

HERMANO MONTEIRO DE BARROS PEREIRA

**PHYLOGENY AND RECOMBINATION OF NEW WORLD
BEGOMOVIRUSES**

Dissertação apresentada à
Universidade Federal de Viçosa,
como parte das exigências do
Programa de Pós-Graduação em
Fitopatologia, para obtenção do
título de *Magister Scientiae*

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Claudine Márcia Carvalho

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Francisco Murilo Zerbini Júnior
(Orientador)

*“O coração do homem planeja seu caminho, mas o Senhor lhe dirige os passos”
Provérbios 16:9.*

*A Deus,
Aos meus pais Eugênia Augusta e Victor Geraldo e a minha irmã Louisiana,*

Dedico.

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BIOGRAFIA

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RESUMO

PEREIRA, Hermano Monteiro de Barros, M.Sc., Universidade Federal de Viçosa, fevereiro de 2017. **Phylogeny and recombination of New World begomoviruses.** Orientador: Francisco Murilo Zerbini Júnior. Coorientadora: Camila Geovana Ferro.

O gênero *Begomovirus* (família *Geminiviridae*) é constituído por vírus que apresentam um ou dois componentes genômicos de DNA circular de fita simples (ssDNA), infectam plantas dicotiledôneas e são transmitidos naturalmente pela mosca-branca *Bemisia tabaci* (Homoptera: Aleyrodidae). Os begomovírus constituem um importante grupo de patógenos de plantas responsáveis por perdas severas em diversas culturas de importância econômica, principalmente em regiões tropicais e subtropicais. Com base em relacionamento filogenético e organização do genoma, os begomovírus podem ser divididos em dois grupos: Velho Mundo (VM) e Novo Mundo (NM). Os begomovírus do NM possuem em sua maioria dois componentes genômicos, denominados DNA-A e DNA-B. Recentemente, a associação de alguns begomovírus do NM com DNAs satélites foi demonstrada. Begomovírus evoluem a taxas comparáveis às de vírus que possuem genoma de RNA. Embora se saiba que a mutação e a recombinação são os principais mecanismos geradores de variabilidade para esses vírus, ainda falta uma maior compreensão das forças evolucionárias que atuam sob as populações virais. Os objetivos deste estudo foram: (i) obter mais informações sobre os mecanismos evolucionários que influenciam a variabilidade genética dos begomovírus no NM; (ii) avaliar, por meio de filogenia, o efeito de recombinação na evolução dos begomovírus do NM; (iii) mensurar, por meio de análise filogeográfica, o efeito da migração na evolução dos begomovírus do NM. Sequências-referência de todos os DNA-A e DNA-B de begomovírus do NM foram obtidas do GenBank para montagem dos conjuntos de dados. Análises de recombinação evidenciaram um relacionamento entre begomovírus e alfassatélites do NM, sugerindo que a recombinação é um importante mecanismo evolucionário afetando a evolução do DNA-A, e em particular do gene *Rep* (presente em ambos os agentes). A retirada de blocos recombinantes mostrou-se desnecessária para a construção de filogenias, uma vez que não alterou o sinal filogenético. Foi encontrado um forte agrupamento entre as espécies baseado nos seus locais de origem, sugerindo que a introdução/migração de genomas oriundos de diferentes áreas contribui grandemente para o aumento dos eventos de recombinação e pseudo-recombinação, o que aumentaria a probabilidade do

surgimento de novas variantes virais mais bem adaptadas que seus parentais. Resultados da filogeografia também indicam que DNA-A e DNA-B possuem histórias evolutivas distintas, já que as análises sugerem ancestrais diferentes. Em conjunto, os resultados indicam um importante papel da recombinação na diversificação dos begomovírus do NM.

ABSTRACT

PEREIRA, Hermano Monteiro de Barros, M.Sc., Universidade Federal de Viçosa, February, 2017. **Phylogeny and recombination of New World begomoviruses.** Advisor: Francisco Murilo Zerbini Júnior. Co-adviser: Camila Geovana Ferro.

The genus *Begomovirus* (family *Geminiviridae*) includes viruses with mono- and bipartite genomes of circular, single-stranded DNA (ssDNA), which infect dicot plants and are transmitted by the whitefly *Bemisia tabaci*. Begomoviruses constitute an important group of plant pathogens responsible for severe losses in several crops of economic importance, mainly in tropical and subtropical regions. Based on phylogenetic relationships and genomic organization, begomoviruses can be divided into New World (NW) and Old World (OW) groups. NW begomoviruses have mostly bipartite genomes, with the two components named DNA-A and DNA-B. Recently, the association of a small number of NW begomoviruses with alphasatellites was demonstrated. Begomoviruses evolve at high rates, compared to viruses with RNA genomes. Although it is well established that mutation and recombination are the main sources of genetic variability for these viruses, a better understanding of the evolutionary forces that act upon begomovirus populations is still lacking. The objectives of this study were: (i) to obtain more detailed information on the evolutionary mechanisms that influence the genetic variability of NW begomoviruses; (ii) to assess, phylogenetically, the effect of recombination on the evolution of NW begomoviruses; (iii) to measure, by phylogeographic analysis, the effect of migration on the diversification of NW begomoviruses. Datasets including all DNA-A and DNA-B reference sequences of NW begomoviruses were obtained from GenBank. Recombination analysis revealed a relationship between NW begomoviruses and alphasatellites, suggesting that recombination is an important evolutionary mechanism affecting DNA-A evolution, particularly of the *Rep* gene (present in both agents). Removal of recombinant blocks from the datasets was shown to be unnecessary, as it did not affect the phylogenetic signal when reconstructing phylogenies. Clusters among species were observed based on their locals of origin, suggesting that the introduction/migration of genomes from different areas greatly contributes to recombination and reassortment, which would increase the probability of the emergence of new variants better adapted than their parental viruses. Phylogeography results suggest that the DNA-A and DNA-B have distinct evolutionary histories, since they have

different common ancestors. Together, the results indicate an important role of recombination in the diversification of NW begomoviruses.

INTRODUÇÃO GERAL

Os vírus classificados na família *Geminiviridae* possuem genoma composto de DNA fita simples (ssDNA) circular, envolto por um único tipo de proteína capsidial que confere à partícula uma morfologia icosaédrica geminada (Zerbini *et al.*, 2017). A família é composta por nove gêneros (*Begomovirus*, *Becurtovirus*, *Capulavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Mastrevirus*, *Topocuvirus* e *Turncurtovirus*), definidos com base no tipo de inseto vetor, gama de hospedeiros, organização genômica e relacionamento filogenético (Varsani *et al.*, 2014; Varsani *et al.*, 2017; Zerbini *et al.*, 2017).

O gênero *Begomovirus* inclui vírus de plantas de grande relevância para a agricultura, devido à severidade das doenças por eles causadas, principalmente em regiões tropicais e subtropicais (Patil e Fauquet, 2009; Rocha *et al.*, 2013; Sattar *et al.*, 2013; Mabvakure *et al.*, 2016; Zaidi *et al.*, 2016). Os vírus classificados nesse gênero possuem um ou dois componentes genômicos, infectam plantas dicotiledôneas e são transmitidos naturalmente pela mosca-branca *Bemisia tabaci* (Homoptera: Aleyrodidae) (Brown *et al.*, 2015). Com base em estudos filogenéticos e características do genoma, os begomovírus podem ser divididos em dois grupos: Velho Mundo (VM; Europa, África e Ásia) e Novo Mundo (NM; as Américas) (Rybicki, 1994; Padidam *et al.*, 1999; Paximadis *et al.*, 1999; Briddon *et al.*, 2010).

A maioria dos begomovírus do VM possui genoma monossegmentados e estão normalmente associados a DNAs satélites (Mansoor *et al.*, 2003). Os begomovírus encontrados no Novo Mundo possuem genoma bissegmentado, com exceção do *Tomato leaf deformation virus* (ToLDeV), encontrado no Peru e no Equador (Melgarejo *et al.*, 2013; Sanchez-Campos *et al.*, 2013). Os componentes genômicos dos begomovírus

bissegmentados são denominados DNA-A (que contém genes envolvidos na replicação, supressão de respostas de defesa da planta e encapsidação do genoma viral) e DNA-B (que contém genes envolvidos no movimento viral intra- e intercelular) (Rojas *et al.*, 2005).

Três tipos de DNAs satélites podem estar associados a begomovírus: alfassatélites (anteriormente denominados DNA-1), betassatélites (anteriormente DNA- β) e deltassatélites (Zhou, 2013; Lozano *et al.*, 2016). Esses agentes são dependentes do begomovírus auxiliar para movimento, encapsidação e transmissão pelo vetor e, no caso dos beta- e deltassatélites, também para a replicação (Zhou, 2013; Fiallo-Olivé *et al.*, 2016). Os alfassatélites ocorrem majoritariamente associados a complexos begomovírus/betassatélites no Velho Mundo (Leke *et al.*, 2015), entretanto, recentemente foram encontrados em associação com begomovírus bissegmentados do Novo Mundo (Brasil, Cuba e Venezuela) (Paprotka *et al.*, 2010; Romay *et al.*, 2010; Jeske *et al.*, 2014).

Os alfa- e betassatélites constituem-se de uma molécula de ssDNA de aproximadamente 1300 nucleotídeos. Ambos possuem organização genômica distinta dos begomovírus, entretanto possuem uma estrutura em forma de grampo, idêntica à dos begomovírus, que é fundamental para a replicação por círculo rolante. O genoma dos betassatélites compartilha apenas a sequência conservada presente na alça do grampo (5'-TAATATTAC-3') com o begomovírus auxiliar. Outras características do genoma desses agentes são uma região altamente conservada entre os betassatélites ("satellite conserved region", SCR), em posição análoga à região comum dos begomovírus; uma região rica em adenina; e um único gene, *betaC1*, codificado no sentido complementar (Bridson *et al.*, 2008). A proteína betaC1 atua como determinante de patogenicidade, supressora de silenciamento gênico transcricional e pós-transcricional (Cui *et al.*, 2004; Yang *et al.*, 2011; Li *et al.*, 2014). No genoma dos alfassatélites é encontrada uma estrutura em forma

de grampo que contém o nanonucleotídeo 5'-TAGTATTAC-3', idêntico ao dos vírus classificados na família *Nanoviridae* (Zhou, 2013). Também é identificado um único gene, *alpha-Rep*, que codifica uma proteína essencial à replicação. Dessa forma, os alphassatélites são capazes de replicar de forma independente do begomovírus auxiliar, embora necessitem do vírus para encapsidação, movimento na planta e transmissão pelo vetor (Zhou, 2013).

A terceira classe de DNAs satélites associados aos begomovírus são os deltassatélites, constituídos por uma molécula de ssDNA com tamanho aproximado de 700 nucleotídeos. O genoma possui uma estrutura em forma de grampo com a sequência conservada 5'-TAATATTAC-3', sequências similares aos iterons de begomovírus, uma região rica em adenina e uma pequena região com alta identidade com a SCR dos betassatélites (Fiallo-Olivé *et al.*, 2012). Essa classe de satélite foi encontrada inicialmente em associação a begomovírus bissegmentados em plantas não-cultivadas em Cuba (Fiallo-Olivé *et al.*, 2012), e posteriormente foram relatados em batata-doce (*Ipomoea batatas*) e *Ipomoea indica* na Espanha e *Merremia dissecta* na Venezuela (Lozano *et al.*, 2016). Os possíveis efeitos da presença dessas moléculas na infecção por begomovírus é desconhecido.

Os geminivírus, incluindo os begomovírus, possuem elevada variabilidade genética, equivalente à dos vírus com genoma de RNA (Ge *et al.*, 2007; Prasanna *et al.*, 2010; Rocha *et al.*, 2013). Isso é devido principalmente aos frequentes eventos de recombinação (Padidam *et al.*, 1999; Lima *et al.*, 2013; Lefeuvre e Moriones, 2015), à pseudo-recombinação entre os vírus de genoma bissegmentado (Andrade *et al.*, 2006), e à alta taxa de mutação (Duffy e Holmes, 2008; Duffy e Holmes, 2009; Lima *et al.*, 2017).

A recombinação ocorre durante o processo de replicação viral e consiste na incorporação de um fragmento da fita de DNA ou RNA na fita de um indivíduo diferente

(Padidam *et al.*, 1999). Esse processo contribui grandemente para a diversidade genética dos begomovírus, aumentando seu potencial evolucionário e adaptação local (revisado por Lefeuvre e Moriones, 2015).

A estratégia de replicação dependente de recombinação (RDR) (Jeske *et al.*, 2001; Preiss e Jeske, 2003), em adição à replicação por círculo rolante (RCR) (Saunders *et al.*, 2001), pode explicar a elevada frequência de eventos de recombinação envolvendo os begomovírus. Além disso, as infecções mistas podem contribuir para uma maior variabilidade genética, principalmente em plantas não-cultivadas que constantemente são encontradas infectadas por vírus que apresentam um relacionamento filogenético distante (Torres-Pacheco *et al.*, 1996; Harrison *et al.*, 1997; Sanz *et al.*, 2000; Pita *et al.*, 2001; Ribeiro *et al.*, 2003; García-Andrés *et al.*, 2006; Davino *et al.*, 2009). Dessa forma, nessas plantas há uma maior probabilidade de recombinação entre espécies pouco relacionadas, o que pode gerar begomovírus com maior grau de adaptabilidade em comparação a seus progenitores (García-Andrés *et al.*, 2006; Alabi *et al.*, 2008; Silva *et al.*, 2012).

Os mecanismos precisos que controlam a recombinação em begomovírus permanecem desconhecidos (Lefeuvre e Moriones, 2015). No entanto, é conhecido que sítios recombinantes não são uniformemente distribuídos ao longo do genoma, com a existência de sítios frequentes ("hot spots") e não-frequentes ("cold spots") de recombinação (Stanley, 1995; Fauquet *et al.*, 2005; García-Andrés *et al.*, 2007; Lefeuvre *et al.*, 2009). Análises bioinformáticas para detectar vírus recombinantes ocorrendo naturalmente revelaram que a origem de replicação viral é um sítio frequente de recombinação (Gutierrez, 1999; Hanley-Bowdoin *et al.*, 1999).

Embora o papel da recombinação na geração de variabilidade de begomovírus esteja claro, uma análise detalhada de 15 conjuntos de dados de sequências de begomovírus, incluindo vírus mono- e bissegmentados no NM e VM, demonstrou que em

todos os casos a mutação é o mecanismo que contribui em maior grau para a variabilidade genética (Lima *et al.*, 2017). Os autores concluíram também que não existe relação entre as frequências relativas desses dois processos e suas contribuições relativas para a variabilidade genética das populações de begomovírus.

A filogeografia pode ser utilizada com a finalidade de avaliar a evolução das espécies. Essa ferramenta avalia como os eventos históricos ajudaram a formar a distribuição geográfica atual dos genes, populações e espécies. Além disso, visa entender como as populações vêm comportando-se ao longo do tempo e inferir padrões de variação em seu tamanho efetivo para interpretá-los sob o ponto de vista evolutivo e biogeográfico. Deter o conhecimento e entender esses mecanismos é de grande importância quando se trata da conservação da diversidade genética das espécies (Avise, 2000; Templeton, 2004; Avise, 2009).

O objetivo deste estudo foi obter mais informações sobre os mecanismos evolucionários que influenciam a variabilidade genética dos begomovírus no NM. Análises de recombinação evidenciaram um relacionamento entre begomovírus e alfassatélites do NM, sugerindo que a recombinação é um importante mecanismo evolucionário afetando a evolução do DNA-A, e em particular do gene *Rep* (presente em ambos os agentes). Foi encontrado um forte agrupamento entre as espécies baseado nos seus locais de origem, sugerindo que a introdução/migração de genomas oriundos de diferentes áreas contribui grandemente para o aumento dos eventos de recombinação e pseudo-recombinação, o que aumentaria a probabilidade do surgimento de novas variantes virais mais bem adaptadas que seus parentais.

Literatura Citada

- Alabi, O.J.; Ogbe, F.O.; Bandyopadhyay, R.; Kumar, P.L.; Dixon, A.G.O.; Hughes, J.D.; Naidu, R.A. Alternate hosts of African cassava mosaic virus and East African cassava mosaic Cameroon virus in Nigeria. **Archives of Virology**, v. 153, p. 1743-1747, 2008.
- Andrade, E.C.; Manhani, G.G.; Alfenas, P.F.; Calegario, R.F.; Fontes, E.P.B.; Zerbini, F.M. *Tomato yellow spot virus*, a tomato-infecting begomovirus from Brazil with a closer relationship to viruses from *Sida* sp., forms pseudorecombinants with begomoviruses from tomato but not from *Sida*. **Journal of General Virology**, v. 87, p. 3687-3696, 2006.
- Avise, J.C. **Phylogeography: the history and formation of species**. Cambridge, MA, USA: Harvard University Press, 2000.
- Avise, J.C. Phylogeography: retrospect and prospect. **Journal of Biogeography**, v. 36, p. 3-15, 2009.
- Briddon, R.W.; Brown, J.K.; Moriones, E.; Stanley, J.; Zerbini, F.M.; Zhou, X.; Fauquet, C.M. Recommendations for the classification and nomenclature of the DNA-beta satellites of begomoviruses. **Archives of Virology**, v. 153, p. 763-781, 2008.
- Briddon, R.W.; Patil, B.L.; Bagewadi, B.; Nawaz-ul-Rehman, M.S.; Fauquet, C.M. Distinct evolutionary histories of the DNA-A and DNA-B components of bipartite begomoviruses. **BMC Evolutionary Biology**, v. 10, p. 97, 2010.
- Brown, J.K.; Zerbini, F.M.; Navas-Castillo, J.; Moriones, E.; Ramos-Sobrinho, R.; Silva, J.C.; Fiallo-Olive, E.; Briddon, R.W.; Hernandez-Zepeda, C.; Idris, A.; Malathi, V.G.; Martin, D.P.; Rivera-Bustamante, R.; Ueda, S.; Varsani, A. Revision of *Begomovirus* taxonomy based on pairwise sequence comparisons. **Archives of Virology**, v. 160, p. 1593-1619, 2015.
- Cui, X.F.; Tao, X.R.; Xie, Y.; Fauquet, C.M.; Zhou, X.P. A DNA beta associated with *Tomato yellow leaf curl China virus* is required for symptom induction. **Journal of Virology**, v. 78, p. 13966-13974, 2004.
- Davino, S.; Napoli, C.; Dellacroce, C.; Miozzi, L.; Noris, E.; Davino, M.; Accotto, G.P. Two new natural begomovirus recombinants associated with the tomato yellow leaf curl disease co-exist with parental viruses in tomato epidemics in Italy. **Virus Research**, v. 143, p. 15-23, 2009.
- Duffy, S.; Holmes, E.C. Phylogenetic evidence for rapid rates of molecular evolution in the single-stranded DNA begomovirus *Tomato yellow leaf curl virus*. **Journal of Virology**, v. 82, p. 957-965, 2008.
- Duffy, S.; Holmes, E.C. Validation of high rates of nucleotide substitution in geminiviruses: phylogenetic evidence from East African cassava mosaic viruses. **Journal of General Virology**, v. 90, p. 1539-1547, 2009.
- Fauquet, C.M.; Sawyer, S.; Idris, A.M.; Brown, J.K. Sequence analysis and classification of apparent recombinant begomoviruses infecting tomato in the Nile and Mediterranean basins. **Phytopathology**, v. 95, p. 549-555, 2005.
- Fiallo-Olivé, E.; Martínez-Zubiaur, Y.; Moriones, E.; Navas-Castillo, J. A novel class of DNA satellites associated with New World begomoviruses. **Virology**, v. 426, p. 1-6, 2012.
- Fiallo-Olivé, E.; Tovar, R.; Navas-Castillo, J. Deciphering the biology of deltasatellites from the New World: maintenance by New World begomoviruses and whitefly transmission. **New Phytologist**, v. 212, p. 680-692, 2016.
- García-Andrés, S.; Monci, F.; Navas-Castillo, J.; Moriones, E. Begomovirus genetic diversity in the native plant reservoir *Solanum nigrum*: evidence for the presence of a new virus species of recombinant nature. **Virology**, v. 350, p. 433-442, 2006.

- García-Andrés, S.; Tomas, D.M.; Sanchez-Campos, S.; Navas-Castillo, J.; Moriones, E. Frequent occurrence of recombinants in mixed infections of tomato yellow leaf curl disease-associated begomoviruses. **Virology**, v. 365, p. 210-219, 2007.
- Ge, L.M.; Zhang, J.T.; Zhou, X.P.; Li, H.Y. Genetic structure and population variability of tomato yellow leaf curl China virus. **Journal of Virology**, v. 81, p. 5902-5907, 2007.
- Gutierrez, C. Geminivirus DNA replication. **Cellular and Molecular Life Sciences**, v. 56, p. 313-329, 1999.
- Hanley-Bowdoin, L.; Settlage, S.B.; Orozco, B.M.; Nagar, S.; Robertson, D. Geminiviruses: models for plant DNA replication, transcription, and cell cycle regulation. **Critical Reviews in Plant Sciences**, v. 18, p. 71-106, 1999.
- Harrison, B.D.; Zhou, X.; Otim Nape, G.W.; Liu, Y.; Robinson, D.J. Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. **Annals of Applied Biology**, v. 131, p. 437-448, 1997.
- Jeske, H.; Kober, S.; Schäfer, B.; Strohmeier, S. Circumstances of Cuban geminiviruses reveals the first alphasatellite DNA in the Caribbean. **Virus Genes**, v. 49, p. 312-324, 2014.
- Jeske, H.; Lutgemeier, M.; Preiss, W. DNA forms indicate rolling circle and recombination-dependent replication of *Abutilon mosaic virus*. **EMBO Journal**, v. 20, p. 6158-6167, 2001.
- Lefevre, P.; Lett, J.M.; Varsani, A.; Martin, D.P. Widely conserved recombination patterns among single-stranded DNA viruses. **Journal of Virology**, v. 83, p. 2697-2707, 2009.
- Lefevre, P.; Moriones, E. Recombination as a motor of host switches and virus emergence: geminiviruses as case studies. **Current Opinion in Virology**, v. 10, p. 14-19, 2015.
- Leke, W.N.; Mignouna, D.B.; Brown, J.K.; Kvarnheden, A. Begomovirus disease complex: emerging threat to vegetable production systems of West and Central Africa. **Agriculture & Food Security**, v. 4, p. 1, 2015.
- Li, F.; Huang, C.; Li, Z.; Zhou, X. Suppression of RNA silencing by a plant DNA virus satellite requires a host calmodulin-like protein to repress *RDR6* expression. **PLoS Pathogens**, v. 10, p. e1003921, 2014.
- Lima, A.T.M.; Silva, J.C.F.; Silva, F.N.; Castillo-Urquiza, G.P.; Silva, F.F.; Seah, Y.M.; Mizubuti, E.S.G.; Duffy, S.; Zerbini, F.M. The diversification of begomovirus populations is predominantly driven by mutational dynamics. **Virus Evolution**, v. 3, p. vex005, 2017.
- Lima, A.T.M.; Sobrinho, R.R.; Gonzalez-Aguilera, J.; Rocha, C.S.; Silva, S.J.C.; Xavier, C.A.D.; Silva, F.N.; Duffy, S.; Zerbini, F.M. Synonymous site variation due to recombination explains higher genetic variability in begomovirus populations infecting non-cultivated hosts. **Journal of General Virology**, v. 94, p. 418-431, 2013.
- Lozano, G.; Trenado, H.P.; Fiallo-Olive, E.; Chirinos, D.; Geraud-Pouey, F.; Briddon, R.W.; Navas-Castillo, J. Characterization of non-coding DNA satellites associated with sweepoviruses (genus *Begomovirus*, *Geminiviridae*): definition of a distinct class of begomovirus-associated satellites. **Frontiers in Microbiology**, v. 7, p. 162, 2016.
- Mabvakure, B.; Martin, D.P.; Kraberger, S.; Cloete, L.; van Brunschot, S.; Geering, A.D.; Thomas, J.E.; Bananej, K.; Lett, J.M.; Lefevre, P.; Varsani, A.; Harkins, G.W. Ongoing geographical spread of *Tomato yellow leaf curl virus*. **Virology**, v. 498, p. 257-264, 2016.
- Mansoor, S.; Briddon, R.W.; Zafar, Y.; Stanley, J. Geminivirus disease complexes: an emerging threat. **Trends in Plant Science**, v. 8, p. 128-134, 2003.
- Melgarejo, T.A.; Kon, T.; Rojas, M.R.; Paz-Carrasco, L.; Zerbini, F.M.; Gilbertson, R.L. Characterization of a new world monopartite begomovirus causing leaf curl disease of tomato in Ecuador and Peru reveals a new direction in geminivirus evolution. **Journal of Virology**, v. 87, p. 5397-5413, 2013.

- Padidam, M.; Sawyer, S.; Fauquet, C.M. Possible emergence of new geminiviruses by frequent recombination. **Virology**, v. 265, p. 218-224, 1999.
- Paprotka, T.; Metzler, V.; Jeske, H. The first DNA 1-like alphasatellites in association with New World begomoviruses in natural infections. **Virology**, v. 404, p. 148-157, 2010.
- Patil, B.L.; Fauquet, C.M. Cassava mosaic geminiviruses: actual knowledge and perspectives. **Molecular Plant Pathology**, v. 10, p. 685-701, 2009.
- Paximadis, M.; Idris, A.M.; Torres-Jerez, I.; Villarreal, A.; Rey, M.E.C.; Brown, J.K. Characterization of tobacco geminiviruses in the Old and New world. **Archives of Virology**, v. 144, p. 703-717, 1999.
- Pita, J.S.; Fondong, V.N.; Sangare, A.; Otim-Nape, G.W.; Ogwal, S.; Fauquet, C.M. Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. **Journal of General Virology**, v. 82, p. 655-665, 2001.
- Prasanna, H.C.; Sinha, D.P.; Verma, A.; Singh, M.; Singh, B.; Rai, M.; Martin, D.P. The population genomics of begomoviruses: global scale population structure and gene flow. **Virology Journal**, v. 7, p. 220, 2010.
- Preiss, W.; Jeske, H. Multitasking in replication is common among geminiviruses. **Journal of Virology**, v. 77, p. 2972-2980, 2003.
- Ribeiro, S.G.; Ambrozecius, L.P.; Ávila, A.C.; Bezerra, I.C.; Calegario, R.F.; Fernandes, J.J.; Lima, M.F.; Mello, R.N.; Rocha, H.; Zerbini, F.M. Distribution and genetic diversity of tomato-infecting begomoviruses in Brazil. **Archives of Virology**, v. 148, p. 281-295, 2003.
- Rocha, C.S.; Castillo-Urquiza, G.P.; Lima, A.T.M.; Silva, F.N.; Xavier, C.A.D.; Hora-Junior, B.T.; Beserra-Junior, J.E.A.; Malta, A.W.O.; Martin, D.P.; Varsani, A.; Alfenas-Zerbini, P.; Mizubuti, E.S.G.; Zerbini, F.M. Brazilian begomovirus populations are highly recombinant, rapidly evolving, and segregated based on geographical location. **Journal of Virology**, v. 87, p. 5784-5799, 2013.
- Rojas, M.R.; Hagen, C.; Lucas, W.J.; Gilbertson, R.L. Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. **Annual Review of Phytopathology**, v. 43, p. 361-394, 2005.
- Romay, G.; Chirinos, D.; Geraud-Pouey, F.; Desbiez, C. Association of an atypical alphasatellite with a bipartite New World begomovirus. **Archives of Virology**, v. 155, p. 1843-1847, 2010.
- Rybicki, E.P. A phylogenetic and evolutionary justification for three genera of *Geminiviridae*. **Archives of Virology**, v. 139, p. 49-77, 1994.
- Sanchez-Campos, S.; Martinez-Ayala, A.; Marquez-Martin, B.; Aragon-Caballero, L.; Navas-Castillo, J.; Moriones, E. Fulfilling Koch's postulates confirms the monopartite nature of tomato leaf deformation virus: a begomovirus native to the New World. **Virus Research**, v. 173, p. 286-293, 2013.
- Sanz, A.I.; Fraile, A.; García-Arenal, F.; Zhou, X.; Robinson, D.J.; Khalid, S.; Butt, T.; Harrison, B.D. Multiple infection, recombination and genome relationships among begomovirus isolates found in cotton and other plants in Pakistan. **Journal of General Virology**, v. 81, p. 1839-1849, 2000.
- Sattar, M.N.; Kvarneden, A.; Saeed, M.; Briddon, R.W. Cotton leaf curl disease - an emerging threat to cotton production worldwide. **Journal of General Virology**, v. 94, p. 695-710, 2013.
- Saunders, K.; Bedford, I.D.; Stanley, J. Pathogenicity of a natural recombinant associated with ageratum yellow vein disease: implications for geminivirus evolution and disease aetiology. **Virology**, v. 282, p. 38-47, 2001.

- Silva, S.J.C.; Castillo-Urquiza, G.P.; Hora-Junior, B.T.; Assunção, I.P.; Lima, G.S.A.; Pio-Ribeiro, G.; Mizubuti, E.S.G.; Zerbini, F.M. Species diversity, phylogeny and genetic variability of begomovirus populations infecting leguminous weeds in northeastern Brazil. **Plant Pathology**, v. 61, p. 457-467, 2012.
- Stanley, J. Analysis of African cassava mosaic virus recombinants suggest strand nicking occurs within the conserved nonanucleotide motif during the initiation of rolling circle DNA replication. **Virology**, v. 206, p. 707-712, 1995.
- Templeton, A.R. Statistical phylogeography: methods of evaluating and minimizing inference errors. **Molecular Ecology**, v. 13, p. 789-809, 2004.
- Torres-Pacheco, I.; Garzón-Tiznado, J.A.; Brown, J.K.; Becerra-Flora, A.; Rivera-Bustamante, R. Detection and distribution of geminiviruses in Mexico and the Southern United States. **Phytopathology**, v. 86, p. 1186-1192, 1996.
- Varsani, A.; Navas-Castillo, J.; Moriones, E.; Hernández-Zepeda, C.; Idris, A.; Brown, J.K.; Zerbini, F.M.; Martin, D.P. Establishment of three new genera in the family *Geminiviridae*: *Becurtovirus*, *Eragrovirus* and *Turncurtovirus*. **Archives of Virology**, v. 159, p. 2193-2203, 2014.
- Varsani, A.; Roumagnac, P.; Fuchs, M.; Navas-Castillo, J.; Moriones, E.; Idris, A.; Briddon, R.W.; Rivera-Bustamante, R.F.; Zerbini, F.M.; Martin, D.P. *Capulavirus* and *Grablovirus*: two new genera in the family *Geminiviridae*. **Archives of Virology**, v. 162, p. 1819-1831, 2017.
- Yang, X.L.; Xie, Y.; Raja, P.; Li, S.Z.; Wolf, J.N.; Shen, Q.T.; Bisaro, D.M.; Zhou, X.P. Suppression of methylation-mediated transcriptional gene silencing by betaC1-SAHH protein interaction during geminivirus-betasatellite infection. **PLoS Pathogens**, v. 7, p. e1002329, 2011.
- Zaidi, S.S.; Martin, D.P.; Amin, I.; Farooq, M.; Mansoor, S. Tomato leaf curl New Delhi virus: a widespread bipartite begomovirus in the territory of monopartite begomoviruses. **Molecular Plant Pathology**, p. doi: 10.1111/mpp.12481., 2016.
- Zerbini, F.M.; Briddon, R.W.; Idris, A.; Martin, D.P.; Moriones, E.; Navas-Castillo, J.; Rivera-Bustamante, R.; Varsani, A.; ICTV Consortium. ICTV Virus Taxonomy Profile: *Geminiviridae*. **Journal of General Virology**, v. 98, p. 131-133, 2017.
- Zhou, X. Advances in understanding begomovirus satellites. **Annual Review of Phytopathology**, v. 51, p. 357-381, 2013.

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PHYLOGENY AND RECOMBINATION OF NEW WORLD BEGOMOVIRUSES

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Summary

Begomoviruses are whitefly-transmitted, single-stranded DNA plant viruses that constitute one of the largest and most important groups of emerging plant viruses, growing in importance as the number of species characterized and their economic impact continues to increase. Most New World (NW) begomoviruses have two genomic components designated as DNA-A and DNA-B, and their genomes have the capacity to evolve quickly via mutation, reassortment and recombination. Datasets including all DNA-A and DNA-B reference sequences of NW begomoviruses were obtained from GenBank and analyzed to understand begomovirus evolution in the NW. Recombination was confirmed as a very important evolutionary mechanism acting on DNA-A evolution, particularly upon the *Rep* gene. The analysis revealed a relationship between NW begomoviruses and alphasatellites. Removal of recombinant blocks from the datasets was shown to be unnecessary, as it did not affect the phylogenetic signal when reconstructing phylogenies. Clustering of species was found based on their local of origin, suggesting that the introduction/migration of genomes from different areas greatly contributes to the increase of recombination and reassortment events, which would increase the probability of the emergence of new variants better adapted than their parents. Phylogeography results suggest that the DNA-A and DNA-B have distinct evolutionary histories, since they have different common ancestors.

Introduction

The family *Geminiviridae* includes plant viruses with a genome comprised of one or two molecules of circular, single-stranded DNA (ssDNA) encapsidated by a single structural protein in twinned quasi-icosahedral particles [1]. Members of this family infect a broad range of plants species causing devastating diseases, mainly in subtropical and tropical countries [2-6]. The family is divided into nine genera (*Begomovirus*, *Becurtovirus*, *Capulavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Mastrevirus*, *Topocuvirus* and *Turncurtovirus*) based on the type of insect vector, host range, genome organization and phylogenetic relationships [1, 7].

Begomoviruses infect dicot plants and are transmitted by whiteflies of the *Bemisia tabaci* cryptic species complex (Hemiptera: Aleyrodidae). These viruses constitute the largest and one of the most important groups of plant viruses, growing in importance as the number of species characterized and their economic impact continues to increase [8, 9]. Members of this genus are further subdivided into two groups: Old World (OW; Europe, Africa, Asia and Oceania) and New World (NW; the Americas) based on genome features, phylogeny and geographical distribution [10-15].

Begomoviruses in the New World are mostly bipartite (DNA-A and DNA-B), except for *Tomato leaf deformation virus* (ToLDeV), an indigenous NW monopartite virus [16]. The DNA-A contains five open reading frames (ORFs) which encode proteins with functions in viral replication, suppression of host defenses and particle formation, while the two ORFs in the DNA-B encode proteins which are involved in intra- and intercellular movement in the plant, host range determination and suppression of host defenses [14, 17-19].

Commonly, two classes of circular single-stranded DNA molecules have been described in association with geminiviruses, the alphasatellites and the betasatellites [20]. Recently, a third class of DNA satellites, the deltasatellites, has been reported in association with begomoviruses [21].

Betasatellites have so far only been identified in the Old World, associated with mono- and bipartite begomoviruses [22]. Alphasatellites have also been found in association with NW bipartite begomoviruses, in Brazil, Cuba and Venezuela [23-26]. Both are ssDNA molecules of approximately half the size of begomovirus DNAs and code a single gene, β C1 and *alpha-Rep* respectively [20, 27]. These molecules are dependent on a helper virus for movement, encapsidation and transmission by the insect vector, and in the case of betasatellites also for replication [28, 29]. The deltasatellites have 700 nucleotides, do not encode proteins, and their effects in infections by begomoviruses are unknown [21].

Begomovirus genomes are capable of evolving rapidly through both mutation and recombination, having levels of genetic variability resembling those of viruses with RNA genomes. Recombination greatly contributes to the genetic diversity of begomoviruses, increasing their evolutionary potential and local adaptation (revised by Lefeuvre and Moriones [30]). Pseudo-recombination (or reassortment) is another source of genetic variability, which may occur by the exchange and acquisition of genomic components between bipartite viruses and satellites [10, 14, 31-33]. However, and despite the significant contribution of recombination and pseudo-recombination in the generation of genetic variability in begomoviruses, their diversification is driven primarily by mutational dynamics [34].

Mixed infections by begomoviruses are common in non-cultured hosts, and these hosts can contribute for greater genetic variability since they are often infected by viruses

that present a distant phylogenetic relationship [26, 35-44]. Therefore, in these plants there is a greater probability of recombination between distantly related begomoviruses, which can generate new viruses with a greater degree of adaptability in comparison to its progenitors [37, 38].

Frequent recombination events, pseudo-recombination in bipartite viruses, and high mutation rates all contribute significantly to the emergence of new begomoviruses, increasing their potential of adaptation to different hosts and environmental conditions [33]. The expanded geographical range of *B. tabaci* populations [specially the Mediterranean (MED) and Middle East-Asia Minor 1 (MEAM1) species, which are more invasive and polyphagous] has facilitated the dissemination of begomoviruses beyond their normal geographical ranges [5], as well as the horizontal transfer of indigenous begomoviruses into crop plants [2, 45].

The recent description of a number of highly divergent gemini-like viruses in several countries, including Brazil [46-49], the detection of alphasatellites and the discovery of a new group of DNA satellites in association with NW begomoviruses [21, 23-26], all indicate that we are only beginning to recognize the full extent of the diversity of this group of pathogens. As much as we understand the evolutionary forces that act upon begomovirus populations, it is still not clear how these forces interact to drive the evolution of these viruses and of their associated DNA satellites.

The objective of this study was to obtain more detailed information about the evolutionary mechanisms that influence the genetic diversity of begomoviruses in the New World. Network analysis evidenced the relationship between NW begomoviruses and alphasatellites, suggesting that recombination directly affects the evolution of the DNA-A, particularly the *Rep* gene. We show that begomoviruses cluster phylogenetically based on their respective places of origin, suggesting that the introduction/migration of

viruses from different areas greatly contributes to the increase of recombination and pseudo-recombination, which could increase the probability of the appearance of new viral variants better adapted than their progenitors.

Methods

Begomovirus datasets

All reference sequences of DNA-A and DNA-B of NW begomoviruses were obtained from GenBank (www.ncbi.nlm.nih.gov/refseq/). The dataset also included sequences of some OW bipartite begomoviruses which are more similar to NW than OW begomoviruses [50, 51]. To confirm assigned taxonomy, the sequences were analyzed using Species Demarcation Tool v. 1.2 [52].

Sequences were classified according to their sampling sites, using natural geographic features to divide the NW into five regions: Eastern South America (ESA; Argentina, Bolivia, Brazil, Paraguay and Uruguay), Western South America (WSA; Chile, Colombia, Ecuador and Peru), Central America (CA; Costa Rica, Guatemala, Honduras, Nicaragua and Panama), North America (NA; Mexico and the United States) and the Caribbean (CAR; Cuba, Jamaica, Puerto Rico and Venezuela).

Multiple sequence alignments were prepared for the full-length DNA-A and DNA-B and for the *CP*, *Rep*, *MP* and *NSP* genes of each viral species using the MUSCLE algorithm [53] implemented in MEGA6 [54].

Network and recombination analyses

Evidence of non-tree-like evolution was assessed for DNA-A, DNA-B, *CP*, *Rep*, *NSP* and *MP* datasets using the Neighbor-Net method [55] implemented in SplitsTree v. 4.10 [56]. Network images were edited using CorelDraw.

Recombination analysis was performed using the Rdp, Geneconv, Bootscan, Maximum Chi Square, Chimaera, SisterScan and 3Seq methods implemented in Recombination Detection Program (RDP) v. 4.77 [57]. Alignments were scanned with default settings for the different methods. Statistical significance was inferred by *p*-values lower than a Bonferroni-corrected cut-off of 0.05. Only recombination events detected by at least three different methods were considered to be reliable. To avoid the interference of recombination in the phylogenetic analysis, recombinant blocks in multiple sequence alignments were replaced by question marks (lost data) and later applied to the phylogeny in these data.

Phylogenetics analysis

Phylogenetic trees for the full-length DNA-A were inferred using the maximum likelihood (ML) method with RAxML v. 7.0.3 [58]. Phylogenetic trees for the full-length DNA-B and for the *CP*, *Rep*, *MP* and *NSP* genes were estimated using Bayesian inference with the program MrBayes v. 3.0b4 [59]. The models were generated with MrModeltest v. 2.2 [60] on the Akaike Information Criterion (AIC). Phylogenetic trees were visualized with the program FigTree (tree.bio.ed.ac.uk/software/figtree) and edited using CorelDraw.

Phylogeographic analysis

Spatial dispersion of NW begomoviruses was estimated separately for the DNA-A and DNA-B by Bayesian inference with the program BEAST v. 2.4.4 [61]. The geographic matrix was constructed based on the sampling locations of each species. The dataset was analyzed under demographic models of constant size, exponential growth and Bayesian Skyline. Strict and relaxed molecular clocks were used for all the models applied, assuming reversible substitutions. To analyze the effective size of the sample, the parameters used were analyzed using TRACER v. 1.4 (beast.bio.ed.ac.uk/Tracer), to verify if they converged. The MCMC tree was summarized using TreeAnnotator v. 2.4.4 (beast.bio.ed.ac.uk/TreeAnnotator), visualized with FigTree and edited with CorelDraw.

Results

Begomovirus diversity in the New World

Datasets containing a total of 125 and 97 full-length DNA-A and DNA-B sequences, respectively, were assembled, in which each sequence represents a NW begomovirus species (Suppl. Table S1). Although most begomoviruses naturally occurring in the NW have bipartite genomes, only the DNA-A is used to assign taxonomy [62]. Therefore, bipartite begomoviruses for which only the DNA-A sequence is available were included, leading to the higher number of DNA-A sequences retrieved. Using pairwise comparisons of the DNA-A sequences and the $\geq 91\%$ nucleotide identity criterion established by *Geminiviridae* Study Group of the ICTV [8], we confirmed that all 125 DNA-A sequences corresponded to different species, with nucleotide identity percentages ranging from 65% to 90%. Percent identities for the full-length DNA-B sequences ranged from 65% to 98% (data not shown).

Non-tree-like evolution

Network analysis based on the DNA-A (Fig. 1a) revealed four main clusters (I, II, III and IV), with the remaining viruses constituting an undefined group. The clusters are mostly associated with geography: clusters I and II include viruses from CA/NA/CAR (plus two NW-like OW viruses, CoGMV and CoYVV), while cluster III is comprised entirely of viruses from ESA (*Oxalis yellow vein virus*, although reported in the United States, was found in ornamental plants originating from Brazil). The exception is cluster IV, which contains viruses from all regions.

A complex recombination pattern was observed, based on several ramifications in the network, with strong evidence of a recombinant origin for the viruses in cluster IV (Fig. 1a). Interestingly, we also found evidence of a recombinant origin for viruses in cluster II (Fig. 1a), which contains the two NW-like OW viruses. Cluster IV displays a higher degree of divergence compared to the other clusters, suggesting a strong recombination event that diverts the viruses in this cluster from the remaining ones in the dataset.

Networks based on the *CP* and *Rep* genes (Figs. 1b and 1c, respectively) are deeply incongruent, visualized across different patterns. The *Rep* network shows a similar pattern to the DNA-A, suggesting that this clustering is strongly influenced by recombination occurring mostly in the *Rep* gene. In the *Rep* network (Fig. 1c), the evidence of recombination in cluster II is stronger than in the network based on the DNA-A (Fig. 1a).

Due to the complex recombination pattern presented by cluster IV (Figs. 1a and 1c) and the fact that some of these viruses are associated with alphasatellites (EuYMV and MCIMV), a new dataset was generated which included three sequences of NW

alphasatellites. Analyzing the network, it is possible to recognize a distinct pattern of recombination between alphasatellites and divergent viruses (Fig. 1d), indicating that viral and alphasatellite *Rep* sequences share a common recombination event.

Network analysis based on the DNA-B indicated a weak recombination signal. The network revealed three main clusters (I, II and III), with support for cluster I (Fig. 1e). Cluster I was also observed in the DNA-A network (Fig. 1a). Networks based on the *NSP* and *MP* genes were highly congruent (Figs. 1f and 1g) and exhibited the same patterns visualized in the DNA-B network, with the same three clusters.

Recombination analysis

RDP results for the DNA-A dataset confirmed the complex pattern of recombination indicated by network analysis (100 events - *data not shown*), emphasizing the high divergence present in cluster IV. Fifteen recombination events were detected involving viruses in cluster IV and with breakpoints occurring mostly in the *Rep* gene (Table 1), confirming the involvement of this gene in the origin of these divergent viruses.

Because of the high divergence involving the 20 viruses in cluster IV, we prepared a dataset without their sequences. RDP results of this dataset (Suppl. Table S2) showed 70 recombination events involving sequences from clusters I, II and III. Viruses in cluster I share a recombination event with breakpoints located in the *CP* and *Rep* genes (events 5 and 50; Suppl. Table S2). Interestingly, all viruses belonging to cluster II share at least one recombination event with breakpoints in the *Rep* gene (events 13, 14, 20, 33, and 48; Suppl. Table S2). For the full-length DNA-B, 39 unique recombination events were observed, with breakpoints occurring in the *NSP* and *MP* genes (Suppl. Table S3).

Phylogenetics analysis

A maximum likelihood phylogenetic tree based on the DNA-A (without the 20 divergent viruses) shows the same clusters found in network analysis (Fig. 2a; clusters I, II, III). Cluster II indicates a strong phylogenetic relationship between NW viruses and the two NW-like viruses from Vietnam (CoGMV and CoYVV). Cluster III includes only viruses from ESA (as noted above, OxYVV is actually a ESA virus). On the contrary, cluster I contains viruses from all geographic regions except ESA.

Although the ML phylogenetic tree (Fig. 2a) exhibits the same topology observed in the network tree (Fig. 1a), the ML tree shows a very low bootstrap support for several branches, in particular the most basal ones. This is probably due to the several recombination events involving the DNA-A. Regardless, it complicates the interpretation of this tree.

To investigate the effects of recombination events in the DNA-A phylogeny, a ML phylogenetic tree based on an alignment without recombinants blocks was built (Fig. 2b). The recombination-free tree displays the same three clusters (I, II and III) observed in the original DNA-A tree (compare Figs. 2a and 2b), indicating that the absence of recombinant blocks in the alignment did not restore the phylogenetic signal. Bootstrap support was again low for several branches, indicating the unfeasibility of using the complete DNA-A for the construction of reliable phylogenetic trees of NW begomoviruses.

Using datasets comprised of *CP* and *Rep* gene nucleotide sequences, both without the 20 divergent sequences, phylogenetic trees were built using Bayesian inference. The *CP* and *Rep* phylogenetic trees (Figs. 2c and 2d, respectively) are deeply incongruent.

The *Rep* tree is similar to the DNA-A tree (compare Figs. 2a and 2d) and displays two well-supported clades (clusters I and II) that are not resolved in the *CP* tree.

A Bayesian tree based on DNA-B sequences (Fig. 3a) displays three main clusters (I, II, III). The clustering observed in the phylogenetic tree is very similar to that found in the network analysis (compare Figs. 1e and 3a). Differently from the DNA-A tree, all clades were robustly supported in the DNA-B tree, indicating a high reliability of the analysis. Interestingly, the *NSP* and *MP* Bayesian trees (Figs. 3b and 3c, respectively) are not incongruent and both are very similar to the DNA-B tree. All clades observed for the DNA-B tree were also present in the *NSP* tree, but with lower posterior probability values. Clades are very well supported in the *MP* tree.

Recombinants blocks detected by RDP analysis (Suppl. Table S3) were removed from the DNA-B alignment and a recombination-free phylogenetic tree was built by Bayesian inference (Fig. 3d). The same clusters (I, II, III) were found (compare Figs. 3a and 3d). In cluster III it is possible to observe a small difference involving SiMBoV1 and TRYLCV. These two sequences cluster together in the recombination-free phylogenetic tree (Fig. 3d), which is not observed in the original tree (Fig. 3a).

Phylogeography

Using Bayesian Skyline demographic models, phylogeographic relationships were constructed based on DNA-A (excluding the 20 divergent viruses) and DNA-B sequences. In the DNA-A tree (Fig. 4a), the same clusters observed in the ML phylogenetic tree are present (clusters I, II and III; compare Figs. 2a and 4a). Cluster I includes sequences from CA/NA/CAR, plus BDMV from WSA. This suggests migration among the different biogeographical regions, thus allowing a greater probability of

recombination. The analysis also suggests that ESA viruses originated from NA/CAR viruses.

Phylogeographic analysis was also performed based on a recombination-free DNA-A alignment (Fig. 4b). The same clusters (I, II, and III) were found, with the same ancestor relationships (viruses from ESA originating from NA/CAR). However, the SA viruses were split into two clades (Fig. 4b; clusters IIIa and IIIb).

For the DNA-B, the trees based on the complete and recombination-free alignments (Figs. 4c and 4d, respectively) exhibited the same three clusters observed in the Bayesian phylogenetic tree (Fig. 3a). This great similarity can be explained based on the lower occurrence of recombination in the DNA-B dataset compared with the DNA-A dataset. However, the common ancestor for the DNA-B was mapped to South America, differently from what was found for the DNA-A (compare Figs. 4a and 4c) and suggesting that the DNA-A and DNA-B have distinct evolutionary histories.

Discussion

Differently from most plant viruses, which group together based on host range, begomovirus populations segregate strongly based on geographical origin [2]. NW and OW begomoviruses form clearly distinct phylogenetic clades [63], and within these two major groups, viruses from the same geographical region are closer to each other, irrespective of their hosts, than to viruses infecting the same hosts in different regions (for example, begomoviruses from South America that infect solanaceous hosts are closer to other begomoviruses from SA that infect leguminous or malvaceous hosts than to begomoviruses that infect solanaceous hosts in the Indian subcontinent). Besides mutation and selection, recombination is an important generation of variability acting on the macroevolution of begomovirus populations [34, 64-66]. Here, we decided to assess

the effect of recombination in NW begomovirus evolution in greater detail. Our results confirm that recombination is a very important mechanism affecting DNA-A evolution. More interestingly, we detected recombination in the *Rep* genes of begomoviruses and alphasatellites, suggesting that these two agents may share a common evolutionary history in the NW. Evidence of migration among the different biogeographical regions was also detected.

Pairwise comparisons indicated that the DNA-B displays a higher range of nucleotide identities. Molecular variability in the DNA-A has been associated with recombination events occurring mostly in the *Rep* gene [67, 68]. We observed also that this region is highly recombinant, and a much lower number of recombination events were observed in the DNA-B dataset. Therefore, the greater molecular variability of DNA-B components observed here cannot be explained by recombination.

Network analysis based on the DNA-A evidenced several recombination events, and identified a group of 20 divergent viruses most likely due to frequent recombination (cluster IV in Fig. 1a).

Network analysis of the DNA-B indicated that recombination is not responsible for the greater variability exhibited by this component. The higher variability of the DNA-B may result from a combination of factors, including the absence of overlapping genes and the smaller number of *cis*- and *trans*-interactions between its protein products. Thus, the DNA-B would be more permissive to variation, evolving mostly in response to the host. An alternative explanation is that the DNA-B could have a distinct origin from the DNA-A, initially arising as a satellite that was captured by a monopartite progenitor and later evolved to become an integral part of the genome [10, 69].

Network analysis using a dataset comprised of viral and alphasatellite *Rep* sequences indicated an intrinsic relationship between each other. The same group of 20

divergent begomoviruses (cluster IV) identified in the network analysis is also involved in the recombination with alphasatellites. Interestingly, this group includes two of the three viruses that have been recently associated with these subviral agents: *Euphorbia yellow mosaic virus* (EuYMV) and *Melon chlorotic mosaic virus* (MCIMV). Previous studies have indicated that these viruses comprise a distinct phylogenetic lineage of NW begomoviruses, which remarkably is not associated with geographical location [2, 70]. While the group is comprised mostly of begomovirus found in CA and NA, it includes EuYMV and a few other viruses from ESA. The network analysis suggests a possible involvement of *alpha-Rep* in promoting speciation of this divergent group of NW begomoviruses. Interestingly, *alpha-Rep* is believed to have originated from the DNA-R component of nanoviruses (family *Nanoviridae*) [20], a group of viruses that has never been detected in the NW.

Phylogenetic trees exhibited a pattern similar to the clustering of network analysis, almost entirely coincidental with the places of origin of each virus. A relationship among CA and NA viruses is evident in cluster I. Also, the clade comprised of ESA viruses (cluster III) show a specific subclustering within this large region. Rocha *et al.* [2] reported equivalent results, showing evidence of a geographical segregation of Brazilian begomoviruses. However, the DNA-A phylogenetic tree displayed very low bootstrap scores, indicating insufficient phylogenetic signal in the dataset. This was observed even when a recombination-free dataset was analyzed (and even when the 20 divergent sequences from cluster IV were removed from the dataset), suggesting that the full-length DNA-A is inappropriate for phylogenetic reconstruction of NW begomoviruses. This could be due to the high genetic variability of these begomoviruses, which prevents a proper multiple sequence alignment.

The *Rep* and *CP* phylogenetic trees are incongruent, but their clades are very well supported. This suggests that these two genes may have different evolutionary histories, which could explain the low statistical support of the phylogenetic analysis based on the complete DNA-A. Interestingly, phylogenetic relationships inferred from *Rep*, but not from *CP*, reflect the phylogeny based on the complete DNA-A.

The *CP* gene is the most conserved among begomoviruses [64, 71]. This gene has been used for provisional classification of begomovirus species, and it is the only viral gene involved in insect vector transmission and in particle formation [72, 73]. These features could impose severe structural constraints on the CP protein, and in fact several studies indicate that begomovirus CPs are under strong negative selection [67, 71, 74]. Thus, the CP may be a poor surrogate for the detection of evolutionary relationships among begomoviruses.

The *Rep* gene, on the other hand, displays considerably greater genetic variability and is a recombination hotspot [64, 65]. The N-terminal region of the Rep protein includes conserved motifs essential for rolling-circle replication [75-77]. Conservation of the integrity of these elements is critical for successful infection cycles, despite the variation introduced by frequent recombination. Indeed, studies have provided evidence that the high nucleotide variability in the 5'-terminal portion of the *Rep* gene is accompanied by strong purifying selection that preserves the amino acid sequence [2, 67]. In our results, several of the recombination events detected did not involve the *Rep* 5'-terminal region, indicating the conservation of these elements. Nevertheless, the variability inserted by recombination in *Rep* may contribute to the diversification of the DNA-A.

Interesting results were obtained for the DNA-B datasets. Phylogenetic trees based on the full-length DNA-B and on *NSP* and *MP* sequences exhibited similar clustering, even though the DNA-B is more variable than the DNA-A [2]. Moreover, the

recombination-free phylogenetic tree of the DNA-B exhibited the same clusters found in the original tree. Although this could be due to the low number of recombination events detected for this component, a more likely explanation is that the presence of recombinant blocks in the alignment does not affect the phylogenetic signal. Indeed, all clades were very well-supported with high bootstrap scores in both phylogenetic trees (with and without recombinant blocks).

Previous studies have suggested that the DNA-B can tolerate a greater number of mutations since it does not contain overlapping genes, and that the distinct origin of the DNA-B, possibly from a satellite molecule captured by a monopartite begomovirus, may contribute to an increase in variability [10, 78]. In spite of its greater variability (or maybe because of it), the great similarity among the trees based on the two coding regions of the DNA-B and the tree based on the entire component confers greater reliability for using a DNA-B dataset to reconstruct the phylogeny of NW begomoviruses.

Phylogeographic analysis provided additional evidence for the clustering of NW begomoviruses based on geographical location. The DNA-A tree without the 20 divergent sequences indicated a common ancestor from North America. All viruses from Eastern South America clustered together in the same clade, suggesting an ecological barrier effect, most likely of the Amazon rainforest and the Andes mountains. This is well illustrated by the fact that *Bean golden mosaic virus* (BGMV) and *Bean golden yellow mosaic virus* (BGYMV), two begomoviruses that infect the same hosts, have been kept separated since their description in the 1960's. BGMV is found from the eastern boundaries of the Amazon rainforest to Argentina, and BGYMV is found from southern Mexico to Colombia [79, 80].

Cluster I in the DNA-A tree contains viruses from all regions except Eastern South America. We believe it represents an evolutionary lineage separated from other NW

begomoviruses. Therefore, begomoviruses placed in cluster I may represent a source of genetic variability not available (due to geographical isolation) to begomoviruses in South America. Introduction of these viruses could greatly affect begomovirus evolution in SA as they can recombine and introduce new features into South American begomoviruses.

Nevertheless, we found evidence that some long distance spread occurred between these areas. Evidence of migration can be observed for *Oxalis yellow vein virus* (OxYVV), which was originally described in North America [81] infecting a vegetatively-propagated ornamental plant which is native to South America [82, 83]. This virus clustered in the Eastern South America clade, indicating that it is related to viruses from that region rather than from North America.

The phylogeographic analysis indicated that recombination influences the spread of begomoviruses. The recombination-free tree showed a different topology compared to the original tree. Nevertheless, posterior probability values are lower in the recombination-free tree.

For the DNA-B, the analysis also indicated segregation based on geographical location, although forming distinct clades compared to the DNA-A tree. Segregation based on geography can be explained by the requisite relationship between DNA-A and DNA-B components of bipartite begomoviruses, both being necessary for the virus to establish a systemic infection and be transmitted under natural conditions [84]. Clade III of the DNA-B tree contains mostly viruses from Eastern South America, but some sequences from other regions are present (albeit with low posterior probability values). The same topology was observed in the recombination-free tree, suggesting that recombination does not affect the spread of the DNA-B.

This study confirmed the role of recombination as a mechanism of variability in NW begomoviruses. Nevertheless, the phylogenetic signal is maintained in the presence

of recombinants blocks. Migration was detected to be very frequent into the different biogeographical regions, with its effect being highlighted by recombination, which was responsible for inserting new molecular features to indigenous begomoviruses and for mixing the genetic variability already present. Although migration and recombination deeply affect the evolution of NW begomoviruses, the maintenance of distinct clusters despite overlapping geographical regions and hosts was observed. These clusters represent sources of genetic variability, which can be transferred to begomoviruses in other clusters by migration and recombination.

References

1. Zerbini FM, Briddon RW, Idris A, Martin DP, Moriones E, Navas-Castillo J *et al.* ICTV Virus Taxonomy Profile: *Geminiviridae*. J Gen Virol 2017;98:131-133.
2. Rocha CS, Castillo-Urquiza GP, Lima ATM, Silva FN, Xavier CAD, Hora-Junior BT *et al.* Brazilian begomovirus populations are highly recombinant, rapidly evolving, and segregated based on geographical location. J Virol 2013;87:5784-5799.
3. Sattar MN, Kvarnheden A, Saeed M, Briddon RW. Cotton leaf curl disease - an emerging threat to cotton production worldwide. J Gen Virol 2013;94:695-710.
4. Patil BL, Fauquet CM. Cassava mosaic geminiviruses: actual knowledge and perspectives. Mol Plant Pathol 2009;10:685-701.
5. Mabvakure B, Martin DP, Kraberger S, Cloete L, van Brunschot S, Geering AD *et al.* Ongoing geographical spread of *Tomato yellow leaf curl virus*. Virology 2016;498:257-264.
6. Zaidi SS, Martin DP, Amin I, Farooq M, Mansoor S. Tomato leaf curl New Delhi virus: a widespread bipartite begomovirus in the territory of monopartite begomoviruses. Mol Plant Pathol 2016;doi: 10.1111/mpp.12481.
7. Varsani A, Roumagnac P, Fuchs M, Navas-Castillo J, Moriones E, Idris A *et al.* *Capulavirus* and *Grablovirus*: two new genera in the family *Geminiviridae*. Arch Virol 2017;162:1819-1831.
8. Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JC *et al.* Revision of *Begomovirus* taxonomy based on pairwise sequence comparisons. Arch Virol 2015;160:1593-1619.
9. Varma A, Malathi VG. Emerging geminivirus problems: A serious threat to crop production. Ann Appl Biol 2003;142:145-164.
10. Briddon RW, Patil BL, Bagewadi B, Nawaz-ul-Rehman MS, Fauquet CM. Distinct evolutionary histories of the DNA-A and DNA-B components of bipartite begomoviruses. BMC Evol Biol 2010;10:97.
11. Harrison BD, Robinson DJ. Natural genomic and antigenic variation in whitefly transmitted geminiviruses (begomoviruses). Annu Rev Phytopathol 1999;39:369-398.

12. Padidam M, Sawyer S, Fauquet CM. Possible emergence of new geminiviruses by frequent recombination. *Virology* 1999;265:218-224.
13. Paximadis M, Idris AM, Torres-Jerez I, Villarreal A, Rey MEC, Brown JK. Characterization of tobacco geminiviruses in the Old and New world. *Arch Virol* 1999;144:703-717.
14. Rojas MR, Hagen C, Lucas WJ, Gilbertson RL. Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. *Annu Rev Phytopathol* 2005;43:361-394.
15. Rybicki EP. A phylogenetic and evolutionary justification for three genera of *Geminiviridae*. *Arch Virol* 1994;139:49-77.
16. Melgarejo TA, Kon T, Rojas MR, Paz-Carrasco L, Zerbini FM, Gilbertson RL. Characterization of a new world monopartite begomovirus causing leaf curl disease of tomato in Ecuador and Peru reveals a new direction in geminivirus evolution. *J Virol* 2013;87:5397-5413.
17. Hanley-Bowdoin L, Bejarano ER, Robertson D, Mansoor S. Geminiviruses: Masters at redirecting and reprogramming plant processes. *Nat Rev Microbiol* 2013;11:777-788.
18. Mahajan N, Parameswari C, Veluthambi K. Severe stunting in blackgram caused by the Mungbean yellow mosaic virus (MYMV) KA27 DNA B component is ameliorated by co-infection or post-infection with the KA22 DNA B: MYMV nuclear shuttle protein is the symptom determinant. *Virus Res* 2011;157:25-34.
19. Brustolini OJ, Machado JP, Condori-Apfata JA, Coco D, Deguchi M, Loriato VA *et al.* Sustained NIK-mediated antiviral signalling confers broad-spectrum tolerance to begomoviruses in cultivated plants. *Plant Biotechnol J* 2015;13:1300-1311.
20. Zhou X. Advances in understanding begomovirus satellites. *Annu Rev Phytopathol* 2013;51:357-381.
21. Lozano G, Trenado HP, Fiallo-Olive E, Chirinos D, Geraud-Pouey F, Briddon RW *et al.* Characterization of non-coding DNA satellites associated with sweepviruses (genus *Begomovirus*, *Geminiviridae*) - definition of a distinct class of begomovirus-associated satellites. *Front Microbiol* 2016;7:162.
22. Briddon RW, Mansoor S, Bedford ID, Pinner MS, Saunders K, Stanley J *et al.* Identification of DNA components required for induction of cotton leaf curl disease. *Virology* 2001;285:234-243.
23. Paprotka T, Metzler V, Jeske H. The first DNA 1-like alphasatellites in association with New World begomoviruses in natural infections. *Virology* 2010;404:148-157.
24. Jeske H, Kober S, Schäfer B, Strohmeier S. Circomics of Cuban geminiviruses reveals the first alphasatellite DNA in the Caribbean. *Virus Genes* 2014;49:312-324.
25. Romay G, Chirinos D, Geraud-Pouey F, Desbiez C. Association of an atypical alphasatellite with a bipartite New World begomovirus. *Arch Virol* 2010;155:1843-1847.
26. Ferro CG, Silva JP, Xavier CAD, Godinho MT, Lima ATM, Mar TB *et al.* The ever increasing diversity of begomoviruses infecting non-cultivated hosts: new species from *Sida* spp. and *Leonurus sibiricus*, plus two New World alphasatellites. *Ann Appl Biol* 2017;10.1111/aab.12329.
27. Zhou X, Xie Y, Tao X, Zhang Z, Li Z, Fauquet CM. Characterization of DNAbeta associated with begomoviruses in China and evidence for co-evolution with their cognate viral DNA-A. *J Gen Virol* 2003;84:237-247.
28. Briddon RW, Bull SE, Amin I, Idris AM, Mansoor S, Bedford ID *et al.* Diversity of DNA beta, a satellite molecule associated with some monopartite begomoviruses. *Virology* 2003;312:106-121.

29. Briddon RW, Bull SE, Amin I, Mansoor S, Bedford ID, Rishi N *et al.* Diversity of DNA 1: a satellite-like molecule associated with monopartite begomovirus–DNA β complexes. *Virology* 2004;324:462-474.
30. Lefeuvre P, Moriones E. Recombination as a motor of host switches and virus emergence: Geminiviruses as case studies. *Curr Opin Virol* 2015;10:14-19.
31. Rey MEC, Ndunguru J, Berrie LC, Paximadis M, Berry S, Cossa N *et al.* Diversity of dicotyledenous-infecting geminiviruses and their associated DNA molecules in Southern Africa, including the South-West Indian Ocean islands. *Viruses* 2012;4:1753-1791.
32. Saunders K, Bedford ID, Stanley J. Adaptation from whitefly to leafhopper transmission of an autonomously replicating nanovirus-like DNA component associated with ageratum yellow vein disease. *J Gen Virol* 2002;83:907-913.
33. Seal SE, Van den Bosch F, Jeger MJ. Factors influencing begomovirus evolution and their increasing global significance: Implications for sustainable control. *Crit Rev Plant Sci* 2006;25:23-46.
34. Lima ATM, Silva JCF, Silva FN, Castillo-Urquiza GP, Silva FF, Seah YM *et al.* The diversification of begomovirus populations is predominantly driven by mutational dynamics. *Virus Evol* 2017;3:vex005.
35. Torres-Pacheco I, Garzón-Tiznado JA, Brown JK, Becerra-Flora A, Rivera-Bustamante R. Detection and distribution of geminiviruses in Mexico and the Southern United States. *Phytopathology* 1996;86:1186-1192.
36. Sanz AI, Fraile A, García-Arenal F, Zhou X, Robinson DJ, Khalid S *et al.* Multiple infection, recombination and genome relationships among begomovirus isolates found in cotton and other plants in Pakistan. *J Gen Virol* 2000;81:1839-1849.
37. Pita JS, Fondong VN, Sangare A, Otim-Nape GW, Ogwal S, Fauquet CM. Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *J Gen Virol* 2001;82:655-665.
38. García-Andrés S, Monci F, Navas-Castillo J, Moriones E. Begomovirus genetic diversity in the native plant reservoir *Solanum nigrum*: Evidence for the presence of a new virus species of recombinant nature. *Virology* 2006;350:433-442.
39. Davino S, Napoli C, Dellacroce C, Miozzi L, Noris E, Davino M *et al.* Two new natural begomovirus recombinants associated with the tomato yellow leaf curl disease co-exist with parental viruses in tomato epidemics in Italy. *Virus Res* 2009;143:15-23.
40. Harrison BD, Zhou X, Otim Nape GW, Liu Y, Robinson DJ. Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. *Ann Appl Biol* 1997;131:437-448.
41. Silva SJC, Castillo-Urquiza GP, Hora-Junior BT, Assunção IP, Lima GSA, Pio-Ribeiro G *et al.* Species diversity, phylogeny and genetic variability of begomovirus populations infecting leguminous weeds in northeastern Brazil. *Plant Pathol* 2012;61:457-467.
42. Tavares SS, Ramos-Sobrinho R, Gonzalez-Aguilera J, Lima GSA, Assunção IP, Zerbini FM. Further molecular characterization of weed-associated begomoviruses in Brazil with an emphasis on *Sida* spp. *Planta Dan* 2012;30:305-315.
43. Fiallo-Olivé E, Navas-Castillo J, Moriones E, Martinez-Zubiaur Y. Two novel begomoviruses belonging to different lineages infecting *Rhynchosia minima*. *Arch Virol* 2010;155:2053-2058.
44. Fiallo-Olivé E, Navas-Castillo J, Moriones E, Martinez-Zubiaur Y. Begomoviruses infecting weeds in Cuba: Increased host range and a novel virus infecting *Sida rhombifolia*. *Arch Virol* 2012;157:141-146.

45. Ribeiro SG, Ávila AC, Bezerra IC, Fernandes JJ, Faria JC, Lima MF *et al.* Widespread occurrence of tomato geminiviruses in Brazil, associated with the new biotype of the whitefly vector. *Plant Dis* 1998;82:830.
46. Loconsole G, Saldarelli P, Doddapaneni H, Savino V, Martelli GP, Saponari M. Identification of a single-stranded DNA virus associated with citrus chlorotic dwarf disease, a new member in the family *Geminiviridae*. *Virology* 2012;432:162-172.
47. Basso MF, Silva JC, Fajardo TV, Fontes EP, Zerbini FM. A novel, highly divergent ssDNA virus identified in Brazil infecting apple, pear and grapevine. *Virus Res* 2015;210:27-33.
48. Ma Y, Navarro B, Zhang Z, Lu M, Zhou X, Chi S *et al.* Identification and molecular characterization of a novel monopartite geminivirus associated with mulberry mosaic dwarf disease. *J Gen Virol* 2015;96:2421-2434.
49. Al-Rwahnih M, Dave A, Anderson MM, Rowhani A, Uyemoto JK, Sudarshana MR. Association of a DNA virus with grapevines affected by red blotch disease in California. *Phytopathology* 2013;103:1069-1076.
50. Ha C, Coombs S, Revill P, Harding R, Vu M, Dale J. Molecular characterization of begomoviruses and DNA satellites from Vietnam: Additional evidence that the New World geminiviruses were present in the Old World prior to continental separation. *J Gen Virol* 2008;89:312-326.
51. Ha C, Coombs S, Revill P, Harding R, Vu M, Dale J. Corchorus yellow vein virus, a New World geminivirus from the Old World. *J Gen Virol* 2006;87:997-1003.
52. Muhire B, Martin DP, Brown JK, Navas-Castillo J, Moriones E, Zerbini FM *et al.* A genome-wide pairwise-identity-based proposal for the classification of viruses in the genus *Mastrevirus* (family *Geminiviridae*). *Arch Virol* 2013;158:1411-1424.
53. Edgar RC. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinf* 2004;5:1-19.
54. Tamura K, Stecher G, Peterson D, Filipinski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 2013;30:2725-2729.
55. Bryant D, Moulton V. Neighbor-Net: An agglomerative method for the construction of phylogenetic networks. *Mol Biol Evol* 2004;21:255-265.
56. Huson DH, Bryant D. Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol* 2006;23:254-267.
57. Martin DP, Lemey P, Lott M, Moulton V, Posada D, Lefevre P. RDP3: a flexible and fast computer program for analyzing recombination. *Bioinformatics* 2010;26:2462-2463.
58. Stamatakis A. RAxML-VI-HPC: Maximum Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinf Appl Note* 2006;22:2688-2690.
59. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 2003;19:1572-1574.
60. Nylander JAA. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University 2004.
61. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 2007;7:214.
62. Brown JK, Fauquet CM, Briddon RW, Zerbini FM, Moriones E, Navas-Castillo J. Family *Geminiviridae*. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. *Virus Taxonomy - Ninth Report of the International Committee on Taxonomy of Viruses*. London, UK: Elsevier Academic Press; 2012. p. 351-373.

63. Stanley J, Bisaro DM, Briddon RW, Brown JK, Fauquet CM, Harrison BD *et al.* Family *Geminiviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, editors. *Virus Taxonomy - Eighth Report of the International Committee on Taxonomy of Viruses*. San Diego: Elsevier Academic Press; 2005. p. 301-326.
64. Lefeuvre P, Lett JM, Varsani A, Martin DP. Widely conserved recombination patterns among single-stranded DNA viruses. *J Virol* 2009;83:2697-2707.
65. Lefeuvre P, Martin DP, Hoareau M, Naze F, Delatte H, Thierry M *et al.* Begomovirus 'melting pot' in the south-west Indian Ocean islands: Molecular diversity and evolution through recombination. *J Gen Virol* 2007;88:3458-3468.
66. Martin DP, Lefeuvre P, Varsani A, Hoareau M, Semegni JY, Dijoux B *et al.* Complex recombination patterns arising during geminivirus coinfections preserve and demarcate biologically important intra-genome interaction networks. *PLoS Pathog* 2011;7:e1002203.
67. Lima ATM, Sobrinho RR, Gonzalez-Aguilera J, Rocha CS, Silva SJC, Xavier CAD *et al.* Synonymous site variation due to recombination explains higher genetic variability in begomovirus populations infecting non-cultivated hosts. *J Gen Virol* 2013;94:418-431.
68. Silva FN, Lima ATM, Rocha CS, Castillo-Urquiza GP, Alves M, Zerbini FM. Recombination and pseudorecombination driving the evolution of the begomoviruses *Tomato severe rugose virus* (ToSRV) and *Tomato rugose mosaic virus* (ToRMV): two recombinant DNA-A components sharing the same DNA-B. *Virol J* 2014;11:66.
69. Nawaz-UI-Rehman MS, Fauquet CM. Evolution of geminiviruses and their satellites. *Febs Letters* 2009;583:1825-1832.
70. Castillo-Urquiza GP, Beserra Jr. JEA, Bruckner FP, Lima ATM, Varsani A, Alfenas-Zerbini P *et al.* Six novel begomoviruses infecting tomato and associated weeds in Southeastern Brazil. *Arch Virol* 2008;153:1985-1989.
71. De Bruyn A, Harimalala M, Zinga I, Mabvakure BM, Hoareau M, Ravigne V *et al.* Divergent evolutionary and epidemiological dynamics of cassava mosaic geminiviruses in Madagascar. *BMC Evol Biol* 2016;16:182.
72. Briddon RW, Pinner MS, Stanley J, Markham PG. Geminivirus coat protein gene replacement alters insect specificity. *Virology* 1990;177:85-94.
73. Brown JK, Idris AM, Torres-Jerez I, Banks GK, Wyatt SD. The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of begomoviruses. *Arch Virol* 2001;146:1581-1598.
74. Ramos-Sobrinho R, Xavier CAD, Pereira HMB, Lima GSA, Assunção IP, Mizubuti ESG *et al.* Contrasting genetic structure between two begomoviruses infecting the same leguminous hosts. *J Gen Virol* 2014;95:2540-2552.
75. Ilyina TV, Koonin EV. Conserved sequence motifs in the initiator proteins for rolling circle DNA replication encoded by diverse replicons from eubacteria, eucaryotes and archaeobacteria. *Nucleic Acids Res* 1992;20:3279-3285.
76. Koonin EV, Ilyina TV. Geminivirus replication proteins are related to prokaryotic plasmid rolling circle DNA replication initiator proteins. *J Gen Virol* 1992;73:2763-2766.
77. Nash TE, Dallas MB, Reyes MI, Buhman GK, Ascencio-Ibanez JT, Hanley-Bowdoin L. Functional analysis of a novel motif conserved across geminivirus Rep proteins. *J Virol* 2011;85:1182-1192.
78. Ho ES, Kuchie J, Duffy S. Bioinformatic analysis reveals genome size reduction and the emergence of tyrosine phosphorylation site in the movement protein of New World bipartite begomoviruses. *PLoS ONE* 2014;9:e111957.

79. Costa AS. Three whitefly-transmitted virus diseases of beans in São Paulo, Brazil. *Plant Prot Bull FAO* 1965;13:2-12.
80. Morales FJ. History and current distribution of begomoviruses in Latin America. *Adv Virus Res* 2006;67:127-162.
81. Herrera F, Aboughanem-Sabanadzovic N, Valverde RA. A begomovirus associated with yellow vein symptoms of *Oxalis debilis*. *Eur J Plant Pathol* 2015;142:203-208.
82. Luo S, Zhang D, Renner SS. *Oxalis debilis* in China: distribution of flower morphs, sterile pollen and polyploidy. *Ann Bot* 2006;98:459-464.
83. Lourteig A. *Oxalis* L. subgéneros *Monoxalis* (Small) Lourt., *Oxalis* y *Trifidus* Lourt. *Bradea* 2000;7:201-629.
84. Stanley J. Infectivity of the cloned geminivirus genome requires sequences from both DNAs. *Nature* 1983;305:643-645.

Table 1. Putative recombination events involving the DNA-A components of a set of 20 divergent New World begomoviruses.

Events	Breakpoint ^a		Genomic region	Recombinants	Putative parents		Methods ^b	P-value ^c
	Begin	End			Minor	Major		
1	2625	2010	IR	AbMBV NC_016574	SiYBV NC_020254 SiMAIV NC_016573	CuLCrV NC_002984	RGBMCS3	1.03E-43
2	2023	2641	Rep	SiYLCV EU710750	ToCmMV NC_010835	SiYMV NC_004639	RGBMCS3	2.04E-40
3	25	1912	CP	DeLDV NC_008494	CoYSV NC_008492 MacGMV NC_010952 SiGMoV NC_014130	Unknown	RGBMCS3	3.38E-32
4	869	1944	CP/Rep	ToSLCV NC_004642	ToChLPV NC_005843	Unknown	RGBMCS3	1.83E-29
5	1972	2619	Rep	TRYLCV NC_020257	EuMV NC_008304 SMLCuV NC_004645 ToMYLCV NC_009490	TrYMV BR-Msj1-10A SiMoV NC_004637 SiYNV NC_020253	RGBMCS3	2.88E-26
6	1933	11	Rep	ToCmMV NC_010835	EuMV NC_008304 MCLCuV NC_003865	TrYMV BR-Msj1-10A OMoV NC_011181 SiYNV NC_020253	RGBMCS3	7.94E-26
7	1964	2491	Rep	CLCrV NC_004580	Unknown Unknown Unknown Unknown Unknown Unknown	BChMV NC_022005 AbMV NC_001928 BDMV NC_001931 OYMV NC_014066 SiGMCRV NC_004657 SiGMV NC_002046	RGBMCS3	1.70E-24
8	1928	24	Rep/CR	CabLCuV NC_003866 RhGMYuV NC_012481	CuLCrV NC_002984	Unknown	RGBMCS3	6.82E-25
9	1960	165	Rep/IR	BCaMV NC_003504 MCLCuV NC_003865	Unknown	BGYMV NC_001439	RGBMCS3	7.59E-26

Table 1 (cont.)

Events	Breakpoint		Genomic region	Recombinants	Putative parents		Methods	P-value
	Begin	End			Minor	Major		
10	1809	101	Rep/IR	MCIMV NC_014380	Unknown	BleICV NC_019035	RGBMCS3	1.56E-21
11	1870	2572	Rep	PepGMV NC_004101	Unknown	ToChLPV NC_005843	RGBMCS3	4.43E-18
12	1801	2585	Rep	SqLCV NC_001936	Unknown	HeYMV BR-Mac7-10A	RGBMCS3	2.39E-23
13	2076	2440	Rep	EuYMV NC_012553 EuMV NC_008304 SqLCV NC_001936 SMLCuV NC_004645 ToMYLCV NC_009490 ToSLCV NC_004642	Unknown	DaChMV NC_018716	RGBMCS3	1.66E-11
14	601	1005	CP	SqLCV NC_001936	RhGMYuV NC_012481	MCLCuV NC_003865	RGBMCS3	9.64E-03
15	1032*	1145	CP	CabLCuJV DQ178614 CarYSYV NC_022007 RhGMYuV NC_012481	RhGMV NC_010294 MacMPRV NC_004097	BCaMV NC_003504	RGBMCS3	2.47E-04

^a* breakpoint could not be precisely pinpointed.

^bR, RDP; G, GeneConv; B, Bootscan; M, MaxChi; C, Chimera; S, SisScan; 3, 3SEQ.

^cThe reported *P* values are for the methods indicated in red, and they are the lowest *P* values calculated for the region in question.

Figure legends

Figure 1. Phylogenetic evidence for recombination based on (a) full-length DNA-A, (b) *CP*, (c) *Rep*, (d) *Rep* of begomoviruses and alphasatellites, (e) full-length DNA-B, (f) *NSP* and (g) *MP* nucleotide sequences of New World begomoviruses. Isolates from Eastern South America (ESA) are indicated in green, Western South America (WSA) in yellow, Central America (CA) in blue, North America (NA) in red, the Caribbean (CAR) in purple and Old World (OW) in black. Neighbor-Net network analysis was performed using SplitsTree4. Formation of a reticular network rather than a single bifurcated tree is suggestive of recombination.

Figure 2. Phylogenetic relationships among New World begomoviruses based on DNA-A sequences. (a) Maximum likelihood tree based on the full-length DNA-A, (b) Maximum likelihood tree based on a recombination-free alignment of DNA-A sequences (c) Bayesian tree based on the *CP* gene, (d) Bayesian tree based on the *Rep* gene. Isolates from Eastern South America (ESA) are indicated in green, Western South America (WSA) in yellow, Central America (CA) in blue, North America (NA) in red, the Caribbean (CAR) in purple and Old World (OW) in black.

Figure 3. Phylogenetic relationships among New World begomoviruses based on DNA-B sequences. Bayesian trees based on (a) full-length DNA-B, (b) *NSP* gene, (c) *MP* gene, (d) recombination-free alignment of DNA-B sequences. Isolates from Eastern South America (ESA) are indicated in green, Western South America (WSA) in yellow, Central America (CA) in blue, North America (NA) in red, the Caribbean (CAR) in purple and Old World (OW) in black.

Figure 4. Phylogeographic clustering of New World begomoviruses based on **(a)** DNA-A, **(b)** recombination-free DNA-A, **(c)** DNA-B, **(d)** recombination-free DNA-B. Isolates from Eastern South America (ESA) are indicated in green, Western South America (WSA) in yellow, Central America (CA) in blue, North America (NA) in red, the Caribbean (CAR) in purple and Old World (OW) in black.

Figure 1a

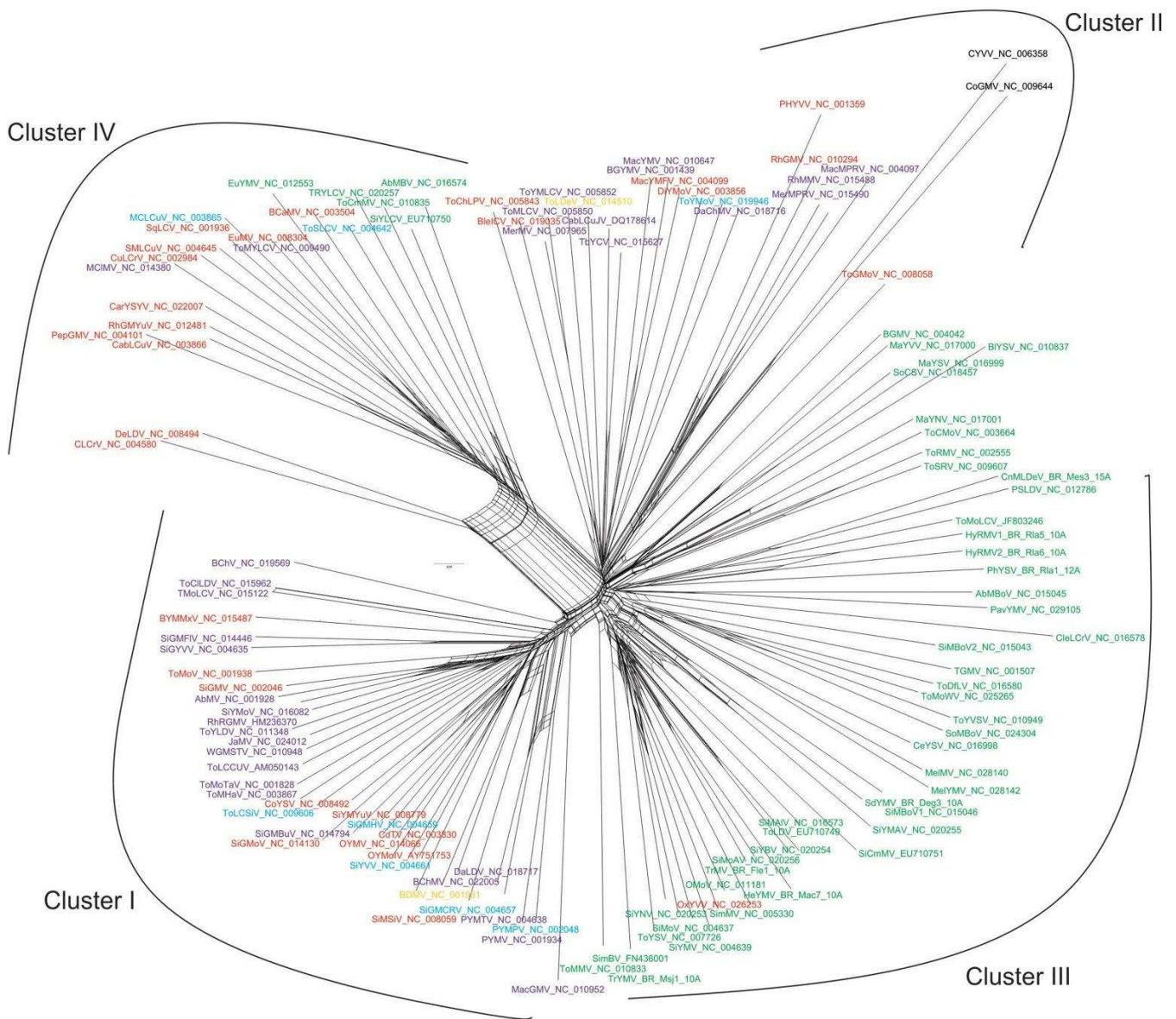


Figure 1b

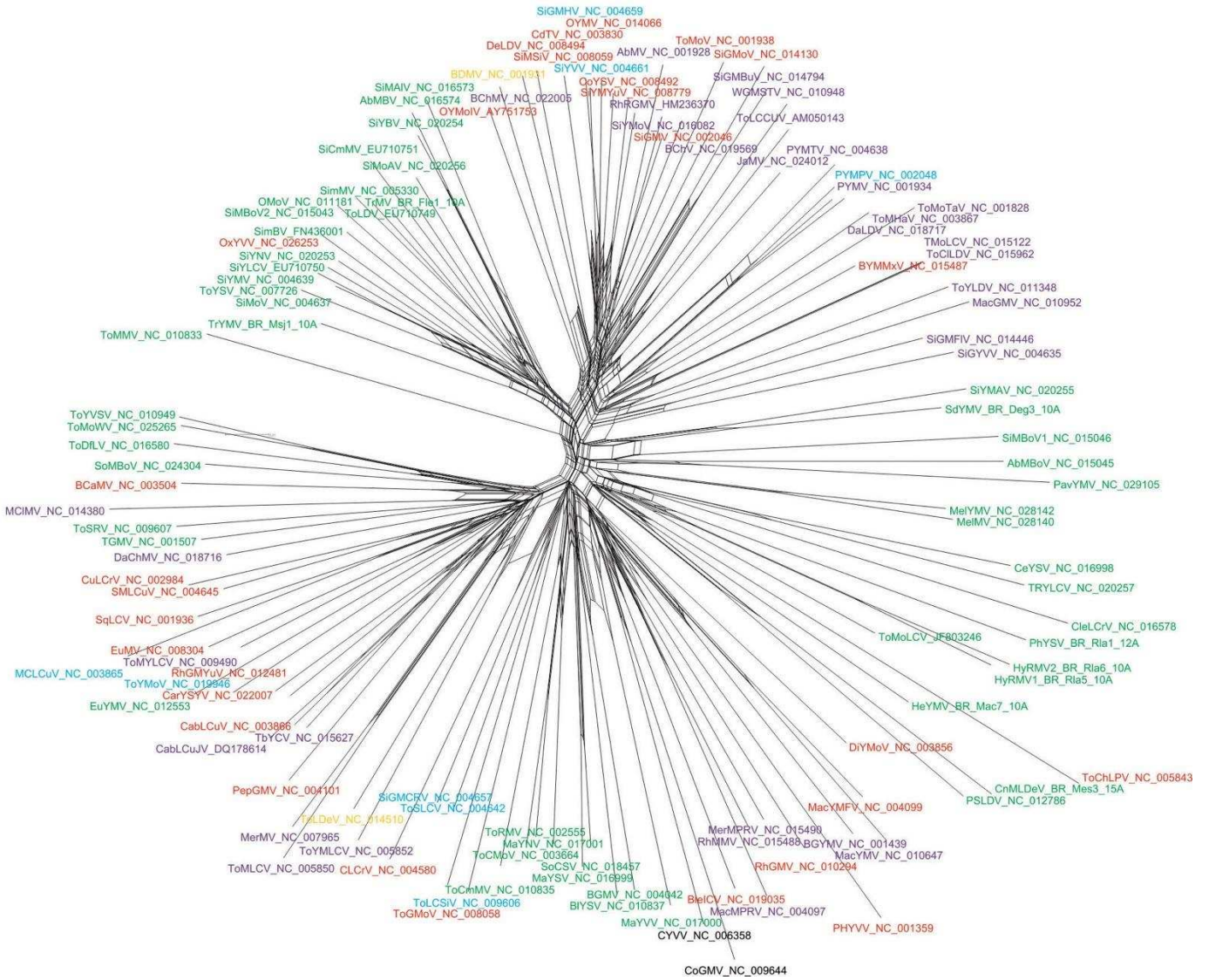


Figure 1c

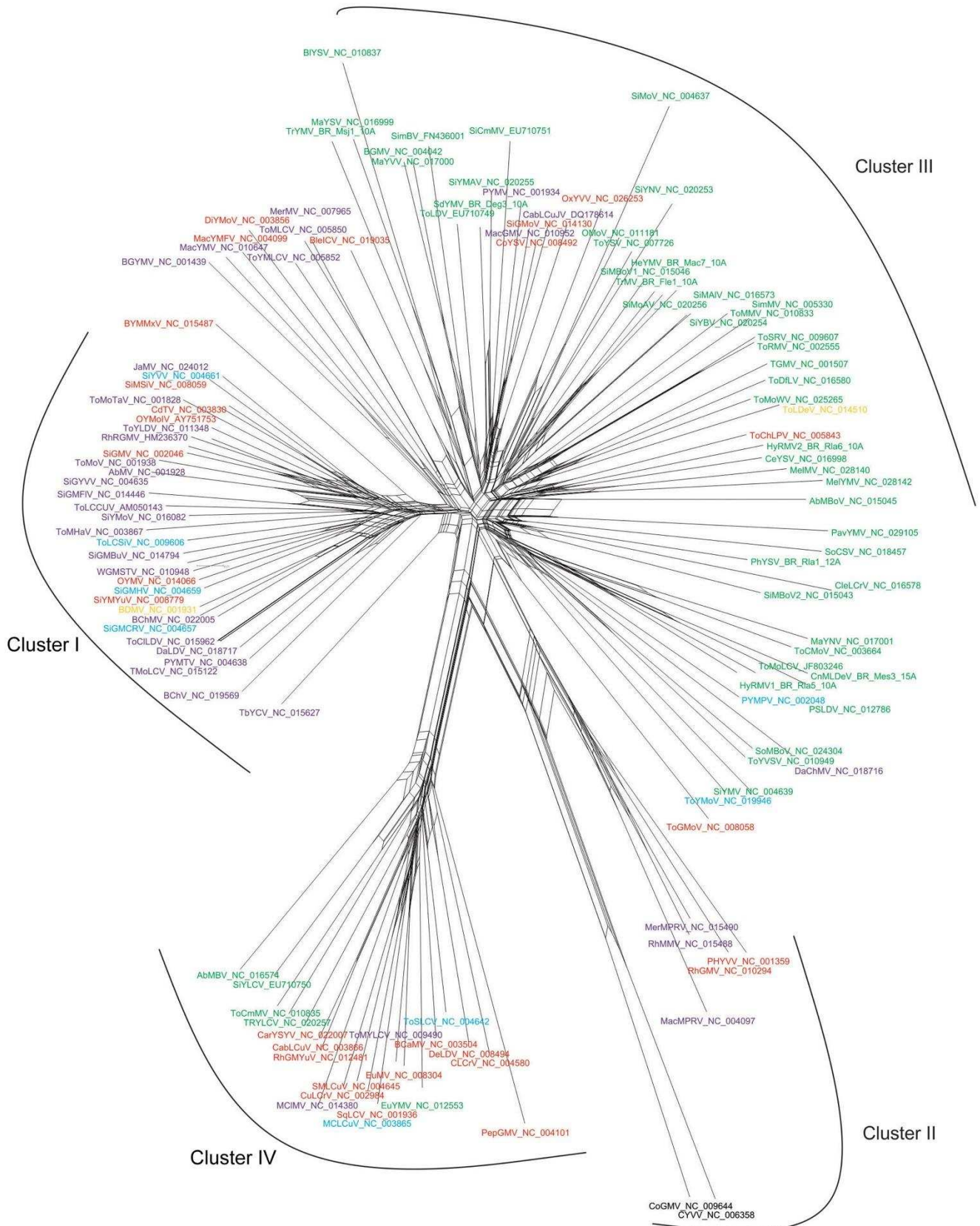


Figure 1f

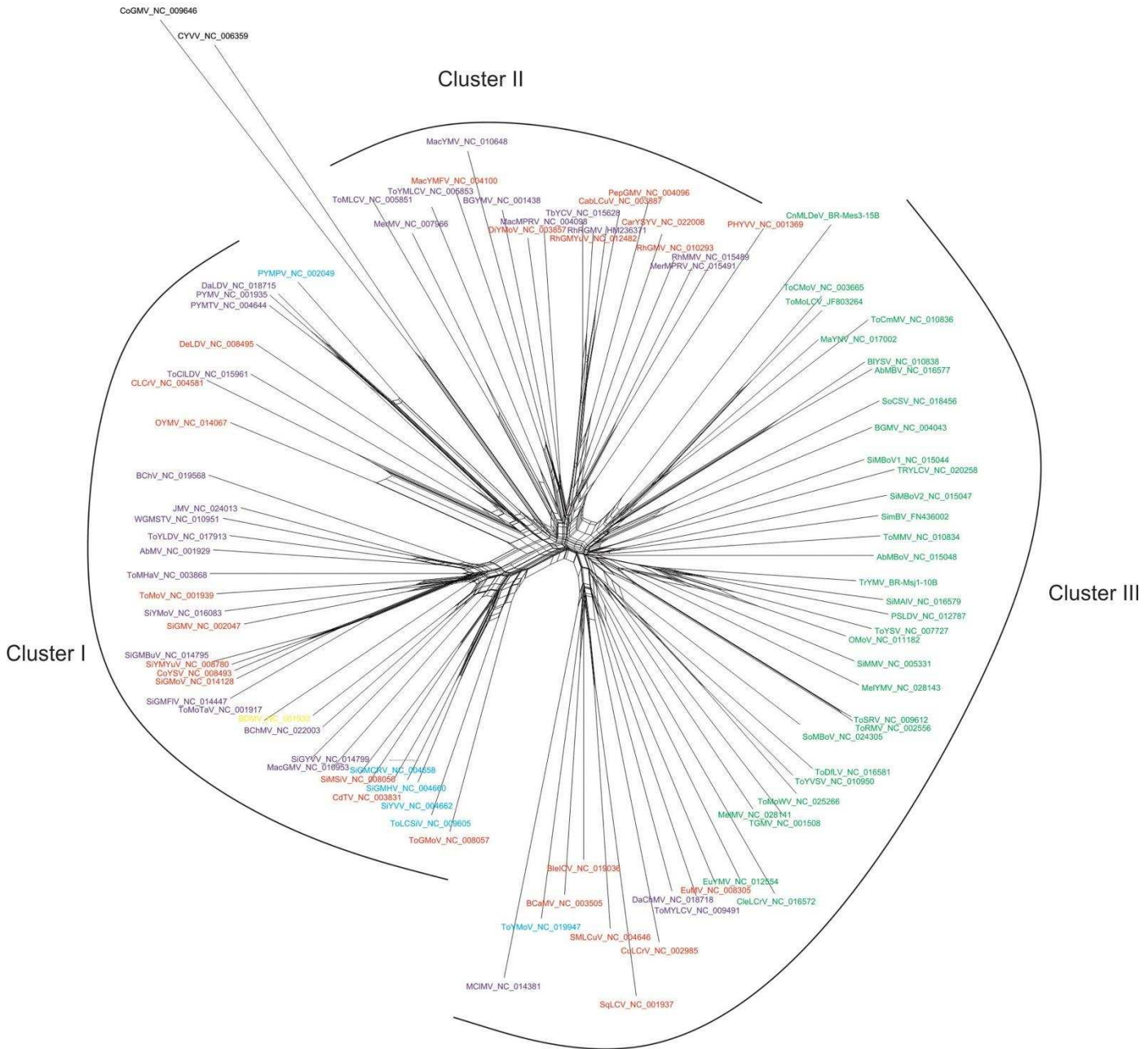


Figure 1g

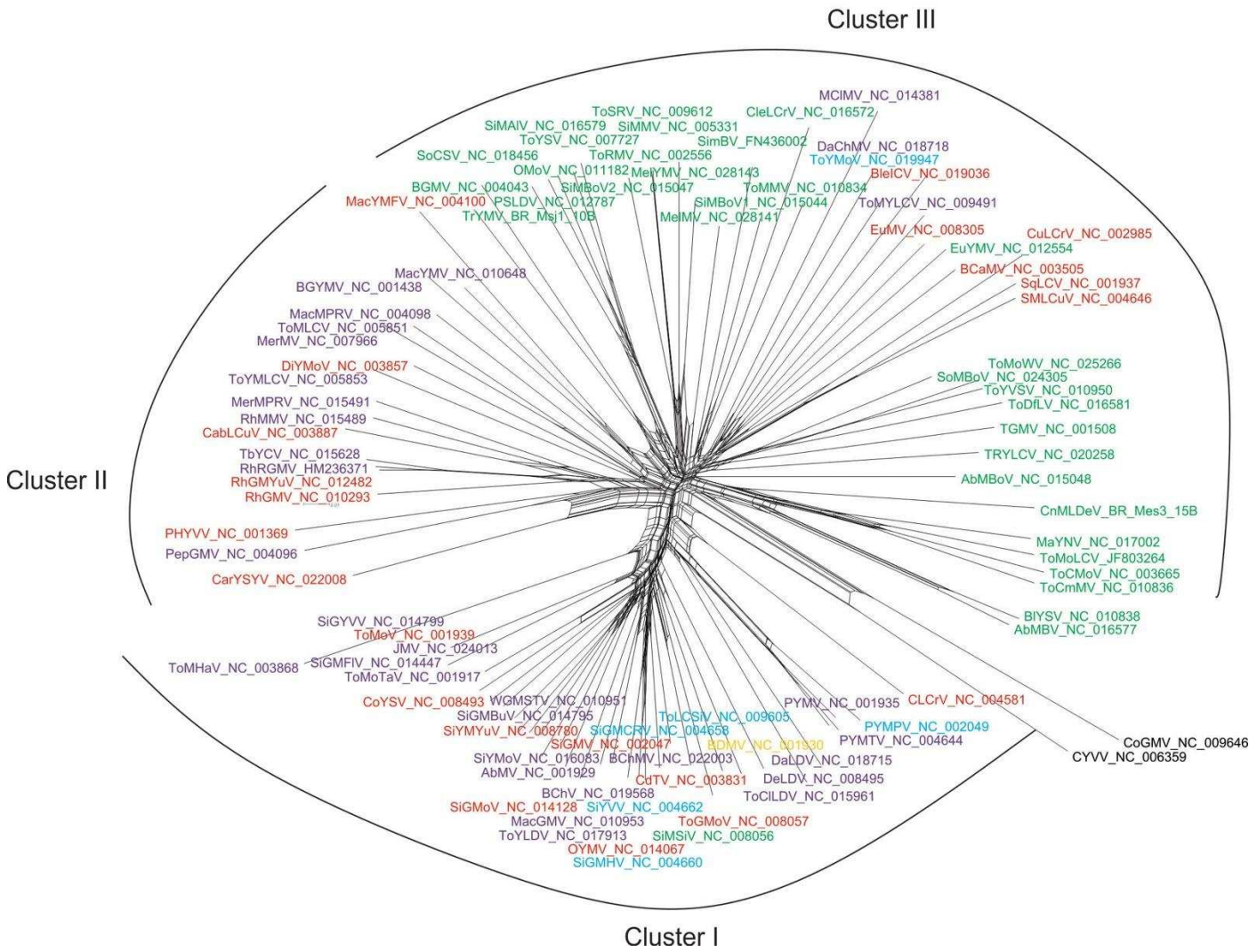


Figure 2a

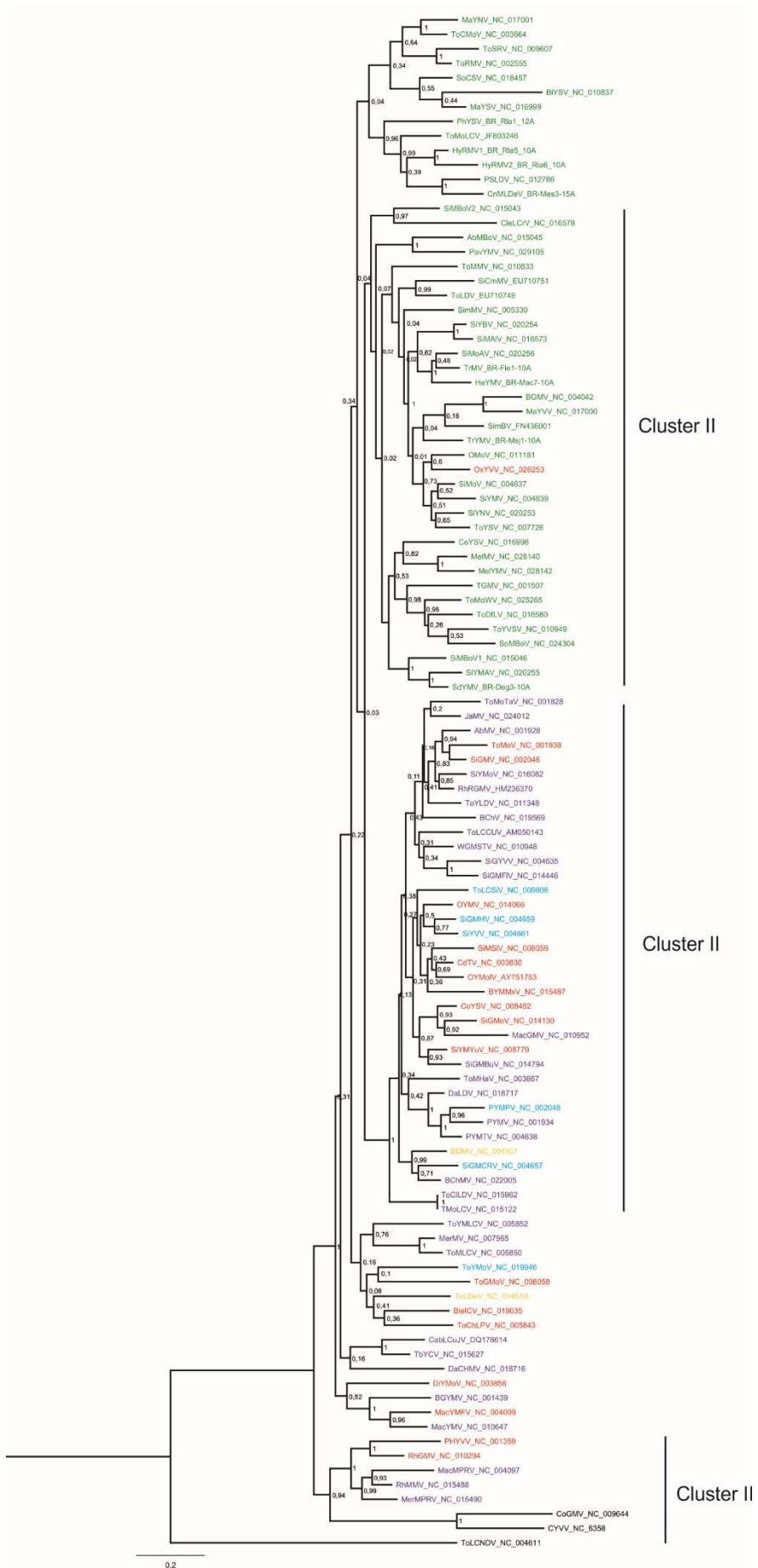


Figure 2b

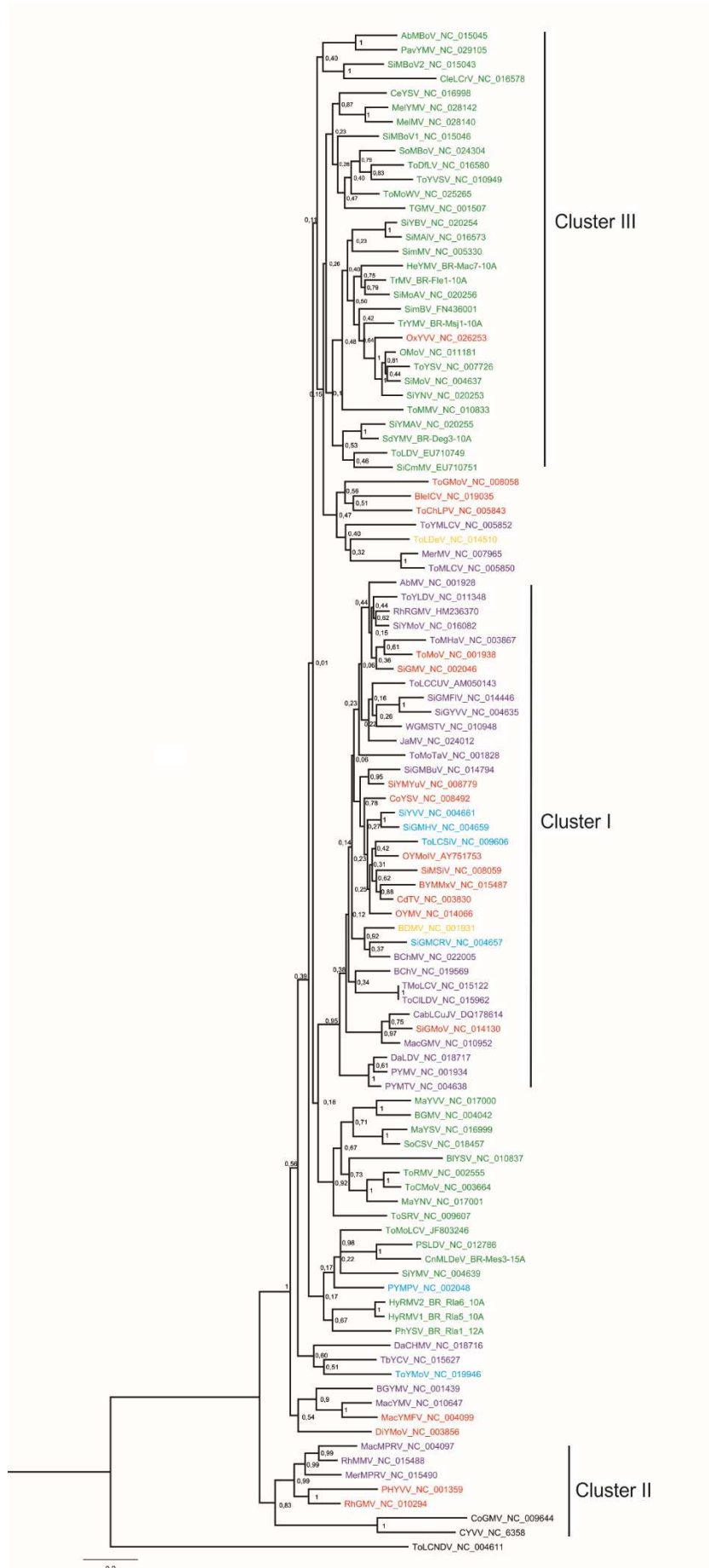
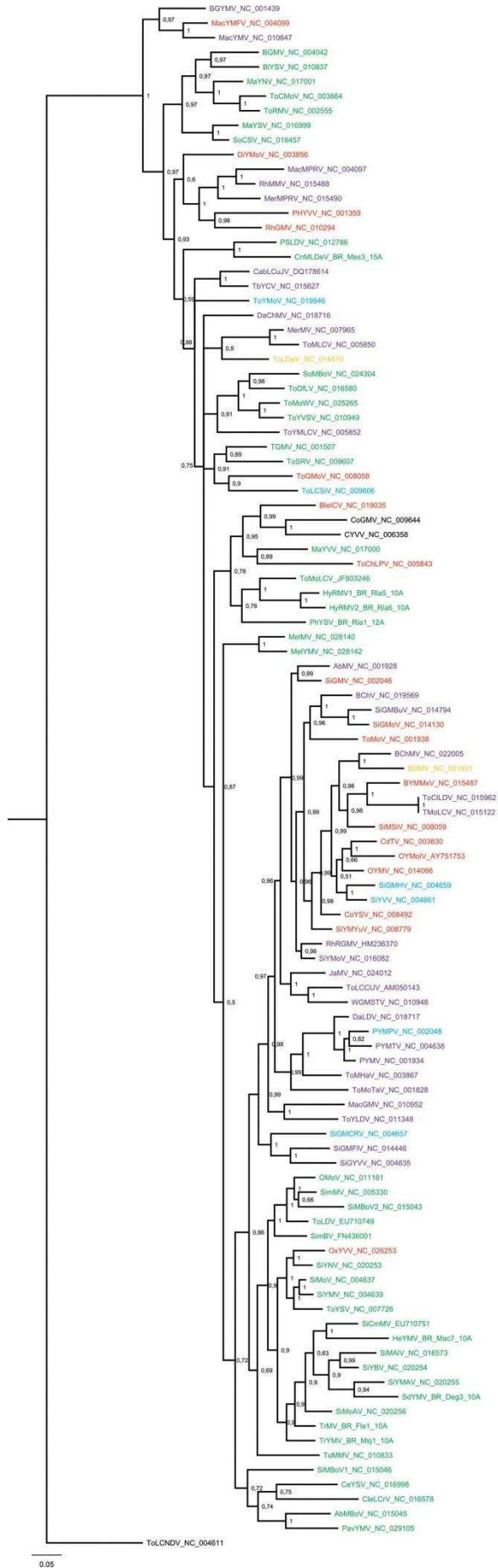


Figure 2c



Cluster I

0.05

Figure 2d

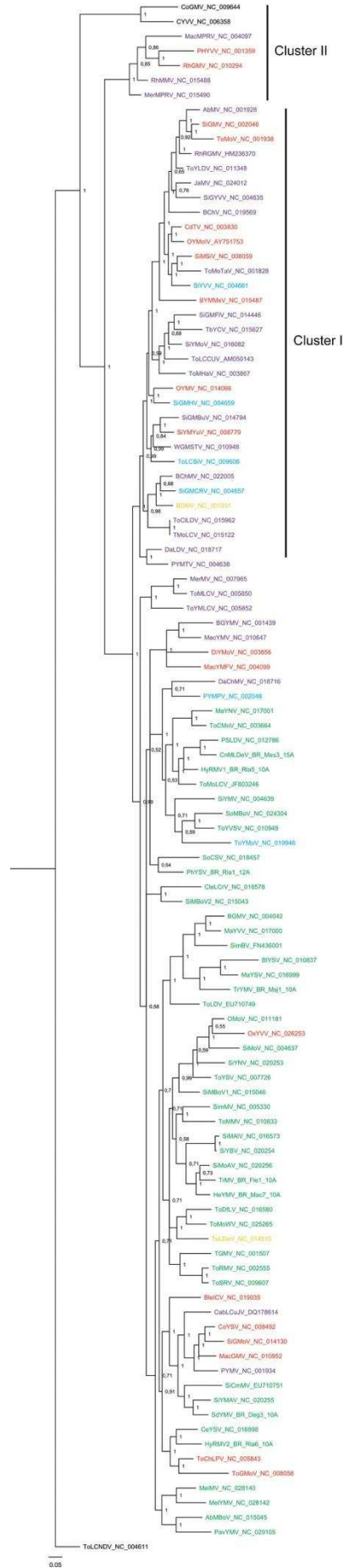


Figure 3a

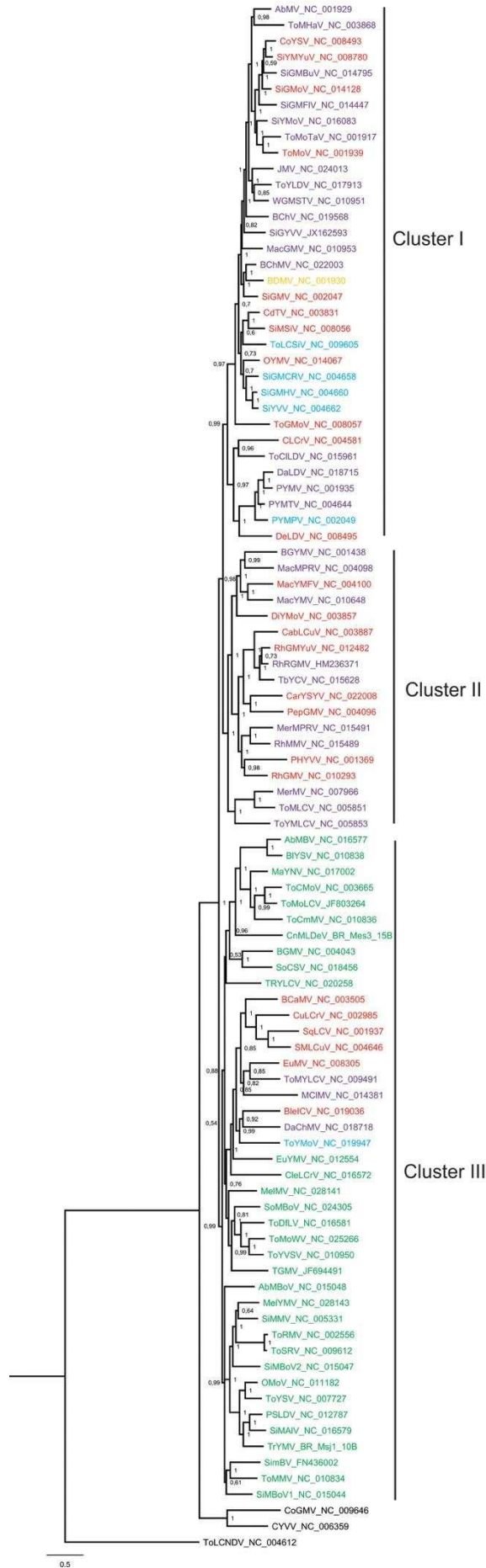


Figure 3b

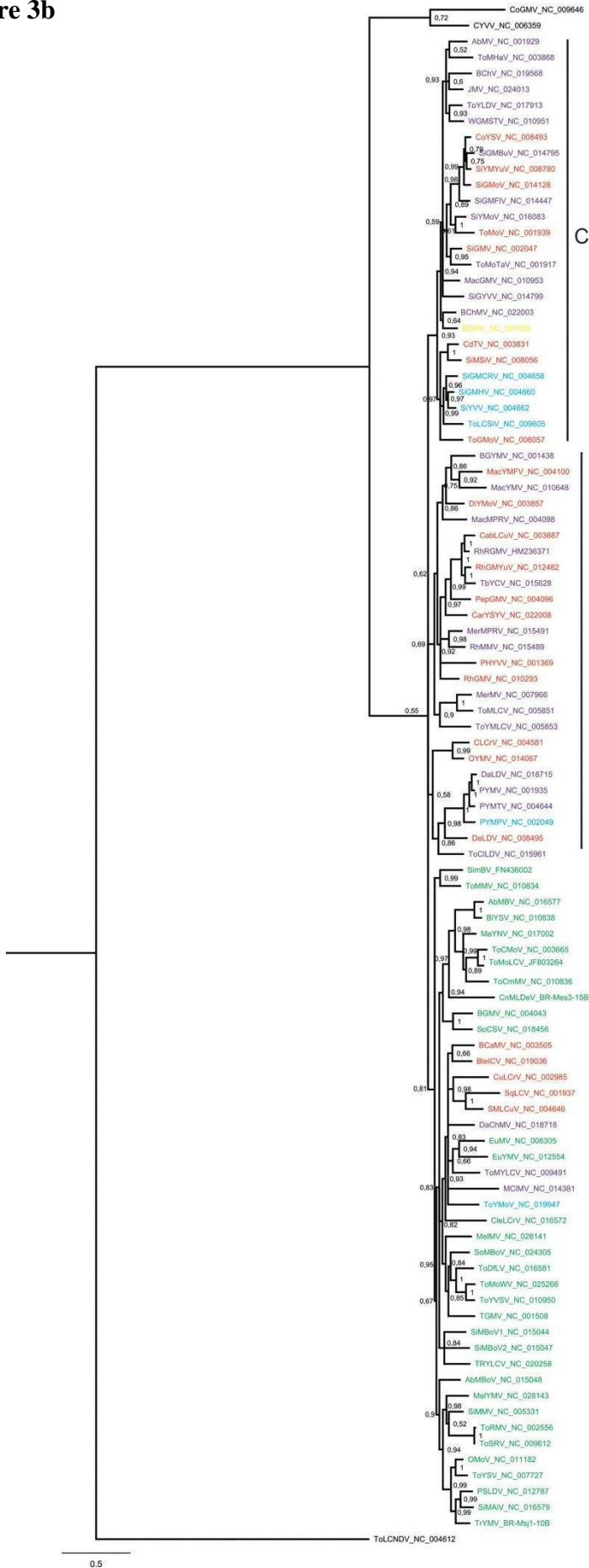


Figure 3c

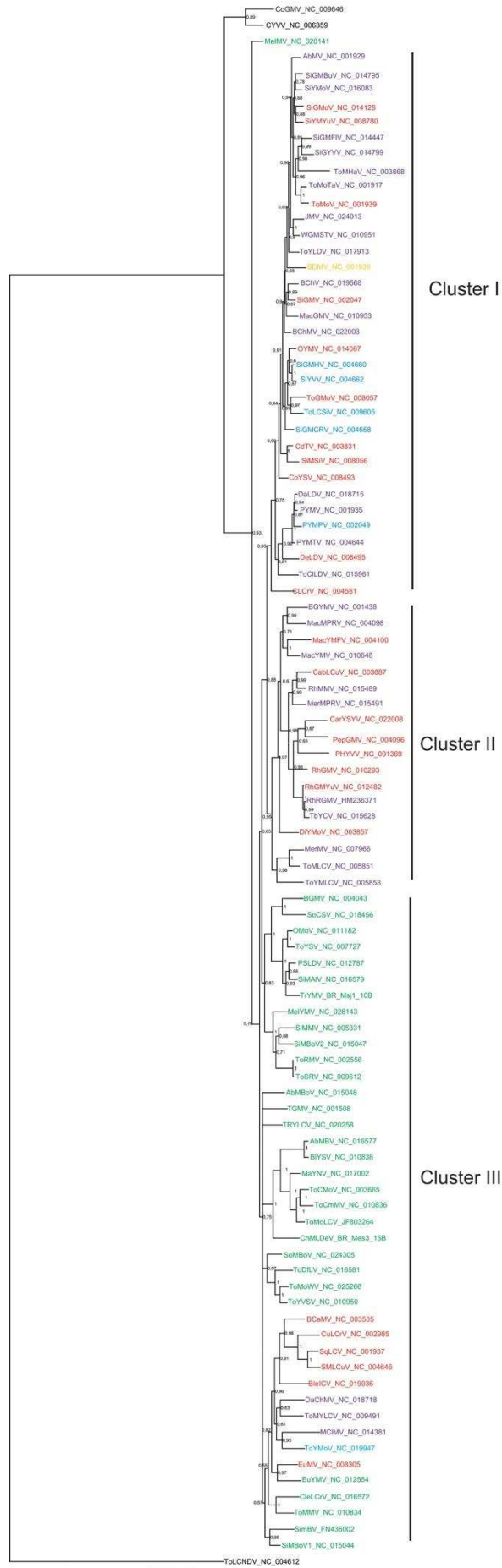


Figure 3d

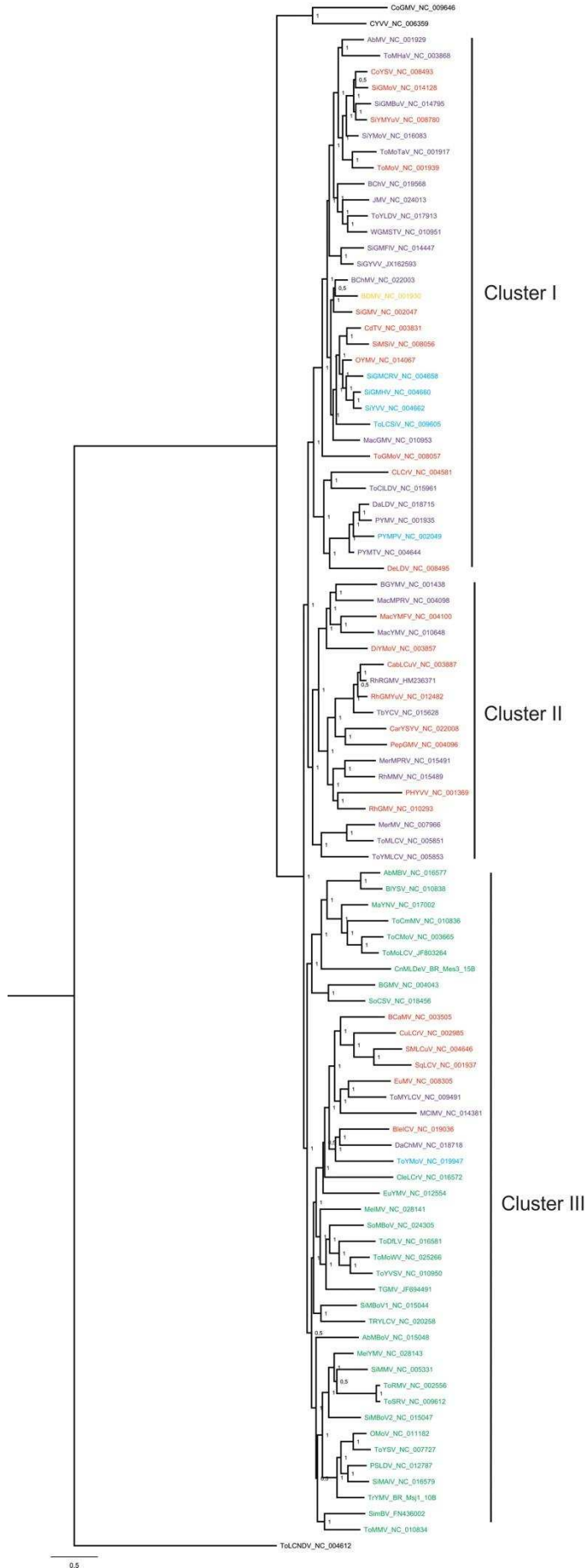


Figure 4a

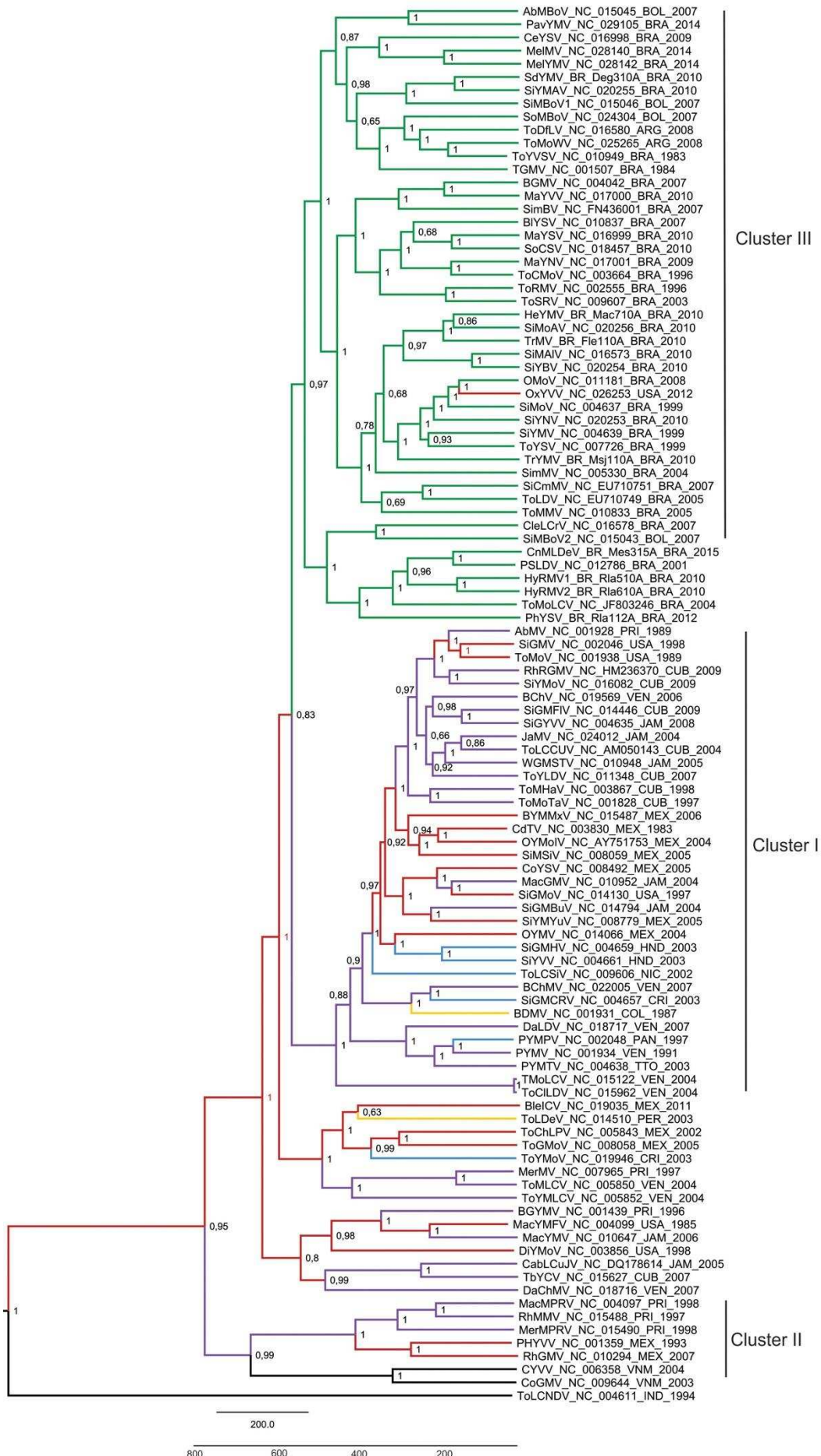


Figure 4b

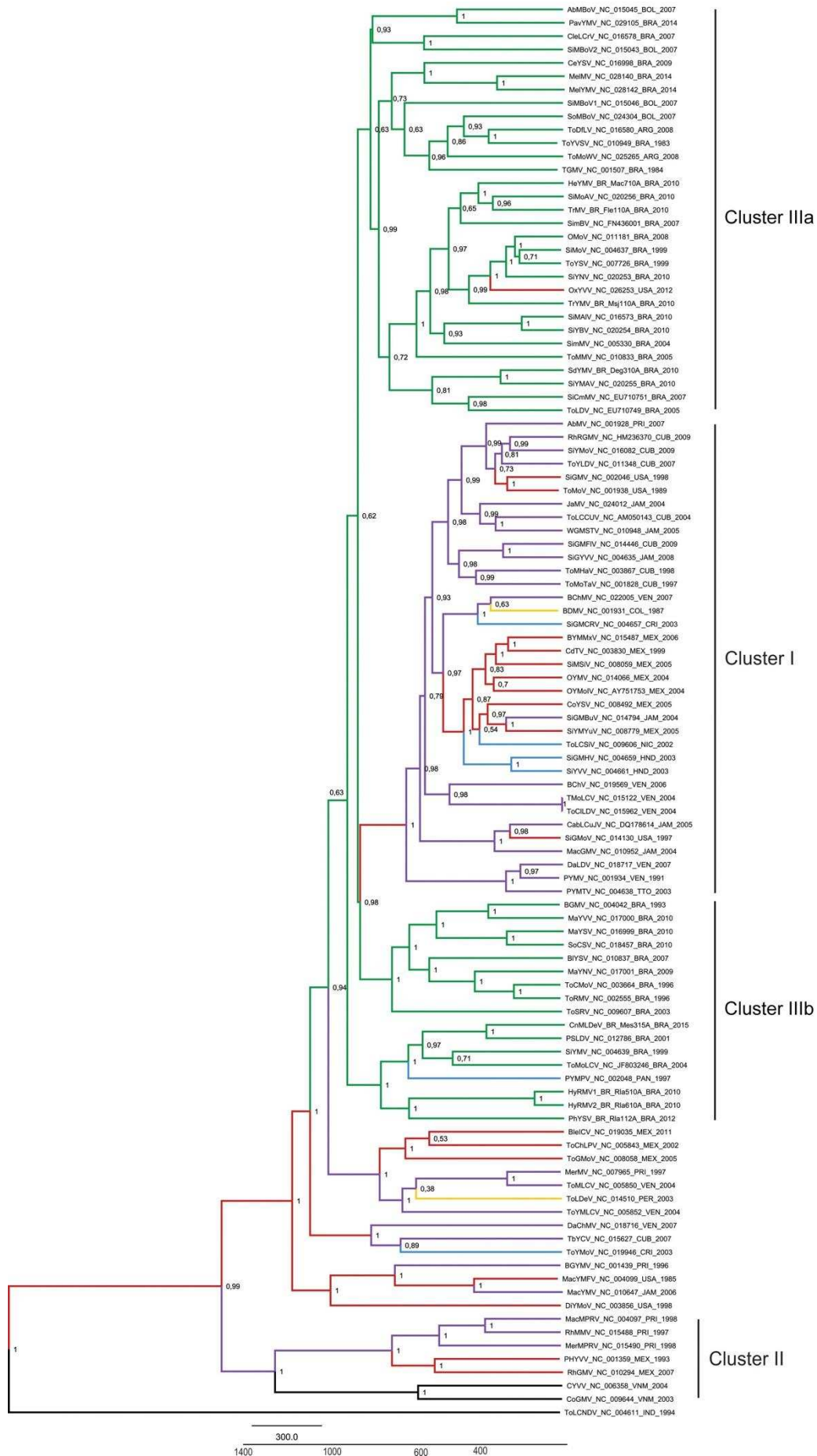


Figure 4c

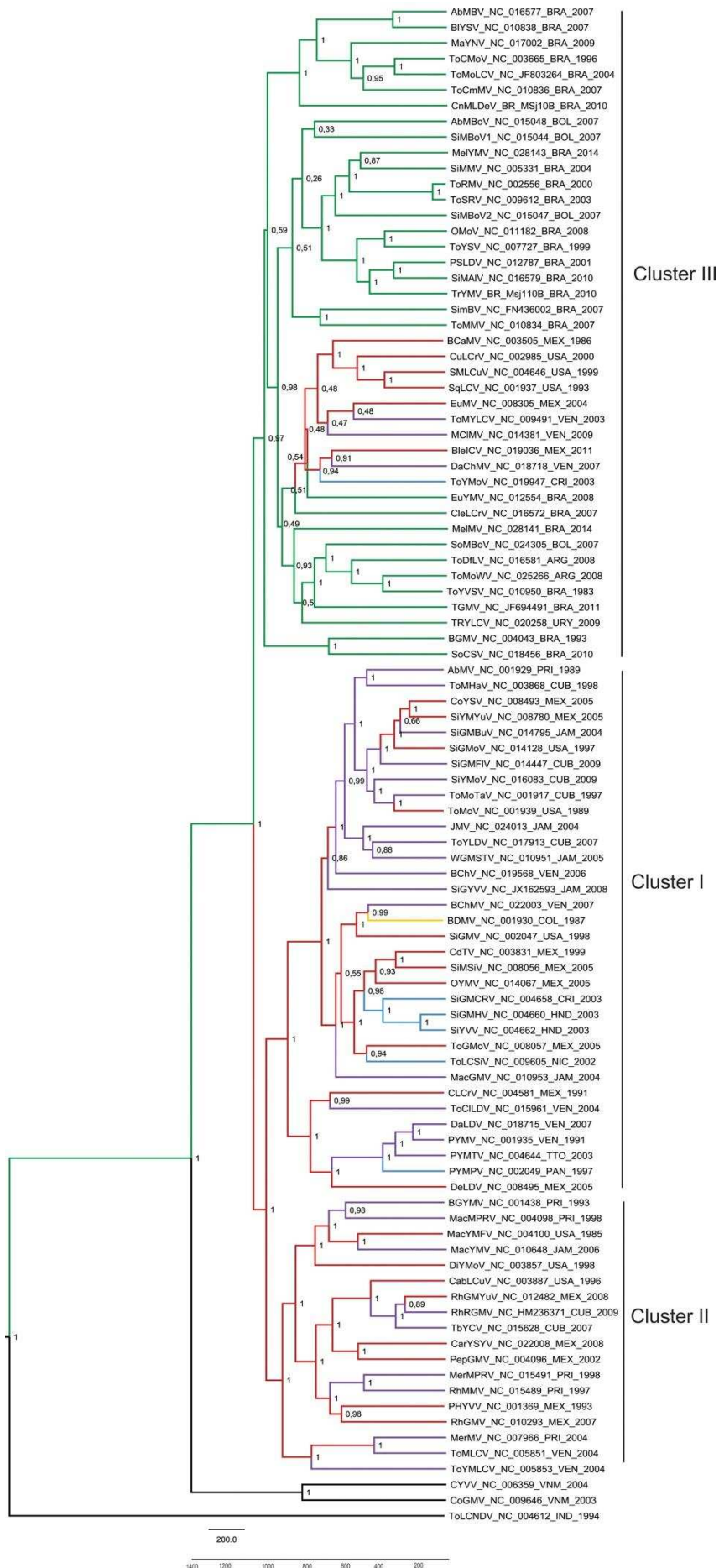
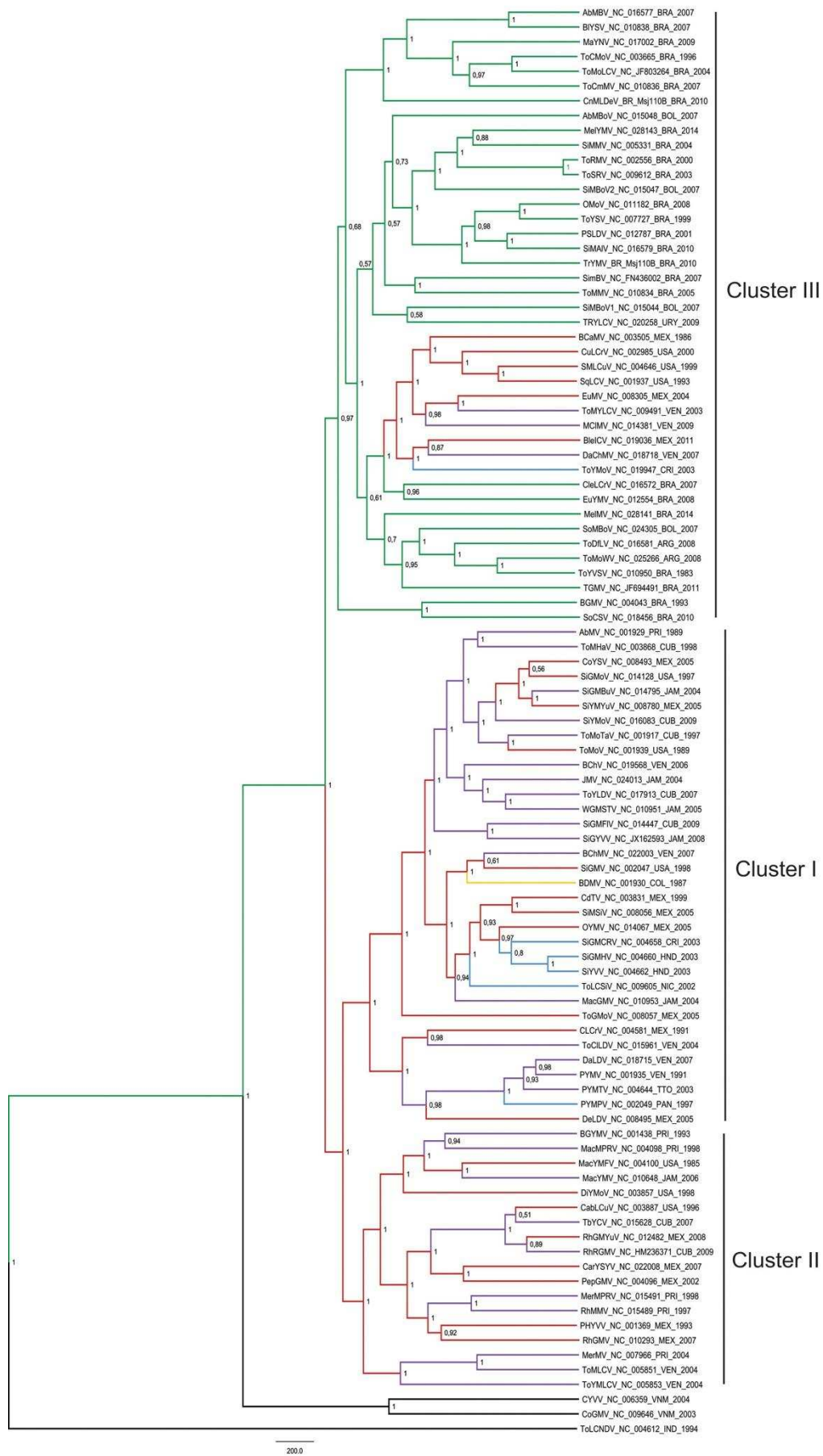


Figure 4d



Supporting Information

Suppl. Table S1. New World begomovirus sequences retrieved from GenBank's non-redundant nucleotide database.

N°	Species	Accession #		Acronym	Location	Date
		DNA-A	DNA-B			
<i>Eastern South America</i>						
1	<i>Abutilon mosaic Bolivia virus</i>	NC_015045	NC_015048	AbMBoV	Bolivia	2007
2	<i>Abutilon mosaic Brazil virus</i>	NC_016574	NC_016577	AbMBV	Brazil	2007
3	<i>Bean golden mosaic virus</i>	NC_004042	NC_004043	BGMV	Brazil	1993
4	<i>Blainvillea yellow spot virus</i>	NC_010837	NC_010838	BIYSV	Brazil	2007
5	<i>Centrosema yellow spot virus</i>	NC_016998	---	CeYSV	Brazil	2009
6	<i>Cleome leaf crumple virus</i>	NC_016578	NC_016572	CleLCrV	Brazil	2007
7	<i>Euphorbia yellow mosaic virus</i>	NC_012553	NC_012554	EuYMV	Brazil	2007
8	<i>Macropodium yellow net virus</i>	NC_017001	NC_017002	MaYNV	Brazil	2009
9	<i>Macropodium yellow spot virus</i>	NC_016999	---	MaYSV	Brazil	2010
10	<i>Macropodium yellow vein virus</i>	NC_017000	---	MaYVV	Brazil	2010
11	<i>Melochia mosaic virus</i>	NC_028140	NC_028141	MelMV	Brazil	2014
12	<i>Melochia yellow mosaic virus</i>	NC_028142	NC_028143	MelYMV	Brazil	2014
13	<i>Okra mottle virus</i>	NC_011181	NC_011182	OMoV	Brazil	2008
14	<i>Passionfruit severe leaf distortion virus</i>	NC_012786	NC_012787	PSLDV	Brazil	2001
15	<i>Pavonia yellow mosaic virus</i>	NC_029105	---	PavYMV	Brazil	2014
16	<i>Sida Brazil virus</i>	FN436001	FN436002	SimBV	Brazil	2007
17	<i>Sida common mosaic virus</i>	EU710751	---	SiCmMV	Brazil	2007
18	<i>Sida micrantha mosaic virus</i>	NC_005330	NC_005331	SiMMV	Brazil	2004
19	<i>Sida mosaic Alagoas virus</i>	NC_016573	NC_016579	SiMAIV	Brazil	2010

Suppl. Table S1. (cont.)

N°	Species	Accession #		Acronym	Location	Date
		DNA-A	DNA-B			
20	<i>Sida mosaic Bolivia virus 1</i>	NC_015046	NC_015044	SiMBoV1	Bolivia	2007
21	<i>Sida mosaic Bolivia virus 2</i>	NC_015043	NC_015047	SiMBoV2	Bolivia	2007
22	<i>Sida mottle Alagoas virus</i>	NC_020256	---	SiMoAV	Brazil	2010
23	<i>Sida mottle virus</i>	NC_004637	---	SiMoV	Brazil	1999
24	<i>Sida yellow blotch virus</i>	NC_020254	---	SiYBV	Brazil	2010
25	<i>Sida yellow leaf curl virus</i>	EU710750	---	SiYLCV	Brazil	2007
26	<i>Sida yellow mosaic Alagoas virus</i>	NC_020255	---	SiYMAV	Brazil	2010
27	<i>Sida yellow mosaic virus</i>	NC_004639	---	SiYMV	Brazil	1999
28	<i>Sida yellow net virus</i>	NC_020253	---	SiYNV	Brazil	2010
29	<i>Solanum mosaic Bolivia virus</i>	NC_024304	NC_024305	SoMBoV	Bolivia	2007
30	<i>Soybean chlorotic spot virus</i>	NC_018457	NC_018456	SoCSV	Brazil	2010
31	<i>Tomato chlorotic mottle virus</i>	NC_003664	NC_003665	ToCMoV	Brazil	1996
32	<i>Tomato common mosaic virus</i>	NC_010835	NC_010836	ToCmMV	Brazil	2007
33	<i>Tomato dwarf leaf virus</i>	NC_016580	NC_016581	ToDfLV	Argentina	2008
34	<i>Tomato golden mosaic virus</i>	NC_001507	NC_001508	TGMV	Brazil	1984
35	<i>Tomato leaf distortion virus</i>	EU710749	---	ToLDV	Brazil	2005
36	<i>Tomato mild mosaic virus</i>	NC_010833	NC_010834	ToMMV	Brazil	2005
37	<i>Tomato mottle leaf curl virus</i>	JF803246	JF803264	ToMoLCV	Brazil	2004
38	<i>Tomato mottle wrinkle virus</i>	NC_025265	NC_025266	ToMoWV	Argentina	2008
39	<i>Tomato rugose mosaic virus</i>	NC_002555	NC_002556	ToRMV	Brazil	1996
40	<i>Tomato rugose yellow leaf curl virus</i>	NC020257	NC_020258	TRYLCV	Uruguay	2009
41	<i>Tomato severe rugose virus</i>	NC_009607	NC_009612	ToSRV	Brazil	2003
42	<i>Tomato yellow spot virus</i>	NC_007726	NC_007727	ToYSV	Brazil	1999
43	<i>Tomato yellow vein streak virus</i>	NC_010949	NC_010950	ToYVSV	Brazil	1983
44	<i>Cnidoscopus mosaic leaf deformation virus</i>	BR-Mes3-15A	BR-Msj1-10B	CnMLDeV	Brazil	2015

Suppl. Table S1. (cont.)

N°	Species	Accession #		Acronym	Location	Date
		DNA-A	DNA-B			
45	<i>Herissantia yellow mosaic virus</i>	BR-Mac7-10A	---	HeYMV	Brazil	2010
46	<i>Herissantia rugose mosaic virus 1</i>	BR_Rla5_10A	---	HyRMV1	Brazil	2010
47	<i>Herissantia rugose mosaic virus 2</i>	BR_Rla6_10A	---	HyRMV2	Brazil	2010
48	<i>Physalis yellow spot virus</i>	BR_Rla1_12A	---	PhYSV	Brazil	2012
49	<i>Sidastrum yellow mosaic virus</i>	BR-Deg3-10A	---	SdYMV	Brazil	2010
50	<i>Triumfetta mosaic virus</i>	BR-Fle1-10A	---	TrMV	Brazil	2010
51	<i>Triumfetta yellow mosaic virus</i>	BR-Msj1-10A	BR-Msj1-10B	TrYMV	Brazil	2010
<i>Western South America</i>						
52	<i>Bean dwarf mosaic virus</i>	NC_001931	NC_001930	BDMV	Colombia	1987
53	<i>Tomato leaf deformation virus</i>	NC_014510	---	ToLDeV	Peru	2003
<i>Central America</i>						
54	<i>Melon chlorotic leaf curl virus</i>	NC_003865	---	MCLCuV	Costa Rica	1998
55	<i>Potato yellow mosaic Panama virus</i>	NC_002048	NC_002049	PYMPV	Panama	1997
56	<i>Sida golden mosaic Costa Rica virus</i>	NC_004657	NC_004658	SiGMCRV	Costa Rica	2003
57	<i>Sida golden mosaic Honduras virus</i>	NC_004659	NC_004660	SiGMHV	Honduras	2003
58	<i>Sida yellow vein virus</i>	NC_004661	NC_004662	SiYVV	Honduras	2003
59	<i>Tomato leaf curl Sinaloa virus</i>	NC_009606	NC_009605	ToLCSiV	Nicaragua	2002
60	<i>Tomato severe leaf curl virus</i>	NC_004642	---	ToSLCV	Guatemala	1996
61	<i>Tomato yellow mottle virus</i>	NC_019946	NC_019947	ToYMoV	Costa Rica	2003

Suppl. Table S1. (cont.)

N°	Species	Accession #		Acronym	Location	Date
		DNA-A	DNA-B			
<i>Caribbean</i>						
62	<i>Abutilon mosaic virus</i>	NC_001928	NC_001929	AbMV	Porto Rico	1989
63	<i>Bean chlorosis virus</i>	NC_019569	NC_019568	BChV	Venezuela	2006
64	<i>Bean chlorotic mosaic virus</i>	NC_022005	NC_022003	BChMV	Venezuela	2007
65	<i>Bean golden yellow mosaic virus</i>	NC_001439	NC_001438	BGYMV	Puerto Rico	1996
66	<i>Cabbage leaf curl Jamaica virus</i>	DQ178614	---	CabLCuJV	Jamaica	2005
67	<i>Dalechampia chlorotic mosaic virus</i>	NC_018716	NC_018718	DaChMV	Venezuela	2007
68	<i>Datura leaf distortion virus</i>	NC_018717	NC_018715	DaLDV	Venezuela	2007
69	<i>Jatropha mosaic virus</i>	NC_024012	NC_024013	JaMV	Jamaica	2004
70	<i>Macropodium golden mosaic virus</i>	NC_010952	NC_010953	MacGMV	Jamaica	2004
71	<i>Macropodium mosaic Puerto Rico virus</i>	NC_004097	NC_004098	MacMPRV	Puerto Rico	1998
72	<i>Macropodium yellow mosaic virus</i>	NC_010647	NC_010648	MacYMV	Jamaica	2006
73	<i>Melon chlorotic mosaic virus</i>	NC_014380	NC_014381	MCIMV	Venezuela	2009
74	<i>Merremia mosaic Puerto Rico virus</i>	NC_015490	NC_015491	MerMPRV	Puerto Rico	1998
75	<i>Merremia mosaic virus</i>	NC_007965	NC_007966	MerMV	Puerto Rico	1997
76	<i>Potato yellow mosaic Trinidad virus</i>	NC_004638	NC_004644	PYMTV	Trinidad and Tobago	2003
77	<i>Potato yellow mosaic virus</i>	NC_001934	NC_001935	PYMV	Venezuela	1991
78	<i>Rhynchosia mild mosaic virus</i>	NC_015488	NC_015489	RhMMV	Puerto Rico	1997
79	<i>Rhynchosia rugose golden mosaic virus</i>	HM236370	HM236371	RhRGMV	Cuba	2009
80	<i>Sida golden mosaic Backup virus</i>	NC_014794	NC_014795	SiGMBuV	Jamaica	2004
81	<i>Sida golden mosaic Florida virus</i>	NC_014446	NC_014447	SiGMFIV	Cuba	2009
82	<i>Sida golden yellow vein virus</i>	NC_004635	NC_014799	SiGYVV	Jamaica	2008
83	<i>Sida yellow mottle virus</i>	NC_016082	NC_016083	SiYMoV	Cuba	2009

Suppl. Table S1. (cont.)

N°	Species	Accession #		Acronym	Location	Date
		DNA-A	DNA-B			
84	<i>Tobacco leaf curl Cuba virus</i>	AM050143	---	ToLCCUV	Cuba	2004
85	<i>Tobacco yellow crinkle virus</i>	NC_015627	NC_015628	TbYCV	Cuba	2007
86	<i>Tomato chlorotic leaf distortion virus</i>	NC_015962	NC_015961	ToCILDV	Venezuela	2004
87	<i>Tomato mild yellow leaf curl Aragua virus</i>	NC_009490	NC_009491	ToMYLCV	Venezuela	2003
88	<i>Tomato mosaic Havana virus</i>	NC_003867	NC_003868	ToMHaV	Cuba	1998
89	<i>Tomato mosaic leaf curl virus</i>	NC_005850	NC_005851	ToMLCV	Venezuela	2004
90	<i>Tomato mottle leaf curl Zulia virus</i>	NC_015122	---	TMoLCV	Venezuela	2004
91	<i>Tomato mottle Taino virus</i>	NC_001828	NC_001917	ToMoTaV	Cuba	1997
92	<i>Tomato yellow leaf distortion virus</i>	NC_011348	NC_017913	ToYLDV	Cuba	2007
93	<i>Tomato yellow margin leaf curl virus</i>	NC_005852	NC_005853	ToYMLCV	Venezuela	2004
94	<i>Wissadula golden mosaic St Thomas virus</i>	NC_010948	NC_010951	WGMSTV	Jamaica	2005
<i>North America</i>						
95	<i>Bean calico mosaic virus</i>	NC_003504	NC_003505	BcaMV	Mexico	1986
96	<i>Bean yellow mosaic Mexico virus</i>	NC_015487	---	BYMMxV	Mexico	2006
97	<i>Blechum interveinal chlorosis virus</i>	NC_019035	NC_019036	BleICV	Mexico	2011
98	<i>Cabbage leaf curl virus</i>	NC_003866	NC_003887	CabLCuV	USA	1996
99	<i>Capraria yellow spot Yucatan virus</i>	NC_022007	NC_022008	CarYSYV	Mexico	2007
100	<i>Chino del tomate virus</i>	NC_003830	NC_003831	CdTV	Mexico	1999
101	<i>Corchorus yellow spot virus</i>	NC_008492	NC_008493	CoYSV	Mexico	2005
102	<i>Cotton leaf crumple virus</i>	NC_004580	NC_004581	CLCrV	Mexico	1991
103	<i>Cucurbit leaf crumple virus</i>	NC_002984	NC_002985	CuLCrV	USA	2000
104	<i>Desmodium leaf distortion virus</i>	NC_008494	NC_008495	DeLDV	Mexico	2005

Suppl. Table S1. (cont.)

N°	Species	Accession #		Acronym	Location	Date
		DNA-A	DNA-B			
105	<i>Dicliptera yellow mottle virus</i>	NC_003856	NC_003857	DiYMoV	USA	1998
106	<i>Euphorbia mosaic virus</i>	NC_008304	NC_008305	EuMV	Mexico	2004
107	<i>Macroptilium yellow mosaic Florida virus</i>	NC_004099	NC_004100	MacYMFV	USA	1985
108	<i>Okra yellow mosaic Mexico virus</i>	NC_014066	NC_014067	OYMV	Mexico	2004
109	<i>Okra yellow mottle Iguala virus</i>	AY751753	---	OYMoIV	Mexico	2004
110	<i>Oxalis yellow vein virus strain</i>	NC_026253	---	OxYVV	USA	2012
111	<i>Pepper golden mosaic virus</i>	NC_004101	NC_004096	PepGMV	Mexico	1996
112	<i>Pepper huasteco yellow vein virus</i>	NC_001359	NC_001369	PHYVV	Mexico	1993
113	<i>Rhynchosia golden mosaic virus</i>	NC_010294	NC_010293	RhGMV	Mexico	2007
114	<i>Rhynchosia golden mosaic Yucatan virus</i>	NC_012481	NC_012482	RhGMYuV	Mexico	2006
115	<i>Sida golden mosaic virus</i>	NC_002046	NC_002047	SiGMV	USA	1998
116	<i>Sida golden mottle virus</i>	NC_014130	NC_014128	SiGMoV	USA	1997
117	<i>Sida mosaic Sinaloa virus</i>	NC_008059	NC_008056	SiMSiV	Mexico	2005
118	<i>Sida yellow mosaic Yucatan virus</i>	NC_008779	NC_008780	SiYMYuV	Mexico	2005
119	<i>Squash leaf curl virus</i>	NC_001936	NC_001937	SqLCV	USA	1993
120	<i>Squash mild leaf curl virus</i>	NC_004645	NC_004646	SMLCuV	USA	1979
121	<i>Tomato chino La Paz virus</i>	NC_005843	---	ToChLPV	Mexico	2002
122	<i>Tomato golden mottle virus</i>	NC_008058	NC_008057	ToGMoV	Mexico	2005
123	<i>Tomato mottle virus</i>	NC_001938	NC_001939	ToMoV	USA	1989
<i>Old World and outgroup</i>						
124	<i>Corchorus golden mosaic virus</i>	NC_009644	NC_009646	CoGMV	Vietnam	2003
125	<i>Corchorus yellow vein virus</i>	NC_006358	NC_006359	CoYVV	Vietnam	2000
126	<i>Tomato leaf curl New Delhi virus</i>	NC_004611	NC_004612	ToLCNDV	India	1994

Suppl. Table S2. Putative recombination events detected among begomoviruses from the New World, based on the full-length DNA-A.

Events	Breakpoints ^a		Genomic regions	Recombinants	Putative parents		Methods ^b	P-values ^c
	Begin	End			Minor	Major		
1	1517	2599	Rep	ToRMV_NC_002555	ToSRV_NC_009607	ToCMoV_NC_003664	RGBMCS3	4.87E-62
2	2631	1708	Rep/CP	SiYMV_NC_004639	SiMoV_NC_004637	Unknown	RGBMCS3	1.02E-52
3	1952	76	Rep/IR	HyRMV1_BR_Rla5_10A	PSLDV_NC_012786	HyRMV2_BR_Rla6_10A	RGBMCS3	6.09E-43
4	1911	104	Rep/IR	PYMV_NC_001934	Unknown	DaLDV_NC_018717	RGBMCS3	2.86E-41
5	117	1762	CP/Rep	PYMPV_NC_002048	DaLDV_NC_018717	Unknown	RGBMCS3	5.89E-34
6	19	1838	CP/Rep	CabLCuJV_DQ178614	TbYCV_NC_015627	CoYSV_NC_008492 SiGMoV_NC_014130	RGBMCS3	3.96E-32
7	1	1908	CP/Rep	SiGMoV_NC_014130	SiGMBuV_NC_014794	MacGMV_NC_010952	RGBMCS3	4.48E-33
8	1837	2473	Rep	MaYSV_NC_016999	BIYSV_NC_010837	SoCSV_NC_018457 MaYNV_NC_017001	RGBMCS3	2.52E-23
9	1749	24	Rep	CoYSV_NC_008492	MacGMV_NC_010952	OYMV_NC_014066	RGBMCS3	1.10E-22
10	1907	2140	Rep	ToSRV_NC_009607 ToRMV_NC_002555	SimMV_NC_005330 ToLDV_EU710749 ToMMV_NC_010833	SoMBoV_NC_024304 ToYVSV_NC_010949	RGBMCS3	2.08E-20
11	1891	2115	Rep	SiYMoV_NC_016082 ToLCCUV_AM050143	TbYCV_NC_015627	RhRGMV_HM236370 AbMV_NC_001928 CdTV_NC_003830 OYMoIV_AY751753	RGBMCS3	1.54E-18

Suppl. Table S2(cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
						SiGMV_NC_002046 ToYLDV_NC_011348		
12	1768	129	Rep/IR	DaLDV_NC_018717	BChMV_NC_022005 BDMV_NC_001931 SiGMCRV_NC_004657	PYMTV_NC_004638	RGBMCS3	9.01E-17
13	1950	157	Rep/IR	MacMPRV_NC_004097	Unknown	RhMMV_NC_015488	RGBMCS3	3.54E-16
14	1959	2418	Rep	RhMMV_NC_015488 MerMPRV_NC_015490	BChMV_NC_022005 AbMV_NC_001928 BDMV_NC_001931 DaLDV_NC_018717 JaMV_NC_024012 OYMoIV_AY751753 SiGMCRV_NC_004657 SiGMHV_NC_004659 SiGMV_NC_002046 SiYVV_NC_004661 TMoLCV_NC_015122 ToCILDV_NC_015962 ToMHaV_NC_003867 ToMoV_NC_001938 WGMSTV_NC_010948	Unknown	RGBMCS3	5.01E-16
15	2217	2493	Rep	MelYMV_NC_028142	Unknown	MelMV_NC_028140 CeYSV_NC_016998 SdYMV_BR_Deg3_10A	RGBMCS3	5.06E-16

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
15						SiMBoV1_NC_015046 SiMoAV_NC_020256 ToMoWV_NC_025265 ToYSV_NC_007726		
16	1973	2222*	Rep	SiMoV_NC_004637 CeYSV_NC_016998 HyRMV2_BR_R1a6_10A OMoV_NC_011181 OxYVV_NC_026253	Unknown	MaYNV_NC_017001 ToCMoV_NC_003664	RGBMCS3	1.70E-16
17	1878	2593	Rep	SimBV_FN436001	Unknown	HeYMV_BR_Mac7_10A SiMAIV_NC_016573 SiMoAV_NC_020256 SiYBV_NC_020254	RGBMCS3	6.97E-16
18	1925	2397	Rep	ToYMLCV_NC_005852	BChMV_NC_022005 BDMV_NC_001931 SiGMCRV_NC_004657 SiYVV_NC_004661 TMoLCV_NC_015122 ToCILDV_NC_015962 WGMSTV_NC_010948	Unknown	RGBMCS3	1.73E-15
19	1653	2597	Rep	BYMMxV_NC_015487	Unknown	CdTV_NC_003830	RGBMCS3	2.57E-15
20	1955	2373	Rep	RhGMV_NC_010294	BChMV_NC_022005 BDMV_NC_001931 CdTV_NC_003830	Unknown Unknown Unknown	RGBMCS3	4.67E-15

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
20					DaLDV_NC_018717	Unknown		
					OYMoIV_AY751753	Unknown		
					OYMV_NC_014066	Unknown		
					PYMTV_NC_004638	Unknown		
					RhRGMV_HM236370	Unknown		
					SiGMBuV_NC_014794	Unknown		
					SiGMCRV_NC_004657	Unknown		
					SiGMFIV_NC_014446	Unknown		
					SiGMHV_NC_004659	Unknown		
					SiGYVV_NC_004635	Unknown		
					SiYMYuV_NC_008779	Unknown		
					SiYVV_NC_004661	Unknown		
					TbYCV_NC_015627	Unknown		
					TMoLCV_NC_015122	Unknown		
					ToCILDV_NC_015962	Unknown		
					ToLCSiV_NC_009606	Unknown		
					ToMHaV_NC_003867	Unknown		
					ToMLCV_NC_005850	Unknown		
					ToYLDV_NC_011348	Unknown		
					WGMSTV_NC_010948	Unknown		
21	1912	2419	Rep	ToCMoV_NC_003664	PSLDV_NC_012786	BGMV_NC_004042	RGBMCS3	6.01E-17
					CnMLDeV_BR_Mes3_15A	MaYVV_NC_017000		
22	2498	1838	Rep/CP	ToMoWV_NC_025265	ToYVSV_NC_010949	ToLDV_EU710749	RGBMCS3	3.25E-21
23	1891	2308	Rep	ToMLCV_NC_005850	BChMV_NC_022005	Unknown	RGBMCS3	2.72E-14
				MerMV_NC_007965	BChV_NC_019569			
					BDMV_NC_001931			

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
23					DaLDV_NC_018717 OYMV_NC_014066 RhRGMV_HM236370 SiGMCRV_NC_004657 SiGMFIV_NC_014446 SiGMHV_NC_004659 SiYVV_NC_004661 ToLCSiV_NC_009606 WGMSTV_NC_010948			
24	2167	2540	Rep	TrYMV_BR_Msj1_10A	Unknown	HeYMV_BR_Mac7_10A SiMoAV_NC_020256 TrMV_BR_Fle1_10A	RGBMCS3	6.73E-14
25	2308	28	Rep/IR	SiYNV_NC_020253	TrMV_BR_Fle1_10A SiMoAV_NC_020256	ToYSV_NC_007726	RGBMCS3	5.12E-13
26	953	1403	CP/Rep	MacGMV_NC_010952	Unknown Unknown	SiYMAV_NC_020255 SdYMV_BR_Deg3_10A	RGBMCS3	3.69E-13
27	2207	27	Rep/IR	ToLCNDV_NC_004611	ToMMV_NC_010833 AbMBoV_NC_015045 BChMV_NC_022005 BChV_NC_019569 BGYMV_NC_001439 CdTV_NC_003830 CleLCrV_NC_016578	CoGMV_NC_009644 CYVV_NC_006358	RGBMCS3	3.89E-13

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
27					DaChMV_NC_018716			
					DaLDV_NC_018717			
					DiYMoV_NC_003856			
					HeYMV_BR_Mac7_10A			
					JaMV_NC_024012			
					MacGMV_NC_010952			
					MacYMFV_NC_004099			
					MacYMV_NC_010647			
					MelMV_NC_028140			
					OYMoIV_AY751753			
					OYMV_NC_014066			
					PavYMV_NC_029105			
					PhYSV_BR_Rla1_12A			
					PHYVV_NC_001359			
					PYMTV_NC_004638			
					RhRGMV_HM236370			
					SdYMV_BR_Deg3_10A			
					SiCmMV_EU710751			
					SiGMBuV_NC_014794			
					SiGMHV_NC_004659			
					SiMAIV_NC_016573			
					SiMBoV1_NC_015046			
					SiMBoV2_NC_015043			
					SimMV_NC_005330			
					SiMoAV_NC_020256			
					SiYMoV_NC_016082			
					SiYVV_NC_004661			
					SoCSV_NC_018457			
					TbYCV_NC_015627			

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values	
	Begin	End			Minor	Major			
27					ToDfLV_NC_016580 ToLCCUV_AM050143 ToLCSiV_NC_009606 ToLDeV_NC_014510 ToLDV_EU710749 ToMHaV_NC_003867 ToMoLCV_JF803246 ToMoV_NC_001938 ToSRV_NC_009607 ToYLDV_NC_011348 ToYSV_NC_007726 TrMV_BR_Fle1_10A				
28	1915	2186	Rep	SoMBoV_NC_024304 ToYVSV_NC_010949	Unknown	ToDfLV_NC_016580	RGBMCS3	7.37E-13	
29	2019	2412	Rep	TbYCV_NC_015627 SiGMFIV_NC_014446	ToYLDV_NC_011348 BChV_NC_019569 CdTV_NC_003830 JaMV_NC_024012 OYMoIV_AY751753 RhRGMV_HM236370 SiGMBuV_NC_014794 SiGMV_NC_002046 SiGYVV_NC_004635 SiMSiV_NC_008059 SiYMYuV_NC_008779 SiYVV_NC_004661	ToYMoV_NC_019946	RGBMCS3	1.73E-16	

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
29					ToLCSiV_NC_009606 ToMoTaV_NC_001828 ToMoV_NC_001938 WGMSTV_NC_010948			
30	69	1000	CP	ToYLDV_NC_011348	BChV_NC_019569	SiYMoV_NC_016082 RhRGMV_HM236370	RGBMCS3	2.08E-11
31	2371	20	Rep/IR	MerMV_NC_007965 ToMLCV_NC_005850	JaMV_NC_024012 MacGMV_NC_010952 SiMSiV_NC_008059 ToLCCUV_AM050143 ToMoTaV_NC_001828	Unknown Unknown Unknown Unknown	RGBMCS3	3.94E-11
32	2013	2250*	Rep	SiYMV_NC_004639 CnMLDeV_BR_Mes3_15A	CYVV_NC_006358 CoGMV_NC_009644	SiYNV_NC_020253 AbMBoV_NC_015045 AbMV_NC_001928 CoYSV_NC_008492 DaLDV_NC_018717 HeYMV_BR_Mac7_10A MacGMV_NC_010952 MelYMV_NC_028142 PYMTV_NC_004638 RhRGMV_HM236370 SiCmMV_EU710751 SiGMBuV_NC_014794 SiGMCRV_NC_004657 SiGMHV_NC_004659	RGBMCS3	5.87E-14

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
32						SiGMoV_NC_014130 SiGMV_NC_002046 SiMBoV1_NC_015046 SiMoAV_NC_020256 ToCILDV_NC_015962 ToLDeV_NC_014510 ToMMV_NC_010833 TrMV_BR_Fle1_10A		
33	1986	2338	Rep	PHYVV_NC_001359	BDMV_NC_001931 BChMV_NC_022005 DaLDV_NC_018717 OYMV_NC_014066 PYMTV_NC_004638 SiGMBuV_NC_014794 SiGMCRV_NC_004657 SiGMHV_NC_004659 SiYMYuV_NC_008779 TMoLCV_NC_015122 ToCILDV_NC_015962 ToLCSiV_NC_009606	Unknown Unknown Unknown	RGBMCS3	6.71E-11
34	1954	2104	Rep	CoYSV_NC_008492 CabLCuJV_DQ178614 MacGMV_NC_010952 PYMV_NC_001934 SiGMoV_NC_014130	MaYVV_NC_017000 BGMV_NC_004042 SimBV_FN436001	JaMV_NC_024012 AbMV_NC_001928 BChV_NC_019569	RGBMCS3	6.24E-11

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
34						CdTV_NC_003830 OYMoIV_AY751753 RhRGMV_HM236370 SiGMV_NC_002046 SiGYVV_NC_004635 SiMSiV_NC_008059 SiYVV_NC_004661 ToMoTaV_NC_001828 ToMoV_NC_001938 ToYLDV_NC_011348		
35	1920	2248	Rep	SoCSV_NC_018457	PhYSV_BR_Rla1_12A	MaYVV_NC_017000 BGMV_NC_004042 SimBV_FN436001	RGBMCS3	8.01E-11
36	1814	2042	Rep	SiGMHV_NC_004659	SiMAIV_NC_016573 SiYBV_NC_020254	SiYVV_NC_004661 CdTV_NC_003830 JaMV_NC_024012 OYMoIV_AY751753 RhRGMV_HM236370 SiGMV_NC_002046 SiMSiV_NC_008059 ToYLDV_NC_011348	RGBMCS3	1.08E-10
37	1465	1555	Rep	SiCmMV_EU710751	Unknown	CeYSV_NC_016998 HyRMV2_BR_Rla6_10A MelMV_NC_028140 MelYMV_NC_028142	RGBMCS3	1.32E-10

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
37						OMoV_NC_011181 OxYVV_NC_026253 PavYMV_NC_029105 SdYMV_BR_Deg3_10A SiMBoV1_NC_015046 SiMBoV2_NC_015043 SiMoAV_NC_020256 SiMoV_NC_004637 SoMBoV_NC_024304 TrMV_BR_Fle1_10A TrYMV_BR_Msj1_10A		
38	184	1642	CP	OMoV_NC_011181	SimMV_NC_005330	SiMoV_NC_004637 OxYVV_NC_026253 SiYMV_NC_004639 ToYSV_NC_007726	RGBMCS3	1.46E-10
39	2231*	2616	Rep	ToYSV_NC_007726	MelMV_NC_028140	OMoV_NC_011181 OxYVV_NC_026253	RGBMCS3	8.19E-11
40	2453	78	Rep/IR	BleICV_NC_019035	SiMSiV_NC_008059 MacGMV_NC_010952	Unknown Unknown	RGBMCS3	5.13E-10
41	1881	2088	Rep	SiYMYuV_NC_008779 SiGMBuV_NC_014794	Unknown Unknown	SiGMV_NC_002046 AbMV_NC_001928	RGBMCS3	6.49E-10
42	27	622	CP	SiYMAV_NC_020255 MelMV_NC_028140 MelYMV_NC_028142 SdYMV_BR_Deg3_10A	Unknown	SiMSiV_NC_008059 OYMV_NC_014066 SiGMHV_NC_004659 SiGMoV_NC_014130	RGBMCS3	1.42E-09

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
43	2024	2178	Rep	SiMBoV1_NC_015046 TGMV_NC_001507	ToMMV_NC_010833	Unknown Unknown	RGBMCS3	4.68E-09
44	1426	1887*	Rep	RhGMV_NC_010294	Unknown	ToYMoV_NC_019946	RGBMCS3	5.27E-15
45	1447	1709*	Rep	BChV_NC_019569	Unknown	CoYSV_NC_008492 CdTV_NC_003830 OYMoIV_AY751753 OYMV_NC_014066 SiGMBuV_NC_014794 SiGMHV_NC_004659 SiGMoV_NC_014130 SiGMV_NC_002046 SiMSiV_NC_008059 SiYMYuV_NC_008779 SiYVV_NC_004661 ToLCSiV_NC_009606 ToMHaV_NC_003867	RGBMCS3	9.94E-08
46	2416	2542	Rep	SiMSiV_NC_008059 ToMoTaV_NC_001828	JaMV_NC_024012 CabLCuJV_DQ178614 CoYSV_NC_008492	SiGMV_NC_002046 ToMHaV_NC_003867 ToMoV_NC_001938	RGBMCS3	2.66E-08
47	1831	2566	Rep	OYMV_NC_014066	Unknown	CdTV_NC_003830	RGBMCS3	2.45E-10
48	1247	1915	Rep	RhMMV_NC_015488 MacMPRV_NC_004097	CYVV_NC_006358	ToChLPV_NC_005843 AbMV_NC_001928 BGMV_NC_004042	RGBMCS3	7.83E-10

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
48						BIYSV_NC_010837 CleLCrV_NC_016578 CnMLDeV_BR_Mes3_15A HeYMV_BR_Mac7_10A JaMV_NC_024012 MacYMFV_NC_004099 MacYMV_NC_010647 MaYVV_NC_017000 MelMV_NC_028140 MelYMV_NC_028142 OYMoIV_AY751753 PavYMV_NC_029105 PhYSV_BR_Rla1_12A PYMTV_NC_004638 RhRGMV_HM236370 SdYMV_BR_Deg3_10A SiGMFIV_NC_014446 SiGMV_NC_002046 SiGYVV_NC_004635 SiMBoV1_NC_015046 SiMBoV2_NC_015043 SimMV_NC_005330 SiMoAV_NC_020256 SiYBV_NC_020254 SiYNV_NC_020253 SiYVV_NC_004661 TbYCV_NC_015627 TGMV_NC_001507 ToCILDV_NC_015962 ToDfLV_NC_016580		

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
48						ToLDeV_NC_014510 ToLDV_EU710749 ToMHaV_NC_003867 ToMMV_NC_010833 ToMoTaV_NC_001828 ToMoV_NC_001938 ToSRV_NC_009607 ToYLDV_NC_011348 ToYSV_NC_007726 TrMV_BR_Fle1_10A WGMSTV_NC_010948 ToLCSiV_NC_009606		
49	2212*	2305	Rep	MaYVV_NC_017000 BGMV_NC_004042 SimBV_FN436001	Unknown Unknown Unknown	OxYVV_NC_026253 BChV_NC_019569 CdTV_NC_003830 JaMV_NC_024012 OYMoIV_AY751753 PYMTV_NC_004638 RhRGMV_HM236370 ToChLPV_NC_005843 ToMoTaV_NC_001828	RGBMCS3	2.68E-07
50	1957	2102*	Rep	JaMV_NC_024012 AbMV_NC_001928 BChV_NC_019569 CdTV_NC_003830 OYMoIV_AY751753 RhRGMV_HM236370 SiGMV_NC_002046 ToYLDV_NC_011348	Unknown	ToCILDV_NC_015962 TMoLCV_NC_015122	RGBMCS3	3.32E-07

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
50				SiYVV_NC_004661 ToMoTaV_NC_001828 ToMoV_NC_001938				
51	350	622*	CP	SiYMAV_NC_020255	SiMAIV_NC_016573 SiYBV_NC_020254	SdYMV_BR_Deg3_10A SiMBoV1_NC_015046	RGBMCS3	4.11E-07
52	1967	2082	Rep	ToGMoV_NC_008058	BYMMxV_NC_015487	ToChLPV_NC_005843	RGBMCS3	6.02E-07
53	2144	2494	Rep	MacYMV_NC_010647	Unknown	MacYMFV_NC_004099	RGBMCS3	7.52E-07
54	2446	2599	Rep	SiYMoV_NC_016082 RhRGMV_HM236370 ToYLDV_NC_011348	Unknown	SiGMV_NC_002046	RGBMCS3	9.28E-07
55	170*	256	IR/CP	TGMV_NC_001507	RhGMV_NC_010294	SiGMFIV_NC_014446	RGBMCS3	9.32E-07
56	2177*	2461	Rep	TrMV_BR_Fle1_10A HeYMV_BR_Mac7_10A SiMoAV_NC_020256 SiYNV_NC_020253	ToMoV_NC_001938 SiGMCRV_NC_004657 SiGMV_NC_002046	Unknown	RGBMCS3	1.56E-06
57	2504	2616*	Rep	SdYMV_BR_Deg3_10A SiYMAV_NC_020255	JaMV_NC_024012 MacGMV_NC_010952	Unknown Unknown	RGBMCS3	2.74E-07
58	756	1074	CP	ToMoWV_NC_025265	ToSRV_NC_009607	AbMBoV_NC_015045	RGBMCS3	5.57E-06

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
59	2486*	2635	Rep	TrMV_BR_Fle1_10A HeYMV_BR_Mac7_10A SiMBoV1_NC_015046 SiMoAV_NC_020256	Unknown	SiGMFIV_NC_014446 SiGYVV_NC_004635 ToMHaV_NC_003867	RGBMCS3	5.25E-10
60	245	1685	CP/Rep	ToLDV_EU710749	OxYVV_NC_026253	Unknown	RGBMCS3	1.93E-18
61	1357	1516	Rep	RhRGMV_HM236370 AbMV_NC_001928 BChMV_NC_022005 BDMV_NC_001931 BYMMxV_NC_015487 CdTV_NC_003830 CoYSV_NC_008492 DaLDV_NC_018717 JaMV_NC_024012 OYMoIV_AY751753 OYMV_NC_014066 PYMTV_NC_004638 SiGMCRV_NC_004657 SiGMFIV_NC_014446 SiGMHV_NC_004659 SiGMV_NC_002046 SiMSiV_NC_008059 SiYMoV_NC_016082 SiYMYuV_NC_008779 SiYVV_NC_004661 ToLCCUV_AM050143 ToMHaV_NC_003867 ToMoTaV_NC_001828	Unknown Unknown	SiYBV_NC_020254 OxYVV_NC_026253	RGBMCS3	1.07E-05

Suppl. Table S2 (cont.)

Events	Breakpoints		Genomic regions	Recombinants	Putative parents		Methods	P-values
	Begin	End			Minor	Major		
62	2252*	35	Rep/IR	ToMoLCV_JF803246	CnMLDeV_BR_Mes3_15A PSLDV_NC_012786	HyRMV2_BR_Rla6_10A	RGBMCS3	3.86E-06
63	1940	2196	Rep	AbMBoV_NC_015045	ToLDV_EU710749	PavYMV_NC_029105	RGBMCS3	7.73E-07
64	403	1062	CP	MacYMFV_NC_004099 BGYMV_NC_001439 MacYMV_NC_010647	Unknown	PavYMV_NC_029105 AbMBoV_NC_015045	RGBMCS3	1.40E-05
65	2520	2619*	Rep	MacYMFV_NC_004099	SiGYVV_NC_004635	Unknown	RGBMCS3	1.40E-07
66	2486	11	Rep/IR	CnMLDeV_BR_Mes3_15A PSLDV_NC_012786	SiGYVV_NC_004635	BIYSV_NC_010837	RGBMCS3	1.72E-07
67	2407	2536	Rep	JaMV_NC_024012	MacGMV_NC_010952 PYMV_NC_001934	SiGMV_NC_002046	RGBMCS3	5.79E-07
68	1739	1964*	Rep	ToYLDV_NC_011348 BChV_NC_019569 CdTV_NC_003830 RhRGMV_HM236370 ToMoTaV_NC_001828	SiYMV_NC_004639	ToMHaV_NC_003867	RGBMCS3	5.18E-05
69	264*	791	CP	SimMV_NC_005330	SiMBoV2_NC_015043	Unknown	RGBMCS3	1.93E-06
70	166	249	IR/CP	SiGMFIV_NC_014446	Unknown	JaMV_NC_024012	RGBMCS3	4.02E-04

a *, breakpoint could not be precisely pinpointed.

b R, RDP; G, GeneConv; B, Bootscan; M, MaxChi; C, Chimera; S, SisScan; 3, 3SEQ.

c The reported - values are for the methods indicated in red, and they are the lowest *P* values calculated for the region in question.

Suppl. Table S3. Putative recombination events detected among begomoviruses from the New World, based on the full-length DNA-B.

Events	Breakpoint ^a		Genomic regions	Recombinants	Putative parents		Methods ^b	P-value ^c
	Begin	End			Minor	Major		
1	79	667	NSP	OMoV_NC_011182	SiMMV_NC_005331	ToYSV_NC_007727	RGBMCS3	2.31E-31
2	592	1356	NSP	OYMV_NC_014067	CLCrV_NC_004581	BChV_NC_019568	RGBMCS3	4.29E-29
3	2406	2522	IR	PYMV_NC_001935	CoYSV_NC_008493 SiGMoV_NC_014128	PYMTV_NC_004644	RGBMCS3	6.91E-16
4	2391	27	IR	RhGMYuV_NC_012482	ToMYLCV_NC_009491	TbYCV_NC_015628	RGBMCS3	4.31E-14
5	2170	2393*	IR	PYMTV_NC_004644	PYMPV_NC_002049	PYMV_NC_001935	RGBMCS3	1.09E-15
6	2603	73	IR	BChV_NC_019568	SiMBoV2_NC_015047	BChMV_NC_022003	RGBMCS3	4.71E-11
7	102	1416	NSP/MP	SiGMFIV_NC_014447	CoYSV_NC_008493	Unknown	RGBMCS3	6.55E-11
8	2446*	2544	IR	DaLDV_NC_018715	Unknown	PYMTV_NC_004644	RGBMCS3	2.18E-12
9	2074	129	MP/IR	CabLCuV_NC_003887	Unknown	TbYCV_NC_015628	RGBMCS3	5.94E-10
10	288	1538	NSP/MP	SiYMoV_NC_016083	Unknown	CoYSV_NC_008493	RGBMCS3	6.92E-11
11	103	345	IR	SiGMBuV_NC_014795	Unknown	SiYMYuV_NC_008780	RGBMCS3	4.63E-13
12	29	62*	IR	ToRMV_NC_002556	Unknown	ToSRV_NC_009612	RGBMCS3	3.55E-09
13	2137	2298	MP	DaLDV_NC_018715	Unknown	PYMPV_NC_002049	RGBMCS3	4.38E-09
14	1645	1978	MP	CoYSV_NC_008493	DeLDV_NC_008495	SiGMoV_NC_014128	RGBMCS3	1.94E-07

Suppl. Table S3 (cont.)

Events	Breakpoint		Genomic regions	Recombinants	Putative parents		Methods	P-value
	Begin	End			Minor	Major		
15	2643	80	IR	SiMBoV1_NC_015044	SiMAIV_NC_016579	Unknown	RGBMCS3	1.68E-09
16	2102	2414	MP	WGMSTV_NC_010951	BDMV_NC_001930	ToYLDV_NC_017913	RGBMCS3	4.06E-07
17	381	2122	NSP/MP	TbYCV_NC_015628	CarYSYV_NC_022008	PYMTV_NC_004644	RGBMCS3	4.27E-07
18	2387	2491	MP/IR	SiMSiV_NC_008056	Unknown	CdTV_NC_003831	RGBMCS3	5.14E-07
19	69	179	IR	TrYMV_BR_Msj1_10B	MelMV_NC_028141	Unknown	RGBMCS3	4.26E-06
20	2350	28*	IR	MerMV_NC_007966	ToMLCV_NC_005851	Unknown	RGBMCS3	4.82E-11
21	697	2452	NSP/MP	MelYMV_NC_028143	Unknown	OMoV_NC_011182	RGBMCS3	9.70E-27
22	2470*	26	IR	MacYMFV_NC_004100	WGMSTV_NC_010951	CarYSYV_NC_022008	RGBMCS3	9.76E-06
23	736	1345	NSP	SiGMV_NC_002047	BDMV_NC_001930	SiGMBuV_NC_014795 CoYSV_NC_008493 SiYMYuV_NC_008780	RGBMCS3	3.87E-09
24	2459	33	IR	JMV_NC_024013	ToMLCV_NC_005851	Unknown	RGBMCS3	5.51E-06
25	2427*	72*	IR	OYMV_NC_014067	SiMMV_NC_005331	SiYVV_NC_004662 SiGMHV_NC_004660	RGBMCS3	1.04E-05
26	2462	306	MP/IR	ToYVSV_NC_010950	SiMBoV2_NC_015047	Unknown	RGBMCS3	2.49E-12
27	2390	60	IR	TbYCV_NC_015628	ToMoV_NC_001939	Unknown	RGBMCS3	5.50E-05

Suppl. Table S3 (cont.)

Events	Breakpoint		Genomic regions	Recombinants	Putative parents		Methods	P-value
	Begin	End			Minor	Major		
28	1542	2003	MP	CabLCuV_NC_003887	RhMMV_NC_015489	RhRGMV_HM236371 RhGMYuV_NC_012482	RGBMCS3	6.62E-05
29	2464	13	IR	CnMLDeV_BR_Mes3_15B	ToYLDV_NC_017913	CLCrV_NC_004581	RGBMCS3	3.90E-05
30	1045	1285	NSP	SiGMHV_NC_004660 SiGMCRV_NC_004658 SiYVV_NC_004662	RhMMV_NC_015489	SiGMoV_NC_014128 CoYSV_NC_008493 SiGMBuV_NC_014795 SiYMYuV_NC_008780	RGBMCS3	5.86E-04
31	85	222	IR/NSP	MeIMV_NC_028141	MeIYMV_NC_028143	ToYMoV_NC_019947	RGBMCS3	1.62E-04
32	2409	293*	IR	ToDfLV_NC_016581	SiMBoV2_NC_015047	Unknown	RGBMCS3	2.63E-06
33	2278	16*	MP/IR	ToCmMV_NC_010836	TRYLCV_NC_020258	BIYSV_NC_010838	RGBMCS3	6.51E-05
34	1645	2117*	MP	SiGYVV_JX162593	Unknown	SiGMBuV_NC_014795	RGBMCS3	1.17E-05
35	1105	1593	NSP	WGMSTV_NC_010951	AbMV_NC_001929	ToYLDV_NC_017913	RGBMCS3	7.40E-04
36	1114	2090*	MP	ToLCSiV_NC_009605	PYMPV_NC_002049	Unknown	RGBMCS3	3.64E-05
37	42*	1062*	NSP	PYMTV_NC_004644	OMoV_NC_011182 ToYSV_NC_007727	SiGMHV_NC_004660 SiYVV_NC_004662	RGBMCS3	2.04E-04
38	1328	2113	MP	DeLDV_NC_008495	ToMoV_NC_001939	ToYVSV_NC_010950	RGBMCS3	3.33E-06
39	1019	1274	NSP	RhMMV_NC_015489	BChV_NC_019568	Unknown	RGBMCS3	1.29E-04

a * breakpoint could not be precisely pinpointed.

b R, RDP; G, GeneConv; B, Bootscan; M, MaxChi; C, Chimera; S, SisScan; 3, 3SEQ.

c The reported *P*-values are for the methods indicated in red, and they are the lowest *P* values calculated for the region in question.