

1 **Mating strategy is determinant of Adenovirus prevalence in European bats**

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5

6 **Abstract**

7 Adenoviruses are double-strained DNA viruses found in a great number of vertebrates,
8 including humans. In order to understand their transmission dynamics, it is crucial, even from a
9 human health perspective, to investigate how host traits influence their prevalence. Bats are
10 important reservoirs for Adenoviruses, and here we use the results of recent screenings in
11 Western Europe to evaluate the association between characteristic traits of bat species and their
12 probability of hosting Adenoviruses, taking into account their phylogenetic relationships. Across
13 species, we found an important phylogenetic component in the presence of Adenoviruses and
14 mating strategy as the most determinant factor conditioning the prevalence of Adenoviruses
15 across bat species. Contrary to other more stable mating strategies (e.g. harems), swarming could
16 hinder transmission of Adenoviruses since this strategy implies that contacts between individuals
17 are too short. Alternatively, bat species with more promiscuous behavior may develop a stronger
18 immune system. Outstandingly high prevalence of Adenoviruses was reported for the Iberian
19 species *Pipistrellus pygmaeus*, *P. kuhlii* and *Nyctalus lasiopterus* and we found that in the latter,
20 males were more likely to be infected by Adenoviruses than females, due to the
21 immunosuppressing consequence of testosterone during the mating season. As a general trend
22 across species, we found that the number of Adenoviruses positive individuals was different
23 across localities and that the difference in prevalence between populations was correlated with
24 their geographic distances (*P. pygmaeus*). These results increase our knowledge about the
25 transmission mechanisms of Adenoviruses.

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29 **Author Summary**

30 Adenoviruses are DNA viruses with a wide range of vertebrate hosts, including humans,
31 causing ocular, respiratory and gastrointestinal diseases. Here, we focus on the prevalence of
32 Adenoviruses in bats, which are known to be natural reservoir of many viruses, using the results
33 of recent screenings for prevalence of these viruses in 33 European bat species. Our aim is to
34 find association between Adenoviruses prevalence and biological and behavioral host traits,
35 considering the heterogeneity both between and within species in order to have a deeper
36 understanding of mechanisms of viral transmission.
37 Our results highlight the importance of mating strategy: bats species using swarming as mating
38 strategy are less likely to be infected by Adenoviruses. Moreover, we found that locality of
39 capture can explain a higher prevalence of Adenovirus within species. However, no general
40 pattern has been found in the analysis at individual level, suggesting a strong species specificity
41 and complex viral transmission dynamics.

42

43 **Introduction**

44 Some viruses have the potential for cross-species transmission, with spillover episodes
45 from humans to wildlife and vice versa, a phenomenon referred to as zoonosis [1]. Current
46 research on the dynamics behind host/virus interactions and inter- and intra-specific
47 transmissions is of scientific interest and important implications for emerging zoonoses and
48 consequently for public health.

49 The heterogeneity (understood as the inter- and intra-specific variation) in the ability of
50 hosts to transmit pathogens is among the most fundamental concepts in disease dynamics and
51 crucial in the design of disease control strategies [2]. The potentiality of cross-species or cross-
52 populations transmission depends on the characteristics of host and virus traits and is affected

53 directly or indirectly by a variety of ecological, social, or socioeconomic changes [3] that can
54 lead to new conditions boosting the expansion of the viruses to new host species or populations.
55 Recent studies have revealed that traits such as host phylogenetic relatedness, conservation
56 status, and geographical overlap are critical in the potential for cross-species transmission of
57 viruses [4,5].

58 The highest proportion of zoonotic viruses in mammals is found in bats, primates and
59 rodents [6]. Specific bat characteristics like their ability to fly, unique among mammals, or their
60 migratory capacity [7], increase their potential role as vectors of diseases. Bats are natural
61 reservoirs for many viruses, such as Coronavirus, Nipah virus and Ebola related to new emerging
62 diseases that have received attention in the last years due to the potential risk they pose for
63 human pandemic events [8–10] or with important health and economic consequences world-
64 wide, such as rabies outbreaks [11,12]. The capacity to coexist with viruses (in the absence of
65 disease) is probably linked to the bats' immune-system which seems be different to that of other
66 mammals, [7,13] and related to the development of altered mitochondrial genomes [14]. This
67 pathogen control in bat hosts has favored ancient events of coevolution or parallel evolution
68 [15,16] between bats and viruses.

69 Additionally, bats are systematically very diverse and form the second largest order of
70 mammals [17]. In fact, bats occupy all kinds of habitats throughout all continents except
71 Antarctica, showing a surprising ecological breadth that implies large variation in trait
72 characteristics either in their morphology: e.g. size variation across species [18]; social behavior:
73 e.g. differences in mating system [19]; or habitat requirements: e.g. roosts preferences from trees
74 to caves [18]. For all these reasons, bats provide a good model to inspect the correlation of host
75 trait characteristics with the prevalence of viruses.

76 Adenoviruses (AdVs) are non-enveloped dsDNA viruses with a broad range of vertebrate
77 hosts, including humans. These viruses have been grouped into five genera [20]: *Mastadenovirus*

78 (mammals), *Atadenovirus* (mammals, birds and reptiles), *Aviadenovirus* (birds), *Siadenovirus*
79 (poultry and amphibians) and *Ichtadenovirus* (fish). Symptoms in humans include respiratory,
80 eye infections and intestinal or digestive illness and seldom lead to mortality [21,22]. Since the
81 first detection of an AdV in a fruit bat from Japan [23], a high diversity of AdVs has been
82 isolated from bats from America, Africa and Asia, all grouped within the genus *Mastadenovirus*
83 [24]. The first AdV in a European bat was isolated from a common pipistrelle (*Pipistrellus*
84 *pipistrellus*) in Germany [25]. Later, Vidovszky *et al.* [26] obtained partial sequences from as
85 many as 28 presumably new AdVs hosted by 12 different bat species in Germany and Hungary.
86 Iglesias-Caballero *et al.* [16] conducted a country-wide survey in Spain constituting the largest
87 screening for AdVs in bats to date, checking >1,000 individuals belonging to 28 species and
88 focusing not only on the analysis of fecal samples and internal tissues -as in previous studies-
89 but also on the analysis of the oropharyngeal swabs. AdVs are detected in almost half of the
90 Iberian bat species studied, in both feces and in the upper respiratory tract (for the first time in
91 bats), establishing a possible fecal-oral transmission route in two *Pipistrellus pygmaeus*.
92 Moreover, they found a surprisingly high prevalence in this species together with the co-generic
93 *P. kuhlii* and the closely related giant noctule, *Nyctalus lasiopterus*, which accounted for the vast
94 majority of viruses detected in the study. These results have presented us with the opportunity to
95 analyze the abiotic factors or biotic traits determining prevalence of AdVs in European bats at
96 two levels: across-species and among individuals within a species.

97 The analysis of morphological and behavioral traits and presence of AdVs in bats is also
98 useful for the understanding of viral transmission dynamics. In fact, little is known about the
99 transmission mechanisms of AdVs and no study has focused on transmission in bats. A handful
100 of studies in humans suggest that transmission may need direct contact or at least a droplet
101 spraying (such as those produced by coughing or sneezing) or aerosol [27–29]. The strong host
102 specificity and the parallelism between host and AdVs phylogenies found for bats [16,30] and

103 for primates [31] strongly suggest that cross-species switching of mastadenovirus are not
104 frequent events although they have been detected in AdVs evolution [30].

105 No study has focused so far on the analyses of the determinant factors influencing
106 prevalence of viruses on bats at both species and individual levels. Across species, Webber *et al.*
107 [32] found that viral richness was positively correlated with group size as predicted by the
108 contact-rate hypothesis. In turn, at the intra-specific level Dietrich *et al.* [33] found an important
109 seasonal shift in prevalence with a significant increase in AdVs shedding during reproduction
110 while studying AdVs prevalence in two bats (*Miniopterus natalensis* and *Rousettus aegyptiacus*).
111 The aim of this study is to advance our understanding of heterogeneity in the prevalence of
112 AdVs in bats, testing the association between traits and presence of AdVs at both the among-
113 species and within-species levels. Among species, and according to the contact-rate hypothesis,
114 higher prevalence of AdVs is expected in species roosting in large groups, sharing refuge with
115 other species and/or mating in swarming aggregations. In addition, we hypothesize a strong
116 phylogenetic signal given the strong species-specificity found in the presence of AdVs. Within
117 species, we predict a higher prevalence in females because of their concentration in large
118 numbers in nursery colonies [34], with high contact rate and a high concomitant risk of infection.

119 In summary, our aims were to: 1) Test for significant phylogenetic component to the
120 presence of AdVs in European bats; 2) test the importance of ecological characteristics at a
121 species-level for the presence of AdVs taking into account phylogenetic relationships; 3)
122 investigate whether some individual characteristics are determinant to explain the differences in
123 the prevalence of AdVs within species.

124

125 **Results**

126 The final working database consisted of 1,985 bats sampled and checked for AdVs,
127 belonging to 10 genera (*Barbastella*, *Eptesicus*, *Hypsugo*, *Miniopterus*, *Myotis*, *Nyctalus*,

128 *Pipistrellus*, *Plecotus*, *Rhinolophus*, *Vespertilio*) and representing 33 of the 45 European bat
 129 species. This database included a total of 1,612 bats belonging to 27 Iberian species surveyed for
 130 the study (Fig 1). The inclusion in the analyses of the published results from Vidovszky *et al.*
 131 [26] and Sonntag *et al.* [25] for Germany and Hungary, allowed the addition of seven European
 132 species (*Eptesicus nilssonii*, *Myotis brandtii*, *M. dasycneme*, *M. nattereri*, *Pipistrellus nathusii*
 133 and *Vespertilio murinus*), not included in the Iberian database.

134

135 **Table 1: Number of individuals screened as positive or negative for Adenoviruses and**
 136 **percentage of positives for each species taken into account in this study.**

Species	N° Positives	N° Negatives	Percentage (%)	Lower limit (%)	Upper limit (%)
<i>Barbastella barbastellus</i>	0	46	0.00		
<i>Eptesicus nilssonii</i>	1	5	16.67	0.85	58.18
<i>Eptesicus isabellinus</i>	4	18	18.18	6.46	36.91
<i>Eptesicus serotinus</i>	2	20	9.09	1.64	25.95
<i>Hypsugo savii</i>	3	50	5.66	1.56	13.98
<i>Miniopterus schreibersii</i>	0	163	0.00		
<i>Myotis alcathoe</i>	0	4	0.00		
<i>Myotis bechsteinii</i>	1	38	2.56	0.13	11.60
<i>Myotis blythii</i>	1	35	2.78	0.14	12.51
<i>Myotis brandtii</i>	0	7	0.00		
<i>Myotis capaccinii</i>	0	15	0.00		
<i>Myotis crypticus</i>	0	3	0.00		
<i>Myotis dasycneme</i>	2	3	40.00	7.64	81.07
<i>Myotis daubentonii</i>	0	102	0.00		
<i>Myotis emarginatus</i>	4	59	6.35	2.20	13.94
<i>Myotis escaleraei</i>	0	23	0.00		
<i>Myotis myotis</i>	5	88	5.38	2.14	10.97
<i>Myotis mystacinus</i>	1	29	3.33	0.17	14.86
<i>Myotis nattereri</i>	0	13	0.00		
<i>Nyctalus lasiopterus</i>	24	210	10.26	7.17	14.12
<i>Nyctalus leisleri</i>	5	40	11.11	4.48	21.95
<i>Nyctalus noctula</i>	24	60	28.57	20.55	37.77
<i>Pipistrellus kuhlii</i>	22	208	9.57	6.56	13.38
<i>Pipistrellus nathusii</i>	2	13	13.33	2.42	36.34
<i>Pipistrellus pipistrellus</i>	10	85	10.53	5.82	17.20
<i>Pipistrellus pygmaeus</i>	30	268	10.07	7.34	13.41
<i>Plecotus auritus</i>	1	26	3.70	0.19	16.40
<i>Plecotus austriacus</i>	0	15	0.00		

<i>Rhinolophus euryale</i>	7	40	14.89	7.20	26.16
<i>Rhinolophus ferrumequinum</i>	9	95	8.65	4.59	14.62
<i>Rhinolophus hipposideros</i>	1	10	9.09	0.47	36.44
<i>Rhinolophus mehelyi</i>	0	1	0.00		
<i>Vespertilio murinus</i>	1	14	6.67	0.34	27.94

137

138 Data from Vidovszky *et al.* [26] and Sonntag *et al.* [25] included. Lower and upper limits of
139 95% confidence intervals of the percentage have been included.

140

141 The number of individuals sampled within species varied considerably and 5 species
142 (*Myotis daubentonii*, *Miniopterus schreibersii*, *Nyctalus lasiopterus*, *Pipistrellus kuhlii*, *P.*
143 *pygmaeus*), accounted for 50% of the total number of individuals sampled for this study. On the
144 other hand, the species *Eptesicus nilssonii*, *Myotis alcathoe*, *M. brandtii*, *M. crypticus*,
145 *Rhinolophus mehelyi* were represented by fewer than 10 individuals and were consequently
146 discarded from the proportion-based analyses of prevalence. Values of proportion of positive
147 samples varied considerably across species, averaging 7.02% (Table 1). The species targeted for
148 the individual-level analyses were: *Nyctalus lasiopterus* (10.26% prevalence), *Pipistrellus*
149 *pygmaeus* (10.07% prevalence) and *Pipistrellus kuhlii* (9.57% prevalence).

150

151 **Phylogenetic Signal**

152 The fully resolved phylogeny for all the studied species with proportional branch lengths
153 was used to estimate the different phylogenetic component in AdVs presence (S1 Fig). Fritz's *D*
154 parameter was 0.482, and we detected a marginally significant departure from Brownian motion
155 structure ($p = 0.058$), whereas the probability of *D* resulting from Brownian motion structure was
156 $p = 0.165$. The value of Pagel's λ when analyzing the distribution of proportion of AdVs across
157 the phylogeny was $\lambda = 0.99$ and highly significant ($p = 0.0003$) and Blomberg' *K* was $K = 0.74$ (p

158 =0.002). Thus, the distribution of the AdVs infection across the studied European bats showed a
159 strong phylogenetic component following Brownian motion.

160 **Trait association with AdVs prevalence**

161 The phylogenetic linear regression analysis showed that regardless of the phylogenetic
162 model used (although Brownian motion had the lowest AIC score, all three models tested were
163 within $\Delta\text{AIC} \leq 3$), the variable MATING STRATEGY showed a significant (and negative) effect
164 , as shown in Fig 2 (model=Brownian motion, $t = -2.8920$, $p = 0.0093$; all model results
165 presented in S1 Table). Moreover, the same result was recovered when using AdVs presence as a
166 binomial dependent variable (S2 Table), indicating a significantly higher AdVs presence in bat
167 species not engaging in swarming for mating.

168 At individual level and for the three species considered, the complete models were used
169 since no model was selected by AIC ($\Delta\text{AIC} < 3$). The GLMM analysis revealed a significantly
170 higher AdVs presence for males ($z = 2.067$, $p = 0.039$) in *N. lasiopterus* (Fig 3A), and a trend
171 towards higher AdVs prevalence for individuals with smaller forearm (Fig 3B) for *P. kuhlii* ($z = -$
172 1.656 , $p = 0.098$). The variance of the model explained by only the random variable was 4% for
173 *N. lasiopterus*, 62% for *P. pygmaeus* and 16% for *P. kuhlii*. Results for all the variables selected
174 are available in supplementary material S3 Table.

175 **Mantel test**

176 The Mantel tests showed no significant correlation for all pairwise comparisons between
177 geographic distances and differences in the proportion of AdVs presence across localities for *N.*
178 *lasiopterus* ($r = -0.3186$; $p = 0.941$) and *P. kuhlii* ($r = 0.1132$; $p = 0.113$) (S2 Fig), but this
179 correlation was significant for *P. pygmaeus* ($r = 0.3364$; $p = 0.036$) indicating that with greater
180 distance, there will be a greater difference in the proportion of individuals affected by AdVs (S2
181 Fig).

182

183 Discussion

184 For the first time to our knowledge, Adenovirus prevalence and its correlation with host
185 traits is studied across a wide range of bats both at the species and individual level. The
186 understanding of which and how host traits affect the presence of viruses in bats is a key step to
187 the understanding of the transmission mechanisms and evolutionary strategies of viruses. Such
188 mechanisms are key in the process of host switching that cause the appearance of emerging
189 diseases and therefore its understanding is important to improve disease managements [35]. Our
190 results show that Adenoviruses seem to be quite common in European bats since they were
191 found across all the studied tribes and in most genera (except for *Barbastella* and *Miniopterus*)
192 but their prevalence varied considerably among species [16]. The three most abundant species
193 showing the highest frequency of adenovirus infection (*Nyctalus lasiopterus*, *Pipistrellus*
194 *pygmaeus* and *P. kuhlii*) experienced an average of 9.97% prevalence. In contrast, other species
195 showed very low Adenovirus presence and was completely absent in others despite their large
196 sample sizes (e.g.: *Miniopterus schreibersii*) but in other species, the absence of positive results
197 could be due to the low number of sampled individuals (generally the rarest bats in Europe).

198 Fritz's D , Pagel's λ and Blomberg's K parameters all pointed to a strong phylogenetic
199 signal in the presence of AdVs across European bats and consequently, the need for accounting
200 for phylogenetic relationships in all subsequent models. This phylogenetic component of the
201 distribution pattern of AdVs in bats was also recently suggested by Iglesias-Caballero *et al.* [16]
202 as the sequences of new mastadenoviruses were clustering generally in agreement with the host
203 bat families or even with the bat species.

204 Contrary to a previous study on the factors influencing viruses on bats [32], we have not
205 found significant effect of the bats' group size on the presence of Adenoviruses for European
206 species. In their study, Webber *et al.* [32] had a wider perspective and focused on overall viral
207 richness in bats, whereas this study is centered exclusively on AdVs and so our differing results

208 may indicate that Adenoviruses use different transmission pathways than other viruses.
209 Transmission of respiratory AdVs in humans requires close contact although it can possibly
210 occur through droplet spray (such as those produced by coughing or sneezing) and/or aerosols,
211 but data are still limited [27–29]. In general, little is known about transmission mechanisms of
212 Adenoviruses and to our knowledge, no study has focused on this particular aspect across bats,
213 although the host species specificity found for most of the Adenoviruses in bats [16] points to
214 cross-species contacts as rare events.

215 Counterintuitively, species engaging in swarming behavior were found to have
216 significantly lower prevalence of Adenoviruses than other bats despite having theoretically
217 greater chances of contact. Swarming related to mating is shown mainly by forest species and is
218 described as the gathering of bats, commonly in caves and underground sites, during a few hours
219 after dusk and for a few days [36]. Two hypotheses could explain this finding. Firstly, bat
220 species engaging in swarming seem to be in contact for only a short time, which would imply a
221 lower chance of transmission compared to bats with polygynous mating systems, like harems,
222 which stay in contact for longer periods of time, sometimes all year around [37]. A second
223 plausible hypothesis is based on the fact that mammals with high infection risk, especially those
224 with promiscuous behavior, develop a stronger immune system compared to species with low
225 infection risk [38,39]. Thus, it seems reasonable that bats with swarming behavior may show
226 stronger immune response protecting them from infections, despite being very costly and even
227 represent a trade-off with other life-history traits [39].

228 When analyzing the effect on the AdVs presence of within-species variance in traits, we
229 found that the site of capture explains an important part of the variance of all models suggesting
230 an underlying general pattern across species and supporting the contact-rate hypothesis. This
231 trend is particularly clear for *Pipistrellus pygmaeus*, whose sampling localities are more
232 widespread along the Iberian Peninsula than those for *Nyctalus lasiopterus* and *P. kuhlii* (Fig 4).

233 Following the contact-rate hypothesis, bats from a specific locality interact with other individuals
234 of the same area, favoring the virus transmission locally [7]. A similar pattern was already found
235 for the viruses responsible for avian influenza, Marburg or Ebola [40–42]. In this direction, the
236 Mantel’s test supported a significant geographic component in the incidence of the Adenoviruses
237 for *Pipistrellus pygmaeus* which is typically sedentary species [34].

238 A higher prevalence of Adenoviruses was found in males for *Nyctalus lasiopterus*, the
239 species in which the different sexes were best represented. This result is particularly interesting
240 since it could affect the general dynamics of the virus given the generally predominant role
241 played by males in dispersal [43]. The possibility of a sex byes infection as a general pattern
242 should be explored further in other bat species and could be related to the trade-off between
243 immune function and reproduction, making males more susceptible to virus infections during
244 reproductive season due to the immune-suppressant effect of testosterone production [44].

245 Our results show that, individuals with smaller forearm are more likely to be infected by
246 Adenoviruses for *Pipistrellus kuhlii*. Subadult bats usually have slightly longer forearms [34] and
247 the lower prevalence in bats with larger measures could be explained by the influence of the
248 antibody loads transferred to them through their mother’s milk in still developing bats, making
249 them less suitable to Adenoviruses prevalence [45]. This is just a possibility and further studies
250 should help understand the relationships between Adenoviruses presence and age.

251 In sum, our findings highlight a common occurrence of Adenoviruses in European bats
252 and provide evidence of the importance of mating behavior in the prevalence of Adenoviruses,
253 contrary to what has been suggested generally for viruses. Besides, the three European bats
254 species that had higher Adenovirus prevalence do not show any common pattern, pointing to
255 Adenoviruses transmission as a complex process. Our study highlights the importance of
256 combining behavioral with ecological traits in explaining viral richness and transmission.

257

258 **Materials and Methods**

259 **Ethics Statement**

260 Non-lethal sampling was based on permits 201710730002961/IRM/MDCG/mes issued
261 by Dirección General de Gestión del Medio Natural y Espacios Protegidos (Consejería de Medio
262 Ambiente, Junta de Andalucía, Spain), 10/085545.9/17.9/17 issued by Consejería de Medio
263 Ambiente, Administración Local y Ordenación del Territorio, Comunidad de Madrid, and
264 PNSNG_SG_2018_0093 issued by Servicio Territorial de Medio Ambiente de Segovia, Junta de
265 Castilla y León. The techniques used meet the guidelines published by the American Society of
266 Mammalogists on the use of wild mammals in research [46]

267

268 **Data collection**

269 Most of the information used in this study was obtained during a surveillance program for
270 bat rhabdovirus and lyssavirus carried out between 2004 and 2008, 2016 and 2018. Bats were
271 mist-netted near roosts or over water and released in the same collecting point after sampling.
272 Each animal was identified, sexed, measured and weighed. Sampling consisted of obtaining a
273 membrane wing-punch, saliva with oropharyngeal swabs and stool samples (when this was
274 possible). Swabs were stored in 1.5 mL tubes filled with lysis buffer. All samples were aliquoted
275 and stored at -80 °C prior to analysis. Samples were screened for AdVs following published
276 protocols [16,47,48]. Our own database was completed with the published data of AdVs
277 presence in bats from Germany and Hungary [25,26]. The identification of bats belonging to
278 species complexes was confirmed through PCR amplification of a diagnostic mtDNA fragment
279 following Ibañez *et al.*, [49] and Kaňuch *et al.* [50].

280 **Phylogeny and phylogenetic signal**

281 A fully resolved phylogeny containing all the study species was not available from the
282 literature and is notoriously difficult to reconstruct for this group, [51] and so a hybrid approach
283 was taken whereby, published trees were grafted to obtain a full-solved topology whereas branch
284 lengths were estimated using molecular sequence data. The published phylogenetic studies for
285 the four families existing in Europe, used to manually construct a topology of all species in this
286 study were Guillén-Servent *et al.*, [52] for relationship in the family Rhinolophidae; Ruedi *et al.*,
287 [53] and Stadelmann *et al.* [54] for the subfamily Myotinae within Vespertilionidae; and Hofer
288 and Bussche [55] for the rest of this last family and for the family Miniopteridae. Complete
289 sequences of the mitochondrial genes *cytochrome b* (*CYTB*) and *NADH dehydrogenase 1* (*ND1*)
290 were obtained from GenBank for all available European species and were used to estimate
291 branch length in the constructed topology. This dataset needed to generate sequences *de novo* for
292 the species *Miniopterus schreibersii* for which these markers were not available. The new
293 sequences are deposited in GenBank under the accession numbers MK737740 and MK737741.
294 For each locus sequences were aligned with the software ‘ALTER’ from
295 <http://darwin.uvigo.es/software/> [56] and MEGA [57], and partitionfinder v2.1.1 [58] was run on
296 the concatenated alignment (1805 base pairs in length) to obtain the optimal partitioning scheme
297 and substitution models. Partitionfinder was set to use unlinked branch lengths, search only
298 BEAST models with the ‘greedy’ algorithm and AICc as the model selection criterion. A
299 maximum of six possible partitions were allowed; the three codon positions of the two loci.
300 BEAST v2.4.7 [59] was then fed the alignment and the fixed topology, and using a relaxed clock
301 log normal model with exponential priors on the mean (mean=10) and standard deviation
302 (mean=0.33), was allowed to estimate the branch lengths over 20 million MCMC iterations,
303 storing every 1000th. Chain diagnostics was performed using Tracer v1.7 [60] to ensure
304 sufficient mixing and parameter convergence and a maximum clade credibility tree was
305 generated using TreeAnnotator v.2.4.7 (part of BEAST package) using a 10% burn-in and
306 median node heights.

307 Fritz's D , Pagel's λ and Blomberg's K parameters were used to estimate the strength of
308 the phylogenetic signal affecting the pattern of AdVs presence across European bats. Fritz's D
309 was used for binary data (presence/absence) so that values close to one indicate that the
310 distribution of the binary trait is random with respect to the given phylogeny and values close to
311 zero indicate the trait is distributed as expected under a Brownian motion model of evolution
312 [61]. When the variable estimated was the proportion of infected bats in each species, Pagel's λ
313 and Blomberg's K parameters were used. Values of λ range from zero to one where $\lambda = 0$
314 indicates that related species do not share similar values for the trait (percentage of infection) and
315 $\lambda = 1$ indicates a pattern fully explained by the phylogenetic relationships under Brownian
316 motion, with related species showing similar values for the given trait. Finally, K is scaled so that
317 zero indicates no phylogenetic signal, $K=1$ is the expected value for trait evolution under
318 Brownian motion and values higher than one suggest stronger phylogenetic signal than predicted
319 by Brownian motion [62,63].

320

321 **Variables Selection and modelling**

322 Ecological and behavioural traits were selected considering their '*a priori*' importance for
323 the virus transmission and their availability for the European bat species from the bat literature
324 and general revisions [64], identification guides [34] (S4 Table). For the comparative analysis
325 across all European bats, the following variables were recorded for each species: (1) GROUP
326 SIZE, defined as the upper bound of individuals found usually in summer roosts; (2)
327 FOREARM: defined as the average range of forearm length (in mm) reported for each species;
328 (3) SOCIABILITY defined as the chance of sharing summer roost with other species and
329 considering just two categories: species that have never been found sharing their roost with other
330 species and species that sometimes or always share roost with other species; (4) MATING
331 STRATEGY: considered as whether mating takes place in seasonal swarming or not; (5)

332 MIGRATION: defined as whether the bat species is known to perform long-distance movements
333 (longer than 100 km), regional (between 10 and 100 km) or no movements; (6) ROOST TYPE:
334 considered in three categories: roosts in caves, trees or crevices. At the individual level, the
335 analyses focused on the three species that showed the highest AdVs presence combined with a
336 large enough sample size (> 200 individuals). The recorded variables were: (1) LOCALITY: site
337 of capture; (2) SEX: male or female; (3) FOREARM: Measured in mm.

338 For among-species comparisons, AdVs prevalence was analyzed as the arcsin-
339 transformed proportion of positive samples over the total number of samples for each species.
340 Species with fewer than 10 samples were eliminated from the matrix and from the phylogeny.
341 The analyses were also run considering presence/absence of AdVs as a dependent variable and
342 including in this case all sampled species. The analyses were performed with R statistical
343 computing packages [65] and scripts are available upon request. Fritz's D, Pagel's λ and
344 Blomberg's K parameters were estimated with the packages '*Caper*' [66] and '*Phytools*' [67].
345 Phylogenetic linear regressions were carried out with the package '*Phyloilm*' [68] that respects
346 the shared evolutionary histories of species [69]. Models of trait evolution were compared
347 assuming correlation structures under either Brownian motion, Ornstein-Uhlenbeck or Pagel
348 models [70] and the best model was selected based on the Akaike Information Criterion (AIC)
349 [71]. As a threshold value for model selection, a model showing $\Delta AIC > 3$ was taken as having
350 greater support [72]. Complementary, a binary phylogenetic generalized linear model was run
351 for each species, with presence of AdVs as the dependent variable and using the '*Ape*' package
352 [73].

353 Within species, we used general linear mixed models implemented in the '*Lme4*' package
354 [74] to test for associations between traits and presence of AdVs, and considering AdVs presence
355 as a binary dependent variable. In order to account for uncontrolled spatial variation,
356 LOCALITY was included in the models as a random variable. The best linear mixed model was

357 again selected based on AIC and same threshold criterion. As a confirmatory analysis for the
358 models, the variance explained by only the fixed variables was compared to the variance
359 obtained including the random variables using the package ‘*Mumin*’ [75]

360 At both among-species and within-species levels, inter-correlation was were checked
361 using a Pearson’s correlation test for continuous variables and an ANOVA for categorical
362 variables. The spatial distribution of the AdVs positive bats was inspected by performing a
363 Mantel test between the matrix of geographic distances and the matrix of differences in
364 proportion of infection between sites with the package ‘*Ade4*’ [76,77].

365

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367

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372

373

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375

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579

580

581 **Figure captions**

582

583 **Fig 1. Study sites in Spain where bats were screened for AdVs.** Blue dots indicate localities
584 with at least one AdVs positive samples, orange dots indicate localities with negative samples for
585 AdVs. (Modified from HansenBCN Miguillen - Own work, Public Domain,
586 <https://commons.wikimedia.org/w/index.php?curid=10577766>)

587 **Fig 2. Boxplot of the percentage of positive individuals for species engaging in swarming**
588 **and species not engaging in swarming.** 0 indicates species not using swarming as mating
589 strategy, 1 indicates species using swarming as mating strategy. ($N= 160$).

590 **Fig 3. Results of within-species analysis.** (A) Barplot by sex of number of individuals positive
591 (blue) and negative (red) to Adenovirus, for *Nyctalus lasiopterus*. (B) Boxplot for individuals
592 screening negative for AdVs (0) and positive for AdVs (1) in relation to forearm length for
593 *Pipistrellus kuhlii*.

594 **Fig 4. Sampling localities with percentage of AdVs positive samples.** Portion of AdVs
595 positive sample is indicated in blue, AdVs negative samples in orange for (A) *Nyctalus*
596 *lasiopterus*, (B) *Pipistrellus pygmaeus* (C) *Pipistrellus kuhlii*. Only localities with at least 5
597 recorded individuals are shown. (Modified from HansenBCN Miguillen - Own work, Public
598 Domain, <https://commons.wikimedia.org/w/index.php?curid=10577766>)

599

600 **Supporting information**

601 **S1 Table. Result of the percentage models.** Models under Brownian motion, Pagel and
602 Ornstein-Uhlenbeck structures.

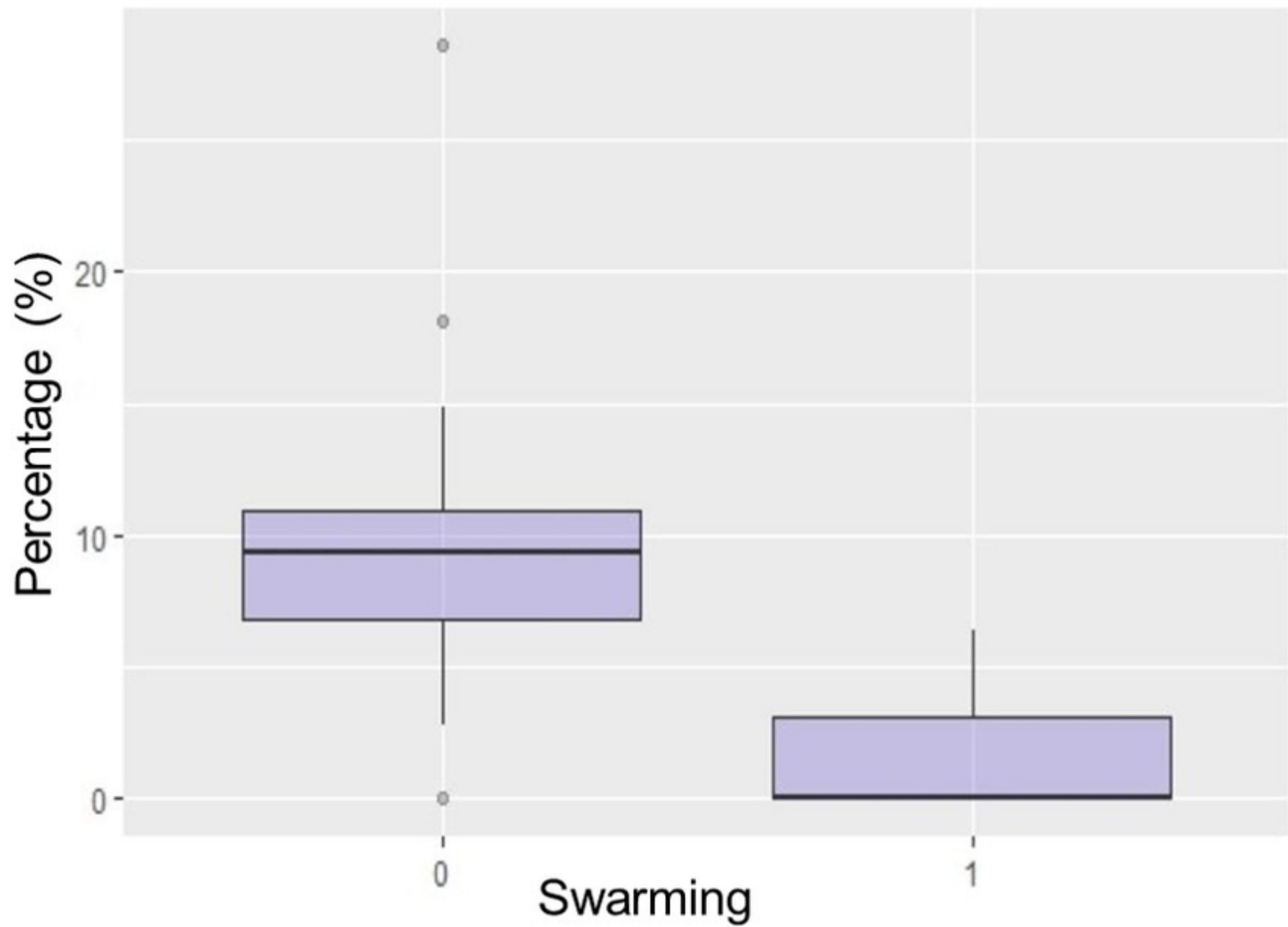
603 **S2 Table. Results of absence/presence model.** Estimate value, standard error, Z-score and p-
604 value included.

605 **S3 Table. Result of GLMM model (individual level) for each species taken into account.** A:
606 *Nyctalus lasiopterus*. B: *Pipistrellus pygmaeus*. C: *Pipistrellus kuhlii*.

607 **S4 Table. Traits used in the model for each species.** Information taken from: 1: Dietz *et*
608 *al.*[34], 2: Action Plan for the Conservation of All Bat Species in the European Union [64].
609 Values indicated with asterisk have been obtained by personal communication of Jens Rydell
610 and Jesús Nogueras Montiel.

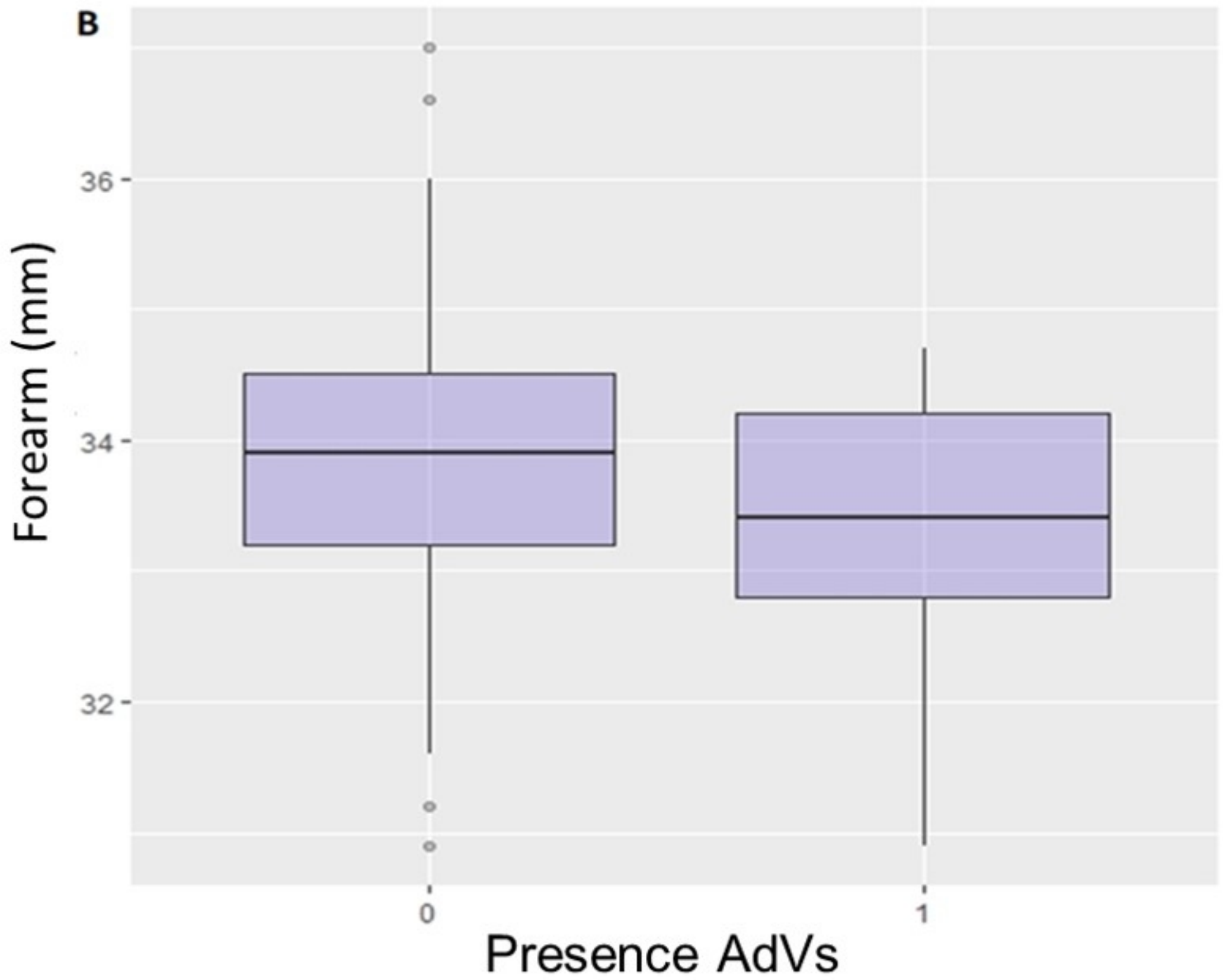
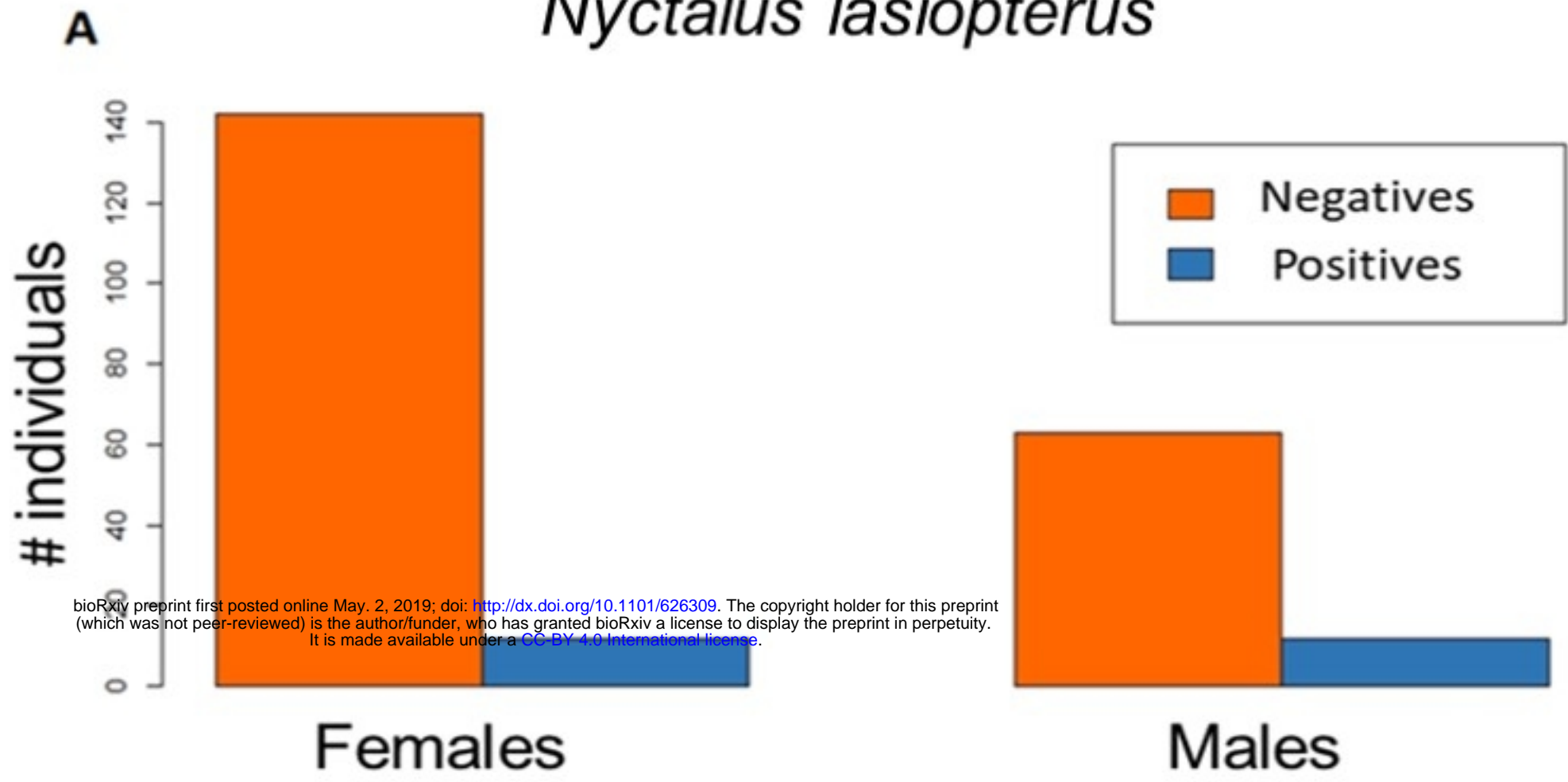
611 **S1 Fig. Evolutionary relationships of bat species considered in this study.** Based on the
612 phylogenetic hypotheses of: Guillén-Servent *et al* [52].; Hooper and Bussche [55]; Ruedi *et al.* [53]
613 and Stadelmann *et al.* [54]. Branch lengths reflect mitochondrial sequence divergences. Tip
614 labels indicate species screening positive (black) or negative (white) for AdVs.

615 **S2 Figure. Scatterplot showing the results of the Mantel test.** Mantel test between the matrix
616 of differences in percentages of AdVs presence and the matrix of geographic distances for (A)
617 *Nyctalus lasiopterus* (B) *Pipistrellus pygmaeus* and (C) *P. kuhlii*.



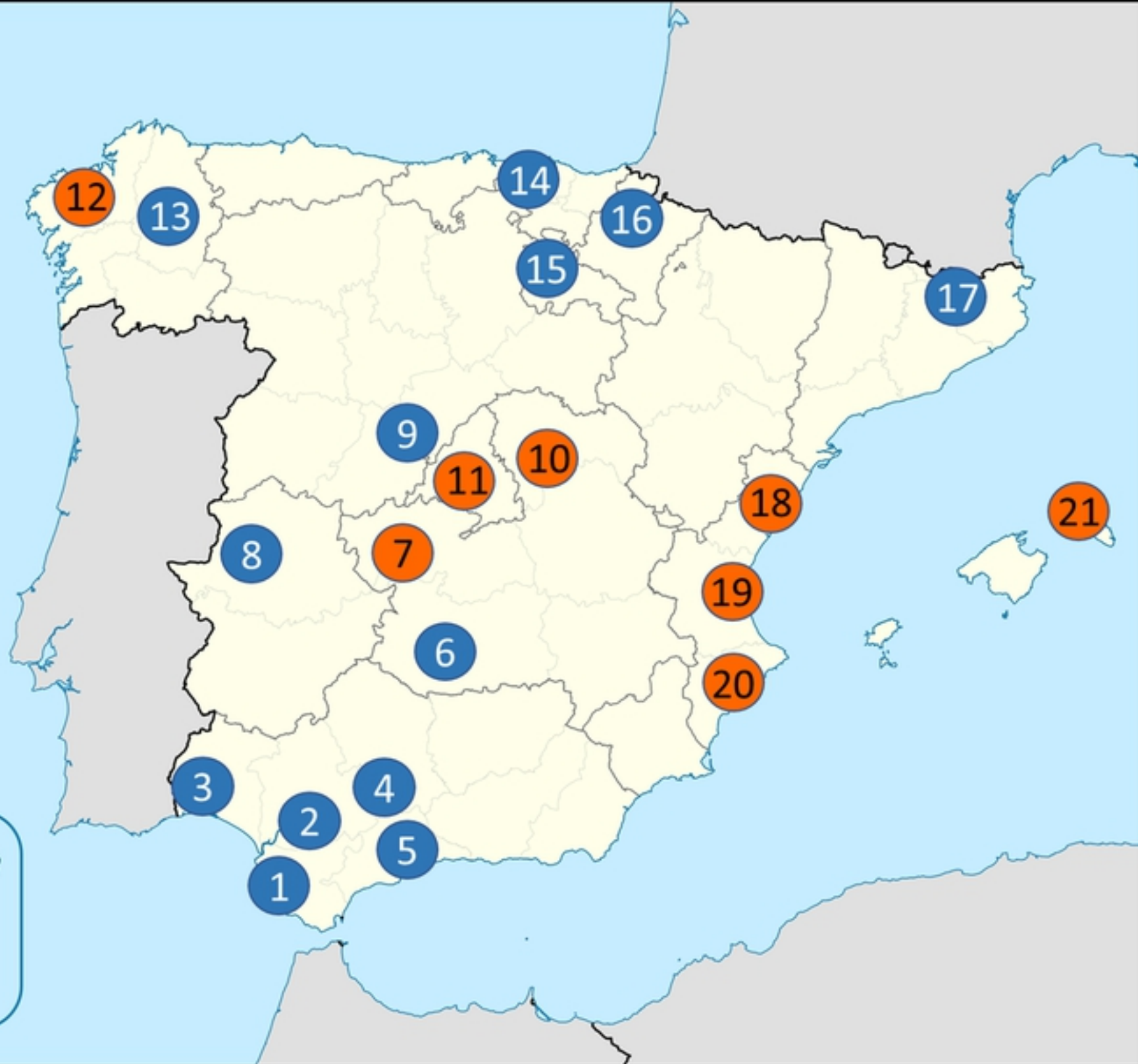
Figure

Nyctalus lasiopterus

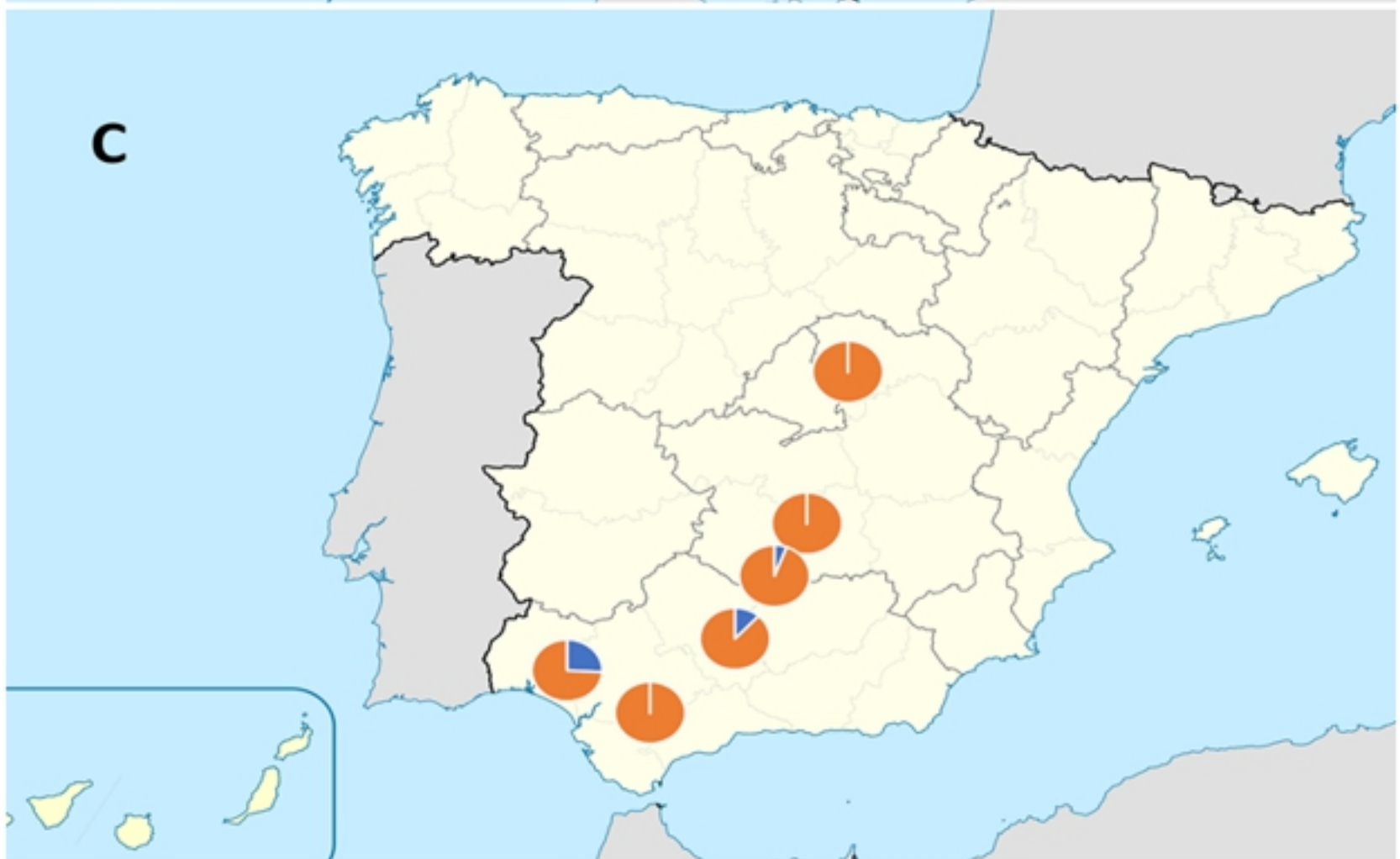
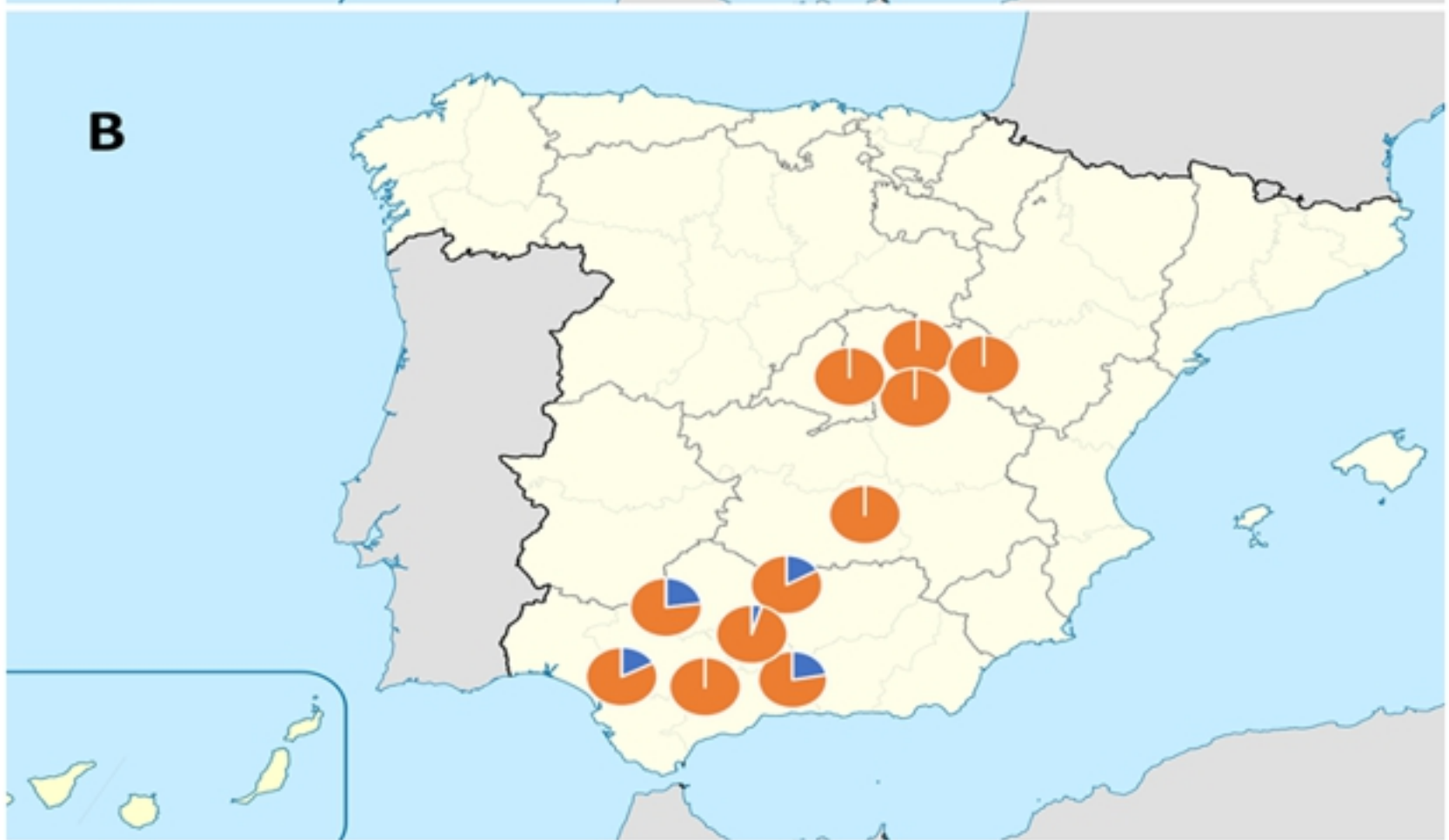
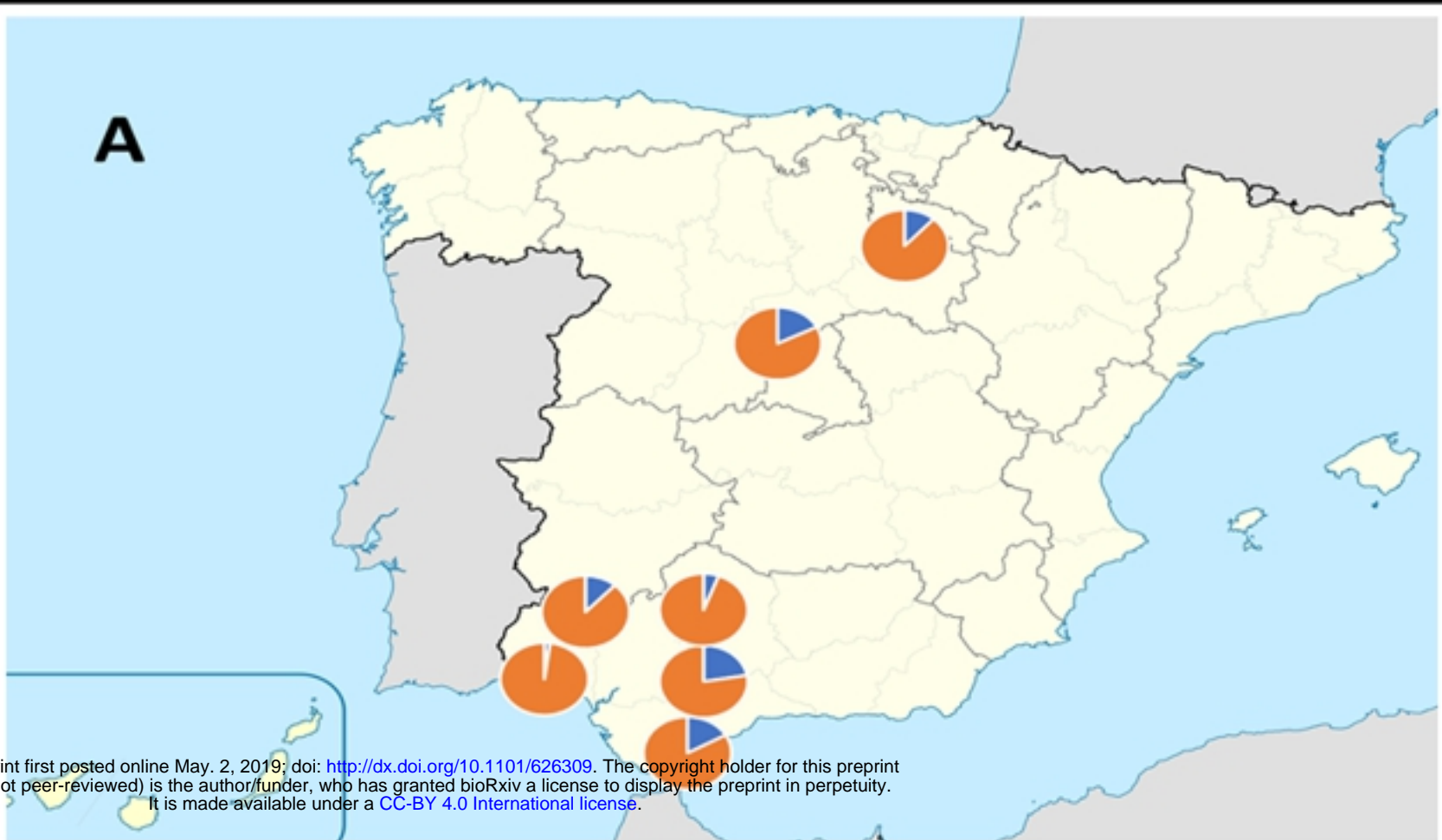


Figure

	Locality
1	Cádiz
2	Seville
3	Huelva
4	Córdoba
5	Málaga
6	Ciudad Real
7	Toledo
8	Cáceres
9	Segovia
10	Guadalajara
11	Madrid
12	A Coruña
13	Lugo
14	Biscay
15	La Rioja
16	Navarra
17	Gerona
18	Castellón
19	Valencia
20	Alicante
21	Menorca



Figure



Figure