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:	***2016.006aP***	(to be completed by ICTV officers)

Short title: Two new species in the genus *Umbravirus*, family *Tombusviridae*
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 11 are required)	**2** ☒

Author(s):

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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 Study Group

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

Date first submitted to ICTV:	July 6, 2016
Date of this revision (if different to above):	July 12, 2016

ICTV-EC comments and response of the proposer:

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	***2016.006aP***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Umbravirus***
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: (only 1 per species please)**	**GenBank sequence accession number(s)**
Ethiopian tobacco bushy top virus	18-2	KJ918748
Opium poppy mosaic virus	PHEL5235	EU151723

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 11

Ethiopian tobacco bushy top virus (ETBTV) combined with the polerovirus potato leaf roll virus and satellite RNA-E (satRNA-E) were found in diseased tobacco (*Nicotiana tabacum*) in Ethiopia [1]. This virus/helper virus/satRNA combination is distinct from the complex of the umbravirus tobacco bushy top virus (TBTV), polerovirus tobacco vein distorting virus (TVDV), and satRNAs producing similar symptoms in tobacco found in Zimbabwe and China [2, 3]. Both disease complexes are transmitted by the same aphid, *Myzus persicae*. *Nicandra physalodes* (shoofly plant or apple of Peru) is also a natural host for the complex of ETBTV, PLRV, and satRNA-E. Mechanical inoculation supports accumulation of ETBTV and satRNA-E, and produces symptoms in *N. occidentalis*, *N. hesperis*, *N. clevelandii*, and *Physalis floridana* while *N. rustica*, *N. tabacum*, and *Solanum lycopersicum* produced latent infections [1]. Groundnut (*Arachis hypogaea* L) was not infected via mechanical inoculation [1].

Opium poppy mosaic virus (OPMV) was found in New Zealand on *Papaver somniferum* (opium poppy), *P. rhoeas* and *Tropaeolum majus* L (nasturtium) plants showing leaf mosaic and mottling symptoms [4]. Sap inoculations produced varying systemic symptoms in *N. benthamiana*, *N. clevelandii*, and *N. tabacum* while local necrotic spots developed on *Gomphrena globosa*. A unique partial sequence of a virus related to viruses in the family *Luteoviridae* was also detected in field samples, suggesting that the helper virus, tentatively named opium poppy mosaic-associated virus, is unrelated to sequenced luteovirids [4].

Complete sequences of ETBTV and OPMV genomic RNAs (gRNAs) have been determined, and their sequences and genome structures (Appendix, Fig. 1) indicate they are umbraviruses, including expression of the RNA dependent RNA polymerase (RdRp) coding region via a -1 frameshift (-1 fs) [1, 4]. OPMV is most closely related to TBTV with 59.3% nt sequence identity [4], while ETBTV is most closely related to groundnut rosette virus (GRV), with 70.7% nt sequence identity [1]. GRV is transmitted by *Aphis craccivora* Koch causing disease in groundnuts in a complex with groundnut rosette assistor virus (unassigned luteovirid) and satellite RNAs (reviewed in [5]).

The following species demarcation criteria [6] were established before genus *Umbravirus* was moved into family *Tombusviridae*:

Natural host range
dsRNA band pattern (bearing in mind that some bands may represent satellite RNA species)
Nucleotide sequence identity less than 70%
Little or no hybridization with cDNA probes representing most parts of the genome

For coat protein-encoding members of the family *Tombusviridae,* the committee has found that the phylogenetic relationship of the replicase proteins provides the most robust division of species and genera [7]. The advent of improved RNA/DNA sequencing methods that allow complete viral genomes or genome segments to be sequenced and assembled from total RNA of infected plant material readily allows vRNAs from unencapsidated or low titre viruses to be determined, so the criteria related to hybridizations is no longer very useful.

We propose the species demarcation criteria be revised as follows:

at least two of the three following criteria shall be met
natural host range (including insect vector and helper virus as well as plant species)
less than 75% aa sequence identity of the viral replicase
less than 75% aa sequence identity of cell-to-cell movement protein (MP)

Protein homologies (Appendix, Tables 1-4) and phylogenetic trees (Appendix, Figs. 2-4) show the relationships of ETBTV and OPMV with established umbraviruses, and support their inclusion in the genus as unique umbravirus species.

Note that two ORF1 annotations are missing from GenBank and three RdRps have errors associated with the frameshift in the current GenBank submissions, so five sequences not available in initial GenBank entries are included in the annex. We are trying to get submitters to correct these sequences. Additionally, since none of the submitters for PEMV 2 (U03563) can be found, the group responsible for viral genomes at NCBI Reference Sequences have corrected PEMV 2 annotation in NC_003853.1 for its RdRp (NP_620846.3).

Acknowledgements

The author thanks study group members D’Ann Rochon, Luisa Rubino, Giovanni Martelli, and K. Andrew White for discussions on revising species demarcation criteria and for proposal review.

MODULE 11: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1 Abraham, A., Menzel, W., Bekele, B., Winter, S., 2014. A novel combination of a new umbravirus, a new satellite RNA and potato leafroll virus causes tobacco bushy top disease in Ethiopia. Archives of Virology 159, 3395-3399.

2 Mo, X.H., Chen, Z.B., Chen, J.P., 2010. Complete nucleotide sequence and genome organization of a Chinese isolate of tobacco vein distorting virus. Virus Genes 41, 425-431.

3 Mo, X.H., Qin, X.Y., Wu, J., Yang, C., Wu, J.Y., Duan, Y.Q., Li, T.F., Chen, H.R., 2003. Complete nucleotide sequence and genome organization of a Chinese isolate of tobacco bushy top virus. Arch Virol 148, 389-397.

4 Tang, J., Lebas, B., Liefting, L., Veerakone, S., Wei, T., Ward, L., 2016. Opium poppy mosaic virus, a new umbravirus isolated from *Papaver somniferum* in New Zealand. Arch Virol 161, 197-201.

5 Naidu, R.A., Kimmins, F.M., Deom, C.M., Subrahmanyam, P., Chiyembekeza, A.J., van der Merwe, P.J.A., 1999. Groundnut rossette: a virus disease affecting groundnut production in sub-Saharan Africa. Plant Disease 83, 700-709.

6 Ryabov, E.V., Taliansky, M.E., Robinson, D.J., Waterhouse, P.M., Murant, A.F., de Zoeten, G.A., Falk, B.W., Vetten, H.J., Gibbs, M.J., 2011. Genus *Umbravirus*, 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. 1191-1195.

7 Scheets, K., White, K.A., Rubino, L., Martelli, G., Rochon, D.A., 2015. ICTV taxonomic proposal 2015.007a-rP.A.v1.split_Carmovirus. Divide the genus *Carmovirus* into three new genera: *Alphacarmovirus*, *Betacarmovirus*, and *Gammacarmovirus*.

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 identities[a] of ORF1-encoded proteins of ETBTV (yellow) and OPMV (green) with those of recognized umbraviruses.

	CMoMV	PEMV 2	GRV	TBTV	ETBTV	OPMV
CMoV	39.0	26.1	31.2	30.1	32.4	28.4
CMoMV		26.5	31.6	27.7	34.8	25.1
PEMV 2			33.8	29.1	31.3	28.9
GRV				34.4	50.7	38.0
TBTV					33.1	41.7
ETBTV						36.9

Table 2. Percent amino acid identities of RdRps of ETBTV (yellow) and OPMV (green) with those of recognized umbraviruses.

	CMoMV	PEMV 2	GRV	TBTV	ETBTV	OPMV
CMoV	58.0	47.6	49.6	50.5	50.3	50.1
CMoMV		47.6	50.6	49.9	50.4	46.8
PEMV 2			50.6	51.4	50.4	51.1
GRV				59.6	72.0	58.1
TBTV					59.0	64.2
ETBTV						57.2

Table 3. Percent amino acid identities of long distance movement proteins (LD MPs) of ETBTV (yellow) and OPMV (green) with those of recognized umbraviruses.

	CMoMV	PEMV 2	GRV	TBTV	ETBTV	OPMV
CMoV	33.5	18.3	17.0	12.7	18.3	21.0
CMoMV		21.8	18.3	17.9	20.0	21.1
PEMV 2			33.6	38.0	35.2	31.4
GRV				36.0	50.2	36.0
TBTV					39.0	38.8
ETBTV						39.4

Table 4. Percent amino acid identities of cell-to-cell movement proteins (MPs) of ETBTV (yellow) and OPMV (green) with those of recognized umbraviruses.

	CMoMV	PEMV 2	GRV	TBTV	ETBTV	OPMV
CMoV	56.5	38.4	38.6	44.0	39.7	38.3
CMoMV		39.1	34.9	41.1	35.7	36.2
PEMV 2			54.8	59.2	55.2	51.2
GRV				60.1	77.9	55.9
TBTV					62.6	63.9
ETBTV						57.4

[a] based on MUSCLE

Table 5. Virus abbreviations and accession numbers for proposal

	Alphacarmovirus	
CarMV	Carnation mottle virus	X02986
	Alphanecrovirus	
TNVA	Tobacco necrosis virus A	M33002
	Aureusvirus	
CLSV	Cucumber leaf spot virus	EU127904
	Avenavirus	
OCSV	Oat chlorotic stunt virus	X83964
	Betacarmovirus	
TCV	Turnip crinkle virus	M22445
	Betanecrovirus	
TNVD	Tobacco necrosis virus D	U62546
	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
	Gammacarmovirus	
MNSV	Melon necrotic spot virus	M29671
	Macanavirus	
FNSV	Furcraea necrotic streak virus	FJ768020
	Machlomovirus	
MCMV	Maize chlorotic mottle virus	X14736
	Panicovirus	
PMV	Panicum mosaic virus	U55002
	Pelarspovirus	
PLPV	Pelargonium line pattern virus	AY613852
	Tombusvirus	
TBSV	Tomato bushy stunt virus	M21958
	Umbravirus	
CMoMV	Carrot mottle mimic virus	U57305
CMoV	Carrot mottle virus	FJ188473
ETBTV	Ethiopian tobacco bushy top virus (proposed)	KJ918748
GRV	Groundnut rosette virus	Z69910
OPMV	Opium poppy mosaic virus (proposed)	EU151723
PEMV 2	Pea enation mosaic virus 2	U03563
TBTV	Tobacco bushy top virus	AF402620
	Zeavirus	
MNeSV	Maize necrotic streak virus	AF266518
	other viral sequences	
YFV	Yellow fever virus NS5 protein	NP_776009.1

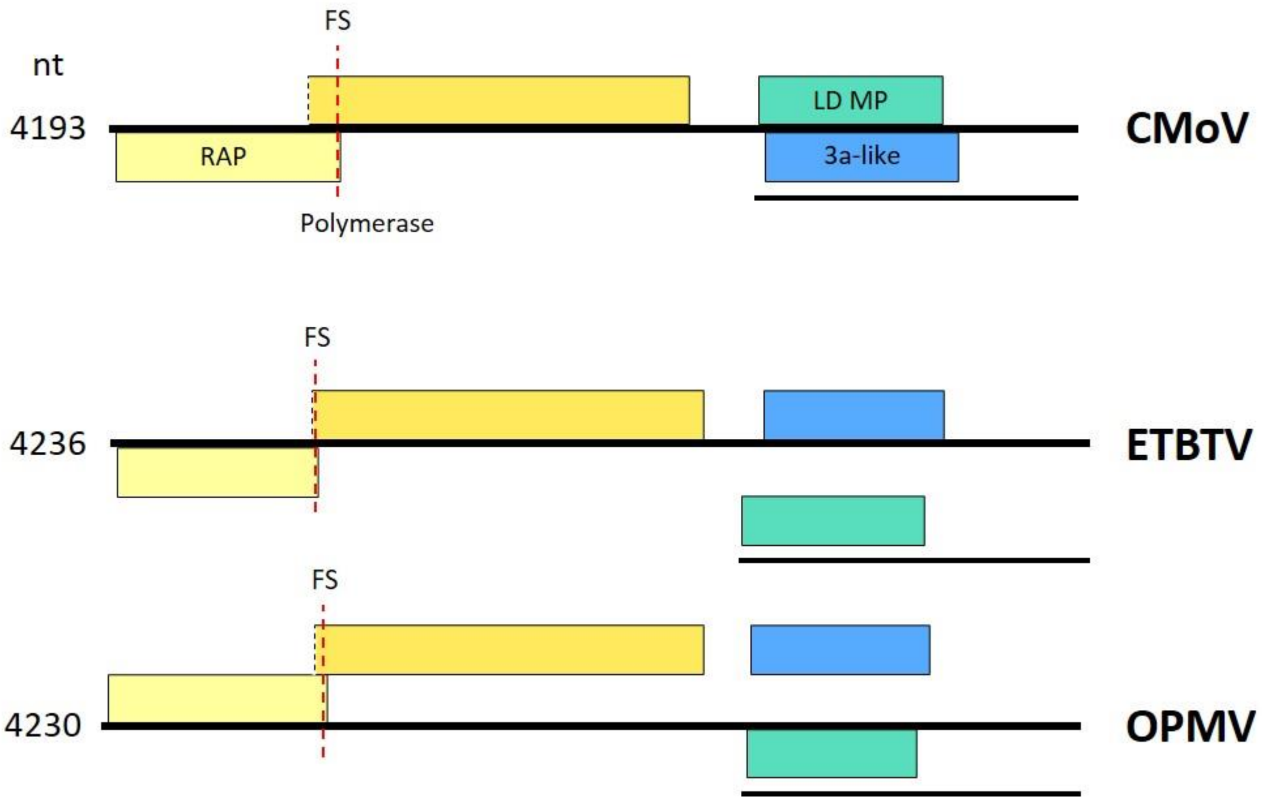

Figure 1. Genome structures of ETBTV and OPMV compared to carrot mottle virus (CMoV) (type species). Heavy lines mark gRNA while the thinner lines indicate a subgenomic RNA. The red dotted line marks the -1 fs location. ORF1 likely codes a replicase-associated protein (RAP), the frameshift product encodes the RdRp, ORF3 encodes a long distance (LD) MP, while ORF4 encodes a 3a-like MP.

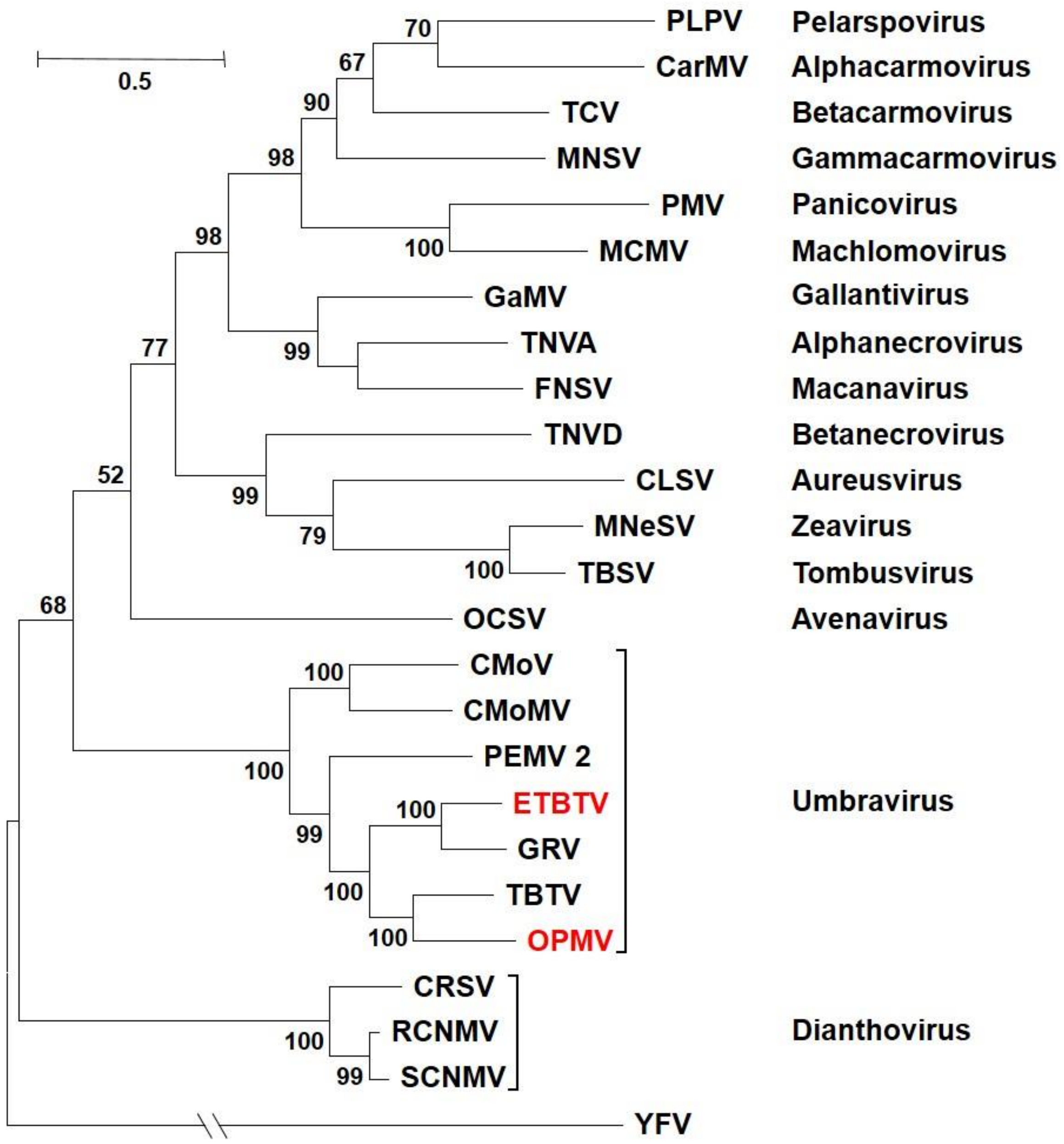

Figure 2. Phylogenetic (distance) analysis of the RdRps of umbraviruses compared to dianthoviruses and type species for genera that use readthrough expression of RdRp. Alignments were made using MUSCLE, and the initial tree was generated using Neighbor-Join and BioNJ algorithms with a matrix of pairwise distances estimated using a JTT model with the Maximum Likelihood (ML) algorithm. Numbers at branches show the percentage bootstrap support $\geq$ 50 % for 1000 replicates for the tree with highest log likelihood. Positions with < 50% site coverage were eliminated, leaving 776 positions in the final dataset. Yellow fever virus (YFV) RdRp (NS5NP) was used as the outgroup. Brackets mark monophyletic lineages. Red font indicates proposed species. Evolutionary analyses were conducted in MEGA5. Virus abbreviations are listed in Table 5.

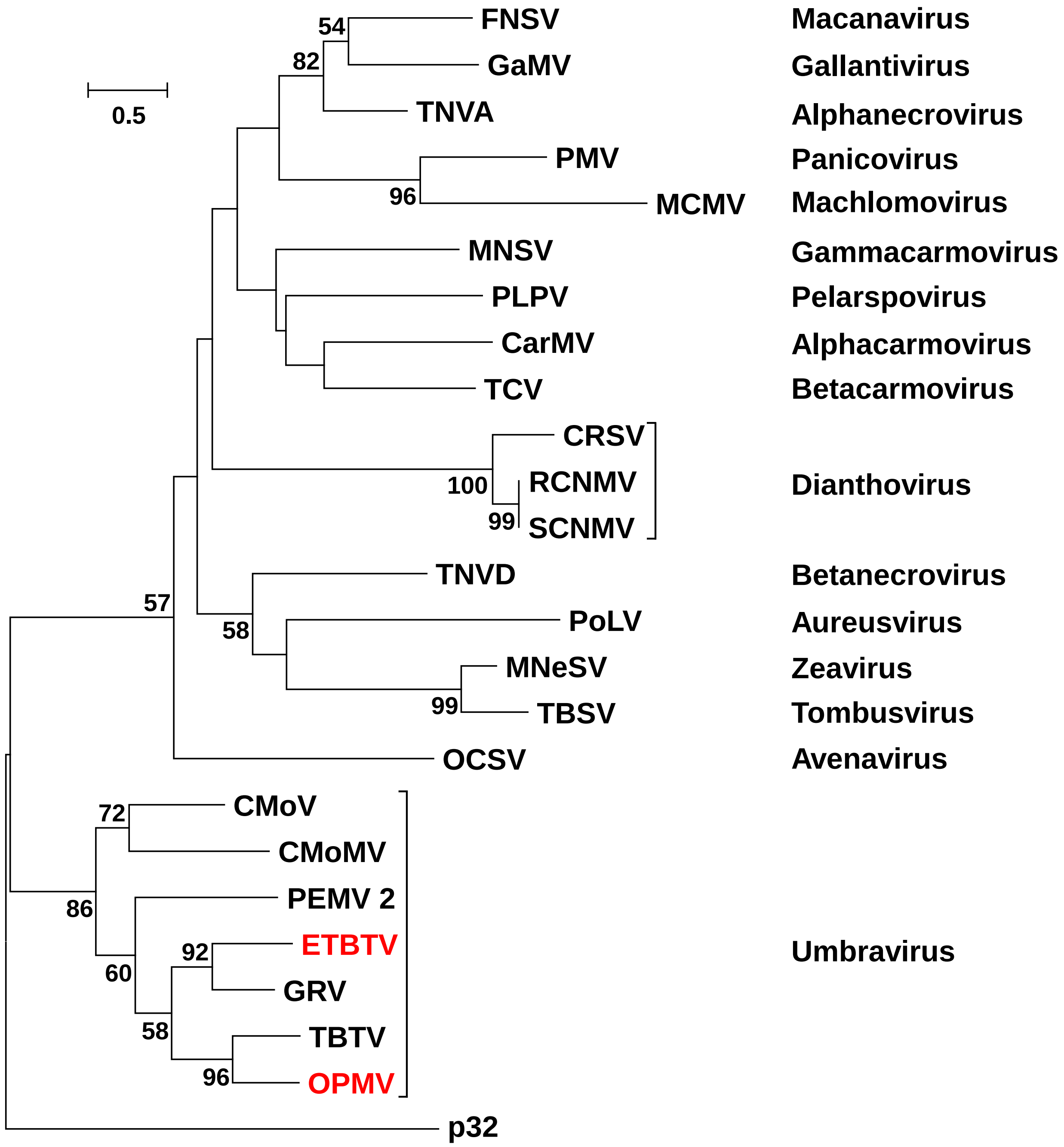

Figure 3. Phylogenetic analysis of the ORF 1-encoded proteins of umbraviruses compared to dianthoviruses and type species of genera that use readthrough expression of RdRp. Alignment, tree building methods, and labelling is the same as Fig. 2. There were 253 positions in the final dataset, and MCMV p32 was used as the outgroup.

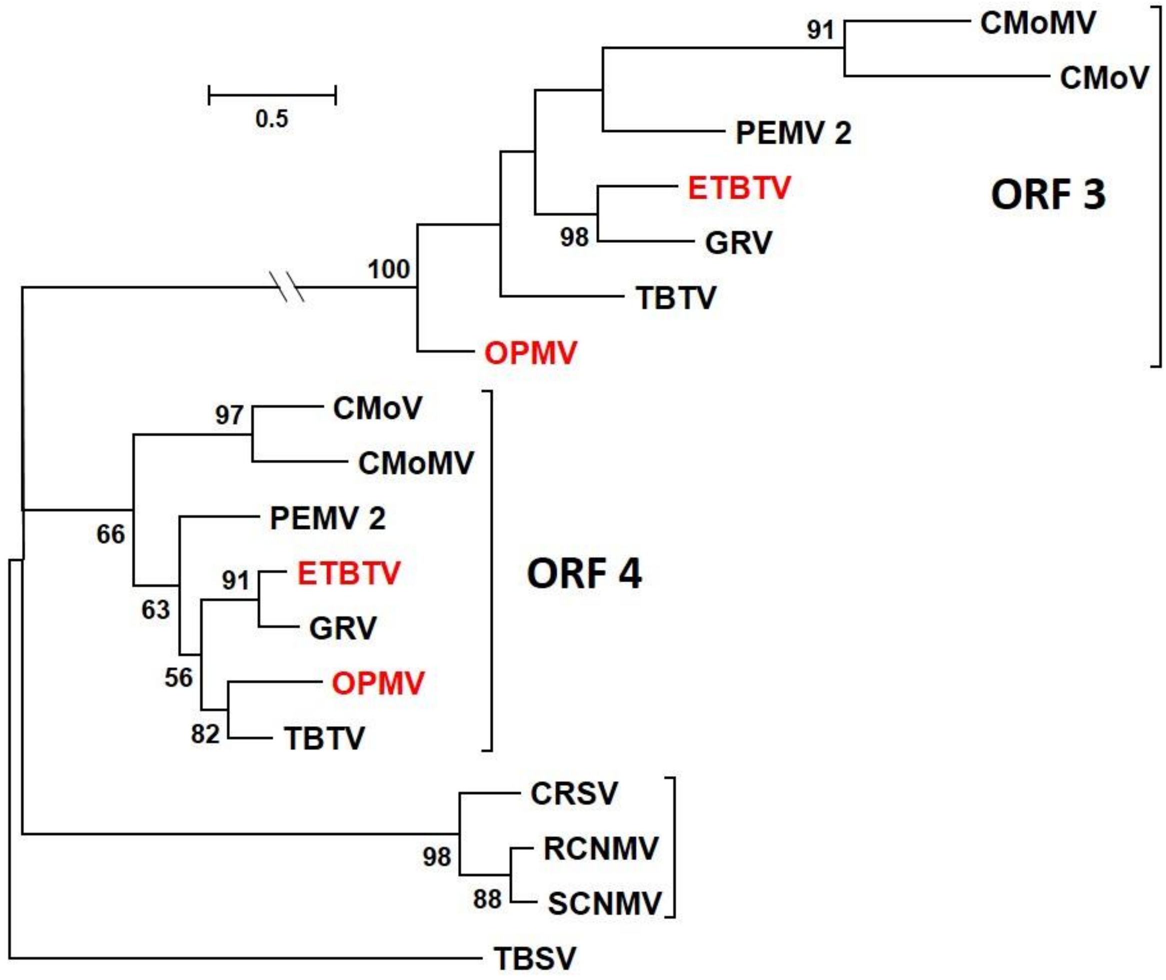

Figure 4. Phylogenetic analysis of umbravirus MPs [ORF 3 (systemic) and ORF 4 (type 3A cell-to-cell)] compared to dianthovirus 3A MPs. Alignment, tree building methods, and labelling is the same as Fig. 2 except MEGA6 was used. There were 260 positions in the final dataset, and TBSV MP p22 was used as the outgroup.

Protein sequences used for phylogenetic analyses that are not in original GenBank entries:

```
>CMoV ORF1 not in GenBank; derived from FJ188473.1
MSFLKRILKHLDSQFSPAPGVYNRDVCAELYGAEWGVLITQERMTRALSVEHERWYTGRVETDVYIPVAQ
YVDPRDVEAEPTSQQGNSTGVDNPVNNCSGTVPTMPATEVIPVDGLGGRRVGVVPAPEGPIPCEGNVYAI
LRMADPTADASESSDDEEDTCCPAYIHAASNSVAEHSANAVPPPMEGVHPGTSPDGVPSSSSAVGISLAV
DVTHVNTVDASTEMPTKVEGPDEGVSTSPLQEAGPSMAFVDASSVAMELRARFGYRSKNPANLELGGRVA
RDILKGCGAVRSENYYLAQLAVQLWFAPTLLDLVLRGPVQDFC

>ETBTV ORF1 not in GenBank; derived from KJ918748
MAIRAITNWLRLDYSPAPYIKSRRRCNEBYGEXWGLLITQARMTESAGHHMMAWYEGSAEVNGLIPGHDD
QGLADPAPPPVMEVTTPPNEEVSCPGPSBDHGVEWVENLPSPPSPKDQRNRLPTLXEVLGADRLQIIPYT
GGHRVLSEXEVRPRAQGVILLPAPRYRPSLLERALDALVFAWRPASQALDALVRSQPQEDGLPASLKLQE
EGAPXEYITAHMIAMELRSMFGWQLNTPANRELGNRVARDILRDRCGANRENTWYLSSLGVQMWFQPTLC
DLAVKAGPQNF

>CMoV RdRp in FJ188473.1 has an error; corrected here
MSFLKRILKHLDSQFSPAPGVYNRDVCAELYGAEWGVLITQERMTRALSVEHERWYTGRVETDVYIPVAQ
YVDPRDVEAEPTSQQGNSTGVDNPVNNCSGTVPTMPATEVIPVDGLGGRRVGVVPAPEGPIPCEGNVYAI
LRMADPTADASESSDDEEDTCCPAYIHAASNSVAEHSANAVPPPMEGVHPGTSPDGVPSSSSAVGISLAV
DVTHVNTVDASTEMPTKVEGPDEGVSTSPLQEAGPSMAFVDASSVAMELRARFGYRSKNPANLELGGRVA
RDILKGCGAVRSENYYLAQLAVQLWFAPTLLDLVLRGPVQDFLLGGVLVREGVCTSVIPKVLSPSLTIRP
ADRPRPVSRVSYQIDAVRPLADYGVHNNSLKNLVRGINERVFYIDNVGTKPVQPSGHGFSSIRDDIKAFR
VVPWTMEQVVASYSGSQQTRYNTAMQSIYEKPFNRDDARVSTFIKAEKINFTAKPDPAPRVIQPRDPRFN
ICFAKFIKPLEPMLYKALGRLYKHPCIAKGFNAFQTGDIIAKKWRSFRDPVCVGLDASRFDQHVSVAALK
FTHKVYKRFVRDPEFDRLLQLMYTNKGRGTAKDGVVKYKVKGCRMSGDMDTALGNCVLMVPMTRELCKRL
EIPHELMDNGDDCIVIFDKEHLSKFQRAVKPYFKDLGFSMKVEAPVFKLEQIEFCQTQPVFDGTQWRMVR
QVNSIAKDLCSVISWEQLLYWWDAIGTCGLALAGGIPVLHSFYKWLCRIGTRGSNVDKHPLFKCGMVYLS
LGMDSASKRITPEGRLSFASAFGISPAMQVALEGIYDKMGNPKGSSVCQVAQSHDFNDWFTPDSRYKELD
SGCQDLTTVESTAQVEFPIYGGLYLPHIEA

>CMoMV RdRp in U57305 has an error; corrected here
MDHITKFLRMDFSPKDGVLTRRQCAERFDDTWGLLITQARMTTAHTADMCAWYEGSRETNQYLPLEQFVD
SRDASKEAEDKVDKLSSPPTRGCGGTTVNADGGLVENPLPTNRDEMPASSMQMNAPSGGIAVASVNSEVA
RWVFALPRELDEPIMETPVDPSAPTEAERELARVLNNTVAVGDTASALSTIQEEPQHEVIAVPGTAVGVV
HQEDPAPVEGSPSTRDAAMEGDAGHSAHAGDDTDASVEMPTTVEAAGPLMEEGPSMAHLTSYTVAMELRS
RFGLRPASPANIELGARVAREILKTAGARRQDNYYLAQQAVKFFLTPTLLDVVYSTPIRDFLLGDVIARD
GVCTKTVPIILSPSLHIRPSARPRPVKRVSYQIDAVRPSADFGVHNNSLVNLVRGINERVFYTDNRGSLP
VRPTPGRFDRLTVDIKHFKINAWSMEDVVNSYTGRQRARYEQAMLSIMERPLERRDARVSTFIKAEKINF
SAKPDPAPRVIQPRDPRFNVVFAKYIKPLEPMLYKALGRLYEQPCVAKGFNAFQTGEIIRKKWDSFRNPV
CVGLDASRFDQHVSVEALKFTHSVYKRFNSSKEFVDLLNQMYVNRGLGTCKDGLVKYKVKGCRMSGDMDT
ALGNCVLMVLMTRNLCLDLSIPHELMDNGDDCIVIFEKEHLQRFNAAVKPYFAALGFTMKVEAPVYQFEK
VEFCQTQPVFDGTQWRMVRQIPSIAKDLCSVIDWEQLESWWHAIGNCGQSLAGGLPVFGSFYRWLCRIGK
ANTKVTEHPLYKCGMVNLSRGMDARDKPITTEGRLSFSLAFGLSIPMQIALEGIYDNLKRPGRVVPGACE
RRGFNDWFIPDVQCLNPSRSAVHTDEVEVEVDFLYPPPVSAC

>PEMV 2 RdRp in U03563 has an error; NP_620846.3 is the correct protein.
MNCVARIKRWFTPDFTPGEGVKSRAQLERELDPTWALLVCQERARRDADSIANEWYEGSMECNLLIPRPT
TEDVFGPSIAPEPVALVEETTRSRAPCVDVPAEESCKSAEIDPVDLAKFDSLHRRLLAEANPCREMVLWV
PPGLPAERDVLPRARGVIMIPEVPASAHTLSVKVMEAVRLAQEVLASLAKRALEKRSTPTLTAQAQPEAT
LSGCDYPYQETGAAAAWITPGCIAMELRAKFGVCKRTPANLEMGSRVARELLRDNCVTCRETTWYTSAIA
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ARDSILSNPLSPKDARVKMFVKAEKINFTAKPDPAPRVIQPRDPRSNIVLAKYIKPLEPMLYKALGKLYK
YPAVAKGFNAVETGEIIAGKWRCFKDPVVVGLDASRFDQHVSVEALQFTHAVYRGFIKSREFNNLLQMMY
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SLCAQC
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