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Development of new primers based on *gapdh* gene for *Cercospora* species and new host and fungus records for Iran

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Abstract: ITS region and protein coding genes such as *actA*, *cmdA*, *gapdh*, *his3*, *rpb2*, *tef1* and *tub2* have been applied to investigate the molecular phylogeny of *Cercospora* species in recent years. Although *gapdh* is an informative gene for species delimitation in the genus, difficult amplification of this locus with available primers limits its use for *Cercospora*. Therefore, in this study, novel primers including GpdF-Cer (5'-TTC ATY GAG CCM CAC TAC GCT-3') and GpdR-Cer (5'-RTC GGT GAC KRC GAG VAC-3') were developed to supplement previously published primers for the amplification of *gapdh*. Besides, in a taxonomic survey on the genus *Cercospora* in Iran based on consolidated species concept, leaf samples with leaf spot symptoms were collected and *Cercospora* isolates were characterized based on a combination of morphological features and sequence data from the ITS, *actA*, *cmdA*, *gapdh*, *his3* and *tef1* loci. Seventeen species of the genus *Cercospora* were recognized, of which *C. mercurialis* on *Mercurialis annua* is confirmed for the first time, for Iran (Asia) mycobiota using sequence data of six genomic loci. Several new hosts are recorded for *C. apii* (one), *C. beticola* (one), *C.* cf. *flagellaris* (10), *C. gamsiana* (one), *C. iranica* (one), *Cercospora* sp. G (three) and *Cercospora* sp. T (four). Thus, new host families were added to the host range of *C. beticola* (Brassicaceae), *C.* cf. *flagellaris* (Lamiaceae, Polygonaceae, Vitaceae), and *Cercospora* sp. T (Lamiaceae, Plantaginaceae, Rosaceae) in the world.

Keywords: Biodiversity, cercosporoid fungi, leaf spot, Mycosphaerellaceae, new primer.

INTRODUCTION

Fungi in the genus *Cercospora* (Mycosphaerellaceae, Capnodiales) are known as serious plant pathogens, causing major losses on a wide range of crop plants worldwide, including sugar beet (Weiland & Koch 2004; Bakhshi et al. 2011; Vaghefi et al. 2018), beans (Chand et al. 2015; Duangsong et al. 2016), faba beans (Kimber & Paull 2011), corn (Crous et al. 2006), carrots (Kushalappa et al. 1989), sesame (Bakhshi & Zare 2020) and soybean (Soares et al. 2015; Bakhshi & Zare 2020) as well as many vegetable and ornamental species. Several taxa are also considered potential biocontrol agents of weeds (Tessmann et al. 2001; Praveena & Naseema 2004).

Correct identification of *Cercospora* species has a crucial role in order to understand the epidemiology of the diseases caused by these taxa and to develop effective control measures. Due to the lack of useful morphological characters and high levels of intraspecific variation, morphology does not provide sufficient and informative characters for accurate identification of *Cercospora* species (Groenewald et al. 2013; Bakhshi et al. 2012b, 2015a, 2015b). Therefore, traditional identification systems in *Cercospora* relied heavily on host plant association (Crous & Braun 2003). Molecular studies of *Cercospora* spp. in recent years revealed that many taxa have broader host ranges (Groenewald et al. 2013; Bakhshi et al. 2015a, 2018); consequently, relying only on host data in *Cercospora* taxonomy has proven to be problematic.

Phylogenetic analyses based on DNA sequences have led to momentous progress in the systematics of the genus. In this regard, the phylogenetic performance of sequence data of eight genomic loci, including ITS, *actA*, *cmdA*, *gapdh*, *his3*, *rpb2*, *tef1* and *tub2*, were assessed for *Cercospora* species based on the inter-/intraspecific distance ratio and clade recovery (Groenewald et al. 2013; Bakhshi et al. 2015a, 2018; Bakhshi 2019). According to these results, none of the genes analyzed provides an effective barcode on its own across the entire genus. However, Bakhshi et al. (2018) showed that, *gapdh* is

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a strong candidate for improved species delimitation in *Cercospora* and this gene provided better insight, especially into species complexes. The amplification of *gapdh* with available primers (Berbee et al. 1999; Myllys et al. 2002) was, however, not easy and indicated the need for new *gapdh* primer designation in *Cercospora*.

Therefore, our primary aim was to designate an additional primer set for amplification of *gapdh* in *Cercospora*. In addition, our secondary aim was to characterize *Cercospora* species gained from the infected leaves of several plant species collected from different provinces of Iran, based on morphology, cultural characteristics and phylogenetic analyses of DNA sequence data.

MATERIALS AND METHODS

Samples and morphology

Plant samples with *Cercospora* leaf spot symptoms were collected from seven provinces of Iran, including Ardabil, Golestan, Guilan, Hormozgan, Khuzestan, Mazandaran and North Khorasan, during the growing seasons 2017–2019, taken to the laboratory, and examined under a Nikon SMZ 445 stereo-microscope to observe sporulation. Fungal strains were isolated in pure culture by direct spore transfer from a single leaf spot onto plates containing 2% malt extract agar (MEA; Fluka, Hamburg, Germany) with a sterile, fine-pointed needle as explained in Bakhshi et al. (2011). Representative samples of diseased specimens were dried in a plant press and deposited in the Fungal Herbarium of the Iranian Research Institute of Plant Protection (IRAN F). Representative isolates of the fungi were deposited in the Culture Collection of the Iranian Research Institute of Plant Protection (IRAN C), Tehran, Iran.

Morphological descriptions are based on structures from dried material. Diseased leaf tissues were examined under a Nikon SMZ 445 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 an Olympus-BX51 (Olympus, Tokyo, Japan) light microscope and photographed using a mounted Olympus DP 25 high-definition color 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 brackets.

DNA isolation, PCR amplification and sequencing

Mycelium from actively growing fungal cultures was scraped from the surface of MEA using a sterile scalpel blade and DNA was isolated using the protocol of Möller et al. (1992). The DNA samples were subsequently diluted 50–100 times in preparation for further DNA amplification reactions. The primers V9G (de Hoog & Gerrits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify part of the nuclear rRNA operon (ITS) spanning the 3' end of 18S rRNA gene, the first internal transcribed spacer, the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene. Part of the actin gene (*actA*) was amplified using the primer set ACT-512F (Carbone & Kohn 1999) and ACT2Rd (Groenewald et al. 2013), whereas the primer set EF1-728F (Carbone & Kohn 1999) and EF-2 was used to amplify part of the translation elongation factor 1-alpha (*tef1*) gene. Primers employed for the amplification of calmodulin gene (*cmdA*) included CAL-228F and CAL-737R (Carbone & Kohn 1999) or CAL-2Rd (Groenewald et al. 2013), while the primer set CylH3F and CylH3R (Crous et al. 2004) was used to amplify part of the histone H3 gene (*his3*). The PCR amplifications were performed in a total volume of 25 µL on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California). The protocols and conditions for standard PCR amplification of the loci followed Bakhshi & Arzanlou (2017) and subsequent sequencing was performed in both directions using the PCR primers by Microsynth company (Balgach, Switzerland).

To amplify part of the *gapdh*, a new primer set was designated here. For this purpose, the available sequences of *gapdh* for *Cercospora* spp. were retrieved from National Center for Biotechnology Information (NCBI) GenBank sequence database and were aligned with the MEGA v.7 (Molecular Evolutionary Genetics Analysis) software (Kumar et al. 2016). The forward and reverse primers were designed in regions showing similarity between different sequences using the OligoCalc (Oligonucleotide Properties Calculator) online software (http://biotools.nubic.northwestern.edu/OligoCalc.html) (Kibbe 2007). Synthesis of primers was carried out by Microsynth company. The different PCR mixtures and conditions were tested using the new primers to set the best condition and PCR mixture for amplification of part of the *gapdh*. Finally, the resulting fragments were sequenced in both directions using the PCR primers.

Sequence alignment and phylogenetic inference

The raw trace files were inspected and edited with MEGA v.7 software (Kumar et al. 2016), and consensus sequences were manually generated from the forward and reverse sequences. The newly generated sequences were blasted against the NCBI's GenBank sequence database using MegaBLAST to identify closely related taxa. The obtained sequences from GenBank, together with the novel sequences generated during this study, were initially aligned with the MAFFT v.7 online interface using default settings (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013) for each gene.

For phylogenetic comparison, Bayesian inference (BI) analyses on individual *gapdh* gene and concatenated ITS, *actA*, *cmdA*, *gapdh*, *his3* and *tef1*

loci were performed with MrBayes 3.2.6 (Ronquist et al. 2012). The best evolutionary model for each data partition was obtained using the software MrModelTest v. 2.3 (Nylander 2004). The heating parameter was set at 0.15 and the Markov Chain Monte Carlo (MCMC) analysis of four chains was started in parallel from a random tree topology and lasted until the average standard deviation of split frequencies came below 0.01. Trees were saved each 1 000 generations and the first 25% of saved trees were discarded as the 'burn-in' phase and posterior probabilities (PP) determined from the remaining trees. The resulting phylogenetic tree was printed with Geneious v. 5.6.7 (Drummond et al. 2012). Sequences derived from this study were lodged at NCBI's GenBank nucleotide database (http://www.ncbi.nlm.nih.gov; Table 1).

RESULTS AND DISCUSSION

Field survey

During the field survey of this study, leaf spot symptoms of various species of *Cercospora* were associated with different plant species, including important crops and vegetables such as sugar beet (*Beta vulgaris*), celery (*Apium graveolens*), alfalfa (*Medicago sativa*), kohlrabi (*Brassica oleracea*), radish (*Raphanus sativus*), basil (*Ocimum basilicum*) and mint (*Mentha longifolia*), ornamentals such as *Gazania* sp. and Boston ivy (*Parthenocissus tricuspidata*), medical plants and or weeds such as mallow (*Malva* sp.), camel thorn (*Alhagi maurorum*), hemp-agrimony (*Eupatorium cannabinum*), sticky nightshade (*Solanum sisymbriifolium*), creeping cinquefoil (*Potentilla reptans*) etc. (Fig. 1).

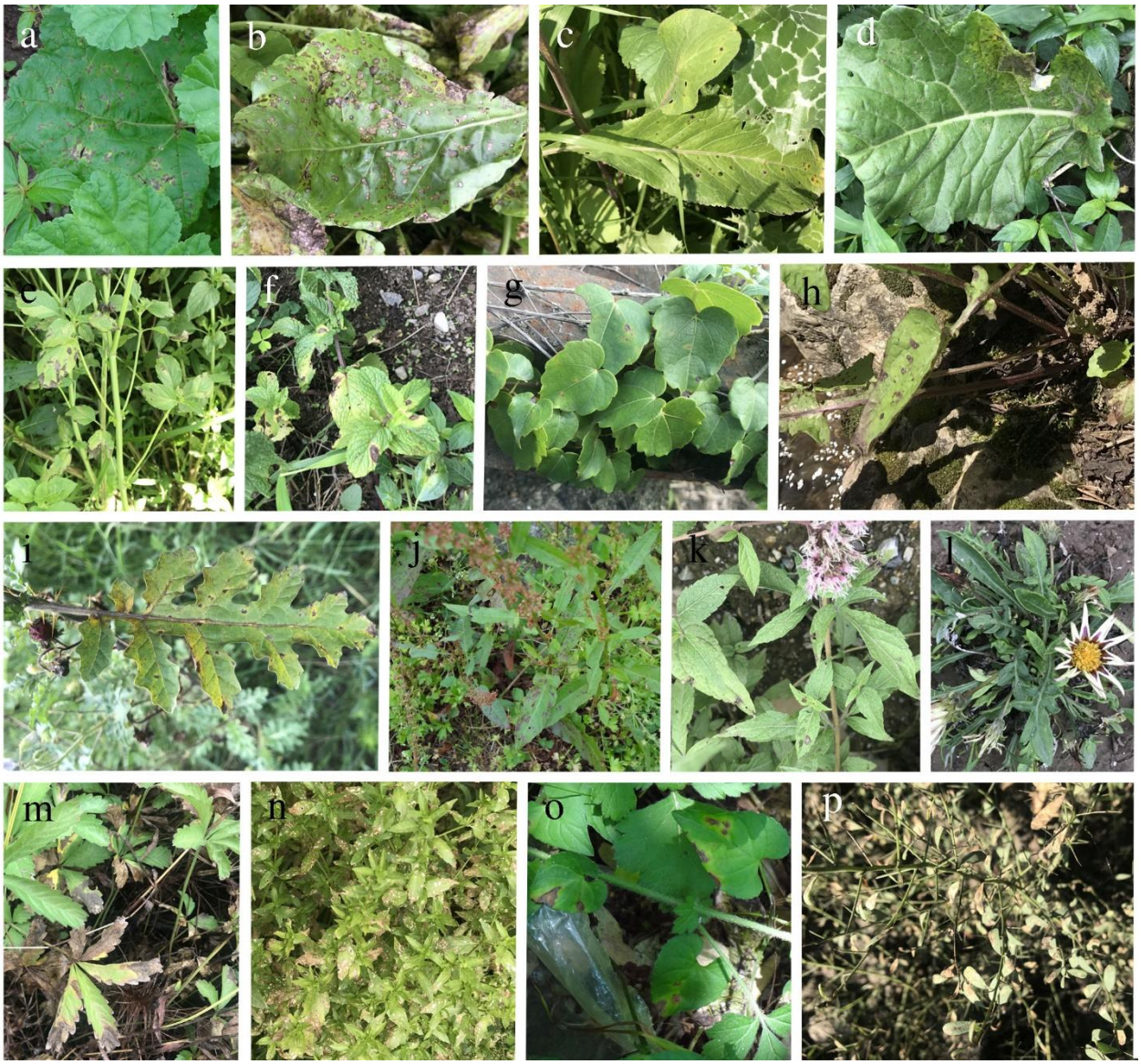

Fig. 1. Disease symptoms associated with *Cercospora* spp. in the field. **a.** *C. althaeina* on *Malva* sp.; **b.** *C. beticola* and *C. gamsiana* on *Beta vulgaris*; **c.** *C. beticola* on *Raphanus sativus*; **d–i.** *C.* cf. *flagellaris* on **d.** *Brassica oleracea*; **e.** *Ocimum basilicum*; **f.** *Mentha longifolia*; **g.** *Parthenocissus tricuspidata*; **h.** *Lapsana* sp.; **i.** *Solanum sisymbriifolium*; **j.** *C. rumicis* on *Rumex* sp.; **k, l.** *Cercospora* sp. G on **k.** *Eupatorium cannabinum*; **l.** *Gazania* sp.; **m.** *Cercospora* sp. T on *Potentilla reptans*; **n.** *C. mercurialis* on *Mercurialis annua*; **o.** *C. violae* on *Viola* sp.; **p.** *C. zebrina* on *Alhagi maurorum.*

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

Species	Culture accession number	Host	Host Family	Origin	GenBank accession numbers					
					ITS	*tef1*	*actA*	*cmdA*	*his3*	*gapdh*
C. althaeina	IRAN 3920C	*Malva* sp.	Malvaceae	Mazandaran, Amol**	_	MT843584	MT843607	MT843631	MT843658	MT843686
C. apii	IRAN 3921C	*Apium graveolens*	Apiaceae	Guilan, Paresar, Pilembra	MT804377	MT843585	MT843608	MT843632	MT843659	MT843687
	IRAN 3922C	*Ipomoea hederacea**	Convolvulaceae	Golestan, Galikesh	MT804378	MT843586	MT843609	MT843633	MT843660	MT843688
C. beticola	P 631 I2	*Beta vulgaris*	Amaranthaceae	Ardabil, Moghan	_	_	_	MT843634	MT843661	MT843689
	IRAN 3923C	*Beta vulgaris*	Amaranthaceae	Ardabil, Moghan	_	_	_	MT843635	MT843662	MT843690
	IRAN 3924C	*Beta vulgaris*	Amaranthaceae	Mazandaran, Kelardasht, Goharkela	_	_	MT843610	MT843636	MT843663	MT843691
	P 656 R2	*Beta vulgaris*	Amaranthaceae	Mazandaran, Marzanabad, Foshkour	_	_	MT843611	MT843637	MT843664	MT843692
	IRAN 3925C	*Raphanus sativus**	Brassicaceae*	Khuzestan, Shush-Dezful	_	MT843587	MT843612	MT843638	MT843665	MT843693
C. bizzozeriana	IRAN 3926C	*Cardaria draba*	Brassicaceae	North Khorasan, Bojnourd	_	MT843588	MT843613	MT843639	MT843666	MT843694
C. conyzae-canadensis	IRAN 3927C	*Conyza canadensis*	Asteraceae	Mazandaran, Sangdeh**	_	_	_	_	_	MT843695
	IRAN 3928C	*Conyza canadensis*	Asteraceae	Mazandaran, Amol, Baudeh	_	_	_	_	_	MT843696
C. cylindracea	IRAN 3929C	*Cichorium intybus*	Asteraceae	Mazandaran, Galugah-Sefidchah**	_	MT843589	MT843614	MT843640	MT843667	MT843697
	IRAN 3930C	*Cichorium intybus*	Asteraceae	North Khorasan, Eshghabad, Raz**	MT804379	MT843590	MT843615	MT843641	MT843668	MT843698
C. cf. *flagellaris*	IRAN 3931C	*Conyza canadensis**	Asteraceae	Guilan, Rasht	_	_	_	MT843642	_	_
	IRAN 3932C	*Ocimum basilicum**	Lamiaceae*	Golestan, Gorgan**	MT804380	MT843591	MT843616	MT843643	MT843669	_
	IRAN 3933C	*Plantago major**	Plantaginaceae	Mazandaran, Tonekabon, Sehezar Road	_	_	_	MT843644	_	_
	IRAN 3934C	*Abutilon theophrasti*	Malvaceae	Mazandaran, Babol, Tazehabad	_	_	_	_	_	MT843699
	IRAN 3935C	*Brassica oleracea**	Brassicaceae	Guilan, Shaft, Siahmazgi	_	_	_	_	_	MT843700
	IRAN 3936C	*Brassica oleracea*	Brassicaceae	Guilan, Shaft, Siahmazgi	_	_	_	_	_	MT843701
	IRAN 3937C	*Calendula* sp.	Asteraceae	Mazandaran, Tonekabon, Sehezar Road	_	_	_	_	_	MT843702
	IRAN 3938C	*Fallopia convolvulus**	Polygonaceae*	Guilan, Talesh, Jokandan	_	_	_	_	_	MT843703

Table 1. Continue…

Species	Culture accession number	Host	Host Family	Origin	GenBank accession numbers					
					ITS	*tef1*	*actA*	*cmdA*	*his3*	*gapdh*
	IRAN 3939C	*Fallopia convolvulus*	Polygonaceae	Guilan, Astara, Havigh	_	_	_	_	_	MT843704
	IRAN 3940C	*Lapsana* sp.*	Asteraceae	Guilan, Shaft, Siahmazgi	_	_	_	_	_	MT843705
	IRAN 3941C	*Mentha longifolia**	Lamiaceae	Mazandaran, Tonekabon, Sehezar Road	_	_	_	_	_	MT843706
	P 682 I2	*Mentha longifolia*	Lamiaceae	Mazandaran, Tonekabon, Sehezar Road	_	_	_	_	_	MT843707
	IRAN 3942C	*Parthenocissus tricuspidata**	Vitaceae*	Guilan, Paresar, Pilembra	_	_	_	_	_	MT843708
	IRAN 3943C	*Solanum sisymbriifolium**	Solanaceae	Guilan, Rasht, Saravan	_	_	_	_	_	MT843709
	IRAN 3944C	*Sonchus* sp.*	Asteraceae	Mazandaran, Babol, Tazehabad	_	_	_	_	_	MT843710
	IRAN 3945C	Unknown	Unknown	Mazandaran, Tonekabon, Dohezar Road	_	_	_	_	_	MT843711
C. gamsiana	IRAN 3946C	*Beta vulgaris**	Amaranthaceae	Mazandaran, Kelardasht, Goharkela	_	_	MT843617	MT843645	MT843670	MT843712
	IRAN 3947C	*Malva* sp.	Malvaceae	Hormozgan, Minab**	_	_	MT843618	MT843646	MT843671	MT843713
C. iranica	IRAN 3948C	*Bidens tripartita**	Asteraceae	Guilan, Siahkal	_	MT843592	MT843619	MT843647	MT843672	MT843714
C. mercurialis	IRAN 3949C	*Mercurialis annua*	Euphorbiaceae	Golestan, Gorgan	MT804381	MT843593	MT843620	MT843648	MT843673	MT843715
	IRAN 3950C	*Mercurialis annua*	Euphorbiaceae	Golestan, Gorgan	_	MT843594	MT843621	MT843649	MT843674	_
C. plantaginis	IRAN 3951C	*Plantago lanceolata*	Plantaginaceae	North Khorasan, Eshghabad, Raz**	_	MT843595	_	MT843650	MT843675	MT843716
	IRAN 3952C	*Plantago lanceolata*	Plantaginaceae	Azerbaijan-Iran border, Ardabil, Mil-Mughan Water Reservoir	_	MT843596	_	MT843651	MT843676	MT843717
C. rumicis	IRAN 3953C	*Rumex* sp.	Polygonaceae	Mazandaran, Amol, Najarmahalleh**	_	_	_	_	_	MT843718
Cercospora sp. G	IRAN 3954C	*Eupatorium cannabinum**	Asteraceae	Guilan, Shaft, Siahmazgi	_	_	_	_	_	MT843719
	IRAN 3955C	*Gazania* sp.*	Asteraceae	Guilan, Rasht	_	_	_	_	_	MT843720
	IRAN 3956C	*Lapsana* sp.*	Asteraceae	Mazandaran, Tonekabon, Dohezar**	_	_	_	_	_	MT843721
	IRAN 3957C	*Lapsana* sp.	Asteraceae	Mazandaran, Tonekabon, Dohezar	_	_	_	_	_	MT843722
Cercospora sp. T	IRAN 3958C	*Helianthus tuberosus**	Asteraceae	Mazandaran, Salmanshahr**	_	MT843597	MT843622	MT843652	MT843677	MT843723
	IRAN 3959C	*Mentha longifolia**	Lamiaceae*	Guilan, Sowme'eh Sara, Lifshagard	_	_	MT843623	_	_	MT843724

Table 1. Continue …

Species	Culture accession number	Host	Host Family	Origin	GenBank accession numbers					
					ITS	ITS	ITS	ITS	ITS	ITS
	P 686 I1	*Plantago major**	Plantaginaceae*	Mazandaran, Tonekabon, Sehezar	_	MT843598	MT843624	MT843653	MT843678	MT843725
	IRAN 3960C	*Potentilla reptans**	Rosaceae*	Mazandaran, Salmanshahr	_	MT843599	MT843625	_	_	MT843726
C. uwebrauniana	IRAN 3961C	*Heliotropium europaeum*	Boraginaceae	Golestan, Gorgan-Aghghala**	MT804382	MT843600	MT843626	_	MT843679	MT843727
	IRAN 3962C	*Heliotropium europaeum*	Boraginaceae	Mazandaran, Amol, Ejbarkola**	MT804383	MT843601	MT843627	_	MT843680	MT843728
C. violae	IRAN 3963C	*Viola* sp.	Violaceae	Golestan, Gorgan, Shastkola	_	_	_	_	_	MT843729
	IRAN 3964C	*Viola* sp.	Violaceae	Golestan, Gorgan, Shastkola	_	_	_	_	_	MT843730
C. zebrina	IRAN 3965C	*Medicago sativa*	Fabaceae	Golestan, Gorgan	_	MT843602	MT843628	MT843654	MT843681	MT843731
	IRAN 3966C	*Medicago sativa*	Fabaceae	North Khorasan, Ashkhaneh**	_	MT843603	_	MT843655	MT843682	MT843732
	IRAN 3967C	*Medicago sativa*	Fabaceae	Mazandaran, Galugah-Sefidchah	MT804384	MT843604	MT843629	MT843656	MT843683	MT843733
	IRAN 3968C	*Oxalis* sp.	Fabaceae	Golestan, Gorgan, Ghorogh Forest Park**	_	MT843605	MT843630	MT843657	MT843684	MT843734
	IRAN 3969C	*Alhagi maurorum*	Fabaceae	Golestan, Aghghala-Incheboroun, Agh Ghabr	MT804385	MT843606	_	_	MT843685	MT843735

* new host species and family records. ** new locality (province) record

Primer design and experimental setup for *gapdh* gene amplification

Recently eight-gene (ITS, *actA*, *cmdA*, *gapdh*, *his3*, *rpb2*, *tef1* and *tub2*) molecular phylogenetic study on the genus *Cercospora* have revealed that *gapdh* is a strong candidate for improved species delimitation in this genus; however, the amplification of the locus using the available primers was not easy (Bakhshi et al. 2018; Bakhshi 2019). Therefore, during the course of this study, we developed two new primers, namely GpdF-Cer and GpdR-Cer, to amplify fragments of the protein-coding gene *gapdh* in *Cercospora* species. Primer sequences and annealing conditions are presented in Table 2. The primers successfully amplified the target in *Cercospora* species; however, based on their degenerate design, they may also be applied to a broader fungal community.

To obtain the partial *gapdh* sequences, using the novel primer set, we found that the best PCR mixture consisted of 5–10 ng genomic DNA, 1× PCR buffer, 2 mM $MgCl_2$, 56 µM of each dNTP, 0.7 µL DMSO, 0.28 µM of each primer and 0.5 unit *Taq* DNA polymerase in a total volume of 25 µL. As multiple bands were sometimes present, we adapted a touchdown PCR protocol: initial denaturation (94 °C, 5 min), five amplification cycles (94 °C, 45 s; 59 °C, 45 s; 72 °C, 2 min), five amplification cycles (94 °C, 45 s; 57 °C, 45 s; 72 °C, 2 min), 30 amplification cycles (94 °C, 45 s; 52 °C, 45 s; 72 °C,2 min) and a final extension (72 °C, 8 min).

Phylogenetic analysis

***gapdh* phylogeny:** The final aligned *gapdh* dataset contained 125 ingroup taxa with a total of 889 characters, containing 302 unique site patterns and *Septoria provencialis* (GenBank accession JX142538) as the outgroup taxon and a heating parameter set at 0.15. The results of MrModeltest recommended a general time reversible (GTR) substitution model with inverse gamma rates for *gapdh* and dirichlet base frequencies. During the generation of the tree (Fig. 2), a total of 5152 trees were saved, and consensus trees and posterior probabilities were calculated from the remaining 3864 (75%) trees. The isolates of some *Cercospora* species could be identified based on the results of the *gapdh* phylogeny; therefore, there was no need to do multi-gene phylogeny (Fig. 2).

Multi-gene phylogeny: In the multi-gene analyses (gene boundaries of ITS: 1–481, *tef1*: 482–817, *actA*: 818–1033, *cmdA*: 1034–1303, *his3*: 1304–1672 and *gapdh*: 1673–2568) of 199 isolates of *Cercospora* (including 145 taxa from NCBI, and 54 taxa from this study), 2568 characters including the alignment gaps were used and these characters contained 1044 unique site patterns (86, 239, 141, 136, 141 and 301 for ITS, *tef1*, *actA*, *cmdA*, *his3* and *gapdh* respectively). *Septoria provencialis* (CBS 118910) was used as an outgroup in the phylogenetic analyses. The results of MrModeltest recommended a HKY+G with gamma distributed rate variation for ITS, *tef1*, *actA*, *cmdA* and *his3*; while, a GTR+I+G with inverse gamma-distributed rate variation for *gapdh*. All partitions had dirichlet base frequencies. The Bayesian analysis lasted 90175000 generations and generated 180352 trees from which the first 45088 trees (25%), representing the burn-in phase of the analyses, were discarded, and the remaining trees (135264) were used for calculating posterior probability (PP) values in the phylogenetic tree (50% majority rule consensus tree) (Fig. 3).

Taxonomy

During the course of the present research, the Consolidated Species Concept (Quaedvlieg et al. 2014), using a polyphasic approach based on multilocus DNA sequences, host taxonomy, and morphological data, was employed to distinguish species. Seventeen species of *Cercospora* including *C. althaeina*, *C. apii*, *C. beticola*, *C. bizzozeriana*, *C. conyzae-canadensis*, *C. cylindracea*, *C.* cf. *flagellaris*, *C. gamsiana*, *C. iranica*, *C. mercurialis*, *C. plantaginis*, *C. rumicis*, *Cercospora* sp. G & T, *C. uwebrauniana*, *C. violae* and *C. zebrina* were resolved based on the clustering and support in the Bayesian trees obtained from the single *gapdh* phylogeny (Fig. 2) and the combined six-gene (ITS, *actA*, *cmdA*, *gapdh, his3* and *tef1*) phylogeny (Fig. 3). Data are alphabetically summarized in Table 1.

Cercospora mercurialis was confirmed for the first time in Iran (Asia) using multi-gene molecular data. In addition, several new host species and families were recognized for the previously known *Cercospora* species, including *C. apii*, *C. beticola*, *C.* cf. *flagellaris*, *C. gamsiana*, *C. iranica*, *Cercospora* sp. G & T in the world and some species were recorded for the first time in some provinces of Iran. The species are treated as follows.

Cercospora althaeina Sacc., Michelia 1: 269 (1878) (Fig. 4)

Description. Leaf spots distinct, angular to irregular, mostly vein-limited, olivaceous-brown, sometimes grey-brown with dark brown margin, center becoming pale grey with black dots (= stroma with conidiophores). Caespituli amphigenous, mostly epiphyllous. Mycelium internal. Stromata well-developed, emerging through stomatal openings or erumpent through the cuticle. Conidiophores in divergent fascicles (6–18), pale olivaceous-brown at the base, paler upwards, 2–8-septate, straight to mildly curved, (50–)130–170(–250) × 3.5–6 µm, conically narrowed at the apex; loci conspicuous, apical or on shoulders formed by geniculation, 1.5–2 µm. Conidia solitary, obclavate-cylindrical to filiform, not acicular, straight to mildly curved, hyaline, 4–12-septate, obtuse at the apex, subtruncate or obconically truncate at the base, (40–)70–95(–145) × 3–5 µm.

Specimen examined. IRAN, Mazandaran province, Amol, 36°28'31.21" N, 52°27'56.69"E, on leaves of *Malva* sp. (Malvaceae), 2 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3920C, IRAN 17716F).

Notes: Based on available literature (Ershad 2009; Bakhshi et al. 2012a, 2015a, 2018, Ershad et al. 2018), *C. althaeina* is reported here for the first time from Mazandaran Province.

Table 2. Details of primers developed for *gapdh* in this study.

Primer name	Primer sequence (5' to 3')	Orientation	Tm (ºC)	%GC	Annealing temperature
GpdF-Cer	TTCATYGAGCCMCACTACGCT	Forward	59.5	48–57	59→57→52
GpdR-Cer	RTCGGTGACKRCGAGVAC	Reverse	53.8	50–72	

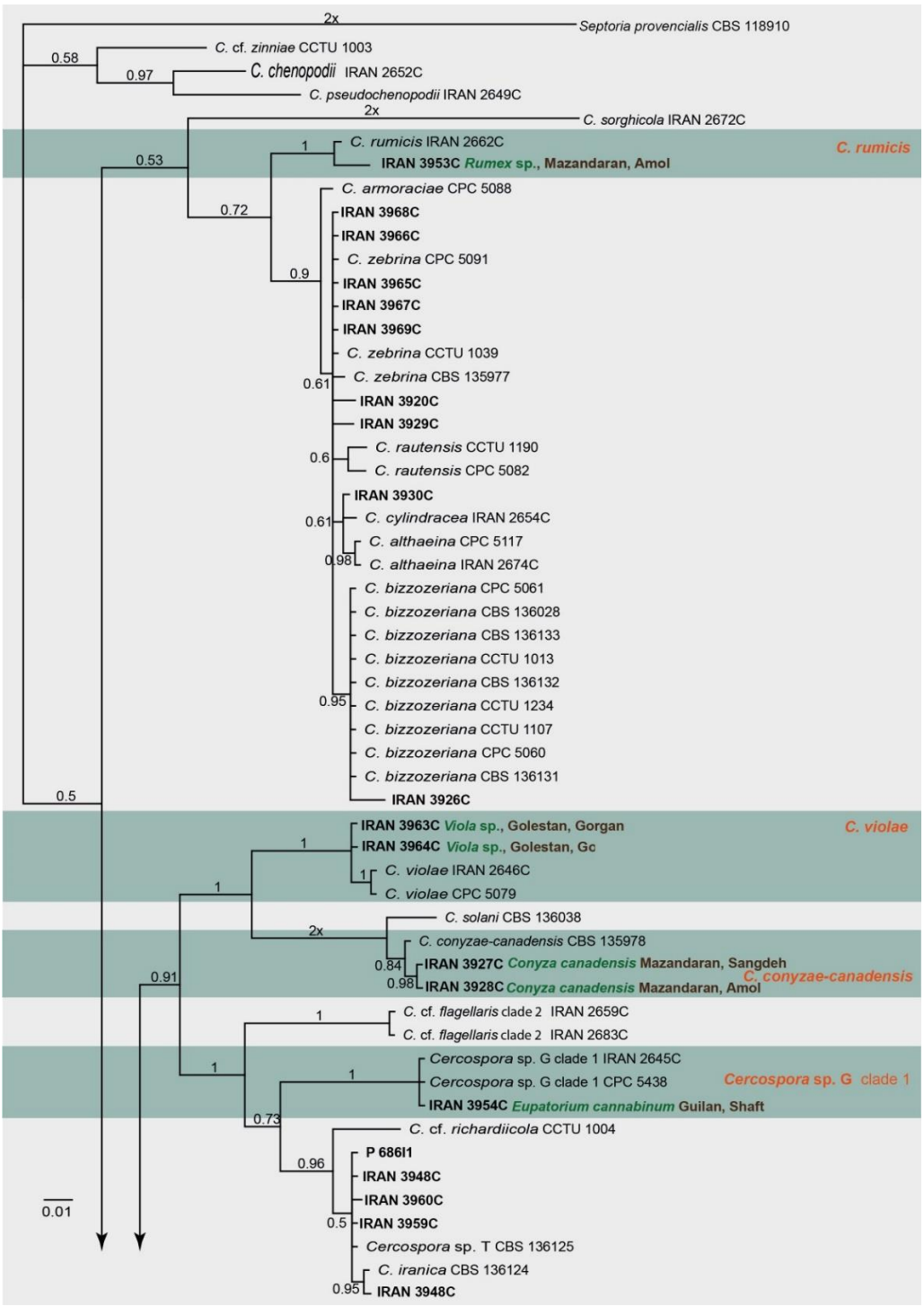

Fig 2. Part 1

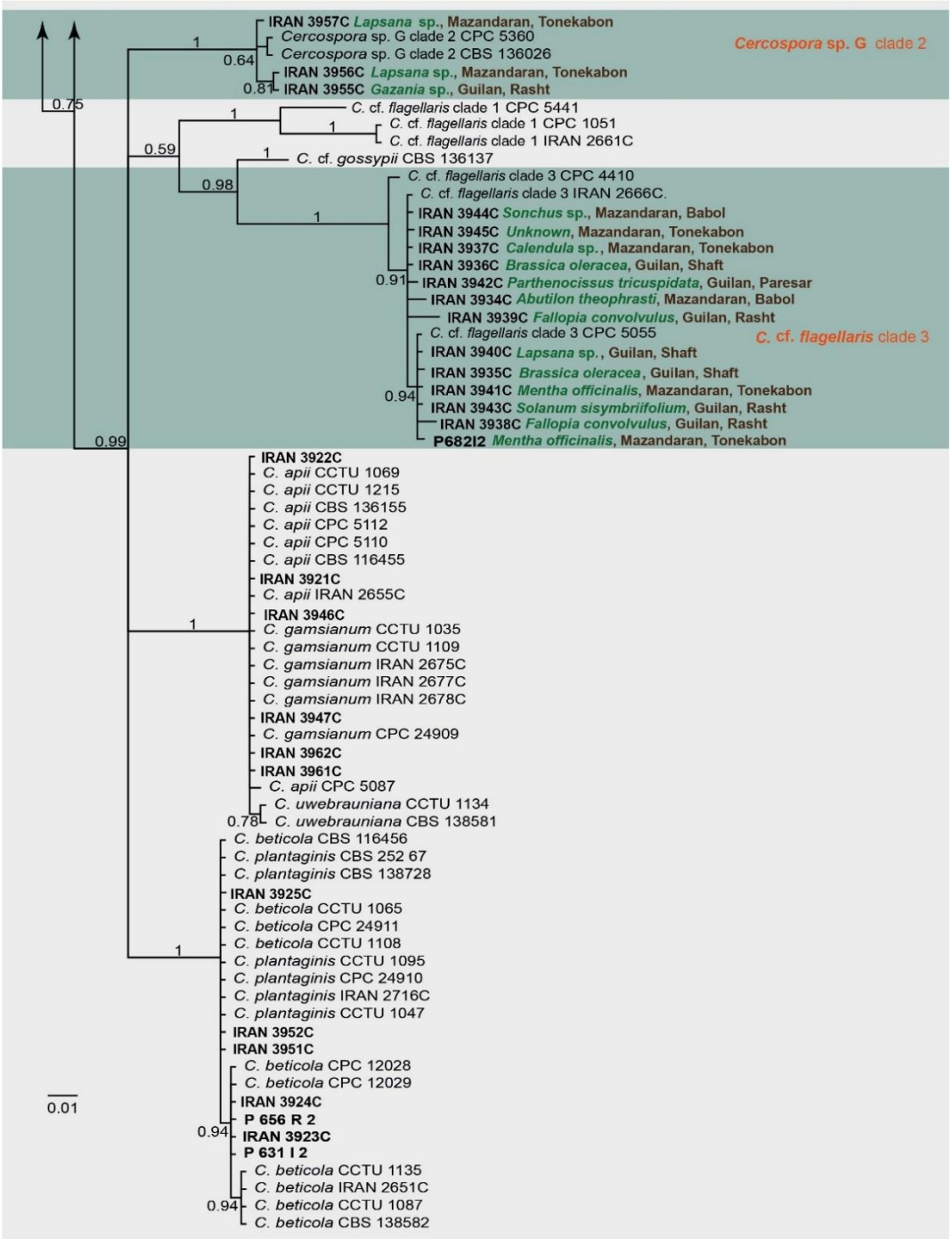

Fig. 2. part 2. Phylogenetic tree inferred by Bayesian analysis of the *gapdh* sequence alignment using MrBayes v.3.2.6. The scale bar indicates 0.01 expected changes per site. *Cercospora* species could be identified based on the results of the *gapdh* phylogeny, are indicated in colored blocks. Hosts and provinces of origin are indicated in green and brown text, respectively.

Cercospora apii Fresen., emend. Groenewald et al., Phytopathology 95: 954 (2005)

Description and illustration: Bakhshi et al. (2018).

Specimens examined. IRAN, Guilan province, Paresar, Pilembra, 37°35'43.51"N, 49°04'51.62"E, on leaves of *Apium graveolens* (Apiaceae), 17 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3921C, IRAN 17717F); Golestan province, Galikesh, 37°16'28.9"N 55°25'33.2"E, on leaves of *Ipomoea hederacea* (Convolvulaceae), 3 Nov. 2017, M. Bakhshi & A. Bahramishad (IRAN 3922C, IRAN 17718F).

Notes. In this investigation, *Cercospora apii* was found for the first time on *Ipomoea hederacea* in the world based on multi-gene phylogeny and morphological data.

Cercospora beticola Sacc., emend. Groenewald et al., Phytopathology 95: 954 (2005)

Description and illustration: Bakhshi et al. (2018).

Specimens examined. IRAN, Ardabil province, Moghan, 39°30'08.27"N, 48°02'38.62"E, on leaves of *Beta vulgaris*, 14 May 2018, M. Bakhshi (IRAN 3923C, IRAN 17720F) (P 631 I2, IRAN 17719F); Mazandaran province, Kelardasht, Goharkela, 36°28'59.04"N, 51°14'58.68"E, on leaves of *B. vulgaris*, 12 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3924C, IRAN 17721F); Mazandaran province, Marzanabad, Foshkour, 36°21'29.2"N 51°11'43.0"E, on leaves of *B. vulgaris*, 12 Aug. 2018, M. Bakhshi & A. Bahramishad (P 656 R2, IRAN 17722F); IRAN, Khuzestan province, Shush-Dezful, 32°15'14.5"N 48°22'46.9"E, on leaves of *Raphanus sativus* (Brassicaceae), 22 Feb. 2018, M. Bakhshi & F. Ghamghami (IRAN 3925C, IRAN 17723F).

Notes. In the present research, *C. beticola* is found for the first time on *Raphanus sativus* in the world, thus a further family, Brassicaceae was added to the host range of this species.

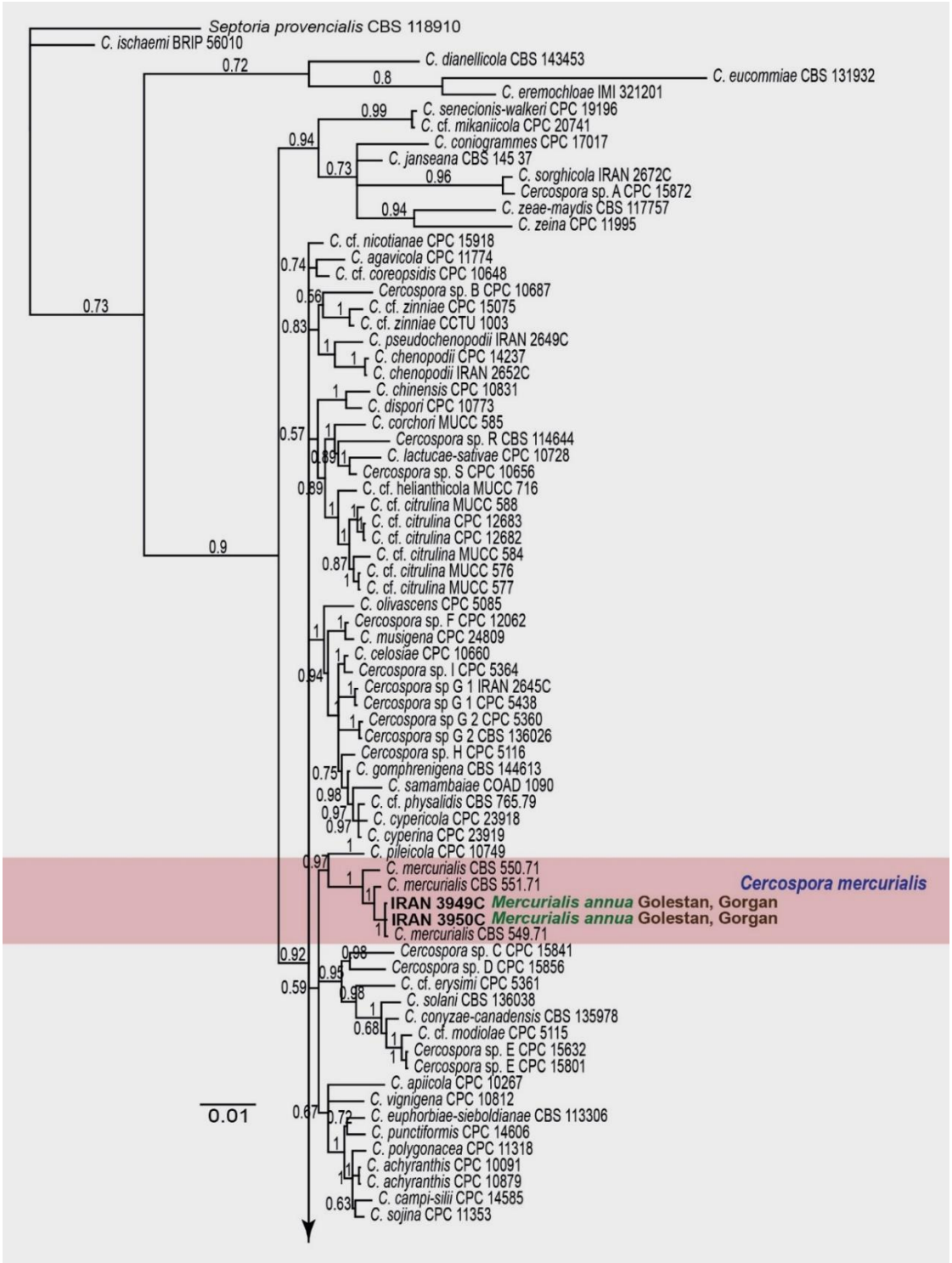

Fig 3. Part 1

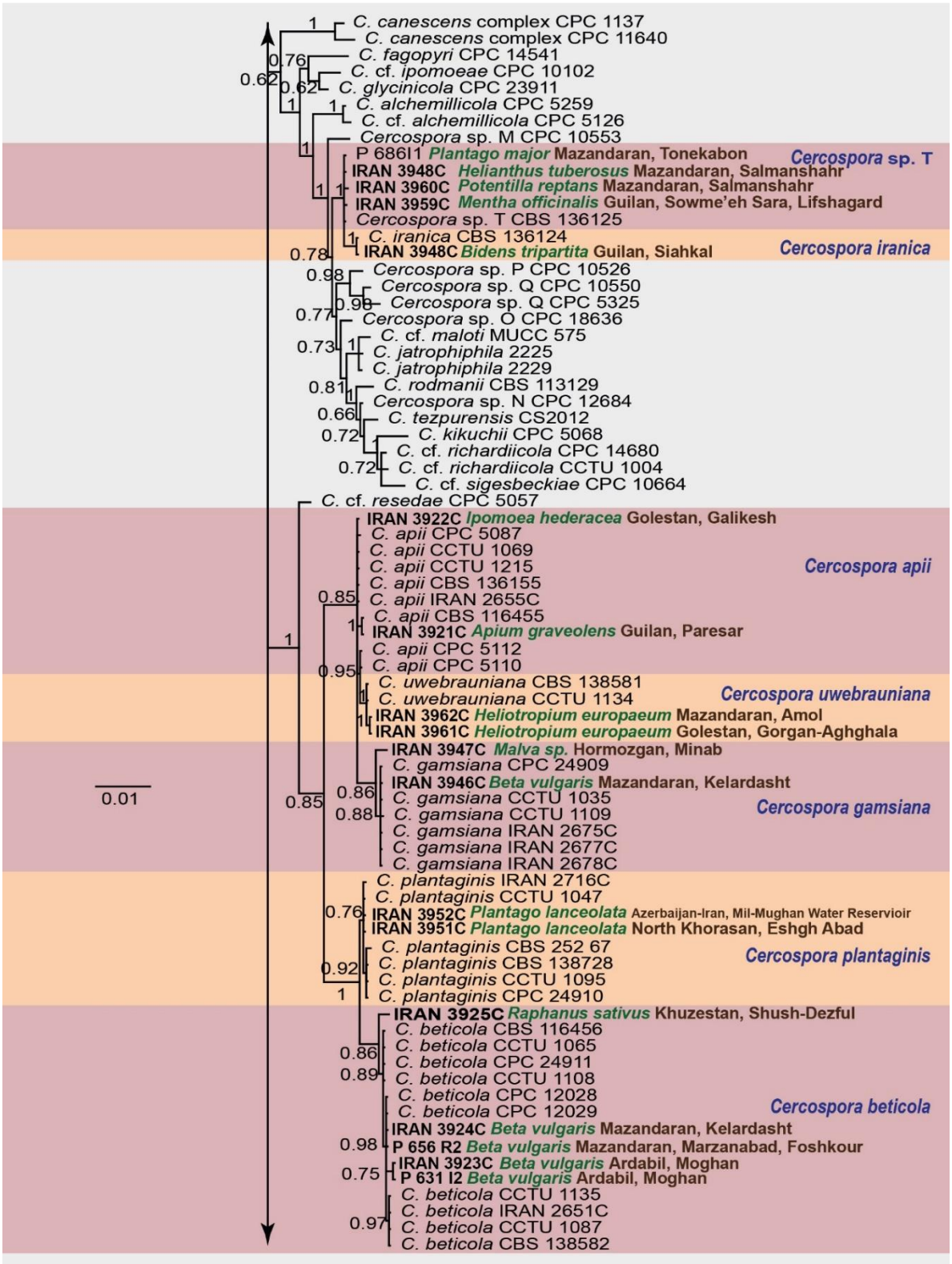
C. canescens complex CPC 1137
C. canescens complex CPC 11640
C. fagopyri CPC 14541
C. cf. ipomoeae CPC 10102
C. glycinicola CPC 23911
C. alchemillicola CPC 5259
C. cf. alchemillicola CPC 5126
Cercospora sp. M CPC 10553
P 686I1 Plantago major Mazandaran, Tonekabon
IRAN 3948C Helianthus tuberosus Mazandaran, Salmanshahr
IRAN 3960C Potentilla reptans Mazandaran, Salmanshahr
IRAN 3959C Mentha officinalis Guilan, Sowme'eh Sara, Lifshagard
Cercospora sp. T CBS 136125
Cercospora sp. T
C. iranica CBS 136124
IRAN 3948C Bidens tripartita Guilan, Siahkal
Cercospora iranica
Cercospora sp. P CPC 10526
Cercospora sp. Q CPC 10550
Cercospora sp. Q CPC 5325
Cercospora sp. O CPC 18636
C. cf. maloti MUCC 575
C. jatrophiphila 2225
C. jatrophiphila 2229
C. rodmanii CBS 113129
Cercospora sp. N CPC 12684
C. tezpurensis CS2012
C. kikuchii CPC 5068
C. cf. richardiicola CPC 14680
C. cf. richardiicola CCTU 1004
C. cf. sigesbeckiae CPC 10664
C. cf. resedae CPC 5057
IRAN 3922C Ipomoea hederacea Golestan, Galikesh
C. apii CPC 5087
C. apii CCTU 1069
C. apii CCTU 1215
C. apii CBS 136155
C. apii IRAN 2655C
C. apii CBS 116455
IRAN 3921C Apium graveolens Guilan, Paresar
C. apii CPC 5112
C. apii CPC 5110
Cercospora apii
C. uwebrauniana CBS 138581
C. uwebrauniana CCTU 1134
IRAN 3962C Heliotropium europaeum Mazandaran, Amol
IRAN 3961C Heliotropium europaeum Golestan, Gorgan-Aghghala
Cercospora uwebrauniana
IRAN 3947C Malva sp. Hormozgan, Minab
C. gamsiana CPC 24909
IRAN 3946C Beta vulgaris Mazandaran, Kelardasht
C. gamsiana CCTU 1035
C. gamsiana CCTU 1109
C. gamsiana IRAN 2675C
C. gamsiana IRAN 2677C
C. gamsiana IRAN 2678C
Cercospora gamsiana
0.01
C. plantaginis IRAN 2716C
C. plantaginis CCTU 1047
IRAN 3952C Plantago lanceolata Azerbaijan-Iran, Mil-Mughan Water Reservioir
IRAN 3951C Plantago lanceolata North Khorasan, Eshgh Abad
C. plantaginis CBS 252 67
C. plantaginis CBS 138728
C. plantaginis CCTU 1095
C. plantaginis CPC 24910
Cercospora plantaginis
IRAN 3925C Raphanus sativus Khuzestan, Shush-Dezful
C. beticola CBS 116456
C. beticola CCTU 1065
C. beticola CPC 24911
C. beticola CCTU 1108
C. beticola CPC 12028
C. beticola CPC 12029
IRAN 3924C Beta vulgaris Mazandaran, Kelardasht
P 656 R2 Beta vulgaris Mazandaran, Marzanabad, Foshkour
IRAN 3923C Beta vulgaris Ardabil, Moghan
P 631 I2 Beta vulgaris Ardabil, Moghan
C. beticola CCTU 1135
C. beticola IRAN 2651C
C. beticola CCTU 1087
C. beticola CBS 138582
Cercospora beticola

Fig 3. Part 2

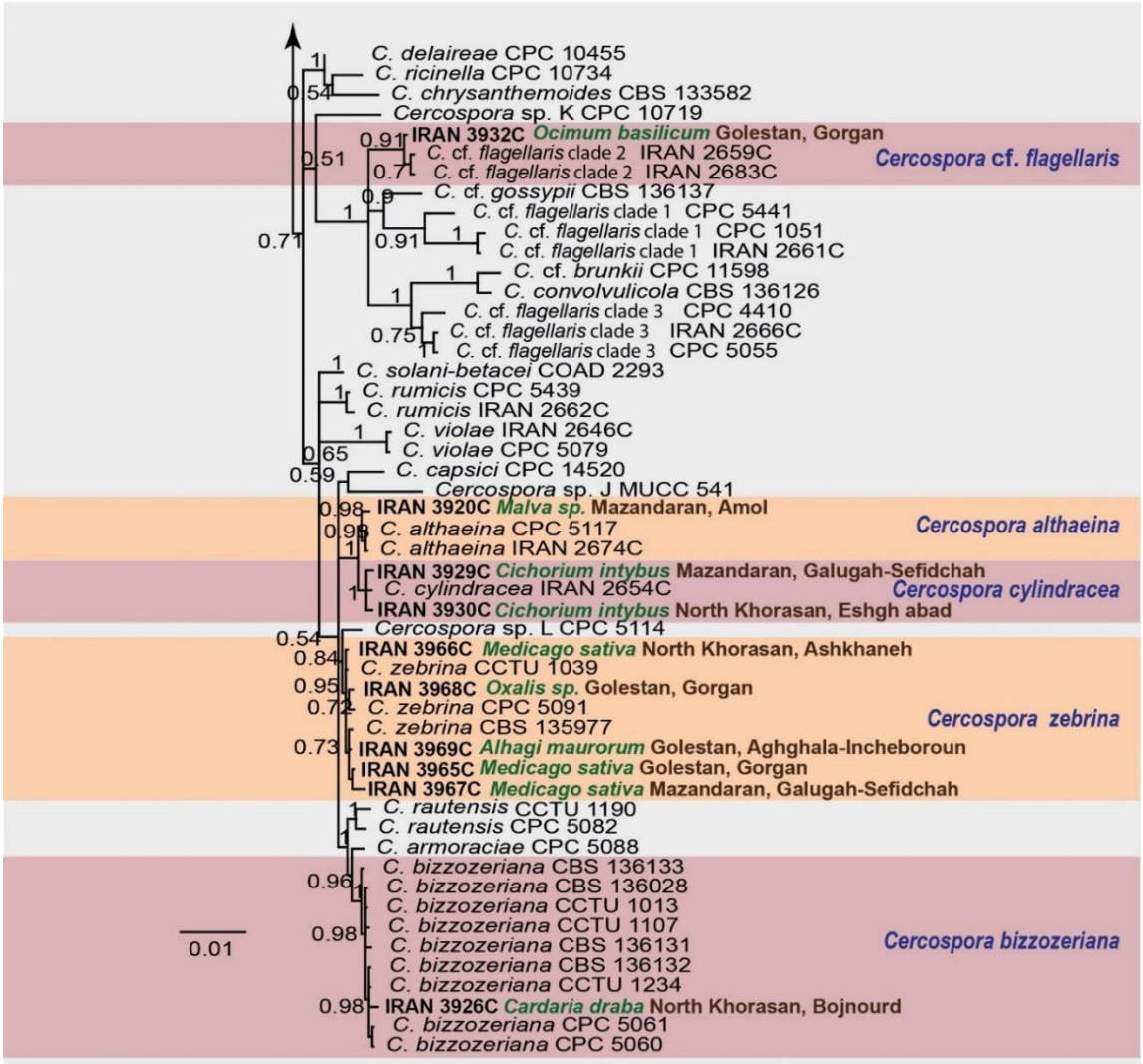

Fig. 3. Part 3. Phylogenetic tree inferred by Bayesian analysis of the combined 6-gene (ITS, *tef1*, *actA*, *cmdA*, *his3* and *gapdh*) sequence alignment using MrBayes v.3.2.6. The scale bar indicates 0.01 expected changes per site. *Cercospora* species could be identified based on the results of the 6-gene phylogeny, are indicated in colored blocks. Hosts and provinces of origin are indicated in green and brown text, respectively.

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

Description and illustration: Bakhshi et al. (2018).

Specimens examined. IRAN, North Khorasan province, Bojnourd, 37°28'35.27"N, 57°19'01.47"E, on leaves of *Cardaria draba* (Brassicaceae), 6 Nov. 2017, M. Bakhshi & A. Bahramishad (IRAN 3926C, IRAN 17724F).

Cercospora conyzae-canadensis M. Bakhshi, Arzanlou, Babai-ahari, Crous & U. Braun, Persoonia 34: 77 (2015a)

Description and illustration: Bakhshi et al. (2015a).

Specimens examined. IRAN, Mazandaran province, Sangdeh, 36°08'05.72"N, 53°12'49.12"E, on leaves of *Conyza canadensis* (Asteraceae), 31 Oct. 2017, M. Bakhshi & A. Bahramishad (IRAN 3927C, IRAN 17725F); Mazandaran province, Amol, Baudeh, 36°34'52.46"N, 52°20'59.88"E, on leaves of *Conyza canadensis*, 3 May 2018, M. Bakhshi & A. Bahramishad (IRAN 3928C, IRAN 17726F).

Notes: *Cercospora conyzae-canadensis* was described recently by Bakhshi et al. (2015a) from Guilan and Zanjan provinces as host-specific to *Conyza canadensis*. Here the species recorded on this host, for the first time from Mazandaran Province.

Cercospora cylindracea M. Bakhshi, Arzanlou, Babai-ahari, Crous & U. Braun, Persoonia 34: 78 (2015a)

Description and illustration: Bakhshi et al. (2015a).

Specimens examined. IRAN, Mazandaran province, Galugah-Sefidchah, 36°41'50.38"N, 53°47'58.84"E, on leaves of *Cichorium intybus* (Asteraceae), 8 Nov. 2017, M. Bakhshi & A. Bahramishad (IRAN 3929C, IRAN 17727F); North Khorasan province, Eshghabad, Raz, 37°41'47.6"N

56°55'08.7"E, on leaves of *Cichorium intybus*, 7 Nov. 2017, M. Bakhshi & A. Bahramishad (IRAN 3930C, IRAN 17728F).

Notes: *Cercospora cylindracea* was described by Bakhshi et al. (2015a) from Ardabil, West Azerbaijan and Zanjan provinces on the host plants, *Cichorium intybus* and *Lactuca serriola* (Asteraceae) based on multi-gene phylogeny and morphological data. The species recorded here for the first time from North Khorasan and Mazandaran Provinces.

Cercospora* cf. *flagellaris

Description and illustration: Bakhshi et al. (2018).

Specimens examined. IRAN, Guilan province, Rasht, 37°11'04.66"N, 49°39'34.09"E, on leaves of *Conyza canadensis*, 14 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3931C, IRAN 17729F); Golestan province, Gorgan, 36°50'26.22"N, 54°27'24.98"E, on leaves of *Ocimum basilicum* (Lamiaceae), 5 July 2017, M. Bakhshi & F. Ghamghami (IRAN 3932C, IRAN 17730F); Mazandaran province, Tonekabon, Sehezar Road, 36°36'14.26"N, 50°50'20.64"E, on leaves of *Plantago major* (Plantaginaceae), 13 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3933C, IRAN 17731F); Mazandaran province, Babol, Tazehabad, 36°33'01.58"N, 52°47'39.56"E, on leaves of *Abutilon theophrasti* (Malvaceae), 11 Oct. 2017, M. Bakhshi & F. Ghamghami (IRAN 3934C, IRAN 17732F); Guilan province, Shaft, Siahmazgi, Livandan, 37°01'19.13"N, 49°16'25.45"E, on leaves of *Brassica oleracea* (Brassicaceae), 16 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3935C, IRAN 17733F) (IRAN 3936C, IRAN 17734F); Mazandaran province, Tonekabon, Sehezar Road, 36°36'14.26"N, 50°50'20.64"E, on leaves of *Calendula* sp. (Asteraceae), 13 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3937C, IRAN 17735F); Guilan province, Talesh, Jokandan, on leaves of *Fallopia convolvulus* (Polygonaceae), 25 Aug. 2019, M. Kermanian (IRAN 3938C, IRAN 17736F); Guilan province, Havigh, Eshikaghasi, on leaves of *Fallopia convolvulus*, 25 Aug. 2019, M. Kermanian (IRAN 3939C); Guilan province, Shaft, Siahmazgi,

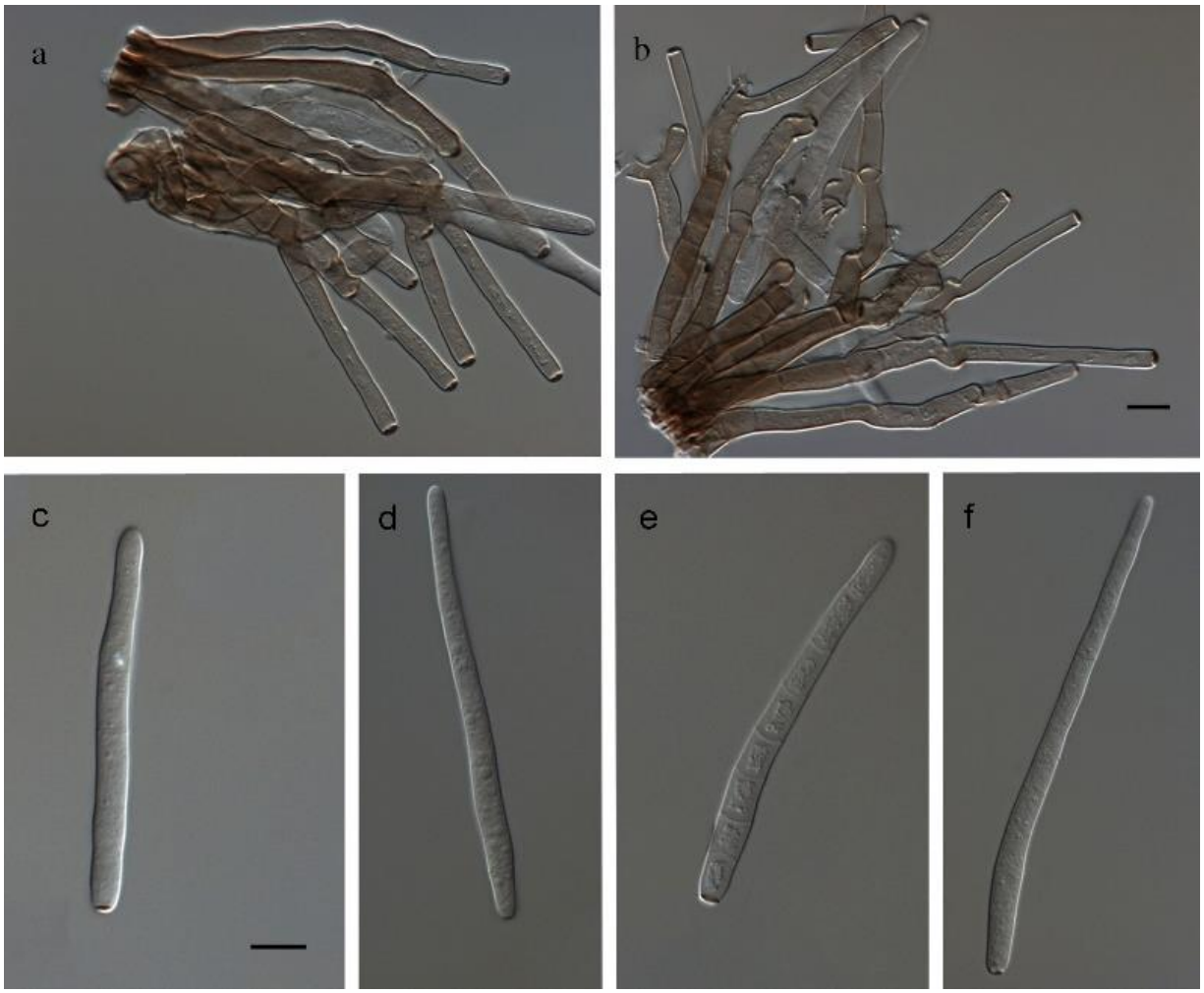

Fig. 4. *Cercospora althaeina*. **a, b.** Fasciculate conidiophores; **c–f.** Conidia. — Scale bars = 10 µm.

Doudvazan Waterfall, 37°01'02.61"N, 49°15'01.21"E, on leaves of *Lapsana* sp. (Asteraceae), 16 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3940C, IRAN 17737F); Mazandaran province, Tonekabon, Sehezar Road, 36°36'14.26"N, 50°50'20.64"E, on leaves of *Mentha longifolia* (Lamiaceae), 13 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3941C, P 682I2, IRAN 17738F); Guilan province, Paresar, Pilembra, 37°35'43.51"N, 49°04'51.62"E, on leaves of *Parthenocissus tricuspidata* (Vitaceae), 17 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3942C, IRAN 17739F); Guilan province, Rasht, Saravan, 37°10'34.52"N, 49°35'50.17"E, on leaves of *Solanum sisymbriifolium* (Solanaceae), 16 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3943C, IRAN 17740F); Mazandaran province, Babol, Tazehabad, 36°33'01.58"N, 52° 47'39.56"E, on leaves of *Sonchus* sp. (Asteraceae), 11 Oct. 2017, M. Bakhshi & F. Ghamghami (IRAN 3944C, IRAN 17741F). Mazandaran province, Tonekabon, Dohezar Road, Barseh, 36°38'28.1"N, 50°43'48.5"E, Unknown, 13 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3945C, IRAN 17742F).

Notes: Recently based on the combination of morphological and multi-gene phylogenetic analysis, it has been demonstrated that *C.* cf. *flagellaris* is a plurivorous species with multiple family-associations in different groups of plants viz. agricultural crops, ornamentals, forest trees and weeds including Acerceae, Amaranthaceae, Araceae, Asteraceae, Balsaminaceae, Brassicaceae, Buxaceae, Caesalpinaceae, Campanulaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Geraniaceae, Hydrangeaceae, Malvaceae, Oleaceae, Onagraceae, Phytolaccaceae, Poaceae, Pontederiaceae, Rutaceae, Salicaceae, Solanaceae and Urticaceae, and is geographically distributed worldwide (Groenewald et al. 2013; Bakhshi et al. 2015a, 2018; Farr & Rossman 2020). Similar to Bakhshi et al. (2015a, 2018), in the present study, *C.* cf. *flagellaris* was the most common species in the country. Additionally, *C.* cf. *flagellaris* is newly recorded here on 10 new hosts, *Brassica oleracea*, *Conyza canadensis*, *Fallopia convolvulus*, *Lapsana* sp., *Mentha longifolia*, *Ocimum basilicum*, *Parthenocissus tricuspidata*, *Plantago major*, *Solanum sisymbriifolium* and *Sonchus* sp. in the world. Thus, three more plant families, including Lamiaceae, Polygonaceae and Vitaceae are here reported as a new host of this species. In addition, it is reported for the first time from Golestan province.

Cercospora gamsiana M. Bakhshi & Crous, IMA Fungus 9: 321 (2018)

Description and illustration: Bakhshi et al. (2018).

Specimens examined. IRAN, Mazandaran province, Kelardasht, Goharkela, 36°28'59.04"N, 51°14'58.68"E, on leaves of *B. vulgaris*, 12 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3946C, IRAN 17743F); Hormozgan province, Minab, 27°06'29.9"N 57°05'28.4"E, on leaves of *Malva* sp. (Malvaceae), 9 March 2018, M. Bakhshi (IRAN 3947C, IRAN 17744F).

Notes: *Cercospora gamsiana* was described recently by Bakhshi et al. (2018) on *Malva* spp., *Rumex crispus*, *Sesamum indicum* and *Sonchus* sp. from north and north-west of Iran. The species reported here for the first time from the south of Iran (Hormozgan province). Furthermore, the report of this species on *Beta vulgaris* is new for the world.

Cercospora iranica M. Bakhshi, Arzanlou, Babai-ahari, Crous & U. Braun, Persoonia 34: 79 (2015a)

Description and illustration: Bakhshi et al. (2015a).

Specimens examined. IRAN, Guilan province, Siahkal, 37°11'58.61"N, 49°55'20.78"E, on leaves of *Bidens tripartite* (Asteraceae), 14 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3948C, IRAN 17745F).

Notes: *Cercospora iranica* was described by Bakhshi et al. (2015a) on *Vicia faba* (Fabaceae) and *Hydrangea* sp. (Hydrangeaceae). Report of this species on *Bidens tripartita* is new for the world.

Cercospora mercurialis Pass., in Thüm., Mycoth. Univ., No. 783. (1877) (Fig. 5)

Description. Leaf spots amphigenous, circular to subcircular, 1–5 mm, grey-brown, with dark brown border. Mycelium internal. Caespituli amphigenous, brown. Conidiophores aggregated in moderately loose fascicles (3–15), arising from a moderately-developed, erumpent, brown stroma, up to 25 µm diam; conidiophores pale to medium brown, aseptate or sparingly septate, straight to geniculate-sinuous due to sympodial proliferation, simple, uniform in width, sometimes constricted at the proliferating point, (15–)30–40(–55) × 3.5–4(–5) µm. Conidiogenous cells intercalary and terminal, sometimes conidiophores reduced to conidiogenous cells, pale brown, proliferating sympodially, 15–30 × 3.5–5 µm, multi-local; loci distinctly thickened, darkened and somewhat refractive, apical, lateral or formed on shoulders caused by geniculation, 2–3 µm diam. Conidia solitary, cylindrical to acicular, straight to slightly curved, hyaline, (25–)55–80(–120) × 2.5–5 µm, (3–)6–9(–15)-septate, with subobtusely rounded apices and subtruncate or obconically truncate bases; hila thickened, darkened, refractive, 1–2 µm diam.

Specimens examined. IRAN, Golestan province, Gorgan, 36°50'26.22"N, 54°27'24.98"E, on leaves of *Mercurialis annua* (Euphorbiaceae), 1 Nov. 2017, M. Bakhshi (IRAN 3949C, IRAN 17746F); on leaves of *M. annua*, 5 May 2018, M. Bakhshi (IRAN 3950C, IRAN 17747F)

Notes: *Cercospora mercurialis* was reported from Iran based on morphological data (Pirnia et al. 2010). To our knowledge, this study is the first molecular confirmation of *C. mercurialis* in Asia. Furthermore, part of the *gapdh* is sequenced for the first time in this species.

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

Description and illustration: Bakhshi et al. (2018).

Specimens examined. IRAN, North Khorasan province, Eshghabad, Raz, 37°41'47.58"N, 56°55'08.65"E, on leaves of *Plantago lanceolata* (Plantaginaceae), 7 Nov. 2017, M. Bakhshi & A. Bahramishad (IRAN 3951C, IRAN 17748F); Azerbaijan-Iran border, Ardabil province, Mil-Mughan Water Reservoir, 39°25'55.7"N, 47°22'16.8"E, on leaves of *Plantago lanceolata*, 15 May 2018, M. Bakhshi (IRAN 3952C, IRAN 17749F).

Notes: Recently, Bakhshi et al. (2018) have designated an epitype for *C. plantaginis* based on the combination of morphological and molecular data and have shown that the species is host-specific to *Plantago lanceolata.* This is the first report of this species from North Khorasan Province.

Cercospora rumicis Pavgi & U.P. Singh, Mycopathol. Mycol. Appl. 23: 191 (1964) (Fig. 6)

Description. Leaf spots circular to subcircular, with grey center and purple-brown margin, 2–8 mm diam. Mycelium internal. Caespituli amphigenous, brown. Conidiophores in divergent fascicles, arising from the upper cells of a moderately to well-developed, intraepidermal and substomatal, brown stroma; conidiophores pale brown to brown, 1–6-septate, straight, sinuous to distinctly geniculate, (40–)58–70 × 4– 5 µm, irregular in width, constricted at the parts of proliferation or at the septa. Conidiogenous cells terminal or intercalary, unbranched, pale brown, smooth, proliferating sympodially, multi-local; loci thickened, darkened, refractive, apical, or formed on the shoulders caused by geniculation. Conidia solitary, subcylindrical to filiform, straight to mildly curved, hyaline, distinctly 2–15-septate, subobtuse at the apex, truncate at the base, (37–)80–110(–160) × 2.5–5 µm; hila thickened, darkened, refractive, 1.5–2.5 µm diam.

Specimen examined. IRAN, Mazandaran province, Amol, Najarmahalleh, 36°26'39.88"N, 52°27'11.02"E, on leaves of *Rumex* sp. (Polygonaceae), 3 May 2018, M. Bakhshi & A. Bahramishad (IRAN 3953C, IRAN 17750F).

Notes: *Cercospora rumicis* recorded here for the first time from Mazandaran Province.

***Cercospora* sp. G** sensu Groenewald et al. (2013)

Description and illustration: Bakhshi et al. (2018).

Specimens examined. IRAN, Guilan province, Shaft, Siahmazgi, Doudvazan Waterfall, 37°01'0 2.61"N, 49°15'01.21"E, on leaves of *Eupatorium cannabinum* (Asteraceae), 16 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3954C, IRAN 17751F); Guilan province, Rasht, 37°11'04.66"N, 49°39'3 4.09"E, on leaves of *Gazania* sp. (Asteraceae), 14 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3955C, IRAN 17752F); Mazandaran province, Tonekabon, Dohezar Road, Barseh, 36°38'28.1"N, 50°43'48.5"E, on leaves of *Lapsana* sp., 13 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3956C, IRAN 17753F) (IRAN 3957C, IRAN 17754F).

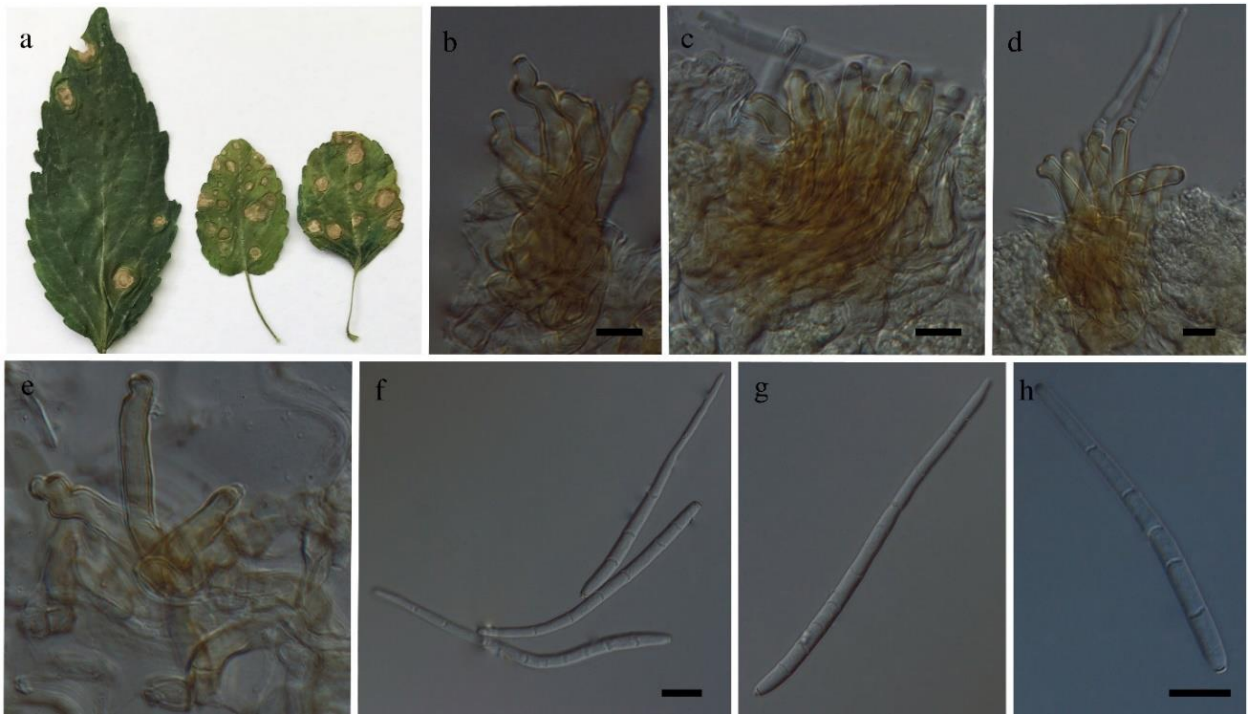

Fig. 5. *Cercospora mercurialis*. **a.** Leaf spot; **b–e.** Fasciculate conidiophores; **f–h.** Conidia. — Scale bars = 10 µm.

Fig. 6. *Cercospora rumicis*. **a–c.** Fasciculate conidiophores; **d–f.** Conidia. — Scale bars = 10 µm.

Notes: *Cercospora* sp. G occurs on a wide host range such as Amaranthaceae, Asteraceae, Cucurbitaceae, Lamiaceae, Malvaceae, Plantaginaceae, Poaceae (Groenewald et al. 2013, Bakhshi et al. 2015a). *Cercospora* sp. G is found in this research on three new hosts, *Eupatorium cannabinum*, *Gazania* sp. and *Lapsana* sp. in the world, and additionally for the first time from Mazandaran province.

***Cercospora* sp. T** sensu Bakhshi et al. (2015a)

Description and illustration: Bakhshi et al. (2015a).

Specimens examined. IRAN, Mazandaran province, Salmanshahr, 36°42'22.4"N, 51°12'44.6"E, on leaves of *Helianthus tuberosus* (Asteraceae), 13 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3958C, IRAN 17755F); Guilan province, Sowme'eh Sara, Lifshagard, 37°19'49.0"N, 49°25'12.6"E, on leaves of *Mentha longifolia*, 17 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3959C, IRAN 17756F); Mazandaran province, Tonekabon, Sehezar Road, 36°36'14.26"N, 50°50'20.64"E, on leaves of *Plantago major*, 13 Aug. 2018, M. Bakhshi & A. Bahramishad (P 686 I1); Mazandaran province, Salmanshahr, 36°42'22.4"N, 51°12'44.6"E, on leaves of *Potentilla reptans* (Rosaceae), 13 Aug. 2018, M. Bakhshi & A. Bahramishad (IRAN 3960C, IRAN 17757F).

Notes: *Cercospora* sp. T was reported on *Coreopsis* sp. (Asteraceae) (Bakhshi et al. 2015a). In this research, *Cercospora* sp. T, is found on four new hosts, *Helianthus tuberosus*, *Mentha longifolia*, *Plantago major* and *Potentilla reptans*; therefore, three more plant families, including Lamiaceae, Plantaginaceae and Rosaceae are newly recorded as the hosts of this species in the world. In addition, it is reported for the first time from Mazandaran province.

Cercospora uwebrauniana M. Bakhshi & Crous, IMA Fungus 9: 317 (2018)

Description and illustration: Bakhshi et al. (2018).

Specimens examined. IRAN, Golestan province, Gorgan-Aghghala, 36°52'15.4"N, 54°25'49.4"E, on leaves of *Heliotropium europaeum* (Boraginaceae), 1 Nov. 2017, M. Bakhshi & A. Bahramishad (IRAN 3961C, IRAN 17758F); Mazandaran province, Amol, Ejbarkola, 36°28'31.2"N 52°27'56.7"E, on leaves of *Heliotropium europaeum*, 14 Oct. 2017, M. Bakhshi & A. Bahramishad (IRAN 3962C).

Notes: *Cercospora uwebrauniana* was described recently by Bakhshi et al. (2018) and appears to be host specific to *Heliotropium europaeum*. Here we report this species for the first time from Golestan and Mazandaran provinces.

Cercospora violae Sacc., Nuovo Giron. Bot. Ital. 8: 187 (1876) (Fig. 7)

Description. Leaf spots circular to irregular, mostly vein-limited, dark brown, with concentric rings (= stroma with conidiophores), 2–8 mm diam. Mycelium internal. Caespituli amphigenous. Stromata lacking to moderately developed, dark brown, intraepidermal, and substomatal. Conidiophores in moderately dense fascicles, irregular in width, slightly attenuated at the upper portion, straight or mildly sinuous-geniculate, straight, simple, rarely branched, pale brown to brown, short conically truncate at the apex, wider at the base, 45–70(–90) × 3.5–4.5 µm, 2–12-septate. Conidiogenous cells integrated, terminal, rarely intercalary, proliferating sympodially, multi-local; loci distinct, thickened, apical, or formed on shoulders caused by geniculation, 2–3.5 µm diam. Conidia solitary, hyaline, subcylindrical to obclavate or acicular, straight to slightly curved, truncate at the base, subobtuse at the apex, 44–95(–132) × 2.5–3.5 µm, 3–14-septate, smooth.

Specimen examined. IRAN, Golestan province, Gorgan, Shastkola, 36°46'59.0"N, 54°21'58.0"E, on leaves of *Viola* sp. (Violaceae), 6 July 2017, M. Bakhshi & F. Ghamghami (IRAN 3963C, IRAN 17759F) (IRAN 3964C, IRAN 17760F).

Cercospora zebrina Pass., Hedwigia 16: 124 (1877) (Fig. 8)

Description. Leaf spots distinct, circular to irregular, brown to dark grey, without definite borders. Caespituli amphigenous. Mycelium internal. Stromata well-developed, intraepidermal or substomatal. Conidiophores in moderately dense fascicles (4–18), brown at the base, paler upwards, 1–6-septate, straight to mildly curved, (30–)50–65(–98) × 3.5–5 µm. Conidiogenous cells mostly terminal, pale brown, proliferating sympodially, uni-local to multi-local; loci conspicuous, thickened, darkened, refractive, apical, 2–3 µm. Conidia solitary, rarely catenate, cylindrical to obclavate-subcylindrical, straight to mildly curved, hyaline, 3–14-septate, obtuse at the apex, subtruncate or obconically truncate at the base, (30–)50–85(–135) × 3–5 µm.

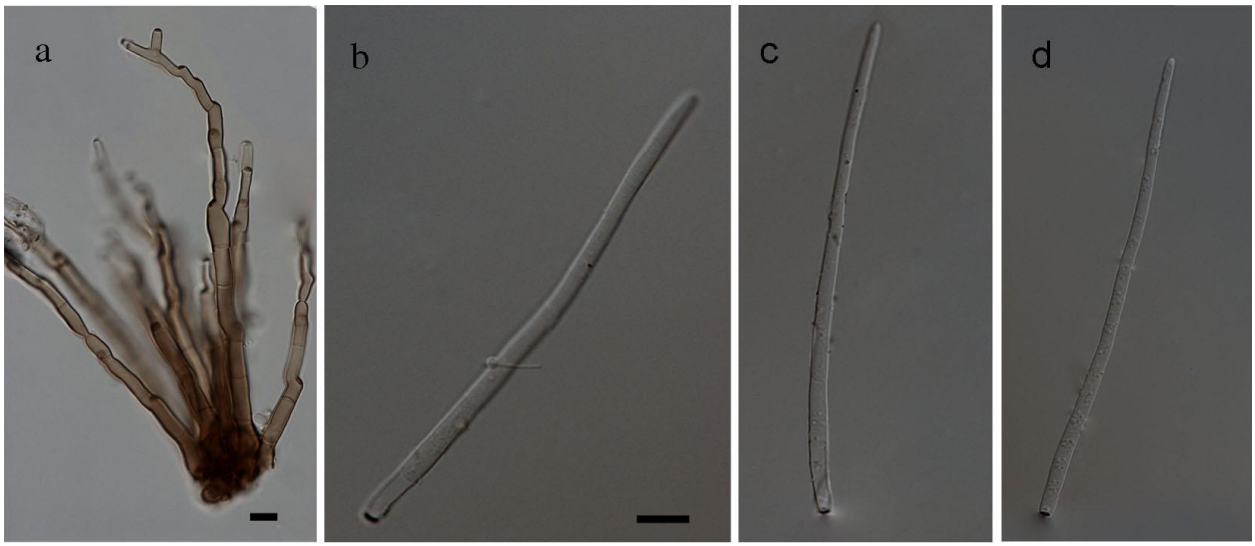

Fig. 7. *Cercospora violae*. **a.** Fasciculate conidiophores; **b–c.** Conidia. — Scale bars = 10 µm.

Fig. 8. *Cercospora zebrina*. **a.** Fasciculate conidiophores; **b–c.** Conidia. — Scale bars = 10 µm.

Specimens examined. IRAN, Golestan province, Gorgan, 36°50'26.2"N 54°27'25.0"E, on leaves of *Medicago sativa* (Fabaceae), 5 July 2017, M. Bakhshi & F. Ghamghami (IRAN 3965C, IRAN 17761F); North Khorasan province, Ashkhaneh, 37°35'13.2"N 56°52'13.7"E, on leaves of *Medicago sativa*, 7 Nov. 2017, M. Bakhshi & A. Bahramishad (IRAN 3966C, IRAN 17762F); Mazandaran province, Galugah-Sefidchah, 36°41'50.38"N, 53°47'58.84"E, on leaves of *Medicago sativa*, 4 May 2018, M. Bakhshi & A. Bahramishad (IRAN 3967C, IRAN 17763F); Golestan province, Gorgan, Ghorogh Forest Park, 36°52'58.5"N, 54°40'47.2"E, on leaves of *Oxalis* sp. (Fabaceae), 7 May 2018, M. Bakhshi & A. Bahramishad (IRAN 3968C, IRAN 17764F); Golestan province, Aghghala–Incheboroun, Agh–Ghabr, 37°00'42.6"N 54°23'43.1"E, on leaves of *Alhagi maurorum* (Fabaceae), 11 Nov. 2019, M. Bakhshi & A. Torabi (IRAN 3969C, IRAN 17765F).

Notes: To our knowledge, here, we report *C. zebrina* for the first time from Golestan and North Khorasan Provinces.

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طراحی آغازگرهای جدید برای تکثیر ژن *gapdh* در جنس سرکوسپورا و گزارش گونه‌ها و میزبان‌های جدید برای ایران

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چکیده: برای مطالعه فیلوژنی مولکولی جنس سرکوسپورا، در سال‌های اخیر، علاوه بر ناحیه ژنی ITS، نواحی رمز کننده پروتئین از قبیل ژن‌های *actA*، *cmdA*، *gapdh*، *his3*، *rpb2*، *tef1* و *tub2* نیز به کار گرفته شده‌اند. با وجود اینکه ناحیه *gapdh*، ژن خوبی برای تفکیک گونه‌های جنس سرکوسپورا شناخته شده است، تکثیر این ژن با استفاده از آغازگرهای موجود در این جنس دشوار است. بنابراین در این تحقیق، آغازگرهای جدید به نام‌های GpdF-Cer (5'-TTC ATY GAG CCM CAC TAC GCT-3') و GpdR-Cer (5'-RTC GGT GAC KRC GAG VAC-3') برای تکثیر این ژن طراحی شد که برای تکثیر ناحیه *gapdh* در این جنس مناسب می‌باشند. علاوه بر این، در ادامه مطالعه آرایه‌های جنس سرکوسپورا در ایران با استفاده از مفهوم ترکیبی گونه، نمونه‌های گیاهی با علایم لکه برگی جمع آوری شدند و جدایه‌های سرکوسپورای بدست آمده بر اساس ترکیب ویژگی‌های ریخت‌شناختی و توالی شش ناحیه ژنی ITS، *actA*، *cmdA*، *gapdh*، *his3* و *tef1* شناسایی شدند. هفده گونه از جنس سرکوسپورا شناسایی شدند و گونه *C. mercurialis* از روی گیاه شنگرفی یک ساله (*Mercurialis annua*) برای اولین بار در قاره آسیا با استفاده از داده‌های توالی شش ناحیه ژنی در ایران شناسایی شد. چندین میزبان گیاهی جدید برای گونه‌های *C. apii* (یک گونه)، *C. beticola* (یک گونه)، *C.* cf. *flagellaris* (۱۰ گونه)، *C. gamsiana* (یک گونه)، *C. iranica* (یک گونه)، *Cercospora* sp. G (سه گونه) و *Cercospora* sp. T (چهار گونه) در دنیا شناسایی شدند. در نتیجه چندین تیره گیاهی به دامنه میزبانی گونه‌های *C. beticola* (Brassicaceae)، *C.* cf. *flagellaris* (Lamiaceae، Polygonaceae، Vitaceae) و *Cercospora* sp. T (Lamiaceae، Plantaginaceae، Rosaceae) در دنیا اضافه شد.

کلمات کلیدی: آغازگر جدید، قارچ‌های سرکوسپوروئید، تنوع زیستی، میکوسفرلاسه، لکه برگی.

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