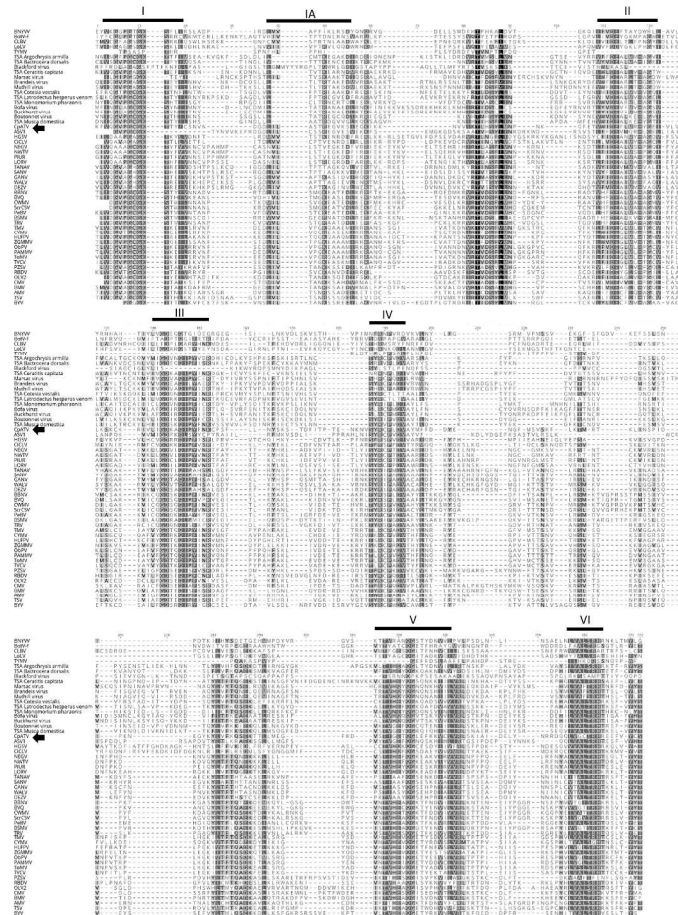


Supplementary Figure 1

Supplementary Figure 1: Distribution of quality scores using Solexa/Illumina scale along read position, for the reads mapped to the CpATV_Ayed (panel A) and CpATV_Jedaida (panel B) genomes after nucleotide trimming of read ends.

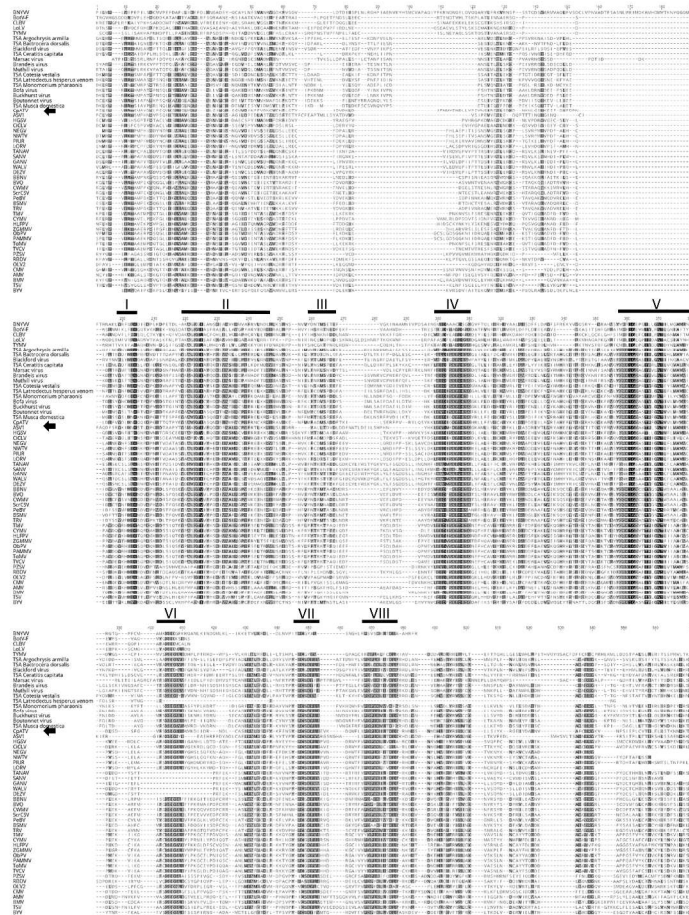
297x420mm (300 x 300 DPI)



Supplementary Figure 2

Supplementary Figure 2: Alignment of conserved helicase domain of the genome of *Culex pipiens* Associated Tunisia Virus (344 aa). Taxon names are virus acronyms. Similarity in amino acids alignments is represented by shades of black (100% in black, 80% in dark gray and 60% in light gray). Arrows indicate sequences of CpATV. Conserved motifs I to VI as defined by Koonin and Dolja in 1993 are indicated above sequences (73). Sequences details and GenBank accessions numbers are available in Supplementary Table 2.

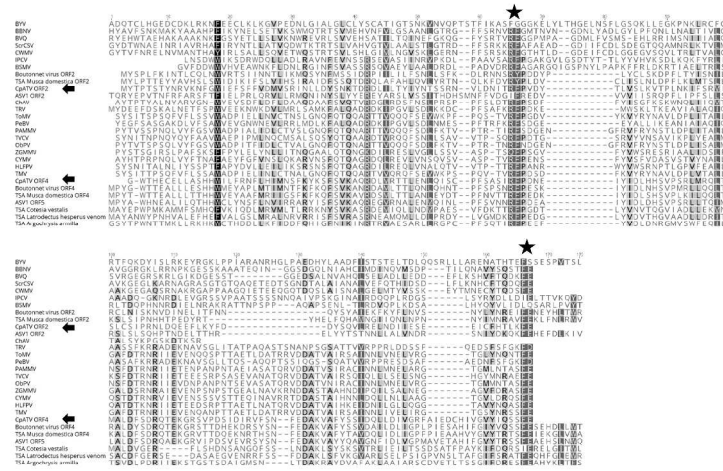
297x420mm (300 x 300 DPI)



Supplementary Figure 3

Supplementary Figure 3: Alignment of conserved RNA-dependent RNA-polymerase domain of the genome of Culex pipiens Associated Tunisia Virus (597 aa). Taxon names are in acronyms. Similarity in amino acids alignments is represented by shades of black (100% in black, 80% in dark gray and 60% in light gray). Arrows indicate sequences of CpATV. Conserved motifs I to VI as defined by Koonin and Dolja in 1993 are indicated above sequences (73). Sequences details and GenBank accessions numbers are available in Supplementary Table 2.

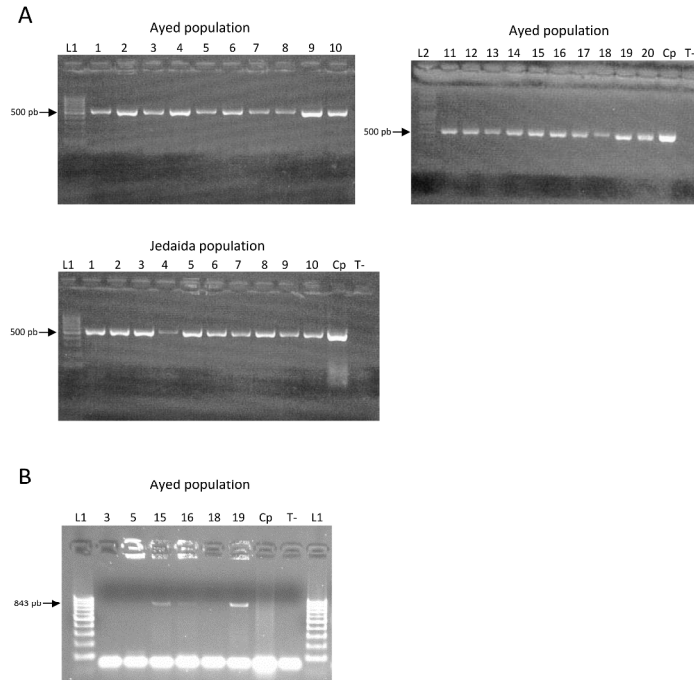
297x420mm (300 x 300 DPI)



Supplementary Figure 4

Supplementary Figure 4: Alignment of conserved capsids domains of the genome of Culex pipiens Associated Tunisia Virus (218 aa). Taxon names are in acronyms. Similarity in amino acids alignments is represented by shades of black (100% in black, 80% in dark gray and 60% in light gray). Arrows indicate sequences of CpATV. Stars indicates the conserved residues as defined by Koonin and Dolja in 1993 which form a functionally important salt bridge (R(+) - E(-)) (73). Sequences details and GenBank accessions numbers are available in Supplementary Table 2.

297x420mm (300 x 300 DPI)



Supplementary figure 5

Supplementary Figure 5: RT-PCR detection of CpATV in wild *Culex pipiens* samples from Ayed and Jedaïda populations. A) Detection of *C. pipiens* *ace-1* gene by RT-PCR for cDNA quality control. B) CpATV detection resulted in 3 positive samples in Ayed population (samples Ayed_15, 16 and 19), all other samples from Ayed or Jedaïda samples were negative. L1: 100 pb DNA Ladder (Jena Bioscience), L2: 100 pb DNA Ladder (Euromedex), Cp: *Culex pipiens* genomic DNA, T-: Ultrapure water.

297x420mm (300 x 300 DPI)

Supplementary Table 1: Origins and characteristics of mosquito samples.

Culex species	Individual name	Country	Location	Year	Latitude	Longitude	SRA Accession number	References
<i>Culex pipiens</i>	GA34I	Algeria	Lac des Oiseaux	2008	36.7715922	8.1092816	SRX565088	(Romiguier, <i>et al</i> , 2014)
	GA34C	Burkina Faso	Ouagadougou	1997	12.364637	-1.5338639	SRX565082	(Romiguier, <i>et al</i> , 2014)
	GA34J	China	Zhu-Shang	2003	24.6446709	102.686988	SRX565089	(Romiguier, <i>et al</i> , 2014)
	GA34H	Costa Rica	Pueto Viero	2006	9.646168	-82.7490229	SRX565087	(Romiguier, <i>et al</i> , 2014)
	GA34F	France	Ganges	2011	43.934614	3.708787	SRX565085	(Romiguier, <i>et al</i> , 2014)
	GA34G	France	Triplet	2011	43.629366	3.860393	SRX565086	(Romiguier, <i>et al</i> , 2014)
	GA35A	USA	California (lab strain)	1950	na	na	SRX1453901	This study
	GA35B	USA	California (lab strain)	1950	na	na	SRX1453908	This study
	GA34E	Israel	Tel Aviv Yatouch	2011	32.070065	34.777508	SRX565084	(Romiguier, <i>et al</i> , 2014)
	GA34B	Réunion island	Saint Benoît	2011	-21.043737	55.717857	SRX565081	(Romiguier, <i>et al</i> , 2014)
	GA34D	Philippine	Manille	2003	14.5995124	120.9842195	SRX565083	(Romiguier, <i>et al</i> , 2014)
	GA34A	Tunisia	Grombalia	2005	36.601541	10.50034	SRX565080	(Romiguier, <i>et al</i> , 2014)
	GA35C *	Tunisia	Ayed	2005	35.626433	10.912933	SRX1457280	This study
	GA35D	Tunisia	Grombalia	2005	36.590900	10.490600	SRX1457281	This study
	GA35E *	Tunisia	Jedaida	2011	36.803817	9.942400	SRX1457282	This study
	GA35F	Tunisia	Kef2	2008	36.159550	8.718633	SRX1457496	This study
GA35G	Tunisia	Azib	2011	37.222633	9.934417	SRX1457498	This study	
GA35H	Tunisia	Utique	2011	37.069600	10.007067	SRX1457500	This study	
<i>Culex hortensis</i>	GA34K	France	Laroque	2011	43.922663	3.723849	SRX565078	(Romiguier, <i>et al</i> , 2014)
	GA34L	France	Mas de Foiton	2004	43.796794	4.593698	SRX565079	(Romiguier, <i>et al</i> , 2014)
<i>Culex torrentium</i>	GA34M	France	Polignac	1996	45.071348	3.859441	SRX565090	(Romiguier, <i>et al</i> , 2014)
	GA34N	Sweden	Uppsala	1994	59.8585638	17.6389267	SRX565091	(Romiguier, <i>et al</i> , 2014)

*Individuals with a full-length viral genome detected

Romiguier J, Lourenco J, Gayral P, Faivre N, Weinert LA, Ravel S, Ballenghien M, Cahais V, Bernard A, Loire E, Keller L, Galtier N. 2014. Population genomics of eusocial insects: the costs of a vertebrate-like effective population size. *J. Evol. Biol.* **27**:593–603.

Supplementary Table 2: Mosquitoes genomic and transcriptomic sequence data used in this study.

BioProject Accession	ID	Project Name	Type of data	Taxonomic ID	Scientific Name	Availability
PRJNA29017	29017	Reference sequences (RefSeq) of the <i>Culex quinquefasciatus</i> genome	Genome and transcriptome	7176	<i>Culex quinquefasciatus</i>	Release April 2014*
PRJNA18751	18751	The vector for <i>West Nile Virus</i>	Genome	7176	<i>Culex quinquefasciatus</i>	
PRJNA246260	246260	<i>Culex pipiens pallens</i> and <i>C. p. quinquefasciatus</i> with different resistant level	Transcriptome	7176	<i>Culex quinquefasciatus</i>	SRR1271734
				7176	<i>Culex quinquefasciatus</i>	SRR1271735
				7176	<i>Culex quinquefasciatus</i>	SRR1271736
				7176	<i>Culex quinquefasciatus</i>	SRR1271737
				42434	<i>Culex pipiens pallens</i>	SRR1271738
				42434	<i>Culex pipiens pallens</i>	SRR1271739
				42434	<i>Culex pipiens pallens</i>	SRR1271740
				42434	<i>Culex pipiens pallens</i>	SRR1271741
				42434	<i>Culex pipiens pallens</i>	SRR1271742
				42434	<i>Culex pipiens pallens</i>	SRR1271743
				42434	<i>Culex pipiens pallens</i>	SRR1271744
				7176	<i>Culex quinquefasciatus</i>	SRR1271745
PRJNA253629	253629	RNA-sequencing of <i>Culex pipiens molestus</i> and <i>C. pipiens quinquefasciatus</i>	Transcriptome	7176	<i>Culex quinquefasciatus</i>	SRR1462324
				233155	<i>Culex pipiens molestus</i>	SRR1462325

*http://www.broadinstitute.org/annotation/genome/culex_pipiens

Supplementary Table 3: *Plasmodium* genomes available in PlasmoDB (release 2015/07/23)

<i>Plasmodium</i> species	Genome version
<i>P. berghei</i> ANKA	2013/03/01
<i>P. chabaudi chabaudi</i>	2013/03/01
<i>P. coatneyi hackeri</i>	2014/07/21
<i>P. cynomolgi</i> strain B	2012/09/19
<i>P. falciparum</i> 3D7	2013/03/01
<i>P. falciparum</i> IT	2013/03/01
<i>P. gallinaceum</i> 8A	2006/12/31
<i>P. knowlesi</i> strain H	2013/03/01
<i>P. reichenowi</i> CDC	2014/09/03
<i>P. vivax</i> Sal-1	2015/05/01
<i>P. yoelii yoelii</i> 17X	2014/02/01
<i>P. yoelii yoelii</i> 17XNL	2005/09/01
<i>P. yoelii yoelii</i> YM	2013/03/01

Supplementary Table 4: Summary statistics (means for each species) of transcriptome assembly quality, ORF prediction and viral homology search.

Genus	<i>Culex</i>		
Species	<i>pipiens</i>	<i>hortensis</i>	<i>torrentium</i>
No. of transcriptomes analyzed	18	2	2
Initial assembly (ABYSS)			
No. million reads	25.6	54.9	58.3
No. contigs (x 1000)	27.2	73.8	90.2
Median length	52	52	51
N50	69	54	52
Final assembly (ABYSS-CAP3)			
No. contigs (x 1000)	3.4	8.3	7.9
Median length	122	118	122
N50	233	196	238
Virus detection			
No. of ORF predicted per species (x 1000)	2.6	6.0	5.8
No. of transcriptomes with full-length viral genome	2	0	0

Supplementary Table 5: Fixed mutations distinguishing CpATV_Ayed and CpATV_Jedaïda genomes.

ORF	Genomic Region	Mutation number	Position on CpATV_Ayed	Nucleotide in CpATV_Ayed	Position on CpATV_Jedaïda	Nucleotide in CpATV_Jedaïda	Mutation Type	Consequence of mutation	Amino acid changes
5' non-coding region	-	1	1	C	-	-	Insertion / Deletion	-	-
		2	2	C	-	-	Insertion / Deletion	-	-
		3	3	T	-	-	Insertion / Deletion	-	-
		4	110	C	107	T	Transition	-	-
ORF 1	No domain	5	498	C	495	T	Transition	Synonymous	AGC (S) <-> AGT (S)
	Viral Helicase	6	765	G	762	T	Transversion	Synonymous	TCG (S) <-> TCG (S)
		7	1,014	C	1,011	T	Transition	Synonymous	GTC (V) <-> GTT (V)
	No domain	8	1,497	A	1,494	G	Transition	Synonymous	CCA (P) <-> CCG (P)
		9	1,608	C	1,605	T	Transition	Synonymous	TTC (F) <-> TTT (F)
		10	1,699	A	1,696	C	Transversion	Synonymous	AGA (R) <-> CGA (R)
	RdRp	11	2,079	T	2,076	A	Transversion	Synonymous	ATT (I) <-> ATA (I)
12		2,580	T	2,577	A	Transversion	Synonymous	CTT (L) <-> CTA (L)	
13		2,739	T	2,736	C	Transition	Synonymous	GCT (A) <-> GCC (A)	
Intergenic region	-	14	4,025	-	4,022	T	Insertion / Deletion	-	-
ORF3	Signal peptide	15	4,111	T	4,109	C	Transition	Non-synonymous	TTT (F) <-> CTT (L)
	No domain	16	4,377	G	4,375	A	Transition	Synonymous	TTG (L) <-> TTG (L)
		17	4,410	T	4,408	C	Transition	Synonymous	TAT (Y) <-> TAC (Y)
		18	4,467	G	4,465	A	Transition	Synonymous	ACG (T) <-> ACA (T)
		19	4,607	G	4,605	A	Transition	Non-synonymous	AGA (R) <-> AAA (K)
		20	5,083	A	5,081	G	Transition	Non-synonymous	AAA (K) <-> GAA (E)
	MSP7_C	21	5,117	C	5,115	T	Transition	Non-synonymous	CCT (P) <-> CTT (L)
		22	5,598	G	5,596	A	Transition	Synonymous	AAG (K) <-> AAA (K)
		23	5,803	G	5,801	A	Transition	Non-synonymous	GTT (V) <-> ATT (I)
		24	5,964	T	5,962	C	Transition	Synonymous	ATT (I) <-> ATC (I)
25		6,072	C	6,070	T	Transition	Synonymous	AAC (N) <-> AAT (N)	
ORF 4	Capsid	26	6,416	C	6,414	T	Transition	Synonymous	GCC (A) <-> GCT (A)

Supplementary Table 6: Details of viruses and viral sequences used in ORF1 (helicase and RNA-dependant RNA polymerase) and ORF2-ORF4 (capsids) phylogenies.

Acronyme	Complete virus name	Viral Family	Viral Genus	Genbank Accession	Host
AMV	<i>Alfalfa mosaic virus</i>	Bromoviridae	<i>Alfavirus</i>	NC_002024, NC_001495	Plant
ASV1	Adelphocoris suturalis-associated virus 1	Not assigned	Not assigned	KX966285	Hemiptera
BBNV	<i>Broad bean necrosis virus</i>	Virgaviridae	<i>Pomovirus</i>	D86636, D86637	Plant
	Blackford Virus	Not assigned	Not assigned	KU754514	Drosophilidae (Diptera)
BMV	<i>Brome mosaic virus</i>	Bromoviridae	<i>Bromovirus</i>	NC_002027, KU726253	Plant
BNRBV	<i>Blueberry necrotic ring blotch virus</i>	Not assigned	<i>Blunervirus</i>	NC_016084, NC_016085	Plant
BNYVV	<i>Beet necrotic yellow vein virus</i>	Benyviridae	<i>Benyvirus</i>	NC_003514	Plant
	Bofa Virus	Not assigned	Not assigned	KU754515	Drosophilidae (Diptera)
BotV-F	<i>Botrytis virus F</i>	Gammaflexiviridae (Tymovirales)	<i>Mycoflexivirus</i>	NC_004063	Fungi
	Boutonnet Virus	Not assigned	Not assigned	KU754539	Drosophilidae (Diptera)
	Brandeis Virus	Not assigned	Not assigned	(1)	Drosophilidae (Diptera)
BSMV	<i>Barley stripe mosaic virus</i>	Virgaviridae	<i>Hordeivirus</i>	MBSRNAGT, MBSARNA, X03854	Plant
	Buckhurst Virus	Not assigned	Not assigned	KU754516	Drosophilidae (Diptera)
BVQ	<i>Beet virus Q</i>	Virgaviridae	<i>Pomovirus</i>	AJ223596, AJ223597	Plant
BYV	<i>Beet yellows virus</i>	Closteroviridae	<i>Closterovirus</i>	NC_001598	Plant
ChAV	<i>Chara australis virus</i>	Not assigned	Not assigned	JF824737	Plant
CiCLV	<i>Citrus leprosis virus C</i>	Not assigned	<i>Cilevirus</i>	DQ157466	Plant (mites transmitted)
CLBV	<i>Citrus leaf blotch virus</i>	Betaflexiviridae (Tymovirales)	<i>Citivirus</i>	NC_003877	Plant
CMV	<i>Cucumber mosaic virus</i>	Bromoviridae	<i>Cucumovirus</i>	NC_002035, KC019299	Plant
CWMV	<i>Chinese wheat mosaic virus</i>	Virgaviridae	<i>Furovirus</i>	AJ012005, AJ012006	Plant
CYMV	<i>Clitoria yellow mottle virus</i>	Virgaviridae	<i>Tobamovirus</i>	NC_016519	Plant
DEZV	<i>Dezidougou virus</i>	Not assigned	Sandewavirus	JQ675604	Insect (Diptera-Culicidae)
GANV	<i>Goutanap virus</i>	Not assigned	Sandewavirus	KF588035	Insect (Diptera-Culicidae)

HGSV	<i>Hibiscus green spot virus</i>	Not assigned	<i>Higrevirus</i>	HQ852052	Plant
HLFPV	<i>Hibiscus latent Fort Pierce virus</i>	Virgaviridae	<i>Tobamovirus</i>	FJ196834, AY250831	Plant
IPCV	<i>Indian peanut clump virus</i>	Virgaviridae	<i>Pecluvirus</i>	AF447397	Plant
LoLV	<i>Lolium latent virus</i>	Alphaflexiviridae (Tymovirales)	<i>Lolavirus</i>	NC_010434	Plant
LORV	<i>Loreto virus</i>	Not assigned	Nelorpivirus	JQ675611	Insect (Diptera-Culicidae)
	Marsac Virus	Not assigned	Not assigned	KU754518	Drosophilidae (Diptera)
	Muthill Virus	Not assigned	Not assigned	KU754517	Drosophilidae (Diptera)
NWTV	<i>Ngewotan virus</i>	Not assigned	Nelorpivirus	JQ686833	Insect (Diptera-Culicidae)
NEGV	<i>Negev virus</i>	Not assigned	Nelorpivirus	JQ675608	Insect (Diptera-Culicidae)
ObPV	<i>Obuda pepper virus</i>	Virgaviridae	<i>Tobamovirus</i>	MTVGRNA	Plant
OLV-2	<i>Olive latent virus 2</i>	Bromoviridae	<i>Oleavirus</i>	NC_003674, NC_003673	Plant
PAMMV	<i>Paprika mild mottle virus</i>	Virgaviridae	<i>Tobamovirus</i>	AB089381	Plant
PCV	<i>Peanut clump virus</i>	Virgaviridae	<i>Pecluvirus</i>	X78602	Plant
PeBV	<i>Pea early browning virus</i>	Virgaviridae	<i>Tobravirus</i>	X14006, X15883	Plant
PIUR	<i>Piura virus</i>	Not assigned	Nelorpivirus	JQ675607	Insect (Diptera-Culicidae)
PZSV	<i>Pelargonium zonate spot virus</i>	Bromoviridae	<i>Anulavirus</i>	NC_003650, AJ272327	Plant
RBDV	<i>Raspberry bushy dwarf virus</i>	Not assigned	<i>Idaeovirus</i>	NC_003739	Plant
SANV	<i>Santana virus</i>	Not assigned	Sandewavirus	JQ675606	Insect (Diptera-Culicidae)
ScrCSV	<i>Sorghum chlorotic spot virus</i>	Virgaviridae	<i>Furovirus</i>	AB033691, AB033692	Plant
TANAV	<i>Tanay virus</i>	Not assigned	Sandewavirus	KF425261	Insect (Diptera-Culicidae)
	<i>TSA Argochrysis armilla</i>	Not assigned	Not assigned	GAXO01029871	Unannotated virus-like (2)
	<i>TSA Bactrocera dorsalis</i>	Not assigned	Not assigned	GAKP01015888	Unannotated virus-like (2)
	<i>TSA Ceratitis capitata</i>	Not assigned	Not assigned	GAMC01007262	Unannotated virus-like (2)
	<i>TSA Cotesia vestalis</i>	Not assigned	Not assigned	GAKG01005025	Unannotated virus-like (2)
	<i>TSA Latrodectus hesperus</i> venom	Not assigned	Not assigned	GBCS01005187	Unannotated virus-like (2)
	<i>TSA Monomorium pharaonis</i>	Not assigned	Not assigned	LA822010	Unannotated virus-like (2)
	<i>TSA Musca domestica</i>	Not assigned	Not assigned	GARN01041480	Unannotated virus-like (2)
TMV	<i>Tobacco mosaic virus</i>	Virgaviridae	<i>Tobamovirus</i>	V01408	Plant

ToMV	<i>Tomato mosaic virus</i>	Virgaviridae	<i>Tobamovirus</i>	X02144	Plant
TRV	<i>Tobacco rattle virus</i>	Virgaviridae	<i>Tobravirus</i>	AF166084, AF034621	Plant
TSV	<i>Tobacco streak virus</i>	Bromoviridae	<i>Ilarvirus</i>	NC_003842, FJ561302	Plant
TVCV	<i>Turnip vein-clearing virus</i>	Virgaviridae	<i>Tobamovirus</i>	BRU03387	Plant
TYMV	<i>Turnip yellow mosaic virus</i>	Tymoviridae (Tymovirales)	<i>Tymovirus</i>	NC_004063	Plant
WALV	<i>Wallerfield virus</i>	Not assigned	<i>Sandewavirus</i>	KF042857	Insect (Diptera-Culicidae)
ZGMMV	<i>Zucchini green mottle mosaic virus</i>	Virgaviridae	<i>Tobamovirus</i>	AJ295949	Plant
CpATV	<i>Culex pipiens</i> Associated Tunisia Virus strain Ayed	Not assigned	Not assigned	MG457154 (This study)	Insect (Diptera-Culicidae)
CpATV	<i>Culex pipiens</i> Associated Tunisia Virus strain Jedaida	Not assigned	Not assigned	MG457155 (This study)	Insect (Diptera-Culicidae)

1. **Webster CL, Waldron FM, Robertson S, Crowson D, Ferrari G, Quintana JF, Brouqui J-M, Bayne EH, Longdon B, Buck AH, Lazzaro BP, Akorli J, Haddrill PR, Obbard DJ.** 2015. The discovery, distribution, and evolution of viruses associated with *Drosophila melanogaster*. PLoS Biol. **13**:e1002210.
2. **Webster C, Longdon B, Lewis S, Obbard D.** 2016. Twenty-five new viruses associated with the Drosophilidae (Diptera). Evol. Bioinforma. **13**.