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:	***2014.006aP***	(to be completed by ICTV officers)

Short title: Create 4 new unassigned species in the family *Tombusviridae*
(e.g. 6 new species in the genus *Zetavirus*)

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

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

Kay Scheets (kay.scheets@okstate.edu), Carmen Hernandez (cahernan@ibmcp.upv.es), Ramon Jordan (Ramon.Jordan@ARS.USDA.GOV), and Andy White (kawhite@yorku.ca)

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)	**Tombusviridae and Umbravirus Study Group**

ICTV-EC or Study Group comments and response of the proposer:

SG comment: The decision to accept this proposal was unanimous. With respect to the genus name, 3 members voted pelarspovirus and 2 members voted pelipavirus, so pelarspovirus was chosen.

EC comment: This proposal generated some discussion among EC members. The SG is commended for its effort to update the taxonomy of tombusviridae. The EC also recognizes the difficulties associated with classifying a group of viruses known to have evolved through multiple recombination events. However, this proposal generated several concerns.

In the taxonomy code, the definition of a genus (or of any taxonomic taxon) stipulates that it should be monophyletic to indicate a common evolutionary origin. While mosaic evolution is acknowledged to complicate classification in many virus family (including the tombusviridae), the study group should decide which protein is the most valid to determine evolutionary relationships for this family and consider a classification scheme supported by phylogenetic trees for this protein. The problem with the proposed pelarspovirus genus is that it does not separate clearly from the carmovirus genus into two separate monophyletic branches for any of the proteins considered. It was also noted that the current genus carmovirus is not monophyletic for any of the proteins considered. This suggests that the taxonomy of the family (especially of species related to the genus carmovirus) may need to be reconsidered.

It was also noted that the number of sgRNAs is generally not considered as a sufficient criterion

for the creation of a new genus. For example, isolates within a single coronavirus species may have different numbers of sgRNAs.

For the reasons mentioned above, the proposal has been coded as Ud and will need to be reconsidered next year after reevaluation of the proposal by the study group.

The EC suggested that the creation of the species could go forward this year if the creation of the genus is removed from the proposal. The species could be created either as unassigned species in the family or possibly as members of the genus carmovirus.

Response to EC comment: The proposal has been revised for inclusion of these 4 viruses in the family *Tombusviridae* but unassigned to a genus.

Date first submitted to ICTV:	June 18, 2014
Date of this revision (if different to above):	September 9, 2014

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	***2014.006aP***	(assigned by ICTV officers)

To create four new species within:

Genus:	unassigned
Subfamily:	
Family:	*Tombusviridae*
Order:	

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:**	**GenBank sequence accession number(s)**
Elderberry latent virus,	ELV	AY038066
Pelargonium chlorotic ring pattern virus,	GR57	AY038069
Pelargonium ringspot virus	DSMZ-PV0304	AY038068
Rosa rugosa leaf distortion virus	MN-3	KC166238

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

Biological properties of the four viruses are summarized as follows:

1. Elderberry latent virus (ELV) [Jones et al., 2000; Jones, 2007; Kinard and Jordan, 1998]
   - Originally isolated from American elder (*Sambucus canadensis*) exhibiting line-pattern symptoms.
   - Mechanically transmissible to *Chenopodium quinoa* (local chlorotic lesions) and *Nicotiana benthamiana* and *N. clevelandii* (symptomless systemic infection).
   - No known vector.
   - Virions are spherical and ~30 nm in diameter.
   - Two dsRNA species of about 4.0 kb and 1.8 kb are detected in infected plants.
   - Serologically distinct from PCRPV, pelargonium line pattern virus (PLPV) and PelRSV.
2. Pelargonium chlorotic ring pattern virus (PCRPV) [Kinard and Jordan, 2002; Lisa et al, 1996]
   - Originally isolated from ornamental geranium (*Pelargonium zonale*) showing chlorotic spots or ringspots, line pattern and vein banding.
   - Mechanically transmissible to *C. quinoa* (local chlorotic lesions and, at high temperatures, systemic chlorotic lesions), *N. benthamiana* (symptomless systemic infection) and *N. clevelandii* (local chlorotic lesions and systemic mottle).
   - No known vector.

- Virions are spherical and ~30-32 nm in diameter.
- Two major dsRNA species of about 4.0 kb and 1.8 kb are detected in infected plants.
- Serologically distinct from ELV, PLPV and PelRSV.

3. Pelargonium ringspot virus (PelRSV) [Jones et al., 2000; Kinard et al., 1996; Kinard and Jordan, 1998]
    - Originally isolated from *Pelargonium peltatum* showing ringspot symptoms.
    - Mechanically transmissible to *C. quinoa* (local chlorotic lesions), *N. benthamiana* and *N. clevelandii* (symptomless systemic infection).
    - No known vector.
    - Virions are spherical and ~30-32 nm in diameter.
    - Two major dsRNA species of about 4.0 kb and 1.6 kb are detected in infected plants.
    - Serologically distinct from ELV, PCRPV and PLPV.
4. Rosa rugosa leaf distortion virus (RrLDV) and rose yellow leaf virus (RYLV; KC166239) [Lockhart et al., 2011; Mollov et al., 2013; Mollov et al., 2014]
    - RrLDV originally found in naturally-infected *Rosa rugosa* cultivars exhibiting stunting, leaf distortion and pale circular lines in early spring growth. RYLV was found in *Rosa* sp. showing blotchy yellow mosaic symptoms.
    - Mechanical transmissibility not reported.
    - No known vector.
    - Virions are spherical and about 33-32 nm in diameter.
    - Detection and analysis of dsRNA not reported.
    - Serological reactions not reported.

Inclusion of viruses in the family *Tombusviridae*:
BLASTp searches of reference proteins with RNA dependent RNA polymerases (RdRps) of ELV, PCRPV, PelRSV and RrLDV in August 2014 produced best hits to PLPV, PCRPV, and RrLDV followed by carmoviruses. (Note that ELV, RYLV, and PelRSV RdRps were not found in reference proteins). These search results are similar to searches with the RdRp of trailing lespedeza virus TGP1 (TLV TGP1) which was submitted in a separate sequence-only proposal as an unassigned tombusvirid (2014.008).

The complete genomes for ELV, PCRPV, PelRSV, and RrLDV, and nearly complete sequence of RYLV were recently released in GenBank. These viruses/isolates have been partially or completely characterized (above) and show characteristics and genome organizations similar to PLPV (Fig. 1) that clearly indicate they are members of the family *Tombusviridae* (Rochon et al., 2012).

A comparison of amino acid identities for the encoded RdRps, coat proteins (CPs), and movement proteins (MP1 and MP2) for ELV, PelRSV, PCRPV, RrLDV and RYLV to current or proposed (TLV TGP1) tombusvirids indicated that RYLV is an isolate of RrLDV, sharing 90% (RdRp), 95% (CP), 82% (MP1) and 86% (MP2) amino acid identities (Tables 1-4). PLPV, PelRSV, and PCRPV have overlapping host ranges, but for all six (current and proposed) unassigned tombusvirids, RdRp identities of 38-70% (Table 1) and CP identities of 29-71% (Table 2) indicate that the pelargonium-infecting viruses as well as ELV and RrLDV are unique species.

Phylogenetic support for inclusion in the family *Tombusviridae*:
Phylogenetic analyses for proteins encoded by four separate ORFs were conducted (Figs. 2-5),

and ELV, PelRSV, PCRPV, RrLDV, and RYLV always cluster with PLPV. For the phylogenies of MP1, MP2 and CP, sequences from TGP carmovirus 3 (TGP Car3) were included, and its proteins also cluster within this group. Since TGP Car3 is a partial sequence from a metagenomic survey [Scheets et al., 2011] we are not currently proposing its recognition by ICTV. ELV, PelRSV, PCRPV, RrLDV, and RYLV are less closely related to TLV TGP1 followed by carmoviruses.

PLPV was previously approved as an unassigned member of the family *Tombusviridae* (2007.124P) due to its obvious relatedness to carmoviruses (RdRp and CP phylogenies) while the presence of a predicted p13 ORF overlapping the readthrough region of RdRp, a 12 kDa protein produced via a -1 frameshift of MP1, a p6 ORF with no AUG, and production of only one subgenomic RNA (sgRNA) indicated it was not a carmovirus [Castaño and Hernandez, 2005]. More recently analyses of the translation strategies and gene functions of PLPV ORFs indicated the predicted p13 ORF is nonfunctional, no MP1 fusion protein is produced, and a 9.7 kDa MP2 is made using a GUG start codon instead of a predicted p6 ORF product [Castaño et al., 2009] (Figs. 1 & 6).

Characteristics that differ from any current tombusviridae genera:
The current description of the genus *Carmovirus* includes the following sentence: "The ORFs 2, 3 and 4 polypeptides are translated from two sgRNAs with sizes of about 1.7 and 1.5 kb" (Rochon et al. 2012). The viruses in this proposal are known (ELV, PCRPV, PelRSV) [Kinard et al, 1996; Kinard and Jordan, 2002] or predicted (RrLDV) [Mollov et al., 2013] to produce only one sgRNA encoding genes in the 3' half of their genomes similar to PLPV [Castaño and Hernandez, 2005], panicoviruses [Turina et al., 2000] and *Maize chlorotic mottle virus* [Scheets, 2000] instead of two sgRNAs (Fig. 1). This expression strategy is supported by the lack of any AUG codons between the MP1 AUG and CP AUG as seen with PLPV [Castaño et al., 2009] and TLV TGP1, whereas 15 carmoviruses have 1-8 AUGs in that region [Scheets et al., 2011]. The MP2 ORFs overlap MP1 ORFs in genome regions similar to carmoviruses but are known to initiate [Castaño et al., 2009] or are predicted to initiate with a noncanonical start codon (GUG, CUG, and/or ACG) [Scheets et al., 2011] (Figs. 1 & 6). Thus, leaky scanning past MP1 and MP2 start codons allows production of three proteins from one sgRNA. These characteristics indicate that, like PLPV, PelRSV, PCRPV, RrLDV, and ELV should be included in the family *Tombusviridae* but remain unassigned to any current genus.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Castaño, A., and Hernandez, C. (2005). Complete nucleotide sequence and genome organization of Pelargonium line pattern virus and its relationship with the family Tombusviridae. Archives of Virology 150(5), 949-65.

Castaño, A., Ruiz, L., and Hernandez, C. (2009). Insights into the translational regulation of biologically active open reading frames of Pelargonium line pattern virus. Virology 386(2), 417-26.

Jones, A. T., McGavin, W. J., Brunt, A. A., and Phillips, S. (2000). Elderberry latent virus: its relationship to Pelargonium ringspot virus and its identification as a distinct member of the genus Carmovirus, family Tombusviridae. Annals of Applied Biology 136(2), 147-152.

Jones, A.T. (2007). Elderberry latent virus. CMI/AAB Descriptions of Plant Viruses #419.

References:

Kinard G.R., Jordan, R.L., Hurtt, S.S. (1996). Partial characterization of Pelargonium line pattern and Pelargonium ringspot viruses. Acta Horticulturae 432:148-155.

Kinard, G. R., and Jordan, R. (2002). Genome organization of pelargonium chlorotic ring pattern virus: further implications for tombusviridae taxonomy. Acta Horticulturae 568, 17-27.

Kinard, G. R., and Jordan, R. L. (1998). Genome organization of pelargonium ringspot and elderberry latent viruses. Phytopathology 88, S48.

Lisa, V., Vaira, A. M., Dellavalle, G., Masenga, V., and Milne, R. G. (1996). Viruses of pelargonium in Italy. Acta Horticulturae 432, 108-117.

Lockhart, B., Zlesak, D., and Fetzer, J. (2011). Identification and partial characterization of six new viruses of cultivated roses in the USA. Acta Horticulturae 901, 139-147.

Mollov, D., Lockhart, B., and Zlesak, D. C. (2013). Complete nucleotide sequence of Rosa rugosa leaf distortion virus, a new member of the family Tombusviridae. Archives of Virology 158(12), 2617-20.

Mollov, D., Lockhart, B., and Zlesak, D. C. (2014). Complete nucleotide sequence of rose yellow leaf virus, a new member of the family Tombusviridae. Archives of Virology.

Rochon, D., Rubino, L., Russo, M., Martelli, G.P., Lommel, S., 2012. Tombusviridae, in: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.),Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier, San Diego, pp. 1111-1138.

Scheets, K. (2000). Maize chlorotic mottle machlomovirus expresses its coat protein from a 1.47-kb subgenomic RNA and makes a 0.34-kb subgenomic RNA. Virology 267(1), 90-101.

Scheets, K., Blinkova, O., Melcher, U., Palmer, M. W., Wiley, G. B., Ding, T., and Roe, B. A. (2011). Detection of members of the Tombusviridae in the Tallgrass Prairie Preserve, Osage County, Oklahoma, USA. Virus Research 160(1-2), 256-63.

Turina, M., Desvoyes, B., and Scholthof, K.-B. G. (2000). A gene cluster encoded by panicum mosaic virus is associated with virus movement. Virology 266(1), 120-128.

Annex:

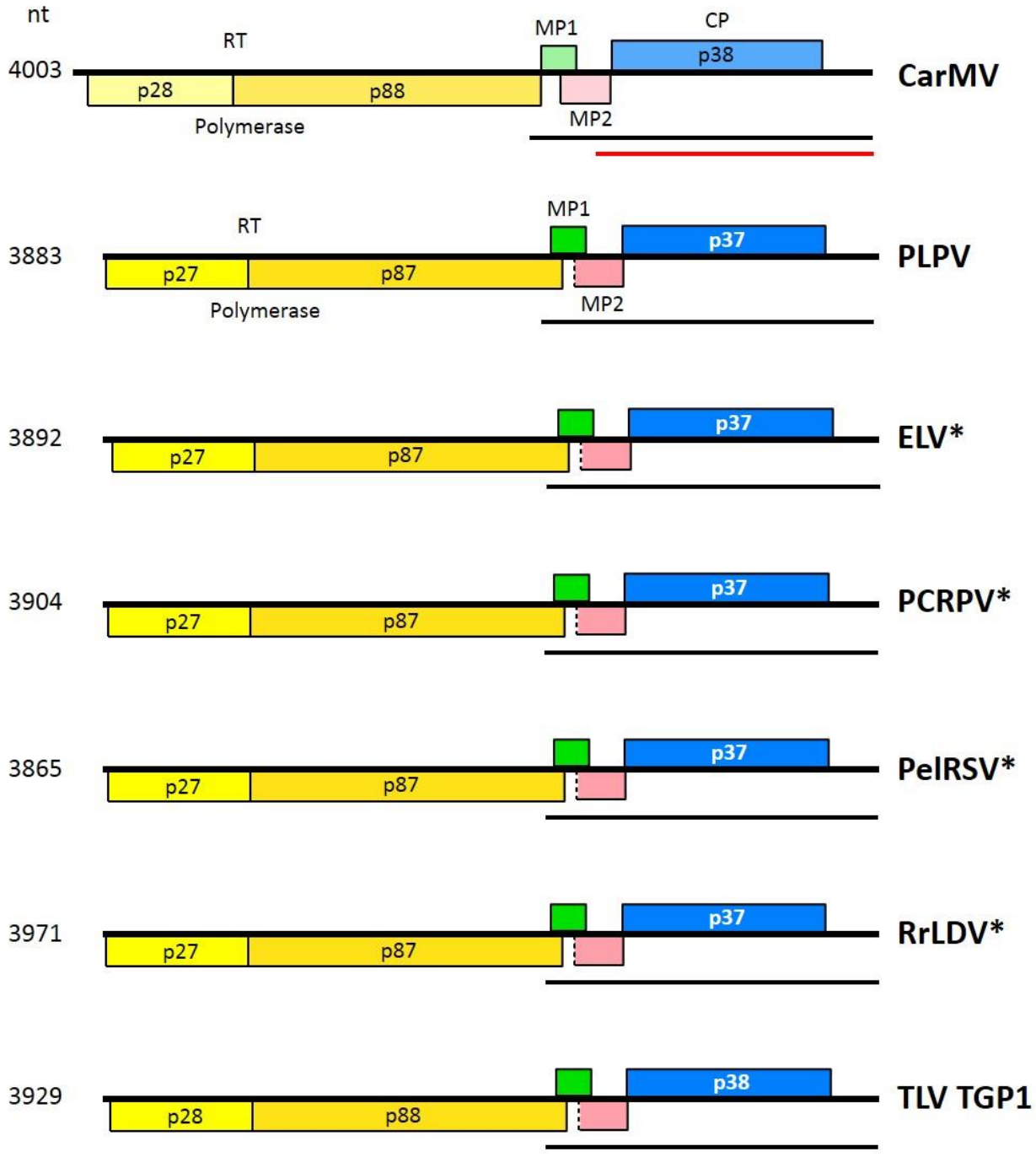

Figure 1. Length and genome organizations of carnation mottle virus (CarMV), pelargonium line pattern virus (PLPV), trailing lespedeza virus TGP1 and unassigned tombusvirids in this proposal*. Thin lines under genomes indicate sgRNAs. Dashed lines of MP2 indicate noncanonical start codons (see Fig. 6). See Table 5 for virus acronyms.

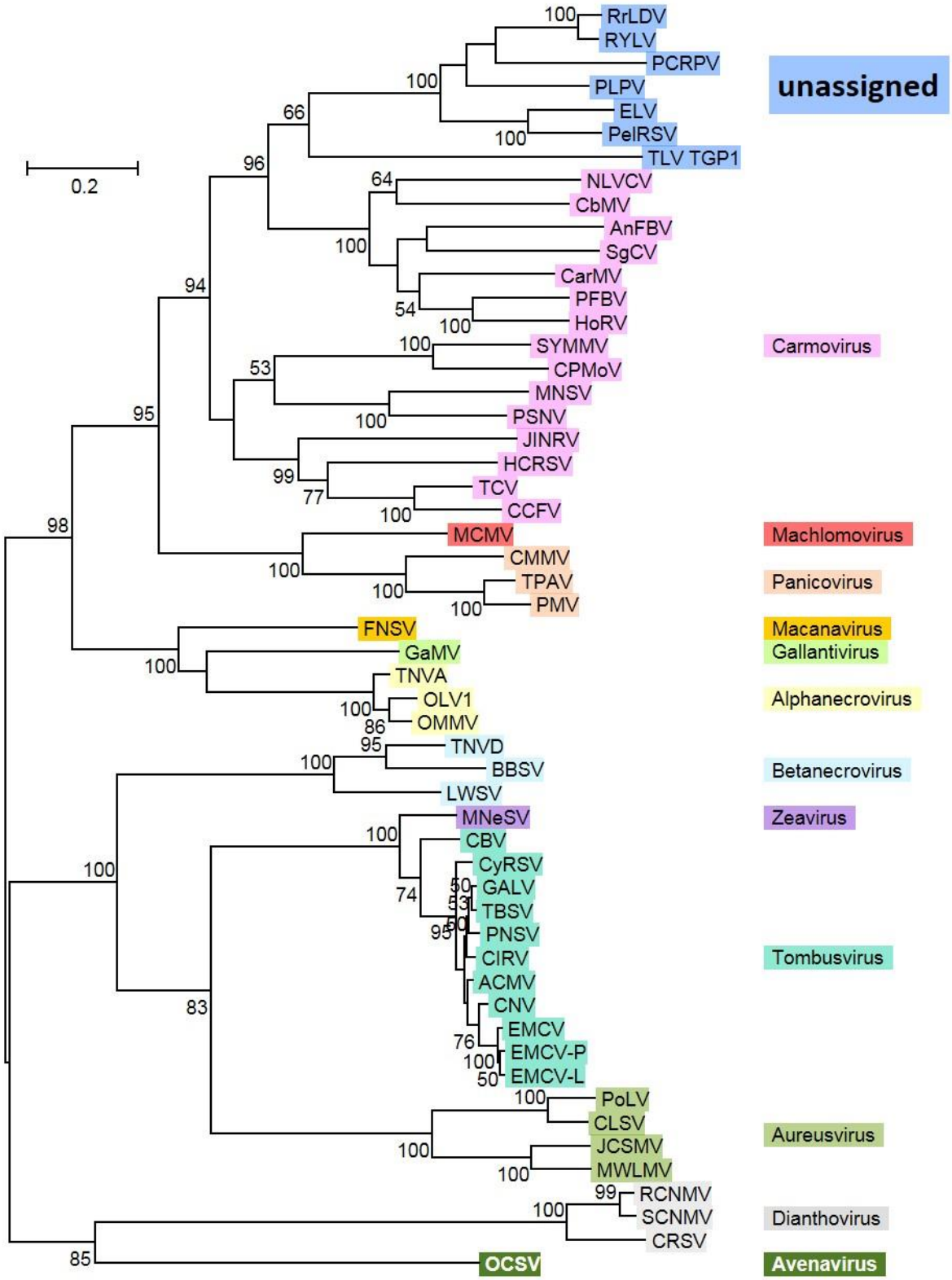

Figure 2. Phylogenetic (distance) analysis of the RdRps of *Tombusviridae* members. Unassigned members (mid-blue) cluster on one branch. Alignments were made using ClustalO while trees were generated with the Maximum Likelihood algorithm using 1000 bootstrap replicates (showing values >50%). See Table 5 for virus acronyms.

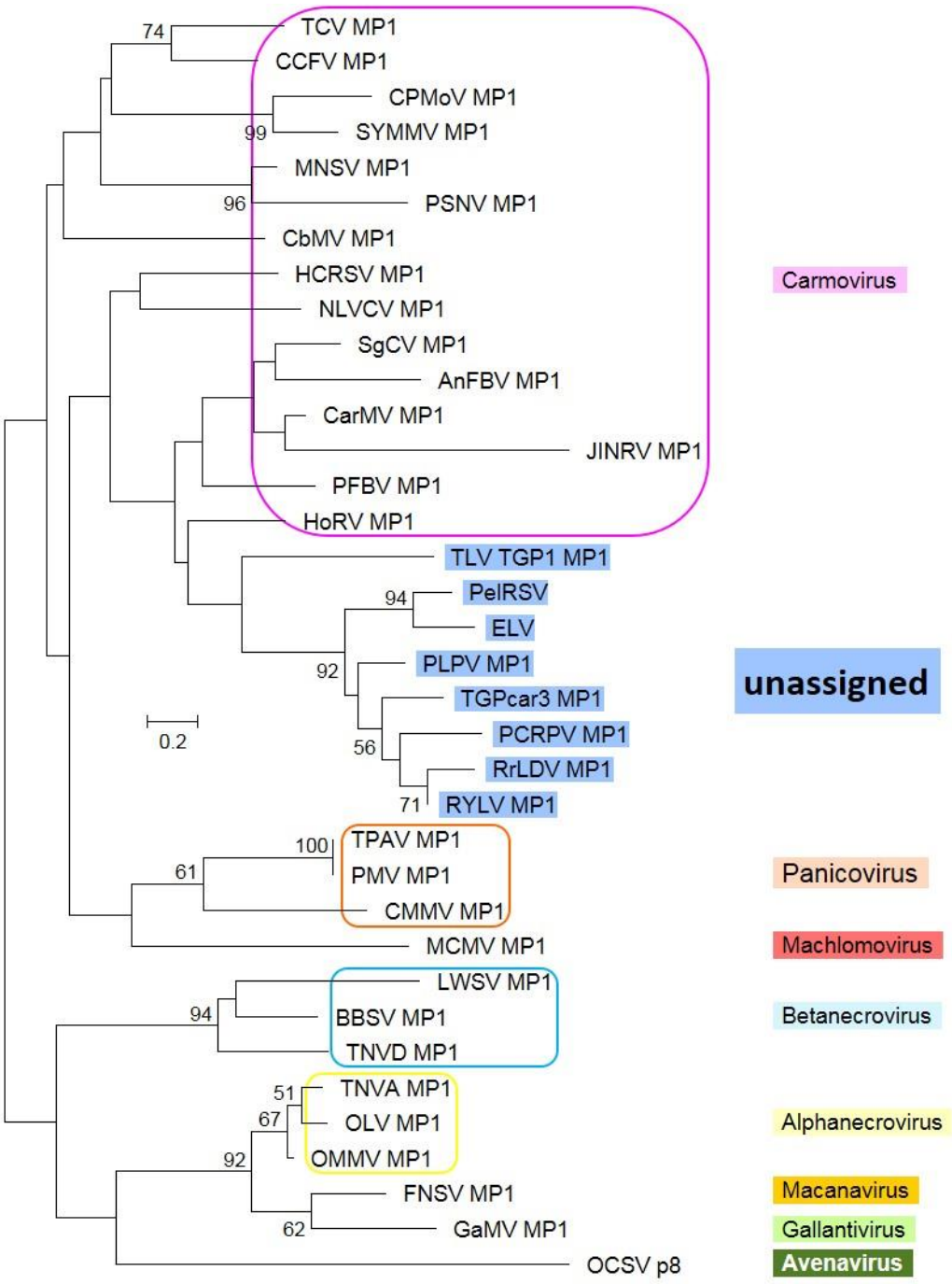

Figure 3. Phylogenetic (distance) analysis of the MP1 proteins of *Tombusviridae* members. Unassigned members (mid-blue) cluster on one branch. Alignments were made by ClustalO while trees were generated with the Maximum Likelihood algorithm using 1000 bootstrap replicates of all sites (showing values >50%). See Table 5 for virus acronyms.

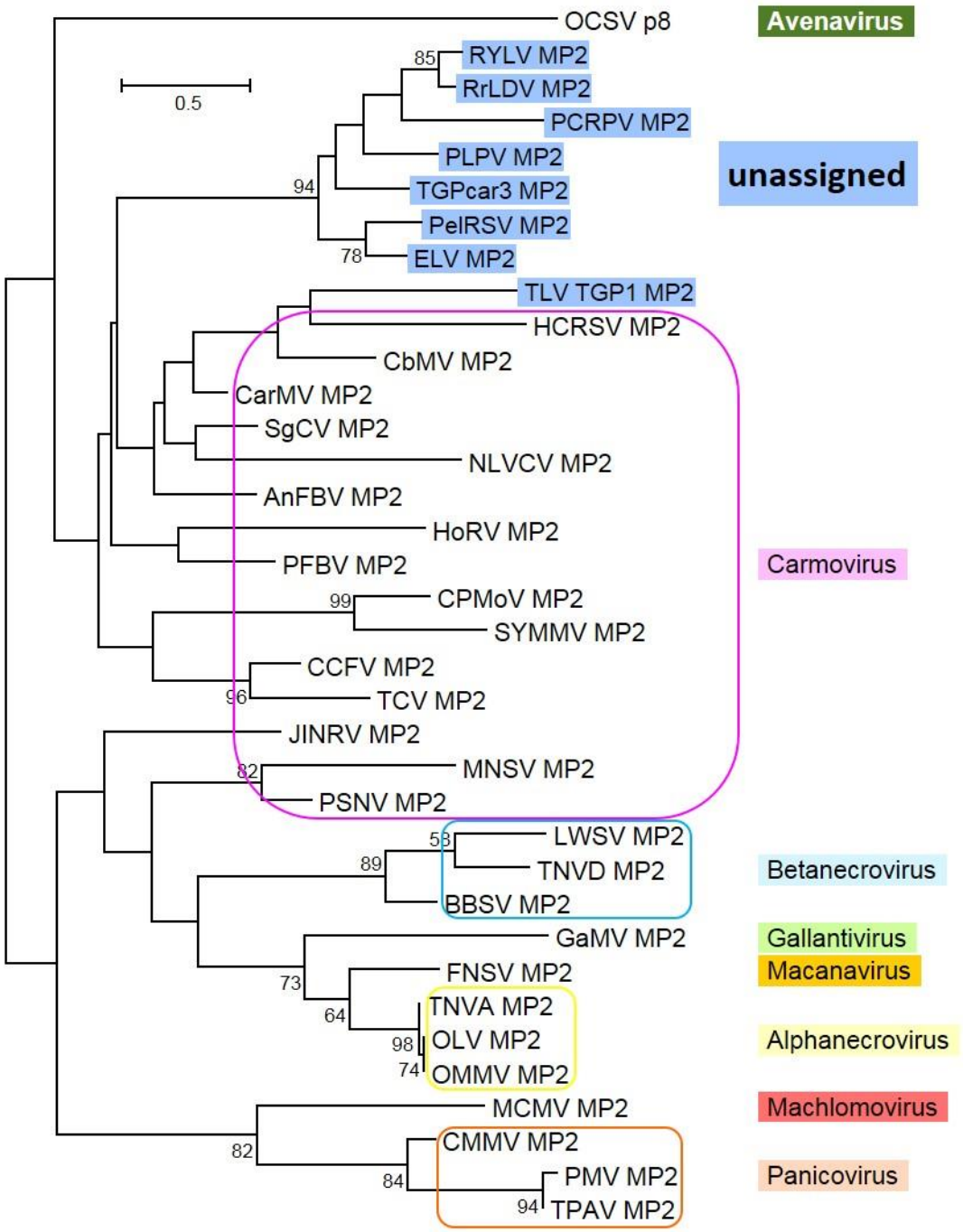

Figure 4. Phylogenetic (distance) analysis of the MP2 of *Tombusviridae* members. Unassigned members (mid-blue), with the exception of TLV TGP1, cluster separately from *Carmovirus* members. Alignments were made by ClustalO while trees were generated with the Maximum Likelihood algorithm using 1000 bootstrap replicates of all sites (showing values >50%). See Table 5 for virus acronyms.

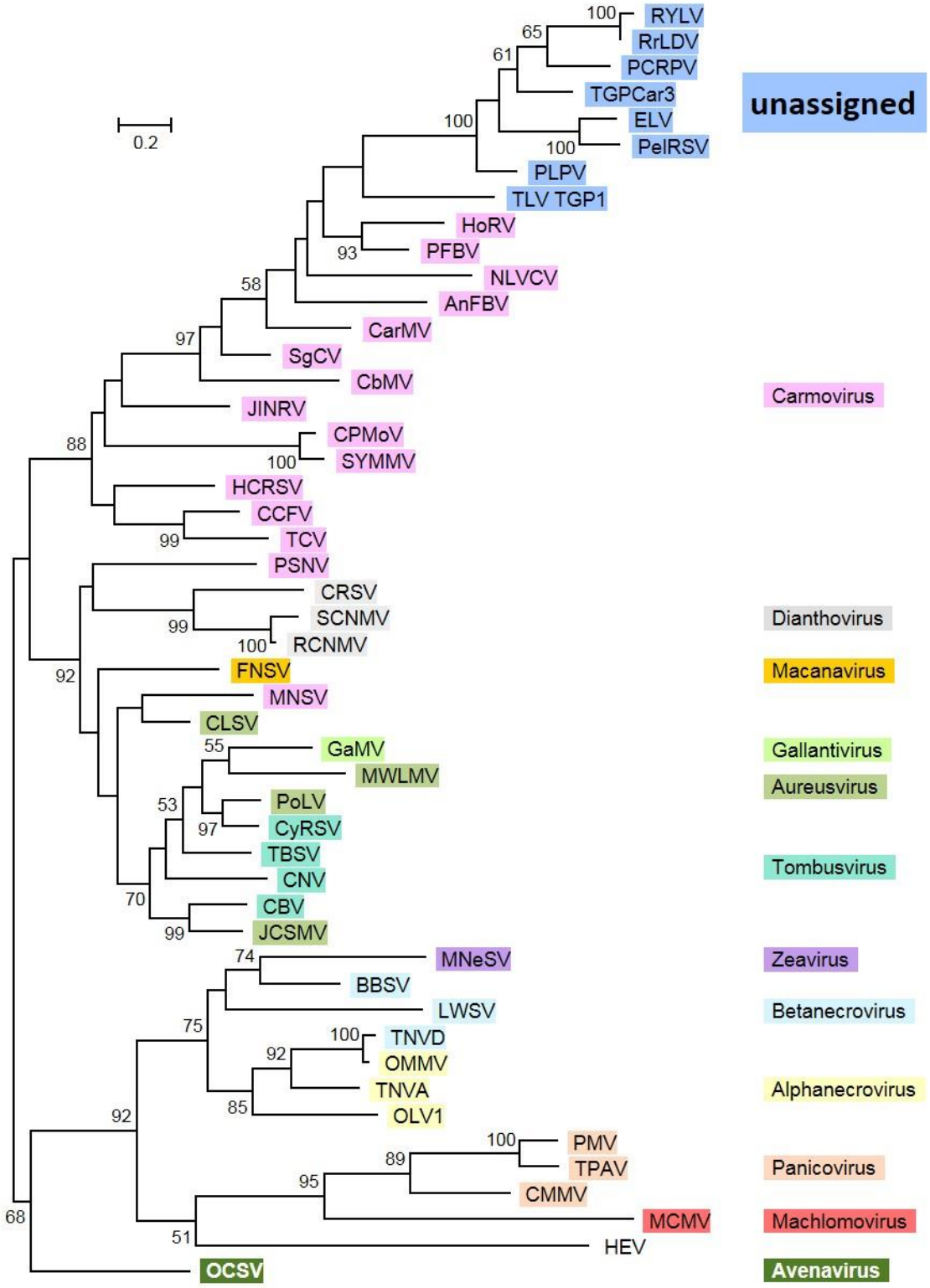

Figure 5. Phylogenetic (distance) analysis of the coat proteins of *Tombusviridae* members. Only four tombusvirus were included. Unassigned members (mid-blue) cluster on one branch. Alignments were made by ClustalO while trees were generated with the Maximum Likelihood algorithm using 1000 bootstrap replicates (showing values >50%). See Table 5 for virus acronyms.

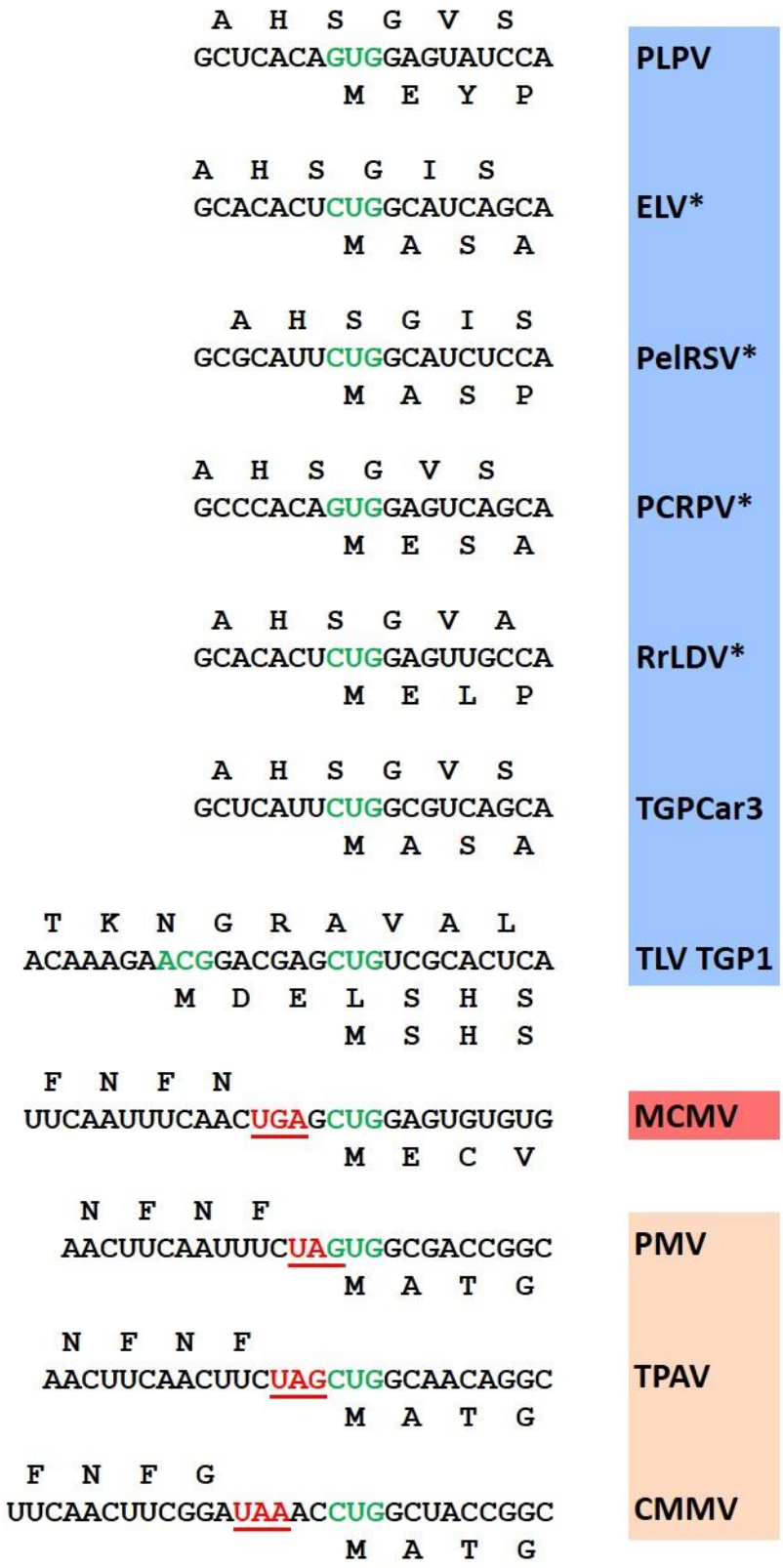

Figure 6. Contexts for MP2 noncanonical start codons (green) for related unassigned viruses, MCMV and panicoviruses. Two alternatives are shown for TLV TGP1. MP1 sequences are above the RNA sequences and are aligned as in ClustalO. See Table 5 for virus acronyms.

Table 1. Percent amino acid sequence identity between the polymerases of unassigned members and those of carmoviruses.

	PCRPV	PelRSV	PLPV	RrLDV	RYLV	TLV TGP1		Carmo-viruses
ELV	55	70	57	58	57	38		37-43
PCRPV		56	58	61	61	39		39-45
PelRSV			57	58	58	38		37-42
PLPV				61	61	40		39-45
RrLDV					**90**	39		40-44
RYLV						39		40-44
TLV TGP1								35-41

Table 2. Percent amino acid sequence identity between the coat proteins of unassigned members and those of carmoviruses.

	PCRPV	PelRSV	PLPV	RrLDV	RYLV	TLV TGP1	TGPCar3	Carmo-viruses
ELV	44	71	50	44	43	33	46	16-34
PCRPV		45	44	49	49	29	55	16-34
PelRSV			48	43	43	32	44	18-34
PLPV				46	46	33	49	18-34
RrLDV					**95**	32	48	17-32
RYLV						33	48	17-33
TLV TGP1							33	17-39
TGPCar3								17-34

Table 3. Percent amino acid sequence identity between MP1 of unassigned members and those of carmoviruses.

	PCRPV	PelRSV	PLPV	RrLDV	RYLV	TLV TGP1	TGPCar3	Carmo-viruses
ELV	48	69	53	47	53	22	55	15-35
PCRPV		52	56	62	68	33	59	16-36
PelRSV			63	56	60	28	50	16-35
PLPV				55	67	31	64	18-39
RrLDV					**82**	34	63	14-37
RYLV						36	67	14-40
TLV TGP1							28	18-36
TGPCar3								17-39

Table 4. Percent amino acid sequence identity between MP2 of unassigned members and those of carmoviruses.

	PCRPV	PelRSV	PLPV	RrLDV	RYLV	TLV TGP1	TGPCar3	Carmo-viruses
ELV	41	69	53	52	52	18	61	11-29
PCRPV		44	43	48	51	26	55	9-23
PelRSV			51	49	46	25	51	11-25
PLPV				58	58	15	56	14-27
RrLDV					**86**	22	55	16-28
RYLV						20	58	14-29
TLV TGP1							20	13-36
TGPCar3								10-28

Table 5. ABBREVIATIONS USED IN THE PROPOSAL

	Alphanecrovirus	
OLV1	*Olive latent virus* 1	X85989
OMMV	*Olive mild mosaic virus*	AY616760
TNVA	*Tobacco necrosis virus* A	M33002
	Aureusvirus	
CLSV	*Cucumber leaf spot virus*	EU127904
JCSMV	*Johnsongrass chlorotic stripe mosaic virus*	AJ557804
MWLMV	*Maize white line mosaic virus*	EF589670
PoLV	*Pothos latent virus*	X87115
	Avenavirus	
OCSV	*Oat chlorotic stunt virus*	X83964
	Betanecrovirus	
BBSV	*Beet black scorch virus*	AF452884
LWSV	*Leek white stripe virus*	X94560
TNVD	*Tobacco necrosis virus* D	U62546
	Carmovirus	
AnFBV	*Angelonia flower break virus*	DQ219415
CbMV	*Calibrachoa mottle virus*	GQ244431
CCFV	*Cardamine chlorotic fleck virus*	L16015
CarMV	*Carnation mottle virus*	X02986
CPMV	*Cowpea mottle virus*	U20976
HCRSV	*Hibiscus chlorotic ringspot virus*	X86448
HoRSV	*Honeysuckle ringspot virus*	HQ677625
JINRV	*Japanese iris necrotic ring virus*	D86123
MNSV	*Melon necrotic spot virus*	M29671
NLVCV	*Nootka lupine vein clearing virus*	EF207438
PSNV	*Pea stem necrosis virus*	AB086951
PFBV	*Pelargonium flower break virus*	AJ514833
SgCV	*Saguaro cactus virus*	U72332
SYMMV	*Soybean yellow mottle mosaic virus*	FJ457015
TCV	*Turnip crinkle virus*	M22445
	Dianthovirus	
CRSV	*Carnation ringspot virus*	L18870, M8858
RCNMV	*Red clover necrotic mosaic virus*	J04357, X08021
SCNMV	*Sweet clover necrotic mosaic virus*	L07884, S4602
	Gallantivirus	
GaMV	*Galinsoga mosaic virus*	Y13463
	Macanavirus	
FNSV	*Furcraea necrotic streak virus*	FJ768020

continued

	Machlomovirus	
MCMV	*Maize chlorotic mottle virus*	X14736
	Panicovirus	
CMMV	*Cocksfoot mild mosaic virus*	EU081018
PMV	*Panicum mosaic virus*	U55002
TPAV	thin paspalum asymptomatic virus (proposed)	JX848617
	Tombusvirus	
AMCV	*Artichoke mottled crinkle virus*	X62493
CIRV	*Carnation Italian ringspot virus*	X85215
CBV	*Cucumber Bulgarian virus*	AY163842
CNV	*Cucumber necrosis virus*	M25270
CyRSV	*Cymbidium ringspot virus*	X15511
EMCV	*Eggplant mottled crinkle virus*	JQ864181
EMCV-P	*Eggplant mottled crinkle virus*-P (pear latent virus)	AY100482
EMCV-L	*Eggplant mottled crinkle virus*-L (lisianthus necrosis virus)	DQ011234
GALV	*Grapevine Algerian latent virus*	AY830918
PNSV	*Pelargonium necrotic spot virus*	AJ607402
TBSV	*Tomato bushy stunt virus*	M21958
	Zeavirus	
MNeSV	*Maize necrotic streak virus*	AF266518
	unassigned	
ELV	elderberry latent virus	AY038066
PCRPV	pelargonium chlorotic ring pattern virus	AY038069
PelRSV	pelargonium ringspot virus	AY038068
PLPV	*Pelargonium line pattern virus*	AY613852
RrLDV	rosa rugosa leaf distortion virus	KC166238
RYLV	rose yellow leaf virus	KC166239
TLV TGP1	trailing lespedeza virus TGP 1 (proposed tombusvirid)	HM640935
	other virus sequences	
HEV	*Hepatitis E virus* CP	AAA45727
TGPCar3	TGP carmovirus 3	JF437874