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:	***2011.014aP***	(to be completed by ICTV officers)

Short title: create species *Olive latent virus 3* in the genus *Marafivirus*, family *Tymoviridae*, order *Tymovirales*

Modules attached	**1** ☒	**2** X	**3** ☐	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☒	

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

T.W. Dreher. theo.dreher@oregonstate.edu (on behalf of the *Tymoviridae* Study Group)

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)	Tymoviridae

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

Date first submitted to ICTV: 20 August 2011
Date of this revision (if different to above):

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	***2011.014aP***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Marafivirus***
Subfamily:	
Family:	***Tymoviridae***
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.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Olive latent virus 3	FJ444852 = NC_013920

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

The family *Tymoviridae* has three genera: *Tymovirus, Marafivirus, Maculavirus,* whose main demarcation criteria are (see 9th ICTV Report, Dreher et al., 2011):
(i) Mechanical transmission. Restricted to *Tymovirus*
(ii) Vector-assisted transmission. Restricted to *Tymovirus* and *Marafivirus*
(iii) Particle type and size. Non-enveloped icosahedral particles 30 nm diameter (all three genera)
(iv) Genome type and size. Positive strand ss-RNA 6.0-6.7 kb, 3 ORFs, presence of "tymobox" (*Tymovirus*); 6.3-6.5 kb, 1 or 2 ORFs, presence of "marafibox" (*Marafivirus*); 7.5 kb, 4 ORFs, no tymobox or marafibox (*Maculavirus*).

Properties of Olive latent virus 3

Origin: found in apparently symptomless olives (*Olea europaea*) in Italy and six other Mediterranean countries (Alabdullah et al., 2009).

Host range: apparently restricted to olive

Genome size and organization (Alabdullah et al., 2010): The genome is a single-stranded positive-sense RNA 7,148 nt in size, excluding the poly(A) tail. It has a high cytosine (39%) and low guanine (17%) content (like all tymovirids) and contains four ORFs (Fig. 1).

ORF1 codes for the replication-associated polyprotein (p222) comprising the conserved signature domains for methyltransferase (MTR), papain-like protease (PRO), helicase (HEL) and polymerase (RdRp) as found in all members of the tymoviridae. The overall identity at the aa level of p222 with comparable proteins of other family members ranges from 35 to 48% (OBDV, marafivirus). The C-terminal region of the p222 ORF contains the sequence CAAGGUCAAUUGCUUG that resembles the marafibox sequence (presumed subgenomic promoter found in marafivirus genomes); it is the most divergent marafibox so far described, differing by 2 nt from that of CSDaV, but is distinct the related

tymobox sequence (Alabdullah et al., 2010).

ORF2 is nested in ORF1 like in MRFV (marafivirus) and encodes the putative movement protein, which is 42% identical with the comparable protein of MRFV (also nested in ORF1).

ORF3 encodes the 28.5 kDa coat protein (CP). Contrary to marafivirus CPs, which are produced in two forms and are encoded at the extreme 3' end of ORF1, there appears to be only one OLV-3 CP expressed from a separate ORF, as with tymo- and maculaviruses. The identity level of OLV-3 CP with CPs of other tymovirids is usually low (14-23%), but reaches 42% with CSDaV and 44% with OBDV (both marafiviruses).

ORF4 codes for a 16 kDa protein (p16) rich in serine and proline. p16 has 50% aa identity with proteins of the marafivirus CSDaV and the maculavirus GFkV both of which have the same size and occupy the same position in the genome (Fig. 1).

Phylogenetic relationships. In phylogenetic trees constructed with the aa sequences of replication proteins and CPs of members of the family *Tymoviridae*, OLV-3 clusters with high bootstrap values with a group comprising the traditional marafiviruses (MRFV, OBDV, BELV) and viruses GSyV-1 (accepted by the 2010 ICTV EC as a new marafivirus) and GRVFV (a potential marafivirus that lacks a peer-reviewed paper) (Figs. 2 and 3). OLV-3, GSyV-1 and GRVFV are clearly somewhat divergent from the traditional marafiviruses, but the most parsimonious interpretation of both replication polyprotein and CP phylogenetic trees (Figs. 2 and 3) is inclusion of all these viruses in a larger marafivirus genome; splitting may ultimately necessitate creation of more than one new genus.

Summary

- The experimental data indicate that OLV-3 is a *bona fide* member of the family *Tymoviridae* that is sufficiently distinct to warrant identification as a new species.
- OLV-3 shares several molecular properties with the marafiviruses: closest sequence relationship, including in phylogenetic trees; ORF organization except for separation of replication/CP ORFs; presence of marafibox.
- OLV-3 also has properties distinct from those of the traditional marfiviruses: a separate CP ORF (rather than fused to the replication protein ORF), an apparently single CP form probably produced from a sgRNA (rather than two forms with different N-termini produced by proteolytic liberation from the replication/CP polyprotein and from a sgRNA), and an unusually large CP bigger than that of any other member of the *Tymoviridae* (28.5 kDa cf. 25 kDa).
- Inclusion of OLV-3 in the *Marifivirus* genus is recommended, necessitating a wider view than that focused on the traditional marafis, which replicate in both plants and insects. These marafiviruses already include some divergence in genome design (see MRFV, OBDV and CSDaV genome diagrams in Fig. 1). One might view OLV-3 as a mutant of a more traditional marafivirus that has acquired a stop codon at the end of the replication polyprotein and is thus able to make only one CP form from a sgRNA; the replication and CP ORFs are in-frame and separated by only 6 nt (the stop codon and a second codon).

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Alabdullah A., Elbeaino T., Minafra A. Digiaro M., Martelli G.P., 2009. Detection and variability of Olive latent virus 3 in the Mediteranenan region. *Journal of Plant Pathology* **91**: 521-525.

Alabdullah A., Minafra A., Elbeaino T., Saponari M. Savino V., Martelli G.P., 2010. Complete nucleotide sequence and genome organization of Olive latent virus 3, a new putative member of the family *Tymoviridae*. *Virus Research* **152**: 10-18.

Annex:

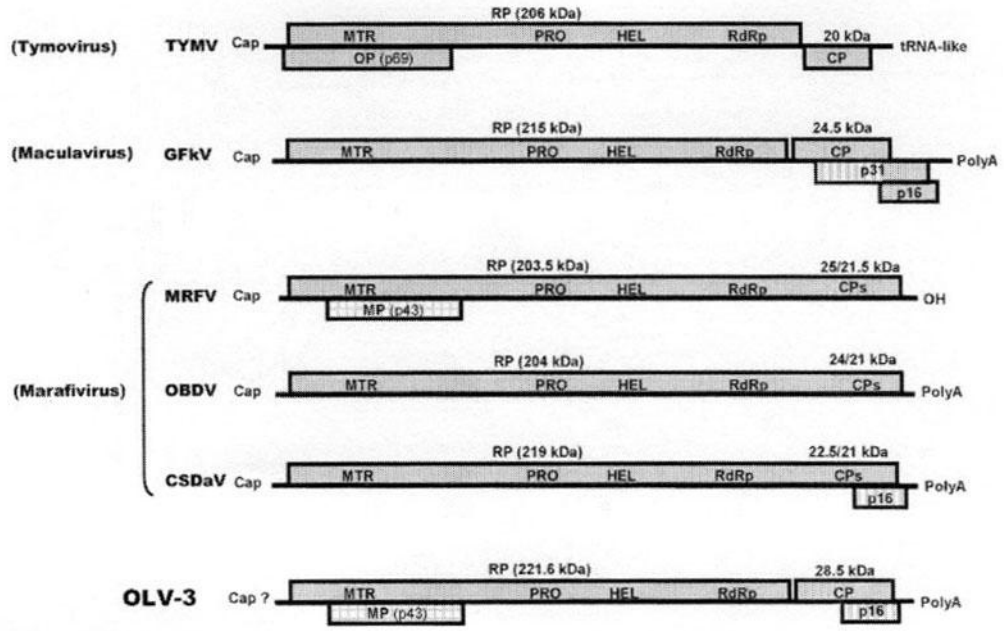

Fig. 1. Genome organization of OLV-3 and viruses of the three genera of the family *Tymoviridae*

Figure 2. Phylogenetic (neighbor-joining) tree showing the relationship of OLV-3 to other species and genera of the family *Tymoviridae.* The tree was constructed in MEGA5 using the maximum composite likelihood method and 10,000 bootstrap replicates (percentage values shown at branches where >60%) and was based on the codon-aligned replication polyprotein nucleotide sequence and rooted with Botrytis virus F, genus *Mycoflexivirus*, family *Gammaflexiviridae*, order *Tymovirales.* Colors are used to show approved members of the 3 genera and the unassigned species.

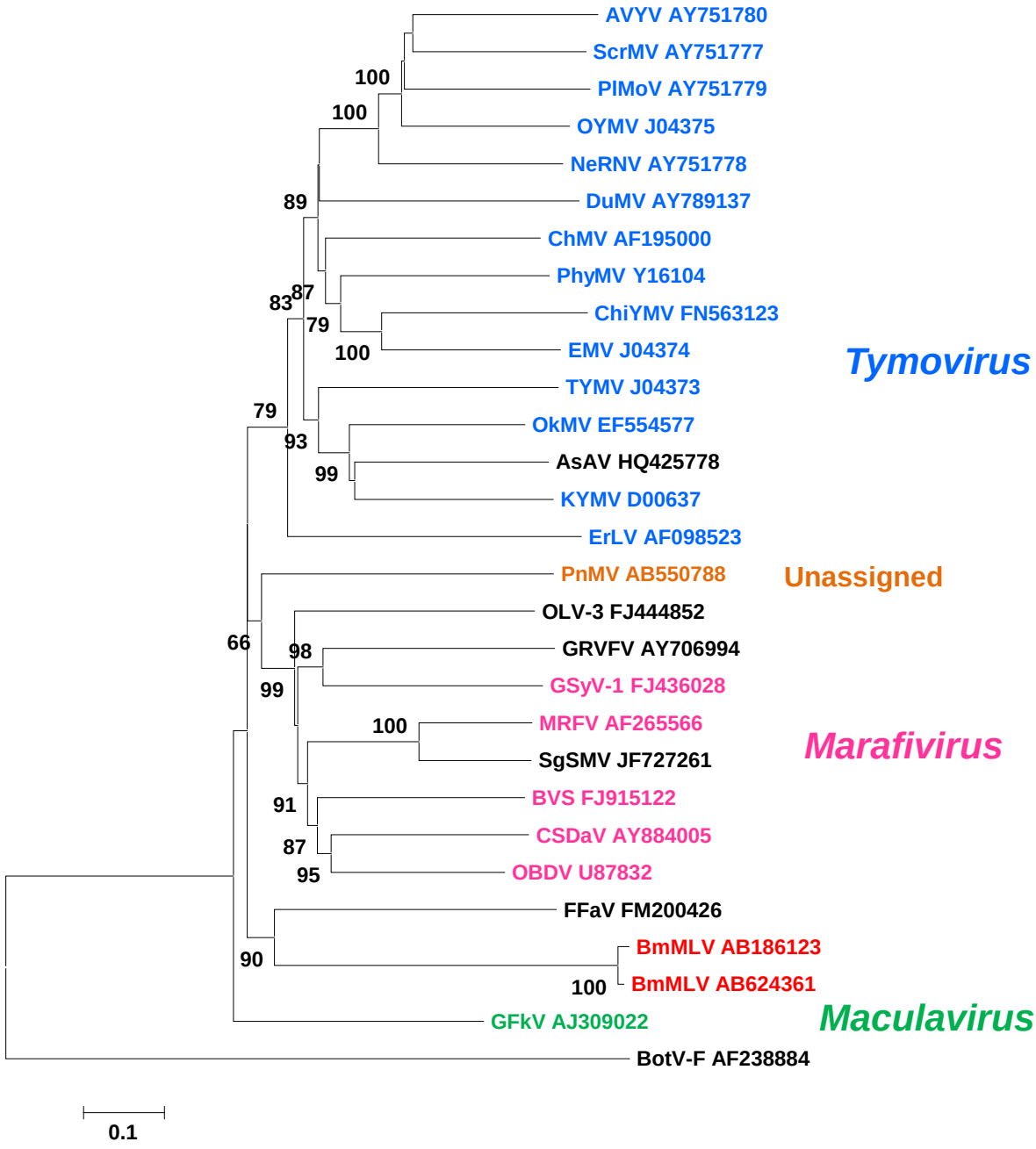

Figure 3. Phylogenetic (neighbor-joining) tree of the codon-aligned **coat protein** nucleotide sequences of members of the family *Tymoviridae* showing their relationship to OLV-3. The unrooted tree was constructed in MEGA5 using the maximum composite likelihood method and 10,000 bootstrap replicates (percentage values shown at branches where >60%).