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First report of *Colletotrichum theobromicola* causing anthracnose on *Anthurium* sp.

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Abstract
Anthurium is one of the most frequently grown commercial tropical flowers. In December 2016, *Anthurium* flowers with circular necrotic spot symptoms were collected in Brazil. Based on morphological and molecular characterisation, the fungus isolates were identified as *Colletotrichum theobromicola*, which were detected for the first time causing anthracnose on *Anthurium* in the world.

Keywords Tropical flowers · Araceae · Fungal disease · Phylogenetic analysis

The Araceae are widely distributed in Brazil, with species occurring from the southern most subtropical areas to the equatorial rainforest in the extreme north of the country. The genus *Anthurium* contains 130 native species from Brazil, which are known most commonly as anthurium, flamingo flower and tailflower (Tombolato and Castro 2005). *Colletotrichum* species are among the most important damaging pathogens infecting economic crops such as vegetables, fruits, flowers and non-cultivated plants. Tropical flowers are expected to have a good appearance, however disease damage affects marketability. In Brazil, information about tropical flower diseases is limited (Figs. 1, 2, 3 and 4).

In December 2016, flowers (*Anthurium* spp.) with circular necrotic spot symptoms were collected in Atalaia, state of Alagoas, Brazil. Initially, small symptomatic flower pieces were cut from the edge of the diseased and healthy sections and were surface sterilised with 70% ethanol for 30 s and 1% NaOCl for 1 min followed by two washes in sterile distilled water, then placed on 2% water agar (*w*/*v*), and incubated at 25 °C for 5 days. Hyphae extending from the flower pieces were transferred to potato dextrose agar (PDA). After incubation at 25 °C for 7 days under 12 h light-dark cycles, initial identification of the isolates was realised according to Sutton (1992). Six *Colletotrichum*-like isolates were obtained by single-spore culture in potato dextrose agar (PDA) and deposited in the Culture Collection Micoteca URM at the Universidade Federal de Pernambuco (URM8227). The isolates produced white cottony aerial hyphae that later became brown. The underside of the colony was uniformly black with a growth rate of 44 mm/day. Conidia hyaline, straight to cylindrical, aseptate and 7.5 to 25.5 (15.5) μm and 3.5 to 6.5 (4.5) μm. The morphological characteristics were consistent with the descriptions of species in the *Colletotrichum gloeosporioides* species complex.

To confirm the identity of the isolates, partial sequences of the internal transcribed spacer (ITS) of rDNA region, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), calmodulin (CAL), chitin synthase (CHS) and actin (ACT) genes were amplified. The reaction mixture and cycling conditions were the same as described by Carbone and Kohn (1999) and Weir et al. (2012). The sequences of URM8227, as a representative isolate, were submitted to GenBank (MH155178,

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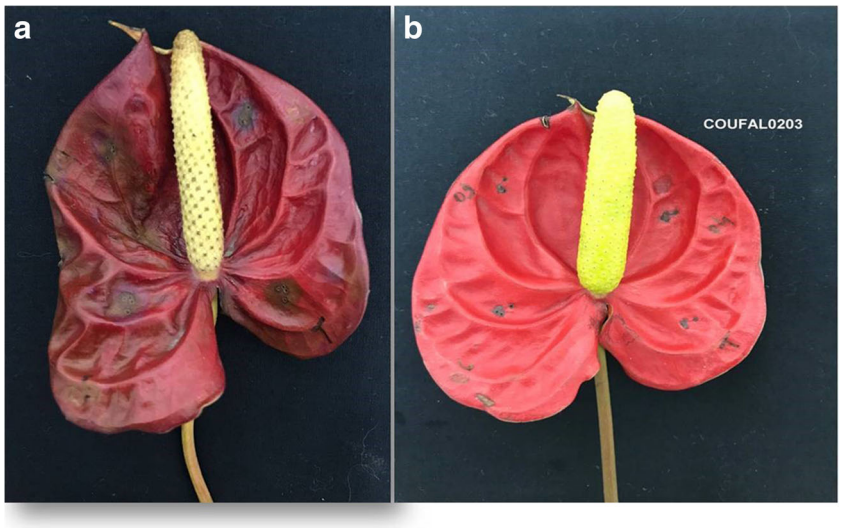

Fig. 1 Lesions, circular necrotic spots on petals caused by *C. theobromicola* in *Anthurium* flowers (**a**) Symptoms on flowers of *Anthurium* with injury 3 days post inoculation; (**b**) Symptoms observed in pathogenicity tests no injury 3 days post inoculation

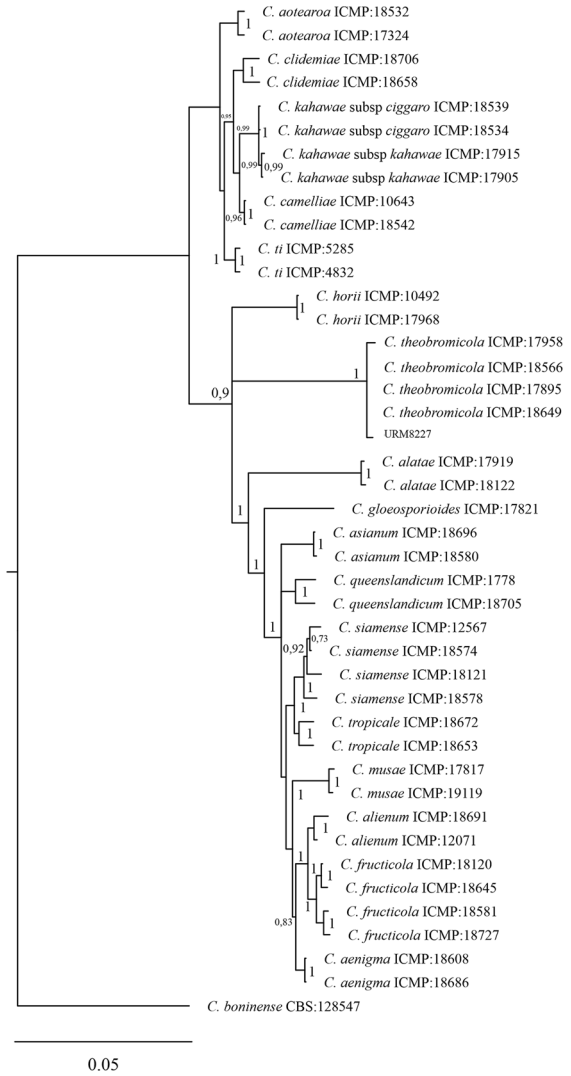

Fig. 2 Phylogenetic tree based on alignment of nucleotides sequences of genes ACT, CHS-1, CAL, GAPDH and ITS region using Bayesian analysis

MH155179, MH155176, MH155177 and MH155175 respectively). In addition, Bayesian inference analysis using concatenated sequences of genes (ITS, GAPDH, CAL, CHS, ACT) the *Colletotrichum gloeosporioides* species complex was performed. Based on Bayesian inference analysis and morphological characteristics, the URM8227 isolate obtained from diseased *Anthurium* flowers was identified as *C. theobromicola* (Rojas et al. 2010).

In order to confirm pathogenicity of *C. theobromicola*, healthy *Anthurium* flowers were first washed with distilled water following the protocol of Bellé et al. (2018). The flowers were inoculated with 10 μL of conidial suspension (10^6 spores/mL) from a 7-day-old culture (grown at 25 °C) on PDA. Some of the *Anthurium* flowers were wounded with a sterile needle and others were not wounded. On control flowers, only sterilised water was used.

Inoculated flowers were kept in a humid chamber for 2 days at 25 °C with photoperiod of 12 h. After 7 days, the flowers showed necrotic spot symptoms. No symptoms were observed on the control flowers. A culture of *C. theobromicola* was re-isolated from diseased flowers, thereby fulfilling Koch's postulates. Currently, The *C. gloeosporioides* species complex comprises more than 22 members, using multi locus phylogenetic analyses (Weir et al. 2012). However, in Brazil there are only reports of *Colletotrichum gloeosporioides* causing anthracnose in tropical flowers. (Warumby et al. 2004). *Colletotrichum theobromicola* was previously reported on *Theobroma cacao*, *Coffea* spp., *Annona reticulata*, *A. muricata* (Rojas et al. 2010; James et al. 2014; Udayanga et al. 2013) and in Brazil was first reported on eucalyptus (Rodrigues et al. 2014). To our knowledge, this is the first report of *C. theobromicola* causing anthracnose on flowers of *Anthurium* sp. in the world.

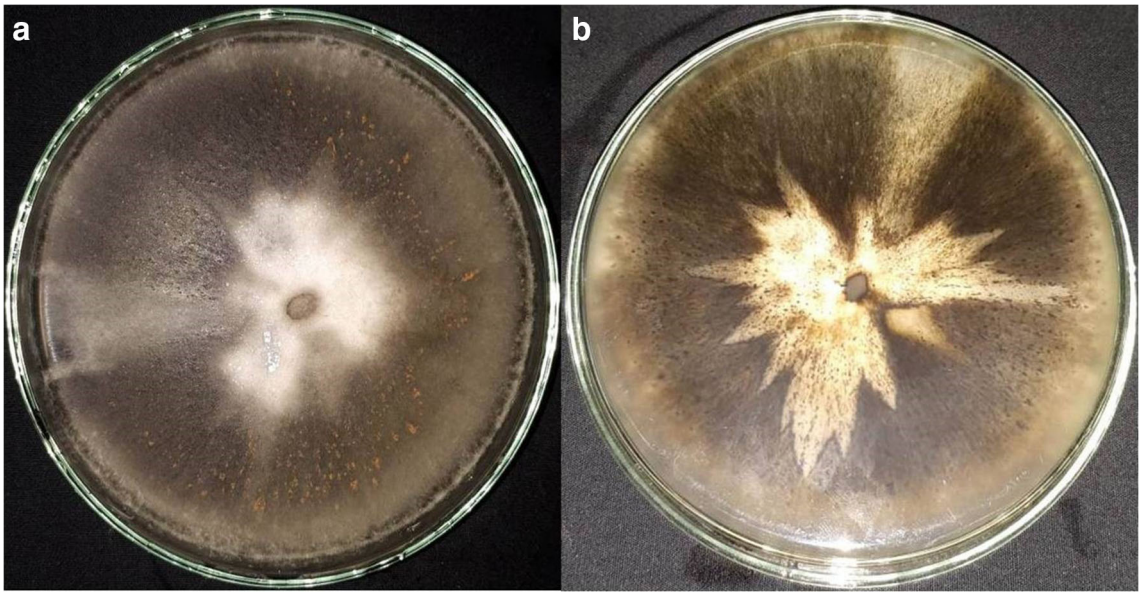

Fig. 3 Colony of *Colletotrichum theobromicola* on PDA 15-day-old (**a**) from above; (**b**) from below

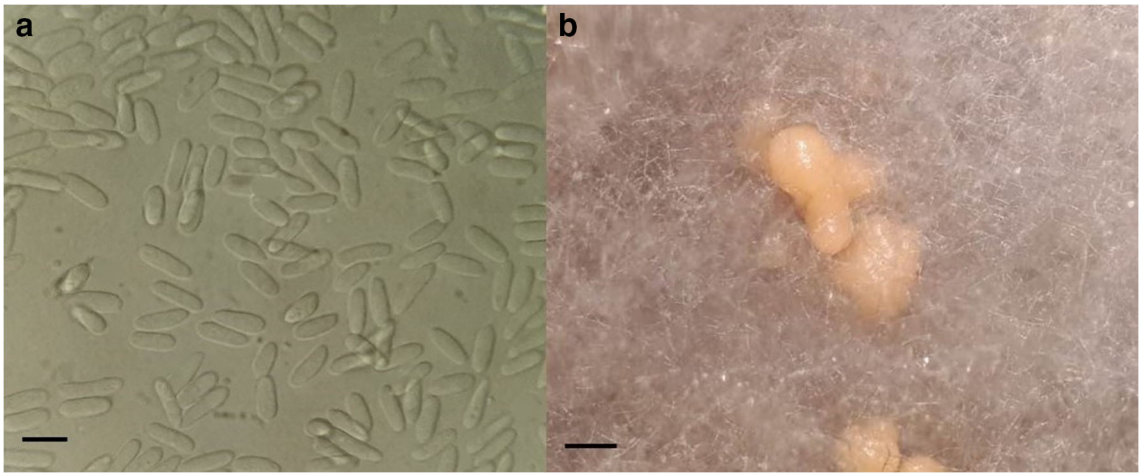

Fig. 4 (**a**) Conidia (×100) of *C. theobromicola*; Scale bar = 15 μm (**b**) conidiophores without setae on PDA 15-days-old; Scale bar = 0.5 cm

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