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:	***2015.005a-dS***	(to be completed by ICTV officers)

Short title: Novel virus species (*Lake Sinai virus 1* and *Lake Sinai virus 2*) in a new proposed virus genus (*Sinaivirus*), which infect the Western honey bee (*Apis mellifera*)
(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** ☒

Author(s):

Katie F. Daughenbaugh, Charles Runckel, Joseph DeRisi, Michelle L. Flenniken

Corresponding author with e-mail address:

Michelle L. Flenniken (michelle.flenniken@montana.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)	There is currently no invertebrate study committee; Elliot Lefkowitz recommended we contact the *Nodaviridae* SG chaired by Toshihiro Nakai, nakaitt@hiroshima-u.ac.jp and Subcommittee chair, Nick Knowles for advice (nick.knowles@pirbright.ac.uk). In addition Yanping (Judy) Chen is familiar with honey bee infecting viruses and is a member of the *Picornavirales* study group (Judy.Chen@ars.usda.gov).

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

Date first submitted to ICTV:	7/21/2011
Date of this revision (if different to above):	16/11/2015

ICTV-EC comments and response of the proposer:

EC47 Decision: Uc. Correct error in list of representative isolates and ambiguous second sentence in the justification for the new species. Scale bar for Fig 1a?

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	***2015.005aS***	(assigned by ICTV officers)

To create new species within:

Genus:	***Sinaivirus*** (new)
Subfamily:	
Family:	
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)**
Lake Sinai virus 1 *Lake Sinai virus 2*	Lake Sinai virus 1 Lake Sinai virus 2	Lake Sinai virus 1 (HQ871931) Lake Sinai virus 2 (HQ888865)

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

- The LSV1 and LSV2 RNA-dependent RNA polymerase (RdRp) amino acid sequence is only 26 and 27% similar to CBPV, respectively. Therefore, although similar, these RdRp sequences are clearly different/distinct.

Phylogenies derived from the RdRp aa sequence place LSV and CBPV on separate branches/clades, which are distinct from fish, insect, and plant infecting nodaviruses and noda-like viruses (Annex Fig. 1). More recent analysis with additional LSV RdRp sequences and a greater number of other virus RdRp aa sequences more clearly distinguish the LSV clade from the CBPV and AACV (Anopheline-associated C virus) containing clade, as well as the the *Nodaviridae* (Annex Fig. 2, 3, and 4).

- The ORF1 gene of LSV1 and LSV2 has low aa similarity with CBPV ORF1 (i.e., 20 and 21% amino acid similarity, respectively).

- In contrast to CBPV, which has a bipartite genome, the LSVs have a monopartite genome.

- The LSVs also differ from CBPV in genome organization, the LSV genomes encode the capsid gene 5' of the RdRp. In addition, the LSV1 capsid predicted to overlap the RdRp with a frameshift, while the LSV2 capsid is predicted to follow the RdRp gene in-frame (Annex Fig. 1).

- The LSV capsid gene has no significant similarity to the other noda-like viruses, but does have similarity by motif prediction (HHPred e-26) to the Nudaurelia capensis beta-tetravirus. BLAST analyses (i.e., tblastn) of the LSV1 capsid sequence returns an alignment with Nudaurelia capensis beta virus complete genome (27% aa coverage, 26% aa identity; LSV2 aligns over 30% aa coverage and 29% identity).

- The LSV1 and LSV2 are distinct and share the following amino acid identities over the specified sections of their genomes: Orf1: 70%, RdRp: 80%, and Capsid: 70%.
Thirty-six isolates of LSV2 and six isolates of LSV1 displayed >95% nucleotide homology and >97% amino acid homology to the consensus of each species, indicating that the two proposed species represent distinct clades and that there is not a continuum of divergence between them.

- Since their discovery in 2011, additional LSVs have been sequenced throughout the globe (i.e., Belgium, Spain, US West Coast, US East Coast). To date, the LSV sequences on NCBI share between 63-85% nucleotide identity, suggesting they are diverse enough to be designated individual species within the Sinaivirus genus, and not strains of one another (Annex Fig. 4).

- LSV1 and LSV2 have been observed to have different seasonal peak abundances, as assessed in a 2010-2011 honey bee colony monitoring project carried out in the Western United States (over 400 honey bee samples were assessed for pathogens using microarray, PCR, and qPCR). Specifically, LSV1 abundance peaked during the summer months and LSV2 was most abundant during the winter months. LSV2 was the most abundant virus in this study, and was the most abundant LSV present in our recent pathogen screen (Annex Fig. 5 and Fig. 6).

- Seven of 20 hives sampled on August 5, 2009 were positive for LSV1 and an additional five hives in the time-course, from July (SD) and January/February (CA), were found to be positive for LSV1, all with >95% nucleotide identity. LSV2 was more prevalent and was detected by PCR in 30 of 197 time-course samples from all three geographic regions (Annex Fig. 5).

- The replicative forms of LSV1 and 2 were detected in time-course samples. Positive-sense RNA viruses, like LSV1 and 2, utilize negative strand template to produce viral genome copies, therefore detection of the negative-strand intermediate is indicative of an actively replicating virus. We used negative-strand specific RT-PCR to detect the replicative forms of both LSV1 and LSV2 (Annex Fig. 7).

- The relative abundance of LSV2 is greatest in the abdomen and gut of infected honey bees, and is more pronounced in bees with high levels of infection (Annex Fig. 8).

- LSV2 was purified from honey bees from an infected colony by cesium chloride gradient, and was visualized by transmission electron microscopy. Purified virions were subjected to SDS-PAGE analysis, and the capsid protein amino acid sequence of the predominant band (~56 kDa) was confirmed to be the LSV2 capsid protein by mass spectrometry (Annex Fig. 9).

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2015.005bS***	(assigned by ICTV officers)

To create a new genus within:

+Subfamily:	
Family:	***unassigned***
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 family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2015.005cS***	(assigned by ICTV officers)

To name the new genus: *Sinaivirus*

Assigning the type species and other species to a new genus

Code	***2015.005dS***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Lake Sinai virus 2	Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

Two species. There are other nearly complete LSV genome sequences, but at this time we feel it is important to designate LSV2 and LSV1; others may be added in the future.

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

All Lake Sinai viruses share homology in all three genes and in having a monopartite genome as compared to related bipartite genome viruses. They differ from one another by 70-80% amino acid identity, with isolates closely matching the consensus sequence of each species. A genus designation is necessary to distinguish the LSVs from the Chronic bee paralysis virus. CBPV is currently unclassified. It clusters near the Sinaiviruses by RdRp-based phylogeny, but is clearly distinct. All LSVs sequenced to date distinctly cluster/clade together (see Annex).

Origin of the new genus name:

Sinaivirus - Derived from the new virus species names Lake Sinai virus 1 and Lake Sinai virus 2 (NCBI now includes LSV1-LSV7, as well as other geographic names for samples isolated in Belgium). LSV2 would be the type species for this group.
Lake Sinai viruses were named according to virus naming conventions for viruses – the name "Lake Sinai" was derived from the name of the lake (Lake Sinai) in South Dakota, USA, which was near the site where the honey bee samples were obtained from which LSVs were originally discovered.

Reasons to justify the choice of type species:

LSV1 and LSV2 are both fully sequenced. However, additional information is available for LSV2, including a purification protocol, TEM image of the virus, and tissue specificity data.

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

Distinct species designation within the proposed genus *Sinaivirus* – should be in line with other virus genera. The guidelines used for virus genera within the *Nodaviridae* family (i.e., *Alphanodavirus* and *Betanodavirus genera*) would likely be suitable for this new genus as well.

Alternatively, and based on our sequence analyses - distinct LSV species within the proposed genus *Sinaiviruses* should share less than 85% amino acid identity in the RdRp gene, Orf1 encoding region, and the capsid genes; and nucleotide identities should be less than 90% over the entire genome.

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Runckel C*, Flenniken ML*, Engel JC, Ruby JG, Ganem D, Andino R, DeRisi, JL. Temporal Analysis of the Honey Bee Microbiome Reveals Four Novel Viruses and Seasonal Prevalence of Known Viruses, Nosema and Crithidia. PLoS One 2011 6(6):e20656

Daughenbaugh KF, Martin M, Brutscher LM, Cavigli I, Garcia E, Lavin M, Flenniken ML. Honey bee infecting Lake Sinai Viruses. Viruses 2015, in review.

Ravoet, J.; De Smet, L.; Wenseleers, T.; de Graaf, D. C. Genome sequence heterogeneity of Lake Sinai Virus found in honey bees and Orf1/RdRP-based polymorphisms in a single host. Virus Res 2015, 201, 67-72

Ravoet, J.; De Smet, L.; Meeus, I.; Smagghe, G.; Wenseleers, T.; de Graaf, D. C. Widespread occurrence of honey bee pathogens in solitary bees. J Invertebr Pathol 2014, 122, 55–58.

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.

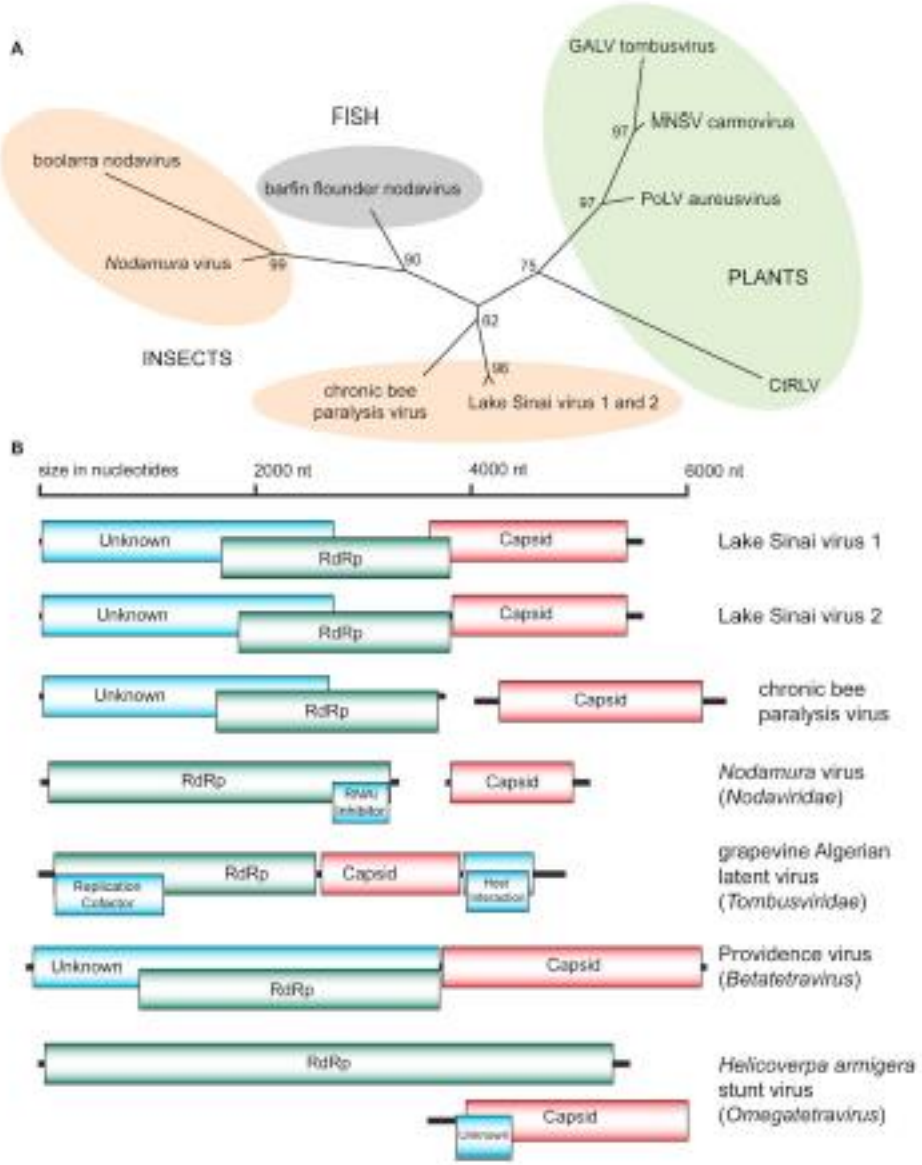


Figure 1 Phylogenetic placement and genome organization of Lake Sinai viruses.

(A) RdRp amino acid phylogeny of the *Nodavirales* superfamily. Lake Sinai virus strain 1 (LSV1; HQ871931), Lake Sinai virus strain 2 (LSV2: HQ888865), chronic bee paralysis virus (CBPV; NC_010711; EU122229), boolarra virus (BoV; NC_004142; AF329080), *Nodamura* virus (NoV; NC_002690; AF174533), barfin flounder nodavirus BF93Hok (BFV; NC_011063; EU826137), grapevine Algerian latent virus (GALV; NC_011535; AY830918), melon necrotic spot virus (MNSV; NC_001504; M29671), pothos latent virus (PoLV; NC_000939; X87115) and carrot red leaf virus (CtRLV; NC_006265; AY695933). Protein sequences were aligned by ClustalW and a tree generated by the Neighbor-Joining method with 100 replicate.

*for scale bar, see phylogenetic tree in Figure 2.

(B) Genome organization of the Lake Sinai viruses, which have a monopartite genome of ~5.5 kb without sub-genomic RNAs (see Northern Blot), and similar RNA viruses.

Figure from Runckel*, Flenniken*, et al PLoS One 2011.

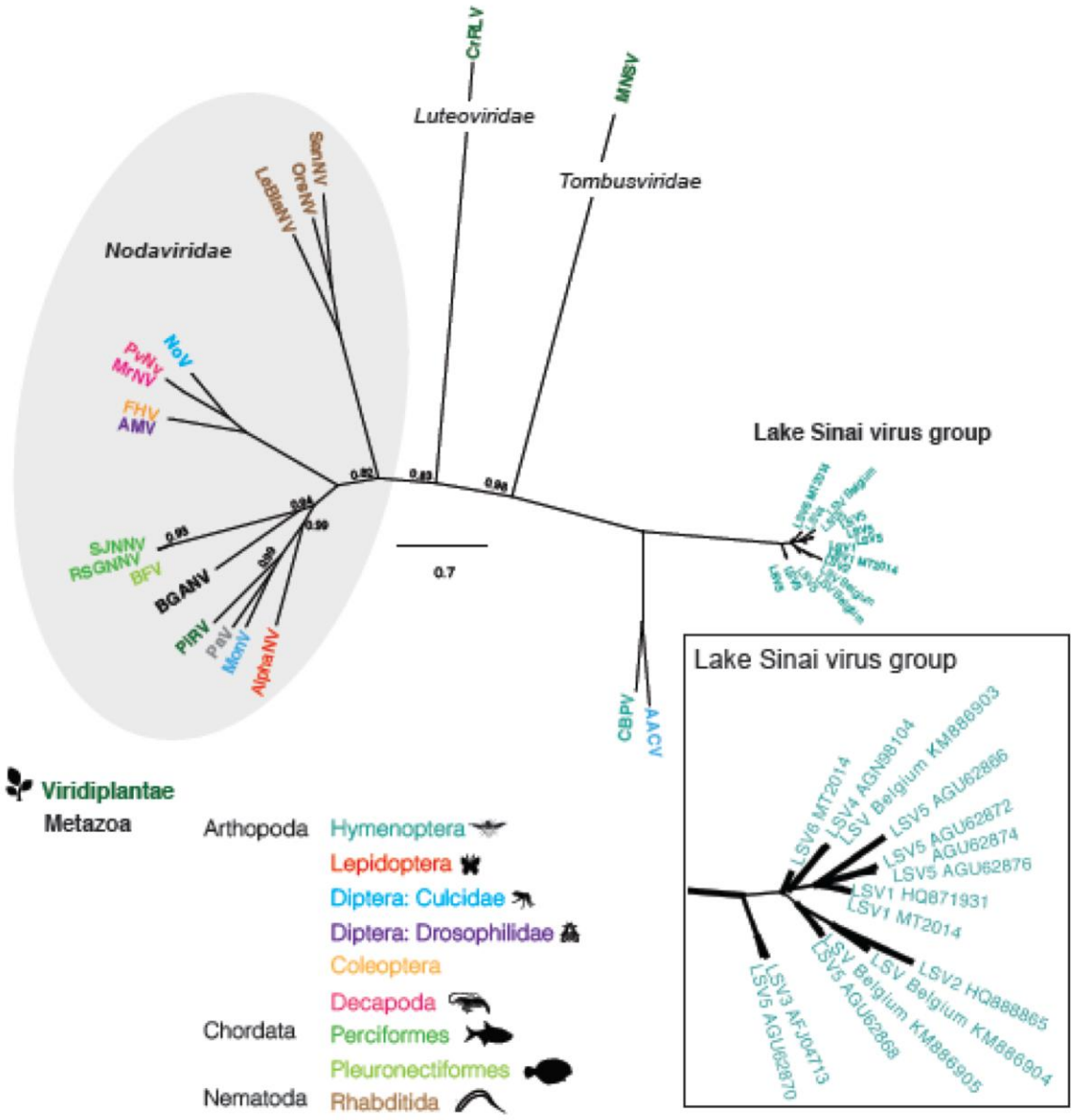
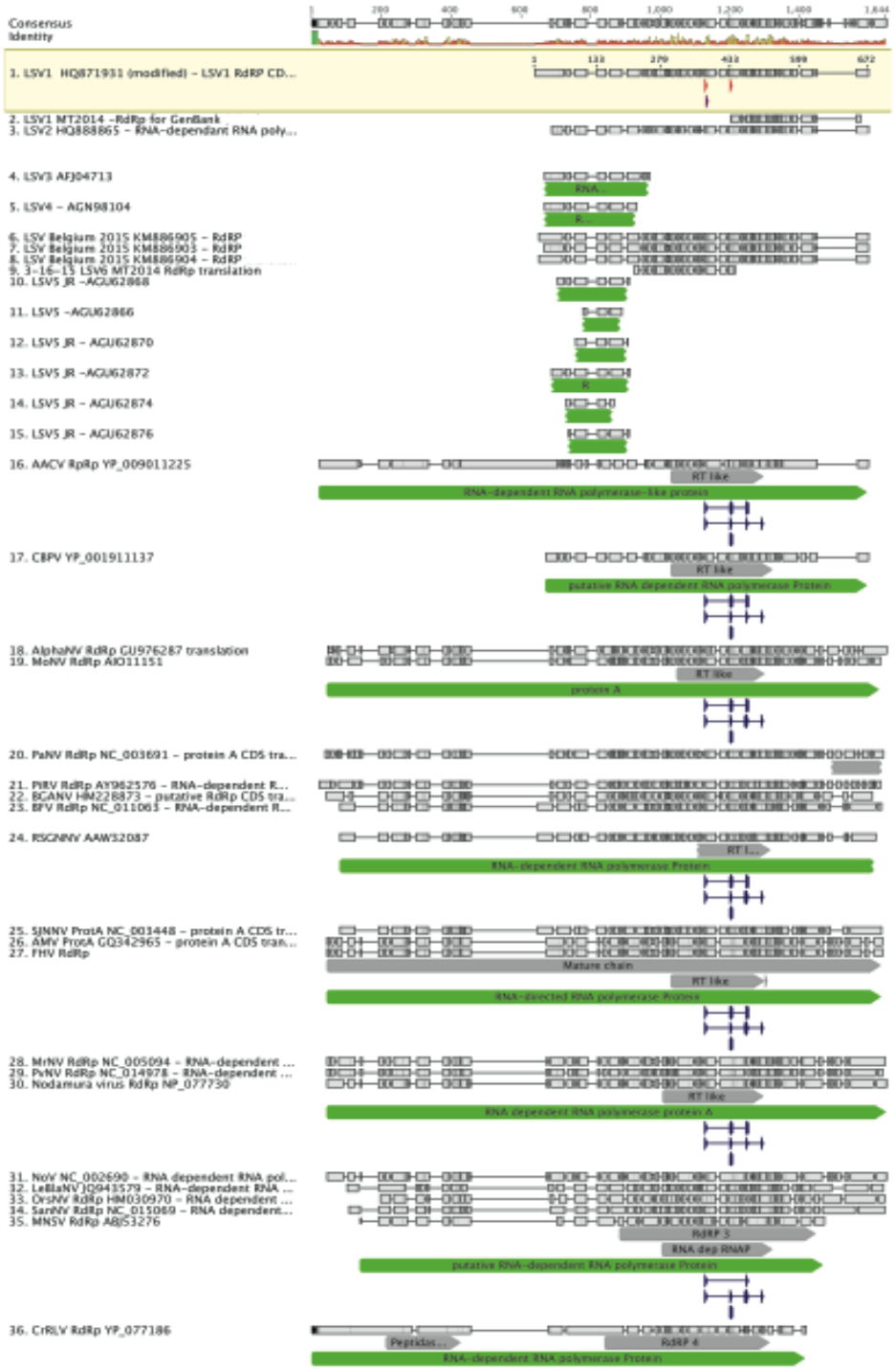


Figure 2:
Lake Sinai virus phylogenetic relationship inferred from RdRp amino acid sequences.

Majority rule Bayesian consensus tree of Lake Sinai viruses derived from Bayesian analysis of an RNA dependent RNA polymerase (RdRp) amino acid alignment implemented in MrBayes v3.1.2 using the WAG amino acid substitution model (Supplemental Figure S6) [39] . Numbers on branches are Bayesian posterior probabilities (0–1). To improve figure clarity only posterior probability values that were less than 1 are shown on the full tree and branch line thickness was used to indicate posterior probabilities (0.5 - 1) in the LSV inset; the scale bar corresponds to the proportion of amino acid change. GenBank accession numbers (in parentheses) for either the RdRp sequences or the genome sequences from where the RdRp sequence obtained are as follows: LSV1, Lake Sinai virus 1 (HQ871931), LSV1 MT2014 (KR021356), LSV2 (HQ888865), LSV3 (AFJ04713), LSV4 - AGN98104, LSV Belgium 2015 (KM886905), LSV Belgium 2015 (KM886903), LSV Belgium 2015 (KM886904), LSV6 MT2014 (KR021357), LSV5 JR (AGU62868), LSV5 JR (AGU62866), LSV5 JR (AGU62870), LSV5 JR (AGU62872), LSV5 JR (AGU62874), LSV5 JR (AGU62876), AACV, Anopheline-associated C virus RpRp

(YP_009011225), CBPV, Chronic bee paralysis virus (YP_001911137), AlphaNV, Alphanodavirus RdRp (GU976287), MoNV, Mosinovirus RdRp (AIO11151), PaV, Pariacoto virus RdRp (NC_003691; AF171942), PiRV, Pieris rapae virus RdRp (AY962576), BGANV, Bat guano associated nodavirus (HM228873), BFV, Barfin flounder nervous necrosis virus RdRp (NC_011063; EU826137), SJNNV, Striped Jack nervous necrosis virus ProtA (NC_003448; AB056571), RSGNNV, Redspotted grouper nervous necrosis virus (AAW32087), AMV,Drosophila melanogaster American nodavirus ProtA (GQ342965), FHV, Flock house virus RdRp (Q66929), MrNV, Macrobrachium rosenbergii nodavirus RdRp (NC_005094; AY222839), PvNV, Penaeus vannamei nodavirus RdRp (NC_014978; HQ259079), NoV Nodamura virus RdRp (NC_002690; AF174533, NP_077730; AAF97860), LeBNV, Le Blanc nodavirus (JQ943579), OrsNV, Orsay nodavirus RdRp (HM030970), SanNV, Santeuil nodavirus RdRp (NC_015069; HM030972), MNSV, Melon necrotic spot virus RdRp (53276), CrRLV, Carrot red leaf virus RdRp (YP_077186); Figure from Daughenbaugh, et al., Viruses, 2015 – in review.

Supplemental Figure S6. Virus RNA-dependent RNA polymerase (RdRp) protein alignment



Supplemental Figure S6. Virus RNA dependent RNA polymerase (RdRp) amino acid sequences were aligned generated in Geneious R8 using the MAFFT alignment plugin. GenBank accession numbers are listed in figure and in the methods section.

Figure 3: Virus RNA-dependent RNA polymerase (RdRp) amino acid alignment. Virus RNA dependent RNA polymerase (RdRp) amino acid sequences were aligned generated in Geneious R8 using the MAFFT alignment plugin. GenBank accession numbers are listed in figure and in the methods section; Figure from Daughenbaugh, et al., Viruses, 2015 – in review.

Supplemental Table S2. Nucleotide alignment of all Lake Sinai virus sequences in NCBI database (March 2015).

Lake Sinai virus nucleotide sequences were aligned using the Geneious Alignment tool with the default cost matrix (65% similarity (5.0/-4.0).

	LSV1 HQ87193 1	LSV1 MT2014	LSV2 - HQ88886 5	LSV3 - JQ480620	LSV4 - JX878492	LSV4 MT2014	LSV5 JR - KC88012 5	LSV6 2014 MT	LSV7 2014MT	LSV Navarra JX045859	LSV 2015 Belgium KM88690 2	LSV Belgium 2015 KM88690 3	LSV Belgium 2015 KM88690 4	LSV Belgium 2015 KM88690 5	LSV5 JR - KC88012 1	LSV5 JR - KC88012 3	LSV5 JR - KC88012 2	LSV5 JR - KC88012 4	LSV5 JR - KC88012 6	LSV 55 - KJ561228	LSV 56 - KJ561229	LSV 324 - KJ561227	LSV Av - KF768350	LSV e31 KF768348	LSV e35 KF768349	LSV e101 - KF768351
LSV1 HQ871931		97.724	69.806	79.117	78.756	82.532	83.103	82.456	63.14	77.655	70.037	74.393	69.337	74.138	82.927	79.931	77.413	82.857	85.321	74.019	73.458	82.21	77.711	77.711	78.112	74.598
LSV1 MT2014	97.724		70.789	0	0	0	0	100	0	0	70.865	71.168	66.161	73.445	0	0	0	0	0	0	0	0	0	0	0	0
LSV2 - HQ888865	69.806	70.789		78.251	76.897	78.72	76.083	75.292	63.636	73.418	77.653	69.578	73.724	72.656	84.878	79.585	75.741	76.484	80.734	88.224	88.598	77.757	75.752	75.752	77.154	81.263
LSV3 - JQ480620	79.117	0	78.251		81.088	81.444	76.581	87.5	0	73.659	76.722	81.362	79.716	80.165	80.244	96.021	77.29	77.033	80.275	76.168	76.542	77.809	94.478	94.679	98.293	75.502
LSV4 - JX878492	78.756	0	76.897	81.088		94.105	77.964	80	0	70.732	76.684	98.618	77.72	77.72	81.951	83.045	75.186	77.802	80.734	74.579	74.393	77.341	79.92	79.719	80.723	75.402
LSV4 MT2014	82.532	0	78.72	81.444	94.105		79.545	89.503	0	63.095	81.09	95.513	80.449	82.372	80.488	82.353	76.957	79.75	82.263	74.713	74.253	77.189	80.631	80.405	81.532	74.662
LSV5 JR -KC880125	83.103	0	76.083	76.581	77.964	79.545		0	0	52.778	68.083	78.36	76.383	76.779	78.322	80.911	76.877	97.736	98.391	70.571	70.571	76.779	75.198	75.593	76.779	67.688
LSV6 2014 MT	82.456	100	75.292	87.5	80	89.503	0		0	0	82.602	82.602	76.462	79.386	0	0	0	0	0	0	0	0	100	100	100	100
LSV7 2014MT	63.14	0	63.636	0	0	0	0	0		76.923	65.124	61.983	64.298	64.628	0	0	0	0	0	0	0	0	0	0	0	0
LSV Navarra JX045859	77.655	0	73.418	73.659	70.732	63.095	52.778	0	76.923		74.485	74.96	77.179	78.605	0	0	77.446	66.429	50	63.784	64.324	95.109	68.116	68.116	68.841	67.029
LSV 2015 Belgium KM886902	70.037	70.865	77.653	76.722	76.684	81.09	68.083	82.602	65.124	74.485		76.31	72.424	79.938	100	77.163	72.958	71.868	73.089	83.738	83.551	74.345	74.699	74.498	75.502	98.695
LSV Belgium 2015 KM886903	74.393	71.168	69.578	81.362	98.618	95.513	78.36	82.602	61.983	74.96	76.31		68.426	73.17	81.951	83.045	75.186	78.022	81.04	74.953	74.766	77.528	80.12	79.92	80.924	75.602
LSV Belgium 2015 KM886904	69.337	66.161	73.724	79.716	77.72	80.449	76.383	76.462	64.298	77.179	72.424	68.426		72.386	85.366	82.699	96.473	76.703	80.122	75.14	75.14	86.891	78.112	77.912	79.518	76.406
LSV Belgium 2015 KM886905	74.138	73.445	72.656	80.165	77.72	82.372	76.779	79.386	64.628	78.605	79.938	73.17	72.386		82.439	83.391	98.948	77.143	80.734	73.458	73.458	88.202	78.514	78.313	80.12	74.799
LSV5 JR -KC880121	82.927	0	84.878	80.244	81.951	80.488	78.322	0	0	0	100	81.951	85.366	82.439		79.024	81.585	79.024	79.512	90.732	90.732	80.976	81.463	80.976	80.976	99.024
LSV5 JR -KC880123	79.931	0	79.585	96.021	83.045	82.353	80.911	0	0	0	77.163	83.045	82.699	83.391	79.024		82.18	81.315	82.353	78.893	78.201	81.315	94.81	95.156	95.848	77.163
LSV5 JR - KC880122	77.413	0	75.741	77.29	75.186	76.957	76.877	0	0	77.446	72.958	75.186	96.473	98.948	81.585	82.18		76.481	80.581	71.79	71.543	87.067	75.681	75.433	77.661	73.082
LSV5 JR - KC880124	82.857	0	76.484	77.033	77.802	79.75	97.736	0	0	66.429	71.868	78.022	76.703	77.143	79.024	81.315	76.481		98.165	72.308	72.747	76.923	76.652	76.872	77.093	71.586
LSV5 JR - KC880126	85.321	0	80.734	80.275	80.734	82.263	98.391	0	0	50	73.089	81.04	80.122	80.734	79.512	82.353	80.581	98.165		76.147	76.758	80.428	79.817	80.122	80.428	72.783
LSV 55 -KJ561228	74.019	0	88.224	76.168	74.579	74.713	70.571	0	0	63.784	83.738	74.953	75.14	73.458	90.732	78.893	71.79	72.308	76.147		98.689	73.084	75.665	75.46	75.869	84.56
LSV 56 -KJ561229	73.458	0	88.598	76.542	74.393	74.253	70.571	0	0	64.324	83.551	74.766	75.14	73.458	90.732	78.201	71.543	72.747	76.758	98.689		72.71	75.256	75.051	75.869	84.151
LSV 324 -KJ561227	82.21	0	77.757	77.809	77.341	77.189	76.779	0	0	95.109	74.345	77.528	86.891	88.202	80.976	81.315	87.067	76.923	80.428	73.084	72.71		77.049	77.049	77.254	74.283
LSV Av -KF768350	77.711	0	75.752	94.478	79.92	80.631	75.198	100	0	68.116	74.699	80.12	78.112	78.514	81.463	94.81	75.681	76.652	79.817	75.665	75.256	77.049		99.799	94.98	75.602
LSV e31 KF768348	77.711	0	75.752	94.679	79.719	80.405	75.593	100	0	68.116	74.498	79.92	77.912	78.313	80.976	95.156	75.433	76.872	80.122	75.46	75.051	77.049	99.799		95.181	75.402
LSV e35 KF768349	78.112	0	77.154	98.293	80.723	81.532	76.779	100	0	68.841	75.502	80.924	79.518	80.12	80.976	95.848	77.681	77.093	80.428	75.869	75.869	77.254	94.98	95.181		76.205
LSV e101 -KF768351	74.598	0	81.263	75.502	75.402	74.662	67.688	100	0	67.029	98.695	75.602	76.406	74.799	99.024	77.163	73.082	71.586	72.783	84.56	84.151	74.283	75.602	75.402	76.205	

Figure 4. **. Nucleotide alignment of all Lake Sinai virus sequences in NCBI database (March 2015).** Lake Sinai virus nucleotide sequences were aligned using the Geneious Alignment tool with the default cost matrix (65% similarity (5.0/-4.0); figure from Daughenbaugh, et al., Viruses, 2015 – in review.

Figure 5. PCR Detection of LSV1 and LSV2 from individual 20 colonies monitored monthly from April 2009 to December 2010 (Mississippi, South Dakota, California U.S.A.), red in circle diagrams = percentage of virus positive samples. Note differential abundance of the two strains. LSV2 incidence surged in April, July and January during which over a third of all 20 monitor hives were infected; Figure from Runckel*, Flenniken*, et al PLoS One 2011.

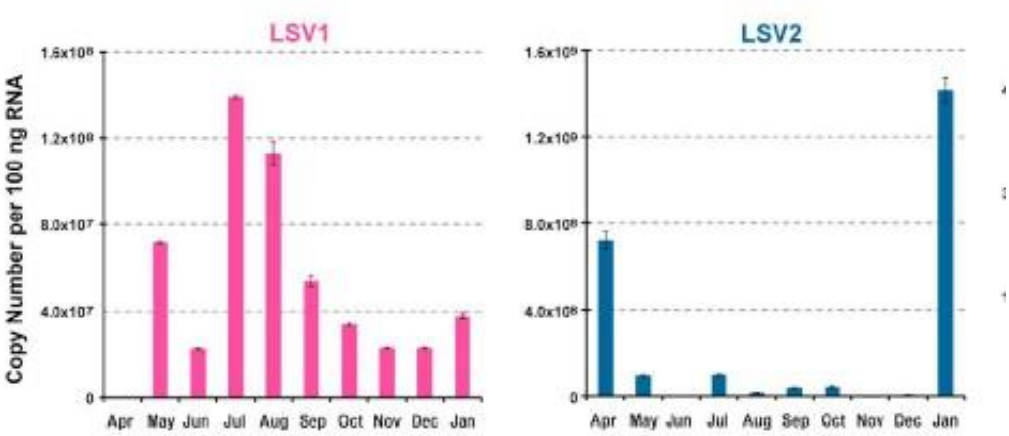


Figure 6. Quantitative PCR of LSV1 and LSV2 from pooled monthly samples of 20 colonies that were sampled each week from April 2009 to December 2010 (Mississippi, South Dakota, California U.S.A.). Strain specific qPCR demonstrated high abundance ($\geq 2x10^6$ copies per 100 ng RNA) of both LSV strains in our monitor colonies throughout the majority of the time-course (see below). LSV1 copy number peaked in July, at $1.39x10^8$ copies per 100 ng of RNA sample (approximately $7.0x10^{10}$ copies per bee). Notably, LSV2 was the most abundant virus detected in this study ($\sim 10^{11}$ copies per bee). Copy number peaked in both April and January, at

$7.22x10^8$ copies per 100 ng of RNA sample (approximately $3.61x10^{11}$ copies per bee) and $1.42x10^9$ copies per 100 ng of RNA sample (approximately $7.1x10^{11}$ copies per bee), respectively; figure from Runckel*, Flenniken*, et al PLoS One 2011.

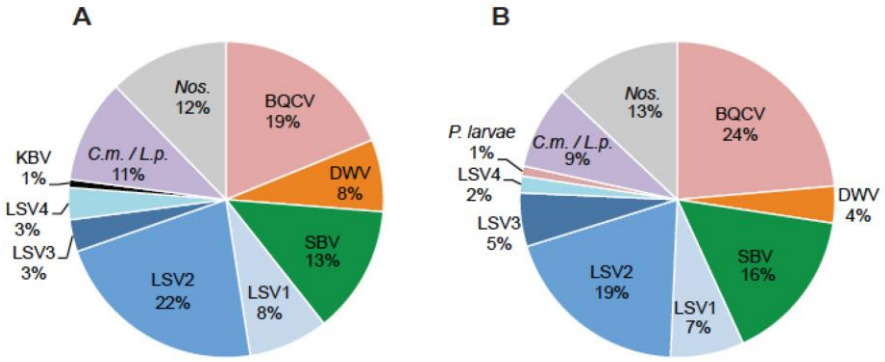


Figure 6: Distribution of honey bee pathogens detected in weak and strong colonies. Honey bee samples were obtained from 60 monitor colonies from October 2013 to April 2014. PCR was used to test for 14 honey bee infecting pathogens including: viruses (*Acute bee paralysis virus* (ABPV), *Black queen cell virus* (BQCV), *Deformed wing virus* (DWV), *Israeli acute paralysis virus* (IAPV), *Kashmir bee virus* (KBV), *Sacbrood virus* (SBV), Lake Sinai virus 1 (LSV1), LSV2, LSV3, and LSV4, microsporidia (*Nosema spp*.), bacteria (*Paenibacillus larvae* and *Melissococcus plutonius)*, and trypanosomatids (*Crithidia mellificae* / *Lotmaria passim)*. The pathogen prevalence in (A) healthy (>9 frames; n=81) or (B) weak (<5 frames; n=41) honey bee colonies is shown as a percentage of the total number of pathogens detected; figure from Daughenbaugh, et al., Viruses, 2015 – in review.

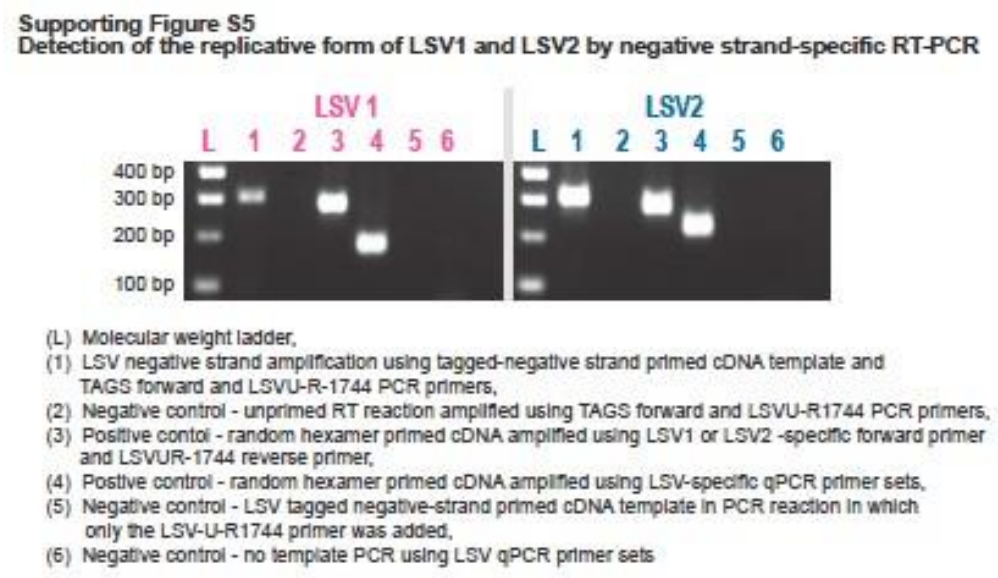


Figure 7. Detection of the replicative intermediate form of LSV1 and LSV1 by negative-strand specific PCR.
We confirmed the presence of the replicative forms of LSV1 and 2 in time-course samples.

Positive sense RNA viruses, like LSV 1 and 2, utilize a negative strand template to produce viral genome copies, therefore detection of the negative-strand intermediate is indicative of an actively replicating infectious virus. We used negative-strand specific RT-PCR to detect the replicative forms of both LSV1 and LSV2 (figure below). cDNA synthesis reactions were performed using tagged negative strand-specific LSV1 and 2 primers followed by exonuclease I digestion of excess unincorporated RT-primers. PCR amplification using a tag-specific forward primer and LSV-specific reverse primers confirmed the presence of the replicative forms of both LSV1 and LSV2 in the July RNA sample; figure from Runckel*, Flenniken*, et al PLoS One 2011.

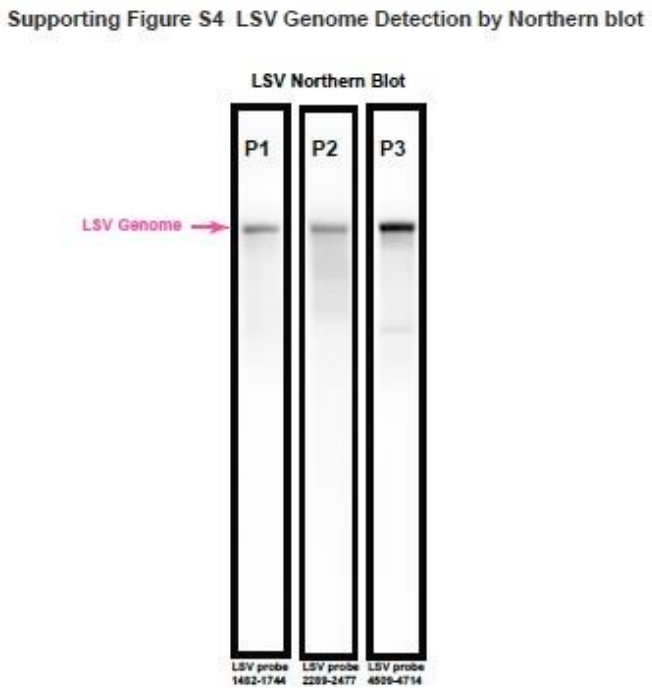


Figure 8. LSV genome detection by Northern blot analysis.
Detection of the LSV genome by denaturing 1.5% agarose gel electrophoresis and Northern blots using three LSV-specific probes. RNA (15 ug) extracted from the supernatants of homogenized honey bees was transferred to a membrane and probed using LSV-specific probes corresponding to different regions of the genome (P1 - 1482-1744, P2 - 2289-2477, and P3 - 4509-4714); figure from Runckel*, Flenniken*, et al PLoS One 2011.

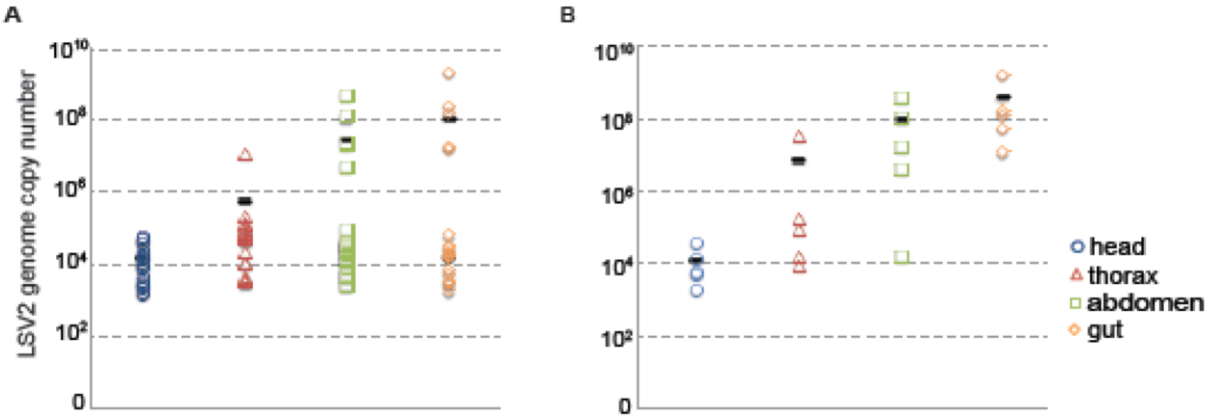


Figure 9. Relative Distribution of LSV2 in honey bees.
LSV2 positive adult honey bees (n=22) were dissected (head, thorax, abdomen, and gut) and the relative abundance of LSV2 was assessed by qPCR. The average copy number per 1 □g RNA of each region (x-axis) is as follows: head - $1.56x10^4$, thorax - $1.96x10^6$, abdomen - $2.72x10^7$, and gut - $1.07x10^8$ (represented by a black dash).
B. Bees with highest LSV2 levels (n=5) harbored the majority of virus in their gut - $4.72x10^8$ and abdomen - $1.19x10^8$ average copy number per 1 □g RNA (black dash), compared to lower copy numbers detected in the thorax - $8.42x10^6$ and head -$1.55x10^5$ regions.
Figure from Daughenbaugh, et al., Viruses, 2015 – in review.

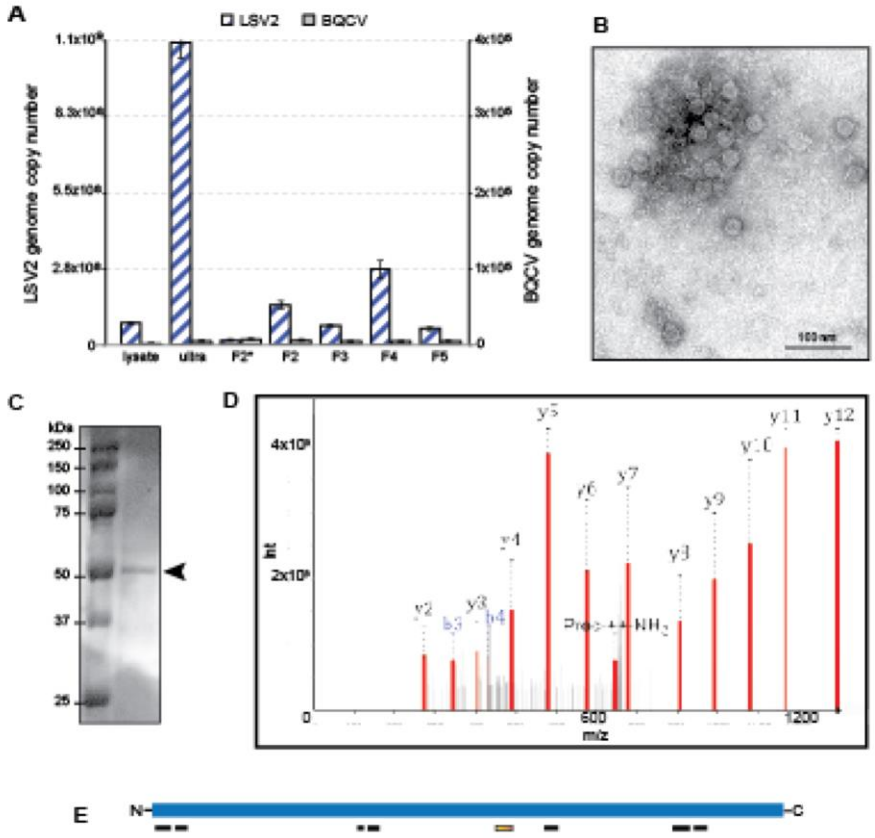


Figure 10. Characterization of Lake Sinai virus 2 (LSV2).
A standard virus purification protocol was used to isolate honey bee associated viruses from bees primarily infected with LSV2; pathogen specific PCR was used to screen samples for additional pathogens (see Supplemental Figure S7). A. The relative abundance of LSV2 and BQCV in several virus purification protocol subsamples including: the initial honey bee lysate (lysate), virus-pellet after ultracentrifugation (ultra), and several fractions from a CsCl gradient (F2*- fraction 2 unconcentrated, F2 - fraction 2 concentrated, F3 - fraction 3 concentrated, F4 – fraction 4 concentrated, and F5 - fraction 5 concentrated), was determined by qPCR. The LSV2 genome copy number per 500 ng RNA for each fraction is as follows: lysate - $8.0x10^8$, ultra – $1.1x10^9$, F2* - $1.8x10^7$, F2 - $1.5x10^8$, F3 - $7.1x10^7$, F4 - $2.7x10^8$, and F5 - $6.0x10^7$. The BQCV genome copy number per 500 ng RNA for each fraction is as follows: lysate - $1.6x10^3$, ultra - $5.4x10^3$, F2* - $7.2x10^3$, F2 - $6.3x10^3$, F3 - $4.6x10^3$, F4 - $4.7x10^3$, and F5 - $5.3x10^3$. B. The viruses in fraction 4 (F4), which contained the most LSV2 genome copies (i.e., $2.7x10^8$ copies/500ng RNA), were imaged using a TEM (37,000x magnification). The icosahedral virus particles have an average

diameter of 27.7 +/- 3.1 nm. C. The proteins contained in fraction 4 were analyzed by SDS-PAGE, a single protein band (arrow) from fraction 4 was visualized by Coomassie staining. D. The putative LSV2 capsid protein (MW 57.3 kDa) band was isolated and analyzed by mass spectrometry. Spectrum and fragment ions from MS peptide1 (NVESSSQTVSSMPR) corresponding to LSV2 capsid protein 286-300 aa (orange rectangle). E. Illustration of peptide matches (rectangles) to the predicted LSV2 capsid protein (blue line). Peptides identified by mass spectrometry covered 18.85% of the LSV2 capsid protein sequence; Supplemental Table S3 includes peptide and LSV2 capsid amino acid sequences. Figure from Daughenbaugh, et al., Viruses, 2015 – in review.