

# 1 Endoparasitoid lifestyle promotes 2 endogenization and domestication of dsDNA 3 viruses

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12  
13 **Abstract** The accidental endogenization of viral elements within eukaryotic genomes can occasionally provide signifi-  
14 cant evolutionary benefits, giving rise to their long-term retention, that is, to viral domestication. For instance, in some  
15 endoparasitoid wasps (whose immature stages develop inside their hosts), the membrane-fusion property of viruses has  
16 been repeatedly domesticated following the ancestral endogenizations of double-stranded DNA viruses. The endoge-  
17 nized genes provide female wasps with a delivery tool to inject virulence factors that are essential to the developmental  
18 success of their offspring. Because all known cases of viral domestication involve endoparasitic wasps, we hypothesized  
19 that this lifestyle, relying on a close interaction between individuals, may have promoted the endogenization and do-  
20 mestication of viruses. By analyzing the composition of 124 Hymenoptera genomes, spread over the diversity of this  
21 clade and including free-living, ecto- and endoparasitoid species, we tested this hypothesis. Our analysis first revealed  
22 that double-stranded DNA viruses, in comparisons with other viral genomic structures (ssDNA, dsRNA, ssRNA), are more  
23 often integrated (that is, endogenized) and domesticated (that is, retained by selection) than expected from their esti-  
24 mated abundance in insect viral communities. Secondly, our analysis indicates that the rate at which dsDNA viruses are  
25 endogenized is higher in endoparasitoids than in ectoparasitoids or free-living hymenopterans, which also translates into  
26 more frequent events of domestication. Hence, these results are consistent with the hypothesis that the endoparasitoid  
27 lifestyle has facilitated the endogenization of dsDNA viruses, in turn increasing the opportunities of domestications that  
28 now play a central role in the biology of many parasitoid lineages.

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## 30 Introduction

31 The recent boom of genome sequencing programs has revealed the abundance of DNA fragments of viral origin within  
32 eukaryotic genomes. These so-called Endogenous Viral Elements (EVEs) stem from endogenization events that not only  
33 involve retroviruses as donors (as could be expected from their natural lifecycle) but also viruses that do not typically  
34 integrate into their host chromosomes [1, 2, 3]. In fact, retroviruses have never been discovered in any insect species,  
35 but various non-retroviral viruses have been identified as being engaged in viral endogenization events : three families of  
36 large double-stranded (ds) DNA viruses, at least 22 families of RNA viruses, and three families of single-stranded (ss) DNA  
37 viruses [4]. Degeneracy and loss is likely the fate of most EVEs, since they do not *a priori* benefit their hosts. Still, several  
38 studies have reported that EVEs can be retained by selection, thus becoming *domesticated* [5]. The functions involved  
39 include defensive properties against related viruses in mosquitoes [6, 7], against macroparasites in some Lepidoptera  
40 [8], or modifications in the expression of genes involved in dispersal in aphids [9]. Interestingly, the membrane fusion

41 capacity of viruses, allowing their entry into host cells, has been repeatedly co-opted in three metazoan clades: mammals,  
42 viviparous lizards and parasitoid wasps. In placental mammals and viviparous Scincidae lizards, domestication of the  
43 *syncytin* protein from retroviruses has allowed the emergence of the placenta, through the development of the syncytium  
44 (composed of fused cells) involved in metabolic exchanges between the mother and the fetus [10, 11]. A similar fusogenic  
45 property was repeatedly co-opted by parasitoids belonging to the Hymenoptera order through the endogenization and  
46 domestication of complex viral machineries deriving from large dsDNA viruses [12, 4]. The numerous retained viral genes  
47 allow parasitoid wasps to produce virus-like structures (VLS) within their reproductive apparatus. These are injected into  
48 the parasitoid's host, together with their eggs, and protect the wasp progeny against the host immune response. This  
49 protection is achieved thanks to the ability of VLS to deliver wasp virulence factors in the form of genes (in which case  
50 VLS are called polydnavirus - PDV) or proteins (in which case VLS are called Virus-like particles - VLPs) to host immune  
51 cells (reviewed in [13, 14]). So far, 5 independent cases of such viral domestication have been detected in parasitoid  
52 wasps, four of them falling within the Ichneumonoidea superfamily [15, 16, 17, 18] and one in the Cynipoidea superfamily  
53 [19]. The four cases where the donor virus family has been unequivocally identified point towards dsDNA viruses. More  
54 specifically, the domesticated EVEs (hereafter, dEVEs) derive from the *Nudiviridae* family in three cases [15, 17, 18] while  
55 the fourth involves a putatively new viral family denoted "LbFV-like" [19]. Notably, all these domestication events took place  
56 in endoparasitoids, that is, in species that deposit their eggs inside the hosts, as opposed to ectoparasitoids that lay on  
57 their surface.

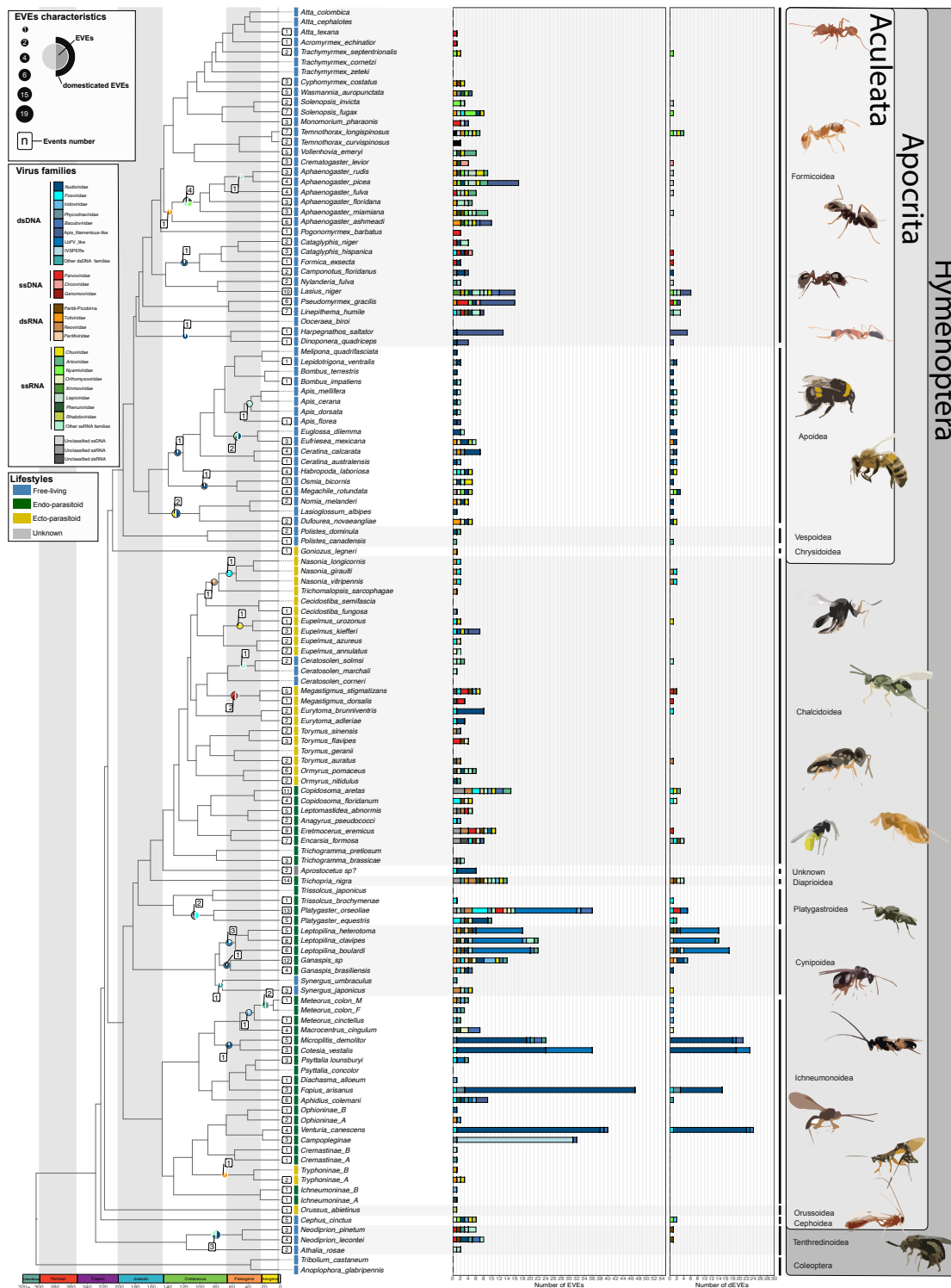
58 Beyond these well characterized events of viral domestication in Hymenoptera, additional cases of endogenization  
59 have been uncovered, in studies that enlarged the taxonomic focus of either the hosts [20, 21, 22] or the viruses that  
60 were considered [23, 20, 24, 25]. Here, we complement this earlier work by expanding the range of both the hosts and  
61 viruses under study, and by further analyzing which endogenization cases have been followed by a domestication event.

62 To this end, we developed a bioinformatic pipeline to detect endogenization events involving any kinds of viruses  
63 (DNA/RNA, single-stranded, double-stranded), at the scale of the whole Hymenoptera order. This analysis first allowed us  
64 to test whether the propensity of viruses to enter Hymenoptera genomes, and to be domesticated, depend on their ge-  
65 nomic structure (in line with the pattern observed so far, where only dsDNA viruses have been involved in domestication  
66 events as described above). We then tested whether the lifestyle of the species (free-living, endoparasitoid, ectoparasitoid)  
67 correlates with their propensity to integrate and domesticate viruses. Our working hypothesis was that the endopara-  
68 sitoid lifestyle may be associated with a higher rate of viral endogenization and / or a higher rate of domestication events,  
69 for two non-exclusive reasons related either to the exposure to new viruses and the adaptive value of the endogenized  
70 elements.

71 First, a higher endogenization rate may simply stem from a higher exposure to viruses. Such an effect could be at play  
72 in endoparasitoids due to the intimate interaction between the parasitoid egg or larva and the host. In other words, the  
73 endo-parasitic way of life may facilitate the acquisition of new viruses deriving from the hosts. Notably, this lifestyle may  
74 also facilitate the maintenance and spread of newly acquired viruses within wasp populations. Indeed, endoparasitoid  
75 wasps often inject not only eggs but also venomous compounds (typically produced in the venom gland or in calyx cells)  
76 where viruses can be present and may thus be vertically transmitted [26]). In addition, the confinement of the several  
77 developing wasps within a single host may facilitate horizontal transmission (e.g. [27]).

78 Second, a higher rate of domestication may also contribute to produce a particularly high abundance of EVEs in en-  
79 doparasitoids. This is expected since these insects are facing the challenge of resisting the host immune system. This  
80 selective pressure may promote the co-option of viral functions such as the above-mentioned membrane fusion activity,  
81 that provide a very effective mean to deliver virulence factors.

82 Our analysis reveals numerous new instances of endogenization events, some of which are also characterized by sig-  
83 natures of molecular domestication. We found a clear enrichment in endogenization events deriving from dsDNA viruses  
84 as compared to those with other genomic structures. While the data did not reveal a significant effect of Hymenoptera  
85 lifestyles on the acquisition of dsRNA, ssRNA or ssDNA viruses, it supports the hypothesis that genes from dsDNA viruses  
86 are more often endogenized and domesticated in endoparasitoids than in free-living and ectoparasitoid species.



**Figure 1. Endogenous Viral Elements and their domestication status in Hymenoptera.** Lifestyles are displayed next to species names (blue: free-living, green: endoparasitoid, yellow: ectoparasitoid, grey: unknown). The number of EVEs and domesticated EVEs (dEVEs) found in each species are represented respectively by the first and second facet of the horizontal histograms. Colors along these histograms indicate the potential donor viral families (where blue tones correspond to viral dsDNA viruses, red tones to ssDNA viruses, orange/yellow tones to dsRNA viruses and green tones to ssRNA genomes). Endogenous Viral elements (EVEs) shared by multiple species and classified within the same event are represented by circles whose size is proportional to their number; those that are considered as domesticated (dEVEs) are surrounded by a black border. Numbers in the white boxes indicate to the number of endogenization events inferred. As an example, *Megastigmus dorsalis* and *Megastigmus stigmatizans* are ectoparasitoids (yellow) sharing a common endogenization event (within the Cluster21304) that likely originated from an unclassified dsRNA virus (grey color in circle), and shows no sign of domestication (no black border around the grey part of the circle). The figure was inspired from the work of [28]. Details on the phylogenetic inference and time calibration can be found in the MM section; bootstrap information can be found in TableS2; details on lifestyle assignment can be found on the github repository under the name : Assembly\_genome\_informations.csv

## 87 Results

88 We screened for EVEs 124 Hymenoptera genome assemblies, including 24 ectoparasitoids, 37 endoparasitoids and 63  
89 free-living species (can be found on the github repository under the name : Assembly\_genome\_informations.csv). EVEs  
90 were identified using a sequence-homology approach based on a comprehensive viral protein database. Different confi-  
91 dence levels (ranging from A to D) were associated with the various EVEs inferred, where the A score indicates a maximal  
92 confidence. This confidence index is based upon sequencing depth combined with information on the genomic environ-  
93 nment of the candidate loci, that is, the presence of eukaryotic genes and/or transposable elements (as detailed in the MM  
94 section). By default, the four categories are included in the analysis, but unless otherwise stated statistical tests based  
95 on the A category only led to the same conclusions (see (FigureS13-7 for more details). Our analysis further included an  
96 inference of the phylogenetic relationships among homologous EVEs, that was used to map endogenization events on  
97 the Hymenoptera species tree. Finally, inferences of domestication events relied upon signatures of purifying selection  
98 in the integrated genes (based on dN/dS estimates) and/or on expression data.

99 An important objective of our analysis is to detect and enumerate not only endogenous viral elements (EVEs) but also  
100 endogenization *events* that can explain the presence of these EVEs. Indeed, an EVE denotes a single gene of viral origin  
101 in a single species. Several neighboring EVEs in a genome may result from the endogenization of a single viral genome,  
102 and homologous EVEs shared by several closely related species may further stem from a single ancestral endogenization  
103 events. This distinction is critical when it comes to examining the effect of various factors on the probability of integrating  
104 EVEs, which implies counting events rather than EVEs. As an example, consider the *Leptopilina* case [19], involving 13 EVEs  
105 shared by 3 closely related species. In this wasp genus, based on previous findings, we expect the 39 EVEs to be grouped  
106 into a single endogenization event. Our pipeline appropriately detected 36 EVEs (out of 39) and correctly aggregated  
107 them into a single endogenization event mapped on the branch leading to the *Leptopilina* genus. Furthermore, because  
108 some of the genes involved are inferred as domesticated, this event is appropriately classified as a domestication event  
109 (see Figure 1 and Figure S14 for more canonical examples). In total, the pipeline correctly detected 88.4% (152/172) of the  
110 EVEs involved in our four "positive controls", previously described as mediating the protection of young wasps against  
111 their host immune system. Among them, 71.82% were inferred as being domesticated. Out of the 152 positive controls  
112 EVEs, 147 were grouped into 4 independent endogenization events, as was expected. The remaining 5 genes had peculiar  
113 histories that led our pipeline to infer two additional spurious events (Table S1). All detailed results regarding EVEs and  
114 dEVEs can be found on the github repository under the name : All\_EVEs\_dEVEs\_informations.txt.

### 115 Endogenizations involve all viral genomic structures

116 A total of 1261 convincing EVEs have been inferred in the whole dataset (TableS2,Figure1). These translate into 367  
117 endogenization events, the majority of which involved ssRNA and dsDNA viruses (41% and 35%, respectively) (TableS2).  
118 Among the 124 species under study, 113 were involved in at least one endogenization event, with a maximum of 14  
119 events per species and a median of 3 (Figure1). Most of the events (331) are specific to a single species and have thus  
120 likely taken place relatively recently, while the remaining 9% are shared by at least two closely related species (TableS2,  
121 Figure1). To assess the validity of the procedure used to aggregate multiple EVEs into a single ancestral endogenization  
122 event, we assessed whether EVEs inferred as homologous shared a common genomic environment. We thus tested for  
123 the presence of homologous loci in different species around the EVEs that had been aggregated (using blastn searches  
124 between the corresponding scaffolds (see details in Materials and methods). Among the 36 endogenization events that  
125 involved at least two species, 31 were found to carry more homologous loci around the insertion sites than expected  
126 by chance (see details in Materials and methods). Notably, the majority of endogenization events involved a single EVE  
127 (a single gene) and only 12 (all from dsDNA viruses), involved the concomitant integration of more than 4 viral genes  
128 (Figure2-C).

129 A total of 40 different viral clades (usually families) were inferred as putative donors. Most of them (34) are known to  
130 infect insects (Figure2-B) and these account for the majority of the endogenization events. However, we found 36 EVEs  
131 (24 endogenization events), including 20 high-confidence ones (A-ranked), that derived from 6 viral families not previ-  
132 ously reported to infect insects (*Phycodnaviridae*, *Herpesviridae*, *Caulimoviridae*, *Asfaviridae*, *Bornaviridae* and *Mypoviridae*).  
133 However, in those cases, the true viral donors may belong to unknown clades that do infect insects. Indeed, although the  
134 homology with viral proteins was convincing (median e-value was 9.4095e-12 [min = 9.212e-129, max = 3.305e-08]), the  
135 average percentage identity was relatively low (38% [min = 23.2%, max = 79.1%], suggesting that these loci may originate  
136 from unknown viruses that are only distantly related to these 6 viral families.

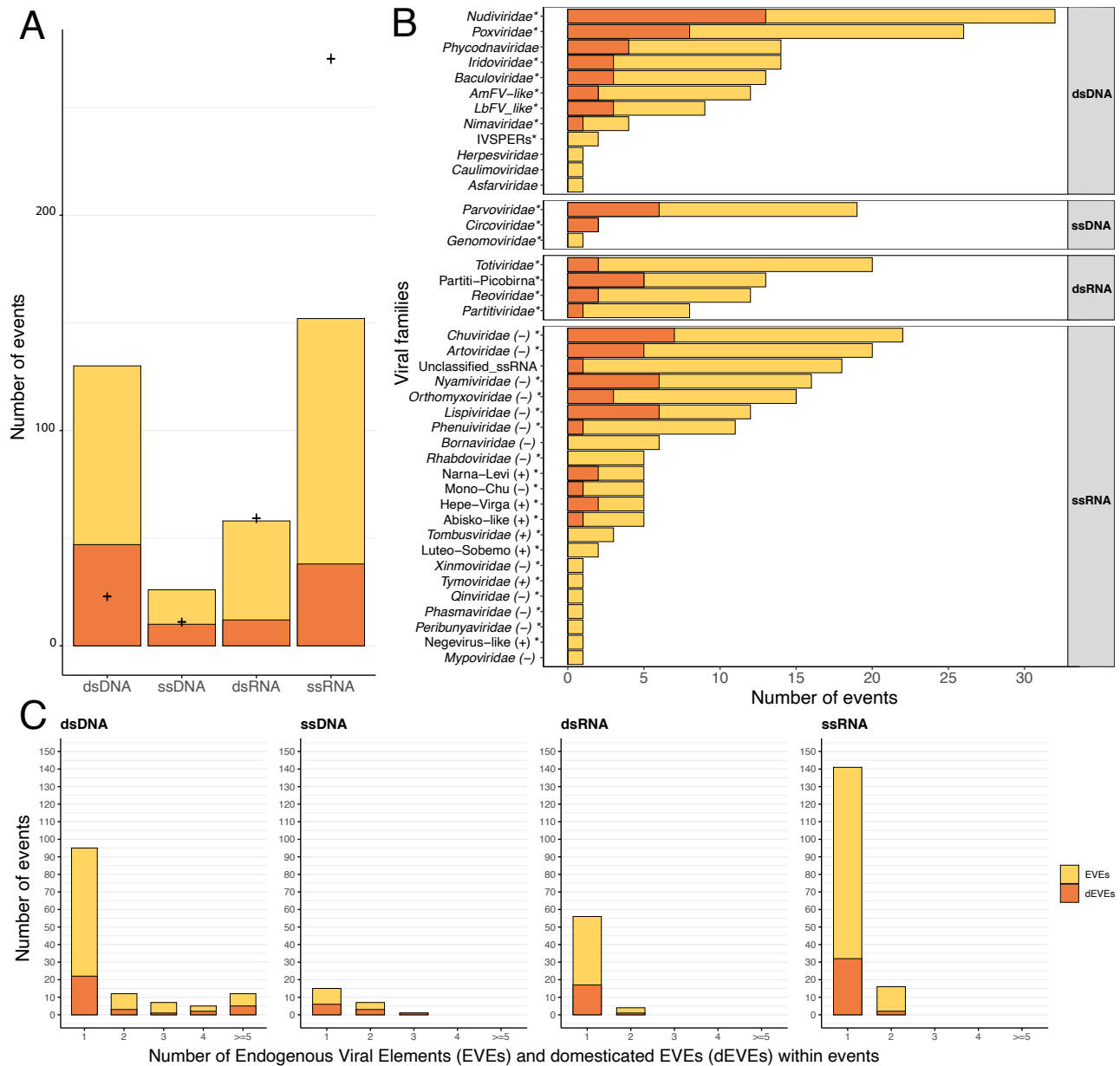


Figure 2. **Endogenization involves all virus genomic structures.** In all three panels, a yellow color indicates endogenization events that have not been followed by domestication, while orange indicates domestication events. **A** : Distribution of the number of events inferred, according to the four categories of viral genomic structures. The crosses refer to the expected number of endogenization events based for each category based on its estimated relative abundance in insects (see details in Materials and methods and virus-infecting data in (Excel tab:All\_virus\_infecting\_insects\_informations)). **B** : Distribution of the various viral families involved in endogenization events. The polarity of ssRNA viruses is displayed next to the family name. Events involving multiple putative families (i.e. where several viral families are present in the same scaffold) have been excluded from the count. The star next to the family name indicates that the viral family is known to infect insects. **C** : Distribution of the number of EVEs per event across viral categories.

### 137 **Double-stranded DNA viruses are over-represented in endogenization events**

138 Most of the endogenization events recorded involve ssRNA and dsDNA viruses. But do these proportions simply mirror  
 139 the diversity and respective abundances of the different kinds of viruses encountered by insects? The analysis summa-  
 140 rized in (Figure2-A) (see details in Materials and methods) indicates this is not the case. More specifically, it shows that  
 141 dsDNA viruses are more frequently endogenized than expected on the basis of their representation in the databases,  
 142 while ssRNA viruses are under-represented ( $\chi^2 = 213.36$  and  $221.38$ , respectively, for endogenization events and domes-  
 143 tication events, d.f. = 3, both p-value <  $2.2e-16$ ). Notably, this result is not purely driven by the presence in our data set  
 144 of the four positive controls (previously described cases of viral domestication, that all involve dsDNA viruses as donors).  
 145 Finally, among endogenization events involving ssRNA viruses, we found an over-representation of negative stranded  
 146 ssRNA compared to their relative abundance in public databases (72.2% compared to 32.6% in the databases,  $\chi^2=145.87$ ,  
 147 d.f.=1, p-value <  $2.2e-16$ ; see supplemental information for a discussion).

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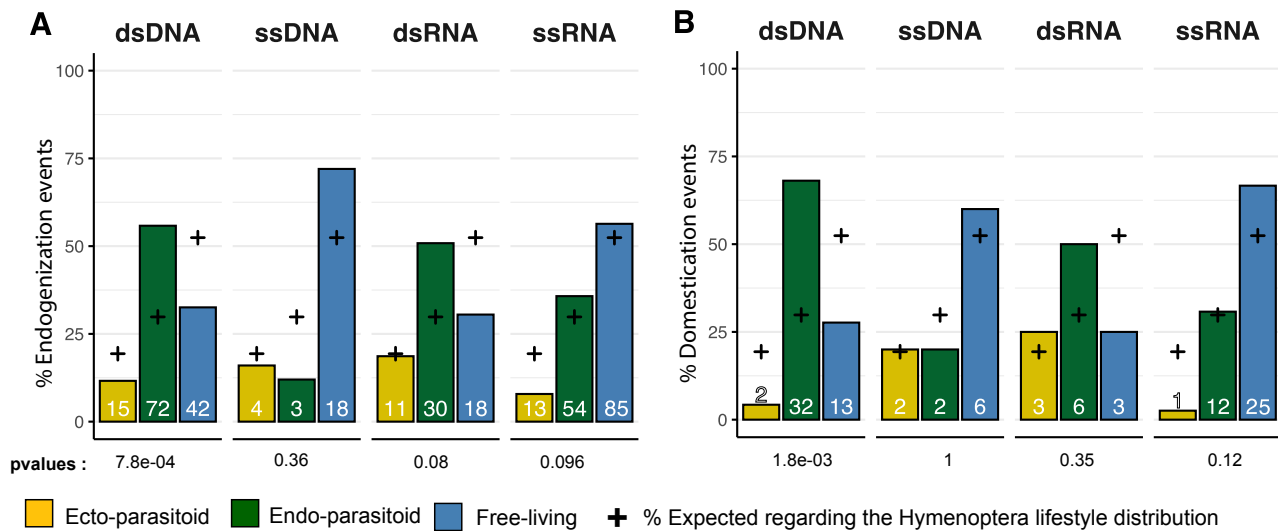


Figure 3. **Endogenization and domestication of dsDNA viruses are most prevalent in endoparasitoid species.** **A:** Distribution of viral endogenization events (Event) and **B** of domestication events (dVEs) across Hymenoptera lifestyles. Crosses indicate the expected proportion of events based on the respective frequencies in our database (ectoparasitoid = 24/124, endoparasitoid = 37/124, free-living = 63/124). The p-values are the results of Fisher's tests comparing the observed and expected distributions. Numbers inside the bars indicate the absolute numbers of events inferred. The ancestral states of the nodes, in terms of lifestyle, were inferred in a Bayesian analysis (see details in Materials and methods)

### 149 **Endogenizations of dsDNA viruses are more frequent in endoparasitoid species**

150 Next, we sought to characterize the factors that could explain the patterns of endogenization events inferred (Figure 1). To  
 151 this end, for each virus genomic structure, we assessed whether endogenization events were evenly distributed among  
 152 the three wasp lifestyles, taking into account their respective frequencies in the dataset. No significant departure from  
 153 the null hypothesis was detected for endogenization involving ssDNA, dsRNA or ssRNA viruses (Fisher exact test p-values  
 154 BH corrected > 0.05). On the contrary, we detected a highly significant enrichment of dsDNA viruses endogenization  
 155 events in endoparasitoid species, and conversely a deficiency in free-living and ectoparasitoid species (corrected p-value  
 156 =  $7.8e-04$ , Figure 3-A).

157 To further test the apparent correlation between Hymenoptera lifestyle and the rate of endogenization events, we  
 158 inferred ancestral lifestyles along the phylogeny using a Bayesian model (see details in Materials and methods). We then  
 159 constructed a generalized linear model where the dependent variable is the number of endogenization events inferred  
 160 on each branch, while branch length and lifestyle are the explanatory variables (see details in Materials and methods).  
 161 Branch length was included as an additive effect to remove the expected effect of time on the number of endogenization  
 162 events, thus allowing the decomposition of the remaining variance according to the lifestyle (free-living, ectoparasitoid  
 163 or endoparasitoid).

164 We first tested whether the rate of endogenization events deriving from any virus (that is, regardless of their genomic  
165 structures) was structured by lifestyles, and found no significant effect (Figure S5-A left side). We then split the dataset  
166 according to the genomic structure of the donor viruses. For RNA or ssDNA virus, the analysis did not reveal evidence of  
167 a correlation between wasps' lifestyles and the rate of endogenization events (for details, see supplemental information,  
168 FigureS5-G,I K). On the contrary, in the case of dsDNA viruses, we found a highly significant effect of the wasp lifestyle:  
169 endogenization rates appear to be 2.43 times higher in endoparasitoids than in free-living species (89% CI [1.53-3.41], Fig-  
170 ure4-A). The corresponding probability of direction (pd, an index representing the confidence in the direction of an effect)  
171 was equal to 99.9%. In contrast, ectoparasitoids did not differ from free-living species (Figure4-A). Accordingly, more than  
172 98% of the MCMC iterations led to a higher coefficient value for endoparasitoids than for ectoparasitoids (so-called  $P_{MCMC}$   
173 in Figure4-A). This effect was consistently found using high confidence scaffolds only (A-ranked scaffolds, FigureS5-C right  
174 side). We also carried out the same analysis without the 4 domestication cases previously mentioned in the literature  
175 (because including them in our data set could have skewed the results) and reached the same conclusion (FigureS5-E  
176 right and left sides). Overall, these results show that dsDNA viruses are more often endogenized in endoparasitoids than  
177 in free-living and ectoparasitoid species.

### 178 **Domestications of dsDNA viruses are most prevalent in endoparasitoid species**

179 We then investigated whether lifestyles may explain the abundance of domestication events. A simple Fisher's exact test  
180 approach revealed an enrichment in endoparasitoid species of domestication events involving dsDNA viruses (corrected  
181 p-value = 1.8e-03), whereas no deviation from the null hypothesis was detected for the other viral genomic structures  
182 (Figure 3-B).

183 We built upon the generalized linear models described above, in a Bayesian framework, to test whether lifestyle could  
184 also be a factor explaining the propensity of Hymenoptera to domesticate (and not simply endogenize) viral genes (see  
185 details in Materials and methods). We found that, domestication of dsDNA viruses are 3.68 times more abundant in  
186 endoparasitoids than in with free-living species (89% CI [1.72-6.17], pd =99.9%, Figure4-B). This effect was also detected  
187 when only high confidence candidates were considered (FigureS5-D right side), or if we removed the four known cases  
188 of domestication (FigureS5-F left and right side). In other viral categories, no convincing effect of the wasp lifestyle was  
189 detected (all pd<99%) (Figure S5-H J) with the only exception higher rate of domestication of ssRNA viruses in free-living  
190 than in ectoparasitoid species (Figure S5-L)

191 Two non mutually exclusive hypotheses may be envisaged to explain the high frequency of dsDNA viruses domestica-  
192 tion in endoparasitoids. First, it may simply stem from the higher rate of endogenization outlined above: a higher rate of  
193 entry would overall translate into a higher rate of domestication. Second, it may result more specifically from differences  
194 in the rate at which viral elements are domesticated after being endogenized. To disentangle these hypotheses, we built  
195 a binomial logistic regression model in a Bayesian framework, focusing on events involving dsDNA viruses, and specify-  
196 ing the number of domesticated events *relative* to the total number of endogenization events inferred. By controlling for  
197 the endogenization input (the denominator), these binomial models make it possible to test whether the probability of  
198 domestication after endogenization of dsDNA viruses is correlated with the lifestyle.

199 Based on this analysis, the probability that an endogenization event will lead to a domestication event is not signifi-  
200 cantly different between endoparasitoids and freelifing species (FigureS10-A, pd=89.18%). However, the probability of do-  
201 mestication was found to be significantly higher in endoparasitoids than in ectoparasitoids (FigureS10-A,  $P_{MCMC}$ =99.81%).  
202 The same trend was observed if we focused on high confidence scaffolds and/or if we removed the controls from the  
203 dataset (FigureS10-B,C D, pd < 86%).

204 Together, these findings show that the endoparasitoid lifestyle is associated with an increased rate of dsDNA viruses  
205 endogenization. Endoparasitoids are also characterized by an elevated frequency of domestication events that does not  
206 appear to be explained by an elevated rate of post-endogenization domestication.

### 207 **New remarkable cases of endogenization and domestication**

208 Here, we describe in more details specific cases identified by our pipeline. We found a massive entry of genes from ds-  
209 DNA viruses in an undescribed species belonging to the Campopleginae subfamily ("Campopleginae sp" in Figure 1). In  
210 Ophioniformes (a clade that includes Campopleginae), two lineages have previously been shown to hosts domesticated  
211 viruses (the Campopleginae species *Hyposoter didymator* [29], and the Banchinae species *Glypta fumiferanae* [30]). It has  
212 been advocated that these so-called ichnoviruses viruses found in *Hyposoter didymator* and *Glypta fumiferanae* may de-

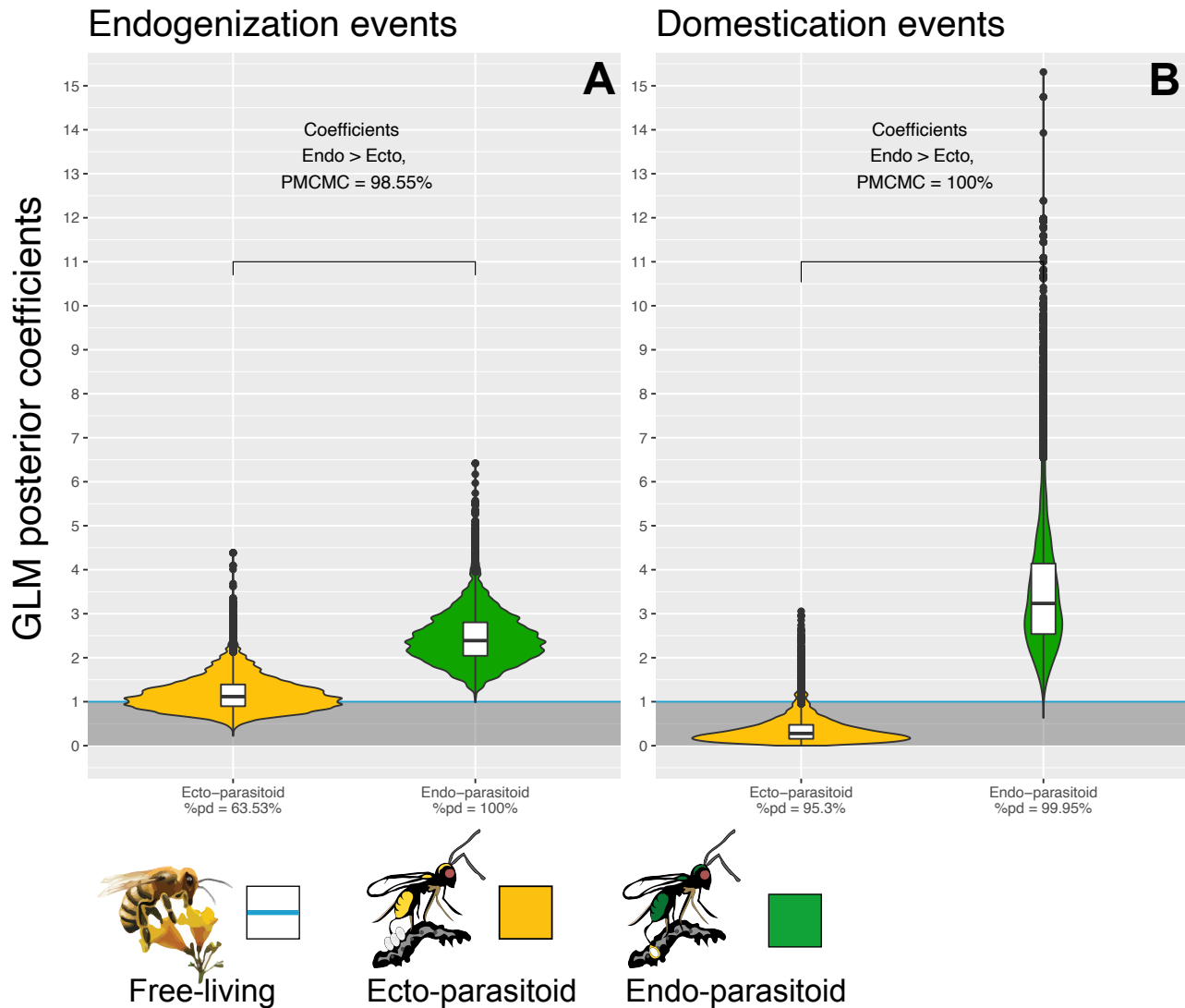


Figure 4. **Endogenization and domestication of dsDNA viruses are more frequent in endoparasitoid species.** Violin plots represent the posterior distribution of the coefficients obtained under the different GLM models (after exponential transformation to obtain a rate relative to free-living species). The coefficients are derived from 1000 independent GLM models, where 1000 probable scenarios of ancestral states at nodes were sampled randomly among the MCMCM iterations (see details in *Materials and methods*). Branches from nodes older than 160 million years were removed from the dataset. The %pd is the probability of direction and indicates the proportion of the posterior distribution where the coefficients have the same sign as the median coefficient.  $P_{MCMCM}$  indicates the proportion of MCMC iterations where the coefficient obtained for endoparasitoid species is higher than for ectoparasitoid species. All statistical summaries of the Bayesian GLM models can be found on the github repository under the name : Lifestyle\_statistical\_analysis\_results.xlsx.



213 rive from the same endogenization event [30]. In our unknown Campopleginae species, we identified homologs of 35  
 214 out of the 40 ichnovirus genes present in the genome of *H. didymator* (so-called IVSPER genes, [16]). Those genes show  
 215 conserved synteny in the two species (Figure S11), strongly suggesting that they derive from the same endogenization  
 216 event. However, our analysis did not identify viral homologs in the two Ophioninae and Cremastinae subfamilies, that  
 217 are internal to the clade including Campopleginae and Banchinae wasps. This result argues against the view of a single  
 218 event at the root of Ophioniformes, and thus supports the alternative view [31] that the so-called IVSPER genes in the  
 219 Campopleginae and Banchinae subfamilies stem from independent events, despite their striking structural similarities.  
 220 (see FigureS12 for illustration). We found no trace of the previously suggested remnants of ichnoviruses in the related  
 221 species *Venturia canescens*, although the clear presence of nudiviral genes in this species was confirmed [17].  
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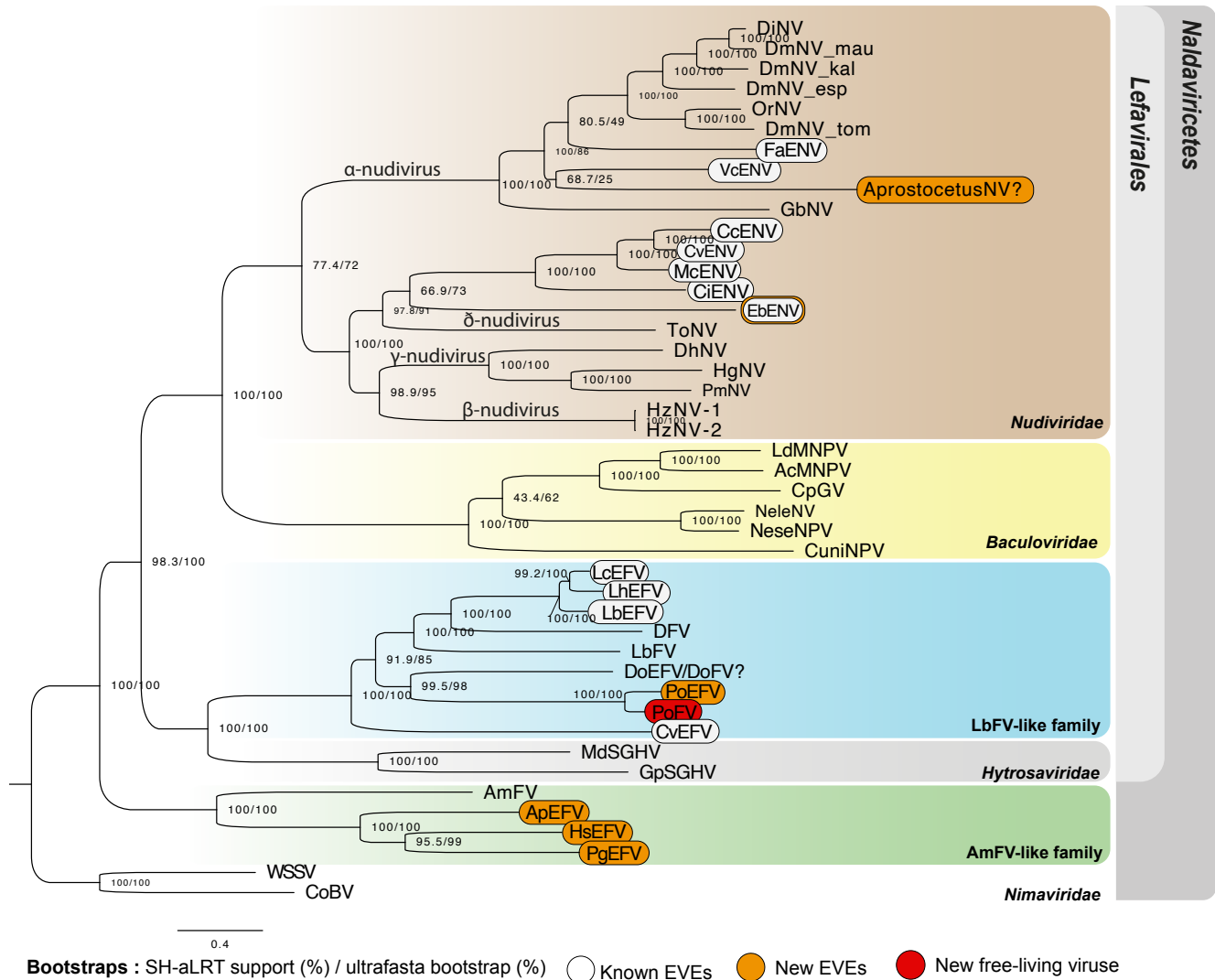


Figure 5. **Phylogenetic relationships among endogenized and "free-living" dsDNA viruses.** Specifically, this figure shows the relationships between *Naldaviricetes* double-stranded DNA viruses and EVEs from hymenopteran species, where at least 3 endogenization events were found. This tree was computed using maximum likelihood in Iqtree (v2) from a 38,293 long protein alignment based on the concatenation of 142 viral genes. Confidence scores (aLRT%/ultra-bootstrap support%) are shown at each node. The scale bar indicates the average number of amino acid substitutions per site. Previously known EVEs are in white, those from the present study in orange, and leaves inferred as free-living viruses are in red. All the best partitioned models can be found on the github repository under the name : dsDNA\_phylogeny\_best\_ML\_partitions.nxs. All free-living dsDNA viruses used in this phylogeny were obtained from published complete viral genomes. More details on the phylogenetic inference can be found in methods.

223 We found 5 new cases of endogenization involving multiple EVEs from dsDNA viruses belonging to *Nudiviridae*, LbFV-  
 224 like and AmFV-like families.

225 Two of them involve parasitoid species, i.e. *Platygaster orseoliae* and an *Aprostocetus* species. For *Aprostocetus*, we  
226 detected 6 EVEs related to nudiviruses branching between the Chalcidoidea and the Diaprioidea superfamilies (Fig. S4).  
227 Among these EVEs we found four with an annotation : *lef-4*, *Ac68/pif-6*, GrBNV\_gp19/60/61-like proteins, and a rep-like  
228 protein, none of them showing sign of domestication since no RNAseq, nore dN/dS analysis was possible. The *P. orseoliae*  
229 case involve the recently characterized putative family of filamentous viruses [32]. The free-living LbFV virus is the only  
230 representative of this putative family and has been identified as a source of adaptive genes in *Leptopilina* wasps that par-  
231 asitize *Drosophila* flies, with 13 virally-derived genes involved in the production of VLPs protecting the wasp's eggs from  
232 encapsulation [19]. In *P. orseoliae*, 15 genes homologous to LbFV were detected (out of 108 ORFs in the LbFV genome;  
233 median E-value = 9.39e-21 [min = 2.617e-76, max = 4.225e-08], Figure S8-A). Among these 15 genes, 5 were also endog-  
234 enized in *Leptopilina* species (named LbFV\_ORF58:DNApol, LbFV\_ORF78, LbFV\_ORF60:LCAT, LbFV\_ORF107 LbFV\_ORF85)  
235 [19]. Assuming the ancestral donor virus contained the same 108 genes as LbFV, the number of shared genes in these two  
236 independent domestication events is higher than expected by chance (one-sided binomial test:  $x = 5$ ,  $n = 15$ ,  $p = 13/108$ ,  
237  $p = 0.02682$ ), suggesting that similar functions could have been retained in both lineages (see additional information in  
238 supplemental information). Notably, we also found within the *P. orseoliae* assembly a new "free-living" virus among scaff-  
239 folds noted as F or X, related to LbFV, that we propose to call PoFV (*Platygaster orseoliae* filamentous virus) (see fig. 5 and  
240 supplemental information for details). This virus is the closest relative to the EVEs found in *P. orseoliae* and is composed  
241 of 136 ORFs FigureS8-B). Using this putative whole genome viral sequence to search for homologous genes in the *P. orse-*  
242 *oliae* genome, we were able to detect a total of 139 convincing EVEs (corresponding to 89/136 PoFV ORFs). 44/89 of these  
243 EVEs presented signs of domestication using paralogs, with dN/dS significantly lower than 1 (see details in supplemental  
244 information). Although functional studies are clearly needed to confirm that these virus-derived genes are involved in  
245 the production of VLPs as in *Leptopilina* [19], we see *Platygaster orseoliae* endogenous viral elements (PoEFVs) as good  
246 candidates for viral domestication, which could possibly be involved in counteracting the immune system of its dipteran  
247 host (from the Cecidomyiidae family [33]). To our knowledge, this is the first report of a massive viral endogenization and  
248 putative domestication within the Platygastroidea superfamily.

249 The other three cases involved ant species : *Harpegnathos saltator* (EsEFV) (12EVEs/6dEVEs), *Pseudomyrmex gracilis*  
250 (PgEFV) (9EVEs/1dEVE), *Aphaenogaster picea* (ApEFV) (7EVEs). These endogenized elements are related to a poorly char-  
251 acterized family of filamentous viruses denoted AmFV [34, 35]. In *H. saltator*, 9 genes deriving from an AmFV-like virus  
252 were detected (including 3 genes that have been previously identified by [23]). Intriguingly, all these 135 genes show nu-  
253 merous paralogs within the genomes (FigureS7 FigureS4), with 22 copies for AmFV\_0062 (*pif-1*), 18 for AmFV\_0102 (*pif-2*),  
254 51 for AmFV\_0090 (*pif-3*), 24 for AmFV\_0044 (*integrase*), 13 for AmFV\_0079 (*p74*), 5 for AmFV\_0047 (*RNA polymerase*), 19  
255 for AmFV\_0126 (Unknown), 23 for AmFV\_0168 (Unknown) and 7 for AmFV\_0154 (Unknown). Most paralogs were found  
256 in scaffolds exceeding the expected size for any virus sequence (min = 23,726bp, mean = 326,262bp, max = 2,693,376bp).  
257 In addition, all scaffolds do include transposable elements and eukaryotic genes making them undoubtedly endogenized.  
258 Accordingly, our pipeline attributed the highest confidence index A for 104 of them (out of 135). The *P.gracilis* genome  
259 revealed 9 EVEs, including homologs of *pif-1*, *pif-3*, *RNA polymerase*, *ac81*, *integrase* and *odv-e56* (FigureS4). Notably, one of  
260 the 9 EVEs (AmFV\_059, of unknown function) shows both a dN/dS < 1 (mean= 0.1747, p-value 5.877e-02), and a very high  
261 TPM value (362836 TPM from whole body tissues). Finally, in *A. picea*, 7 EVEs were detected, including homologs of *pif-1*,  
262 *pif-3*, *integrase*, *odv-e56* and *p74* (Figure S7. No raw reads data were available for this species, precluding coverage-based  
263 inferences). Since there were neither orthologs or paralogs for these genes to compute dN/dS analyses, nor transcrip-  
264 tomic data, it was not possible to infer their domestication status. At this stage it is thus not possible to conclude as to  
265 the functions of these genes in *H. saltator*, *P. gracilis* and *A. picea*, but this surely deserves further attention.

## 266 Discussion

267 All kinds of viruses can integrate arthropod genomes, although the mechanisms underlying these phenomena remain  
268 unclear [1, 4]. Prior to the present analysis, 28 viral families had been described as involved in endogenization in arthro-  
269 pods [4]. Our study of Hymenopteran genomes further revealed the ubiquity of this phenomenon, with at least 40 viral  
270 families (or family-like clades) involved. Of the 1,261 EVEs found, the average identity with the closest known viral proteins  
271 was 36.32% [min = 15.7%, max = 99.1%]. This large overall divergence does not exclude the possibility that some of the  
272 integrations are recent, because free-living descendants of the true donors may be unknown or extinct [36].

273 In the following section, we will first discuss why double-stranded DNA viruses, in comparisons with other viral ge-  
274 nomic structures (ssDNA, dsRNA, ssRNA), are more often endogenized than expected from their estimated abundance in

275 insect viral communities. We will then discuss hypotheses that could explain the pattern we find regarding a higher rate of  
276 endogenization of dsDNA viruses among endoparasitoid wasps compared to ectoparasitoids or free-living hymenopter-  
277 ans, which also translates into more frequent events of domestications.

278

279

### 280 **dsDNA viruses are more frequently involved in endogenization than expected by chance**

281 Despite the observations that all viral genomic structures can be involved in endogenization, we clearly identified dif-  
282 ferences in their propensity to do so. Based on a comparison between the respective proportions of the various viral  
283 categories in the inferred endogenization events and in public databases, we found that dsDNA viruses are much more  
284 represented than expected, while ssRNA viruses are under-represented (Figure 2-A). We acknowledge that current knowl-  
285 edge on the actual diversity of free-living viruses (as approximated through the NCBI taxonomy database) remains incom-  
286 plete, but the strength of the effect reported here makes this conclusion rather robust to variations in the null distribution.  
287 On the basis of current knowledge, RNA viruses, and in particular ssRNA viruses, appear to be much more diversified and  
288 prevalent than DNA viruses in insects. We note that viral-metagenomic studies often focus either on DNA or RNA viruses,  
289 and as such do not provide an accurate and unbiased picture of the extent viral diversity. To gain insights on this topic, we  
290 may thus focus on model systems where long-lasting research efforts have likely produced a more reliable picture. The  
291 Honeybee *Apis mellifera* is probably the most studied of all Hymenopteran species. In honeybees, the great majority of  
292 known viruses belongs to the RNA world [37], with very few exceptions [35]. Similarly, until 2015, only RNA viruses were  
293 known to infect the fruit fly *Drosophila melanogaster*, despite the extensive research conducted on this model system [38].  
294 A very limited set of DNA viruses has now been described from this species [39] but clearly, RNA viruses dominate the  
295 *Drosophila* viral community, both in terms of diversity and prevalence. In support of this view, recent studies revealed the  
296 very elevated absolute diversity of RNA viruses. For instance, a survey of 600 insect transcriptomes recovered more than  
297 1,213 RNA viruses belonging to 40 different families. Although, obviously, this study does not inform on the diversity of  
298 DNA viruses, it shows that the RNA virome of insects is both prevalent (e.g. in this study, 15% of all insects were infected  
299 by a single Mononegales-like virus) and extremely diversified [40]. Actually, this view appears to hold at the larger scale  
300 of eukaryotes [41]. Taking into account this patent abundance of ssRNA viruses in insects, our study indicates they are by  
301 far less frequently endogenized than their dsDNA counterparts in hymenopterans. Notably, a similar trend was recently  
302 reported in a study including a diverse set of eukaryotes [24].

303 Most of the major endogenization events characterized so far in hymenopterans involve dsDNA viruses from the  
304 *Nudiviridae* family [21, 18, 17, 42, 43, 44, 4]. Our study further confirms that this viral family represents a major source  
305 of exogenous and sometimes adaptive genes for Hymenoptera. Indeed, 28 new independent endogenization events in-  
306 volve this family, among which 9 are shared by at least two related species (Figure 2-B, Figure 1). The major contribution  
307 of nudiviruses to endogenization may be explained by their wide host range in arthropods [45]. Their nuclear replica-  
308 tion constitutes another plausible explanatory factor [46], since it may facilitate contact with host DNA. In addition, their  
309 tropism for gonads may favor the endogenization in germinal cells [47]. In fact, nuclear replication is a feature shared  
310 by nearly all families of dsDNA viruses found in our analysis : *Baculoviridae*, *Iridoviridae*, *Phycodnaviridae*, *Nimaviridae*  
311 *Caulimoviridae*, *Herpesviridae*, *Asfaviridae* (at early times) [48, 49, 50, 51, 52], *Apis*-filamentous-like [53] and LbFV-like fami-  
312 lies [54] (the *Poxviridae* viruses, that replicate in the cytoplasm, are thus the only exception). In contrast, most RNA viruses  
313 replicate in the cytoplasm. Nuclear replication may thus constitute a general explanation for the elevated propensity of  
314 DNA viruses to endogenization. Additionally, we may expect that a DNA molecule, rather than an RNA molecule, is more  
315 likely to integrate the insect genome, because the latter requires reverse transcription before possible endogenization.

316 The *Poxviridae* case indicates that cytoplasmic replication does not necessarily impede endogenization. These viruses  
317 do not require nuclear localization to propagate [55, 48] and were nevertheless found to be involved in many endoge-  
318 nization events (n=28) (as found in another paper within ant genomes [23]), especially from entomopoxviruses (18) with  
319 four cases of EVEs shared between several closely related species (Figure 2-B).

320

321

### 322 **Factors behind variations in endogenization and domestication rates**

323 Several recent studies have uncovered abundant EVEs in insect genomes [23, 20, 56], with huge variation in abundance  
324 between species. For instance, in their analysis based on 48 arthropod genomes, Ter Horst A et al., 2019 found that the  
325 number of EVEs ranged between 0 and 502. Although insect genome size and assembly quality may partly explain this

326 variation [4], the underlying biological factors are generally unknown. In this study, we tested the hypothesis that the  
327 insect lifestyle may influence both the integration and domesticating rates. We used a Bayesian approach to reconstruct  
328 ancestral states throughout the phylogeny of Hymenoptera, thus accounting for uncertainty, and found that endopar-  
329 asitoidism, in comparison with other lifestyles, tends to promote dsDNA viral endogenization. Notably, this conclusion  
330 was not the artefactual consequence of differences in genome assembly quality. In fact, the quality of genome assem-  
331 blies was correlated with the lifestyle in our data set, but the genomes of endoparasitoid species were generally less well  
332 assembled than those of free-living species. If anything, this difference should reduce the power for detecting endog-  
333 enization events in endoparasitoids, where our analysis detected an excess of such events. Our estimate of the effect  
334 sizes (with 2.43 times more endogenization events in endoparasitoids than in free-living species) should thus be seen as  
335 conservative. Why do endoparasitoid wasps tend to undergo more endogenization than others? We initially had in mind  
336 two non-exclusive hypotheses that remain plausible explanations for the observed pattern. First, endoparasitoids may  
337 be more intensively exposed to viruses. In addition, or alternatively, endoparasitoids may have a higher propensity to  
338 endogenize and retain viral genes.

339 Several factors come in support of the first hypothesis. Endoparasitoid larvae grow by definition inside their host's  
340 body, and such a close interaction implies that any endoparasitoid individual will also be interacting with its host's viruses.  
341 Accordingly, the best studied cases of viral domestication in wasps involve nudiviruses, that are known to replicate in their  
342 caterpillar hosts [57, 17, 42]. Another putatively important factor is the presence of virus in the venoms that parasitoids  
343 inject into hosts together with their eggs. These are known to protect the offspring against the host's immune response,  
344 and to manipulate the host physiology [58] but this feature could favor the subsequent spread of viruses in wasp popula-  
345 tions: by colonizing the venom-producing tissues (venom gland or calyx, depending on the species biology) viruses may  
346 secure an effective pseudo-vertical transmission and thus maintain themselves efficiently in wasp populations. Numer-  
347 ous endoparasitoid viruses benefit from such pseudo-vertical transmission [54, 59], including some whose relatives have  
348 been endogenized and domesticated by endoparasitoids [19]. The presence of viruses within venoms may also facilitate  
349 horizontal transmission between conspecifics in the case of superparasitism, as observed in the *Drosophila* parasitoid  
350 *Leptopilina bouleardi* [27]. Although this effect may also be at play for some ecto-parasitoid species [60], we expect it to  
351 be more pronounced for endo-parasitoid species since they have a closer interaction from the inside of their hosts. Gen-  
352 erally, endoparasitoids may thus carry a higher load of non-integrated viruses than other hymenopterans. However, if  
353 this effect is at play, we expect to have an "endoparasitoid" effect for all viruses, whatever their genomic structure. For  
354 instance, we would expect such an effect to be detected for ssRNA viruses which are involved in the greatest number of  
355 endogenization events (Figure 2-A). This was not the case since only dsDNA viruses were more frequently endogenized in  
356 endoparasitoids. Thus, we argue that this hypothesis is unlikely to explain the observed pattern.

357 The second hypothesis posits that endoparasitoids are more frequently selected for retaining virally-derived genes  
358 than ectoparasitoid or free-living hymenopterans. In our analysis, domestication events are most frequently observed  
359 in endoparasitoids (over 3 times more frequently than in other hymenopterans). Obviously, this may be at least partly  
360 explained by the higher input discussed above (the higher endogenization rate). Yet, once this effect is controlled for,  
361 a trend towards a higher rate of domestication remains. More specifically, the likelihood of domestication following  
362 endogenization was significantly higher in endoparasitoids than in ectoparasitoids, but was not significantly higher than  
363 in free-living species. This later lack of significant difference may be biologically explained if a single domestication event  
364 precludes the domestication of additional EVEs, while not affecting the rate of non-adaptive endogenization. This would  
365 "dilute" the signal along branches involved in domestication. If this effect is at play, then it reduces considerably the  
366 power of our analysis to detect any difference on the rate of domestication between lifestyles. Indeed, in all known cases,  
367 only one domesticated virus has been documented, suggesting that further domestications are not beneficial once a viral  
368 machinery has been recruited by a wasp lineage.

369 Whether or not the rate of domestication *per se* is higher in endoparasitoids than in other hymenopterans, the se-  
370 lective advantages brought by these viral genes in endoparasitoids should be discussed. It has been demonstrated in a  
371 few model systems that EVEs may confer antiviral immunity against related "free-living" viruses via the piRNA pathway  
372 [7, 61]. Yet, to our knowledge, such an effect has only been demonstrated against RNA viruses, so that it would not  
373 explain the excess of DNA viruses documented here. Furthermore, the sequence identities with known viral sequences,  
374 which is needed for this mechanism to work, is low in our dataset. Accordingly, previous work revealed that EVE-derived  
375 piRNAs studied in 48 arthropod species were also probably too divergent to induce an efficient antiviral response [20].  
376 At that stage, the ability of EVEs to generate PIWI-interacting RNAs that play a functional role in antiviral immunity seems

questionable. Further studies involving small RNA sequencing in hymenopterans would be required to shed light on this issue. Protection of the eggs and larvae against the host immune system is recognized as an important trait where EVEs play a critical role. Because of their peculiar lifestyle, endoparasitoids are all targeted by the host immune system, a matter of life or death to which other hymenopterans are not exposed to. Several cases of endogenization and domestication in endoparasitoids, all involving dsDNA viruses, are thought to be related to this particular selective pressure [42, 16, 17, 18, 19]). The parasitoids appear to have co-opted the viral fusogenic property to address their own proteins (VLPs) or DNA fragments (polydnviruses) to host immune cells, thereby canceling the host cellular immune response. The above-hypothesized high exposure of endoparasitoids to viruses, together with this unique selective pressure, may act in concert to produce the pattern documented here: a strong input, that is, a diverse set of putative genetic novelties, combined with a strong selective pressure for retaining some of them. The observed excess of dsDNA viruses may be an indication that these viruses display a better potential for providing adaptive material in this context. In the cases of polydnviruses (found in some Braconidae and some Campopleginae), it appears that one way to efficiently deliver virulence factors to the host cell is by addressing DNA circles that ultimately integrate into the host immune cells and get expressed [62, 63]. The DNA which is packed into the mature particles typically encodes virulence proteins deriving from the wasp [64]. This means that, at least for these cases, the viral system should be able to pack DNA, which is most likely a feature that DNA viruses may provide. Such an argument does not hold in the VLP systems, where only proteins are packed in viral particles, and it is unclear why EVEs deriving from dsDNA viruses would be more able to fulfill such a function. Here other features of dsDNA viruses come into mind as possibly important factors: their large genome size, and their large capsids and envelopes [65]. These may predispose dsDNA viruses to be domesticated, since abundant quantities of venoms have to be transmitted in order to efficiently suppress the host immune response.

## Materials and methods

### Genome sampling, assembly correction and assembly quality

A bioinformatic pipeline mixing sequence homology search, phylogeny, genomic environment, and selective pressure analysis was built to search for viral endogenization and domestication events in Hymenoptera genomes. We used 133 genome assemblies in total, of which 101 were available on public repositories (NCBI and BIPPA databases) and 32 were produced by our laboratory (all SRA reads and assemblies available under the NCBI submission ID : SUB11373855). Concerning the last 32 samples, DNA was extracted on single individuals (usually one female) or a mix of individuals when the specimens were too small using Macherey-Nagel extraction kit, the DNA was then used to construct a true seq nano Illumina library at Genotoul platform (Toulouse, France). The sequences were generated from HiSeq 2500 or HiSeq 3000 machines (15Gb/sample). The paired-end reads were then quality trimmed using fastqmc (-q15 -qual-mean 30 -D150, github) and assembled using IDBA-UD [66]. All sample information can be found on the github repository under the name : All\_sample\_informations.txt and is available under the NCBI Biosample number : SUB11338872.

The size of the 133 assemblies ranged from 106.14mb to 2102.30mb. We kept only genome assemblies containing at least 70% non-missing BUSCO genes (124/133 genomes, [67]) (all genome information can be found on the github repository under the name : Assembly\_genome\_informations.txt). In addition, when the raw reads were available, we used the MEC pipeline [68] to correct possible assembly errors. Although some genomes were highly fragmented (such as the 32 genomes we generated since they were obtained using short reads only), the N50 values (min: 3542bp) were equal to or larger than the expected sizes of genes known to be endogenized and domesticated (min known domesticated EVE : 165bp) indicating that most of the putative EVEs should be detected entirely.

Out of the 32 samples sequenced by our laboratory for this study, one (corresponding to *Platygaster orseoliae*) gave unexpected results. After assembly and BUSCO analysis, two sets of contigs were identified: one with only 4X coverage on average, and one with 33X on average. The phylogeny of these different BUSCOs gene sets showed that the low-coverage scaffolds likely belong to an early diverging lineage of Chalcidoidea (Figure 1), whereas the 33x scaffolds belong to the target species *P. orseoliae*. This result suggests that the pool of 10 individuals used for sequencing was likely a mix of two species. A phylogenetic study of Ultra Conserved Elements (UCEs) obtained from several species of Chalcidoidea by [69, 70] recovered the unknown species as sister to *Aprostocetus* sp (Eulophidae) (see details in the supporting information and Figure S2). In the figures and tables, the name putative\_ *Aprostocetus* sp was consequently assigned to the unknown sample. However, since the lifestyle and identity of this species are uncertain, we did not include the corresponding scaffolds in the main analysis. The scaffolds belonging to this putative\_ *Aprostocetus* sp. (i.e : all scaffolds with a mean

426 coverage < 10X) were removed from the *P. orseoliae* assembly file hosted in NCBI.

## 427 Pipeline outline

428 EVEs were identified from the 124 Hymenoptera assemblies using a sequence-homology approach against a comprehensive viral protein database (including all categories of viruses : ssDNA, dsDNA, dsRNA and ssRNA). In order to validate viral endogenization within Hymenoptera genomes, we developed an "endogenization confidence index" ranging from 430 A to X (Figure S13-7). This index takes into consideration the presence of eukaryotic genes and/or transposable elements around candidate loci, and scaffolds coverage information (coverage for a valid candidate should be similar to that found 433 in BUSCO containing scaffolds. Finally, the pipeline also included an assessment of the evolutionary history and of the selective regime shaping the candidates (based on dN/dS and/or expression data).

## 435 Hymenoptera phylogeny

436 The phylogenetic reconstruction of the 124 Hymenoptera species was performed based on a concatenation of the 375 BUSCO proteins. The analysis was conducted by maximum likelihood via Iqtree2 [71] selecting the best model [72]. The tree was rooted via two species of the Coleoptera order (*Anoplophora glabripennis* and *Tribolium castaneum*). Bootstrap scores were evaluated using the UFboot approach [73]. The results found were consistent with a previous, more comprehensive study [28].

## 441 Search for viral homology

442 We collected all protein sequences available in NCBI virus database [74], removing phage and polydnavirus (virulence genes from wasp origin found within PDVs) sequences. This database contained 849,970 viral protein sequences (download date : 10/10/2019), to which the 40 putative viral proteins encoded by the *Hyposoter didymator* genome were added (so-called IVSPER sequences, [29]). The sequence homology search was performed with a BlastX equivalent implemented in Mmseqs2 [75] using each genome assembly as queries and the viral proteins collected as database. The result gave a total of 81,953,678 viral hits (max E-value 5e-04 with an average of 660,916 hits per genomes). We kept only candidates with a percentage coverage of the viral protein  $\geq 30\%$ , an identity score  $\geq 20\%$  and an E-value score  $< 5e-04$  (Figure S13-1). The threshold parameters were optimized to maximize the detection of the 13 endogenous viral sequences within the genus *Leptopilina* [19]. Once all the viral hits were recovered, we formed putative EVEs loci (n=238,108) corresponding to the overlap of several viral hits on the same scaffold using the GenomicRanges R package [76] (Figure S13-2). To remove false positives corresponding to eukaryotic genes rather than viral genes, we then performed another generalist sequence homology search against the Nr database (downloaded the 09/11/20) using mmseqs2 search (-s 7.5, E-value max = 0.0001) (Figure S13-3). We did not select our candidate based on the best hit, since it does not necessarily reflect the true phylogenetic proximity. Instead, candidates with more than 25 hits with either eukaryotic non-hymenoptera species or prokaryotic species were removed, except if they also had hits with at least 10 different virus species (bits  $\geq 50$ ). We chose to eliminate Hymenoptera hits from the database because if a real endogenization event concerns both one of the 124 species of our dataset and some species in the NCBI database, then an apparent "Hymenoptera" hit will be detected, possibly leading to its (unfair) elimination. Since viral diversity is poorly known, we also kept sequences with even one single viral hit, as long as it did not have more than 5 eukaryotic or prokaryotic hits. Using these filtering criteria we removed a total of 234,036/238,108 (98,3%) candidate loci leaving 4,072 candidates with convincing homology to viral proteins. Note that among these loci a certain proportion actually corresponded to non-endogenized "free-living" viruses. To study the evolutionary history of these candidate EVEs, we then performed a general protein clustering of all the candidates and the NCBI viral proteins (Figure S13-4, Mmseqs cluster; thresholds : E-value 0.0001, cov% 30, options : -cluster-mode 1 -cov-mode 0 -cluster-reassign -single-step-clustering[77]).

466 We eliminated from the dataset the chuviral glycoproteins that have been captured by LTR retrotransposons [78], as these loci have complex histories mostly linked to the transposition activity after endogenization. For this purpose, we systematically searched among the candidates for the presence of TEs within or overlapping with the EVE (see the file All\_TEs\_overlapping\_with\_EVEs under the github repository). Only one cluster (Cluster4185) was concerned by such a situation (chuviral glycoproteins overlapping to Gypsy/LTRs). It was detected in 89/124 species (1074 total copies, median = 7 copies/species, max = 244, min =1), and was probably similar as the one described in [79].

## 472 **Evolutionary history and selection pressure of endogenous loci**

### 473 **Arguments for endogenization**

474 Among all the candidates for endogenization there were probably false positives that corresponded either to natural  
475 contaminants (infecting viruses sequenced at the same time as the eukaryotic genome) or laboratory contaminants (virus  
476 accidentally added to the samples). One way to filter these cases was to study (i) the genomic environment (are there  
477 other eukaryotic genes or transposable element on the same scaffolds?) and (ii) metrics such as G+C% (used only for  
478 cov/GC plots) and scaffold coverage depth around candidate loci (are they the same as scaffolds containing housekeeping  
479 genes?). All of these data were used to establish confidence in the endogenization hypothesis, scaled from A to X (Figure  
480 S13-7).

481 **(i) Scaffolds sequencing depth (Figure S13-5) :** In order to support the hypothesis that a scaffold containing candi-  
482 date EVEs was part of the Hymenoptera genome, we studied the sequencing depth of the scaffolds. If the sequencing  
483 depth of a candidate scaffold was not different from the depth observed in scaffolds containing BUSCO genes, then this  
484 scaffold was likely endogenized into the Hymenoptera genome. Hence, when DNA reads were available (FigureS1), we  
485 measured this metric by mapping the reads on the assemblies using hisat2 v 2.2.0 [80]. An empirical p-value was then  
486 calculated for each scaffold containing a candidate EVE. To calculate this empirical p-value, we sampled 500 loci of the  
487 size of the scaffold of interest within BUSCO scaffolds. These 500 samples represented a null distribution for a scaffold  
488 belonging to the Hymenoptera genome. The p-value then corresponded to the proportion of BUSCO depth values that  
489 were more extreme than the one observed in the candidate scaffold (two-sided test). We used a threshold of 5% and a  
490 5% FDR (multiply python package [81]).

491 **(i) Genomic environment and scaffold size (Figure S13-6)** Another way to rule out contaminating scaffolds was to  
492 look for the presence of eukaryotic genes and transposable elements in the scaffolds containing candidate EVEs, assum-  
493 ing that their presence in a viral scaffold is unlikely. Indeed, so far, very few viral genomes have been shown to contain  
494 transportable elements [82, 83, 84, 85, 86] because TE insertions are mostly deleterious and are therefore quickly elimi-  
495 nated by negative selection [84, 85]. We searched for transposable elements by a BlastX-like approach (implemented in  
496 Mmseqs2 search -s 7.5), taking as query the scaffolds of interest and as database the protein sequences of the transpos-  
497 able element (TE) available in RepeatModeler database (RepeatPeps) RepeatModeler (v2.0.2) [87]. We only kept hits with  
498 an E-value < 1e-10 and with a query alignment greater than 100aa. We then merged all overlapping hits and counted  
499 the number of TEs for each scaffold. To find eukaryotic genes within genomes we used Augustus 3.3.3 [88] with BUSCO  
500 training and then assigned a taxonomy to these genes via sequence homology with Uniprot/Swissprot database using  
501 mmseqs2 search [75], and only retained genes assigned to insects.

502 Accordingly, the scaffolds were scored as follows (Figure S13-7) :

- 503 • A: scaffolds with a corrected coverage p-value > 0.05 and at least one eukaryotic gene and/or one repeat element,
- 504 • B: scaffolds with at least one eukaryotic gene and/or one repeat element but no coverage data available,
- 505 • C: scaffolds with a corrected coverage p-value > 0.05 and neither eukaryotic gene nor transposable element,
- 506 • D: scaffolds with a corrected coverage p-value < 0.05 and whose coverage value was higher than the average of  
507 the scaffolds containing BUSCOs (as it is difficult to imagine that an endogenized scaffold present a lower coverage  
508 than expected, whereas a higher coverage could correspond to the presence of repeated elements that inflate the  
509 coverage of the scaffold for example) but with at least 5 eukaryotic genes and/or a repeated element (in total),
- 510 • E: scaffolds presenting no DNA seq coverage data available and no eukaryotic gene nor transposable element de-  
511 tected,
- 512 • F: scaffolds presenting a corrected p-value of coverage < 0.05 and less than 5 eukaryotic genes without any trans-  
513 posable elements; this category may rather correspond to free-living viruses.
- 514 • X: scaffolds with a corrected p-value < 0.05 and neither eukaryotic gene nor transposable element; This category  
515 may rather correspond to free-living viruses.

### 516 **Inference of endogenization events**

517 Because several EVEs may derive from the same endogenization event, we sought to aggregate EVEs into unique events.  
518 We aggregated into a single event, firstly (i) all the EVEs present on the same scaffolds, and secondly (ii) all the EVEs that  
519 presented the same taxonomic assignment at the level of the viral family. These two steps were sufficient to aggregate  
520 EVEs in the simplest case of events involving only one species (but possibly several EVEs).

521 To further characterize the endogenization events including more than one species, we also relied on phylogenetic inference. To this end, the protein sequences belonging to each of the clusters (containing both viral proteins and candidate EVEs) were first aligned with clustalo v1.2.4 [89] in order to merge possible candidate loci (which may in fact correspond to various HSPs). All loci (=HSPs) within the same scaffold presenting no overlap in the alignment were thus merged, as they probably correspond to multiple HSPs and not duplications. We then performed a new codon alignment from the augmented sequences in the clusters using the MACSE v2 alignsequence program [90] (Figure S13-8). This alignment allowed us to obtain a protein and nucleotide codon alignment. We used the protein alignment to infer the phylogeny of each cluster with the program Iqtree2 v2.1.2 [71] (-m MFP -alrt 1000 (partitioned))(Figure S13-9). No trimming was performed at the amino-acid level, since this may result in loss of topology information [91, 92]. However, since it can affect branch length, only codon alignment was trimmed at the protein level via Trimal v1.2 (Figure S13-10) (-backtrans -automated1) [93]. We then exploited the information from the cluster phylogenies to form the endogenization events. EVEs potentially deriving from the same event should be supported by the formation of the same well-supported monophyletic clade (bootstrap > 80) both in the gene tree and the Hymenoptera tree (allowing gene losses in 20% of the species concerned by the monophyletic group). EVEs were possibly aggregated within the same event only if the Hymenoptera belonged to the same family. (Figure S13-11). Finally, the clustering of multiple EVEs within the same scaffold in one species was used to aggregate the homologous EVEs found in a related species within the same shared event, even if they were on different scaffolds (Figure S13). For details, see some canonical examples in Figure S14.

538 For events shared by several species, we were also able to analyze gene synteny around putative EVEs. To do this, we conducted the equivalent of an all vs all TblastX (Mmseqs2 search -search-type 4, max E-value =1e-07) between all the candidate loci within a putative event (deduced from the phylogenetic inference), and then looked for hits (HSPs) between homologous EVEs around the insertions. Because it is possible to find homology between two genomic regions that does not correspond to orthology, for example because of the presence of conserved domains, we had to define a threshold to identify with confidence the orthology signal. We therefore conducted simulations to define this value, based on the well assembled genome of *Cotesia congregata* (GCA\_905319865.3) by simply performing the same all vs all blast analysis against itself (as if the two species considered had the same genome). Based on this, we defined two types of simulated EVEs, (i) independently endogenized EVEs in the genomes of the two "species". This is simply simulated by randomly selecting two different regions in the genomes, and (ii) a shared simulated EVE that was acquired by their common "ancestor". This is simulated by selecting the same random genomic location in both "genomes". We then counted the total length of the HSPs found around the simulated insertions all along the corresponding scaffold (i and ii). As the result will obviously depend on scaffold length, we performed these simulations on several scaffold lengths (100000000bp, 10000000bp, 1000000bp, 100000bp and 10000bp). We conducted 500 simulations in each scenario, and we measured the cumulative length of homologous sequences by counting the sum of HSPs (bit score > 50). We then defined a threshold for each windows size in order to minimize for the false-positive (FP) and maximize true-positifs (TP) (thresholds 100000000bp = 172737bp (FP = 0.012, TP= 0.922); 10000000bp = 74262 bp (FP= 0.012, TP=0.878); 1000000bp = 21000 bp (FP=0.014, TP=0.28); 100000bp = 1332 bp (FP= 0.012 TP= 0.198) and 10000bp = 180 bp (FP= 0.008, TP= 0.208)).

556 Events were linked to viral families based on the closest match information between the viral blastx (GenBank accession number and/or viral protein and/or viral species) and the classification proposed in [94].

## 558 Arguments for domestication

559 One way to test for the domestication of an EVEs (dEVEs) was to estimate the ratio (omega) of the number of nonsynonymous substitutions per non-synonymous site (dN), to the number of synonymous substitutions per synonymous site (dS). If EVEs are evolving neutrally, then the ratio is expected to be equal to 1, whereas if the EVE is under purifying selection, dN/dS is expected to be lower than 1. We conducted this analysis on trimmed codon alignments from (Figure S13-11) via the codeml algorithm from PAML [95] implemented in the python 3.3 package [96] (model Muse Gaut [97]). We tested the deviation from the null model in which the branches of the monophyletic group evolved under a neutral scenario ( $\chi^2$  test). The p-values were then corrected by selecting a FDR of 0.05 (multiply python package), and we estimated the standard errors of dN/dS that maximized the likelihood (option getSE = 1). dN/dS with dS greater than 10 were removed, since this indicates substitution saturation (Figure S13-12).

568 The other way we choose to study the domesticated nature of a viral gene was to study their expression profile (Figure S13-13). We reasoned that domesticated genes are likely to be significantly expressed. To test this, when RNAseq reads were available on NCBI (SRA), we mapped them on the assembled genomes (until reaching 300x coverage as far



571 as possible). Using the TPMCalculator program [98], we measured expression in ovaries and whole body if available or  
572 alternatively in any tissue (see supplemental information table :RNA\_seq\_reads\_mapped.txt). An EVE was considered as  
573 domesticated if the gene was expressed with a Transcripts Per Kilobase Million (TPM) index above 1000. This threshold  
574 was chosen based on the median value observed for control EVEs (718.70 TPM), rounded up to 1000TPM to be conser-  
575 vative. We measured the accuracy of this metric using EVEs for which both TPM and dN/dS calculations were possible:  
576 among the 36 genes having a TPM>1000, 33 also had a dN/dS significantly below 1 suggesting that inferring domestication  
577 based on TPM>1000 was consistent with dN/dS test with a 0.9166 probability. Finally, based on the idea that an active  
578 EVE should encode a protein with similar length to the donor virus, we calculated the actual viral protein sequence length  
579 using the orfipy algorithm [99] (Figure S13-14).

580 A possible bias when comparing lifestyles on domesticated elements could come from a difference of RNAseq reads  
581 availability depending on the lifestyle, which may result in a different number of EVEs considered as domesticated. A  
582 GLM binomial analysis did not reveal any correlation between RNAseq data availability and lifestyle (endoparasitoid =  
583 Slope(SE)=0.21(0.62), p=0.73; free-living= Slope(SE)=0.40(0.57), p=0.49 using ectoparasitoid as intercept).

## 584 Sensitivity and specificity of the analysis

585

### 586 Capacity to find Endogenous Viral Elements (EVEs)

587 Among the species included in our dataset, 7 were known to contain a domesticated virus (2 with similar PDV [42], 5 with  
588 different VLPs [17, 18, 19]), corresponding to 4 independent endogenization events. Our pipeline was able to detect the  
589 vast majority of the corresponding virally-derived genes (88.6%, details in table S1). The 11.14% false negatives corre-  
590 sponded to sequences that were too divergent or with a region of similarity too small to be detected by our pipeline. We  
591 found that 88.7% of the control EVEs were located within scaffolds scored as A (i.e. having a depth of coverage falling  
592 within the distribution of those containing BUSCO genes, as well as having one or more eukaryotic genes and/or trans-  
593 posable elements in the vicinity). Since the remaining 11.3% were scored either C (7.64%) or D (3.66%) (table S1), we  
594 considered candidates within the range A-D as valid candidates for endogenization. On the contrary, scaffolds annotated  
595 as F or X were rather considered as free-living viruses since they did not show eukaryotic genes or TE in their vicinity  
596 and had different coverage compared to BUSCO-containing scaffolds. Scaffolds classified as E were of unclear status and  
597 discarded.

598

### 599 Capacity to find domesticated EVEs (dEVEs)

600 An EVE was considered as domesticated if the dN/dS ratio was significantly below 1 or if TPM was above 1000. When  
601 dN/dS computations were possible (for 75/152 control EVEs), our pipeline considered the EVEs as being under purifying  
602 selection in 70.39% of the cases. Overall, by combining the two metrics (dN/dS and TPM), our pipeline identified 69.04%  
603 of the control locus as being domesticated (table S1).

604

605 **Capacity to infer events of endogenization (EVEs events)** Among the control species, the pipeline correctly inferred  
606 the expected 4 independent events: (1) *Leptopilina boulardi/Leptopilina clavipes/Leptopilina heterotoma* [19] (2) *Venturia*  
607 *canescens* [17], (3) *Fopius arisanus* [57], and (4) *Cotesia vestalis/Microplitis demolitor* [15] (table S1). However, in addition to  
608 the expected unique shared event concerning the *M. demolitor* and *C. vestalis* species, our pipeline inferred two additional  
609 events, each specific to one lineage. This was due to the fact that two genes were not detected by our pipeline as shared  
610 by *M. demolitor* and *C. vestalis*, either because they are effectively not shared (for 3 of them: HzNVorf118, like-*pif-4* (*19kda*),  
611 *fen-1*), or because of some false negative in one of the two lineage (for one of them: *p33* (*ac92*)).

## 612 Assessing the distribution of virus infecting insects

613 We estimated the number of viral species infecting insect species based on the virushostdb database (downloaded the  
614 23/05/2022 on <https://www.genome.jp/virushostdb/>) which lists a wide diversity of viral species associated with their pu-  
615 tative hosts. We also added two important exploratory RNA virus search studies [94, 100]. We kept only viruses present  
616 found in interaction with insect in at least one of these databases. Genomic structures were retrieved through the ICTV  
617 report (2021.v1) and information available in ViralZone (all viral species details can be found on the github repository un-  
618 der the name : All\_virus\_infecting\_insects\_informations.csv). We counted the number of viruses per genomic structure,  
619 and viruses from unknown genomic structures were discarded. In total, 2,626 viral species infecting insects were consid-

620 ered (detail : ssRNA(-) = 603sp, ssRNA(+) = 1,241sp, ssDNA = 75sp, dsRNA = 401sp, dsDNA = 155sp, Unknown= 151sp). The  
621 Partiti-Picobirna, Narna-Levi, Mono-Chu, Bunya-Arenao, Luteo-Sobemo, Hepe-Virga and Picorna-Calici clades correspond  
622 to viral clades proposed by [94].

### 623 **Divergence time estimation**

624 We time-calibrated the inferred phylogenetic tree using a Bayesian approach on RevBayes 1.1.1 [101] and information on 5  
625 fossils selected by [28]. Condensation of the supermatrix became necessary to overcome computational limitations when  
626 estimating node ages resulting from the large size of the concatenated BUSCO supermatrix (nsites = 228,009). Therefore,  
627 we first selected 176,648 variable sites from the supermatrix via the snp-sites function [102]. We then generated one  
628 fasta file with a random draw without replacement of 20,000 sites from the supermatrix composed of the variable sites.  
629 Evaluation of the phylogenetic likelihood being the most expensive operation when calculating the posterior density, we  
630 decided to use the method developed in [103] to reduce computational cost and approximate the phylogenetic likelihood  
631 using a two-step approach. In the first step, the posterior distribution of branch lengths measured in expected number of  
632 substitutions is obtained for the fixed unrooted topology of using a standard MCMC analysis (100,000 iterations, 3 chains,  
633 5000 burn-in, tuningInterval=200). The obtained posterior distribution is then used to calculate the posterior mean and  
634 posterior variance of the branch length for each branch of the unrooted topology. In the second step, we date the  
635 phylogeny using a relaxed clock model and calibrations (500,000 iterations, 4 chains, 5000 burn-in, tuningInterval=200).  
636 Calibration of the root was done using a uniform law between 300 and 412 Mya. To verify that MCMC analyses converged  
637 to the same posterior distribution, we applied for both steps the effective sample size and the Kolmogorov-Smirnov test  
638 computed in R v1.4.1717.

### 639 **Ancestral state reconstruction**

640 To explore the dynamics of EVEs gain in relation to lifestyle, we first had to reconstruct the ancestral lifestyle states of  
641 the Hymenoptera used in this study. This was achieved using a Bayesian approach implemented in RevBayes 1.1.1 [101].  
642 The lifestyles of the Hymenoptera species used in this study were deduced from various sources (details and sources in  
643 the table named Assembly\_genome\_informations.csv from the github repository). Since lifestyle characters are probably  
644 not equally likely to change from any one state to any other state, we choose the Mk model with relaxed settings allowing  
645 unequal transition rates. Thus, we assumed 6 different rates with an exponential prior distribution. Before running the  
646 MCMC chains, we made a preliminary MCMC simulation used to auto-tune the moves to improve mixing of the MCMC  
647 analysis with 1000 generations and a tuning interval of 300. We then ran two independent MCMC analyses, each set to  
648 run for 200 000 cycles, sampling every 200 cycles, and discarding the first 50 000 cycles as burn-in. To verify that MCMC  
649 analyses converged to the same posterior distribution, we applied the effective sample size and the Kolmogorov-Smirnov  
650 test computed in R v1.4.1717. After this step and for each likely scenario, each branch was thus assigned a lifestyle (free-  
651 living, endoparasitoid or ectoparasitoid).

### 652 **Test of the lifestyle effect on viral endogenization and domestication**

653 In order to test the lifestyle effect on the propensity to integrate and domesticate viral element, we first randomly sampled  
654 1000 probable ancestral state scenarios to take into account the uncertainty in the estimates of the ancestral states of the  
655 nodes. Because a lot of branches had no EVE endogenization inferred, we ran zero-inflated negative-binomial GLM model,  
656 for each of these 1000 scenarios such that (GLM(NbEVEs free-living + endoparasitoid + ectoparasitoid \* Branch\_length,  
657 family = zero inflated neg binomial). We eliminated all branches older than 160 million years because they are too old for  
658 our analysis to detect events (the oldest event detected by our analysis is around 140 mya) that could artificially inflate the  
659 zero count. The model was implemented in stan language using the R package brms (seed = 12345, thin=5, nchains =4,  
660 niter = 10000) [104, 105]. Posterior predictive check was done using the package brmsfit in order to check that the model  
661 was correctly predicting the proportion of zeros. Indices relevant to describe and characterize the posterior distributions  
662 were computed using the R package BayestestR [106]. Autocorrelation was studied using the effective sample size index  
663 (ESS) with a value greater than 1000 being sufficient for stable estimates [104]. The convergence of Markov chains was  
664 evaluated by a Rhat statistic equal to 1. All the posterior coefficient estimated values were then pooled together (after  
665 checking the convergence of all chains via the GelmanRubin function in R [107]) and compared between the free-living,  
666 endoparasitoid and ectoparasitoid modalities.

667 To calculate the rate of domestication independent of the rate of endogenization, we built a binomial logistic regres-

668 sion model in a Bayesian framework, specifying the number of domesticated EVEs (or Events) (the numerator) relative to  
669 the total number of EVEs or Events inferred by our pipeline (the denominator). These binomial models allowed us to test  
670 whether the probability of domestication after endogenization correlated with lifestyle by controlling for the endogeniza-  
671 tion input (the denominator). Thus, for each of the 1000 lifestyle scenarios, we ran a binomial brms model with a logit  
672 link such that  $\text{brms}(\text{Nb dEVEs/dEvents} \mid \text{trials}(\text{Nb EVEs/Events}) \text{ lifestyle} + \text{Branch length})$ .

673 Before analyzing the data, we checked that the inferences did not depend on the quality of the genomes selected  
674 for analysis. We found a significant effect of the lifecycle on the N50 and percentage of complete+partial BUSCO in the  
675 assemblies (Kruskal-Wallis rank sum test p-values respectively =  $3.192e-10$  and  $1.26e-14$ ). Furthermore, a pairwise Wilcoxon  
676 test with p-values adjusted with Bonferroni method revealed a significantly higher values of N50 and %complete+partial  
677 BUSCO within genome assemblies from free-living species compared to endo and ectoparasitoids species (p-value  $<0.05$ ).  
678 The same test using the total assembly length in bp did not reveal any difference between the three lifestyles (p-value  
679  $>0.05$ ). Overall, free-living species have better assemblies. Because better assembly quality should facilitate the discovery  
680 of endogenous viral elements both by sequence homology detection and by a better assessment of the endogenized  
681 nature of the EVE (scaffolds A,B,C and D), we should thus underestimate the number of EVEs in endo- and ectoparasitoid  
682 species compared to free-living species. Since our analysis led to opposite conclusion, our results cannot be explained  
683 by this feature of the dataset.

## 684 Author contributions

685 BG & JV conceptualized the study. BG coordinated and performed all bio-informatic analysis, wrote the first draft, and  
686 edited the manuscript. BB participated in the Bayesian analysis. DDV developed the scripts used in the phylogenetic  
687 analysis to infer endogenization events. SC, DGN, R. J-Y, S.J, S.M, R.N, C.S, N-B.P and J-T.E provided the specimens. DL  
688 did all the molecular biology work. BG, SC and JV participated in the interpretation of the results. BG, SC & JV wrote the  
689 manuscript and integrated the comments done by all co-authors.

## 690 Data availability

691 All sequencing data are available at NCBI via the BioProject accession number NCBI: PRJNA826991. All scripts and ad-  
692 ditional tables as well as all cluster phylogeny figures are available under the github repository : (BenjaminGuinet/Viral-  
693 domestication-among-Hymenoptera).

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## 701 Declaration of interests

702 The authors declare no competing interests.

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## Supporting Information

### 1- ssRNA endogenization

Although our results show an under-representation of EVEs deriving from ssRNA viruses (relatively to their high abundance in insect virome), they were involved in a high absolute number of endogenization events: 21 viral families/clades were involved in 174 independent endogenization events in the 114 Hymenoptera genomes analyzed, in particular involving *Chuviridae*, *Artoviridae* and *Nyamiviridae* (Figure 2-B). In a recent meta-analysis [4], more than 1876 EVEs involving ssRNA viruses were identified in 37 species distributed in 8 insect orders. Interestingly, the authors noticed that the contribution of negative-stranded RNA viruses was overall high (67%), but was also highly species-specific. In our dataset, the great majority of ssRNA viruses donors were negative-stranded (14/21 viral family/clades) accounting for 78.8% of ssRNA events. The pattern thus seems even more pronounced in Hymenoptera compared to insects in general, and resemble the pattern observed in ticks [56]. The reasons for the asymmetry observed between negative and positive strand RNA viruses in endogenization are unclear. One explanation proposed by [108] posits that the ssRNA(-) have a higher probability of endogenization compared to ssRNA(+) because non-segmented ssRNA(-) usually produce abundant short mRNAs compared to ssRNA(+) which conversely produce lower amount of long mRNAs encoding a single polyprotein [109]. Then, all else being equal, an RNA(-) virus would produce more RNA molecules which increase the likelihood that some of them get reverse-transcribed and ultimately endogenized into the host genome. In support of that, [108] noticed that the NP gene was more often endogenized compared to the other genes encoded by most ssRNA(-), which fits its prediction. This is because for most Mononegavirales species the 3 nucleoprotein (NP) gene is the most abundant RNA [110], due to the polar 3-5 stepwise attenuation of transcription [110]. This pattern was since observed on some systems (i.e. mosquitoes and few mammals genomes [1]) but opposite results were also obtained on others (ticks, [56]). In our dataset, we found two ssRNA(-) non-segmented families showing the expected pattern where the genes closest to the 3' regions were the most endogenized: the Nucleoprotein (N) which is the first transcribed protein in *Nyamiviridae* was endogenized in 26 cases out of 28, and the 3'-unknown protein in *Lispiviridae* (first transcribed) was endogenized in 12 cases out of 15 endogenization events (Figure S3). On the contrary, all the other putative ssRNA families donor presented more EVEs deriving from the middle or the 5' genomic regions: the most endogenized gene from *Artoviridae* was the U2 protein (19/39) which is in the middle of the genome (2nd/3); in *Bornaviridae* and *Rhabdoviridae* the most endogenized gene was the RdRp (L) protein, which is the last ORF in the first genome (out of 8 genes) and the one just before the last gene in *Rhabdoviridae*. Finally, out of 36 EVEs deriving from *Chuviridae*, 26 corresponded to the Nucleoprotein (N) which is the last transcribed protein in the closest viral genomes. These unexpected results under Holmes model may thus lead one to reject the hypothesis, unless peculiar mechanisms of regulation of the transcription are at play for these viruses. Another explanation could come from a strong selective pressure for retaining particular proteins (i.e. Nucleoprotein) in the genome, independently of their level of transcription.

### 2- A new case of virus domestication in *Platygaster orseoliae*

In the assembly of *P. orseoliae*, 12 scaffolds were annotated as free-living viruses (F-X scaffolds). They had a different sequencing depth compared to BUSCO containing scaffolds and encoded 136 complete ORFs for which 21 presented homology with LbFV ORFs (min bit score = 50, min ORF size = 150pb, max overlaps = 23pb). ORF density was 82.7% which is in the range of expected values for related free-living viruses [111]. In order to identify additional scaffolds possibly belonging to this free-living virus, we searched for homology between the 136 putative viral proteins, and the scaffolds obtained from the assembly of *P. orseoliae*. These results allowed us to identify two additional scaffolds (scaffold\_117128 scaffold\_18896). Because the total size of the 14 putative "free-living" scaffolds was within the expected range for a dsDNA virus genome (136,801 bp) and because the average coverage was much higher than BUSCO-containing scaffolds (mean cov = 95.6X [sd=5.05X] compared to 33X in BUSCOs) and homogeneous (Figure S4), we believe that this set of scaffolds corresponds to the whole genome of a new virus, related to LbFV, which we propose to call *Platygaster orseoliae* filamentous virus (PoFV). In order to characterize the relationship of this new virus within dsDNA viruses diversity, we inferred a phylogenetic tree including ORFs of known dsDNA viruses along with the EVEs newly identified here. The phylogenetic reconstruction revealed that PoFV was the closest relative of the endogenized virus found in the same species (PoEFV, Figure 5).

In order to detect possible new viral endogenization from the same "donor virus", we queried the genome of *P. orseoliae* with the 136 predicted proteins of PoFV. This way, we found a total of 139 convincing hits (89 PoFV ORFs), including

the hits to the 15 ORFs with LbFV-homology. All ORFs were encoded by scaffolds with BUSCO-like coverage depth (p-value  $\text{cov} \geq 0.05$ ) and/or containing eukaryotic genes and/or transposable elements (Blastx E-value  $\text{max} = 7.060\text{e-}07$ , bits  $\text{min} = 50$ , with an average percentage of identity of 69.16%). Furthermore, a large proportion of the EVEs (22.7%) presented premature stop codons within the sequences, further suggesting that these virally-derived genes are indeed endogenized since abundant pseudogenization is not expected in free-living virus genomes (Figure S8-A).

Among the 81/139 apparently intact EVEs (with ORF length  $\geq 50\%$  of the PoFV ORF), some are likely implicated in DNA replication (*integrase*), in transcription (*lef-8, lef-9, lef-5, lef-4*), in packaging and envelopment (*ac81, 38k*) and in infectivity (*pif-1, pif-2, pif-3*). Among the 139 PoFV-related EVEs found in *P. orseoliae*, 104 corresponded to putative paralogs. Conversely, none of these 104 ORFs were present in two copies within the PoFV genome, suggesting that a major post-endogenization duplication event occurred or that multiple endogenization events did occur. Among these 104 duplicated EVEs, 78 presented topologies allowing us to calculate dN/dS ratios using Bayesian pairwise estimates (runmode -3 in codeml) or foreground/background tests (codeml) when topologies presented more than 4 leaves. Before running the foreground/background tests, we constrained all paralogs to form a monophyletic group including the PoFV loci as the closest taxa in the phylogenies (all LRT tests did not significantly present differences between constrained and unconstrained trees). Among these 78 paralogs EVEs, 44 presented a complete and intact open reading frame and a dN/dS ratio significantly lower than 1 suggesting that they are under stabilizing selection (Figure S8-A).

Although functional studies are needed to confirm that these virus-derived genes are involved in the formation of VLPs as observed in *Leptopilina* [19], we believe that *P. orseoliae* filamentous virus (PoEFV) is a good candidate for viral domestication, possibly involved in counteracting its Diptera host immune system (from the family Cecidomyiidae [33]).

#### 4- Assignment of the unknown Hymenoptera to species

UCEs along with 400 bp of flanking regions on either side were extracted from the low coverage scaffolds with a custom script. We used a two-step process to assign the unknown sample to species. First, UCEs + flanking regions were analyzed with a set of UCEs + flanking regions obtained from early diverging families of Chalcidoidea by [70, 69] to assign the unknown sample to family. Then, unknown sequences were analysed with a larger set of species belonging to the identified family (Eulophidae; loci taken from [69]). In both cases, only loci that had a sequence for at least 75% of the samples included in the analysis were retained. Loci were aligned with MAFFT (-linsi option; [112]). Positions with > 90% gaps and sequences with > 25% gaps were removed from the alignments using SEQTTOOLS (package PASTA; [113]). The concatenation of all loci was analysed with IQ-TREE v 2.0.6 [71] without partitioning. Best fit models were selected with the Bayesian Information Criterion (BIC) as implemented in ModelFinder ([72]). FreeRate models with up to ten categories of rates were included in tests. The candidate tree set for all tree searches was composed of 98 parsimony trees + 1 BIONJ tree and only the 20 best initial trees were retained for NNI search. Statistical support of nodes was assessed with ultrafast bootstrap (UFBoot) ([114]) with a minimum correlation coefficient set to 0.99 and 1,000 replicates of SH-aLRT tests ([115]). Results of the phylogenetic analyses are presented in Figure S2. Placement of the unknown species in trees shows that samples of *P. orseoliae* were likely mixed up with a species belonging to the genus *Aprostocetus* (Eulophidae, Tetrastichinae). Given its small size, color and abundance (265 species described just in Europe), it seems plausible that one specimen of *Aprostocetus* sp. remained unnoticed in the pool of *P. orseoliae*.

#### Supplementary figures

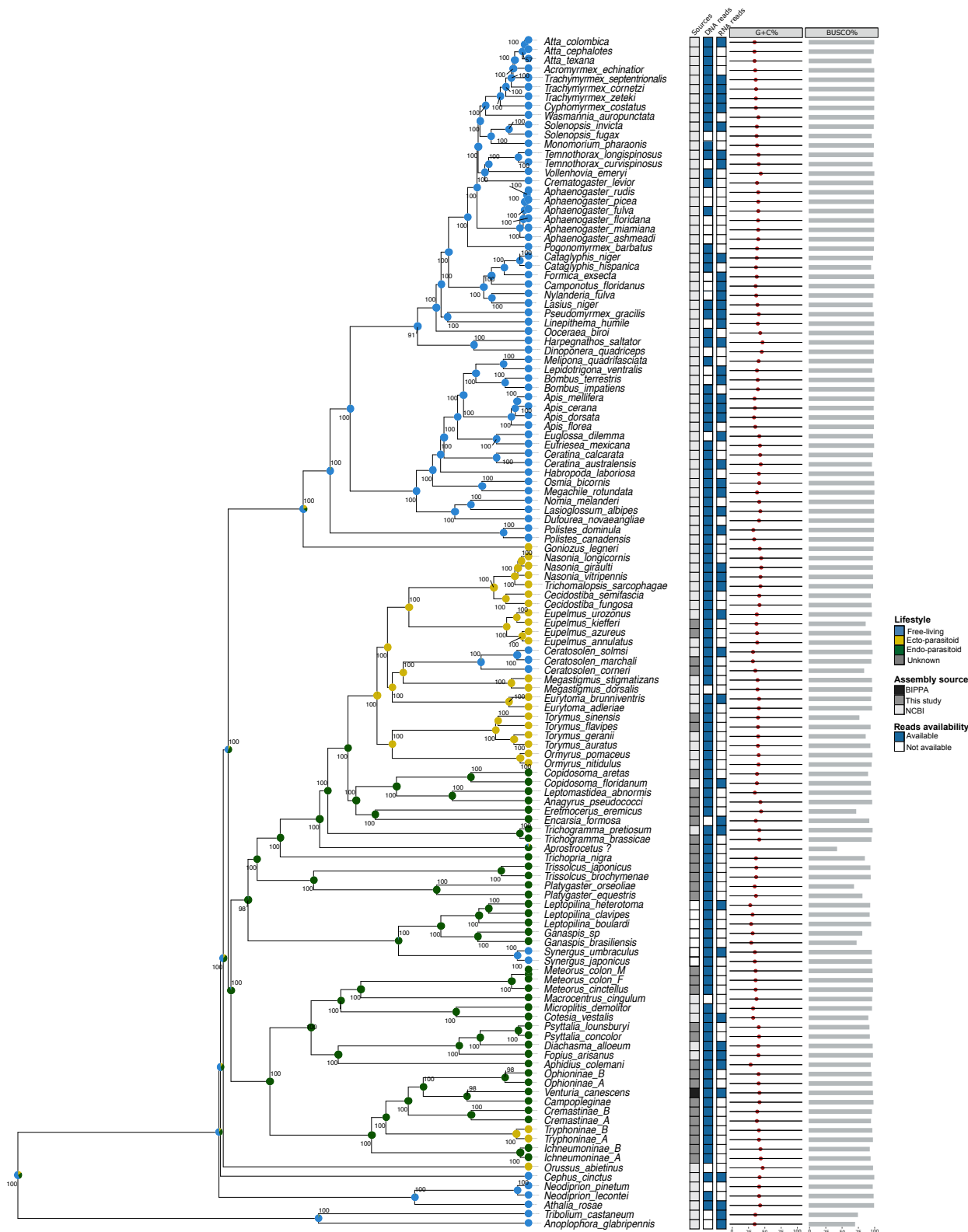


Figure S1. **Source of the datasets and availability of the reads.** Phylogeny of 124 Hymenoptera species. Two Coleoptera species were used to root the tree. The aLRT bootstrap scores are represented along the nodes. The sources refer to the platform or laboratory in which the assemblages come from (This study, BIPPA: Bioinformatics Platform for Agroecosystem Arthropods, NCBI: National Center for Biotechnology Information). The assemblies for which raw DNaseq or RNaseq reads were available are listed in the column DNA or RNA reads. The G+C% column reflects the average G+C rate for each assembly, and the BUSCO% column reflects the rate of complete or partial BUSCOs found via the analysis with BUSCO V3. Posterior Bayesian lifestyle inference distribution for each node and tips are represented by colored pie charts.



Figure S2. **UCE trees built to assign to species the unknown Chalcidoidea sequenced with the pool of *P. orseoliae*.** **A**: Phylogeny of early diverging families of Chalcidoidea (423 UCES and 127,979 bp were analysed to get the tree, best fit model = GTR+F+R10). **B**: Phylogeny of the family Eulophidae to which the unknown sample was inferred to belong to (408 UCES and 77,514 bp were analysed to get the tree, best fit model = GTR+F+R10). For both trees, SH-aLRT/UFBoot are shown at nodes; the number of UCES analyzed for each sample is indicated between bracket and the unknown sample is highlighted in red.

**Table S1. Summary statistics for control cases.** The numerator indicates the numbers of EVEs or dEVEs inferred by our pipeline, and the denominator indicates the number of known EVEs for each case. Analysis on dN/dS was only possible when orthologs or paralogs were available. Controls Endogenous viral elements present in scaffolds probably belonging to the Hymenoptera genome are scored from A to D, and scaffolds probably belonging to free viruses are scored as F or X: (see details in Materials and methods). TPM (Transcripts per kilobase million) values were calculated via RNAseq read mapping when available in the databases (all RNAseq data sources can be found on the github repository under the name : RNA\_seq\_reads\_mapped.txt). \* In addition to the expected unique shared event concerning the *M. demolitor* and *C. vestalis* species, our pipeline inferred two additional events, each specific to one lineage. This was due to the fact that two genes were not detected by our pipeline as shared by *M. demolitor* and *C. vestalis*, either because they are effectively not shared (for 3 of them: HzNVorf118, like-*pif-4* (19kda), *fen-1*), or because of some false negative in one of the two lineage (for one of them: p33 (ac92)).

	<i>V. canescens</i>	<i>F. arisanus</i>	<i>C. vestalis</i>	<i>M. demolitor</i>	<i>L. bouleardi</i>	<i>L. heterotoma</i>	<i>L. clavipes</i>	% Total
---- <b>NB EVEs</b> <sup>1</sup>	<b>36/40</b>	<b>42/47</b>	<b>20/21</b>	<b>18/25</b>	<b>12/13</b>	<b>12/13</b>	<b>12/13</b>	<b>88.4%</b>
Scaffold A	36	42	18	16	11	6	8	90.13%
Scaffold B	0	0	0	0	0	0	0	0%
Scaffold C	0	0	0	0	1	6	4	7.24%
Scaffold- D	0	0	2	2	0	0	0	2.63%
Scaffolds E-F-X	0	0	0	0	0	0	0	0%
---- <b>NB dEVEs</b>	<b>22/36</b>	<b>11/42</b>	<b>20/20</b>	<b>18/18</b>	<b>12/12</b>	<b>12/12</b>	<b>12/12</b>	<b>71.82%</b>
by dN/dS <sup>2</sup>	3/3	0/1	16/20	15/18	12/12	12/12	12/12	70.39%
by TPM 1000	21	11	10	9	1	0	0	43.60%
---- <b>Nb Events</b>	<b>1/1</b>	<b>1/1</b>	<b>3/1*</b>		<b>1/1</b>			.

1 When paralogs were detected on different scaffolds, the best scaffold score was used.

2 Analysis on dN/dS was only possible when orthologs or paralogs were detected (for example, in *V. canescens* this calculation was only possible for 3 genes having paralogs)

**Table S2. Summary table.** Clusters refers to the number of homologous clusters with one or more candidate Endogenous Viral Elements (EVEs). Raw EVEs is the raw number of EVEs (i.e. including all paralogs and orthologs) according to the scaffold categories from A to D. Nb EVEs with complete ORFs corresponds to the number of raw EVEs with an ORF starting with a methionine, without premature stop codons, and ending with a stop codon, distinguishing ORFs whose size is at least equal to or greater than 80% of that of the best viral hit. EVEs is the count of EVEs, i.e. counting the number of genes within each monophyletic group only once (as several EVEs may have undergone post-endogenization duplications or an EVE may be ancestrally acquired and shared by several species). Mean pident is the average of the percentage identity of all EVEs with the best viral hit, the number in brackets corresponds to the standard error. Nb EVEs pident and Nb EVE E-values 1e-20 correspond to the number of refined EVEs showing a hit with more than 80% identity and an E-value below 1e-20 with a viral protein, respectively. Domesticated EVEs (dEVEs) corresponds to the number of refined EVEs with either a dN/dS significantly less than 1 with a complete ORF and without a stop codon and/or a TPM value > 1000 with a complete ORF and without stop codon. Events corresponds to the number of endogenization events that may include one or more genes and involve one or more species. Shared Events is the number of endogenization events shared by at least two species. Viral families corresponds to the number of different putative viral families associated with the best viral hits. dEvents corresponds to the number of endogenization events presenting at least one dEVE.

	dsDNA	ssDNA	dsRNA	ssRNA	Total
Clusters	166	9	18	36	229
Raw EVEs (A B C D)	819 (534 61 102 122)	92 (40 27 6 19)	95 (45 19 26 5)	255 (129 74 34 18)	1261
Compleat raw EVEs ORFs (>80% best viral hit)	694 (409)	74 (39)	76 (26)	223 (76)	1067 (550)
EVEs	366	32	61	162	621
Mean pident (sd)	36.99 (15.35)	36.91 (11.37)	38.32 (13.27)	33.30 (11.82)	36.32
EVE pidents >= 80	22	0	0	0	22
EVE E-values 1e-20	430	29	67	126	652
dEVEs	112	9	12	38	171
Events	130	26	59	152	367
shared Events	17	1	5	13	36
viral families/clades	12	3	4	21	40
dEvents	47	10	12	38	107



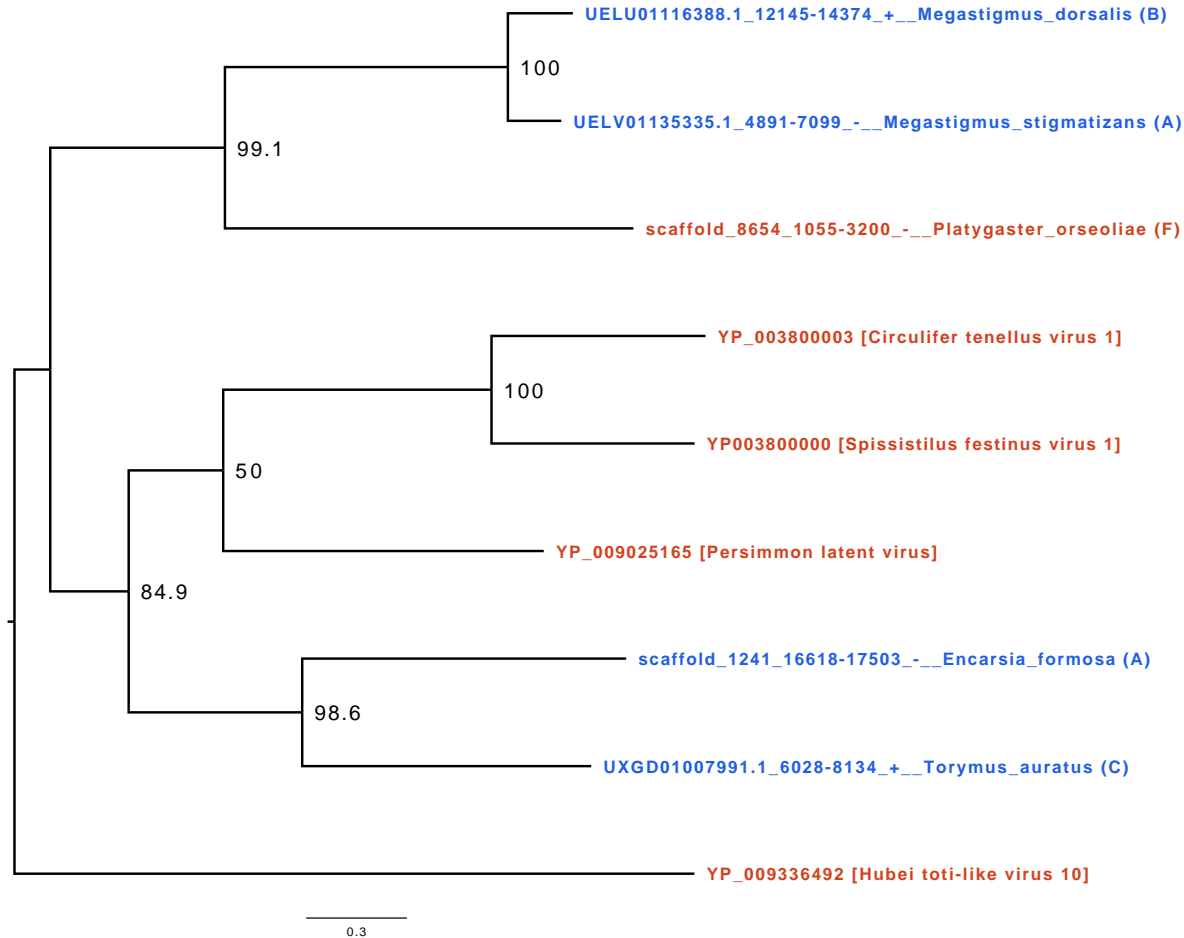
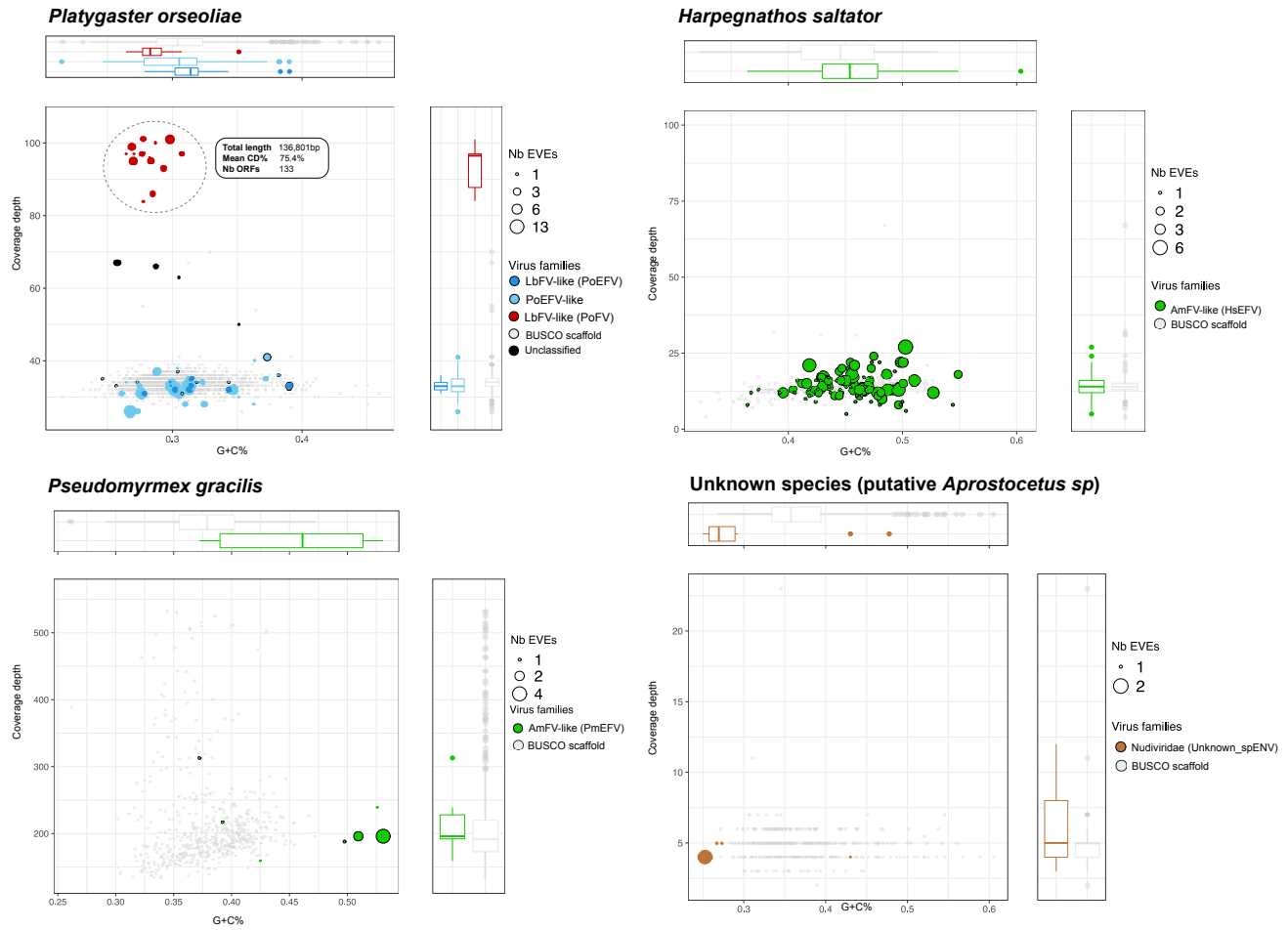


Figure S3. **Example of endogenization events.** The phylogeny of cluster21304 corresponds to the clustering of a set of viral and candidate viral insertion genes sharing a homology. In red are represented the loci of viral origin, and in blue are represented the loci probably endogenized (EVEs). The letter at the end of the taxon label represents the endogenization score assigned to the candidate (see details in Materials and methods). In this example, we found two singular endogenization events in the species endoparasitoid *Encarsia formosa* (annotated A and thus presenting a depth of coverage non-significantly different from the distribution of the BUSOs of the genome as well as at least one transposable element and/or one eukaryotic gene) and ectoparasitoid *Torymus auratus* (annotated C and thus presenting only a depth of coverage non-significantly different from the distribution of the BUSOs of the genome). Since these two species do not share a close common ancestor in the phylogeny and come from two different families, the algorithm therefore assigned them to two independent viral endogenization events. The viral locus found in the assembly of the endoparasitoid species *Platygaster orseoliae* was annotated F, meaning that the depth of coverage deviated significantly from the BUSCO distribution of the genome and that no TEs and less than 5 eukaryotic genes were found in the scaffold containing the candidate insertion. Finally, the two loci belonging to the ectoparasitoid species *Megastigmus stigmatizans* and *Megastigmus dorsalis* both show a score supporting viral endogenization. Furthermore, these species exhibit a doubly monophyletic clade (high bootstrap score) within the gene phylogeny and within the species phylogeny, suggesting that they acquired this viral gene from their closest common ancestor about 20 million years ago. All newick phylogenies are available on the github repository under the name : All<sub>c</sub>luster<sub>p</sub>hylogenies<sub>m</sub>erged.



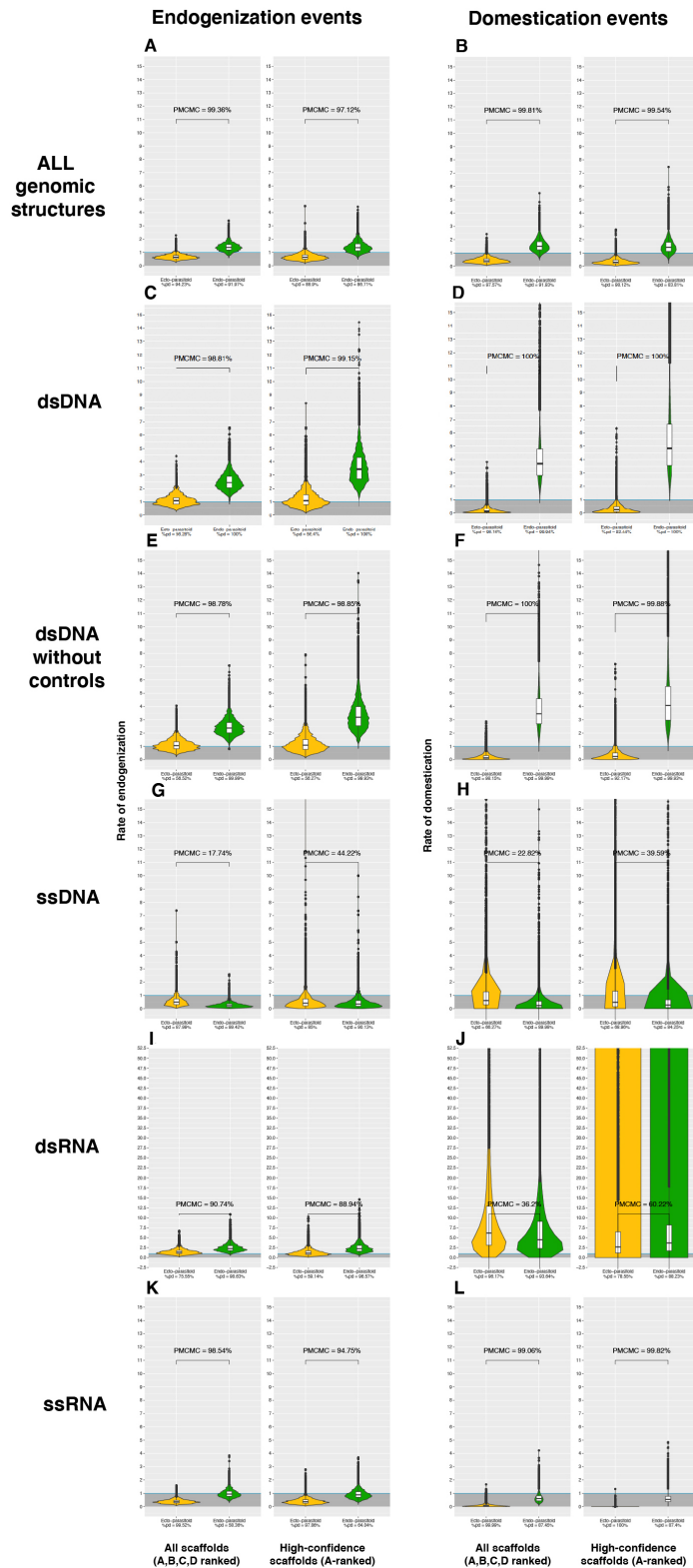


Figure S5. **Violin plots of the posterior distribution of GLM coefficients after exponential transformation in relation to wasp lifestyle.** The ectoparasitoid lifestyle is in yellow, the endoparasitoid lifestyle is in green, and the free-living lifestyle is in blue. Coefficients have been transformed into exponential and correspond to the posterior distribution of the coefficients of a binomial negative zero-inflated GLM model, where the lifestyle free-living stand for the intercept. The Y-axis corresponds to the multiplicative factor of the number of endogenization and/or domestication of EVES and/or events relative to free-living species. The coefficients are derived from 1000 GLM models adjusted on 1000 randomly selected probable scenarios (>90 CI) of ancestral states at nodes. Branches from nodes older than 160 million years have been removed from the dataset. The ROPE% is the percentage of the posterior distribution of coefficients below the intercept. The posterior distribution of the interaction coefficients between lifestyles and branch size were not informative, and the branch size factor was therefore added as an additive effect to the model.

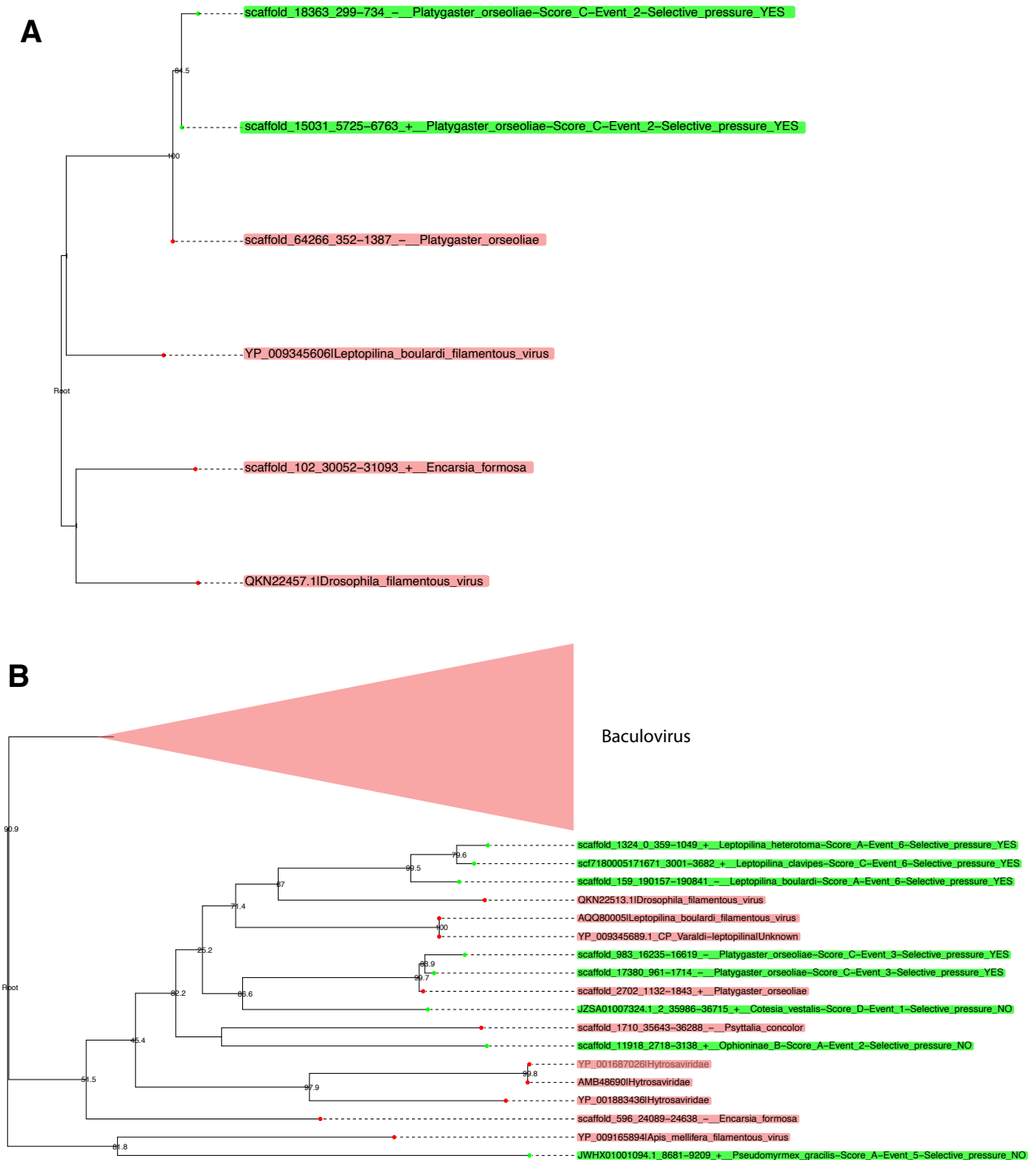
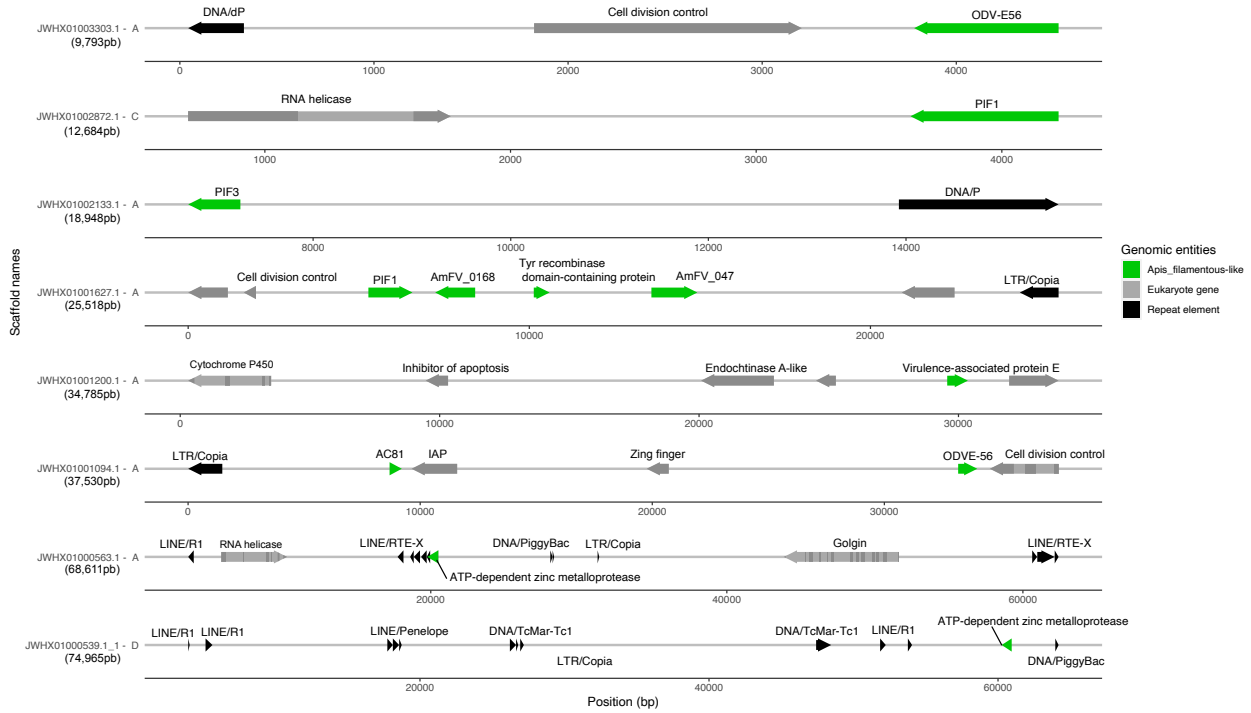


Figure S6. **Phylogenies of LbFV-like proteins under purifying selection in *Platygaster orseoliae* genome.** The panel A represents the Cluster\_25710 which corresponds to the *integrase* protein. The panel B represents the Cluster\_26675 which corresponds to the *ac81* protein. Taxa in red correspond to putative loci belonging to free-living viruses, while green taxa correspond to putative EVEs (the putative assigned family is assigned after the pipe). Green labels indicate the following information : (1) scaffold name, (2) start location, (3) end location, (4) strand, (5) Hymenoptera species, (6) endogenization index, (7) the inferred event number within the cluster phylogeny, and (8) whether the loci are found under purifying selection (YES) or not (NO).

### Genomic environment of : *Pseudomyrmex gracilis*



### Genomic environment of : *Aphaenogaster picea*

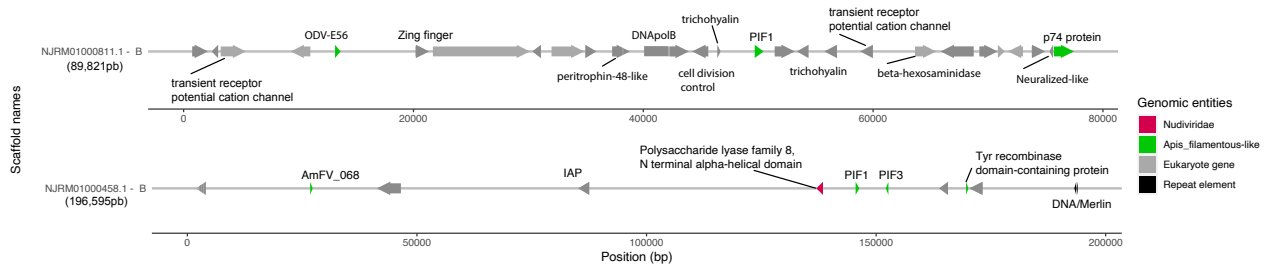


Figure S7. **Candidate EVEs in two ant species.** In grey are displayed the eukaryotic genes predicted by Augustus, with a dark color for exons, and light for introns. In black are displayed the transposable elements predicted by sequence homologies with the RepeatPeps protein database. The size of the scaffold is displayed below the name of each scaffold. The letter followed by the scaffold name refers to the scoring given to the scaffold based on coverage and gene/ET presence information (see details in Materials and methods). For the sake of representation, all scaffolds are represented at the different scale, the exact coordinates of the elements are referred in the abscissa which corresponds to the coordinates in base pairs. Annotation is indicated above the arrows.

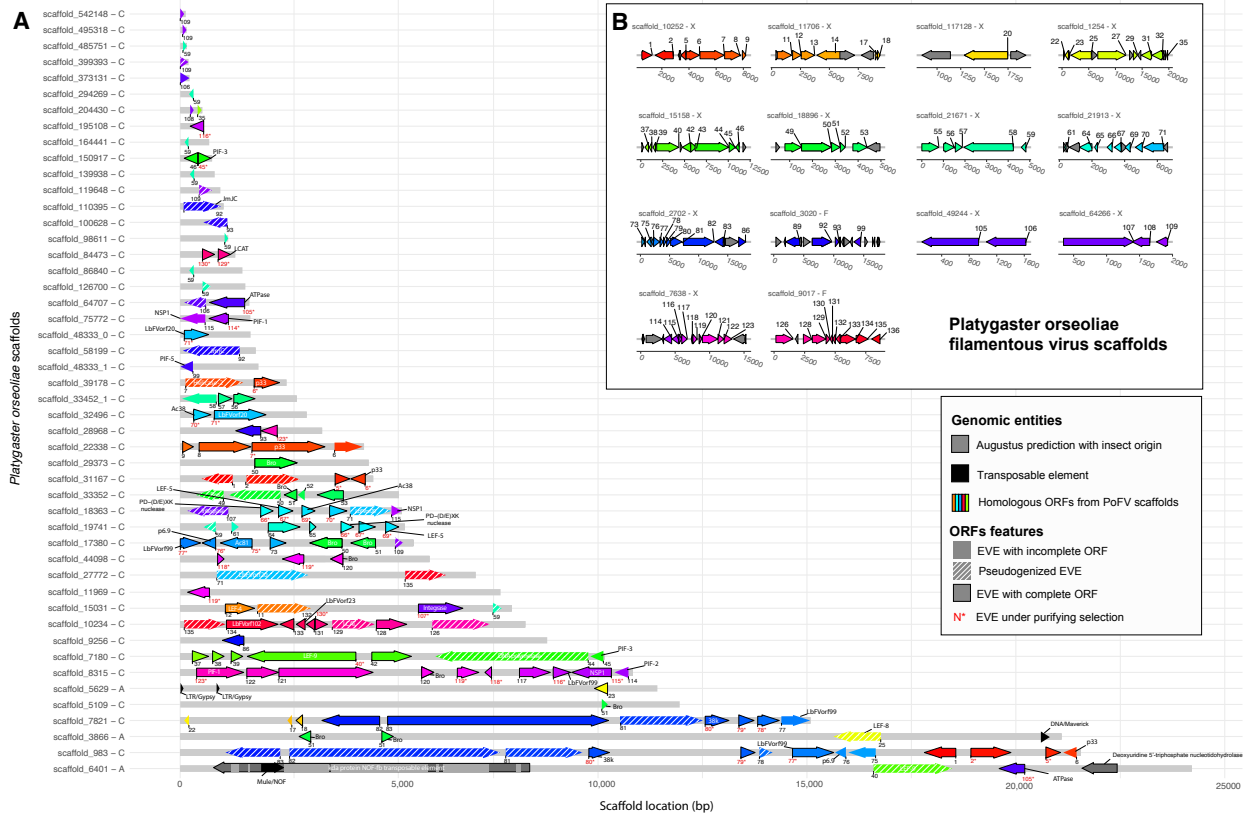


Figure S8. **Genomic environment for the EVEs detected in *Platygaster orseoliae***. The plot show regions homologous to viral ORFs in the *Platygaster orseoliae filamentous virus* (PoFV) genome (A). The colored regions correspond to the predicted ORFs in the PoFV genome (gray ORFs in PoFV scaffolds mean that no homologous EVEs were found in the genome of *P. orseoliae*.) (B), so the closer the colours, the closer the ORFs were initially in the PoFV genome. In grey are displayed the eukaryotic genes predicted by Augustus, with a dark color for exons, and light for introns. In black are displayed the transposable elements predicted by sequence homologies with the RepeatPeps protein database (max E-value =5.866e-18). The letter followed by the scaffold name refers to the scoring given to the scaffold based on coverage and gene/ET presence information (see details in Materials and methods). The exact coordinates of the elements are referred to the x abscissa, which corresponds to the coordinates in base pairs and can be found on the github repository under the name : All\_PoFV\_PoEFV\_loci\_informations.txt. Annotation is indicated in or next to the arrows. The number below each EVE correspond to the homologous ORF number in the PoFV genome. Numbers are colored in red if the EVE has at least one paralog and if the computed dN/dS is below 1, suggesting purifying selection in the *P. orseoliae* genome. Arrow with black borders correspond to EVEs showing a complete ORF (>50% of the size of the best PoFV ORF). Hatched arrows correspond to pseudogenized EVEs (with premature stop codons). The colour difference between black and white for the names of the proteins is for visual purposes only.

**Table S3. EVEs distribution according to putative non-segmented single-stranded RNA virus donor and their genomic position.**

For each EVE, we retrieved the position of the homologous ORF in the virus genome identified after a blastp search (first hit). The information on the position of the ORFs was retrieved either from [94], from the ICTV reports or manually after recovery of the viral assembly in NCBI, ORF annotation with getorf (ORF length min= 150bp) and blastp to confirm the position of the ORFs and their functions. The position of the ORF in the free-living virus genome is reported in the column "ORF position", when the genome was incomplete, we inferred the position of the ORF with respect to the position of the homolog in the closest complete viral genome. The number of EVEs corresponds to the number of EVEs counting paralogs only once (i.e. counting only one EVE per species per gene cluster).

Family	Closest viral blast hit	Protein ID	Protein Name	ORF position	Source	Nb EVEs
<i>Artoviridae</i>	Pteromalus puparum peropuvirus	YP_009505431	Glycosylated matrix protein M (U3)	3 out 5	ICTV	19
<i>Artoviridae</i>	Pteromalus puparum peropuvirus	YP_009505433	RNA pol (L)	5 out 5	ICTV	9
<i>Artoviridae</i>	Beihai rhabdo like virus 1	YP_009333442	Phosphoprotein (U2)	2 out 3	ICTV	1
<i>Bornaviridae</i>	Estrildid finch bornavirus 1	YP_009505428	RNA pol (L)	4 out 4	Manually	8
<i>Chuviridae</i>	Hubei chuvirus like 1	YP_009337906	Nucleoprotein (N)	Incomplete (putative 3 out 3)	Manually	24
<i>Chuviridae</i>	Hubei coleoptera virus 3	YP_009336865	Nucleoprotein (N)	2 out 3	Shi et al 2016	6
<i>Chuviridae</i>	Hubei chuvirus like 3	YP_009337091	Nucleoprotein (N)	3 out 3	Shi et al 2016	2
<i>Chuviridae</i>	Hubei coleoptera virus 3	YP_009336866	RNA pol (L)	3 out 3	Shi et al 2016	2
<i>Chuviridae</i>	Lonestar tick chuvirus	YP_009254003	Nucleoprotein (N)	3 out 3	Manually	1
<i>Chuviridae</i>	Hubei odonate virus 11	YP_009336948	Nucleoprotein (N)	3 out 3	Shi et al 2016	1
<i>Lispiriviridae</i>	Tacheng tick virus 6	YP_009304417	ORF1	1 out 5	Manually	5
<i>Lispiriviridae</i>	Hubei rhabdo like virus 3	YP_009336884	Glyceraldehyde-3-phosphate dehydrogenase	1 out 5	Manually	7
<i>Lispiriviridae</i>	Hubei rhabdo like virus 3	YP_009336887	Glycoprotein (G)	3 out 5	Manually	2
<i>Lispiriviridae</i>	Hubei rhabdo like virus 3	YP_009336889	RNA pol (L)	5 out 5	Manually	1
<i>Nyamiviridae</i>	Midway nyavirus	YP_002905336	Nucleoprotein (N)	1 out 6	ICTV	8
<i>Nyamiviridae</i>	Orinoco orinovirus	YP_009666283	Nucleoprotein (N)	1 out 4	Manually	8
<i>Nyamiviridae</i>	Sierra Nevada nyavirus	YP_009044206	Nucleoprotein (N)	1 out 6	ICTV	5
<i>Nyamiviridae</i>	Nyamanini nyavirus	YP_002905342	Nucleoprotein (N)	1 out 6	ICTV	4
<i>Nyamiviridae</i>	Midway nyavirus	YP_002905331	RNA pol (L)	6 out 6	ICTV	1
<i>Nyamiviridae</i>	Wenzhou Crab Virus 1	YP_009304556	Nucleoprotein (N)	1 out 4	Manually	1
<i>Nyamiviridae</i>	Beihai rhabdo like virus 3	YP_009666292	RNA pol (L)	5 out 5	Shi et al 2016	1
<i>Rhabdoviridae</i>	Wugan Ant virus	YP_009304559	RNA pol (L)	Incomplete (putative 5 out 5)	Manually	2
<i>Rhabdoviridae</i>	Wuhan insect virus 7	YP_009301743	RNA pol (L)	5 out 5	Manually	1
<i>Rhabdoviridae</i>	Sanxia Water Strider Virus 5	YP_009289351	Glycoprotein (G)	4 out 5	Manually	1
<i>Rhabdoviridae</i>	Muscina stabulans sigmavirus	YP_009664711	RNA pol (L)	Incomplete (putative 5 out 5)	Manually	1

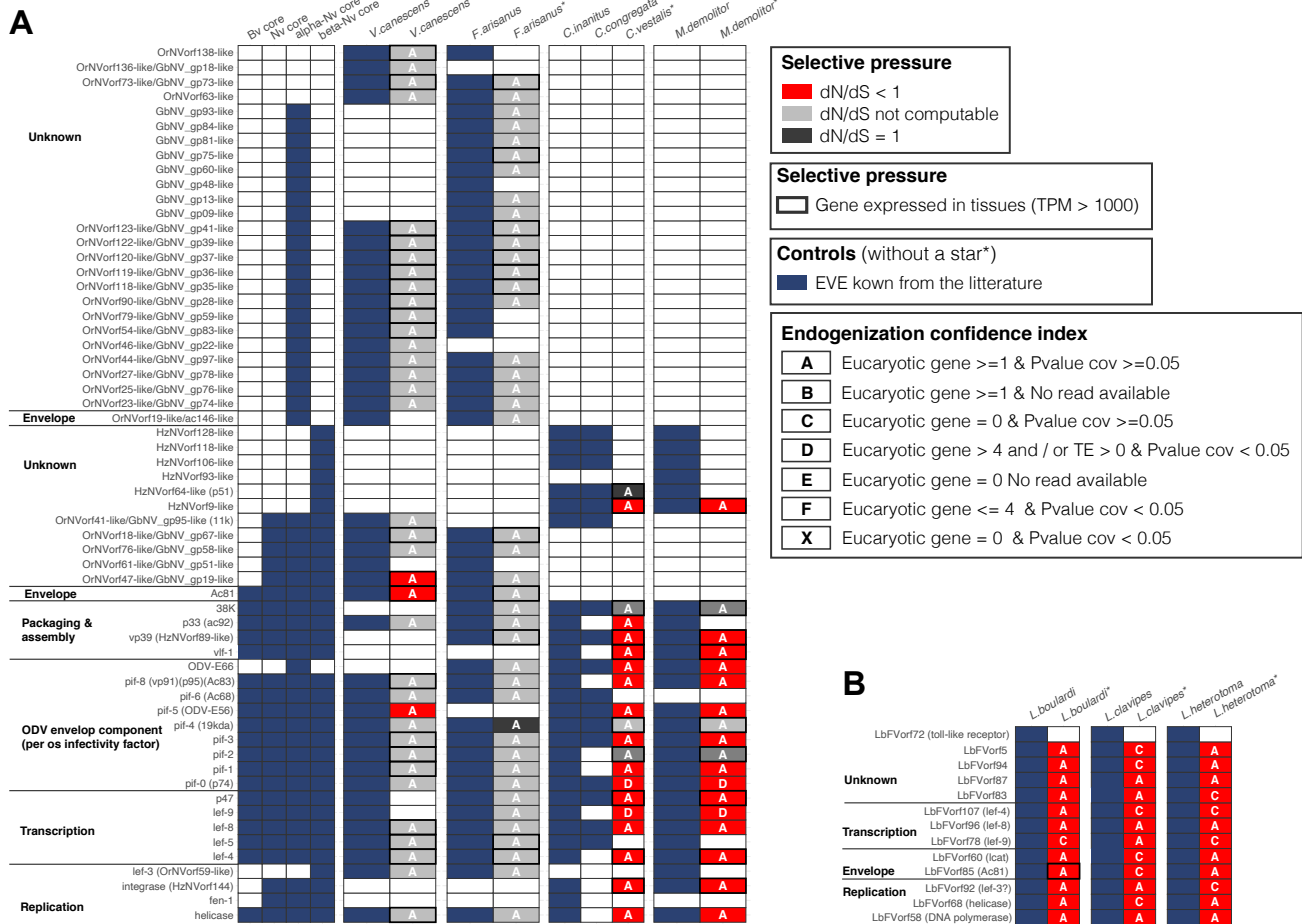


Figure S9. **Heatmap representing the viral genes known to be domesticated by Hymenoptera.** The panel (A) refers to the four known cases (*Venturia canescens*, *Fopius arisanus*, *Cotesia congregata* and *Microplitis demolitor*) involving Nudivirus donors while the panel (B) refers to the known case involving LbFV donors in three *Leptopilina* species. Complete parasitoid wasp genomes information were available for *Microplitis demolitor*, *Venturia canescens*, *Fopius arisanus* and *Cotesia congregata*, while only partial genomic data were available for *Chelonus inanitus*. Each row indicates a gene which has been identified previously as being endogenized in at least one species. In (A), the first four columns indicate whether the gene is a core gene for baculoviruses (Bv), Nudiviruses (Nd), alpha-nudiviruses (alpha-Nv) or beta-nudivirus (beta-nv). The following columns indicate the presence of each gene based on the literature (in blue) and based on our pipeline (columns with a star symbol). The colors indicate the inferred selection pressure acting on each gene (dN/dS) and the letters A, B, C, D, E, and X represent the degree of confidence in the endogenization. Capital letters indicate that this gene is present in a scaffold that contains other candidate genes. When the box is framed in black, it means that the gene is expressed (TPM>1000).



## Post-endogenization domestication rate

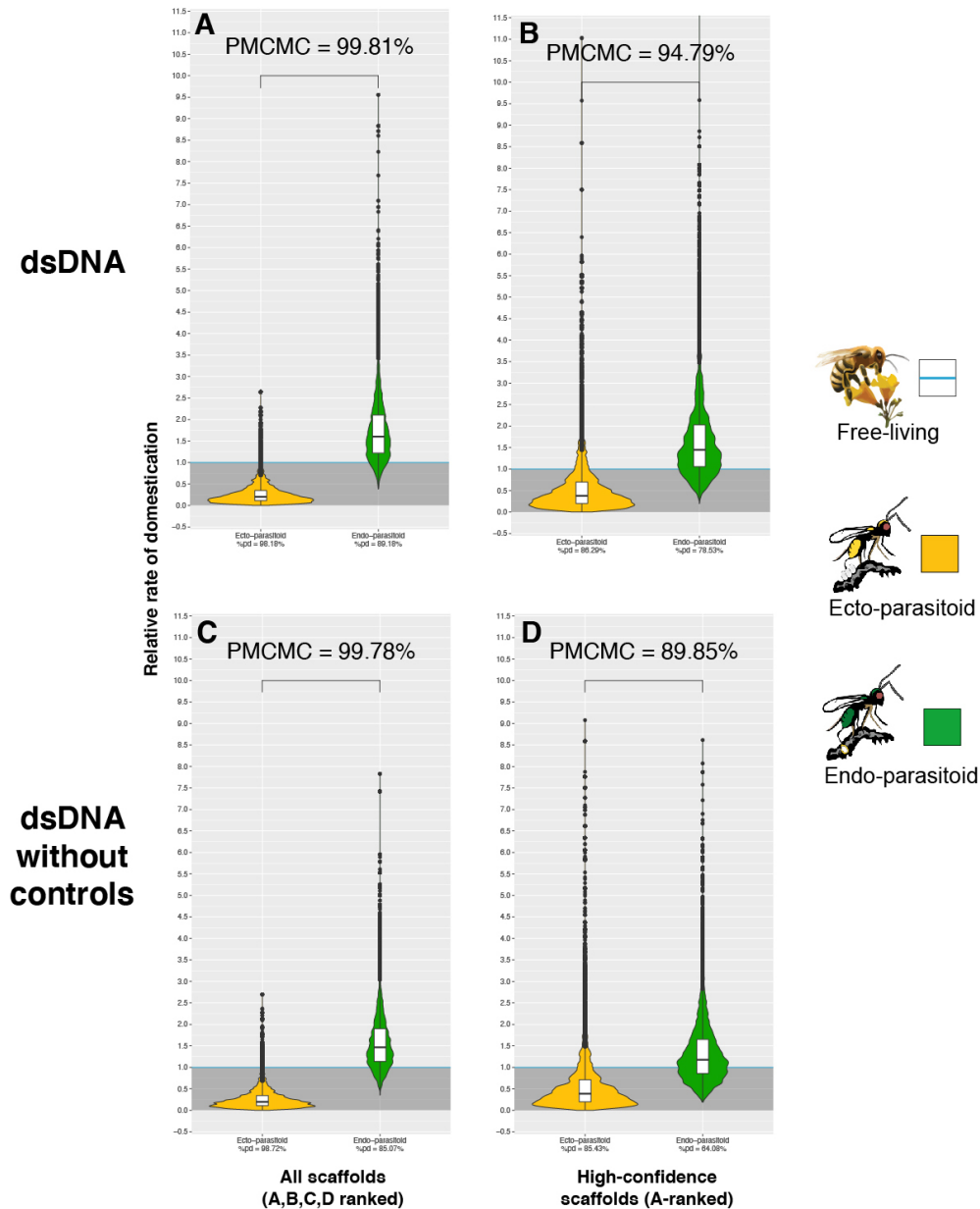


Figure S10. **Violin plots of the posterior distribution of dEVES and dEvents GLM coefficients in relation to wasp lifestyle (corrected for EVEs and Events rates)**. The ectoparasitoid lifestyle is in yellow, the endoparasitoid lifestyle is in green, and the free-living lifestyle is in blue (the intercept). Coefficients have been transformed into exponential and correspond to the posterior distribution of the coefficients of a binomial logistic regression GLM model, where the lifestyle free-living stand for the intercept. The Y-axis corresponds to the multiplicative factor of the number of domestication of dEVES or dEvents (corrected for EVEs and Events rates) correlative to free-living species. The coefficients are derived from 1000 GLM models adjusted on 1000 randomly selected probable scenarios (>90 CI) of ancestral states at nodes. Branches from nodes older than 160 million years have been removed from the dataset. The ROPE% is the percentage of the posterior distribution of coefficients below the intercept. The posterior distribution of the interaction coefficients between lifestyles and branch size were not informative, and the branch size factor was therefore added as an additive effect to the model. A- The corrected coefficient within all number of dEVES and dEvents, B- The corrected coefficient within all number of dEVES and dEvents without the control genomes, C- The corrected coefficient within all number of dEVES and dEvents present in a scaffold annotated with a score A, D- The corrected coefficient within all number of dEVES and dEvents present in a scaffold annotated with a score A and without the control genomes.

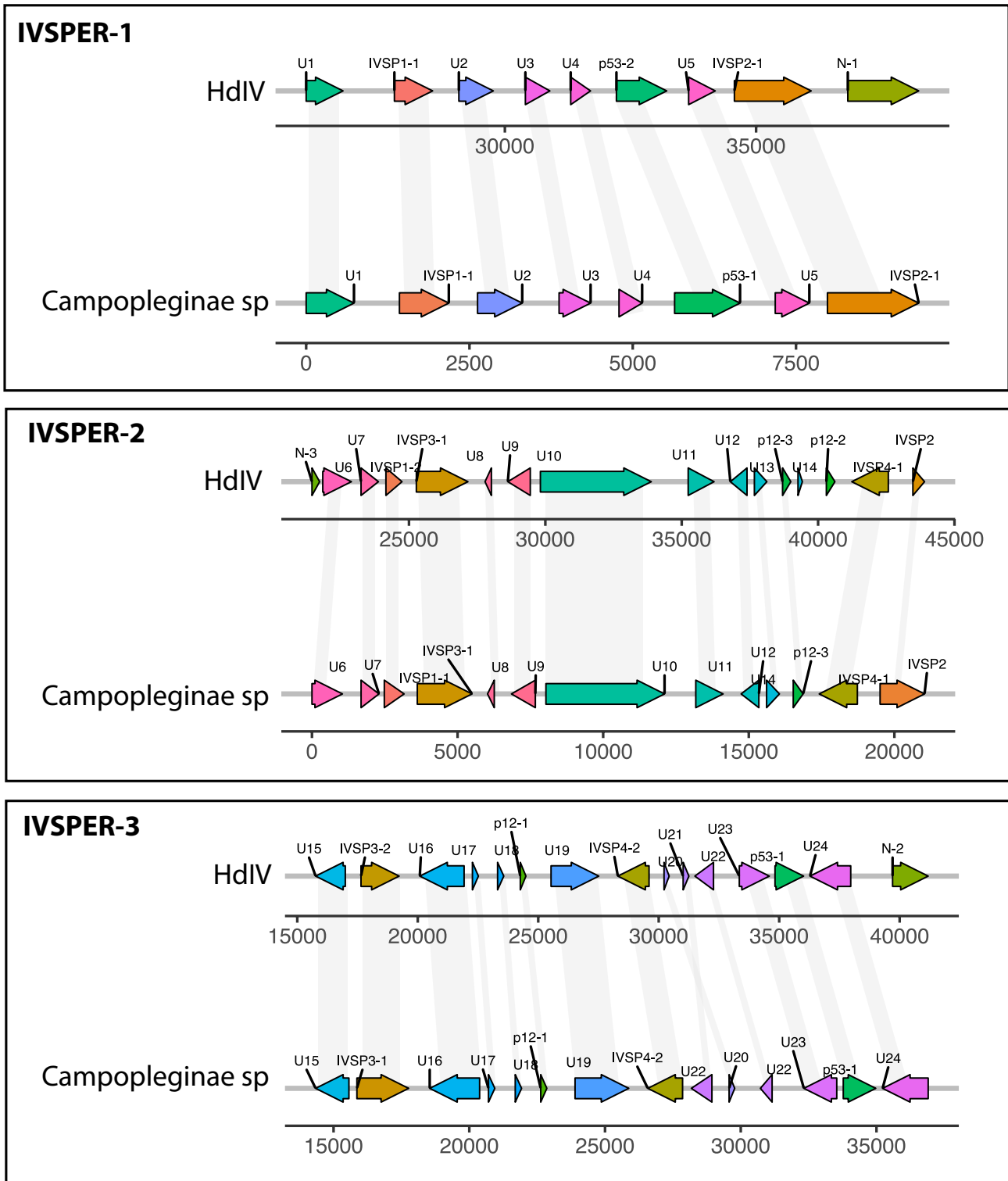


Figure S11. **IVSPER genes identified in the Campopleginae genome.** The figure compares the synteny of the IVSPER between *Hyposoter didymator* ichnovirus (HdIV) and the Campopleginae of our dataset. Homologous genes with syntenicity between the two species are indicated by grey shading. The direction of the arrows corresponds to the sense and anti-sense strand. The color of the boxes is unique to each IVSPER.

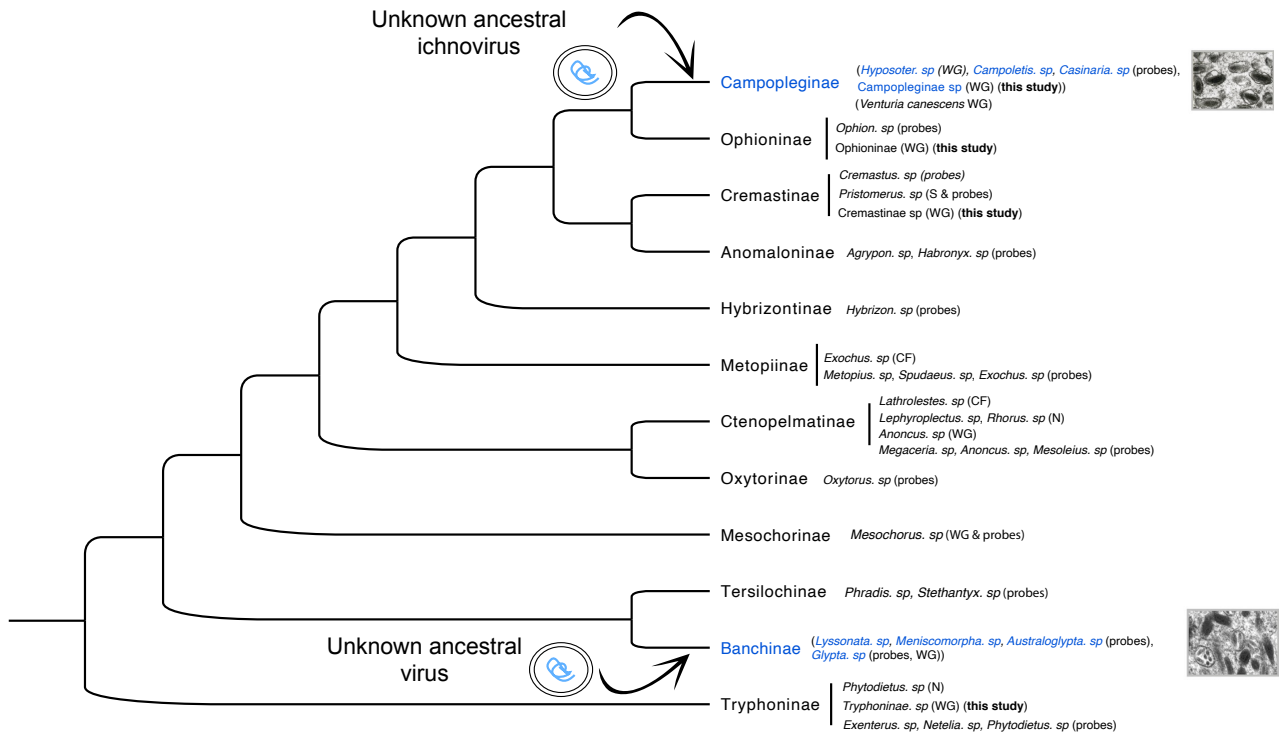


Figure S12. **Cladogram of the Ophioniformes group, illustrating the two independent endogenization events of two unknown viruses in Banchinae and Campopleginae lineages.** The phylogeny includes 12 subfamilies of the Ophioniformes group within the superfamily Ichneumonoidea. Several species of these subfamilies have been examined for the presence of ichnovirus-like polydnviruses: by negative staining of calyx fluid (N), TEM of ovarian sections (S), visual examination of the calyx fluid (CF), probes from ichnovirus replication or structural proteins (probes) or by IVSPER sequence homology on whole genome assemblies (WG). The subfamilies and species in blue correspond to those positive for a dsDNA virus endogenization from unknown origin (ichnovirus-like). The others (in black) were negative for endogenized ichnovirus-like elements. The phylogeny is inspired from [116], and the data reported comes from [116, 117, 118, 119].

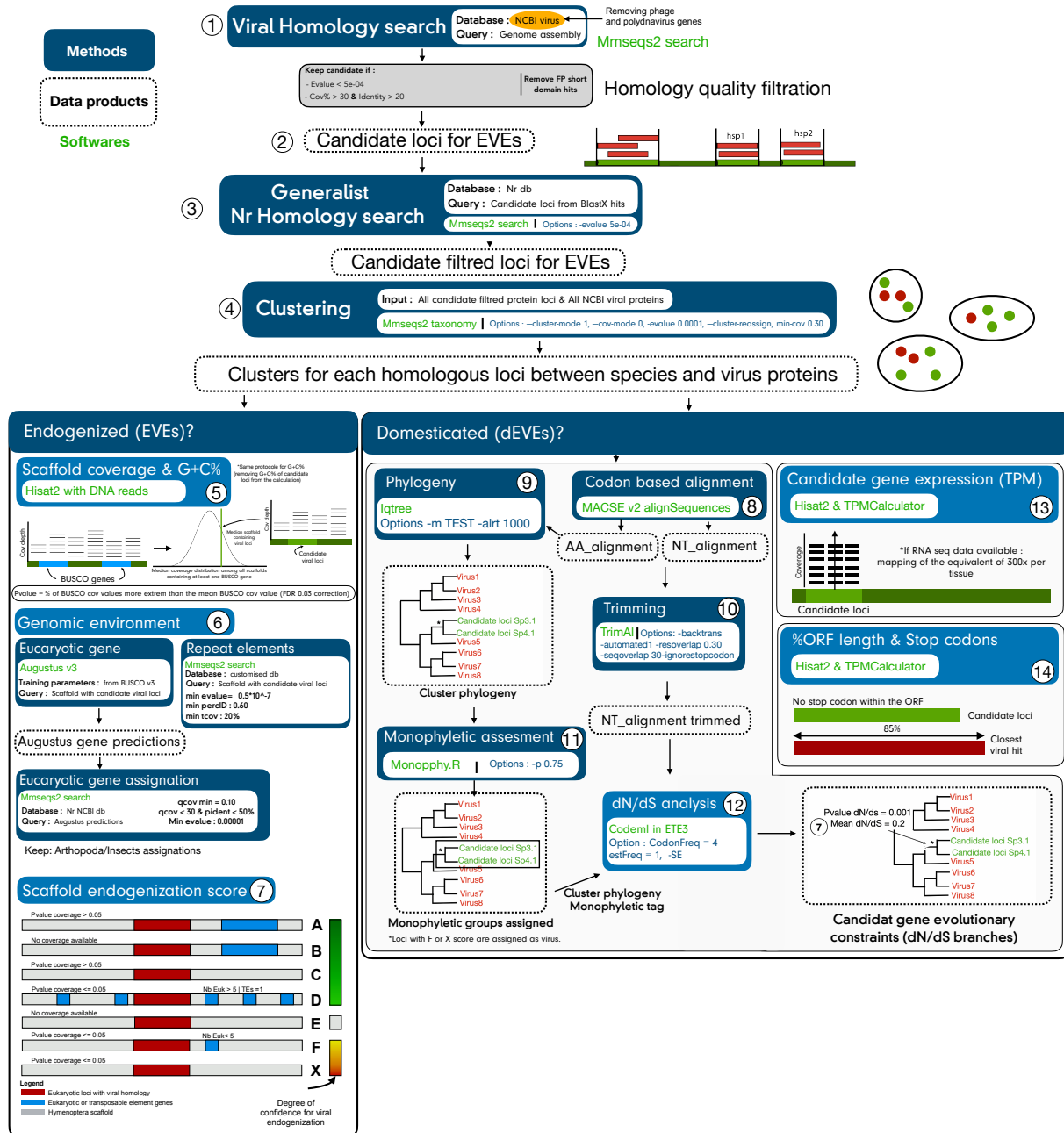
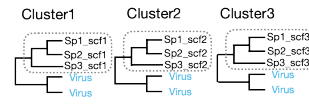


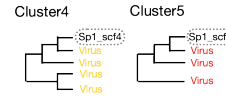
Figure S13. Simplified summary of the bioinformatics pipeline for the detection and validation of candidates for endogenization and domestication.

Sp names	Viral Family	Cluster number	Monophyletic clade number	Event number
Sp1_A		Cluster1	1	1
Sp2_A		Cluster1	1	1
Sp3_A		Cluster1	1	1
Sp1_B		Cluster2	1	1
Sp2_B		Cluster2	1	1
Sp3_B		Cluster2	1	1
Sp1_C		Cluster3	1	1
Sp2_C		Cluster3	1	1
Sp3_C		Cluster3	1	1



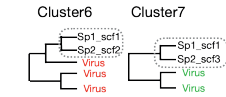
In this example with three clusters (and three distinct genes), three species are involved (SP1,2 and 3). The three species form a monophyletic clade in the species phylogeny of each of the three gene phylogenies and share homology with the same family of blue viruses. As a result, this pattern is consistent with the hypothesis that SP1, 2, and 3 share these genes from their common ancestor. Therefore, we combine these 9 loci into a single event (1) that consists of 3 common EVEs.

Sp names	Viral Family	Cluster number	Monophyletic clade number	Event number
Sp1_scf4		Cluster4	1	2
Sp1_scf4		Cluster5	1	2



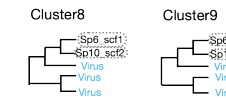
In this example, there are only 1 species and 2 clusters (and consequently 2 different genes) (SP1). We group the SP1\_scf4 and SP1\_scf4 loci together as a single endogenization event (2) because they share homologies with two distinct viral families (red and yellow), but are both present in the same scaffold (scf4). This is done because two independent endogenizations are unlikely to occur at the same place in the genome (note, we restrict this rule to viral families belonging to the same genomic structure).

Sp names	Viral Family	Cluster number	Monophyletic clade number	Event number
Sp1_scf1		Cluster6	1	3
Sp2_scf2		Cluster6	1	3
Sp1_scf1		Cluster7	1	3
Sp2_scf3		Cluster7	1	3



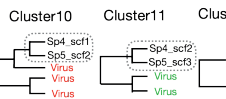
Two species are involved in this example (SP1 and 2), which has two clusters (and thus two different genes). In terms of species phylogeny, the two species are represented by a monophyletic clade in both gene phylogenies. The two loci in SP1 are on the same scaffold (scf1), so we will assume that these two genes were endogenized together in the common ancestor of SP1 and SP2, despite the fact that they show homology to a different viral family (note, we restrict this rule to viral families belonging to the same genomic structure). Therefore, by extension, we combine SP2\_scf3 and SP2\_scf2 in the same endogenization event using the information we gained about SP1 (possibly as a result of better assembly) (3).

Sp names	Viral Family	Cluster number	Monophyletic clade number	Event number
Sp6_scf1		Cluster8	1	4
Sp10_scf2		Cluster8	2	5
Sp6_scf2		Cluster9	1	4
Sp10_scf3		Cluster9	2	5



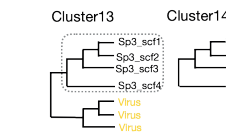
Two species are involved in this example (SP6 and SP10), which has two clusters (and thus two different genes). We treat these two endogenizations as independent in these two species because they do not form a monophyletic clade in terms of species phylogeny (too many species within the same clade lack the EVEs). Contrarily, we detect homology in both species for the same family of blue viruses, leading us to create two independent endogenization events in SP6 and SP10 at two loci each (4 and 5). That the virus diversity has not yet been sampled or that the virus lineages disappeared could be two explanations for the monophyly of these two loci.

Sp names	Viral Family	Cluster number	Monophyletic clade number	Event number
Sp4_scf1		Cluster10	1	6
Sp5_scf2		Cluster10	1	6
Sp4_scf2		Cluster11	1	7
Sp5_scf3		Cluster11	1	7
Sp4_scf3		Cluster12	1	7
Sp5_scf4		Cluster12	1	7



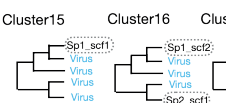
In this instance, which includes 3 clusters (and 3 distinct genes), 2 species are involved (SP4 and 5). According to species phylogeny, the two species SP4 and SP5 form a monophyletic group in the gene Cluster10. These two loci were likely endogenized independently of the other cases in the common ancestor of SP4 and SP5, and they were then attributed to the same event (6). In contrast, for Clusters 11 and 12, the two loci in SP4 and SP5 were probably endogenized in their common ancestor since they both share homology with the same green viral family, we then assign these EVEs to the same endogenization event (7).

Sp names	Viral Family	Cluster number	Monophyletic clade number	Event number
Sp3_scf1		Cluster13	1	8
Sp3_scf2		Cluster13	1	8
Sp3_scf3		Cluster13	1	8
Sp3_scf4		Cluster13	1	8
Sp3_scf5		Cluster14	1	8



In this example, there are only 1 species (SP3) and 2 clusters (so 2 different genes) involved. Since the loci SP3\_scf1,2,3, and 4 are likely multiple paralogs, we take a conservative approach to the number of EVEs and endogenization events and assume that they all result from the same endogenization event. We also placed another SP3 locus (scf5) within the same endogenization event because it exhibits homology with viruses in the yellow family (8).

Sp names	Viral Family	Cluster number	Monophyletic clade number	Event number
Sp1_scf1		Cluster15	1	9
Sp1_scf2		Cluster16	1	9
Sp2_scf1		Cluster16	2	10
Sp1_scf3		Cluster17	1	12
Sp2_scf2		Cluster17	2	11



In this example, which consists of three clusters (i.e., three distinct genes), there are EVEs in two species (SP1 and SP2). Due to their homologies to viruses in the blue family, the SP1 genome's EVE loci SP1\_scf1 and SP1\_scf2 are combined into a single endogenization event (9). The SP2\_scf1 and SP2\_scf2 loci, on the other hand, exhibit homologies with two distinct viral families (blue and green), respectively, and are not present in the same scaffold, so we count them as two separate endogenization events (10 and 11). We count SP1\_scf3 as a single event because it is the only locus in SP1 that shares homology with a family of green viruses (12).

Figure S14. **Canonical examples of endogenization events inferred by our pipeline.** The column "Sp names" contains the species name, followed by the name of the scaffold in which the EVE has been identified. The "Viral family" column refers to the putative viral family that donated the EVEs. The column "Cluster number" corresponds to the number of the corresponding cluster phylogeny (thus the EVE phylogeny). The "Monophyletic clade number" column corresponds to the number of the monophyletic clade within a cluster (can be a single locus or multiple loci). The column "Event number" is the number given to single/multiple EVEs that derive from the same endogenization event.