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:	***2013.001aI***	(to be completed by ICTV officers)

Short title: Two (2) new species in the genus *Iflavirus*, family *Iflaviridae*.
(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** ☒	

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List the ICTV study group(s) that have seen this proposal:

	***Dicistroviridae/Iflaviridae* Study Group**:

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

Comments from ICTV-EC: The only change needed is to remove the hyphen and replace it with a space in the proposed species name. i.e. change to Lygus lineolaris virus 1 and Nilaparvata lugens honeydew virus 1. (dashes are not allowed in a species name)

Response of Proposer:

The hyphen in the species names *Lygus lineolaris virus 1* and *Nilaparvata lugens honeydew virus 1* was removed.

Date first submitted to ICTV: 17 June 13
Date of this revision (if different to above): 16 Aug. 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	***2013.001aI***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Iflavirus***
Subfamily:	
Family:	***Iflaviridae***
Order:	***Picornavirales***

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:**
Lygus lineolaris virus 1	JF720348
Nilaparvata lugens honeydew virus 1	AB766259

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

Species demarcation criteria for the members of the genus *Iflavirus*

- Natural host range: species can be differentiated on the basis of their natural host range
- Sequence identity between the CPs of isolates and strains of a species is above 90%.

The genome sequence of Nilaparvata lugens honeydew virus 1 (NLHV-1) was originally found in the transcriptome data of the brown planthopper, *Nilaparvata lugens* (Delphacidae, Hemiptera, Insecta). Resequencing using the Sanger method and 5'- and 3'-RACE analyses revealed that the NLHV-1 genome consists of 10,937 nucleotides excluding the poly (A) tail with a single large ORF at nucleotide position 1137-10664. Conserved motifs for iflaviral capsid proteins were observed in the N-terminal region of the predicted polyprotein sequence and those for 2C helicase, 3C protease, and 3D polymerase were located in the C-terminal region (Fig. 1). Non-enveloped spherical particles, 30 nm in diameter, containing approximately 11 kb RNA, were detected in a fraction of the density gradient ultracentrifugation. These properties indicate that NLHV-1 is a member of the genus *Iflavirus*. NLHV-1 is the first reported iflavirus from the host insect, *N. lugens*. The host insect of the virus is monophagous for the rice plant and no iflaviruses have been previously reported from insects feeding on rice plants. This indicates that the natural host range of NLHV-1 is differentiated from previously known iflaviruses. Phylogenetic analysis based on amino acid sequence of RNA-dependent RNA polymerase (RdRP) showed that NLHV-1 is closest to *Slow bee paralysis virus* (SBPV) (Fig. 2A), which is isolated from honey bees, *Apis mellifera*. Amino acid sequence identity of capsid proteins between NLHV-1 and SBPV was 29.5% (Fig. 2B) suggesting that NLHV-1 is a distinct species in the genus *Iflavirus*.

- 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

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

Species demarcation criteria for the members of the genus *Iflavirus*

- Natural host range: species can be differentiated on the basis of their natural host range
- Sequence identity between the CPs of isolates and strains of a species is above 90%.

The entity identified to infect the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) has been named *Lygus lineolaris virus 1* (LyLV-1) and described by Perera et al. (2012). The complete genome sequence of LyLV-1 has been determined (GenBank Accession# JF720348) and shows the following features that fulfill the *Iflavirus* genus inclusion criteria:

Genome: Positive-sense, single stranded RNA genome which is approximately 9655 nt long and contains a single open reading frame (ORF). The ORF encodes a polyprotein of 2986 amino acid residues flanked by approximately 603 nt of 5′-UTR and 69 nt of 3′-UTR which terminates in a poly(A) tail. The N-terminal portion of the polyprotein shows homology with the structural CPs of other iflaviruses. The capsid proteins, arranged in the order of VP2-VP4-VP3-VP1, are preceded by a short leader protein (L). The non-structural proteins include an RNA helicase, a 3C-like cysteine protease, and an RNA-dependent RNA polymerase (Fig. 1).

Phylogeny: Phylogenetic analysis with the amino acid sequences of viral non-structural proteins of the C-terminal portion of the polyprotein reveals that LyLV-1 assorts with members of *Iflaviridae* and has closest phylogenetic relationship with Sacbrood virus (SBV) (Fig. 2A). Comparison of the structural CPs (847 amino acids of the N-terminal region of the polyprotein of LvLV-1) with current members of the *Iflavirus* genus also revealed that LyLV-1 has closest phylogenetic relationship with SBV with 12% sequence identity.

Natural host range: Replication was indicated by the presence of full-length negative stranded genomic RNA of LyLV in the fat bodies of the infected host, *Lygus lineolaris*. The virus appeared to be transmitted via eggs (vertically).

These data provide strong support for assignment of LyLV-1 to the iflavirus genus. The physical, genome and phylogenetic characteristics are distinct from any other reported iflaviruses justifying its classification as a novel species in this genus. (*Iflavirus*, family *Iflaviridae).*

- 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

MODULE 9: **APPENDIX**: supporting material

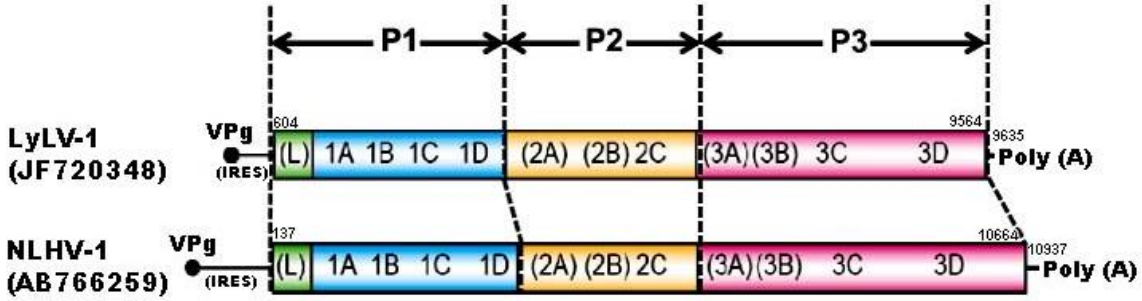

Fig. 1: Genome organization of LyLV-1 and NLHV-1. The genomes are presented according to the L434 nomenclature (Rueckert and Wimmer, 1984). P1 represents viral structural proteins. P2 and P3 represent nonstructural proteins. L, 2A, 2B, 3A, and 3B proteins were shown in parenthesis because sequence motifs for these proteins are not identified in iflaviruses.

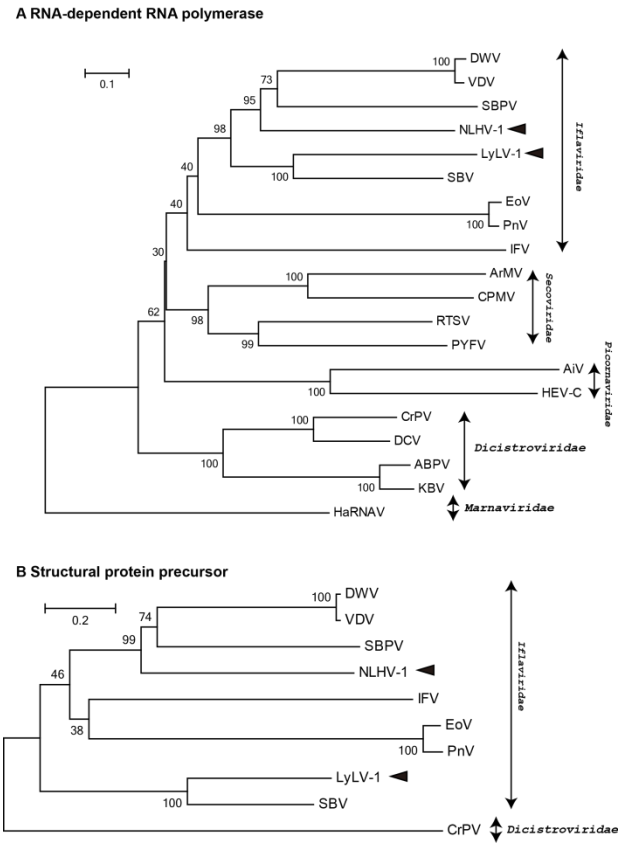

Fig. 2: Phylogenetic status of LyLV-1 and NLHV-1. Arrowheads depict the position of LyLV-1 and NLHV-1. Neighbor-joining trees of the deduced amino acid sequence of RNA-dependent RNA polymerase (A) and the structural proteins (B) were generated via the software MEGA 5.05. Bootstrap values in 1000 replications are shown. Note that some bootstrap values are low but these are caused by diversity of iflaviruses. The cleavage sites of each protein within the polyprotein of iflaviruses are not clear for most members, therefore, the C-terminal sequence starting from RdRp motif I and the sequence between the NXNXFQXG motif in 1A (VP2) protein and the FXRG motif in 1D (VP1) protein were used for analyses. Abbreviations and accession numbers for Iflaviruses; deformed wing virus, DWV, NP_853560; Ectropis obliqua virus, EoV, NP_919029;infectious flacherie virus, IFV, NP_620559; Lygus lineolaris virus-1, LyLV-1, AEL30247; Perina nuda virus, PnV, AAL06289;slow bee paralysis virus, SBPV, NP_003622540; sacbrood virus, SBV, NP_049374; Varroa destructor virus-1, VDV-1, YP_145791 :Dicistroviruses; acute bee paralysis virus, ABPV, AAG13118; cricket paralysis virus, CrPV, NP_647481; Drosophila C virus, DCV, AAC58807; Kashmir bee virus, KBV, AAP32283:Picornaviruses; Aichi virus, AiV, NP_047200; human enterovirus C, HEV-C, NP_041277:Marnavirus; Heterosigma akashiwo RNA virus, HaRNAV, NP_944776:Secoviruses; Arabis mosaic virus, ArMV, YP_053925; cowpea mosaic virus, CPMV, NP_613283; rice tungro spherical virus, RTSV, NP_042507; parsnip yellow fleck virus, PYFV, NP_619734.

References:

Murakami, R., Suetsugu, Y., Kobayashi, Y., Nakashima, N., 2013, The genome sequence and transmission of an iflavirus from the brown planthopper, *Nilaparvata lugens.* Virus Research, in press.

Perera, O.P., Snodgrass, G.L., Allen, K.C., Jackson, R.E., Becnel, J.J., O'Leary, P.F., Luttrell, R.G., 2012. The complete genome sequence of a single-stranded RNA virus from the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). J. Invertebr. Pathol. 109: 11-19.

Rueckert R, Wimmer E (1984). Systematic nomenclature of picornavirus proteins. J Virol 50: 957-959.

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.