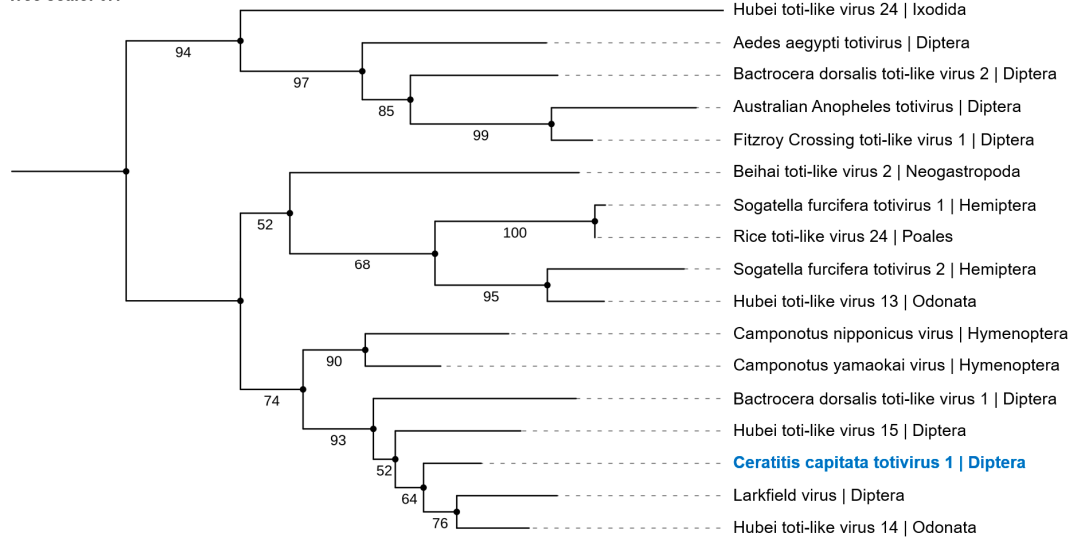


# Supplementary Materials:

Supplementary Figure S1:

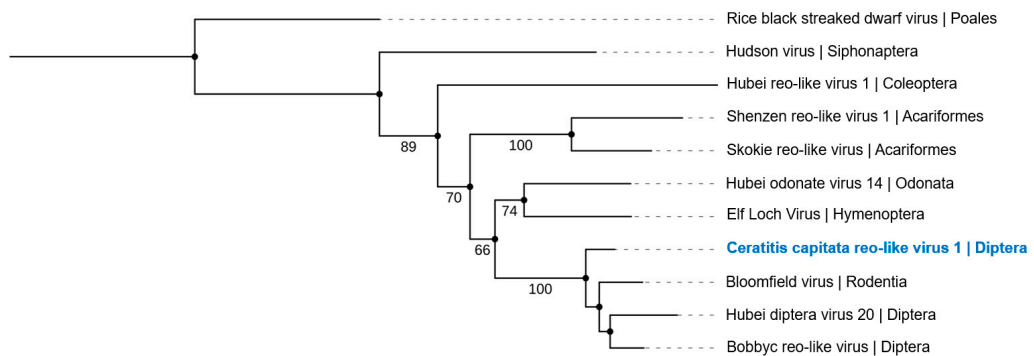
## A) *Ceratitis capitata* totivirus 1

Tree scale: 0.1



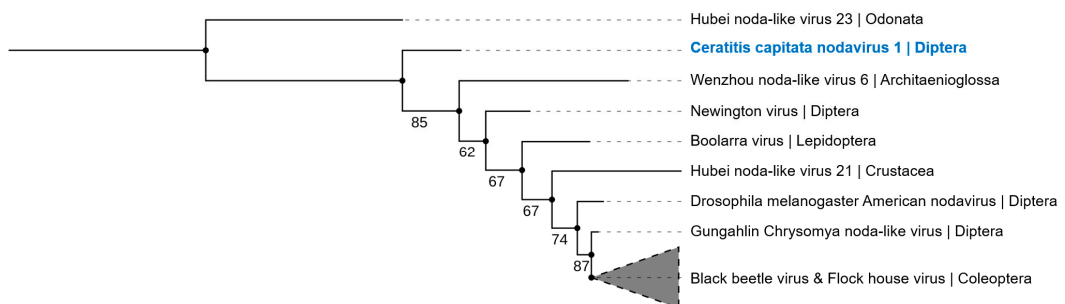
## B) *Ceratitis capitata* reo-like virus 1

Tree scale: 0.5

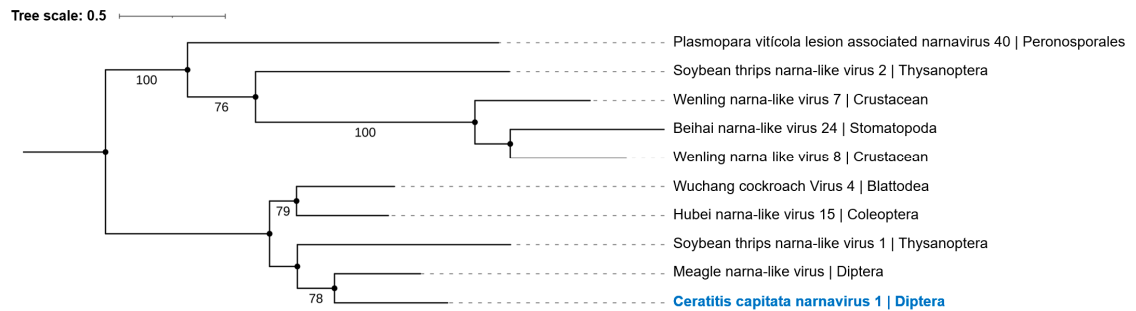


## C) *Ceratitis capitata* nodavirus 1

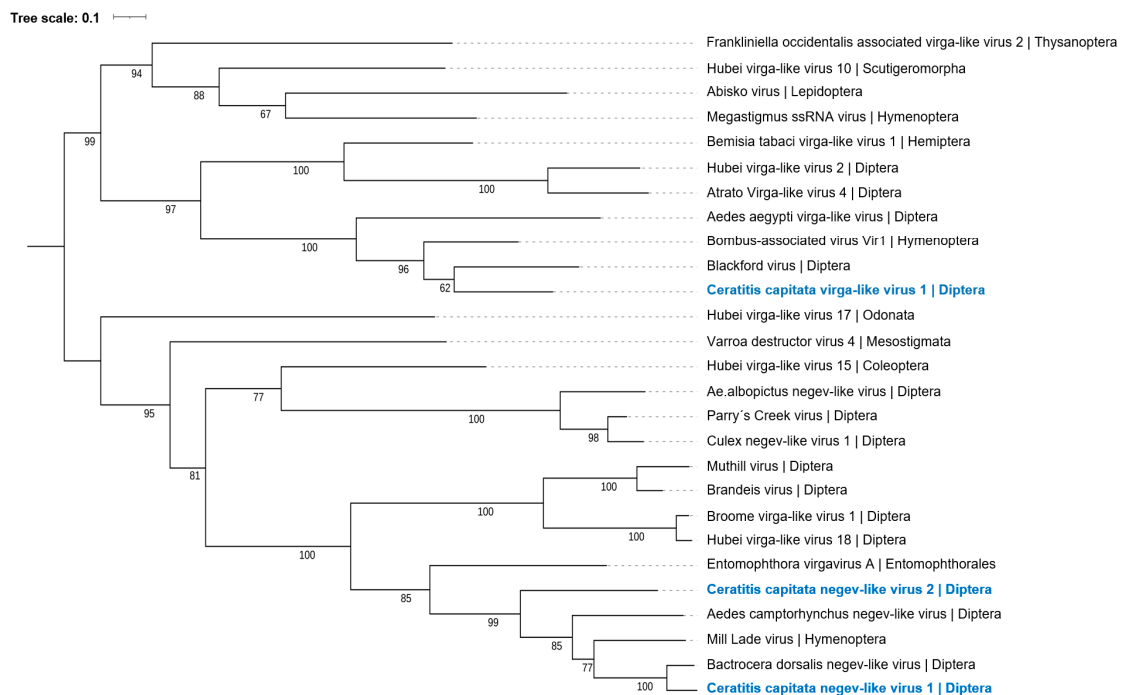
Tree scale: 0.1



D) *Ceratitis capitata* narnavirus 1:

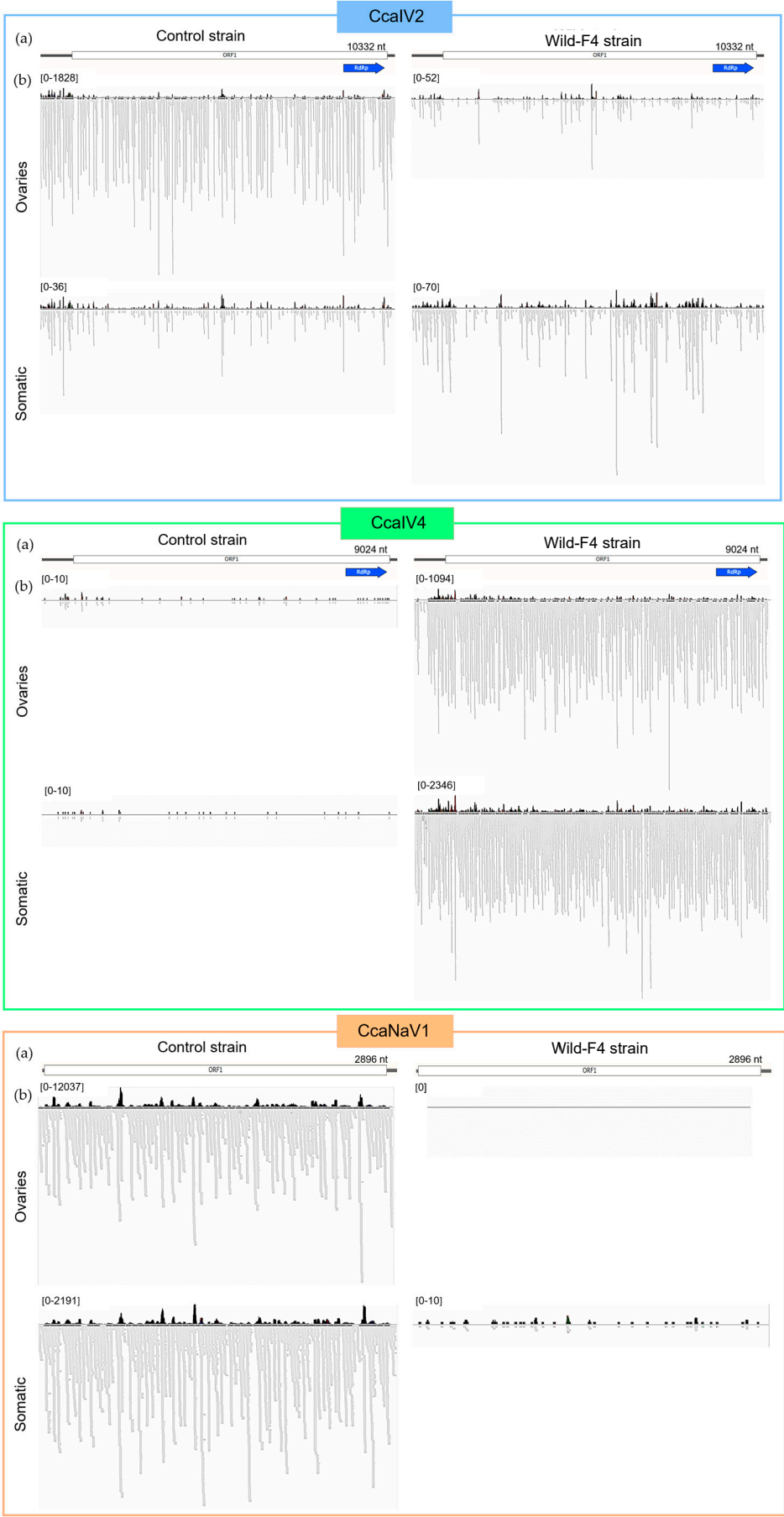


E) *Ceratitis capitata* virga-like virus 1; *Ceratitis capitata* negev-like virus 1, and *Ceratitis capitata* negev-like virus 2:



**Figure S1 Phylogenetic classification of the newly described medfly covert RNA viruses.** Sequences were analyzed at the amino acid level and the closest viral relatives to the medfly viruses were detected using blastp (Table S3). Phylogenetic inferences were calculated using IQtree2 with default settings and 1000 ultrafast bootstrap. RNA viruses infecting medfly are shown in blue in the figures.

Supplementary Figure S2:





*Supplementary Table S1* List of the 13 RNA viruses described in the medfly and the primers designed to amplify them through RT-qPCR.

| <b>Virus</b>    | <b>Genome length (bp)</b> | <b>NCBI accession number</b> | <b>Forward primer</b>     | <b>Reverse primer</b>       | <b>Estimated efficiency (%)</b> |
|-----------------|---------------------------|------------------------------|---------------------------|-----------------------------|---------------------------------|
| <b>CcaIV1</b>   | 10502                     | GAMC01001920.1               | TATGTGTTGTCCACCCCAGC      | TGTGCGCATGGTGATTCTAAC       | -                               |
| <b>CcaIV2</b>   | 10332                     | OL957305                     | CCAAGATAAGAATAGGCTAATGCGT | AACATTTCGCATTACCATTAAACACAG | 110.731                         |
| <b>CcaIV3</b>   | 7370                      | HG994137                     | GATGTTGGTGAGTTCCTC        | CTATTGCCGTTAGTGAGAC         | -                               |
| <b>CcaIV4</b>   | 9023                      | HG994138                     | CGTTGGTTGTTGATTGGAAG      | GTGAAGATTGTTGGCGGAAC        | 131.196                         |
| <b>CcaNaV1</b>  | 2896                      | OL957306                     | GCTCCATCTTGGGTTCATTG      | CTGCCGTCTCCATCTTCC          | 100.707                         |
| <b>CcaNdV1</b>  | 3092                      | OL957308                     | CTAATCGGTATGGGACAC        | GACAAACTGGAGACACAAC         | 114.666                         |
|                 | 1798                      | OL957309                     | -                         | -                           | -                               |
| <b>CcaNeLV1</b> | 10258                     | HG994139                     | GCGGGTAGAAGAGTATTG        | GACATTTCGAGATAACAG          | 118.508                         |
| <b>CcaNeLV2</b> | 10506                     | OL957307                     | CCCATCTAACCCCTACTCC       | GCGTTCTTCTTGTCATTC          | 117.12                          |
| <b>CcaNV</b>    | 12014                     | GAMC01015827.1               | GCTCAAGCAAAAGCAGGCAG      | AGCATTTCAGTCCGCTACGA        | 110.981                         |
| <b>CcaViLV1</b> | 9925                      | GAMC01017950.1               | ATGATACGGTTGACTTTCAG      | GAGGTGGTGACGAGTAAG          | 109.206                         |
| <b>CcaSV</b>    | 12583                     | KR822825.1                   | TACAAACAAACACGAAGC        | CACACCCATAACCTACAG          | -                               |
| <b>CcaRLV1</b>  | 4177                      | OL957310                     | GACTAACAGGTCTCAAGG        | CGCTACTCTAACTTGACC          | 94.234                          |
|                 | 3857                      | OL957311                     | -                         | -                           | -                               |
|                 | 3141                      | OL957312                     | -                         | -                           | -                               |
| <b>CcaTV1</b>   | 6132                      | OL957313                     | CTCAAGGAAGAGACACAG        | GAGATATGTCTGGCGTAG          | -                               |
| <b>L23a</b>     | 991                       | XM004518966                  | GCCGAGAAATCCGCCAAATC      | CTTGCTGCAGCGGGTTAG          | 104.17                          |

Supplementary Table S2 List of 48 medfly sequence read archives (SRAs) queried for viral presence and relative abundance using *in silico* methods.

| NCBI accession number | Sequencing method                         | <i>C. capitata</i> origin | Library details             |                    | SRA accession number |
|-----------------------|---|---------------------------|-----------------------------|--------------------|----------------------|
| SRP133427             | Illumina HiSeq 2000                       | Benakeion                 | 10 adults, whole body       | 10 adult M         | SRR6780762           |
|                       |   |                           |                             | 10 adult F         | SRR6780764           |
| ERP119522             | Illumina NovaSeq 6000; pair end           |                           | Pool of 3 larvae            | Immobilized larvae | ERR4026379           |
|                       |   |                           |                             | Jumping larvae     | ERR4026376           |
| SRP073787             | Illumina HiSeq 2000, single               | Egypt II                  | Gastrointestinal tract      | Virgin female      | SRR3436822           |
|                       |   |                           | Reproductive tract          | Mated male         | SRR3436821           |
|                       |   |                           |                             | Mated male         | SRR3436820           |
|                       |   |                           |                             | Mated female       | SRR3436819           |
|                       |   |                           |                             | Mated female       | SRR3436818           |
|                       |   |                           |                             | Virgin male        | SRR3436817           |
|                       |   |                           |                             | Virgin male        | SRR3436816           |
|                       |   |                           |                             | Virgin female      | SRR3436815           |
|                       |   |                           |                             | Virgin female      | SRR3436814           |
|                       |   |                           | Gastrointestinal tract      | Virgin female      | SRR3436813           |
| SRP021914             | Illumina Genome Analyzer Iix; pair end    | Ispra                     | Adult                       | Male               | SRR836190            |
|                       |   |                           |                             | Female             | SRR836189            |
|                       |   |                           | Embryo                      |                    | SRR836188            |
| ERP124511             | Illumina HiSeq 2000 paired end sequencing | Madrid                    | Whole adult, Control strain | Replicate 1        | ERR4690327           |
| Replicate 2           |   |                           |                             | ERR4690326         |                      |
| Replicate 3           |   |                           |                             | ERR4690325         |                      |
| SRP343008             |   |                           | Whole adult, W-1Kλ strain   | Replicate 1        | SRR16562767          |
|                       |   |                           |                             | Replicate 2        | SRR16562768          |
|                       | Replicate 3                               | SRR16562769               |                             |                    |                      |
| SRP075464             | Illumina Genome Analyzer II               | Toliman                   | Female ovary                |                    | SRR3554596           |
|                       |   |                           | Elongated spermatids        |                    | SRR3554595           |
|                       |   |                           | Round spermatids            |                    | SRR3554594           |
|                       |   |                           | Late spermatocytes          |                    | SRR3554593           |
|                       |   |                           | Early spermatocytes         |                    | SRR3554592           |

|           |                                 |          |                  |                    |            |
|-----------|---------------------------------|----------|------------------|--------------------|------------|
| ERP119522 | Illumina NovaSeq 6000; pair end | Wp       | Pool of 3 larvae | Immobilized larvae | ERR4026385 |
|           |                                 |          |                  | Jumping larvae     | ERR4026382 |
| SRP026213 | Illumina HiSeq 2000             | Vienna 7 | Non-irradiated   | Pupae              | SRR915844  |
|           |                                 |          |                  |                    | SRR915840  |
|           |                                 |          |                  |                    | SRR915839  |
|           |                                 |          |                  | Adult              | SRR915838  |
|           |                                 |          |                  |                    | SRR915837  |
|           |                                 |          |                  |                    | SRR915836  |
|           |                                 |          | Irradiated       | Pupae              | SRR915835  |
|           |                                 |          |                  |                    | SRR915834  |
|           |                                 |          |                  |                    | SRR915833  |
|           |                                 |          |                  | Adult              | SRR915832  |
|           |                                 |          |                  |                    | SRR915831  |
|           |                                 |          |                  |                    | SRR915830  |
| SRP026213 | Illumina HiSeq 2000             | Hawaiian | Whole body       | Wild pupae         | SRR915852  |
|           |                                 |          |                  |                    | SRR915851  |
|           |                                 |          |                  |                    | SRR915850  |
|           |                                 |          |                  | Wild adults        | SRR915848  |
|           |                                 |          |                  |                    | SRR915846  |
|           |                                 |          |                  |                    | SRR915845  |

*Supplementary Table S3 List of viral proteins included in the phylogenetic analysis.*

| Viral name  | Protein accession number | Viral name   | Protein accession number |
|---|--------------------------|--|--------------------------|
| <b>(A) Totiviridae</b>                                    |                          | <b>(D) Narnaviridae</b>                                  |                          |
| Aedes aegypti totivirus                                   | BBM96510.1               | Beihai narna-like virus 24                               | YP_009333245.1           |
| Australian Anopheles totivirus                            | ASU43981.1               | Ceratitis capitata narna-like virus 1                    | *OL957306                |
| Bactrocera dorsalis toti-like virus 1                     | QMU95572.1               | Hubei narna-like virus 15                                | YP_009337783.1           |
| Bactrocera dorsalis toti-like virus 2                     | QMU95575.1               | Meagle narna-like virus                                  | QIJ70070.1               |
| Beihai toti-like virus 2                                  | APG75984.1               | Plasmopara viticola lesion associated narnavirus 40      | QIR30319.1               |
| Camponotus nipponicus virus                               | YP_009230208.1           | Soybean thrips narna-like virus 1                        | QQP18719.1               |
| Camponotus yamaokai virus                                 | YP_009143313.1           | Soybean thrips narna-like virus 2                        | QQP18720.1               |
| Ceratitis capitata totivirus 1                            | *OL957313                | Wenling narna-like virus 7                               | YP_009337166.1           |
| Fitzroy Crossing toti-like virus 1                        | QLJ83495.1               | Wenling narna-like virus 8                               | APG77263.1               |
| Hubei toti-like virus 13                                  | YP_009336780.1           | Wuchang cockroach Virus 4                                | YP_009342297.1           |
| Hubei toti-like virus 14                                  | APG76023.1               | <b>(E) Negeviridae</b>                                   |                          |
| Hubei toti-like virus 15                                  | YP_009336859.1           | Abisko virus   | YP_009408586.1           |
| Hubei toti-like virus 24                                  | YP_009336908.1           | Ades albopictus negev-like virus                         | QGN03453.1               |
| Larkfield virus   | AWA82248.1               | Aedes aegypti virga-like virus                           | BBN20999.1               |
| Rice Toti-like virus                                      | QKN84381.1               | Aedes camptorhynchus negev-like virus                    | YP_009388601.1           |
| Sogatella furcifera totivirus 1                           | YP_009552726.1           | Atrato Virga-like virus 4                                | QHA33744.1               |
| Sogatella furcifera totivirus 2                           | YP_009553182.1           | Bactrocera dorsalis negev-lik virus                      | QMU95554.1               |
| <b>(B) Reoviridae</b>                                     |                          | Bemisia tabaci virga-like virus 1                        | QNJ34552.1               |
| Bloomfield virus  | AWB14602.1               | Blackford virus  | KU754514.2               |
| Bobbyc reo-like virus                                     | QIJ70114.1               | Bombus-associated virus Vir1                             | QAY29261.1               |
| Ceratitis capitata reo-like virus 1                       | *OL957310                | Brandeis virus   | AVZ66283.1               |
| Elf Loch virus  | QAY29252.1               | Broome virga-like virus 1                                | QLJ83498.1               |
| Hubei diptera virus 20                                    | APG79173.1               | Ceratitis capitata Negev-like virus 1                    | *HG994139                |
| Hubei odonate virus 14                                    | APG79163.1               | Ceratitis capitata Negev-like virus 2                    | *OL957307                |
| Hubei reo-like virus 1                                    | APG79117.1               | Ceratitis capitata Paretaria Mottle-like virus           | *GAMC01017950.1          |
| Hudson virus  | QED21522.1               | Culex negev-like virus 1                                 | YP_009388585.1           |
| Rice black streaked dwarf virus                           | QFO48930.1               | Entomophthora virgavirus A                               | QED42956.1               |
| Shenzhen reo-like virus 1                                 | QPN36950.1               | Frankliniella occidentalis associated virga-like virus 2 | QNM37806.1               |
| Skokie reo-like virus                                     | QPG92977.1               | Hubei virga-like virus 10                                | APG77802.1               |
| <b>(C) Nodaviridae</b>                                    |                          | Hubei virga-like virus 15                                | YP_009337693.1           |
| Black beetle virus  | YP_053043.1              | Hubei virga-like virus 17                                | YP_009337715.1           |
| Boolarra virus  | NP_689439.1              | Hubei virga-like virus 18                                | YP_009333286.1           |
| Ceratitis capitata nodavirus 1                            | *OL957308                | Hubei virga-like virus 2                                 | YP_009337412.1           |
| Drosophila melanogaster American nodavirus (ANV) SW-2009a | ACU32794.1               | Megastigmus ssRNA virus                                  | QDZ71189.1               |
| Flock House virus   | NP_689444.1              | Mill Lade virus  | QAY29259.1               |
| Gungahlin Chrysomya noda-like virus                       | QIJ70031.1               | Muthill virus  | AMO03223.1               |
| Hubei noda-like virus 21                                  | APG76486.1               | Parry's Creek negev-like virus 1                         | QLJ83503.1               |
| Hubei noda-like virus 23                                  | APG76332.1               | Varroa destructor virus 4                                | QGA69815.1               |
| Newington virus   | AMO03244.1               |  |                          |
| Wenzhou noda-like virus 6                                 | APG76600.1               |  |                          |

\*Nucleotide accession numbers were provided when protein accession numbers were not available. Nucleotide sequences were translated into protein using the standard code.



*Supplementary Table S4. Total number of sRNA reads mapping to each RNA virus and the percentage they represent respect to the total number of reads.*

|          | <i>C. capitata</i> Control strain            |       |                                       |       | <i>C. capitata</i> Wild-F4 strain            |      |                                       |      |
|----------|--|-------|---------------------------------------|-------|--|------|---------------------------------------|------|
|          | <b>Somatic tissue</b><br>(322209 sRNA reads) |       | <b>Ovaries</b><br>(665240 sRNA reads) |       | <b>Somatic tissue</b><br>(454562 sRNA reads) |      | <b>Ovaries</b><br>(286966 sRNA reads) |      |
|          | Aligned reads                                | %     | Aligned reads                         | %     | Aligned reads                                | %    | Aligned reads                         | %    |
| CcaIV1   | 5  | 0     | 41                                    | 0,010 | 11   | 0    | 5                                     | 0    |
| CcaIV2   | 511  | 0,160 | 7118                                  | 1,070 | 1563   | 0,34 | 465                                   | 0,16 |
| CcaIV3   | 1  | 0     | 37                                    | 0,010 | 3  | 0,   | 1                                     | 0,00 |
| CcaIV4   | 29   | 0,010 | 57                                    | 0,010 | 20182  | 4,44 | 10116                                 | 3,53 |
| CcaNaV1  | 6479   | 2,010 | 13672                                 | 2,060 | 46   | 0,01 | 78                                    | 0,03 |
| CcaNdV1  | 91   | 0,030 | 313                                   | 0,050 | 40351  | 8,88 | 11451                                 | 3,99 |
| CcaNeLV1 | 1269   | 0,390 | 2738                                  | 0,410 | 2261   | 0,50 | 1226                                  | 0,43 |
| CcaNeLV2 | 2  | 0     | 2                                     | 0     | 1  | 0    | 0                                     | 0    |
| CcaNV    | 0  | 0     | 2                                     | 0     | 67   | 0,01 | 28                                    | 0,01 |
| CcaRLV1  | 1  | 0     | 0                                     | 0     | 92   | 0,02 | 0                                     | 0    |

Four datasets were analyzed, representing somatic tissue and ovaries samples from “Control” and “Wild-F4” medfly strains. The distribution of these reads along the genomes of CcaIV2, CcaIV4, CcaNaV1, CcaNdV1, CcaNeLV1, and CcaNV is displayed in Figure S2. No sRNA reads mapping to CcaSV, CcaTV1, and CcaViLV1 were found.