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BRIEF REPORT

Molecular and biological characterization of corchorus mottle virus, a new begomovirus from Brazil

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Abstract A begomovirus infecting Orinoco jute (*Corchorus hirtus*) from Brazil was characterized. Molecular analysis revealed a bipartite genomic organization, which is typical of the New World begomoviruses. Sequence analysis and phylogenetic data showed that both genomic components have the closest relationship with abutilon mosaic Brazil virus, with an identity of 87.3 % for DNA-A, indicating that this virus is a member of a new begomovirus species for which the name "Corchorus mottle virus" (CoMoV) is proposed. *Sida rhombifolia* plants inoculated by biolistics with an infectious clone of CoMoV showed systemic vein chlorosis, mottling and leaf deformation symptoms, while *Nicotiana benthamiana* and tomato plants had symptomless infection. CoMoV is the first corchorus-infecting begomovirus reported in Brazil.

Begomoviruses are plant pathogens that cause high yield losses in many important crops worldwide [31, 33, 43]. They belong to the family *Geminiviridae*, which is additionally composed of six other genera, *Curtovirus*, *Mastrevirus*, *Becurtovirus*, *Eragrovirus*, *Turncurtovirus*, and *Topocuvirus*. Begomoviruses are transmitted by the whitefly *Bemisia tabaci* in a circulative manner to dicots through either a bipartite or monopartite genome. Bipartite viruses have a genome consisting of two circular single-stranded DNA (ssDNA) molecules (DNA-A and DNA-B), while monopartite viruses have only one ssDNA component. Satellite molecules (alphasatellites and betasatellites) may be associated with begomoviruses, especially Old World monopartite viruses [6, 8]. Begomovirus DNA-A encodes proteins responsible for particle encapsidation (CP/ORF AV1), viral replication (Rep/ORF AC1 and Ren/ORF AC3), regulation of gene expression, and for some viruses, suppression of gene silencing (TrAP/ORF AC2). AC4 has been implicated in modulation of symptom development and may also act as a suppressor of gene silencing. AV2 is only present in begomoviruses that originated in the Old World. The proteins NSP (ORF BV1) and MP (ORF BC1) located in DNA-B encode two proteins involved in intracellular and intercellular virus movement [27, 29]. The DNA-A and DNA-B components share a common region (CR) of approximately 200 bp with high sequence identity (usually 80-100 %) [7, 24, 30, 37]. The CR harbours a putative stem-loop structure containing the nonanucleotide TAATATT↓AC, which is highly conserved and functions in the initiation of the rolling-circle replication. The CR also contains cis elements (CA-, TATA- and G-box) involved in the virus replication and transcription processes [11, 12].

In Brazil, begomoviruses are major pathogens and constraint factors for common bean and tomato production [13, 36]. Members of different begomoviruses species have also been reported infecting weed plants belonging to the families Euphorbiaceae, Solanaceae, Compositae, Capparaceae, Fabaceae and Malvaceae [3, 9, 15, 34, 39, 40]. Weeds and wild plants are believed to serve as reservoirs

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for begomoviruses where evolutionary-driving processes such as recombination and pseudo-recombination may occur, thus making these species intermediate hosts in the adaptation process that allows these viruses to infect economically important crops [32, 36]. Characterization of viruses infecting weed plants may shed light on the evolutionary and ecological dynamics of begomoviruses. Nevertheless, little attention has been paid to these aspects or to surveying begomoviruses infecting weed plants.

Orinoco jute (*Corchorus hirtus* L.) is an annual malvaceous herbaceous plant native to the Americas that grows in the West Indies, the southern United States, Mexico, Central and South America [42]. In Brazil, *C. hirtus* is found distributed in all geographical regions, occupying the Amazon, Caatinga, Cerrado, Mata Atlântica and Pantanal phytogeographical domains [5]. In these areas, it grows either as a ruderal or an invading weed that infests pasture preferably, but it may also occur in agricultural crops such as cotton, cassava, maize and beans [18, 28]. Begomoviruses have been isolated from different species of *Corchorus* collected in Mexico, Vietnam and India [16, 17, 19, 20, 23]; however, *Corchorus* spp. have not yet been described as begomovirus hosts in Brazil.

In this study, we report the molecular and biological characterization of a proposed new bipartite begomovirus infecting *Corchorus hirtus* in Brazil, which we tentatively named corchorus mottle virus (CoMoV).

A *C. hirtus* plant showing mottle symptoms (Fig. 1A) was collected in the vicinity of a sweet potato field in the rural area of Batateiras District, São Joaquim dos Montes, in the Northeastern state of Pernambuco, Brazil. Total DNA was extracted using a CTAB protocol [10]. PCR using primers pAL1v1978 and pAR1c496 [38] resulted in a fragment of ~1.2 kb, which was cloned in pGEM®-T Easy Vector. Comparison of the sequences of eight independent clones showed 98-99 % identity among them, and BLAST search for similar sequences confirmed begomoviral infection. The viral genome was amplified by the rolling-circle amplification (RCA) method using *Φ*29 DNA polymerase (TempliPhi, GE Healthcare) according to Inoue-Nagata *et al*. [26]. RCA multimeric products were initially digested with the four-base cutter restriction enzyme *Msp*I. The sum of the restriction fragments obtained was approximately 5.3 kb, which is indicative of an infection with a single New World bipartite begomovirus [21]. RCA products were then digested with a set of restriction endonucleases (*Eco*RI, *Bam*HI, *Sma*I, *Sac*I, *Kpn*I, *Xho*I and *Hind*III). A complete digestion of the RCA products was obtained with *Kpn*I and putative viral full-length monomers of ca. 2.6 kb were cloned into the pBluescript SK+ vector linearized with the same enzyme. Five clones harboring fragments of the expected size were recovered, and digestion with *Msp*I rendered two different restriction patterns. All recovered clones were initially sequenced with pBluescript SK+ standard primers. Since the sequences of the clones with the same restriction pattern were nearly identical over a 1.6-kb sequence fragment (99 % identity), the clones 62K2 and 62K3 were chosen as representative of DNA-A and DNA-B, respectively, and fully sequenced by primer walking (Macrogen Inc., South

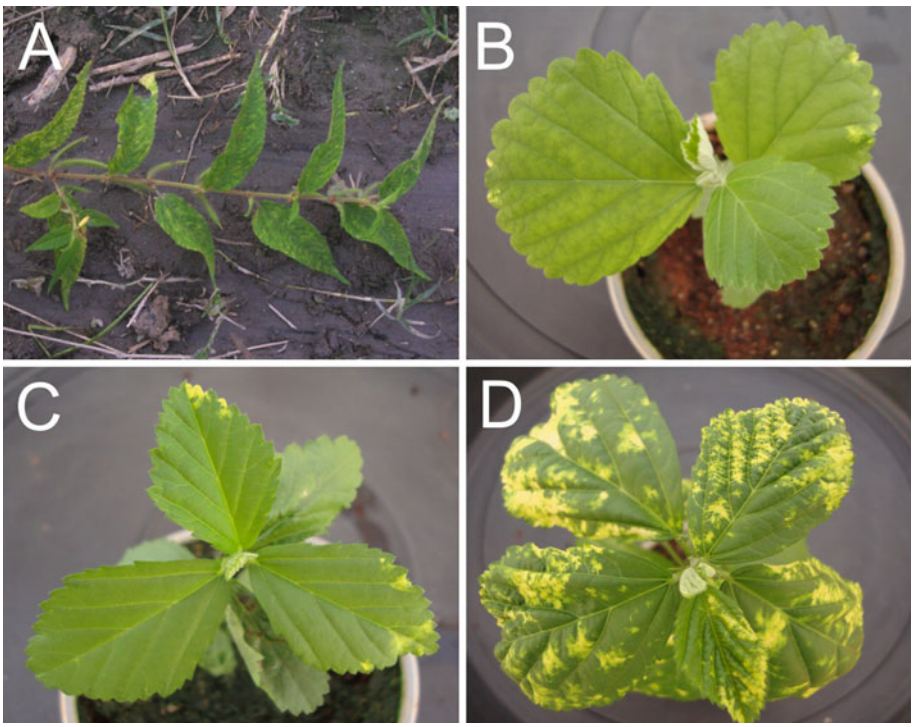


Fig. 1 Symptoms induced by natural or experimental CoMoV infection. (**A**) Mottling in a naturally infected *Corchorus hirtus* plant. (**B**) Diffuse local chlorotic lesions in inoculated leaves of *Sida glasiovii* at 10 days post-inoculation (dpi). (**C**) Chlorotic local spots and diffuse local chlorotic lesions in *S. rhombifolia* at 10 dpi. (**D**) Vein chlorosis, mottling and leaf deformation in *S. rhombifolia* at 22 dpi

Korea). Contigs were assembled using the Sequence Assembly function of the DNAMAN program (Lynnon Corporation).

Nucleotide sequences of clones 62K2 and 62K3 were analyzed by BLAST to search for sequence similarity with other begomoviruses and for preliminary species assignment. The ClustalV algorithm (with default parameters) implemented in the MegAlign software (DNASTAR, WI, USA) was used for multiple sequence alignment and pairwise comparisons [14]. Phylogenetic analysis and tree graphics were generated by Mega v.5 (maximum-likelihood method, 1000 bootstrap replicates) [41]. Promoter analysis was performed using GPMiner (http://gpminer.mbc.nctu.edu.tw/index.php).

DNA-A of CoMoV is 2650 nucleotides in length with a genomic structure characteristic of begomoviruses from the New World. Pairwise comparison with other begomoviruses showed highest identity (87.3 %) to abutilon mosaic Brazil virus (AbMBV-[BR:BA]) [35]. According to the current demarcation threshold for begomovirus species (<89 % identity for DNA-A) recommended by the *Geminiviridae* Study Group of the International Committee on Taxonomy of Viruses [14], CoMoV should be regarded as a member of a tentative new bipartite begomovirus species.

The intergenic region of CoMoV-A consisted of 250 bp, containing several cis-acting elements, including TATA boxes and CAA repeats typically found in begomoviruses. Using tools available in GPMiner, three TATA motifs were found at positions 2522-2527, 2594-2597 and 199-202, one CCAAT box (GCCAAT) was predicted at position 128-133, and one GC box (GGCGGC) was found at position 45-50.

The complete nucleotide sequence of clone of 62K3, which represents the B component of CoMoV, consisted of 2611 bp, with greatest nucleotide sequence identity (66.6 %) to AbMBV-[BR:BA]-DNA-B.

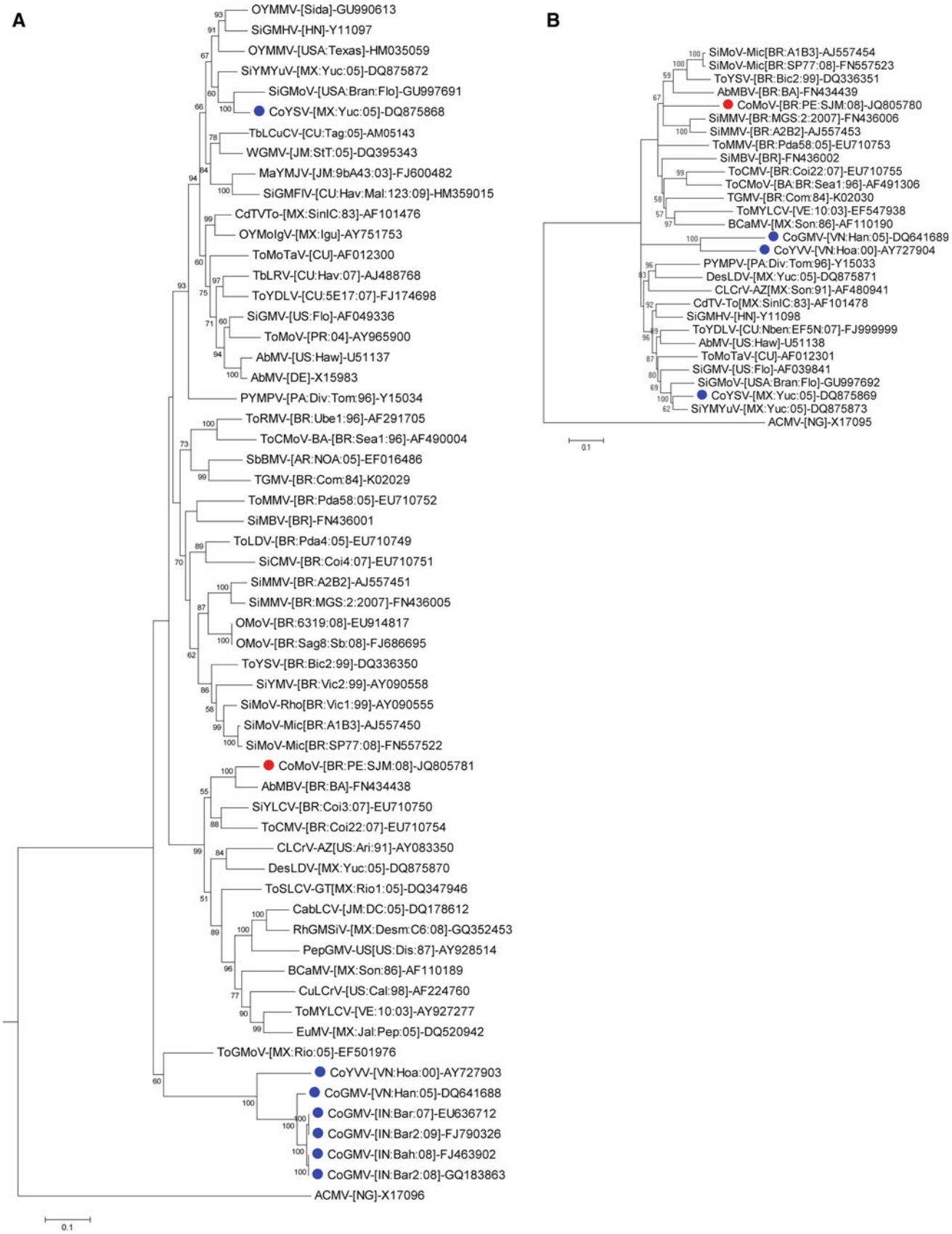
Detailed nucleotide (nt) and deduced amino acid (aa) sequence comparisons between CoMoV and closely related and corchorus-infecting begomovirus sequences are presented in Table 1. As expected, the highest degree of identity of CoMoV genes was with AbMBV-[BR:BA], except for the AC2 nt sequence, which had highest identity (88.1 %) to AC2 of tomato common mosaic virus (ToCMMV-[BR:Coi22:07]-EU710754) [9]. With the exception of the CoMoV-AV1-derived aa sequence, which had the highest similarity score of 96.4 % with the CP of tomato leaf distortion virus (ToLDV-[BR:Pda4:05]-EU710749) [9], all other protein sequences were more similar to those of AbMBV-[BR:BA]. For DNA-B, BV1 had the highest sequence identity for both nt and aa to AbMBV-[BR:BA]. For BC1, the maximum nt sequence identity was with sida mosaic Brazil virus-[BR], while the derived aa sequence had highest similarity to that of abutilon mosaic Brazil virus-[BR:BA]. An incongruent nucleotide sequence identity for different segments of the viral genome may be indicative of a recombinant origin. However, further studies are needed to verify if there were any recombination events involving CoMoV AC2 and BC1 to confirm a possible recombinant nature of CoMoV.

CoMoV DNA-A and DNA-B intergenic regions share a common region (CR) of 189 bp with an identity of 89.4 %. This identity score is more divergent than what is commonly observed for CRs of a cognate pair of DNA-A and DNA-B. However, similar and even lower CR identity scores have several precedents in the literature [7, 22, 24, 25, 30]. The CoMoV CR contains the geminivirus conserved nonanucleotide sequence (TAATATT↓AC) and two core iteron [1] sequences, GGAGTCC and GGACTCC.

Although CoMoV and the closely related AbMBV-[BR:BA] share the same iterons, the number and position of the iterated sequences differ between the two viruses. Whereas CoMoV iterons were found four times upstream of the TATA box in DNA-A, five times in DNA-B, and once downstream of the TATA box in both components, in the AbMBV-[BR:BA] CR, the iterons are present four times in both DNA-A and DNA-B, only downstream of the TATA box (Supplementary Figure S1). Interestingly, because of their partially palindromic sequence, the iteron repeats can be perceived either as direct or inverted repeats (Supplementary Figure S1).

Phylogenetic maximum-likelihood trees were constructed using a multiple alignment of selected bipartite New World and corchorus-infecting begomoviruses. CoMoV and AbMBV DNA-A are sister taxa in a group that also includes sida yellow leaf curl virus (SiYLCV) and ToCMMV from Brazil. This group clusters along a large group of viruses isolated from weed and crop plants, mainly from Mexico and the USA, in agreement with previous reports [9, 35] (Fig. 2). CoMoV DNA-B also clustered with other begomoviruses from Brazil, but in contrast to DNA-A, it was more distantly related to the DNA-B of AbMBV (Fig. 2). These results are in accordance with the pairwise sequence identity data (Table 1). No close relationship was found between CoMoV and other corchorus-infecting viruses reported from Mexico, India or Vietnam [19, 20, 23].

For infectivity tests and partial host range determination, single copies of DNA-A and DNA-B genomes were released from the *Kpn*I site of pBluescript SK^+ vector and self-ligated as described [37]. For particle bombardment inoculation, DNA (2.5 μg) of each component was combined and precipitated with 3.0 mg of tungsten particles (M10). Plants showing one to two fully expanded true leaves were placed in a helium-driven particle bombardment device, at a distance of 2-5 cm from the macrocarrier, which was launched by a helium discharge of 850 PSI, in a


A
OYMMV-[Sida]-GU990613
SiGMHV-[HN]-Y11097
OYMMV-[USA:Texas]-HM035059
SiYMYuV-[MX:Yuc:05]-DQ875872
SiGMoV-[USA:Bran:Flo]-GU997691
CoYSV-[MX:Yuc:05]-DQ875868
TbLCuCV-[CU:Tag:05]-AM05143
WGMV-[JM:StT:05]-DQ395343
MaYMJV-[JM:9bA43:03]-FJ600482
SiGMFIV-[CU:Hav:Mal:123:09]-HM359015
CdTVTo-[MX:SinIC:83]-AF101476
OYMoIgV-[MX:Igu]-AY751753
ToMoTaV-[CU]-AF012300
TbLRV-[CU:Hav:07]-AJ488768
ToYDLV-[CU:5E17:07]-FJ174698
SiGMV-[US:Flo]-AF049336
ToMoV-[PR:04]-AY965900
AbMV-[US:Haw]-U51137
AbMV-[DE]-X15983
PYMPV-[PA:Div:Tom:96]-Y15034
ToRMV-[BR:Ube1:96]-AF291705
ToCMoV-BA-[BR:Sea1:96]-AF490004
SbBMV-[AR:NOA:05]-EF016486
TGMV-[BR:Com:84]-K02029
ToMMV-[BR:Pda58:05]-EU710752
SiMBV-[BR]-FN436001
ToLDV-[BR:Pda4:05]-EU710749
SiCMV-[BR:Coi4:07]-EU710751
SiMMV-[BR:A2B2]-AJ557451
SiMMV-[BR:MGS:2:2007]-FN436005
OMoV-[BR:6319:08]-EU914817
OMoV-[BR:Sag8:Sb:08]-FJ686695
ToYSV-[BR:Bic2:99]-DQ336350
SiYMV-[BR:Vic2:99]-AY090558
SiMoV-Rho[BR:Vic1:99]-AY090555
SiMoV-Mic[BR:A1B3]-AJ557450
SiMoV-Mic[BR:SP77:08]-FN557522
CoMoV-[BR:PE:SJM:08]-JQ805781
AbMBV-[BR:BA]-FN434438
SiYLCV-[BR:Coi3:07]-EU710750
ToCMV-[BR:Coi22:07]-EU710754
CLCrV-AZ[US:Ari:91]-AY083350
DesLDV-[MX:Yuc:05]-DQ875870
ToSLCV-GT[MX:Rio1:05]-DQ347946
CabLCV-[JM:DC:05]-DQ178612
RhGMSiV-[MX:Desm:C6:08]-GQ352453
PepGMV-US[US:Dis:87]-AY928514
BCaMV-[MX:Son:86]-AF110189
CuLCrV-[US:Cal:98]-AF224760
ToMYLCV-[VE:10:03]-AY927277
EuMV-[MX:Jal:Pep:05]-DQ520942
ToGMoV-[MX:Rio:05]-EF501976
CoYVV-[VN:Hoa:00]-AY727903
CoGMV-[VN:Han:05]-DQ641688
CoGMV-[IN:Bar:07]-EU636712
CoGMV-[IN:Bar2:09]-FJ790326
CoGMV-[IN:Bah:08]-FJ463902
CoGMV-[IN:Bar2:08]-GQ183863
ACMV-[NG]-X17096
0.1
B
SiMoV-Mic[BR:A1B3]-AJ557454
SiMoV-Mic[BR:SP77:08]-FN557523
ToYSV-[BR:Bic2:99]-DQ336351
AbMBV-[BR:BA]-FN434439
CoMoV-[BR:PE:SJM:08]-JQ805780
SiMMV-[BR:MGS:2:2007]-FN436006
SiMMV-[BR:A2B2]-AJ557453
ToMMV-[BR:Pda58:05]-EU710753
SiMBV-[BR]-FN436002
ToCMV-[BR:Coi22:07]-EU710755
ToCMoV-[BA:BR:Sea1:96]-AF491306
TGMV-[BR:Com:84]-K02030
ToMYLCV-[VE:10:03]-EF547938
BCaMV-[MX:Son:86]-AF110190
CoGMV-[VN:Han:05]-DQ641689
CoYVV-[VN:Hoa:00]-AY727904
PYMPV-[PA:Div:Tom:96]-Y15033
DesLDV-[MX:Yuc:05]-DQ875871
CLCrV-AZ[MX:Son:91]-AF480941
CdTV-To[MX:SinIC:83]-AF101478
SiGMHV-[HN]-Y11098
ToYDLV-[CU:Nben:EF5N:07]-FJ999999
AbMV-[US:Haw]-U51138
ToMoTaV-[CU]-AF012301
SiGMV-[US:Flo]-AF039841
SiGMoV-[USA:Bran:Flo]-GU997692
CoYSV-[MX:Yuc:05]-DQ875869
SiYMYuV-[MX:Yuc:05]-DQ875873
ACMV-[NG]-X17095
0.1

◄**Fig. 2** Maximum-likelihood phylogenetic trees based on complete DNA-A (**A**) and DNA-B (**B**) alignments of selected New World and corchorus-infecting begomoviruses. Bootstrap scores above 50 % (1000 replicates) are placed at the tree nodes. Genomic component sequences of an African cassava mosaic virus (ACMV) isolate were used as outgroups. Red dot indicates CoMoV; and blue dots, sequences from other corchorus-infecting viruses. The scale bar represents the number of nucleotide substitutions per site. Accession numbers are placed beside the virus name abbreviation. Supplementary Table S2 shows virus names and acronyms used throughout this paper

vacuum chamber (25 inHg). Immediately after, inoculated plants were placed in a humid chamber for 2 days and then transferred to whitefly-proof cages and kept in a greenhouse at 19-28 °C. Eight plants of each tested host species (*Nicotiana benthamiana*, *Sida glaziovii, S. rhombifolia, S. cordifolia and Solanum lycopersicum*) were inoculated. Mock-inoculated plants were used as negative controls. Symptoms were recorded, and total DNA was extracted 22 days post-inoculation (dpi). CoMoV infection was

Table 1 Nucleotide (nt) and deduced amino acid (aa) sequence identities (%) between corchorus mottle virus and other selected begomoviruses

Virus	Genomic region										
	DNA-A	DNA-B	IR [a]		AV1	AC3	AC2	AC1	AC4	BV1	BC1
Abutilon mosaic Brazil virus-[BR:BA]	**87.3**[b]	**66.6**[b]	**84.9**[b]	nt	**89.4**[b]	**90.2**[b]	87.7	**87.6**[b]	**90.3**[b]	**78.5**[b]	75.7
				aa	95.6	**88.6**[b]	**85.3**[b]	**90.4**[b]	**78.6**[b]	**82.8**[b]	**84.6**[b]
Sida yellow leaf curl virus-[BR:Coi3:07]	78.3	n.a[c]	62.6	nt	84.8	88.2	85.9	76.8	77.3	n.a	n.a
				aa	93.6	84.8	80.6	81.7	56.5		
Tomato common mosaic virus-[BR:Coi22:07]	77.8	55.6	61.8	nt	80.3	86.5	**88.1**[b]	77.9	75.7	66.3	70.2
				aa	90.4	87.1	79.7	83.7	54.4	69.1	82.3
Desmodium leaf distortion virus-[MX:Yuc:05]	74.2	54.0	46.7	nt	81.7	79.2	73.8	76.1	80.1	64.1	72.0
				aa	90.4	81.1	67.4	80.9	59.5	68.0	81.2
Tomato leaf distortion virus-[BR:Pda4:05]	73.9	n.a	61.8	nt	87.7	87.7	85.1	63.8	45.5	n.a	n.a
				aa	**96.4**[b]	87.1	80.6	64.0	25.3		
Tomato severe leaf curl virus-GT[MX:Rio1:05]	72.3	n.a	39.9	nt	82.1	75.7	71.8	75.1	78.7	n.a	n.a
				aa	92.8	75.8	58.6	81.0	62.0		
Euphorbia mosaic virus-B[MX:Jal:Pep:05]	72.1	n.a	41.4	nt	79.0	72.4	71.3	77.1	74.6	n.a	n.a
				aa	92.4	73.5	65.1	83.4	58.4		
Sida mottle virus-Rho[BR:Vic1:99]	70.2	n.a	57.4	nt	86.6	88.2	87.9	60.8	49.2	n.a	n.a
				aa	93.6	87.1	83.7	59.3	25.6		
Sida mottle virus - Mic[BR:SP77:08]	70.3	54.5	51.3	nt	83.6	88.0	85.9	64.04	42.0	77.3	75.4
				aa	92.8	84.1	82.9	65.2	23.1	80.1	80.9
Sida mottle virus-Mic[BR:A1B3]	70.5	55.0	51.3	nt	84.8	88.5	86.2	63.9	45.0	73.0	71.3
				aa	93.6	84.1	**85.3**[b]	64.6	20.0	79.3	80.2
Tomato yellow spot virus-[BR:Bic2:99]	70.4	55.7	49.5	nt	83.7	86.7	84.4	61.7	45.0	72.0	72.0
				aa	92.8	84.8	80.6	63.2	25.9	77.3	79.2
Sida mosaic Brazil virus-[BR]	69.7	57.9	55.4	nt	84.5	83.5	79.7	60.1	43.0	67.4	**76.1**[b]
				aa	94.4	83.3	73.6	63.2	17.6	72.7	84.0
Corchorus yellow spot virus-[MX:Yuc:05]	68.8	51.3	50.9	nt	83.1	83.7	75.1	61.0	45.8	62.9	72.6
				aa	92.8	84.8	69.8	62.1	26.4	67.6	82.6
Sida micranta mosaic virus-[BR:MGS:2:2007]	68.7	55.5	50.3	nt	85.2	85.2	83.6	59.8	45.7	72.0	72.2
				aa	94.8	84.8	80.6	61.8	23.5	79.3	82.9
Sida yellow mosaic virus-[BR:Vic2:99]	65.7	n.a	55.1	nt	83.5	85.7	83.8	61.3	43.4	n.a	n.a
				aa	86.9	73.5	71.3	60.1	22.4		
Corchorus golden mosaic virus-[VN:Han:05]	51.8	42.3	22.8	nt	74.7	54.2	51.8	46.0	36.6	47.4	62.2
				aa	86.3	44.6	44.2	45.2	18.0	51.8	71.9
Corchorus yellow vein virus-[VN:Hoa:00]	50.1	42.9	25.3	nt	75.9	53.7	51.5	45.0	41.2	51.3	62.1
				aa	86.9	46.2	47.3	45.5	16.7	54.5	74.0

[a] The intergenic region (IR) sequence is derived from DNA- A

[b] Maximum identities are in bold

[c] n.a: not available

Table 2 Experimental hosts and symptoms displayed by plants inoculated by particle bombardment with CoMoV clones

Host plant	Infectivity[a]	Symptoms[b]
Sida rhombifolia	8/8	CS, VC, Mo, LD
Sida glaziovii	1/8	LCS
Sida cordifolia	0/8	NI
Solanum lycopersicum 'Santa Clara'	4/8	SI
Nicotiana benthamiana	8/8	SI

[a] Number of infected plants /number of inoculated plants

[b] CS, chlorotic spots; LCS, local chlorotic spots; LD, leaf deformation; Mo, mottle; VC, veinal chlorosis; SI, symptomless infection; NI, no infection

confirmed by amplifying and sequencing virus fragments with primers PAL1v1978 and PAR1c496 [38].

A range of plants proved to be experimental hosts of CoMoV (Table 2). All *S. rhombifolia* plants initially showed very small chlorotic spots, which evolved to larger and more diffuse spots by 10 dpi (Fig. 1C). At 22 dpi, extensive vein chlorosis, mottling and leaf deformation could be observed (Fig. 1D). In one of the eight inoculated *S. glaziovii,* similar diffused local chlorotic spots could be seen in inoculated leaves, and the virus could be PCR-amplified from DNA extracted from these lesions (Fig. 1B). However, the infection did not spread, and the virus could not be recovered from new, non-symptomatic leaves. Although none of the *N. benthamiana* or tomato plants showed symptoms, CoMoV fragments could be amplified from systemic non-inoculated leaves from the eight *N. benthamiana* and four tomato plants that were inoculated. *S. cordifolia* plants did not become infected by CoMoV.

Transmission of weed-infecting Brazilian begomoviruses to crop plants such as tomato by grafting and/or biolistics has been reported previously [2, 4, 34]. Although present at high titers in the weed host, usually these viruses are poorly transmitted to tomatoes, and symptoms are milder or absent, as in the case of CoMoV-infected tomato plants. Barreto *et al.* [4] suggest that viruses originating from weeds are less adapted to tomatoes. Nevertheless, viruses such as CoMoV may pose a potential threat to crop plants, since adaptation to the cultivated host may occur by mutation, recombination or pseudo-recombination and emerge as a new tomato disease.

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