FUSE

Fungal Systematics and Evolution

VOLUME 3
JUNE 2019
PAGES 157–163

doi.org/10.3114/fuse.2019.03.08

Epitypification of *Cercospora rautensis*, the causal agent of leaf spot disease on *Securigera varia*, and its first report from Iran

M. Bakhshi*

Department of Botany, Iranian Research Institute of Plant Protection, P.O. Box 19395-1454, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

*Corresponding author: mounesbakhshi@gmail.com

Key words:
Cercospora armoraciae
complex
cercosporoid
leaf spot
Mycosphaerellaceae
new epitype

Abstract: *Cercospora* is a well-studied and important genus of plant pathogenic species responsible for leaf spots on a broad range of plant hosts. The lack of useful morphological traits and the high degree of variation therein complicate species identifications in *Cercospora*. Recent studies have revealed multi-gene DNA sequence data to be highly informative for species identification in *Cercospora*. During the present study, *Cercospora* isolates obtained from Crownvetch (*Securigera varia*) in Iran and Romania were subjected to an eight-gene (ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, *rpb2* and *gapdh*) analysis. By applying a polyphasic approach including morphological characteristics, host data, and molecular analyses, these isolates were identified as *C. rautensis.* To our knowledge, this is the first record of *C. rautensis* from Iran (Asia). In addition, an epitype is designated here for *C. rautensis*.

Effectively published online: 13 March 2019.

INTRODUCTION

Crownvetch (*Securigera varia* ≡ *Coronilla varia*), is a herbaceous, perennial legume with creeping stems belonging in the family *Fabaceae* which is native to the Mediterranean region of Europe, southwest Asia, and northern Africa (Roland 1998). It is an intercropping plant in many orchards in the world, with many benefits, including controlling weeds, decreasing soil erosion, increasing soil enzyme activities, improving the soil micro-ecological environment and, like other *Fabaceae*, it is a nitrogen fixer (Qian *et al*. 2015, Zheng *et al.* 2016). However, this plant may become weedy or invasive in some regions or habitats and may displace desirable vegetation if not properly managed (Randall & Marinelli 1996, Kaufman & Kaufman 2013).

The cosmopolitan genus *Cercospora* is species-rich (2 522 legitimate species names listed in MycoBank, accessed 20 Feb. 2019) that belongs to the family *Mycosphaerellaceae* in the order *Capnodiales*. The genus comprises numerous destructive plant pathogens, for instance *C. apii* on celery (Groenewald *et al.* 2006), *C. beticola* on sugar beet (Weiland & Koch 2004), *C. zonata* on faba beans (Kimber & Paull 2011), *C. zeae-maydis* and *C. zeina* on maize (Crous *et al.* 2006) and *C. carotae* on carrots (Kushalappa et al. 1989). *Cercospora* was established by Fuckel (Fungi Rhen. Exs., no. 117, 1863; as Fresen. ex Fuckel, see Braun & Crous 2016), and *C. apii* was later designated as conserved type of the genus under the International Code of Nomenclature for algae, fungi, and plants, Art. 14.9 (Braun & Crous 2016). The systematics of *Cercospora* has been problematic for a long time, as there are only few distinctive morphological characters useful for species discrimination and since specialised as well as plurivorous species are involved (Crous & Braun 2003, Groenewald *et al.* 2013, Bakhshi *et al.* 2015, 2018). Molecular techniques are commonly used to overcome taxonomic problems posed by the limitations of morphological characteristics. In this regard, ex-type cultures are essential for the study of *Cercospora*, because the current systematic scheme is based on multilocus phylogeny (Groenewald *et al.* 2013, Nguanhom *et al.* 2015, Soares *et al.* 2015, Bakhshi *et al.* 2015, 2018, Albu *et al.* 2016, Guillin *et al.* 2017, Guatimosim *et al.* 2017) and DNA can rarely be extracted from herbarium samples. Therefore, it is important to typify and epitypify species within this genus to stabilise the names for future studies, and provide connections between specimens assessed through molecular and morphological methods.

In an eight-gene molecular DNA sequence analysis employed for *Cercospora s. str.*, Bakhshi *et al.* (2018) revealed cryptic species within several species complexes. Therefore, besides introducing some new species, epitypes have been designated for some species which were previously regarded as synonyms of other species based on previously published five-gene phylogenies (Groenewald *et al.* 2013, Bakhshi *et al.* 2015, 2018). The objective of the present study was therefore to confirm the taxonomy and DNA phylogeny of the *Cercospora* isolates obtained from *S. varia* from Iran and Romania, which were previously synonymised under *C. armoraciae* based on a five-gene DNA dataset (Groenewald *et al.* 2013, Bakhshi *et al.* 2015).

MATERIAL AND METHODS

Specimens and isolates

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 Westerdijk Fungal Biodiversity Institute), and the culture collection of Tabriz University (CCTU), Tabriz, Iran.

Fungal Systematics and Evolution is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License

© 2019 Westerdijk Fungal Biodiversity Institute

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	Origin	Collector	GenBank accession numbers[2]						
						ITS	*tef1*	*actA*	*cmdA*	*his3*	*tub2*	*rpb2*	*gapdh*
C. armoraciae	**CBS 250.67; CPC 5088 (TYPE)**	*Armoracia rusticana*	*Brassicaceae*	Romania, Fundulea	O. Constantinescu	JX143545	JX143299	JX143053	JX142807	JX142561	MH496351	–	MH496181
C. bizzozeriana	CCTU 1013	–	–	Iran, East Azerbaijan	M. Torbati	KJ886414	KJ886253	KJ885931	KJ885770	KJ886092	MH496362	MH511855	MH496192
	CCTU 1022; CBS 136028	–	–	Iran, East Azerbaijan	M. Torbati	KJ886415	KJ886254	KJ885932	KJ885771	KJ886093	MH496363	MH511856	MH496193
	CCTU 1127; CBS 136133	*Capparis spinosa*	*Capparidaceae*	Iran, Khuzestan	E. Mohammadian	KJ886420	KJ886259	KJ885937	KJ885776	KJ886098	MH496364	MH511857	MH496194
	CCTU 1117; CBS 136132	*Lepidium draba*	*Brassicaceae*	Iran, West Azerbaijan	M. Arzanlou	KJ886418	KJ886257	KJ885935	KJ885774	KJ886096	MH496365	MH511858	MH496195
	CCTU 1234	*Lepidium draba*	*Brassicaceae*	Iran, West Azerbaijan	M. Arzanlou	KJ886419	KJ886258	KJ885936	KJ885775	KJ886097	MH496366	MH511859	MH496196
	CCTU 1107	–	–	Iran, Zanjan	M. Bakhshi	KJ886417	KJ886256	KJ885934	KJ885773	KJ886095	MH496367	MH511860	MH496197
	CBS 258.67; CPC 5061 (TYPE)	*Lepidium draba*	*Brassicaceae*	Romania, Fundulea	O. Constantinescu	JX143546	JX143300	JX143054	JX142808	JX142562	MH496368	–	MH496198
	CBS 540.71; IMI 161110; CPC 5060	*Lepidium draba*	*Brassicaceae*	Romania, Hagieni	O. Constantinescu	JX143548	JX143302	JX143056	JX142810	JX142564	MH496369	–	MH496199
	CCTU 1040; CBS 136131	*Tanacetum balsamita*	*Asteraceae*	Iran, Zanjan	M. Bakhshi	KJ886416	KJ886255	KJ885933	KJ885772	KJ886094	MH496370	MH511861	MH496200
C. rautensis	CCTU 1190; CBS 136134	*Securigera varia*	*Fabaceae*	Iran, West Azerbaijan	M. Arzanlou	KJ886422	KJ886261	KJ885939	KJ885778	KJ886100	**MK531769**	**MK564169**	**MK531771**
	CBS 555.71; IMI 161117; CPC 5082 (TYPE)	*Securigera varia*	*Fabaceae*	Romania: Hagieni	O. Constantinescu	JX143550	JX143304	JX143058	JX142812	JX142566	**MK531770**	–	**MK531772**
C. sorghicola	**CCTU 1173; CBS 136448; IRAN 2672C (TYPE)**	*Sorghum halepense*	*Poaceae*	Iran, Guilan	M. Bakhshi	KJ886525	KJ886364	KJ886042	KJ885881	KJ886203	MH496471	MH511961	MH496301

[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 Westerdijk Fungal Biodiversity Institute; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran.

[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 DNA-directed RNA polymerase II second largest subunit; *gapdh*: partial glyceraldehyde-3-phosphate dehydrogenase gene.

Dried specimens are preserved in the Fungal Herbarium of the Iranian Research Institute of Plant Protection (IRAN F), Tehran, Iran, and the Westerdijk Fungal Biodiversity Institute (CBS H), Utrecht, The Netherlands.

DNA extraction, PCR amplification and sequencing

DNA samples comprised those previously extracted by Bakhshi *et al.* (2015) and Groenewald *et al.* (2013). Three additional partial nuclear genes were sequenced for each isolate. The primers Gpd1-LM and Gpd2-LM (Myllys *et al.* 2002) were used to amplify part of the glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene. Part of the β-tubulin (*tub2*) gene was amplified using the primer set BT-1F and BT-1R (Bakhshi *et al.* 2018), whereas the primer set RPB2-C5F and RPB2-C8R (Bakhshi *et al.* 2018) was used to amplify part of the DNA-directed RNA polymerase II second largest subunit (*rpb2*) gene. All amplification mixtures and conditions were performed in a total volume of 12.5 µL as described by Bakhshi *et al.* (2018). PCR products were visualised by electrophoresis using a 1.2 % agarose gel, stained with GelRed™ (Biotium, Hayward, CA, USA) and viewed under ultra-violet light. Size estimates were made using a HyperLadder™ I molecular marker (Bioline).

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

Phylogenetic analyses

The raw DNA sequences of *tub2*, *rpb2* and *gapdh* 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. In addition, sequences of isolates from the *C. armoraciae* complex (Groenewald *et al.* 2013, Bakhshi *et al.* 2015, 2018) corresponding to the ITS locus (including ITS1, 5.8S, ITS2), together with parts of seven protein coding genes, *viz.* translation elongation factor 1-alpha (*tef1*), actin (*actA*),

© 2019 Westerdijk Fungal Biodiversity Institute

calmodulin (*cmdA*), histone H3 (*his3*), *tub2*, *rpb2* and *gapdh*, were retrieved from the NCBIs GenBank nucleotide database and included in the analyses (Table 1). Sequences were aligned with the MAFFT online interface using default settings (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013), and adjusted manually where necessary using MEGA v. 6. Sequences of *C. sorghicola* (CBS 136448 = IRAN 2672C) were used as outgroup.

Phylogenetic analyses were based on Bayesian inference (BI). For this purpose, the best nucleotide substitution model was selected independently for each locus using MrModeltest v. 2.3 (Nylander 2004). 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. Phylogenetic reconstruction under optimal criteria per partition was performed using Bayesian inference (BI) Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.2.6 (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. The first 25 % of saved trees were discarded as the "burn-in" phase and posterior probabilities (Rannala & Yang 1996) were calculated from the remaining trees. The resulting phylogenetic tree was printed with Geneious v. 5.6.7 (Kearse *et al.* 2012). Newly generated sequences in this study were deposited in NCBIs GenBank nucleotide database (www.ncbi.nlm.nih.gov; Table 1) and alignments and respective phylogenetic trees in TreeBASE, study number 24021 (www.TreeBASE.org).

Morphology

To examine morphological characteristics, diseased leaf tissues were observed 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 at ×1000, and 95 % confidence intervals were derived for the 30 measurements with extreme values given in parentheses. High-resolution photographs of microscopic fungal structures were captured with a Nikon digital sight DS-f1 high definition colour camera mounted on the above-mentioned light microscope and the layout of acquired images and photographic preparations was carried out in Adobe Photoshop CS5. Colony macro-morphology on MEA was determined after 20 d at 25 °C in the dark in duplicate and colony colour was described using the mycological colour charts of Rayner (1970).

Allele group designation

The isolates of this study along with the other isolates from the *C. armoraciae* species complex (Bakhshi *et al.* 2018), including *C. armoraciae s. str*. and *C. bizzozeriana*, were compared using the individual alignments of the eight single loci in MEGA v. 6. Allele groups were established for each locus based on sequence identity, i.e., each sequence with one or more nucleotide difference from the other sequences was regarded as a different allele.

RESULTS AND DISCUSSION

Phylogenetic analyses

The final concatenated alignment contained 12 aligned sequences of the isolates from the *C. armoraciae* species complex (Groenewald *et al.* 2013, Bakhshi *et al.* 2015, 2018) and 4 084 characters including alignment gaps. 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*, 1 213–1 568 bp for *his3*, 1 573–1 982 bp for *tub2*, 1 987–3 215 bp for *rpb2,* and 3 220–4 084 bp for *gapdh*. Four sets of four Ns were used in the alignment to separate adjacent loci and were excluded from the phylogenetic analyses.

Based on the results of MrModeltest, a SYM-gamma model with a gamma distributed rate variation for ITS, a K80-gamma with a gamma distributed rate variation for *tef1*, *actA* and *cmdA*, a HKY+G with gamma-distributed rates for *his3*, a GTR+G model with a gamma distributed rate variation for *tub2* and *rpb2* were applied while *gapdh* required GTR+I+G with inverse gamma distributed rate variation. The ITS, *tef1*, *actA* and *cmdA* partitions had fixed (equal) base frequencies, whereas the remaining partitions (*his3*, *tub2*, rpb2 and *gapdh*) had dirichlet base frequencies. From this alignment, 4 056 characters were

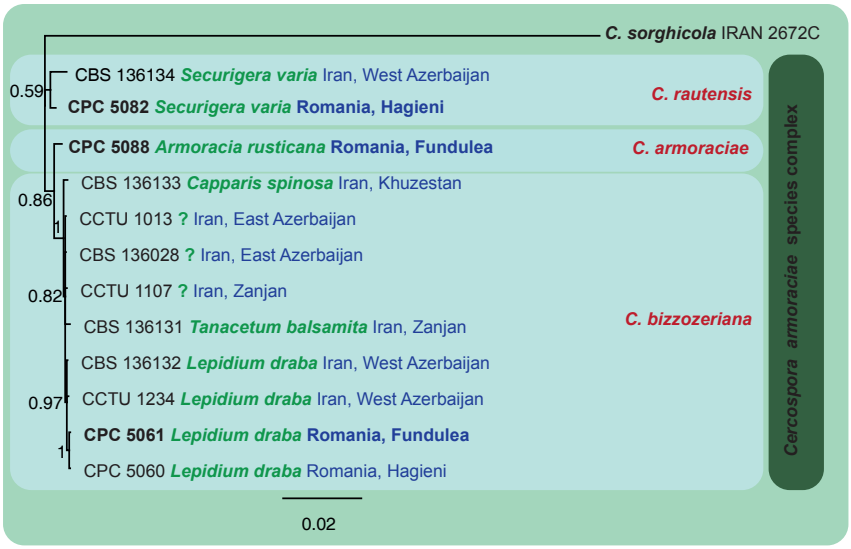

Fig. 1. Consensus phylogram (50 % majority rule) of 850 trees resulting from a Bayesian analysis of the combined eight-gene (ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, *rpb2* and *gapdh*) sequence alignment using MrBayes v. 3.2.6. The scale bar indicates 0.02 expected changes per site. The tree was rooted to *Cercospora sorghicola* (CBS 136448 = IRAN 2672C).

© 2019 Westerdijk Fungal Biodiversity Institute

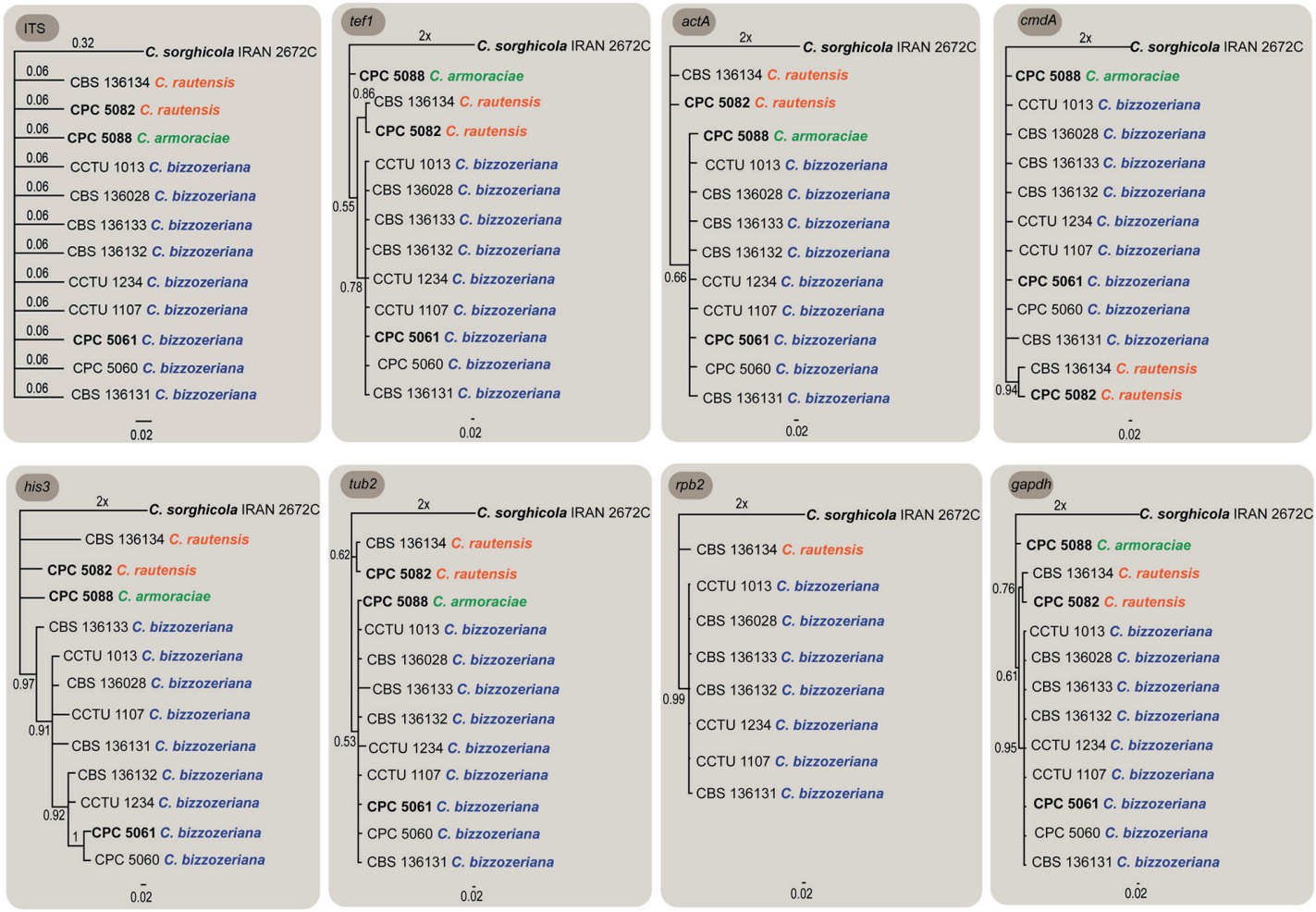

Fig. 2. Bayesian 50 % majority rule consensus trees of the individual gene loci using MrBayes v. 3.2.6. The scale bar indicates 0.02 expected changes per site. The trees were rooted to *Cercospora sorghicola* (CBS 136448 = IRAN 2672C).

used for the Bayesian analysis; these contained 200 unique site patterns (7, 26, 17, 16, 32, 25, 32 and 45 for ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, rpb2 and *gapdh*, respectively). The Bayesian analysis lasted 565 000 generations and generated a total of 1 132 trees. After discarding the first 25 % of sampled trees for burn-in the phylogenetic tree (50 % majority rule consensus tree) and posterior probabilities were calculated from the remaining 850 trees (Fig. 1).

All genes were also assessed individually using Bayesian analyses under the above-mentioned substitution models, for each data partition (Fig. 2). Based on individual gene tree assessments, the isolates obtained from *Securigera varia* are supported as a clade of its own based on phylogenies derived from the *tef1*, *cmdA*, *tub2*, and *gapdh* alignments (Fig. 2).

Taxonomy

The Consolidated Species Concept (Quaedvlieg *et al*. 2014) accepted in recent revisions of the taxonomy of the genus *Cercospora* (e.g., Groenewald *et al.* 2013, Bakhshi *et al.* 2015, 2018) was employed in this study to distinguish the isolates of the genus *Cercospora* obtained from *Securigera varia*. These isolates were previously recognised as *C. armoraciae* based on the five-gene phylogenetic tree (ITS, *tef1*, *actA*, *cmdA* and *his3*) (Groenewald *et al.* 2013, Bakhshi *et al.* 2015). Recently Bakhshi *et al.* (2018) re-assessed species of the genus *Cercospora* using a combined approach based on the evaluation of an eight-gene (ITS, *tef1*, *actA*, *cmdA*, *his3*, *tub2*, *rpb2* and *gapdh*) molecular DNA sequence dataset, host, and morphological data. The robust eight-gene phylogeny revealed several novel clades within the existing *Cercospora* species complexes, such as *C. armoraciae*, and the *C. armoraciae s. lat*. isolates were distributed over two clades, *C. armoraciae s. str*. and *C. bizzozeriana* (Bakhshi *et al.* 2018). In this study, the eight-gene phylogeny of the *Cercospora* isolates obtained from *Securigera varia* (as *C. armoraciae* in Groenewald *et al.* 2013, and Bakhshi *et al.* 2015) revealed that the clade comprising these two strains is completely distinct from *C. armoraciae s. str*. and *C. bizzozeriana* (*C. armoraciae s. lat*. complex) clades. Based on a literature survey and morphological similarities, the species name *C. rautensis* was assigned to this clade.

Cercospora rautensis C. Massal., Madonna Verona, Boll. Mus. Civico Verona **3**: 19. 1909. Fig. 3.
Synonyms: *Cercospora coronillae-scorpioidis* Ferraris, Fl. Ital. Cryptog. I, Fungi, Hyphales: 893. 1910.
Cercospora coronillae-variae Lobik, Bolezni Rastenij **17**: 194. 1928.

Description in planta: *Leaf spots* distinct, circular to irregular, grey-brown, without definite border, 1–5 mm diam. *Mycelium*

© 2019 Westerdijk Fungal Biodiversity Institute

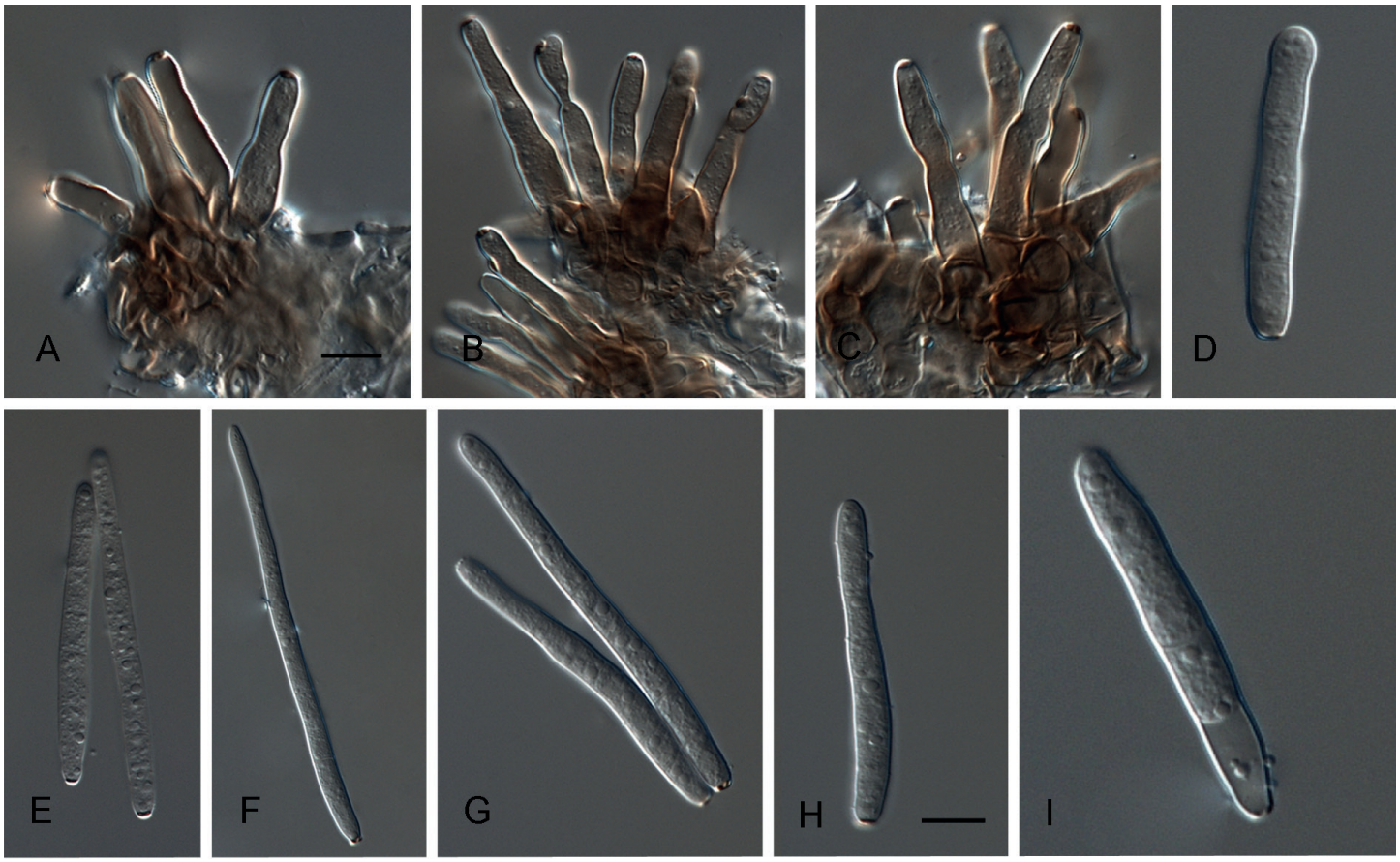

Fig. 3. *Cercospora rautensis* (CBS 136134). **A–C.** Fasciculate conidiophores. **D–I.** Conidia. Scale bars = 10 µm.

internal. *Caespituli* amphigenous, brown. *Conidiophores* in moderately dense fascicles (4–25), arising from the upper cells of a well-developed, intraepidermal and substomatal, brown stroma, up to 45 µm diam; conidiophores pale brown to brown, 0–3-septate, straight to mildly geniculate, flexuous, unbranched, (30–)45–65 × 4.5–6 µm, somewhat irregular in width, becoming narrower towards the apex, *Conidiogenous cells* terminal or integrated, brown, smooth, proliferating sympodially, 15–40 × 3.5–5 µm, mono-local or multi-local; loci thickened, darkened, refractive, protuberant, mostly apical, sometimes lateral, 2–3.5 µm diam. *Conidia* solitary, subcylindrical to cylindrical, straight to mildly curved, hyaline, thin-walled, smooth, distinctly 3–9-septate, obtuse at the tip, truncate to obconically truncate at the base, (38–)65–80(–130) × (4–)4.5–6 µm; hila thickened, darkened, refractive, 2–3.5 µm diam.

Typus: **Italy**, "Nel bosco "delle Raute" presso il paesetto di Cogolo, on *Securigera varia* [≡ *Coronilla varia*] (*Fabaceae*), Aug., *C. Massalongo* (**holotype** VER, n.v.). **Romania**, Hagieni, on *S. varia*, 20 Jul. 1970, *O. Constantinescu* (**epitype designated here** CBS H-9861, MBT 385978), ex-epitype culture CBS 555.71 = IMI 161117 = CPC 5082.

Additional material examined: **Iran**, West Azerbaijan Province, Khoy, Firouragh, on *Securigera varia*, Jul. 2012, *M. Arzanlou* (IRAN 17180F, CCTU 1190 = CBS 136134).

Culture characteristics: Colonies on MEA reaching 60 mm diam after 20 d at 25 °C in the dark; flat with smooth, even margins and moderate aerial mycelium; surface olivaceous grey, reverse iron-grey.

Distribution: Italy, Hungary, Lithuania, Poland, Romania, Ukraine (Europe), Russia (European part), Georgia, Pennsylvania (USA) (Crous & Braun 2003, Farr & Rossman 2019) and Iran (Asia) (this study).

Notes: Based on the results of the eight-gene phylogenetic tree, the isolates obtained from *S. varia* from Iran and Romania, previously recognised as *C. armoraciae* based on five-gene phylogenetic tree (Groenewald *et al.* 2013, Bakhshi *et al.* 2015), clustered in a clade, distinct from the ex-type isolate of *C. armoraciae* (CBS 250.67 = CPC 5088) (Fig. 1). Therefore, this species must be regarded as a separate species and appears to be specific to *S. varia*. *Cercospora rautensis* is the only species of *Cercospora* known from *S. varia.* The collection from Iran agrees morphologically well with Chupp's (1954) description of *C. rautensis* (conidiophores 20–100 × 3–5 µm, conidia acicular to cylindrical, straight to mildly curved, 35–100 × 3–5 µm, base truncate to obconically truncate, tip subobtuse). It also perfectly agrees with type material of *C. coronillae-variae* (LE 158151), which has been reduced to synonymy with *C. rautensis* (conidiophores 15–65 × 4–8 µm, conidia cylindrical, subcylindrical to slightly obclavate, 40–100 × (3.5–)4–5(–6) µm, base truncate to somewhat obconically truncate) (examined by Uwe Braun). It is unclear whether Chupp (1954) had seen and examined the type material of *C. rautensis*. A long time ago, U. Braun (pers. commun.) received the information that Massalongo's types are maintained at VER, but a loan was not possible and is not possible until now. However, as long as the existence of type material of *C. rautensis* at VER must be assumed, neotypification is not justified to solve the issue. Since one European isolate from *S. varia* in Romania (CBS 555.71 =

© 2019 Westerdijk Fungal Biodiversity Institute

Table 2. Results from allele group designation per locus for *C. armoraciae*, *C. bizzozeriana* and *C. rautensis* (*C. armoraciae s. lat.*) isolates.

Species	Culture accession number	Allele group per locus							
		ITS	*tef1*	*actA*	*cmdA*	*his3*	*tub2*	*rpb2*	*gapdh*
C. armoraciae	CBS 250.67; CPC 5088 (TYPE)	I	I	I	I	I	I	–	I
C. bizzozeriana	CCTU 1013	I	II	I	I	III	I	I	II
	CCTU 1022; CBS 136028	I	II	I	I	III	I	I	II
	CCTU 1040; CBS 136131	I	III	I	II	VI	I	II	II
	CCTU 1107	I	II	I	I	VII	I	I	II
	CCTU 1117; CBS 136132	I	II	I	I	V	I	I	II
	CCTU 1234	I	II	I	I	V	III	I	II
	CCTU 1127; CBS 136133	I	II	I	I	IV	II	III	II
	CBS 540.71; CPC 5060	I	II	I	I	II	I	–	II
	CBS 258.67; CPC 5061 (TYPE)	I	II	I	I	II	I	–	II
C. rautensis	CCTU 1190; CBS 136134	I	IV	II	III	VIII	IV	IV	III
	CBS 555.71; CPC 5082 (TYPE)	I	IV	II	III	IX	IV	–	IV

IMI 161117 = CPC 5082) (as *C. rautensis* until Jul. 2013; see Groenewald *et al.* 2013) also resides in this clade, I designate it here as epitype for this species, and fix the application of the name *C. rautensis* to this clade.

Allele group designation

The results of allele group designation of the isolates of *C. rautensis* and other isolates in *C. armoraciae* complex are summarised in Table 2. The allele group for the *tef1*, *actA*, *cmdA* and *tub2* sequences for both strains of *C. rautensis* from Iran (CBS 136134) and Romania (CBS 555.71) was similar and also different from the allele group of the *C. armoraciae* and *C. bizzozeriana* isolates. For ITS, the allele group of these two isolates was the same as *C. armoraciae* and *C. bizzozeriana* isolates, while for *his3* and *gapdh*, these two isolates had a different allele group which was also distinct from the allele group of the *C. armoraciae* and *C. bizzozeriana* isolates (Table 2).

CONCLUSIONS

Extensive studies of *Cercospora* and related genera in Iran have generated records of numerous species (Hesami *et al.* 2012, Pirnia *et al.* 2012, Bakhshi *et al.* 2012, 2015, 2018). However, *C. rautensis* has not been detected in Iran before. Therefore, this is the first report of *C. rautensis* infection of Crownvetch in Iran. Since one European isolate was included in this study, I was able to designate an epitype here for this species as well, which was necessary to determine the application of the name *C. rautensis*.

In recent years, two significant advances in the understanding of *Cercospora* have been achieved. First, with the comprehensive molecular examination of *Cercospora s. str*. based on a multi-locus DNA sequence dataset of five genomic loci of the large sampling of species conducted by Groenewald *et al.* (2013), a backbone phylogeny was achieved for *Cercospora*. Second, an eight-gene molecular DNA sequence analysis of *Cercospora s. str*. was conducted by Bakhshi *et al.* (2018), which revealed cryptic species within several species complexes. One important finding of these studies is that it was not always possible to apply North American or European names to African or Asian strains and *vice versa*. Therefore, type specimens are essential for molecular analyses of *Cercospora* species for correct applications of such species names. Unfortunately, many (epi-)type cultures are lacking for a significant number of *Cercospora* species. These species will have to be recollected from their original hosts and continents from where they were described. These collections are necessary to stabilise the application of the names to facilitate subsequent taxonomic work on *Cercospora*.

ACKNOWLEDGEMENTS

The Iran National Science Foundation (INSF) and the Research Deputy of the Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO) are acknowledged for financial support. Prof. dr Uwe Braun (Martin Luther University, Halle, Germany) is thanked for his helpful advice concerning typification of *Cercospora rautensis* and his valuable comments on the manuscript.

REFERENCES

Albu S, Schneider R, Price P, *et al.* (2016). *Cercospora* cf. *flagellaris* and *Cercospora* cf. *sigesbeckiae* are associated with Cercospora leaf blight and purple seed stain on soybean in North America. *Phytopathology* **106**: 1376–85.

Bakhshi M, Arzanlou M, Babai-Ahari A (2012). Comprehensive checklist of Cercosporoid fungi from Iran. *Plant Pathology and Quarantine* **2**: 44–55.

Bakhshi M, Arzanlou M, Babai-ahari A, *et al.* (2015). Application of the consolidated species concept to *Cercospora* spp. from Iran. *Persoonia* **34**: 65–86.

Bakhshi M, Arzanlou M, Babai-ahari A, *et al.* (2018). Novel primers improve species delimitation in *Cercospora*. *IMA Fungus* **9**: 299–332.

Braun U, Crous PW (2016). (2415) Proposal to conserve the name *Cercospora* (*Ascomycota*: *Mycosphaerellaceae*) with a conserved type. *Taxon* **65**: 185.

Chupp C (1954). *A Monograph of the Fungus Genus Cercospora*. Ithaca, New York.

© 2019 Westerdijk Fungal Biodiversity Institute

Crous PW, Braun U (2003). *Mycosphaerella* and its anamorphs: 1. Names published in *Cercospora* and *Passalora*. *CBS Biodiversity Series* **1**: 1–571.

Crous PW, Groenewald JZ, Groenewald M, *et al.* (2006). Species of *Cercospora* associated with grey leaf spot of maize. *Studies in Mycology* **55**: 189–197.

Farr DF, Rossman AY (2019). Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Available online at https://nt.ars-grin.gov/fungaldatabases

Groenewald JZ, Nakashima C, Nishikawa J, *et al.* (2013). Species concepts in *Cercospora*: spotting the weeds among the roses. *Studies in Mycology* **75**: 115–170.

Groenewald M, Groenewald JZ, Braun U, *et al.* (2006). Host range of *Cercospora apii* and *C. beticola* and description of *C. apiicola*, a novel species from celery. *Mycologia* **98**: 275–285.

Guatimosim E, Schwartsburd PB, Barreto RW, *et al.* (2017). Novel fungi from an ancient niche: cercosporoid and related sexual morphs on ferns. *Persoonia* **37**: 106–41.

Guillin EA, de Oliveira LO, Grijalba PE, *et al.* (2017). Genetic entanglement between *Cercospora* species associating soybean purple seed stain. *Mycological Progress* **16**: 593–603.

Hesami S, Khodaparast S, Zare R (2012). New reports on *Cercospora* and *Pseudocercospora* from Guilan province. *Rostaniha* **13**: 95–100.

Katoh K, Standley DM (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.

Kaufman SR, Kaufman W (2013). Invasive plants: guide to identification and the impacts and control of common North American species. Stackpole Books.

Kearse M, Moir R, Wilson A, *et al.* (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647–1649.

Kimber RBE, Paull JG (2011). Identification and genetics of resistance to Cercospora leaf spot (*Cercospora zonata*) in faba bean (*Vicia faba*). *Euphytica* **177**: 419–429.

Kushalappa AC, Boivin G, Brodeur L (1989). Forecasting incidence thresholds of Cercospora blight in carrots to initiate fungicide application. *Plant Disease* **73**: 979–983.

Maddison WP, Maddison DR (2011). Mesquite: a modular system for evolutionary analysis. Version 2.75. http://mesquiteproject.org.

Myllys L, Stenroos S, Thell A (2002). New genes for phylogenetic studies of lichenized fungi: glyceraldehyde-3-phosphate dehydrogenase and beta-tubulin genes. *Lichenologist* **34**: 237–246.

Nguanhom J, Cheewangkoon R, Groenewald JZ, *et al.* (2015). Taxonomy and phylogeny of *Cercospora* spp. from Northern Thailand. *Phytotaxa* **233**: 27–48.

Nylander JAA (2004). *MrModeltest. Version 2.0.* Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.

Pirnia M, Zare R, Zamanizadeh HR, *et al.* (2012). New records of cercosporoid hyphomycetes from Iran. *Mycotaxon* **120**: 157–169.

Qian X, Gu J, Pan HJ, *et al.* (2015). Effects of living mulches on the soil nutrient contents, enzyme activities, and bacterial community diversities of apple orchard soils. *European Journal of Soil Biology* **70**: 23–30.

Quaedvlieg W, Binder M, Groenewald JZ, *et al*. (2014). Introducing the Consolidated Species Concept to resolve species in the *Teratosphaeriaceae*. *Persoonia* **33**: 1–40.

Randall JM, Marinelli J (1996). Invasive plants: weeds of the global garden (Vol. 149). Brooklyn Botanic Garden.

Rannala B, Yang Z (1996). Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* **43**: 304–311.

Rayner RW (1970). A Mycological Colour Chart. Kew, UK: Commonwealth Mycological Institute.

Roland AE (1998). *Roland's Flora of Nova Scotia, 3rd edition*. Nimbus Publishing Ltd. and Nova Scotia Museum, Halifax, Nova Scotia.

Ronquist F, Teslenko M, van der Mark P, *et al.* (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.

Soares APG, Guillin EA, Borges LL, *et al.* (2015). More *Cercospora* species infect soybeans across the Americas than meets the eye. *PloS One* **10**: e0133495.

Tamura K, Stecher G, Peterson D, *et al.* (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* **30**: 2725–2729.

Weiland J, Koch G (2004). Sugarbeet leaf spot disease (*Cercospora beticola* Sacc.). *Molecular Plant Pathology* **5**: 157–166.

Zheng W, Li Y, Gong Q, *et al.* (2017). Improving yield and water use efficiency of apple trees through intercrop-mulch of crown vetch (*Coronilla varia* L.) combined with different fertilizer treatments in the Loess Plateau. *Spanish Journal of Agricultural Research* **14**: 1207.

© 2019 Westerdijk Fungal Biodiversity Institute