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

Code assigned:	***2013.010a,bP***

Short title: Transfer of the *Umbravirus* genus from "unassigned genus" to *Tombusviridae* family.

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:

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Giovanni Martelli
Luisa Rubino
Kay Scheets
Andy White

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

Tombusviridae study group	

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

Your proposal was discussed at the EC and generated quite a bit of discussion. Some members of the EC, felt that perhaps the creation of an Umbraviridae family would be granted, while others were in support of the proposal. In general, the group felt it would be prudent to wait a year until approval of the proposal to allow the proposal to be posted and let the community input comments if they had any objections. We thought that perhaps it may be a good idea to also consult with other SGs working with related viruses (perhaps the luteovirus SG) and see if they also support the proposal. For these reasons, the proposal was given a Ud status. It will be posted on the ICTV website for a year and will be reconsidered next year.

Before it is posted however, the EC recommended a few editorial changes to improve the proposal.

1. On Figure 1, the labeling should read "Umbravirus, unassigned genus" rather than "Umbravirus, unassigned family"
2. Also on Fig. 1, it may be a good idea to show the frameshift in the Dianthovirus diagram in the same manner as shown for the umbravirus diagram.
3. Fig. 2, please give info on program used to generate alignment and tree
4. Fig. 2, many wondered why you used cytochrome C as an outgroup. It was suggested that using a sequence closer to the sequences aligned (e.g. using a flavivirus) would be a better idea.

Response:

Each of the suggested modifications were made. We thank Helene and the remaining Executive Committee members for their comments.

D'Ann Rochon, Tombusviridae Study Group Chair

Date first submitted to ICTV: June 17, 2013	June 2013
Date of this revision (if different to above):	Oct 2 2013

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	***2013.010aP***	(assigned by ICTV officers)

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

Umbravirus

The present taxonomic position of these taxon/taxa:

Genus:	
Subfamily:	
Family:	**Unassigned**
Order:	

Reasons to justify the removal:

Phylogenetic analyses indicate that the general genome structure and RDRP of the members of *Umbravirus* genus clearly groups within the *Tombusviridae*.

Part (b) re-assign to a higher taxon

Code	***2013.010bP***	(assigned by ICTV officers)

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

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

Reasons to justify the re-assignment:

The main justification to assign the *Umbravirus* genus to the *Tombusviridae* family is that the RDRP clusters within the *Tombusviridae*. Consistent with this assignment is the genome structure (i.e., (+)ssRNA genome encoding the RdRp, an uncapped, non-polyadenylated genome and the possession of several similar functional RNA elements. We note that members of the *Umbravirus* genus do not encode a coat protein. This differs from a major characteristic of the *Tombusviridae* which is the possession of a spherical capsid with either rough or smooth morphology corresponding to a coat protein with or without a protruding domain. However, members of the *Tombusviridae* study group feel that the RdRp phylogenetic results are compelling and should override the lack of a coat protein. Correspondingly, the study group recognizes that this descriptive feature of the

Tombusviridae should be adjusted to reflect inclusion of the *Umbravirus* genus should this proposal be accepted. The Appendix in Module 9 provides background and a more detailed justification.

References:

1. Virus Taxonomy: 9th Report of the International Committee on the Taxonomy of Viruses. (*Tombusviridae* and *Umbravirus* chapters)
2. Wang Z, Treder K, Miller WA. (2009) Structure of a viral cap-independent translation element that functions via high affinity binding to the eIF4E subunit of eIF4F. J Biol Chem. 284:14189-202.
3. Wang Z, Kraft JJ, Hui AY, Miller WA (2010) Structural plasticity of Barley yellow dwarf virus-like cap-independent translation elements in four genera of plant viral RNAs. Virology. 402:177-86
4. Gao F, Kasprzak W, Stupina VA, Shapiro BA, Simon AE. (2012) A ribosome-binding, 3'-translational enhancer has a T-shaped structure and engages in a long-distance RNA-RNA interaction. J Virol. 86:9828-42.
5. Nicholson BL, White KA (2011) 3' Cap-independent translation enhancers of positive-strand RNA plant viruses. Curr Opin Virol. 1:373-80.
6. Simon AE, Miller WA. (2013) 3' Cap-Independent Translation Enhancers of Plant Viruses. Annu Rev Microbiol. [Epub ahead of print]
7. Tajima Y, Iwakawa HO, Kaido M, Mise K, Okuno T. (2011) A long-distance RNA-RNA interaction plays an important role in programmed -1 ribosomal frameshifting in the translation of p88 replicase protein of Red clover necrotic mosaic virus. Virology. 417:169-78.
8. Cimino PA, Nicholson BL, Wu B, Xu W, White KA. Multifaceted regulation of translational readthrough by RNA replication elements in a tombusvirus. PLoS Pathog. 7(12):e1002423. doi: 10.1371/journal.ppat.1002423.
9. Na H, White KA. (2006) Structure and prevalence of replication silencer-3' terminus RNA interactions in Tombusviridae. Virology. 345(2):305-16.

Annex:

Genome structure and function

The *Umbraviruses* [type member = Carrot mottle virus (CMoV)] are plus sense, single-stranded RNA viruses that do not encode a coat protein (1). Their genomes are encapsidated by the coat protein of "helper" viruses in the aphid-transmitted *Enamo*- and *Polerovirus* genera of the *Luteoviridae*. Encapsidation enables aphid-transmission. Complete genome sequences for 5 umbraviruses are known along with partial or complete genome sequences of 3 of the helper viruses (Table1). The genome consists of a single molecule of RNA which is 4.0-4.2 kb in length. Similar to tombusvirids, the 5' terminus is not capped and the 3' terminus is not polyadenylated.

The umbravirus genome, as depicted by CMoV in Fig. 1, contains 4 ORFs. ORF1 encodes a putative product of 31-37 kDa and ORF2, which overlaps the 3' end of ORF1, encodes a product of 63-65 kDa that lacks an AUG codon and is probably translated following a frameshift event during translation of ORF1. The 63-65 kDa region contains amino acid

sequences typical of RdRps. ORF3 and ORF4 overlap extensively. ORF3 encodes an approximate 26-29 kDa protein involved in long distance movement. No similarity exists between this protein and tombusvirid encoded proteins or to any other proteins in the databases. ORF4 encodes an approximate 28 kDa movement protein which is similar to the *Cucumovirus* 3A movement protein family. Members of the *Tombusviridae* have one of 4 distinct types of movement proteins with the *Dianthovirus* genus encoding a 3A-like movement protein similar to that of the umbraviruses. ORF3 and ORF4 are probably encoded by a subgenomic RNA since a dsRNA from infected plants of carrot mottle mimic virus (CMoMV) has been shown to contain ORFs 3 and 4 and the 3'UTR, and probably represents the dsRNA form of the subgenomic RNA. A subgenomic strategy for expression of downstream ORFs is demonstrated by all members of the *Tombusviridae*. In addition, the frameshifting strategy putatively utilized by the umbraviruses is a strategy employed by the dianthoviruses (see below). The genome organizations of several *Tombusviridae* members are shown in Fig. 2 to show that the general genome organization of umbraviruses is similar to that of several tombusvirids, especially the tombusviruses and the aureusviruses. It is also similar to that of zeaviruses and gallantiviruses of the *Family Tombusviridae* (not shown) with the notable absence of a coat protein ORF in the umbraviruses.

Functional RNA elements

In terms of functional RNA elements, the umbraviruses share many striking similarities with tombusvirids. First, the uncapped and non-polyadenylated umbraviruses possess predicted and functionally-confirmed 3'-cap-independent translational enhancers (3'CITEs) in their 5'UTR (2,3,4). This unusual translational mechanism is also employed by nearly all genera in the *Tombusviridae* (5,6) (Fig. 1).

Second, umbraviruses are predicted to express their RdRp via a frameshift event (Fig. 1). Sequence and structure analysis of the RNA genomes of umbraviruses predicts a long-range RNA base-pairing interaction involving the loop sequence of the 3'-terminal stem loop in their genomes with a bulge in a large stem loop RNA structure located just 3' to the predicted frame-shift sequence (K.A. White, unpublished data). Notably, a corresponding long-range RNA base-pairing interaction was shown to be essential for frameshift-mediated RdRp production in the *Dianthovirus* RCNMV (*Family Tombusviridae*) (7) and equivalent long-distance RNA interactions are critical for readthrough-mediated RdRp production in all other genera in the *Tombusviridae* (8).

Third, the umbraviruses possess a 3'-terminal –CCC-$_{OH}$ sequence that is also present in tombusvirids. In tombusviruses this sequence pairs with an internal RNA structure referred to as replication silencer element and sequence and structure analysis of umbraviruses has predicted the formation of similar pseudoknot-type structures at the 3' ends of their genomes (9). Collectively, the presence of three different and important types of RNA elements in umbraviruses, all of which are closely associated with current tombusvirids, lends additional support for their inclusion within this family.

Phylogenetic analysis

The dendrogram in Fig. 2 shows that the RdRp of the umbraviruses forms a cohesive group within the *Tombusviridae* lineage. This observation provides compelling evidence for inclusion of the *Umbravirus* genus in the *Tombusviridae.*

Table 1. List of approved *Umbravirus* species and associated helper viruses[1].

Umbravirus	**Acronym**	**Helper virus**	**Taxon of Helper Virus**
Carrot mottle virus (Type member)	CMoV	Carrot red leaf virus (CRLV)	*Polerovirus* (*Family Luteoviridae*)
Carrot mottle mimic virus	CMoMV	Carrot red leaf virus (CRLV)	*Polerovirus* (*Family Luteoviridae*)
Lettuce speckles mottle	LSMV	Beet western yellows virus (BWYV)	*Polerovirus* (*Family Luteoviridae*)
Pea enation mosaic virus-2	PEMV-2	Pea enation mosaic virus-1 (PEMV-1)	*Enamovirus* (*Family Luteoviridae*)
Tobacco bushy top	TBTV	Tobacco vein distorting virus (TVDV)	*Polerovirus* (*Family Luteoviridae*)
Tobacco mottle virus	TMoV	Tobacco vein distorting virus (TVDV)	*Polerovirus* (*Family Luteoviridae*)
Groundnut rosette virus	GRV	Groundnut rosette assistor virus	Unassigned genus in *Family Luteoviridae*

[1] See Ref. 1

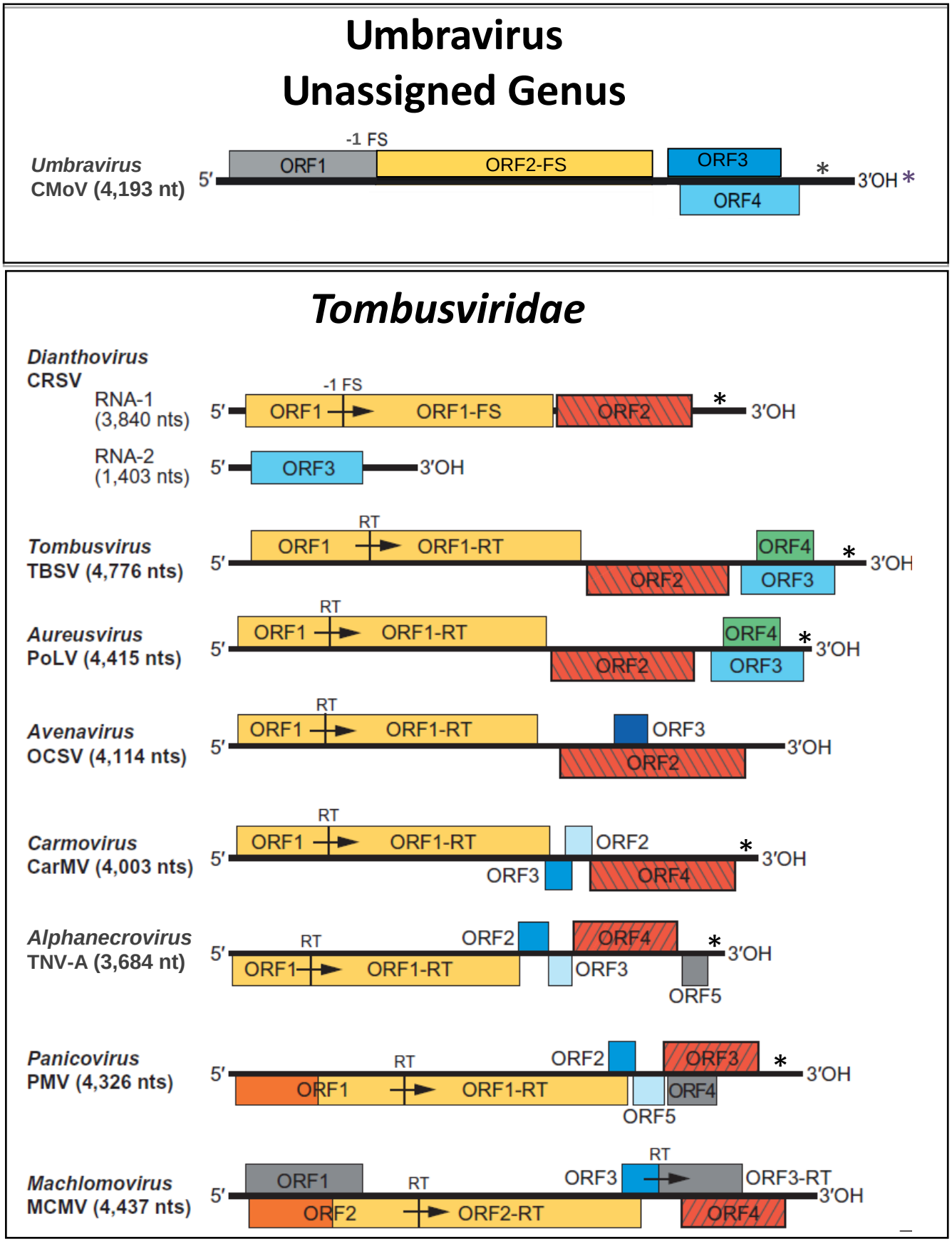
Umbravirus
Unassigned Genus
Umbravirus
CMoV (4,193 nt)
-1 FS
ORF1
ORF2-FS
ORF3
ORF4
5′
3′OH
Tombusviridae
Dianthovirus
CRSV
RNA-1
(3,840 nts)
RNA-2
(1,403 nts)
ORF1-FS
Tombusvirus
TBSV (4,776 nts)
Aureusvirus
PoLV (4,415 nts)
Avenavirus
OCSV (4,114 nts)
Carmovirus
CarMV (4,003 nts)
Alphanecrovirus
TNV-A (3,684 nt)
ORF5
Panicovirus
PMV (4,326 nts)
Machlomovirus
MCMV (4,437 nts)
RT
ORF1-RT
ORF2-RT
ORF3-RT
Polymerase
Movement protein(s)
Coat protein
3′ Cap independent translational element

Figure 1

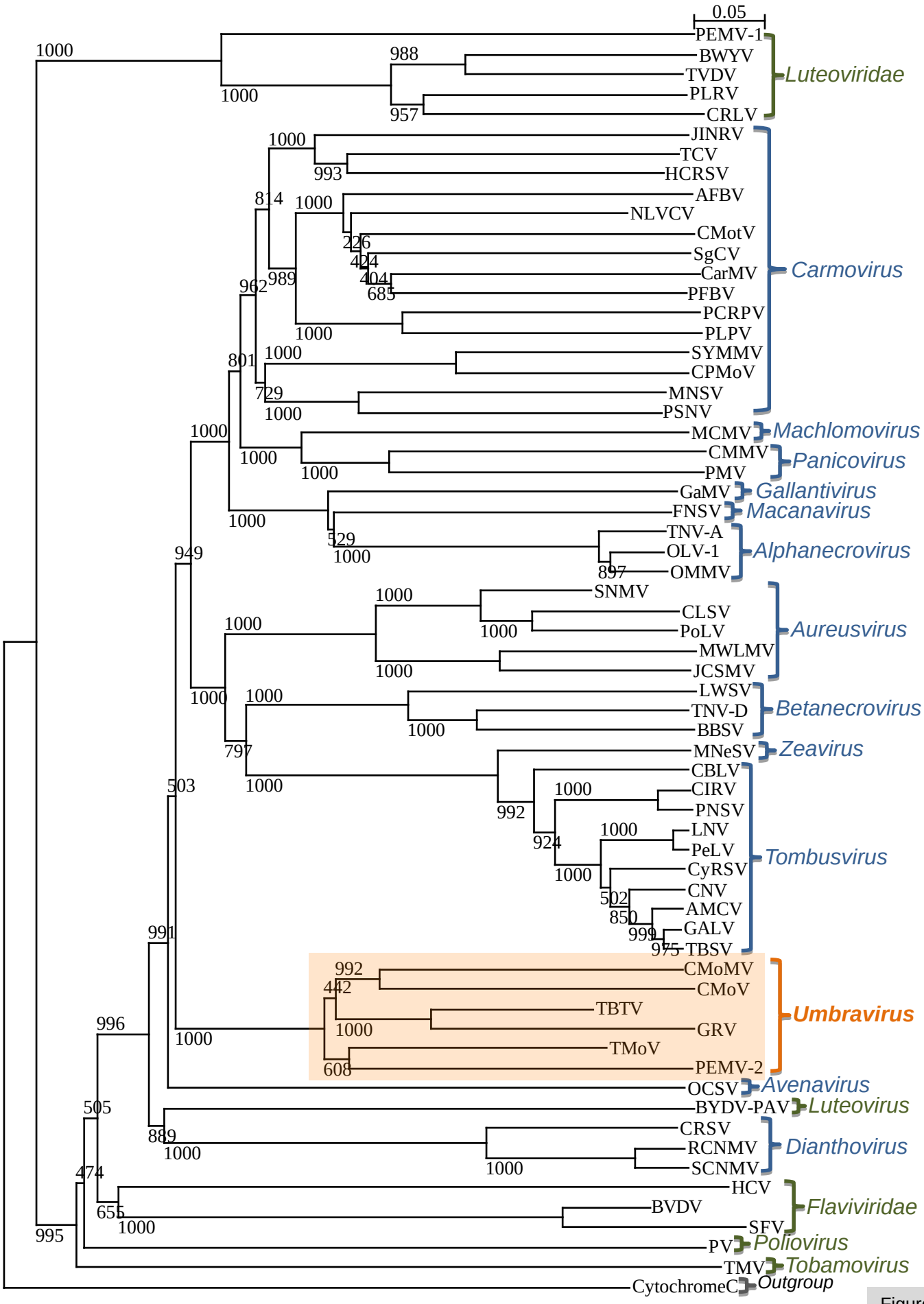
0.05
PEMV-1
BWYV
TVDV
PLRV
CRLV
Luteoviridae
JINRV
TCV
HCRSV
AFBV
NLVCV
CMotV
SgCV
CarMV
PFBV
PCRPV
PLPV
SYMMV
CPMoV
MNSV
PSNV
Carmovirus
MCMV
Machlomovirus
CMMV
PMV
Panicovirus
GaMV
Gallantivirus
FNSV
Macanavirus
TNV-A
OLV-1
OMMV
Alphanecrovirus
SNMV
CLSV
PoLV
MWLMV
JCSMV
Aureusvirus
LWSV
TNV-D
BBSV
Betanecrovirus
MNeSV
Zeavirus
CBLV
CIRV
PNSV
LNV
PeLV
CyRSV
CNV
AMCV
GALV
TBSV
Tombusvirus
CMoMV
CMoV
TBTV
GRV
TMoV
PEMV-2
Umbravirus
OCSV
Avenavirus
BYDV-PAV
Luteovirus
CRSV
RCNMV
SCNMV
Dianthovirus
HCV
BVDV
SFV
Flaviviridae
PV
Poliovirus
TMV
Tobamovirus
CytochromeC
Outgroup

Figure 2

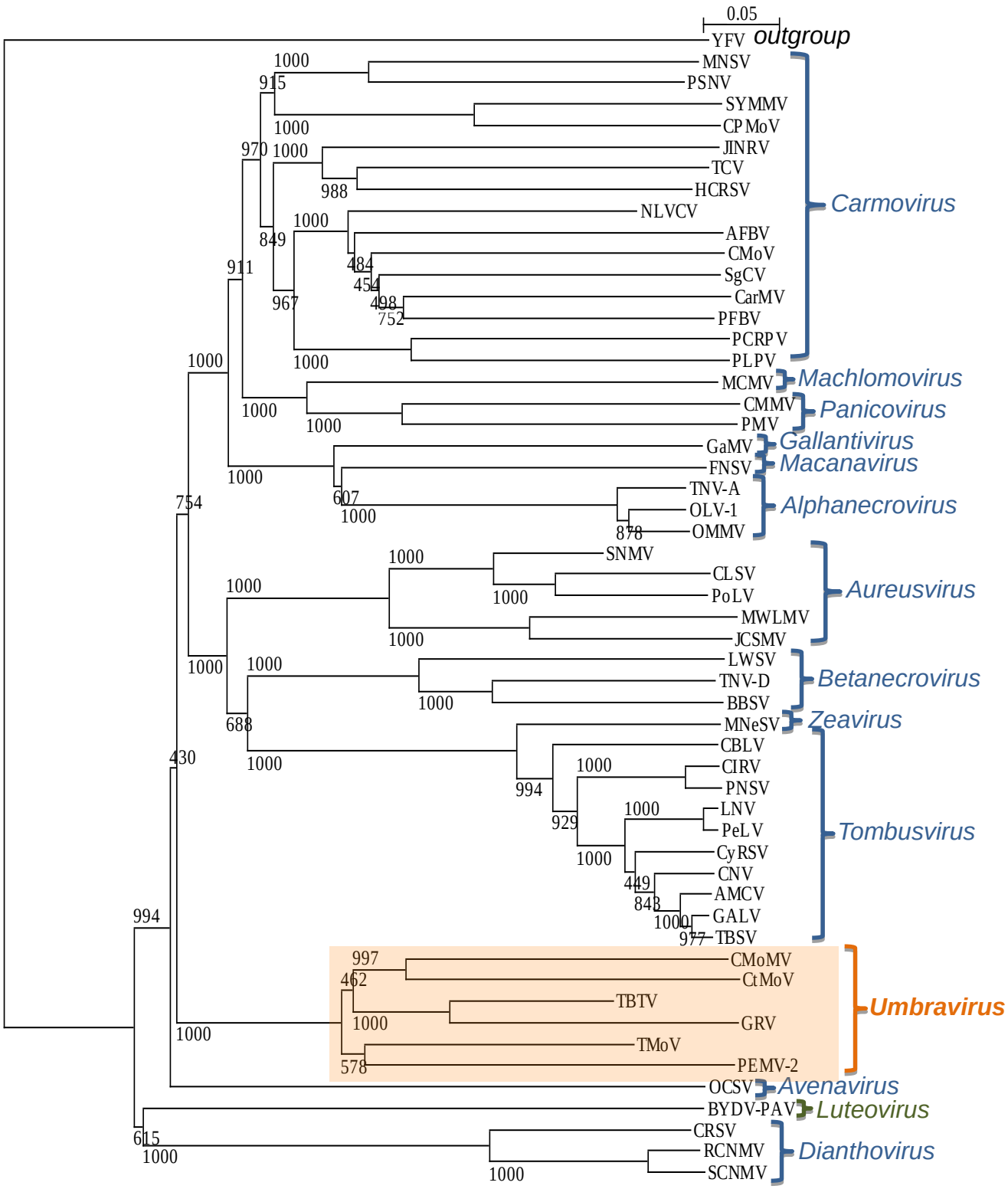
0.05
YFV
outgroup
MNSV
PSNV
SYMMV
CPMoV
JINRV
TCV
HCRSV
NLVCV
AFBV
CMoV
SgCV
CarMV
PFBV
PCRPV
PLPV
Carmovirus
MCMV
Machlomovirus
CMMV
PMV
Panicovirus
GaMV
Gallantivirus
FNSV
Macanavirus
TNV-A
OLV-1
OMMV
Alphanecrovirus
SNMV
CLSV
PoLV
MWLMV
JCSMV
Aureusvirus
LWSV
TNV-D
BBSV
Betanecrovirus
MNeSV
Zeavirus
CBLV
CIRV
PNSV
LNV
PeLV
CyRSV
CNV
AMCV
GALV
TBSV
Tombusvirus
CMoMV
CtMoV
TBTV
GRV
TMoV
PEMV-2
Umbravirus
OCSV
Avenavirus
BYDV-PAV
Luteovirus
CRSV
RCNMV
SCNMV
Dianthovirus

Figure 3

Figure Legends

Figure 1. Genome organization of the type member of the *Umbravirus* genus (CMoV=Carrot mottle virus) and type members of several *Tombusviridae* genera. As indicated, the yellow bars correspond to replicase associated proteins, the light or dark blue bars to the 1 or sometimes 2 movement proteins encoded by members of some genera and the red bars, the coat protein. Gray bars indicate proteins with unknown function. ORF4 of the tombusviruses and aureusviruses encodes a suppressor of silencing (also encoded by either the movement protein or coat protein of other viruses). The orange boxes in panicum mosaic virus (PMV) and maize chlorotic mottle virus (MCMV) are part of the viral replicase The asterisk indicates the presence of a 3' cap independent translational enhancer (CITE) present in 1 or more members of the indicated genera. Abbreviations: CRSV=Carnation ringspot virus; TBSV=Tomato bushy stunt virus; PoLV=Pothos latent virus; OCSV=Oat chlorotic stunt virus; CarMV= Carnation mottle virus; TNV-A= Tobacco necrosis virus-A; PMV=Panicum mosaic virus; MCMV= Maize chlorotic mottle virus. Genome structures were adapted from reference 1.

Figure 2. Dendrogram showing the relationships of the RdRps of several umbraviruses to the *Tombusviridae*. Genera of the *Tombusviridae* are colored blue, the umbravirus genus is colored orange and as well its members are highlighted in orange. Remaining virus genera or families are in green. Note that the 6 sequenced umbravirus members form a cohesive group within the *Tombusviridae*, suggesting that these viruses form a genus in the *Family Tombusviridae*. Note also that the RdRps of the umbraviruses do not show any significant relationship to those of their assistor viruses from the *Luteoviridae* family (PEMV-1 (an *Enamovirus*), TVDV and CRLV (*Poleroviruses*). Abbreviations: PEMV-1=Pea enation mosaic virus-1; BWYV=Beet western yellows virus; TVDV=Tobacco vein distorting virus; PLRV=Potato leafroll virus; CRLV= Carrot red leaf virus, CMoMV=Carrot mottle mimic virus; CMoV=Carrot mottle virus; TBTV= Tobacco

bushy top virus; GRV=Groundnut rosette virus; TMoV=Tobacco mottle virus; PEMV-2=Pea enation mosaic virus 2; HCV=Hepatitis C virus; BYDV-PAV=Barley yellow dwarf virus, a luteovirus whose RDRP has previously been shown to group with the *Tombusviridae* (see 9th Edition of Virus Taxonomy); BVDV=Bovine viral diarrhea virus; SFV=Classical Swine fever virus; PV=poliovirus (*Picornaviridae*) ; TMV=Tobacco mosaic virus (*Virgaviridae*). Abbreviations and accession numbers for members of the *Tombusviridae* can be found in reference 1. Accession numbers for the remaining viruses follow: PEMV-1=NP_620026.2; BWYV=ADR74377.1; TVDV=YP_001931931.2; PLRV=P11623.2; CRLV=YP_077186.1; CMoMV= ACJ03575.1; CMoV=YP_002302259; TBTV=CAR85733.1; GRV=NP_619714.1; TMoV=AAG02571.1; PEMV-2=NP_620846.2; BYDV-PAV=CAA30498; HCV=ADC54804; BVDV=BAC55962; SFV=NP075354.1; PV=AAA46912; TMV=CAA24688.1; Cytochrome C NP_865439.1. Sequences were aligned using the ClustalX 2.1 algorithm and the Gonnet protein weight matrix and trees were generated by the Neighbor Joining method using 1000 bootstrap replicates.

Figure 3. Dendrogram showing the relationships of the RdRps of several umbraviruses to the *Tombusviridae*. As in Fig. 2 but the *Luteoviruses* PEMV-1, BWYV, TVDV and PLRV are omitted as well as the *Flaviviridae* members HCV, BVDV and SFV. Poliovirus (PV) and TMV were also omitted. This allowed the construction of a phylogenetic tree in which the *Flavivirus* YFV (NP_04176.1) could be used as an outgroup.