This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

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:	***2015.012a-dP***	(to be completed by ICTV officers)

Short title: 1 new species in a new genus in the family *Alphaflexiviridae* order *Tymovirales*
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 10 are required)	**1** ☒	**2** ☒	**3** ☒	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☐	**10** ☒

Author(s):

Mike Adams & Jan Kreuze on behalf of the Flexiviruses SG

Corresponding author with e-mail address:

Jan Kreuze, j.kreuze@cgiar.org

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)	Flexiviruses SG

ICTV Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV:	June 2015
Date of this revision (if different to above):	July 28, 2015

ICTV-EC comments and response of the proposer:

EC comment: In figure 1, the drawing at the top of the figure could be clarified to remove the impression of a white box in the +1 frame.

Response: Done.

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	***2015.012aP***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Platypuvirus* (new)**
Subfamily:	
Family:	***Alphaflexiviridae***
Order:	***Tymovirales***

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.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Donkey orchid symptomless virus	Mariginiup11	KC923234

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

Donkey orchid symptomless virus (DOSV) was initially identified using a high-throughput sequencing approach from asymptomatic plants of Australian terrestrial orchid *Diuris longifolia* (Common donkey orchid) growing in a remnant forest patch near Perth, western Australia. DOSV was identified in two *D. longifolia* plants of 264 tested, and from at least one plant of 129 *Caladenia latifolia* (pink fairy orchid). The complete sequence of isolate Mariginiup11 was then confirmed by sequencing of RT-PCR products and RACE to determine the genome extremities. The genome of 7838 nt (KC923234; Wylie et al., 2013) was predicted to encode seven proteins of apparently disparate origins (Annex Fig. 1). A 69- kDa protein (ORF1) that overlapped the replicase shared low identity with MPs of tymoviruses (*Tymoviridae*). A 157-kDa replicase (ORF2) and 22-kDa coat protein (ORF4) shared 32% and 40% amino acid identity, respectively, with homologous proteins encoded by members of the family *Alphaflexiviridae*. A 44-kDa protein (ORF3) shared low identity with myosin and an autophagy protein from Squirrelpox virus. A 27-kDa protein (ORF5) shared no identity with described proteins. A 14-kDa protein (ORF6) shared limited sequence identity (26%) over a limited region of the envelope glycoprotein precursor of mammal-infecting Crimea-Congo hemorrhagic fever virus (*Bunyaviridae*). The putative 25-kDa movement protein (MP) (ORF7) shared limited (27%) identity with 3A-like MPs of members of the *Tombusviridae* and *Virgaviridae*. In inoculation experiments DOSV systemically infected *Nicotiana benthamiana* plants causing leaf distortion, mosaic and stunting; infection was confirmed by RT-PCR and sequencing. The structure and organization of the domains within the putative replicase of DOSV suggests a common evolutionary origin with 'potexvirus-like' replicases of viruses

within the *Alphaflexiviridae* and *Tymoviridae*, and the CP appears to be ancestral to CPs of allexiviruses (*Alphaflexiviridae*). The MP shares an evolutionary history with MPs of dianthoviruses, but the other putative proteins are distant from plant viruses.

In a separate study, a further isolate of a similar virus (Capel isolate) was discovered in another wild Australian orchid, *Drakaea elastica*. Its genome was completely sequenced (KP760463) and amino acid sequence identities for the replicase and CP with the corresponding regions of the genomes of DOSV isolates were 78 and 87%, respectively. These values are only marginally below (replicase) or above (CP) the species demarcation limit (80% identity) used for viruses within the family *Alphaflexiviridae*, so it seems best to treat this as another isolate of the same species (Ong et al., 2015).

This virus is not easy to classify but clearly belongs in the order *Tymovirales*. The replicase could be considered as an outlier in the family *Alphaflexiviridae* (Annex Fig. 2) and the CP is related to those of members of the same family (Annex Fig. 3). Until more related viruses are discovered it therefore seems best to place it as the only species in a new genus in the family *Alphaflexiviridae*.

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2015.012bP***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Alphaflexiviridae***
Order:	***Tymovirales***

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 family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2015.012cP***	(assigned by ICTV officers)

To name the new genus: *Platypuvirus*

Assigning the type species and other species to a new genus

Code	***2015.012dP***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Donkey orchid symptomless virus

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

1

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

See module 2: this virus is quite distinct in genome organization and the phylogeny of the replicase from any of the existing genera.

Origin of the new genus name:

From platypus because it appears to combine features of different virus lineages.

Reasons to justify the choice of type species:

Only member

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

Not applicable

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Ong JWL, Phillips RD, Dixon KW, Jones MGK, Wylie SJ (2015). Characterisation of the first two viruses described from wild populations of hammer orchids (*Drakaea* spp.) in Australia. Plant Pathol., in press, DOI: 10.1111/ppa.12396

Wylie SJ, Li H, Jones MGK (2013) Donkey Orchid Symptomless Virus: A Viral 'Platypus' from Australian Terrestrial Orchids. PLoS ONE 8(11): e79587. doi:10.1371/journal.pone.0079587

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.

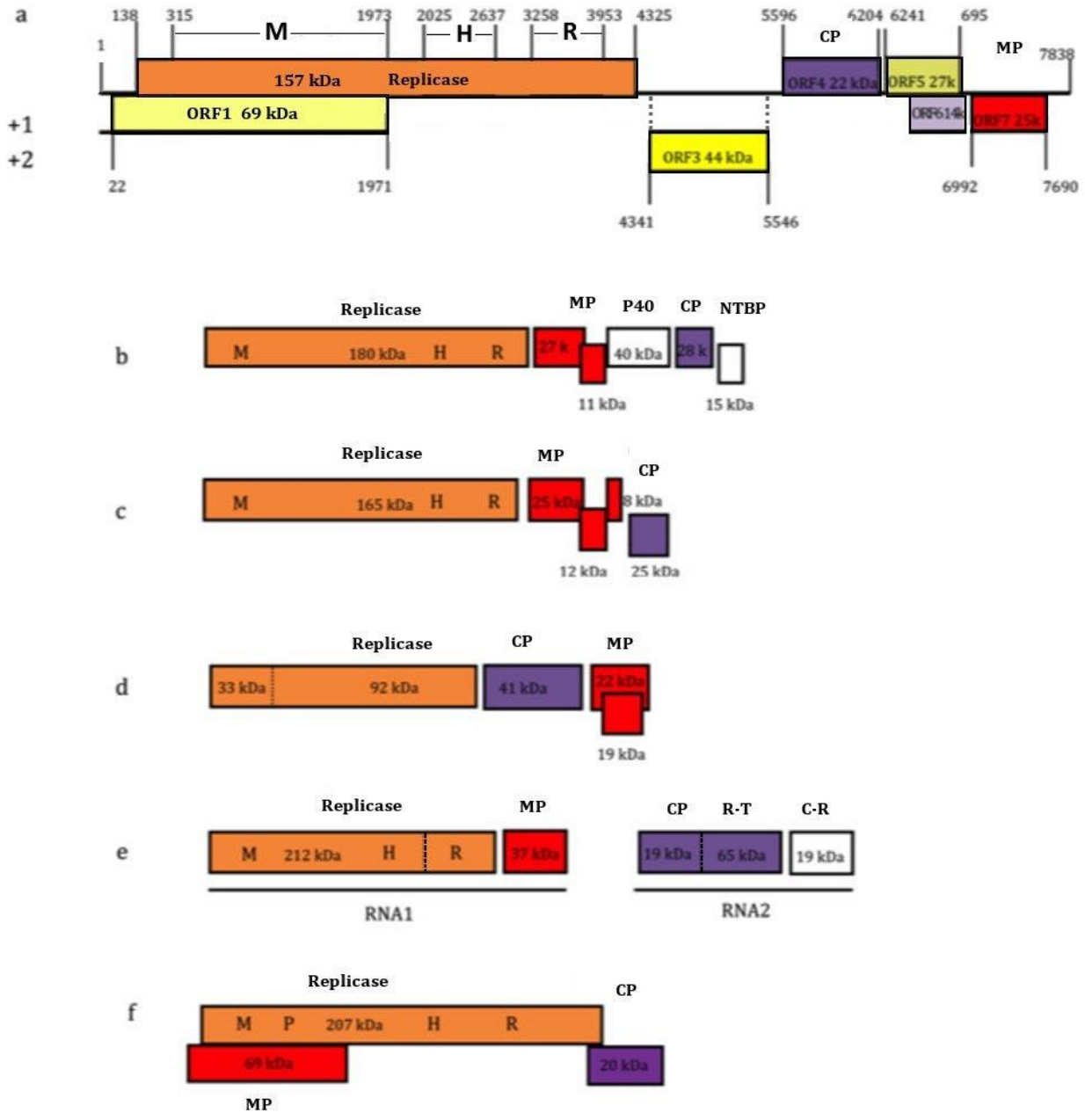

Figure 1. Genome organization of Donkey orchid symptomless virus. Genome organization of Donkey orchid symptomless virus isolate Mariginiup11 (a), showing the nucleotide positions of open reading frames (ORF) including putative coat protein (CP), movement protein (MP), and untranslated regions (UTR), and calculated sizes of putative proteins. Open reading frames were designated with respect to the replicase (ORF1). Diagrams of genome organization of (b) *Garlic virus A* (family *Alphaflexiviridae*, genus *Allexivirus*) encodes a 40-kDa protein (P40) and nucleotide binding protein (NTBP), (c) *Plantago asiatica mosaic virus* (*Alphaflexiviridae*, *Potexvirus*), (d) Tomato bushy stunt virus (TBSV) (*Tombusviridae*, *Tombusvirus*), (e) Sorghum chlorotic spot virus (SCSV) (*Tombusviridae*, *Furovirus*), and (f) *Turnip yellow mosaic virus* (*Tymoviridae*, *Tymovirus*) are shown (not drawn to scale). Within the replicase, the papain-like protease (P), methyltransferase (M), helicase (H), and RNA-dependent RNA polymerase (RdRp) domains are indicated where present. In TBSV and SCSV, read-through opal stop codons are indicated by a dotted line, R-T = read-through region, and C-R = cysteine-rich protein. Adapted from Wylie et al., 2013.

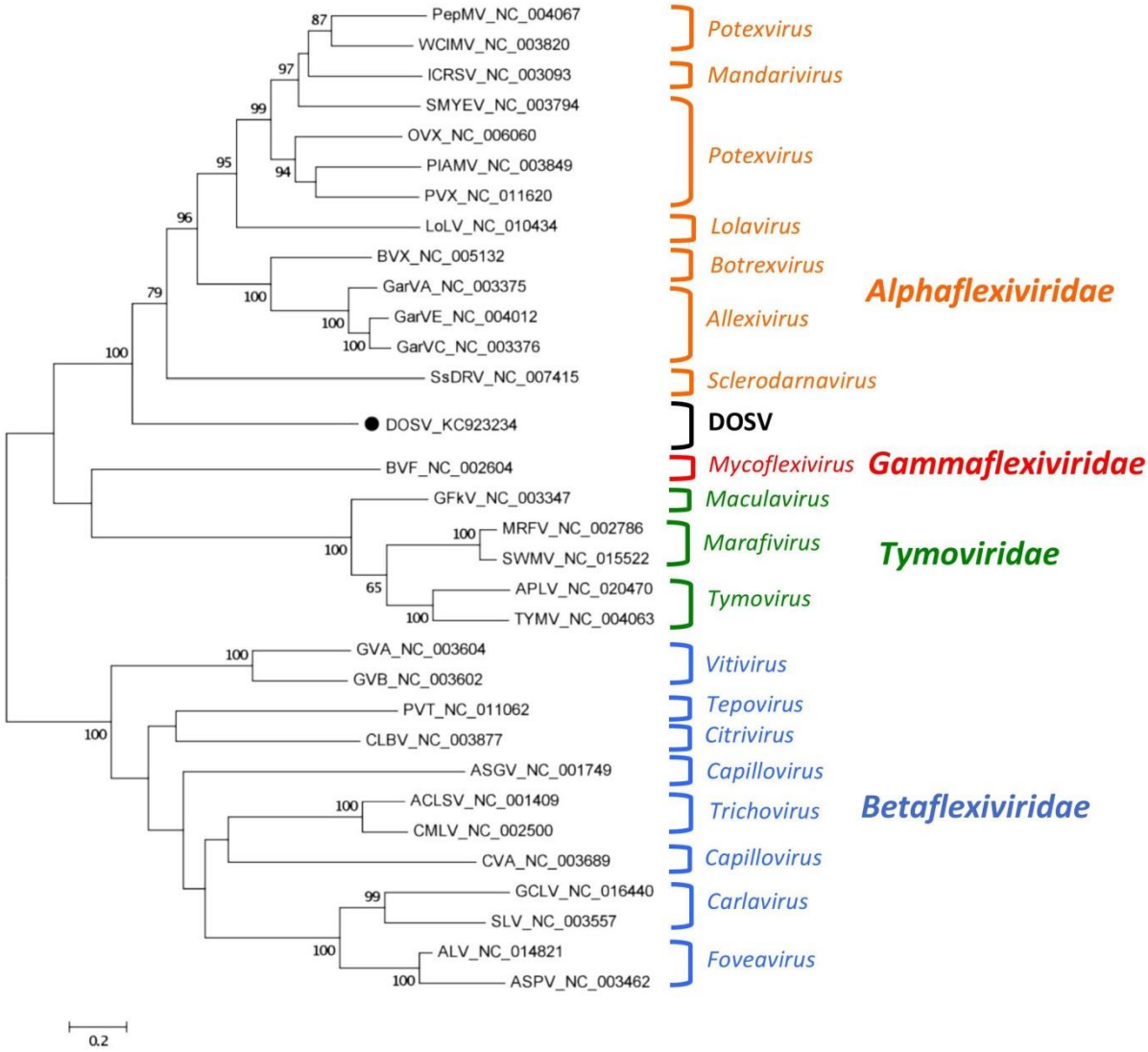

Figure 2. Phylogenetic relationships between the donkey orchid symptomless virus (DOSV) replicase and homologous proteins of representative viruses within the order *Tymovirales*. Relationships were inferred for amino acid sequences using the Maximum Likelihood method. The abbreviated names of the viruses used and their accession codes are shown. Genus and family names are given on the right. ACLSV, *Apple chlorotic leaf spot virus*; ALV, *Apricot latent virus*; APLV, *Andean potato latent virus*; ASGV, *Apple stem grooving virus*; ASPV, *Apple stem pitting virus*; BVF, *Botrytis virus F*; BVX, *Botrytis virus X*; CLBV, *Citrus leaf blotch virus*; CMLV, *Cherry mottle leaf virus*; CVA, *Cherry virus A*; GarVA, *Garlic virus A*; GarVC, *Garlic virus C*; GarVE, *Garlic virus E*; GCLV, *Garlic common latent virus*; GFkV, *Grapevine fleck virus*; GVA, *Grapevine virus A*; GVB, *Grapevine virus B*; ICRSV, *Indian citrus ringspot virus*; LoLV, *Lolium latent virus*; MRFV, *Maize rayado fino virus*; OVX, *Opuntia virus X*; PepMV, *Pepino mosaic virus*; PlAMV, *Plantago asiatica mosaic virus*; PVT, *Potato virus T*; PVX, *Potato virus X*; SLV, *Shallot latent virus*; SMYEV, *Strawberry mild yellow edge virus*; SsDRV, *Sclerotinia sclerotiorum debilitation-associated RNA virus*; SWMV, Switchgrass mosaic virus; TYMV, *Turnip yellow mosaic virus*; WClMV, *White clover mosaic virus*. The percentage of replicate trees above 60 % in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Units are amino acid substitutions per site. Adapted from Wylie et al., 2013.

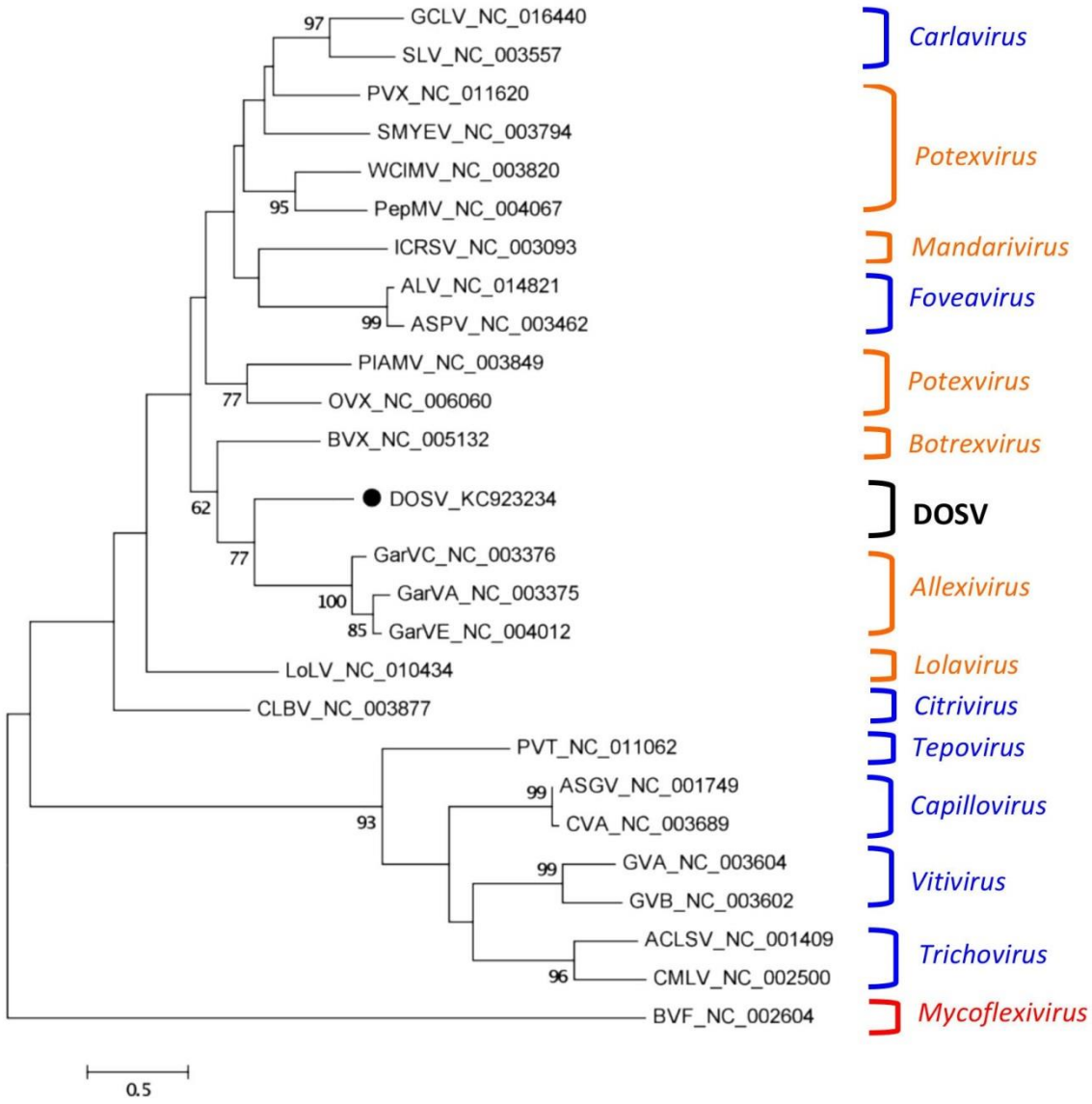

Figure 3. Phylogenetic relationships between the coat protein amino acid sequences of donkey orchid symptomless virus (DOSV) and those of representative flexiviruses. Relationships were inferred for amino acid sequences using the Maximum Likelihood method. The abbreviated names of the viruses used and their accession codes are shown. Genus names are given on the right. Other details as in Figure 2. Adapted from Wylie et al., 2013.