



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:	2015.001aD	(to be completed by ICTV officers)			
Short title: One new species, <i>Agrotis segetum granulovirus</i>, in the genus <i>Betabaculovirus</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 checked="" 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"/>

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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 Study Group

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

Date first submitted to ICTV: 6/22/15
Date of this revision (if different to above): 9/23/15

ICTV-EC comments and response of the proposer:

9/23/15: The tree in Figure 2 was replaced with a comparable tree showing that the proposed species is different and distinct from the other two *Betabaculovirus* species discussed at EC47, *Errinyis ello granulovirus* and *Spodoptera frugiperda granulovirus*.

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	2015.001aD	(assigned by ICTV officers)
To create 1 new species within:		
Genus:	<i>Betabaculovirus</i>	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.
Subfamily:		
Family:	<i>Baculoviridae</i>	
Order:		
Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)
<i>Agrotis segetum granulovirus</i>	Agrotis segetum granulovirus DA (AgseGV-DA)	KR584663

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

- Isolates of the proposed species can be identified as belonging to the genus *Betabaculovirus* on the basis of three or more of the following criteria: host species of the insect order Lepidoptera; circular double-stranded DNA genome ranging in size from 110–170 kbp with a gene content characteristic of other betabaculoviruses; relationships to other betabaculoviruses as inferred by molecular phylogeny; granule-shaped occlusion bodies formed within the nucleus of infected cells with a characteristic size and shape; and rod-shaped virions consisting of nucleocapsids enveloped singly. Figure 2 shows the relationships of the isolate of the proposed species to other recognized isolates in the *Baculoviridae*, as determined from the concatenated alignment of the predicted amino acid sequences of 37 baculovirus core genes (Garavaglia et al., 2012).
- *Agrotis segetum granulovirus* was originally isolated from infected *Agrotis segetum* larvae. One isolate was described (Lipa & Ziemnicka, 1971; Bashir et al., 1979), which showed a different restriction profile to isolates from *Agrotis segetum nucleopolyhedrovirus* and *Agrotis ipsilon multiple nucleopolyhedrovirus* (Wennmann et al., 2015a). Eventually, two complete genome sequences of *Agrotis segetum granulovirus* from China, namely AgseGV-XJ and AgseGV-L1 were published (Zhang et al., 2014). The isolate presented in this proposal, AgseGV-DA, showed an overlapping host range with the nucleopolyhedroviruses AgseNPV and AgipNPV and the potential for interactions in mixed infections (Wennmann et al., 2015b) with one of these isolates.
- Complete genome sequencing of AgseGV-DA, AgseGV-L1, and AgseGV-XJ revealed significant differences in nucleotide sequence and open reading frame composition to other viruses from the genus *Betabaculovirus*. Proposed species demarcation criteria for baculoviruses were published, including pairwise nucleotide distance estimates based on the Kimura 2-parameter model of nucleotide substitution (Jehle et al., 2006). According to this criterion, if nucleotide distances between two baculovirus isolates, estimated from alignments of partial *lef-8*, *lef-9*, and *polh/gran* genes, are less than 0.015 substitutions/site, the two baculoviruses are considered to be the same species. If pairwise nucleotide distances between two viruses at these loci are greater than 0.05 substitutions/site, the viruses are considered to be different species. If the nucleotide distances lie between 0.015 and 0.050 substitutions/site,

additional characteristics of the two viruses are considered. The proposed criterion were originally based on comparisons of sequences from 117 separate baculovirus isolates, and researchers have applied this criterion to other isolates to identify potential new baculovirus species and variants of currently recognized species. Pairwise Kimura 2-parameter (K2P) distances for reference isolates of the proposed species are listed in Table 1. The K2P distances of the three fully sequenced isolates of *Agrotis segetum granulovirus*, AgseGV-DA; AgseGV-L1 and -XJ, were larger than 0.3388 to that of any other isolates of other species in the genus *Betabaculovirus*, indicating that a separate species should be established to include these isolates. The K2P distances between AgseGV-DA; AgseGV-L1 and -XJ, were smaller than 0.0014, indicating that the three isolates should belong to the same species.

- Finally, *Agrotis segetum granulovirus* is different and distinct from the other two new *Betabaculovirus* species proposed in 2015, *Errinyis ello granulovirus* and *Spodoptera frugiperda granulovirus*.

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

- Bashir, H.S., Zethner, O., Gul, H., Chaudry, M.I., 1979. Control experiments using *Agrotis segetum granulovirus* against *Agrotis ipsilon* [Lep.: Noctuidae] on tobacco seedlings in Northern Pakistan. *BioControl* 24(4):393-401.
- Cuartas, P.E., Barrera, G.P., Belaich, M.N., Barreto, E., Ghiringhelli, P.D., Villamizar, L.F., 2015. The complete sequence of the first *Spodoptera frugiperda* betabaculovirus genomes: a natural multiple recombinant virus. *Viruses* 7:394-421; doi:10.3390/v7010394
- Garavaglia, M.J., Miele, S.A.B., Iserte, J.A., Belaich, M.N., Ghiringhelli, P.D., 2012. The ac53, ac78, ac101, and ac103 genes are newly discovered core genes in the family *Baculoviridae*. *J. Virol.* 86:12069-79.
- Jehle, J.A., Lange, M., Wang, H., Hu, Z., Wang, Y., Hauschild, R., 2006. Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera. *Virology* 346:180-93.
- Lipa, J., Ziemnicka, J., 1971. Studies on the granulosis virus of cutworms *Agrotis* spp. (Lepidoptera, *Noctuidae*). *Acta Microbiol. Pol.* 3:354-60.
- Wennmann, J.T., Gueli Alletti, G., Jehle, J.A., 2015a. The genome sequence of *Agrotis segetum* nucleopolyhedrovirus B (AgseNPV-B) reveals a new baculovirus species within the *Agrotis* baculovirus complex. *Virus Genes* 50:260-76.
- Wennmann, J.T., Köhler, T., Gueli Alletti, G., Jehle J. A., 2015b. Mortality of cutworm larvae is not enhanced by *Agrotis segetum* granulovirus and *Agrotis segetum* nucleopolyhedrovirus B coinfection relative to single infection by either virus. *Appl. Environ. Microbiol.* 81(8):2893-9.
- Zhang, X., Liang, Z., Yin, X., Wang, J., Shao, X., 2014. Complete genome sequence of *Agrotis segetum* granulovirus Shanghai strain. *Arch. Virol.* 159:1869-72.

Table 1: Baculovirus genomes used in the phylogenetic analysis of concatenated *polh/gran*, *lef-8*, and *lef-9* genes

Baculovirus isolates	Acc. Number	Abbreviation
<i>Adoxophyes orana</i> granulovirus	NC_005038	AdorGV
<i>Agrotis segetum</i> granulovirus XJ	NC_005839	AgseGV-XJ
<i>Agrotis segetum</i> granulovirus L1	KC994902	AgseGV-L1
<i>Agrotis segetum</i> granulovirus DA	KR584663	AgseGV-DA
<i>Cryptophlebia leucotreta</i> granulovirus (CV3)	NC_005068	CrleGV
<i>Cydia pomonella</i> granulovirus (M1)	NC_002816	CpGV
<i>Helicoverpa armigera</i> granulovirus	NC_010240	HearGV
<i>Phthorimaea operculella</i> granulovirus	NC_004062	PhopGV
<i>Plutella xylostella</i> granulovirus (K1)	NC_002593	PlxyGV
<i>Pseudaletia unipunctata</i> granulovirus (Hawaiian)	NC_013772	PsunGV
<i>Xestia c-nigrum</i> granulovirus (alpha4)	NC_002331	XecnGV
<i>Agrotis segetum</i> nucleopolyhedrovirus A (renamed)	NC_007921	AgseNPV-A
<i>Agrotis ipsilon</i> nucleopolyhedrovirus	NC_011345	AgipNPV
<i>Autographa californica</i> multiple nucleopolyhedrovirus (C6)	NC_001623	AcMNPV

Neodiprion lecontei nucleopolyhedrovirus
 Neodiprion sertifer nucleopolyhedrovirus

NC_005906
 NC_005905

NeleNPV
 NeseNPV

Table 2: Pairwise nucleotide distances of concatenated partial sequences of *polh/gran*, *lef-8* and *lef-9* using Kimura-2-parameter (K-2-P) model of betabaculovirus isolates representing their species. Distances of the three *Agrotis segetum granulovirus* isolates are highlighted in red. In addition to other criteria, two baculoviruses are recognized to belong to different species when the K-2-P distance of these loci is larger than 0.05 substitutions/site. K-2-P distances smaller than 0.015 between two isolates have been proposed to indicate isolates are definitely members of the same species (Jehle et al., 2006).

	AdorGV	AgseGV-XJ	AgseGV-L1	AgseGV-DA	ClGV	CpGV	HearGV	PhopGV	PxGV	PsunGV
AgseGV-XJ	0.3756									
AgseGV-L1	0.3734	0.0014								
AgseGV-DA	0.3734	0.0014	0.0000							
CrleGV	0.3241	0.3410	0.3388	0.3388						
CpGV	0.3351	0.3575	0.3553	0.3553	0.2635					
HearGV	0.3902	0.3983	0.3984	0.3984	0.3919	0.3749				
PhopGV	0.3443	0.3643	0.3621	0.3621	0.3186	0.3043	0.3701			
PlyyGV	0.3710	0.3955	0.3932	0.3932	0.3910	0.3815	0.3969	0.3033		
PsunGV	0.3964	0.4135	0.4136	0.4136	0.3969	0.3907	0.1518	0.3797	0.4232	
XecnGV	0.3904	0.3970	0.3972	0.3972	0.3858	0.3761	0.0151	0.3761	0.4031	0.1526

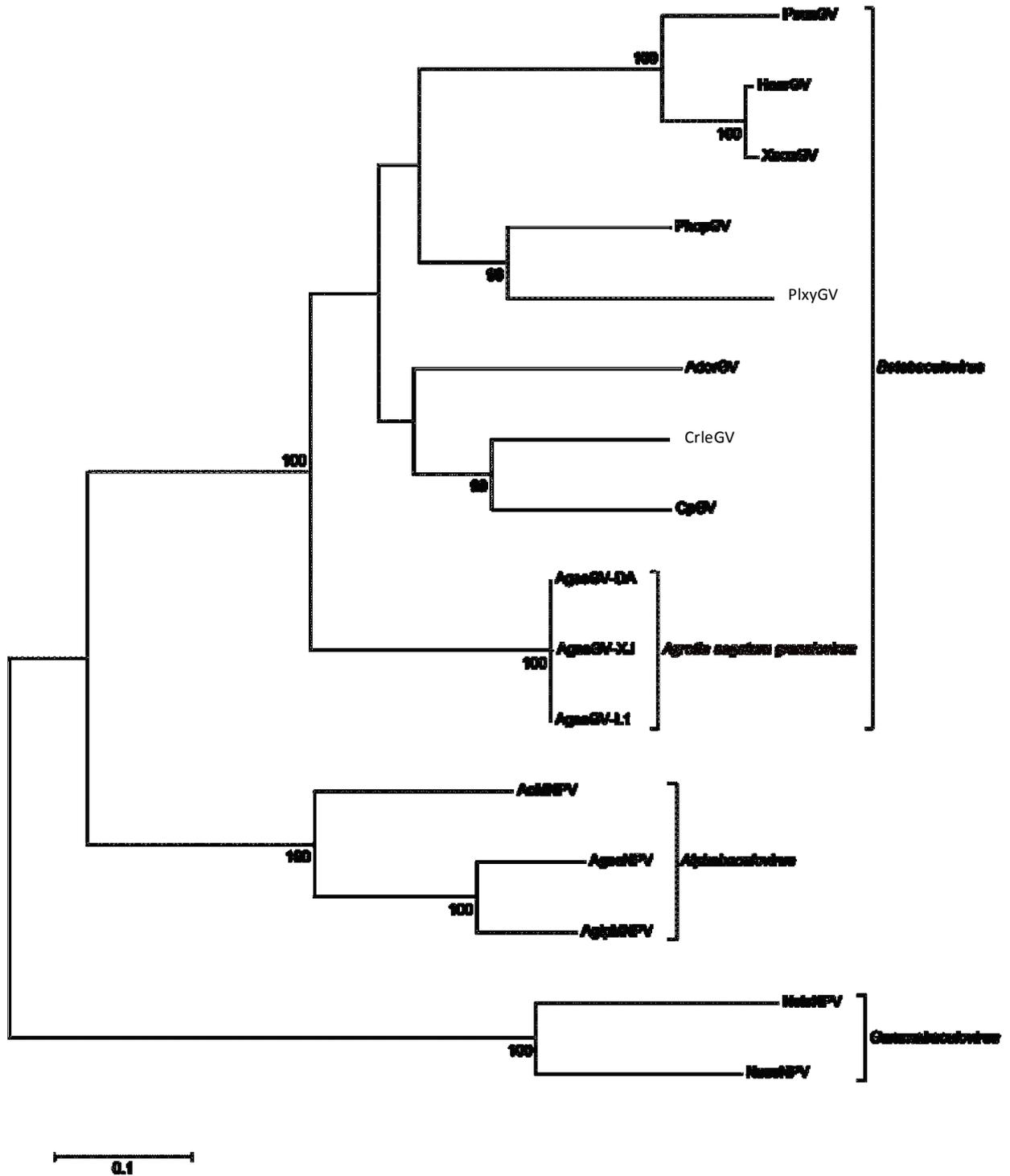


Figure 1: Maximum-Likelihood tree for *Agrotis segetum granulovirus* isolates and other selected baculoviruses. Phylogram is based on a concatenation built with partial sequence alignments of *gran/polh*, *lef-8* and *lef-9* (Jehle et al., 2006). The phylogenetic tree was inferred using MEGA 6 program. Numbers on the branches indicate bootstrap support of 1000 replicates. *CrleGV* and *PlyxGV* are represented as *CIGV* and *PxGV*, respectively, in this figure. The proposed new species is shown in red.

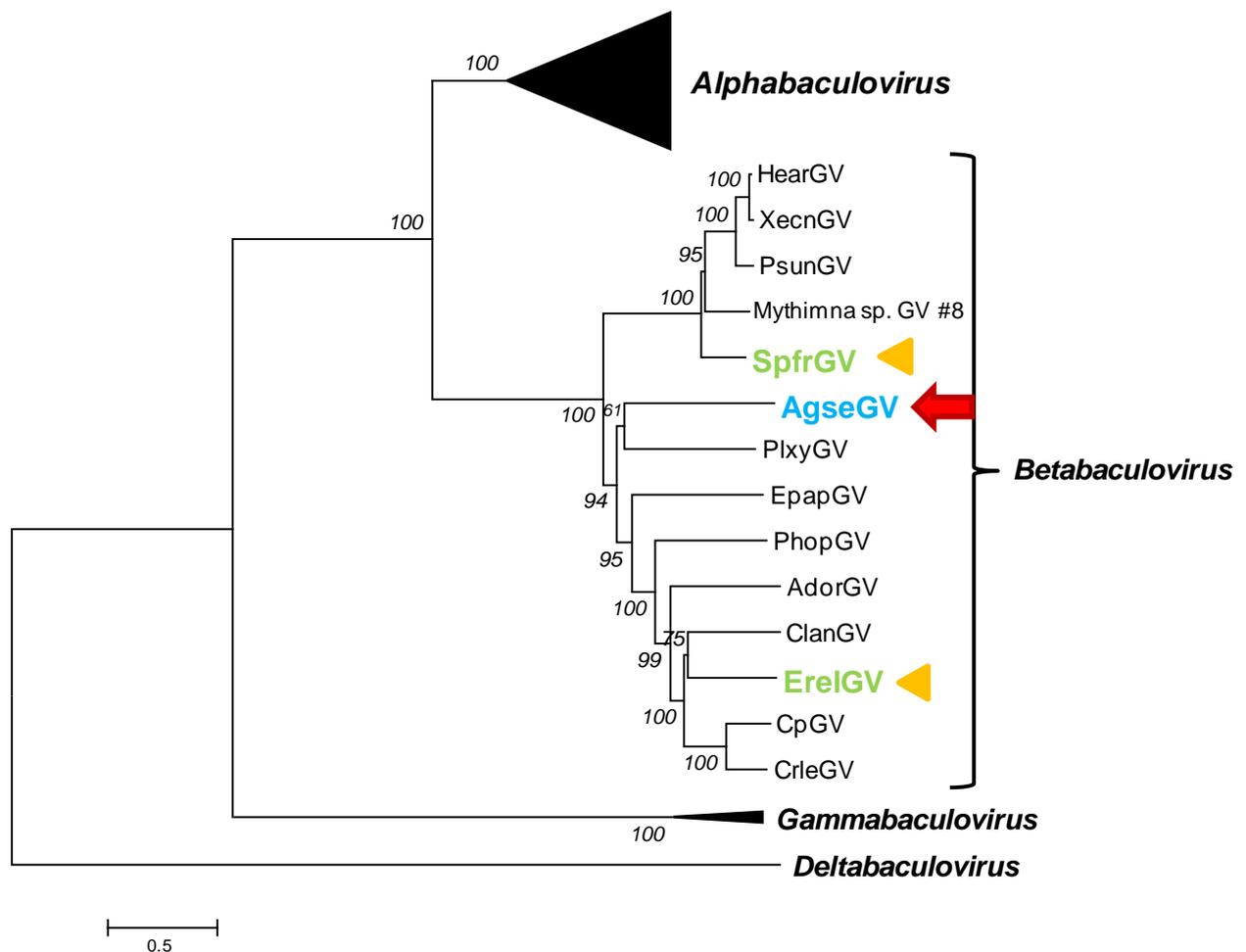


Figure 2. Relationships of baculovirus species and other isolates, inferred from the predicted amino acid sequences of baculovirus core genes. The phylogenetic tree was constructed from the concatenated alignments of 34 baculovirus core gene amino acid sequences using the minimum-evolution method using 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), *Pseudalattia 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 *Mythemna sp. granulovirus* #8 (Mythemna sp. #8).

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.