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NEW DISEASE REPORT

First report of *Colletotrichum agaves* causing anthracnose in *Agave angustifolia* in Brazil

E.R.M.C. Araújo[1,*] | **K.F.B. Oliveira**[1,*] | **E.S. Sousa**[1] | **B.M. Barguil**[2] | **K.S. Matos**[3] | **J.E.A. Beserra Jr.**[1]

[1] Departamento de Fitotecnia, Universidade Federal do Piauí (UFPI), Teresina, PI, Brazil

[2] Departamento de Biologia, Universidade Estadual do Piauí (UESPI), Teresina, PI, Brazil

[3] Departamento de Fitotecnia, Universidade Federal de Roraima (UFRR), Boa Vista, RR, Brazil

Correspondence
Jose Evando Beserra, Departamento de Fitotecnia, Universidade Federal do Piauí (UFPI), 64049–550, Teresina, PI, Brazil.
Email: evando@ufpi.edu.br

[*]The first two authors contributed equally to this report.

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Agave angustifolia is primarily an ornamental species in Brazil, often used in landscaping (Lorenzi & Souza, 2001). During August 2018, symptoms including brown to pale-brown spots at the leaf margin (Figure 1a-b), which became depressed as the disease progressed, were observed on all (10 plants) of *A. angustifolia* in a garden in Teresina, Piauí, Brazil. Leaf fragments c. 5 mm from the leading edge of infection were dipped in 70% ethanol, followed by 1% sodium hypochlorite for one minute, and rinsed twice in sterile distilled water. Fragments were transferred to Petri dishes containing potato dextrose agar media and cultures were incubated for six days at 26°C, 12 hr photoperiod. Single-spore isolates were deposited in the culture collection at the Phytopathology Laboratory of the Universidade Federal do Piauí, Brazil (Accession Nos. COUFPI 217 and COUFPI 219).

The colonies were cream coloured on the upper side, brown on the reverse, with concentric growth and smooth margins (Figure 2). Conidia (n = 30) were hyaline, aseptate, cylindrical, 16.3 μm (12.2-20.8) × 3.9 μm (3.0-4.2) (Figure 3a). Appressoria (n = 30) had entire margins, sometimes lobed, 10.0 μm (6.7-14.7) x of 6.3 μm (4.9-10.4) (Figure 3b). Perithecia, ascospores and setae were not observed. The isolates were identified as a *Colletotrichum* sp. (Cannon et al., 2012). For identification by molecular phylogeny, the internal transcribed spacer (ITS), large subunit ribosomal ribonucleic acid (LSU) and actin (ACT) were selected. Two phylogenetic analyses (GenBank Accession Nos. ITS: MZ396971-MZ39672; LSU: MZ396974-MZ396975) and (ACT: MZ409081-MZ409082) were performed by Bayesian inference according to Sousa et al. (2018). Concatenated analysis of ITS and LSU confirmed the isolates identity as *Colletotrichum agaves* (Figure 4). Phylogenetic analysis of the ACT gene showed that the isolates did not cluster with any other *Colletotrichum* species (Figure 5). The absence of ACT sequences or other housekeeping genes from *C. agaves* holotypes does not allow for a more accurate phylogenetic analysis.

Pathogenicity was confirmed by spraying a suspension of conidia (10^5 ml^{-1}) on artificially wounded leaves, followed by incubation in a humid chamber at 26°C for 48 hours. Ten healthy three-month-old *A. angustifolia* plants were inoculated. Five control plants were sprayed with sterile water and incubated under identical conditions. Symptoms were first observed two days after inoculation. On the third day, small circular and depressed spots were observed (Figure 1c) and on the eighth day these spots coalesced (Figure 1d). The leaves had severe symptoms 15 days after inoculation. All inoculated plants showed

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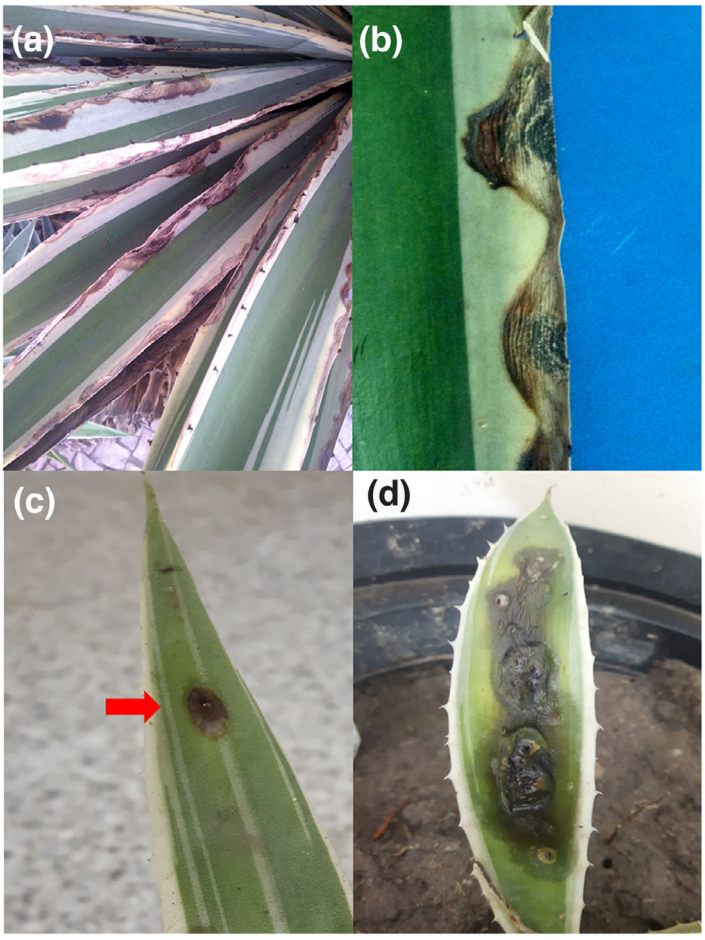


FIGURE 1 Symptoms on leaves of *Agave angustifolia* naturally infected with *Colletotrichum agaves* (COUFPI 219) (a, b), and on inoculated leaves three (c) and eight (d) days after inoculation

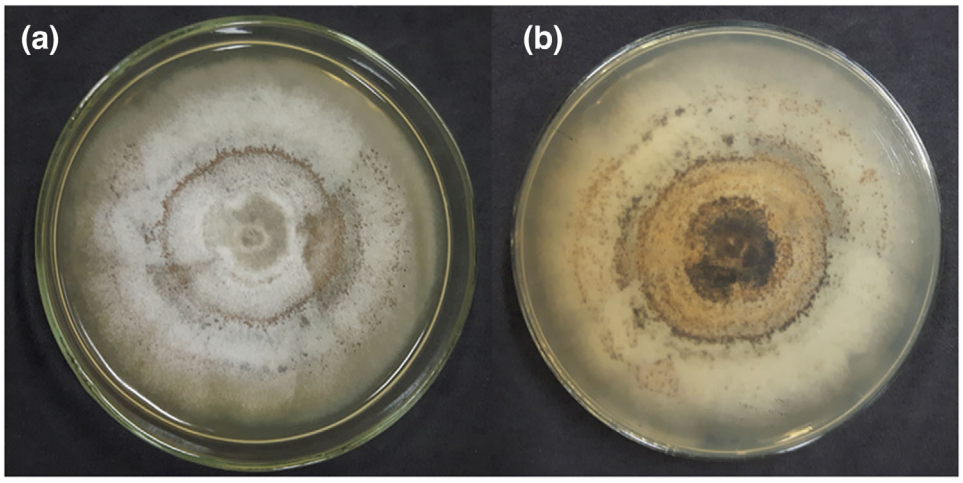


FIGURE 2 *Colletotrichum agaves* (COUFPI 219): top (a) and reverse (b) views of the fungus in potato dextrose agar media

anthracnose, while control plants were symptomless. *Colletotrichum agaves* was consistently re-isolated from inoculated plants, fulfilling Koch's postulates

Colletotrichum agaves was previously reported to cause anthracnose in *Agave* spp. in The Netherlands and Mexico (Farr et al., 2006). This is the first report of *C. agaves* in Brazil.

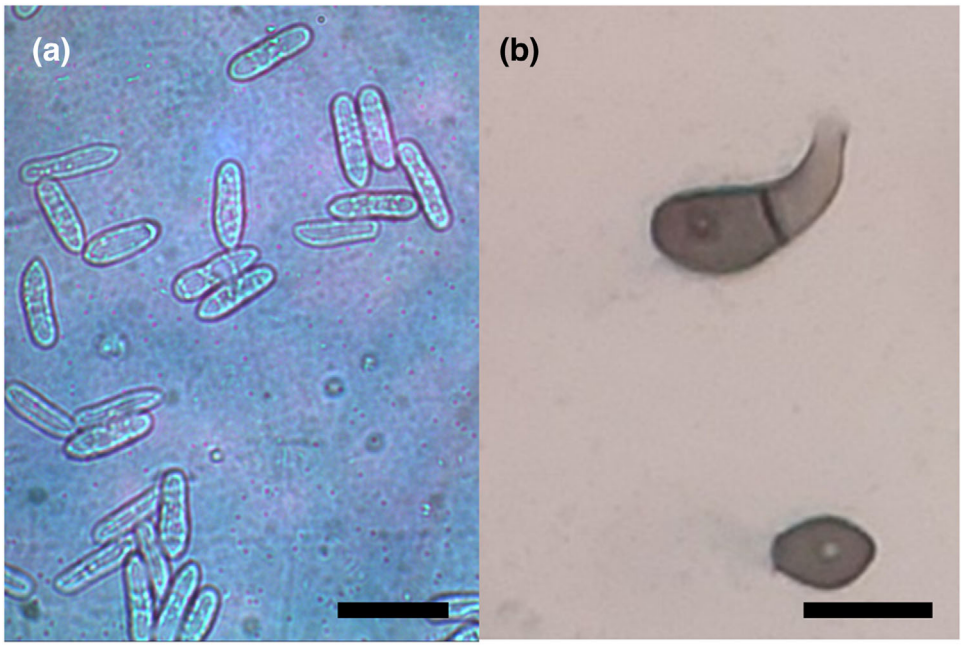


FIGURE 3 Conidia (A) and appressoria (D) of *Colletotrichum agaves* (COUFPI 219). Bars: 20 μm (a); 10 μm (b)

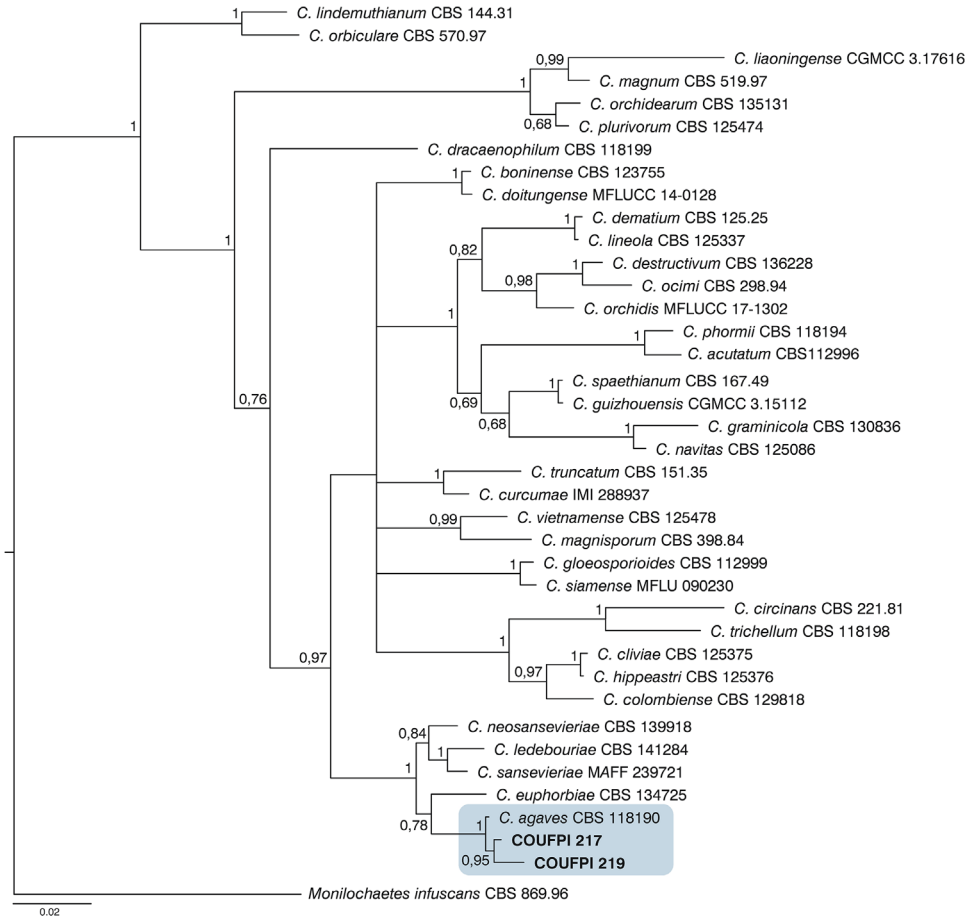


FIGURE 4 Bayesian phylogenetic tree of concatenated sequences of the ITS and LSU regions of *Colletotrichum agaves* isolates from *Agave angustifolia*. Bayesian posterior probability values are indicated above the nodes. Analysis was done with sequences from ex-type or authenticated cultures (sequences from this study in bold). The nucleotide substitution model used was GTR+I+G. The scale bar (0.02) represents nucleotide substitutions per site. Tree rooted with *Monilochaetes infuscans*

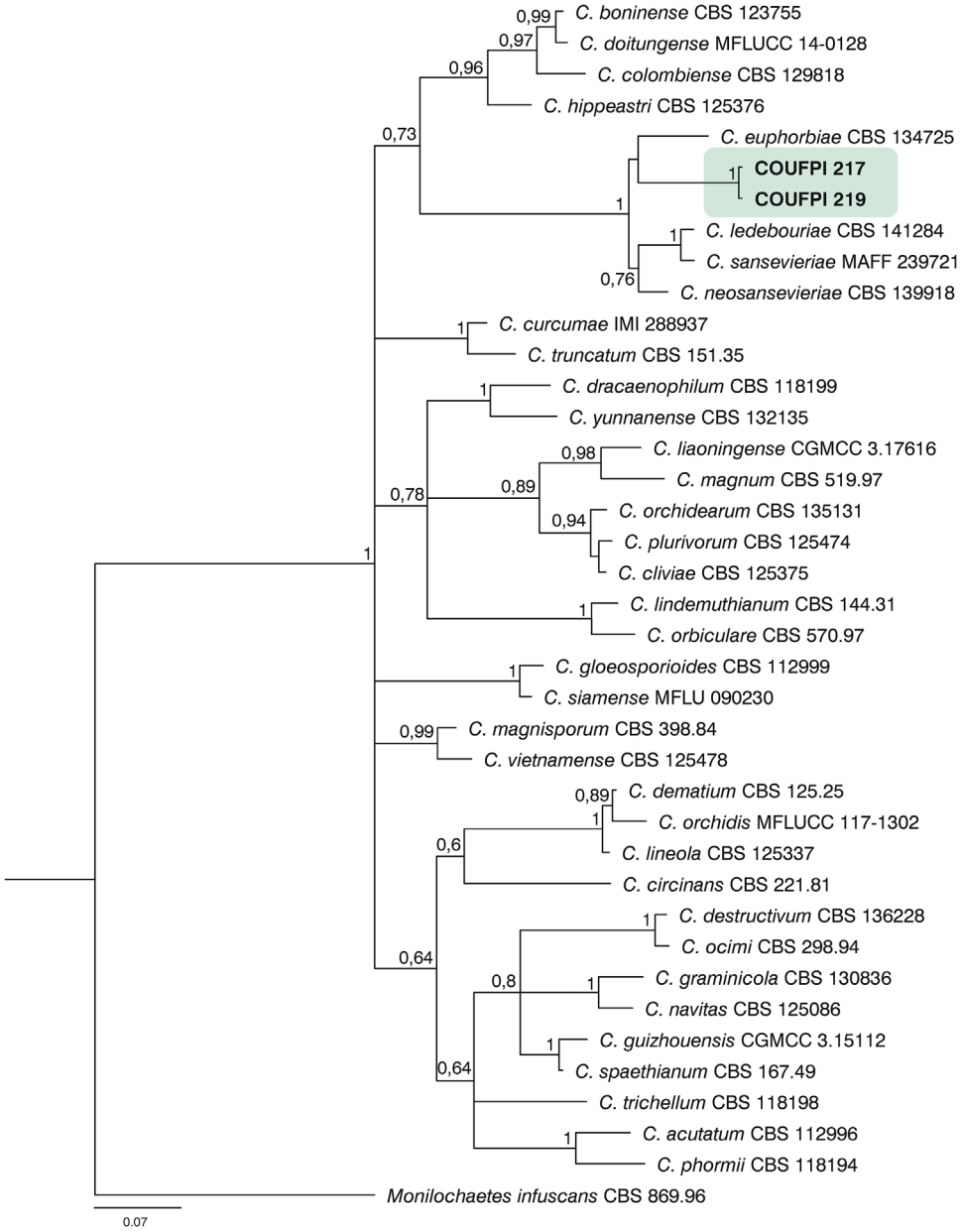


FIGURE 5 Bayesian phylogenetic tree of ACT gene sequences of *Colletotrichum agaves* isolates from *Agave angustifolia*. Bayesian posterior probability values are indicated above the nodes. Analysis was done with sequences from ex-type or authenticated cultures (sequences from this study in bold). The nucleotide substitution model used was HKY+I+G. The scale bar (0.07) represents nucleotide substitutions per site. Tree rooted with *Monilochaetes infuscans*

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ORCID

J.E.A. Beserra Jr. https://orcid.org/0000-0002-0490-9612

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