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ANNOTATED SEQUENCE RECORD

Complete genome sequence of a new bipartite begomovirus infecting *Macroptilium lathyroides* in Brazil

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Abstract A distinct bipartite begomovirus was isolated in northeastern Brazil infecting *Macroptilium lathyroides* showing symptoms of yellow mosaic. The complete genome (DNA-A and DNA-B) of the virus was cloned using rolling circle amplification and subsequently sequenced. Clones presented the typical genomic organization of a New World bipartite begomovirus. Based on the current taxonomic criteria established for the genus *Begomovirus*, the virus corresponds to a new species, showing highest nucleotide identity with other Brazilian begomoviruses that infect leguminous hosts. In phylogenetic analysis the virus clustered with bean golden mosaic virus. Recombination events were not detected. We propose the name Macroptilium common mosaic virus (MacCMV) for the virus reported in this study.

Begomoviruses (genus *Begomovirus*, family *Geminiviridae*), are circular single-stranded DNA viruses which infect dicotyledonous plants and are packaged in twinned icosahedral particles 20 × 30 nm in size [3]. These viruses are transmitted in a persistent circulative manner by whiteflies of the *Bemisia tabaci* cryptic species complex (Hemiptera: Aleyrodidae) [13]. Begomoviruses found in the New World have two genomic components denominated DNA-A and DNA-B, with DNA-A encoding proteins responsible for replication, encapsidation and vector transmission and DNA-B encoding proteins with movement related functions. The two components, each about 2.6 kb in size, do not have sequence identity to each other, except for a region of approximately 200 nucleotides denominated the common region (CR) which is highly conserved between the two components of the same species (generally above 90% sequence identity). The CR includes the recognition sequence for the viral Replication associated protein (Rep) as well as regions responsible for transcription of viral genes [9].

Non-cultivated plants of different botanical families can be infected by a high diversity of begomoviruses [4, 21], which allows the maintenance of these pathogens in the absence of their cultivated host [19]. *Macroptilium lathyroides* L. Urban is a forage legume common in South and Central America, which has become a weed in the Brazilian northeast. Until now, in the Americas, *M. lathyroides* has been reported as a host of nine begomoviruses - besides the six species named after it, *M. lathyroides* is also infected by bean golden mosaic virus [5], bean golden yellow mosaic virus [2], sida golden mosaic Jamaic virus [17] and the BR-Mur1-09 isolate of tomato interveinal chlorosis virus, originally named macroptilium yellow net virus [2, 19]. Non-cultivated plants can act as natural reservoirs of begomoviruses and as sources of inoculum for crop plants, favoring the occurrence of epidemics [15]. In this work we describe a new begomovirus species naturally infecting *M. lathyroides* in northeastern Brazil.

The sequences reported in this paper have been deposited in GenBank under the accession numbers KX691396, KX691397 and KX691412.

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Two samples of *M. lathyroides* with typical symptoms of begomovirus infection (Fig. 1A) were collected in 2008 (Limoeiro, state of Ceará) and 2009 (Teresina, state of Piauí), and maintained at -80 °C. Total DNA from the samples was extracted [6] and used as a template for rolling circle amplification (RCA) using the phi29 DNA polymerase [10]. Amplification products were individually cleaved with *Apa*I, *Bam*HI and *Sac*I restriction endonucleases, ligated into the pBluescript KS + vector (Stratagene) previously cleaved with the same enzymes and transformed in *Escherichia coli* DH5α [18]. Viral inserts were completely sequenced by primer walking (Macrogen, Inc.).

Sequences were initially analyzed using the BLAST*n* algorithm [1], and identities with the closest begomoviruses were calculated with SDT (Species Demarcation Tool) v.1.2 [12]. Genomic sequences were aligned with MUSCLE [7] implemented in MEGA v. 6.0 [20]. Phylogenetic trees based on DNA-A and DNA-B alignments were generated by Bayesian inference using MrBayes v.3.0b4 [16] with the GTR + G+I nucleotide substitution model selected by MrModeltest v. 2.2 [14] in the Akaike Information Criterion (AIC). Analyses were carried out for 20,000,000 generations and the sampling was done at every 1,000 steps to produce the distribution tree. The first 4,000 trees were discarded. To detect putative recombination events, the software RDP3 v.3.4 [11] was used after alignment with MUSCLE for the sequences selected with SWeBLAST (with a window size of 200 and a step size of 200) [8]. Recombination events were considered reliable only if they were detected by at least four of the seven methods implemented in the program.

Three clones were sequenced (two DNA-A and one DNA-B). The BR-Ter1-09A (2632 nt, KX691396) and BR-Lim1-08A (2629 nt, KX691397) clones (of different samples) showed 97% sequence identity amongst themselves and a maximum sequence identity of 87% with the DNA-A of Macroptilium yellow vein virus (MacYVV) (JN419021) and bean golden mosaic virus (BGMV) (M88686), two legume-infecting begomoviruses (Fig. 1B). Based on the begomovirus species demarcation criterion, which considers the minimum value of 91% identity for complete DNA-A, a new species was thus identified, for which we propose the name "*Macroptilium common mosaic virus*" with individual isolates being referred to as macroptilium common mosaic virus (MacCMV).

The DNA-B clone BR-Ter1-09B (2598 nt, KX691412) was obtained from the same sample from which the corresponding DNA-A (BR-Ter1-09A) was cloned. Sequence identity of the common region (CR) of 95% between the components DNA-A and DNA-B, as well as the presence of identical iterons (GAGTG) between these DNA-A and DNA-B clones indicate that they are cognate pair. Thus, BR-Ter1-09B corresponds to the DNA-B of MacCMV.

The three DNA components have the typical genomic organization of New World begomoviruses, with six ORFs (including AC5) in the DNA-A and two ORFs in the DNA-B (Suppl. Table S1). The CR of the isolates possesses a conserved nonanucleotide (5'-TAATATTAC-3') as part of a stem-loop at the origin of replication.

A Bayesian phylogenetic tree based on complete DNA-A nucleotide sequences (Fig. 2) confirmed the close genetic relationship among MacCMV and BGMV and MacYVV, two Brazilian legume-infecting begomoviruses. Similar to DNA-A, the DNA-B of MacCMV was phylogenetically close to that of BGMV (M88687) (Suppl. Fig. S1). Recombination analysis using RDP3 revealed no evidence of significant recombination events (data not shown).

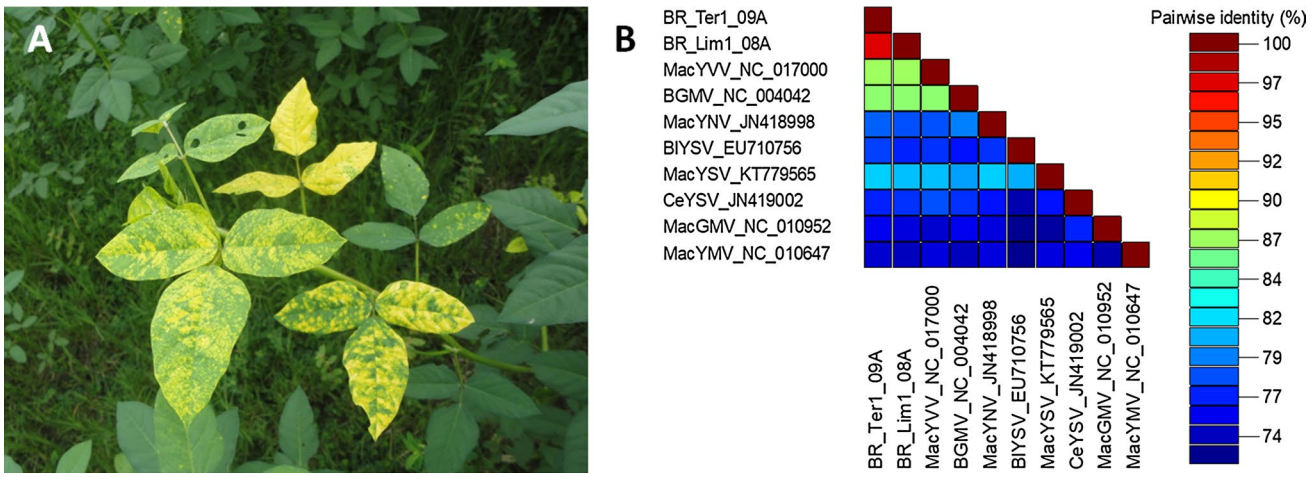

Fig. 1 **A.** Symptoms of macroptillium common mosaic virus (MacCMV) infection in *Macroptilium lathyroides* collected in Teresina, state of Piauí, Brazil. **B.** Pairwise nucleotide sequence identities of the complete DNA-A of MacCMV with the most closely related begomoviruses. Identities were calculated with SDT v. 1.2 [11]. Full virus names are listed in Fig. 2

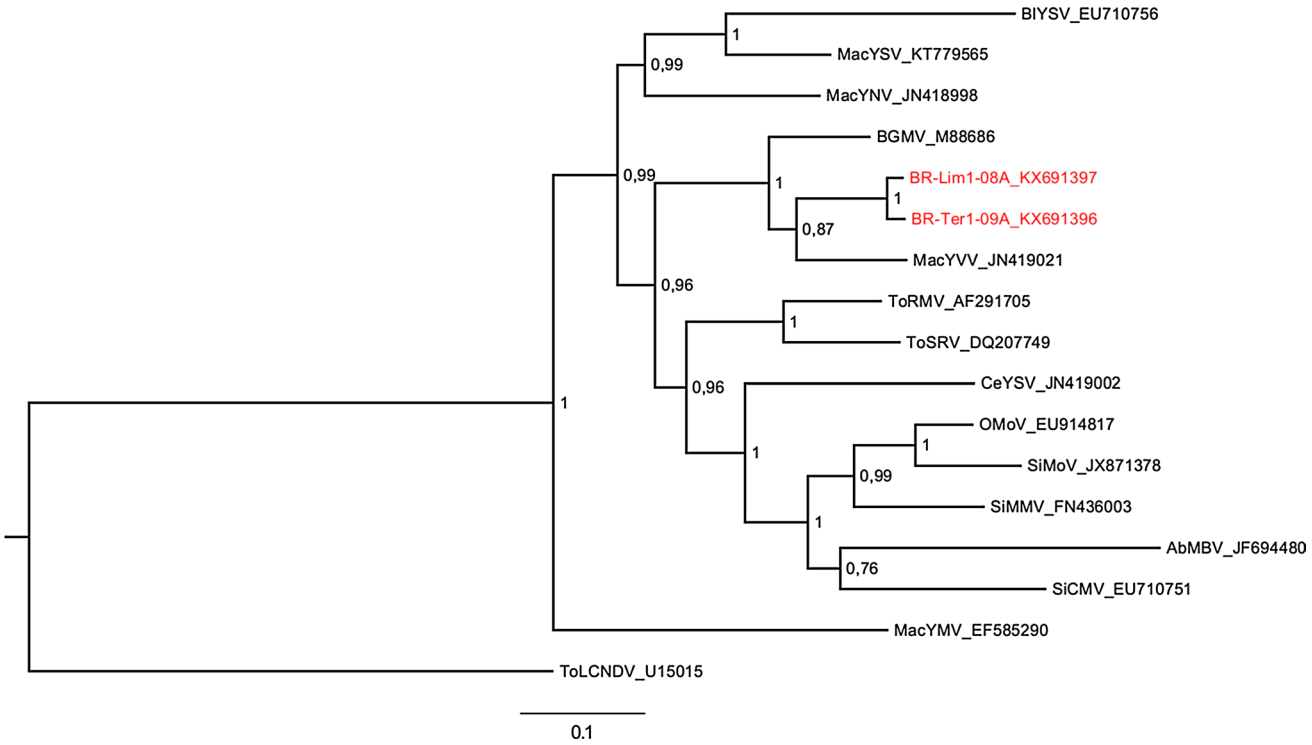

Fig. 2 Phylogenetic reconstruction using Bayesian inference. The tree is based on the complete DNA-A sequences of the begomovirus described in this work and other begomoviruses from the New World. Sequences corresponding to isolates of the new species are highlighted in red. An Old World begomovirus (ToLCNDV, tomato leaf curl New Delhi virus) was used as outgroup. Numbers at the nodes indicate Bayesian posterior probabilities. AbMBV, abutilon mosaic Brazil virus; BGMV, bean golden mosaic virus; BlYSV, blainvillea yellow spot virus; CeYSV, centrosema yellow spot virus; MacYMV, macroptilium yellow mosaic virus; MacYNV, macroptilium yellow net virus; MacYSV, macroptilium yellow spot virus; MacYVV, macroptilium yellow vein virus; OMoV, okra mottle virus; SiCMV, sida common mosaic virus; SiMMV, sida micrantha mosaic virus; SiMoV, sida mottle virus; ToRMV, tomato rugose mosaic virus; ToSRV, tomato severe rugose virus

We report a member of a new begomovirus species naturally infecting *M. lathyroides*, further demonstrating the role of this non-cultivated host as a reservoir of begomovirus diversity in the Americas.

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Compliance with ethical standards

Conflict of interest We declare that the authors have no conflicts of interest.

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

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