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First report of citrus chlorotic spot virus infecting the succulent plant *Agave desmettiana* Jacob.

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Endemic in the Americas, citrus leprosis (CL) is a viral disease that reduces yields and threatens the sustainability of citrus orchards. The disease is caused by a heterogeneous group of ssRNA viruses assigned to the genera *Dichorhavirus* (family *Rhabdoviridae*, order *Mononegavirales*) or *Cilevirus* (unassigned genus) (Freitas-Astúa et al. 2018). The typical dichorhavirus genome encompasses two (-)ssRNA molecules that encode six ORFs (Dietzgen et al. 2018). Isolates of the tentative dichorhavirus citrus chlorotic spot virus (CiCSV) were previously identified in Teresina, Piauí, Brazil (Chabi-Jesus et al. 2018). Besides affecting sweet orange (*Citrus sinensis*) trees producing symptoms resembling early lesions of a typical CL, CiCSV also infects beach hibiscus (*Talipariti tiliaceum* syn. *Hibiscus tiliaceus* L.) plants. In 2018, a dozen of the ornamental *Agave desmettiana* Jacob plants (*Agavaceae*) showing chlorotic lesions were detected in the urban region of Teresina city within an area of approx. 30 km² including the region where the original CiCSV-infected plants have been described. Analyses of symptomatic agaves' tissues by transmission electron microscopy revealed the presence of viroplasm in the nucleus of the infected parenchymal cells, as a typical dichorhavirus infection (Dietzgen et al. 2018). Moreover, eight mites that were collected during the examination of the symptomatic leaves were morphologically identified as *Brevipalpus* aff. *yothersi*, the same species found in CiCSV-infected citrus (Chabi-Jesus et al. 2018). Total RNA from leaf lesions of three symptomatic agave plants (isolates Trs4, -5 and -6) were extracted using TRIzol Reagent (Life Technologies, Foster City, CA, USA), and the RT-PCR assays performed using all of the primer pairs available for the detection of known dichorhaviruses (Dietzgen et al. 2018) proved the presence of CiCSV. Additionally, the same RNA extracts reacted with a Digoxigenin-labeled probe derived from the *N* gene of CiCSV_Tr1 in a RNA dot-blot assay (Roche Diagnostics GmbH, Mannheim, Germany). Ninety-five percent of the isolate Trs4's genome was recovered after the Sanger sequencing of the overlapping amplicons generated by using 15 primers pairs (Chabi-Jesus et al. 2018). Globally, RNA1 (6082 nt, GB: MH595621) and RNA2 (5688 nt, MH595622) shows 96.4% nucleotide sequence identity with the type virus CiCSV_Tr1 (KY700685 and KY700686), and less than 75% nucleotide sequence identity with the dichorhaviruses orchid fleck virus (OFV; AB244417 and AB244418), coffee ringspot virus (CoRSV; KF812525 and KF812526), citrus leprosis virus N (CiLV-N; KX982176 and KX982179) and Clerodendrum chlorotic spot virus (CICSV; MG938506 and MG938507). Comparisons of the deduced amino acid sequences of the putative proteins N, P, MP, M, G, and L (encoded by ORFs 1-6, respectively) of CiCSV_Tr4 show 95–99% identity with CiCSV_Tr1 and 28-90% identity with OFV, CoRSV, CiLV-N, and CICSV. Sequences of the generated amplicons corresponding to *G* and *L* genes from the isolates Trs5 and Trs6 (MH802037, MH802038, MH802039 and MH802040, respectively) showed >93% nucleotide sequence identity with CiCSV_Tr1 (KY700685 and KY700686) and with isolate Trs4. Comprehensively, results indicate that CiCSV_Tr4, -5 and -6 are isolates of the tentative species citrus chlorotic spot virus of the genus *Dichorhavirus*, whose transmission has been previously associated with the mite *B. aff. yothersi*. This is the first report of CiCSV naturally infecting the

succulent plant *A. desmettiana* and, to our knowledge, it is also the first report of a (-)ssRNA virus infecting plants of this genus.

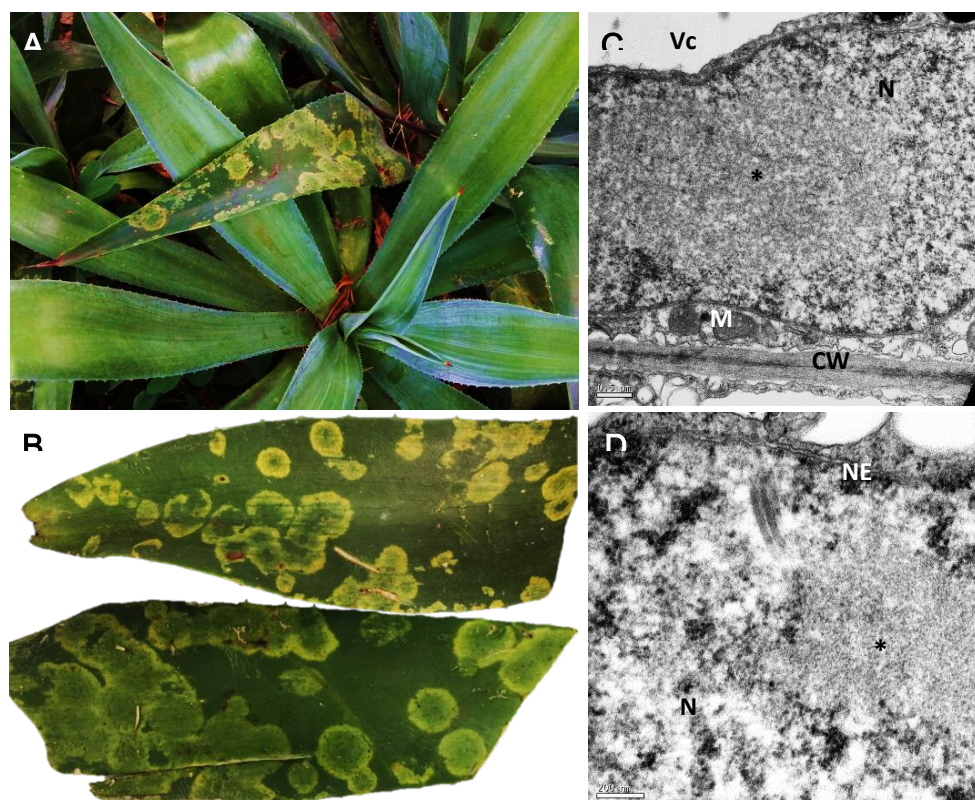
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Supplementary Figure S1: **A** and **B**. Localized chlorotic symptoms in *Agave desmettiana* leaves infected with citrus chlorotic spot virus (CiCSV) collected in Teresina, State of Piauí, Brazil, 2018; **C** and **D**. Transmission electron micrographs of thin sections from lesions; **C**. Parenchymal cell showing a nucleus with electron lucent viroplasm (*); **D**. Similar to B, with the presence of viroplasm (*) in the nucleus (N). Chloroplast (C), cell wall (CW), mitochondrion (M) and vacuole (Vc).



Supplementary Figure S2. Differential interference contrast (DIC) micrographs showing the diagnostic characters of a female *Brevipalpus* aff. *yothersi* mite found in symptomatic *Agave desmettiana* plants infected with the dichorhavirus citrus chlorotic spot virus. **A.** Oval spermatheca vesicle with a distal stipe; **B.** Ventral cuticle between coxae II-IV, genital and ventral plates with typical reticulation pattern; **C.** Dorsal overview showing the propodosomal and opisthosomal cuticle.