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1 Cover page

2 **Genomic characterization of viruses associated with the parasitoid**

3 ***Anagyrus vladimiri* (Hymenoptera: Encyrtidae)**

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19 **Running title:** The Virome of a Biological Control Agent Parasitoid

20 Abstract

21 The knowledge on symbiotic microorganisms of insects has increased in recent years, yet
22 relatively little data is available on non-pathogenic viruses. Here we studied the virome of the
23 parasitoid wasp *Anagyrus vladimiri* (Hymenoptera: Encyrtidae), a biocontrol agent of mealybugs.
24 By high-throughput sequencing of viral nucleic acids, we revealed three novel viruses, belonging
25 to the families *Reoviridae* (provisionally termed AnvRV [*Anagyrus vladimiri* reovirus]),
26 *Iflaviridae* (AnvIFV) and *Dicistroviridae* (AnvDV). Phylogenetic analysis further classified the
27 AnvRV in the genus *Idnoreovirus*, and the AnvDV in the genus *Triatovirus*. The genome of
28 AnvRV is comprised of 10 distinct genomic segments ranging in length from 1.5 to 4.2 Kbp, but
29 only two out of the 10 open reading frames (ORFs) have a known function. AnvIFV and AnvDV
30 each have one polypeptide ORF, which is typical to iflaviruses but very un-common among
31 dicistroviruses. AnvRV was found to be fixed in a mass-reared population of *A. vladimiri*, whereas
32 it's prevalence in field-collected wasps was ~15%. Similarly, the prevalence of AnvIFV and
33 AnvDV were much higher in the mass rearing population than in the field population.
34 Transmission electron micrographs of females' ovaries revealed clusters and viroplasm of
35 *Reovirus*-like particles in follicle cells. AnvRV was not detected in the mealybugs, suggesting that
36 this virus is truly associated with the wasps. The possible effects of these viruses on *A. vladimiri*'s
37 biology, and on biocontrol agents in general are discussed. Our findings identify RNA viruses as
38 potential players involved in the multitrophic system of mealybugs, their parasitoids and other
39 members of the holobiont.

40 Importance

41 Different biological control approaches use industrially mass-reared natural enemy insects to
42 reduce damage of arthropod pests. Such mass-reared cultures may be positively and/or negatively
43 affected by various microorganisms, including viruses. Yet, current knowledge on virus diversity,
44 especially in arthropods, is limited. Here, we provide the first virome characterization of a member
45 of the wasps family Encyrtidae - the member being the parasitoid *Anagyrus vladimiri* - a
46 commercially important natural enemy of mealybug pests. We describe the genome of three
47 previously unknown RNA viruses, co-inhabiting this parasitoid, and elaborate on the prevalence
48 of those viruses in individual wasps of both mass-reared and environmental origins. Microscopy
49 images suggest that at least one of the viruses is transmitted maternally via the ovaries. We discuss

50 the genomic structure of the viruses and the possible relationship between those viruses and the *A.*
51 *vladimiri* host, with implications on improvement of biocontrol of mealybug pests.

52 Introduction

53 Numerous arthropod species host symbiotic microorganisms that are integral to their life history,
54 ecology and evolution (Zchori-Fein and Bourtzis, 2011). Such microorganisms engage in a wide
55 range of symbiotic relationships, from parasitism to obligate mutualism, and may affect various
56 biological features of their hosts (Zchori-Fein and Bourtzis, 2011; Drew et al., 2021). While the
57 diverse interactions of bacteria, and to a lesser extent of fungi, with insects have been extensively
58 studied, viruses associated with insects are much less explored. This is mainly due to paucity of
59 knowledge of a great fraction of virus diversity as attested by recent large-scale or more focused
60 surveys that unravelled numerous new virus lineages (Shi et al., 2016; Webster et al., 2016; Wu et
61 al., 2020).

62 Among insects, it appears that endoparasitoids have special relationships with viruses. Those
63 insects develop within an arthropod host, ultimately causing its death (Godfray, H. J., 1994). Most
64 parasitoids belong to the order Hymenoptera. Apart from pathogenic viruses, they often harbour
65 inherited viruses that may affect their phenotype. For instance, some viruses may affect their
66 behaviour, as observed in the solitary parasitoid *Leptopilina boulardi*. In that case, the virus forces
67 infected females to superparasitize (i.e., laying eggs in already parasitized hosts), thus favouring
68 its horizontal transmission within the superparasitized host (Varaldi et al., 2003, 2005). The ssRNA
69 iflavivirus DcPV is also injected by the parasitoid *Dinocampus coccinellae* into its ladybeetle host
70 during oviposition. The virus then replicates in the beetle's brain which may participate in turning
71 the ladybeetle into a 'zombie' that guards the parasitoid cocoon from predation (Dheilly et al.,
72 2015). Other true free-living viruses, such as the entomopoxvirus DIEPV found in some parasitoid
73 wasps, may protect the wasp offspring from host immune reaction (Coffman et al., 2020). On the
74 farther end of the pathogenic-mutualist axis, are some domesticated viruses that became integrated
75 into the insect genome. The best-known example of this phenomenon is the case of polydnviruses
76 (PDVs) which are associated with wasps that parasitize caterpillars of butterflies and moths. The
77 endogenized viral genes allow the production of viral-like particles which are injected together
78 with the eggs inside the caterpillar host and suppress its immune system, hence allowing the
79 successful development of the wasp larvae (Herniou et al., 2013). Evidently then, viruses have

80 diverse and important effects on the phenotype and ecology of endoparasitoids, and there are likely
81 many more virus-insect associations that are yet to be unveiled. In the current study the viruses
82 associated with *Anagyrus vladimiri* Triapitsyn (Hymenoptera: Encyrtidae), a solitary
83 endoparasitoid wasp attacking mealybugs (Hemiptera: Pseudococcidae), were identified and
84 characterized. *Anagyrus vladimiri* is mass-reared for commercial use in biological control
85 programs to manage two major global pest species, which attack plants in dozens of families: the
86 citrus mealybug, *Planococcus citri* (Risso) and the vine mealybug *P. ficus* (Signoret) (Bugila et
87 al., 2015). The female parasitoid lays an egg into the body of the mealybug host, which continues
88 to develop while the wasp larva feeds and develops inside it. After a few days the mealybug host
89 dies, its cuticle hardens and turns into a ‘mummy’ and the parasitoid larva pupates inside.
90 Eventually, the adult parasitoid chews a hole in the mummified host and emerges (Avidov et al.,
91 1967).

92 Recently, we reported an analysis of the fungal and bacterial portion of microbiome inhabiting this
93 population of *A. vladimiri*. We found that the symbiont *Wolbachia* is dominating the bacterial
94 community and under some conditions it has minor negative effects on the fecundity of the
95 parasitoid (Izraeli et al., 2020). The current research was launched to characterize the virome of *A.*
96 *vladimiri*, annotate the viral genomes, assess their phylogenetic position, and test the prevalence
97 of the various viruses in lab and field populations of the wasp.

98 Results

99 *Electrophoresis characterization of Anagyrus vladimiri virome*

100 Viral nucleic acids (VNAs) were purified from a pool of adults (from the mass-reared line, see
101 Table 1 for details) and treated by RNaseA and DNaseI. At least nine bands ranging in size from
102 ~1.5Kbp to ~4Kbp were clearly observed when VNAs were separated on non-denaturing gel by
103 electrophoresis (Fig. 1, lane 3). VNAs were successfully digested by RNaseA (Fig. 1, lanes 8-11),
104 but not by DNaseI (Fig. 1, lanes 13), suggesting that the virome of *A. vladimiri* is composed of
105 RNA viruses only. For both our positive control (Phi6 dsRNA) and the VNAs from *A. vladimiri*,
106 the RNase digestion was mitigated in high concentration of sodium citrate buffer (=high ionic
107 strength) with low concentration of RNase, which is the expected outcome when the RNA is
108 double-, and not single-stranded (Sorrentino et al., 1980; Khramtsov et al., 1997). These results
109 suggest that the tested strain of *A. vladimiri* harbours at least one, dsRNA virus with nine segments
110 (or more, since segments of similar size may co-migrate at the same position), or several non-
111 segmented or segmented dsRNA viruses.

112 *Sequencing the RNA virome of A. vladimiri*

113 After reverse transcription, the VNAs of *A. vladimiri* were sequenced on an Illumina platform. We
114 obtained 16 million of high quality paired-end reads. High sequence duplication levels were
115 observed (>96%), suggesting that the genome sequence is complete (except for the extremities of
116 untranslated regions), and as expected from the apparent low complexity of the sample (Fig. 1)
117 which totalled approximately 20-30Kbp. De novo assembly of this dataset, resulted in only 30
118 contigs, 18 of which were shorter than 1,500bp.

119 Homology (by amino acid sequence identity) of 11 out of the 12 contigs longer than 1,500bp, as
120 identified by BLASTx and PSI-BLASTp, were found to three RNA virus families; *Dicistroviridae*,
121 *Iflaviridae* (both positive strand ssRNA, order: *Picornavirales*) and *Reoviridae* (segmented linear
122 dsRNA, order: *Reovirales*) (Table 2, Fig. 2).

123 One contig was most similar to a *Dicistroviridae* member named Black queen cell virus infecting
124 honeybees, with an average of 41% amino acid sequence identity. The contig is 8,726bp long,
125 which is in the expected range for *Dicistriviridae* in general (8.5-10.2kb) and very close to the
126 genome length of Black queen cell virus (8,550bp). We propose to name this newly discovered
127 virus: *Anagyrus vladimiri dicistrovirus* (AnvDV).

128 One contig, 10,031bp long, had the highest similarity to different members of the family
129 *Iflaviridae*, with 46% amino acid identity with the iflavirus member *Dinocampus coccinellae*
130 paralysis virus used as a reference. The contig length is well within the expected range for
131 *Iflaviridae* (8.8-9.7kb). We propose to name this newly discovered virus: Anagyrus vladimiri
132 iflavirus (AnvIFV).

133 Nine contigs were identified as homologous to nine different segments of a reovirus infecting the
134 winter moth *Operophtera brumata* (ObIRV). Protein alignment to the known ObIRV segments as
135 a reference by BLASTp, revealed that the coverage between the nine contigs to the nine segments
136 ranges between 20-98%, while the amino acid sequence identity ranges between 19% and 37% in
137 all segments (Table 2). The one remaining contig out of the 12 ‘long’ ones, had no similarity
138 according to BLASTx and Psi-BLASTp searches (contig k199_18). However, its length was
139 similar to other contigs assigned to the reovirus, therefore it was added to the screening assay for
140 all reovirus segments in individuals. Results of that assay (see below) strongly support the
141 assumption that this contig is part of the reovirus genome, and together with the nine contigs
142 identified by BLAST, this genome consists of 10 segments. We propose to name this newly
143 discovered segmented virus: Anagyrus vladimiri reovirus (AnvRV).

144 Six out of the 18 contigs shorter than 1,500bp (contigs k119_1, k119_3, k119_5, k119_8, k119_9
145 and k119_13; Fig. 2), were also assigned to the *Iflaviridae* family by the BLASTx search (Table
146 2, Fig. 2). Aligning those to the long AnvIFV contig showed that the small contigs are actually
147 parts of this long contig with >96% nucleotide identity. They were discarded from the analysis.

148 The nearly-complete genome sequence of the three novel RNA viruses is available in GeneBank
149 under BioProject PRJNA641546, with the following BioSample accession numbers ___.

150 *Annotation*

151 Analysis of the recovered contigs predicted that all 12 ‘long’ (>1,500bp) ones have a large open
152 reading frame (ORF), covering >90% of their length, suggesting that they encode true functional
153 genes. In contrast, most of the 18 ‘short’ (<1,500bp) contigs had large portions of un-translated
154 regions (UTRs), with the ORFs covering on average less than 60% (Table 2, Fig. 2).

155 Interestingly, the contig identified as AnvDV harbours only one ORF, unlike most known viruses
156 of this family, which harbour two ORFs. Furthermore, no ‘internal ribosome entry site’ (IRES) -
157 an RNA element typically found between the two ORFs of dicistroviruses (and enables the
158 translation initiation of the second ORF) - was found along this contig. The genomes of the three

159 RNA viruses were further annotated by searching the predicted ORFs for conserved domains using
160 the NCBI CDD tool. Both the AnvIFV and AnvDV were found to each harbour five domains
161 encoding for: RNA-dependent RNA polymerase (RdRP) (accession: cd01699), RNA helicase
162 (accession: pfam00910), capsid proteins (CRPV_capsid superfamily, found in many
163 dicistroviruses) (accession: cl07393) and two ‘rhv-like’ domains (part of a capsid protein, named
164 as ‘drug-binding pocket’ found in poliovirus, also a member of *Picornavirales* order) (accession:
165 cd00205) (Fig. 3a).

166 No conserved domains were found for the 10 AnvRV segments. Nevertheless, two genes were
167 inferred from the BLASTx searches by homology with the ObIRV genome: RdRP on segment
168 no.1 and a polyhedrin protein on segment no.4 (Fig. 3b).

169 One conserved sequence motif (GAAGAKC) was found in the 3’ end termini of the five out of 10
170 contigs assigned to AnvRV (supp. fig 2). No conserved sequences were found in the 5’ end,
171 suggesting that a few bases in the extremities of the contigs may be missing in our current
172 assembly.

173 *Phylogeny*

174 *AnvRV*. Phylogenetic analysis of the RdRP encoding segment of the AnvRV revealed that its
175 closest known relative is the ‘Zoersel tick virus’, identified from the tick *Ixodes ricinus*. This virus,
176 together with three other close relatives, have no genus taxonomic classification. The closest
177 classified virus is the ObIRV, which belongs to the genus *Idnoreovirus* (Fig. 4). Pairwise
178 alignments of the AnvRV to those five viruses reveals a 34-43% RdRP amino acid sequence
179 identity, while it’s amino acid identity with all other viruses compared in the phylogenetic analysis
180 was 27% and lower. As the common cut-off between ICTV verified genera is 30% amino acid
181 identity (Matthijssens et al., 2022), this suggests that the AnvRV, together with the four other
182 unclassified relatives, belongs to the genus *Idnoreovirus* (Fig. 4).

183 *AnvDV*. The phylogenetic analysis for the AnvDV clearly show that this virus belongs to the genus
184 *Triatovirus*, together with the Black queen cell virus (51% amino acid RdRP identity) and other
185 relatives (Fig. 5).

186 *AnvIFV*. The phylogenetic analysis for the AnvIFV place this virus near the *Nasonia vitripennis*
187 virus [accession ACN94442.1], *Aedes vexans* iflavirus [QGW51140.1](61-62% amino acid RdRP
188 identity), and others. The unique *Dinocampus coccinellae* paralysis virus [YP_009111311.1], also
189 clusters on the same branch with AnvIFV, and has 58% amino acid RdRP identity with it (Fig. 5).

190 *Prevalence of viruses in lab and field samples*

191 To assess the prevalence of the three RNA virus candidates in the different *A. vladimiri*
192 populations, diagnostic PCRs with specific primers were used (Supp. table 1) on 20 individual
193 females from each of the *Wolbachia*-carrying (W^+) and *Wolbachia*-free (W^-) lab-reared lines
194 (Izraeli et al., 2020). In addition, 24 field-collected individuals were screened (see Table 1 for
195 details).

196 All 40 lab-reared individuals harboured the AnvRV. The AnvIFV and the AnvDV were found in
197 33 and 25 specimens out of 40 individuals respectively (Fig. 6). All three viruses were also detected
198 in field individuals, although the prevalence was lower, with three females carrying AnvRV
199 (12.5%), 10 carrying AnvDV (42%), and two carrying AnvIFV (8%) out of 24 individuals (Fig.
200 6).

201 Interestingly, out of 10 field females carrying the AnvDV, only three lacked *Wolbachia*, and seven
202 out of the eight females carrying *Wolbachia*, also carried the AnvDV. The co-occurrence of those
203 two microbes was statistically significantly correlated (Fisher's exact test: $p=0.002$), suggesting a
204 positive interaction between *Wolbachia* and AnvDV within *A. vladimiri* populations. However,
205 AnvDV was present both in the *Wolbachia* positive and the *Wolbachia* negative strains in the lab.

206 *Screening for the 10 Reovirus segments in individual A. vladimiri wasps*

207 To test whether all the 10 Reovirus segments (nine identified by sequence homology, and one -
208 contig K119_18 - suspected by its length and UTR/contig length ratio) belong to the same AnvRV
209 genome, rather than to different RNA viruses, 24 individuals from two wasp lines were screened
210 by a set of diagnostic PCRs. Those included 16 individuals from the W^+RV^+ line - a line harbouring
211 the AnvRV as confirmed by PCR with primers targeting the RdRP segment, and 8 individuals
212 from the 'Field-RV-' line, as negative controls. Generally, the results were as expected (table 3);
213 in 9 W^+RV^+ individuals all the segments were amplified, while in 6 'Field-RV-' individuals, none
214 were amplified, suggesting that all 10 segments belong to the same virus. However, in 7
215 individuals, one or two segments failed to amplify, and in two individuals from the negative
216 population one single segment was detected (Table 3).

217 *Presence of viruses in the mealybugs*

218 To test whether any of the three RNA viruses originated from the mealybug hosts, four pools were
219 tested for their presence: i) Mass-reared (MR) W^+ *A. vladimiri*, ii) Field-collected *A. vladimiri* line
220 without the AnvRV (RV^-), iii) un-parasitized *P. citri*, and iv) *P. citri*, four days after parasitization

221 by MR *A. vladimiri*, (see Table 1 for details). AnvRV was not detected in the un-parasitized
222 mealybugs, nor in the Field-RV⁻ *A. vladimiri*, which were reared under the same conditions as the
223 MR *A. vladimiri*, indicating that this virus was not acquired from the mealybugs, and is associated
224 with *A. vladimiri*. In contrast, AnvIFV and AnvDV were detected in both *A. vladimiri* and un-
225 parasitized mealybugs (albeit the bands for the mealybug samples were weak, they were confirmed
226 by Sanger sequencing, with >99% identity), suggesting that those viruses may be acquired from
227 the mealybugs or from the rearing environment, possibly the potato sprouts that the mealybugs
228 feed on (Cheng et al., 2021), or even the various fungi that develop on this substrate
229 (supplementary Fig. 1).

230 *Virus particles in A. vladimiri ovaries*

231 To validate the presence of viruses in the females' reproductive tissues, transmission electron
232 microscopy (TEM) was carried on sections of *A. vladimiri* ovaries (from the W⁺RV⁺ line, see Table
233 1 for details). Images obtained from the three specimens tested showed clusters of round shaped
234 viral particles, 60-65nm in diameter, organized either in viroplasm structures, or in dense clusters
235 in the perinucleus area in the cytoplasm of follicle cells (Fig. 7). This pattern is typical to
236 *Reoviridae* viruses (Shah et al., 2017).

237 Discussion

238 The viral community of the parasitoid *A. vladimiri*, an agriculturally important biological control
239 agent of mealybugs, is dominated by two positive ssRNA viruses from the order *Picornavirales*,
240 which have sequence similarity to viruses of economically important insects, and a dsRNA
241 segmented genome, assigned to the *Idnoreovirus* genus of the *Reoviridae* family. The latter was
242 localized in the ovaries of the *A. vladimiri*, and was not found in the mealybug hosts, suggesting it
243 specifically infects the wasp.

244 As most insect viromes are yet to be explored, it was not surprising to discover new viruses in this
245 host (e.g. Shi et al. 2016). Nevertheless, to the best of our knowledge, other than this study there
246 is only one available detailed report on viruses in wasps of the family Encyrtidae, which reported
247 the genome sequence of one Picorna-like virus in the parasitoid *Diversinervus elegans* (Wu et al.,
248 2021).

249 Most reoviruses reported to infect insects were found in Dipterans and in Lepidopterans (most of
250 them in species that are important agricultural pests), and are assigned to the genera *Cypovirus* (16
251 accepted species, which include >75 isolates), and *Idnoreovirus* (7 accepted species, >10 isolates)
252 (Matthijnsens et al., 2022). Some are reported to cause chronic diarrheal disease to larvae, with
253 mild-severe symptoms, while many others have no apparent symptoms on their host
254 (Matthijnsens et al., 2022).

255 Out of the few reports on reoviruses infecting parasitoids, even fewer have tested for phenotypic
256 effects of the virus on its host. Renault et al., (2005) and Graham et al., (2008) both found no
257 evidence for phenotypic effects of reoviruses inhabiting *Diadromus pulchellus* and *Phobocampe*
258 *tempestiva* respectively. However, in some cases, reoviruses evidently cause significant effects on
259 their parasitoid host. Renault et al., (2003) reported that during parasitization *Diadromus*
260 *pulchellus* females inject a reovirus into the lepidopteran host together with the egg, where like the
261 PDV effect, it inhibits the immune response and allows the full development of the parasitoid
262 larva. In contrast, the cypovirus that infects both *Campoletis sonorensis* parasitoid and its
263 lepidopteran host (in which it cannot replicate), seems to be beneficial to the lepidopteran as it
264 reduces percent of mortality from parasitism (Deacutis, 2012).

265 Viruses of the families *Dicistroviridae* (15 accepted species, which include many more isolates)
266 and *Iflaviridae* (15 accepted species, >80 isolates) are more commonly associated with insects, but
267 similarly to reoviruses, in most cases their phenotype in the insect host is unknown (Valles et al.,
268 2017a, 2017b). Reported effects of viruses from those families are mostly pathogenic (e.g., Acute
269 bee paralysis virus, Drosophila C virus, Cricket paralysis virus, for *Dicistroviridae*, and Varroa
270 destructor virus1, Deformed wing virus for *Iflaviridae*). Conversely, at least one symbiotic
271 relationship of those viruses with parasitoids was reported. The iflavirus DcPV aids the
272 *Dinocampus coccinellae* to paralyze its ladybeetles host, as it replicates in the ladybeetle brain
273 possibly leading to behaviour changes that protect the developing parasitoid (Dheilly et al., 2015).
274 Another symbiotic relationship was found between a cripavirus (*Dicistroviridae*) and *Drosophila*
275 flies, where the virus seems to increase the fecundity of individuals that carry it (Zhang et al.,
276 2021).

277 As mentioned above, in most cases the relationship of the virus with its insect host is unknown.
278 As *A. vladimiri* wasps are reared in our lab for many generations, with no apparent disease
279 symptoms, it can be assumed that under the tested rearing conditions (especially the AnvRV,

280 which is fixed in the mass-reared population), the virus is at least not pathogenic, or even may be
281 beneficial to the wasp. Nonetheless, all scenarios are to be taken into consideration when
282 speculating on the relationship of those three novel viruses with *A.vladimiri*, including the
283 possibility that those viruses don't have any phenotypic effect, and are spread in the population
284 through efficient vertical and or horizontal transmission (e.g. Lüthi et al., 2020; Martinez et al.,
285 2016; Perreau & Moran, 2022) . Future study should address these possibilities.

286 Screening the wasp populations showed that while the two ssRNA viruses are not present in all
287 individuals, the AnvRV is fixed in the lab-reared lines, suggesting either efficient transfer of this
288 virus, whether vertical, horizontal or both (e.g. Varaldi et al., 2006). It may also suggest that the
289 specific rearing conditions favour the carrying of the virus (e.g. Himler et al., 2011; Coffman et
290 al., 2020). However, due to the low prevalence of the AnvRV in field populations of *A. vladimiri*,
291 we conclude that the interaction between the two is not obligatory. Additionally, while the two
292 ssRNA viruses were detected in virion extractions of unparasitized mealybugs, the AnvRV was
293 not, meaning it is a wasp symbiont. On the other hand, the two ssRNA viruses may originate from
294 the mealybug, or they can infect both the wasp and its host (e.g. Zhang et al., 2021)).

295 The TEM images of virus particles in follicle cells of *A. vladimiri*, resemble the typical pattern
296 found in viruses from the family Reoviridae, which are known to be found in the cytoplasm, to be
297 round shaped, 60-70nm long, and may be occluded in viroplasms (Shah et al., 2017). All species
298 of this genus were detected only in insects (Matthijssens et al., 2022) .Such a localization of a
299 microbe in the ovary, strongly suggests vertical maternal transfer, which in turn may hint on long
300 evolutionary relationships, and perhaps mutualism, between the two (Zchori-Fein and Bourtzis,
301 2011; Perlmutter and Bordenstein, 2020).

302 The evolutionary arms race between parasitoids and their hosts have led for several defence
303 mechanisms that hosts use to escape parasitism. In response to invasion by an endo-parasitoid egg
304 (or other foreign organisms), immune haemocytes in mealybugs may aggregate around the inserted
305 egg, and form a melanized capsule, which eliminates its development (Blumberg, 1997). The rates
306 of this encapsulation response to *A. vladimiri* parasitism is known to differ between *P. citri* and *P.*
307 *ficus* hosts species (Blumberg et al., 1995; Suma et al., 2012). it can be speculated that AnvRV
308 aids the wasp to overcome encapsulation, like DpRV2 does in the *Diadromus pulchellus* -
309 *Acrolepiopsis assectella* system (Renault et al., 2003), but with different efficiency in different
310 *Planococcus* hosts. If this speculation is indeed true then the different prevalence between lab and

311 field lines can be explained by the fact that the field population originated in vineyards where *P.*
312 *ficus* is the main host for *A. vladimiri*, whereas the lab population develops in *P. citri* hosts.

313 Overall results of the screening of all ten segments in *A. vladimiri* specimens, were conclusive and
314 indicate that all segments belong to the same virus. However, four segments failed to amplify in
315 between one-four out of 16 specimens tested. This might suggest that those four segments have
316 lower copy numbers than the other five. The detection of segment number four (k119_19) in two
317 specimens of the negative population can be explained by either technical error, or by
318 contamination.

319 From the pattern of *Wolbachia* and AnVDV prevalence in the 24 field collected females, where the
320 presence of those two microorganisms is somewhat correlated, it can be speculated that some
321 interaction takes place between those two. Reports on *Wolbachia* interaction with viruses focus on
322 associations with human viral pathogens vectored by insects and insect diseases caused by viruses.
323 For example, *Wolbachia* protects *Drosophila* flies from the Drosophila C virus (Family:
324 *Dicistroviridae*) (e.g. Hedges et al., 2008), and inhibits the Dengue Fever and Chikungunya
325 pathogens in humans (DENV and CHIKV, both also positive ssRNA viruses), (e.g. Hoffmann et
326 al., 2011; Walker et al., 2011). This effect is also detected in natural populations of *Drosophila*
327 and is however virus-specific (Cogni et al., 2021). Those inhibitions were generally correlated with
328 lower viral copy number of the viruses. In other cases, endosymbionts do correlate positively with
329 viral presence and transmission (e.g. Gottlieb et al., 2010; Kliot et al., 2014), such as reported here
330 for *A. vladimiri*, where a significant positive correlation was found between the presence of the
331 bacterium and the AnVDV in the field population, and also a higher prevalence in the lab strain
332 infected by *Wolbachia*. This is intriguing since it could suggest a positive effect of *Wolbachia* on
333 virus transmission, may further indicate that this virus and *Wolbachia* interact and clearly needs to
334 be further investigated.

335 Interestingly, in oppose to most species of *Dicistroviridae* members, which have a ‘Di-cistronic’
336 genome (the trait which is the source of the family’s name), only one polypeptide ORF could be
337 identified in AnVDV. This is more similar to other families in the *Picornavirales* order, such as
338 *Iflaviridae* and *Picornaviridae*, however in those families the genes are in opposite order to the
339 order that was found in the AnVDV, where non-structural proteins are closer to the 3’ end (Bonning
340 and Miller, 2010). As far as we know, such genomic arrangement was found only in a few viruses,
341 most of which are from aquatic invertebrates (Shi et al., 2016, see supplementary data #33; Cheng

342 et al., 2021; Wu et al., 2021). This rare finding suggests that this family has a broader diversity
343 than previously known.

344

345 **Conclusion**

346 Most studies on viruses in insects focus on pathogenic interactions or transient in vectors to plants
347 or animals. This is one of the first attempts to characterize the virome of a biocontrol agent, that
348 overall seems to be healthy. The discovery of three new RNA viruses in a single population with
349 no apparent diseases, highlights the richness of this hidden community of viruses. This community
350 certainly deserves further investigation in order to clarify its transmission along generations and
351 the phenotypic effects associated. If one (or more) of the three viruses influences the efficiency of
352 *A. vladimiri* as a natural enemy, whether positively or negatively, this knowledge can be applied
353 to improve the biological control of mealybug pests.

354 Methods

355 *Insect rearing and field collections of parasitoids*

356 *Anagyrus vladimiri* wasps were obtained from 'BioBee Sde Eliyahu Ltd' mass-rearing (MR)
357 facility (Sde Eliyahu, Israel), and used to establish a *Wolbachia*-positive line (W^+), from which a
358 *Wolbachia*-free line (W^-) was established by antibiotic treatments (Izraeli et al., 2020). The two
359 lines were reared under controlled conditions of $26\pm 1^\circ\text{C}$, $60\pm 20\%$ RH, and 16L:8D photoperiod
360 regime on the citrus mealybugs *Planococcus citri*, feeding on sprouted potatoes *Solanum*
361 *tuberosum*.

362 Additional *A. vladimiri* individuals were collected in the field during the summer of 2020, using
363 funnel insect traps placed in an unsprayed vineyard in northern Israel (32.7200N, 35.1903E). The
364 traps ($n=10$), baited with mealybugs-infested potatoes, were retrieved from the field after one week
365 and parasitized mealybugs were incubated in the lab. The *A. vladimiri* adults which emerged
366 (emergence was achieved from three out of the 10 traps, the other seven were empty), were used
367 either for screening viruses' prevalence ($n=24$, results in Fig. 3), and others were let to parasitize
368 in the lab, establishing the 'Field' line ($n\sim 20$ female foundresses). One generation later, eight
369 females of this Field line were individually placed in cups and allowed to oviposit in mealybugs.
370 Mothers and three of their emerged offspring were then tested for the presence of the AnvRV and
371 *Wolbachia*. All the cups in which both symbionts were not detected ($n=7$, since *Wolbachia* was
372 detected in one mother) were pooled to establish an uninfected *A. vladimiri* line (termed hereafter
373 'Field-RV-' line).

374 The origins of the five wasp lines, and the various experiments they were used for are summarized
375 in Table 1.

376 *Enzymatic characterization of A. vladimiris' virome*

377 To characterize the viral community inhabiting *A. vladimiri*, viral nucleic acids (VNAs) were
378 purified following (Martinez et al., 2016). Briefly, viral capsids were purified from 0.5 g of MR
379 *A. vladimiri* wasps (pool of $>1,500$ individuals), by filtration, nuclease treatment (RNaseA and
380 DNaseI) and centrifugation. To classify the genome type of potential viruses (RNA/DNA,
381 double/single strand), VNAs were then extracted by SDS, subjected to different nuclease
382 treatments, and then examined on an 0.8% agarose gel (Martinez et al., 2016). A non-treated VNA

383 sample, a dsRNA ladder (Phi6, Invitrogen, MA, USA), and a DNA ladder (1Kb+, Invitrogen, MA,
384 USA) were used as controls.

385 *Sequencing strategy*

386 To further reveal all viral candidates inhabiting the wasp, VNAs were extracted from purified viral
387 capsids using the All-In-One DNA/RNA Miniprep Kit (Biobasic, ON, Canada). Since the gel
388 images produced in this process showed no indication of viral DNA molecules, only samples with
389 RNA VNAs were further examined and sequenced. Libraries were prepared by the TruSeq RNA
390 Ribo-Zero kit (Illumina, CA, USA) and sequenced by Illumina novaseq 6000 with 100 bp paired-
391 end reads.

392 Since the sequence yield was very high (~16 million paired-end reads, where suspected viruses'
393 genome size is up to 30 thousand bp), the data was randomly subsampled to three smaller datasets,
394 including 10^5 paired reads, 10^6 paired reads and 2×10^6 paired reads. The subsampled datasets
395 were *de-novo* assembled using 'Megahit' version 3.4 with default parameters. Comparing
396 assembly results of the three subsampled datasets to the original full dataset, revealed that most of
397 the contigs are identical, with a few additional small contigs and the 10^6 paired reads dataset was
398 chosen as the most suitable for further analysis (average fold (sometimes termed 'depth') per bp =
399 4.2×10^4). The resulting contigs were searched for homologous similarity against both the nr and
400 the swissprot databases using BLASTx, targeting viruses, bacteria and Hymenoptera (taxids
401 10239, 2, 7399 respectively). The following parameters were changed from default to increase
402 sensitivity: matrix – BLOSUM45, Gap costs – 12;2. An additional PSI-Blastp analysis, using the
403 protein sequences predicted by the ORFfinder online tool (RRID:SCR_016643), was used to
404 identify similarity of contigs that Blastx failed to identify.

405 *Annotation*

406 All contigs were subjected to an open reading frame (ORF) search using ORFfinder online tool
407 (RRID:SCR_016643), with start codon including alternative initiation codons. The length of the
408 un-translated regions (UTR), included the 5' and 3'UTRs, was calculated as contig length minus
409 the ORF length. As the ORFfinder tool may predict also false proteins, a calculation of UTR
410 divided by the contig length was done to estimate the likeliness of the contig to harbour a 'true'
411 protein, the higher the value the lower chance to have a 'true' protein.

412 The UTRs of the ten identified segments of AnvRV were searched for conserved sequence motifs
413 in the 5' and 3' end termini. This was done by subjecting those UTR sequences to a BLAST search
414 against themselves, with the automatic adjusted parameters for short sequences of NCBI BLASTn
415 suite.

416 Further annotation analysis of the viral genomes was done by subjecting all the ORFs to the NCBI
417 batch-CDD tool (Marchler-Bauer et al., 2015) , against the CDD v.3.19 database to search for
418 conserved domains. For relevant viruses a search for internal ribosome entry sites (IRES) was done
419 by the IRESite online tool (IRESite: The database of experimentally verified IRES structures,
420 2022) using the NUC4.4 matrix.

421 *Phylogeny*

422 To construct phylogenetic trees of the three novel viruses, the RdRP amino acid sequence of each
423 virus was aligned together with ~20 sequences of other viruses from the corresponding family.
424 Representative sequences were chosen from a BLASTp search and from the supplementary
425 material of Shi et al. 2016, to represent the whole family (or subfamily for the AnvRV). As the
426 AnvDV and AnvIFV belong to the same *Picornavirales* order, one tree was constructed for both
427 of them together. Multiple sequence alignment was conducted by Clustal Omega and a maximum
428 likelihood tree was constructed with the LG substitution model using PhyML V.3 (Sievers et al.,
429 2011) . Branch support was measured by approximate likelihood ratio tests (SH-aLRT; Guindon
430 et al., 2010). The outgroups chosen for the AnvRV tree were two mammal infecting viruses from
431 the subfamily *Sedoreovirinae*, and a poliovirus sequence was used for the *Picornavirales* tree.

432 *Virion purification from Anagyrus vladimiri and Planococcus citri*

433 To test whether any of the three RNA viruses originate from the mealybug hosts, virions were
434 purified from pools of: i) Mass-reared (MR) W⁺ *A. vladimiri* (~0.7 g), ii) Field-collected *A.*
435 *vladimiri* line without the AnvRV (RV⁻) (~0.2 g), iii) un-parasitized *P. citri*, (~0.7 g) and iv) *P.*
436 *citri*, four days after parasitization by MR *A. vladimiri*, (~0.3 g) (see Table 1 for details).
437 Purification was done according to Luria et al., (2020), with minor modifications. Briefly, the
438 insects were homogenized in a TBE buffer, supernatant was filtered through a 0.45 µm membrane,
439 placed on a 30% sucrose cushion, and subjected to ultracentrifugation at 242,922 g for 2 h. Then,
440 RNA was extracted from the pellet using the Viral RNA extraction kit (Bioneer, Daejeon, South

441 Korea). Detection of the three viruses (AnvRV, AnvIFV and AnvDV) in those samples was carried
442 by cDNA synthesis and PCRs as described below.

443 *PCR detections of the three viruses*

444 Detection of RNA viruses in *A. vladimiri* individuals was obtained by RNA extraction (RNeasy
445 plus kit, Qiagen, Düsseldorf, Germany) according to manufacturer protocol, with addition of
446 Dithiothreitol to homogenizing buffer, to inhibit degradation by RNase. RNA samples were
447 reverse-transcribed to cDNA using the RT-PCRbio kit (PCR Biosystems, London, UK), with no-
448 template and no-enzyme controls. cDNA was used as templates for diagnostic PCR to identify
449 presence/absence of the three viruses. Specific primers were designed for each virus and each viral
450 genomic segment according to the contig sequences identified from the RNA-seq data (Supp. table
451 1).

452 *Transmission electron microscope (TEM)*

453 *Anagyrus vladimiri* females from the W⁺RV⁺ line (see Table 1 for details) were used to test for the
454 presence of viruses in the reproductive tissues of the parasitoids. Dissected *A. vladimiri* ovaries
455 were fixed overnight with 1% Paraformaldehyde, 2.5% Glutaraldehyde in 0.1 M Cacodylate buffer
456 pH 7.4 containing 5 mM CaCl₂, washed in 0.1 M cacodylate, post fixed for 1 hour in with 1%
457 OsO₄ and 5 mM CaCl₂ in Cacodylate buffer. Then the samples went through dehydration in
458 ethanol and embedded in epon 812 (EMS). The blocks were cut with a diamond knife using a UC7
459 ultramicrotome (Leica, Wetzlar, Germany). Sections of ~75nm were collected on grids, contrast
460 stained for 10 min with 2% uranyl acetate and visualized in a TEM (L120C, Talos, Thermo Fisher
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599 Tables

Table 1. Summary of *Anagyrus vladimiri* lines used in this study.

<i>line name</i>	<i>source</i>	<i>experiments used for</i>
MR	‘Biobee’ mass-rearing facility	Virome analysis
W⁺RV⁺	‘Biobee’ mass-rearing facility, and further reared in our lab. Wasps carry <i>Wolbachia</i> and AnvRV.	Virus prevalence screening, Reovirus segment screening, Transmission electron microscopy
W⁻RV⁺	MR wasps that fed on antibiotics (Izraeli <i>et al.</i> 2020) Wasps carry AnvRV but not <i>Wolbachia</i> .	Virus prevalence screening
Field	Field collections, north Israel	Virus prevalence screening, Reovirus segment screening (negative controls)
Field-RV⁻	Iso-female lines of the field collected wasps	Presence of viruses in mealybugs

600 **Table 2.** Virome analysis of *A. vladimiri*. Details for all 30 contigs resulted from RNAseq.

contig name	contig length	ORF length	UTRs length	UTR/contig length	BLASTx hit	taxonomic group	amino acid identity (%)
k119_28	10031	9173	858	0.085535	YP_009111311.1	Iflaviridae	47
k119_29	8726	7829	897	0.102796	NP_620564.1	Dicistroviridae	38
k119_27	4250	4148	102	0.024	YP_392501.1	Reoviridae	33
k119_21	3936	3788	148	0.037602	QBA09478.1	Reoviridae	29
k119_25	3620	3521	99	0.027348	APG79179.1	Reoviridae	25
k119_19	3388	3257	131	0.038666	YP_392504.1	Reoviridae	45
k119_14	2163	1985	178	0.082293	YP_392505.1	Reoviridae	22
k119_18	1963	1388	575	0.292919		Reoviridae*	
k119_22	1880	1814	66	0.035106	QYV43128.1	Reoviridae	
k119_20	1865	1739	126	0.06756	AWA82241.1	Reoviridae	24
k119_12	1620	1526	94	0.058025	YP_392507**	Reoviridae	
k119_17	1535	1418	117	0.076221	QBA09478.1	Reoviridae	34
k119_24	1067	107	960	0.899719	XP_017796731.1	Hymenoptera	
k119_30	887	223	664	0.748591		NA	
k119_9	809	743	66	0.081582	YP_009342053.1	Iflaviridae	39
k119_10	782	311	471	0.602302		NA	
k119_16	711	0	711	1		NA	
k119_8	701	611	90	0.128388	YP_009342053.1	Iflaviridae	40
k119_15	571	437	134	0.234676	CAB0037418.1	Hymenoptera	
k119_2	564	389	175	0.310284	XP_015596585.1	NA	
k119_4	560	230	330	0.589286	EZA52908.1	Hymenoptera	
k119_7	451	320	131	0.290466	CAA84692.1	Phage	
k119_13	432	203	229	0.530093	YP_009342053.1	Iflaviridae	
k119_6	412	83	329	0.798544		NA	
k119_5	394	356	38	0.096447	QFP98431.1	Iflaviridae	36
k119_3	375	371	4	0.010667	YP_009342053.1	Iflaviridae	
k119_0	358	143	215	0.600559	ABD33943.1	Picornaviriales	
k119_23	351	86	265	0.754986	AGF91671.1	Phage	
k119_26	306	296	10	0.03268	QGJ03578.1	Phage	
k119_1	218	164	54	0.247706	YP_009342053.1	Iflaviridae	

601 *concluded as part of the genome of AnvRV by the segment screening assay (see table 3)

602 **this hit was found only by using the PSI-BLASTp algorithm

603 **Table 3.** Screening of ten AnvRV segments in 24 individuals from two lines of *A. vladimiri*; 16
 604 females from the MR population, and eight from Field population. ‘0’ denotes absence, ‘+’ denotes
 605 presence of the viral genomic segment by diagnostic PCR. Empty boxes denote untested samples.

Contig	Homologous ObIRV segment	Mass-reared population																Field population							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	1	2	3	4	5	6	7	8
k119_27	segment1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
k119_21	segment2	+	+	+	0	+	+	+	+	+	+	0	+	+	+	0	0	0	0	0	0	0	0	0	0
k119_25	segment3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
k119_19	segment4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	+
k119_14	segment5	+	+	+	0	+	+	+	0	+	+	+	+	0	+	+	+	0	0	0	0	0	0	0	0
k119_20	segment6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
k119_12	segment7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
k119_17	segment8	+	+	+	+	+	0	+	+	+	+	+	+	0	+	+	+	0	0	0	0	0	0	0	0
k119_22	segment10	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
k119_18	Unknown	+	+	+	+	+	+	+	+	+				0	+	+	+	0	0	0	0	0	0	0	0

606

607 Figure legends

608 **Fig 1.** Electrophoresis pattern (using a 0.8% agarose gel) of VNAs extracted from *A. vladimiri*,
609 which were treated by RNase and DNase separately. ‘AV’ denotes VNAs of *A. vladimiri*. ‘SSC’
610 denotes sodium citrate buffer, which was used in 0.01x and 2x ionic strengths. ‘10’/‘0.1’ denotes
611 high/low concentration ($\mu\text{g/ml}$) of RNase respectively. Details by lane numbers: 1-3; no digestion
612 treatment. 1; 1Kb ladder (1KB+, Invitrogen, MA, USA), 2; Phi6 – a ladder made of viral dsRNA
613 (Invitrogen, MA, USA), 3; *A. vladimiri* VNA. 4-11; Digestion by RNase A in four buffer/enzyme
614 concentrations. 4-7; Phi6 ladder as a control for the enzymatic activity of RNase. 8-11; AV
615 digestion. 12-13; Digestion by DNase I, 12; 1Kb – a DNA ladder as a control for the enzymatic
616 activity of DNase. 13; AV digestion.

617 **Fig 2.** Virome analysis of *A. vladimiri*. The assembled 30 contigs were assigned to seven
618 taxonomic groups according to the best BLASTx hit. **A)** Average depth per taxonomic group,
619 calculated as: $((\text{sum number of reads} * 100\text{bp}) / \text{sum length of contigs})$. **B)** Columns represent the size of
620 each of the 30 contigs (bp length). Purple crosses represent the proportion of both 5’ and 3’
621 untranslated regions (UTRs) to the contigs’ full length, as predicted by the ORF finder. High
622 proportion suggests insufficient protein products, while low proportion suggests a true protein is
623 translated from this contig. See table 2 for more details about each contig.

624 **Fig 3.** Genome annotation of the three novel RNA viruses. **A)** AnvDV and AnvIFV. White box indicates
625 location of ORF, colored boxes indicate identified protein domains. Numbers on scale are in bp according
626 to the full contig. RdRP, RNA-dependent RNA polymerase. **B)** Annotation of the segmented AnvRV.
627 The ten segments are drawn to scale, with boxes indicating the ORF location on the full segment, colors
628 represent the proteins encoded from those segments as assigned from homology to known sequences of the
629 ObIRV genome.

630 **Fig 4.** Phylogenetic analysis of AnvRV (colored), based on amino acid sequence similarities of the RdRP
631 genome segment (~1,200aa long). Representative viruses of all nine genera in the subfamily
632 Spinareovirinae are shown. The icons of organisms on the tips represent the taxonomic group of the host in
633 which the virus was found. The ● symbol indicate viruses that have not been classified to the genus level.
634 The human Rotavirus B and the bovine Bluetongue virus (Family: *Reoviridae*, subfamily: *Sedoreovirinae*)
635 were used as an outgroup. The tree was inferred by maximum-likelihood using the LG substitution model,
636 the numbers on branches indicate the results of approximate likelihood ratio tests for branch support (aLRT-
637 SH test; Guindon et al. 2010). Scale bar: 0.7 substitutions per site.

638 **Fig 5.** Phylogenetic analysis of *A. vladimiri* iflavivirus (AnvIFV) and *A. vladimiri* dicistrovirus
639 (AnvDV) (both colored), based on amino acid sequence similarities of the RdRP gene (~300aa
640 long). The human poliovirus (order: *Picornavirales*, family: *Picornaviridae*) was used as an out-
641 group. The icons of organisms on the tips represent the taxonomic group of the host in which the
642 virus was found (however, in some cases the viruses can be found in hosts from additional arthro-
643 pod orders, such as Cricket paralysis virus). The ‘star of David’ symbol signs the virus that is
644 reported to have a mutualistic relationship with its insect host. The phenotype of all other viruses
645 is either unknown or pathogenic. The tree was inferred by maximum-likelihood using the LG

646 substitution model, the numbers on branches indicate the results of approximate likelihood ratio
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648 **Fig 6.** The multi-infection status of three lines of *A. vladimiri* individuals by the three RNA viruses
649 and *Wolbachia*. Boxes on the same horizontal axis denote the infection status of the four microbes
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651 a segmented genome, was done with the primers targeted to the largest segment, encoding for the
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653 line, W-: wasps from *Wolbachia*-uninfected line.

654 **Fig 7.** Transmission electron microscope images of AnvRV particles in *A. vladimiri* follicle cells.
655 **A)** a cluster of virus particles (black arrow), **B)** a viroplasm (Vp), **C)** high magnification of a small
656 cluster of virions. N, nucleus; M, mitochondria. Scale bars; A) 500 nm, B) 500nm, C) 100nm.

657 Data availability

658 **Accession numbers to be received.**

659 Conflict of interest declaration

660 Author SS was employed by BioBee Sde Eliyahu Ltd. The remaining authors declare that the
661 research was conducted in the absence of any commercial or financial relationships that could be
662 construed as a potential conflict of interest.

663 Acknowledgements

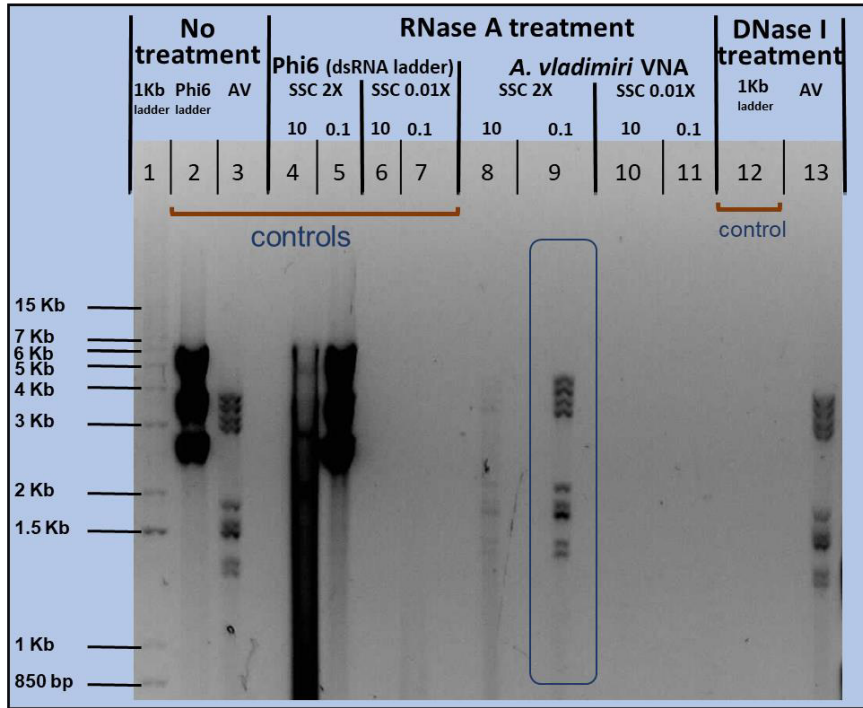
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1 Figures

2 Fig 1



3

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 9 control for the enzymatic activity of DNase. 13; AV digestion.

10

11

Fig 2

A

Taxonomic Group	Average Depth
Reoviridae	8
Iflaviridae	12621
NA	234
Hymenoptera	16
Phage	348
Dicistroviridae	307

B

Contig ID	Contig Length (bp)	UTR/contig_length
k119_28	~10000	~0.1
k119_29	~9000	~0.1
k119_27	~4000	~0.1
k119_21	~3800	~0.1
k119_25	~3500	~0.1
k119_19	~3200	~0.1
k119_14	~2000	~0.15
k119_18	~1800	~0.25
k119_22	~1600	~0.1
k119_20	~1500	~0.1
k119_12	~1400	~0.1
k119_17	~1300	~0.1
k119_24	~1000	~0.9
k119_30	~800	~0.1
k119_9	~700	~0.1
k119_10	~600	~0.55
k119_16	~500	~0.9
k119_8	~400	~0.15
k119_15	~300	~0.25
k119_2	~250	~0.3
k119_4	~200	~0.55
k119_7	~150	~0.25
k119_13	~100	~0.5
k119_6	~100	~0.75
k119_5	~100	~0.15
k119_3	~100	~0.1
k119_0	~100	~0.55
k119_23	~100	~0.65
k119_26	~100	~0.1
k119_1	~100	~0.25

12

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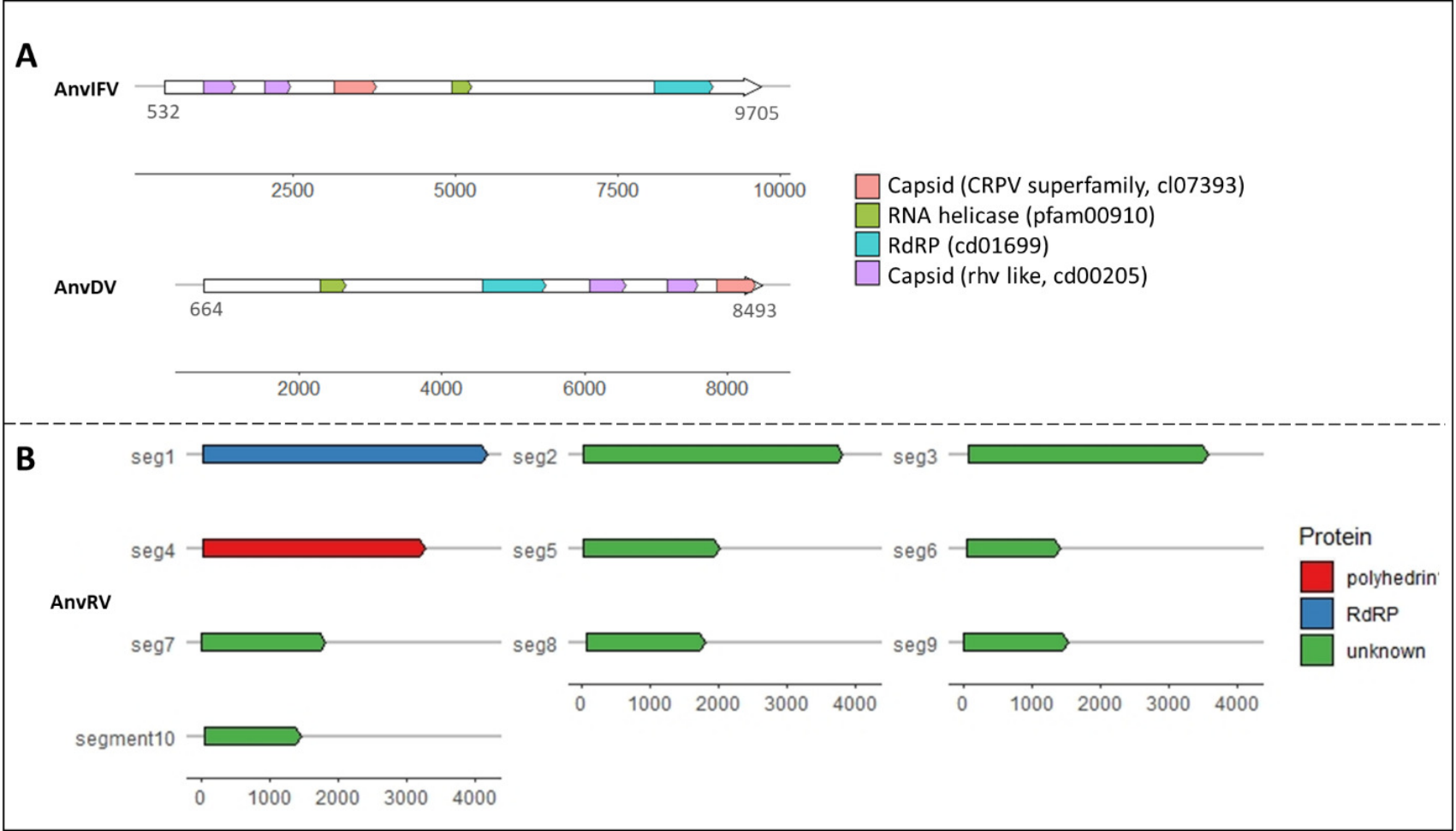
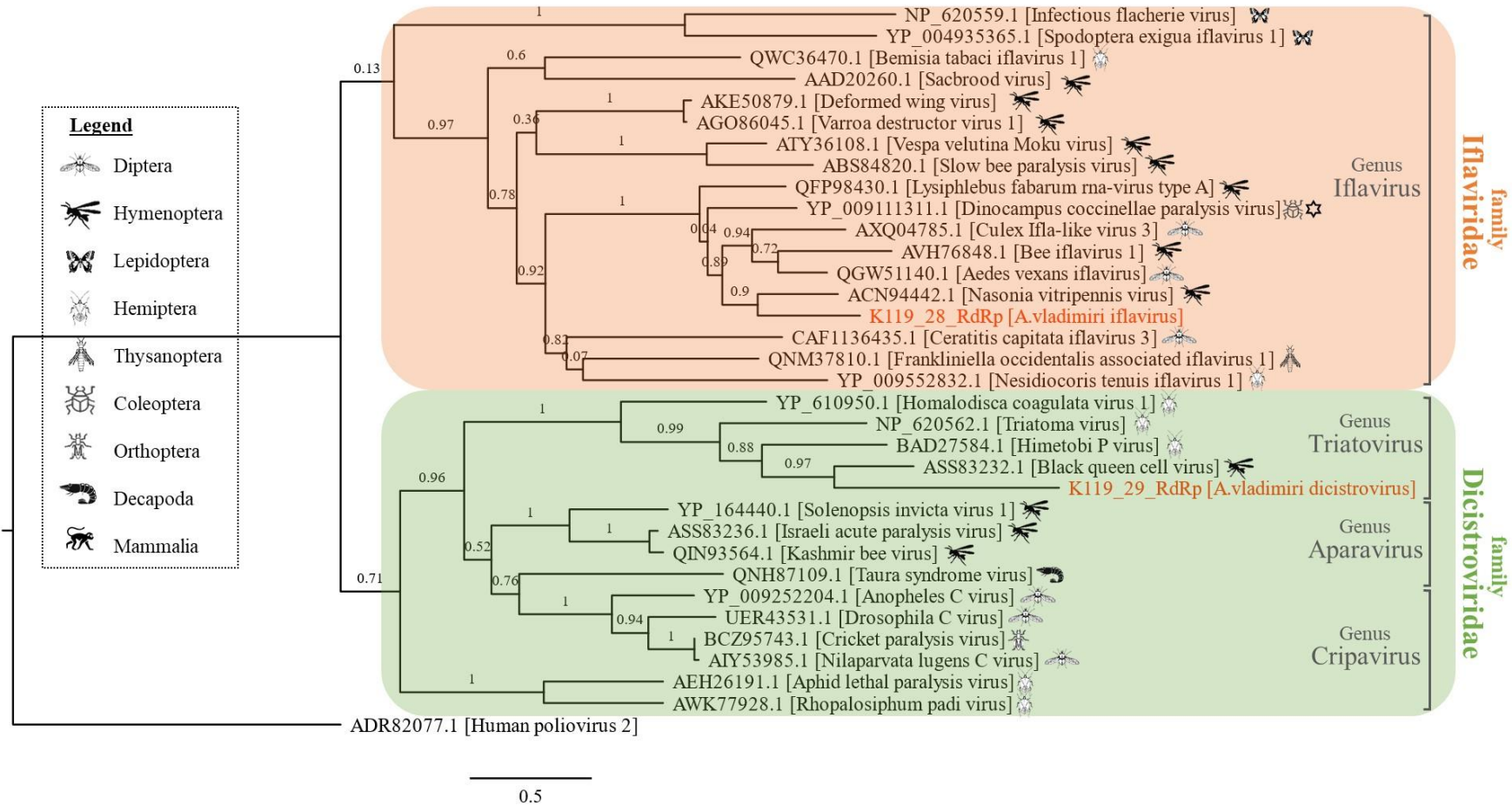
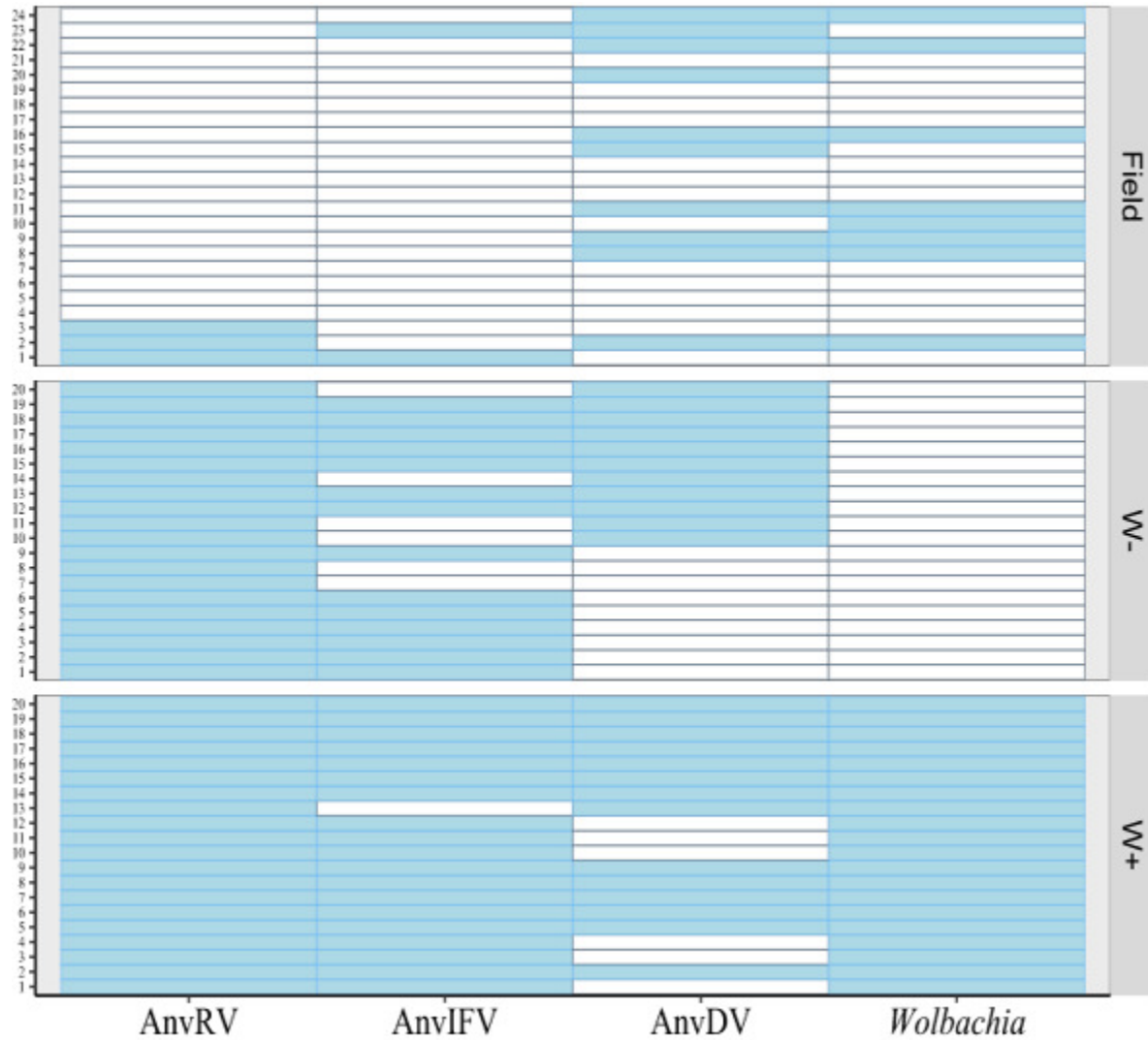


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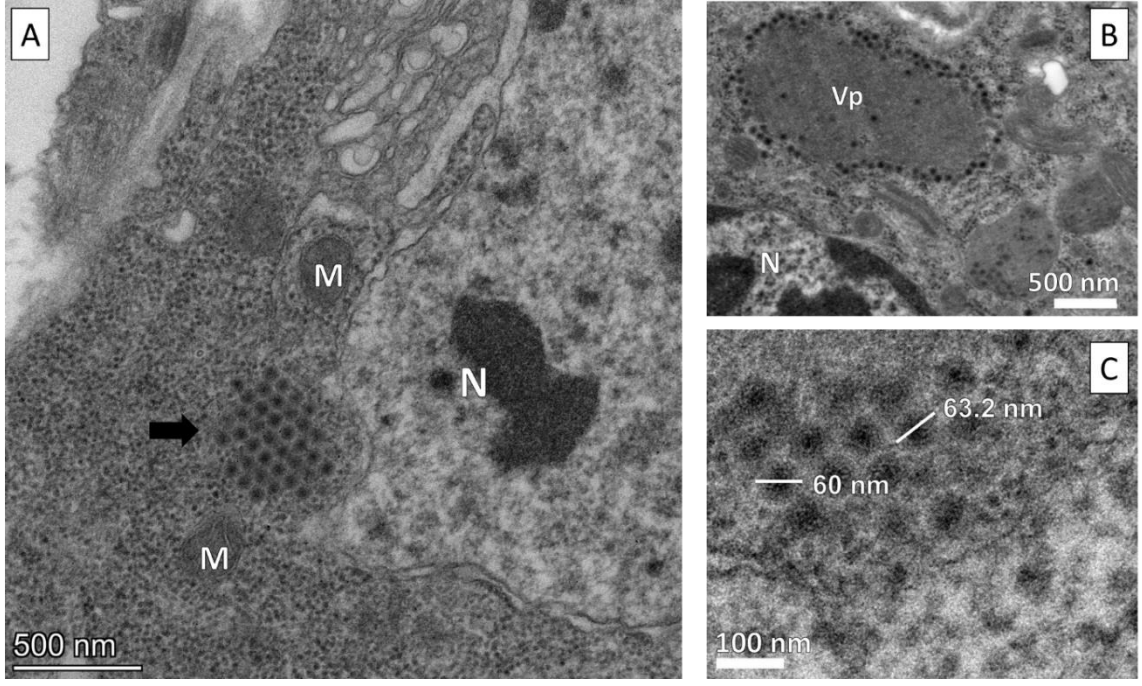
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46

Fig 7



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48

49

Fig 7. Transmission electron microscope images of AnvRV particles in *A. vladimiri* follicle cells. **A)** a cluster of virus particles (black arrow), **B)** a viroplasm (Vp), **C)** high magnification of a small cluster of virions. N, nucleus; M, mitochondria. Scale bars; A) 500 nm, B) 500nm, C) 100nm.

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