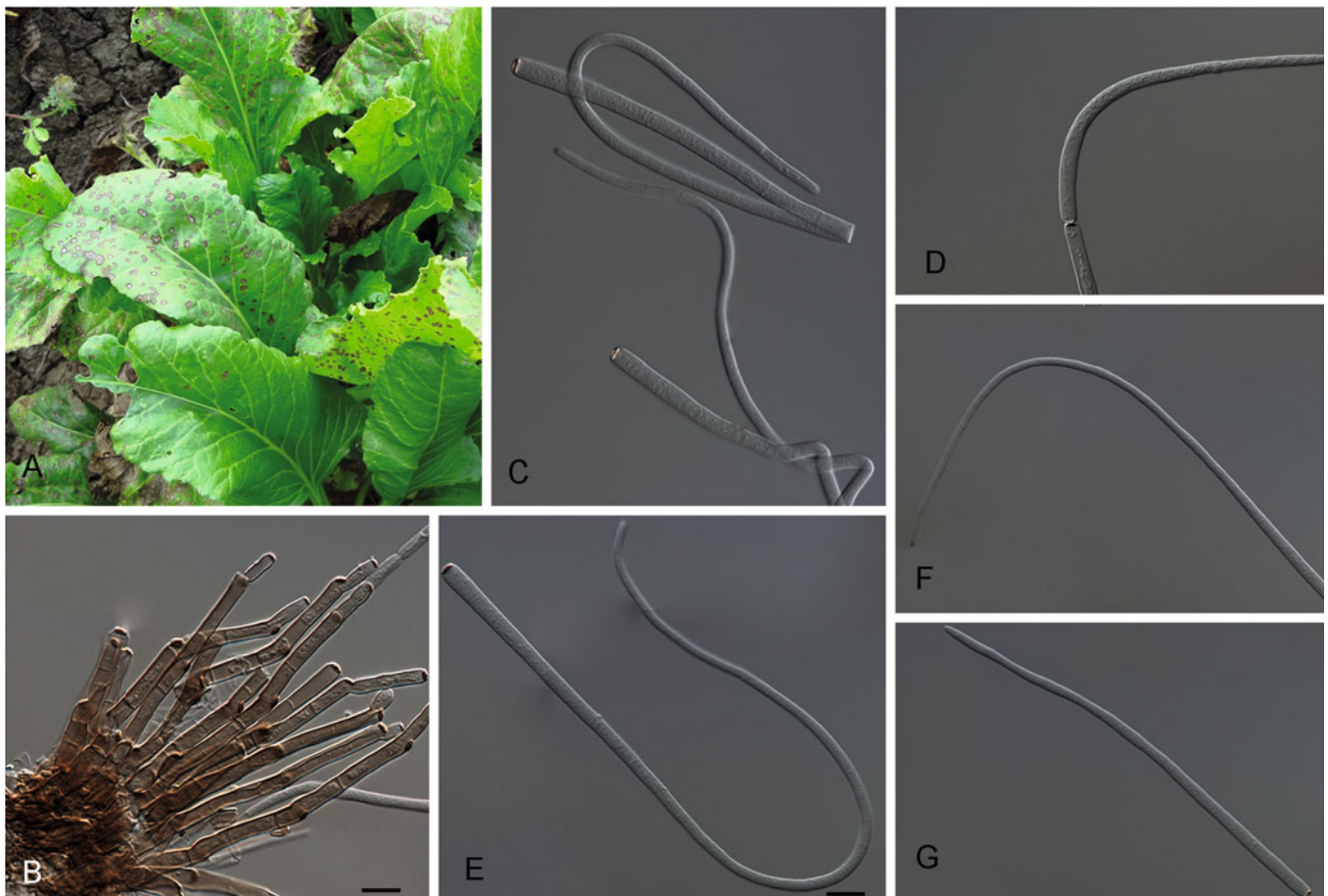


Fig. 6. *Cercospora beticola* (CCTU 1135). A. Leaf spots. B. Fasciculate conidiophores. C–G. Conidia. Bars = 10 μ m.

Table 7. Results from allele group designation per locus for *Cercospora beticola* s. lat. isolates in this study. Abbreviations of loci and collection accession numbers follow Table 1.

Species	Culture accession number	Host	Allele group per locus							
			ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>
<i>C. beticola</i>	CCTU 1057; IRAN 2651C	<i>Chenopodium</i> sp.	I	I	I	I	I	I	II	III
	CCTU 1065	<i>Chenopodium</i> sp.	I	I	I	I	I	I	II	II
	CCTU 1087	<i>Chenopodium</i> sp.	I	I	I	I	I	I	II	III
	CCTU 1088; CBS 138582	<i>Sonchus asper</i>	I	I	I	I	I	I	II	III
	CCTU 1089; CPC 24911	<i>Plantago lanceolata</i>	I	I	I	I	I	II	II	II
	CCTU 1108	<i>Plantago lanceolata</i>	I	I	I	I	I	I	II	II
	CBS 116456; CPC 11557 (TYPE)	<i>Beta vulgaris</i>	I	I	I	I	I	I	I	I
	CCTU 1135	<i>Beta vulgaris</i>	I	I	I	I	I	I	II	III
	CPC 12028	<i>Beta vulgaris</i>	I	I	I	I	I	I	II	III
	CPC 12029	<i>Beta vulgaris</i>	I	I	I	I	I	I	II	III
<i>C. gamsiana</i>	CCTU 1035	<i>Malva sylvestris</i>	I	I	I	I	I	I	III	IV
	CBS 144962; CCTU 1074; CPC 24909 (TYPE)	<i>Malva neglecta</i>	I	I	I	I	I	I	III	IV
	CCTU 1109	<i>Malva sylvestris</i>	I	I	I	I	I	I	III	IV
	CCTU 1199; CBS 136128; IRAN 2675C	<i>Rumex crispus</i>	I	I	I	I	I	I	II	IV
	CCTU 1205; CBS 136127; IRAN 2677C	<i>Sesamum indicum</i>	I	I	I	I	I	I	II	IV
	CCTU 1208; IRAN 2678C	<i>Sonchus</i> sp.	I	I	I	I	I	I	II	IV

walled, straight to geniculate-sinuous, unbranched, uniform in width, (30–)80–110(–185) \times 4–5(–6) μ m. *Conidiogenous cells* integrated, terminal or lateral, unbranched, brown,

smooth, proliferating sympodially, 10–30 \times 3.5–5.5 μ m, mostly multi-local, sometimes mono-local; loci apical or formed on shoulders caused by geniculation, thickened, darkened,

refractive, 1.5–2 µm diam. *Conidia* solitary, subcylindrical, filiform to acicular, straight to variously curved, hyaline, 3–15(–29)-septate, apex subacute to acute, base truncate to subtruncate, (40–)90–140(–300) × 2–5 µm; hila thickened, darkened, refractive, 1.5–2.5 µm diam.

Note: This clade includes the ex-epitype culture of *C. beticola* (CBS 116456 = CPC 11557), therefore we fixed the application of the name *C. beticola* s. str. to this clade.

Additional specimens examined: **Egypt**, on *B. vulgaris*, 15 Apr. 2004, *M. Hasem* (CPC 12028, CPC 12029). – **Iran:** *Guilan Province:* Talesh, Khotbeh Sara, on leaves of *B. vulgaris*, Jun. 2012, *M. Bakhshi* (IRAN 17020F, CCTU 1135). *Zanjan Province:* Tarom, Mamalan, on *P. lanceolata*, Oct. 2011, *M. Bakhshi* (IRAN 17023F, CCTU 1108). *Ardabil Province:* Moghan, on *P. lanceolata*, Oct. 2011, *M. Bakhshi* (CCTU 1089 = CPC 24911); Moghan, on *Chenopodium* sp. (*Chenopodiaceae*), Oct. 2011, *M. Bakhshi* (IRAN 17021F, IRAN 17022F, CCTU 1057 = IRAN 2651C, CCTU 1065, CCTU 1087); Moghan, on *Sonchus asper* (*Asteraceae*), Oct. 2011, *M. Bakhshi* (IRAN 17024F, CCTU 1088 = CBS 138582).

***Cercospora gamsiana* M. Bakhshi & Crous, sp. nov.**
MycoBank MB827522
(Fig. 7)

Etymology: Dedicated to the recently deceased Walter Gams to honour his contribution to mycology.

Diagnosis: Morphologically distinct from species of the *C. apii* complex in the irregularly constricted, often conical and attenuated at the apex conidiophores, and conidia with long obconically truncate bases; sporulation is restricted to the terminal part of conidiophores.

Type: **Iran:** *Ardabil Province:* Moghan, on leaves of *Malva neglecta* (*Malvaceae*), Oct. 2011, *M. Bakhshi* (IRAN 17011F – holotype; CBS 144962 = CCTU 1074 = CPC 24909– ex-type culture).

Description: *Leaf spots* amphigenous, circular to irregular, 3–8 mm diam, grey to brown. *Mycelium* internal. *Caespituli* amphigenous, brown. *Conidiophores* aggregated in moderately dense fascicles, arising from a well-developed, intraepidermal and substomatal, brown stroma, to 45 µm diam; conidiophores pale brown, 1–5-septate, geniculate-sinuuous, irregularly constricted, unbranched, moderately thin-walled, irregular in width, often conical and attenuated at the apex, sporulation is restricted at the terminal part of conidiophores, 45–60(–110) × 4–5 µm. *Conidiogenous cells* integrated, terminal, pale brown to olivaceous-brown, proliferating sympodially, 10–25 × 3.5–5 µm, uni- or multi-local; loci distinctly thickened, darkened and somewhat refractive, apical, circumspered, 1.5–2 µm diam. *Conidia* solitary, subcylindrical to obclavate or somewhat narrowed towards the tip, straight to slightly curved, hyaline, thin-walled, (27–)49–62(–100) × 2–4 µm, distinctly 3–10-septate, subobtuse at the apex and long obconically truncate at the base; hila distinctly thickened, darkened, refractive, 1.5–2.5 µm diam.

Notes: Until now, 14 species names in *Cercospora* have been introduced from these host species, including *C. apii*, *C. althaeina*, *C. beticola*, *C. hyalospora* (*C. apii* s. lat. complex), *C. malvarum* (*C. apii* s. lat. complex), *C. malvicola*, *C. sigesbeckiae*, *C. peckiana* (*C. apii* s. lat. complex), *C. rumicis*, *C. sonchi* (*C. apii* s. lat. complex), *C. sonchicola* (*C. apii* s. lat. complex), *C. sonchifolia*, *C. sesami* (*C. apii* s. lat. complex), and *C. sesamigena* (Crous & Braun 2003, <https://nt.ars-grin.gov/fungaldatabases/>). *Cercospora gamsiana* is phylogenetically clearly distinguishable from *C. apii*, *C. althaeina*, *C. beticola*, *C. sigesbeckiae* and *C. rumicis* (Bakhshi *et al.* 2015a) (Fig. 1, part 2). It is morphologically well distinguished from species of the *C. apii* complex and other species of *Cercospora* by its irregularly constricted, thin-walled, often conical and attenuated at the apex conidiophores and, conidia with long obconically truncate bases; sporulation is restricted at the terminal part of conidiophores.

Additional specimens examined: **Iran:** *Zanjan Province:* Tarom, Zehar Abad, on leaves of *Malva sylvestris*, Sep. 2011, *M. Bakhshi* (CCTU 1035); Tarom, Mamalan, on leaves of *M. sylvestris*, Oct. 2011, *M. Bakhshi* (CCTU 1109). *Ardabil Province:* Moghan, on leaves of *Sonchus* sp., Oct. 2012, *M. Bakhshi* (IRAN 17072F, CCTU 1208 = IRAN 2678C); Moghan, on leaves of *Sesamum indicum* (*Pedaliaceae*), Oct. 2012, *M. Bakhshi* (CCTU 1205 = IRAN 2677C = CBS 136127). *Guilan Province:* Ramsar, on leaves of *Rumex crispus* (*Polygonaceae*), Sep. 2012, *M. Bakhshi* (CCTU 1199 = IRAN 2675C = CBS 136128).

***Cercospora* cf. *flagellaris* complex**

The 61 isolates previously recognised as *C. cf. flagellaris* based on a five-gene phylogenetic tree (Groenewald *et al.* 2013, Bakhshi *et al.* 2015a) cluster into at least four distinct phylogenetic clades based on the eight-gene phylogenetic tree including *C. cf. gossypii*, *C. cf. flagellaris* clades 1, 2 and 3 (Fig. 1, part 3). Three, four, six, seven, seven, seven, two and nine allele groups were distinguished for the ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, *rpb2* and *gapdh* sequences, respectively (Table 8).

***Cercospora* cf. *gossypii* Lall *et al.*, *Indian Phytopath.*
14: 116 (1962) ["1961"].**
(Fig. 8)

Description: *Leaf spots* amphigenous, circular to subcircular, 1–4 mm diam, with grey-brown centre and purple-brown margins. *Mycelium* internal. *Caespituli* amphigenous, brown. *Conidiophores* aggregated in dense fascicles, arising from the upper cells of a well-developed, intraepidermal and substomatal, brown stroma, to 65 µm diam; conidiophores pale brown to brown, simple, rarely branched, 1–4-septate, straight or flexuous caused by sympodial proliferation, almost uniform in width, often constricted at proliferating point, (35–)60–75(–110) × 4–5 µm. *Conidiogenous cells* terminal or integrated, pale brown, smooth, proliferating sympodially, 10–45 × 3.5–5 µm, multi-local; loci thickened, darkened, refractive, apical, lateral, circumspered, 1.5–2.5 µm diam. *Conidia* solitary, smooth, subcylindrical to obclavate, straight or mildly curved, successively tapering towards the apex, hyaline, 1–7-septate, apex subacute to subobtuse, base truncate to short obconically



Fig. 7. *Cercospora gamsiana* (CPC 24909 = CBS 144962). **A.** Leaf spots. **B–C.** Fasciculate conidiophores. **D–H.** Conidia. Bars = 10 μ m.

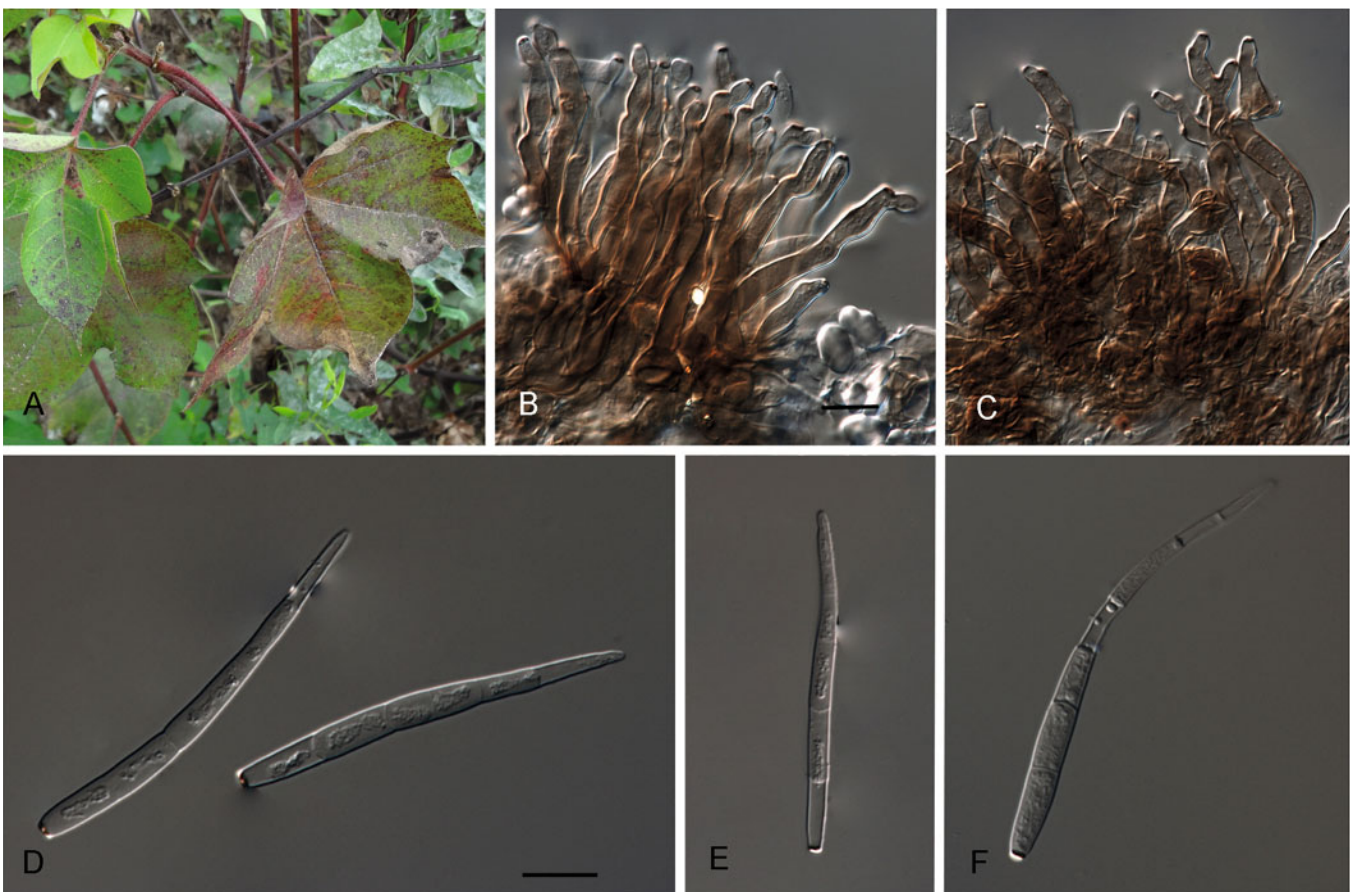


Fig. 8. *Cercospora* cf. *gossypii* (CBS 136137). **A.** Leaf spots. **B–C.** Fasciculate conidiophores. **D–F.** Conidia. Bars = 10 μ m.

Table 8. Results from allele group designation per locus for *Cercospora* cf. *flagellaris* isolates in this study. Abbreviations of loci and collection accession numbers follow Table 1.

Species	Culture accession number	Host	Allele group per locus							
			ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>
<i>Cercospora</i> cf. <i>gossypii</i>	CCTU 1055; IRAN 2650C	<i>Hibiscus trionum</i>	I	I	I	III	III	I	II	IV
	CCTU 1070; CBS 136137	<i>Gossypium herbaceum</i>	I	I	I	III	III	I	II	IV
<i>Cercospora</i> cf. <i>flagellaris</i> clade 1	CCTU 1007; CBS 136031	<i>Hydrangea</i> sp.	II	I	I	I	I	I	II	V
	CCTU 1027; CBS 136034	<i>Lepidium sativum</i>	I	I	I	I	I	I	I	V
	CCTU 1031; CBS 136036; IRAN 2648C	<i>Urtica dioica</i>	II	I	II	I	I	I	II	V
	CCTU 1120	<i>Raphanus sativus</i>	II	I	I	I	I	I	II	V
	CCTU 1128; CBS 136141; IRAN 2661C	<i>Phaseolus vulgaris</i>	II	I	I	I	I	I	II	V
	CCTU 1159; CBS 136148	<i>Arachis hypogaea</i>	II	I	I	I	I	I	II	V
	CCTU 1162; IRAN 2670C	<i>Citrullus lanatus</i>	II	I	I	I	I	I	I	V
	CCTU 1168	<i>Phaseolus vulgaris</i>	II	I	I	II	I	I	I	V
	CCTU 1171	<i>Raphanus sativus</i>	II	I	I	I	I	I	II	V
	CPC 1051	<i>Populus deltoides</i>	II	I	III	II	I	I	II	V
	CBS 132653; CPC 10884	<i>Dysphania ambrosioides</i>	II	I	I	III	VII	VI	II	VII
	CPC 5441	<i>Amaranthus</i> sp.	I	III	V	III	IV	III	I	VI
	<i>Cercospora</i> cf. <i>flagellaris</i> clade 2	CCTU 1059; CBS 136136	<i>Ecballium elaterium</i>	II	I	I	I	I	I	I
CCTU 1068		<i>Xanthium spinosum</i>	II	IV	III	III	III	I	II	VIII
CCTU 1085		<i>Xanthium strumarium</i>	II	I	I	III	V	V	II	VIII
CCTU 1115; CBS 136139; IRAN 2659C		<i>Cercis siliquastrum</i>	II	I	I	I	I	I	I	VIII
CCTU 1195		<i>Datura stramonium</i>	I	I	I	I	I	I	II	IX
CCTU 1198; CBS 136151		<i>Acer velutinum</i>	II	I	I	II	I	I	II	IX
CCTU 1204		<i>Abutilon theophrasti</i>	I	I	I	II	I	I	II	IX
CCTU 1216		<i>Ecballium elaterium</i>	II	I	I	I	I	I	II	VIII
CCTU 1223; CBS 136154; IRAN 2683C		<i>Eclipta prostrata</i>	I	I	I	II	I	I	II	VIII
CBS 132667; CPC 11643		<i>Celosia argentea</i> var. <i>cristata</i> (≡ <i>C. cristata</i>)	III	I	VI	III	III	I	II	VIII
<i>Cercospora</i> cf. <i>flagellaris</i> clade 3	CCTU 1005; IRAN 2644C	<i>Xanthium strumarium</i>	II	I	II	I	I	I	II	I
	CCTU 1006; CBS 136030	<i>Impatiens balsamina</i>	II	I	I	I	I	II	II	I
	CCTU 1010; CBS 136032	<i>Pelargonium hortorum</i>	II	I	I	I	I	I	I	I
	CCTU 1021; CBS 136033	<i>Amaranthus retroflexus</i>	II	I	I	I	I	I	I	II
	CCTU 1029; CBS 136035; IRAN 2647C	<i>Cucurbita maxima</i>	I	I	I	VI	I	I	II	II
	CCTU 1048; CBS 136029	<i>Xanthium strumarium</i>	II	I	I	I	I	I	I	I
	CCTU 1064	<i>Amaranthus retroflexus</i>	II	I	II	I	I	I	II	II
	CCTU 1072; IRAN 2653C	<i>Amaranthus blitoides</i>	II	I	I	I	I	I	II	I
	CCTU 1075	<i>Raphanus sativus</i>	I	I	I	VII	I	II	II	I
	CCTU 1084; CBS 136156	<i>Amaranthus</i> sp.	II	I	I	I	I	II	I	I
	CCTU 1118; CBS 136140; IRAN 2660C	<i>Populus deltoides</i>	II	I	II	II	I	I	I	II
	CCTU 1130; CBS 136142	<i>Olea europaea</i>	II	I	I	I	I	I	II	I
	CCTU 1136	<i>Cucurbita pepo</i>	II	I	I	I	I	I	II	I
	CCTU 1138; IRAN 2664C	<i>Phaseolus vulgaris</i>	I	I	I	II	I	I	II	II
	CCTU 1139; IRAN 2665C	<i>Phaseolus vulgaris</i>	I	I	II	I	I	I	II	I
	CCTU 1140; CBS 136143; IRAN 2666C	<i>Calendula officinalis</i>	II	I	II	I	I	I	II	II
	CCTU 1141; CBS 136144	<i>Tagetes patula</i>	I	I	II	I	I	VII	II	II
	CCTU 1142; IRAN 2667C	<i>Phaseolus vulgaris</i>	II	II	III	IV	II	I	I	I

Table 8. (Continued).

Species	Culture accession number	Host	Allele group per locus							
			ITS	tef1	actA	cmdA	his3	tub2	rpb2	gapdh
	CCTU 1143; CBS 136145	<i>Datura stramonium</i>	II	I	I	I	I	I	I	I
	CCTU 1147	<i>Urtica dioica</i>	I	I	I	II	I	I	I	II
	CCTU 1150	<i>Buxus microphylla</i>	II	I	I	II	I	I	II	II
	CCTU 1154; CBS 136147	<i>Abutilon theophrasti</i>	II	I	I	I	I	I	I	II
	CCTU 1155.11	<i>Phaseolus vulgaris</i>	II	I	I	I	I	I	I	I
	CCTU 1156	<i>Xanthium strumarium</i>	II	I	II	II	I	I	I	II
	CCTU 1158; IRAN 2668C	<i>Xanthium strumarium</i>	II	I	I	I	I	I	I	I
	CCTU 1160; CBS 136149	<i>Vicia faba</i>	II	I	I	I	I	I	II	I
	CCTU 1161; IRAN 2669C	<i>Phaseolus vulgaris</i>	II	I	I	I	I	I	II	I
	CCTU 1167; CBS 136150	<i>Anubias</i> sp.	II	I	II	I	I	I	I	I
	CCTU 1172	<i>Oenothera biennis</i>	I	I	I	V	I	I	I	I
	CCTU 1175; IRAN 2673C	<i>Phaseolus vulgaris</i>	II	I	I	II	I	I	I	I
	CCTU 1209; CBS 136152	<i>Glycine max</i>	II	I	II	I	I	I	II	I
	CCTU 1210; IRAN 2679C	<i>Glycine max</i>	II	I	II	I	I	I	II	II
	CCTU 1211	<i>Glycine max</i>	II	I	I	I	I	I	I	I
	CCTU 1212; CBS 136153; IRAN 2680C	<i>Silybum marianum</i>	II	I	I	I	I	I	II	II
	CCTU 1218; IRAN 2682C	<i>Hibiscus trionum</i>	I	I	I	I	I	I	I	I
	CBS 115482; CPC 4410	<i>Citrus</i> sp.	II	I	IV	III	VI	IV	I	III
	CBS 143.51; CPC 5055	<i>Bromus</i> sp.	II	I	I	I	I	IV	II	I

truncate, (30–)65–90(–160) × 2–4 µm; hila distinctly thickened, darkened, refractive, 1–2 µm diam.

Notes: This clade includes two isolates obtained from *G. herbaceum* and *Hib. trionum*, both in the *Malvaceae* (Fig. 1, part 3). *Cercospora althaeina*, *C. fagopyri*, *C. malayensis* (*C. apii* s. lat.), *C. gossypii*, *C. gossypicola*, *C. gossypina* and *C. Ihuillieri* (*C. apii* s. lat.) are six *Cercospora* species which have been reported until now on *Gossypium* and *Hibiscus* host genera (Crous & Braun 2003, <https://nt.ars-grin.gov/fungal-databases/>). This species is phylogenetically distinct from *C. althaeina* (Fig. 1) and *C. fagopyri* (Groenewald et al. 2013, Bakhshi et al. 2015a). *Cercospora gossypina* is distinguished from this species in that it induces wider leaf spots (0.5–10 mm), and has unbranched, longer and wider conidiophores (75–250 × 4–6.5 µm) (Hsieh & Goh 1990). *Cercospora malayensis* is distinguished from *C. cf. gossypii* in that it has elliptical, yellow to tan leaf spots; unbranched, 1–8-septate conidiophores and mostly terminal conidiogenous cells and somewhat longer conidia (50–270 × 2.5–4 µm) (Little 1987). *Cercospora gossypicola* (Narayan et al. 2001) and *C. Ihuillieri* (Montegut 1967) resemble *C. apii* (with acicular conidia), but are different. They do not have stromata, and form less conidiophores per fascicle. The description of *C. gossypii* (Lall et al. 1961) is rather close to this taxon. The type of *C. gossypii* is from India. Thus, fresh material is needed from India to resolve the application of the name *C. gossypii*.

Specimens examined: Iran: Ardabil Province: Moghan, on *Gossypium herbaceum* (*Malvaceae*), Oct. 2011, M. Bakhshi (IRAN 17073F, CCTU 1070 = CBS 136137); Moghan, on *Hibiscus trionum*

(*Malvaceae*), Oct. 2011, M. Bakhshi (IRAN 17074F, CCTU 1055 = IRAN 2650C).

***Cercospora* cf. *flagellaris* Ellis & G. Martin, *Am. Nat.* 16: 1003 (1882).**

Clade 1; Clade 2; Clade 3

In view of the overlap between the morphological characters of these three clades, we provide a single over-arching description here.

Description: Mycelium internal. *Caespituli* amphigenous, brown. *Conidiophores* aggregated in loose to dense fascicles, arising from a weakly to well-developed, intraepidermal and substomatal, brown stroma; conidiophores pale brown to brown, 2–18-septate, straight, sinuous to distinctly geniculate, flexuous, simple, unbranched or rarely branched, uniform or irregular in width, sometimes constricted at septa and proliferating point, (75–)130–165(–300) × 4–5.5 µm in clade 1; (30–)80–120(–210) × 3.5–5.5 µm in clade 2; (25–)60–95(–230) × 3.5–5.5 µm in clade 3. *Conidiogenous cells* integrated, terminal, proliferating sympodially, mono- or multi-local; loci thickened, darkened, apical, lateral or circumsperised, 1.5–2.5 µm diam. *Conidia* solitary, hyaline, subcylindrical, filiform to obclavate, straight to slightly curved, with truncate to obconically truncate base and subacute to subobtuse apices, (60–)125–170(–300) × 3–5 µm, 5–20-septate in clade 1; (25–)60–95(–260) × 2.5–4.5 µm, (2–)8–11(–25)-septate in clade 2; (30–)100–155(–320) × 2–5 µm, (2–)10–14(–28)-septate in clade 3; hila distinctly thickened, darkened, refractive, 1–2 µm diam.

Notes: Screening the remaining isolates of *C. cf. flagellaris*, with three more genomic loci in this study (*tub2*, *rpb2* and *gapdh*), clusters them into at least three distinct clades in the eight-gene phylogenetic tree (Fig. 1, part 3); clade 1 is sister to *C. cf. gossypii*; clade 3 is sister to *C. convolvulicola* and clade 2 is sister to the clade including *C. cf. flagellaris* clade 3 and *C. convolvulicola*. However, there is a high level of variation in morphological characteristics between different isolates of these three clades. In addition, several isolates originating from diverse hosts and families reside in these three clades and there is also overlap between host ranges among them. Different names can therefore be applied to these clades, and therefore we prefer to simply regard them as distinct phylogenetic species for now. To resolve their taxonomy, fresh collections authentic for the names (based on host and country) need to be recollected and included in future studies.

***Cercospora cf. flagellaris* Clade 1**

Specimens examined: **Fiji:** on *Amaranthus* sp. (*Amaranthaceae*), C.F. Hill (CPC 5441). – **Iran:** *Guilan Province:* Talesh, Khotbeh Sara, on leaves of *Phaseolus vulgaris* (*Fabaceae*), Oct. 2012, *M. Bakhshi* (CCTU 1128 = IRAN 2661C = CBS 136141); Talesh, Jamakuh, on leaves of *Raphanus sativus* (*Brassicaceae*), Nov. 2011, *M. Bakhshi* (IRAN 17042F, CCTU 1120); Talesh, Dulbin, on *Hydrangea* sp. (*Hydrangeaceae*), Jul. 2011, *M. Bakhshi* (IRAN 17039F, CCTU 1007 = CBS 136031). *Guilan Province:* Kiashahr, on leaves of *Ph. vulgaris*, Aug. 2012, *M. Bakhshi* (CCTU 1168 = IRAN 2715C); Kiashahr, on leaves of *R. sativus*, Aug. 2012, *M. Bakhshi* (IRAN 17041F, CCTU 1171); Kiashahr, on leaves of *Arachis hypogea* (*Fabaceae*), Aug. 2012, *M. Bakhshi* (CCTU 1159 = CBS 136148); Sowme'eh Sara, Dowgur, on leaves of *Urtica dioica* (*Urticaceae*), Jun. 2012, *M. Bakhshi* (IRAN 17043F, CCTU 1031 = IRAN 2648C = CBS 136036); Chamkhaleh, on leaves of *Lepidium sativum* (*Brassicaceae*), Jun. 2012, *M. Bakhshi* (IRAN 17040F, CCTU 1027 = CBS 136034); Lahijan, Rudboneh, on leaves of *Citrullus lanatus* (*Cucurbitaceae*), Aug. 2012, *M. Bakhshi* (IRAN 17038F, CCTU 1162 = IRAN 2670C). – **South Africa:** *Limpopo Province:* Messina, 30 Apr. 1995, on *Populus deltoides* (*Salicaceae*), P.W. Crous (CPC 1051). – **South Korea:** Jeju, on *Dysphania ambrosioides* (syn. *Chenopodium ambrosioides*) (*Chenopodiaceae*), 12 Nov. 2003, H.D. Shin (CBS 132653 = CPC 10884) (as *C. chenopodii-ambrosioidis*).

***Cercospora cf. flagellaris* Clade 2**

Specimens examined: **Iran:** *Ardabil Province:* Moghan, on leaves of *Xanthium spinosum* (*Astraceae*), Oct. 2011, *M. Bakhshi* (IRAN 17049F, CCTU 1068); Moghan, on leaves of *Xanthium strumarium* (*Asteraceae*), Oct. 2011, *M. Bakhshi* (IRAN 17050F, CCTU 1085); Moghan, on leaves of *Ecballium elaterium* (*Cucurbitaceae*), Oct. 2011, *M. Bakhshi* (IRAN 17047F, CCTU 1059 = CBS 136136); Moghan, on leaves of *E. elaterium*, Oct. 2012, *M. Bakhshi* (IRAN 17048F, CCTU 1216 = IRAN 2717C); Moghan, on leaves of *Abutilon theophrasti* (*Malvaceae*), Oct. 2012, *M. Bakhshi* (IRAN 17044F, CCTU 1204). *Guilan Province:* Astara, on leaves of *Cercis siliquastrum* (*Caesalpinaceae*), Oct. 2012, *M. Bakhshi* (CCTU 1115 = IRAN 2659C = CBS 136139); Talesh, Khotbeh Sara, on leaves of *Eclipta prostrata* (*Astraceae*), Oct. 2012, *M. Bakhshi* (CCTU 1223 = IRAN 2683C = CBS 136154); Talesh, on leaves of *Datura*

stramonium (*Solanaceae*), Oct. 2012, *M. Bakhshi* (IRAN 17046F, CCTU 1195). *Mazandaran Province:* Ramsar, on leaves of *Acer velutinum* (*Aceraceae*), Sep. 2012, *M. Bakhshi* (IRAN 17045F, CCTU 1198 = CBS 136151). – **South Korea:** Hoengseong, on *Celosia argentea* var. *crispata* (syn. *C. crispata*) (*Amaranthaceae*), 11 Oct. 2004, H.D. Shin (CBS 132667 = CPC 11643).

***Cercospora cf. flagellaris* Clade 3**

Specimens examined: **Iran:** *Guilan Province:* Rudsar, on leaves of *Cucurbita maxima* (*Cucurbitaceae*), Oct. 2012, *M. Bakhshi* (CCTU 1029 = IRAN 2647C = CBS 136035); Rudsar, Korjehposht, on leaves of *Tagetes patula* (*Asteraceae*), Aug. 2012, *M. Bakhshi* (IRAN 17065F, CCTU 1141 = CBS 136144); Talesh, Khotbeh Sara, on leaves of *Cucurbita pepo* (*Cucurbitaceae*), Jun. 2012, *M. Bakhshi* (CCTU 1136); Khotbeh Sara, on leaves of *Vicia faba* (*Fabaceae*), Oct. 2012, *M. Bakhshi* (IRAN 17067F, CCTU 1160 = CBS 136149); Khotbeh Sara, on leaves of *Calendula officinalis* (*Asteraceae*), Jun. 2012, *M. Bakhshi* (IRAN 17058F, CCTU 1140 = IRAN 2666C = CBS 136143); Talesh, Khalif Abad, on *Ph. vulgaris*, Jul. 2012, *M. Bakhshi* (CCTU 1142 = IRAN 2667C); Talesh, Dulbin, on leaves of *X. strumarium*, Jul. 2011, *M. Bakhshi* (IRAN 17069F, CCTU 1005 = IRAN 2644C); Dulbin, on leaves of *Impatiens balsamina* (*Balsaminaceae*), Jul. 2011, *M. Bakhshi* (IRAN 17062F, CCTU 1006 = CBS 136030); Dulbin, on leaves of *Pelargonium hortorum* (*Geraniaceae*), Aug. 2011, *M. Bakhshi* (CCTU 1010 = CBS 136032); Talesh, Jowkandan, on leaves of *Po. deltoides*, Oct. 2012, *M. Bakhshi* (CCTU 1118 = IRAN 2660C = CBS 136140); Talesh, Jowkandan, on leaves of *Oenothera biennis* (*Onagraceae*), Oct. 2012, *M. Bakhshi* (IRAN 17051F, CCTU 1172); Talesh, on leaves of *D. stramonium*, Oct. 2012, *M. Bakhshi* (IRAN 17059F, CCTU 1143 = CBS 136145); *Guilan Province:* Astara, Chubar, on leaves of *Ph. vulgaris*, Jun. 2012, *M. Bakhshi* (CCTU 1138 = IRAN 2664C, CCTU 1139 = IRAN 2665C); Rasht, Khomam, on leaves of *X. strumarium*, Aug. 2012, *M. Bakhshi* (IRAN 17068F, CCTU 1156); Khomam, on leaves of *Ab. theophrasti*, Aug. 2012, *M. Bakhshi* (IRAN 17052F, CCTU 1154 = CBS 136147); Langarud, Otaqvar, on leaves of *X. strumarium*, Aug. 2012, *M. Bakhshi* (CCTU 1158 = IRAN 2668C); Lahijan, Rudboneh, on leaves of *Ph. vulgaris*, Aug. 2012, *M. Bakhshi* (CCTU 1161 = IRAN 2669C); *Guilan Province:* Fuman, on leaves of *Ph. vulgaris*, Aug. 2012, *M. Bakhshi* (CCTU 1155.11); Fuman, on leaves of *Buxus microphylla* (*Buxaceae*), Jul. 2012, *M. Bakhshi* (IRAN 17057F, CCTU 1150); Fuman, on leaves of *Amaranthus retroflexus*, Sep. 2011, *M. Bakhshi* (IRAN 17054F, CCTU 1021 = CBS 136033); Sowme'eh Sara, Dowgur, on leaves of *Ph. vulgaris*, Aug. 2012, *M. Bakhshi* (CCTU 1175 = IRAN 2673C); Sowme'eh Sara, Bahambar, on leaves of *R. sativus*, Aug. 2012, *M. Bakhshi* (IRAN 17063F, CCTU 1075); Kiashahr, on leaves of *Anubias* sp. (*Araceae*), Oct. 2012, *M. Bakhshi* (IRAN 17056F, CCTU 1167 = CBS 136150); Masal, on leaves of *U. dioica*, Aug. 2012, *M. Bakhshi* (IRAN 17066F, CCTU 1147). *Zanjan Province:* Tarom, Pasar, on leaves of *X. strumarium*, Sep. 2011, *M. Bakhshi* (IRAN 17070F, CCTU 1048 = CBS 136029); Tarom, on leaves of *Olea europaea* (*Oleaceae*), Nov. 2011, *M. Torbati* (CCTU 1130 = CBS 136142). *Ardabil Province:* Moghan, on leaves of *Silybum marianum* (*Astraceae*), Oct. 2012, *M. Bakhshi* (IRAN 17064F, CCTU 1212 = IRAN 2680C = CBS 136153); Moghan, on leaves of *A. retroflexus*, Oct. 2011, *M. Bakhshi* (IRAN 17053F, CCTU 1064); Moghan, on leaves of *Amaranthus* sp., Oct. 2011, *M. Bakhshi* (IRAN 17055F, CCTU 1084 = CBS 136156); Moghan, on leaves of *Amaranthus blitoides*, Oct. 2011, *M. Bakhshi* (CCTU 1072 = IRAN

2653C); Moghan, on leaves of *Glycine max* (Fabaceae), Oct. 2012, *M. Bakhshi* (IRAN 17060F, CCTU 1209 = CBS 136152, CCTU 1210 = IRAN 2679C, CCTU 1211); Moghan, on leaves of *Hib. trionum*, Oct. 2012, *M. Bakhshi* (IRAN 17061F, CCTU 1218 = IRAN 2682C). – **South Africa:** Limpopo Province: Messina, on *Citrus* sp. (Rutaceae), *M.C. Pretorius* (CBS 115482 = CPC 4410). **Unknown**, on *Bromus* sp. (Poaceae), *M.D. Whitehead* (CBS 143.51 = CPC 5055).

Cercospora sp. G complex

The 16 isolates previously recognised as *Cercospora* sp. G based on a five-gene phylogenetic tree (Groenewald et al. 2013, Bakhshi et al. 2015a) cluster into two distinct phylogenetic clades based on the eight-gene phylogenetic tree (Fig. 1, part 1). One, four, one, two, two, two, three and two allele groups were detected for the ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, *rpb2* and *gapdh* sequences, respectively (Table 9).

Cercospora sp. G Clade 1

Description: Mycelium internal. *Caespituli* amphigenous, brown. *Conidiophores* aggregated in loose fascicles, arising from a moderately developed, intraepidermal and substomatal, brown stroma, to 35 µm diam; conidiophores pale brown to brown, 2–11-septate, straight to flexuous, simple, unbranched, uniform in width, (55–)110–150(–260) × 3.5–5 µm. *Conidiogenous cells* integrated, terminal, proliferating sympodially, mono- and multi-local; loci thickened, darkened, apical or formed on shoulders caused by sympodial proliferation, 1.5–2.5 µm diam. *Conidia* solitary, hyaline, subcylindrical, filiform to obclavate, straight to slightly curved, with truncate to obconically truncate base and subacute to subobtuse apices, (40–)75–100(–165) × 2–4 µm, 4–15-septate; hila distinctly thickened, darkened, refractive, 1–2 µm diam.

Specimens examined: Iran: Guilan Province: Talesh, Dulbin, on leaves of *Plantago major*, Jul. 2011, *M. Bakhshi* (IRAN 17085F, CCTU 1015 = IRAN 2645C = CBS 136024); Talesh, Kishonben, on leaves of *Bidens tripartita* (Asteraceae), Sept. 2012, *M. Bakhshi* (IRAN 17084F, CCTU 1197). – **New Zealand:** Manurewa, on *Salvia viscosa* (Lamiaceae), *C.F. Hill* (CPC 5438) (as *C. salviicola*).

Cercospora sp. G Clade 2

Description: Mycelium internal. *Caespituli* amphigenous, brown. *Conidiophores* aggregated in loose to dense fascicles, arising from a weakly to well-developed, intraepidermal and substomatal, brown stroma, to 50 µm diam; conidiophores pale brown to brown, 3–11-septate, straight to flexuous, simple, unbranched, uniform in width, (30–)65–105(–240) × 2.5–5 µm. *Conidiogenous cells* integrated, terminal, proliferating sympodially, 10–30 × 2.5–5 µm, mono- or multi-local; loci distinctly thickened, darkened and somewhat refractive, apical or formed on shoulders caused by sympodial proliferation, 1.5–2.5 µm diam. *Conidia* solitary, subcylindrical, filiform to obclavate, straight to slightly curved, hyaline, (25–)75–110(–200) × 3.5–5.5 µm, (3–)8–15(–20)-septate, with subacute to subobtuse apices and truncate to obconically truncate bases; hila thickened, darkened, refractive, 1–2 µm diam.

Notes: Isolates of *Cercospora* sp. G clustered in two distinct clades with high posterior probability in the eight-gene phylogenetic tree (Fig. 1, part 1). However, several isolates from diverse host families cluster in these two clades, to which different names can be applied. Moreover, there is also overlap between host ranges of the two clades. On the other hand, there is no morphological basis to divide them into two distinct species. Based on the gene loci screened in the present study, we were unable to resolve the taxonomy of

Table 9. Results from allele group designation per locus for *Cercospora* sp. G isolates in this study. Abbreviations of loci and collection accession numbers follow Table 1.

Species	Culture accession number	Host	Allele group per locus							
			ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>	<i>tub2</i>	<i>rpb2</i>	<i>gapdh</i>
<i>Cercospora</i> sp. G clade 1	CCTU 1015; CBS 136024; IRAN 2645C	<i>Plantago major</i>	I	IV	I	I	I	I	III	II
	CCTU 1197	<i>Bidens tripartita</i>	I	IV	I	I	I	I	I	II
	CPC 5438	<i>Salvia viscosa</i>	I	II	I	I	II	II	–	II
<i>Cercospora</i> sp. G clade 2	CCTU 1002	<i>Celosia cristata</i>	I	I	I	I	I	I	I	I
	CCTU 1020; CBS 136023	<i>Sorghum halepense</i>	I	I	I	I	I	I	II	I
	CCTU 1030; CBS 136026	<i>Bidens tripartita</i>	I	III	I	I	II	I	I	I
	CCTU 1046	<i>Plantago major</i>	I	I	I	I	I	I	I	I
	CCTU 1053; CBS 136027	<i>Cichorium intybus</i>	I	III	I	I	II	I	I	I
	CCTU 1054	<i>Amaranthus</i> sp.	I	I	I	I	I	I	I	I
	CCTU 1058	<i>Helminthotheca echioides</i>	I	I	I	I	I	I	I	I
	CCTU 1079; CBS 136025	<i>Amaranthus retroflexus</i>	I	I	I	I	I	I	I	I
	CCTU 1090	<i>Abutilon theophrasti</i>	I	I	I	I	I	I	I	I
	CCTU 1116	<i>Plantago major</i>	I	I	I	I	I	I	I	I
	CCTU 1122	<i>Amaranthus</i> sp.	I	I	I	I	I	I	I	I
	CCTU 1144; CBS 136130	<i>Cucurbita maxima</i>	I	I	I	II	I	I	I	I
CBS 115518; CPC 5360	<i>Bidens frondosa</i>	I	IV	I	I	I	I	–	I	

these isolates and for now prefer to treat them as unresolved phylogenetic species. As with *C. cf. flagellaris*, in order to resolve their taxonomy, fresh collections from the same host and country as the original material need to be recollected and included in future studies.

Specimens examined: **Iran:** *Zanjan Province:* Tarom, Pasar, on leaves of *P. major*, Oct. 2011, *M. Bakhshi* (CCTU 1046); Tarom, Pasar, on leaves of *P. major*, Nov. 2011, *M. Bakhshi* (IRAN 17093F, CCTU 1116). *Guilan Province:* Talesh, Kishonben, on leaves of *Bi. tripartita*, Oct. 2012, *M. Bakhshi* (IRAN 17091F, CCTU 1030 = CBS 136026); Talesh, on leaves of *Sorghum halepense* (*Poaceae*), Sep. 2011, *M. Bakhshi* (IRAN 17094F, CCTU 1020 = CBS 136023); Talesh, Dolbin, on leaves of *Celosia cristata*, Jul. 2011, *M. Bakhshi* (IRAN 17092F, CCTU 1002). *Ardabil Province:* Moghan, on leaves of *A. retroflexus*, Oct. 2011, *M. Bakhshi* (IRAN 17088F, CCTU 1079 = CBS 136025); Moghan, on leaves of *Amaranthus* sp., Oct. 2011, *M. Bakhshi* (IRAN 17089F, CCTU 1054); Moghan, on leaves of *Helminthotheca echioides* (*Asteraceae*), Oct. 2011, *M. Bakhshi* (IRAN 17086F, CCTU 1058); Moghan, on leaves of *Ab. theophrasti*, Oct. 2012, *M. Bakhshi* (IRAN 17087F, CCTU 1090). *Guilan Province:* Talesh, Jamakuh, on leaves of *Amaranthus* sp., Nov. 2011, *M. Bakhshi* (IRAN 17090F, CCTU 1122); Masal, on leaves of *Cu. maxima*, Jul. 2012, *M. Bakhshi* (CCTU 1144 = CBS 136130); Sowme'eh Sara, Dowgur, on leaves of *Cichorium intybus* (*Asteraceae*), Jun. 2012, *M. Bakhshi* (CCTU 1053 = CBS 136027). – **New Zealand:** Kopuku, on *Bidens frondosa* (*Asteraceae*), *C.F. Hill* (CBS 115518 = CPC 5360).

Identification of the best-performing DNA barcode

Kimura-2-parameter values

The Kimura-2-parameter distribution graphs (Fig. 9) visualise the inter- and intraspecific distances per locus corresponding to the barcoding gap (Hebert *et al.* 2003, Schoch *et al.* 2012). A useful barcoding locus should have no overlap between the inter- and intraspecific K2P distances and generally should have an average interspecific distance that is at least ten times as high as the average intraspecific distance of that locus (Quaedvlieg *et al.* 2012, Verkley *et al.* 2013, Stielow *et al.* 2015).

The eight tested loci showed varying degrees of overlap in their K2P distribution between inter- and intraspecific variation graphs (Fig. 9). In this dataset, the average interspecific variation in ITS dataset was very low (0.002) compared to its intraspecific variation (0.0005), leading to a very low inter- to intraspecific variation ratios of 4:1 for this locus (Fig. 9, Table 4). This low ratio is far below the recommended 10:1 ratio, indicating a general lack of natural variation within the ITS locus, making it ill-suited for effective identification of the individual species of *Cercospora*. Due to the presence of introns in the seven protein coding loci, these genes provide much higher interspecific variation than the more conserved ITS locus. These protein coding genes had K2P inter- to intraspecific variation ratios of 127:1 for *tef1*, 76:1 for *cmdA*, 74:1 for *rpb2*, 71:1 for *tub2*, 44:1 for *gapdh*, 15:1 for *actA* and 13:1 for *his3* (Table 4), making them all suitable for reliable species resolution of *Cercospora* spp. As the *tef1*, *cmdA*, *rpb2*, *tub2* and *gapdh* have the largest barcoding gap, these loci should give the highest species resolution. However,

all of these genes do have overlap between the inter- and intraspecific K2P distances (as is evident in the graphs of Fig. 9), suggesting that no one of them can serve as a single ideal barcoding locus for *Cercospora* spp.

Molecular phylogenetic resolution (clade recovery)

Based on the results of the individual gene tree assessments, no single gene region was found which could reliably distinguish all species, and occurrences of the same sequence(s) shared between multiple species were observed in each locus.

The ITS phylogeny had low resolution and was only able to distinguish *C. chenopodii*, *C. solani* and *C. sorghicola* from the other included species. The remaining loci had different levels of resolution. The *gapdh* region was more effective and could resolve 61 % of 28 lineages, whereas *his3*, *tub2*, *actA*, *tef1*, *cmdA* and *rpb2* had respectively 48, 43, 43, 39, 32 and 32 % clade recovery. Based on the *gapdh* region, we were able to distinguish 17 of the 28 species clades, including *C. althaeina*, *C. armoraciae*, *C. bizzozeriana*, *C. chenopodii*, *C. conyzae-canadensis*, *C. cf. flagellaris* clade 1, *C. cf. flagellaris* clade 2, *C. cf. gossypii*, *C. pseudochenopodii*, *C. cf. richardiicola*, *C. rumicis*, *C. solani*, *C. sorghicola*, *Cercospora* sp. G clade 1, *Cercospora* sp. G clade 2, *C. violae* and *C. cf. zinnia*; whereas, 13 species clades including *C. althaeina*, *C. chenopodii*, *C. conyzae-canadensis*, *C. cylindracea*, *C. pseudochenopodii*, *C. cf. richardiicola*, *C. rumicis*, *C. solani*, *C. sorghicola*, *C. uwebrauniana*, *C. violae*, *C. zebrina* and *C. cf. zinniae* were distinguished in the *his3* phylogeny; 12 species clades including *C. althaeina*, *C. chenopodii*, *C. conyzae-canadensis*, *C. cylindracea*, *C. iranica*, *C. pseudochenopodii*, *C. cf. richardiicola*, *C. solani*, *C. sorghicola*, *Cercospora* sp. T, *C. uwebrauniana* and *C. cf. zinniae* were distinguished in the *tub2* phylogeny; 12 species clades including *C. althaeina*, *C. chenopodii*, *C. convolvulicola*, *C. conyzae-canadensis*, *C. cylindracea*, *C. pseudochenopodii*, *C. cf. richardiicola*, *C. solani*, *C. sorghicola*, *C. violae*, *C. zebrina* and *C. cf. zinniae* were distinguished in the *actA* phylogeny; 11 species clades including *C. bizzozeriana*, *C. chenopodii*, *C. conyzae-canadensis*, *C. pseudochenopodii*, *C. cf. richardiicola*, *C. rumicis*, *C. solani*, *C. sorghicola*, *C. uwebrauniana*, *C. violae* and *C. cf. zinniae* were distinguished in the *tef1* phylogeny; nine species clades including *C. convolvulicola*, *C. conyzae-canadensis*, *C. iranica*, *C. cf. richardiicola*, *C. solani*, *C. sorghicola*, *Cercospora* sp. T, *C. violae* and *C. cf. zinniae* were distinguished in the *cmdA* phylogeny; and nine species clades including *C. bizzozeriana*, *C. chenopodii*, *C. conyzae-canadensis*, *C. pseudochenopodii*, *C. cf. richardiicola*, *C. solani*, *C. sorghicola*, *C. zebrina* and *C. cf. zinniae* were distinguished in the *rpb2* phylogeny.

Therefore, the *gapdh* phylogeny displayed a high resolution and had the highest clade recovery and was responsible for resolving most of the cryptic taxa within *C. apii*, *C. armoraciae*, *C. beticola*, *Cercospora* sp. G, and *C. cf. flagellaris*.

DISCUSSION

In this study, we re-assessed species of the genus *Cercospora* using a combined approach based on the evaluation of an

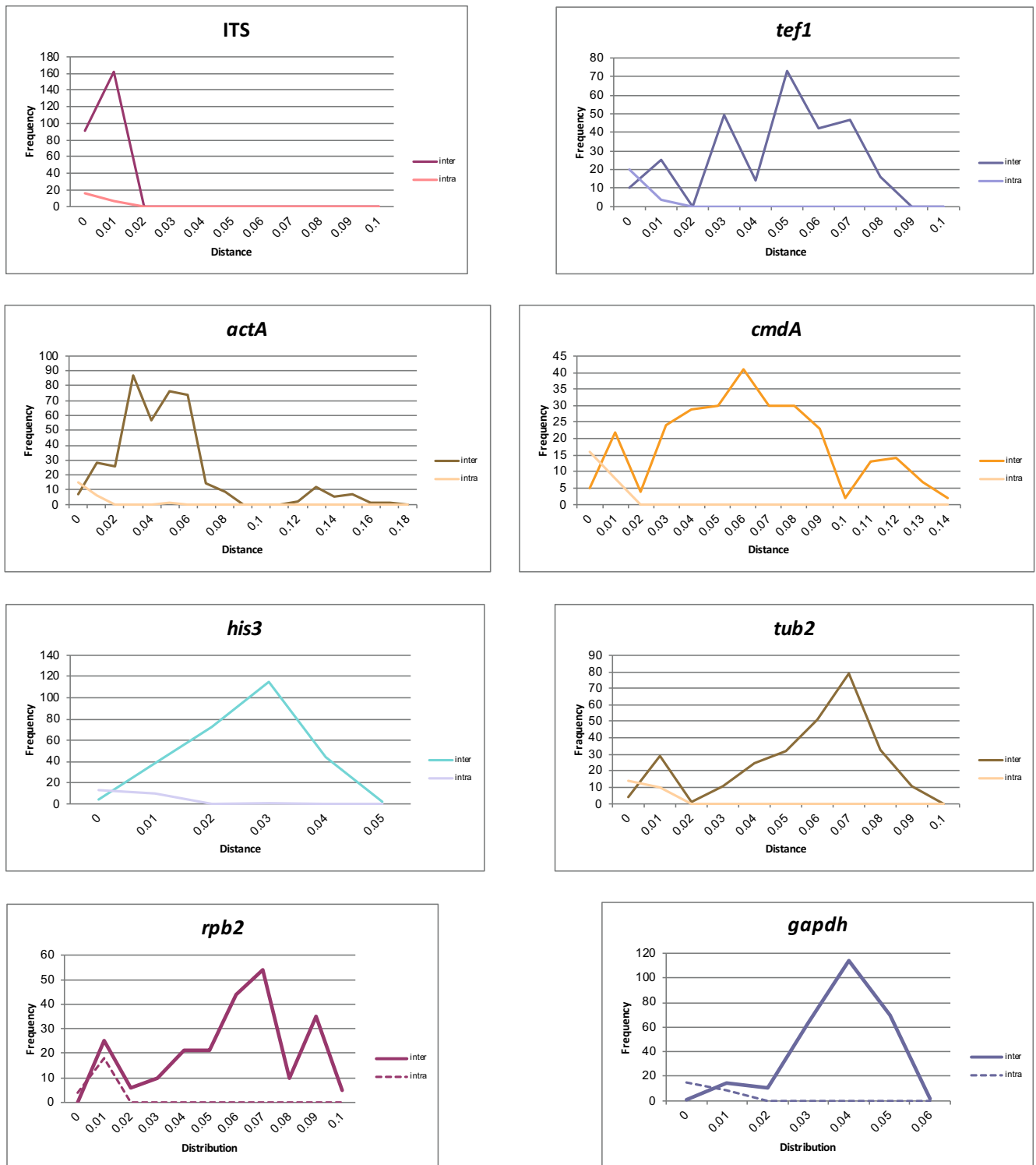


Fig. 9. Frequency distributions of the Kimura-2-parameter distance (barcoding gap) for the eight loci.

eight-gene molecular DNA sequence dataset, host, and morphological data (in those cases where morphological variation was present). In recent years, the rapid advance of molecular techniques has brought about the possibility of a more precise species delimitation and a better consideration of the evolution of fungi. It is well-known that many fungal taxa based on morphology or on sequence data of the commonly used fungal barcode ITS region of the nrDNA operon (Schoch et al. 2012) hide cryptic species complexes when molecular data from multiple gene regions are considered (Lombard et

al. 2010, Cabral et al. 2012, Crous et al. 2013, Groenewald et al. 2013, Quaedvlieg et al. 2013, Woudenberg et al. 2013). This is most likely an underestimation for many fungal taxa. For instance, the *Colletotrichum acutatum* species complex, once considered to be a single species, has been shown to include at least 31 cryptic taxa (Damm et al. 2012). In the present study, phylogenetic inference also revealed cryptic species complexes that could not be distinguished based on geography, host association, morphology, or ITS sequence data alone.

Before this study, Groenewald *et al.* (2013) and Bakhshi *et al.* (2015a) inferred phylogenies of *Cercospora* based on sequence data of five genomic loci (ITS, *tef1*, *actA*, *cmdA* and *his3*). Their results showed the importance of all five loci in a combined analysis for *Cercospora* taxonomy (Groenewald *et al.* 2013, Bakhshi *et al.* 2015a). Despite this, the sequences of these five loci were too conserved in *Cercospora*, and it was not possible to identify a single gene as the best DNA barcoding locus. In addition, several species complexes remained unresolved (Groenewald *et al.* 2013, Bakhshi *et al.* 2015a). To overcome these deficiencies, three more potential candidate gene regions, *tub2*, *rpb2*, and *gapdh*, were amplified and sequenced for *Cercospora* isolates previously investigated by Bakhshi *et al.* (2015a) and some related reference isolates investigated by Groenewald *et al.* (2013). Phylogenetic performance of the eight loci (ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, *rpb2* and *gapdh*) were assessed based on the inter-/intraspecific distance ratio and clade recovery. With the final classification presented here, none of the genes we analysed provides an effective barcode on its own across the entire genus. However, *gapdh* emerged as a strong candidate for improved species delimitation in *Cercospora* and provides better insight, especially into species complexes. Groenewald *et al.* (2013) evaluated this gene in the *Cercospora* sp. Q species complex and their results also showed high variation in this gene. The performance of *gapdh* in species delimitation has been also reported in other fungal groups, including *Alternaria* (Woudenberg *et al.* 2013) and the *Colletotrichum gloeosporioides* species complex (Weir *et al.* 2012). Additionally, when using the *gapdh* gene, *cmdA* sequences are crucial to distinguish some species of *Cercospora*. We therefore recommend *gapdh* as the gene for species delimitation in *Cercospora*. However, it needs to be combined with *cmdA*, *tef1* and *tub2* to obtain a robust species identification. In addition, data from the ITS, *actA*, *rpb2*, and *his3*, have been useful, and were at times necessary, to provide clear evidence of multi-gene phylogenetic concordance to separate cryptic species.

The amplification of *gapdh* with the available primers was not, however, easy, and we need to design new primer sets for *gapdh* in *Cercospora* derived from the sequences generated. On the other hand, lack of ex-type or reliable sequences in public databases is a serious problem in the accurate molecular identification of *Cercospora* species, and it is essential to also amplify at least the *gapdh* and *tub2* genes for all of the reference isolates used by Groenewald *et al.* (2013) in the future.

One of the main goals of this project was to generate an eight-gene DNA dataset for species of the genus *Cercospora*. In this regard, one of the achievements of this research was that the sequencing of additional loci revealed new clades within some taxa which were found to actually represented a species complex (in the eight-gene phylogenetic tree) rather than a single species, while the five-gene phylogenetic tree (Bakhshi *et al.* 2015a) was unable to resolve them. The phylogenetic tree based on the combined eight-gene dataset resolved at least four, three, two, two and two well-supported clades respectively within the species complexes *C. cf. flagellaris*, *C. apii*, *C. beticola*, *C. armoraciae*, and *Cercospora* sp. G.

Some of the species revealed by the eight-gene phylogeny in this study can be distinguished based on their morphology or host range. For example, as explained in the notes for *C. uwebrauniana*, characteristics of the conidia in this species, which clustered in the *C. apii* complex based on the five-gene phylogenetic tree (see Bakhshi *et al.* 2015a), are clearly distinguishable from those of *C. apii*. However, some species cannot be separated using morphological characters. For instance, the *C. cf. flagellaris* species complex included at least three distinct clades and there is considerable overlap between morphological features and host ranges of the clades 1, 2, and 3. In addition, pursuant to high levels of intraspecific variation in these three clades, the distinction between these clades is only possible based on molecular data. It is conceivable that some members of these three clades represent new species, yet to be described. This is also true for the *Cercospora* sp. G species complex.

Another problem arises because many morphological features change according to the host plant and different weather conditions. Such differences in morphological characters under different conditions have also been seen in other groups of fungi, such as *Colletotrichum* species (Weir *et al.* 2012). Because we do not yet have access to sequence data of most species of the *Cercospora*, we have chosen to consider these clades as different clades of *C. cf. flagellaris* and *Cercospora* sp. G rather than introduce new species names. Recent molecular studies on the *Cercospora* species associated with *Cercospora* leaf blight and purple seed stain on soybean, have revealed several *Cercospora* species, including *C. cf. flagellaris* as one of the most important agents (Bakhshi *et al.* 2015a, Soares *et al.* 2015, Albu *et al.* 2016). In this regard, Guillin *et al.* (2017) studied the genetic entanglement between *Cercospora* species infecting soybean and provided evidence that revealed interspecific gene flow played a significant role in the evolutionary dynamics of *Cercospora* species. Taking into consideration the shared host range that exists between different clades of *C. cf. flagellaris*, our data also provide more support for this hypothesis.

Furthermore, we found that all of the isolates of *C. apii* obtained from *Plantago lanceolata* from different localities clustered in clade 2 of this species in the eight-gene phylogenetic tree. Additionally, isolates of *C. beticola* and *C. apii* which intermix with *P. lanceolata*, had a common allele in *gapdh*. Thus, it seems that the *gapdh* gene might play a role in pathogenicity or host range, and has the potential to reflect this phylogenetically; however, that remains to be tested.

This study emphasises the complex nature of the evolutionary pathways that have been traversed within the genus *Cercospora*. Speciation has taken place much more prolifically than had previously been suspected in this genus, and it seems likely that the *C. apii sensu* Crous & Braun (2003) species complex is still rapidly evolving. The emergence of new species is doubtlessly encouraged by the opportunities for mixing gene pools that are provided by modern global agricultural practices, and indiscriminate use of fungicides combined with imperfect phytosanitary regulation.

The present study provides the first eight-gene phylogenetic overview of *Cercospora* species. We hope that this dataset will provide a stable platform to accommodate the numerous undescribed species that still await description,

and the recollection and epitypification of already named species. Moreover, it seems that *Cercospora* should still be subjected to a more detailed analysis based on yet additional gene loci to provide a more vigorous phylogenetic basis for species delimitation.

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