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:	***2013.006aV***	(to be completed by ICTV officers)

Short title: *Eilat virus*, a new species in the genus *Alphavirus*
(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:

Farooq Nasar (corresponding author; fanasar@utmb.edu)

Scott C. Weaver (co-corresponding author and member of the ICTV Togaviridae Study Group; sweaver@utmb.edu)

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)	Togaviridae Study Group Nicole C. Arrigo, Chair ncarrigo@gmail.com

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

Date first submitted to ICTV:	June, 2013
Date of this revision (if different to above):	August, 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.

Code	***2013.006aV***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Alphavirus***
Subfamily:	
Family:	***Togaviridae***
Order:	***Unassigned***

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:**
Eilat virus	**JX678730.1**

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

PROPOSAL OVERVIEW: To designate a new species for Eilat virus within the genus *Alphavirus* based on genetic, serologic, and phenotypic data. The name is based on one of the collection sites during the survey of the Negev desert in Israel during 1982-1984 (1).

Background and overview of justification for creating a new designation for Eilat as a new alphavirus species:

The genus *Alphavirus* currently includes 29 species grouped into 10 complexes based on antigenic and/or genetic similarities (2-5). Most alphaviruses infect terrestrial vertebrates via mosquito-borne transmission and exhibit a broad host range infecting many different vertebrates including birds, rodents, equids, humans, and nonhuman primates as well as mosquito species encompassing at least eight genera (4, 5). Alphaviruses are maintained in endemic transmission cycles and occasionally spill over into humans and domesticated animals to cause disease. Human infections with Old World alphaviruses such as Ross River, chikungunya, and Sindbis viruses are typically characterized by fever, rash, and polyarthritis, whereas infections with the New World alphaviruses Venezuelan, eastern, and western equine encephalitis viruses can cause fatal encephalitis (4, 5).

Host restricted "insect-only" viruses have not previously been described for alphaviruses, but have been identified in other arbovirus families. In the family *Flaviviridae*, Cell-fusing agent, Kamiti River, Culex flavivirus, Nounané, Lammi, and Marisma Mosquito virus have been isolated from mosquitoes (*Aedes* spp., *Culex* spp., *Uranotaenia* spp. and *Ochlerotatus* spp.) (6-11). Sigma and Moussa viruses have been isolated from *Drosophila melanogaster* and *Culex decens*, respectively, in the family *Rhabdoviridae* (12, 13). Lastly, a new bunyavirus named Gouleako virus was isolated from several mosquito species (*Anopheles* spp., *Culex* spp., *and Uranotaenia* spp.) (14).

Eilat virus (EILV) was isolated from a pool of *Anopheles coustani* mosquitoes collected in the Negev desert of Israel (1). Genetic analysis showed major sequence divergence at both nucleotide and amino acid levels from other alphaviruses (2). Phylogenetic analyses placed EILV as a sister to the western equine encephalitis antigenic complex within the main clade of mosquito-borne alphaviruses (2). Electron microscopy revealed that, like other alphaviruses, EILV virions are spherical, 70 nm in diameter, and bud from the plasma membrane (2). EILV readily infects a variety of insect cells with little overt cytopathic effect (2). However, in contrast to typical mosquito-borne alphaviruses, EILV apparently cannot infect mammalian or avian cell lines (2). EILV is the first mosquito-borne alphavirus reported to be restricted to insects.

Currently the ICTV definition of a virus species is a "a monophyletic group of viruses whose properties can be distinguished from those of other species by multiple criteria." (15). These criteria may include, but are not limited to, natural and experimental host range, cell and tissue tropism, pathogenicity, vector specificity, antigenicity, and the degree of relatedness of their genomes or genes (15). Our data meet many of the criteria as defined by ICTV and support the classification of Eilat virus a new species within the genus *Alphavirus*.

Summary of evidence supporting Eilat virus as a new alphavirus species:

1. *Genetic, serologic, phylogenetic and phenotypic analyses*:
    a. Nucleotide and amino acid sequence identity of EILV compared with other alphaviruses ranges from 57-43% and 58-28%, respectively (Module 9: Appendix, Table 1).
    b. Amino acid comparisons of each individual protein show major sequence divergence relative to other mosquito-borne viruses. Amino acid divergence for the two most conserved genes, nsP2 and ns P4, ranges from 35-51% and 23-32%, respectively, and from 50-58% for E1 and 58-68% for the E2 glycoproteins, respectively (Module 9: Appendix, Table 2).
    c. Complement fixation (CF) and hemagglutination inhibition (HI) assays demonstrate minimal cross-reactivity with other mosquito-borne alphaviruses (Module 9: Appendix, Table 3).
    d. Neighbor-joining, maximum-likelihood, and Bayesian methods place EILV within the mosquito-borne clade sister to WEEV complex, with far greater divergence from other alphaviruses than is observed within any other alphavirus species (Module 9: Appendix, Figure 1).
    e. Electron microscopy reveals that, like other alphaviruses, EILV virions are spherical, 70 nm in diameter, and bud from the plasma membrane of mosquito cells in culture. (Module 9: Appendix, Figure 2)
    f. In contrast to all other mosquito-borne alphaviruses, EILV was unable to infect and replicate in cell lines representing six vertebrate species, but could readily infect and replicate in insect cell lines (Module 9: Appendix, Figure 3-5). This is a major phenotypic and functional difference from all other mosquito-borne alphaviruses and likely indicates a difference in host range and/or cell/tissue tropism.
    g. EILV genomic RNA is incapable of replication in five vertebrate cell lines and the lack of observed replication is not due to temperature sensitivity or inefficient electroporation of RNA (Module 9: Appendix, Figure 6-7). This suggests that in contrast to all other mosquito-borne alphaviruses, EILV host-range is very likely restricted to insects.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. Samina I, Margalit J, Peleg J. Isolation of viruses from mosquitoes of the Negev, Israel. Trans R Soc Trop Med Hyg. 1986;80(3):471-2.
2. Nasar F, Palacios G, Gorchakov RV, Guzman H, Da Rosa AP, Savji N, Popov VL, Sherman MB, Lipkin WI, Tesh RB, Weaver SC. Eilat virus, a unique alphavirus with host range restricted to insects by RNA replication. Proc Natl Acad Sci U S A. 2012 Sep 4;109(36):14622-7.
3. Forrester NL, Palacios G, Tesh RB, Savji N, Guzman H, Sherman M, Weaver SC, Lipkin WI. Genome-scale phylogeny of the alphavirus genus suggests a marine origin. J Virol. 2012 Mar;86(5):2729-38.

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5. Strauss JH, Strauss EG. The alphaviruses: gene expression, replication, and evolution. Microbiol Rev. 1994 Sep;58(3):491-562.
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12. Longdon B, Obbard DJ, Jiggins FM. Sigma viruses from three species of Drosophila form a major new clade in the rhabdovirus phylogeny. Proc Biol Sci. 2010 Jan 7;277(1678):35-44.
13. Quan P-L, Junglen S, Tashmukhamedova A, Conlan S, Hutchinson SK, Kurth A, Ellerbrok H, Egholm M, Briese T, Leendertz FH, Lipkin WI, 2010. Moussa virus: a new member of the Rhabdoviridae family isolated from *Culex decens* mosquitoes in Côte d'Ivoire. Virus Res 147: 17-24.
14. Marklewitz M, Handrick S, Grasse W, Kurth A, Lukashev A, Drosten C, Ellerbrok H, Leendertz FH, Pauli G, Junglen S. Gouleako virus isolated from West African mosquitoes constitutes a proposed novel genus in the family *Bunyaviridae*. J Virol. 2011 Sep;85(17):9227-34.
15. http://ictvonline.org/codeOfVirusClassification.asp.

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.

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	EILV	TROV	AURAV	WHATV	SINV	WEEV	EEEV	VEEV	CHIKV	RRV	UNAV	SFV	MIDV	BFV	NDUV	SESV	SPDV
EILV	-	52	43	58	44	37	36	37	49	48	39	28	39	38	49	47	28
TROV	53	-	43	57	43	38	38	39	51	52	39	28	39	38	51	47	29
AURAV	55	57	-	47	65	38	39	51	41	41	41	30	40	39	41	37	21
WHATV	57	56	61	-	55	39	39	39	53	54	41	31	41	40	53	49	30
SINV	56	57	61	70	-	39	39	52	40	41	41	31	41	39	41	37	22
WEEV	52	54	55	58	57	-	70	46	40	40	40	30	40	39	40	38	21
EEEV	51	53	53	54	54	64	-	47	41	41	40	30	40	40	40	38	21
VEEV	51	54	54	55	54	58	60	-	41	40	40	30	40	39	40	38	22
CHIKV	52	53	53	55	54	53	54	54	-	66	48	36	46	42	58	53	30
RRV	51	53	54	55	55	55	55	54	62	-	49	38	47	42	60	53	30
UNAV	52	54	54	54	55	53	54	53	62	64	-	59	46	42	44	39	22
SFV	53	53	54	55	55	54	55	54	62	65	66	-	36	33	33	29	29
MIDV	52	53	54	55	54	53	54	54	60	62	61	63	-	59	44	39	22
BFV	52	52	53	54	53	53	54	53	56	57	56	58	58	-	42	39	22
NDUV	51	52	53	54	53	53	53	53	58	58	57	59	59	57	-	53	29
SESV	50	51	52	52	51	52	52	52	54	54	54	54	54	54	54	-	29
SPDV	43	44	44	44	45	44	45	45	45	46	45	46	45	45	43	43	-

Table 1. Comparison of nucleotide and amino acid identity of EILV structural and nonstructural ORFs with other alphaviruses. Upper diagonal displays percent amino acid identity; lower diagonal contains percent nucleotide identity. EILV – Eilat Virus; TROV – Trocara virus; AURAV – Aura virus; WHATV – Whataroa virus; SINV – Sindbis virus; WEEV – Western equine encephalitis virus; EEEV – Eastern equine encephalitis virus; VEEV – Venezuelan equine encephalitis virus; CHIKV – chikungunya virus; RRV – Ross River virus; UNAV – Una virus; SFV – Semliki Forest virus; MIDV – Middelburg virus; BFV – Barmah Forest virus; NDUV – Ndumu virus; SESV – Southern elephant seal virus; SPDV – Salmon pancreas disease virus. Copyright © 2012, Farooq Nasar and Scott C. Weaver. All Rights Reserved.

Virus	nsP1	nsP2	nsP3	nsP4	capsid	E3	E2	6k	E1
TROV	64	58	30	72	49	41	34	41	46
AURAV	73	60	36	74	53	46	36	36	47
WHATV	72	65	36	74	50	42	43	40	49
SINV	71	65	34	77	53	45	40	45	50
WEEV	57	49	29	68	43	44	42	38	49
EEEV	56	50	29	69	43	42	36	40	47
VEEV	56	51	29	68	40	47	34	39	45
CHIKV	56	53	34	69	44	43	36	44	42
RRV	60	52	30	69	41	45	35	25	42
UNAV	58	53	32	71	42	45	36	28	43
SFV	58	53	36	69	42	53	35	28	43
MIDV	59	53	37	70	42	46	37	32	42
BFV	58	52	35	70	45	42	32	34	42
NDUV	60	52	32	70	42	50	33	18	42
SESV	53	51	28	65	44	47	30	42	42
SPDV	41	38	21	52	31	25	24	26	36

Table 2. Comparison of individual EILV proteins with other alphaviruses. Percent amino acid identities are shown. EILV – Eilat Virus; TROV – Trocara virus; AURAV – Aura virus; WHATV – Whataroa virus; SINV – Sindbis virus; WEEV – Western equine encephalitis virus; EEEV – Eastern equine encephalitis virus; VEEV – Venezuelan equine encephalitis virus; CHIKV – chikungunya virus; RRV – Ross River virus; UNAV – Una virus; SFV – Semliki Forest virus; MIDV – Middelburg virus; BFV – Barmah Forest virus; NDUV – Ndumu virus; SESV – Southern elephant seal virus; SPDV – Salmon pancreas disease virus. Copyright © 2012, Farooq Nasar and Scott C. Weaver. All Rights Reserved.

A)

MIAF	Eilat Antigen Ht/Ho*
TROV	8/≥256
AURAV	8/≥256
SINV	16/≥256
WHATV	<8/16
WEEV	<8/≥128
FMV	<8/≥256
HJV	<8/64
EEEV	8/≥256
VEEV	16/≥256
EVEV (VEE II)	<8/≥256
MUCV (VEE IIIA)	<8/≥256
PIXV (VEE IV)	<8/≥256
MAYV	<8/≥256
UNAV	<8/≥256
BEBV	<8/≥256
RRV	<8/≥256
GETV	<8/≥256
CHIKV	<8/≥256
ONNV	<8/≥256
SFV	<8/≥256
MIDV	<8/≥256
NDUV	<8/≥256
BFV	<8/≥256

B)

Antigen	Eilat Antibody Ht/Ho*
TROV	20/10240
SINV	40/5120
WEEV	40/2560
EEEV	20/2560
AURAV	<20/5120
MAYV	<20/5120
UNAV	<20/5120
GETV	<20/10240
CHIKV	<20/5120
SFV	<20/2560

Table 3. Complement fixation (A) and hemagglutination-inhibition (B) tests with Eilat virus and other alphavirus antigens and hyperimmune mouse ascitic fluids (MIAF). *Reciprocal of heterologous titer/reciprocal of homologous titer. TROV – Trocara virus; AURAV – Aura virus; WHATV – Whataroa virus; SINV – Sindbis virus; WEEV – Western equine encephalitis virus; FMV – Ft. Morgan virus; HJV – Highlands J virus; EEEV – Eastern equine encephalitis virus; VEEV – Venezuelan equine encephalitis virus; EVEV – Everglades virus; MUCV – Mucambo virus; PIXV – Pixuna virus; MAYV – Mayaro virus; CHIKV – chikungunya virus; ONNV – O'nyong-nyong virus; RRV – Ross River virus; UNAV – Una virus; SFV – Semliki Forest virus; MIDV – Middelburg virus; BFV – Barmah Forest virus; NDUV – Ndumu virus. Copyright © 2012, Farooq Nasar and Scott C. Weaver. All Rights Reserved.

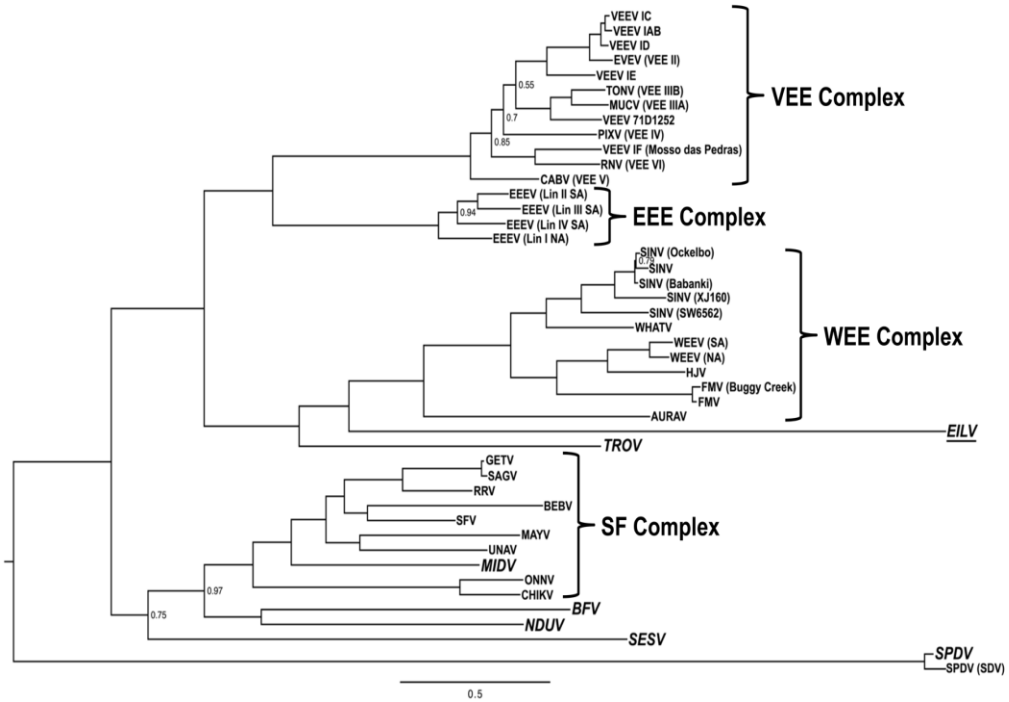

Figure 1. Bayesian phylogenetic tree based on nucleotide sequences of the alphavirus structural ORF. A midpoint rooted tree is shown with all posterior probabilities <1 shown on major branches. Alphavirus complexes are denoted in bold and italic. EILV – Eilat Virus; TROV – Trocara virus; AURAV – Aura virus; WHATV – Whataroa virus; SINV – Sindbis virus; WEEV – Western equine encephalitis virus; EEEV – Eastern equine encephalitis virus; VEEV – Venezuelan equine encephalitis virus; EVEV – Everglades virus; MUCV – Mucambo virus; PIXV – Pixuna virus; RNV – Rio Negro virus; MAYV – Mayaro virus; CHIKV – chikungunya virus; RRV – Ross River virus; UNAV – Una virus; SFV – Semliki Forest virus; MIDV – Middelburg virus; BFV – Barmah Forest virus; NDUV – Ndumu virus; SESV – Southern elephant seal virus; SPDV – Salmon pancreas disease virus. Copyright © 2012, Farooq Nasar and Scott C. Weaver. All Rights Reserved.

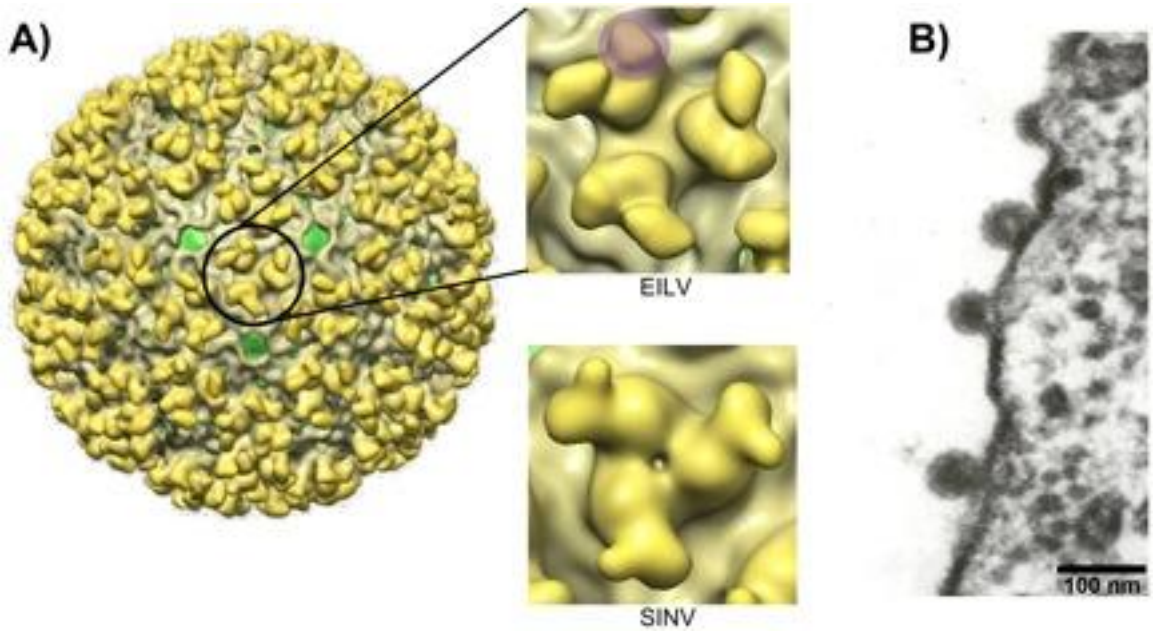

Figure 2. Eilat virion morphology determined by cryo-electron microscopy and transmission electron microscopy. 20 Å cryo-EM reconstruction of EILV glycoprotein spikes on the virion surface (A). The protrusion possibly representing the E3 protein is highlighted in purple. SINV glycoprotein spikes are shown as a comparison (147).

EILV virions are shown budding from the surface of C7/10 cells (B). SINV – Sindbis virus; EILV – Eilat virus. Copyright © 2012, Farooq Nasar and Scott C. Weaver. All Rights Reserved.

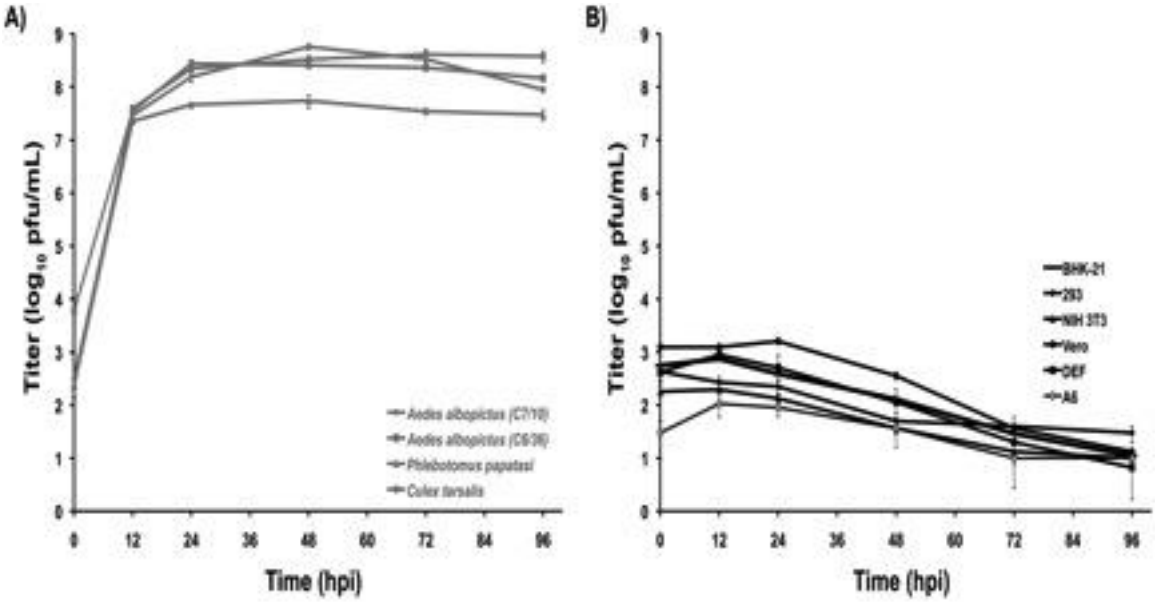

Figure 3. Replication kinetics of Eilat virus (EILV) on representative insect (grey, 28°C) (A) and vertebrate (black, 37°C) (B) cell lines. Monolayers were infected at MOI of 10 PFU/cell. Supernatants were collected at indicated intervals post-infection and titrated on C7/10 cell monolayers. Each data point represents the mean titer of samples taken from triplicate infections +/-SD. A6 cells were incubated at 28°C. Copyright © 2012, Farooq Nasar and Scott C. Weaver. All Rights Reserved.

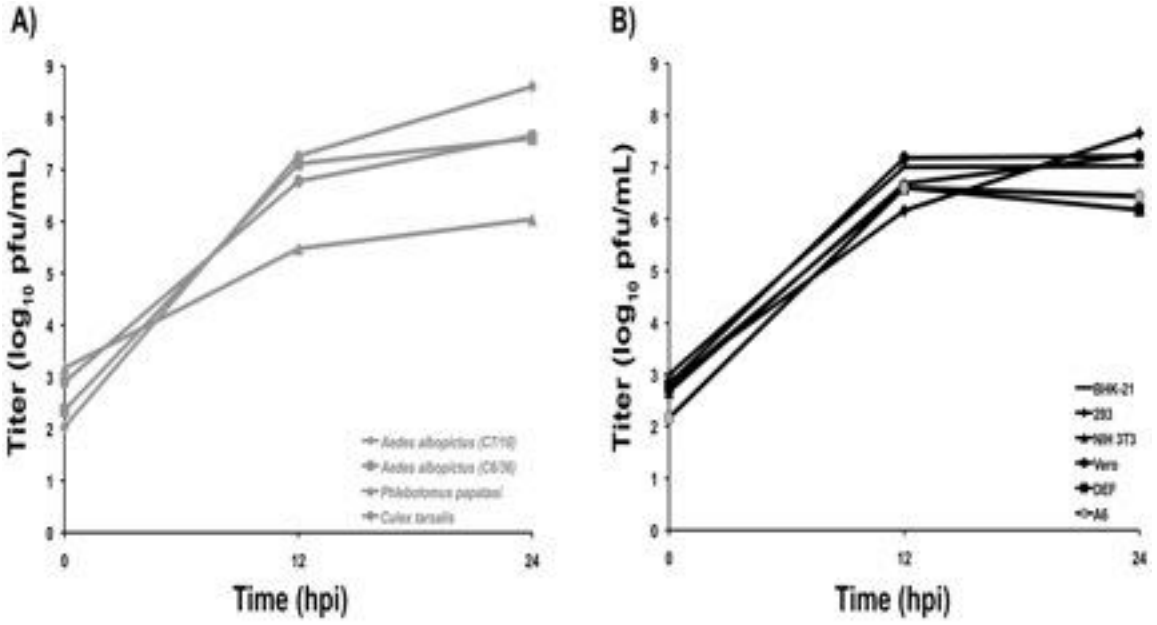

Figure 4. Replication kinetics of Sindbis virus (SINV) on representative insect (grey, 28°C) (A) and vertebrate (black, 37°C) (B) cell lines. Monolayers were infected at MOI of 10 PFU/cell. Supernatants were collected at indicated intervals post-infection and titrated on C7/10 cell monolayers. A6 cells were incubated at 28°C. Hpi – hours post infection; PFU – plaque forming units. Copyright © 2012, Farooq Nasar and Scott C. Weaver. All Rights Reserved.

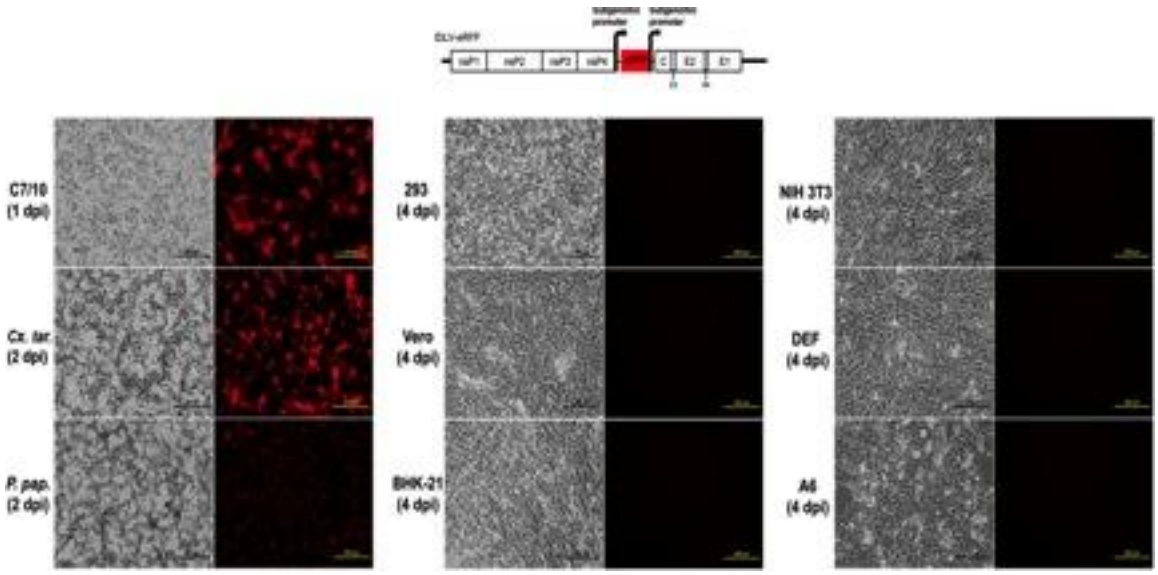

Figure 5. Infection of representative vertebrate (37°C) and insect (28°C) cell lines with EILV-eRFP. 50% confluent monolayers were infected at MOI of 10 PFU/cell, phase contrast and fluorescent field photographs were taken at various points post-infection. A6 cells were incubated at 28° C. EILV – Eilat virus; dpi – days post infection. Copyright © 2012, Farooq Nasar and Scott C. Weaver. All Rights Reserved.

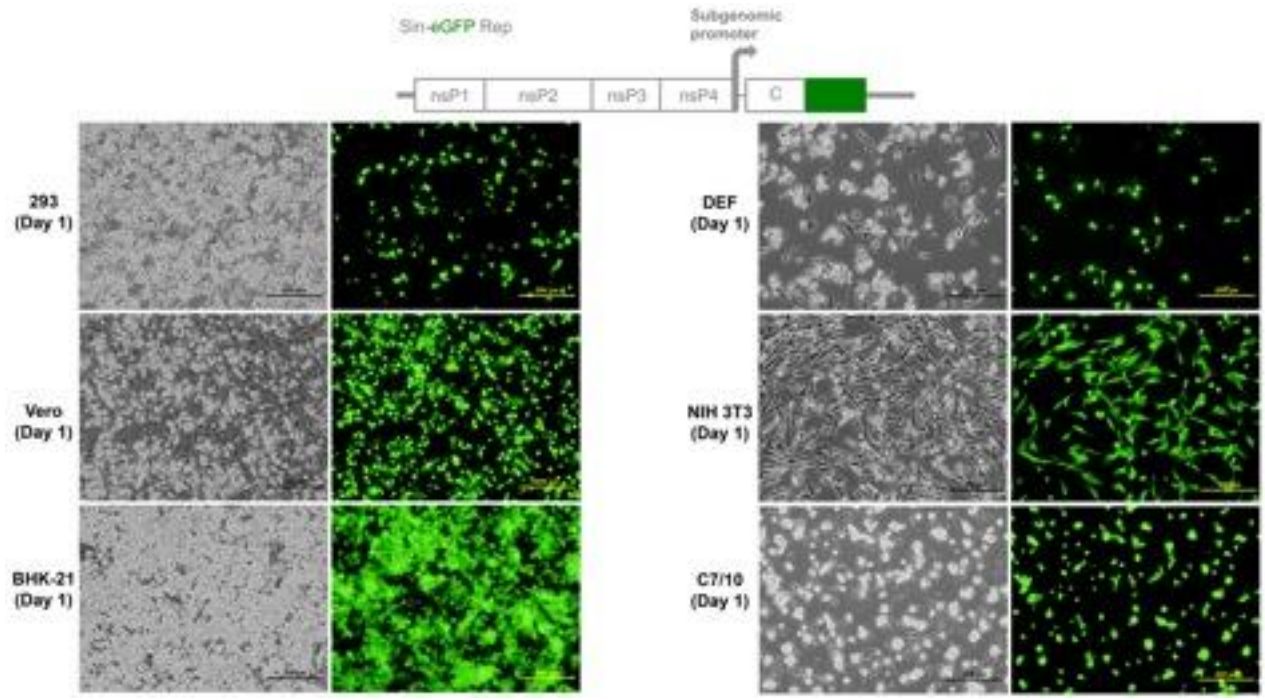

Figure 6. Replication of SINV-eGFP replicon genomic RNA in vertebrate (37° C) and insect (28° C) cell lines. SINV-eGFP replicon genomic RNA was transcribed in vitro and ≈10 μg aliquots of RNA were electroporated into vertebrate and insect cells. Phase contrast and fluorescent field photographs were taken at 4 dpe. SINV – Sindbis virus.

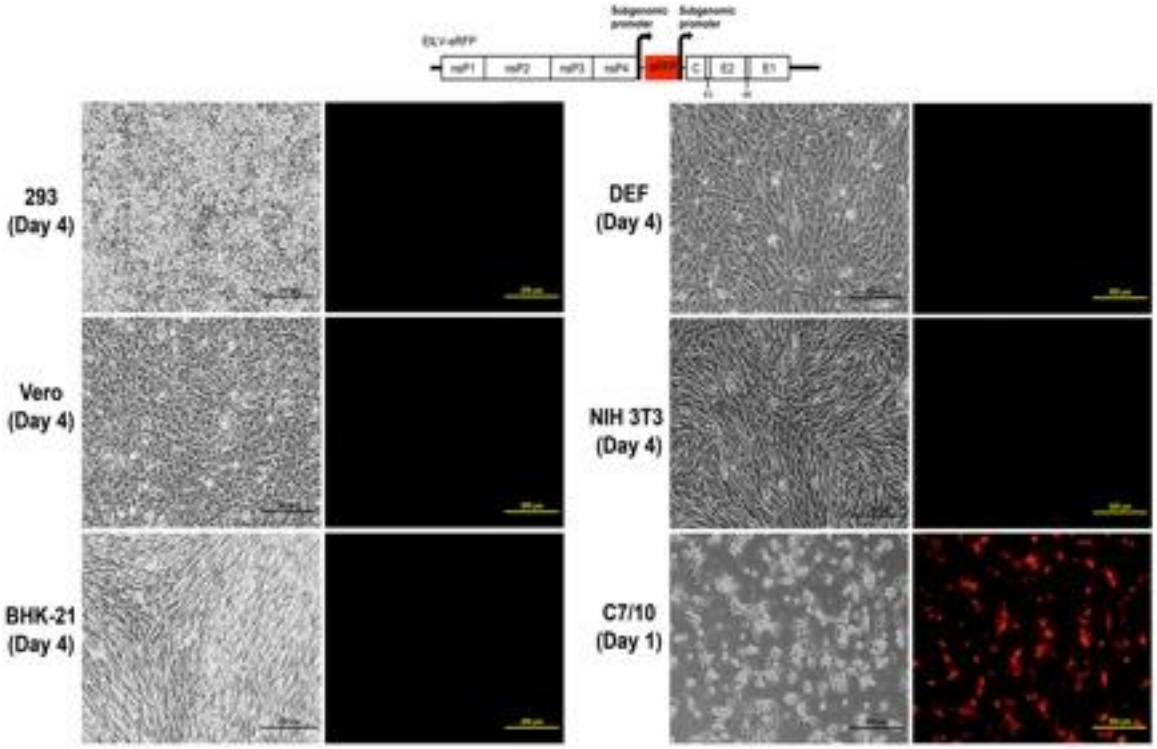

Figure 7. Replication of Eilat virus (EILV) genomic RNA in vertebrate (37° C) and insect (28° C) cell lines. RNA was transcribed in vitro from the cDNA clone and ≈10 μg aliquots of RNA were electroporated into vertebrate and insect cells. Phase contrast and fluorescent field photographs were taken at day 4-post-electroporation. Copyright © 2012, Farooq Nasar and Scott C. Weaver. All Rights Reserved.