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:	***2010.009aP***	(to be completed by ICTV officers)

Short title: 2 new species in the genus Nanovirus
(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:

Joe Vetten (heinrich-josef.vetten@jki.bund.de) on behalf of the Nanoviridae SG

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

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

Date first submitted to ICTV:
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	***2010.009aP***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Nanovirus***
Subfamily:	
Family:	***Nanoviridae***
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:**
Faba bean necrotic stunt virus *Pea necrotic yellow dwarf virus*	GQ150778 to GQ150785 GU553134 (DNA-R only)

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

Species demarcation criteria published in the 8th report are:

- Overall nt sequence identity of <75% is generally indicative of a distinct species,
- Different reactions to antibodies to individual species,
- Differences in CP aa sequences of >15%,
- Differences in natural host range, and
- Differences in the number and types of vector aphid species.

Two geographically and genetically distinct isolates of a hitherto undescribed nanovirus, referred to as faba bean necrotic stunt virus (FBNSV), have been recently described, one from Holetta, Ethiopia (Grigoras et al., 2009) and one from El Jadida, Morocco (Abraham et al., 2009). The genome of the FBNSV isolates consists of eight circular ssDNA components ranging in size from 980 to 1003 (Ethiopia; GenBank acc. nos. GQ150778 to GQ150785) and from 952 to 1005 (Morocco; GQ274031 to GQ274038). When comparing the eight FBNSV DNAs with the homologous DNAs of all other known nanovirus isolates (Figure 1), FBNSV was more closely related to faba bean necrotic yellows virus (FBNYV) and milk vetch dwarf virus (MDV) than to subterranean clover stunt virus (SCSV). FBNSV, FBNYV and MDV were about equally distant from each other (72% to 75% overall identity). The percent identity between the three viruses varied from 57% (DNA-U2 and –U4) to 90% (DNA-R) depending on component, and all three shared identities ranging from 48% (DNA-M) to 79% (DNA-R) with SCSV (Table 1), while all four nanoviruses were only 35% (DNA-M) to 59% (DNA-R) identical to BBTV, ABTV or CBDV, three members of the genus *Babuvirus*. FBNSV, FBNYV and MDV appeared to be similarly equidistant also when only their protein amino acid sequences (coding sequences represent 49% of the total genome) were considered (Table 1). The M-Rep was the most conserved protein between FBNSV, FBNYV and MDV (93-97% amino acid sequence identity) as well as among all nanoviruses (83% amino acid sequence identity with SCSV and 54-57% with babuviruses). FBNSV, FBNYV and MDV shared amino acid sequence identities of 71-84% in the CP, MP and Clink protein and less than 70% in the proteins with unknown functions (encoded by DNA-U1, -U2, and -U4). In contrast, the two FBNSV isolates

differ from each other in these less conserved DNA components (DNA-U1, -U2, and -U4) by only 15-22% and in overall nucleotide sequences by only ~13% (Table 1).

Although FBNSV has been shown to infect a range of legume species and to be transmitted efficiently by *Acyrthosiphon pisum* and *Aphis craccivora* (Grigoras et al., 2009), there is no information on any biological properties other than symptoms (e.g., vector specificity; host range) in which FBNSV differs strikingly from FBNYV and MDV. However, it differs in serological properties from other nanoviruses, by reacting only from weak to intermediate with antiserum to FBNYV (Vetten, unpublished), by failing to react with two monoclonal antibodies (MAbs) raised against FBNYV (Grigoras et al., 2009), and by eliciting several MAbs that react strongly with FBNSV but not (or only weakly) with FBNYV (Grigoras et al., 2009; Abraham et al., 2010). This is substantiated by significant differences in coat protein sequences between FBNSV and other nanoviruses. Most notably, however, FBNSV isolates differ from other nanoviruses not only in coat protein amino acid sequences (identities of 57-84%) but also in overall nucleotide sequences (identities of 59-73%). Thus, they fully meet the two molecular criteria for nanovirus species demarcation.

Table 1. Total nucleotide and predicted amino acid (coding) sequence identities (%) between DNAs of the Moroccan isolate (Mor5) of FBNSV [GQ274031-8] and other nanovirus isolates, including the Ethiopian isolate of FBNSV (FBNSV-Eth).

Isolate / Virus [§]	DNA components (nt) / encoded protein (aa)																Mean identity	
	R / M-Rep		S / CP		M / MP		C / Clink		N / NSP		U1		U2		U4			
	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
FBNSV-Eth	**95.1**	**96.5**	**88.4**	**98.8**	**83.0**	**85.7**	**90.8**	**89.9**	**86.3**	**96.1**	**88.6**	**84.7**	**83.8**	**80.2**	**77.3**	**78.3**	**86.7**	**88.8**
FBNYV-Eg	90.1	93.0	77.6	84.3	65.2	74.1	76.7	76.3	79.0	88.9	70.1	64.5	60.0	54.5	60.2	52.4	72.4	73.5
FBNYV-Sy	90.4	93.0	77.4	83.7	67.0	75.9	76.6	74.6	78.7	88.9	70.6	67.8	65.4	55.4	60.3	54.3	73.3	74.2
MDV	88.7	93.7	77.7	83.7	63.8	75.9	76.9	71.0	77.5	88.9	72.0	69.3	56.8	57.9	57.2	58.5	71.3	74.9
SCSV	79.2	83.2	56.4	57.4	47.8	45.0	59.0	46.9	57.4	67.3	54.2	43.8	—*	—	—	—	[59.0]*	[57.3]

* Dashes (—) indicate that comparison was not possible because a homologous DNA component has not (yet) been identified from this nanovirus. Mean identities values for viruses with less than eight genome components are shown in parentheses. Values for the closest relative of Mor5 are in bold.

[§] The following acc. nos. were used for comparisons: FBNYV isolates from Syria (Syr) [Y11405-9, AJ005965, AJ005967, AJ749903] and Egypt (Eg) [AJ132179-84, AJ132186, AJ749902]**,** Ethiopian (Eth) isolate of FBNSV [GQ150778-85], MDV [AB000923-7, NC_003648], SCSV [NC_003812-13, NC_003815-17, NC_003819].

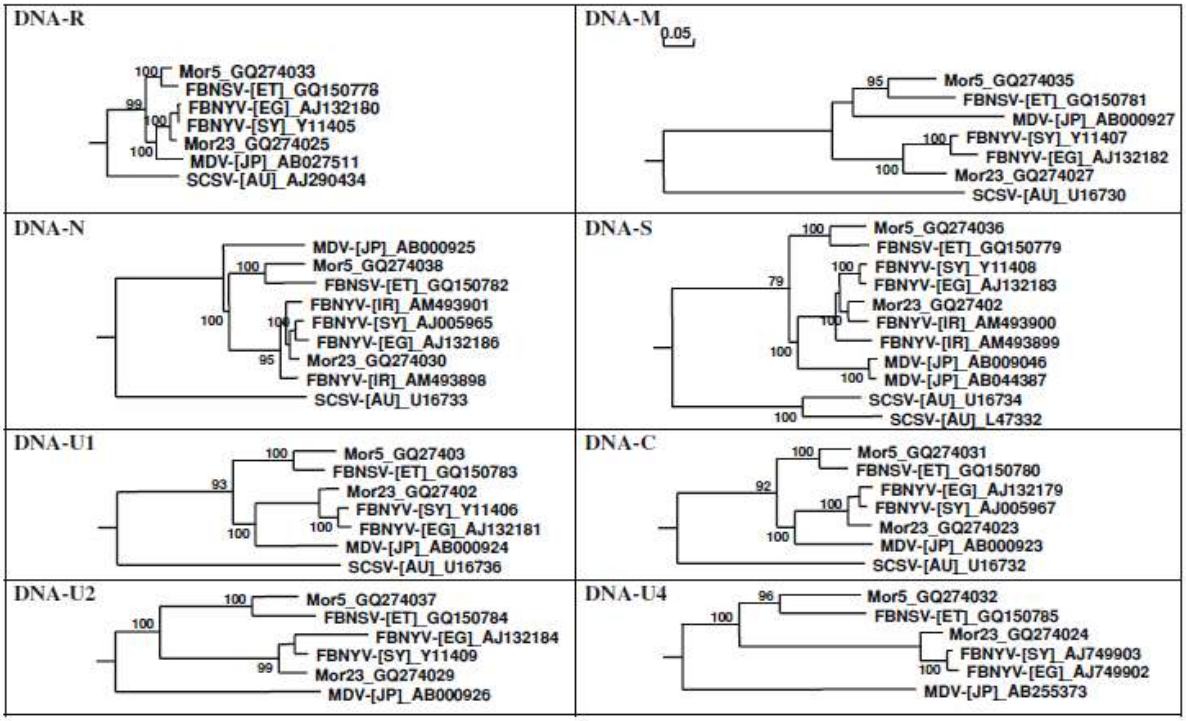

Fig. 1. Phylogenetic trees illustrating the relationship between the eight DNAs of the Moroccan isolate (Mor5) of FBNSV (GQ274031-8) and the corresponding DNAs of the Ethiopian isolate of faba bean necrotic stunt virus (FBNSV-[ET]) and virus isolates (from Australia [AU], Egypt [EG], Syria [SY], Iran (IR), Morocco [Mor23] and Japan [JP]) assigned to *Faba bean necrotic yellows virus* (FBNYV), *Milk vetch dwarf virus* (MDV), *Subterranean clover stunt virus* (SCSV), assigned species of the genus *Nanovirus*. Horizontal branch length are scaled (see bar) according to the number of base substitutions per site. Bootstrap values (1000 replicates) higher than 70% are shown at nodes.

During the growing season of 2009, a disease consisting of leaf rolling, top yellows, and plant stunting affected pea (*Pisum sativum*) in fields near Aschersleben, Saxony-Anhalt, Germany. Analysis of 23 samples from symptomatic pea plants collected in July 2009 showed that two of them contained a new nanovirus which was persistently transmitted by the pea aphid (*Acyrthosiphon pisum*) and caused severe yellowing and stunting in pea and faba bean, sometimes followed by necrosis. This nanovirus cross-reacted weakly with polyclonal antibodies (PAbs) against faba bean necrotic yellows virus (FBNYV) in DAS-ELISA and gave weak to strong TAS-ELISA reactions with six of 26 monoclonal antibodies raised against FBNYV and faba bean necrotic stunt virus (FBNSV). Following rolling circle amplification of total DNA extracted from symptomatic leaves and restriction of the amplified DNA in a nanovirus iteron-specific manner by *Aat*II endonuclease, a predominant and abundant product of ~1 kb was obtained only from infected plants (containing pea isolate D15). Sequence comparisons of eight cloned DNAs of 1,002 nucleotides long unequivocally identified them as complete DNA-R component of a new member of the genus *Nanovirus* (Figure 2). The DNA-R sequence (GenBank No. GU553134) of isolate D15 was nearly equidistant from the DNA-R sequences of FBNYV (Y11405), FBNSV (GQ150778), milk vetch dwarf virus (MDV) (AB027511) and subterranean clover stunt virus (SCSV) (AJ290434), sharing with them respective sequence identities of 79, 78, 79, and 73%. Moreover, it was more distinct from the DNA-R sequences of FBNYV, FBNSV, and MDV than the three latter are from each other (86 to 91%) (Figure 2). Based on unpublished data, all 8 DNA segments that typically form a nanovirus genome have meanwhile been identified from D15. They range in size from 978 (DNA-U1) to 1002 (DNA-R) nucleotides. Analysis of the seven DNAs [other than DNA-R] confirmed that they are also genetically very distinct from all other known nanovirus sequences. Isolate D15 shared with other members of the genus *Nanovirus* overall nucleotide sequence identities of only 61-64%, with DNA-R and -N being the

most conserved DNA components (identities of 73-79% and 59-68%, respectively) and DNA-U1, -U2, and -U4 being the least conserved genome segments (44-54%). Coat protein (CP) amino acid sequence identities of D15 with other nanoviruses ranged only from 51 to 57%. Since the nanovirus isolate D15, referred to as pea necrotic yellow dwarf virus (PNYDV), clearly meets the molecular criteria for nanovirus species discrimination [overall nt sequence identity of <75% and differences in CP aa sequences of >15%], we propose the name *Pea necrotic yellow dwarf virus* for this new nanovirus species, isolates of which naturally infect pea in Germany.

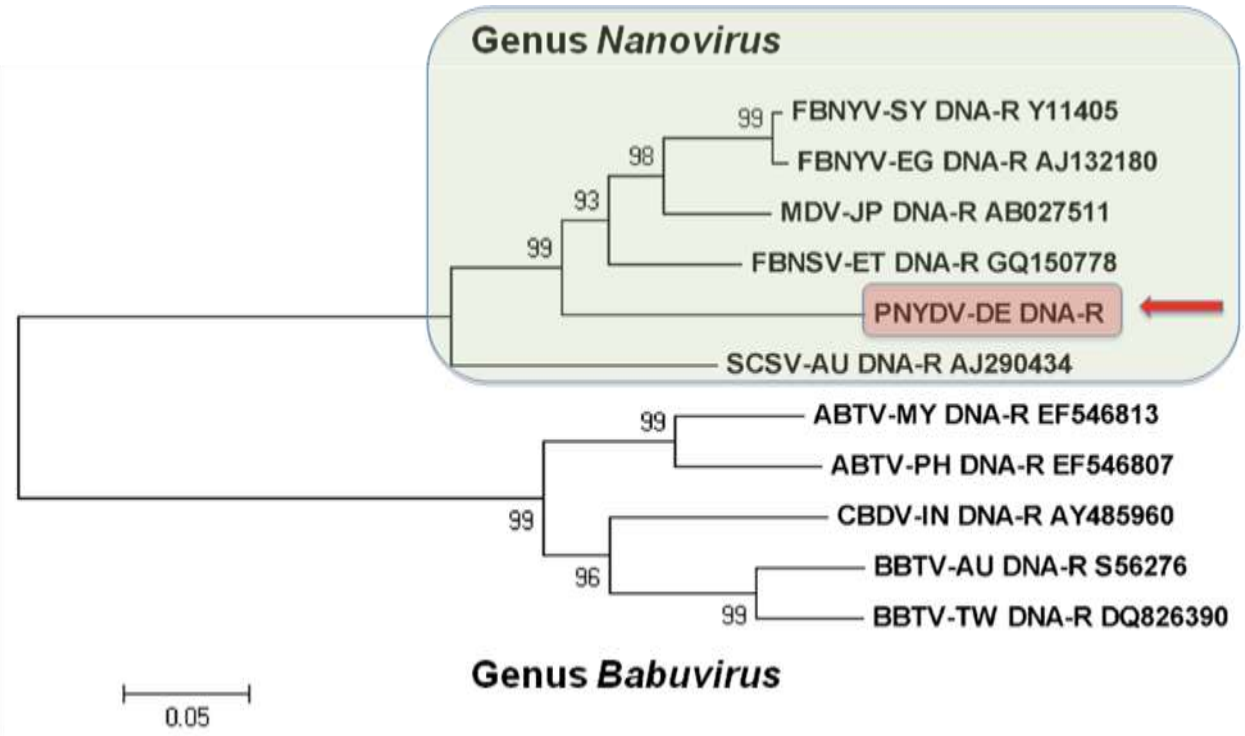

Figure 2. Neighbor-joining dendograms illustrating the phylogenetic relationships between the German isolate of pea necrotic yellow dwarf virus (PNYDV; GenBank No. GU553134) and other nano- and babuviruses on the basis of their DNA-R sequences. Horizontal branch lengths are scaled according to the number of base substitutions per site. Vertical branch lengths are arbitrary. Bootstrap per cent (500 replicates) are shown next to the branches. GenBank accession numbers are shown in the figure.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Grigoras, I., T. Timchenko, L. Katul, A. Grande-Pérez, H.J. Vetten & B. Gronenborn (2009). Reconstitution of authentic nanovirus from multiple cloned DNAs. J. Virol. 83, 10778–10787.

Abraham, A.D., B. Bencharki, V. Torok, L. Katul, M. Varrelmann & H.J. Vetten (2010). Two distinct nanovirus species infecting faba bean in Morocco. Arch Virol 155, 37-46.

Grigoras, I., B. Gronenborn & H.J. Vetten (2010). First report of a nanovirus disease of pea in Germany. Plant Disease 94, 643.

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.