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:	***2013.005a-gP***	(to be completed by ICTV officers)

Short title: Establishment of the family *Amalgaviridae*, the genus *Amalgavirus* and inclusion of four species in the genus
(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:

I.E. Tzanetakis on behalf of the Plant virus and *Totiviridae* SG; E-mail: itzaneta@uark.edu and S. Sabanadzovic on behalf of the *Partitiviridae* SG; E-mail: ss501@msstate.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)	Plant and Fungal SC

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

EC comments (July 21, 2013):

1. At the top of module 2, please provide GenBank number rather than RefSeq number (NC011591) for Southern tomato virus. This is because RefSeq numbers can change over time, while GenBank numbers are assigned permanently and are stable.
2. In general, names that are easy to pronounce and remember are preferred. While the name Amalgamaviridae is acceptable, the EC wondered if you would consider a simpler name such as "Amalgaviridae" and "Amalgavirus".

Plant Virus Chair comments (July 26, 2013):

Both comments have been addressed to satisfaction in this revised version.

Date first submitted to ICTV:	May 31th , 2013
Date of this revision (if different to above):	July 23rd , 2013

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	***2013.005aP***	(assigned by ICTV officers)

To create 4 new species within:

Genus:	***Amalgavirus* (new)**
Subfamily:	
Family:	***Amalgaviridae* (new)**
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.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Southern tomato virus	EF442780
Blueberry latent virus	EF442779
Rhododendron virus A	HQ128706
Vicia cryptic virus M	EU371896

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

A new group of related plant viruses have been identified in the last decade. The genome comprises of dsRNA containing two overlapping ORFs (Martin et al., 2006; 2011; Liu and Chen, 2009; Sabanadzovic et al., 2009; 2010). These viruses share several properties and common evolutionary history making them warrant of the official recognition as a separate taxon among dsRNA viruses.

Properties of STV, BBLV, VCVM and RhVA

Virus particles: virus particles could not be purified or observed in infected tissue for any of these viruses. However, in case of blueberry latent virus (BBLV), the putative CP was detected in the plant cytosol using specific antibodies raised against the recombinant product of ORF 1 (Isogai et al., 2011) (Figure 1).

Genome: The genome of southern tomato virus (STV) and related viruses consists of a single molecule of dsRNA of ca. 3.5 kbp, containing two partially overlapping ORFs (Martin et al., 2011; Liu and Chen 2009; Sabanadzovic et al., 2009; 2010). The 5'proximal ORF encodes a 42-44 kDa protein with a putative CP function. The predicted product of ORF2 is identified as viral RNA-dependent RNA polymerase as it contains characteristic motifs for these enzymes (Martin et al., 2011). Expression of the OFR2 is likely via +1 translational frameshift or stoprestart mechanism resulting in a fusion protein of ~ 120 kDa similar to "*gag-pol*" proteins (Figure 2).

Double stranded RNAs are abundant and easily detectable in infected tissue (Martin et al., 2006; 2011; Liu and Chen, 2009; Sabanadzovic et al., 2009; 2010). Northern hybridization tests carried out for STV, BBLV and rhododendron virus A (RhVA) did not reveal any 3' co-terminal subgenomic molecule (Figure 3).

Phylogenetic relationships: The four proposed members cluster together (Appendix) and form a distinct group of dsRNA viruses distantly related to members of the family *Partitiviridae*. This grouping is supported with high bootstrap value (Figure 4).

Biological and epidemiological properties:

All four viruses have been reported associated with symptomless infections in their respective hosts (Martin et al., 2006, 2011; Liu and Chen 2009, Sabanadzovic et al., 2009; 2010). *In situ* hybridization studies showed that BBLV is present in the palisade, spongy mesophyll and in vascular tissue of the infected leaves (Isogai et al., 2011).

Repeated attempts to transmit the virus via grafting and mechanical inoculation have failed (Martin et al., 2006; Sabanadzovic et al., 2009). Nevertheless, STV and BBLV are very efficiently transmitted vertically via seed (and possibly pollen) (Martin et al., 2011; Sabanadzovic et al., 2009).

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2013.005bP***	(assigned by ICTV officers)

To create a new genus within: Amalgaviridae

Subfamily:		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
Family:	*Amalgaviridae* (new)	
Order:		

naming a new genus

Code	***2013.005cP***	(assigned by ICTV officers)

To name the new genus: *Amalgavirus*

Assigning the type species and other species to a new genus

Code	***2013.005dP***	(assigned by ICTV officers)

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

Southern tomato 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:**

4

Reasons to justify the creation of a new genus:

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

A new group of closely related viruses has recently been identified in several plant species. The genome comprises of single bicistronic dsRNA molecule of ~ 3.5 Kbp, containing two partially overlapping cistrons (Martin et al., 2006; 2011; Liu and Chen, 2009; Sabanadzovic et al., 2009; 2010). This genomic organization is reminiscent of that of viruses belonging to the family *Totiviridae* (Wickner et al., 2011) (Figure 2). However, phylogenetic analyses, independent of the method used (i.e. Maximum Likelihood, Neighbour Joining), consistently place members of the group in a clade evolutionarily closer to the *Partitiviridae* than to *Totiviridae* (Figure 4 and not shown) indicating a distinct lineage of dsRNA viruses (Martin et al., 2011; Sabanadzovic et al., 2009; 2010).

Therefore, members of the putative new taxon share common properties with extant totiviruses and partitiviruses suggesting that they may represent an evolutionary link between these two families as proposed by several authors (Martin et al., 2011; Liu and Chen, 2009; Sabanadzovic et al., 2009; 2010).

Similarities with the extant taxa: These viruses share monopartite dsRNA genome with totiviruses, as well as bi-cistronic organization (Wickner et al., 2011). Moreover, the two ORFs present on the positive strand molecule are partially overlapping which suggest that the putative RdRp encoded by ORF2 is expressed as a fusion protein via ribosomal frameshift mechanism as in case of

totiviruses.
Similar to partitiviruses (especially those reported from plants - cryptoviruses), STV and allied viruses apparently do not cause any visible effect on their hosts (Ghabrial et al., 2011). The members of proposed genus *Amalgavirus* in the family *Amalgaviridae* are efficiently transmitted vertically in their hosts, as cryptoviruses do. Furthermore, amalgaviruses are not graft transmissible - another common trait shared with cryptoviruses. Finally, the members of the proposed new taxon are evolutionarily closer to partitiviruses than to totiviruses. However, unlike partitiviruses, which have their genome split on more than one dsRNA segment, amalgaviruses contain monopartite genome.

Origin of the new genus name:

Amalgam (noun): blend, mix

Reasons to justify the choice of type species:

First virus of the genus fully characterized molecularly and biologically

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.

Proposed species demarcation criteria for the genus *Amalgavirus*:

1. Amino acid sequence divergence of polymerase is >25%.
2. Differences in natural host range

MODULE 5: **NEW FAMILY**

creating and naming a new family

Code	***2013.005eP***	(assigned by ICTV officers)

To create a new family containing the subfamilies and/or genera listed below within the Order:

If there is no Order, write "**unassigned**" here.
If the Order has yet to be created (in Module 6) please write **"(new)"** after the proposed name.

Code	***2013.005fP***	(assigned by ICTV officers)

To name the new family: *Amalgaviridae*

assigning subfamilies, genera and unassigned species to a new family

Code		(assigned by ICTV officers)

To assign the following subfamilies (if any) to the new family:
You may list several subfamilies here. For each subfamily, please state whether it is new or existing.

- If the subfamily is new, it must be created in Module 4
- If the subfamily already exists, please complete Module 7 to 'REMOVE' it from its existing family

Code	***2013.005gP***	(assigned by ICTV officers)

To assign the following genera to the new family:
You may list several genera here. For each genus, please state whether it is new or existing.

- If the genus is new, it must be created in Module 3
- If the genus already exists, please state whether it is currently unassigned or is to be removed from another family. If the latter, complete Module 7 to 'REMOVE' it from that family

Amalgavirus

The new family will also contain any other new species created and assigned to it (Module 3) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of unassigned species that the family will contain (those NOT within any of the genera or subfamilies listed above):**

0

Reasons to justify the creation of the new family:
Additional material in support of this proposal may be presented in the Appendix, Module 9

General biological and molecular properties, along with distinct evolutionary history, indicate that the recently discovered group of related viruses typified by Southern tomato virus (STV) represents a new taxon, as repeatedly suggested in literature (Martin et al., 2011; Sabanadzovic et al., 2009; 2010). Based on acquired knowledge on these viruses it appears obvious that a new virus family needs to be formed. Martin and collaborators in 2011, informally proposed the name *Amalgamaviridae* (Martin et al., 2011) that we put forward in a simplified version (*Amalgaviridae*) for consideration by the ICTV. This name is proposed to symbolize the fact that viruses of the proposed new taxon blend the properties of the extant families *Partitiviridae* (phylogeny) and the *Totiviridae* (genome organization) (Ghabrial et al, 2011; Wickner et al., 2011).

Details about taxa in this family are listed in detail in Modules 2 and 3.

Origin of the new family name:

Amalgam (noun): blend, mix

APPENDIX: supporting material

additional material in support of this proposal

References:

Ghabrial SA, Nibert ML, Maiss E, Lesker, T., Baker, T.S. and Tao, Y.J., 2011. Family Partitiviridae. In: King MQ, Adams MJ, Carstens EB and Lefkowitz EJ (eds) Virus Taxonomy, pp. 522-534.

Isogai, M., Nakamura, T., Ishii, K., Watanabe, M., Yamagishi, N., Yoshikawa, N. , 2011. Histochemical detection of Blueberry latent virus in highbush blueberry plant. J. Gen. Plant Pathol., 77, 304–306.

Liu, W., Chen J., 2009. A double-stranded RNA as the genome of a potential virus infecting *Vicia faba*. Virus Genes 39, 126-131.

Martin R.R., Tzanetakis, I.E., Sweeney, M., Wegener, L., 2006. A virus associated with blueberry fruit drop disease. Acta Hortic. 715, 497-501.

Martin, R.R., Zhou, J. and Tzanetakis, I.E., 2011. Blueberry latent virus: An amalgam of the *Partitiviridae* and the *Totiviridae*. *Virus Res.* 155, 175-180.

Sabanadzovic, S., Valverde, R.A., Brown, J.K., Martin, R.R., Tzanetakis, I.E., 2009. Southern tomato virus, The link between the families *Totiviridae* and *Partitiviridae*. Virus Res. 140, 130-137.

Sabanadzovic S., Abou Ghanem-Sabanadzovic N., Valverde, R.A., 2010. A novel monopartite dsRNA virus from rhododendron. Arch. Virol. 55, 1859–1863

Wickner, R.B., Ghabrial, S.A., Nibert, M.L., Patterson, J.L. and Wang, C.C., 2011. Family Totiviridae. In: King MQ, Adams MJ, Carstens EB and Lefkowitz EJ (eds) Virus Taxonomy, pp. 639-650.

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.

Figure 1. Western blot detection of the ORF1-encoded protein of BBLV (from Isoagai et al., 2011). The ~45-48 kDa protein (arrow) is present in BBLV-infected plants (lanes 1 and 8-10), but not in BBLV-free plants (lanes 2-7). BBLV infections were verified by virus-specific RT-PCR.

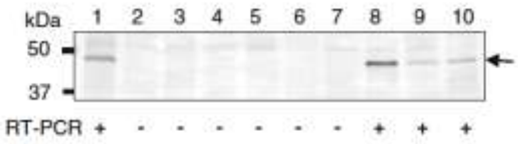

Figure 2. Genome organization of putative members of the genus *Amalgavirus* (fam. *Amalgaviridae*) compared to those of members of families *Totiviridae* and *Partitiviridae* (from Sabanadzovic et al., 2009).

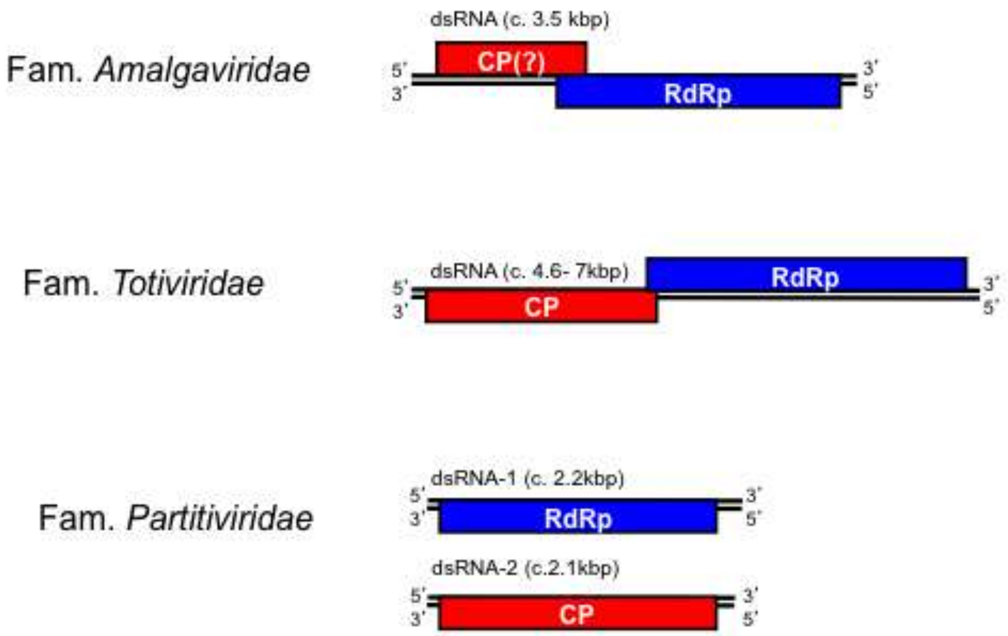

Figure 3. Agarose gel electrophoresis of dsRNAs extracted from BBLV-infected blueberries (panel A, lanes 1and 2). The same dsRNA preparations were blotted and hybridized with a 3'-terminal DIG-labeled probe producing only one visible signal corresponding to full genomic size molecule (panel B) indicating the absence of any 3'co-terminal subgenomic molecule. (reproduced from Martin et al., 2011). Similar results were also reported for STV and RhVA (not shown)

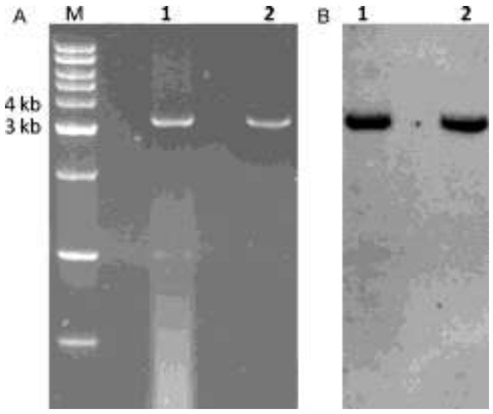

Figure 4. Maximum likelihood-based phylogenetic tree constructed on the alignment of putative viral RNA dependent RNA polymerases of members of families *Chrysoviridae*, *Partitiviridae* and *Totiviridae* and four members of the proposed new family *Amalgaviridae*. The distinct clade grouping four putative members of the *Amalgaviridae* represents a new lineage among dsRNA viruses distantly related to partiti- toti- and chrysoviruses. The clade is supported by 99.9% bootstrap value.

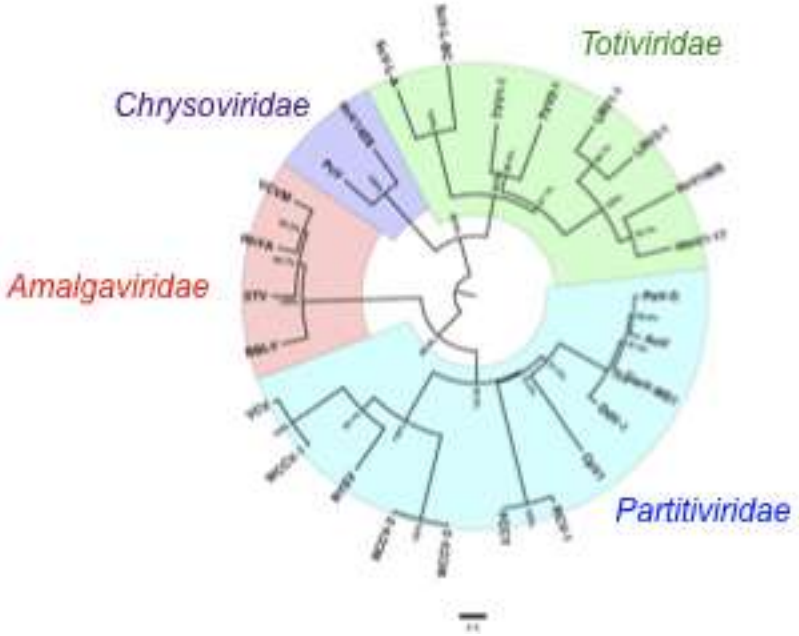

Table 1. Pairwise percentage (%) identities among putative proteins encoded by the reference strains of four proposed species in the genus *Amalgavirus*, fam. *Amalgaviridae*. Identities of putative coat proteins and RNA-dependent RNA polymerases are reported below and above the diagonal, respectively.

	STV	RhVA	VCVM	BBLV
STV		20	17	18
RhVA	47		21	20
VCVM	49	49		17
BBLV	45	45	43	