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:	***2015.013aD***	(to be completed by ICTV officers)

Short title: One new species, *Spodoptera frugiperda granulovirus,* **in the genus** ***Betabaculovirus***
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 10 are required)	**1** ☒	**2** ☒	**3** ☐	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☐	**10** ☒

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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)	Baculoviridae/Nudiviridae Study Group

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

ICTV has the following rule for distinguishing between names of virus isolates and names of species: Species names are entirely italicized, while the names of virus isolates and their genome sequences are entirely in regular type (e.g. no part is italicized). While historically baculovirologists have italicized the host genus and species in the names of virus isolates, this is no longer an accepted convention.

Use of the abbreviation SpfrGV should be consistent throughout the proposal.

June 2015 – Module 2 justification has been re-written.

The SG corrected the virus abbreviation name throughout the proposal. The virus names have been corrected in regular type.

Date first submitted to ICTV:	April 12, 2015
Date of this revision (if different to above):	Sept 23, 2015

ICTV-EC comments and response of the proposer:

7/21/2015: Check and (if necessary) correct scale on Figure 2.

9/22/2015: The Figure 2 tree was replaced with a comparable tree showing that the new species of this proposal is different and distinct from the other two new *Betabaculovirus* species discussed at EC47, *Errinyis ello granulovirus* and *Agrotis segetum granulovirus*. The scale bar was generated by the software that constructed the tree (MEGA 6) and is consistent with the degree of divergence among the genera of *Baculoviridae* and the species of *Betabaculovirus*

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	***2015.013aD***	(assigned by ICTV officers)

To create new species within:

Genus:	***Betabaculovirus***
Subfamily:	
Family:	***Baculoviridae***
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.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Spodoptera frugiperda granulovirus	Spodoptera frugiperda granulovirus VG008 (SpfrGV VG008)	KM371112

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 group of viruses from the host insect *Spodoptera frugiperda* (order Lepidoptera, family Noctuidae) appear to be members of a previously undescribed species, *Spodoptera frugiperda granulovirus*, in genus *Betabaculovirus* of family *Baculoviridae* (Jehle *et al*., 2006a, b; Cuartas *et al*., 2014).

Occlusion bodies produced by these viruses possess an ovocylindrical shape with dimensions characteristic of betabaculoviruses and containing a single rod-shaped nucleocapsid (Figure 1; Cuartas *et al*., 2014; Herniou *et al*., 2011). The genome sequence of a representative isolate (Spodoptera frugiperda granulovirus VG008; SpfrGV VG008) was determined and found to contain all the core genes found in viruses of family *Baculoviridae*. Phylogenetic inference with concatenated alignments of the predicted core gene amino acid sequences place SpfrGV VG008 among other viruses of genus *Betabaculovirus* (Figure 2).

A proposed baculovirus species demarcation criterion based on pairwise nucleotide distances estimated with the Kimura 2-parameter model (Jehle et al, 2006b) states that two baculoviruses are considered to represent different species if the pairwise nucleotide distances between these viruses, calculated from alignments of partial *polh*, *lef8* and *lef9* genes, are greater than 0.05 substitutions/site. Nucleotide distances between SpfrGV VG008 and the representative isolates of other betabaculovirus species are much greater than the threshold of 0.05 substitutions/site (Figure 3, Table 1). In addition, the isolates of SpfrGV can be distinguished from representative isolates of other betabaculovirus species on the basis of host range, genome size, and gene composition and order (see Appendix). We conclude that SpfrGV VG008 should be classified as a virus in the new species, *Spodoptera frugiperda granulovirus*, in the genus *Betabaculovirus* within family *Baculoviridae.*

This new species is also different and distinct from the two other *Betabaculovirus* species proposed in 2015, *Errinyis ello granulovirus* and *Agrotis segetum granulovirus* (Figure 2).

Background information: The fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797; Lepidoptera: Noctuidae), is a polyphagous insect with a wide geographical distribution, considered the most important pest in maize (*Zea mays* L.) in the Americas (Clark *et al*., 2007). Isolates of S. frugiperda granulovirus (SpfrGV) have been reported previously, but they have not been completely characterized (Jehle *et al*., 2006a, b; Steinhaus, 1957; Valicente, 1989).

Two baculovirus morphologies from the baculovirus genera *Alphabaculovirus* and *Betabaculovirus* were reported in *S. frugiperda* larvae collected in a pasture crop in Colombia (Gómez *et al*., 2010). Then, the Colombian S. frugiperda granulovirus (VG008) was characterized morphologically, biologically and molecularly and was compared with a reference granulovirus isolate (from the same insect species) from Brazil (VG014) (Cuartas *et al*., 2014).

OBs morphology: The VG008 OBs were ovoidal with a mean size of 434.8 (±25.8) nm by 161 (±10.6) nm. The ultrastructural morphology showed granular-shaped OBs (ovoid or elliptical) and single virion (**Figure 1**), corresponding with the OB sizes described for the *Betabaculovirus* genus, with sizes falling between 400 and 600 nm in length and 160 and 300 nm in width (Caballero *et al*., 2001; Miller, 1997; Slack & Arif, 2007).

Granulin protein: The characterization of proteins of VG008 was performed by 12% polyacrylamide gel electrophoresis (SDS-PAGE). The estimated molecular weight of VG008 granulin (principal protein of the granulovirus OBs) was 27.2 kDa which falls within the range of 27–31 kDa betabaculovirus granulins (Finnerty *et al*., 2000; Sciocco-Cap *et al*., 2001; Tweeten *et al*., 1981; Wang *et al*., 2008). This datum was supported with the subsequently obtained nucleotide information revealing that the theoretical protein encoded by *granulin* gene is 29.2 kDa.

Genome and phylogenetic analyses: Complete genome sequence of SpfrGV VG008 (KM371112.1) showed that the single circular double-stranded DNA molecule contained in the nucleocapsids has 140,913 bp with 146 putative ORFs, values included within the corresponding ranges from viruses in the *Betabaculovirus* (119–183 kbp in size, 116–181 ORFs) (Miele *et al*., 2011). The phylogenetic inference revealed closer relationships with the other betabaculoviruses in preference than with alpha-, gamma- and deltabaculoviruses based on the baculovirus core protein comparison (Garavaglia *et al*., 2012). Particularly, the closest relationships are with Helicoverpa armigera GV (HearGV), Xestia c-nigrum GV (XecnGV), Pseudaletia unipuncta GV (PsunGV) and Spodoptera litura GV (SpliGV) (**Figure 2).** However, SpfrGV shows big difference in genome size (140,913 bp *vs.* 169,794 bp for HearGV, 178,733 bp for XecnGV, 176,677 bp for PsunGV and 124,121 bp for SpliGV), gene content (146 ORFs *vs.*136 for HearGV, 181 for XecnGV, 183 for PsunGV and 136 for SpliGV), protein identity (best value is 83.3% for SpfrGV ORF103 *vs*. XecnGV ORF121) and genome colinearity (presence of 14 unique genes and different genome organization) (Cuartas *et al*., 2015).

Isolates of the new species: The species *Spodoptera frugiperda granulovirus* could also include the other two isolates of granuloviruses infecting *S. frugiperda* with partial genome sequence available, including SpfrGV VG014 (KJ698696, KJ698694, KJ698692) and SpfrGV A12-4 (AY706677.1)] since previous analysis of Kimura-2 parameter nucleotide distances on lef8-lef9-granulin concatemer revealed that the three isolates are genotypes of the same species (Cuartas *et al.*, 2014).

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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.

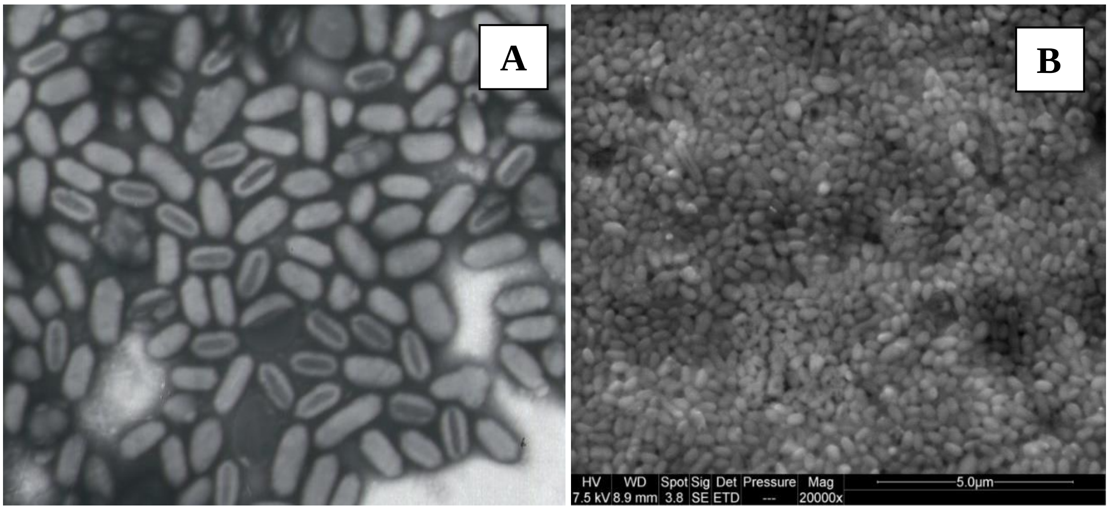

Figure 1. Electron micrographs of OBs from the betabaculovirus of *S. frugiperda* VG008. (A) Transmission electron microscopy (TEM); (B) Scanning electron microscopy (SEM). One hundred microliters of the OB suspension (1 x 10^8 OBs/mL) was fixed overnight at 4ºC in 4% v/v formaldehyde and 1% v/v glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). For TEM, negative staining was made with 1% phosphotungstic acid (pH 6.0) and the viral suspension was placed on copper grids for analysis (TEM CM 10 microscopy). For SEM, the sample was sputter coated with gold following fixation and observed in a Phillips 510 microscope.

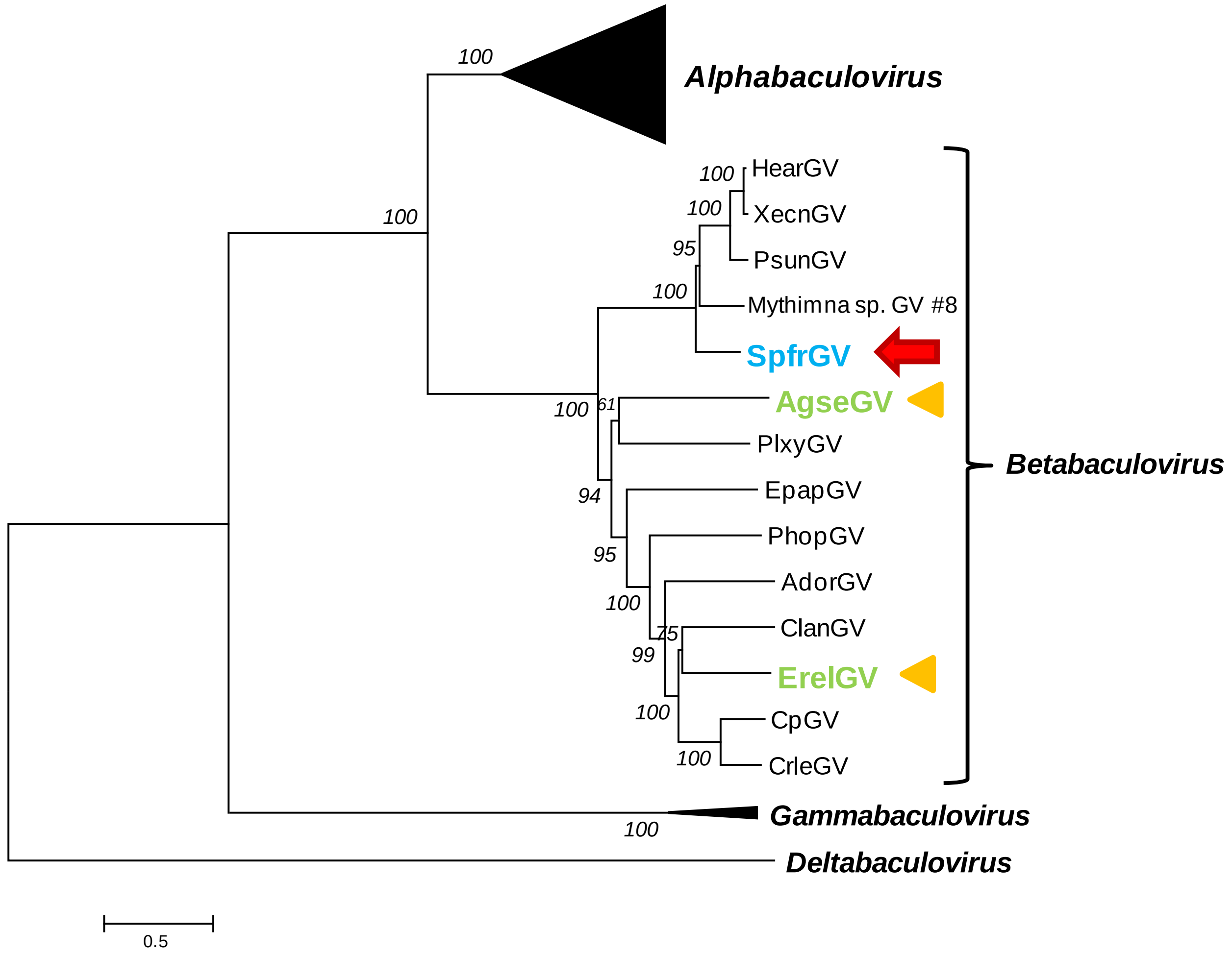

Figure 2. Relationships of baculovirus species and other isolates, inferred from the predicted amino acid sequences of baculovirus core genes. The phylogenetic tree was constructed from the concatenated alignments of 34 baculovirus core gene amino acid sequences using the minimum-evolution method using the James-Taylor-Thornton substitution model and a gamma parameter of 0.85 (estimated from the *dnapol* alignment). Bootstrap values are shown for each node. Clades corresponding to the four genera of *Baculoviridae* are indicated. The proposed species is listed in blue font and denoted with a red arrow. Additional new *Betabaculovirus* species discussed at EC47 are listed in green font and denoted with an orange arrowhead. *Betabaculovirus* taxa include the representative isolates for species *Adoxophyes orana granulovirus* (AdorGV), *Agrotis segetum granulovirus* (AgseGV), *Cryptophlebia leucotreta granulovirus* (CrleGV), *Cydia pomonella granulovirus* (CpGV), *Errinyis ello granulovirus* (ErelGV), *Helicoverpa armigera granulovirus* (HearGV), *Phthorimaea operculella granulovirus* (PhopGV), *Plutella xylostella granulovirus* (PlxyGV), *Pseudalatia unipuncta granulovirus* (PsunGV), *Spodoptera frugiperda granulovirus* (SpfrGV), *Xestia c-nigrum granulovirus* (XecnGV), as well as isolates Clostera anachoreta granulovirus (ClanGV), Epinotia aporema granulovirus (EpapGV) and Mythimna sp. granulovirus #8 (Mythimna sp. #8).

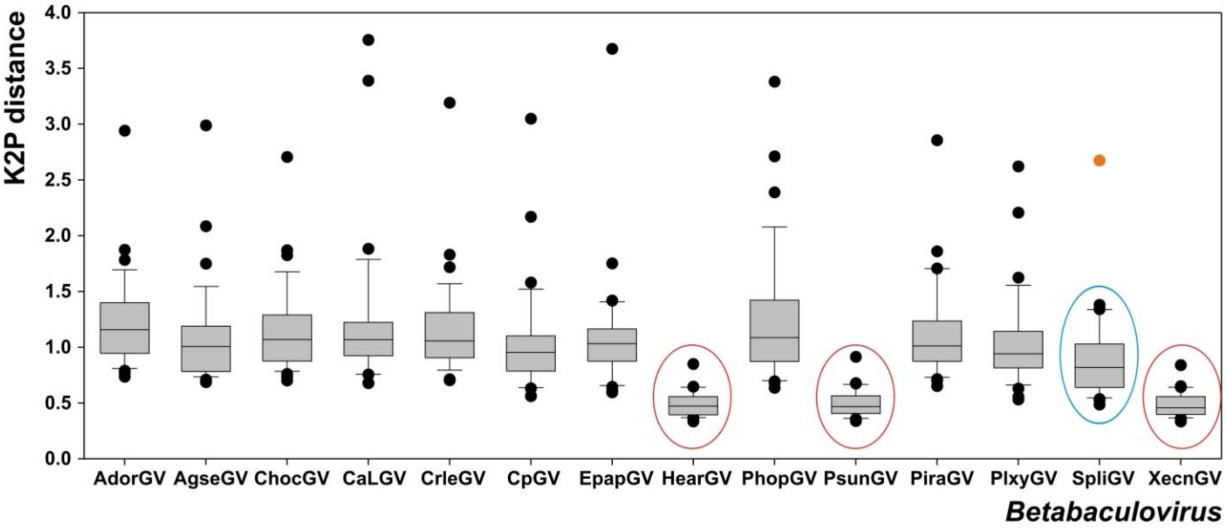

Figure 3. K2P distance analyses of SpfrGV VG008 core genes. The box plot shows the Kimura 2 parameter distances of the 37 core genes present in SpfrGV VG008 with respect to the orthologs located in the other betabaculoviruses (names are indicated). The boundary of boxes closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars above and below boxes indicate the 90th and 10th percentiles, respectively. The filled circles indicate outlying points. Red ovals indicate the closest neighbors, and blue oval indicates Spodoptera litura GV (orange outlier corresponds to *desmoplakin* gene). Virus abbreviations are as defined in Figure 2; additional taxa include Choristoneura occidentalis granulovirus (ChocGV), Clostera anastomosis granulovirus (CalGV), Pieris rapae granulovirus (PiraGV), and Spodoptera litura granulovirus (SpliGV).

Table 1. Kimura 2 parameter distances of a concatemer of 37 core genes

	SpfrGV	AdorGV	AgseGV	ChocGV	CaLGV	CrleGV	CpGV	EpapGV	HearGV	PhopGV	PsunGV	PiraGV	PlxyGV	SpliGV
AdorGV	1.124													
AgseGV	0.987	0.954												
ChocGV	1.084	0.701	0.888											
CaLGV	1.075	0.891	1.060	0.777										
CrleGV	1.104	0.719	0.890	0.600	0.821									
CpGV	0.953	0.815	0.977	0.699	0.716	0.493								
EpapGV	1.006	0.906	0.950	0.864	0.946	0.894	0.834							
HearGV	0.486	1.051	0.945	0.993	1.117	1.015	1.010	1.002						
PhopGV	1.106	0.799	0.956	0.713	0.904	0.723	0.747	0.878	1.044					
PsunGV	0.494	1.066	0.943	0.988	1.128	0.995	1.026	1.010	0.215	1.043				
PiraGV	1.049	0.682	0.863	0.489	0.721	0.562	0.619	0.834	0.958	0.677	0.963			
PlxyGV	0.934	1.013	0.939	0.975	1.057	1.000	0.937	0.932	0.939	1.011	0.938	0.931		
SpliGV	0.788	1.009	0.884	0.930	1.110	0.936	0.997	0.980	0.770	1.013	0.772	0.909	0.887	
XecnGV	0.487	1.052	0.945	0.993	1.112	1.014	1.010	1.007	0.027	1.052	0.214	0.960	0.942	0.771

Color numbers show the K2P distances between SpfrGV VG008 and each one of the other betabaculoviruses. In red are showed the closest neighbors and in blue the other ones. Virus abbreviations are as defined in Figures 2 and 3.