



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:	2015.010aP	(to be completed by ICTV officers)			
Short title: Create two species in the genus <i>Nanovirus</i> , family <i>Nanoviridae</i> (e.g. 6 new species in the genus <i>Zetavirus</i>)					
Modules attached (modules 1 and 10 are required)	1 <input checked="" type="checkbox"/>	2 <input type="checkbox"/>	3 <input type="checkbox"/>	4 <input type="checkbox"/>	5 <input type="checkbox"/>
	6 <input type="checkbox"/>	7 <input type="checkbox"/>	8 <input type="checkbox"/>	9 <input type="checkbox"/>	10 <input checked="" type="checkbox"/>

Author(s):

John E Thomas and H-J Vetten, on behalf of the Nanoviridae Study Group

Corresponding author with e-mail address:

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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)

Nanoviridae Study Group

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

Date first submitted to ICTV:

June 2015

Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

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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	2015.010aP	(assigned by ICTV officers)
To create two new species within:		
Genus:	<i>Nanovirus</i>	Fill in all that apply. <ul style="list-style-type: none"> • 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.
Subfamily:		
Family:	<i>Nanoviridae</i>	
Order:		
Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)
<i>Black medic leaf roll virus</i> <i>Pea yellow stunt virus</i>	BMLRV - [AZ;47] PYSV- [AT;15]	KC978949 - KC978956 KC979054 - KC979059

<p>Reasons to justify the creation and assignment of the new species:</p> <ul style="list-style-type: none"> • Explain how the proposed species differ(s) from all existing species. <ul style="list-style-type: none"> ○ 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
<p>Criteria used as guidelines for Nanovirus species demarcation in the 9th report [1] are:</p> <ul style="list-style-type: none"> • Differences in natural host range • Differences in the number and types of vector aphid species • Different reactions to antibodies to individual species • Differences in CP aa sequences of >15%, and/or • Overall nt sequence identity of <75% is generally indicative of a distinct species. <p>The report states that:</p> <p>“Since several nanovirids are now known to have overlapping host ranges and to be transmitted by a similar range of aphid species, biological criteria appear no longer useful for species discrimination within a genus. Although species-specific monoclonal antibodies (where available) can be used for species discrimination, preference should nowadays be given to the molecular criteria specified above.”</p> <p>Two genetically distinct and previously unrecognised nanoviruses have been described from surveys in Europe, and for which the names black medic leaf roll virus (BMLRV) and pea yellow stunt virus (PYSV) are proposed [2]. This proposal is based on the properties described for a single isolate of PYSV and three isolates of BMLRV.</p> <p>A single isolate of PYSV has been reported [2], originating from Austria. This isolate, PYSV-[AT;15], has a genome consisting of eight circular, single-stranded DNA components, ranging in size from 970 nt to 1002 nt, and with a total genome size of 7825 nt. All eight DNAs appear to be structurally similar to nanoviruses in having a common stem loop sequence and one major open reading frame (ORF) that potentially encodes one of the eight distinct nanovirus proteins. Compared with all other known nanoviruses, the overall genome of PYSV shares less than 75% identity (Fig. 1) and the coat protein amino acid sequence less than 60% identity (Fig. 2). PYSV is phylogenetically related to, but distinct from, all other nanoviruses (Figs 1 and 2). It most closely related to <i>Pea necrotic yellow dwarf virus</i> (PNYDV) and together they constitute the most basal branch of the nanoviruses from Eurasia/Africa (Fig 1). This isolate of PYSV is unusual in having five variants of DNA U2, three of which are recombinants.</p>

Single isolates of BMLRV were obtained from Austria [AT;3], Azerbaijan [AZ;47] and Sweden [SE;153]. These isolates were all >85% identical across the whole genome, and 91-95% identical in coat protein amino acid sequence, and hence are all clearly isolates of the same virus species according to ICTV guidelines. Two distinct populations of DNA-U2 molecules were identified from the population of genomic DNAs of the Austrian isolate BMLRV-[AT;3], one of which appears to be the genuine DNA-U2, the other is both a recombinant and a reassortant [2]. An alphasatellite was also present in isolates BMLRV-[AZ;47] (1030 nt, KC978957), and BMLRV-[AT;3], (1021 nt, KC978948).

A representative BMLRV isolate, BMLRV-[AZ;47] from Azerbaijan, has a genome consisting of eight circular, single-stranded DNA components, ranging in size from 1008 to 1019 nt, and with a total genome size of 8016 nt. All eight DNAs appear to be structurally similar to nanoviruses in having a common stem loop sequence and one major open reading frame (ORF) that potentially encodes one of the eight distinct nanovirus proteins. The overall genome sequence shares ca 70% identity to other described nanoviruses (Fig 1.) and each genome component is phylogenetically related to, but distinct from, other nanoviruses (Fig 2). The coat protein amino acid sequence is ca 61% identical to its closest match (Faba bean necrotic stunt virus, KC978993). The eight cloned genomic components were shown together to be infectious and to be transmissible by the aphid species *Acyrtosiphum pisum* and *Aphis craccivora*.

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

- [1] Vetten, H.J., Dale, J.L., Grigoras, I, Gronenborn, B., Harding, R., Randles, J.W., Sano, Y., Thomas, J.E., Timchenko, T. and Yeh, H.-H. 2011. Family *Nanoviridae*, pp. 395-404. In: King, A.M.Q., Adams, M.J., Carstens, E.C, and Lefkowitz, E.J. (eds). *Virus Taxonomy*, Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, London.
- [2] Grigoras I, Ginzo AldC, Martin DP, Varsani A, Romero J, Mammadov AC, Huseynova IM, Aliyev JA, Kheyr-Pour A, Huss H, Ziebell H, Timchenko T, Vetten H-J, Gronenborn B (2014) Genome diversity and evidence of recombination and reassortment in nanoviruses from Europe. *J Gen Virol* 95:1178-1191

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.

Supporting data in the form of phylogenetic trees is attached, from Grigoras et al. (2014) *Journal of General Virology* 95, 1178–1191.

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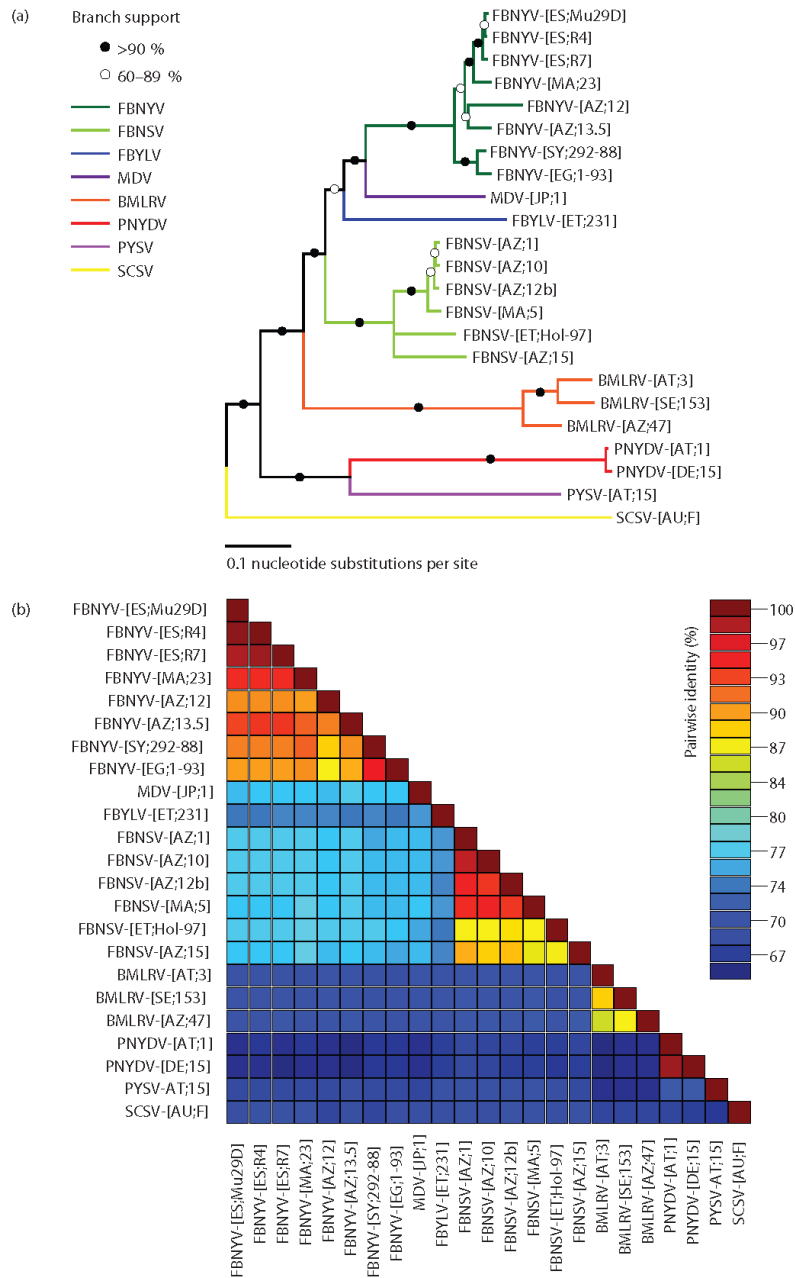
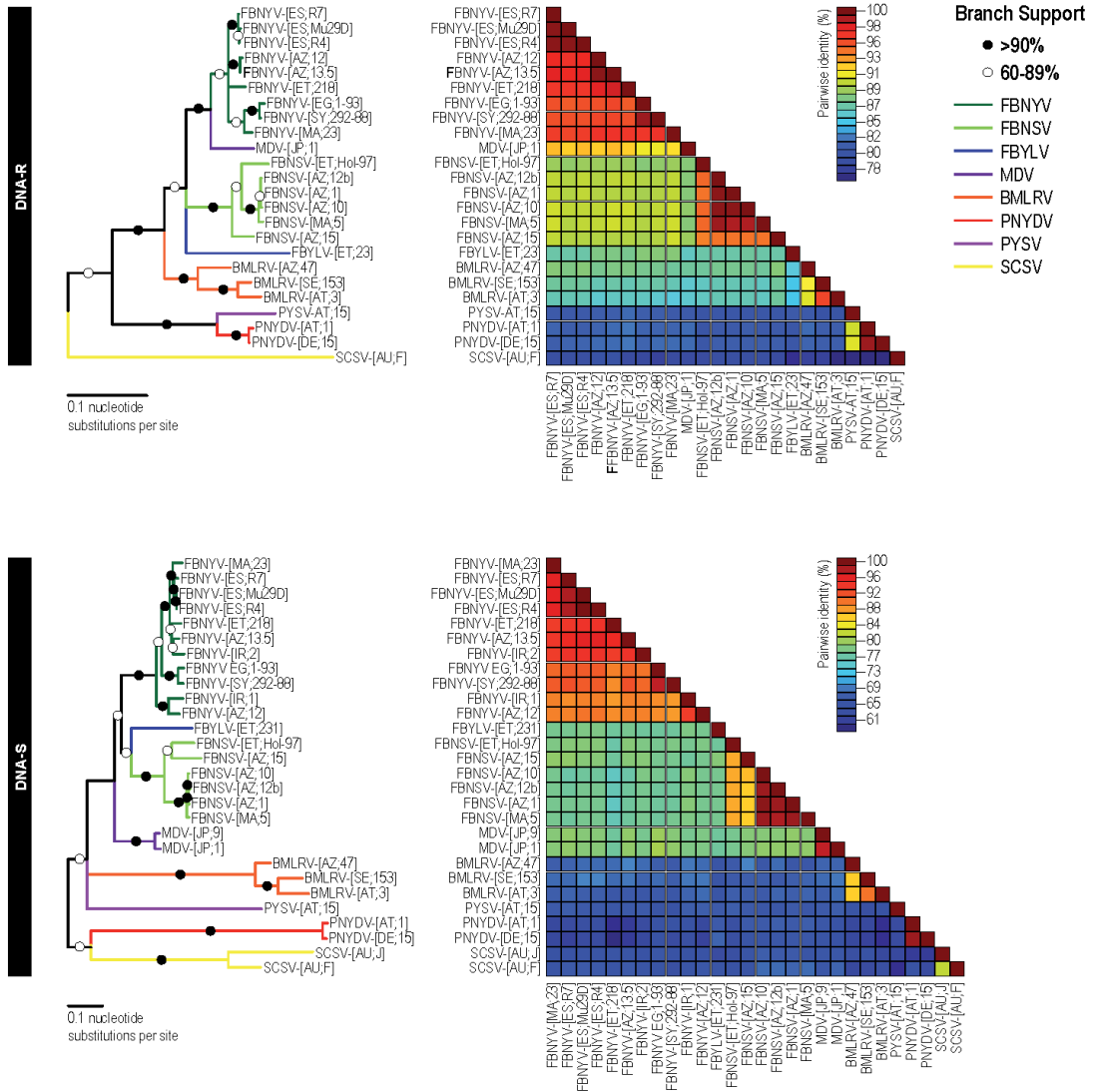
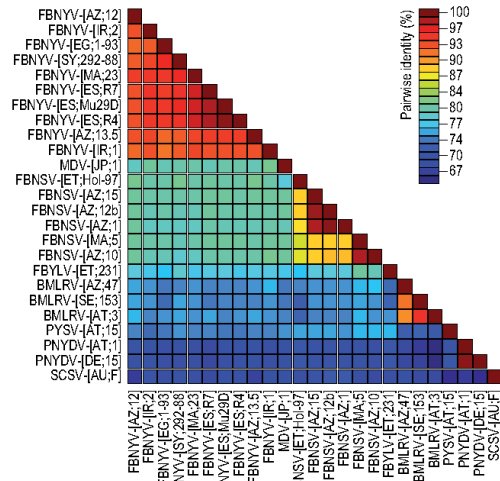
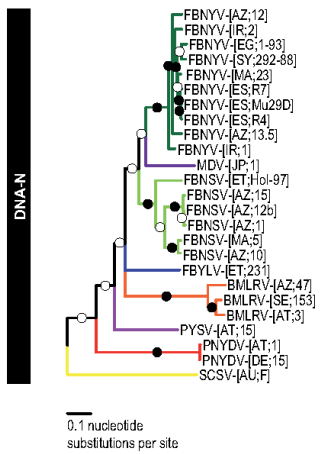
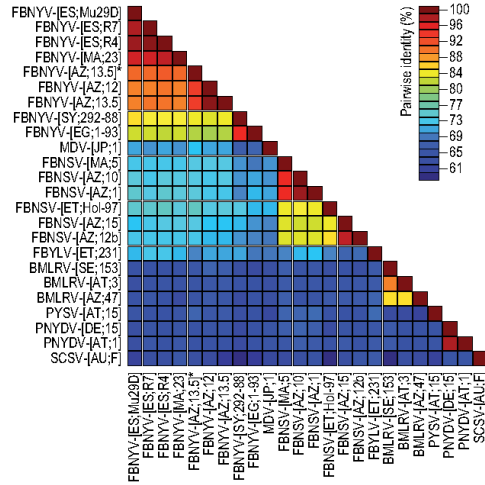
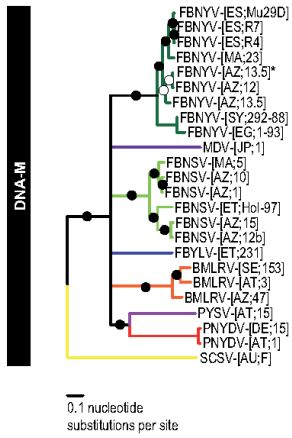
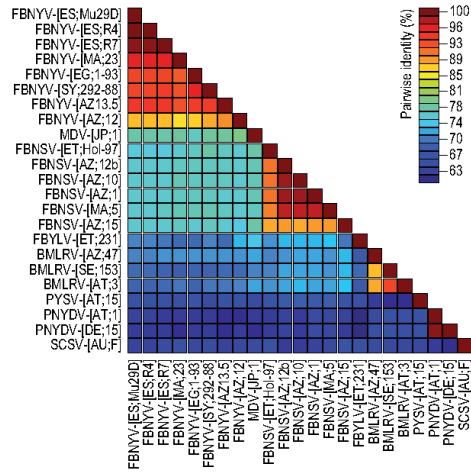
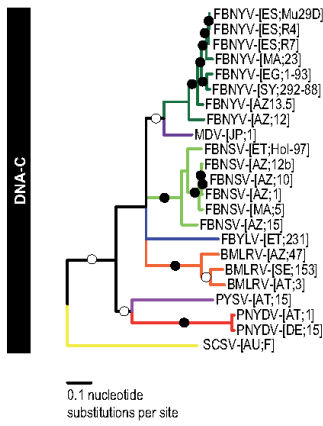


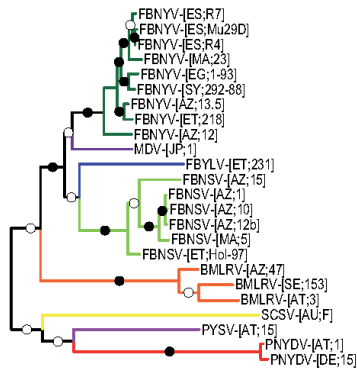
Fig. 1. Evolutionary relationships and similarities among nanoviruses. (a) Maximum-likelihood phylogenetic tree of nanovirus genomes, based on genome components concatenated in the order DNA-R, -S, -C, -M, -N, -U1, -U2 and -U4. Branches with > 90 % bootstrap support are indicated by filled black circles and those with 60–89 % support by open black circles. Branches with < 60 % bootstrap support have been collapsed. (b) Graphical illustration of pairwise nucleotide identities. From Grigoras et al. (2014) *Journal of General Virology* 95, 1178–1191

Fig. 2. Maximum likelihood phylogenetic trees of individual nanovirus genome components and pairwise nucleotide identity scores.
 From Grigoras et al. (2014) Journal of General Virology 95, 1178–1191

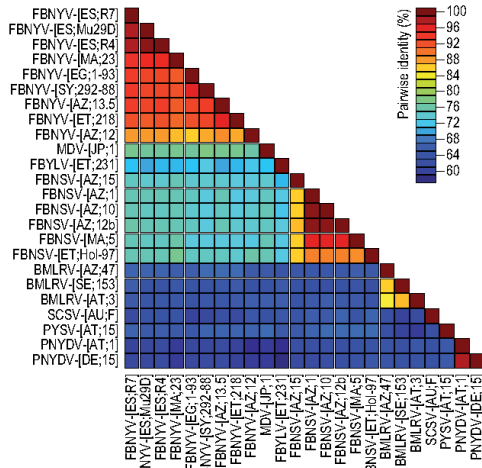




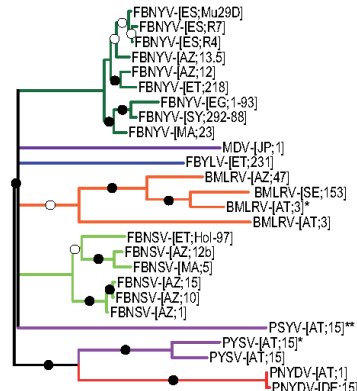
DNA-U1



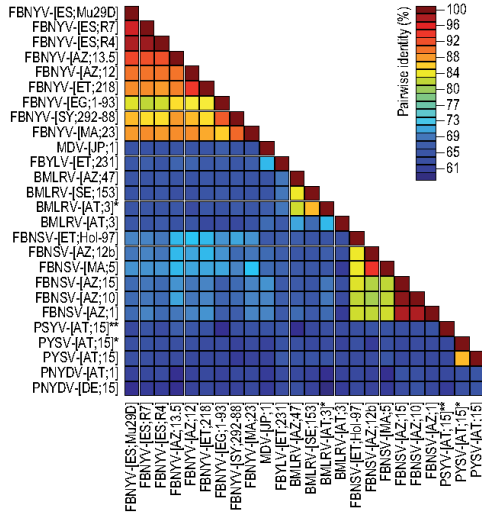
0.1 nucleotide substitutions per site



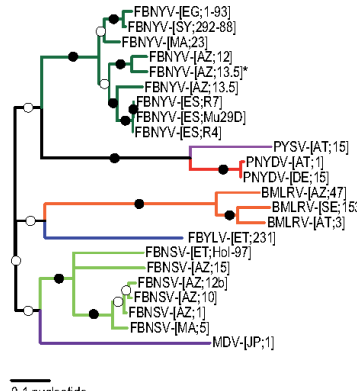
DNA-U2



0.1 nucleotide substitutions per site



DNA-U4



0.1 nucleotide substitutions per site

