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:	***2014.006b-fP***	(to be completed by ICTV officers)

Short title: Create the genus *Pelarspovirus* in the family *Tombusviridae*
(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):

Kay Scheets, Ramon Jordan, K. Andrew White, and Carmen Hernandez

Corresponding author with e-mail address:

Kay Scheets kay.scheets@okstate.edu

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

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

The study group supports the creation of this new genus.

Date first submitted to ICTV: June 18, 2014
Date of this revision (if different to above): June 5, 2015

ICTV-EC comments and response of the proposer:

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2014.006bP***	(assigned by ICTV officers)

To create a new genus within:

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

naming a new genus

Code	***2014.006cP***	(assigned by ICTV officers)

To name the new genus: *Pelarspovirus*

Assigning the type species and other species to a new genus

Code	***2014.006dP***	(assigned by ICTV officers)

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

Pelargonium line pattern 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:**

5

Reasons to justify the creation of a new genus:

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

Revision of genus demarcation criteria and division of genus *Carmovirus*

In 2014 we submitted a similar proposal (2014.006a-fP.N.v1.Pelarspovirus) to create this genus with six members. The EC did not support its creation at that time due to the placement of the pelarspovirus genus branch within a larger monophyletic lineage of many of the members of the carmovirus genus which have very similar genome organizations. The EC suggested a revision of the family with particular emphasis on the carmovirus genus, and the Tombusviridae Study Group is submitting a separate proposal that divides the current carmovirus genus into three new genera. Additionally, the genus demarcation criteria have been revised in the carmovirus proposal to include transcriptional characteristics (number of subgenomic RNAs (sgRNAs) and to emphasize that the complete RNA dependent RNA polymerase (RdRp) is the most useful protein for providing phylogenetic trees that define the genera. The division of carmovirus and the revised demarcation criteria provide a new framework for examining the creation of genus *Pelarspovirus*.

Revised genus demarcation criteria in the family *Tombusviridae*

- Structural criteria: spherical virions with either a smooth or granular appearance.
- Genomic criteria: genome organization, number of genome segments, size of genome, number of subgenomic RNAs.

- Polymerase criteria: gene interrupted by a termination codon or a -1 ribosomal frameshifting element that is periodically read through; differential branching of phylogenetic trees based on the complete RdRp.

Coding and translation characteristics of proposed pelarspoviruses

Elderberry latent virus (ELV)*, Pelargonium chlorotic ring pattern virus* (PCRPV)*, Pelargonium ringspot virus* (PelRSV), *Rosa rugosa leaf distortion virus* (RrLDV) [Scheets et al., 2014] and *Pelargonium line pattern virus* (PLPV) (2007.124P) are unassigned members of the family *Tombusviridae* that have very similar genome sizes (3865-3971 nt). They encode five ORFs; p27, its readthrough product p87 which encodes the RdRp, two small movement proteins MP1 (6.1 to 6.9 kDa) and MP2 (8.9 to 9.7 kDa), and a 37 kDa coat protein (CP) (Fig. 1). Although these viruses have genomes similar to the 15 sequenced viruses in the current carmovirus genus that will become three new genera (Fig. 1), a discriminating characteristic is that pelarspoviruses produce only one subgenomic RNA (sgRNA) to express all three 3'-proximal ORFs [Scheets et al., 2015] compared to the two sgRNAs transcribed by carmoviruses. This characteristic was noted as the reason PLPV was initially given "unassigned" status instead of proposing to add it to genus *Carmovirus* (2007.124P). The transcription strategy is aided by leaky scanning past the MP1 AUG which has poor translational context to allow occasional initiation at the noncanonical MP2 start codon (GUG or CUG) or the CP AUG, which has the best context (Fig. 2). *Trailing lespedeza virus* 1 (TLV1) is another unassigned carmovirus-like family member, and it is predicted to have a transcription strategy similar to pelarspoviruses since its MP2 ORF has a noncanonical start codon [Scheets et al., 2011; Scheets and Melcher, 2014]. Panicoviruses and *Maize chlorotic mottle virus* use similar noncanonical MP2 start codons to allow expression of four ORFs from a single sgRNA (Fig. 2). All the viruses described above have no AUG codons in any frame between the MP1 and CP start codons, and this characteristic is not shared by current carmoviruses. A detailed description of the proposed genus *Pelarspovirus* is currently in press [Scheets et al., 2015].

Phylogenetic support for genus *Pelarspovirus*

Proteins encoded by RdRp, MP1, MP2, and CP ORFs were analyzed for CP-encoding tombusvirids using MUSCLE and ClustalOmega alignment programs, and phylogenetic trees were built using two statistical approaches (maximum likelihood or neighbor-joining) with two or three methods for treating gaps and missing data (Figs. 3-8 [Scheets et al., 2015] for ClustalOmega trees and data not shown). This generated 10 trees for RdRp and CP, but not all combinations of treatments were capable of analyzing MP1 (9 trees) and MP2 (6 trees) due to their generally low similarities. With the exception of one MP2 tree (Fig. 7), phylogenetic trees for all pelarspovirus proteins formed a monophyletic branch, which was not the case for the three proposed genera created from current carmoviruses (Figs. 3-8; [Scheets et al., 2015] and data not shown). Additionally, TLV1 proteins did not consistently group with pelarspoviruses for any protein, and TLV1 RdRp and CP were as likely to cluster near alphacarmoviruses as they were to group with pelarspoviruses (Figs. 3-8 and data not shown), so we do not consider it a candidate for inclusion in the pelarspovirus genus.

Whole genome sequence comparison is a useful tool for defining taxonomic hierarchies for some viruses [Bao et al., 2014]. When PAirwise Sequence Comparison (PASC) tool at the NCBI Viral Genomes web site was applied to tombusvirid reference sequences under conditions suggested by Yiming Bao, the five pelarspoviruses were identified as a unique genus while the current carmoviruses were divided into eight genera (Fig. 4). We consider this additional support for creation of pelarspovirus as a separate genus from any of the other carmovirus-like family members.

Genetic distances of viral proteins
The % amino acid identities of RdRps among the five pelarspoviruses ranged from 55-69.4% (Table 1). Alphacarmoviruses were the next most similar proteins (38.1-46.5%) followed by betacarmoviruses, gammacarmoviruses, and TLV1 (Table 1). For sequenced viruses in the other 11 genera of CP-encoding viruses, maximum and minimum identities were for PCRPV vs. MCMV (39%) and PLPV vs. CRSV (26.3%) (data not shown).

Origin of the new genus name:

Sigla Pelargonium ringspot virus

Reasons to justify the choice of type species:

PLPV is the first virus in this genus to be recognized by ICTV as an unassigned species in the family *Tombusviridae* (2007.124P). There are papers or abstracts describing hosts, dsRNAs, virion EMs, coat protein size estimates [Kinard et al., 1996], and serological comparisons [Lesemann and Adam, 1994]. It is the best characterized with the first complete genome appearing in GenBank 30-APR-2004 with two additional complete genomes. Additionally there are publications on sgRNA mapping [Castaño and Hernandez, 2005], work on an infectious transcript cDNA [Castaño and Hernandez, 2007], analyses of gene function and translational mechanisms [Castaño et al, 2009; Pérez-Cañamas et al., 2015], and molecular variability [Castaño et al., 2011]. The genus name is derived from PelRSV since it was the first of these viruses to be named and described [Hollings 1957]. The genus name was proposed in 1998 by Kinard and Jordan [1998], and has been used extensively (1999-2015) in abstracts, papers, and books, and it appears in a commercial web site.

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.

<75% amino acid sequence identity in RdRps and
<75% amino acid sequence identity in CPs
Natural host range

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

To remove the following taxon (or taxa) from their present position:

Elderberry latent virus, Pelargonium chlorotic ring pattern virus, Pelargonium line pattern virus, Pelargonium ringspot virus, Rosa rugosa leaf distortion virus

The present taxonomic position of these taxon/taxa:

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

Fill in all that apply.

If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right

Reasons to justify the removal:
Explain why the taxon (or taxa) should be removed

These species will be moved into the new genus *Pelarspovirus*

Part (b) re-assign to a higher taxon

Code	***2014.006fP***	(assigned by ICTV officers)

To re-assign the taxa listed in Part (a) as follows:

Genus:	***Pelarspovirus*** **(new)**
Subfamily:	
Family:	***Tombusviridae***
Order:	

Fill in all that apply.
- If the higher taxon has yet to be created write "**(new)**" after its proposed name and complete relevant module to create it.

If no genus is specified, enter "**unassigned**" in the genus box.

Reasons to justify the re-assignment:
- If it is proposed to re-assign species to an existing genus, please 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.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

See module 3.

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Bao, Y., Chetvernin, V., Tatusova, T., 2014. Improvements to pairwise sequence comparison (PASC): a genome-based web tool for virus classification. Arch .Virol. 159, 3293-3304.

Castaño, A., Hernández, C., 2005. Complete nucleotide sequence and genome organization of pelargonium line pattern virus and its relationship with the family Tombusviridae. Arch.Virol. 150, 949-65.

Castaño, A., Hernández, C., 2007. Biological activity of transcripts from cDNA of pelargonium line pattern virus. Acta Virologica 51, 271-4.

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

Castaño, A., Ruiz, L., Elena, S.F., Hernández, C., 2011. Population differentiation and selective constraints in pelargonium line pattern virus. Virus Res. 155, 274-282.

Hollings, M., 1957. Pelargonium ring spot. Plant Pathology 6, 17-18.

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

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

Lesemann, D.E., Adam, G., 1994. Electron microscopical and serological studies on four isometrical *Pelargonium* viruses. Acta Hort. 377:41-54.

Pérez- Cañamás, M., Hernández, C., 2015. Key importance of small RNA binding for the activity of a glycine/tryptophan (GW) motif-containing viral suppressor of RNA silencing. J. Biol. Chem. 290:3106-3120

Scheets, K., Blinkova, O., Melcher, U., Palmer, M.W., Wiley, G.B., Ding, T., Roe, B.A. 2011. Detection of members of the *Tombusviridae* in the Tallgrass Prairie Preserve, Osage County, Oklahoma, USA. Virus Res 160: 256-263.

Scheets, K., Hernández, C., Jordan, R., White, A. 2014. ICTV taxonomic proposal 2014.006aP.A.v2.Tombusviridae_4sp. Create 4 new species unassigned in the family *Tombusviridae*. http://www.ictvonline.org/proposals-14/2014.006aP.A.v2.Tombusviridae_4sp.pdf

Scheets, K., Jordan, R., White, K.A., Hernández, C. 2015. *Pelarspovirus*, a proposed new genus of plant viruses in the family *Tombusviridae*. Arch.Virol. (in press)

Scheets, K., Melcher, U. 2014. ICTV taxonomic proposal 2014.008aP.A.v3.Tombusviridae_sp. Create 1 new species unassigned in the family *Tombusviridae*. http://www.ictvonline.org/proposals-14/2014.008aP.A.v3.Tombusviridae_sp.pdf

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.

Table 1. Percent amino acid sequence identity[a] ranges between RdRps of carmovirus-like genera members.

	Alpha-carmovirus	Beta-carmovirus	Gamma-carmovirus	Pelarspo-virus	TLV1
Alpha-carmovirus	**47.9-64.6**				37.8-41.6
Beta-carmovirus	39.5-41.8	**47.1-67.0**			36.8-38.9
Gamma-carmovirus	37.9-42.0	39.9-45.0	**42.9-64.7**		34.8-37.1
Pelarspo-virus	38.1-46.5	38.0-44.0	37.2-41.1	**55.0-69.4**	37.7-40.3

[a] based on MUSCLE alignment

Table 2. Virus abbreviations and accession numbers for 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
YSV	Yam spherical virus (proposed)	KF482072
	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 (2014)	
	Alphacarmovirus (proposed)	
AnFBV	Angelonia flower break virus	DQ219415
CbMV	Calibrachoa mottle virus	GQ244431
CarMV	Carnation mottle virus	X02986
HoRSV	Honeysuckle ringspot virus	HQ677625
NLVCV	Nootka lupine vein clearing virus	EF207438
PFBV	Pelargonium flower break virus	AJ514833
SgCV	Saguaro cactus virus	U72332
	Betacarmovirus (proposed)	
CCFV	Cardamine chlorotic fleck virus	L16015
HCRSV	Hibiscus chlorotic ringspot virus	X86448
JINRV	Japanese iris necrotic ring virus	D86123
TCV	Turnip crinkle virus	M22445
	Gammacarmovirus (proposed)	
CPMV	Cowpea mottle virus	U20976
MNSV	Melon necrotic spot virus	M29671
PSNV	Pea stem necrosis virus	AB086951
SYMMV	Soybean yellow mottle mosaic virus	FJ457015
	move to unassigned (proposed)	
AWV	Ahlum waterborne virus	na
BMMV	Bean mild mottle virus	na
CSBV	Cucumber soil-borne virus	na
WWV	Weddel waterborne virus	na
	Dianthovirus	
CRSV	Carnation ringspot virus	L18870, M88589
RCNMV	Red clover necrotic mosaic virus	J04357, X08021
SCNMV	Sweet clover necrotic mosaic virus	L07884, S46028
	Gallantivirus	
GaMV	Galinsoga mosaic virus	Y13463
	Macanavirus	
FNSV	Furcraea necrotic streak virus	FJ768020
	Machlomovirus	
MCMV	Maize chlorotic mottle virus	X14736

(cont.)

Table 2 (cont.)

	Panicovirus	
CMMV	Cocksfoot mild mosaic virus	EU081018
PMV	Panicum mosaic virus	U55002
TPAV	Thin paspalum asymptomatic virus	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
GALV	Grapevine Algerian latent virus	AY830918
MPV	Moroccan pepper virus	JX197071
PNSV	Pelargonium necrotic spot virus	AJ607402
TBSV	Tomato bushy stunt virus	M21958
	Zeavirus	
MNeSV	Maize necrotic streak virus	AF266518
	unassigned Tombusviridae	
TLV1	Trailing lespedeza virus 1	HM640935
	Pelarspovirus (proposed)	
ELV	Elderberry latent virus	AY038066
PCRPV	Pelargonium chlorotic ring pattern virus	AY038069
PLPV	Pelargonium line pattern virus	AY613852
PelRSV	Pelargonium ringspot virus	AY038068
RrLDV	Rosa rugosa leaf distortion virus	KC166238
	other viral sequences	
HCV	Hepatitis C virus RdRp	ADC54804.1
HEV	Hepatitis E virus CP	AAA45727.1

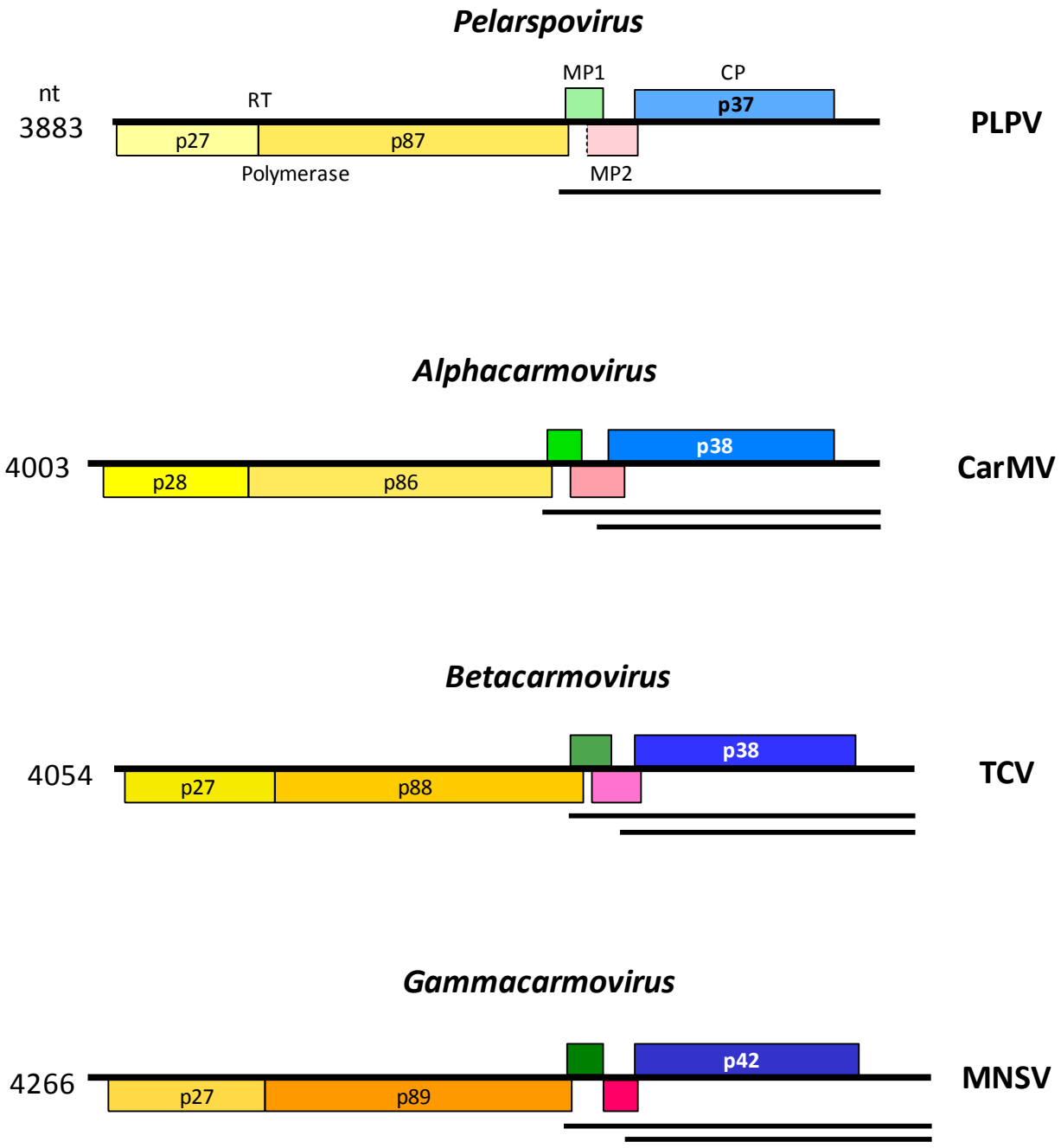

Figure 1. Lengths, proposed genus names, and genome organizations of type viruses for genus *Pelarspovirus* and proposed genera for carmovirus division. RT marks the read through stop codon. ORFs with similar functions are shaded in similar color-families. Dashed line of PLPV MP2 marks a noncanonical start codon. Thin lines under genomes indicate sgRNAs. See Table 2 for virus acronyms.

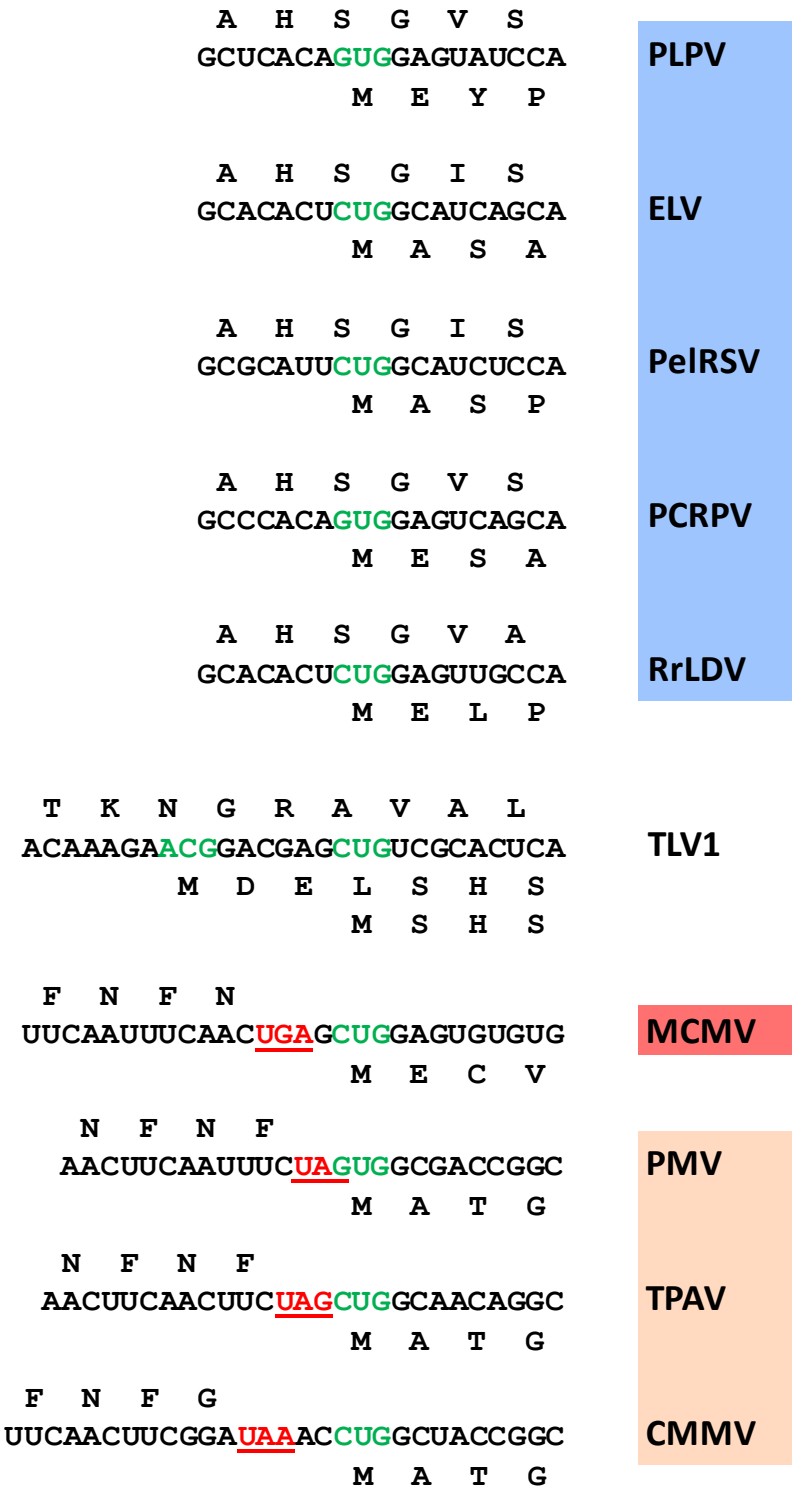

Figure 2. Contexts for MP2 noncanonical start codons (green) for pelarspoviruses, TLV1, MCMV and panicoviruses. Alternative start codons are shown for TLV1. MP1 sequences are above the RNA with stop codons in red. See Table 2 for virus acronyms.

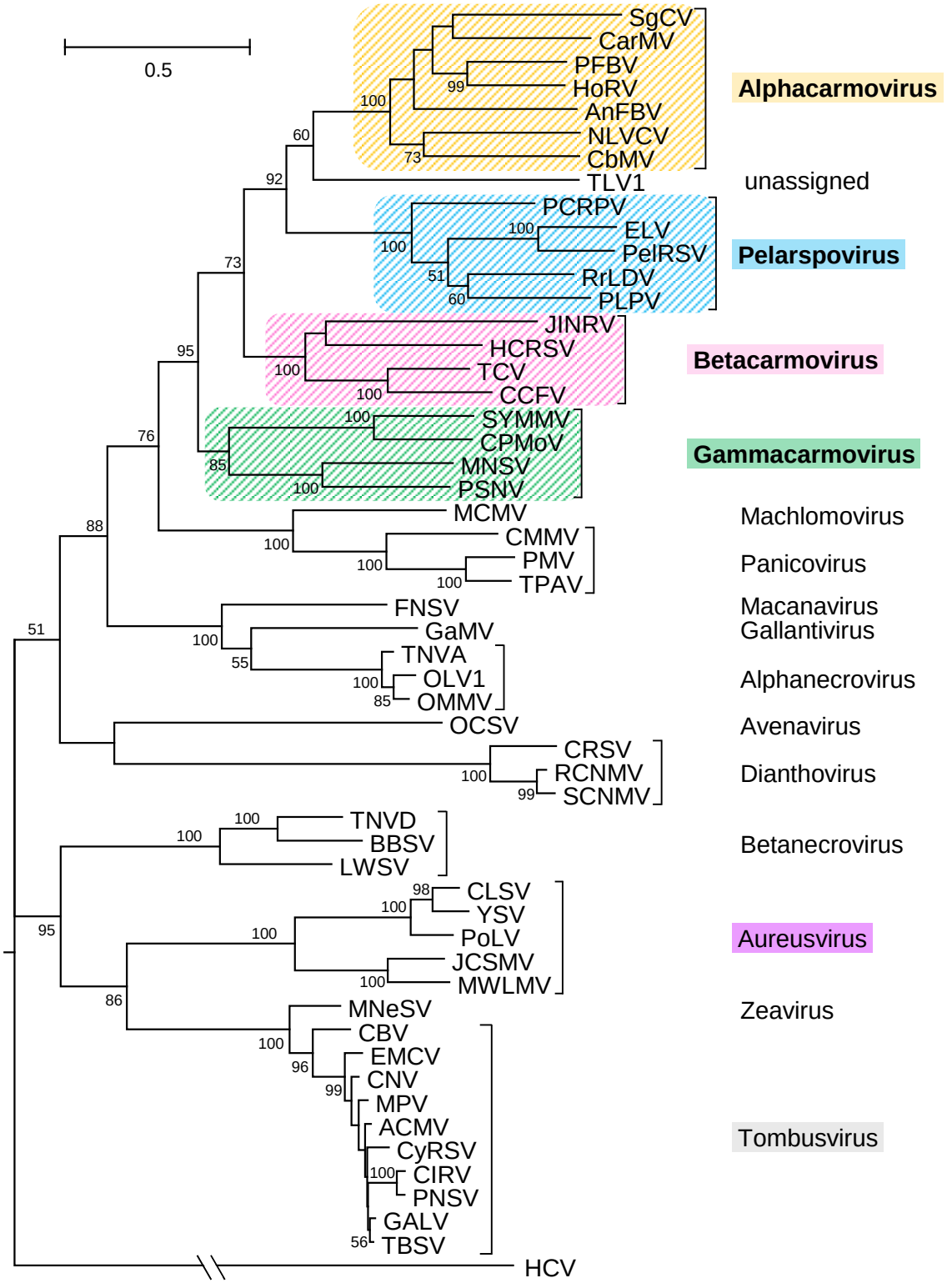

Figure 3. Phylogenetic (distance) analysis of the RdRps of CP-encoding tombusvirids. Alignments were made using MUSCLE while trees were generated with the Maximum Likelihood (ML) algorithm using 1000 bootstrap replicates (showing values >50%). Positions with < 50% site coverage were eliminated, leaving 765 positions in the final dataset. Hepatitis C virus (HCV) RdRp was used as the outgroup. Brackets mark monophyletic lineages.

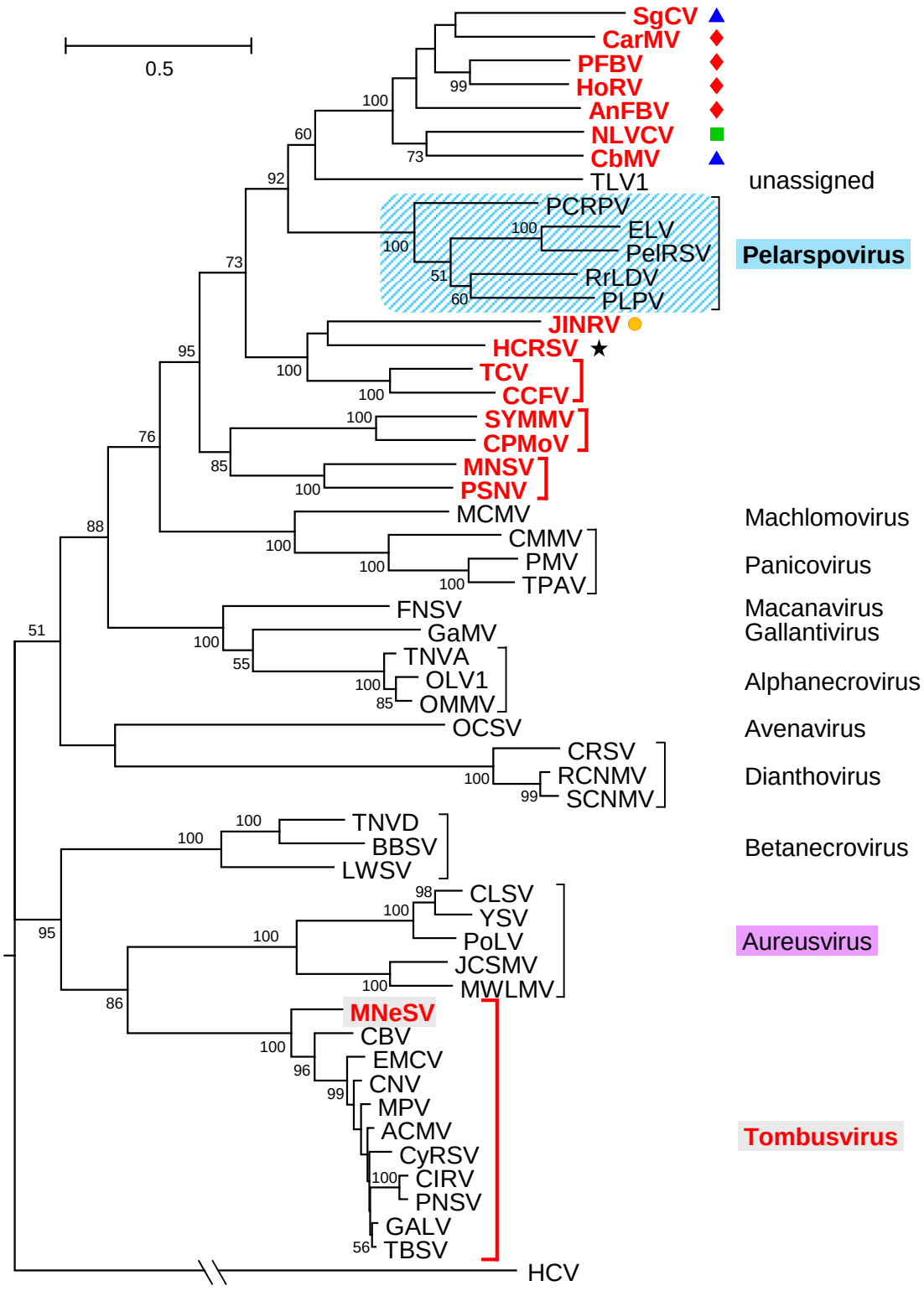

Figure 4. Phylogenetic tree from Fig. 2 with genera identified by using PASC analysis with the following nt % identity cutoffs: merge species above 82%; separate species below 80%; merge genera above 45%; separate genera below 44%. Species in alternative genera have bold red font. Brackets mark monophyletic lineages. Species that would represent alternative genera that are monotypic or not monophyletic are marked with color- and shape-specific symbols.

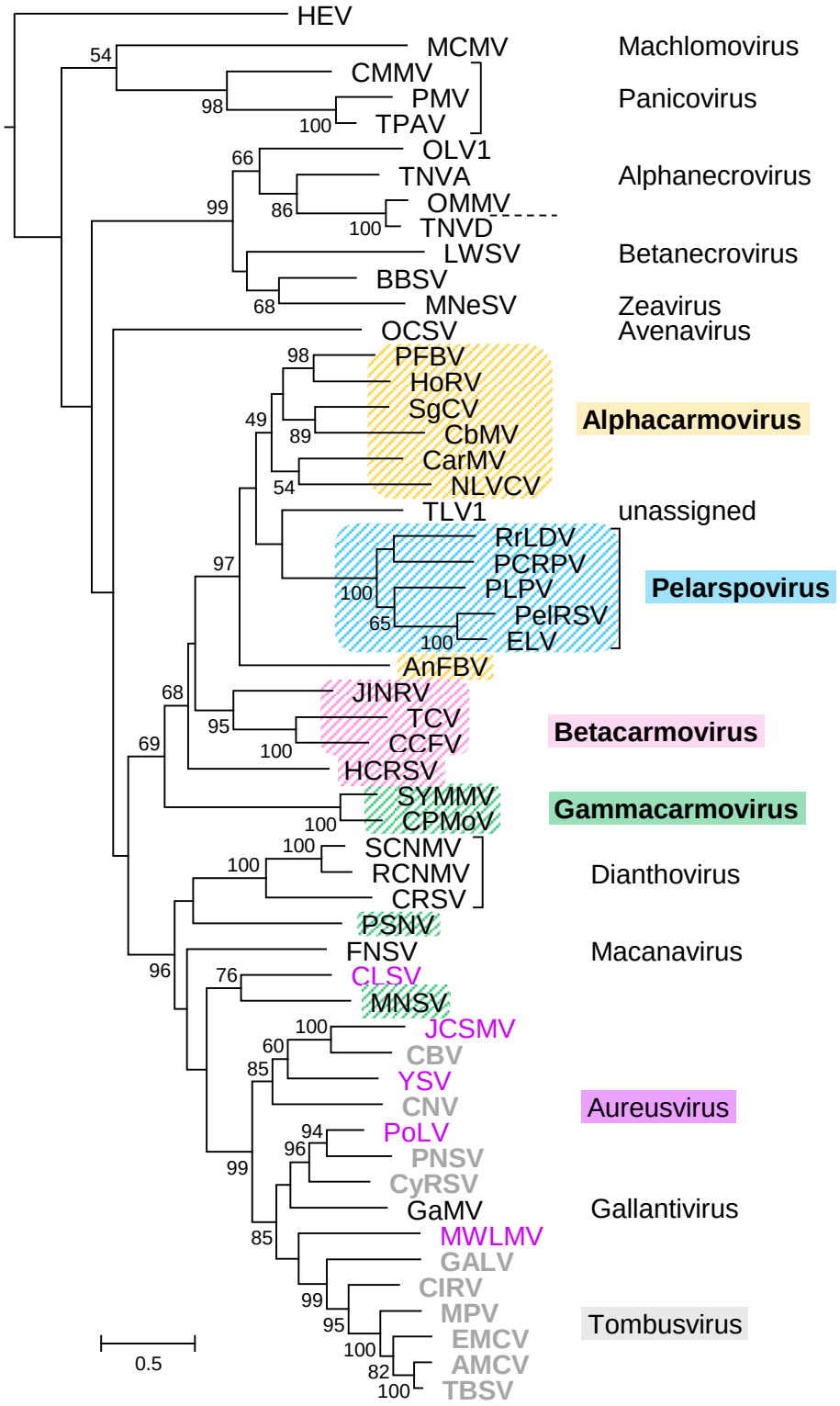

Figure 5. Phylogenetic (distance) analysis of the CPs of CP-encoding tombusvirids. Trees were generated using the same conditions as Fig. 2. There were 356 positions in the final dataset. Hepatitis E virus (HEV) CP was used as the outgroup. Brackets mark monophyletic lineages.

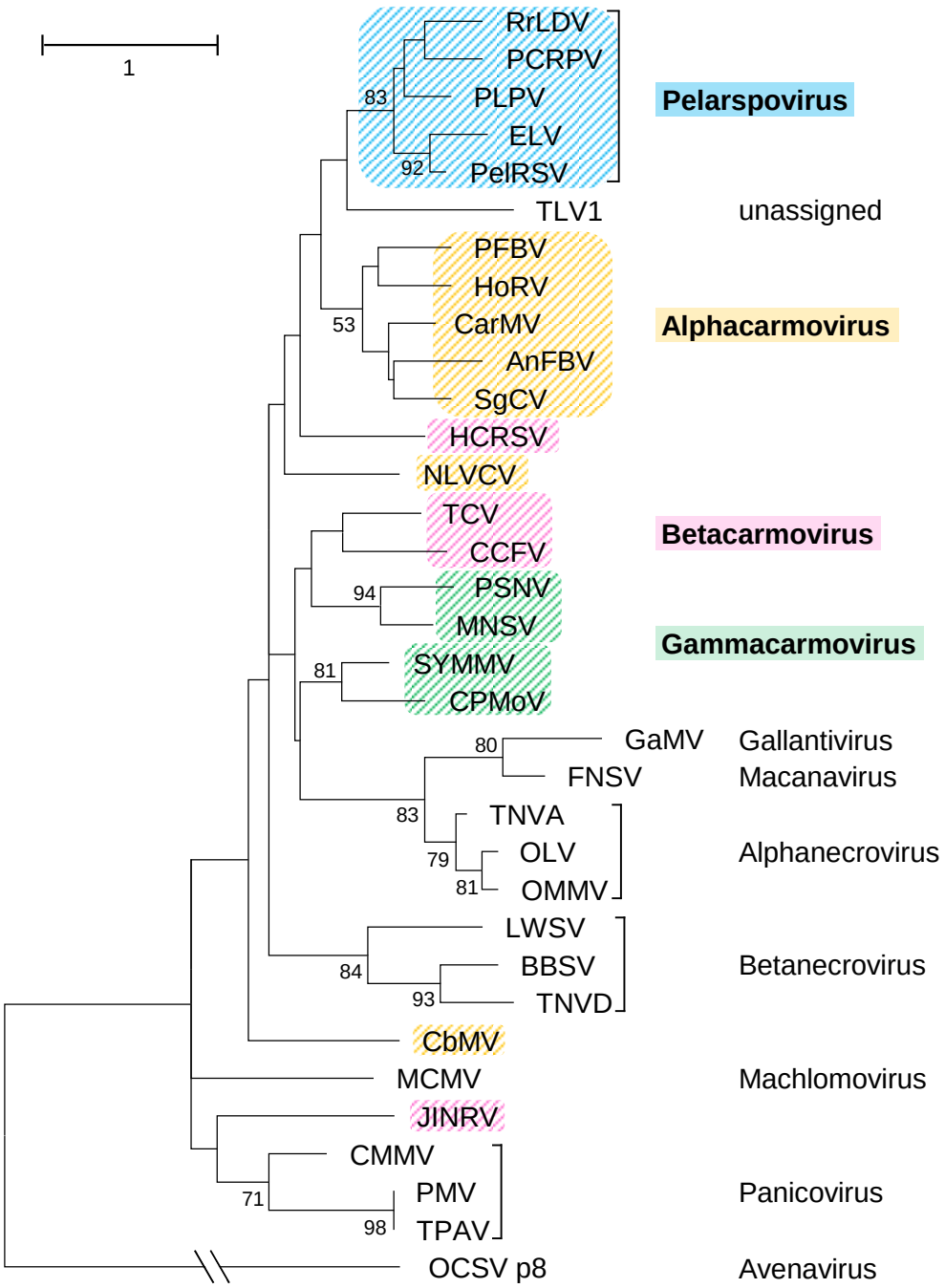

Figure 6. Phylogenetic (distance) analysis of the MP1s of CP-encoding tombusvirids. Trees were generated using the same conditions as Fig. 2. Positions with < 50% site coverage were eliminated, leaving 70 positions in the final dataset. Oat chlorotic stunt virus (OCSV) p8 was used as the outgroup. Brackets mark monophyletic lineages.

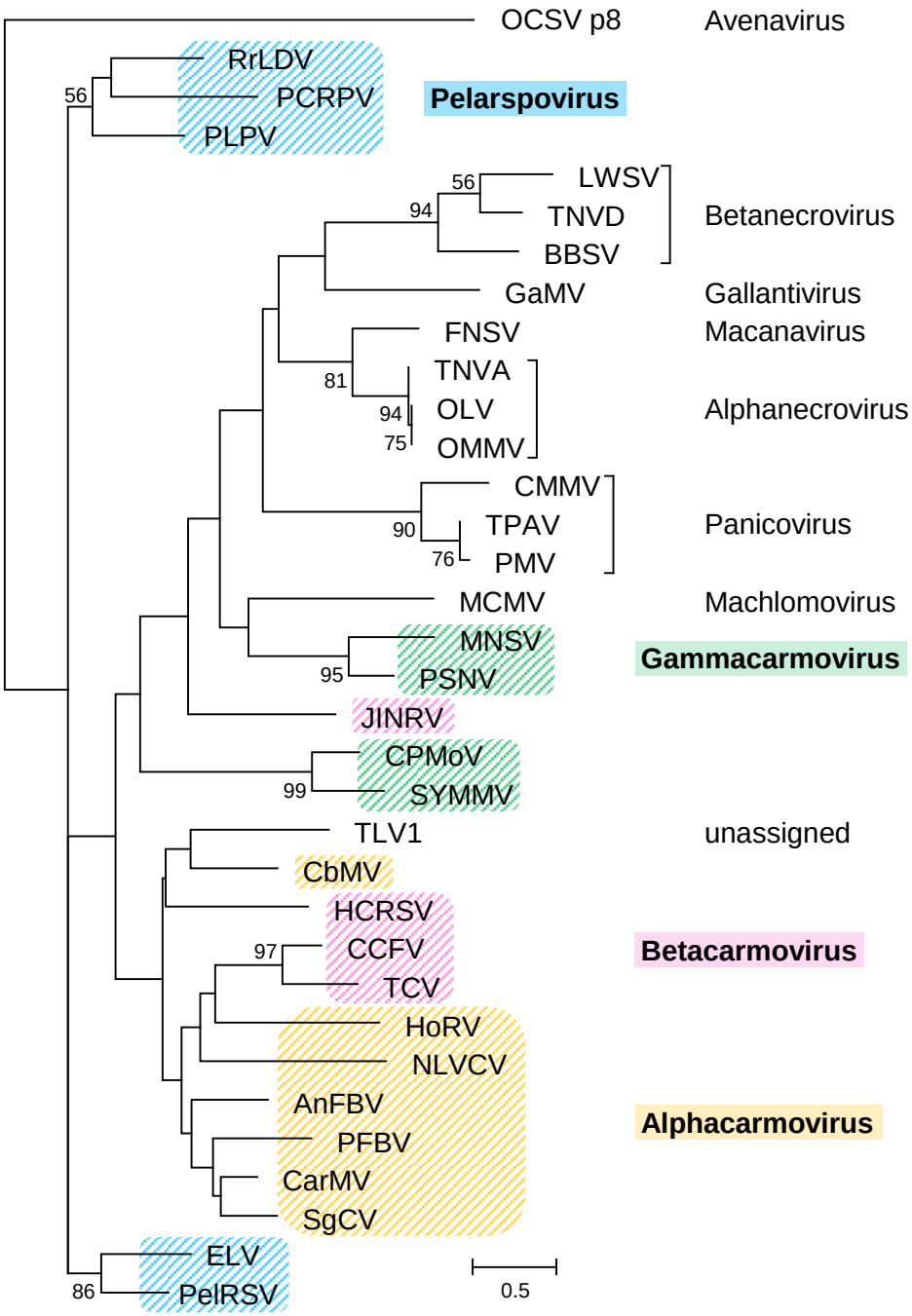

Figure 7. Phylogenetic (distance) analysis of the MP2s of CP-encoding tombusvirids. Trees were generated using the same conditions as Fig. 2. Positions with < 50% site coverage were eliminated, leaving 81 positions in the final dataset. OCSV p8 was used as the outgroup. Brackets mark monophyletic lineages.

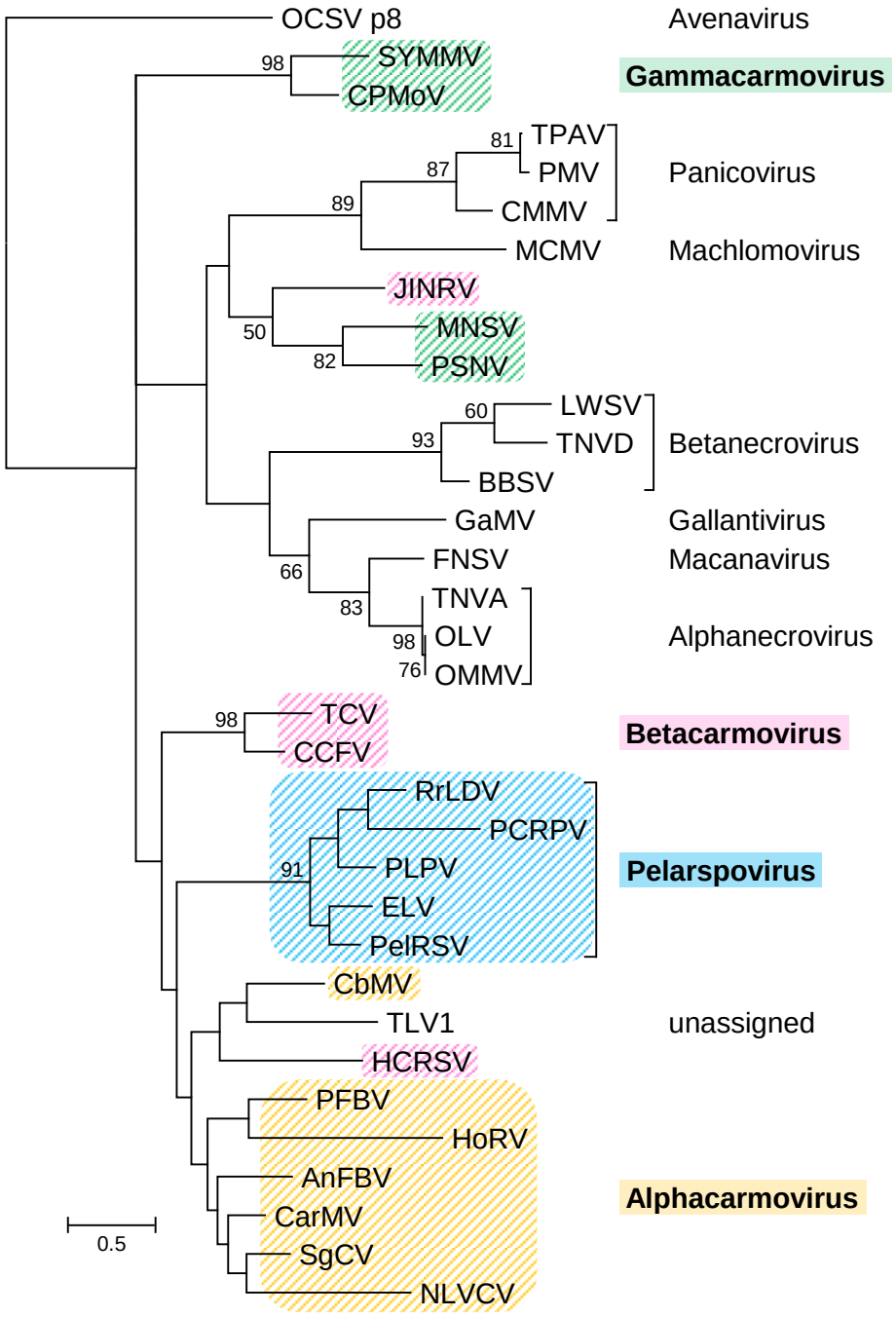

Figure 8. Phylogenetic (distance) analysis of the MP2s of CP-encoding tombusvirids. Trees were generated using the same conditions as Fig. 2 except initial alignment was performed with ClustalOmega. Positions with < 50% site coverage were eliminated, leaving 83 positions in the final dataset. OCSV p8 was used as the outgroup. Brackets mark monophyletic lineages.