Received: 26 February 2024 | Revised: 26 May 2024 | Accepted: 10 June 2024

DOI: 10.1111/efp.12869

SHORT COMMUNICATION

Forest Pathology WILEY

First report of anthracnose caused by *Colletotrichum theobromicola* on *Myrciaria dubia* in Brazil

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Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Abstract

Plants of the genus *Myrciaria* are commonly cultivated in the northern region of Brazil for fruit production. Symptoms of leaf spot in camu-camu (*Myrciaria dubia*) trees are frequently observed. The objective of this study was to identify the etiological agent associated with anthracnose in camu-camu using the concept of morphological and phylogenetic analyses. Leaves with symptoms of anthracnose were collected from camu-camu plants in the state of Roraima, Brazil. Morphological identification; sequencing analysis of *CAL*, *GAPDH*, *CHS-1* and *TUB2* gene regions; a pathogenicity test and reisolation of the fungus from symptomatic artificially inoculated plants confirmed *C. theobromicola* as the causal agent of the disease. This is the first record of *C. theobromicola* causing anthracnose in camu-camu in Brazil.

KEYWORDS
Amazon fruit tree, anthracnose, Camu-camu, Myrtaceae

1 | INTRODUCTION

Camu-camu (*Myrciaria dubia* [Kunth] McVaugh) is a native tree of Amazon region that produces fruit with high content of vitamin C, phenolic compounds and carotenoids (Grigio et al., 2021). Due to the importance of the economic potential of this species, studies have been carried out to domesticate and select camu-camu clones with superior initial development in the northern region of Brazil (Sakazaki et al., 2022).

The main disease affecting camu-camu trees is anthracnose, which is caused by *Colletotrichum* spp. and detected by sunken brown circular spots. At present only two species, *Colletotrichum aeschynomenes* and *C. tropicale*, have been recorded causing anthracnose on camu-camu in Brazil (Matos et al., 2020). However, information about the diversity of *Colletotrichum* species that cause disease and damage this crop is scarce. Studies on disease diagnosis with detailed identification of the causal agent in camu-camu are fundamental for disease management and for genetic improvement programs, aiming to obtain resistant genotypes.

In 2020, an unknown leaf spot disease characterized by anthracnose-like lesions was observed on native camu-camu trees. Thus, this study aimed to identify *Colletotrichum* isolates associated with leaf anthracnose in camu-camu. Morpho-cultural and molecular phylogenetic characterization was performed for species identification, in addition to the pathogenicity test.

2 | MATERIALS AND METHODS

Symptomatic leaves of camu-camu trees in a natural population have been observed in Rorainópolis, Roraima, Brazil (0°54′128″ N, 60°36′226″ W). In May 2020, the leaves were collected and leave

pieces were obtained from the transition area between diseased and healthy tissue. The surfaces of the fragments were sterilized with 70% ethanol and 2.5% sodium hypochlorite, both for 1 min, and rinsed three times in sterile distilled water. Four leave pieces were transferred to Petri dishes containing potato dextrose agar (PDA), and cultures were incubated for 48 h at 25 ± 2°C under a 12-h photoperiod. Single-spores cultures were deposited in the collection at the Phytopathology Laboratory of the UFRR, Boa Vista, Brazil. Preliminary identification was based on morphological characteristics. The isolates originate from single-spore were cultured on PDA for 14 days at 25 ± 2°C under 12 h photoperiod. Colony colour and shape were observed and photographed with a Canon EOS Rebel t7 camera. Measurements and morphological descriptions of conidia and appressoria were based on 20 observations of each structure/isolate under a Leica DM2500 microscope.

DNA sequencing followed by phylogenetic analyses were used to confirm identification. Genomic DNAs were extracted from 7 days of pure cultures grown on PDA at 25 ± 2°C under 12 h photoperiod using a Wizard® Genomic DNA Purification Kit (Promega Corporation) according to the manufacturer's recommendations. Gene sequences were obtained from four gene regions, calmodulin (*CAL*) was amplified with primers CL1 (5′-GARTWCAAGGAGGCCTTCTC-3′) and CL2A (5′-TTTTTGCATCA TGAGTTGGAC-3′), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) with GDF (5′-GCCGTCAACGACCCCTTCATTGA-3′) and GDR (5′-GGGTGGAGTCGTACTT GAGCATGT-3′) and chitin synthase (*CHS-1*) with CHS-79F (5′-TGGGGCAAGGATG CTTGGAAGAAG-3′) and CHS-345R (5′-TGGAAGAACATCTGTGAGAGTTG-3′), and β-tubulin 2 (*TUB2*) was amplified with primers T1 (5′-AACATGCGTGAGATTGT AAGT-3′) and T2 (5′-TAGTGACCCTTGGCCCAGTTG-3′). The reagents and PCR amplification conditions for *CAL*, *GAPDH*, *CHS-1* and *TUB2* are detailed in Weir et al. (2012). The PCR products were purified and sequenced with the Sanger—ABI 3500XL Genetic Analyser (Thermo Fisher Scientific) at the Molecular Biology laboratory, Embrapa Amazônia Ocidental, Manaus, Amazonas, Brazil. Sequences were analysed and edited in the program SeqAssem v. 1.0 and were compared in the GenBank database, National Center for Biotechnological Information (NCBI), using the BLASTn tool. The sequences obtained were aligned with additional sequences retrieved from GenBank using the multiple sequence alignment program MUSCLE® implemented in the MEGA v. 7 software. Bayesian Inference concatenated analyses were performed as described by Sousa et al. (2018) at the CIPRES web portal. Trees were visualized using Figtree v 1.4.4 and exported to a graphics program. The best nucleotide substitution models selected according to the Akaike information criterion (AIC), were GTR + G for *CAL* and *TUB2*, HKY + I for *GAPDH* and K80 + I + G for *CHS*. The sequences obtained in this study were deposited in GenBank (*CAL*: PP455359, PP455360, PP455361 and PP455362; *GAPDH*: PP434424, PP434425, PP434426 and PP434427; *CHS-1*: PP449018, PP449019, PP449020 and PP449021; *TUB2*: PP455355, PP455356, PP455357 and PP455358).

Pathogenicity tests were conducted in a randomized design with four camu-camu genotypes (UAT0796-3, UAT1596-7, UAT1796-7 and UAT1896-7), two isolates (JFM01 and JFM03), five replications (five seedlings 120-day-old clonal of each genotype/isolate and control), totalling 60 plants. The standard substrate consisted of soil and sand, in a 3:1 ratio. The clones were produced by rooting piles in sub-irrigation chambers. Conidial suspensions from 10-day cultures were used at the concentration of 1×10^6 spores mL^{-1} and sprayed (15 mL/plant) on the seedlings. The seedlings were maintained in a moist chamber using plastic bags for 24 h in the greenhouse under 28 ± 2°C, 70%–96% relative humidity and 12 h of photoperiod. Disease development was observed until 15 days after inoculation. The control seedlings were sprayed with sterile distilled water. To complete Koch's postulate, the fungi were reisolated from the inoculated leaves that showed disease symptoms (Figure 1a), transferred to the culture medium PDA and cultures were incubated for 48 h at 25 ± 2°C under a 12-h photoperiod.

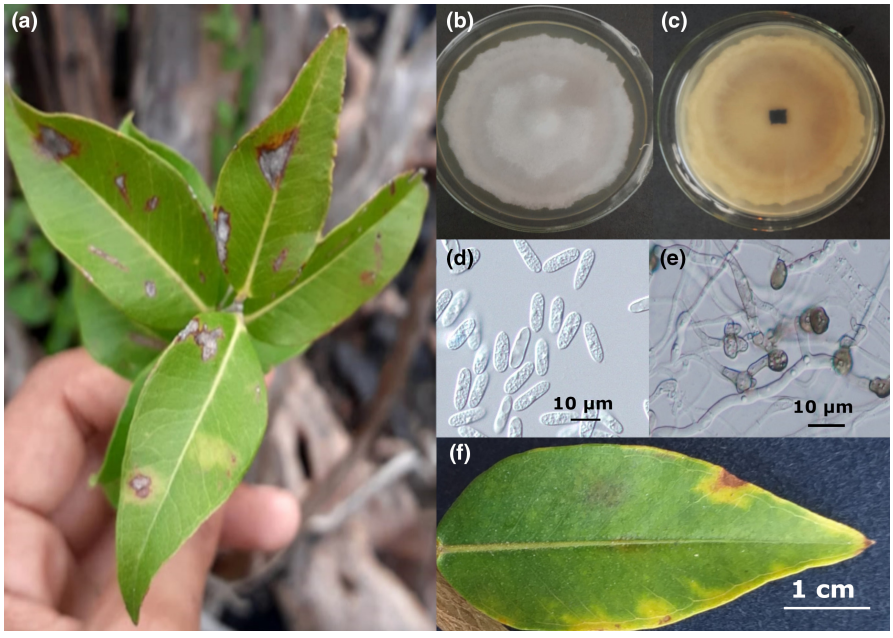

FIGURE 1 *Colletotrichum theobromicola* in camu-camu (*Myrciaria dubia*). (a) Symptoms on leaves with naturally occurring anthracnose symptoms; (b) colony of isolate JFM01 cultured on PDA for 14 days and (c) reverse side; (d) conidia; (e) appressoria; (f) symptoms in leaf at 15 days after inoculation.

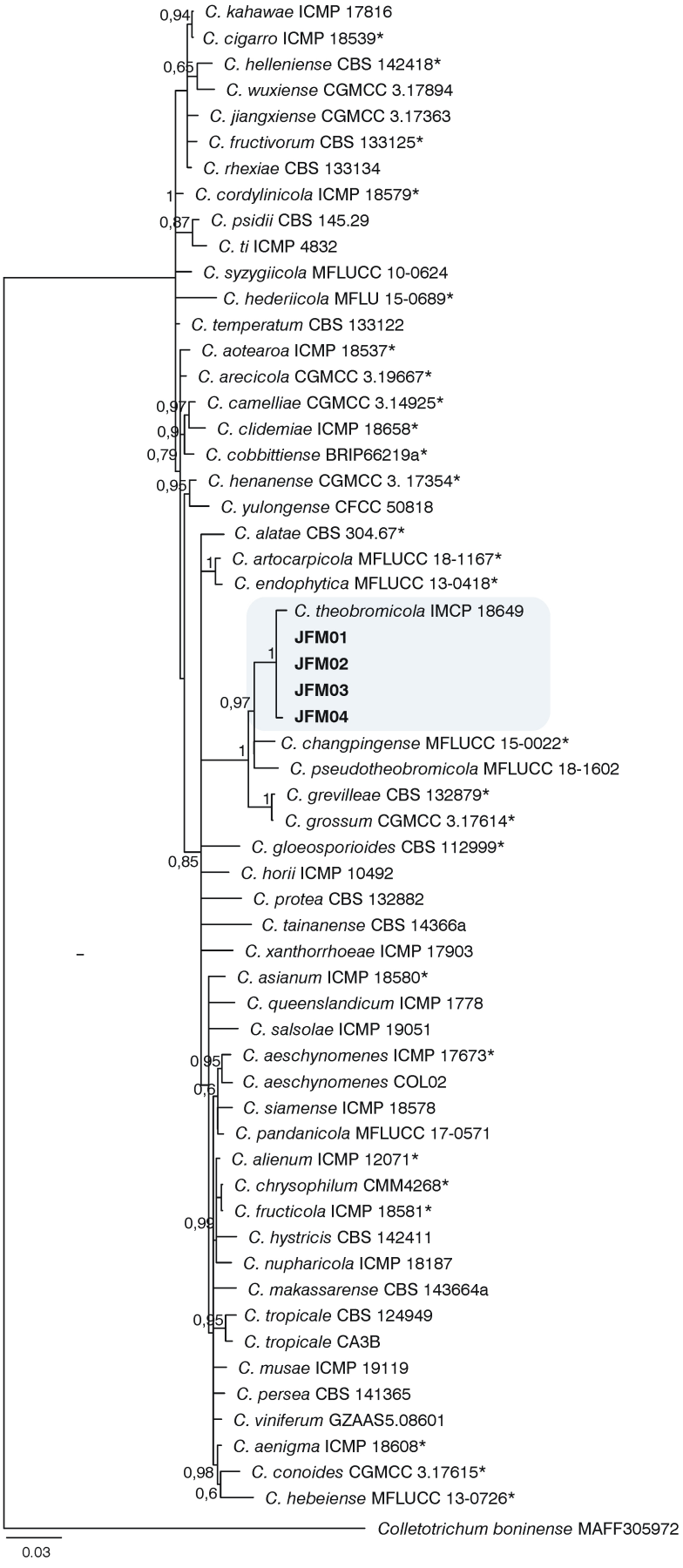

FIGURE 2 Phylogenetic tree constructed by Bayesian Inference of sequences of *CAL*, *GAPDH*, *CHS-1* and *TUB2* regions showing the phylogenetic relationship between *Colletotrichum gloeosporioides* species complex from the leaves of plants of camu-camu (*Myrciaria dubia*) exhibiting anthracnose. Posterior probability values are represented in the junctions of the figure. The isolates used in this study are highlighted in bold. Sequences from ex-type or ex-epitype cultures are indicated (*). The species *Colletotrichum boninense* was used as outgroup.

3 | RESULTS AND DISCUSSION

The morphology of colonies, conidia and appressoria was identical for the four fungal isolates (JFM01, JFM02, JFM03 and JFM04) from camu-camu anthracnose. After 14 days of incubation on PDA, the colonies were characterized as dense white mycelium with a light grey centre on the upper surface (Figure 1b), while the reverse side was grey to dark grey after 2 weeks of incubation and orange-coloured spore masses (Figure 1c). Conidia were hyaline, one-celled, straight, smooth-walled and cylindrical with obtuse to slightly rounded ends (Figure 1d), $15.2 \pm 0.8 \times 6.1 \pm 1.3\,\mu m$ (n = 20), which is typical of the *Colletotrichum gloeosporioides* species complex (Weir et al., 2012). Appressoria were ovoid, nailed and irregular, brown to dark brown (Figure 1e), $6.7 \pm 1.6 \times 5.2 \pm 1.4\,\mu m$ ($n = 20$).

For the molecular phylogenetic studies, the *CAL* sequence dataset gave total alignments of 806 bp, the *GAPDH* sequence dataset with 309 bp, the *CHS-1* sequence dataset with 300 bp and *TUB2* sequence dataset with 729 bp, including gaps. Phylogenetic Bayesian inference analysis based in a combined data set (concatenated sequences of *CAL*, *GAPDH*, *CHS-1* and *TUB2* = 2144 bp) showed that the isolates clustered with the ex-type specimen of *C. theobromicola* (ICMP 18649) with high support (posterior probability = 1) (Figure 2).

The pathogenicity test showed that spots appeared on the sixth day after inoculation, while no lesions were observed on control seedlings. The leaves exhibited small brown to dark spots initially surrounded by a yellow halo, which enlarged and coalesced into regular or irregular brown necrotic lesions (Figure 1f). Symptoms were similar to those observed in the field (Figure 1a). The four camu-camu genotypes showed similar reactions and no resistance to both isolates of the *C. theobromicola*. The UAT0796-3 genotype was not resistant, but showed lower disease severity when inoculated with both fungal isolates. The disease assay was replicated twice for consistency. The same fungal isolates based on morphological analyses were reisolated from the infected leaves and identified as *C. theobromicola*.

The *C. gloeosporioides* species complex consists of *C. gloeosporioides* and 51 closely related species, which may be plant pathogens and endophytes (Jayawardena et al., 2021). *Colletotrichum theobromicola* assigned to the *Gloeosporioides* complex is a broad host range pathogen known to cause anthracnose leaf spot worldwide (Weir et al., 2012). In the Myrtaceae family, *C. theobromicola* was reported on *Eucalyptus urophylla* × *E. grandis*, *Campomanesia phaea*, *Acca sellowiana* and *Psidium guajava* (Farr & Rossman, 2024). To our knowledge, this is the first report of *C. theobromicola* causing anthracnose in camu-camu.

The correct identification of phytopathogenic fungi is important for the adoption of efficient control methods; therefore, different studies are necessary to understand how factors related to the host, the pathogen and the environment affect the incidence and severity of the disease. This pathogen can cause camu-camu yield losses and become a source of a pathogen inoculum and can cause significant damage to other crops.

ACKNOWLEDGEMENTS

Financial support for this study came from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for which we are grateful. We also thank Embrapa Amazônia Ocidental and Embrapa Roraima for technical support, without which this study would not have been possible.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Sachet, M. T. G., de Farias Machado, J., Rincón, D. X. V., da Silva, G. F., Sousa, T. F., Chagas, E. A., da Conceição da Rocha Araújo, M., & da Silva Matos, K. (2024). First report of anthracnose caused by *Colletotrichum theobromicola* on *Myrciaria dubia* in Brazil. *Forest Pathology*, *54*, e12869. https://doi.org/10.1111/efp.12869