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Cnidoscolus blistering yellow mosaic virus: a new begomovirus isolated from *Cnidoscolus urens* in Brazil

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Abstract

Members of the genus *Begomovirus* have circular single-strand DNA genome encapsidated into quasi-icosahedral particles, which are transmitted by the *Bemisia tabaci* complex, with non-cultivated hosts acting as reservoirs for these viruses. In this study, a new begomovirus has been chacarterized infecting *Cnidoscolus urens* (Euphorbiaceae) from Brazil. The complete DNA-A sequence was used for species demarcation, phylogenetic and recombination analyzes with other previously reported begomoviruses. The DNA-A (2585 nt) has a genome organization that is typical of the New World bipartite begomovirus, most closely related to tomato yellow spot virus, with 80.6% nucleotide identity. Based on the identity criterion for DNA-A established by the *Geminiviridae* Study Group of the *International Committee on Taxonomy of Viruses* of ≥ 91%, this isolate should be considered as a member of a new species, for which the name "*Cnidoscolus* blistering yellow mosaic virus" (CnBYMV) is proposed. Phylogenetic analysis revealed that CnBYMV grouped with begomoviruses described in cultivated and non-cultivated plants from Brazil. Results of recombination analysis indicated that the novel begomovirus was a recombinant, with cnidoscolus mosaic leaf deformation virus and sida mottle virus as probable parents.

Keywords: *Geminiviridae*, non-cultivated plants, species demarcation

The family *Geminiviridae* includes viruses with circular single-stranded DNA genome (ssDNA) encapsidated in geminated quasi-icosahedral particles (Brown et al., 2015). Based on their host range, the type of vector, genome organization and phylogenetic relationships, this family is divided into the genera *Becurtovirus*, *Begomovirus*, *Capulavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Mastrevirus*, *Topocuvirus* and *Turncurtovirus* (Varsani et al., 2017; Zerbini et al., 2017). Begomoviruses have one (monopartite) or two (bipartite) genomic components, known as DNA-A and DNA-B. They are transmitted by whiteflies of the *Bemisia tabaci* cryptic species complex (Zerbini et al., 2017) and cause damage to economically important crops in tropical and subtropical regions of the world (Rojas et al., 2018).

Non-cultivated hosts of different botanical families are considered as a source and reservoir of new viruses. Begomovirus species have been reported in non-cultivated plants in the family Euphorbiaceae: *Euphorbia mosaic virus* (EuMV; Hernández-Zepeda et al., 2007), *Croton yellow vein virus* (CYVV; Hussain et al., 2011), *Euphorbia yellow mosaic virus* (EuYMV; Fernandes et al., 2011), *Dalechampia chlorotic mosaic virus* (DCMV; Fiallo-Olivé et al., 2013), *Jatropha mosaic virus* (JMV; Simmonds-Gordon et al., 2014), *Cnidoscolus mosaic leaf deformation virus* (CnMLDV; Melo et al., 2016) and *Croton golden mosaic virus* (CroGMV; Vaca-Vaca et al., 2018). In this study we describe the molecular characterization of a new begomovirus isolate infecting *Cnidoscolus urens*, a non-cultivated plant naturally found in northeastern Brazil.

A leaf sample of *C. urens* showing typical symptoms of begomovirus infections such as yellow mosaic and blistering was collected in 2018 in Teresina, Piauí State, Brazil (Figure 1). Total DNA was extracted using the CTAB method (Doyle & Doyle, 1990) and begomovirus presence was confirmed by PCR with degenerate

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primers, PAR1c496/PAL1v1978 (Rojas et al., 1993). The full-length DNA-A component was amplified by using rolling-circle amplification (RCA; Inoue-Nagata, 2004), individually cleaved with *Apa*I restriction endonuclease. A putative genomic component of approximately 2.6 kbp was cloned into the pBluescript KS+ (Stratagene) vector and transformed into *Escherichia coli* DH10B. The obtained clone was commercially sequenced via *primer walking* (Macrogen Inc., Seoul, South Korea).

Figure 1. *Cnidoscolus urens* with yellow mosaic and blistering on leaves, typical symptoms of begomovirus infection.

The nucleotide sequences obtained were assembled using the CodonCode *Aligner* v. 4.1.1 (www.codoncode.com) and analyzed using the BLAST*n* algorithm (Altschul et al., 1990) and the GenBank non-redundant nucleotide database (www.ncbi.nlm.nih.gov/genbank) to determine viral species with which they shared greatest identity. Multiple nucleotide sequence alignments for the DNA-A dataset of the *C. urens* isolate and other begomoviruses from South America (Supplementary Table S1) were prepared using the MUSCLE in MEGA6 (Tamura et al., 2013). Pairwise sequence identity comparisons were performed using the SDT (*Sequence Demarcation Tool*) v. 1.2 program (Muhire et al., 2013). Phylogenetic analyzes were performed on the CIPRES *web portal* (Miller et al., 2010) using MrBayes v. 3.2.3 (Ronquist et al., 2012). The evolutionary model GTR+I+G was used for DNA-A dataset. Two replicates with four chains each for 10 million generations and sampling every 1,000 generations were employed in the analysis (a total of 10,000 trees). The first 2,500 trees were discarded as a burn-in phase. Trees were visualized and edited using the software FigTree v. 1.4 (ztreebio.ed.acuk/software/figtree).

Recombination analysis was conducted using the RDP, GENECONV, BootScan, MaxChi, Chimera, SiScan and 3Seq methods implemented in the Recombination Detection Program (RDP) v.4.0 (Martin et al., 2015). Only recombination events detected by at least four different methods were considered reliable.

The complete DNA-A sequence was determined to be 2585 nt in length and showed the typical genome organization of bipartite New World begomoviruses (GenBank accession MT553995), encoding one protein (CP; 254 amino acids) in the virion-sense strand, and four in the complementary-sense strand (Rep, TrAP, REn and AC4; 359, 129, 132 and 85 amino acids, respectively). The conserved nonanucleotide (5' TAATATT/AC 3') was detected in the common region (CR), which is part of the stem-loop that contains the viral replication origin. We also identified repeated *GGAG* sequences (*iterons*), which are essential to initiate viral replication.

The DNA-A component shares the highest nucleotide sequence identity (80.6%) with tomato yellow spot virus *(KJ742419)*, begomovirus isolated from chia in Argentina (Celli et al., 2014) (Figure 2). Using pairwise comparisons and the sequence ≥91% nucleotide identity criteria for complete DNA-A sequences established by the *Geminiviridae* Study Group of *International Committee on Taxonomy of Viruses* (ICTV) for the demarcation of species within the *Begomovirus* genus (Brown et al., 2015), the isolate obtained herein was classified as a putative new species of *Begomovirus*. We propose that this virus species could be named "*Cnidoscolus* blistering yellow mosaic virus" (CnBYMV).

The phylogenetic tree was constructed for the complete DNA-A dataset from the nucleotide sequence of CnBYMV; with the most related begomoviruses from cultivated and non-cultivated plants from South America (Figure 3). The analysis showed that CnBYMV clustered in a branch that includes the PSLDV (FJ972767) from passionfruit, CnMLDV (NC038982) from *C. urens*, and MeIMV (KT201151) from *Melochia* sp., all of which have already been reported in Brazil (Melo et al., 2016; Fiallo-Olivé et al., 2015; Ferreira et al., 2010).

RDP4 analysis revealed evidence of a single recombination event occurring in the DNA-A of CnBYMV, with recombination breakpoint (nt 1921-2561) located in the *Rep* gene (Supplementary Table S2). CnMLDV (NC038982), the first begomovirus isolated from *C. urens* in Brazil (Melo et al., 2016), was identified as the putative major parent and sida mottle virus (SiMoV; JX871377) as its minor parent.

Cultivated and non-cultivated plants belonging to the family Euphorbiaceae are known to host a high diversity of begomovirus species (Hernández-Zepeda et al., 2007; Hussain et al., 2011; Fernandes et al., 2011; Fiallo-

Olivé et al., 2013; Simmonds-Gordon et al., 2014; Melo et al., 2016; Vaca-Vaca et al., 2018). Here, we report the natural occurrence of a new begomovirus species causing mosaic and blistering symptoms from *C. urens*.

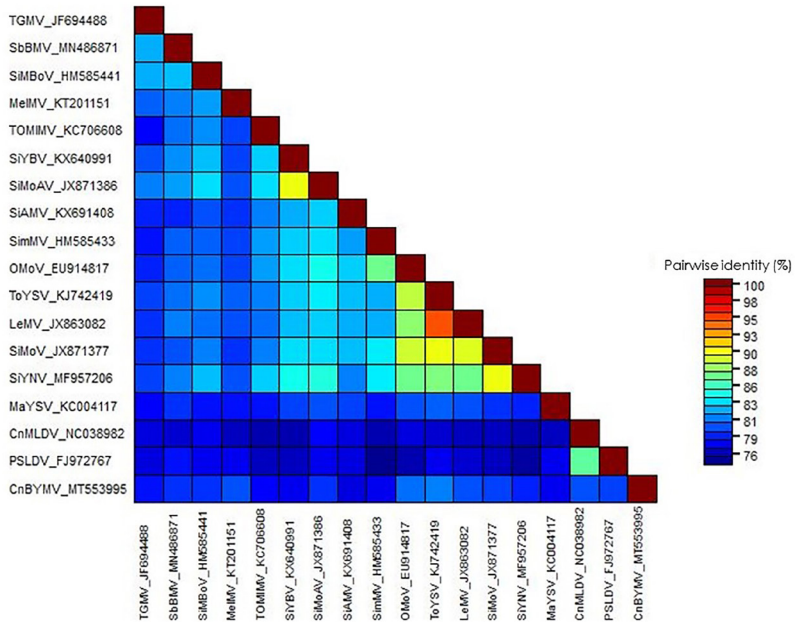

Figure 2. Pairwise comparison of the nucleotide sequences among full-length DNA-A of *Cnidoscolus* blistering yellow mosaic virus and the most closely related begomoviruses obtained by BLASTn analysis.

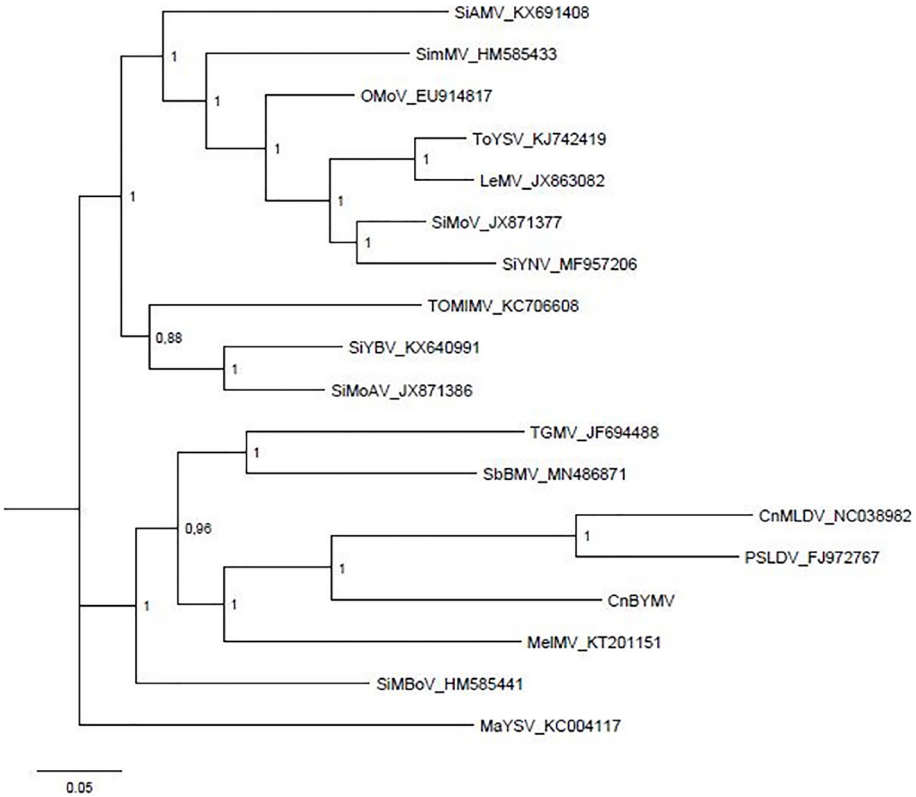

Figure 3. Phylogenetic tree of DNA-A of *Cnidoscolus* blistering yellow mosaic virus and the most closely related begomoviruses from Brazil and other countries in the South America.

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