A Bipartite Begomovirus Infecting *Boerhavia erecta* (Family Nyctaginaceae) in the Dominican Republic Represents a Distinct Phylogenetic Lineage and has a High Degree of Host Specificity

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ABSTRACT

Boerhavia erecta plants in and around agricultural fields in the Azua Valley of the southeastern Dominican Republic often show striking golden mosaic symptoms. Leaf samples from *B. erecta* plants showing these symptoms were collected in 2012 and 2013, and PCR tests with degenerate primers revealed begomovirus DNA-A and DNA-B components. The complete sequences of the DNA-A and DNA-B components of four isolates show a high degree of sequence identity (>96%) and a genome organization typical of New World (NW) bipartite begomoviruses. Sequence comparisons and phylogenetic analyses revealed that these isolates composed a new phylogenetic lineage of NW bipartite begomoviruses. The most closely related begomovirus is *Merremia mosaic virus*, a weed-infecting species from Puerto Rico. Because DNA-A sequence identities are well below the 91% threshold, these isolates represent a new begomovirus species, for

which the name *Boerhavia golden mosaic virus* (BoGMV) is proposed. Infectious cloned BoGMV DNA-A and DNA-B components induced golden mosaic symptoms in agroinoculated *B. erecta* plants, thereby fulfilling Koch's postulates for this disease. Agroinoculation and mechanical transmission experiments revealed that BoGMV has an unusually narrow host range, limited to members of the family Nyctaginaceae and not including the permissive host *Nicotiana benthamiana*. The inability of BoGMV to infect *N. benthamiana* was due to a deficiency in cell-to-cell movement but not to a unique amino acid residue in the movement protein.

Keywords: *Bemisia tabaci*, etiology, geminivirus, *Mirabilis expansa*, viral evolution, virology

Members of the family *Geminiviridae* have a circular single-stranded DNA genome encapsidated in twinned quasi-isometric virions measuring approximately 18 to 30 nm (Zerbini et al. 2017). This family has been recently expanded to nine genera: *Becurtovirus*, *Begomovirus*, *Curtovirus*, *Eragrovirus*, *Mastrevirus*, *Topocuvirus*, *Turncurtovirus*, *Capulavirus*, and *Grablovirus* (Varsani et al. 2017). Members of the genus *Begomovirus* are transmitted by the whitefly species complex *Bemisia tabaci*, and constitute the largest number of species in the family *Geminiviridae* (approximately 90%). Collectively, begomoviruses infect a wide range of cultivated and non-cultivated dicotyledonous plants, and cause numerous economically important diseases of fiber and vegetable crops (Rojas et al. 2018). Begomovirus diseases occur in tropical and subtropical regions throughout the world, and their spread and emergence has been mediated by the *B. tabaci* supervector (Gilbertson et al. 2015). Phylogenetic analyses of begomoviruses have revealed a phylogeographic distribution, which reflects ancient lineages that likely emerged before continental drift. Thus, the majority of begomoviruses in the Old World (OW) have a monopartite genome of approximately 2.8 kb and are commonly associated with different types of satellite and satellite-like DNAs (Zhou 2013). In contrast, most species from the New World (NW) have a bipartite genome composed of DNA-A and DNA-B components that are each approximately 2.6 kb. The DNA-A component of bipartite begomoviruses is homologous to the genomic DNA of monopartite begomoviruses (Gilbertson et al.

2015; Hanley-Bowdoin et al. 2013; Rojas et al. 2005). The sequences of the DNA-A and DNA-B components of bipartite begomoviruses are different except for an approximately 150- to 200-nucleotide (nt) common region (CR), which has high sequence identity (≥90%) between cognate components of a species. The CR contains the viral origin of replication, including a highly conserved stem-loop structure containing the invariant nonanucleotide TAATATTAC sequence, which is the nicking site for initiation of rolling-circle replication of the viral genome (Hanley-Bowdoin et al. 2013). Also contained within the CR are high affinity binding sites for interaction with the replication-associated protein (Rep) and maintenance of the bipartite genome, and cis elements (TATA- and G-box) involved in virus replication and transcription (Argüello-Astorga and Ruiz-Medrano 2001; Gutierrez 1999; Hanley-Bowdoin et al. 1999, 2013).

In addition to infecting crop plants, begomoviruses infect a diversity of weed species, often inducing striking golden or yellow mosaic symptoms in leaves of infected plants. In South and Central America, numerous bipartite NW begomovirus species have been reported infecting weed species in the families Asteraceae, Capparaceae, Convolvulaceae, Euphorbiaceae, Fabaceae, Malvaceae, and Solanaceae (Barreto et al. 2013; Blawid et al. 2013; da Silva et al. 2011; Ferro et al. 2017; Fiallo-Olivé et al. 2017; Hernández-Zepeda et al. 2007b; Mar et al. 2017; Melgarejo et al. 2015; Melo et al. 2016; Nascimento et al. 2016; Passos et al. 2017; Pinto et al. 2016; Romay et al. 2016; Stewart et al. 2014; Tavares et al. 2012). These weed-infecting begomoviruses also show a high level of genetic diversity (Lima et al. 2013; Melgarejo et al. 2015; Rocha et al. 2013; Sobrinho et al. 2014).

It has long been hypothesized that begomoviruses infecting noncultivated plants serve as (i) inoculum sources for crop-infecting begomoviruses and (ii) progenitors for the evolution and emergence of such viruses (Barreto et al. 2013; Martínez-Ayala et al. 2014; Sánchez-Campos et al. 2013). Several lines of evidence support the latter hypothesis. First, molecular studies have shown

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that weed- and crop-infecting begomoviruses are usually genetically distinct. Second, there are relatively few reports of weed-infecting begomoviruses extensively infecting crop plants and causing economic losses (Inoue-Nagata et al. 2016), although host range studies have demonstrated that some weed-infecting begomoviruses can also infect crop plants (Barbosa et al. 2011; Blawid et al. 2013; Melgarejo et al. 2015; Pramesh et al. 2013; Tahir et al. 2015). Third, the well-established phenomenon of local evolution of most crop-infecting begomoviruses is more consistent with weed-infecting begomoviruses serving as progenitors of these crop-infecting viruses (Idris et al. 2019; Melgarejo et al. 2013; Zhou et al. 2008). Together, these findings indicate the importance of host adaptation of begomoviruses, and help explain the high degree of sequence divergence between weed- and crop-infecting viruses (Ambrozewicz et al. 2002; Barboza et al. 2018; Lima et al. 2013; Silva et al. 2012).

Boerhavia erecta is an annual plant in the family Nyctaginaceae and is native to the United States, Mexico, Central America, and western South America (<https://plants.sc.egov.usda.gov/java/>). It is now found worldwide in tropical and subtropical regions, including Africa and Asia, where it is considered an invasive weed species (Chen and Wu 2007; Struwig and Siebert 2013). In the Azua Valley of the southeastern Dominican Republic, *B. erecta* is a common weed in and around agricultural fields and disturbed areas (Bastidas et al. 2008). In this region, it is not uncommon to find *B. erecta* plants with striking golden mosaic symptoms, suggestive of begomovirus infection. Similar symptoms in *B. coccinea* plants in Mexico were associated with infection by a distinct NW begomovirus species, *Boerhavia yellow spot virus* (BoYSV) (Hernández-Zepeda et al. 2007a).

In the present study, we describe the biological and molecular properties of a new bipartite begomovirus associated with golden mosaic symptoms in *B. erecta* plants in the Azua Valley of the Dominican Republic, for which the name *Boerhavia golden mosaic virus* (BoGMV) is proposed. Isolates of BoGMV constituted a distinct and strongly supported cluster, revealing a new lineage of NW begomoviruses. Full-length infectious DNA-A and DNA-B clones of BoGMV induced golden mosaic symptoms in agro-inoculated *B. erecta* plants, fulfilling Koch's postulates for this disease. We further show that BoGMV has a very narrow host range, restricted to members of the family Nyctaginaceae and not including the permissive host, *Nicotiana benthamiana*. Finally, pseudorecombination (PR), replication, and mutational analyses were used to investigate the BoGMV–host interactions.

MATERIALS AND METHODS

Virus source, sample preparation, DNA extraction, and detection of begomovirus DNA. As part of surveys to identify potential reservoir hosts of *Tomato yellow leaf curl virus* in the Azua Valley, leaf samples with golden mosaic symptoms were collected from *B. erecta* plants from four locations in and around processing tomato fields in 2012 and 2013. Sample B1 was collected in March 2012, sample B2 was collected in February 2013, and samples B3-1 and B3-2 were collected in June 2013. In the Dominican Republic, sap was prepared from leaf tissue (approximately 3.0 g) by grinding in plastic bags containing approximately 3.0 ml of LYL1 buffer (Agdia, Elkhart, IN, U.S.A.), and Agdia absorption strips were inserted into the sap for 30 s, which allowed the absorbent pad to become saturated. The strips were allowed to dry overnight at room temperature, and then transported to the University of California at Davis (UC Davis). Total DNA was extracted from the dried plant sap on the absorbent pads according to the method of Dellaporta et al. (1983).

Begomovirus DNA-A and DNA-B components were detected by PCR with the degenerate primer pairs PAL1v1978/PAR1c496 and PCRc1/B1200F (Inoue-Nagata et al. 2004; Rojas et al. 1993), respectively. PCR-amplified DNA-A and DNA-B fragments were

purified with the QIAquick gel extraction kit (Qiagen, Germantown, MD, U.S.A.) and directly sequenced with the PAL1v1978 and B1200F primers, respectively, at the UC Davis DNA Sequencing Facility.

Cloning of full-length begomovirus DNA components. Rolling circle amplification (RCA) with Φ -29 DNA polymerase (TempliPhi; GE Healthcare, Piscataway, NJ, U.S.A.) was used to generate linear double-stranded concatemers of begomovirus DNA (Inoue-Nagata et al. 2004). The resulting RCA products were digested with various restriction enzymes to identify single sites in each component (i.e., that generated linear approximately 2.6-kb fragments). Putative full-length DNA-A and DNA-B fragments were obtained with *EcoRI* and *NdeI*, respectively. The resulting putative monomeric components were gel purified and cloned into pSL1180 (Promega Corp., Madison, WI, U.S.A.). Recombinant plasmids were identified by restriction enzyme digestion and DNA sequence analyses.

Sequencing and phylogenetic analyses. Sequences of the putative full-length DNA-A and DNA-B components were determined at the UC Davis DNA Sequencing Facility, and analyzed with the Vector NTI Advance software (Invitrogen, Carlsbad, CA, U.S.A.). Pairwise nucleotide sequence alignments were performed with Muscle within the species demarcation tool (SDT) v.1.2 (Muhire et al. 2014). SDT analysis with full-length DNA-A component sequences was then used to determine the relationship of the begomovirus isolates from *B. erecta* from the Dominican Republic with (i) the 18 most closely related begomoviruses identified by a BLASTn search and (ii) selected species representing the four major lineages of NW bipartite begomoviruses: *Abutilon mosaic virus*, Brazilian begomoviruses, *Squash leaf curl virus* (SLCuV), and *Bean golden yellow mosaic virus* (BGYMV). Comparisons were also made with sequences of the full-length DNA-B components, the CR, and individual open reading frames (ORFs) from both components with those of the 11 most closely related begomoviruses.

For phylogenetic analyses, we included complete DNA-A and DNA-B sequences of begomovirus isolates from *B. erecta* leaf samples B1, B2, B3-1, and B3-2; the 18 most closely related begomoviruses; and selected species representing the four lineages of NW bipartite begomoviruses. In this analysis, we also included the complete DNA-A sequence of BoYSV from Mexico (there is no DNA-B component sequence available). Nucleotide sequence alignments were performed with the Muscle program in MEGA v.7 (Kumar et al. 2016), and the aligned sequences were edited and exported as nexus files. Phylogenetic trees were constructed using Bayesian inference performed with MrBayes v.3.2.4 (Ronquist et al. 2012). The best-fit model of nucleotide substitution was determined with the program jModelTest v.2.1 (Darriba et al. 2012). The Bayesian analysis was carried out by running 2.5 M generations, and trees were sampled every 100th generation, resulting in 25,000 trees. After discarding the first 20% of samples as burn-in, the remaining 20,000 trees were used for calculating posterior probabilities (PPs) in the consensus tree. Trees for the DNA-A and DNA-B sequences were generated in FigTree v.1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree>) and rooted with the DNA-A and DNA-B components, respectively, of the OW bipartite begomovirus *Tomato leaf curl New Delhi virus* (ToLCNDV).

Non-tree-like relationships indicative of recombination were assessed in the data set of DNA-A component sequences used in the phylogenetic analysis by the construction of a phylogenetic network with the Neighbor-Net method (Bryant and Moulton 2004) and the Pairwise Homoplasy Index (PHI) test (Bruen et al. 2006) implemented in the program SplitsTree v.4.14.4 (Huson and Bryant 2005). Recombination events, breakpoints, and parental sequences were also assessed in this DNA-A data set with the Recombination Detection Program (RDP) v. 4.46 (Martin et al. 2015), with default settings and a Bonferroni-corrected *P* value cutoff of 0.001. To minimize the probability of false positives, only

recombination events detected with all eight methods implemented in RDP were considered likely to be real.

Production of multimeric clones and an agroinoculation system. To determine infectivity of the cloned DNA-A and DNA-B components of the B1 isolate (hereafter referred to as B1), multimeric clones were generated in the pCambia1300 binary vector (Hajdukiewicz et al. 1994). For the DNA-A component, an approximately 1.8-kb *EcoRI*-*Bam*HI fragment containing the CR was cloned into pCambia1300 to generate a 0.7-mer pC-B1-A0.7. The full-length monomer (*EcoRI* fragment) was then cloned into the *EcoRI* site of pC-B1-A0.7 to generate the 1.7-mer pC-B1-A1.7. For the DNA-B component, an approximately 1.8-kb *NdeI*-*XbaI* fragment containing the CR was cloned into pSL1180 to generate a 0.7-mer pSL-B1-B0.7. The full-length monomer (*NdeI* fragment) was then cloned into the *NdeI* site of pSL-B1-B0.7 to generate the 1.7-mer pSL-B1-B1.7. This plasmid was then digested with *EcoRI* and *Hind*III, and the resulting fragment with the 1.7-mer was cloned into pCambia1300 to generate pC-B1-B1.7. Recombinant plasmids having the multimeric clones were identified by restriction enzyme digestion and transformed into competent *Agrobacterium tumefaciens* cells (strain C58C1) with the freeze-thaw method (Wise et al. 2006).

Determination of infectivity and host range. Infectivity of the cloned B1 multimeric DNA components was initially assessed by agroinoculation of *B. erecta* seedlings (2 weeks old) and *N. benthamiana* plants at the three- to five-leaf stage (3 weeks old). Plants were inoculated with a mixture of cell suspensions of *A. tumefaciens* strain (optical density at 600 nm = 1.0) with the DNA-A and DNA-B multimeric clones of B1 by needle puncture inoculation of the stem just beneath the shoot apex.

Mechanical (sap) inoculation of *B. erecta* and *N. benthamiana* plants was performed as previously described (Gilbertson et al. 1991). Sap was prepared from leaf tissue of 4-week-old *B. erecta* and *N. benthamiana* plants.

Host range determination was performed by agroinoculation and mechanical inoculation of *N. tabacum* 'Samsun', *N. benthamiana*, *N. glutinosa*, *Datura stramonium*, *Solanum lycopersicon* 'Glamour', *Capsicum annuum* 'Cayenne long', *Phaseolus vulgaris* 'Topcrop', *Cucurbita pepo* 'Small sugar', *Chenopodium quinoa*, *C. amaranticolor*, and *Ocimum basilicum* seedlings. In addition to *B. erecta*, two species in the family Nyctaginaceae, *Mirabilis jalapa* and *M. expansa*, were also inoculated.

Inoculated plants were maintained in a growth chamber and monitored on a daily basis, with the final recording of disease incidence and symptoms done at 21 to 24 days postinoculation (dpi). For selected symptomatic and all symptomless plants, the presence of DNA-A and DNA-B components in newly emerged (noninoculated) leaves was determined by PCR with primers specific for each component (Supplementary Table S1).

PR experiments. *N. benthamiana* plants were agroinoculated with mixtures of cell suspensions of *A. tumefaciens* strains with multimeric clones of the DNA-A and DNA-B components of *Jatropha mosaic virus* (JMV) Az-4 strain (this strain was used throughout the study) (Melgarejo et al. 2015), and the B1 multimeric DNA-A and DNA-B clones. The negative control consisted of plants agroinoculated with an *A. tumefaciens* strain with the empty pCambia1300 vector (EV). All inoculated plants were maintained in a growth chamber, and symptoms were recorded at 21 to 24 dpi. The presence of viral DNA components in newly emerged leaves of agroinoculated plants was evaluated as described above with component-specific primers.

Leaf disk replication assays. The replication of the DNA-A and DNA-B components of B1 was assessed in *B. erecta* and *N. benthamiana* leaf disks as described by Kon et al. (2009). Total DNA was extracted from agroinfiltrated leaf patches 5 days after agroinfiltration (daa) and from newly emerged leaves 15 days later. Approximately 2.5 µg of total DNA was fractionated in 1.0% agarose gels in Tris-borate-EDTA buffer and transferred to

Hybond-N+ nylon membranes (Amersham Pharmacia, Piscataway, NJ, U.S.A.). Blots were hybridized with specific probes for B1: an approximately 2.0-kb DNA-A fragment spanning nucleotides 174 to 2,183, and an approximately 1.9-kb DNA-B fragment spanning nucleotides 163 to 2,051. DNA probes were labeled with digoxigenin (DIG) and the random primed labeling technique (Sigma Aldrich, Deisenhofen, Germany).

Replication of the PRs was assessed in infiltrated *N. benthamiana* leaf disks. In this case, blots were hybridized with JMV- or B1-specific DNA-A probes, nucleotides 235 to 2,140 for JMV DNA-A and as previously described for B1; or DNA-B-specific probes, nucleotides 125 to 2,051 for JMV DNA-B and as previously described for B1. In these experiments, the amount of DIG-labeled DNA probes was standardized based upon quantification of labeling efficiency according to the manufacturer's instructions.

Cell-to-cell movement assays. Movement of begomovirus DNA-A and DNA-B components outside of infiltrated patches in *N. benthamiana* leaves was assessed by RCA and restriction enzyme digestion. *N. benthamiana* leaves were infiltrated with *A. tumefaciens* strains with the multimeric DNA-A and DNA-B components and leaf disks of 0.7-cm diameter were collected from inside and outside (approximately 5 mm away) of the infiltrated leaf patch at 4, 7, and 10 daa. Total DNA extraction and RCA reactions were performed as previously described. The RCA products were digested with *Bam*HI (B1) or *Bsr*BI (JMV). For B1, *Bam*HI digestion produces an approximately 2.6-kb DNA-A fragment and approximately 0.6- and 2.0-kb DNA-B fragments, respectively, whereas, for JMV, *Bsr*BI digestion produces an approximately 2.6-kb DNA-A fragment and approximately 1.1- and approximately 1.5-kb DNA-B fragments, respectively.

Mutational analysis. Mutations were introduced into the BC1 ORF, which encodes the movement protein (MP), of the DNA-B components of B1 and JMV. The recombinant plasmids pC-B1-B1.7 and pC-Az-4-B1.6 (Melgarejo et al. 2015) were used as templates for site-directed mutagenesis with phosphorylated primers. The mutations were double-nucleotide substitutions (AGT to GTT at nucleotides 1,305 and 1,306 for B1 or GTT to AGT at nucleotides 1,304 and 1,305 for JMV). This resulted in Thr260 to Asn260 and Asn260 to Thr260 substitutions in the MPs of B1 and JMV, respectively. PCR was performed according to the protocol of the Q5 High-Fidelity DNA polymerase (New England BioLabs, Ipswich, MA, U.S.A.). Following mutagenesis, the PCR-amplified DNA components were self ligated; digested with *NdeI* or *ApaI* for B1 or JMV, respectively; and cloned into *NdeI*- or *ApaI*-digested pSL1180. Recombinant plasmids were recovered, and the presence of the introduced mutation (as well as the lack of any other introduced mutations) was verified by sequence analysis. Monomers confirmed to have the mutations were used to generate multimeric clones for agroinoculation. For the B1 DNA-B Thr260Asn mutant, the multimeric clone was constructed as previously described to generate the pC-B1-B1.7 mutant construct; and, for the JMV DNA-B Asn260Thr mutant, an approximately 1.5-kb *ApaI*-*NdeI* fragment containing the CR was cloned into pSL1180 to generate a 0.6-mer pSL-Az-4-B0.6. The full-length monomer (*ApaI* fragment) was then cloned into the *ApaI* site of pSL-Az-4-B0.6 to generate the 1.6-mer pSL-Az-4-B1.6. This plasmid was then digested with *EcoRI* and *Hind*III, and the resulting fragment with the 1.6-mer was cloned into pCambia1300 to generate the pC-Az-4-B1.6 mutant.

RESULTS

Detection and characterization of a new bipartite begomovirus associated with golden mosaic disease of *B. erecta* in the Dominican Republic. In PCR tests performed with degenerate DNA-A and DNA-B primer pairs, the expected-size approximately 1.1-kb and approximately 1.2-kb DNA fragments were amplified from extracts prepared from absorption

strips with sap of leaf samples from *B. erecta* plants with golden mosaic symptoms collected from four locations in the Azua Valley in 2012 (B1 sample) and 2013 (B2, B3-1, and B3-2 samples). A BLASTn search performed with the sequences of these fragments revealed highest identities of 81 and 75% with DNA-A and DNA-B sequences, respectively, of previously characterized NW bipartite begomoviruses (data not shown). These results indicated that the *B. erecta* plants with golden mosaic symptoms were infected with an uncharacterized NW bipartite begomovirus.

Full-length clones of the DNA-A and DNA-B components of the bipartite begomovirus isolate infecting each of the four *B. erecta* leaf samples (B1, B2, B3-1 and B3-2) were obtained by digestion of RCA products with *Nde*I or *Eco*RI and cloning the resulting approximately 2.6-kb fragments (monomers). Sequence analyses revealed that the DNA-A components of all four isolates are 2,600 nt, whereas the DNA-B components are 2,575 nt. The genome size (approximately 2,600 nt) and organization of the DNA-A components (five ORFs: AV1, AC1, AC2, AC3, and AC4 encoding the capsid protein, Rep, transcription activator protein, replication enhancer, and AC4 proteins, respectively) are typical of NW bipartite begomoviruses. The size and genome organization of the DNA-B components are typical of those of bipartite begomoviruses (two ORFs: BV1 and BC1 encoding the nuclear shuttle protein [NSP] and MP, respectively). The complete nucleotide sequences of the DNA-A and DNA-B components of the begomovirus isolates from the four *B. erecta* leaf samples were 98 to 99% and 96 to 97% identical, respectively (Table 1). Consistent with these results, the AV1, AC1, AC2, AC3, BV1, and BC1 ORFs of these components all had very high identities (≥ 97 to 98% nt and ≥ 95 to 98% amino acid [aa]), whereas identities for the AC4 ORF were slightly lower (98% nt and ≥ 90 to 93% aa). Together, these results indicated that the DNA-A and DNA-B components cloned from the four samples represent isolates of a bipartite begomovirus species.

Pairwise comparisons performed with the DNA-A and DNA-B sequences of the B1 isolate and those of other begomovirus species revealed the highest nucleotide identities for both the complete

sequences and individual ORFs, with NW bipartite begomoviruses from Latin America, including many weed-infecting viruses from the Caribbean Basin (Table 1; Supplementary Fig. S1). For the DNA-A component, the highest nucleotide identities were 77% with *Merremia mosaic virus* (MerMV) from Puerto Rico and 76% with JMV from the Dominican Republic and a weed-infecting isolate of *Tobacco yellow crinkle virus* (TbYCV) from Jamaica, whereas the identity with the DNA-A component of BoYSV from Mexico was 74%. These identities are well below the 91% threshold for describing a new begomovirus species (Brown et al. 2015). Thus, the four isolates from *B. erecta* plants with golden mosaic symptoms in the Dominican Republic represent a new species of NW bipartite begomovirus for which the name BoGMV is proposed. The designation of the four BoGMV isolates [in brackets] and GenBank accession numbers for DNA-A and DNA-B components (in parentheses) are as follows: [DO-Azua-B1-2012] (KY971533 and KY971534), [DO-Azua-B2-2013] (KY971535 and KY971536), [DO-Azua-B3-1-2013] (KY971537 and KY971538), and [DO-Azua-B3-2-2013] (KY971539 and KY971540).

The DNA-A and DNA-B components of each BoGMV isolate share a CR of 159 to 165 nt, with 91 to 93% identity (data not shown). The CR of all four isolates has the characteristic geminivirus stem-loop structure with the conserved nonanucleotide sequence TAATATT↓AC, Rep high-affinity binding sites (iterons), and the Rep TATA box and G-box (Gutierrez 1999; Hanley-Bowdoin et al. 1999). The CR of both components of all four isolates has the same iteron: GGAGG. The DNA-A CRs have a CCCCC inverted repeat, whereas the DNA-B CRs have a CCTCC inverted repeat. The iteron-related domain (IRD) in the N terminus of the Rep protein, which interacts with the iterons in the CR, is MPRKGAFSIN for all four isolates. Interestingly, this IRD is predicted to recognize the GGKGG iteron sequence (K = G or T) (Argüello-Astorga and Ruiz-Medrano 2001), which does not include the GGAGG iteron of BoGMV.

Phylogenetic analyses. In the phylogenetic tree generated with complete DNA-A sequences, the four BoGMV isolates were

TABLE 1. Nucleotide (nt) identities for total (Tot) and common region (CR) sequences and nucleotide and amino acid (aa) identities and similarities (in parenthesis) of individual open reading frames (ORFs) for the DNA-A and DNA-B components of the B1 isolate of *Boerhavia golden mosaic virus* (BoGMV) with those of three other BoGMV isolates from the Dominican Republic (DO) and the most closely related begomoviruses^a

Species	Year ^b	Loc ^c	DNA-A component												DNA-B component					
			Tot	CR	ORFs										Tot	ORFs				
					AV1		AC1		AC2		AC3		AC4			BV1		BC1		
					nt	aa	nt	aa	nt	aa	nt	aa	nt	aa		nt	aa	nt	aa	
BoGMV (Az-B1)	2012	DO	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
BoGMV (Az-B2)	2013	DO	98	93	98	99 (99)	98	99 (99)	98	96 (97)	98	97 (99)	98	92 (92)	96	97	97 (98)	97	97 (98)	
BoGMV (Az-B3-1)	2013	DO	98	96	98	99 (99)	98	99 (99)	98	97 (97)	98	97 (99)	98	93 (93)	96	97	95 (96)	97	96 (98)	
BoGMV (Az-B3-2)	2013	DO	99	95	99	99 (99)	98	99 (99)	99	98 (99)	98	98 (99)	98	90 (91)	97	98	97 (98)	97	97 (98)	
MerMV (PR80-H3)	ND	PR	77	76	77	83 (88)	80	82 (88)	70	59 (63)	66	54 (71)	ND	ND	70	67	64 (77)	75	82 (90)	
JMV (CI-10-Jatropha)	2010	DO	76	69	78	85 (90)	76	78 (85)	70	58 (64)	67	56 (69)	84	64 (71)	68	69	67 (82)	74	80 (89)	
TbYCV (Rhynchosia)	ND	JM	76	64	76	85 (91)	79	80 (87)	68	55 (67)	65	55 (68)	86	62 (68)	69	69	69 (80)	72	80 (86)	
PYMV (Tomato)	1996	TT	76	59	76	83 (89)	79	80 (86)	69	58 (62)	69	55 (69)	87	71 (73)	69	67	62 (74)	74	80 (88)	
ToMHaV (Quivican)	ND	CU	75	48	78	84 (90)	76	75 (84)	69	55 (62)	70	55 (69)	86	64 (68)	69	67	64 (77)	73	69 (78)	
BChV (LaB459)	2006	VE	75	52	77	84 (89)	74	73 (81)	70	55 (62)	66	54 (70)	85	64 (66)	67	65	60 (76)	75	82 (90)	
ToYLDV (Sidastru603)	2011	CU	75	52	76	85 (91)	76	80 (87)	69	55 (70)	68	55 (68)	88	60 (67)	67	65	62 (75)	74	80 (90)	
SiGMFIV (Hav-Mal-11)	2009	CU	74	48	76	84 (90)	76	76 (83)	70	60 (65)	66	51 (65)	83	62 (70)	67	65	60 (75)	72	79 (88)	
MacYMV (Mona)	2006	JM	74	49	78	83 (87)	74	76 (83)	68	56 (64)	66	54 (70)	84	68 (78)	69	65	65 (77)	74	82 (89)	
MaBYMV (Malvastr.)	2014	US	73	65	75	81 (89)	77	75 (83)	67	55 (62)	67	56 (69)	90	78 (80)	62	69	67 (79)	73	80 (88)	
JacYVV (Jacquemol.)	2010	VE	69	58	78	84 (89)	66	63 (71)	68	60 (68)	67	55 (68)	59	27 (40)	61	64	59 (76)	71	71 (83)	

^a Based on a BLASTn analysis, the most closely related begomoviruses were various New World bipartite begomoviruses. The begomoviruses used for comparison and their corresponding GenBank accession numbers are as follows: *Merremia mosaic virus* (MerMV) (DQ644557 and DQ644560), *Jatropha mosaic virus* (JMV) (KJ174331 and KJ174335), *Tobacco yellow crinkle virus* (TbYCV) (KP641345 and KP641346), *Potato yellow mosaic virus* (PYMV) (AF039031 and AF039032), *Tomato mosaic Havana virus* (ToMHaV) (Y14874 and Y14875), *Bean chlorosis virus* (BChV) (JN848770 and JN848771), *Tomato yellow leaf distortion virus* (ToYLDV) (KU232891 and KU232892), *Sida golden mosaic Florida virus* (SiGMFIV) (HM003779 and HM003778), *Macroptilium yellow mosaic virus* (MacYMV) (EF582840 and EF582841), *Malvastrum bright yellow mosaic virus* (MaBYMV) (KU058853 and KU058860), and *Jacquemontia yellow vein virus* (JacYVV) (KY617094 and KY617095). ND = not determined.

^b Year collected.

^c Geographic location: CU = Cuba, BR = Brazil, JM = Jamaica, PR = Puerto Rico, TT = Trinidad and Tobago, US = United States and VE = Venezuela.

placed together in a strongly supported cluster (PP of 1.0). This BoGMV lineage is distinct from the four previously identified lineages of NW bipartite begomoviruses (Fig. 1). The BoGMV cluster was placed in a larger, strongly supported clade (PP of 0.9) with the two isolates of MerMV from Puerto Rico. The isolate of BoYSV from Mexico was placed in the SLCuV lineage with other Mexican begomoviruses, consistent with it representing a species distinct from BoGMV. In the phylogenetic tree generated with complete DNA-B sequences, the BoGMV isolates were also placed together in a strongly supported cluster (PP of 1.0). This cluster was also placed in a larger strongly supported clade (PP of 0.8) with the MerMV isolates from Puerto Rico (Supplementary Fig. S2). As with the DNA-A phylogenetic tree, the BoGMV DNA-B lineage was distinct from the four known NW begomovirus lineages. These results are fully consistent with BoGMV representing a new species and a new phylogenetic lineage of NW bipartite begomoviruses.

Recombination analysis. Phylogenetic network analysis provides visual evidence of recombination. The topology of the network generated with the BoGMV DNA-A sequence and those of the most closely related begomoviruses was non-tree-like, and the star-shaped topology is suggestive of an evolutionary history involving recombination (Supplementary Fig. S3). Indeed, the PHI test indicated a highly significant probability of recombination ($P < 0.001$). Consistent with this result, the RDP analysis identified a strongly supported recombination event in the DNA-A component of the BoGMV B1 isolate, which involved part of the 5' end of the AC1 (Rep) ORF, the entire overlapping AC4 ORF, and part of the left side of the CR (breakpoints mapped to nucleotide positions 2,043 and 2,500) (Supplementary Fig. S4). The P values were 2.5×10^{-7} , 8.0×10^{-18} , 1.3×10^{-19} , 4.3×10^{-07} , 7.7×10^{-13} , 1.1×10^{-17} , 1.5×10^{-12} , and 3.3×10^{-35} for the RDP, Geneconv, BootScan, MaxChi, Chimaera, SiScan, 3Seq, and LARD methods, respectively. An isolate of *Malvastrum bright yellow mosaic virus* (MaBYMV), associated with golden mosaic symptoms in the weed

Malvastrum spp. in South Texas, United States (Alabi et al. 2016), was identified as a possible minor parent involved in this recombinant event.

Infectivity, symptomatology, and host range of BoGMV.

B. erecta seedlings agroinoculated with the cloned multimeric DNA-A and DNA-B components of the B1 isolate of BoGMV were stunted and developed golden mosaic symptoms in newly emerged leaves by 14 dpi (Fig. 2A), whereas equivalent plants agroinoculated with the EV control did not develop symptoms (Fig. 2B). The BoGMV DNA-A and DNA-B components were detected in newly emerged leaves of *B. erecta* plants with golden mosaic symptoms in PCR tests with BoGMV component-specific primer pairs, and not from leaves of symptomless EV control plants (Table 2). These results established that the cloned DNA-A and DNA-B components of BoGMV (B1 isolate) were infectious and induced golden mosaic symptoms in *B. erecta*, thereby fulfilling Koch's postulates for this disease. Furthermore, when sap prepared from leaves of *B. erecta* plants with golden mosaic symptom was mechanically inoculated onto the first true leaves of *B. erecta*

Fig. 1. Bayesian phylogenetic consensus tree generated from an alignment of the complete nucleotide sequences of the DNA-A component of four isolates of *Boerhavia golden mosaic virus* (BoGMV) from the Dominican Republic (shown in gray) and the most closely related begomoviruses (identified by a BLASTn search); selected begomoviruses representing the *Abutilon mosaic virus* (AbMV), *Bean golden yellow mosaic virus* (BGYMV), Brazil and *Squash leaf curl virus* (SLCuV) lineages of New World begomoviruses; and the DNA-A component of *Boerhavia yellow spot virus* (BoYSV) from Mexico. The DNA-A component of the Old World bipartite begomovirus *Tomato leaf curl New Delhi virus* (ToLCNDV) was used as an out-group. Branch strengths were evaluated by Bayesian posterior probabilities. The scale bar represents the numbers of nucleotide substitutions per site. GenBank accession numbers are indicated.

Fig. 2. Disease symptoms induced by the cloned DNA-A and DNA-B components of the B1 isolate of *Boerhavia golden mosaic virus* (BoGMV). **A**, Golden mosaic symptoms induced in a *Boerhavia erecta* plant 21 days post-agroinoculation (dpi). **B**, *B. erecta* plant agroinoculated with the empty vector control and showing no symptoms 21 dpi. **C**, Yellow vein spotting symptoms induced in a leaf of a *Mirabilis jalapa* plant 18 dpi (top), compared with a leaf of a negative control plant agroinoculated with the empty vector (bottom). **D**, Southern blot hybridization analyses showing replicative forms of BoGMV DNA-A and DNA-B components in *B. erecta* and *Nicotiana benthamiana* leaf patches 5 days after agroinfiltration (daa), and in newly emerged (non-inoculated) leaves 21 daa. Blots in the upper and lower panels were hybridized with BoGMV DNA-A and DNA-B probes, respectively. Lane M = size marker (kb) and lane EV = empty vector. Open circular (OC), single-stranded (SS), and covalently closed circular (CCC) DNA forms are indicated.

seedlings, golden mosaic symptoms, indistinguishable from those in agroinoculated plants, developed in newly emerged leaves of all inoculated plants by 12 dpi (Table 2). This result established that BoGMV is sap transmissible to *B. erecta* plants.

Unexpectedly, none of the *N. benthamiana* plants agroinoculated with the infectious cloned BoGMV DNA-A and DNA-B components developed symptoms by 24 dpi (Table 2). When newly emerged leaves of these plants were tested by PCR with BoGMV component-specific primer pairs, the DNA-A but not the DNA-B component was detected. Furthermore, when *N. benthamiana* plants were mechanically inoculated with sap prepared from (i) leaves of *N. benthamiana* plants infected with BoGMV DNA-A only or (ii) leaves of *B. erecta* plants with golden mosaic symptoms (infected with BoGMV DNA-A and DNA-B), no symptoms developed, nor was either DNA component detected in newly emerged leaves of these plants by PCR with the BoGMV component-specific primer pairs.

The host range of BoGMV was next investigated by agroinoculation and mechanical inoculation of the plant species shown in Table 2. With agroinoculation, the only other species infected by BoGMV were *M. jalapa* and *M. expansa*, which are also members of the family Nyctaginaceae. Agroinoculated *M. jalapa* plants developed yellow spots in veins of newly emerged leaves by 14 dpi (Fig. 2C), whereas no symptoms developed in agroinoculated *M. expansa* plants. In PCR tests of newly emerged leaves, BoGMV DNA-A and DNA-B components were detected in the symptomatic *M. jalapa* plants, whereas both components were detected in 50% (14 of 28) of the symptomless *M. expansa* plants (Table 2). None of the *M. jalapa* and *M. expansa* plants mechanically inoculated with sap prepared from BoGMV-infected *B. erecta* leaves developed symptoms or were infected by BoGMV based on PCR tests of newly emerged leaves. Thus, only *B. erecta* plants were infected with BoGMV following mechanical inoculation (Table 2). These results indicated that BoGMV has an extremely restricted host range, limited to species in the family Nyctaginaceae and not including the permissive host *N. benthamiana*.

Leaf disk replication and cell-to-cell movement assays.

We next investigated the replication properties of BoGMV in leaves of *B. erecta* and *N. benthamiana* plants. Replicative forms of the BoGMV DNA-A and DNA-B components were detected in agroinfiltrated leaf patches of *B. erecta* and *N. benthamiana* by Southern blot hybridization analyses with DNA-A- and

DNA-B-component-specific probes (Fig. 2D). Thus, BoGMV replicated in *B. erecta* and *N. benthamiana* leaves, although higher levels of replication were consistently detected in *B. erecta* leaves (in three independent experiments). These results are in agreement with results of plant infectivity experiments in which symptomless DNA-A-only infections were detected in newly emerged leaves of *N. benthamiana* plants agroinoculated with BoGMV DNA-A and DNA-B. These results also indicated that the DNA-A-only infections were not due to a deficiency in DNA-B replication in this host. Instead, these infections were due to the agroinoculation method, which facilitates the systemic movement of DNA-A alone, as previously reported for other bipartite begomoviruses in this host.

We next tested the hypothesis that the inability of BoGMV to systemically infect and cause symptoms in *N. benthamiana* was due to a deficiency in cell-to-cell movement by determining the capacity of BoGMV to move out of agroinfiltrated leaf patches (Fig. 3A). As a positive control, we used JMV, which systemically infects and causes severe symptoms (stunting, distorted growth, and crumpling, epinasty, and mottling of leaves) in *N. benthamiana* plants, and is also sap transmissible to this host (Melgarejo et al. 2015). In this experiment, restriction enzyme digestion analysis of begomovirus DNA components, amplified by RCA, was used to detect BoGMV and JMV DNA-A and DNA-B components (Supplementary Fig. S5). For JMV, the expected-size fragments of DNA-A (approximately 2.6 kb) and DNA-B (approximately 1.1 kb and approximately 1.5 kb) were detected inside and outside of infiltrated patches at 4, 7, and 10 daa, indicating replication and cell-to-cell movement in *N. benthamiana* leaves (Fig. 3B). In patches agroinfiltrated with JMV DNA-A only, the approximately 2.6-kb DNA-A fragment was detected inside but not outside of these patches (Fig. 3B). This is consistent with the capacity of JMV DNA-A to replicate in *N. benthamiana* leaves, and the requirement for the DNA-B component for cell-to-cell movement. In the case of BoGMV, the expected-size fragments of DNA-A (approximately 2.6 kb) and DNA-B (approximately 0.6 kb and approximately 2.0 kb) were detected inside but not outside of the agroinfiltrated patches at 4, 7, and 10 daa (Fig. 3B). This indicated an inability of BoGMV to move cell to cell in leaves of *N. benthamiana* plants. In patches agroinfiltrated with BoGMV DNA-A only, the expected-size approximately 2.6-kb DNA-A fragment was detected inside but not outside of these patches. This is consistent with the capacity of the BoGMV DNA-A component

TABLE 2. Host range and symptomatology of the B1 isolate of *Boerhavia golden mosaic virus* (BoGMV) from the Dominican Republic^a

Species	Symptoms ^c		Infectivity ^b				Symptom type
			Agro		Sap		
	Agro	Sap	DNA-A	DNA-B	DNA-A	DNA-B	
<i>Boerhavia erecta</i>	22/22	22/22	22/22	22/22	14/14	14/14	Golden mosaic, stunting
<i>Mirabilis jalapa</i>	11/21	0/25	11/21	11/21	0/14	0/14	Yellow spotting of veins
<i>M. expansa</i>	0/28	0/19	14/28	14/28	0/15	0/15	Symptomless
<i>Nicotiana benthamiana</i>	0/42	0/39	42/42	0/42	0/21	0/21	Symptomless
<i>N. glutinosa</i>	0/32	0/17	0/32	0/32	0/14	0/14	No infection
<i>N. tabacum</i> ‘Samsun’	0/32	0/17	0/32	0/32	0/14	0/14	No infection
<i>Datura stramonium</i>	0/32	0/16	0/32	0/32	0/12	0/12	No infection
<i>Solanum lycopersicon</i> ‘Glamour’	0/33	0/33	0/33	0/33	0/12	0/12	No infection
<i>Phaseolus vulgaris</i> ‘Topcrop’	0/26	0/14	0/26	0/26	0/14	0/14	No infection
<i>Cucurbita pepo</i> ‘Small sugar’	0/33	0/11	0/33	0/33	0/11	0/11	No infection
<i>Chenopodium quinoa</i>	0/31	0/17	0/31	0/31	0/17	0/17	No infection
<i>C. amaranticolor</i>	0/32	0/17	0/32	0/32	0/15	0/15	No infection
<i>Capsicum annuum</i> ‘Cayenne Long Red’	0/47	0/25	0/47	0/47	0/14	0/14	No infection
<i>Ocimum basilicum</i>	0/15	0/12	0/15	0/15	0/12	0/12	No infection

^a Plants were inoculated with the cloned DNA-A and DNA-B components of the B1 isolate of BoGMV by agroinoculation (Agro) or by mechanical inoculation with sap prepared from symptomatic *B. erecta* leaves collected from plants infected following agroinoculation (Sap).

^b Number of infected plants/total number inoculated. Infections were determined in newly emerged leaves in all symptomless plants and a subset of symptomatic plants by PCR with BoGMV-specific DNA-A and DNA-B primer pairs 20 to 24 days postinoculation (dpi). Results represent data from three independent experiments.

^c Number of symptomatic plants/total number inoculated. Symptoms were visually evaluated 12 to 24 dpi. Results represent data from three independent experiments.

to replicate in *N. benthamiana*. In patches agroinfiltrated with the JMV or BoGMV DNA-B components, no replication or cell-to-cell movement was detected. Together, these results revealed that BoGMV is deficient in cell-to-cell movement in *N. benthamiana* leaves, whereas JMV readily moved cell to cell, consistent with the capacity of this virus to systemically infect and induce severe symptoms in this host.

Fig. 3. Cell-to-cell movement assay of *Boerhavia golden mosaic virus* (BoGMV) isolate B1 and *Jatropha mosaic virus* (JMV) strain Az-4, and replication assay of pseudorecombinants (PRs) generated with the infectious cloned DNA components of BoGMV and JMV in leaves of *Nicotiana benthamiana* plants. **A**, *N. benthamiana* leaves showing the agroinfiltrated patch (I) and the area outside of the patch (OI, approximately 5 mm away from the border of the infiltrated patch), and the pattern and size of the leaf disks collected for detection of viral DNA components by rolling circle amplification (RCA) and restriction enzyme digestion. Leaf disk samples were taken at 4, 7, and 10 days after agroinfiltration (daa) with the DNA-A and DNA-B components or the DNA-A or DNA-B component only. **B**, Restriction fragments of BoGMV B1 and JMV Az-4 DNA components following RCA, restriction enzyme digestion, and agarose gel electrophoresis. Lanes 1, 2, and 3 = 4, 7, and 10 daa, respectively. White arrows indicate the expected size fragments for the DNA-A and DNA-B components. **C**, Southern blot hybridization analyses showing replicative forms and levels of the DNA-A and DNA-B components of BoGMV B1 and JMV Az-4 and PRs formed with the infectious cloned DNA-A and DNA-B components of these viruses in leaf patches of *N. benthamiana* plants 5 daa. Blots in the upper and lower panels were hybridized with DNA-A and DNA-B probes, respectively. Far left and right lanes = size marker (kb) and lane EV = empty vector. Open circular (OC), single-stranded (SS), and covalently closed circular (CCC) DNA forms are indicated. Total DNA was extracted from each of two plants (lanes 1 and 2) per treatment.

To map the viral factor or factors involved in cell-to-cell movement properties of BoGMV and JMV in *N. benthamiana*, we next investigated whether viable PRs could be generated between the infectious cloned DNA components of these viruses. When agroinoculated into *N. benthamiana* plants, the JMV-DNA-A/BoGMV-DNA-B and BoGMV-DNA-A/JMV-DNA-B PRs did not induce symptoms. As expected, plants agroinoculated with JMV DNA-A and DNA-B developed severe symptoms, whereas those agroinoculated with BoGMV DNA-A and DNA-B were symptomless. PCR tests of newly emerged leaves revealed DNA-A-only infections in all plants agroinoculated with both PRs but DNA-B was not detected in any of these plants. Thus, these plants had symptomless DNA-A-only infections (Supplementary Table S2), similar to those previously described for plants agroinoculated with BoGMV DNA-A and DNA-B. Equivalent PCR tests revealed DNA-A and DNA-B components in symptomatic leaves of plants agroinoculated with JMV DNA-A and DNA-B, whereas the expected symptomless DNA-A-only infections were detected in plants agroinoculated with BoGMV DNA-A and DNA-B. Leaf disk replication assays with the PRs further revealed that the BoGMV DNA-A component did not mediate the replication of the JMV DNA-B component, nor did the JMV DNA-A component mediate the replication of the BoGMV DNA-B component (Fig. 3C). Thus, although JMV is one of the begomoviruses most closely related to BoGMV (Table 1), the failure to generate viable PRs between these viruses likely reflects sequence divergence in the CR, including different iterons and the Rep IRDs. These results are also consistent with BoGMV and JMV representing distinct species in different phylogenetic lineages. Thus, we could not use PR experiments to map the viral determinants of cell-to-cell movement of these two begomoviruses.

The MP, encoded by the BC1 ORF of the DNA-B component, plays an essential role in begomovirus cell-to-cell movement. Therefore, it was hypothesized that amino acid differences in the MPs of BoGMV and JMV underlie the different cell-to-cell movement capacities of these viruses in *N. benthamiana*. The MP amino acid sequences of JMV and BoGMV are 79% identical, and analysis of an alignment of these sequences revealed that 62 of 293 aa are different (21%, data not shown). To narrow down possible differences involved in cell-to-cell movement, an alignment of MP amino acid sequences of 15 NW begomoviruses, selected on the basis of their differential virulence in *N. benthamiana*, was generated. The virulence properties of these viruses are as follows: SL-A, symptomless DNA-A-only infection (e.g., BoGMV); SL-A/B, symptomless DNA-A and DNA-B infection (e.g., *Cucurbit leaf crumple virus* [CuLCrV], *Bean golden mosaic virus* [BGMV], and BGYMV) (Garrido-Ramirez et al. 2000a; Hagen et al. 2008; Petty et al. 2000); and SI-A/B, symptomatic DNA-A and DNA-B infection. Eight regions of the MP alignment were examined based on previous studies showing a role in cell-to-cell movement and pathogenicity (Ingham et al. 1995; Noueiry et al. 1994). Inspection of these aligned regions revealed a single amino acid difference at position 260, where the SL-A virus (BoGMV) has a Thr residue, the SL-A/B viruses (CuLCrV, BGMV, and BGYMV) have Thr or Ser residues, and the 11 SI-A/B viruses have Asn or His residues (Supplementary Fig. S6).

To test the hypothesis that amino acid differences at position 260 were responsible for the inability of BoGMV to move cell to cell in *N. benthamiana* leaves, a mutation in the BoGMV BC1 sequence was introduced that changed Thr260 to Asn260, and the corresponding mutation was introduced into the JMV BC1 sequence that changed Asn260 to Thr260. As expected, control *N. benthamiana* plants agroinoculated with BoGMV DNA-A and DNA-B were symptomless, whereas those agroinoculated with JMV DNA-A and DNA-B were stunted and developed crumpling, epinasty, and mottling of leaves. *N. benthamiana* plants agroinoculated with BoGMV DNA-A and the DNA-B Thr260Asn mutant were symptomless, whereas plants agroinoculated with

JMV DNA-A and the DNA-B Asn260Thr mutant were stunted and developed leaf crumpling, epinasty, and mottling, indistinguishable from wild-type JMV infection (Supplementary Table S3). PCR tests revealed symptomless DNA-A-only infections in plants agroinoculated with BoGMV DNA-A and the DNA-B Thr260Asn mutant, whereas DNA-A and DNA-B components were detected in symptomatic leaves of plants agroinoculated with JMV DNA-A and the DNA-B Asn260Thr mutant. Thus, this single amino acid difference in the BoGMV and JMV MPs was not the determinant of cell-to-cell movement capacity of these viruses in *N. benthamiana*.

DISCUSSION

In tropical and subtropical regions of the world, noncultivated plants, especially weeds, are commonly infected by begomoviruses and often show striking golden yellow mosaic symptoms. To date, most begomoviruses characterized from noncultivated plants have been genetically distinct from those infecting crops, and are only very rarely detected in crops (and vice versa). Because begomovirus-infected weeds of multiple species are often observed in and around agricultural fields, such plants are widely believed to harbor crop-infecting viruses. Thus, the relationship between weed- and crop-infecting begomoviruses remains unclear. There are two major hypotheses: (i) weeds serve as reservoirs for crop-infecting begomoviruses and (ii) weed-infecting begomoviruses serve as progenitors for local evolution of crop-adapted viruses (Gilbertson et al. 2015; Inoue-Nagata et al. 2016; Jones 2009). Therefore, molecular characterization of weed-infecting begomoviruses provides important practical information in terms of management of crop-infecting begomoviruses, as well as insight into viral evolution.

In the present study, we describe a new NW bipartite begomovirus associated with golden mosaic symptoms in *B. erecta* plants in the Azua Valley of the southeastern Dominican Republic. Because this weed is commonly found in and around agricultural fields in the Dominican Republic (Santos et al. 2008), it is important to know the viruses harbored in this plant and their potential to infect crops. The sequence of the DNA-A component of the begomovirus from *B. erecta* had identities of <80% with those of previously characterized begomoviruses, including those infecting crops and weeds in the Caribbean Basin and BoYSV from Mexico. The current International Committee on Taxonomy of Viruses criteria for the demarcation of begomovirus species is <91% nt identity with the DNA-A components of recognized species. Therefore, the begomovirus infecting *B. erecta* in the Dominican Republic is a new species, and the name BoGMV is proposed based on the striking disease symptoms observed in the field. Furthermore, the development of golden mosaic symptoms in *B. erecta* plants agroinoculated with the cloned multimeric DNA-A and DNA-B components of BoGMV fulfilled Koch's postulates for this disease and confirmed that these symptoms are caused by this bipartite begomovirus.

The genome organization of BoGMV is typical of NW bipartite begomoviruses but this virus possesses several notable properties. Phylogenetic analyses established that the BoGMV isolates from the Dominican Republic constitute a new and distinct lineage of NW bipartite begomoviruses. Most likely, this lineage originated following local evolution and adaptation of a progenitor begomovirus to infect and cause symptoms in *B. erecta*. This hypothesis was supported by the finding that the closest relatives of BoGMV are weed-infecting bipartite begomoviruses from the Caribbean Basin (e.g., MerMV from Puerto Rico, JMV from the Dominican Republic, and TbYCV from Jamaica). However, the relatively low sequence identities (<80%) of these begomoviruses with BoGMV indicate a long period of diversification and host adaptation. Further evidence for this came from the inability to make viable BoGMV/JMV PRs, which reflected divergence in the iterons

and Rep IRD sequences (Argüello-Astorga and Ruiz-Medrano 2001; Fontes et al. 1994). Furthermore, BoGMV has a distinct iteron not previously predicted to be recognized by its IRD, and this may be a feature of the BoGMV lineage. Together, these results suggest that BoGMV diverged from (i) a common progenitor virus over a long period of time or (ii) from one of multiple divergent progenitors that independently evolved to infect different weed species in the Caribbean Basin. Independent evolution from genetically distinct progenitors explains the high degree of divergence between BoGMV from the Dominican Republic and BoYSV from Mexico.

Most begomoviruses have relatively narrow host ranges, which typically include species in only one to three families (e.g., Cucurbitaceae, Fabaceae, and Solanaceae). A few have broader host ranges (e.g., ToLCNDV and *Tomato severe rugose virus*, which infect plant species in five and six families, respectively) (Barbosa et al. 2011; Moury et al. 2017; Zaidi et al. 2017). Our results revealed that the host range of BoGMV is limited to *B. erecta* and two other species in the family Nyctaginaceae. This indicates a high degree of host adaptation that likely occurred over a long period of time. Furthermore, the high degree of sequence identity among the four BoGMV isolates collected from different locations and times (years) is consistent with a high degree of host adaptation and the placement of these isolates in a distinct phylogenetic lineage. A similar degree of host adaptation and specificity has been reported for the OW legume-infecting *Dolichos yellow mosaic virus*, which infected only *Lablab purpureus* out of 36 species tested. Furthermore, isolates of this virus were also placed in a distinct phylogenetic lineage (Maruthi et al. 2006; Qazi et al. 2007).

The adaptation of BoGMV to *B. erecta* likely involved the multiple evolutionary forces that shape genetic diversity in virus populations (i.e., mutation, PR, and recombination) (Holmes 2009). The high degree of divergence across the entire BoGMV genome, even with the most closely related bipartite NW begomoviruses, indicates extensive evolution via mutation, a major means of begomovirus diversification (Duffy and Holmes 2009; Duffy et al. 2008). In addition, a recombination event was identified in the BoGMV DNA-A component, and included the entire AC4 ORF. A possible donor of this genomic region was identified by RDP as the weed-infecting begomovirus MaBYMV from South Texas. Following the evolution and host adaptation of BoGMV, subsequent plant-to-plant spread and maintenance in *B. erecta* would have been mediated by local whitefly vector populations (Bastidas et al. 2008; De Marchi et al. 2017). The introduction and establishment of the polyphagous MEAM1 species (biotype B) of *Bemisia tabaci* in the Dominican Republic could change the prevalence or even host range of BoGMV, depending on the preference of these whiteflies for *B. erecta*.

In terms of crop production in the Dominican Republic, the low degree of genetic diversity and high degree of host specificity (narrow host range) of BoGMV minimizes the likelihood that this virus will infect crop plants or serve as a progenitor for crop-infecting begomoviruses. There are few crops in the Nyctaginaceae family (Van Zant et al. 2018), and none of these are grown in the Dominican Republic. This is in contrast to JMV, which has a higher degree of genetic diversity and a wider host range, which includes the crop plants common bean and tobacco (Melgarejo et al. 2015). Therefore, BoGMV can be considered a specialist, whereas JMV is more of a generalist, with greater potential to cause disease in crops or evolve into a crop-infecting begomovirus (Elena 2017; Jones 2009). Thus, the specialist begomovirus BoGMV does not appear to pose a threat to agriculture or warrant implementation of management strategies such as roguing symptomatic *B. erecta* plants. In contrast, *B. erecta* is a reservoir host for the invasive *Tomato chlorotic spot virus* (TCSV), an economically important tomato-infecting tospovirus recently introduced in the Caribbean Basin (Gilbertson et al. 2015). In the Dominican Republic and Puerto Rico, TCSV induces stunting and chlorosis symptoms in *B. erecta* and such plants may be an inoculum source for this virus

(Batuman et al. 2014; Estévez de Jensen et al. 2017; Gilbertson et al. 2015).

Another notable feature of BoGMV was the interaction with *N. benthamiana*. Unlike most begomoviruses, BoGMV did not induce a typical infection in this permissive host (i.e., replication, systemic spread of both components, and development of disease symptoms). The BoGMV DNA-A-only infection detected in *N. benthamiana* plants agroinoculated with the DNA-A and DNA-B components is a well-known phenomenon associated with this inoculation method, and is characterized by no symptoms, low virus titers, and inability to be mechanically transmitted (Buragohain et al. 1994; Evans and Jeske 1993; Frischmuth et al. 1993; Galvao et al. 2003; Garrido-Ramirez et al. 2000a; Hou et al. 1998; Klinkenberg and Stanley 1990). However, the DNA-A-only infection did reveal the capacity for BoGMV to replicate in *N. benthamiana*, and this was confirmed with the leaf disk replication assays. These assays further showed that BoGMV DNA-A replicated the cognate DNA-B component in *N. benthamiana* leaves, which suggested that a deficiency in viral movement was responsible for the inability of BoGMV to infect *N. benthamiana*. Indeed, this was demonstrated by the failure to detect the BoGMV DNA components in leaf disks taken from outside of agroinfiltrated patches in *N. benthamiana* leaves. In contrast, the DNA-A and DNA-B components of JMV were consistently detected in leaf disks taken from outside of the agroinfiltrated patches, which revealed cell-to-cell movement and is consistent with systemic infection and symptom development in this host (Melgarejo et al. 2015).

Because the failure of BoGMV to infect *N. benthamiana* was due to a deficiency in cell-to-cell movement, we next analyzed amino acid sequences of the MP for candidate amino acid residues possibly involved in this process. The amino acid alignment of MP sequences of begomoviruses with differential virulence in *N. benthamiana* revealed a Thr residue at position 260 for BoGMV, whereas begomoviruses that systemically infect and cause symptoms in *N. benthamiana* mostly have an Asn residue at this position. Furthermore, mutation of this residue in the MP of SLCuV abolished infectivity in *N. benthamiana* plants and the mutant MP had an altered subcellular localization and ability to relocate the NSP protein in transient expression studies (Ingham et al. 1995; Sanderfoot and Lazarowitz 1995); thus, we hypothesized that the amino acid difference at this position was involved in adaptation of BoGMV to *B. erecta* and the inability to move cell to cell in *N. benthamiana*. However, this hypothesis was rejected because the BoGMV mutant expressing the Thr260Asn MP did not systemically infect and cause symptoms in *N. benthamiana*, whereas the JMV mutant expressing the Asn260Thr MP did not lose this capacity. Thus, the altered MPs retained their respective cell-to-cell movement properties. It is likely that the host adaptation of BoGMV to *B. erecta* is more complex and involves other amino acid changes in the MP or other viral proteins or non-coding regions (Garrido-Ramirez et al. 2000b; Ingham et al. 1995; Petty et al. 2000; Zhou et al. 2007).

In conclusion, we have characterized a new NW bipartite begomovirus, BoGMV, which induces golden mosaic symptoms in *B. erecta* plants in the Dominican Republic. We further established that BoGMV has a high degree of host adaptation, only infecting members of the family Nyctaginaceae, especially *B. erecta*. BoGMV isolates collected from different locations and times had a low level of genetic diversity and constitute a new lineage of NW begomoviruses. Together, this suggests that BoGMV has become well-adapted to *B. erecta*, most likely over a long period of time and involving extensive mutation and at least one recombination event. The high degree of host specificity and the inability to infect plant species in five other families, including several crop plants, indicates that BoGMV does not pose a threat to agricultural production in the Dominican Republic. Finally, the inability of BoGMV to infect the permissive laboratory host *N. benthamiana* was due to a deficiency in cell-to-cell movement but this deficiency was not due to an amino acid difference at position 260 in the MP.

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