



Epitypification of *Cercospora coffeicola* and its involvement with two different symptoms on coffee leaves in Brazil

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Abstract *Cercospora coffeicola* is the causal agent of brown eye spot, an important disease of coffee (*Coffea arabica*) in Brazil. However, atypical symptom as darker and larger lesions, named black spot, has been reported in field. In this study, we tested the hypothesis that the causal agent of black spot belongs to the same species pathogenic to brown eye spot. Nineteen strains obtained from diseased coffee found in the five largest coffee-producing states of Brazil were characterized by a combination of molecular phylogenetic methods, using a multi-locus approach (internal transcribed spacer regions and intervening 5.8S nrRNAs, *actin*, *calmodulin*, *histone H3*, and *translation elongation factor 1-alpha*),

analyses of morphological markers and pathogenicity. Strains from brown eye spot and black spot disease on coffee leaves formed a clade with *C. coffeicola* strain from Japan. All strains showed same morphological characteristics and caused brown eye spot symptoms in greenhouse. These results confirm that the species associated with brown eye spot and black spot disease on coffee leaves is *C. coffeicola*. Additionally, an epitype is proposed for *C. coffeicola*.

Keywords Black spot · Brown eye spot · *Coffea arabica* · Epitype · Multilocus phylogeny · Plant disease

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Introduction

Coffee (*Coffea arabica*) is widely cultivated in Brazil, and it is one of the most important agricultural commodities to the Brazilian economy (Souza et al. 2011). *Cercospora coffeicola* is the causal agent of brown eye spot (BES) disease, which occurs from nursery-to-field plantings, and severe epidemics are associated with coffee yield losses when management is not well-conducted (Souza et al. 2012). It causes lesions on leaves as necrotic spots consisting of a light-colored center (sometimes surrounded by a purple-brown ring with yellow borders), defoliation, fruit injuries, reduced productivity, and lower beverage quality (Godoy et al. 1997; Pozza et al. 2010; Dell'Acqua et al. 2011; Souza et al. 2011, 2012; Lima et al. 2012). An atypical symptom characterized by darker and larger lesions on leaves, known as black spot (BS) or black cercosporiosis (Nelson 2008) have often been reported in coffee growing areas in Brazil.

Cercospora coffeicola was originally described by Berkeley and Cooke (Cook 1881) on coffee leaves in Jamaica. In Brazil, the first report dates back to 1901 (Noack 1901). This species has different synonyms, such as *Ramularia goeldiana*, *Cercospora coffeae*, and *Cercospora herrerana*. The sexual morph is known as *Mycosphaerella coffeicola*. Although type material of *C. coffeicola* being preserved in the herbarium of the Royal Botanic Gardens, Kew, UK, as K(M) 255,271 and IMI 846, the collections concerned are old, dating back to 1881, so that they are insufficient for novel taxonomic studies including molecular phylogenetic analyses. According to Ariyawansa et al. (2014) dried specimens collected prior to about 1950 cannot be successfully sequenced or sequencing of older specimens requires special, adapted methods as recently shown by Bradshaw and Tobin (2020) for old powdery mildew specimens. Furthermore, some herbaria do not allow samples to be removed for DNA extraction, above all when very type collections are involved. In such cases, epityfication being the methods of choice. An epitype of *C. coffeicola* with ex-epitype sequences will allow the application of modern molecular methods for identification purposes based on living culture.

The taxonomy of *Cercospora* species was traditionally based on host plant association (Chupp 1954; Ellis 1971). Moreover, Crous and Braun (2003) introduced the concept of “compound species” which consisted of morphologically indistinguishable species. Thus, the

taxonomy of this genus has proven highly problematic, because morphological features in species of *Cercospora* are generally rather uniform, and there is a high level of intraspecific variation (Groenewald et al. 2013; Bakhshi et al. 2015). Therefore, gene sequence data have been used to solve taxonomic problems related to *Cercospora* species delimitation (Groenewald et al. 2013; Bakhshi et al. 2015, 2018). According to Groenewald et al. (2013) “many (epi-) type cultures and adequate sequence data are lacking for a significant number of *Cercospora* species”, which also applies to *C. coffeicola*, a phytopathologically relevant species that is urgently in need of proper molecular characterization.

This study aims to verify if BES and BS have the same etiological agent. To achieve this aim, we sequenced the internal transcribed spacer regions and intervening 5.8S nrRNAs (ITS), *actin* (*actA*), *calmodulin* (*cmdA*), *histone H3* (*his3*), and *translation elongation factor 1-alpha* (*tef1*), and used multilocus phylogenetic analyses to define the phylogenetic position of the isolates within the genus *Cercospora*, supplemented by a proposed epitype for *Cercospora coffeicola* with corresponding ex-epitype reference sequences.

Materials and methods

Fungal isolates and culture conditions

The isolation was made directly from coffee leaves with BES and BS symptoms collected in the five largest coffee-producing states of Brazil: Minas Gerais, São Paulo, Paraná, Espírito Santo, and Bahia (Table 1). Reproductive structures were collected using sterile needle to gently scrap the lesions on leaves, then suspended in sterile water, and spread on the surface of malt extract agar (20 g malt extract L⁻¹, 20 g agar L⁻¹; Himedia Laboratories, Mumbai, India) in a Petri dish. Fungi were incubated at 25 °C for 72 h with a 12-h photoperiod (Santos et al. 2014). *Cercospora* isolates derived from single conidia were cultured on V8® medium (100 mL V8® plus 900 mL distilled water, 17 g agar, 2 g calcium carbonate), at 20 °C. Nineteen strains were deposited in the Coleção Micológica de Lavras (CML) of the Federal University of Lavras (UFLA), Minas Gerais, Brazil (<http://www.dfp.ufla.br/cml>). Dried plant material with leaf spot containing fungal structure is deposited in the herbarium of the Universidade Federal de Viçosa (Herbarium VIC).

Table 1 *Cercospora coffeicola* strains obtained in this study

Accession no. ^a	Symptom ^b	Geographic origin ^c	GenBank accession no. ^d				
			ITS	<i>tef1</i>	<i>actA</i>	<i>cmdA</i>	<i>his3</i>
CML2984	BES	MG - Brazil	KU203739	KU203758	KU203682	KU203701	KU203720
CML2985	BS	MG - Brazil	KU203743	KU203762	KU203686	KU203705	KU203724
CML2986	BES	MG - Brazil	KU203733	KU203752	KU203676	KU203695	KU203714
CML2988	BES	ES - Brazil	KU203746	KU203765	KU203689	KU203708	KU203727
CML2990	BS	MG - Brazil	KU203738	KU203757	KU203681	KU203700	KU203719
CML3342	BES	BA - Brazil	KU203745	KU203764	KU203688	KU203707	KU203726
CML3376	BS	MG - Brazil	KU203740	KU203759	KU203683	KU203702	KU203721
CML3391	BES	MG - Brazil	KU203741	KU203760	KU203684	KU203703	KU203722
CML3392	BES	MG - Brazil	KU203735	KU203754	KU203678	KU203697	KU203716
CML3393	BES	PA - Brazil	KU203748	KU203767	KU203691	KU203710	KU203729
CML3394	BES	SP - Brazil	KU203751	KU203770	KU203694	KU203713	KU203732
CML3395	BES	MG - Brazil	KU203750	KU203769	KU203693	KU203712	KU203731
CML3396	BES	ES - Brazil	KU203747	KU203766	KU203690	KU203709	KU203728
CML3397	BES	BA - Brazil	KU203736	KU203755	KU203679	KU203698	KU203717
CML3398	BES	ES - Brazil	KU203742	KU203761	KU203685	KU203704	KU203723
CML3399	BES	PA - Brazil	KU203737	KU203756	KU203680	KU203699	KU203718
CML3412	BES	MG - Brazil	KU203744	KU203763	KU203687	KU203706	KU203725
CML3413	BS	BA - Brasil	KU203749	KU203768	KU203692	KU203711	KU203730
CML3414	BES	SP - Brazil	KU203734	KU203753	KU203677	KU203696	KU203715

^a CML: Coleção Micológica de Lavras, Departamento de Fitopatologia, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. ^b BES: brown eye spot, plant showing conventional disease symptoms; BS: black spot, plant showing symptoms of black cercosporiosis. ^c States of Brazil: MG = Minas Gerais, ES = Espírito Santo; PA = Paraná; BA = Bahia; SP = São Paulo. ^d ITS: internal transcribed spacers and intervening 5.8S nrRNAs; *tef1*: translation elongation factor 1-alpha; *actA*: actin; *cmdA*: calmodulin; *his3*: histone H3

DNA extraction, PCR and sequencing

Genomic DNA was isolated from fungal mycelium grown on 2% malt extract broth medium (Himedia, Mumbai, India) under agitation on a rotary shaker (100 rpm). Genomic DNA extracted with a Wizard® Genomic DNA purification kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. PCR reactions were performed in a total volume of 25 µL by using a GoTaq Colorless Master Mix kit (Promega, Madison, WI, USA) in a My Cycler thermal cycler (Bio-Rad, Hercules, CA, USA). Primer sets used for PCR and sequencing reactions are listed in Online Resource 1. All isolates were sequenced using primers for *actin* (*actA*, Carbone and Kohn 1999), *calmodulin* (*cmdA*, Carbone and Kohn 1999), *histone H3* (*his3*, Crous et al. 2004), internal transcribed spacer regions and intervening 5.8S nrRNAs (ITS, White et al. 1990), and *translation elongation factor 1-alpha* (*tef1*, Carbone and Kohn 1999). PCR conditions

consisted of an initial denaturation step of 5 min at 95 °C, followed by 35 cycles of 30 s at 95 °C; 30 s at 52 °C (*actA*), 49 °C (*cmdA*), 53 °C (*his3*), 60 °C (ITS), 48 °C (*tef1*); and 45 s at 72 °C, then 5 min at 72 °C. Amplified fragments were cleaned with an Wizard SV gel and PCR Clean-Up System kit (Promega, Madison, WI, USA), and sequenced by Macrogen (Rockville, MD, USA).

Phylogenetic analyses

Consensus sequences were assembled from forward and reverse traces using SeqAssem version 07/2008 software (SequentiX - Digital DNA Processing, Klein Raden, Germany). Sequences from the reference strains of *Cercospora* spp. (Feau et al. 2006; Dell'Acqua et al. 2011; Groenewald et al. 2013) available in GenBank were added to the analyses. *Septoria provencialis* (CPC 12226) was selected as outgroup taxon based on previous studies (Groenewald et al. 2013; Bakhshi et al.

2015, 2018; Nguanhom et al. 2015; Guatimosim et al. 2017). Sequences were aligned using Muscle software operated on MEGA version 7 software (Kumar et al. 2016). The concatenate dataset was comprised of *actA* (18 parsimony-informative positions/195 bp), *cmdA* (27/ 308 bp), *his3* (7/ 363 bp), ITS (2/ 453 bp), *tef1* (12/292 bp). Maximum parsimony (MP) and Maximum likelihood (ML) analyses were conducted with MEGA version 7 software (Kumar et al. 2016). Phylogenetic analysis of each individual dataset and the concatenate dataset (*actA*- *cmdA*- *his3*- ITS- *tef1*) was conducted using MP. The consistency index (CI) and the retention index (RI) were estimated as measures for homoplasy in the dataset and tree length was calculated. ML-based analyses of concatenated datasets were inferred with the general-time-reversible model with Gamma distributed with Invariant sites (GTR + G + I). Clade support was inferred from 1000 bootstrap replications for MP and ML. Bayesian phylogenetic (BI) trees were inferred using MrBayes 3.2 software (Ronquist et al. 2012) to calculate posterior probabilities for concatenated datasets. Models of sequence evolution for each partition were determined using jModeltest (Darriba et al. 2012). Models chosen by the Bayesian Information Criterion were: TPM + G for *actA*, TrN + I + G for *cmdA*, HKY + G for *his3*, and K80 + G for ITS and *tef1*. Phylogenetic reconstruction under per partition criteria was performed with a combined dataset. Gene boundaries were 01–207 bp for *actA*, 208–515 bp for *cmdA*, 516–885 bp for *his3*, 886–1347 bp for ITS, and 1348–1695 bp for *tef1*. A Markov Chain Monte Carlo algorithm of four chains was run for 1×10^5 generations and sampled every 100 generations. Fifty percent majority-rule consensus trees were constructed after discarding 25% of the initial trees. Phylogenetic trees were visualized using FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>) and edited with Corel Draw X5 (Corel Corporation, Ottawa, ON, Canada). Sequences generated in this study were deposited in GenBank (Table 1). Aligned datasets were deposited in TreeBASE under the accession number 26812 (<http://purl.org/phylo/treebase/phyloids/study/TB2:S26812?x-access-code=3f81ae4c3e3450b592840c5806d6395e&format=html>).

Examination of morphological markers

Mycelial disks (0.5 mm in diameter) from colony borders were transferred to 20 mL V8® broth medium

(100 mL v8® plus 900 mL distilled water) and incubated under agitation (120 rpm) at 25 °C, for 4 days. Then, the suspension was spread on the surface of 2% water-agar in a Petri dish. Plates were kept open at a distance of 40 cm below a set of 40 W white fluorescent grow lux lamps, in a 12 h photoperiod at 25 °C for 4 days to mycelial mass drying. Mycelia were hydrated with 10 mL distilled water and the suspension was filtered through a layer of cheesecloth (Souza et al. 2011). Microscopic observations were made from clear lactic acid mounts. Measurements were taken from at least 30 conidia and conidiophores for each strain. Photodocumentation was performed by using DM 2000 epifluorescence microscope (Leica, Jena, Germany). The strains were cultivated in triplicates on V8® for determination of growth rates and colony characters in the dark at 25 °C.

Pathogenicity tests

The pathogenicity test was conducted with all 19 strains studied. Coffee tree seedlings cultivar Mundo Novo 376/4 with approximately seven months old of age and showed six pairs of leaves were inoculated with a modified version of the methodology proposed by Souza et al. (2012). Eight mycelial discs, each six mm in diameter, were isolated from 15-day-old *C. coffeicola* colonies. They were macerated in 400 µL sterile distilled water. The macerated mycelium from each strain was placed in Erlenmeyer flasks containing 20 mL liquid V8® culture medium (100 mL V8 in 900 mL distilled water) and agitated at 100 rpm, for 4 days, at room temperature. The liquid containing the mycelium was poured into plates with water-agar medium. Plates were incubated in BOD until all the liquid evaporated (about 4 days). After the drying step, 10 mL sterile water was added to each plate and conidia were removed using a Drigalski spatula. The liquid containing the conidia was filtered through gauze to remove residue and sporulation was quantified with a Neubauer counting chamber. The suspension used for inoculation was adjusted to 3×10^4 conidia mL⁻¹ and was sprayed on all coffee leaves. Uninoculated plant served as a negative control, which leaves were inoculated with sterile water. Each treatment was composed of four repetitions arranged in a completely randomized design. Occurrence of foliar symptoms was evaluated.

Results

Phylogenetic analysis

A collection of 19 field isolates were obtained from *Coffea arabica* showing BES or BS-diseased leaves collected in different coffee-producing states of Brazil. Of these 15 were of leaves with symptoms BES and four of leaves with symptoms of BS. The combined tree (*actA-cmdA-his3-ITS-tef1*) obtained using ML, MP and BI contained the same major clades, and the BI tree is shown in Fig. 1. *Cercospora* strains from coffee leaves this study formed a monophyletic group together with *Cercospora coffeicola* of *Coffea arabica* strain from Japan MUCC 771. Individual phylogenetic performance of the five loci indicated that they have limited resolution to delimitation *C. coffeicola* from other *Cercospora* species (Online Resources 2–6). These results reinforce the importance of multilocus phylogenetic analyses for more precise species delimitation.

Taxonomy

Cercospora coffeicola Berk. & Cooke, Grevillea 9(51): 99. 1881.

= *Sphaerella coffeicola* Cooke, Grevillea 9(49): 11. 1880.

= *Cercospora coffeae* Zimm., Bericht über Land- und Forstwirtschaft in Deutsch-Ostafrika 2: 35. 1904.

= *Cercosporina coffeicola* (Berk. & Cooke) Speg., Boletín de la Academia Nacional de Ciencias en Córdoba 23(3–4): 589. 1918.

= *Ramularia goeldiana* Sacc., Sylloge Fungorum 10: 554. 1892.

= *Cercospora herrerana* Farneti, Atti Ist. Bot. Univ. Pavia, Ser. 2: 37. 1904.

= *Mycosphaerella coffeicola* (Cooke) J.A. Stev. & Wellman, J. Wash. Acad. Sci. 34: 262. 1944.

= *Mycosphaerella coffeicola* (Cooke) Cif., Atti dell'Istituto Botanico della Università e Laboratorio Crittogamico di Pavia 19: 118. 1962.

Typification: Holotype of *Cercospora coffeicola*: JAMAICA, on *Coffea* sp., Jan 1881, Morris (K(M) 255,271). Isotype: IMI 846. Epitype of *Cercospora coffeicola* (Mycobank MBT52640, hic designatus): BRAZIL, Minas Gerais, Bonfinópolis de Minas, on leaves of *Coffea arabica* with brown eye spot symptoms, 2012, César Elias Botelho (VIC 47409); Ex-epitype culture CML 2984 = CCDCA 10745 = COAD 3146. GenBank accession

numbers for DNA sequences derived from the ex-epitype strain: KU203682 (*actA*), KU203720 (*his3*), KU203739 (*ITS*), KU203701 (*cmdA*), KU203758 (*tef1*).

Characteristics in vitro: Colonies on V8® medium attaining 20 mm diameter after two weeks in the dark at 25 °C, white to olivaceous grey, even margins and sparse to moderate aerial mycelium, purple diffuse pigment surrounding culture (Fig. 2a). **Characteristics in vivo:** Conidiophores aggregated, 30–140 × 4–7 µm, pale brown, simple, unbranched, 0–4-septate, thin-walled, smooth, straight or flexuous caused by sympodial proliferation (Fig. 2c–d). Conidiogenous cells terminal or intercalary, unbranched, pale brown, smooth, proliferating sympodial, conidiogenous loci thickened, darkened, protuberant, apical, lateral, 2.5–4 µm wide (Fig. 2c–d). Conidia solitary, hyaline, acicular to obclavate, straight or curved, 51–270 × 1–5 µm, 0–11-septate, thin-walled, smooth, apex pointed or subobtuse, base truncated to obconically truncated, hila thickened and darkened, 2–4 µm wide (Fig. 2e–g).

Additional specimens examined: BRAZIL, Minas Gerais, Três Pontas, *Coffea arabica*, 2012, Alvarenga L, CML 2985; Minas Gerais, Lavras, *Coffea arabica*, 2012, Botelho DMS, CML 2986; Minas Gerais, Bonfinópolis de Minas, *Coffea arabica*, 2012, Botelho C, CML 2990; Minas Gerais, Machado, *Coffea arabica*, 2012, Botelho C, CML 3376; Minas Gerais, Turmalina, *Coffea arabica*, 2012, Mendonça AC, CML3391; Minas Gerais, São Sebastião do Paraíso, *Coffea arabica*, 2012, Botelho C, CML 3392; Minas Gerais, Manhuaçu, *Coffea stenophylla*, 2013, Resende MLV, CML 3395; Minas Gerais, Monte Carmelo, *Coffea arabica*, 2012, Alvarenga L, CML 3412; Espírito Santo, Marechal Floriano, *Coffea arabica*, 2013, Almeida JAM, CML2988; Espírito Santo, Venda Nova do Imigrante, *Coffea arabica*, 2013, Botelho C, CML3396; Espírito Santo, Alegre, *Coffea canephora*, 2012, Belan LL, CML3398; Bahia, São Desidério, *Coffea arabica* 2012, Almeida JAM, CML3342; Bahia, Luiz Eduardo Magalhães, *Coffea arabica*, 2012, Pozza EA, CML3397; Bahia, Luiz Eduardo Magalhães, *Coffea arabica*, 2012, Pozza EA, CML3413; Paraná, Jaboti, *Coffea arabica*, 2013, Custódio A, CML3393; Paraná, Londrina, *Coffea arabica*, 2012, Custódio A, CML3399; São Paulo, Garçae, *Coffea arabica*, 2013, Vasco G, CML3394; São Paulo, Arandu, *Coffea arabica*, 2012, Botelho C, CML3414.

Phylogenetic differentiation: This species is differentiated from allied species in phylogenetic analyses by concatenate dataset (*actA-cmdA-his3-ITS-tef1*).

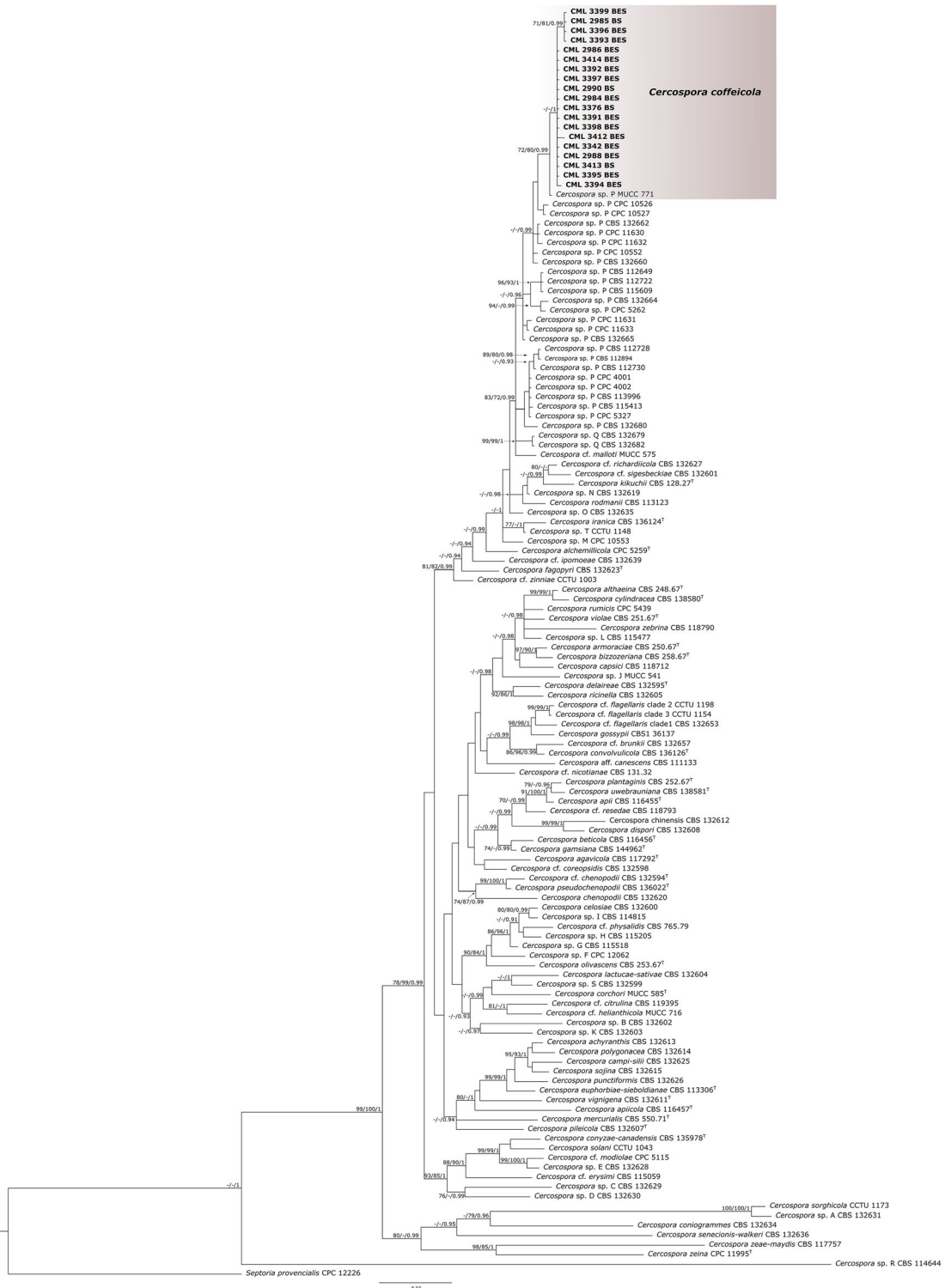


Fig. 1 Majority-rule consensus Bayesian phylogram based on concatenated (*actA-his3-cmdA-ITS-tef1*) sequences showing relationships among *Cercospora* species. Strains from this study are highlighted in bold. Bootstrap values and posterior probabilities

(ML/ MP/ BI) are shown at the internodes. A minus sign (–) refers to support values lower than 70% bootstrap or 0.90 posterior probability. BES: strains from leaves with Brown eye spot. BS: strains from leaves with Black spot

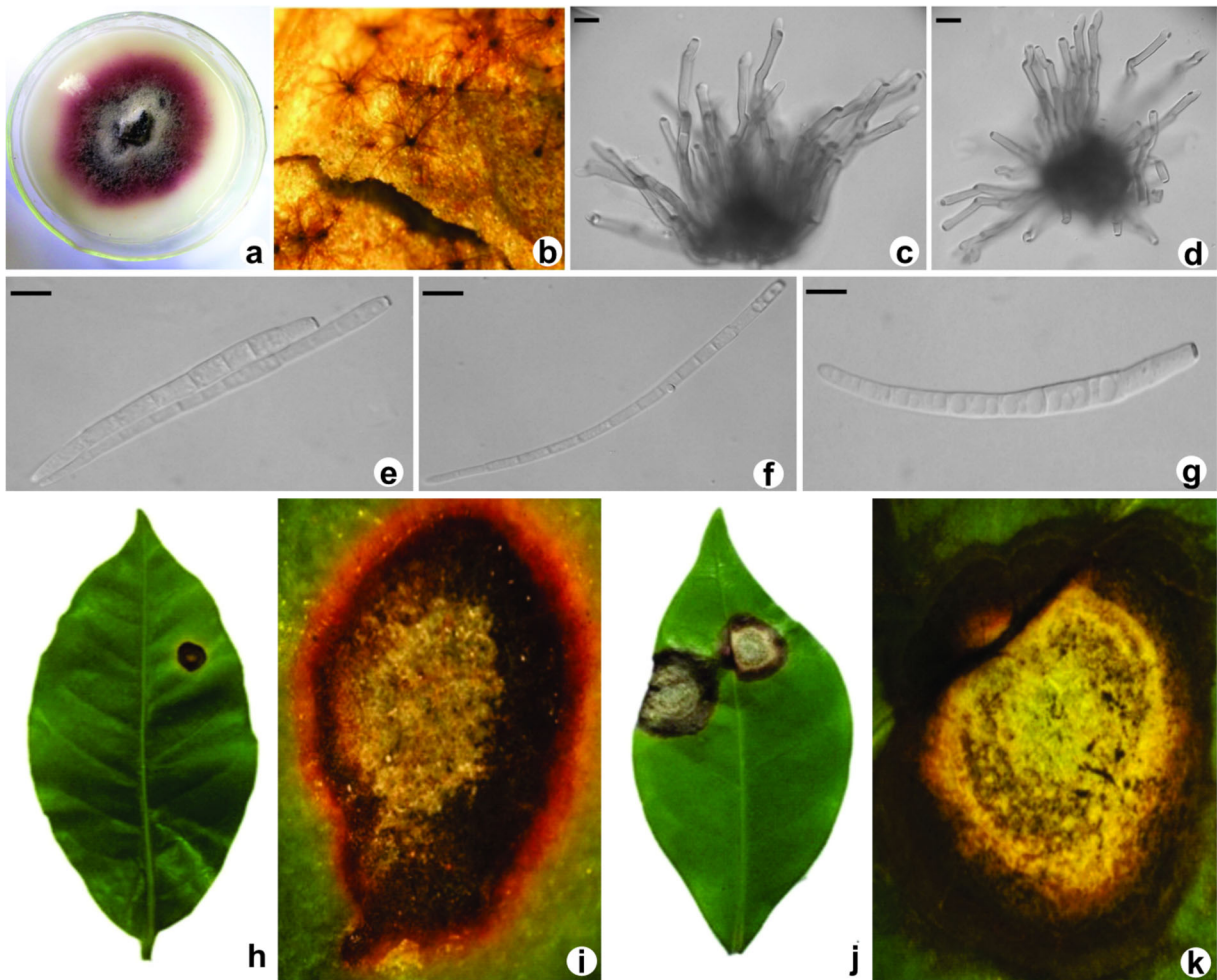


Fig. 2 Photographs (**a**, **h** and **j**), Stereomicrographs (**i** and **k**) and Photomicrographs (**c** – **g**). **a** Morphological characteristic of *Cercospora coffeicola*, CML 2984 – Epitype colony on V8@ after

two weeks. **b** Production of reproductive structures on the lesion. **c-d** Conidiophores. **e-g** Conidia. **h-i** Brown eye spot symptom. **j-k** Black spot symptom. Scale bar = 10 µm

Description of symptoms caused by C. coffeicola in the field: Brown eye spot (BES), necrotic leaf spots amphigenous, subcircular to irregular, with light-colored centers surrounded by a purple-brown ring with yellow edges (Fig. 2**h-i**). Black spot (BS), necrotic leaf spots amphigenous, subcircular to irregular, grey to pale brown, surrounded by a large, brown border (Fig. 2**j-k**). BES and BS lesions, small to fairly large, 2–9 mm in diameter.

Pathogenicity tests

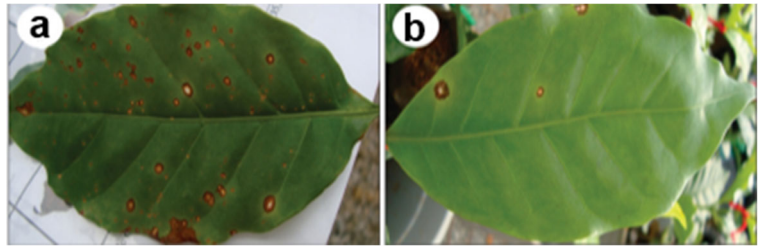
All *C. coffeicola* strains induced typical symptoms of BES approximately 30 days after inoculation (Fig. 3). Control plants did not develop symptoms. To fulfill

Koch's postulates, the pathogen was re-isolated from inoculated plants.

Discussion

This work confirms that the *Cercospora* strains causing BES and BS on *Coffea arabica* in Brazil belong to a single species in phylogenetic analyses (Fig. 1). Multilocus analyses showed that all 19 strains from leaves with BES and BS symptoms, including the MUCC 771 strain of *Coffea arabica* from Japan (Fig. 1), form a single clade, suggesting the involvement of a single species. The strain from Japan, named as *Cercospora coffeicola*, clusters within *Cercospora* sp.

Fig. 3 Leaf symptoms induced by *Cercospora coffeicola* strains in greenhouse. **a.** CML 2988. **b** CML 2990



P clade, according to Groenewald et al. (2013). In this clade, sequences retrieved from cultures isolated from many hosts, such as *Acacia*, *Cajanus*, *Citrus*, *Coffea*, *Dioscorea*, *Hibiscus* and *Ricinus*, cluster together. The authors reported that the gene loci screened were unable to resolve the taxonomy these isolates and they preferred to treat them as an unresolved species complex. In this study, we included in phylogenetic analyses all strains from P clade, and we verified that strains isolated from *Coffea* formed a clade phylogenetically distinct from the *Cercospora* sp. P clade based on concatenated dataset. However, the ITS region was not sufficient to clarify the delimitation of *Cercospora* species. Some strains obtained in this study cluster together with *C. coffeicola* (as *Mycosphaerella coffeicola*, Feau et al. 2006) and other *C. coffeicola* strains from Brazil (Dell' Acqua et al. 2011), while other strains clustered with different species of *Cercospora* (Online Resource 2). Other loci led to various levels of success in species recognition (Online Resources 3, 4, 5 and 6).

Cercospora coffeicola strains showed high morphological plasticity with large variations in dimensions of particular structures, covering the characteristics and measurements given in the original description of Berkeley and Cooke (Cooke 1881). However, morphological parameters alone are insufficient to distinguish most *Cercospora* species with acicular conidia belonging to the *C. apii* complex (Crous and Braun 2003). The recognition of such *Cercospora* species require the application of multilocus phylogenetic analyzes (Groenewald et al. 2013; Bakhshi et al. 2015, 2018). However, the type material of *Cercospora coffeicola* (Cooke 1881) is very old and not suitable and available for molecular analyses. In order to overcome these obstacles and to determine the name *C. coffeicola* genetically, an epitype with ex-epitype sequences is proposed according to the rules of the International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code). Epitypes should usually be from the type locality

or a region close to and from the same substrate or host as in the original type. However, this is not always possible as, for example, demonstrated in the case of *Colletotrichum circinans*, the cause of smudge in onion (Walker 1925), which could not be recollected from the original site as it is now a housing estate (Hyde and Zhang 2008). Thus, the selection of an epitype isolated from coffee leaves in Brazil is reasonable, above all since a *Cercospora* species of cultivated hosts being involved, with unknown origin, occurring wherever coffee is grown. Thus, strain CML 2984 is designated here as epitype for *C. coffeicola*. The availability of the ex-epitype reference culture, as well as its DNA sequences will be useful for comparison and identification purposes of this species.

In Brazil, the increase in the incidence of **cercospora leaf spot** in coffee plantations in the late 2000s, coincided with the expansion of coffee cultivation from traditional areas to other regions with different environmental conditions. Furthermore, climate change, cultivation of new coffee varieties and utilization of different cultural practices may have favored coffee pathogens (Fazuoli et al. 2002; Souza et al. 2015). Based on observations made in different coffee growing fields, we believe that environmental changes may influence the occurrence of different symptoms. According to Nelson (2008), environmental factors such as high altitudes, cloudiness and high humidity are favorable conditions for foliar spots to become leaf blights, causing more damage. In addition, increase of the productive capacity of coffee plantations may lead to a nutritional imbalance and leaves may become more susceptible to colonization by *C. coffeicola* (Nelson 2008; Souza et al. 2015).

The present study clarifies the involvement of a single species, *C. coffeicola*, as causative agent of two distinct symptoms on coffee leave in Brazil. Moreover, the fresh collection of *Cercospora* strains obtained from *Coffea arabica* in Brazil helped to resolve the phylogenetic

position of *C. coffeicola* within of the genus *Cercospora* and confirmed its status as a species of its own.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10658-020-02170-y>.

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Compliance with ethical standards

Ethical approval The authors declare that have followed the guidelines of the Ethical Standards requested by EJPP, and this manuscript is original and not published elsewhere.

Conflict of interest The authors declare no conflict of interest.

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