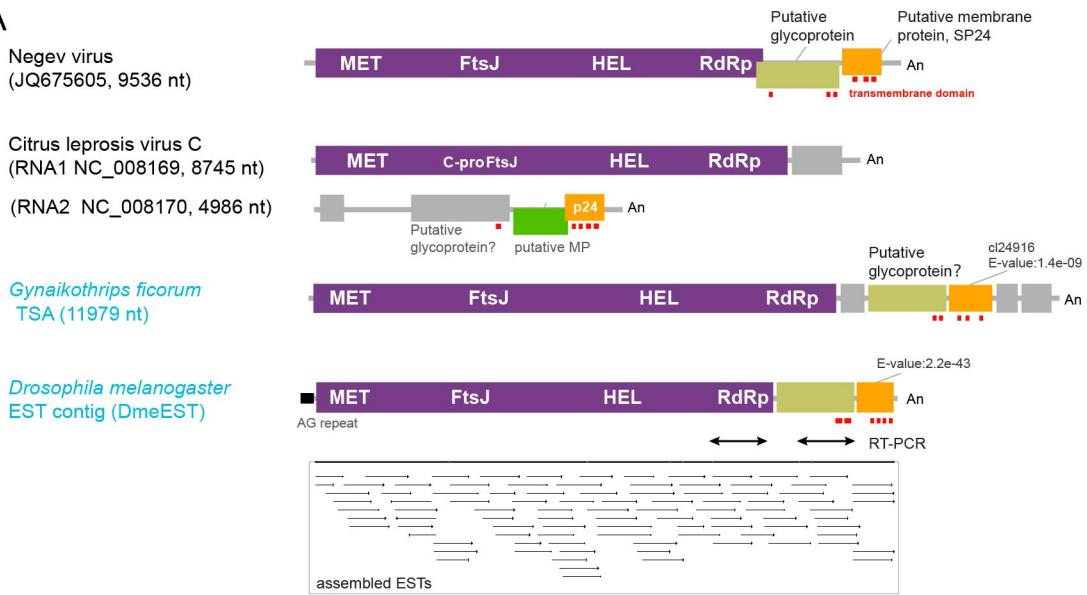
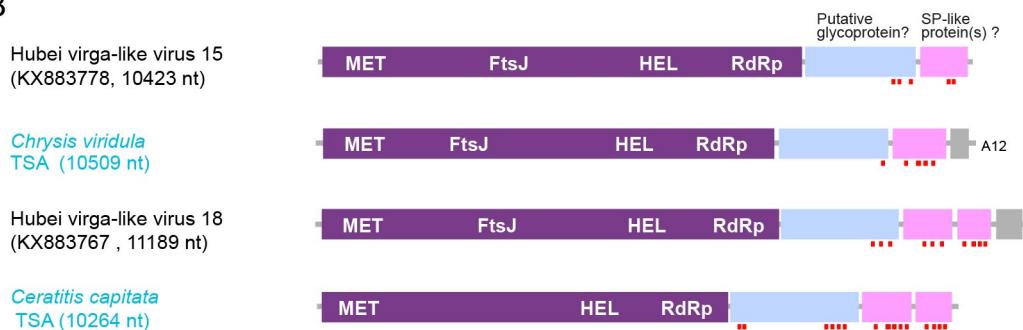


Supplementary Figures

A



B



C

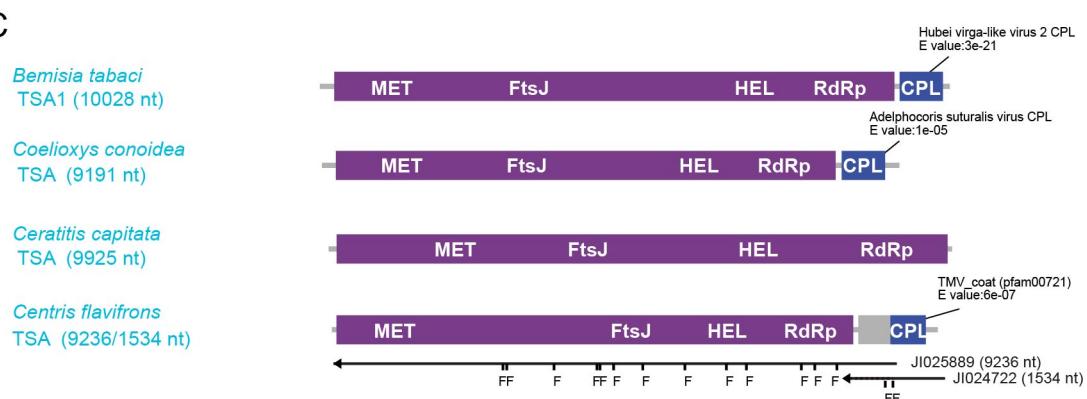


Fig. S1. Putative genome structure of insect virga/nege-related viruses and virus-like TSAs. (A–C) Schematic representations of virus-like insect TSAs (or an EST contig) related to nege- and cileviruses (A), Hubei virga-like virus 15 (B) and Hubei virga-like virus 1 (C, see also Fig. 3A). A virus-like sequence contig (named DmeEST) assembled from *Drosophila melanogaster* ESTs derived from Schneider L2 cell culture is shown in (A). List of the *D. melanogaster* ESTs for DmeEST construction (A): GenBank accession, BI638919, BG635657, BI638482, AI532995, AI534252, BG637461, BI141725, BI639062, BI638906, AI544395, BG640747, AI533274, BI636597,

BG635757, BG641116, AI542032, BI142081, AI532201, BI635191, BI639542, AI542337, AI533639, AI533639, BI141910, BG640612, AI543524, AI530962, BG635737, AI532854, BI636200, AI534776, AI532083, AI532473, AI542186, AI532583, AI542621, AI534897, AI530958, BI636474, AI533948, BI640491, BI639403, BI635232, BG635707, BI636250, AI541916, AI542073, AI531793, BG635866, BG635772, EL872030, EL872030, AI546576, AI532485, BI639356, AI534952, BI635674, BI640165, AI544345, BG640392, AI534985, BI639679, BI632687, BI641883, BI142109, BG635714, AI541862, BI636567, BI640187, AI534771, BI638192, BI641563, BG640363, BI635234, AI542213, BI637338, AI533282, BI636119, BI636730, BI636683, BI641632, BI636623, BI637714, AI533430, BI632540, BI633908, BI638733, BI640451, BG637012, BG640577, BG635825, BG640481, EL884146, BI141775 and AI531240. Two *Centris flavifrons* TSA sequences were used to construct CfITSA (C).

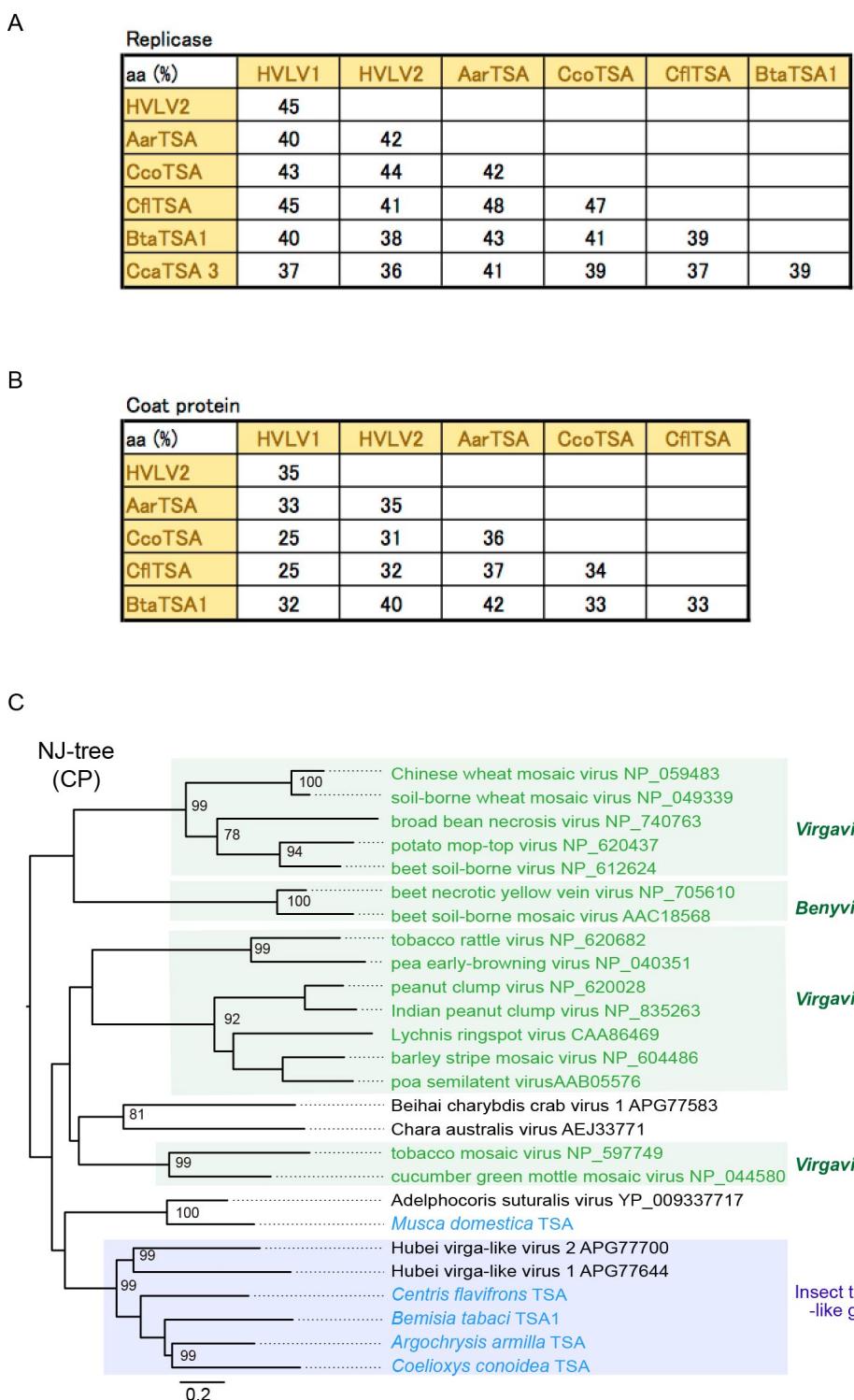


Fig. S2. Sequence identities among insect tobamo-like viruses. Replicase (**A**) and coat protein like (CPL) (**B**) amino acid sequences of insect viruses and virus-like TSA accessions were compared in pairs using BLAST. (**C**) The neighbor-joining (NJ) tree based on CP and CP-like (CPL) amino acid sequences of plant virga- and benyviruses (highlighted by green boxes), insect virga-like viruses and virus-like TSAs (highlighted by blue box).

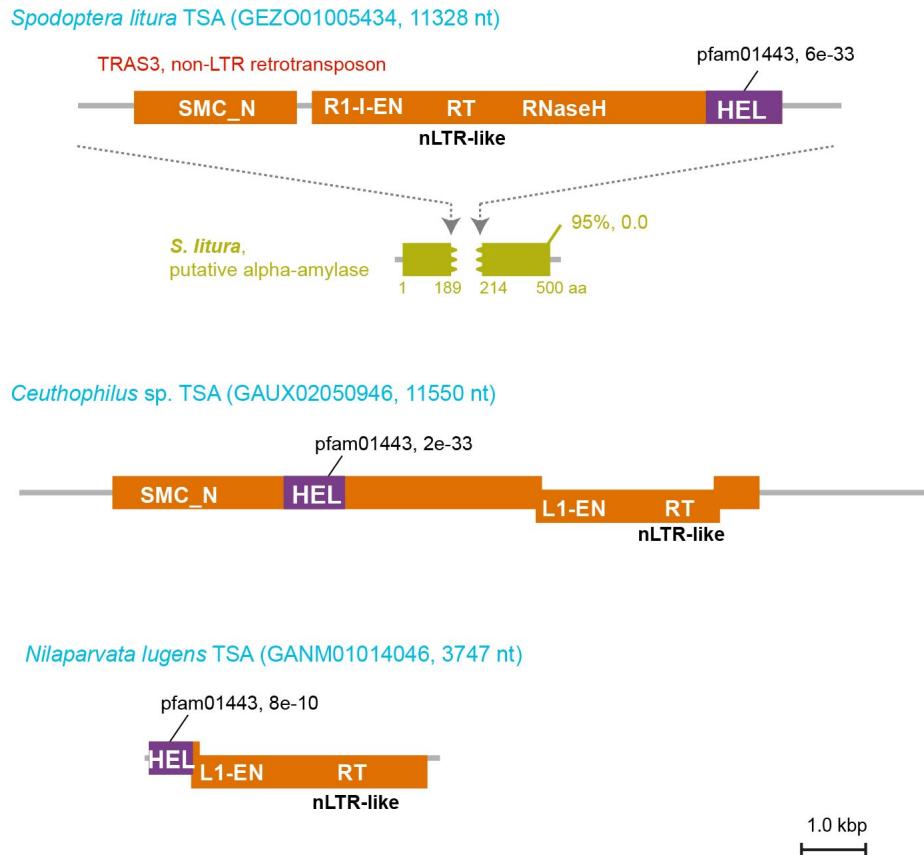


Fig. S3. Schematic representations of insect TSAs derived from putative retrotransposons carrying HEL-like domains (highlighted by purple) in a lepidopteran species, *Spodoptera litura* (A), a cave cricket (*Ceuthophilus* sp.) (B), and a hemipteran (*N. lugens*) (C). The E-values against putative HEL domains are shown above each ORF. The retrotransposon are inserted into a putative alpha amylase coding region (yellow green) in the *S. litura* TSA.

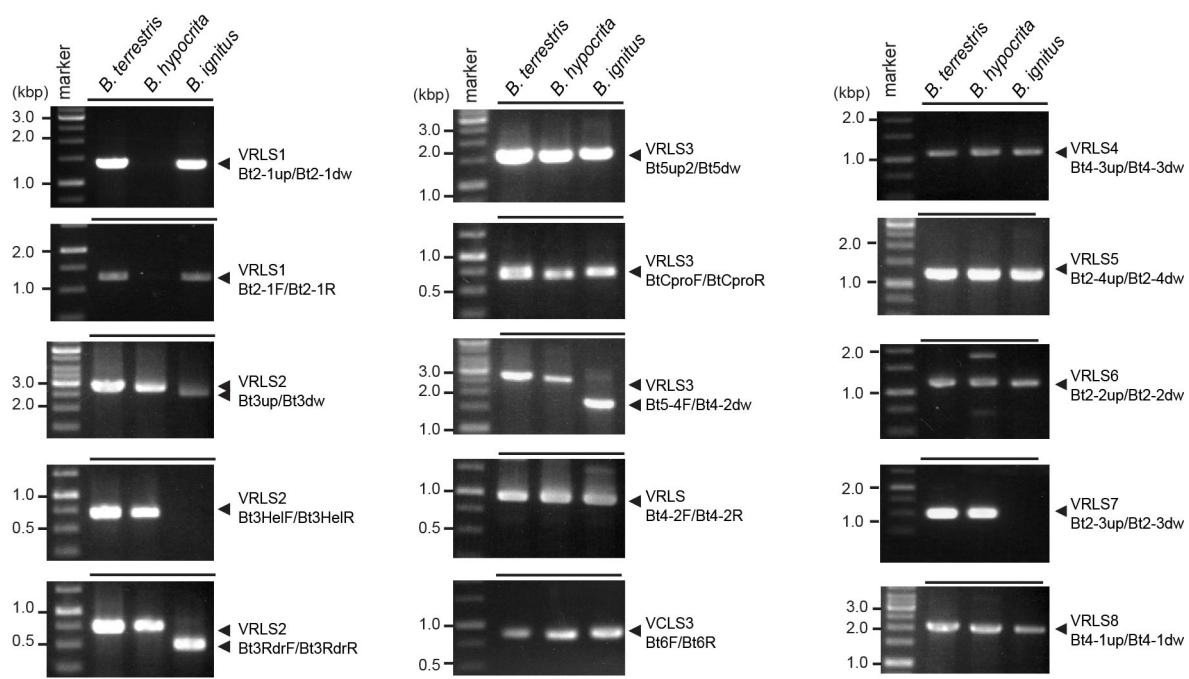


Fig. S4. PCR amplification of virga/negevirus-related EVE candidates in the genome of the *Bombus* genus. Genomic DNA from *B. terrestris*, *B. hypocrita* and *B. ignitus* were used for amplification of virgavirus replicase- or coat protein-related sequences (VRLSs/VCLSs). Primer positions for each VRLS/VCLS are shown in Fig. 5 and S1.

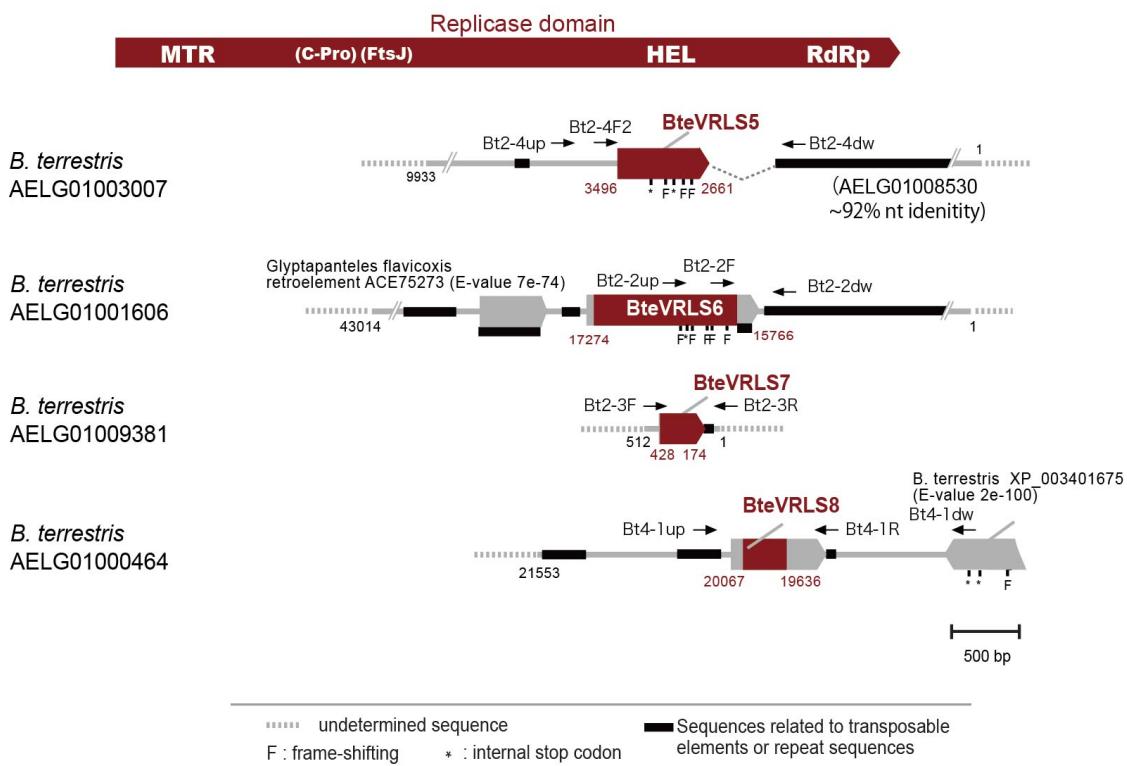


Fig. S5. Virgavirus replicase-related sequences (VRLS) identified in the *Bombus terrestris* genome. A schematic diagram of replicase sequence of Huber virga-like virus 1 used as a query is shown in the top. VRLSs are positioned according to the corresponding sequences in the query and the potential coding regions of *Bombus* EVEs are shown as dark red colored boxes. The WGS-assembled sequences and undetermined sequences are represented with solid and dashed thin-lines, respectively. Arrows and the texts above indicate the position of primer used for PCR amplification. The symbols referring to nucleotide mutations are shown below the grey line.

CLUSTAL format alignment by MAFFT (v7.310)

Fig. S6. Multiple alignments of the *Bombus* EVEs (EVE1, VRLS1; EVE2, VRLS2; EVE3, VRLS3+VCLS3, see Fig. 5). The *Bombus* EVEs and their variants sequenced in this study were labeled with “asterisks” and “a/b”, respectively.

BteEVE3 ttcttcgagatatacgcgttgcggaaatggat
BteEVE3* ttcttcgagatatacgcgttgcggaaatggat
BhyEVE3* ttcttcgagatatacgcgttgcggaaatggat
BigEVE3* ttcttcgagatatacgcgttgcggaaatggat

BteEVE3 actgttgtactgtcgactataatgtcgtactgttat
BteEVE3* actgttgtactgtcgactataatgtcgtactgttat
BhyEVE3* actgttgtactgtcgactataatgtcgtactgttat
BigEVE3* actgttgtactgtcgactataatgtcgtactgttat

BteEVE3 gagggtttcttgatacccttgcggaaatgtcgttagacgacg
BteEVE3* gagggtttcttgatacccttgcggaaatgtcgttagacgacg
BhyEVE3* gagggtttcttgatacccttgcggaaatgtcgttagacgacg
BigEVE3* gagggtttcttgatacccttgcggaaatgtcgttagacgacg

BteEVE3 gaacgactgggtgcacagctgtcgcagaaggccggaaaaggatgttat
BteEVE3* gaacgactgggtgcacagctgtcgcagaaggccggaaaaggatgttat
BhyEVE3* gaacgactgggtgcacagctgtcgcagaaggccggaaaaggatgttat
BigEVE3* gaacgactgggtgcacagctgtcgcagaaggccggaaaaggatgttat

BteEVE3 catccgactgaatcgttggccggagggtgttgcggccataacatca
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BigEVE3* aaaaagaagaaaaaggagactgttgcggccggagggtgttgcggccataacatca

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BteEVE3* gctagtacaaaggactgttgcggccggagggtgttgcggccataacatca
BhyEVE3* gctagtacaaaggactgttgcggccggagggtgttgcggccataacatca
BigEVE3* gctagtacaaaggactgttgcggccggagggtgttgcggccataacatca

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BhyEVE3* ggagaagtaattaaatcccgccgttgcggccggagggtgttgcggccataacatca
BigEVE3* ggagaagtaattaaatcccgccgttgcggccggagggtgttgcggccataacatca

BteEVE3 ctgggttgcggccgttgcggccggagggtgttgcggccataacatca
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BigEVE3* ctgggttgcggccgttgcggccggagggtgttgcggccataacatca
**** ***
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BhyEVE3*	naaggcattaccggccaatcttagccaaacactgcctttacacatgc-----acatccaa
BigEVE3*	aaaggcctaccggccaatcttagccggacactgcctttacacatgcagtgtaatccccaa
	***** * .*****
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BhyEVE3*	tcaataaaacccacacatatttctaaagggttagacaatagtataactcgaggcag-a
BigEVE3*	tcaataaaacccacacatatttctaaagggttagacaatagtataactgaggcaga
	***** *****.*****
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BteEVE3*	atacttgcagacttayaagcttacaataagaactgaagttgcgcgtatgtataaaaca
BhyEVE3*	ntacttgcacarctt-----
BigEVE3*	atacttgcagacttcacaagcttacaataagaactgaagttcscgga-----
	***** ***.
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BteEVE3*	aggacaacaatgtctaaaccaggccatcgagaaccctccacaatcatacttgcctaaacc
BhyEVE3*	-----
BigEVE3*	-----
BteEVE3	ccccacaaccggaaattcattgtttattgagaacaacaaccatgtatatacccaact
BteEVE3*	ccccacaaccggaaattcattgtttattgagaacaacaaccatgtatatacccaact
BhyEVE3*	-----
BigEVE3*	-----
BteEVE3	ggcaataaaacgttattaaaaaaaaaaagaacattatgaaagaaggactcggtctcgccgt
BteEVE3*	ggcaataaaacgttattaaaaaaaaaaagaacattatgaaagaaggactcggtctcgccgt
BhyEVE3*	-----
BigEVE3*	-----
BteEVE3	cacgaaagttaaagcagcgtcgccacagacagtacttggatgagttacccatctttta
BteEVE3*	cacgaaagttaaagcagcgtcgccacagacagtacttggatgagttacccatctttta
BhyEVE3*	-----
BigEVE3*	-----

A

nt (%)	BteEVE1	BigEVE1a*			
BteEVE1*	100				
BigEVE1a*	96				
BigEVE2*	96	99			
nt (%)	BteEVE2	BteEVE2a*	BteEVE2b*	BhyEVE2*	BigEVE2a*
BteEVE2a*	98				
BteEVE2b*	97	99			
BhyEVE2*	97	97	97		
BigEVE2a*	95	95	95	96	
BigEVE2b*	95	95	95	96	99
nt (%)	BteEVE3	BigEVE1a*	BhyEVE3*		
BteEVE3*	99				
BhyEVE3*	97	97			
BigEVE3*	95	95	95		

B

aa (%)	MTR	FtsJ			HEL			RdRp			CP			HEL/RD/CP	
		BimEVE9	BteEVE3-1	BhyEVE3-1*	BteEVE1	BteEVE2	BhyEVE2*	BigEVE2-1*	BteEVE3-1	BhyEVE3-1*	BigEVE3-1*	BteEVE3-2	BhyEVE3-2*	BigEVE3-2*	
MTR	BimEVE9														41
	BimEVE10	75													45
HEL	BteEVE1				57	58	57								49
	BigEVE1a*			93	56	58	57								48
HEL-RdRp	BteEVE2														54
	BhyEVE2*				95										55
FtsJ-RdRp	BigEVE2a*				90	92									49
	BteEVE3-1/3-2				65	66	71								39/57/40
/CP	BteEVE3-1/3-2*	93			64	66	71	95			84				39/56/33
	BigEVE3-1/3-2*	91	91		69	71	72	91	92		85	89			38/62/34
	BimEVE11-1/11-2				70	69	67	69	67	65	60	48	48		*/52/35

Fig. S7. Sequence identities among virga/negevirus-related EVE candidates. Nucleotide (A) and amino acid sequences (B) of the selected *Bombus* EVEs (EVE1, VRLS1; EVE2, VRLS2; EVE3, VRLS/VCLS3; EVE9, VRLS9; EVE10, VRLS10; EVE11, VRLS/VCLS11, see Fig. 5) were compared in pairs using BLAST. The *Bombus* EVEs and their variants sequenced in this study were labeled with “asterisks” and “a/b” (see Fig. S6), respectively. Sequence identity scores are shown in %. A wasp virgavirus-like TSA (*Argochrysis armilla*, AarTSA) was also included in B.