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:	***2013.009aP***	(to be completed by ICTV officers)

Short title: A new species in the genus *Anulavirus*
(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:

Simon W. Scott, sscott@clemson.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)	Bromoviridae

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

Comment from the SC Chair:
The ICTV-EC had two requests following initial review of the proposal.

1. The authors should provide better trees with bootstrap analysis. This has now been done, and I think that the two trees provided convincingly support the creation of a new species.

2. The study group should define species demarcation criteria for the genus, now that there are two species. The study group is actively working on defining criteria and while it has not yet reaching a consensus, it is anticipated that species demarcation criteria will be in place by next year. Considering that the proposed new species in this particular proposal is clearly very different from existing species, it is likely that it will meet species demarcation criteria once they are finalized. I would like to propose that this proposal could go ahead at this time, but I will continue to encourage the SG to develop species demarcation criteria as soon as possible.

Date first submitted to ICTV:	June 11, 2013
Date of this revision (if different to above):	August 13, 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	***2013.009aP***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Anulavirus***
Subfamily:	
Family:	***Bromoviridae***
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:**
Amazon lily mild mottle virus	AB724113, AB724114, AB724115

Reasons to justify the creation and assignment of the new species:

Amazon lily mild mottle virus

Pelargonium zonate spot virus (PZSV) is currently the type species, and sole member of the genus *Anulavirus*.

Amazon lily mild mottle virus (ALMMV) as described by Fuji et al (2013) has biological and morphological properties similar to viruses of the family *Bromoviridae*. Based on molecular analysis, the virus has been identified as a member of a new species in the genus *Anulavirus*. Whereas PZSV can infect plants of three *Nicotiana* species (*N. glutinosa, N. clevelandii* and *N. tabacum*), *Cucumis sativus* and *Cucurbita pepo*, ALMMV did not. ALMMV has 3 genomic RNAs similar in size and organization to PZSV but distinct from the other 5 genera within the *Bromoviridae*. In other genera of the family *Bromoviridae* the RNA 2 (2,593–3,035 nt in length) and the RNA3 (2,438-2,073 nt) are so different in size as to be distinguishable in gel electrophoresis. PZSV and ALMMV have an RNA 2 (2,435-PZSV, 2,507-ALMMV) slightly smaller than their RNA 3 (2,569 - PZSV, 2530 - ALMMV). In gel electrophoresis this results in the appearance of 2 bands rather than the 3 bands of genomic RNA typically viewed in preparations of viruses that are members of other genera of the *Bromoviridae*. There was no evidence of the presence of an encapsidated subgenomic RNA 4 or other subgenomic molecules in preparations of ALMMV. BLAST searches with the nucleotide sequences of the individual genomic molecules of ALMMV show a distant relationship with the corresponding molecules of PZSV (RNA1, 54.5 %; RNA2, 54.3 %; RNA3, 49.1 %) but failed to match genomic sequences for other members of the *Bromoviridae*. The putative p2 protein coded for by the RNA 2 is correspondingly smaller than p2 proteins coded for by other bromovirids.

Phylogenetic analysis (Figure 1 and Figure 2) clearly shows ALMMV clustering with PZSV with the two viruses forming a clade distinct from other genera of the *Bromoviridae*.

There are no defined criteria for the demarcation of a new species within the family *Bromoviridae*. Species in the genera, *Alfamovirus, Bromovirus*, *Cucumovirus,* and some species in the genus *ilarvirus* were established some time ago on the basis of host range and serological reaction. The genera *Oleavirus* and *Anulavirus* are recent creations based on marked differences from the model in which the viruses possess a tripartite genome and express the 3b ORF through a subgenomic RNA 4. Development of sequence data for members of the *Bromoviridae* have both supported the existing separations and revealed erroneous/inconsistent nomenclature. Alfalfa mosaic virus (AMV) is regarded as unique because of its aphid transmission although it shares many molecular properties with some of the ilarviruses. The CP of AMV will activate the genomes of Ilarviruses and vice versa. Development of sequence data for the majority of Ilarviruses has allowed a revision of the taxonomy, eliminating many of the subgroups proposed in the mid 90"s and correcting previous erroneous nomenclature (King et al, 2012). Gay feather mild mottle virus is the most recent addition to the genus *Cucumovirus.*
The differences between ALMMV and PZSV (difference in host range, degree of identity of nucleotide and putative protein sequences) are similar to differences used to separate other members of the family *Bromoviridae* and thus create ALMMV as a new species in the genus *Anulavirus*.

Figure 1. An unrooted phylogenetic tree for currently accepted members of the family *Bromoviridae* plus ALMMV generated using the putative amino acid sequences of the coat proteins. The alignment was completed using ClustalX2 and the tree was drawn using NJPLOT.

Figure 2. An unrooted phylogenetic tree for currently accepted members of the family Bromoviridae plus ALMMV generated using the polymerase signature (Candresse T, Morch MD, Dunez J , 1990. Res Virol 141:315–329). The alignment was completed using ClustalX2 and the tree was drawn using NJPLOT.

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MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Candresse T, Morch MD, Dunez J (1990) Multiple alignment and hierarchical clustering of conserved amino acid sequences in the replication-associated proteins of plant RNA viruses. Res Virol 141: 315-329

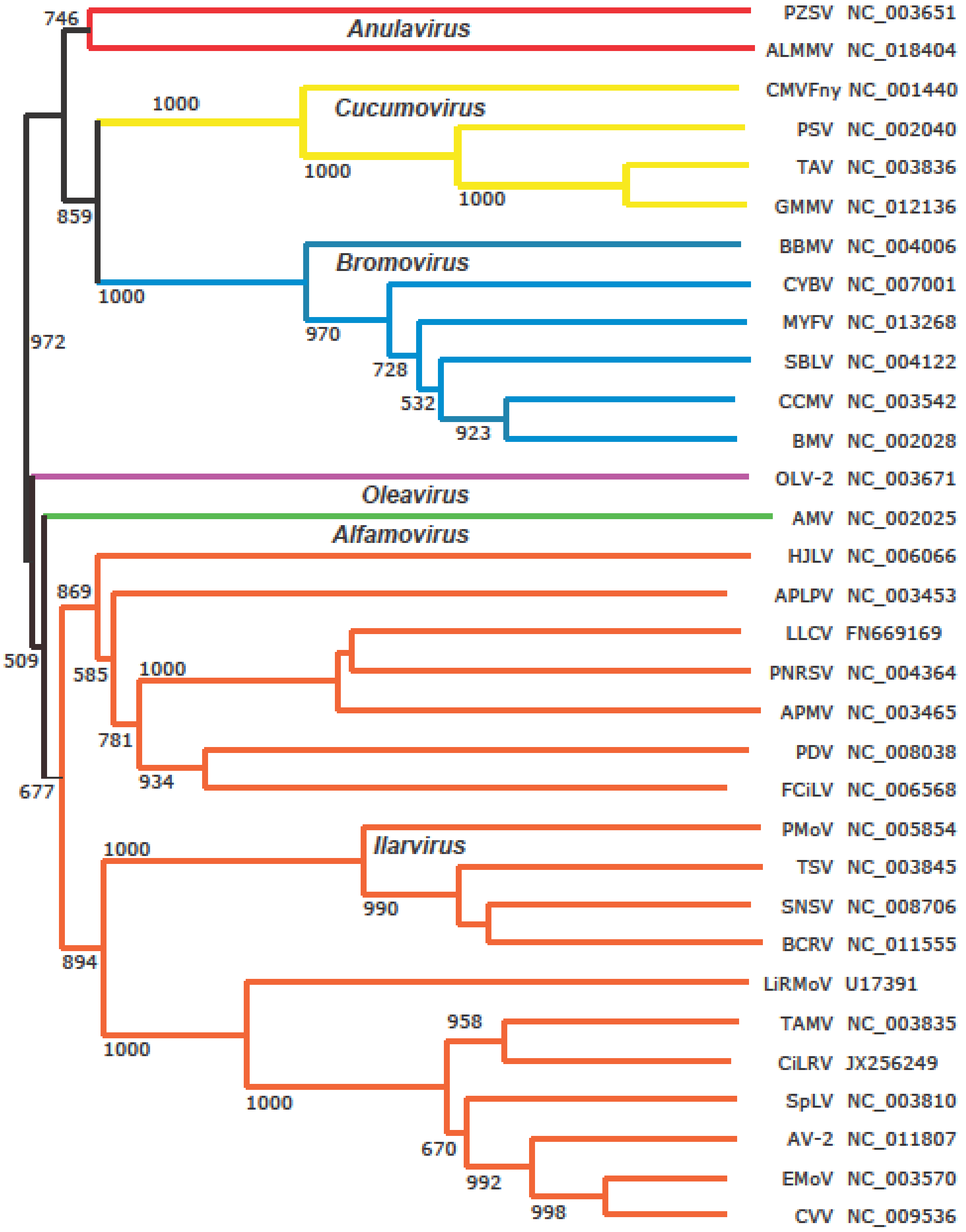
Fuji S, Kikuchi M, Ueda S, Toda T, Furuya H, Fukumoto F, Hanada K. (2013) Characterization of a new Anulavirus isolated from Amazon lily plants.
Arch Virol. 158: 201-6

King, A. M. Q., Adams, M. J., Carstens, E. B., & Lefkowitz, E. J. (2012). In Ninth report of the international committee on taxonomy of viruses, London: Elsevier.

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.

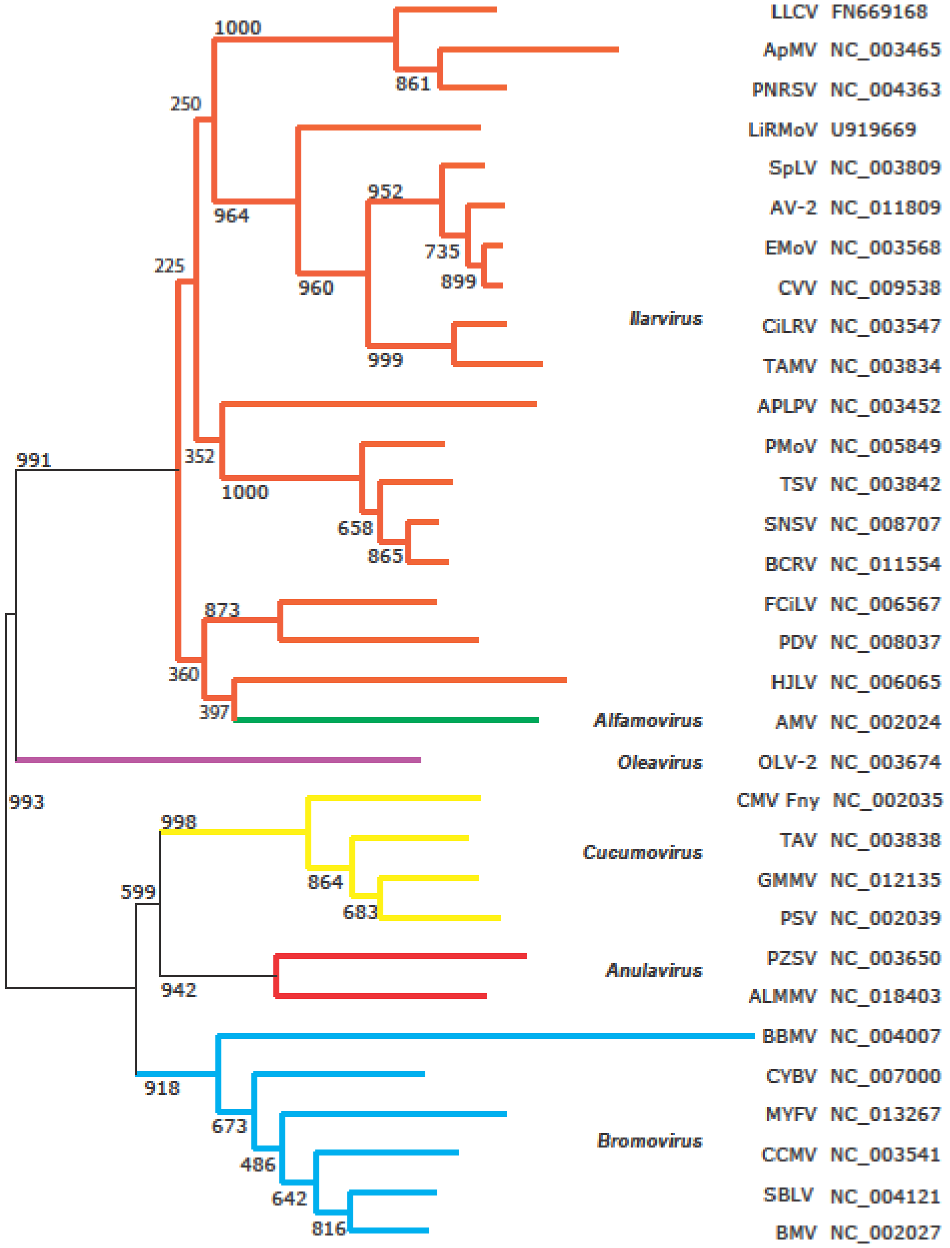
Figure 1



An unrooted phylogenetic tree for currently accepted members of the family *Bromoviridae* plus ALMMV generated using the putative amino acid sequences of the coat proteins. The alignment was completed using ClustalX2 and the tree was drawn using NJPLOT.

Viral acronyms conform to the 9th report of ICTV.
GenBank accession numbers are indicated to the right of the acronym.
Viral species within each genus share the same colored branches.

Figure 2



An unrooted phylogenetic tree for currently accepted members of the family *Bromoviridae* plus ALMMV generated using the polymerase signature (Candresse T, Morch MD, Dunez J, 1990. Res Virol 141:315–329).
The alignment was completed using ClustalX2 and the tree was drawn using NJPLOT.

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