Discovery of new endogenous viral elements in campoplegine wasps (Ichneumonidae, Campopleginae, Campoplex) suggests a widespread nudivirus domestication event

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Abstract

Viral endogenization is a widespread phenomenon that generally results in non-functional viral elements. In parasitoid wasps, nudiviruses have been involved in three independent domestication events and play a key role in parasitism success. In Campopleginae (Ichneumonidae), endogenized ichnovirus presence is widespread, with the exception of *Venturia canescens* (Ichneumonidae Campopleginae), which is known to harbour an endogenous nudivirus that enables the wasp to produce Virus-Like-Particles (VLPs). So far, in Campopleginae, the nudivirus endogenization event was an isolated case restricted to *V. canescens.* Using third generation sequencing technologies, microscopy and mass spectrometry, we described new cases of domesticated nudiviruses in campoplegine wasps of the genus *Campoplex*. We found endogenized viruses in *Campoplex* genomes which belong to the Alphanudivrius genus and derive from the same endogenization event as the virus found in *V. canescens,* suggesting that this integration event could be much more widespread in Campopleginae than previously thought. These nudiviruses are organised in highly conserved clusters, but this organisation appears to have changed relatively to free viruses during the domestication process. We showed that *Campoplex capitator* produces in its ovaries VLPs that are similar morphologically to *V. canescens* VLPs, with almost the same protein content, except for a strikingly different virulence proteins, a sign of different evolutionary paths taken to respond to distinct evolutive pressure. While ichnovirus remnants have been previously

found in *V. canescens*, no trace of ichnovirus could be detected in *Campoplex* genomes, meaning that the evolutionary history of viral integrations in Campopleginae is more complex than previously imagined.

Keywords: Nudivirus, Viral domestication, Campopleginae, Virus-Like-Particle, Parasitoid wasp, Ichnovirus

Introduction

The endogenization of viral sequences within eukaryotic genomes is a common phenomenon in the evolutionary history of eukaryotic organisms (Feschotte & Gilbert, 2012; Holmes, 2011). These viral sequences, known as endogenous viral elements (EVEs), involve all virus types likely to integrate into any eukaryotic genome (Ballinger et al., 2014; Bejarano et al., 1996; Belyi et al., 2010; Crochu et al., 2004; Delaroque et al., 1999; Feschotte & Gilbert, 2012; Irwin et al., 2021; Katzourakis & Gifford, 2010; H. Liu et al., 2010; Maori et al., 2007). A large proportion of EVEs are segmented and non-functional (Arbuckle et al., 2010; Katzourakis & Gifford, 2010; Tarlinton et al., 2006). There are, however, examples of viral integrations providing novel adaptive functions and selective advantage to the eukaryotic organism that carries them. For example, some EVEs can confer protection to their host against genetically related viruses (Armezzani et al., 2014; Frank & Feschotte, 2017; Horie & Tomonaga, 2019).

54 Other EVEs are also involved in placentation in mammals (Lavialle et al., 2013; Mangeney et
55 al., 2007) and synaptic plasticity in animals (Ashley et al., 2018; Campillos et al., 2006).
56
57 In general, for viral endogenization events that provide hosts with novel functions, co-option
58 typically involves individual viral genes rather than entire viral genomes. However, in some
59 parasitoid wasp species, entire portions of large double-stranded DNA (dsDNA) viruses have
60 been integrated and perform crucial functions to the wasp's life cycle. Indeed, the wasps use
61 the virus-derived particles to deliver virulence proteins that suppress the immune response of
62 the wasp’s host and spark other physiological changes that ensure successful parasitization by
63 the wasp larva (Bézier, Herbinière, et al., 2009; Burke et al., 2013; J.-M. Drezen et al., 2017;
64 Strand & Burke, 2012, 2014; Volkoff et al., 2010).
65 At least seven viral endogenization events occurred in independent wasp lineages, each
66 involving the domestication of large dsDNA viruses (Burke, 2019; Burke et al., 2021; Di
67 Giovanni et al., 2020; J.-M. Drezen et al., 2022; Guinet et al., 2023). These viral endogenization
68 events can be classified into different categories based on the nature of the virus that has
69 been endogenized. The first one involves endogenized nudivirus sequences, leading to the
70 production of bracoviruses (BVs) and Virus-Like-Particles (VLPs; particles made of viral
71 envelopes containing wasp proteins and no DNA) (Bézier, Herbinière, et al., 2009; Burke et al.,

72 2018; Pichon et al., 2015). The second one involves the endogenization of a viral ancestor
73 (characterised by a conserved set of genes) from a still uncharacterized virus family in two
74 distinct Ichneumonidae lineages (Banchinae and Campopleginae), producing Ichnovirus (IV)
75 particles (Béliveau et al., 2015; Gundersen-Rindal et al., 2013; Strand & Burke, 2014; Volkoff
76 et al., 2010; Volkoff & Cusson, 2020). The production of these particles involves the expression
77 of several intronless genes tightly clustered into wasp genome regions called IVSPERs
78 (Ichnovirus Structural Protein Encoding Regions) (Lorenzi et al., 2019; Volkoff et al., 2010,
79 2012). The last viral endogenization category involves filamentous viruses related to
80 hytrosaviruses, probably allowing wasps to produce VLPs (Burke et al., 2021; Di Giovanni et
81 al., 2020; Guinet et al., 2023; Wey et al., 2020).

82

83 So far, three of the seven endogenization events described in parasitoid wasps correspond to
84 the integration of nudivirus sequences. The Nudiviridae family, which diverged 220 MA from
85 the well-known Baculoviridae family, comprises viruses infecting arthropods (Abd-Alla et al.,
86 2008; Jehle et al., 2013; Thézé et al., 2011; Y. Wang et al., 2012). Integration of nudivirus
87 sequences have been described in genome of arthropods including several insects (Bézier et
88 al., 2015; Burand et al., 2012; R.-L. Cheng et al., 2014, 2020; S. Liu et al., 2020, 2021; Y. Wang
89 et al., 2007; Y. Zhang et al., 2020), but, except in parasitoid wasps, these integrations were not

90 reported as virus domestication since the function of any of the viral sequences has not been
91 studied.

92 The integration of sequences from a deltanudivirus (Petersen et al., 2022) in the ancestor of
93 Braconidae Microgastrinae wasps 100 MYA led this lineage, currently comprising at least
94 46000 species, to produce BV particles, which are composed of a viral envelope enclosing
95 capsids containing dsDNA circular molecules harbouring virulence genes (Bézier, Annaheim,
96 et al., 2009; Herniou et al., 2013; Murphy et al., 2008; Thézé et al., 2011). More recently, the
97 integration of alphanudivirus sequences was demonstrated in the braconid *Fopius arisanus*
98 (Burke et al., 2018) and more unexpectedly in the ichneumonid *Venturia canescens* (Pichon et
99 al., 2015). In these parasitoid wasps, the endogenization event led to the production of VLPs
100 (Burke et al., 2018; Burke, 2019; Pichon et al., 2015). Several lines of evidence have suggested
101 that the alphanudivirus integration event in *V. canescens* was more recent than the
102 deltanudivirus integration event in an ancient braconid wasp (100 MYA) (Murphy et al., 2008;
103 Pichon et al., 2015; Thézé et al., 2011). Indeed, first, the nudivirus integration event has been
104 described up to now in a single campoplegine wasp species. Second, the viral genes integrated
105 in *V. canescens* are less dispersed in the wasp genome than the BV genome in braconid wasps
106 (Bézier, Annaheim, et al., 2009; Burke et al., 2014; Mao et al., 2023; Pichon et al., 2015),
107 possibly because fewer genomic rearrangements had time to occur in Campopleginae. It has
108 also been shown that in VcENV, losses of certain core nudivirus functions (*i.e.*: proteins

109 involved in capsid formation) are due to pseudogenization involving accumulation of
110 mutations rather than complete loss of genes or genomic regions (Leobold et al., 2018). The
111 fact that pseudogenized nudiviral sequences have been maintained in VcENV and are not
112 completely eroded could be an indication that the nudivirus integration is more recent in this
113 species (Leobold et al., 2018).

114

115 So far, *Venturia canescens* was the only described representative of Campopleginae wasps
116 with an integrated alphanudivirus (Burke, 2019; Burke et al., 2021; Pichon et al.,
117 2015). Because *V. canescens* belongs to the subfamily Campopleginae, known to harbour
118 ichnoviruses, initially the VLPs were thought to derive from IVSPER expression (Reineke et al.,
119 2006). However, sequencing of the wasp's genome identified only a few pseudogenized
120 IVSPER genes, and it was formally demonstrated that the VLPs are in fact of nudiviral origin
121 (Pichon et al., 2015). The alphanudivirus integrated in the Campopleginae wasp *V. canescens*,
122 named "Venturia canescens Endogenous Nudivirus" (VcENV), allows the production VLPs,
123 which consist of a membrane enveloping virulence proteins that protect the wasp eggs from
124 the lepidopteran host immune system after oviposition (Bedwin, 1979; Feddersen et al., 1986;
125 Pichon et al., 2015; Reineke et al., 2006; Rotheram, 1967, 1973; Salt, 1965). Three virulence
126 proteins have been described in VcVLPs: VLP1, VLP2 and VLP3, which are respectively a Rho
127 GTPase activating protein, a phospholipid hydroperoxide glutathione peroxidase and a

128 neprilysin (Asgari et al., 2002; Hellers et al., 1996; Pichon et al., 2015; Reineke et al., 2002;
129 Theopold et al., 1994). Hence, whilst two genera of Nudivirus have been endogenized in
130 different wasp lineages, the evolutionary trajectories they have undertaken since integration
131 have given rise to strikingly different particles but with similar functions (Burke, 2019; J.-M.
132 Drezen et al., 2017).

133

134 In Campopleginae, nudiviral integration is only known from *V. canescens*. Since whole genome
135 data is available only for a few species, it is still unclear whether this event is really restricted
136 to one or a few species or if it is more widespread in campoplegine wasps. To investigate this
137 issue, we selected a genus, *Campoplex*, that is putatively closely related to *V. canescens*
138 (Santos et al., 2022).

139 In this study, we obtained the complete genome sequence of two *Campoplex* species, one of
140 which corresponds to *Campoplex capitator*, the main parasitoid of the vineyard pest *Lobesia*
141 *botrana* (J. Moreau et al., 2010; Papura et al., 2016; Thiéry et al., 2011). These species and
142 their endogenized nudiviruses offered the opportunity to compare domesticated virus
143 genomes and the produced particles to better understand the early mechanisms at the basis
144 of viral domestication and whether these domestication events followed the same
145 evolutionary trajectories.

146 # Results

147 ## Two new Campopleginae wasp genomes of the *Campoplex* genera

148 To investigate mechanisms involved in nudiviral domestication in Campopleginae wasps, we
149 aimed to obtain genomes of *Campoplex* species, which are phylogenetically close to *V.*
150 *canescens*. For this purpose, we sequenced the genome of *C. capitator* and *Campoplex nolae*
151 using long-read and short-read sequencing technologies. The genome assembly generated for
152 *C. capitator* is 261 Mb long and composed of 630 contigs (generated by PacBio sequencing),
153 while that of *C. nolae* is 218 Mb long and composed of 4 593 contigs (generated by Illumina
154 and Nanopore sequencing) (see detailed information in supplementary data Table S1). The
155 quality of these genomes, with low levels of fragmentation (N50 = 7,8 Mb for *C. capitator* and
156 1,8 Mb for *C. nolae*; see Table S1) and a good genome completeness (96% and 94% of
157 complete hymenopteran BUSCOs for *C. capitator* and *C. nolae* respectively; see Table S2)
158 allowed the description of integrated viruses (see next paragraph). Gene models of *C.*
159 *capitator* were predicted using transcriptomic data obtained after RNA sequencing of wasp
160 samples. As no transcriptomic data was available for *C. nolae*, gene models were generated
161 using protein sequences of *C. capitator* predicted previously. In total, 11 288 and 13 929 genes
162 models have been predicted in *C. capitator* and *C. nolae* respectively.

163

164 ## The endogenized nudivirus genomes within the *Campoplex* genomes

165 We identified 47 genes of viral origin in both wasp genomes (Table1 and Table S3). Out of the
166 32 nudivirus core genes, 23 have been found in the *Campoplex* genomes (Table 1). Among
167 these genes, some encode key viral functions in baculoviruses such as viral transcription (*p47*
168 and *lef* genes), DNA amplification (*helicase*), envelope composition/infectiosity (*Ac81, p33* and
169 *pif* genes) and virion morphogenesis (*vlf-1*). These 47 genes are grouped into 4 clusters in both
170 genomes (Figure 1 for *C. capitator* and Figure S1 for *C. nolae*). These clusters are dense in viral
171 genes and are identical in gene content and order between the two species (Figure1, Figure
172 S1 and Table 2).

173 In *V. canescens*, remnants of some nudivirus genes, notably those involved in DNA
174 amplification and capsid formation, have been found as pseudogenes (Leobold et al., 2018).
175 Therefore, for each missing nudivirus gene, we searched for remnant sequences in both
176 *Campoplex* genomes. Several pseudogenes have been identified, some located inside or
177 around the virus clusters (Figure 1 and Figure S1), others such as *38K* located outside (Table
178 1). Genes that are typically involved in DNA amplification (*integrase, FEN-1* and *DNApol*) and
179 capsid formation (*p6.9, 38K* and *vp39*) in baculoviruses have been found pseudogenized in
180 both *Campoplex* species (Table 2), except for *38K* which couldn't be found in *C. nolae*, probably
181 due to the higher fragmentation level of this genome.

182 ## No traces of Ichnovirus remnants in *Campoplex* genomes

183 As remnants of Ichnovirus were found in *V. canescens* (Pichon et al., 2015), we also searched
184 for sequences of IVSPER genes in the *Campopex* genomes. In both *Campoplex* genomes, no
185 trace of ichnovirus could be found using the IVSPER protein sequences (see material and
186 method for details).

187

188 ## The endogenized nudiviruses in *Campoplex* correspond to Alphanudiviruses

189 Viral sequences found in both *Campoplex* genomes share high similarity with nudivirus
190 sequences (Table S3). We therefore aimed to place these new endogenized viruses in the
191 context of the phylogeny of Naldaviricetes, a class of virus comprising Baculoviridae,
192 Nudiviridae, Hytrosaviridae and Nimaviridae. Using protein sequences from 34 genes (21
193 baculoviruses core genes and 13 nudivirus core genes, see Table S4 for more details), the
194 phylogenetic analysis places these endogenized viruses inside the Nudivirus family and as
195 sister to VcENV, which belongs to the Alphanudivirus genus (Figure 2). Due to their placement
196 in the Nudivirus family, we called these genomes CcapiENV and CnolaENV for Campoplex
197 capitator Endogenous Nudivirus and Campoplex nolae Endogenous Nudivirus respectively.
198 These two new virus genomes are from now on the closest relatives of VcENV.

199 ## Evidence for a single nudivirus endogenization event in Campopleginae wasps

200 Given the phylogenetic position of these integrated alphanudiviruses in *Campoplex* (Figure 2),
201 we then investigated whether these nudiviruses resulted from the same integration event as
202 the one for VcENV. Wasp genes around the virus clusters were found to be the same in both
203 *C. capitator* and *V. canescens* species, indicating that the endogenization event occurred at
204 the same place in both genomes (Figure 3). This observation confirms that these viruses come
205 from the same endogenization event.

206

207 ## Evolution of the endogenized nudiviruses in Campopleginae wasps

208 We compared the three endogenized nudivirus genomes in Campopleginae wasps to gain
209 insight on the mechanisms that are operating during the early processes of virus
210 domestication. The gene content in these viruses is almost identical, with only a few
211 differences lying in the gene position, in the number of copies for certain genes and in the
212 identified pseudogene (Table 2). However, while all other genes are present in all genomes,
213 *vlf-1* was found only in CcapiENV and CnolaENV, this gene being found in a pseudogenized
214 form in VcENV (Table 2). In baculoviruses, VLF-1 is a nucleocapsid protein that participates in
215 virion maturation, in the integration and excision of the viral genome and in viral transcription
216 (McLachlin & Miller, 1994; Vanarsdall et al., 2006; Yang & Miller, 1998, 1999). Duplicated

217 copies of *Ac81* and *OrNVorf47-like* have been found only in VcENV (Table 2), indicating that
218 those copies could have originated from duplication events occurring after speciation. Copies
219 of *pif-5* have been found in all genomes, some in similar locations inside the clusters (Table 2
220 and Figure 3), indicating that these copies might come from a duplication event that had
221 happened in the common ancestors of all these wasps. The organisation in clusters is also
222 highly preserved among these endogenized viruses in Campopleginae wasps, with CcapiENV
223 clusters 1 and 2 being almost identical to the VcENV clusters 3 and 2 (Figure 3). CcapiENV
224 cluster 4 and its VcENV equivalent (cluster 4) seems to have endured some genomic shuffling,
225 but not enough to erase syntenies between the two genomes (Figure 3).

226

227 Impact of endogenization on nudivirus gene organisation

228 Integrated alphanudivirus genomes were compared to free virus genomes in order to evaluate
229 the impact of viral domestication after integration. Gene order among free nudiviruses is fairly
230 well conserved (Figure 4 A), but gene order was globally lost following viral endogenization
231 (Figure 4 B), suggesting that loss of certain syntenic regions might be part of the viral
232 domestication process.

233 We also compared the endogenized alphanudiviruses in Campopleginae wasps to other
234 endogenized alphanudiviruses such as FaENV (Fopius arisanus Endogenous Nudivirus) and

235 NlENV (Nilaparvata lugens Endogenous Nudivirus). First, as described before, the viral genome
236 structure in Campopleginae wasps is conserved after integration (Figure 4 C). However, for
237 each independent alphanudivirus integration, the genomes seem to have been shuffled
238 differently (Figure 4 C). Viral genome shuffling after integration might be part of a viral
239 domestication process, as developed in Burke and collaborators (Burke et al., 2018; see
240 discussion).

241

242 Selection pressures acting on nudivirus genes

243 To estimate the selection pressures acting on *C. capitator*, *C. nolae* and *V. canescens* genes,
244 dN/dS ratios were calculated for 8 787 cellular genes and the 41 endogenized nudivirus genes.
245 For the vast majority of genes (13152 out of 13351 values), the dN/dS ratio is lower than 1
246 (Figure 5). Nonetheless, dN/dS ratios obtained for nudivirus genes are higher than the ones of
247 cellular genes, with an average dN/dS ratio of 0.17 for cellular genes and 0.73 for nudivirus
248 genes. Focusing on nudivirus gene evolution according to their function, genes putatively
249 involved in DNA amplification (*helicase*) and viral transcription (*lefs* and *p47*) have a lower
250 dN/dS ratio than genes putatively involved in infectivity and envelope protein production (*pifs*,
251 *vp91*, *p33* and *p74*) (Figure 5). Gauthier and colleagues had also found equivalent results on
252 nudiviral genes associated with Braconid wasps (Gauthier et al., 2018, 2021). Furthermore,

253 duplicated nudiviral genes in the three Campopleginae species have a rather high dN/dS ratio,
254 notably the copies of *OrNVorf41-like* and *pif-5* (Table S5).

255

256 CcapiENV gene expression

257 Virus gene expression activity within the wasps has been investigated through transcriptome
258 analyses performed on *C. capitator* samples of pools of organs from male and female
259 individuals. Because virus genes participate in the production of virus-derived particles in the
260 ovaries for several wasp species, notably in *V.canescens* (J. Drezen et al., 2006; Pichon et al.,
261 2015; Stoltz, 1990), we assumed that CcapiENV genes would be mostly expressed in *C.*
262 *capitator* ovaries. As expected, all CcapiENV genes are expressed in adult wasp ovaries (Figure
263 S2 and Table S6). Transcripts from some nudivirus genes are also visible in venom glands. The
264 head-thorax samples of female wasps appear to be almost free of viral transcripts, with only
265 minute amounts of transcripts being measured. CcapiENV genes are practically not expressed
266 in males, neither in testis nor in head-thorax samples, although testis samples show a slightly
267 higher number of transcripts than head-thorax samples (Table S6). The *pif-2* gene appears to
268 be an exception as it is weakly expressed in head-thorax samples of both males and female
269 wasps.

270 Regulatory sequences have also been studied in *C. capitator* in order to understand regulation
271 mechanisms leading to the expression of endogenized nudivirus genes. However, no
272 congruent results could be observed as the type of promoter (baculovirus early, baculovirus
273 late, VcENV late) does not necessarily match the expected expression kinetics of the CcapiENV
274 genes based on the transcription kinetic described for their VcENV orthologs (Cerqueira de
275 Araujo et al., 2022; Table S7).

276

277 *C. capitator* produces VLPs that are similar in morphology to those produced by 278 *V. canescens*

279 After identifying the endogenized nudivirus in the *Campoplex* genomes, we investigated
280 whether *C. capitator* produces VLPs as described in *V. canescens*. Electron microscopy
281 revealed the presence of particles in the calyx region of *C. capitator* ovaries, a specialised
282 portion of the ovaries that produces virus-derived particles in braconid wasps and
283 Ichneumonid wasps (Bézier, Annaheim, et al., 2009; Burke & Strand, 2012; Pichon et al., 2015;
284 Volkoff et al., 2010). These particles in *C. capitator* are referred to as CcapiVLPs (Figure 6), and
285 are almost morphologically identical to VcVLPs (Pichon et al., 2015). CcapiVLPs are composed
286 of a membrane enclosing an electron-dense body (Figure 6E) and are massively present in the
287 lumen (Figure 6 A and B) while only a few are visible in the cytoplasm and cell nuclei (Figure 6

288 C). Virogenic stromas, electron-dense masses composed of proteins that form viral particles
289 in other wasps (Bézier, Herbinière, et al., 2009; Pichon et al., 2015), are visible in calyx cell
290 nuclei (Figure 6C), along with VLPs and empty envelopes (Figure 6F). As described for VcVLPs
291 (Pichon et al., 2015), CcapiVLPs appear to be be produced inside the nucleus by virogenic
292 stromas and transit through the nuclear membrane and then through the cytoplasmic
293 membrane by budding to finally end in the calyx lumen (Figure 6 D,E and F).

294

295 *C. capitator* and *V. canescens* VLPs have a similar composition but the virulence 296 protein contents are different

297 We proceeded to determine whether the protein composition of the VLPs and their content
298 were the same between *C. capitator* and *V. canescens*. For CcapiVLPs, 28 proteins of nudivirus
299 origin were detected by mass spectrometry (Table S8). In the majority of cases, proteins
300 contained in VcVLPs deriving from VcENV genes were also found in CcapiVLPs. For instance,
301 all the VcVLP proteins involved in envelope formation in baculoviruses were found in purified
302 CcapiVLPs. These include all the PIF proteins, P74, VP91 and P33 proteins. However,
303 OrNvorf46-like and a copy of PIF-5 proteins are present in VcVLPs but not in CcapiVLPs (Table
304 S8). Similarly, OrNVorf18-like, OrNVorf47-like, OrNVorf79-like, OrNVorf90-like and

305 OrNVorf120-like proteins appear to be present in CcapiVLPs but were not detected in VcVLPs
306 (Table S8).

307 A large number of non-viral proteins, which should include virulence proteins, could be
308 identified by mass spectrometry within the VLPs (Table S9). Proteins described in VcVLPs were
309 first sought in the CcapiVLP protein dataset to determine whether the same proteins are
310 enveloped in VLPs in both wasp species. The VLP1 (PHGPx), VLP2 (RhoGAP) and VLP3
311 (Neprilysin) proteins of VcVLPs were not found in CcapiVLPs. Candidate VLP virulence proteins
312 were selected according to their peptide abundance (PSM for Peptide-Spectrum Matches)
313 value and only proteins with a PSM value higher than 50 were retained (Table S10). Some
314 proteins that have been described in parasitoid wasp venoms (Inwood et al., 2023; S. Moreau
315 & Asgari, 2015; Poirié et al., 2014), such as a serpin, a peroxiredoxin, a calreticulin and an
316 aminopeptidase, have been found in CcapiVLPs. Further investigation on the serpin showed
317 that the gene encoding this protein is a member of a multigenic family, which comprises 15
318 members in *Campoplex spp*. and 4 in *V. canescens*. While the four copies found in *V. canescens*
319 have orthologous sequences in *Campoplex spp.*, the serpin identified in CcapiVLPs is more
320 distant, with only one ortholog found in *C. nolae* (Figure S3). This serpin appears to be specific
321 to *Campoplex* species and therefore represents an interesting virulence protein candidate.

322 ## Discussion

323 The sequencing of two new genomes of *Campoplex* wasps has provided the opportunity to
324 describe, together with the previous sequencing of the *V. canescens* wasp genome (Mao et
325 al., 2023; Pichon et al., 2015), the early events involved in the domestication of
326 alphanudiviruses that these wasps have endogenized. Our results convincingly show that the
327 endogenized nudiviruses in the *Campoplex* species correspond to the same endogenization
328 event as the one first described in *V. canescens*. The few and compact viral gene clusters,
329 together with the presence of visible traces of pseudogenes, strongly suggest that this
330 endogenization event is more recent than the one described in Microgastrinae braconid
331 wasps. Furthermore, strong synteny is evident between the three endogenized
332 alphanudiviruses, which contrasts with total lack of synteny with the known free nudiviruses
333 for which sequences are available. This result suggests that a very early process required in
334 viral domestication involves major genomic rearrangements, in accordance with similar
335 observations made in the case of FaENV (Burke et al., 2018). Comparisons between the
336 endogenized nudiviruses in Campopleginae wasps reveal not only conserved synteny of genes
337 but also very high gene similarities, suggesting that evolutionary constraints may be acting to
338 conserve gene functions, to enable coordinated expression leading to VLP formation. Finally,
339 VLPs produced by *V. canescens* and *C. capitator* wasps are very similar, but genes coding for
340 the VLP proteins involved in infectiosity are undergoing higher selection pressures and the VLP

341 virulence protein contents are totally different, illustrating that selection pressures exerted by
342 wasp's host are most probably acting and resulting in tailored-virulence protein contents
343 within VLPs.

344

345 A recent and unique endogenization event of an alphanudivirus in 346 Campopleginae wasps

347 CcapiENV and CnolaENV, the nudiviruses integrated in *C. capitator* and *C. nolae* genomes
348 respectively, are composed of 47 nudiviral genes each. These viruses belong to the Nudivirus
349 family, especially to alphanudiviruses, and are the closest known relative of VcENV, the only
350 endogenized nudivirus that had been previously described in Campopleginae wasps (Figure
351 2). Comparing the insertion location of CcapiENV and VcENV, the same wasp genes in the same
352 order have been found around the viral clusters in both species (Figure 3). These observations
353 indicate that these two new viruses come from the same endogenization event as the one of
354 VcENV, which occurred in the common ancestor of the wasps harbouring them.

355 The integration of the alphanudivirus in Campopleginae appears to have occurred more
356 recently than the endogenisation of BV in Braconidae. First, the wasp phylogeny indicates that
357 the subfamily Campopleginae is a younger lineage than the microgastroid complex (Burke et
358 al., 2021; Murphy et al., 2008; Sharanowski et al., 2021) and not all representatives of the

359 Campopleginae family appear to possess nudivirus (such as wasps of the genus *Hyposoter* or
360 *Dusona*) (Burke et al., 2021), indicating that nudivirus integration is not ancestral to the
361 Campopleginae and occurred later in a specific Campopleginae lineage. Furthermore, in both
362 *Campoplex* species, the distribution of the endogenized virus genes shows that the viral
363 genome is not very dispersed, forming four highly conserved clusters within the wasp
364 genomes (Figure 1). This organisation in clusters has been previously described in VcENV (Mao
365 et al., 2023; Pichon et al., 2015) and in FaENV (Burke et al., 2018), which have five and nine
366 clusters respectively. In bracoviruses, nudivirus clusters have also been characterised but virus
367 genes are more dispersed in the wasp genomes (Bézier, Annaheim, et al., 2009; Burke et al.,
368 2014, 2018; Gauthier et al., 2021). These observations would indicate that alphanudiviruses
369 integrated in parasitoid wasps have undergone fewer rearrangement events than the BV
370 integrated in the Braconidae. Also, it was possible to find pseudogenized nudiviral genes in
371 *Campoplex* wasps and in *V. canescens* (Leobold et al., 2018), whereas no pseudogenes were
372 characterised for BVs, which again suggests that the alphanudivirus integration event in
373 Campopleginae wasps is more recent because traces of gene loss are still detectable.

374

375 ## Genome rearrangement seems to be part of the viral domestication process

376 Genomic rearrangements appear to be a part of the viral domestication process in parasitoid
377 wasps. Indeed, the viral organisation of nudivirus genes of the Alphanudivirus genus is very
378 well conserved among free Alphanudiviruses viruses infecting arthropods (Figure 4 A). While
379 this viral organisation is kept in free viruses, the genomes of endogenized nudiviruses in
380 Campopleginae wasps appear to have lost this well conserved viral organisation, with their
381 genome being rearranged in their host genome (Figure 4 B). This phenomenon of genomic
382 rearrangement after integration is shared by the viruses that have been independently
383 endogenized in different lineages of insects and produce integrated viruses with distinct
384 organisations (Figure 4 C). A similar observation was made in FaENV where the viral genome
385 has been shuffled after endogenization in the wasp genome (Burke et al., 2018). It has been
386 suggested that these early rearrangement events occurring after integration could lead to the
387 deactivation of the virus replication process by scattering the virus genome and could
388 correspond to a founder effect in the process of domestication (Burke et al., 2018).

389

390 ## Key viral functions and the organisation in clusters were kept after domestication

391 CcapiENV, CnolaENV and VcENV share almost exactly the same content in genes (Table 2). The
392 22 core nudivirus genes found in VcENV were also identified in *Campoplex* endogenized

393 viruses (Table 1). Genes necessary for VLP production were kept in these Campopleginae
394 wasps. It has been shown for example that genes such as *lef* genes that are involved in viral
395 transcription are particularly well conserved among wasps that have endogenized a nudivirus
396 (Bézier, Annaheim, et al., 2009; Bézier, Herbinière, et al., 2009; Burke, 2019; Burke et al., 2018;
397 Burke & Strand, 2012; J.-M. Drezen et al., 2022; Pichon et al., 2015; Strand & Burke, 2012).
398 Silencing of these genes leads to the suppression or reduction of particle production in *V.*
399 *canescens* and *Microplitis demolitor* (Burke et al., 2013; Cerqueira de Araujo et al., 2022).
400 Furthermore, in Campopleginae, genes involved in viral transcription (*p47* and *lef* genes) and
401 DNA amplification (*helicase*) are under strong conservative selection pressures compared to
402 genes typically involved in envelope formation and infectiosity (*p33*, *Ac81* and *pif* genes)
403 (Figure 5). The same observation was made in braconid wasps harbouring a BV and in
404 *Leptopilina* wasps harbouring an endogenized filamentous virus (Di Giovanni et al., 2020;
405 Gauthier et al., 2021), showing that the viral transcription function is globally maintained in
406 all these viral domestication events. Genes coding for envelope components (such as *pif*
407 genes) have also been conserved in BV, FaENV and Campopleginae endogenized nudiviruses,
408 although their sequences differ more than the ones of *lef* genes for instance (Bézier,
409 Annaheim, et al., 2009; Bézier, Herbinière, et al., 2009; Burke, 2019; Burke et al., 2018; Burke
410 & Strand, 2012; Di Giovanni et al., 2020; J.-M. Drezen et al., 2022; Gauthier et al., 2021; Pichon
411 et al., 2015; Strand & Burke, 2012). In baculoviruses, PIFs proteins are involved in the entry of

412 viral particles into the cells of the lepidopteran host's digestive tract (Boogaard, 2018; Kikhno
413 et al., 2002; Zheng et al., 2017). As they form the interface between the particle and the host
414 cells, these proteins are therefore likely to diverge depending on the host that the particles
415 infect.

416 Regarding the viral genome structure, the endogenized virus genome structure is organised in
417 clusters of viral genes in Campopleginae wasps, in which the gene order was preserved after
418 wasp diversification. VcENV, CcapiENV and CnolaENV are all composed of three main
419 conserved nudiviral clusters, with observable syntenies between species (Figure 3, Figure 4).
420 For instance, the composition and gene order of the clusters are the same in both CcapiENV
421 and CnolaENV (Figure 1 and Figure S1). Comparing CcapiENV with VcENV, genes of CcapiENV
422 clusters 1 and 2 have exactly the same order as genes in VcENV clusters 3 and 2 (Figure 3).
423 Nonetheless, the order of viral genes between CcapiENV cluster 4 and VcENV clusters 4 and 5
424 is less conserved, indicating that certain recombination events took place after speciation of
425 the parasitoid wasps hosting them (Figure 3). In contrast, pseudogenes identified in
426 *Campoplex* and *V. canescens* genomes are dispersed compared to functional genes (Table S3)
427 (Leobold et al., 2018; Mao et al., 2023).

428 ## Loss of genes not essential for VLP production

429 Remnants of the majority of genes that have been pseudogenized in VcENV can be found in
430 CcapiENV and CnolaENV. Some pseudogenes, such as *OrNVorf130-like*, were not found in
431 CcapiENV or CnolaENV. As pseudogenes can be very degraded, it can sometimes be difficult
432 to detect them, but it is also possible that these genes have been lost by deletion as described
433 in bracoviruses (Bézier, Annaheim, et al., 2009; Burke et al., 2014).

434 Genes involved in capsid formation in baculoviruses have been lost for the most part (see *vlf-*
435 *1* below) in all Campopleginae wasps (Table 2). More specifically, FaENV and BVs have kept
436 genes such as *38K* and *vp39* (Bézier, Annaheim, et al., 2009; Burke et al., 2014, 2018), genes
437 that are involved in baculoviruses in nucleocapsid assembly and DNA packaging (Danquah et
438 al., 2012; Katsuma & Kokusho, 2017; Lai et al., 2018; Wu et al., 2006), but these genes are
439 pseudogenized in *Campoplex* wasps and *V. canescens* (Leobold et al., 2018; present study).
440 Genes involved in DNA packaging such as *integrase* can be found in BVs but were lost by
441 pseudogenization in Campopleginae wasps producing VLPs, which are devoid of DNA (Burke,
442 2019; Burke et al., 2013, 2018; J.-M. Drezen et al., 2022; Pichon et al., 2015).

443

444 ## Specific adaptations of Campopleginae wasps to produce VLPs

445 During virus domestication, the alphanudivirus genome has been well conserved among
446 Campopleginae wasps. Gene content and genome structure are similar between
447 CcapiENV/CnolaENV and VcENV. However, notable differences can be observed between
448 these genomes, probably being hallmarks of different adaptation to the wasp's hosts.

449 ### *Vlf-1* is present in *Campoplex,* but not in *V. canescens*

450 One of the major differences between the two new genomes and VcENV is the presence of
451 *vlf-1* in CcapiENV and CnolaENV, while this gene has been found pseudogenized in VcENV
452 (Table 2). In baculoviruses, *vlf-1* is involved in several processes. The VLF-1 protein is an
453 integrase that binds to specific DNA sites, catalyses DNA rearrangements and participates in
454 the integration and excision of the viral genome (McLachlin & Miller, 1994). This protein,
455 which is included in the nucleocapsid (Yang & Miller, 1998), appears to be necessary for virion
456 maturation (Vanarsdall et al., 2006) and is involved in the transcription of genes expressed at
457 very late stages of the viral infection (Yang & Miller, 1999). In CcapiENV, this gene does not
458 appear to code for a structural VLP protein (Table 1 and Table S8), but is expressed specifically
459 in wasp ovaries (Figure S2 and Table S6). The *vlf-1* gene may have a function in the
460 transcription of late expressed genes and could possibly explain why we are not able to
461 identify the same promoter sequences in CcapiENV and VcENV.

462 ## Duplication of nudivirus genes

463 In giant viruses—dsDNA viruses which exhibit a high level of gene duplications (Filée, 2018;
464 Legendre et al., 2018) the expansion and reduction of the number of genes seems to be an
465 adaptive process to respond to new constraints imposed by the host (Boyer et al., 2011; Elde
466 et al., 2012). Among nudivirus duplicated genes in Campopleginae wasps (Table 2), *Ac81* and
467 *OrNVorf47-like* have been found duplicated only in VcENV (Mao et al., 2023; Pichon et al.,
468 2015). In addition, two extra copies of *pif-5* were found in VcENV and an extra copy of
469 *OrNVorf41-like* was identified in CcapiENV and CnolaENV (Figure 1, Figure S1 and Figure 3).
470 The *OrNVorf41-like* gene belongs to the 11K gene family, which comprises the *Ac145* and
471 *Ac150* genes of the Autographa californica nucleopolyhedrovirus (AcMNPV). *Ac145* and *Ac150*
472 appear to be involved in oral infectivity, although they are not essential for infection (J.-H.
473 Zhang et al., 2004). The action of these genes is host-dependent: the deletion of *Ac145*
474 reduces the infectivity of AcMNPV in the host *Trichoplusia ni,* but not in the host *Heliothis*
475 *virescens*. The deletion of *A150* alone has no effect on either host. By deleting both *Ac145* and
476 *Ac150*, the infectivity of AcMNPV was decreased in both *T. ni* and *H. virescens* (Lapointe et al.,
477 2004). It has been proposed that *Ac145* is specifically involved in the initiation of primary
478 infection while *Ac150* is more involved in the systemic spread of the infection (Beperet et al.,
479 2015). The extra copy of *OrNVorf41-like* identified in CcapiENV and CnolaENV may therefore
480 enable infection of different wasp's hosts compared to *V. canescens*.

481 CcapiVLPs and VcVLP do not share the same VLP content

482 Regarding the virulence proteins contained in VLPs, *V. canescens* produces VLPs containing
483 three virulence proteins: VLP1, VLP2 and VLP3. In *C. capitator*, no VcVLP virulence protein
484 counterparts, nor protein of similar functions, could be found inside CcapiVLPs. Wasp
485 virulence proteins can be encoded from IV and BV circles or be produced in venom glands
486 (Burke & Strand, 2014; J.-M. Drezen et al., 2017; Poirié et al., 2009). We therefore searched
487 for virulence protein candidates which have similar functions to virulence proteins from other
488 domesticated viruses or venom proteins. Of these virulence protein candidates, we found a
489 serine protease inhibitor (serpin), which is a protein that has also been detected in venom of
490 several parasitoid wasps and in MdBV (Microplitis demolitor Bracovirus) (Beck & Strand, 2007;
491 Inwood et al., 2023; Moreau & Asgari, 2015; Poirié et al., 2014; Quicke & Butcher, 2021). These
492 proteins have been shown to inhibit the proteolytic cascade leading to the production of
493 phenoloxidase (PO), involved in the melanisation reaction (Beck & Strand, 2007; Colinet et al.,
494 2009).

495 Other proteins described in wasp venoms have also been identified in the proteomic analysis
496 of CcapiVLP components such as a peroxiredoxin, a calreticulin, and an aminopeptidase.
497 Peroxiredoxin enzymes protect against oxidative stress and have also been identified in the
498 venom of the parasitoid wasp *Anisopteromalus calandrae* (Perkin et al., 2015). In the context
499 of parasitoid wasp-host interactions, this type of enzyme may protect the parasitoid eggs from

500 detrimental reactive oxygen species generated during oviposition and the encapsulation
501 response. Calreticulins have been identified in several parasitoid wasps (Cha et al., 2015; De
502 Graaf et al., 2010; Etebari et al., 2011; Fang et al., 2011; Perkin et al., 2015; L. Wang et al.,
503 2013; G. Zhang et al., 2006). Several studies have suggested calreticulin works as an
504 antagonist, competing for binding sites with host calreticulins, thus inhibiting hemocyte
505 encapsulation (Cha et al., 2015; L. Wang et al., 2013; G. Zhang et al., 2006). Aminopeptidase
506 activity or proteins have been detected in the venoms of several parasitoid wasps
507 (Becchimanzi et al., 2020; Dani et al., 2005; Inwood et al., 2023; Mathé-Hubert et al., 2016;
508 Teng et al., 2017), although the functions of these enzymes in the interaction are not clear
509 they could be involved in general degradation of host tissues.

510

511 It has often been described that genes encoding virulence proteins are part of multigenic
512 families expanded in species harbouring them. For instance, protein tyrosine phosphatases
513 (PTP) genes have been found largely duplicated in several BVs in which they code for virulence
514 proteins (Serbielle et al., 2012). In parasitoid wasps, some venom proteins are derived from
515 duplicated genes (Casewell et al., 2013; Colinet et al., 2014; Fry et al., 2009; Wong & Belov,
516 2012), conferring a selective advantage linked to the diversification of venom proteins. Among
517 all possible candidates (Table S10), we investigated whether these proteins are encoded from
518 genes included in expanded gene families in *Campoplex* species, with no obvious counterparts

519 in *V. canescens* genome. In CcapiVLPs, the serpin is the only protein that meets the criteria
520 described above (see results), establishing it as a major candidate. Thus, it would be
521 interesting to investigate its function using RNA interference to validate its virulence protein
522 function.

523

524 No support for ichnovirus replacement hypothesis in *Campoplex* species

525 In the study of Pichon et al. (2015), sequences that appeared to correspond to degraded
526 remnants of the ichnovirus replication machinery (IVSPERs) were detected in *V. canescens*
527 genome; this result, combined with a preliminary phylogeny inferred from 28S rRNA
528 sequences, led the authors to hypothesise that *V. canescens* may have undergone a process
529 of "endogenous virus replacement", in which an ancestrally present ichnovirus was lost
530 following the acquisition of the endogenous alphanudivirus. In the present study, we were
531 unable to corroborate these results (Pichon et al., 2015) as we could not find remnants of a
532 past ichnovirus integration in the *Campoplex* genomes. Similarly, Burke and collaborators
533 (Burke et al., 2021) did not find IVSPER sequences in a genome of *Dusona sp.*, one of the
534 closest relative to *Campoplex* species (Sharanowski et al., 2021). Previous results and our
535 results suggest that the ichnovirus integration might have happened in the Hyposoter -

536 Campoletis clade, while the Campoplex - Dusona clade have endogenized an alphanudivirus,
537 which had been partially lost in *Dusona sp.* (see Sharanowski et al. (2021) for phylogeny).
538
539
540 To conclude, using third generation sequencing technology, it has been possible to describe
541 new endogenous viruses in parasitoid wasps. The new genomes of two Campopleginae wasps
542 harbour an alphanudivirus which derive from the same endogenization event as the one of
543 VcENV in the common wasp ancestor. The description of these new endogenized nudiviruses
544 sheds light on early evolutionary processes of viral domestication. Comparison with the closely
545 related *V. canescens* species shows that there are minute differences between the genomes
546 of endogenized nudiviruses in Campopleginae wasps. However, major differences can be
547 seen, such as the virulence protein content of VLP which appears to be strikingly different
548 between *C. capitator* and *V. canescens*, showing early adaptation to wasp's hosts in the
549 context of viral domestication.

550 # Material and methods

551 ## Parasitoid model

552 *Campoplex capitator* wasps were obtained after emergence from the lepidopteran host
553 *Lobesia botrana*, a vineyard pest that can grow on *Daphne gnidium* (Loni et al., 2016).
554 Caterpillars of *L. botrana* were sampled from *D. gnidium* shrubs in the natural reserve of
555 Migliarino-San Rossore-Massaciuccoli (Tuscany, Italy). Samplings were carried out in early July
556 of 2019 and 2021, with wasps emerging between June and August (Scaramozzino et al., 2018).
557 *L. botrana* nests were placed individually in test tubes and each tube was monitored every
558 day. Emerging *C. capitator* were used for experiments.

559 To produce enough wasps for our experiments, a *C. capitator* rearing was maintained as
560 described in (Benelli et al., 2020; Lucchi et al., 2018): *L. botrana* larvae were kept in plastic
561 boxes (20cm D x 15cm W x 10cm H) with a nutritious substrate (30g of agar, 60g of sugar, 50g
562 of alfalfa, 36g of Brewer yeast, 25g of Wesson salt, 180g of wheat sprout, 80g of casein, 4g of
563 sorbic acide, 15g of Wanderzahnt vitamins, 2.5g of cholesterol, 2.5g of tetracyclin, 5mL of
564 propionic acid (99,5%), 2mL of linoleic acid (95%), 5mL of olive oil and 1.5mL of distilled water).
565 After the emergence, adults were kept in the same box for reproduction where the substrate
566 was removed and droplets of honey were added. Once eggs were visible on the box surface,
567 adults were removed and nutritious substrate was added to feed the future larvae. *Campoplex*

568 *capitator* females and males were meanwhile kept in plexiglass chambers (40cm D x 25cm W
569 x 30cm H) with honey to allow their reproduction. L2 to L4 host larvae were put in the
570 chambers for two hour parasitism periods and then individually reared into small boxes (5cm
571 Ø x 5cm H) with nutritious substrate. These boxes were monitored every day to check for wasp
572 emergence and honey was added when larvae were about to pupate. All insects were kept at
573 25°C and 45% of humidity, with a 16:8 day/night photoperiod.

574 Meanwhile, a female specimen of *Campoplex nolae* was collected in the wild by active
575 sweeping at Powdermill Nature Reserve, Rector county, Pennsylvania, USA, and kept in liquid
576 nitrogen until the time of DNA isolation.

577

578 ## DNA extraction and sequencing

579 For *C. capitator*, DNA was extracted from one haploid male at emergence using the
580 MagAttract® HMW DNA Kit (Qiagen). The male wasp tissues were disrupted in a 2 mL tube
581 containing 200 µL of 1X DPBS. 20 µL of proteinase K, 4 µL of RNAse A and 150µL of buffer AL
582 were added to the tube. The tube was then mixed carefully and incubated at 56°C at 900 rpm
583 for 2 hours. The manufacturer's protocol was followed from step 4 to step 16. Total DNA was
584 finally eluted in 100 µL of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) and quantified using
585 the Qubit™ DNA HS assay kit (Invitrogen) and the Qubit2.0 fluorometer (Invitrogen). DNA

586 quality and contamination were checked by measurement of 260/280 and 260/230 OD ratios
587 using the Varian Cary® 50 Scan spectrophotometer. Total DNA was then stored at 4°C. The
588 library was prepared and sequenced using the PacBio Sequel II technology by the Gentyane
589 platform (France).

590 For *C. nolae*, extraction was performed using the MagAttract® HMW DNA Kit (Qiagen),
591 following the manufacturer's protocol with the whole specimen immersed in extraction buffer
592 and proteinase K overnight. An additional purification step using Agencourt AMPure XP beads
593 (Beckman Coulter, Brea, U.S.A.) at 0.5X was used to remove small fragments. Genomic
594 libraries were prepared for parallel sequencing in short-read (Illumina) and long-read
595 (Nanopore) platforms: the Illumina library was prepared using the Kapa HyperPrep kit (Kapa
596 Biosystems, Wilmington, MA). A-tailing, end-repair and ligation reactions were performed at
597 a quarter volume relative to the standard protocol, while library amplification was performed
598 at full volume. Custom, dual-indexing adapter-primers were used to allow for in silico de-
599 multiplexing of each sample (Glenn et al., 2016). Following stub ligation and PCR, adapter-
600 dimers were removed by performing a 0.8X bead cleaning using AMPure beads. The library
601 was pooled with other samples at equimolar concentrations and sequenced at 4 nM as single
602 lanes on Illumina NovaSeq 6000 S4 platform (2x150; Illumina Inc., San Diego, CA). The library
603 for long-read data was prepared using the Nanopore ligation kit, complemented by reagents
604 from the NEBNext Ultra II library prep kit (New England Biolabs, Ipswich, Massachusetts,

605 U.S.A.), and then sequenced at a MinION platform (Oxford Nanopore Technologies, Oxford,
606 U.K.) using the manufacturer's specifications.

607 For both wasp species, raw data are available in NCBI (accession number of
608 the BioProject: PRJNA936130).

609

610 RNA extraction and sequencing

611 Emerging *C. capitator* wasps were dissected in order to obtain ovaries where CcapiVLP are
612 produced. Venom glands and head-thorax of female adults and testis and head-thorax of male
613 adults were also dissected in order to investigate expression of CcapiENV genes outside of the
614 calyx region and in males. For all samples, two pools were analysed. Pools were composed of
615 5 individuals for head-thorax for both males and females, 21 female venom glands, 5 ovary
616 pairs and 15 male testis pairs. Total RNA was extracted from the 10 pools in total
617 using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. Only the
618 isopropanol step was replaced by cold absolute ethanol (3 volumes per sample) and total RNA
619 was finally resuspended in 30µL of RNAse free water. RNA quantity of each pool was
620 quantified using the Qubit™ RNA HS assay kit (Invitrogen) designed for the Qubit2.0
621 fluorometer (Invitrogen). Samples of extracted RNA were then stored at -80°C.

622 For genome annotation, total RNA was extracted from two pools of four entire emerging
623 female wasps using the NucleoSpin®RNA kit (Macherey-Nagel). Total RNA was finally
624 resuspended in 40µL of RNAse free water. RNA quantity of the two pools was quantified using
625 the Qubit™ RNA HS assay kit (Invitrogen) designed for the Qubit2.0 fluorometer. Samples of
626 extracted RNA were then stored at -80°C.

627 The sequencing of library preparations and the generation of paired-end reads were
628 performed on an Illumina platform by the Novogene company (UK). Raw data are available in
629 NCBI (accession number of the BioProject: PRJNA936130).

630

631 Genome assembly and annotation

632 For *C. capitator*, genome and K-mer sizes were estimated beforehand using KmerGenie (Chikhi
633 & Medvedev, 2014). Raw reads were then corrected and trimmed using respectively Canu–
634 correct and Canu–trim modules (Canu software v1.8) (Koren et al., 2017) in order to improve
635 the base accuracy and to retrieve high quality sequence portions only. The genome was then
636 assembled using the Canu–assemble module. The newly assembled genome was polished
637 using Racon (v1.4.21) with the longest 80X raw reads to further improve the base accuracy
638 using the longest raw reads. In parallel, *C. nolae* raw reads from Nanopore sequencing were
639 filtered using Filtlong (v.0.2.1) before being assembled using Flye (v.2.9-b1768) (Kolmogorov

640 et al., 2020). The produced assembly was then polished with Hypo (v.1.0.3.flye) using the
641 Illumina reads. Scripts for *C. nolae* genome assembly are available at
642 https://gitlab.in2p3.fr/marie.cariou/campoplex_assembly/.

643 The assembled genome completeness was assessed with BUSCO (Benchmarking Universal
644 Single-Copy Orthologs, v5.4.2) (Manni et al., 2021) using Insecta and Hymenoptera lineage
645 datasets (odb10) and genome contamination was checked with Blobtools (v1.1.1) (Laetsch &
646 Blaxter, 2017) for both genomes.

647 For both genomes, repeated elements were annotated *de novo* using the TEdenovo pipeline
648 comprised in the REPET pipeline (v2.5) (Flutre et al., 2011). Two rounds of the TEannot pipeline
649 (contained in the REPER pipeline) were then used to annotate repeated elements using
650 existing databases. Repeated elements were then masked using Bedtools (v.2.29.1) (Quinlan
651 & Hall, 2010) before gene prediction. Gene models were generated for *C. capitator* using
652 BRAKER-2 (v2.1.6) (Brůna et al., 2021) with assembled transcriptomes by spades (v3.15.5)
653 (Bushmanova et al., 2019). Models were then functionally annotated using InterProScan
654 (v5.53-87.0) (Blum et al., 2021) and blastp (NCBIblast+ v2.13.0) with the nr database
655 (downloaded the 14th of April 2023), before being polished by AGAT (v1.0.0) (Dainat et al.,
656 2023). Proteins were extracted from gene models using the agat_sp_extract_sequences.pl
657 tool from AGAT software. Finally, the genome completeness was assessed again using the
658 protein sequences with BUSCO.

659 In total, 44 CcapiENV genes were annotated with the automated annotation process. Using
660 the gene models predicted previously, additional viral genes were then annotated by
661 reciprocal blasts: translated sequences from predicted genes were aligned using blast tools on
662 the NCBI nr database filtered for sequences of endogenous and exogenous nudiviruses.
663 Aligned sequences were then extracted and realigned on the complete NCBI nr database. The
664 final Blast outputs were filtered to keep only sequences which aligned on sequences of
665 endogenous and exogenous nudiviruses reported in the database.

666 To annotate nudivirus genes on the *C. nolae* genome, open reading frames were predicted
667 using orfipy (v0.0.4) (Singh & Wurtele, 2021). Nudivirus genes were then annotated by
668 reciprocal blasts using translated sequences from predicted ORFs. Miniprot (v0.9-0) (H. Li,
669 2023) was also used to align *C. capitator* proteins on *C. nolae* genome and validate previously
670 annotated nudivirus genes. Miniprot gene models were also used for gene clustering in the
671 dN/dS ratio analysis.

672 Pseudogenized genes were identified by reciprocal blast: tblastn was used first to map
673 nudivirus proteins on the wasp genomes. Hits were then verified by blastx to map potential
674 nudiviral nucleotide sequences on the NCBI nr database. Proteins corresponding to Nucleotide
675 sequences mapping back on nudivirus were kept and incomplete and non-transcribed
676 sequences were considered as pseudogenes.

677 Ichnovirus sequences were searched using a home-made pipeline where genomic IVSPER
678 sequences of *Hyposoter dydimator* and *Glypta fumiferanae* were first aligned on *Campoplex*
679 genome portions (the genomes were segmented into chunks of 500,000 bases). Aligning
680 chunks were then aligned on the NCBI nr database. These hits were filtered to keep only hits
681 aligning on IVSPER proteins that are absent from parasitoid wasps which do not bear an
682 Ichnovirus. These "IVSPER" hits were then aligned back on the nr database and only the first
683 hit per query was retained.

684

685 CO1 and nudivirus gene alignments

686 Because the endogenized nudivirus genomes were highly similar in both *Campoplex* species,
687 we collected evidences to confirm that the *Campoplex* wasps sequenced for this study are
688 part of different species. To determine if the genomes of *C. capitator* and *C. nolae* come from
689 distinct species, whole genome alignments were performed using D-Genies (v1.4.0)
690 (Cabanettes & Klopp, 2018). A CO1 (cytochrome oxydase 1) sequence alignment was also
691 performed in order to measure divergence between the two genomes. CO1 orthologs were
692 searched for in *C. capitator* and *C. nolae* genomes by aligning ORFs on CO1 sequence of
693 *Campoplex* species reported in BOLD database (Ratnasingham & Hebert, 2007). Candidates
694 were then aligned together (*C. capitator* candidates vs *C. nolae* candidates) before being

695 aligned on the NCBI database. Endogenized nudivirus were also aligned by blastp in order to
696 assess the protein divergence between the two species. All results for this part are available
697 in the supplementary materials.

698

699 Endogenized nudivirus evolution

700 To perform the virus phylogeny, protein sequences of one nimavirus, three hytrosaviruses,
701 sixty-nine baculoviruses and twenty nudiviruses (including CcapiENV and *C. nolae* sequences)
702 were first aligned together in order to find orthologous genes. Results were then manually
703 corrected using the knowledge found in the literature (Bézier, Annaheim, et al., 2009; Bézier
704 et al., 2015; Bézier, Herbinière, et al., 2009; Burand et al., 2012; Burke, 2019; J.-M. Drezen et
705 al., 2022). Protein sequences of orthologous genes were then aligned using mafft (v7.487)
706 (Katoh et al., 2019) with the following parameters: maxiterate=1000 (maximum number of
707 iterations), genafpair (Altschul algorithm (Altschul, 1998)), ep=0 (offset value). Alignments
708 were then trimmed by TrimAL (v1.4.rev15) (Capella-Gutiérrez et al., 2009) with the gap
709 threshold parameter fixed at 0.6. For each gene, a tree was finally generated with IQ-TREE 2
710 (v2.1.4) (Minh et al., 2020) with the following parameters: m=TEST (use the best model found),
711 B=1000 (number of bootstrap iterations), alrt=1000 (number of SH-aLRT replicates). Protein
712 sequences of genes that were not supporting the accepted phylogeny (at the family level)

713 were removed from the further analysis. Finally, 21 baculovirus core genes and 13
714 alphanudivirus core genes were kept for the virus tree construction. Protein sequences of
715 these genes were aligned and trimmed as described previously. Sequences were then
716 concatenated using FASconCAT-G (v1.0) (Kück & Longo, 2014) and a tree was constructed with
717 iqtree2 with the same parameters as previously described. The tree image was then built using
718 ape (v5.7-1), treeio (v1.24.0) (L.-G. Wang et al., 2020) and ggtree (v3.8.0) (Yu et al., 2017)
719 packages with R (v4.2.1).

720 A whole genome synteny analysis was performed between *V. canescens*, *C. capitator* and *C.*
721 *nolae* using D-Genies. The cluster synteny figure (Figure 3) between *C. capitator* and *V.*
722 *canescens* was built using R and parity plots (Figure 4) showing syntenies among nudiviruses
723 were generated with Excel (Microsoft v16).

724 To estimate the evolutionary constraint occurring on groups of genes according to their
725 function, protein sequences of *C. capitator*, *V. canescens* and *C. nolae* were first clustered
726 using OrthoMCL (v2.0.9) (L. Li et al., 2003) and scripts written by Arun Seetharam
727 (https://github.com/ISUgenomics/common_scripts). Clustered protein sequences were then
728 aligned using ClustalOmega (v1.2.4) and aligned sequences were converted into codon
729 alignement using PAL2NAL (v14.1) (Suyama et al., 2006). Phylip trees were generated using
730 IQ-TREE 2 with the following parameters: -m JTT (Jones-Taylor-Thornton model (Jones et al.,
731 1992)). Codeml (Paml v4.10.6) was finally used to calculate dN and dS values.

732 ## Regulatory sequences in CcapiENV

733 In the same fashion as described in Cerqueira de Araujo et al. (2022), promoter sequences
734 regulating the early and late expression of viral genes in baculoviruses (Chen et al., 2013;
735 Passarelli & Guarino, 2007; Rohrmann, 2014) were sought in the 300 bp upstream of CcapiENV
736 genes. The "early" promoters described in Baculoviruses include a TATA box
737 (TATA[A/T]T[A/T]) and a sequence motif (CA[G/T]T or CGTCG) placed among the 40
738 nucleotides after the TATA box. The late promoters include the baculovirus motif
739 ([A/T/G]TAAG) and the Heliothis zea nudivirus 1 motif (TTATAGTAT) (C.-H. Cheng et al., 2002).
740 We also considered the "late" motif ([G/T][A/T][A/G]A[A/T]ATAG[T/A]) described in Alexandra
741 Cerqueira 2022 for which 2 mismatches were allowed in the sequence search.

742

743 ## CcapiENV gene expression

744 Paired raw reads from ovaries, head-thorax, testes and venom gland of *C. capitator* wasps
745 were first trimmed using Trimmomatic (v 0.38) (Bolger et al., 2014) and TruSeq3-PE adaptors.
746 A quality check was then performed on trimmed reads using fastqc (v 0.11.9) before mapping.
747 Reads were mapped on the genome using Hisat-2 (v2.2.1-3n) (Kim et al., 2019) after genome
748 indexation. Mapped reads were then sorted using samtools (v1.16). Sorted reads were
749 converted into counts by FeatureCounts from the Rsubread package (v2.14.1) (Liao et al.,

750 2019). Using the edgeR package (v 3.42.2) (Robinson et al., 2010), genes with a count being
751 equal to less than 15 per sample were filtered from the dataset in order to remove non-
752 expressed genes. . The dataset was then normalised by converting the read counts into TPM
753 (transcripts per million). A Spearman correlation heatmap was performed to verify duplicate
754 consistency (Figure S4).

755

756 CcapiVLP purification and protein sequencing

757 In order to purify Virus-Like-Particles, 61 adult female wasps of *C. capitator* were dissected 2
758 days after emergence. In total, 122 calyces were obtained and pooled in 1X DPBS. Calyces
759 were then disrupted thanks to a pellet pestle and CcapiVLPs were purified as described in
760 Pichon et al. (2015): disrupted calyces were centrifuged at 770 g, at 4°C for 10 minutes in order
761 to separate CcapiVLPs and calyx fluid (supernatant) from cellular waste (pellet). The
762 supernatant was then retrieved and transferred into a new tube. The new tube was then
763 centrifuged at 15 400 g, at 4°C for 10 minutes. The supernatant was discarded and the pellet
764 containing CcapiVLPs was suspended in 20 µL of 1X DPBS and stored at -20°C.

765 CcapiVLP proteins were then separated by SDS-polyacrylamide gel electrophoresis (12.5%
766 polyacrylamide gel) under denaturing conditions. Separated bands were incubated for 1h with
767 a Coomassie blue solution (10% acetic acid, 50% ethanol, 0.1% R250 Coomassie blue, q.s. to

768 1L of milliQ water) and 9 slices were cut from the gel. Slices were chopped into 1mm^3 cubes
769 before being stored at -20°C and analysed by the platform PIXANIM (France) using a
770 nanoUPLC_LTQ-VElos Pro Orbitrap Mass Spectrometer.

771 For each protein identified by the mass spectrometry, sites and domains were searched for
772 using InterProScan and gene ontology terms were attributed using sequence homology with
773 curated proteins from the Uniprot SWISSprot database (downloaded the 18th November
774 2022). Proteins with a peptide abundance higher than 50 were then retained as VLP
775 candidates.

776

777 Electronic microscopy of the calyx region

778 The calyx region of adult *C. capitator* wasp ovaries was observed under electronic microscopy.
779 Wasp ovaries were dissected two days after emergence and fixed in PrOx/EPON epoxy resin.
780 Samples were fixed, cut and observed with the same protocol as one used in Cerqueira de
781 Araujo and collaborators (Cerqueira de Araujo et al., 2022). The protocol is available in
782 Supplementary materials.

783

Acknowledgements

The authors would like to thank Cosci Francesca, Filippo Di Giovanni and Augusto Loni from the Department of Agriculture, Food and Environment (DAFE) of the University of Pisa (Italy) for the help provided during field wasp sampling. The authors would like to thank also Amine Chebbi who helped the first author on the transposable element annotation pipeline. We would like to thank the IBiSA Electron Microscopy Facility of the University of Tours and the University Hospital of Tours for their assistance. Research funding was provided by an ADS Research Grant from the Smithsonian Institution to BFS, who was also supported by a GGI Peter Buck postdoctoral fellowship during early stages of this work.

Data availability

Raw reads and assemblies are deposited on the NCBI database under BioProject PRJNA936130.

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1304 Table 1: Summary table of CcapiENV gene information. Putative functions were predicted from gene functions described in baculoviruses.
1305 Functional nudivirus core genes are indicated in bold and pseudogenized nudivirus core genes are indicated with an asterisk. Names between
1306 parentheses correspond to alternative names of the genes found in the literature. TPMs and PSM correspond to transcripts per million and
1307 peptide-spectrum matches respectively. A complete version of this table is available in supplementary data (Table S3).

Function in baculoviruses	Gene name	Contig	Nudiviral cluster	Position in the contig	Gene length in bp	Mean of normalized TPMs in ovaries	Presence in VLPs	Peptide abundance (PSM)	Protein size in aa
DNA amplification	***helicase***	contig 13	2	complement(6986111 - 6989746)	3636	60.10636905			1212
Viral transcription	***p47***	contig 2	1	complement(15493852 - 15494922)	1071	202.0627154			357
	lef-4	contig 13	2	complement(6977003 - 6978238)	1236	307.9753009			412
	lef-8	contig 13	2	6982207 - 6985356	3150	190.7332664			1050
	lef-5	contig 328	4	complement(708144 - 708470)	327	390.8433835			109
	lef-9	contig 328	4	complement(727668 - 729752)	2085	380.7544624			695
Envelope components and infectiosity	***pif-4 (19 Kda)***	contig 328	4	706840 - 707595	756	10234.6896	X	39	252
	p33 (ac92)	contig 2	1	15496092 - 15497036	945	4562.742796	X	49	315
	pif-2	contig 13	2	complement(6971652 - 6972776)	1125	1950.200664	X	51	375
	pif-6 (ac68)	contig 328	4	complement(690595 - 691005)	411	4524.153106	X	13	137
	p74 (pif-0)	contig 328	4	695558 - 697498	1941	1979.354037	X	23	647
	vp91 (pif-8)	contig 328	4	complement(713819 - 715891)	2073	1898.483467	X	71	691
	pif-5-1 (odv-e56)	contig 328	4	725333 - 726583	1251	8407.359441	X	104	417
	pif-5-2 (odv-e56)	contig 328	4	733696 - 734952	1257	2238.100222	X	127	419
	pif-3	contig 328	4	744312 - 744905	594	5389.304417	X	33	198
	pif-1	contig 13	2	6978638 - 6980113	1476	1358.325472	X	24	492
	Ac81	contig 328	4	701318 - 701800	483	870.8774304			161
Morphogenesis and particle assembly	***vlf-1***	contig 328	4	709361 - 710638	1278	785.8570436			426
Unknown	*OrNVorf59-like*	contig 328	4	707602 - 708123	522	9224.822369	X	43	174

OrNVorf54-like	contig 2	1	complement(15472150 - 15473337)	1188	3589.443084	X	49	396
OrNVorf41-like-1 (11K)	contig 2	1	15475263 - 15475550	288	32236.6679	X	13	96
OrNVorf41-like-2 (11K)	contig 2	1	15476320 - 15476727	408	20804.23563	X	33	136
OrNVorf41-like-3 (11K)	contig 2	1	15479909 - 15480358	450	41424.23901			150
OrNVorf41-like-4 (11K)	contig 2	1	15481182 - 15481586	405	55874.69226	X	13	135
OrNVorf44-like	contig 2	1	15483923 - 15484489	567	11612.46173	X	66	189
OrNVorf46-like	contig 2	1	complement(15486115 - 15487905)	1791	151.0870697			597
OrNVorf136-like	contig 2	1	complement(15488716 - 15489168)	453	3212.888481			151
OrNVorf23-like	contig 2	1	15489783 - 15490574	792	261.6013813	X	47	264
OrNVorf19-like	contig 2	1	complement(15494837 - 15495235)	399	420.6676754			133
OrNVorf47-like	contig 13	2	6975948 - 6976769	822	2839.655997	X	18	274
OrNVorf73-like	contig 13	2	6992759 - 6993544	786	242.6407675			262
OrNVorf41-like-5 (11K)	contig 326	3	complement(2612826 - 2613125)	300	11433.84229			100
OrNVorf41-like-6 (11K)	contig 326	3	complement(2613850 - 2614170)	321	12768.01194			107
OrNVorf138-like	contig 328	4	complement(686054 - 686347)	294	2268.386529			98
OrNVorf61-like	contig 328	4	complement(689155 - 689526)	372	10869.81904	X	25	124
OrNVorf76-like	contig 328	4	691432 - 691659	228	978.3012378			76
OrNVorf25-like	contig 328	4	698675 - 700330	1656	3713.565782	X	191	552
OrNVorf27-like	contig 328	4	710904 - 712094	1191	2834.742838	X	20	397
OrNVorf90-like	contig 328	4	complement(717857 - 721093)	3237	225.2549736	X	35	1079
OrNVorf63-like	contig 328	4	complement(723007 - 723255)	249	2050.01484			83
OrNVorf79-like	contig 328	4	731525 - 731737	213	1646.832857	X	11	71
OrNVorf118-like	contig 328	4	736112 - 736948	837	3367.630323	X	97	279
OrNVorf119-like	contig 328	4	736958 - 738058	1101	7250.849857	X	80	367
OrNVorf120-like	contig 328	4	complement(738811 - 740787)	1977	1880.881495	X	120	659
OrNVorf122-like	contig 328	4	741734 - 742414	681	364.2703585			227
OrNVorf123-like	contig 328	4	743586 - 743909	324	6143.331885	X	17	108
OrNVorf18-like	contig 328	4	746646 - 748046	1401	6663.775966	X	109	467

Pseudogenes	pseudo vp39 *	contig 2	1	15465017 - 15465368
	pseudo OrNVorf99-like	contig 4	NA	6410523 - 6410915
	pseudo OrNVorf117-like	contig 9	NA	1382035 - 1382601
	pseudo OrNVorf139-like	contig 9	NA	6965367- 6965623
	pseudo FEN-1 *	contig 12	NA	726667 - 726882, 727180 - 727562
	pseudo DNApol *	contig 13	NA	507908 - 507956, 508176 - 508868
	pseudo OrNVorf99-like	contig 13	NA	5114850 - 5115368
	pseudo pif-4 *	contig 13	2	6989881 - 6990024
	pseudo pif-1 *	contig 13	2	6994508 - 6994810
	pseudo helicase *	contig 13	2	complement(6995640 - 6995816)
	pseudo OrNVorf62-like	contig 13	NA	7033194 - 7033540
	pseudo 38K *	contig 94	NA	29063 - 29227
	pseudo integrase *	contig 322	NA	7794008 - 7794318, 7794375 - 7794869
	pseudo OrNVorf22-like (p6.9) *	contig 322	NA	12698893 - 12699488
	pseudo OrNVorf128-like	contig 322	NA	14126845 - 14127067
	pseudo OrNVorf27-like	contig 326	3	2618461 - 2618893
	pseudo lef-8 *	contig 504	NA	3017 - 3559
	pseudo 38K *	contig 508	NA	23980 - 24171

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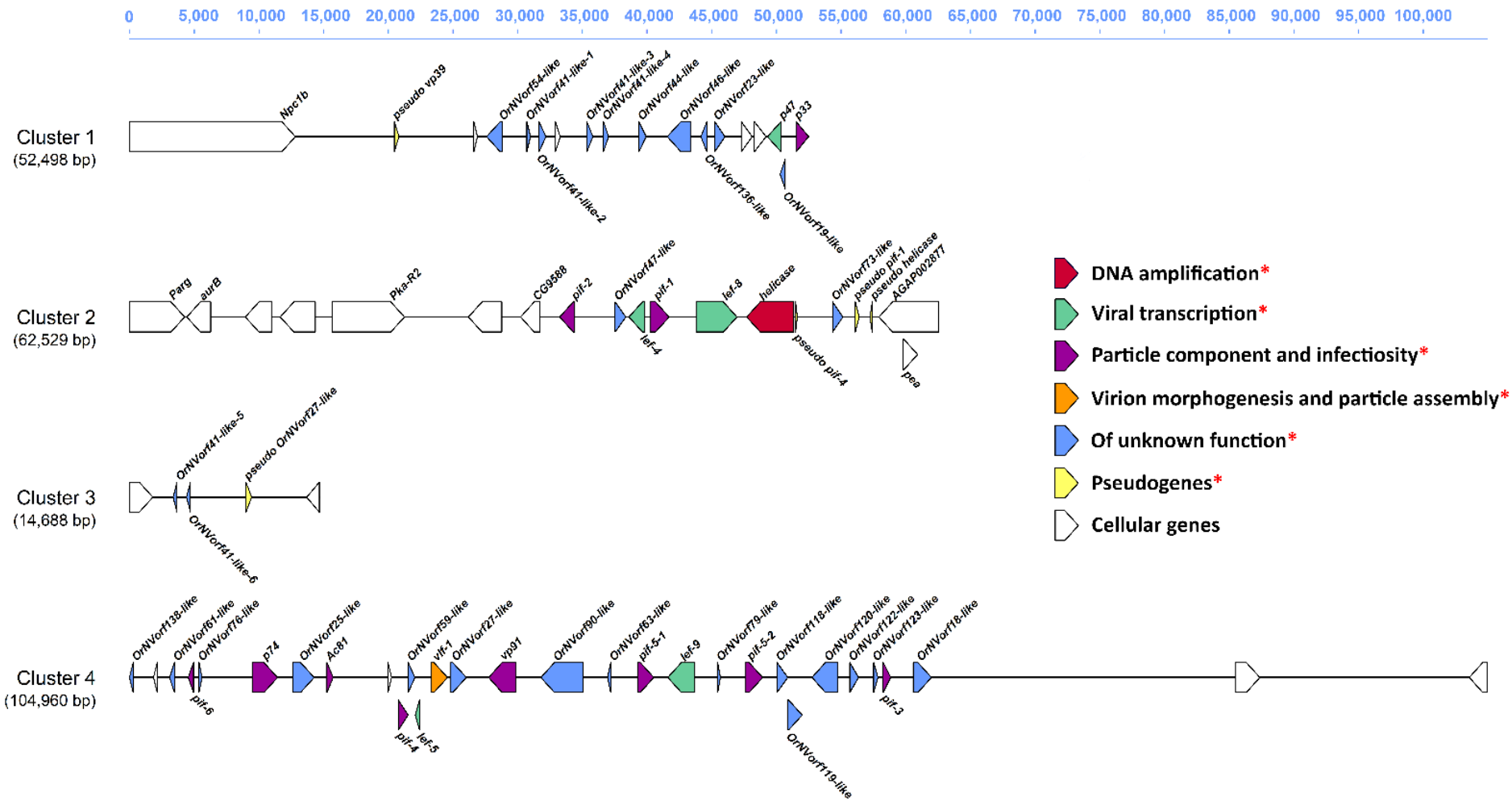


Figure 1: The nudiviral clusters of CcapiENV. Sequences of nudiviral origin are indicated by a red asterisk in the legend. Nudiviral genes were colourised according to their function described in baculoviruses. Cellular genes correspond to genes of non-nudiviral origin (with no nudiviral hit). The scale represents the cluster length in base pairs

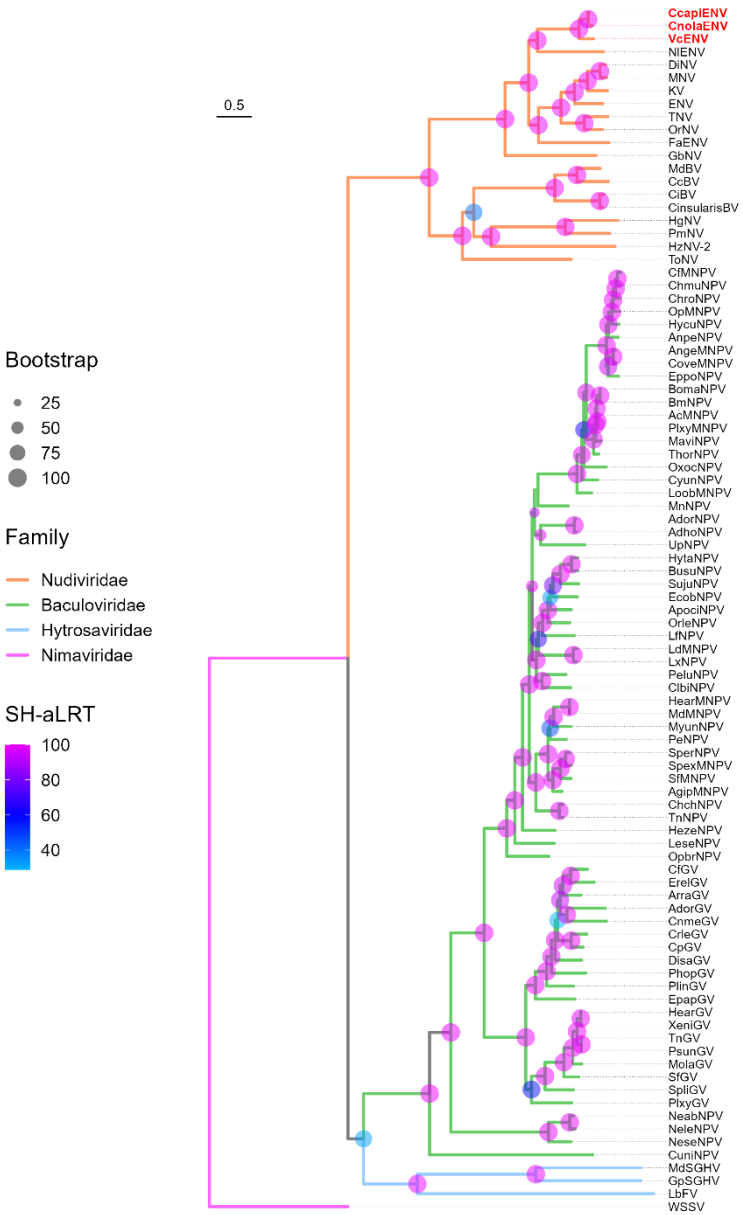


Figure 2: Phylogenetic tree of Naldaviricetes viruses. The tree includes 20 nudiviruses, 69 baculoviruses, 3 hytrosaviruses (including LbFV for Leptopilina boulardi filamentous virus) and a nimavirus (WSSV for White spot syndrome virus) set as the outgroup. In total, 34 genes (21 baculovirus core genes and 13 alphanudivirus core genes) were used for the tree construction, using the maximum likelihood criterion. Branch supports are given by the SH-aLRT (Shimodaira-Hasegawa approximate likelihood ratio test), corresponding to the colour of the node circles and bootstrap values, corresponding to their size. The scale bar represents the phylogenetic distance (percentage of genetic variation). Campoplex capitator Endogenous Nudivirus and Campoplex nolae Endogenous Nudivirus are called respectively CcapiENV and CnolaENV. Nudiviruses endogenized in Campopleginae wasps are mentioned in red. Virus and sequence information is given in supplementary data (Table S4).

1323 Table 2: Nudivirus genes in the *C. capitator, C. nolae* and *V. canescens* genomes. Genes found
1324 in *C. capitator* (in orange) and *C. nolae* (in blue) were compared with those of VcENV (in green).
1325 Genes and pseudogenes are indicated with solid dots and crossed dots respectively. Copies
1326 are indicated by multiple dots. "na" indicates that no copy of the corresponding gene was
1327 found. Alternative gene names are given between parentheses.

Function in baculoviruses	Gene name	CcapiENV	CnolaENV	VcENV
DNA amplification	*DNApol*	⊗	⊗	⊗
	FEN-1	⊗	⊗	⊗
	helicase	•⊗	•	•
	helicase2	na	na	na
	integrase	⊗	⊗	⊗
Viral transcription	*lef-4*	•	•	•⊗
	lef-5	•	•	•
	lef-8	•⊗	•	•
	lef-9	•	•	•
	p47	•	•	•
Envelope components, infectiosity	*ac81*	•	•	•••
	p33 (ac92)	•	•	•
	p74 (pif-0)	•	•	•
	pif-1	•⊗	•	•⊗
	pif-2	•	•	•
	pif-3	•	•	•
	pif-4 (19 Kda)	•⊗	•⊗	•
	pif-5 (odv-e56)	••	••	••••
	pif-6 (ac68)	•	•	•
	vp91(pif-8)	•	•	•
Morphogenesis, particle assembly	*38K*	⊗	na	⊗
	vlf-1	•	•	⊗
	vp39	⊗	⊗	⊗
	OrNVorf22-like (p6.9)	⊗	⊗	⊗
Nucleotid metabolism	*tk1*	na	na	na
	tk2	na	na	na
	tk3	na	na	na
Unknown	*OrNVorf118-like*	•	•	•
	OrNVorf119-like	•	•	•
	OrNVorf120-like	•	•	•
	OrNVorf122-like	•	•	•
	OrNVorf123-like	•	•	•
	OrNVorf136-like	•	•	•
	OrNVorf138-like	•	•	•

	OrNVorf18-like	●	●	●
	OrNVorf19-like	●	●	●
	OrNVorf23-like	●	●	●
	OrNVorf25-like	●	●	●
	OrNVorf27-like	●⊗	●⊗	●
	OrNVorf41-like (11K)	●●●●●●	●●●●●●	●●●●●
	OrNVorf44-like	●	●	●
	OrNVorf46-like	●	●	●
	OrNVorf47-like	●	●	●●●
	OrNVorf54-like	●	●	●
	OrNVorf59-like	●	●⊗	●
	OrNVorf63-like	●	●	●
	OrNVorf73-like	●	●	●
	OrNVorf76-like	●	●	●
	OrNVorf79-like	●	●	●
	OrNVorf90-like	●	●	●
	OrNVorf2-like	na	na	⊗
	OrNVorf9-like	na	⊗	⊗
	OrNVorf62-like	⊗	⊗	⊗
	OrNVorf99-like	⊗	⊗	⊗
	OrNVorf117-like	⊗	⊗	⊗
	OrNVorf128-like	⊗	⊗	⊗
	OrNVorf130-like	na	na	⊗
	OrNVorf139-like	⊗	⊗	⊗

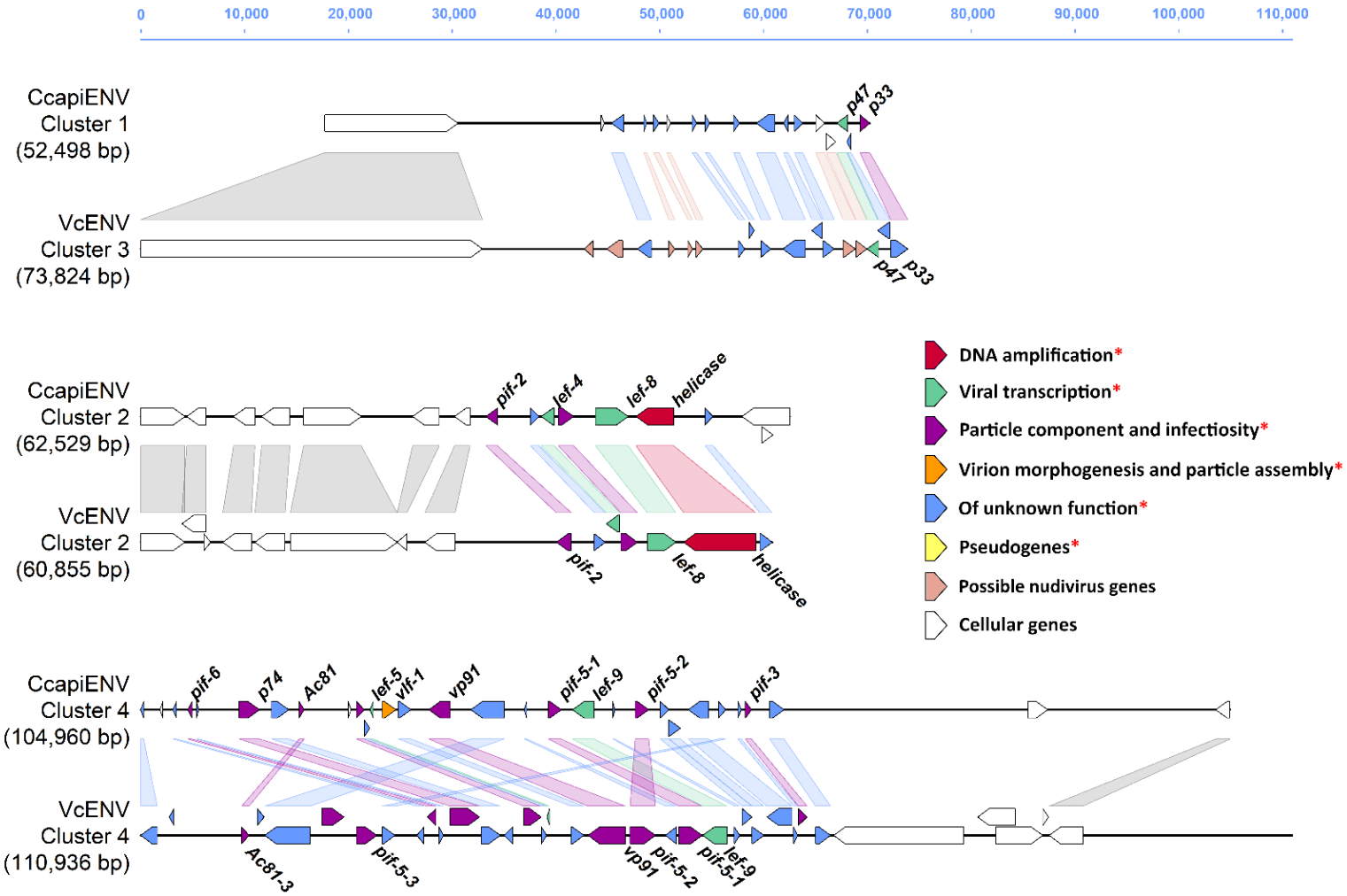
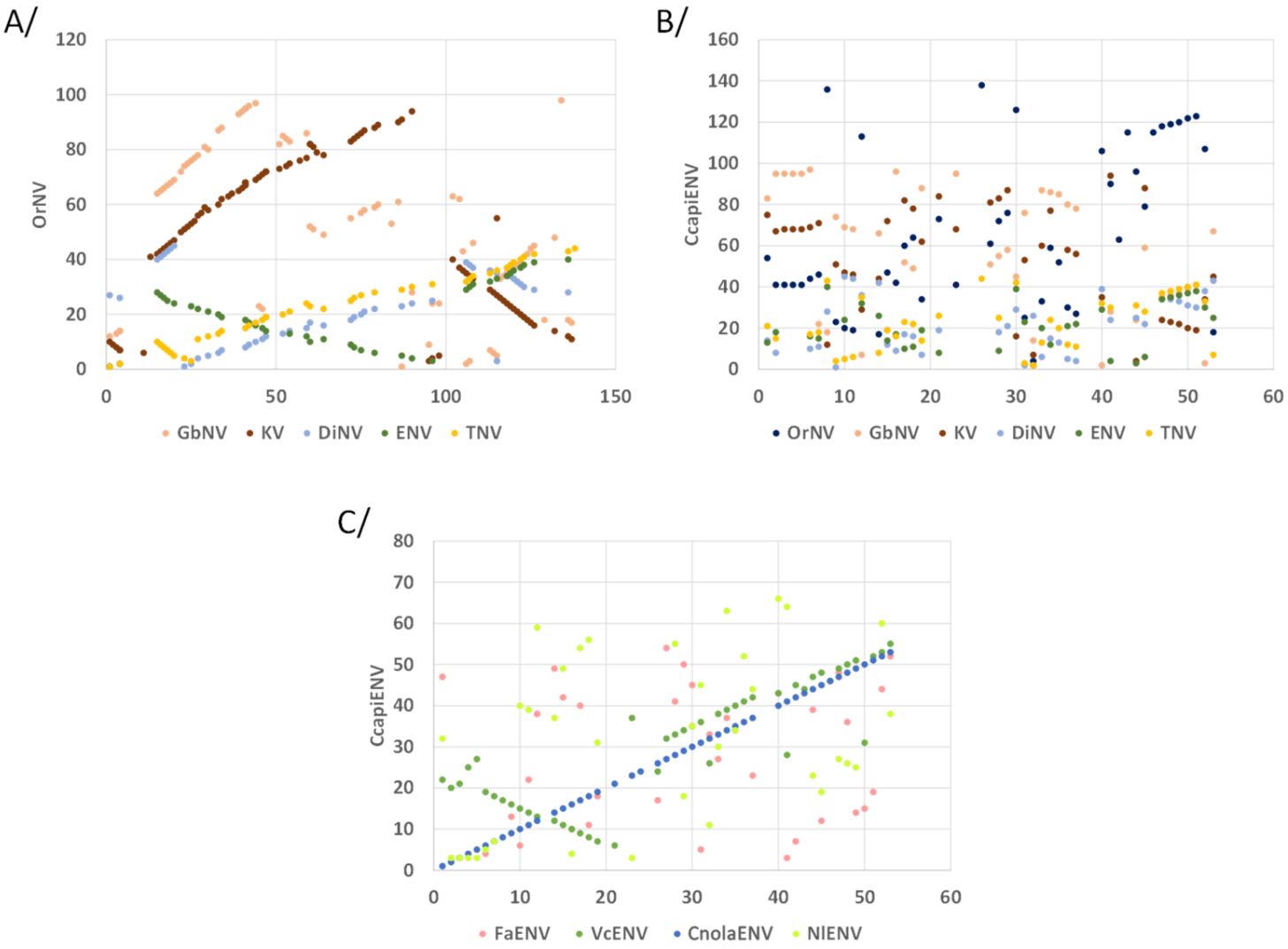


Figure 3: Syntenies between the CcapiENV clusters and VcENV clusters (from Mao et al., 2023). Models were colourised according to their function. Light pink models correspond to genes annotated in Mao and collaborators (Mao et al., 2023) which have no match in the NCBI database, but have nevertheless been annotated as potential virus genes. The scale represents the length in base pairs.



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1339 Figure 4: Parity plots comparing nudivirus genes order. **A** - Parity plot between the genomes
1340 of Oryctes rhinoceros nudivirus (OrNV) on the horizontal axis and other exogenous nudiviruses
1341 on the abscissa. The other nudiviruses are Gryllus bimaculatus nudivirus (GbNV), Kallithea
1342 virus (KV) Drosophila innubia nudivirus (DiNV), Esparto virus (ENV) and Tomelloso virus (TNV).
1343 **B** - Parity plot between the genomes of CcapiENV (ordinate) and the previous exogenous
1344 nudiviruses (abscissa). **C** - Parity plot between the genomes of CcapiENV and other
1345 endogenized nudiviruses (abscissa). The other endogenized nudiviruses are Fopius arisanus
1346 endogenous nudivirus (FaENV), VcENV, CnolaENV and Nilaparvata lugens endogenous
1347 nudivirus (NlENV).

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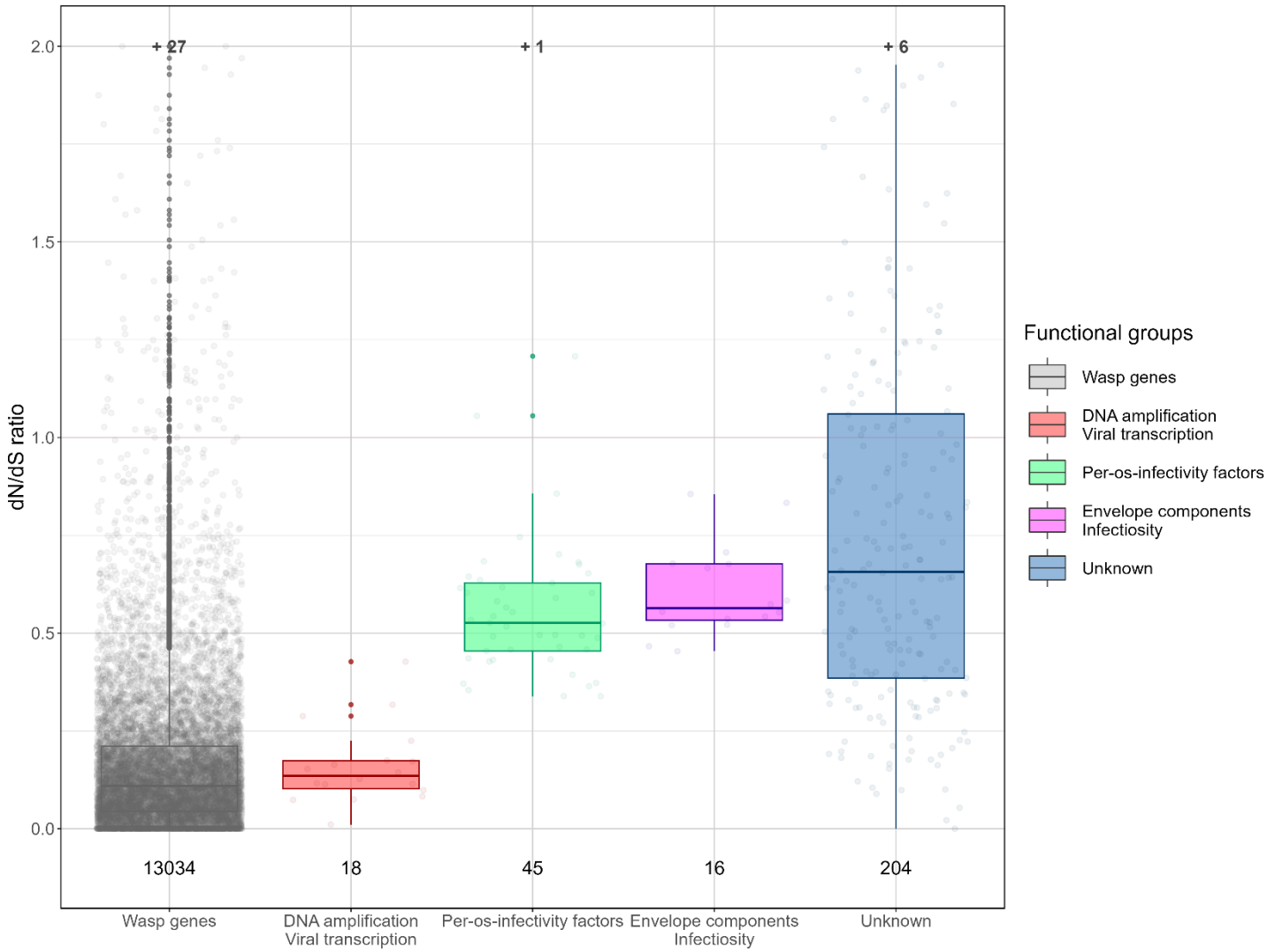

Figure 5: Estimation of selective pressures acting on wasp and endogenized nudivirus genes. dN/dS ratio values were calculated from wasp (in grey) and nudivirus genes of *C. capitator*, *C. nolae* and *V. canescens*. Numbers below each boxplot represent the number of sequences used for dN/dS ratio calculation for each category. Numbers above each boxplot represent the number of values higher than 2, which could not be plotted. In red are the dN/dS ratio values for *helicase*, *p47* and *lef* genes, in green are the values for *pif* (*per-os-infectivity factor*) genes, in pink the values for *Ac81*, *vp91* and *p33* genes, and in blue the values for *OrNVorf-like* genes.

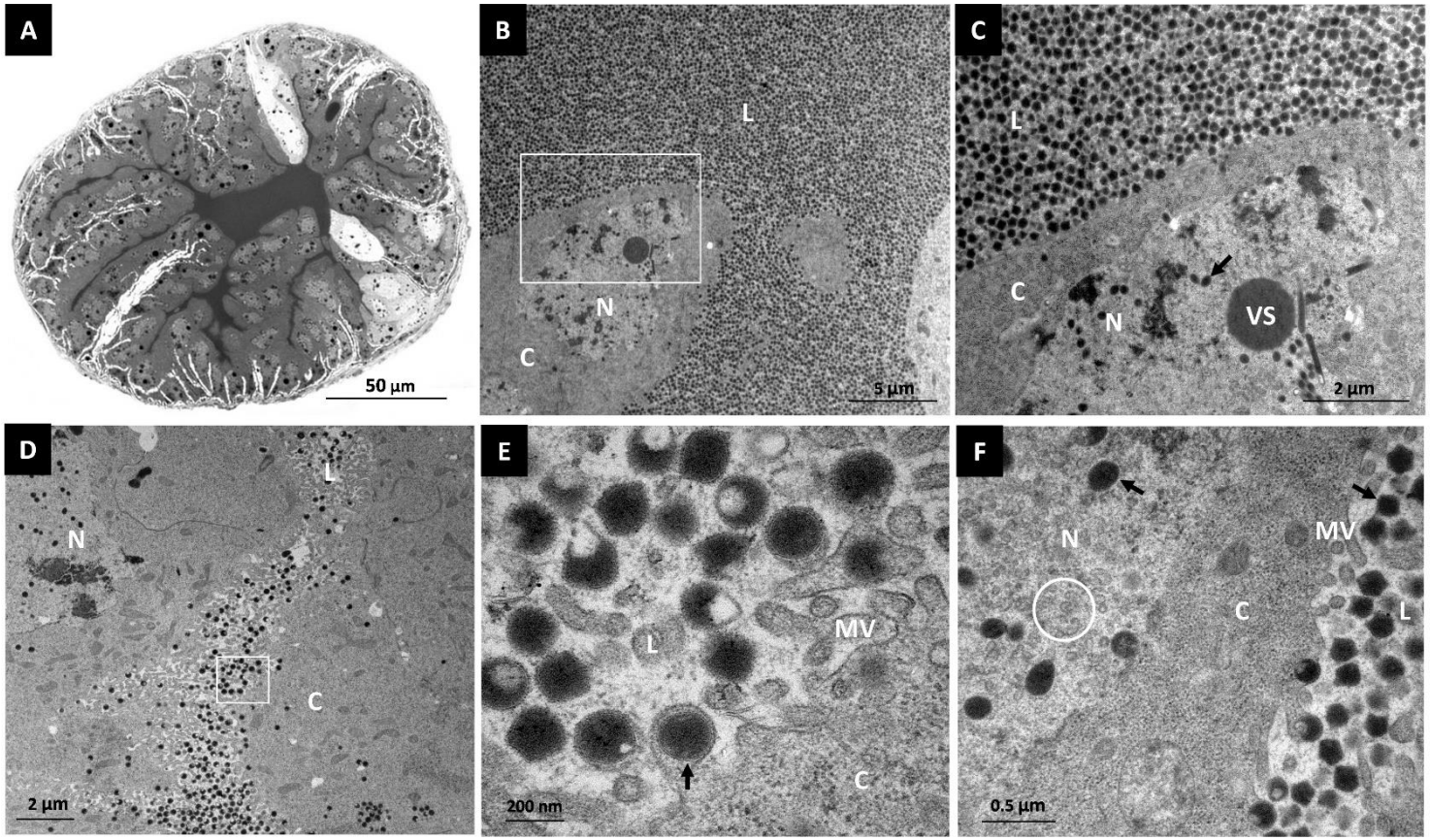

Figure 6: The calyx region of a *C. capitator* ovaries of adult wasp observed using light and electron microscopy. A - light microscopic photograph of a semi-thin section of the calyx region, stained with toluidine blue. B, D - electron microscopy photographs of the calyx region shown in A. C, E - enlarged areas (white frames) of photographs B and D, respectively. Virogenic stromas (VS) and VLPs (dark arrow) are visible in cell nuclei (N). While virogenic stromas can be seen only in the nuclei, VLPs appear to travel from the nucleus to the lumen (L) where they accumulate, passing through the cytoplasm (C) and the membrane microvilli (MV). F - electron microscopy photograph showing the nucleus-cytoplasm-lumen interface where empty VLP envelopes can be seen in the nucleus (white circle), as described in *V. canescens* (Pichon et al., 2015).