



This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections).

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Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	<i>2013.015a,bP</i>	(to be completed by ICTV officers)
Short title: New species and revised taxonomy proposal for the genus <i>Begomovirus</i> (Geminiviridae): phylogenetic and pairwise distance analysis using the same approach as implemented for the genera <i>Mastrevirus</i> and <i>Curtovirus</i> in the same family (submitted in 2012 and approved in 2013)		
Modules attached (modules 1 and 9 are required)	1 <input checked="" type="checkbox"/> 2 <input checked="" type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5 <input type="checkbox"/> 6 <input type="checkbox"/> 7 <input checked="" type="checkbox"/> 8 <input type="checkbox"/> 9 <input checked="" type="checkbox"/>	

Author(s) with e-mail address(es) of the proposer:

J.K. Brown jbrown@ag.arizona.edu (on behalf of the Geminiviridae Study Group)

List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at http://www.ictvonline.org/subcommittees.asp . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	Geminiviridae
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ICTV-EC or Study Group comments and response of the proposer:

Date first submitted to ICTV: June 14, 2013
Date of this revision (if different to above):

MODULE 2: NEW SPECIES

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be “unassigned” within a subfamily or family. Wherever possible, provide sequence accession number(s) for one isolate of each new species proposed.

Code	<i>2013.015aP</i>	(assigned by ICTV officers)
To create 100 new species within:		
Genus:	<i>Begomovirus</i>	Fill in all that apply. • If the higher taxon has yet to be created (in a later module, below) write “ (new) ” after its proposed name. • If no genus is specified, enter “ unassigned ” in the genus box.
Subfamily:		
Family:	<i>Geminiviridae</i>	
Order:		
And name the new species: See Table 1 for the species and isolate names.		GenBank sequence accession number(s) of reference isolate: See GB accessions in Table 1.

Table 1. Proposed new species, isolate name, and corresponding GenBank accession number for accessions deposited in the NCBI-GenBank database, since 2009. The sequences and respective Accession number refer to a bipartite DNA-A component or entire monopartite genome (referred to as, DNA-A); the DNA-B component of bipartite type viruses is not taxonomically informative.

Species name	Isolate name	GenBank Access #
<i>Ageratum leaf curl Cameroon virus</i>	Ageratum leaf curl Cameroon virus - [Cameroon:AGFG14:2009]	FR873229
<i>Abutilon Brazil virus</i>	Abutilon Brazil virus - [Brazil:Bahia:2007]	FN434438
<i>Abutilon mosaic Bolivia virus</i>	Abutilon mosaic Bolivia virus - [Bolivia:2007]	HM585445
<i>Abutilon mosaic Brazil virus</i>	Abutilon mosaic Brazil virus - [Brazil:C21]	JF694480
<i>Allamanda leaf curl virus</i>	Allamanda leaf curl virus - [China:Guandong 10:2006]	EF602306
<i>Bean chlorosis virus</i>	Bean chlorosis virus - [Venezuela:La Barinesa 459:2006]	JN848770
<i>Bean yellow mosaic Mexico virus</i>	Bean yellow mosaic Mexico virus - [Mexico:2006]	FJ944023
<i>Bhendi yellow vein Bhubhaneswar virus</i>	Bhendi yellow vein Bhubhaneswar virus - [India:Orissa:2003]	FJ589571
<i>Bhendi yellow vein Haryana virus</i>	Bhendi yellow vein Haryana virus - [India:Haryana:2003]	FJ561298
<i>Bhendi yellow vein India virus</i>	Bhendi yellow vein India virus - [India:Tumkur:OYTumkur:2006]	GU112049
<i>Blainvillea yellow spot virus</i>	Blainvillea yellow spot virus - [Brazil:Coi25:2007]	EU710756
<i>Blechum interveinal chlorosis virus</i>	Blechum interveinal chlorosis virus - [Mexico:Campeche:2011]	JX827487
<i>Centrosema yellow spot virus</i>	Centrosema yellow spot virus - [Brazil:Car1:2009]	JN419002
<i>Chino del tomate Amazonas virus</i>	Chino del tomate Amazonas virus - [Brazil:AM10:2007]	HM357461
<i>Cleome golden mosaic virus</i>	Cleome golden mosaic virus - [Brazil:BA05:2007]	HQ396465
<i>Cleome leaf crumple virus</i>	Cleome leaf crumple virus - [Brazil:C75]	JF694461
<i>Dalechampia chlorotic mosaic virus</i>	Dalechampia chlorotic mosaic virus - [Venezuela:Albarico 1020:2007]	JN848775
<i>Datura leaf distortion virus</i>	Datura leaf distortion virus - [Venezuela:Rubio 933:2007]	JN848773
<i>Eclipta yellow vein virus</i>	Eclipta yellow vein virus - [Pakistan:Faisalabad:2006]	GQ478343
<i>Euphorbia yellow mosaic virus</i>	Euphorbia yellow mosaic virus - Brazil:Goiás:2008]	FJ619507
<i>Hollyhock leaf curl virus</i>	Hollyhock leaf curl virus - [Pakistan:20-4:2006]	FR772082
<i>Jacquemontia mosaic Yucatan virus</i>	Jacquemontia mosaic Yucatan virus - [Mexico:Yucatan:2007]	JQ821386
<i>Jatropha mosaic India virus</i>	Jatropha mosaic India virus - [India:Lucknow:SK2:2009]	HM230683
<i>Kenaf leaf curl virus</i>	Kenaf leaf curl virus - [India:Bhraich:2007]	EU366903

<i>Leonurus mosaic virus</i>	Leonurus mosaic virus - [Brazil:PR88:2008]	JQ429791
<i>Macroptilium golden mosaic virus</i>	Macroptilium golden mosaic virus - [Jamaica:August Town:Wissadula:2009]	EU158096
<i>Macroptilium yellow net virus</i>	Macroptilium yellow net virus - [Brazil:Mur1:2009]	JN418998
<i>Macroptilium yellow spot virus</i>	Macroptilium yellow spot virus - [Brazil:Agf1:2010]	JN419013
<i>Macroptilium yellow vein virus</i>	Macroptilium yellow vein virus - [Brazil:Mac4:10]	JN419021
<i>Malvastrum yellow mosaic Helshire virus</i>	Malvastrum yellow mosaic Helshire virus - [Jamaica:2005]	FJ600483
<i>Malvastrum yellow mosaic Jamaica virus</i>	Malvastrum yellow mosaic Jamaica virus -[Jamaica:179A73:2003]	FJ601917
<i>Malvastrum yellow vein Changa Manga virus</i>	Malvastrum yellow vein Changa Manga virus - [Pakistan:Changa Manga:2009]	FR715681
<i>Malvastrum yellow vein Honghe virus</i>	Malvastrum yellow vein Honghe virus - [China:Yunnan 249:2004]	FN552749
<i>Melon chlorotic mosaic virus</i>	Melon chlorotic mosaic virus - [Venezuela:2009]	HM163576
<i>Merremia mosaic Puerto Rico virus</i>	Merremia mosaic Puerto Rico virus - [Puerto Rico:PR89:1998]	FJ944021
<i>Okra enation leaf curl virus</i>	Okra enation leaf curl virus [India:Sonipat:EL10:2006]	GU111996
<i>Okra leaf curl Cameroon virus</i>	Okra leaf curl Cameroon virus - [Cameroon:GRec17F:2009]	FR717137
<i>Okra mottle virus</i>	Okra mottle virus - [Brazil:okra:2008]	EU914817
<i>Papaya leaf crumple virus</i>	Papaya leaf crumple virus - [India:Nirulas:2007]	HM140368
<i>Passionfruit severe leaf distortion virus</i>	Passionfruit severe leaf distortion virus - [Brazil:LNS2:Passionfruit:2001]	FJ972767
<i>Pepper leaf curl Yunnan virus</i>	Pepper leaf curl Yunnan virus - [China:Yunnan:YN323]	EU585781
<i>Rhynchosia golden mosaic Havana virus</i>	Rhynchosia golden mosaic Havana virus-[Cuba:Havana:28:2007]	HM236368
<i>Rhynchosia golden mosaic Yucatan virus</i>	Rhynchosia golden mosaic Yucatan virus - [Mexico:Rh239-1:2007]	EU021216
<i>Rhynchosia mild mosaic virus</i>	Rhynchosia mild mosaic virus - [Puerto Rico:PR79:1997]	FJ944019
<i>Rhynchosia rugose golden mosaic virus</i>	Rhynchosia rugose golden mosaic virus-[Cuba:Camaguey:171:2009]	HM236370
<i>Rhynchosia yellow mosaic virus</i>	Rhynchosia yellow mosaic virus - [Pakistan:Lahore:2007]	AM999981
<i>Rose leaf curl virus</i>	Rose leaf curl virus - [Pakistan:Faisalabad:2006]	GQ478342
<i>Sida golden mosaic Braco virus</i>	Sida golden mosaic Braco virus-[Jamaica:Liguanea:2008]	JX162595
<i>Sida golden mosaic Backup virus</i>	Sida golden mosaic Backup virus-[Jamaica:St. Elizabeth:2004]	HQ008338
<i>Sida golden mosaic Liguanea virus</i>	Sida golden mosaic Liguanea virus-[Jamaica:1:2008]	HQ009522
<i>Sida golden mottle virus</i>	Sida golden mottle virus - [USA:Florida:2007]	GU997691

<i>Sida mosaic Alagoas virus</i>	Sida mosaic Alagoas virus - [Brazil:C59:2007]	JF694471
<i>Sida mosaic Bolivia virus 1</i>	Sida mosaic Bolivia virus 1 - [Bolivia:2007]	HM585441
<i>Sida mosaic Bolivia virus 2</i>	Sida mosaic Bolivia virus 2 - [Bolivia:2007]	HM585443
<i>Sida mosaic Sinaloa virus</i>	Sida mosaic Sinaloa virus - [Mexico:Guasave:2005]	DQ520944
<i>Sida mottle Alagoas virus</i>	Sida mottle Alagoas virus - [Brazil:Vsa2:2010]	JX871385
<i>Sida yellow blotch virus</i>	Sida yellow blotch virus - [Brazil:Rla1:2010]	JX871380
<i>Sida yellow mosaic Alagoas virus</i>	Sida yellow mosaic Alagoas virus - [Brazil:Vsa3:2010]	JX871383
<i>Sida yellow mottle virus</i>	Sida yellow mottle virus - [Cuba:Sancti Spiritus159-1:2009]	JN411687
<i>Sida yellow net virus</i>	Sida yellow net virus - Brazil:Vic2:2010]	JX871376
<i>Soybean chlorotic spot virus</i>	Soybean chlorotic spot virus - [Brazil:Jai9254:2010]	JX122965
<i>Soybean mild mottle virus</i>	Soybean mild mottle virus - [Nigeria:Sb17:2007]	GQ472984
<i>Sweet potato leaf curl Sao Paulo virus</i>	Sweet potato leaf curl Sao Paulo virus - [Brazil:Sao Paulo:Alvares Machado:2009]	HQ393477
<i>Sweet potato leaf curl South Carolina virus</i>	Sweet potato leaf curl South Carolina virus - [United States:South Carolina:648-B9:2006]	HQ333144
<i>Sweet potato leaf curl Uganda virus</i>	Sweet potato leaf curl Uganda virus - [Uganda:Kampala:2008]	FR751068
<i>Sweet potato mosaic virus</i>	Sweet potato mosaic virus - [Brazil:Brasilia1:2007]	FJ969831
<i>Tobacco leaf curl Pusa virus</i>	Tobacco leaf curl Pusa virus - [India:Pusa:tobacco 10:2009]	HQ180391
<i>Tobacco leaf curl Thailand virus</i>	Tobacco leaf curl Thailand virus - [Thailand:Tomato:2005]	DQ871221
<i>Tobacco leaf rugose virus</i>	Tobacco leaf rugose virus - [Cuba:Havana:2007]	AJ488768
<i>Tobacco mottle leaf curl virus</i>	Tobacco mottle leaf curl virus - [Cuba:Sancti Spiritus:2007]	FM160943
<i>Tobacco yellow crinkle virus</i>	Tobacco yellow crinkle virus - [Cuba:2007]	FJ213931
<i>Tomato chlorotic leaf distortion virus</i>	Tomato chlorotic leaf distortion virus - [Venezuela:Zulia:2004]	HQ201952
<i>Tomato common mosaic virus</i>	Tomato common mosaic virus - [Brazil:Co122:2007]	EU710754
<i>Tomato dwarf leaf virus</i>	Tomato dwarf leaf virus - [Argentina:Pichanal 397:2008]	JN564749
<i>Tomato golden vein virus</i>	Tomato golden vein virus - [Brazil:Ita1220:2003]	JF803254
<i>Tomato leaf curl Anjouan virus</i>	Tomato leaf curl Anjouan virus - [Comoros:Ouani:2004]	AM701758
<i>Tomato leaf curl Cebu virus</i>	Tomato leaf curl Cebu virus - [Philippines:Nueva Ecija:P2-1:2006]	EU487025
<i>Tomato leaf curl Diana virus</i>	Tomato leaf curl Diana virus -	AM701765

	[Madagascar:Namakely:2001]	
<i>Tomato leaf curl Ghana virus</i>	Tomato leaf curl Ghana virus - [Ghana:Akumadan:2006]	EU350585
<i>Tomato leaf curl Hainan virus</i>	Tomato leaf curl Hainan virus - [China:Hainan:HaNHK7:2008]	FN256261
<i>Tomato leaf curl Hanoi virus</i>	Tomato leaf curl Hanoi virus - [Vietnam:Hanoi:Tomato:2010]	HQ162270
<i>Tomato leaf curl Iran virus</i>	Tomato leaf curl Iran virus - [Iran:Iranshahr]	AY297924
<i>Tomato leaf curl Kumasi virus</i>	Tomato leaf curl Kumasi virus - [Ghana:Kumasi:GOTB2-2:2008]	EU847739
<i>Tomato leaf curl Mindanao virus</i>	Tomato leaf curl Mindanao virus – [Philippines:Mindanao:P162:2007]	EU487046
<i>Tomato leaf curl Moheli virus</i>	Tomato leaf curl Moheli virus - [Comoros:Fomboni:2005]	AM701763
<i>Tomato leaf curl Namakely virus</i>	Tomato leaf curl Namakely virus - [Madagascar:Namakely:2001]	AM701764
<i>Tomato leaf curl Nigeria virus</i>	Tomato leaf curl Nigeria virus - [Nigeria:2006]	FJ685621
<i>Tomato leaf curl Oman virus</i>	Tomato leaf curl Oman virus - [Oman:Alb22:2005:	FJ956700
<i>Tomato leaf curl Toliara virus</i>	Tomato leaf curl Toliara virus - [Madagascar:Miandrivazo:2001]	AM701768
<i>Tomato leaf deformation virus</i>	Tomato leaf deformation virus - [Peru:PT1:Tomato:2003]	GQ334472
<i>Tomato leaf distortion virus</i>	Tomato leaf distortion virus - [Brazil:Pda4:2005]	EU710749
<i>Tomato mild mosaic virus</i>	Tomato mild mosaic virus - [Brazil:Pda58:2005]	EU710752
<i>Tomato mosaic leaf curl virus</i>	Tomato mosaic leaf curl virus - [Venezuela:Trujillo:2003]	AY508991
<i>Tomato mottle leaf curl virus</i>	Tomato mottle leaf curl virus - [Brazil:Jai13:2008]	KC706615
<i>Tomato rugose yellow leaf curl virus</i>	Tomato rugose yellow leaf curl virus - [Uruguay:Salto Grande:2009]	JN381819
<i>Tomato yellow leaf distortion virus</i>	Tomato yellow leaf distortion virus - [Cuba:5E17:2007]	FJ174698
<i>Tomato yellow mottle virus</i>	Tomato yellow mottle virus - [Costa Rica:2003]	KC176780
<i>West African Asystasia virus 1</i>	West African Asystasia virus 1 - [West Africa:Asy1:2011]	JF694484
<i>West African Asystasia virus 2</i>	West African Asystasia virus 2 - [West Africa:Asy2:2011]	JF694486
<i>Wissadula golden mosaic virus</i>	Wissadula golden mosaic virus - [Jamaica:St. Thomas:2005]	DQ395343

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
 - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria.**
 - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

1. All have distinctively begomovirus-like genome organization.
2. All are associated with the whitefly vector *Bemisia tabaci* (Genn.)
3. Phylogenetically, they all group within the *Begomovirus* genus. Based on maximum likelihood analysis with best-fit model (GTR+I+G4) selected by Modeltest (Posada, 2008; Guindon *et al.*, 2010; Figure 2). Phylogenetic trees (ML) for which branch length is related to genetic distance and where the reliability of branching is subject to some test of reliability (bootstrapping) (see Excel file, by tab).
4. The PASC approach (pairwise distances between each possible sequence pair under consideration as species/strains) was applied to establish the working cutoff, or upper threshold, for species demarcation. This is the same approach that was applied for the other three genera in the Geminiviridae for which >1 species has been established (see 2012.018a-pP.A.v4.Geminiviridae.pdf available at ICTVonline.org), and for which this step by step method has been made available for use by the geminivirology community (Muhire *et al.*, 2013) for the purpose of producing results that are comparable, regardless of the individual carrying out the analysis, or the DNA sequence under investigation.
5. Three rather weak troughs were evident in the PASC analysis, 94, 91, and 86%. The deepest trough was observed at 86%, however, species that have distinct biological features, mainly symptomatology in the host or origin, could possibly be grouped together if the 86% or 91% cutoff was implemented, though we do not present the 86% results here. The exceptions to the 91% cutoff make it difficult to arrive at a robust conclusion for all sequences/taxa.
6. Most taxa proposed as new species meet the herein proposed <91% (previously, <89%) demarcation criteria when considering the DNA-A component or monopartite genome of the isolate, when implementing a more robust method (than Clustal V) to compute pairwise identity comparisons (one minus Hamming distances of pairwise aligned sequences with pairwise deletion of gaps (Figure 1).

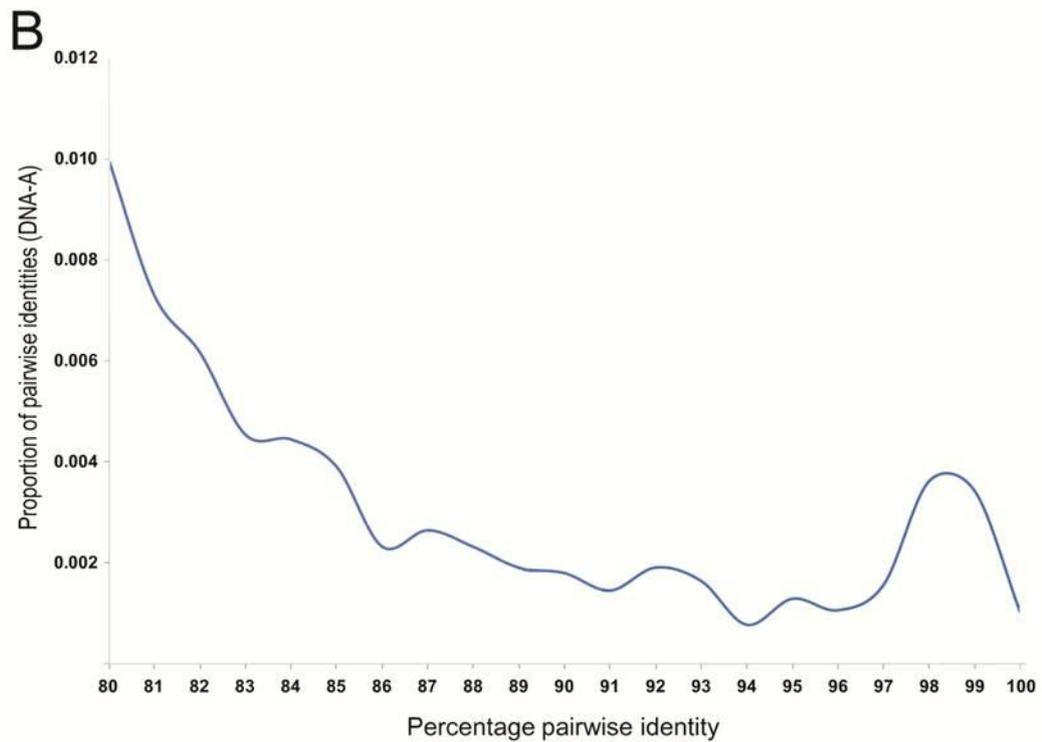
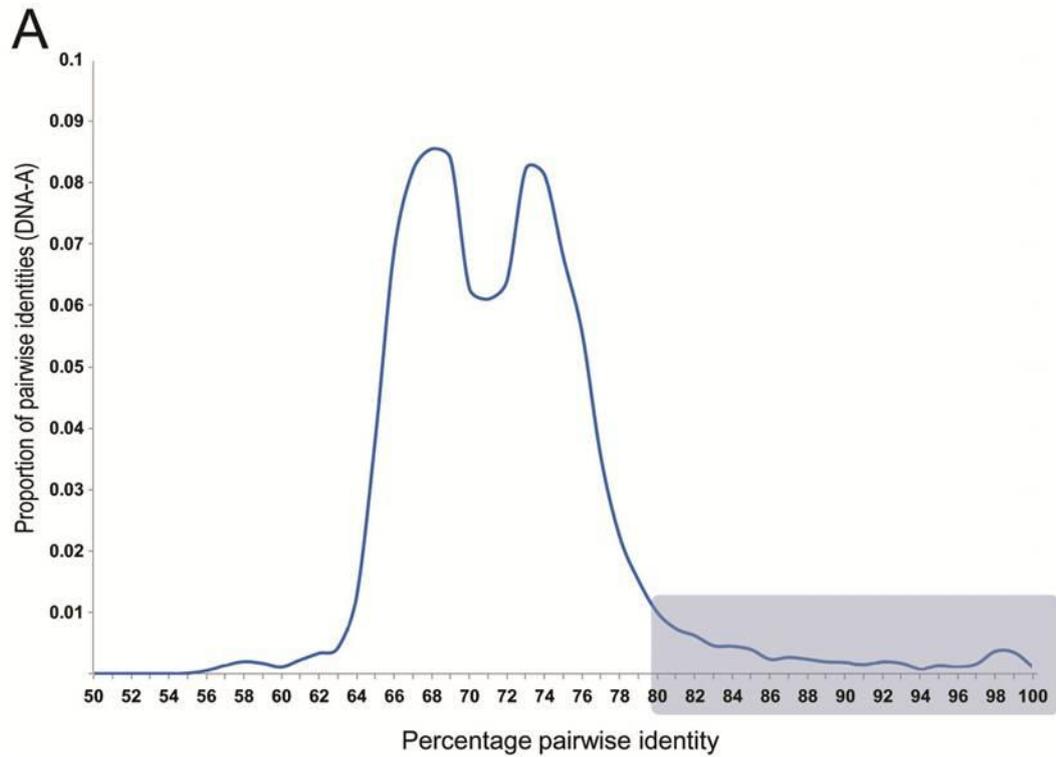


Figure 1. A. Distribution of begomovirus full-length DNA-A component pairwise % nucleotide sequence identity scores. The shaded area is magnified in **B**. The 2,342,530 begomovirus sequence pairs were individually, pairwise aligned to one another using Muscle (Edgar, 2004). The pairwise Hamming distances (equivalent to p-distances calculated in MEGA 5.0) were calculated with pairwise deletion of gaps. Similarity scores were calculated as one minus the Hamming distances. The same calculation can be performed using SDT 1.0 (Muhire *et al.*, 2013).



Figure 2. Maximum-likelihood phylogenetic trees (see Appendix2Trees) based on the complete monopartite genome for monopartite viruses and DNA-A component of bipartite begomoviruses. Bootstrap values (3,000 replications) are represented as filled circles when >60% and as open circles when between 50% and 59%. **A (left)** Old World begomoviruses; and **B (right)** New World begomoviruses.

MODULE 7: REMOVE and MOVE

Use this module whenever an existing taxon needs to be removed:

- *Either* to abolish a taxon entirely (when only part (a) needs to be completed)
- *Or* to move a taxon and re-assign it e.g. when a species is moved from one genus to another (when BOTH parts (a) and (b) should be completed)

Part (a) taxon/taxa to be removed or moved

Code	2013.015bP	(assigned by ICTV officers)
To remove the following taxon (or taxa) from their present position:		
<i>Ipomoea yellow vein virus</i> <i>Sweet potato leaf curl Lanzarote virus</i> <i>Sweet potato leaf curl Spain virus</i> <i>Tomato leaf curl Mayotte virus</i>		
The present taxonomic position of these taxon/taxa:		
Genus:	<i>Begomovirus</i>	Fill in all that apply.
Subfamily:		
Family:	<i>Geminiviridae</i>	
Order:		
If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right		YES

Reasons to justify the removal:

Explain why the taxon (or taxa) should be removed

According to the new species demarcation criteria:

1. *Ipomoea yellow vein virus*, *Sweet potato leaf curl Lanzarote virus*, and *Sweet potato leaf curl Spain virus* are isolates/strains of the species *Sweet potato leaf curl virus*.
2. Isolates of *Tomato leaf curl Mayotte virus* belong to the species *Tomato leaf curl Comoros virus*.

MODULE 9: APPENDICES:

Excel spread sheet showing trees and pairwise distances for all begomoviral taxa included in the analyses (**Appendix I Trees**).

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

How the analysis was performed

1. Phylogenetic analysis. Both neighbor joining and then maximum likelihood analyses were employed.

First, the DNA-A component or monopartite DNA genome sequences were aligned for all unique haplotypes of proposed new species and selected reference sequences (previously accepted species) that grouped with the proposed new species. This was done to reconstruct trees that were manageable in size and legible, given the large number of begomoviral sequences representing a large number of isolates, comprising species and strains.

In Dec. 31, 2012 a total of 3,123 full-length begomovirus sequences were available in the NCBI-GenBank database, corresponding to 283 species according to the 89% species demarcation criteria (for comparison, see the total number reported in the *Geminiviridae* chapter, 9th ICTV Report, which lists 193 species in the genus). To reduce computing time, only the oldest sequences (DNA-A) from groups of sequences that shared >99.5% nt identity were included in the analysis. To the best of our knowledge, the analysis includes all ICTV-recognized and unrecognized begomovirus species for which at least one full-length sequence has been deposited in GenBank (for many there are multiple sequences per species/strain). Using this data set (1,826 sequences), a preliminary phylogeny using the neighbor-joining method was reconstructed (Figure 3) (this figure content had to be reduced to fit the page size; a separate file with the tree at full resolution is provided). The purpose of the NJ analysis was not to reconstruct the final phylogeny, but rather to identify groups of most closely related sequences that could be combined for maximum likelihood analysis.

Based on the NJ trees, 38 groupings were recognized that contained sequences that did not obviously correspond to the same viral species, but also did not obviously correspond to distinct species. This was easier than it sounds, because most trees included several groups of sequences for isolates (haplotypes) of the same species, and so many could be removed to simplify the final analyses. The approach was therefore employed to expedite the alignment and visualization of 'subclade trees' to more easily delineates distinct groups. Some groups comprised as few as 2-3 sequences, whereas, others were represented by >30 sequences (See Figure 4, for examples). It was anticipated that sequences included in the 'test' groups might be more closely relate to one another than to sequences included in the initial groups, and when this was confirmed, they were regrouped and re-analyzed. Details of the analyses are described in the figure legends below.

The maximum likelihood analysis was carried out by aligning the DNA-A or full-length genome sequences using Muscle as implemented in Mega 5.2. The phylogenetic tree was reconstructed using RAxML (Stamatakis, 2006) and 3,000 bootstrap iterations. Bootstrap values (>70%) were placed at major nodes of each tree. The outgroups for sequences that grouped in Old or New World clades were *African cassava mosaic virus* (genus *Begomovirus*), *Tomato pseudo-curly top virus* (TPCTV; genus *Topocuvirus*, family *Geminiviridae*), respectively.

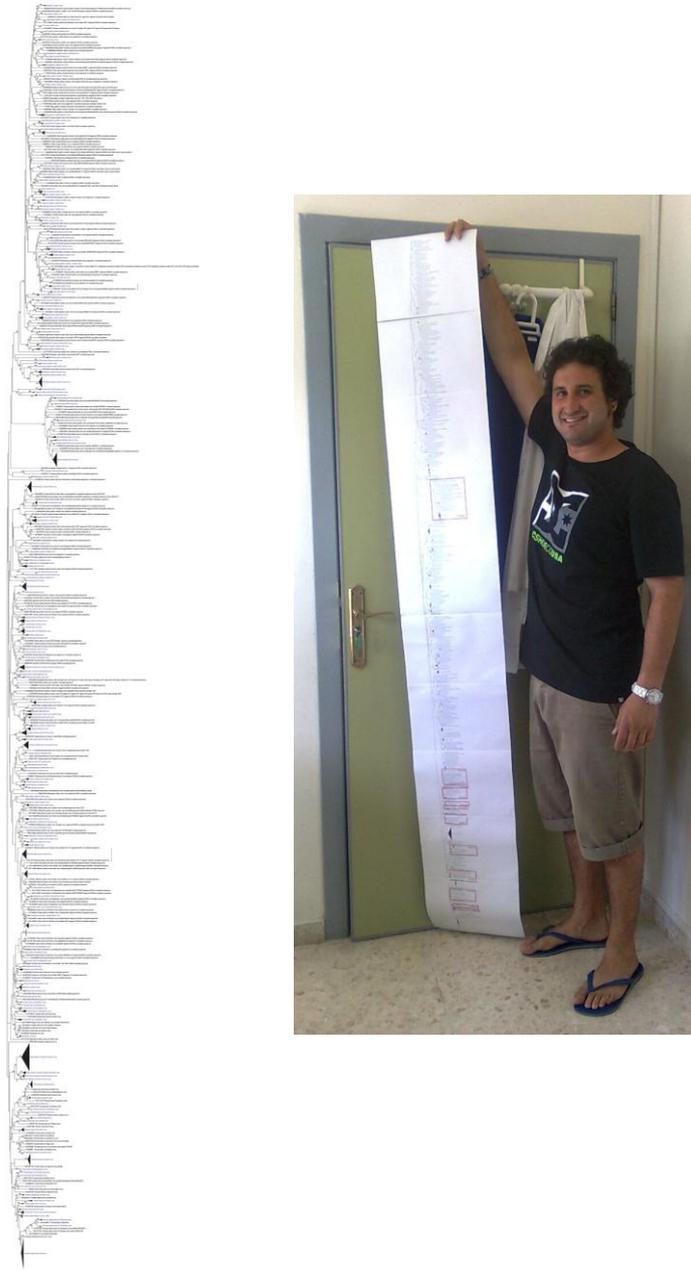


Figure 3. Phylogenetic reconstruction (and student holding the pasted together version) of the genus *Begomovirus* using 1,826 DNA-A sequences available in GenBank on Dec. 31st, 2012; this figure also illustrates the unmanageability of the sequences when all available sequences are aligned and analyzed, necessitating a several step process that requires subdividing the sequences that group together with their closest relatives and conducting multiple, separate analyses.

isolate with which it shares the highest degree of identity"; Muhire *et al.*, 2013). An analysis of each of the 38 groups is available in the accompanying Excel file (Appendix1Trees).

Based on the PASC results, we determined which sequences corresponded to isolates of the same species, and which were isolates of distinct species. For this, we looked for a cut-off value that placed each sequence into a given species without conflicting with threshold (some sequences displayed identity levels above the cut-off value with sequences in two or more groups). Two examples are provided in Figure 5 (the Excel spreadsheet with the analysis of all 38 groups is provided). Also, NJ trees were generated for each group, providing support for the sequence comparisons. Analysis of all 38 groups indicated that the best cut off value was 91%, which is the proposed species demarcation criteria that does not alter the classification of historically 'perceived' species and strains/isolates.

This cut off value yielded a much lower number of sequences that did not conflict with the 91% threshold compared to as 86% or 94%. Phylogenetic analysis resulted in the clustering of isolates as the same species defined by the PASC analysis, among the in the 38 groups. The 91% cut off value appears to be conservative, as is indicated by the trees for groups 3, 5, 7, 11, 16, 30 and 33 (please see the accompanying Excel file). However, several groups of sequences (1, 2, 6, 27, 34 and 36 in the accompanying Excel file) will require further analysis because the PASC and phylogenetic results are conflicting (possibly due to recombination), and so the SG is not proposing new species in these cases at this time to allow further study. We realize that if there are many of these instances (exceptions) and/or recombination cannot be documented as the cause for the conflict for those we do have then it will not be possible to satisfy the proposed species threshold.

To contend with outliers, the same approach was adopted as that described by Muhire *et al.* (2013) for the genus *Mastrevirus*: "The new isolate should be considered as belonging to the species containing the isolate with which it shares the highest degree of identity." Of course any cut off value will yield a number of outliers. By adopting the four conflict-resolution criteria established out by Muhire *et al.* (2013), all outliers were easily placed into species, and we are confident that any future outliers will be as well.

2. The new species demarcation criteria of <91% nt sequence identity (DNA-A) is more stringent than the previous one (<89%).

At first, the higher value, at 91%, compared to the previously accepted 89% working cutoff for species demarcation, gives the impression of a more relaxed species demarcation, which might be expected to results in an even greater number of begomovirus species. However, this was not found to be the case. Rather, the pairwise cut off value at 91% is a consequence of the implementation of a more robust approach (and standardized for the entire Geminiviridae) for calculating pairwise identities: true pairwise alignments (compared to global alignment-based pairwise identities), with the absence of gaps. In practice, 91% is more stringent than the previous analysis that yielded a working cut-off of 89%, which employed a multiple sequence alignment with each gap treated as a character. Therefore, the species proposed herein accurately reflect an unexpected extent of biodiversity of this group of ssDNA plant viruses. In addition, the SG has adopted the standardized use of a powerful suite of algorithms developed to uniformly evaluate viral diversity e.g. biodiversity (Haible *et al.*, 2006). One group that has been affected most by applying the revised analysis are the swepoviruses, or a divergent grouping of sequences, that share in common the ability to infect sweet potato, known as the "sweepoviruses". Previously, the group was proposed to contain 17 species (Albuquerque *et al.*, 2012). The new system reduces the number of species by more than half, by delineating a total number of 8 species (see "Group 16" in the accompanying Excel file).

3. Results of pairwise sequence comparisons for delimiting species often reflected viral biology (when known).

It has been said that begomovirus species are "artificial" because they are defined based on sequence alone. In other words, they do not reflect the biology of these organisms. This is a misconception. Sequence-based taxonomy is possible only because it relies on the knowledge of the biological properties of the viruses. Therefore, sequence comparisons accurately reflect the biology of begomoviruses (as well as that of all viruses). Several examples can be brought forward to make this case. One well-known example involves bean golden mosaic, an important disease of beans in Latin America. The disease is caused by at least two different well-characterized begomoviral species, *Bean golden mosaic virus* (BGMV), from Brazil and *Bean golden yellow mosaic virus* (BGYMV) from Central America and the Caribbean Basin. The symptoms of the disease are indistinguishable, the whitefly vector is the same for both pathogens, and the economic importance with respect to crop loss is comparable. In fact, initially the disease was thought to have identical etiology. However, when the causal agents were sequenced from plants collected in Puerto Rico (USA) and from Brazil the results indicated that they were different viruses (Gilbertson *et al.*, 1991, 1993). Eventually, it was demonstrated that the two agents differed in at least one relevant biological property: tissue tropism. BGMV is phloem-restricted in bean, while BGYMV is not. The previously implemented 89% species cutoff provided a robust and reliable measure to separate the different species. In this proposal, the SG has shown that the previous cut-off of 89% that used older, now less-robust algorithms for pairwise distance analysis, was accurate in the context of the then available tools for PASC, and that the outcome of the approach using what is considered more robust, produces nearly the same results, with the slight modification, at 91%.

The most obvious benefit of the new algorithm is that there are fewer species and strains at the interface between the cut-off and the next lower or higher % nt identity. As such, applying the proposed 91% cut off will facilitate d reliability owing to the robust stringency.

In addition, the relationships depicted by the PASC analysis, were supported by phylogenetic analysis using ML (or Bayesian analysis when carried out; data not shown).

4. Why so many begomoviruses?

The genus *Begomovirus* has the largest number of species among all genera of plant-, animal- and prokaryote-infecting viruses. The 9th Report lists 192 species, and we are proposing the creation of another 96. Why so many? Are these species "artificial", the result of a flawed taxonomic structure? This is the opinion of many highly respected virologists. However, the existence of this large number of species can be explained by natural order relationships and based on the characteristics of this genus that set it apart from many other viral genera.

Begomoviruses are transmitted by a cryptic whitefly species that colonizes herbaceous hosts (unusual among whiteflies), the "whitefly" sibling species group *Bemisia tabaci* (Genn.) (Brown, 2010; Brown *et al.*, 1995; Gill and Brown, 2010). This (cryptic) sibling species group is distributed worldwide and occurs on plant species belonging to many plant families. *B. tabaci* has emerged as a major threat to agricultural systems in many world regions, particularly since the 1970-1980's (Brown, 1990; 1994; Brown and Bird, 1992), and reports of begomovirus infection have often followed unprecedented *B. tabaci* infestations that result in outbreaks of previously undescribed viruses, and the apparent extinction of others (Brown, 2007). Because *B. tabaci* colonizes so many plant species (Gill and Brown, 2010), it potentiates the transfer of begomoviruses between non-cultivated and cultivated hosts (most studied to date by plant virologists). That most begomoviruses isolated from cultivated hosts have most probably evolved

from viruses originally occurring in a non-cultivated host, is beyond the scope of this proposal, but the observations go a long way to add credibility to multiple mechanisms that can be invoked to explain the existence of a currently large number of extant begomoviruses, larger than would seem to make sense, if host-virus-vector 'equilibrium' had been reached (co-evolved to a baseline or 'prudent' number of sustainable complexes).

In addition, the number of begomoviruses detected in non-cultivated hosts has increased significantly since the mid-2000, and a number of the novel species have been detected in these unmanaged, endemic or exotic, more recently established plant hosts.

Why so many isolates?

Geminivirologists (anyone working with small circular DNA genomes) gained powerful new tools to sample virus populations: rolling circle-amplification (RCA), and more recently, deep sequencing (NGen) approaches.

The impact of RCA in the field of geminivirology cannot be overstated (for example, see Haible *et al.*, 2004; 2006). It deemed possible to amplify the complete genome of *any* begomovirus from minute amounts of total plant DNA extracted under suboptimal conditions. Presently, tissue samples can be collected, press-dried, and stored for months at room temperature, and complete begomovirus genomes will be readily amplified using RCA following a quick DNA extraction. In the 1990's it took months to clone one full-length begomovirus genome, whereas, 100 genomes can now be cloned in two weeks time. Because RCA uses random non-sequence specific primers, it is expected that minimal bias will occur in reactions run to detect of most or all molecules present in the sample. As a result, new begomoviruses and more distantly related geminiviruses have been discovered that will probably lead to the creation of additional genera in the family (for example, Krenz *et al.*, 2012; Loconsole *et al.*, 2012).

Likewise, the extent of diversity within this genus is expected to represent the tip of the iceberg. Metagenomics is becoming increasingly affordable, and will inevitably lead to the discovery of, literally, hundreds of new virus families, not to mention species (Edwards and Rohwer, 2005; Ng *et al.*, 2011).

6. Different cut off values must be used for the different genera in the *Geminiviridae*.

The approach implemented to demarcate species in the genus, *Begomovirus* was the same as that used and approved by the ICTV for the genera, *Becurtovirus*, *Curtovirus*, and *Mastrevirus* (see 2012.018a-pP.A.v4.Geminiviridae.pdf available at ICTVonline.org). For each genus, the working cutoff often differed by genus for species demarcation, even though the method applied to determine the cut-off was the same. For example mastrevirus species are demarcated using a 78% cut off was used to separate species, whereas, for the begomoviruses this cut off was determined to be 91%. It should be pointed out that this value was arrived at, following an exhaustive analysis using dozens of groups of begomoviral isolates, as explained above. The 78% species cutoff value for the mastreviruses is demonstrated in the pairwise distance distribution plot (Figure 6), in which a clear "valley" is apparent at the 78% frequency. Such a "valley (trough)" was observed in the vicinity of 91%, in the pairwise frequency plot for the begomoviruses (Figure 1B). We further conducted analysis of all haplotype sequences (proposed new species and reference species), two taxa at a time. This analysis also identified a 91% threshold, or working species cutoff, that best separate the species in the genus, and these groupings proved to be phylogenetically well supported (Figure 2; see also Appendix 1 Trees).

Several other genera have species thresholds somewhat near, but still, lower than begomoviruses, including Parvoviruses (85%), Microviridae (80-85%), Sobemoviruses and Picornas (60-85),

Pospiviroidae (80-85%). It appears that a uniform approach for computing the thresholds does not exist and that various groups use different algorithms, sometimes, for specific genes or sets of genes, instead of complete genomes, and other times, by amino acid comparisons. The Geminivirus Study Group was among the first to implement PASC to establish pairwise comparison graphs, and relate the percent nucleotide identity values to phylogenetic groupings.

The previous calculated threshold for begomovirus species demarcation, at 89%, was calculated using Clustal V (previously, earlier versions when initially introduced), our current threshold of 91% is calculated using the same algorithm that was applied to the other genera in the family, and the latter result produced a tighter distribution than did the Clustal analysis (as anticipated, owing to the robustness of algorithms and computational capacity available very recently). Even though the threshold is relatively high, in comparison to other virus genera and family averages, the resultant groups make phylogeographical sense. Important caveats that the SG cannot effectively address besides insect vector, genotype, and major host type, is that the biology of the majority of begomovirus species is not thoroughly understood (host range for a main example), symptom phenotype can be caused by a single amino acid, and the same phenotype can be caused by different viruses or mixture of them in the same plant, and other such factors that could be drawn upon to recognize the 'distinctiveness of a species'.

7. A step-by-step approach for classifying new begomovirus species, strains, and isolates.

Approval of the approach and therefore of the species defined herein, will lead to the adoption of a standardized step-by-step means of determining the taxonomic placement of unique, newly discovered begomoviral species, strains, and isolates. Taxonomic classification will implement the "protocol" established by Muhire et al. (2013) for calculating pairwise identities of mastreviruses, together with a phylogenetic evaluation (NJ, but preferably a ML or Bayesian algorithm). The establishment of a standardized approach such as that already proposed for one genus of the Geminiviridae will "substantially minimise the degree of classification inconsistency that is permissible under the current system", by providing clear and easy-to-follow guidelines to calculate pairwise sequence identities.

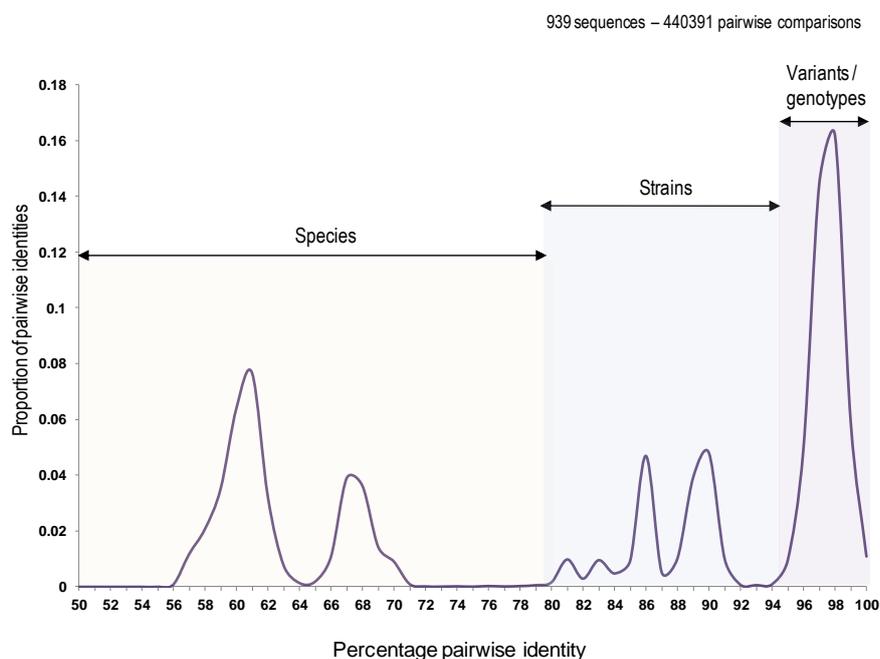


Figure 6. Distribution of mastrevirus full genome pairwise sequence identity scores. Note the "valley (or trough)" at 72-78%.

Specific responses to the ICTV Review Committee 2009-2012 proposal reviews.

Species thresholds sampled across other virus families.

Several other genera have species cut-offs somewhat near but lower than the begomoviruses, including Parvoviruses (85%), Microviridae (80-85%), Sobemoviruses and Picornas (60-85), Pospiviroidae (80-85%). By what method are these values calculated? The previously calculated threshold for begomoviruses, at 89%, was calculated using Clustal, our current threshold of 91% is calculated using the same algorithm that was applied to the other genera in the family, and produces a tighter distribution than did the formerly implemented Clustal V analysis. Even though the threshold is high, it seems to make biological and geographical sense. One cannot support combining biologically different viruses into one species when host range, together with symptomatology, distribution, and other such factors differ. This would result in arbitrary groupings that do not have biological relevance.

Finally, it is not true that the Geminiviridae thresholds (genera) are 'out of step' with those observed for other virus genera/families. The threshold for the genus, *Begomovirus* is 'out of step' both with the other genera in its own family, and with other families. We do not even understand why the threshold is not more like those computed for their closest relatives, the first step in illuminating the answer to this excellent question. Above, we speculate that they are in a 'hyper-diversification' state, and further, provided some reasons based on sound observations why this could be so; this hypothesis is based on numerous lines of evidence. Only time and additional sampling will expose additional information about population structure, differentiation rates, and whether the number and diversity of species will remain high as it is now, or if future environmental shifts will alter differentiation, perhaps slowing it, while at the same time a large number of species will become extinct faster than new ones are being established/selected.

Recombination.

The Committee indicated that viruses whose sequences have undergone (predicted) recombination can be used to infer on those grounds, that they are therefore the same species, is not valid for asexually producing organisms such as viruses. This assumption /criterion has been considered valid for sexually reproducing species, but not for asexually reproducing species. In any case, the ability to identify overtly and subtly recombinant fragments in viral genomes cannot not be ascertained with much certainty using any existing program, particularly, for a data set as large as the begomoviruses, even if only unique haplotypes were considered. Even so, using the most robust programs available, we have obtained different results, depending on the particular algorithm used. Further, there seems to be no logic in the statement that recombination is a main confounding factor behind the resultant computed 89 (previously) – 91% cutoff (tough between peaks). How could this statement be proven or disproven? Certainly, it is agreed that recombination signals in sequences can be confounding in both pairwise comparisons and phylogenetic reconstructions. Per above, in addition to getting different results with different algorithms, the removal of recombinatorial fragments and alignment of the viral backbone sequence does not always yield a better-resolved tree. This suggests other factors are in play including perhaps previous recombination events or mutations that confound the algorithms and/or are not detectable with statistical confidence.

Evolution-real time is never equitable across all virus groups or all groups within a particular environment.

The SG hypothesizes that the extant evolutionary timeframe reflects an unusual period of ‘hyper differentiation’ for the genus, *Begomovirus*, which prior to 1970’s was not represented by a particularly unusual number of perceived species or strains, when compared to other groups of plant viruses, regardless of the degree to which any group was well studied, taxonomically. We propose that the hyper differentiation of begomoviruses into a large number of discrete species, as is reflected by the results of pairwise comparison analysis, could be due to a confluence of short-term ‘positive’ effects overriding influenced by human activities that have resulted in increased whitefly vector population sizes and prevalence in agricultural and urban-agricultural interface systems. Not only has this scenario influenced the selection of robust vector haplotypes/biotypes, but also these unique, differentiating vector haplotypes are shaping begomovirus population structure and concomitant diversification across the genus. The degree to which these patterns of differentiation will continue to occur is unpredictable, but it seems that each group of organisms has passed through various scenarios of flux and change, at times diversifying extensively, while in other scenarios, surviving as bottlenecked or isolated but repeatedly founding populations, or at the other extreme, becoming extinct. Therefore it is not possible to state unequivocally how many species of any organism should be represented at one time in one space, therefore, underscoring the greater importance of keen observations that give rise to hyper-diversification over extinction, and the use of the dynamic range of rates and mechanisms to study virus diversification, selection, and evolution.

Among examples of factors that have affected whitefly vector-begomovirus diversification and genomic differentiation are those that influence the ability of virus-vector complexes to thrive in habitats proximal to human activities, including haplotypes adept at host-range and host-shifting, benefit from long-distance transport and ability to establish in new environments where they may have escaped their predators and pathogens, or the propensity to develop insecticide resistance. Also witnessed are haplotypes that exhibit increased fitness traits enabling their persistence specifically in monoculture (cassava), mixed cropping (cotton-vegetable), at the urban-rural interface (food/fiber-horticultural/ornamental), or in controlled environment production systems (artificial, year-round). In addition, rapid diversification is clearly fostered by the decline of natural habitats that once supported benign whitefly vector haplotypes, making these niches hyper-vulnerable to invasion by exotic or emerging, endemic vector and virus haplotypes.

In addition begomovirus population structure has clearly been shaped by host plant genetic-viral interactions, including the generalized, widespread practice of growing genetically uniform varieties (low diversity, lowered disease/stress resistance replaced by agronomic or desirable horticultural traits), or for example, the introgression of virus resistance genes that stimulate viral diversification to overcome host resistance barriers (*Cotton leaf curl virus* complexes, Pakistan-India). Finally, the timeframe over which begomovirus-whitefly complexes have become widely prevalent is literally, ‘within our lifetime’, coinciding with the onset of larger-scale environmental alterations, the massive expansion of cultivation of arable lands, and climate-related changes.

The SG predicts that we are witnessing an unusual rampant diversification/speciation in light of these changes that will present the opportunity for a comparative approach over time, during which continued sampling, as has been ongoing and accelerating since approximately the 1900’s, will reveal a point at which species number will decline and reach an equilibrium so to speak, much as we envisions genera such as the genus, *Curtovirus* (working species cut-off @ 75%) that has a much lower frequency of differentiation than the genus *Begomovirus*, when the same approaches are employed for PASC and phylogenetic analyses.

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