Begomovirus Caboniensis: a New Bipartite Begomovirus Isolated From *Cnidoscolus Urens* in Brazil

Lívia Francyne Gomes Chaves (liviagomezzz@gmail.com)
Universidade Federal de Alagoas https://orcid.org/0000-0002-3893-0348
Mayra M.M. Ferro
Universidade Federal de Alagoas
Mayara O. de Lima
Universidade Federal de Alagoas
Irraildes P. Assunção
Universidade Federal de Alagoas
Gaus S.A. Lima
Universidade Federal de Alagoas
Sarah J.C. da Silva
Universidade Federal de Alagoas

Research Article

Keywords: Begomovirus Caboniensis, New Bipartite Begomovirus, Cnidoscolus urens, Euphorbiaceae, Brazil

Posted Date: August 4th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-769819/v1

Abstract

A novel bipartite begomovirus infecting *Cnidoscolus urens* (Euphorbiaceae) from Pernambuco State, Brazil has been characterized. The complete DNA-A (2657 to 2692 nt) and DNA-B (2622 nt) components of the viral isolates showed a typical genome organization of New World bipartite begomoviruses. DNA-A of the isolates had the highest percentage of nucleotide identity (88.6–88.9%) with the *Cnidoscolus mosaic leaf deformation virus* (NC_038982). Based on the current classification criteria for the genus *Begomovirus*, a new member infecting *C. urens* was reported, and the name *Begomovirus caboniensis* was proposed for these viruses, adopting the standardized binomial system.

Main Text

The family *Geminiviridae* comprises viruses with a circular single-stranded DNA genome (2.6–5.2 kb) encapsulated in geminated quasi-icosahedral particles [1,2]. This family is divided into 14 genera based on their genome organization, type of vector, phylogenetic relationship, host range, and nucleotide sequence identity [3]. The genus *Begomovirus* includes more than 400 species currently recognized by the *International Committee on the Taxonomy of Viruses* (ICTV, http://www.ictvonline.org/virusTaxonomy.asp), emphasizing its importance within the family [3]. Begomoviruses have one or two genomic components known as DNA-A and DNA-B, are transmitted by whiteflies of the *Bemisia tabaci* complex [4,5], and infect a wide range of cultivated and non-cultivated hosts, mainly in tropical and subtropical regions [6].

Several species of begomoviruses have been reported in non-cultivated plants in the family Euphorbiaceae [7,8,9,10,11,12,13]. In *Cnidoscolus urens,* a wild host largely found in northeastern Brazil, only two viruses were reported: *Cnidoscolus mosaic leaf deformation virus* (CnMLDV) [14] and *Cnidoscolus blistering yellow mosaic virus* (CnBYMV) [not published]. These reports reinforce the role of non-cultivated plants as viral reservoirs. In this study, the molecular characterization of a new bipartite begomovirus infecting *Cnidoscolus urens* in Pernambuco, Brazil was described.

A sample of *C. urens,* showing mild mosaic and leaf deformation symptoms, was collected in 2019 in Cabo de Santo Agostinho, Pernambuco State, Brazil (Figure 1). Total DNA was extracted using the CTAB method [15], and begomovirus infection was confirmed via PCR using the degenerate primers PAR1c496/PAL1v1978 [16]. Complete viral genomes were amplified by rolling-circle amplification (RCA) [17], cleaved with restriction enzyme *Cla*I, ligated into the pBluescript KS+ (Stratagene) plasmid vector, and transformed into *Escherichia coli* DH10B by heat shock [18]. Viral inserts were sequenced commercially by primer walking (Macrogen Inc., Seul, South Korea).

The complete DNA-A and DNA-B genomic components were assembled using CodonCode Aligner v. 6.0.2 (www.codoncode.com) and initially analyzed using the BLASTn algorithm [19] to determine the viral species that shared the highest nucleotide identity. Pairwise sequence identity comparisons were performed using Sequence Demarcation Tool software (SDT) v.1.2 [20].

Multiple sequence alignments were prepared using the MUSCLE algorithm implemented in MEGA6 [21]. Phylogenetic analyses were performed on the CIPRES Science Gateway [22] using MrBayes v.3.3.3 [23]. The best nucleotide substitution model (GTR+I+G) was used for the DNA-A and DNA-B component datasets. The analysis was carried out using two replicates with four chains each for 10 million generations and sampling every 1000 steps (a total of 10,000 trees). The first 2500 trees were discarded as a burn-in phase. Trees were viewed and edited using Figtree v.1.4 (ztreebio.ed.acuk/software/figtree).

A total of five sequences were obtained: three for the DNA-A component and two for the DNA-B component. The sequences ranged from 2657 to 2692 nt in length for DNA-A (GenBank accession MZ465527, MZ465586, and MZ465587) and 2622 nt in length for DNA-B (GenBank accession MZ465585 and MZ46526). They showed a typical New World begomovirus gene organization, with DNA-A encoding one protein (CP) in the virion-sense strand and four in the complementary-sense strand (Rep, TrAP, Ren, and AC4), while DNA-B encoded two proteins, NSP in the virion-sense strand and MP in the complementary-sense strand (Supplementary Table S1). Analysis of the common region (CR) showed a conserved nona-nucleotide (5'-TAATATT/AC-3') and identified three putative iterons (GGGT), one of which was inverted (Supplementary Figure S1). The CR (200 nt) showed 96% nucleotide sequence identity between the two components, suggesting that they are a cognate pair.

Using pairwise comparisons and established criteria for species demarcation within the genus *Begomovirus* [1], clones BR_Ca96_19A (GenBank accession MZ465527), BR_Ca112_19A (MZ465586), and BR_Ca113_19A (MZ465587) showed a nucleotide identity between them ranging from 98.4 to 100%. They shared 79.8% similarity with CnBYMV (MT553995) and 88.6–88.9% similarity with CnMLDV (NC_038982; Supplementary Figure S2), which are begomoviruses isolated from *C. urens* in the Piauí and Alagoas States, respectively [14]. Therefore, these isolates represent a new begomovirus species, and the name *Begomovirus caboniensis* is proposed for this virus, adopting the binomial nomenclature system established by ICTV [24].

Bayesian inference phylogenetic trees were constructed from nucleotide sequences of the DNA-A and DNA-B components obtained in the present study, with the most related begomoviruses from cultivated and non-cultivated plants from South America (Figure 2). The analysis for the DNA-A component indicated that the *B. caboniensis* isolates cluster with CnMLDV (NC_038982) and CnBYMV (MT553995) isolates, both obtained from *C. urens* from the northeast region of Brazil. For the DNA-B component, the isolates cluster with the CnMLDV isolate (KT966772), thus reinforcing the phylogenetic relationship between the isolates obtained from *C. urens.*

Recombination analysis of DNA-A and DNA-B genomic components of the *B. caboniensis* isolates and the complete sequences from the database were conducted using RDP, GENECONV, BootScan, MaxChi, Chimera, SiScan, and 3Seq methods implemented in the Recombination Detection Program (RDP) v.4.0 [25]. However, no evidence of recombination events was observed.

The occurrence of begomoviruses in *C. urens* has been reported for over ten years in Alagoas State [26]. However, it was only in 2016 that the first complete genome of a begomovirus occurring naturally in this

host was characterized, which was identified as CnMLDV [14]. Recently, a new species, CnBYMV, was reported in *C. urens* in Piauí State. In the present study, a new bipartite begomovirus was characterized, demonstrating the potential of *C. urens* as a natural reservoir of begomoviruses.

Declarations

Acknowledgments:

The authors thank the Universidade Federal de Alagoas. M.M.M.F was the recepient of a CAPES postdoctoral fellowship. L.F.G.C was the recepient of a FAPEAL doctoral fellowship.

Funding:

The Fapeal (Fundação de Amparo à Pesquisa do Estado de Alagoas) for funding the research.

Conflict of interest:

All authors declare no conflict of interest

Availability of data and material:

'Not applicable' for that section.

Code availability:

'Not applicable' for that section.

Ethics approval:

This article does not contain any studies with human participants or animals perfomed by any of the authors

Consent to participate:

'Not applicable' for that section.

Consent for publication:

'Not applicable' for that section.

Compliance with ethical standards

Conflict of interest:

All authors declare no conflict of interest

References

1. Brown JK, Zerbini FM, Navas-Castillo J et al (2015) Revision of Begomovirus taxonomy based on pairwise sequence comparisons. Arch Virol 160:1593-1619
2. Gilbertson RL, Batuman O, Webster CG, Adkins S (2015) Role of the insect supervectors Bemisia tabaci and Frankliniella occidentalis in the emergence and global spread of plant viruses. Annu Rev Virol 2:67-93
3. Zerbini FM, Briddon RW, Idris A et al (2017) ICTV virus taxonomy profle: Geminiviridae. J Gen Virol 98:131–133
4. Barbosa LF, Marubayashi JM, Marchi BR et al (2014) Indigenous American species of the *Bemisia tabaci* complex are still widespread in the Americas. Pest Manag Sci 70:1440–1445
5. Navas-Castillo J, Fiallo-Olivé E, Sanchez-Campos S (2011) Emerging virus diseases transmitted by whiteflies. Annu Rev. Phytopathol 49: 219-248
6. Rojas MR, Macedo MA, Maliano MR et al (2018) World Management of Geminiviruses. Annu Rev Phytopathol 56: 637-677
7. Hernandez Z, Idris AM, Carnevali G et al (2007) Molecular characterization and experimental host range of Euphobia mosaic virus-Yucatan Peninsula a begomovirus species in the Squash leaf curl virus. Plant Pathol 56:763–770
8. Hussain K, Hussain M, Mansoor S et al (2011) Complete nucleotide sequence of a begomovirus and associated betasatellite infecting croton (*Croton bonplandianus*) in Pakistan. Arch Virol 156:1101-1105
9. Fernandes FR, Albuquerque LC, Oliveira, CL et al (2011) Molecular and biological characterization of a new Brazilian begomovirus, euphorbia yellow mosaic virus (EuYMV), infecting *Euphorbia heterophylla* plants. Arch Virol 156:2063-2069
10. Fiallo-Olivé E, Chirinos DT, Pouey FG et al (2013) Complete genome sequences of two begomoviruses infecting weeds in Venezuela. Arch Virol 158:277–280
11. Simmonds-Gordon RN, Collins-Fairclough AM, Stewart CSS et al (2014) First report of a complete genome sequence for a begomovirus infecting *Jatropha gossypifolia* in the Americas. Arch Virol 159: 2815-8
12. Srivastava A, Kumar S, Jaidi M, et al (2015) Molecular characterization of a new begomovirus associated with leaf yellow mosaic disease of Jatropha curcas in India. Arch Virol 160:1359-1362
13. Vaca-Vaca JC, Jara-Tejada F, Lopez-Lopez K (2018) Croton golden mosaic virus: a new bipartite begomovirus isolated from *Croton hirtus* in Colombia. Arch Virol 163: 3199–3202.
14. Melo AM, Silva SJC, Ramos-Sobrinho R, Ferro MMM, Assunção IP, Lima GSA (2016) *Cnidoscolus mosaic leaf deformation virus*: a novel begomovirus infecting euphorbiaceous plants in Brazil. Arch Virol 161:2605- 2608.
15. Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13-15

16. Rojas MR, Gilbertson RL, Russell DR et al (1993) Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Disease 77:340-347
17. Inoue-Nagata AK, Albuquerque LC, Rocha WB, Nagata T (2004) A simple method for cloning the complete begomovirus genome using the bacteriophage phi29 DNA polymerase. J Virol Methods 116:209–211
18. Sambrook J, Russel D (2001) Molecular Cloning -A Laboratory Manual (3ª ed.). Cold Spring Harbor Laboratory Press
19. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403-410
20. Muhire BM, Varsani A, Marti DP (2014) SDT: uma ferramenta de classificação de vírus baseada no alinhamento de sequência de pares e cálculo de identidade. PLoS One 9(9): e108277
21. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood evolutionary distance and maximum parsimony methods. Mol Biol Evol 28:2731–2739
22. Miller MA, Holder MT, Vos R, Midford PE, Liebowitz T, Chan L, Hoover P, Warnow T (2010) The CIPRES portals. CIPRES.
23. Ronquist F, Teslenko M, Van der Mark P et al (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. Sys Bio Adv 61: 539-42.
24. Walker PJ, Siddell SG, Lefkowitz EJ et al (2021) Changes to virus taxonomy and to the International Code of Virus Classifcation and Nomenclature ratifed by the International Committee on Taxonomy of Viruses. Arch Virol X:1-16
25. Martin DP, Murrell B, Golden M et al (2015) RDP4: detection and analysis of recombination patterns in virus genomes. Virus Evol 1:vev003
26. Assuncao IP, Listik AF, Barros MCS, Amorim EPR, Silva SJC, Silva IO, Ramalho-Neto CE, Lima GSA (2006) Diversidade genetica de Begomovirus que infectam plantas invasoras na regia ˜o nordeste. Planta Daninha 24:239–244

Figures

Figure 1

Symptoms milds of mosaic and leaf deformation in Cnidoscolus urens.

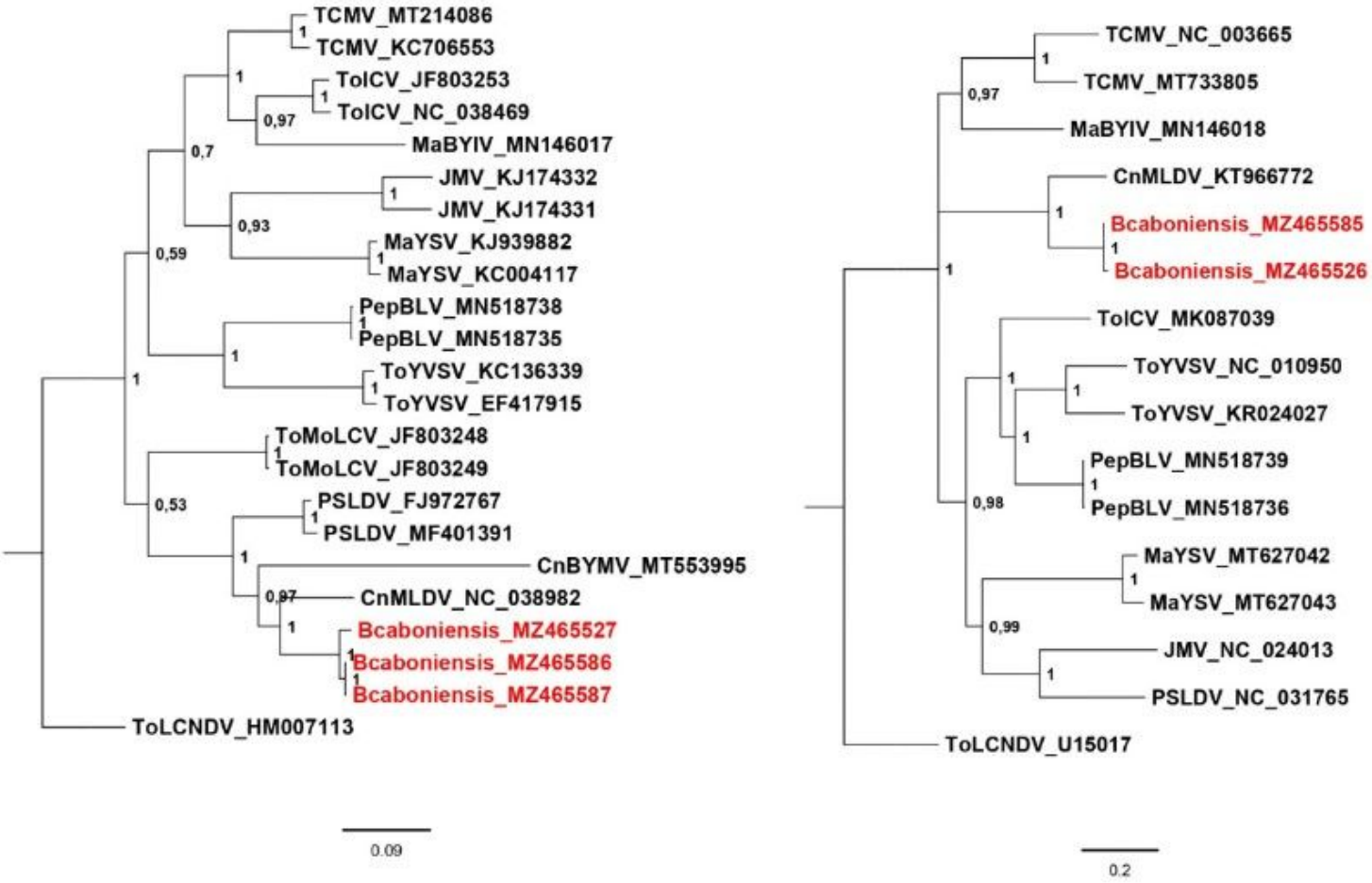

Figure 2

Phylogenetic reconstruction of DNA-A (a) and DNA-B (b) of B. caboniensis (highlighted in red) and the most closely related begomoviruses. Tomato leaf curl new delhi virus (ToLCNDV) was used as an outgroup. The phylogenetic trees were constructed using the Bayesian inference method.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFig.S1.doc
- SupplementaryFig.S2.doc
- SupplementaryTab.1.doc