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ORIGINAL ARTICLE

# New *Cercospora* species on *Jatropha curcas* in central Brazil

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**Abstract** A new cercosporoid hyphomycete, *Cercospora jatrophiphila*, herein described and molecularly characterized, was found in Planaltina, DF, Brazil on *Jatropha curcas* (Euphorbiaceae).

**Keywords** Fungal taxonomy · Mycodiversity · Brazilian cerrado · Leaf spots

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## Introduction

*Jatropha curcas* (*Euphorbiaceae*) is a potential biodiesel source and one of several species being studied in Brazil within a nationwide project to replace fossil fuel. In a field trial in Planaltina, Distrito Federal, some plants showed conspicuous irregular leaf spotting symptoms. Two well-documented *Cercospora* species are known as pathogenic to *J. curcas*; however, none of them have been molecularly characterized. Now a new *Cercospora* species, previously misidentified as *Cercospora jatrophae-curcas* (Dianese and Dianese 2009), is clearly associated with the lesions and is now properly identified, described, segregated from the known species of *Jatropha*, and molecularly characterized.

## Materials and methods

### Morphology

Leaves of *J. curcas* with light brown to brown leaf spots showing light gray centers containing many conidiophore fascicles, were collected from an experimental area belonging to Embrapa Cerrados in Planaltina, Distrito Federal; they dried for 4 days at±50 °C and deposited in the Mycological Collection of the Herbarium UB. Studies under a stereomicroscope were followed by observations of squash preparations and sections made with a micron freezing microtome. The morphological features were described, measured, and documented using a Leica DM 2500 microscope coupled with a Leica DFC 490 digital camera connected to a microcomputer. Image capture, editing, and structural measurements were managed with Leica QWin V3 software. In some cases, the samples were stained with lacto-glycerol cotton blue and the slides sealed with nail polish; however, most of the

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

| Species | Culture accession number | Host name or isolation source | Host Family | Country | Collector | GenBank accession numbers* | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | ITS | CAL | HIS |
| *Cercospora apii* | CBS 116455 (TYPE) | *Apium graveolens* | *Apiaceae* | Germany: Heilbron | K. Schrameyer | AY840519 | AY840417 | AY840384 |
| *Cercospora apii* | CBS 121.31 | *Beta vulgaris* | *Chenopodiaceae* | Austria: Wien | E.W. Schmidt | AY343371 | AY840411 | AY840378 |
| *Cercospora apiicola* | CBS 116457 (TYPE) | *Apium* sp. | *Apiaceae* | Venezuela: Caripe | N. Pons | AY840536 | AY840434 | AY840401 |
| *Cercospora apiicola* | CPC 11642 | *Apium* sp. | *Apiaceae* | Greece | I. Vloutoglou | DQ233341 | DQ233419 | DQ233441 |
| *Cercospora beticola* | CBS 116456 (TYPE) | *Beta vulgaris* | *Chenopodiaceae* | Italy: Ravenna | V. Rossi | AY840527 | AY840425 | AY840393 |
| *Cercospora beticola* | CPC 15623 | *Beta vulgaris* | *Chenopodiaceae* | Mexico: Texcoco | Ma. de Jesús Yáñez-Morales | JX143555 | JX142817 | JX142571 |
| *Cercospora euphorbiae-sieboldianae* | CBS 113306 (TYPE) | *Euphorbia sieboldiana* | *Euphorbiaceae* | South Korea: Samcheok | H.D. Shin | JX143593 | JX142859 | JX142613 |
| *Cercospora jatrophiphila* | CMHUB 21035 Strain 2225 | *Jatropha curcas* | *Euphorbiaceae* | Brazil | A.C. Dianese | KJ186790 | KJ186792 | KJ186794 |
| *Cercospora jatrophiphila* | CMHUB 21035 Strain 2229 | *Jatropha curcas* | *Euphorbiaceae* | Brazil | A.C. Dianese | KJ186791 | KJ186793 | KJ186795 |
| *Cercospora* cf. *malloti* | MUCC 787 | *Mallotus japonicus* | *Euphorbiaceae* | Japan: Okinawa | C. Nakashima & T. Akashi | JX143626 | JX142893 | JX142647 |
| *Cercospora mercurialis* | CBS 549.71 | *Mercurialis annua* | *Euphorbiaceae* | Romania: Cheia | O. Constantinescu | JX143627 | JX142894 | JX142648 |
| *Cercospora mercurialis* | CBS 551.71 | *Mercurialis ovata* | *Euphorbiaceae* | Romania: Hagieni | O. Constantinescu & G. Negrean | JX143629 | JX142896 | JX142650 |
| *Cercospora mercurialis* | CBS 550.71 (TYPE) | *Mercurialis perennis* | *Euphorbiaceae* | Romania: Cheia | O. Constantinescu | JX143628 | JX142895 | JX142649 |
| *Cercospora ricinella* | CBS 132605 | *Ricinus communis* | *Euphorbiaceae* | South Korea: Chuncheon | H.D. Shin | JX143646 | JX142913 | JX142667 |
| *Cercospora ricinella* | CPC 10104 | *Ricinus communis* | *Euphorbiaceae* | South Korea: Chuncheon | H.D. Shin | JX143647 | JX142914 | JX142668 |
| *Cercospora rodmanii* | CBS 113123 | *Eichhornia crassipes* | *Pontederiaceae* | Brazil: Rio Verde | R. Charudattan | DQ835076 | DQ835149 | DQ835176 |
| *Cercospora rodmanii* | CBS 113126 | *Eichhornia crassipes* | *Pontederiaceae* | Brazil: Oroco | R. Charudattan | DQ835079 | DQ835152 | DQ835179 |
| *Cercospora* sp. P | CBS 132680 | *Ricinus communis* | *Euphorbiaceae* | Mexico: Tamaulipas | Ma. de Jesús Yáñez-Morales | JX143708 | JX142976 | JX142730 |
| *Cercospora* sp. Q | CBS 132681 | *Euphorbia* sp. | *Euphorbiaceae* | Mexico: Tamaulipas | Ma. de Jesús Yáñez-Morales | JX143727 | JX142994 | JX142748 |
| *Cercospora* sp. Q | CPC 15875 | *Euphorbia* sp. | *Euphorbiaceae* | Mexico: Tamaulipas | Ma. de Jesús Yáñez-Morales | JX143731 | JX142998 | JX142752 |
| *Cercospora* cf. *zinniae* | CBS 132676 | — | — | Brazil: Valverde | A.C. Alfenas | JX143757 | JX143027 | JX142781 |
| *Septoria provencialis* | CBS 118910 | *Eucalyptus* sp. | *Myrtaceae* | France | P.W. Crous | DQ303096 | JX143030 | JX142784 |

**ITS* internal transcribed spacers and intervening 5.8S nrDNA, *CAL* calmodulin, *HIS* histone H3 photographic work was done without staining using Nomarski optics. A minimum of 50 replicates of conidia and conidiophore measurements were made.

### Culturing and sequencing

Before drying the exsiccates, two *Cercospora* isolates, strains 2,225 and 2,229, were isolated from leaf spots of a collection of *J. curcas* and deposited in Herbarium UB under accession number UB (Mycol. Col. 21035). Monosporic cultures of the two isolates studied were used for genomic DNA extraction from the mycelium. Species sequences from GenBank were selected to include those of isolates from euphorbiaceus hosts, as well as the Brazilian species available in that database, as previously used by Groenewald et al. (2013). The methods used in this recent paper were in most cases followed in the present publication; however, only three genomic segments were here sequenced which were shown in the same publication to be effective in segregating species within the *C. apii* complex. Genomic DNA was extracted from mycelium of the new *Cercospora* species growing on V8-Medium. Following Groenewald et al. (2005), three genomic loci were sequenced for both isolates: ITS, using the ITS5 and ITS4 primers (White et al. 1990); the Calmodulin (CAL) gene, using primers CAL-228 F e CAL-737R (Carbone and Kohn 1999); and primers CYlH3F and CYlH3R (Crous et al. 2004) were used to amplify the Histone H3 gene (HIS).

A total of six new sequences were generated (Table 1), using *Septoria provencialis* Crous as the outgroup, following Groenewald et al. (2013). All datasets were initially aligned using Clustal X (Higgins and Sharp 1988), then manually adjusted; the alignments were also checked, when possible, at the amino acid level, using Phy-De (Müller et al. 2006), and exported as Nexus files. The phylogenetic reconstruction was performed for each locus under maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). Maximum parsimony analyses were carried using PAUP* v. 4.0b10 for Macintosh (Swofford 2002). Heuristic searches were done with 100 random addition replicates, and tree bisection-reconnection (TBR) branch swapping, saving a maximum of 10,000 trees; all characters were unordered and equally weighted, and gaps were treated as missing data. Maximum likelihood analyses were carried using GARLI v. 0951 for Macintosh (Zwickl 2006). The best-fit model of evolution for each locus was obtained using Modeltest 2.1.1 (Guindon and Gascuel 2003), using the Akaike information criteria. Clade support was evaluated using the non-parametric bootstrap (Felsenstein 1985), with 1,000 replicates. Bayesian inferences were carried using Mr Bayes v. 3.2.1 (Ronquist et al. 2012), four Markov Chain Monte Carlo were run for 5,000,000 generations, sampled every 1,000 generations, in two parallel runs. Convergence was established by ensuring that the average standard deviation of split frequency was < 0.01. Also, the software Tracer 1.5 (Rambaut and Drummond 2013) was used to determine when the tree sampling stabilized. The first 25 % of the trees were discarded as "burning." A majority rule consensus tree was constructed from the resulting trees to estimate the posterior probabilities (Fig. 1).

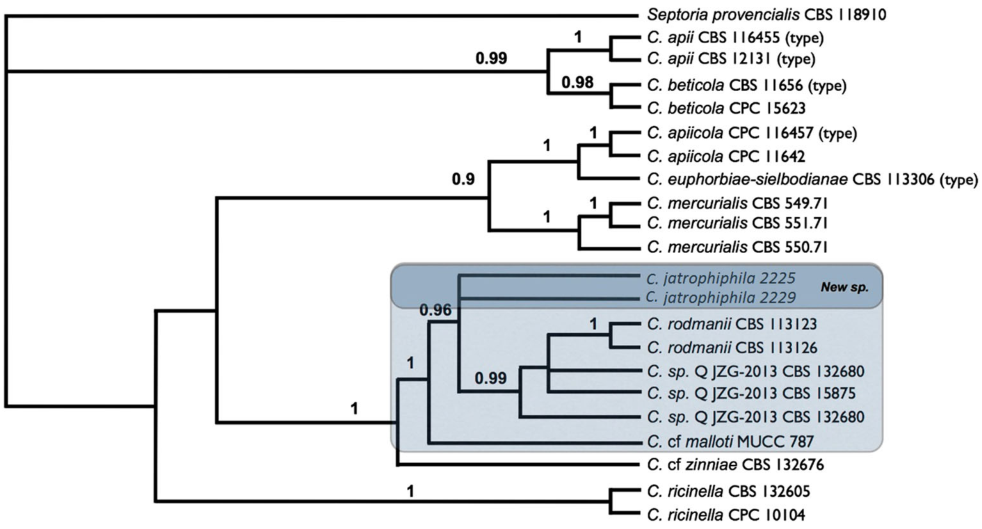
The sequences generated were deposited in NCBI's GenBank (www.ncbi.nlm.nih.gov), with their accession numbers shown in Table 1 together with those of the sequences used in the alignment.

## Results and discussion

### Taxonomy

***Cercospora jatrophiphila*** AC Dianese, Vale, ESC Souza, ZM Chaves, Pereira-Carv. & Dianese **sp. nov**. Fig. 2 (A-I)

**Fig. 1** Consensus tree from Bayesian inference based on all three markers (*ITS*, *CAL* and *HIS*). Numbers above branches are posterior probabilities. Only values above 0.9 are shown



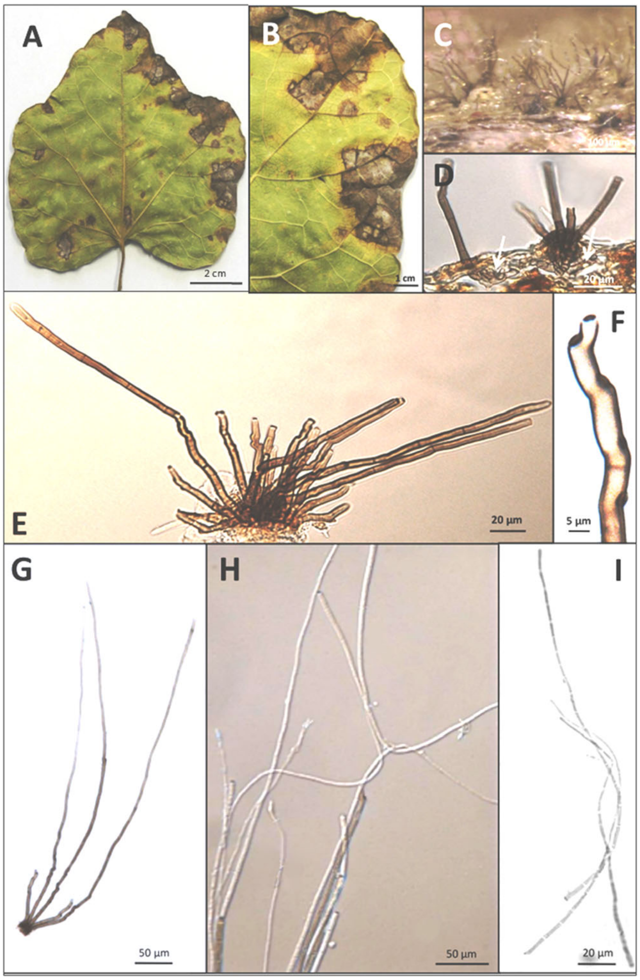


**Fig. 2** (A-I). *Cercospora jatrophiphila* on *Jatropha curcans*, as shown by the holotype (UB Mycol. Col. 21035). *A*, *B* Symptoms consisting of necrotic leaf spots (Scale bars=2 cm, 1 cm, respectively). C Conidiophore fascicles on lesions seen on a Leica stereomicroscope (Scale bar= 100 µm). *D* A fascicle of four conidiophores on a small stroma originated from brown light immerse septate mycelium (*arrows*) (Scale bar= 20 µm). *E* A typical fascicle of conidiophores developed on leaf spots where shorter mature conidiophores apically proliferate to form longer conidiophores (Scale bar=20 µm). *F* Detail view of sympodially elongated conidiophore showing thickened loci (Scale bar=10 µm). *G* Typical combination of long and short conidiophores on the same fascicle (Scale bar=50 µm). *H*, *I* Flexuous cylindrical conidia showing characteristically thickened hylum (Scale bar=20 µm)

MycoBANK: MB 515356

ETYM.: derived from the name of the host genus, *Jatropha*, and -philus (Greek loving).

LESIONS 1–2 mm diam., amphigenous, scattered, irregular, dark brown with light grayish central area containing small black dots, seldom coalescent. COLONIES amphigenous, sparse, brown to dark brown. MYCELIUM immersed, light brown to brown. HYPHAE septate, branched, light brown. STROMATA absent or poorly developed, light brown, 20–25 µm diam. CONIDIOPHORES fasciculate, simple, straight or slightly curved, cylindrical, 79–223 µm long, *ca* 3–4 µm diam., up to 5 µm at the widest part, multi-septate, strongly geniculate, light brown to brown, smooth, thick-walled. CONIDIOGENOUS CELLS integrated, terminal or intercalary, 28–44 µm long, polyblastic, sympodial, clearly geniculate, cylindrical at the lower portion, tapering towards the apex, light brown; CONIDIOGENOUS LOCI very prominent, thick and dark, 1.5–3 µm diam. CONIDIA hyaline, solitary, filiform, cylindrical, acicular, flexuous or straight, 49–272 µm long, 2–4 µm wide, multi-septate hyaline, thin-walled, smooth, truncate at the base, slightly tapering toward the apex; *hilum* not prominent, thick and dark, 1–2 µm wide.

SPECIMEN EXAMINED: **BRAZIL**. DF: Planaltina, Embrapa Cerrados. (CPAC), Km 18, highway Br-20 North, on living leaves of *Jatropha curcas* (*Euphorbiaceae*), 18 Sep. 2009, Alexei de Campos Dianese 97, **holotypus** (UB- Col. Micol. 21035).

COMMENT: Two *Cercospora* and three *Pseudocercospora* species [*P. jatrophae* (G.F. Atk.) A.K. Das & Chattopadh, *P. jatrophae-curcas* (W.Y. Yen) Deighton, and *P. jatropharum* (Speg.) U. Braun] are known on *Jatropha* species (Deighton 1976; Das and Chattopadhyay 1990; Braun 2000; Crous and Braun 2003). Sequence data for the two validly published *Cercospora* species known on *Jatropha*, *C. jatrophicola* (Speg.) Chupp (Chupp 1954) and *C. jatrophigena* U. Braun (Braun 2001), are not yet available in GenBank, but the two species can be easily distinguished from each other because *C. jatrophicola* shows significantly smaller conidia [40–85×2.5–4 µm against 100–300×2.5–5 µm in *C. jatrophigena*] and conidiophores [40–70×4–5 µm against 150–400×3–6 µm in *C. jatrophigena*]. However, considering the morphological features of the *Cercospora* species here described, it is clear that a new species was detected on *J. curcas*. This new *Cercospora* typically shows amphigenous colonies with a large number of strongly geniculate conidiophores (usually 12 or more) per fascicle, while *C. jatrophigena* forms simpler fascicles of non-geniculate conidiophores. In addition, the conidiophores [(79)–132–(223) µm] and conidia [(49)–109–(272) µm] of the new species have dimensions that fit in between those of the structurally larger *C. jatrophigena* and the smaller *C. jatrophicola*. These features clearly place the material examined in a new species here designated as *C. jatrophiphila*.

Species inserted in the *C. apii* complex (*C. apii*, *C. apiicola*, and *C. beticola*), plus all species on euphorbiaceous hosts with sequences of ITS, CAL, and HIS deposited in ICBIGenBank, were compared using Bayesian analysis to generate a phylogeny tree (Fig. 1). This procedure allowed the further characterization of the new species that is not seated within the *C. apii* s. str. and related species, as, for instance, *C. beticola. Cercospora apii* s. lat. is a widely circumscribed complex of plurivorous as well as specialized species established by Crous and Braun (2003).

In Fig. 1 it is shown that the two isolates of the new species *C. jatrophiphila* are located within a well-supported clade, distant from other groups, and from the members of the *C. apii* complex, and also segregated from those present on other euphorbiaceous hosts and *Cercospora* species from Brazil (*Cercospora* cf. *zinnia* and *C. rodmanii*) studied by Groenewald et al. (2013).

The group of species around *C. apii* (*C. apii*, *C. apiicola*, *C. beticola*) proved to be paraphyletic with *C. apiicola* nested within a clade with *C. euphorbiae-sielbodianae*, and also as sister of a clade containing *C. mercurialis*, all with good support. The new species *C. jatrophiphila* belongs in a well-supported different clade containing *C. rodmanii* (on *Eichhornia* – Pontederiaceae from Brazil) and three CBS sequences of unidentified *Cercospora* species on *Euphorbiaceae* from Mexico. *Cercospora maloti*, an isolate from a euphorbiaceous host from Japan, appears as sister to this clade with good support. *Cercospora* cf. *zinniae* is basal to this clade, also with good support. Finally two isolates from *Ricinus* (*C. ricinella*) form a monophyletic unit with good support but its relationship with other the clades is unresolved due to low support.

Thus, considering both the morphological data as well as the molecular phylogeny performed it is justified to describe the *Cercospora* on *Jatropha curcas* from Brazil as new species, namely *C. jatrophiphila*.

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