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Two new begomoviruses infecting tomato and *Hibiscus* sp. in the Amazon region of Brazil

Ayane F. F. Quadros[1,2] · João Paulo Silva[2] · César A. D. Xavier[2] · F. Murilo Zerbini[2] · Alessandra J. Boari[1]

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Abstract

Two begomoviruses were isolated in the northern Brazilian state of Pará, infecting non-cultivated *Hibiscus* sp. and cultivated tomato (*Solanum lycopersicum*). The complete genomes (DNA-A and DNA-B) of the two viruses showed the typical organization of New World bipartite begomoviruses. Based on the species assignment criteria in the genus *Begomovirus*, each virus is a member of a new species. The virus from *Hibiscus* is most closely related to sida yellow mosaic Yucatan virus, while the tomato virus is most closely related to abutilon mosaic Brazil virus and corchorus mottle virus. Recombination events were detected in the DNA-A of the tomato virus, but not in the *Hibiscus* virus genome. We propose the names "hibiscus golden mosaic virus" (HGMV) and "tomato chlorotic leaf curl virus" (ToCLCV) for the viruses reported in this study.

The genus *Begomovirus* (family *Geminiviridae*) includes viruses that infect dicotyledonous plants and whose genomes are composed of one or two genomic components of circular, single-strand DNA (ssDNA) of about 2,700 nucleotides, encapsidated in icosahedral particles and transmitted by whiteflies of the *Bemisia tabaci* cryptic species complex [33]. Begomoviruses constitute an important group of plant pathogens responsible for severe diseases in several crops of economic importance worldwide [27]. The two genomic components of bipartite viruses are designated as DNA-A and DNA-B. The DNA-A contains genes for replication and encapsidation of genetic material. The DNA-B is responsible for intra- and intercellular movement in plant. Both components are required for systemic infection [26].

Brazil is a center of diversity of begomoviruses, with a great number of these viruses reported in non-cultivated hosts [4, 5, 9] as well as in crops [12, 25]. The most important diseases are bean golden mosaic caused by bean golden mosaic virus (BGMV) and golden mosaic/leaf curl diseases caused by a complex of begomoviruses in tomato [27]. The emergence of tomato-infecting begomoviruses in Brazil has been explained by horizontal transfer of indigenous viruses infecting non-cultivated hosts following the introduction of polyphagous *Bemisia tabaci* Middle East Asia Minor 1 (MEAM-1) in the early 1990s [24, 25]. A large diversity of begomoviruses has been sampled in non-cultivated hosts [2, 4, 9, 10, 13, 15, 17, 20–23, 29, 30].

The majority of begomoviruses from Brazil were isolate in the central, northeastern, and southeastern regions of the country [12]. These areas have a climate that favors the buildup of whitefly populations, with a well-defined dry winter season, which is also relatively warm. The northern region, which includes the Amazon forest, comprises almost 50% of the country's area and has an equatorial climate without a dry season. Possibly as a result, whitefly populations rarely reach epidemic levels, and begomoviruses have not been reported. Nevertheless, recent years have seen atypical dry spells in the Amazon, which have been attributed to climate change [8, 32].

In June 2016, two tomato (*Solanum lycopersicum*) samples with mosaic and leaf distortion (Fig. 1A) were

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✉ F. Murilo Zerbini
zerbini@ufv.br

✉ Alessandra J. Boari
alessandra.boari@embrapa.br

[1] Embrapa Amazônia Oriental, Trav. Dr. Enéas Pinheiro s/n, Belém, PA 66095-903, Brazil

[2] Dep. de Fitopatologia/BIOAGRO, Universidade Federal de Viçosa, Viçosa, MG 36570-900, Brazil

Fig. 1 Symptoms of begomovirus infection in plants collected in the state of Pará, northern Brazil. **A.** The tomato plant from which isolate BR-Alt1-16 (tomato chlorotic leaf curl virus) was obtained. **B.** The *Hibiscus* sp. plant from which isolate BR-IgM1-16 (hibiscus golden mosaic virus) was obtained

collected in a field near the city of Altamira (S3°16′094″, W52°23′38.4″), and in August of the same year, one *Hibiscus* sp. plant with yellow mosaic symptoms (Fig. 1B) was collected in the city of Igarapé Miri (S1°58′30″, W48°57′35″), both in the state of Pará, northern Brazil. The identity of the *Hibiscus* sample was confirmed by DNA barcoding using the chloroplast matK and rbcL genes. Total DNA was extracted from each sample [6] and used as a template for rolling-circle amplification (RCA) using the phi29 DNA polymerase [11]. Amplification products were individually cleaved with *Sac*I (tomato samples) and *Eco*RI/*Hind*III (*Hibiscus* sample), ligated into the pBLUESCRIPT-KS+ (pKS+) plasmid vector (Stratagene) previously cleaved with the same enzyme, and introduced into *Escherichia coli* DH5α by transformation. Viral inserts were sequenced commercially (Macrogen, Inc.). Full-length genomes were assembled using Geneious v. 8.1 [14]. Sequences were initially analyzed using the BLASTn algorithm [1], and sequence identity to the closest begomoviruses was calculated with Species Demarcation Tool v.1.2 (SDT) [18]. Full-length genomes of begomoviruses were aligned with MUSCLE [7] implemented in MEGA v. 7.0 [31]. Phylogenetic trees based on DNA-A and DNA-B alignments were generated by Bayesian inference using MrBayes v. 3.2.6 [28] with the GTR + G+I nucleotide substitution model selected by MrModeltest v. 2.2 [19] in the Akaike information criterion (AIC). Analyses were carried out for 20,000,000 generations, and sampling was done at every 1,000 steps to produce the distribution tree. The first 4,000 trees were discarded. The trees were visualized in FigTree v.1.3.1 (http://tree.bio.ed.ac.uk/software/figtree) and edited in CorelDRAW 2017. To detect putative recombination events, the software RDP4 [16] was used. Recombination events were considered reliable only if they were detected by at least four of the seven methods implemented in the program.

DNA-A and DNA-B components were cloned from the *Hibiscus* sp. and tomato samples. All components have the conserved nonanucleotide (5′-TAATATTAC-3′) located at the origin of replication. DNA components from the same sample have identical iteron sequences (GGTGT for the components from the tomato samples; GGTGG for the components from the *Hibiscus* sp. sample), and digestion of the RCA products from each sample with a 4-base cutter restriction enzyme generated fragments with lengths adding up to approximately 5,200 nt (data not shown). These results indicate that they were cognate DNA components, and they were the only two DNA components present in each sample. All components have the typical organization of New World, bipartite begomoviruses, with five ORFs in the DNA-A and two ORFs in the DNA-B.

Three clones (two DNA-A and one DNA-B) were sequenced from the tomato samples. The DNA-A clones corresponding to isolates BR-Alt1-16 (2612 nt; GenBank accession number MK558058) and BR-Alt3-16 (2615 nt; MK558060) (from different samples) showed 99.62% sequence identity amongst themselves and a maximum sequence identity of 84% with the DNA-A of abutilon mosaic Brazil virus (AbMBV; JF694482) and corchorus mottle virus (CoMoV; JQ805781) (Fig. 2A). Based on the begomovirus species demarcation criterion established by the International Committee on Taxonomy of Viruses (ICTV), which considers a 91% identity cutoff for the full-length DNA-A component [3], these isolates should be considered members of a new species, for which

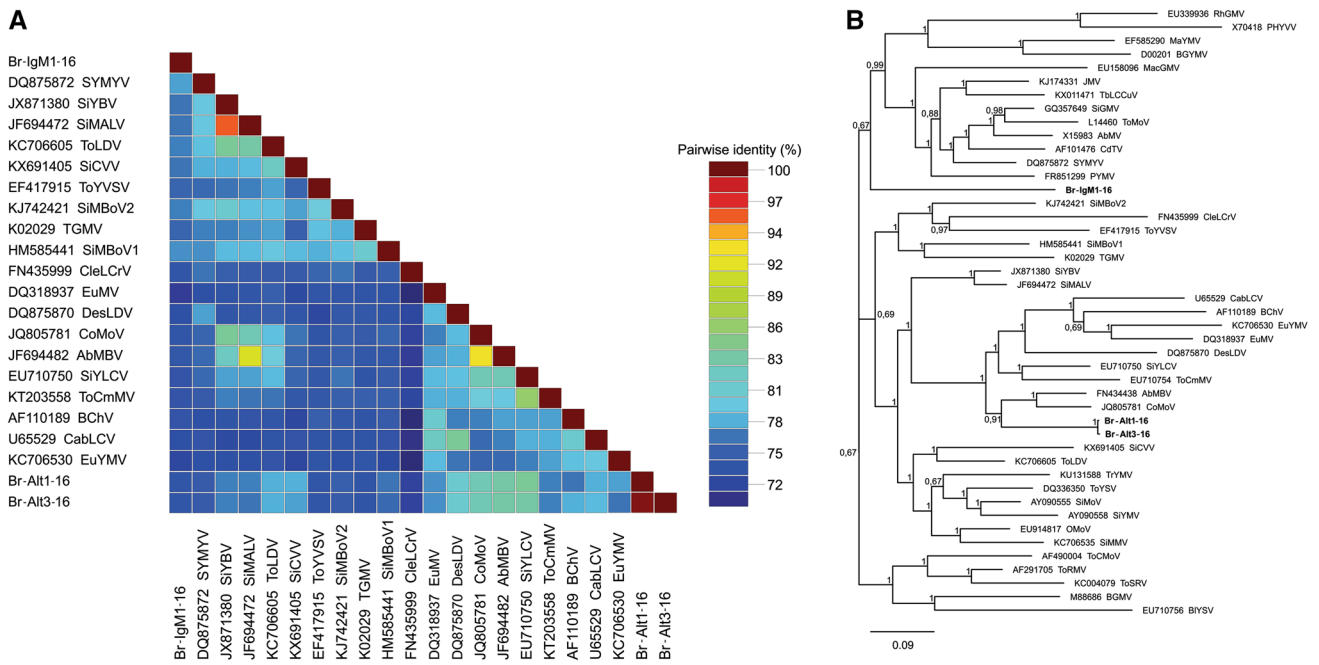

Fig. 2 **A.** Nucleotide identity matrix (DNA-A) of isolates BR-IgM1-16 (hibiscus golden mosaic virus), BR-Alt1-16 and BR-Alt3-16 (tomato chlorotic leaf curl virus) with the most closely related begomoviruses. Identities were calculated with SDT v. 1.2. **B.** Phylogenetic reconstruction using Bayesian inference. The midpoint-rooted tree is based on the complete DNA-A sequences of the begomoviruses described in this work and other New World begomoviruses. Numbers at the nodes indicate Bayesian posterior probabilities

we propose the name "*Tomato chlorotic leaf curl virus*" (ToCLCV). In the DNA-A phylogenetic tree, ToCLCV grouped in a clade with viruses from Central and North America (Fig. 2B), which also includes viruses from Brazil that have been shown to be members of a lineage of New World begomoviruses that is distinct from other Brazilian begomoviruses [25]. The DNA-B tree shows the same topology (data not shown). Recombination analysis indicated a recombination event in the DNA-A of BR-Alt1-16 and BR-Alt3-16, with putative recombination breakpoints at positions 204 and 969, with tomato leaf distortion virus (KC706605) and sida chlorotic vein virus (KX691405) inferred as the major and minor parent, respectively.

Pairwise sequence comparisons with the DNA-A of isolate BR-IgM1-16 (MK558061) from *Hibiscus* sp. indicated a maximum nucleotide sequence identity of 79% to sida yellow mosaic Yucatan virus (SiYMYuV; DQ875872) (Fig. 2A). Thus, the isolate BR-IgM1-16 should be considered a member of a new species, whose proposed name is "*Hibiscus golden mosaic virus*" (HGMV). A phylogenetic tree based on DNA-A nucleotide sequences indicates a distant relationship of HGMV to SiYMYuV and other begomoviruses from Central/North America and the Caribbean (Fig. 2B). The HGMV DNA-B (MK558062) is most closely related to those of blainvillea yellow spot virus and tomato common mosaic virus (data not shown). Recombination analysis revealed no evidence of significant recombination events.

Weeds and wild plants can serve as reservoirs of viruses for cultivated plants, especially between growing seasons. In addition, the great biodiversity of begomoviruses in non-cultivated plants may contribute to the emergence of new viruses in cultivated plants. Therefore, the characterization of begomoviruses in non-cultivated hosts provides information about the possible threat of new epidemics.

Intensive sampling over the last 20 years has led to the description of several new begomoviruses infecting cultivated (especially tomato) and non-cultivated plants in Brazil. However, the Brazilian northern region has been underrepresented in these surveys, and the true extent of begomovirus diversity in this region is likely to have been underestimated as well. The two new viruses described here are more closely related to begomoviruses from Central/North America and the Caribbean than to begomoviruses from South America. Additional sampling in this geographically large region is necessary to illuminate the true richness of begomoviruses present in its different biomes, as well as their evolutionary relationships.

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Compliance with ethical standards

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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