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**The legacy of translocations among populations of the Ibizan Wall Lizard, *Podarcis pityusensis* (Squamata: Lacertidae)**

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### Article

**Citation** (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

**Brown, RP, Perez-Mellado, V and Ramon, MM (2017) The legacy of translocations among populations of the Ibizan Wall Lizard, *Podarcis pityusensis* (Squamata: Lacertidae). *Biological Journal of the Linnean Society*. 121 (1). pp. 82-94. ISSN 0024-4066**

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1 **Full title: The legacy of translocations among populations of the Ibizan Wall Lizard,**  
2 ***Podarcis pityusensis* (Squamata: Lacertidae)**

3

4 Short title: Translocation among populations of *Podarcis pityusensis*

5

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23

24 Abstract

25 The Ibizan wall lizard, *Podarcis pityusensis*, was the subject of several documented  
26 translocations by the German herpetologist Martin Eisentraut, in 1930. He aimed to initiate  
27 long-term experiments into the evolution of melanism and other morphological traits and  
28 accordingly reported introductions into five islets that (he believed) contained no lizards. In  
29 this study, we analysed the genetics and morphological characteristics of individuals we found  
30 there. We found no lizards on two of the islets, namely Escull de Tramuntana or Galera, but  
31 for the first time, detected a large population on a third, Es Vaixell. Analyses of microsatellite  
32 DNA placed individuals from a fourth islet, Dau Gran, with those from the islet of Escull  
33 Vermell. They are also morphologically close to individuals from Escull Vermell. This  
34 suggests that selection pressures could have favoured the Escull Vermell phenotype following  
35 introduction. Eisentraut founded the Es Vaixell population with non- melanic Ibizan  
36 specimens, but the present day population of Es Vaixell was found to be fully melanic. Genetic  
37 markers support a strong similarity between Es Vaixell and Na Gorra, and indicate that, in all  
38 likelihood, the individuals introduced by Eisentraut have left no descendants. It is likely that  
39 Es Vaixell already contained lizards prior to this introduction. For reasons which we discuss,  
40 the translocations have revealed less than Eisentraut would have originally hoped for, although  
41 they do provide some potential insights into lizard morphological evolution following  
42 colonization.

43 Key-words: Balearic Islands - Ibiza - microsatellites - mtDNA

44

45 **Introduction**

46 Evolutionary changes can occur over months or decades in the wild (Kinnison, Unwin  
47 & Quinn, 2008). Unfortunately, there are a lack of empirical data on contemporary adaptive  
48 evolution. In general, morphological traits appear to be able to undergo rapid short-term  
49 changes after a population is exposed to new conditions (Kinnison & Hendry, 2001 for a  
50 review). These rapid evolutionary responses have been demonstrated in several organisms,  
51 from fish (Stockwell & Weeks, 1999) to mammals (Williams & Moore, 1989). Some key  
52 studies have also addressed this topic in reptiles (Kolbe *et al.*, 2008; Kolbe *et al.*, 2012;  
53 Thorpe, Reardon & Malhotra, 2005).

54 Field translocation experiments are employed as a useful tool to test predictions on fast  
55 evolutionary responses and directional selection intensity (Thorpe, Reardon & Malhotra, 2005  
56 and references therein). In this study, instead of designing an experimental procedure to test  
57 some hypotheses, we analysed the results of experimental translocations made eighty-six  
58 years ago by the German herpetologist Martin Eisentraut (Eisentraut, 1949). Several voluntary  
59 or involuntary translocations among insular populations of lizards have been made in Balearic  
60 Islands over the years (Pérez-Mellado, 2009), but those made by Eisentraut were especially  
61 well documented and underpinned by a scientific objective which was to investigate the  
62 evolution of melanism and other morphological traits.

63 Eisentraut (1949) aimed to introduce individuals into islets that contained no lizards,  
64 but, unfortunately, it appears that some of these did in fact contain lizard populations. He was  
65 particularly interested in the adaptive values of these morphological characteristics for island  
66 lizards (Böhme, 2004). His first hypothesis was that melanism is selectively neutral and an  
67 indirect consequence of physiological changes related with dietary shifts in some insular  
68 populations (Eisentraut, 1949). It appears that Eisentraut was also interested in the heritability  
69 of these morphological characteristics and investigated this by introducing males and females  
70 from respective populations with very different morphologies into a new location. It should  
71 be pointed out that the experimental translocations were based on scientific knowledge in the  
72 1930s and so Eisentraut's rationale can only be understood within this context (Böhme,  
73 2004).

74 The model species employed by Eisentraut (1949) was *Podarcis pityusensis* (Boscá,  
75 1883), a lacertid lizard from the western group of Balearic Islands, known as the Pityusic  
76 archipelago. It inhabits the main islands of Ibiza and Formentera, along with 42 of their  
77 associated islets (Pérez-Mellado, 2009), where it shows considerable phenotypic variation  
78 among populations, in terms of size, sexual dimorphism, scalation and coloration (including

79 melanic populations). This has led to the recognition of 23 subspecies (Pérez-Mellado, 2009;  
80 Salvador & Pleguezuelos, 2002). We recently carried out a detailed analysis of its genetic  
81 diversity and historical biogeography and identified two main genetic groupings with some  
82 evidence of recent introgression between them (Rodríguez *et al.* 2013).

83 The five documented translocations (Eisentraut, 1930, 1949; Salvador, 1984) were as  
84 follows (the sex of the lizards was not documented unless stated below):

85 i) Eight melanic adult males from Escull Vermell (a small island off Western coast of Ibiza  
86 Island, Fig. 1) and 20 non-melanic adult females from Ibiza itself were introduced into the  
87 islet of Dau Gran (a small islet, located approximately 1.5 km from the Ibizan coast, Fig. 1).

88 ii) Non-melanic individuals (n= 24) were introduced from Ibiza into Escull de Tramuntana, (a  
89 small islet close to Escull Vermell, Fig. 1).

90 iii) Twenty non-melanic individuals were introduced from Ibiza into Galera, (a very small  
91 islet close to the northern coast of Vedra Island, Fig. 1).

92 iv) Fifty melanic lizards from Bleda Plana (a small island off western coast of Ibiza, Fig. 1)  
93 were introduced into Negra Llevant (an islet near Ibiza harbour, Fig. 1).

94 v) A similar number (n= 51) of non-melanic lizards from Ibiza Island were released in Es  
95 Vaixell, (a small islet close to Na Gorra Islet, Fig. 1).

96 Herpetologists made timely visits and observations in the years following these  
97 translocations. Lizards were observed during some visits to Dau Gran (experiment i) and  
98 Negra Llevant (experiment iv), although other visits seem to have not recorded lizards as being  
99 present. Lizards observed in Dau Gran were reported to have features from both founding  
100 populations. More specifically, their body dimensions and scalation were similar to melanic  
101 lizards from Escull Vermell, and their coloration/colour pattern was intermediate between  
102 Escull Vermell and Ibiza populations (non melanic) (Mayol, 2004). Böhme and Eisentraut  
103 (1981) also reported on the results of the Dau Gran translocation, reaching the conclusion that  
104 hybrids on the islet were larger than parental lizards, with increased variability in this new  
105 population compared with both parental populations. It appears that the present day population  
106 of Dau Gran is derived from the descendants of lizards introduced by Eisentraut in 1930, even  
107 if some author maintain that lizards from 1930 were extinct well before 1962, and that Dau  
108 Gran was later occupied by a more recent introduction in the early 1980s (Cirer, 1987). A very  
109 small population of Negra Llevant (experiment iv) were first recorded in 1979 (Cirer, 1987).  
110 Whether or not these were descendants of Eisentraut's introductions is unknown although  
111 fishermen who had previously visited the island reported not having observed lizards there  
112 prior to this (Cirer, 1987).

113 The islets of Escull de Tramuntana (experiment ii), Galera (experiment iii) and Es  
114 Vaixell (experiment v) were visited during 1979 and 1980 as well as during 1962 and 1985  
115 by J.P. Martínez-Rica (Cirer, 1987), but no lizards were observed in any of these islets.  
116 However, we have recently observed a population of large melanic individuals on Es Vaixell,  
117 which in part provided the motivation for the present analysis.

118 In summary, two of Eisentraut's introductions (Escull de Tramuntana and Galera)  
119 appear to have been unsuccessful (these islets may no longer contain lizards), but there are  
120 current lizard populations on Es Vaixell, Dau Gran and Negra Llevant islets, which may  
121 contain descendants of the introduced individuals. Here, we attempted to evaluate the results  
122 of Eisentraut's translocations by investigating the existence of lizard populations on these five  
123 islets and analysing the genetic and morphological characteristics of the extant populations  
124 that we found.

125

## 126 **Material and methods**

### 127 *Specimens and populations*

128 All lizards were captured by noosing and released after study at the site of capture. Tissue  
129 samples (tail tips) were obtained from 11 specimens from the islets that were subject to  
130 translocations: Es Vaixell, Dau Gran and Negra Llevant (2–5 from each). In addition, in the  
131 genetic analyses we included twenty individuals from Ibiza Main Island and ten islands/islets  
132 (Bleda Plana, Na Bosc, Escull Vermell, Na Gorra, Espartar, Escull d'Espartar, Espardell de  
133 s'Espartar, Bosc, Conillera and Ses Rates) from the western Pityusic archipelago, that is, from  
134 closely related populations to those under study (Rodríguez *et al.*, 2013, see Fig. 1 for exact  
135 location and Table 1).

136 Island/islet ecological characteristics were broadly described in terms of exposed  
137 areas, maximum altitudes and the number of species of vascular plants found there (Rivas,  
138 Costa & Loidi, 1992 and unpublished data, see Table 2).

139

### 140 *DNA isolation and amplification*

141 Total DNA was extracted using a standard phenol-chloroform protocol.

#### 142 *i) Microsatellites*

143 Fifteen microsatellite loci, isolated from the closely-related *Podarcis lilfordi* (Bloor *et al.*,  
144 2011), were genotyped in individuals from introduced populations. Primers, genotyping  
145 and allele assignment were the same as described for *P. lilfordi* in Bloor *et al.* (2011) and

146 previously applied to *P. pityusensis* by Rodríguez *et al.* (2013). Previous genotypes  
147 established in *P. pityusensis* (Dryad Digital Repository. doi: 10.5061/dryad.r1538) for  
148 Ibiza and surrounding islands were included in the analyses (Rodríguez *et al.*, 2013).

149 *ii) Mitochondrial data*

150 Five mtDNA fragments for each specimen were amplified and sequenced. The partial  
151 genes analysed were: 12S rRNA, cytochrome *b* (two regions), part of the control region  
152 and a fragment that included part of the ND1 gene, three tRNA genes (tRNA<sub>Ile</sub>, tRNA<sub>Gln</sub>,  
153 and tRNA<sub>Met</sub>) and part of the ND2 gene (ND). Primers, amplification and sequenced  
154 conditions are the same as those used for *P. lilfordi* (Terrasa *et al.*, 2009) and *P.*  
155 *pityusensis* (Rodríguez *et al.*, 2013). Both heavy and light strands were aligned and  
156 visually checked using BioEdit ver.7.0.5.2 (Hall, 1999).

157 Previous sequences obtained for *P. pityusensis* (GenBank Accessions nos:  
158 EF694768, EF694794, JX852049–1, JX852053, JX852056–8, JX852063–7, JX852069–  
159 0, JX852076, JX852090–1, JX852093–4, JX852099, JX852101, JX852108, JX852118,  
160 JX852121–3, JX852125–6 and JX852130–1) and two *P. lilfordi* (GenBank Accessions  
161 nos: EF 694761, EF 694766, EF 694773, EF 694787, EF 694799, EF 694810, EF 990546,  
162 EU 006730 and EU 006756) were included in the analyses.

163  
164 *Microsatellite analysis*

165 The number of genetically distinct clusters was estimated using STRUCTURE v.2.3.3  
166 (Pritchard, Stephens & Donnelly, 2000) and STRUCTURE HARVESTER v.0.6.94 (Earl  
167 & vonHoldt, 2012). STRUCTURE was run twenty times using a model that incorporates  
168 admixture and correlated allele frequencies among populations, with each run comprising  
169 400000 steps (200000 discarded as burn-in), for all values of K from 1–16. The programs  
170 STRUCTURE and CLUMPP v.1.1.2 (Jakobsson & Rosenberg, 2007) were used to  
171 determine the estimated membership coefficient (*Q*), and assign individuals to  
172 populations. We applied a threshold value of 0.20, as this provides a great efficiency and  
173 accuracy to differentiate between purebreds and hybrids (Vähä & Primer, 2006), so *Q*  
174 values around 0.2 and 0.8 may indicate hybridization between individuals from different  
175 clusters.

176  
177 *Mitochondrial analysis*

178 Haplotypes were identified for the concatenated sequences and a haplotype network  
179 constructed using the program TCS v. 1.21 (Clement, Posada & Crandall, 2000). TCS creates

180 a network using statistical parsimony (Templeton, Crandall & Sing, 1992; Templeton & Sing,  
181 1993) that outputs the 95% plausible set of most parsimonious linkages among sequences.  
182 Bayesian inference of population structure was determined using BAPS v.5.3 (Corander,  
183 Waldmann & Sillanpää, 2003), with an upper bound of  $K=20$ , and without prior information  
184 on geographic location.

185 An alternative phylogenetic analysis was used for comparison with the parsimony  
186 network. The mtDNA tree was obtained using Bayesian inference (BEAST v.1.8.)  
187 (Drummond *et al.*, 2012), with model selection (test AICc) determined by jModelTest v.0.1.1  
188 (Nylander *et al.*, 2004). A lognormal relaxed clock model was used and a coalescent constant  
189 size, tree prior was specified. Two individuals of the sister species *P. lilfordi* from Dragonera  
190 and Aire islands were included as outgroups. Bayesian MCMC analyses were conducted with  
191 random starting trees, run for 50 million generations, and sampled every 1000 generations.  
192 Samples were analysed with Tracer v. 1.5 (Rambaut & Drummond, 2007) and TreeAnnotator  
193 (BEAST package) was used to combine and analyse the trees (trees were combined using the  
194 maximum sum of clade credibilities criterion).

195

#### 196 *Morphometric characters and analysis*

197 The morphologies of 874 captured/museum specimens were also studied from  
198 photographs or measurements of live and preserved specimens (Table 1). All body  
199 dimensions were measured in the field, while scalation characters were recorded in the  
200 field or from digital images taken from each specimen. Additional specimens were  
201 studied from the Herpetological collections of the Alexander Koenig Museum of Natural  
202 History (Bonn, Germany), the Natural History Museum of London (UK), the Animal  
203 Biology Department of the University of Salamanca (Spain), the Zoology Department of  
204 the University of Barcelona (Spain) and the Herpetological Collection of the Natural  
205 History Museum of Madrid (Spain). Six body dimension characters were studied: snout-  
206 vent length (SVL), pileus length (PL), pileus width (PW), head height (HH), intact tail  
207 length (TL) and hind leg length (HLL). All measurements were made with a digital  
208 calliper to the nearest 0.01 mm, with the exception of SVL which was measured with a  
209 steel rule to the nearest 1 mm. Six scalation characters were studied: gularia (GUL),  
210 collaria (COL), dorsalia (DOR), ventralia (VEN), left femoralia (FEM) and left 4<sup>th</sup> digit  
211 lamellae (LAM). Not all characters could be recorded from all individuals (Supporting  
212 Information, Tables S1-S3).

213 Males and females were analysed separately, due to sexual dimorphism. Raw  
214 values were log-transformed for all characters to improve the fit to normality. We  
215 employed a Non-Metric Multidimensional Scaling (NMDS) to establish morphological  
216 divergence among 14 populations of *P. pityusensis*. The main advantage of NMDS is its  
217 suitability for nonlinear metric and even ordinal data. The method aims to depict the  
218 inherent pattern of a dissimilarity matrix in a geometric picture with a minimum number  
219 of dimensions while maintaining a close agreement with the initial data matrix (Clover,  
220 1979). The dissimilarity matrices of Euclidean distances were constructed using the  
221 means of the log-transformed values of twelve morphometric characters (see above). We  
222 used the metaMDS function from the vegan R-package (Oksanen *et al.*, 2015). This  
223 function runs NMDS several times from random starting configurations, compares results  
224 and stops after detecting two similar minimum stress solutions. Finally, it scales and  
225 rotates the solution (Oksanen *et al.*, 2015). To assess the appropriateness of NMDS results  
226 we used a Shepard diagram to visualize the distances among populations in the ordination  
227 plot relative to the original distances. In addition, the goodness-of-fit of the ordination  
228 was assessed by the coefficients of determination ( $R^2$ ) for the linear and non-linear  
229 regressions of the NMDS distances on the original ones (Borcard, Gillet & Legendre,  
230 2011). Finally, we recorded the stress values of NMDS (Zuur, Ieno & Smith, 2007).

231

## 232 **Results**

### 233 *Island/islet ecology*

234 Ecological details are summarized in Table 2. Ibiza is a large and inhabited island and has the  
235 highest ecological complexity within the Pityusic archipelago in terms of microhabitat  
236 diversity, vegetation cover and predation pressure. Vegetation was extremely poor in all of  
237 the islets used for translocations by Eisentraut, even in comparison with islets next to the  
238 Western Ibizan coast (Table 2). The vegetation is comprised of only a few vascular plants that  
239 are strongly adapted to the extreme conditions of these isolated islets. No terrestrial predators  
240 are present in these islets (pers. obs.) while in Ibiza Island feral cats, genets and other lizard  
241 predators are common.

242

### 243 *Dau Gran (experiment i)*

244 A substantial lizard population was found on Dau Gran. Lizards from this population showed  
245 partial melanism, with dorsal colours being dark green and black. In almost half of adult  
246 individuals, we observed narrow dorsolateral lines, continuous or segmented. Dorsolateral

247 lines can be dull golden or greenish. Ventrally, lizards are dark blue with green nuances. In  
248 some males, we observed rounded signal blue ocelli in outer ventral scales. The throat was  
249 profusely reticulated with signal black spots on a greenish or bluish background. Black spots  
250 are more or less aligned on submaxillary scales.

251

252 *Escull de Tramuntana and Galera (experiments ii and iii)*

253 We visited Escull de Tramuntana and Galera islets on spring 2013. Lizards were absent from  
254 both islets. Thus, we confirm previous reports (Cirer, 1987) and conclude that both of  
255 Eisentraut's introductions failed (ii and iii), probably because of a lack of trophic resources  
256 and suitable refuges (Table 2).

257

258 *Negra Llevant (experiment iv)*

259 We compared Negra Llevant lizards with the closest lizards from adjacent sites on Ibiza Island  
260 and lizards from Bleda Plana (the source of Eisentraut's translocated individuals). We found  
261 that adult males from Negra Llevant were significantly larger than those from Ibiza, but  
262 similar in size to those from Bleda Plana (one-way ANOVA of adult male SVL from the three  
263 populations:  $F_{2, 198} = 35.64$ ,  $p = 6.04 \times 10^{-14}$ , see also Table S2). However, lizards from Negra  
264 Llevant showed quite similar patterns to those observed in several lizards from Ibiza Island  
265 and were completely different from the melanic lizards from Bleda Plana. The dorsal area of  
266 males and females were mostly green, with irregular black spots longitudinally arranged in a  
267 vertebral and two dorsolateral stripes. Flanks were greenish or brownish, spotted with black.  
268 Ventrally, lizards had bone-white or cream hues, in several cases with dark brown nuances  
269 and light blue eyespots on outer ventral scales.

270

271 *Es Vaixell (experiment v)*

272 Our first visit to Es Vaixell was in 2010 when we made the first observation of lizards on this  
273 islet. (This was followed by visits in 2013 and 2014). Relatively high population density was  
274 detected (allowing us to study over 65 individuals)..

275 Males and females were melanic, but we observed a light yellowish or dull golden  
276 nuance in the back, clearly contrasted with bluish flanks in 62.5% of individuals (instead of a  
277 uniform very dark blue or fully black colour). We did not observe any greenish dorsal  
278 coloration, as is common in lizards from neighbouring populations, such as those from Na  
279 Bosc islet. In almost all individuals, we observed narrow dorsolateral lines, continuous or  
280 segmented. Dorsolateral lines can be dull bluish or even yellowish or dull golden. In a few

281 individuals we even observed reddish nuances in the anterior third of dorsolateral lines. Flanks  
282 are blackish spotted with cobalt blue rounded points. Ventrally, lizards are ultramarine blue  
283 with a large extension of areas of black blue, particularly, at the upper corners of ventral scales.  
284 Rounded signal blue ocelli in outer ventral scales were seen in some males, instead of greenish  
285 ocelli present in many lizards from Na Bosc and Na Gorra. The throat was profusely  
286 reticulated with signal black spots on an ultramarine blue background. The black colour of the  
287 throat is more intense and with better defined spots than in Na Gorra lizards. In addition, black  
288 spots are more or less aligned on submaxillary scales in lizards from Es Vaixell, but not in  
289 lizards from Na Gorra and Na Bosc.

290

#### 291 *Microsatellite DNA*

292 Only nine individuals (five from Es Vaixell, and two from Negra Llevant and Dau Gran) were  
293 genotyped. The results, together with those from other studied populations (Rodríguez *et al.*,  
294 2013), are shown in Table S3. In general, alleles are shared with other Ibizan populations,  
295 with some exceptions. The following private alleles were detected: allele 167 (Pli18) in both  
296 Dau Gran specimens and alleles 352, 404 (Pli4), 175 (Pli18) and 137 (Pli22) in some Es  
297 Vaixell individuals. Two alleles, 159 (Pli8) and 295 (Pli10) are only shared between Na Gorra  
298 and Es Vaixell, and the allele 246 (Pli12) is common between Es Vaixell and one specimen  
299 from Ibiza.

300 Two clusters were detected using the program Structure ( $\Delta K$  34.006) (Fig. 2A): the  
301 first (Cluster I) included samples from the introduced population of Es Vaixell and the second  
302 (Cluster II) contained the remaining samples, comprising lizards from Ibiza main island and  
303 surrounding islands, as well as the introduced populations of Dau Gran and Negra Llevant. In  
304 our analyses, we found one admixed individual from Na Gorra that presents a slightly lower  
305 membership coefficient ( $Q = 0.725$  to cluster II,  $Q = 0.275$  to cluster I, with samples from Es  
306 Vaixell) ( $Q$  values around 0.2–0.8 are indicative of hybridization between individuals from  
307 different clusters).

308

#### 309 *Mitochondrial DNA*

310 The five mitochondrial fragments provide a total concatenated fragment length of 2383 bp  
311 (cytochrome b, 831 bp; 12S rRNA, 373 bp; ND1, 59 bp; ND2, 415 bp; tRNAs, 211 bp;  
312 control region 481 bp). New sequences have been deposited in GenBank (accession numbers  
313 KT002167–79). Sequences obtained from our previous studies were also used: GenBank

314 accession numbers EF694768, EF694794, JX852058, JX852069–70, JX852076, JX852091,  
315 JX852093–4, JX852101, JX852121–3, JX852130–1.

316 The BAPS analysis defined three clusters (lnL= -757.5806, best visited partition) (Fig.  
317 2B). The first cluster (A) includes samples from Ibiza, Na Gorra, Na Bosc, Bleda Plana, Es  
318 Vaixell, and one specimen from Dau Gran (Dg2). The second cluster (B) contains specimens  
319 from Bosc, Conillera, Ses Rates, Espartar, Espardell de s'Espartar, Escull d'Espartar, Na Bosc,  
320 Negra Llevant, Dau Gran, and one Ibiza sample. Specimens from Escull Vermell, Bleda Plana  
321 and Na Bosc constitute the third cluster (C). Hence, individuals from Ibiza Island (Clusters A,  
322 B), Na Bosc (Clusters A, B and C), Dau Gran (Clusters A and B) and Bleda Plana (Clusters  
323 A and C) are included in different clusters.

324 The TCS network (Fig. 3) revealed two main groups. The samples from Es Vaixell  
325 Island are found in one group only 0-2 mutational steps away from Na Gorra individuals. Two  
326 maternal haplotypes have been observed on Es Vaixell: one of them (present in four  
327 individuals) is shared with a specimen from Na Gorra, and the second one is separated by a  
328 unique mutational step from the first. Only 2 mutational steps or less separate the two  
329 remaining haplotypes from Na Gorra. MtDNA haplotypes from Dau Gran specimens are not  
330 shared by other insular populations, and evidence the existence of two highly differentiated  
331 lineages (>15 mutational steps); one of them (samples Dg1 and Dg3) is similar to Ses Rates  
332 and Na Bosc haplotypes, and the other (Dg2) is close to Ibiza main island specimens and Es  
333 Vaixell-Na Gorra haplotypes. The three mtDNA haplotypes from Negra Llevant are unique  
334 and are separated by three mutational steps from Espartar and by five from one specimen from  
335 Ibiza main island or a Na Bosc individual.

336 Finally, the Bayesian tree (Fig. 4) indicated two well-supported major clades. One of  
337 these clades contains Es Vaixell, Na Gorra, Ibiza Island, and some individuals from Dau Gran,  
338 Bleda Plana and Na Bosc. The second clade comprised the remaining individuals including  
339 individuals from Negra Llevant (which is close to individuals from Espartar). Although some  
340 Dau Gran individuals carry mtDNA that is very similar to that in Ibiza Island (Clade A), other  
341 individuals show similarities with Bosc and Ses Rates (Clade B). In contrast, specimens from  
342 Negra Llevant and Es Vaixell are only found in one clade.

343 *Morphometric characteristics*

344 In the case of non-metric multidimensional scaling, stress values in males and females  
345 (Fig. 5 and Fig. 6) were smaller than 0.05, indicating that both configurations were excellent  
346 and allowed for more detailed inspection (Zuur *et al.*, 2007). The ordination of males (Fig. 5)  
347 and females (Fig. 5) was similar. Ibiza Island and the most remote populations to the west  
348 show the two most extreme (opposing) scores for the first NMDS coordinate, with islet  
349 populations closest to the coast of Ibiza showing intermediate values. In other words, Es  
350 Vaixell and Ibiza Island are found to be morphologically divergent. Negra Llevant was  
351 morphologically divergent from remaining populations for both males and females, including  
352 Bleda Plana. Finally, in the two-dimensional morphological space created by MDS analyses,  
353 Dau Gran is similar to Escull Vermell (the origin of the introduced males) and distant from  
354 Ibiza Island populations (the origin of introduced females) (Tables S2, S3).

355

356

## 357 **Discussion**

358 Negra Llevant individuals are genetically divergent from Bleda Plana: they do not correspond  
359 to the same mtDNA lineage, and the number of shared STR alleles between them is low.  
360 Moreover, their mtDNA clusters with specimens from the main island of Ibiza and other  
361 associated islets (see BAPS results, cluster B). Thus, we do not rule out additional  
362 introductions of lizards from Ibiza at an unknown date, or the existence of a population on the  
363 islet prior to Eisentraut's introduction (Cirer, 1987). On the other hand, lizards from Negra  
364 Llevant are morphologically similar in terms of body dimensions to those from Bleda Plana,  
365 the putative source population for Eisentraut's translocation. This could suggest a genetic  
366 legacy of some Bleda Plana alleles being introduced at that time. However, the Negra Llevant  
367 population, in terms of pattern and colouration, shows greatest similarity with Ibiza main  
368 island populations also supporting the hypothesis that there has been an undocumented  
369 introduction of lizards from this island.

370 Two genetically well-differentiated mitochondrial lineages were detected in Dau Gran  
371 islet, both of which cluster with other Ibizan samples: the source of some of the lizards of  
372 Eisentraut's introduction. Similarities in microsatellite markers and morphology between  
373 individuals from Dau Gran and Escull Vermell individuals are explained by the fact that male  
374 lizards that Eisentraut introduced originated in Escull Vermell. Thus, we found evidence that  
375 the present day population of Dau Gran contains descendants of the two populations  
376 introduced by Eisentraut. However, lizards showed characteristics that most closely resemble

377 one of the source populations suggesting that selection could have favoured the Escull Vermell  
378 over the Ibiza morphology on Dau Gran.

379         Although Zawadzki & Kroniger (2002) suggested that the population of Dau Gran  
380 consists of no more than 12–15 individuals, our results indicate a much larger population size.  
381 Zawadzki & Kroniger (2002) stressed the role of cannibalism and the strong intraspecific  
382 competition, with 85.7% of regenerated tails in lizards from this population. The high  
383 frequency of regenerated tails could indicate strong intraspecific competition, leading to  
384 strong selection pressures due to cannibalism (Mayol, 2004). This would predict a large body  
385 size with average SVLs that are greater than both parental populations. Böhme & Eisentraut  
386 (1981) found that the hybrids on this islet were larger than the parental lizards, and also  
387 showed increased variability. Salvador (1984) observed that lizards from Dau Gran showed  
388 morphological characteristics from two two populations: body size, robustness, body  
389 dimensions and meristic characters were similar to those from lizards from Escull Vermell  
390 while coloration was intermediate between Escull Vermell and Ibiza. He noted a partial  
391 melanism, especially in larger males. Our own results indicate that lizards from Dau Gran are  
392 closer to those of Escull Vermell (Fig. 5 and Fig. 6), with a partial melanism, large body size  
393 and similar scalation characters, both in males and females.

394         Lizards were not observed in Es Vaixell by previous authors (Cirer, 1987), probably  
395 because the highest elevations on this islet, covered with some vegetation (Table 2), are almost  
396 inaccessible without climbing equipment. The shared melanic coloration and genetic affinities  
397 between the Es Vaixell and Na Gorra populations are clearly indicative of their common  
398 ancestry. In contrast, genetic and morphological divergence between Es Vaixell lizards and  
399 those of Ibiza Island indicate that Eisentraut's introduction of lizards from Ibiza Island must  
400 have failed. Thus, contrary to Eisentraut's beliefs, a lizard population already existed on Es  
401 Vaixell. In addition, the genetic affinities between lizards from Es Vaixell and Na Gorra could  
402 indicate a relatively recent origin of Es Vaixell lizards from Na Gorra and, consequently, a  
403 rapid morphological change as a result of strong selection pressures in this tiny and very steep  
404 islet (Table 2). This could explain why lizards from Es Vaixell have a very large body size,  
405 both in males and females, a high number of subdigital lamellae in males, a low number of  
406 femoral pores (femoralia) and a large number of dorsal scales (Tables S2, S3).

407         The intensity of selection acting on translocated lizards was correlated with the  
408 magnitude of ecological change in the case of the Dominican lizard, *Anolis oculatus* (Malhotra  
409 & Thorpe, 1991; Thorpe, Reardon & Malhotra, 2005). We can expect that in our case, strong  
410 changes would be also observed in those translocations between populations with higher

411 ecological differences. It is likely that the greatest differences in ecological conditions were  
412 between Ibiza Island and Es Vaixell or Dau Gran islets (Table 2). As a result, the Ibizan lizards  
413 introduced into Es Vaixell by Eisentraut disappeared without descendants. The remaining Es  
414 Vaixell individuals that we observe today originate from the original population, which  
415 Eisentraut (1949) did not know existed. Lizards from the large heterogenous island of Ibiza  
416 were unable to survive in the extreme environmental conditions on Es Vaixell (Table 2) and  
417 probably disappeared very quickly. They appear to have left no genetic legacy suggesting little  
418 or no successful interbreeding with the native population.

419 In contrast, the admixture of Escull Vermell and Ibiza Island lizards survived in Dau  
420 Gran, but the descendants of the original founders are morphologically closer to lizards from  
421 the remote Escull Vermell islet, which exhibits more similar ecological conditions to Dau  
422 Gran than to Ibiza Island (Table 2). The introduction of a limited number of individuals in a  
423 new population can be a sound strategy to learn about the respective contribution of founder  
424 effects and natural selection (Kolbe *et al.*, 2012) but, in some cases, as probably occurred in  
425 Es Vaixell, Galera and Escull de Tramuntana, the strong selection pressure overwhelmed the  
426 potential founder effects and the population perished.

427 In summary, the translocation experiments conducted by Eisentraut have not revealed  
428 much about the causes of morphological divergence in *P. pityusensis*. Some of them clearly  
429 failed, leaving no surviving populations today (Escull de Tramuntana and Galera). One  
430 translocation is difficult to interpret because the current population appears to contain lizards  
431 of mixed ancestry and is not easy to relate to Eisentraut's description (Negra Llevant). Another  
432 experiment appears to have involved the introduction of lizards to an islet that already  
433 contained a population, but fortunately, they appear to have made little or no contribution to  
434 the gene pool (Es Vaixell). Only the population on the islet of Dau Gran appears to be derived  
435 from Eisentraut's introductions.

436

### 437 **Acknowledgements**

438 Lizards were captured under licenses from the Balearic Islands Government (CAP09/2010  
439 and CEP08/2014 from Conselleria de Medi Ambient, Balear Government). Permits  
440 specifically authorized capture and release of lizards, the morphometric analyses we describe  
441 here and removal of a tail tip for DNA analysis. We conducted all research in compliance with  
442 ethical standards and procedures of the University of Salamanca, University of Balearic  
443 Islands and the Government of Spain. Colin MCarthy (Natural History Museum, London),  
444 Marta Calvo (Natural History Museum, CSIC, Madrid) and Gustavo Llorente (Department of

445 Animal Biology, University of Barcelona), kindly allowed the access and study of  
446 herpetological collections under their care. Jorge Calvo visually discovered the existence of  
447 lizards in Es Vaixell from Na Gorra Islet. Mario Garrido, Zaida Ortega, Abraham Mencía,  
448 Alicia León and Gonzalo Rodríguez helped during field work.

449 This work was financed by the research grants CGL2009-12926, CGL2012-39850 and  
450 CGL2015-68139-C2-2-P of the Spanish Ministerio de Economía y Competitividad and  
451 European Regional Development Fund (ERDF). J.M.B. was funded by FPI fellowships from  
452 the Conselleria d'Educació, Cultura i Universitats (Govern de les Illes Balears), co-financed  
453 by the European Social Fund. A.P.C. was supported by a FPU fellowship from the Spanish  
454 Ministerio de Ciencia e Innovación.

455

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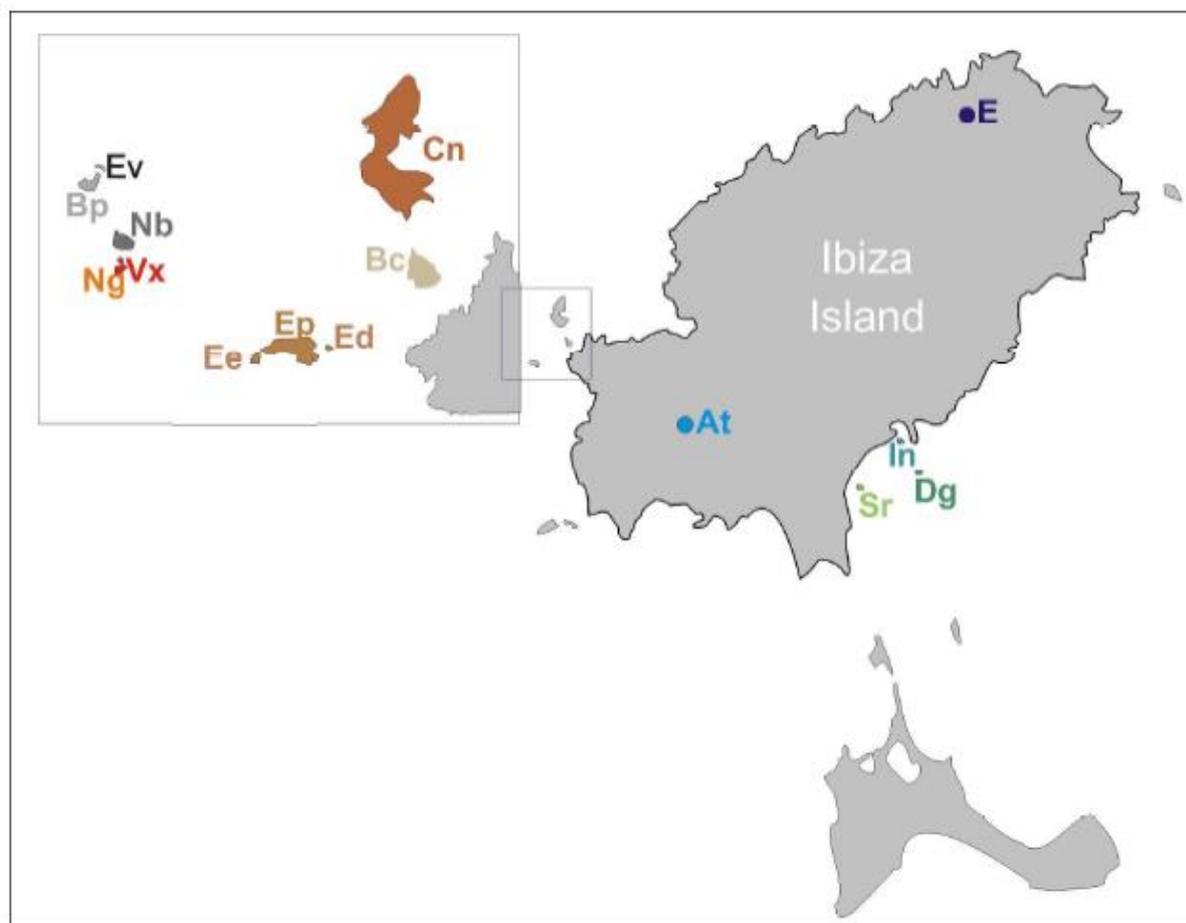
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556 **Figure 1.** Map of the Pityusic archipelago (Balearic Islands), sample localities are indicated.  
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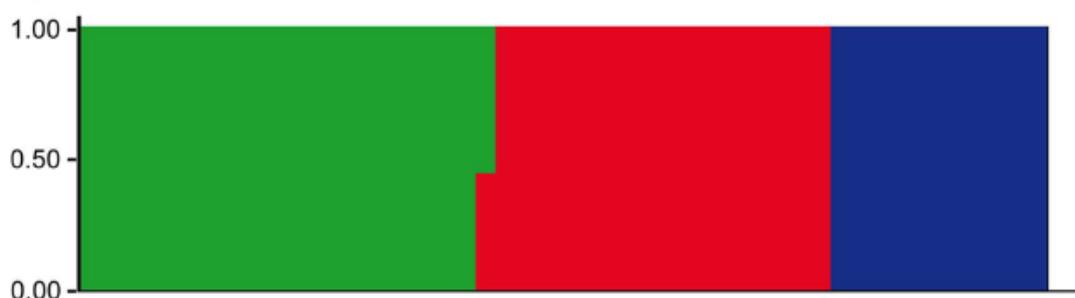
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562 **Figure 2.** Genetic structure inferred from microsatellite DNA and mtDNA. A) Microsatellite-  
563 based Bayesian assignments identified two main groups (cluster I: green and cluster II: red).  
564 The bar plot shows estimated membership coefficient ( $Q$ ); vertical bars represent individuals  
565 and assignment probabilities. B) Mixture analyses for mitochondrial data, estimated by BAPS  
566 software, identified three clusters (cluster A: green, cluster B: red and cluster C: blue). In the  
567 Bar plot, vertical bars represent individuals and proportions of admixture, with different  
568 colours corresponding to different ancestral sources.

A) Microsatellite data

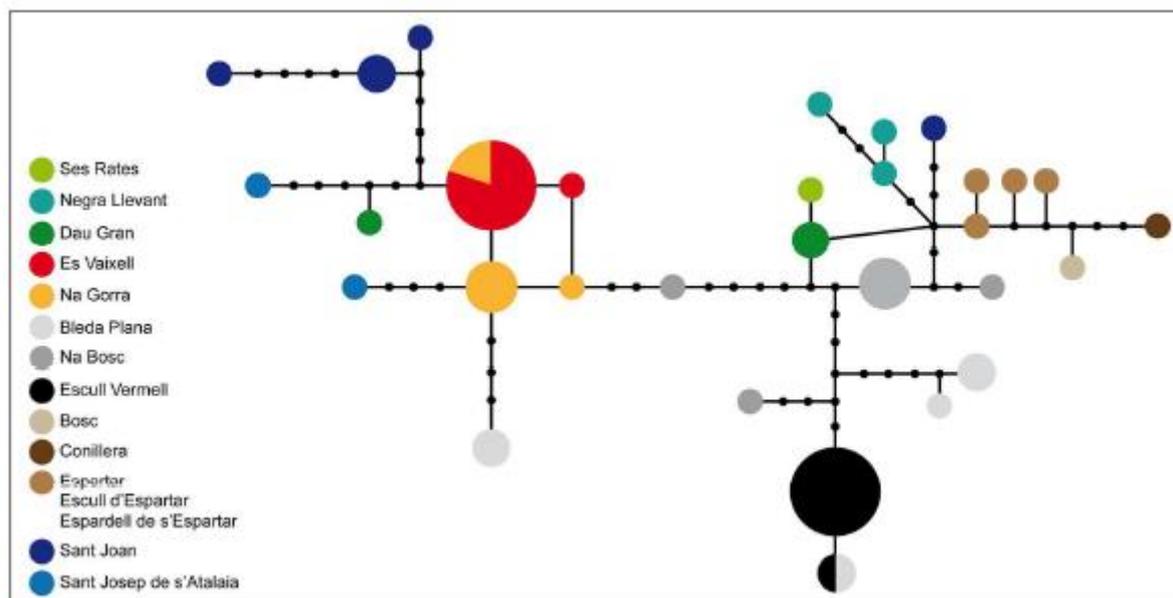


B) Mitochondrial data

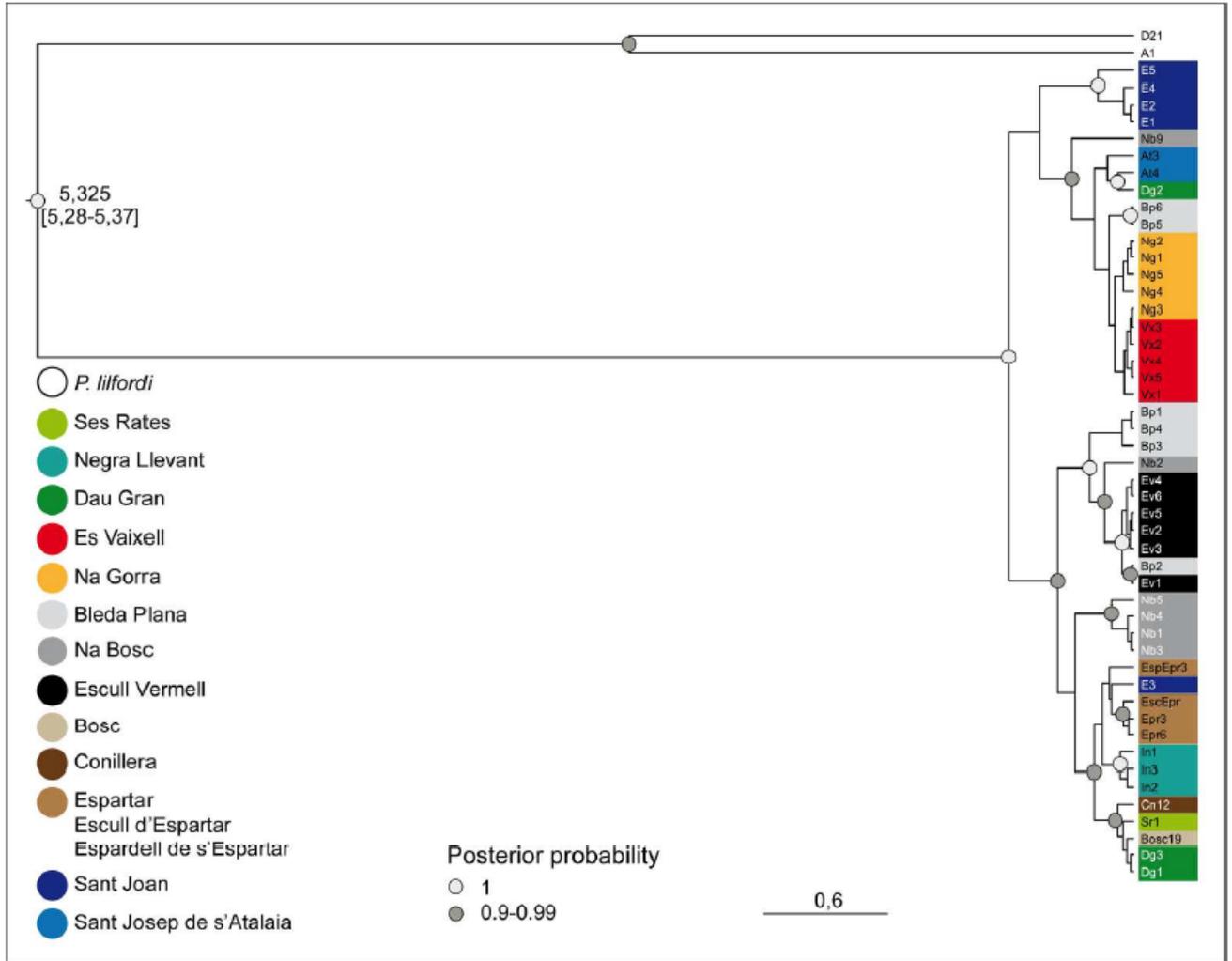


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571 **Figure 3.** MtDNA haplotype network. Specimens from each locality are represented by  
572 individual colours.  
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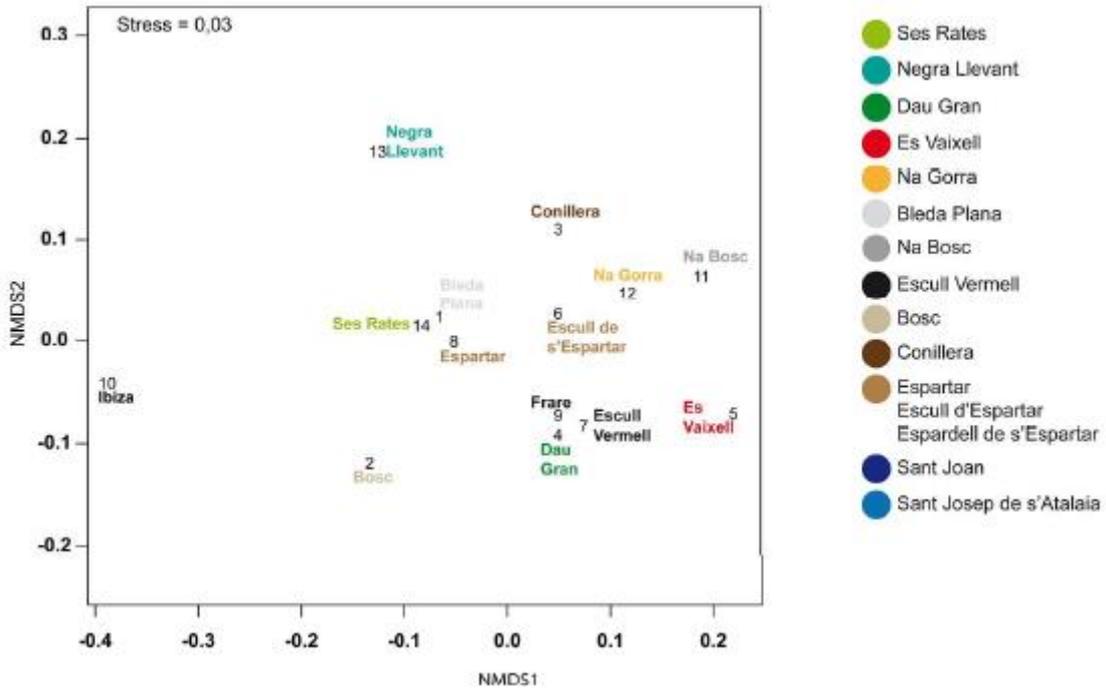
576 **Figure 4.** Mitochondrial phylogenetic tree estimated by BEAST showing relationships  
 577 among *P. pityusensis* haplotypes. Bayesian posterior probabilities (> 0.9) are indicated on the  
 578 tree.



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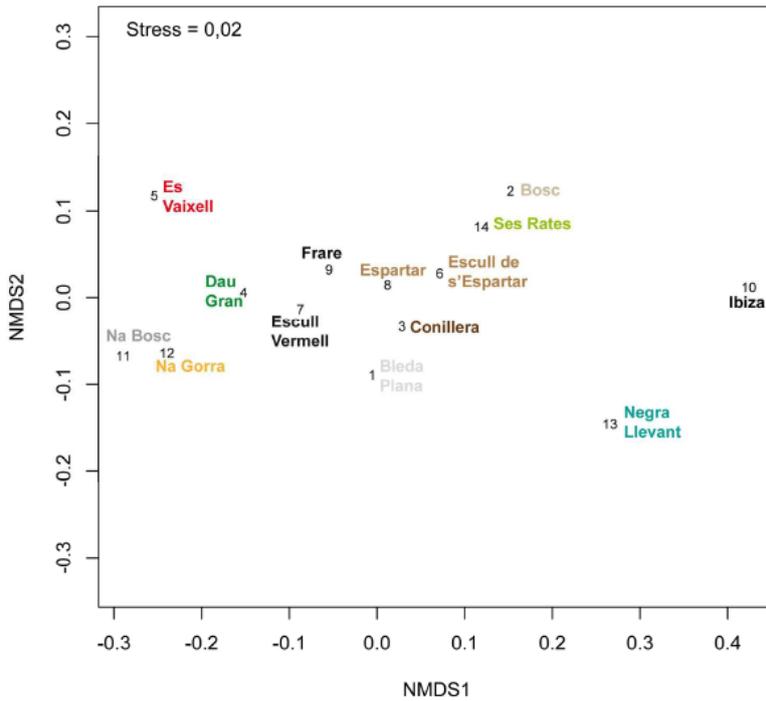
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583 **Figure 5.** Non-Metric multidimensional scaling of adult males  
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**Figure 6.** Non-Metric multidimensional scaling of adult females



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592 **Table 1.** Localities and number of specimens (N) analysed for DNA (microsatellites and  
593 mitochondrial DNA) and for morphometric characters (males and females columns) from *P.*  
594 *pityusensis*. Introduced populations are indicated in bold. Lizards sampled from Ibiza Island  
595 originated from 11 different localities (see Supplementary Material).

n	Id	Locality	DNA	Morphometric characters	
				Males	Females
<i>Islands surrounding Ibiza</i>					
1	Bp	Bleda Plana	1-6	99	82
2	Nb	Na Bosc	1-6	31	27
3	Ev	Escull Vermell	1-6	32	24
4	Ng	Na Gorra	1-5	49	33
5	<b>Vx</b>	<b>Es Vaixell</b>	5	27	27
6	Ep	Espartar	2	61	36
7	Ed	Escull d'Espartar	1	7	12
8	Ee	Espardell de s'Espartar	1	19	26
9	Bc	Bosc	1	23	19
10	Cn	Conillera	1	44	28
11	Sr	Ses Rates	1	12	11
12	<b>Dg</b>	<b>Dau Gran</b>	3	21	34
13	<b>In</b>	<b>Negra Llevant</b>	3	24	4
<i>Ibiza Island</i>					
14		11 localities		78	39
14a	E	Sant Joan	5		
14b	At	Sant Josep de s'Atalaia	1-2		
<b>TOTAL</b>			48	472	402

597 **Table 2.** Ecological characteristics of the localities under study. Introduced populations are  
 598 indicated in bold. Lizards sampled from Ibiza Island originated from 11 different localities  
 599 (see Supplementary Material). (There is no Id entry for the islets of Galera and Escull  
 600 because there are not represented in Fig 1).

<b>n</b>	<b>Id</b>	<b>Locality</b>	<b>Island area (ha)</b>	<b>Maximum altitude (m)</b>	<b>Number of vascular plants</b>
1	Bp	Bleda Plana	3.12	18	13
2	Nb	Na Bosc	3.12	39	31
3	Ev	Escull Vermell	0.04	10	7
4	Ng	Na Gorra	1.56	29	11
5	<b>Vx</b>	<b>Es Vaixell</b>	0.03	20	6
6	Ep	Espartar	18.75	70	131
7	Ed	Escull d'Espartar	0.08	20	6
8	Ee	Espardell de s'Espartar	0.56	20	17
9	Bc	Bosc	18.12	66	98
10	Cn	Conillera	100	66	139
11	Sr	Ses Rates	1.00	12	58
12	<b>Dg</b>	<b>Dau Gran</b>	0.02	12	1
13	<b>In</b>	<b>Negra Llevant</b>	0.37	9.5	16
14		Ibiza Island	57,260	475	921
15		Galera	0.19	6	4
16		Escull de Tramuntana	0.04	10	1