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ANNOTATED SEQUENCE RECORD

Asystasia mosaic Madagascar virus: a novel bipartite begomovirus infecting the weed *Asystasia gangetica* in Madagascar

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Abstract Here, we describe for the first time the complete genome sequence of a new bipartite begomovirus in Madagascar isolated from the weed *Asystasia gangetica* (Acanthaceae), for which we propose the tentative name asystasia mosaic Madagascar virus (AMMGV). DNA-A and -B nucleotide sequences of AMMGV were only distantly related to known begomovirus sequence and shared highest nucleotide sequence identity of 72.9 % (DNA-A) and 66.9 % (DNA-B) with a recently described bipartite begomovirus infecting *Asystasia* sp. in West Africa. Phylogenetic analysis demonstrated that this novel virus from Madagascar belongs to a new lineage of Old World bipartite begomoviruses.

Begomoviruses (genus *Begomovirus*, family *Geminiviridae*) are a group of plant viruses transmitted by the whitefly *Bemisia tabaci* (*Aleyrodidae*) to a large variety of cultivated and uncultivated plant species. They have a circular single-stranded DNA genome encapsidated in twinned icosahedral particles (for review see ref. [9]).

Previous studies have highlighted the diversity of begomoviruses infecting crops in Madagascar. Members of six species of cassava-infecting bipartite begomoviruses (cassava mosaic geminiviruses, CMGs) have been found in Madagascar (Harimalala et al. [5]; De Bruyn et al., submitted), one of them, tentatively called "cassava mosaic Madagascar virus" (CMMGV; [4]), represents a proposed new species. In the past decade, monopartite begomoviruses from tomato crops in Madagascar belonging to at least six established or tentative species have been characterized. These include *Tomato leaf curl Madagascar virus* [2], *Tomato leaf curl Diana virus* [7], *Tomato leaf curl Namakely virus* [7], *Tomato leaf curl Toliara virus* [7], and the proposed species "Tomato leaf curl Ambilobe virus" (unpublished) and "Tomato leaf curl Antsiranana virus" [7]. Finally, a novel begomovirus infecting bean has been characterized and tentatively named "Bean leaf curl Madagascar virus" [7].

While African begomoviruses infecting crops of New World origin are most likely descendants of indigenous viruses that probably co-evolved with African native plants [6], knowledge of begomovirus diversity is still largely restricted to cultivated host plants. Here, we describe for the first time, the complete nucleotide sequences of DNA-A and DNA-B of a new bipartite begomovirus isolated in Madagascar from the wild plant *Asystasia gangetica* (Acanthaceae) and tentatively named asystasia mosaic Madagascar virus (AMMGV). Leaf samples from *A. gangetica* plants showing bright yellow mosaic symptoms (Supplementary Figure 1) were collected in the northern region (sample MG493; Mangabe; 13.7120 S, 48.4256 E; July 2011) and in the south-eastern region (sample MG147; Beretry; 21.7224 S, 48.0006 E; May 2009) of Madagascar

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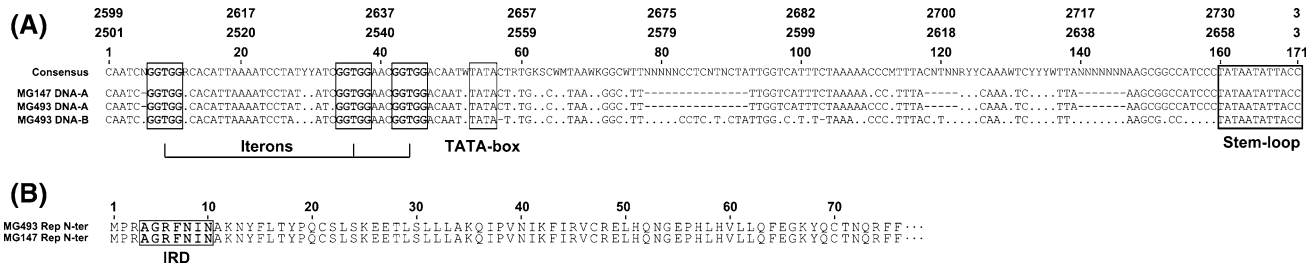

Fig. 1 **A.** Alignment of the common region (CR) of DNA-A and DNA-B sequences of AMMGV isolates. The three repeats of the GGTGG iteron, the TATA-box and the stem-loop are indicated by a black frame. **B.** Alignment of the N-terminal portion of the Rep protein. The iteron-related domain (IRD) is indicated by a black frame

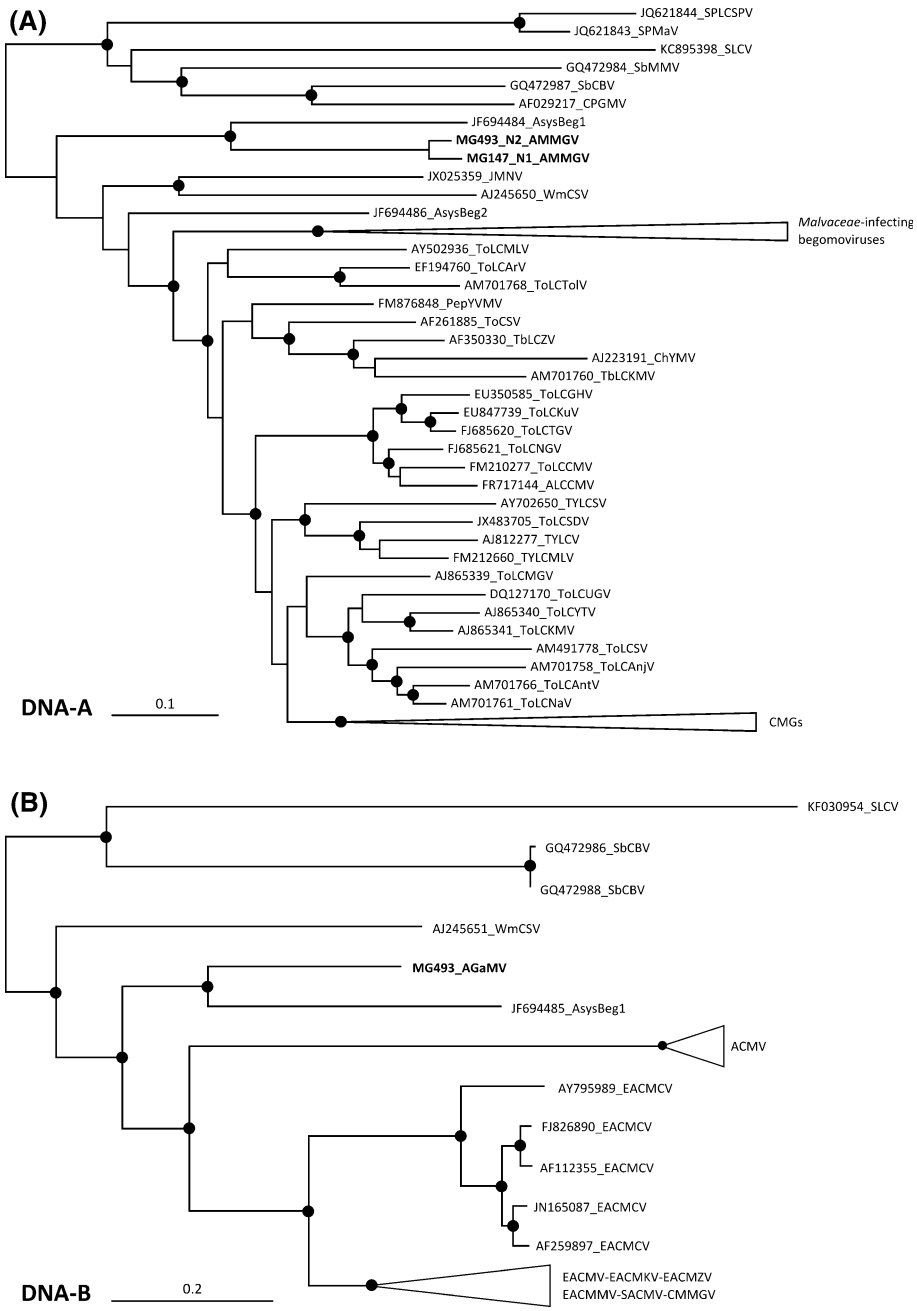

Fig. 2 Maximum-likelihood (ML) trees inferred from alignments of complete DNA-A (**A**) and DNA-B (**B**) sequences of AMMGV and representative begomoviruses. Black circles indicates nodes supported by a SH value above 95 %. For begomovirus acronyms, see Supplementary Table 1

and preserved by dehydration using anhydrous calcium chloride. Total DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN, USA). Viral DNA was amplified by rolling-circle amplification using Phi29 DNA polymerase (TempliPhi, GE Healthcare, USA). Amplified products were digested with *Bam*HI or *Nco*I endonuclease. The monomeric full-length genomes obtained (~2.8 kb) were purified and cloned into the pGEM-3Zf and pGEM-T vectors, respectively, for *Bam*HI and *Nco*I-digested fragments. Cloned DNA was sequenced at Macrogen Inc. (Europe) by primer walking; contigs were assembled using Geneious software (Biomatters, New Zealand).

DNA-A and -B component sequences recovered from sample MG493 displayed features typical of Old World bipartite begomoviruses, while only DNA-A was successfully isolated from sample MG147. The DNA-A components were found to be 2737/2738 nucleotides in length and shared 95.1 % nucleotide sequence identity. These DNA-A components encoded six open reading frames (ORFs): AV1 (254 aa; 95.3 % aa sequence identity) and AV2 (118 aa; 97.5 % aa sequence identity) in the viral sense and AC1 (358 aa; 98.6 % aa sequence identity), AC2 (135 aa; 95.6 % aa sequence identity), AC3 (134 aa; 95.5 % aa sequence identity) and AC4 (65 aa; 89.2 % aa sequence identity) in the complementary sense. The DNA-B component of MG493 has two ORFs: BV1 (254 aa) in the viral sense and BC1 (323 aa) in the complementary sense. Both DNA components isolated from sample MG493 shared a common region (CR) of approximately 150 and 200 nucleotides in length for DNA-A and DNA-B, respectively, with only 72.8 % nucleotide sequence identity. However, the iteron GGTGG was present in the CR of both components and was repeated three times, and the corresponding iteron-related domain of the Rep protein (IRD; Rep N-terminal domain interacting with ori-associated iterons [1]) was identified as MPRA**GRFNIN** (Fig. 1).

DNA-A and -B of AMMGV were only distantly related to known begomoviruses and shared highest nucleotide sequence identity of 72.9 % and 66.9 % with a recently isolated bipartite begomovirus from *Asystasia* sp. in West Africa (JF694484, asystasia begomovirus 1 DNA-A; JF694485, asystasia begomovirus 1 isolate ABgV1 segment DNA-B). Maximum-likelihood phylogenetic trees based on the complete nucleotide sequences of DNA-A and DNA-B of AMMGV isolates and representatives of the genus *Begomovirus* were constructed using PHYML v3.0 [3] (Fig. 2). The ML trees showed the grouping of AMMGV with asystasia begomovirus 1, in a distinct clade only distantly related to other begomoviruses. Analyses of recombination using RDP4 [8] revealed no evidence of a recombination event in any of the genomic components.

Our results show that the bipartite genome of AMMGV is representative of a distinct clade in the phylogeny of African begomoviruses. The grouping of this particular virus with a recently characterized virus infecting the same host in West Africa suggests that these viruses belong to a novel group of bipartite begomoviruses. Except for the cassava mosaic geminiviruses (CMGs) described on cassava, these two viruses are amongst a very few Old World bipartite begomoviruses described in sub-Saharan Africa and SWIO islands.

GenBank accession numbers KP663483, KP663484, KP663485.

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