

First Report of *Colletotrichum aeschynomenes* and *C. tropicale* Causing Anthracnose on *Myrciaria dubia* in Brazil

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Camu-camu (*Myrciaria dubia* (Kunth) McVaugh) is a native fruit tree of Amazon region that produces fruit with high content of vitamin C. The enormous economic potential has encouraged its domestication process in the northern region of Brazil. Since 2016, brown, necrotic, anthracnose-like lesions were observed on 30-40% of the camu-camu plants in an experimental field located in the city of Boa Vista, Roraima, Brazil (2° 52' 20,7" N; 60° 42' 44,2" W). Small pieces of tissue taken from the lesions were surface disinfested previously (1 min in 70% alcohol, 1 min in 1% NaOCl, and rinsed with sterile water). Leaves sections were plated on water agar medium, incubated at 25°C for 4 days and after were transferred to the potato dextrose agar (PDA) medium. After 7 days on PDA at 25°C, the isolate CA3B formed a colony grayish-white. Conidia were hyaline, aseptate, subcylindrical with rounded ends, measuring ($n = 20$) 12.1 to 15.9 × 4.9 to 7.0 µm. Appressoria were brown, subglobose, clavate, measuring 7.4 to 16.7 × 4.6 to 9.9 µm. The isolate COL02 formed light gray colonies. Conidia were hyaline, aseptate, cylindrical, appearing slightly near both ends, measuring ($n = 20$) 12.3 to 16.1 × 4.7 to 6.5 µm. Appressoria were brown, mostly elliptic to subfusoid, deeply lobed, measuring from 8.7 to 15.9 × 5.8 to 9.9 µm. Single spore cultures were deposited in the Microorganisms Culture Collection of EMBRAPA Roraima, Roraima, Brazil. Total DNA was extracted and fragments of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-tubulin 2 (TUB2), glutamine synthetase (GS), manganese-superoxide dismutase (SOD2) genes were amplified (Weir et al. 2012) and sequenced. The obtained sequences showed 94 to 100% identity with sequences published in the GenBank of the *Colletotrichum gloeosporioides* species complex, with a predominance of *C. tropicale*, *C. aeschynomenes* and *C. siamense*. Sequences from the two isolates were deposited in GenBank (accession numbers GAPDH: MK792453, MK792457; GS:

MK792454, MK792458; SOD: MK792455, MK792459; TUB: MK792456, MK792460). Phylogenetic Bayesian inference analysis based in a combined data set (concatenated sequences of GAPDH, TUB2, GS and SOD2) showed that the isolate CA3B clustered with the ex-holotype specimen of *C. tropicale* (CBS 124949) and the isolate COL02 clustered with the ex-type specimen of *C. aeschynomenes* (ICMP 17673) with high support (posterior probability = 1). To confirm the pathogenicity, five seedlings of the camu-camu were sprayed with 10^6 conidia/mL spore suspension for each isolate. Five seedlings were sprayed with sterile water as negative control. All seedlings were covered with plastic bags 24 h after inoculation, and kept at 27°C in a greenhouse with a 12-h photoperiod. After 7 days, depressed and necrotic lesions were observed in the inoculated leaves. In the third treatment both isolated were co-inoculated. Negative control plants showed no symptoms. To fulfill Koch's postulates, the both species were successfully reisolated from the inoculated and co-inoculated leaves by cultural morphology. In Brazil, *C. tropicale* cause anthracnose on different hosts, such as mango and soursop (Lima et al. 2013; Costa et al. 2019). While *C. aeschynomenes* was associated with *Aeschynomene virginica* in the United States (Weir et al. 2012), cassava in Thailand (Sangpueak et al. 2018) and cacao in Brazil (Nascimento et al. 2019). To our knowledge, this is the first report of *C. aeschynomenes* and *C. tropicale* causing anthracnose on *M. dubia* worldwide.

References

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Figure 1. *Colletotrichum aeschynomenes* (COL02): (A) symptoms of anthracnose in leaves from camu-camu with field lesion. (B) symptoms of anthracnose on inoculated

leaves from camu-camu after 7 days. (C) Colony. (D) Conidia. *Colletotrichum tropicale* (CA3B): (E) symptoms of anthracnose in leaves from camu-camu with field lesion. (F) symptoms of anthracnose on inoculated leaves from camu-camu after 7 days. (G) Colony. (H) Conidia. (I) symptoms of anthracnose on co-inoculated leaves from camu-camu after 7 days. (J) Bayesian phylogenetic tree of the concatenated dataset (GAPDH, TUB2, GS and SOD2) showing phylogenetic relationship among *Colletotrichum gloeosporioides* species complex. Posterior probabilities >0.7 are indicated above the nodes. The strains generated in this study are highlighted in bold. Sequences from ex-type and ex-holotype strains are indicated (*). *C. boninense* were used as the outgroup.

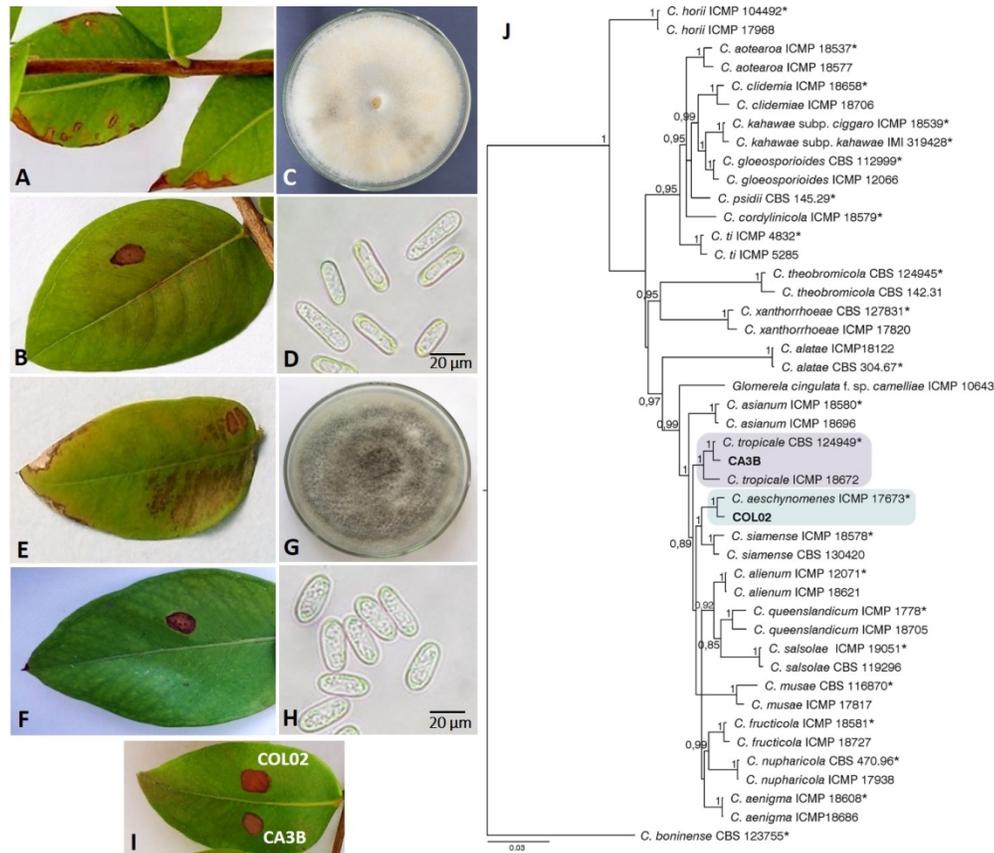


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