First Report of Mulberry Rust Caused by Cerotelium fici on Morus nigra in Brazil

First Report of Mulberry Rust Caused by *Cerotelium fici* on *Morus nigra* in Brazil

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Leaf rust caused by *Cerotelium fici* (Cast.) Arth. is the main disease affecting Moraceae family plants, such as *Ficus* and *Morus* species (Galleti and Rezende 2016; Srikantaswamy et al. 2006). In August 2020, rust symptoms were observed in 100% of mulberry (*Morus nigra* L.) trees in an experimental orchard (Piracicaba, SP, Brazil; 22°42’28”S, 47°37’42”W). Mulberry leaves with high rust severity became yellowish and fell-off prematurely. Pustules were light brown with yellowish halo and presented

mean size of 0.9 mm^2. Uredinial paraphyses (*n* = 50) measured 42.2 ± 0.67 µm long with wall uniformly ca 0.6-1.1 µm thick. Urediniospores were brownish, echinulate, globoid to broadly ellipsoid, and measured 27.1 ± 0.29 × 21.0 ± 0.27 µm with a wall thickness of 0.6 ± 0.01 µm (*n* = 100). The morphology of the urediniospores observed in this study was similar to that reported in the literature for *C. fici* on *Morus alba* and *Ficus* spp. (Gupta et al. 1994; McKenzie 1986; Hennen et al. 2005). We used a low-coverage genome-skimming approach to retrieve genetic information of the rRNA cluster and the mtDNA. Genomic DNA was extracted from 3-4 mg of stored urediniospores at -80 °C, macerated in liquid nitrogen, using a modified cetyl trimethylammonium bromide extraction procedure (Lo Piccolo et al. 2012), and sequenced with 150-bp paired-end reads on Illumina NovaSeq 6000 System. Raw data, (45,761,957 X 2 reads) were assembled with SPAdes v3.15.1 (Bankevich et al., 2012) and the output used to create a custom BLAST database. Loci used for the phylogenetic analyses were identified by BLASTn using, as a query, sequences of *C. fici* from *Ficus* sp. from Australia publicly available: Accession No. MH047210.1 for the rRNA and MW036502.1 for COX3. The retrieved sequences were deposited in GenBank under accession numbers OM296992 and OP797407 for the partial rRNA cluster and COX3, respectively. The Bayesian inference phylogenetic analysis of the three concatenate loci (18S, 28S, and COX3) revealed that the isolate obtained in this study (MN1) was clustered in a well-supported clade with *C. fici* type species. Pathogenicity tests were conducted using mulberry potted plants under greenhouse conditions (25 ± 5 °C). The urediniospores suspension (5 × 10^4 urediniospores ml^{-1}) with 0.05% Tween 20 was sprayed with an airbrush on fully expanded leaves until run-off. As a control, mulberry plants were sprayed with distilled water and kept under the same conditions. Inoculated and mock-inoculated plants were kept in a dark moist chamber at 23 °C (± 2 °C) for 24 h. After this period, plants were moved to the greenhouse. The experimental design was completely randomized with five replicates, each replicate consisted of one potted plant and the experiment was performed twice.

At 12 days post-inoculation, all inoculated plants showed rust symptoms identical to those observed in the field, whereas control plants had no symptoms. The first symptoms were small pustules on the abaxial surface of fully expanded leaves. Small chlorotic lesions were observed on the adaxial leaf surface, which evolved into necrotic lesions. The pathogen was re-inoculated into potted plants, where it was maintained through monthly inoculations. To our knowledge, this is the first report of mulberry rust on *M. nigra* in Brazil. As mulberry leaves are the only natural food for silkworm (*Bombyx mori* L.), rust poses a significant threat to the sericulture industry because the disease can decrease production and quality of mulberry foliage.

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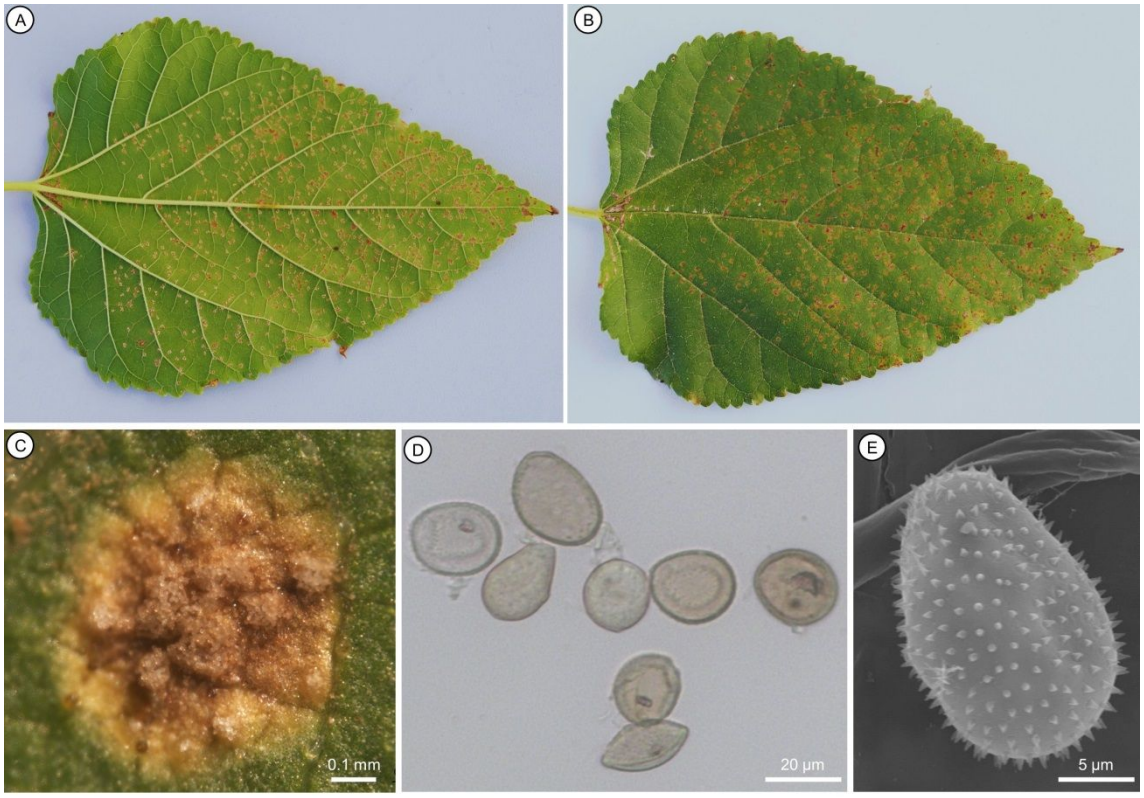
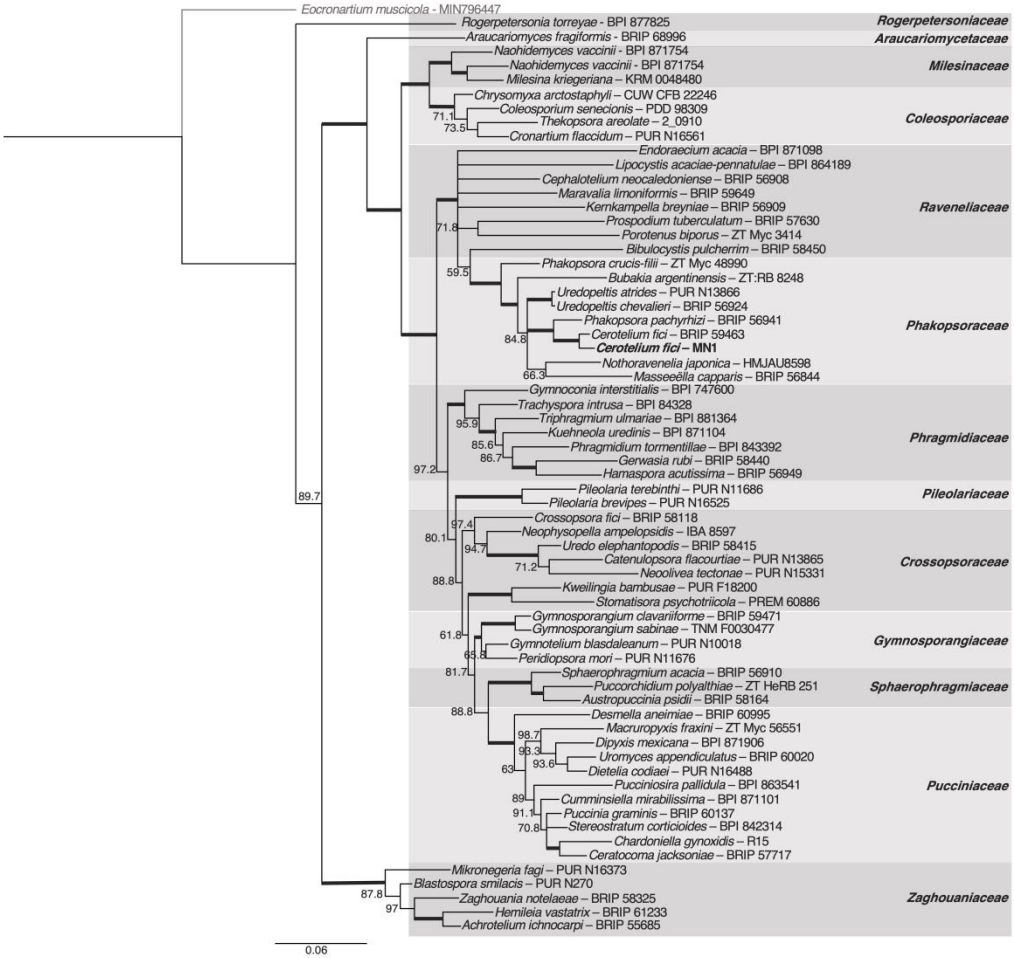
Supplementary material



Supplementary Figure S1. Rust symptoms caused by *Cerotelium fici* in mulberry (*Morus nigra*) leaves; **A**, Uredinia pustules on the abaxial leaf surface; **B**, necrotic lesions on the adaxial leaf surface; **C**, light brown uredinia with a yellowish halo; **D** and **E**, urediniospores of *C. fici* under light and scanning electron microscopy, respectively.



Supplementary Figure S2. Bayesian inference phylogenetic three reconstructed from the combined 28S, 18S, and COX3 sequence alignment of 66 representative isolates of the Pucciniales order. *Eocronartium muscicola* was used as outgroup. Families are indicated by blocks. Thickened nodes represent Bayesian posterior probability (BPP) > 0.99; BPP ≤ 0.99 are shown at the nodes. *Cerotelium fici* strain isolated from *Morus nigra* is emphasized in bold. The scale bar represents the number of expected changes per site.