First Report of Colletotrichum musicola Causing Soybean Anthracnose in Brazil

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1 **First report of *Colletotrichum musicola* causing soybean anthracnose in**

2 **Brazil**

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15 Soybean (*Glycine max* L.) is one of the most important crops worldwide as a

16 source of protein-rich foods and animal feeds. Anthracnose, one of the major

17 limiting factors to soybean production (Dias et al. 2016), is caused by species

18 such as *Colletotrichum truncatum, C. sojae,* and *C. plurivorum* (Damm et al.

19 2019, 2009). In December 2016/2017, soybean plants of cultivars Monsoy 8768

20 and Pioneer y-70 with typical symptoms of anthracnose (necrotic and irregular

21 brown lesions on stems, leaves and pods) were collected in Mato Grosso, Brazil.

22 Commercial fields sampled showed 10 – 15 % of incidence of anthracnose in one

23 hectare in each sampled area. In total, 10 different geographic locations were

24 sampled. *Colletotrichum* strains were isolated and cultured on potato dextrose

25 agar at 25 ºC with a 12-h light photoperiod from surface-disinfected (70 % alcohol

26   - 0.5 % sodium hypochlorite) plant tissues. Among others, three single-spore

27   isolates (LFN0048 from Sinop, LFN0074 and LFN0090 from Lucas do Rio Verde)

28   showed different morphology; isolates LFN0048 and LFN0074 were selected for

29   further characterization. Total DNA was extracted and partial GAPDH

30   (Glyceraldehyde 3-phosphate dehydrogenase), HIS3 (histone H3), and TUB2

31   (beta-tubulin) genes were amplified and sequenced. The sequences were

32   deposited in GenBank (accession numbers MN604249 and MK163893 for HIS3,

33   MN604248 and MK142674 for GAPDH, and MN604250 and MK142675 for TUB)

34   and were compared with most similar reference sequences of *Colletotrichum*

35   (Damm et al. 2019). Both isolates clustered with *C. musicola* epitype (CBS

36   132885), showing 100% and 98.5% similarity in GAPDH, 99.5% and 98.9% in

37   HIS3 and 99.2% in TUB2. On PDA, colonies showed dark-grey aerial mycelium

38   with entire margins, reverse violaceous-black. Conidia and ascospores size and

39   shape match previously described by Damm et al. (2019): 12.12 – 15.86 x 4.93

40   – 6.95 µm and 15.5 – 19.34 x 5 – 7.84 µm, respectively (n = 100). Appressoria

41   (n=50) were single or in loose-groups, violaceous-black with predominant

42   obovoid, truncated and cylindrical shapes, with smooth, undulate or lobate

43   margin, with 9.25 – 29.79 x 7.22 – 21.06 µm in size. Perithecia, paraphyses; and

44   unitunicate 8-spored asci were also observed. Asci were cylindrical to clavate,

45   smooth-walled and 48.12 – 68.78 x 9.59 – 14.47 µm in size (n = 50) (Figure S1).

46   Soybean anthracnose is seed-borne (Rogério et al., 2017; Dias et al., 2018),

47   therefore pathogenicity tests were carried out on pre-germinated seeds. Five

48   seeds of Brasmax 8579 cultivar were inoculated with 10 µL of a conidial

49   suspension (10^6 conidia/mL) that was placed in the emerging radicle while five

50   mock-inoculated seeds were used as control. Seedlings were planted in

51   vermiculite and incubated at 25 ºC with a 12-h photoperiod. After seven days,

52   inoculated plants showed necrotic lesions on the cotyledons, leaflets, and

53   hypocotyl, whereas control plants remained asymptomatic. The experiment was

54   repeated three times. *C. musicola* was re-isolated from the symptomatic tissues

55   and the identity was confirmed by morphology and multilocus phylogeny (Figure

56   S2). Until now, *C. musicola* has been reported to be associated with *Musa* sp.

57   (Damm et al. 2019) and *Colocasia esculenta* (Vásquez-Lopez et al. 2019) in

58   Mexico, and with *Phaseolous lunatus* in Brazil (Cavalcante et al. 2019). To our

59   knowledge, this is the first report of *C. musicola* joining a group of new and

60   emergent species of *Colletotrichum* causing anthracnose in soybean producing

61   regions around the world.

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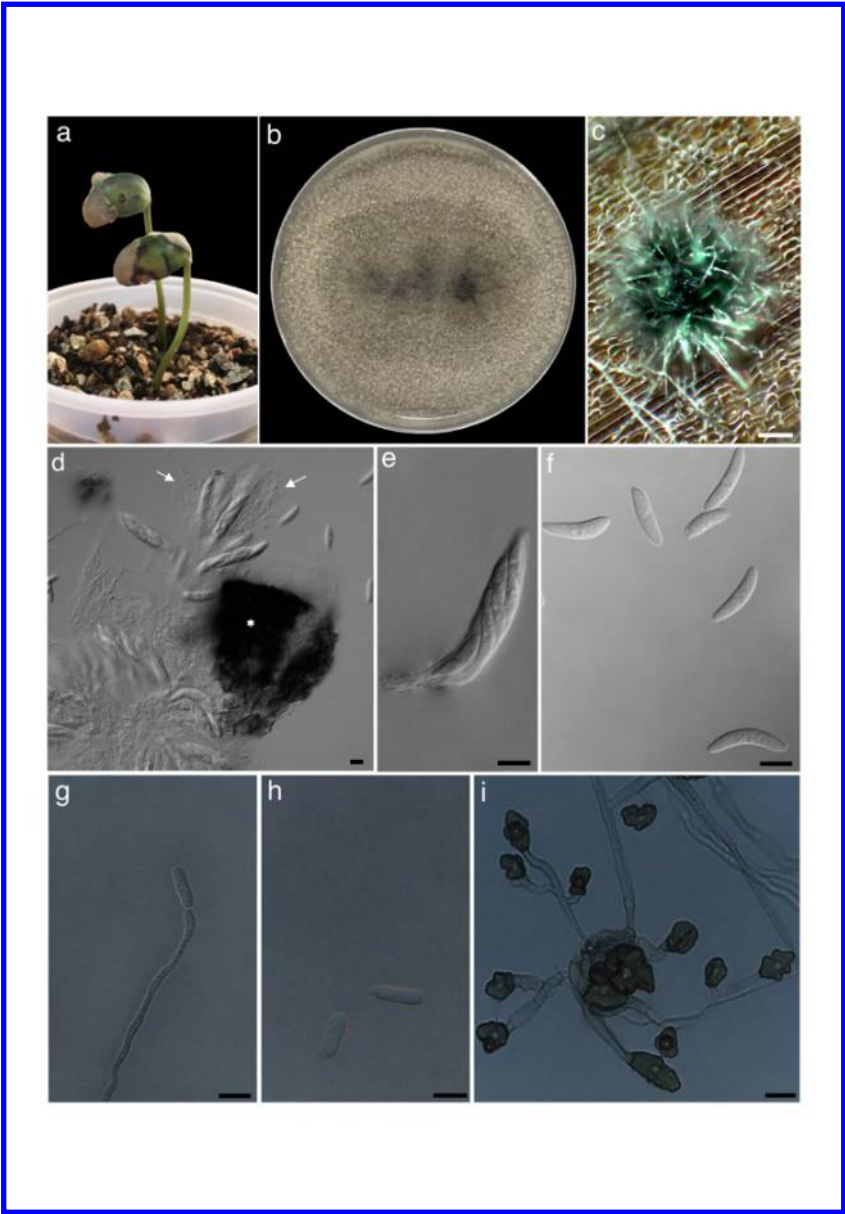78   Rogério, F. et al. 2017. Journ. Of Appl. Microb. 10.1111/jam.13346.

Figure S1: (a) Necrotic lesions on soybean cotyledons seven days after inoculation with *Colletotrichum musicola* conidial suspension. (b) Fourteen-day- old *C. musicola* colony on PDA. (c) Ascomata. (d) Perithecia (*), paraphyses (arrows), asci and ascospores (e) Ascus. (f) Ascospores. (g) Conidia produced in a conidiogenus cell. (h) Conidia. (i) Appressoria. Scale bars: (c) 100 µm. (d-i) 10 µm.
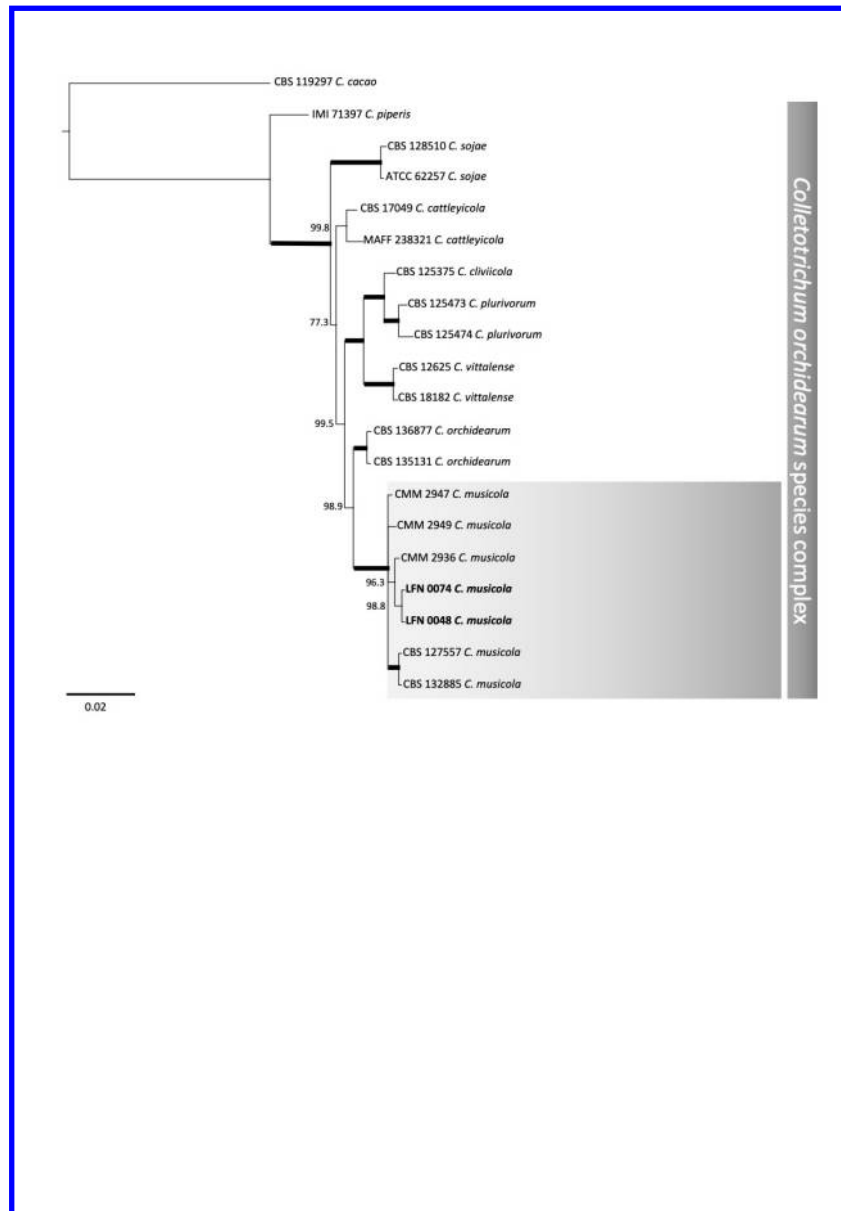
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Figure S2: Bayesian inference phylogenetic tree reconstructed from the combined GAPDH, HIS3, and TUB2 sequence alignment of 19 isolates of the *Colletotrichum orchidearum* species complex. *C. cacao* CBS 119297 was used as outgroup. Thickened nodes represent Bayesian posterior probability (BPP) > 0.9; BPP ≥ 0.9 are shown at the nodes. *Colletotrichum musicola* strains isolated from soybean are emphasized in bold. The scale bar represents the number of expected changes per site. TreeBASE accession number S25689.

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