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DISEASE NOTE

First report of *Cladosporium anthropophilum* causing leaf spots on sourgrass (*Digitaria insularis*) in Brazil

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Abstract Sourgrass (*Digitaria insularis* L. Fedde) is one of the most aggressive agricultural weeds in the Americas, with widespread populations resistant to glyphosate and acetyl coenzyme A carboxylase (ACCase)–inhibiting herbicides. During a survey of the pathogenic mycobiota associated with sourgrass in Brazil, leaf spot symptoms were observed in December 2020 in Uberaba, Minas Gerais. Lesions were initially circular and later became elliptical, dark brown with a light brown center, occurring mainly on the adaxial leaf surface. A fungus was consistently isolated from necrotic leaf tissues and characterized based on morphological and molecular analyses. The isolate (COAD 3621; KDI 0118) exhibited morphological features typical of *Cladosporium*. Sequencing of the ITS, ACT, and TEF gene regions followed by BLASTn and multilocus phylogenetic analyses confirmed the identity of the fungus as *Cladosporium anthropophilum*. Pathogenicity tests conducted on healthy sourgrass plants resulted in severe leaf spot symptoms seven days after inoculation, and the pathogen was successfully reisolated, fulfilling Koch's postulates. *Cladosporium anthropophilum* has been previously reported on other plant hosts; however, to our knowledge, this is the first report of *C. anthropophilum* causing leaf spots on *D. insularis* in Brazil. Studies evaluating the biocontrol potential of this isolate are currently in progress.

Keywords Ascomycota · Cladosporiaceae · Fungal · Taxonomy · Tropical fungi · O weed management

Sourgrass (*Digitaria insularis* L. Fedde) is a graminaceous weed native from tropical and subtropical America. It is presently regarded as one of the most aggressive and damaging agricultural weeds in the Americas. The intensive use of herbicides has resulted in the selection, and widespread occurrence, of sourgrass populations resistant to glyphosate and, more recently, also to acetyl coenzyme A carboxylase (ACCase) inhibitor herbicides (Takano et al. 2020). New management methods are needed to tackle sourgrass infestations. Biological control has never been investigated for this weed species. A survey of the pathogenic mycobiota of sourgrass is under way in Brazil. In December 2020, in an area of approximately 200 m² at Uberaba, MG, Brazil (19°44'52" S, 47°55'55" W), sourgrass plants bearing abundant leaf spots were observed. Such spots were firstly circular and grew becoming elliptical dark brown with a light brown center (2.7–12.5 × 0.7–4 mm), distributed in a large part of the leaf blade and visualized predominantly on the adaxial face. Samples were collected, dried in a plant press, and a representative specimen was selected and deposited in the herbarium at the Universidade Federal de Viçosa under the Accession number VIC 49,447. Isolation was performed from necrotic tissues and a pure culture were obtained by transferring single conidia to plates containing potato dextrose-agar (PDA) with a sterile fine- pointed needle. One isolate, coded as KDI 0118, was selected and deposited at the "Coleção Octávio Almeida Drummond" Universidade Federal de Viçosa under the Accession number COAD 3621. Colonies were: flat, aerial mycelium velvety, green with a thin white margin and a dark green reverse, sporulation very abundant. Morphology after seven days growth in PDA was as follows: conidiophores macronematous, light brown, solitary; ramoconidia cylindrical, pale brown, 3 to 7 × 1.09 - 2.03 µm, aseptate; conidia acrogenous, in branched chains, ellipsoid, obovoid, subhyaline to pale brown, with darkened and thickened hilum, 1.16−5.09 × 1.16–2.76 µm (n=30), aseptate. The fungus on sourgrass is a typical *Cladosporium* (Marin-Felix et al. 2017) (Fig. 1). Genomic DNA

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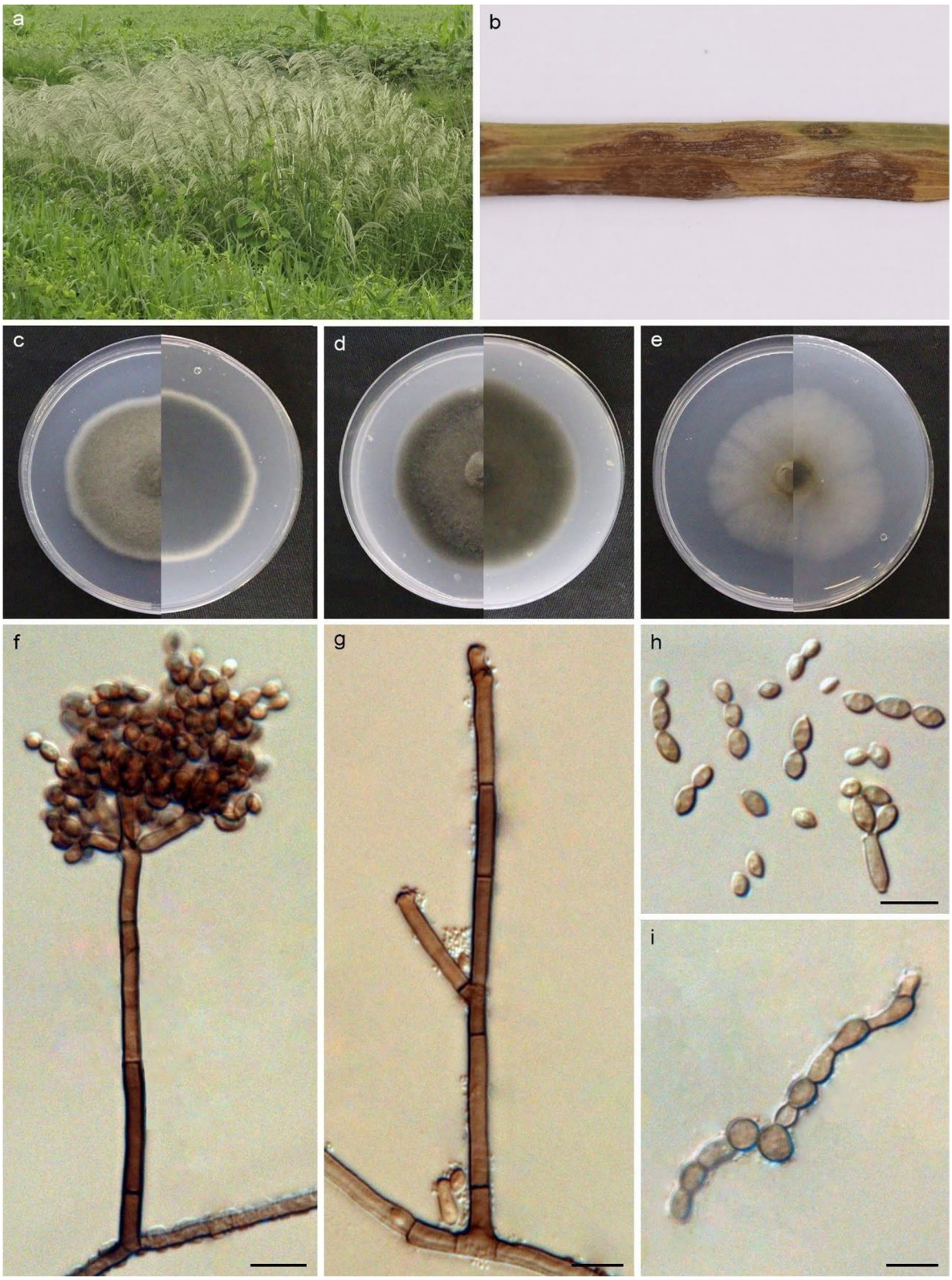

Fig. 1 Morphology and pathogenicity of *Cladosporium anthropophilum*. **a**: *Digitaria insularis* plants in the field; **b**: Necrotic leaf spots; **c-e**: *Cladosporium* colony on PDA, OA and WA; **f**: Conidiophores with conidia; **g**: Branched conidiophores with conidiogenous cells; **h**: Conidia; **i**: Chlamydospores. Scale bars = 5 µm

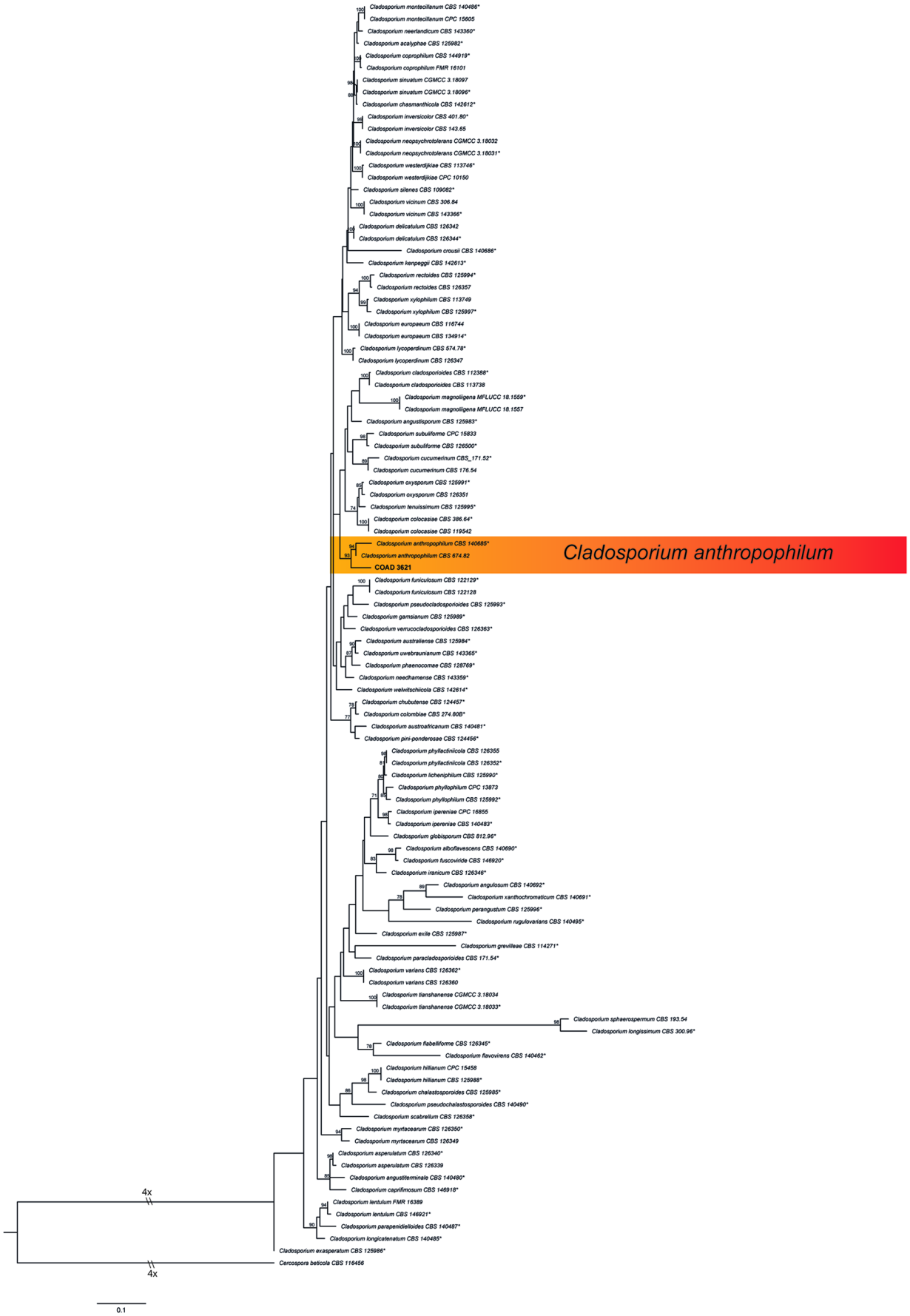
Cladosporium anthropophilum
COAD 3621
4x
4x
0.1

Fig. 2 Multilocus phylogenetic tree of *Cladosporium* specie inferred from RAxML based on combined ITS, TEF and ACT sequences. The bootstrap > 70 are indicated above the nodes. The isolate from the study are highlighted in bold and *** indicates types- isolates. The tree was rooted with *Cercospora beticola* (CBS 116456) isolate

was extracted using the Wizard® genomic DNA purification kit (Promega). The ITS region, TEF and ACT genes were sequenced using ITS1/ITS4 (White et al. al. 1990), EF1- 728 F/ EF1-986R and ACT512F/ACT-783R primer pairs, respectively (Carbone and Kohn 1999). Sequences were deposited in GenBank (accession no. OR473653 for ITS, OR478710 for ACT, and OR478711 for TEF). BLASTn analysis of these sequences showed a high similarity (90 to 99.8%) with the type isolate of *C. anthropophilum* (CBS 140685). Phylogenetic analysis using maximum likelihood based on the combination of sequences from ITS, TEF and ACT indicated that COAD 3621 groups with *C. anthropophilum* with 93% support. Based on morphological and molecular characteristics, the fungus on sourgrass spots was then identified as *C. anthropophilum* (Sandoval-Denis et al. 2016). Five pots containing 10 healthy 20 days-old sourgrass plants were used in inoculation test. 10 mL of a suspension containing 1×10^6 conidia/mL was sprayed until runoff on plants in each pot with a hand-held-spray. Plants in control were sprayed with sterile distilled water and left in a dew chamber for 48 hs.

Afterwards plants were left in a greenhouse bench at ca. 25 °C. All sourgrass leaves inoculated with COAD 3621 developed severe leaf spot lesions 7 days after the inoculation, confirming the pathogenicity of the isolate KDI 0118. Such lesions were equivalent to those observed on diseased plants in the field, whereas no symptoms were observed on the noninoculated plants. Typical *C. anthropophilum* colonies were formed after isolations performed from diseased fragments taken from necrotic – surface sterilized fragments subjected to 1% NaOCl disinfection and plated on PDA and conidia and conidiophores formed on those colonies were typical of *C. anthropophilum*.

Rubus idaeus, *Pinus armandii*, *Phytolacca americana*, *Carya* sp. have been reported as hosts of *C. anthropophilum* (Tibpromma et al. 2019; Cho et al. 2023; Sandoval-Denis et al. 2016). To our knowledge, this is the first report of *C. anthropophilum* causing leaf spots on *D. insularis* in Brazil. Evaluations on biocontrol potential of COAD 3621 (isolate KDI 0118) are being conducted. (Fig. 2)

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Declarations

Ethical approval This article does not contain any studies requiringethical approval.

Conflicts of interest The authors declare that they have no conflicts of interest.

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