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

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

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18

19 Leaf rust caused by *Cerotelium fici* (Cast.) Arth. is the main disease affecting Moraceae
20 family plants, such as *Ficus* and *Morus* species (Galletti and Rezende 2016;
21 Srikantaswamy et al. 2006). In August 2020, rust symptoms were observed in 100% of
22 mulberry (*Morus nigra* L.) trees in an experimental orchard (Piracicaba, SP, Brazil;
23 22°42'28"S, 47°37'42"W). Mulberry leaves with high rust severity became yellowish
24 and fell-off prematurely. Pustules were light brown with yellowish halo and presented

25 mean size of 0.9 mm². Uredinial paraphyses ($n = 50$) measured $42.2 \pm 0.67 \mu\text{m}$ long
26 with wall uniformly ca 0.6-1.1 μm thick. Urediniospores were brownish, echinulate,
27 globoid to broadly ellipsoid, and measured $27.1 \pm 0.29 \times 21.0 \pm 0.27 \mu\text{m}$ with a wall
28 thickness of $0.6 \pm 0.01 \mu\text{m}$ ($n = 100$). The morphology of the urediniospores observed
29 in this study was similar to that reported in the literature for *C. fici* on *Morus alba* and
30 *Ficus* spp. (Gupta et al. 1994; McKenzie 1986; Hennen et al. 2005). We used a low-
31 coverage genome-skimming approach to retrieve genetic information of the rRNA
32 cluster and the mtDNA. Genomic DNA was extracted from 3-4 mg of stored
33 urediniospores at $-80 \text{ }^\circ\text{C}$, macerated in liquid nitrogen, using a modified cetyl
34 trimethylammonium bromide extraction procedure (Lo Piccolo et al. 2012), and
35 sequenced with 150-bp paired-end reads on Illumina NovaSeq 6000 System. Raw
36 data, (45,761,957 X 2 reads) were assembled with SPAdes v3.15.1 (Bankevich et al.,
37 2012) and the output used to create a custom BLAST database. Loci used for the
38 phylogenetic analyses were identified by BLASTn using, as a query, sequences
39 of *C. fici* from *Ficus* sp. from Australia publicly available: Accession No. MH047210.1
40 for the rRNA and MW036502.1 for COX3. The retrieved sequences were deposited in
41 GenBank under accession numbers OM296992 and OP797407 for the partial rRNA
42 cluster and COX3, respectively. The Bayesian inference phylogenetic analysis of the
43 three concatenate loci (18S, 28S, and COX3) revealed that the isolate obtained in this
44 study (MN1) was clustered in a well-supported clade with *C. fici* type species.
45 Pathogenicity tests were conducted using mulberry potted plants under greenhouse
46 conditions ($25 \pm 5 \text{ }^\circ\text{C}$). The urediniospores suspension (5×10^4 urediniospores ml⁻¹)
47 with 0.05% Tween 20 was sprayed with an airbrush on fully expanded leaves until run-
48 off. As a control, mulberry plants were sprayed with distilled water and kept under the
49 same conditions. Inoculated and mock-inoculated plants were kept in a dark moist
50 chamber at $23 \text{ }^\circ\text{C}$ ($\pm 2 \text{ }^\circ\text{C}$) for 24 h. After this period, plants were moved to the
51 greenhouse. The experimental design was completely randomized with five replicates,
52 each replicate consisted of one potted plant and the experiment was performed twice.

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

62

63 *References*

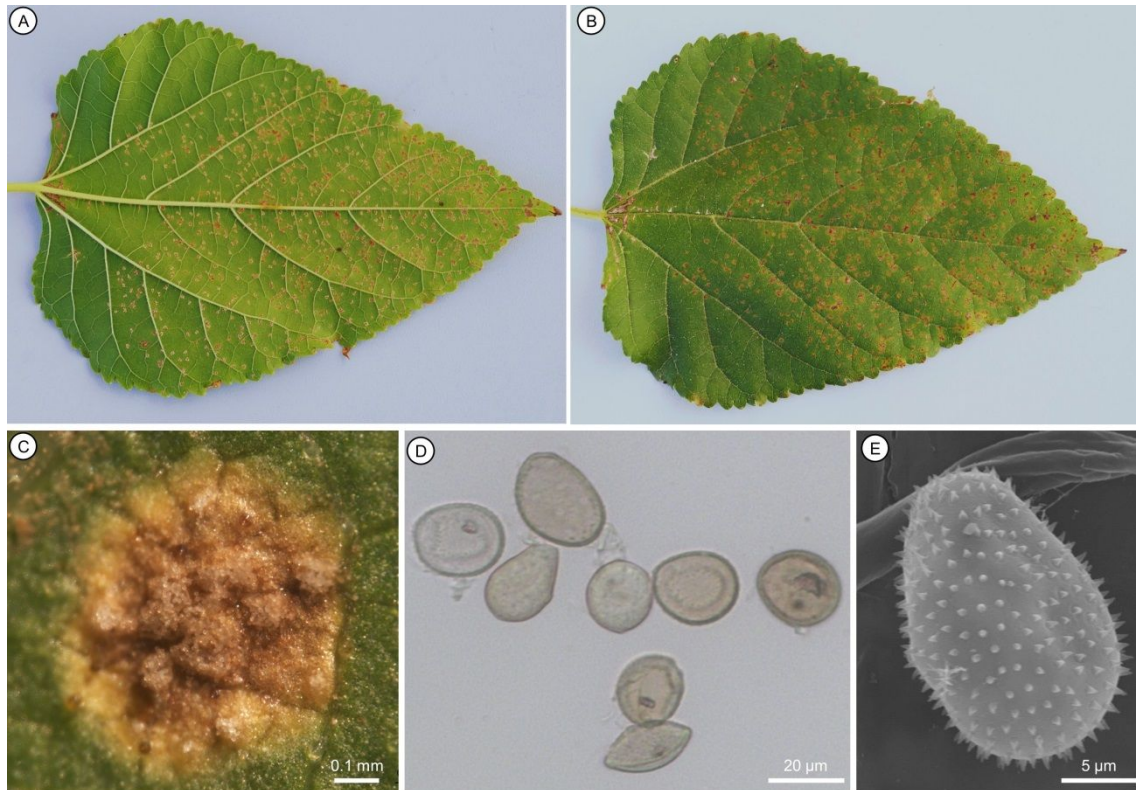
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Supplementary material



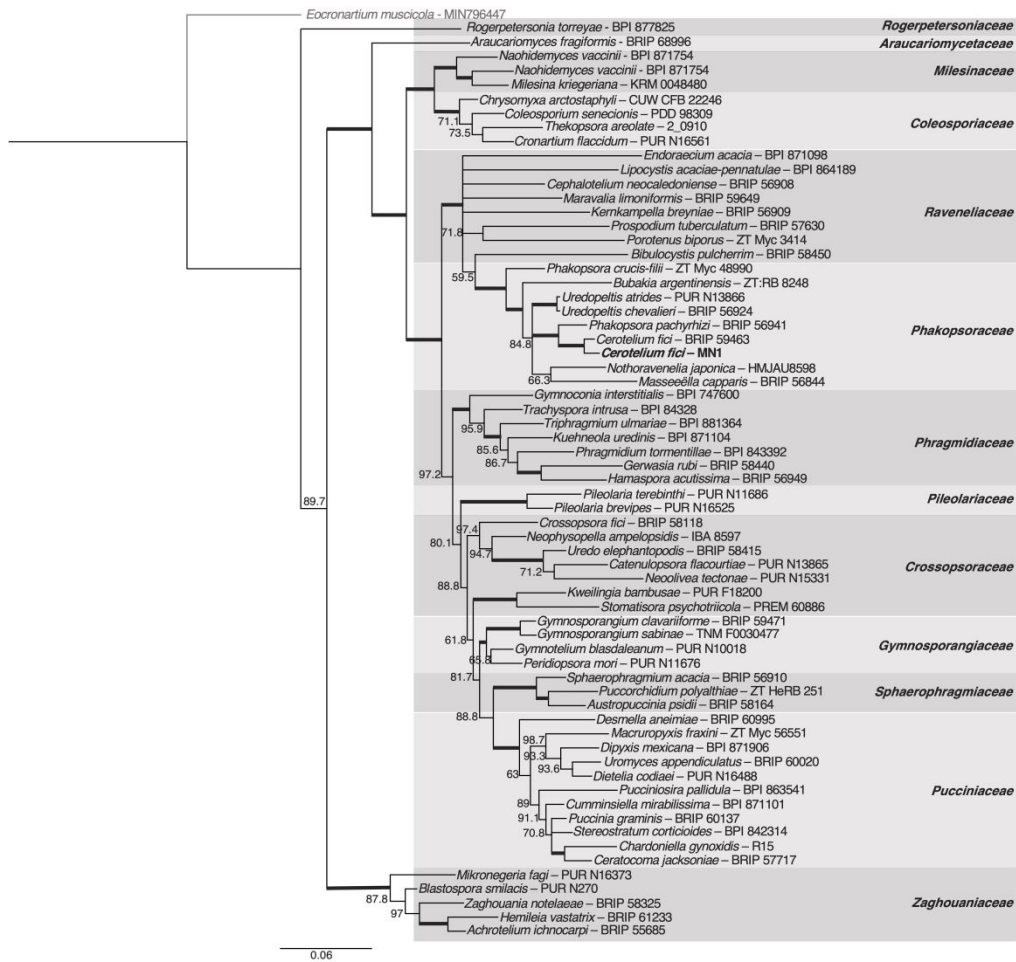
3

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

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13 **Supplementary Figure S2.** Bayesian inference phylogenetic tree
 14 reconstructed from the combined 28S, 18S, and COX3 sequence alignment of
 15 66 representative isolates of the Pucciniales order. *Eocronartium muscicola* was
 16 used as outgroup. Families are indicated by blocks. Thickened nodes represent
 17 Bayesian posterior probability (BPP) > 0.99; BPP ≤ 0.99 are shown at the
 18 nodes. *Cerotelium fici* strain isolated from *Morus nigra* is emphasized in bold.
 19 The scale bar represents the number of expected changes per site.