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

6   **Valentín Pérez-Mellado[1], Virginia Rodríguez [2], Joana M. Buades[2], Richard P. Brown[3],**

7   **Ana Pérez-Cembranos[1], Wolfgang Böhme[4], Bàrbara Terrasa[2], José A. Castro[2],**

8   **Antònia Picornell[2], Cori Ramon[2]**

9

10   [1] Departamento de Biología Animal. Universidad de Salamanca, 37007, Salamanca, Spain

11   [2] Laboratori de Genètica, Departament de Biologia. Universitat de les Illes Balears, 07122

12   Palma de Mallorca, Spain

13   [3] School of Biological and Earth Sciences, Liverpool John Moores University, Liverpool L3

14   3AF, UK

15   [4]Zoologisches Forschunginstitut und Museum Alexander Koenig. D-53113 Bonn, Germany

16

17   Corresponding author: Valentín Pérez-Mellado

18   Departamento de Biología Animal.

19   Universidad de Salamanca

20   Facultad de Farmacia, Campus Miguel de Unamuno, s/n

21   37007, Salamanca, Spain

22   E-mail: valentin@usal.es

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

Introduction

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

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

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

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

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

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

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

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

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

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

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

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

153   and tRNA$_{Met}$) 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 4th 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 x 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 (ΔK 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*

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

Discussion

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

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

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

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

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

455

References

457   Bloor P, Rodríguez V, Terrasa B, Brown R, Pérez-Mellado V, Castro J, Picornell A, Ramon
458   MM. 2011. Polymorphic microsatellite loci for the Balearic Island Lizard *Podarcis lilfordi*
459   (Squamata: Lacertidae). *Conservation Genetic Resources* **3**:323-325.
460   Böhme W. 2004. the German contribution to Mediterranean Herpetology with special
461   reference to the Balearic Islands and their lacertid lizards. In: Pérez-Mellado V, Riera, N &
462   Perera, A, eds. *The biology of lacertid lizards: Evolutionary and ecological perspectives*.
463   Maó, Menorca: Institut Menorquí d'Estudis, 63-82.
464   Böhme W, Eisentraut M. 1981. Vorläufiges Ergebnis eines unter natürlichen Bedingungen
465   angesetzten Kreuzungversuchs bei Pityusen-Eidechsen, *Podarcis pityusensis* (Bosca,
466   1883) (Reptilia: Lacertidae). *Bonner Zoologische Beiträge* **32**: 145-155.
467   Borcard D, Gillet F, Legendre P. 2011. *Numerical Ecology with R*. New York: Springer.
468   Cirer AM. 1987. Revisión taxonómica de las subespecies del lacértido *Podarcis pityusensis*,
469   Boscà, 1883. D. Phil. Thesis, Universitat de Barcelona.
470   Clement M, Posada D, Crandall K. 2000. TCS: a computer program to estimate gene
471   genealogies. *Molecular Ecoloy* **9**: 1657-1659.
472   Clover RC. 1979. Phenetic relationships among populations of *Podarcis sicula* and *P.*
473   *melisellensis* (Sauria: Lacertidae) from islands in the Adriatic Sea. *Systematic Biology* **28**:
474   284-298.
475   Corander J, Waldmann P, Sillanpää MJ. 2003. Bayesian analysis of genetic differentiation
476   between populations. *Genetics* **163**: 367-374.
477   Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with
478   BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**: 1969-1973.
479   Earl D, vonHoldt B. 2012. STRUCTURE HARVESTER: a website and program for
480   visualizing STRUCTURE output and implementing the Evanno method. *Conservation*
481   *Genetic Resources* **4**: 359-361.
482   Eisentraut M. 1930. Beitrag zur Eidechsenfauna der Pityusen und Columbreten.
483   *Mitteilungen aus dem Zoologischen Museum in Berlin* **16**: 397-410.
484   Eisentraut M. 1949. Die Eidechsen der Spanischen Mittelmeerinseln und ihre
485   Rassenaufspaltung im Lichte der Evolution. *Mitteilungen aus dem Zoologischen Museum*
486   *in Berlin* **26**: 1-225.

Hall T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleid Acids Symposium Series* **41**: 95-98.

Jakobsson M, Rosenberg NA. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**: 1801-1806.

Kinnison MT, Hendry AP. 2001. The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica* **112-113**: 145-164.

Kinnison MT, Unwin MJ, Quinn TP. 2008. Eco-evolutionary vs. habitat contributions to invasion in salmon: experimental evaluation in the wild. *Molecular Ecology* **17**: 405-414.

Kolbe JJ, Larson A, Losos JB, de Queiroz K. 2008. Admixture determines genetic diversity and population differentiation in the biological invasion of a lizard species. *Biology Letters* **4**: 434-437.

Kolbe JJ, Leal M, Schoener TW, Spiller DA, Losos JB. 2012. Founder effects persist despite adaptive differentiation: a field experiment with lizards. *Science* **335**: 1086-1089.

Malhotra A, Thorpe RS. 1991. Experimental detection of rapid evolutionary response in natural lizard populations. *Nature* **353**: 347-348.

Mayol J. 2004. Survival of an artificially hybridized population of *Podarcis pityusensis* at Dau Gran: evolutionary implications. In: Pérez-Mellado V, Riera, N & Perera, A, eds. *The biology of lacertid lizards: Evolutionary and ecological perspectives*. Maó, Menorca: Institut Menorquí d'Estudis, 239-244.

Nylander JA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL. 2004. Bayesian phylogenetic analysis of combined data. Systematic Biology **53**: 47-67.

Oksanen J, Guillaume Blanchet F, Kindt R, Legendre O, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2015. Community Ecology Package. R package version 2.2-1. Available at: http://CRAN.R-project.org/package=vegan2015.

Pérez-Mellado V. 2009. *Les sargantanes de les Balears*. Palma de Mallorca: Edicions Documenta Balear.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945-959.

Rambaut A, Drummond AJ. 2007. Tracer v. 1.5. Available from http://tree.bio.ed.ac.uk/software/tracer/.

Rivas S, Costa M, Loidi JJ. 1992. La vegetación de las islas de Ibiza y Formentera (Islas Baleares, España). *Itinera geobotanica* **6**: 99-236.

Rodríguez V, Brown RP, Terrasa B, Pérez-Mellado V, Castro JA, Picornell A, Ramon MM. 2013. Multilocus genetic diversity and historical biogeography of the endemic wall lizard from Ibiza and Formentera, *Podarcis pityusensis* (Squamata: Lacertidae). *Molecular Ecology* **22**: 4829-4841.

Salvador A. 1984. A taxonomic study of the Eivissa wall lizard, *Podarcis pityusensis* Boscà 1883. In: Kuhbier H, Alcover JA, Guerau d' Arellano Tur C, eds. *Biogeography and ecology of the Pityusic Islands*. The Netherlands: Dr W. Junk Publisher, 393-427.

Salvador A, Pleguezuelos JM. 2002. *Reptiles españoles: identificación, historia natural y distribución*. Talavera de la Reina: Canseco Editores.

Stockwell CA, Weeks SC. 1999. Translocations and rapid evolutionary responses in recently stablished populations of western mosquitofish (*Gambusia affinis*). *Animal Conservation* **2**: 103-110.

Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**: 619-633.

535 Templeton AR, Sing CF. 1993. A cladistic analysis of phenotypic associations with
536   haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with
537   cladogram uncertainty and recombination. *Genetics* **134**: 659-669.
538 Terrasa B, Pérez-Mellado V, Brown RP, Picornell A, Castro JA, Ramon MM. 2009.
539   Foundations for conservation of intraspecific genetic diversity revealed by analysis of
540   phylogeographical structure in the endangered endemic lizard *Podarcis lilfordi*. *Diversity*
541   *and Distributions* **15**: 207-221.
542 Thorpe RS, Reardon JT, Malhotra A. 2005. Common garden and natural selection
543   experiments support ecotypic differentiation in the Dominican anole (*Anolis oculatus*).
544   *The American Naturalist* **165**: 495-504.
545 Vähä J-P, Primmer CR. 2006. Efficiency of model-based Bayesian methods for detecting
546   hybrid individuals under different hybridization scenarios and with different numbers of
547   loci. *Molecular Ecology* **15**: 63-72.
548 Williams CK, Moore RJ. 1989. Phenotypic adaptation and natural selection in the rabbit,
549   *Oryctolagus cuniculus*, in Australia. *Journal of Animal Ecology*, **58**: 495-507.
550 Zawadzki M, Kroniger M. 2002. Eidechsenbeobachtungen auf Dado Grande: Ergänzende
551   Bemerkungen zu einem unter natürlichen Bedingungen angesetzten Kreuzungsversuch bei
552   Pityusen-Eidechsen *Podarcis pityusensis* (Boscà, 1883). *Die Eidechse* **13**: 33-42.
553 Zuur AF, Ieno EN, Smith GM. 2007. *Analysing Ecological Data*. New York: Springer.
554
555

Figure 1. Map of the Pityusic archipelago (Balearic Islands), sample localities are indicated.
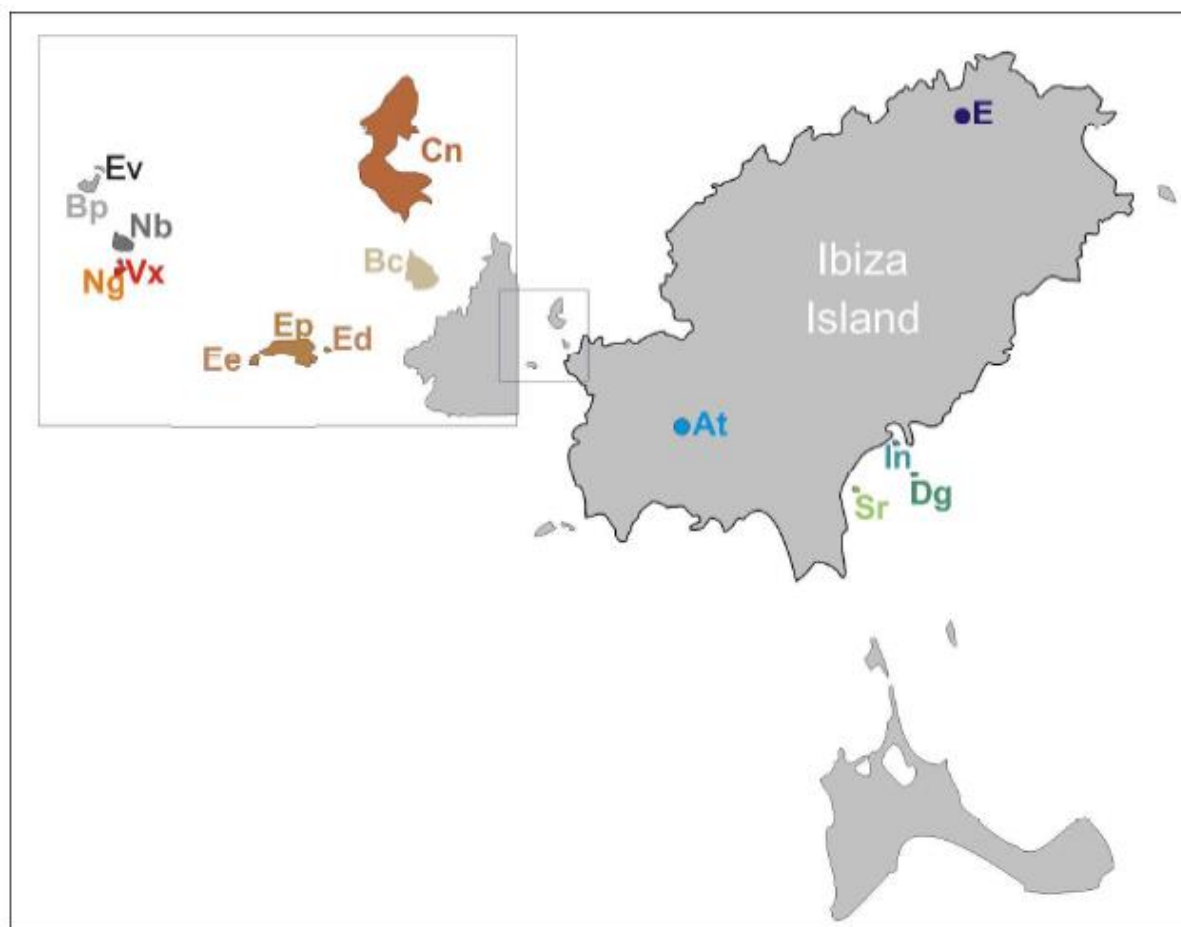
Figure 2. Genetic structure inferred from microsatellite DNA and mtDNA. A) Microsatellite-based Bayesian assignments identified two main groups (cluster I: green and cluster II: red). The bar plot shows estimated membership coefficient (Q); vertical bars represent individuals and assignment probabilities. B) Mixture analyses for mitochondrial data, estimated by BAPS software, identified three clusters (cluster A: green, cluster B: red and cluster C: blue). In the Bar plot, vertical bars represent individuals and proportions of admixture, with different colours corresponding to different ancestral sources.
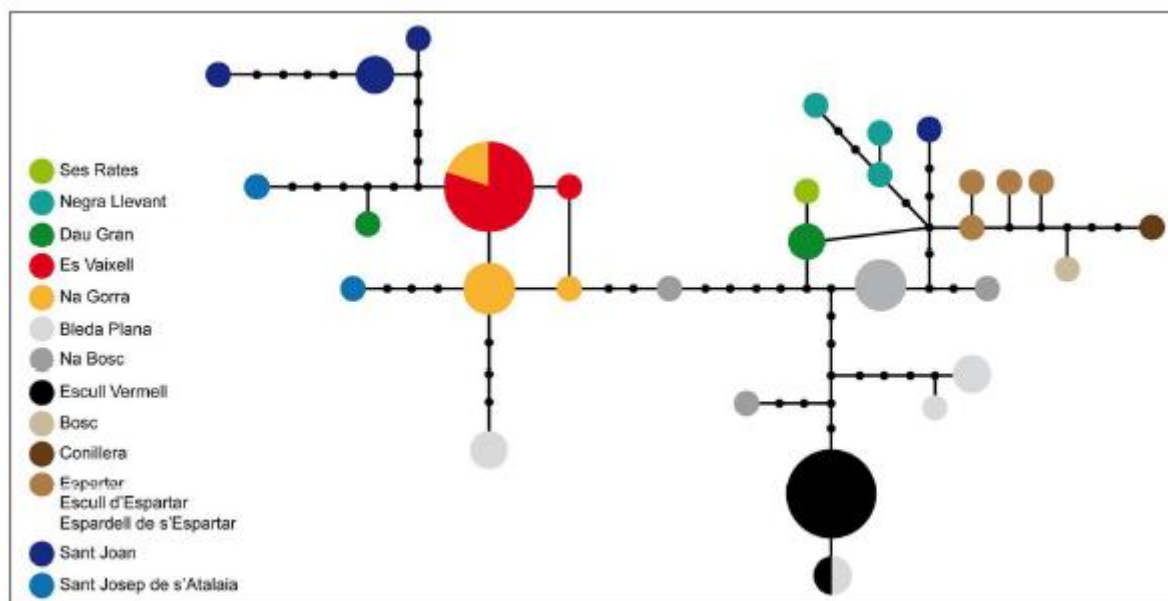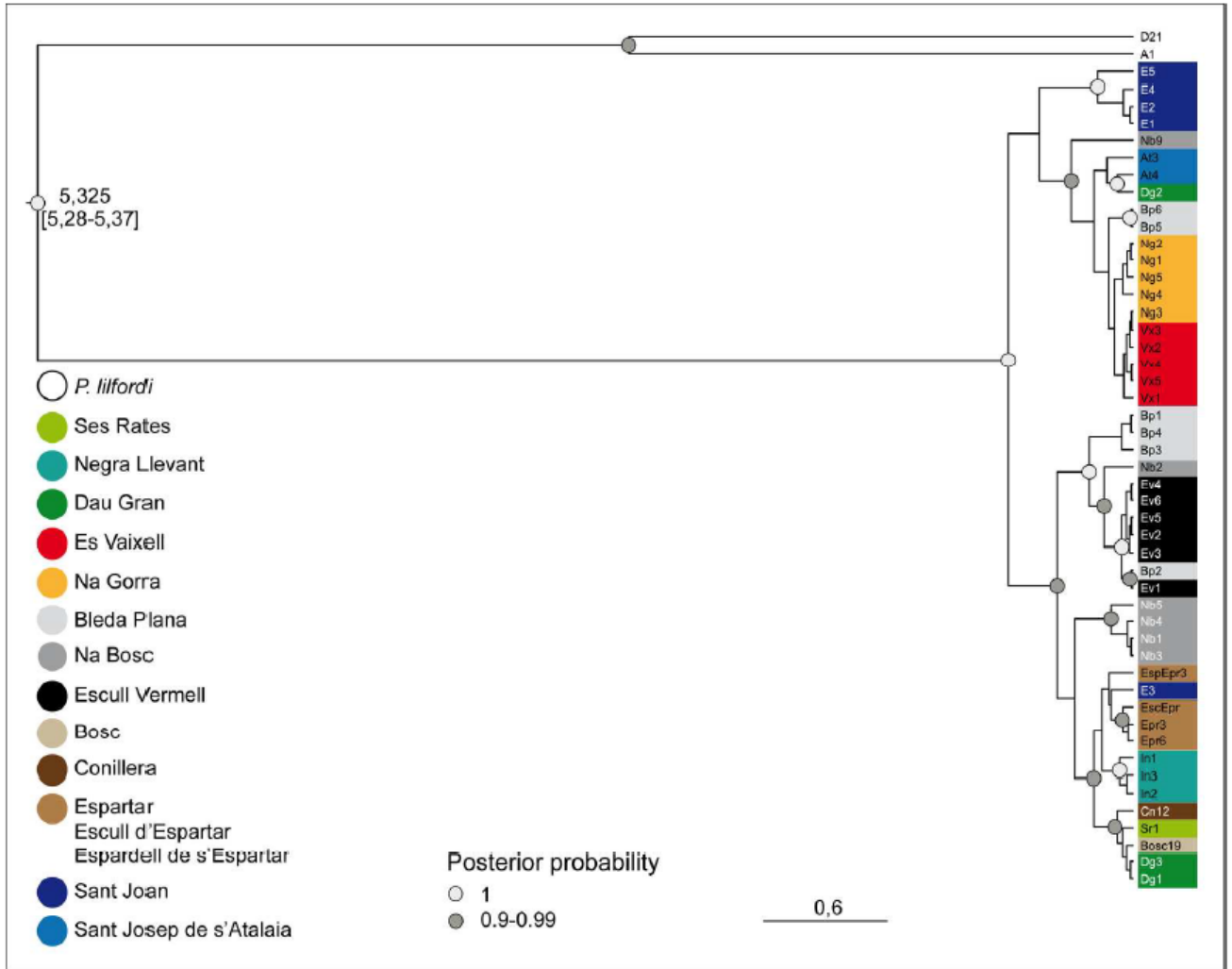
Figure 3. MtDNA haplotype network. Specimens from each locality are represented by individual colours.

579
580

581
582

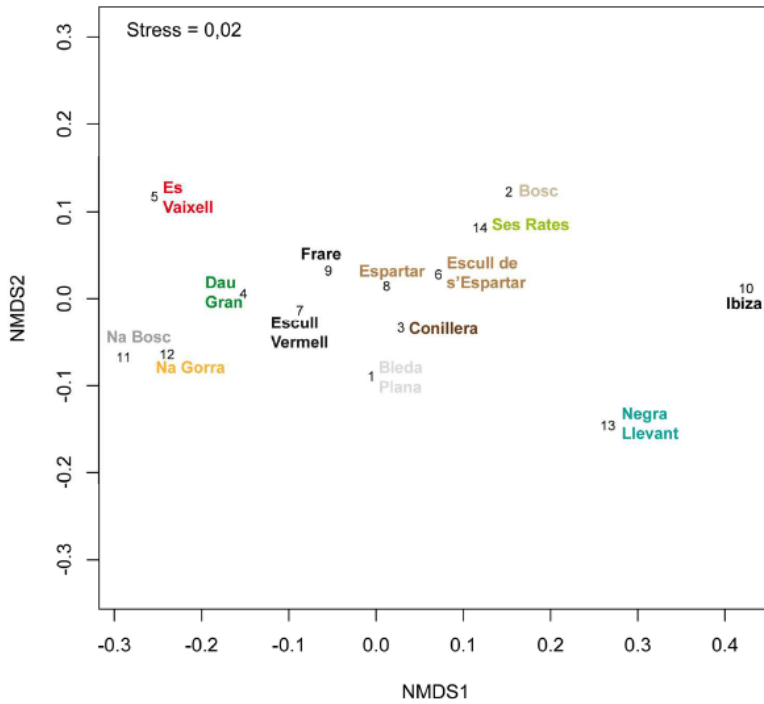583 **Figure 5.** Non-Metric multidimensional scaling of adult males
584
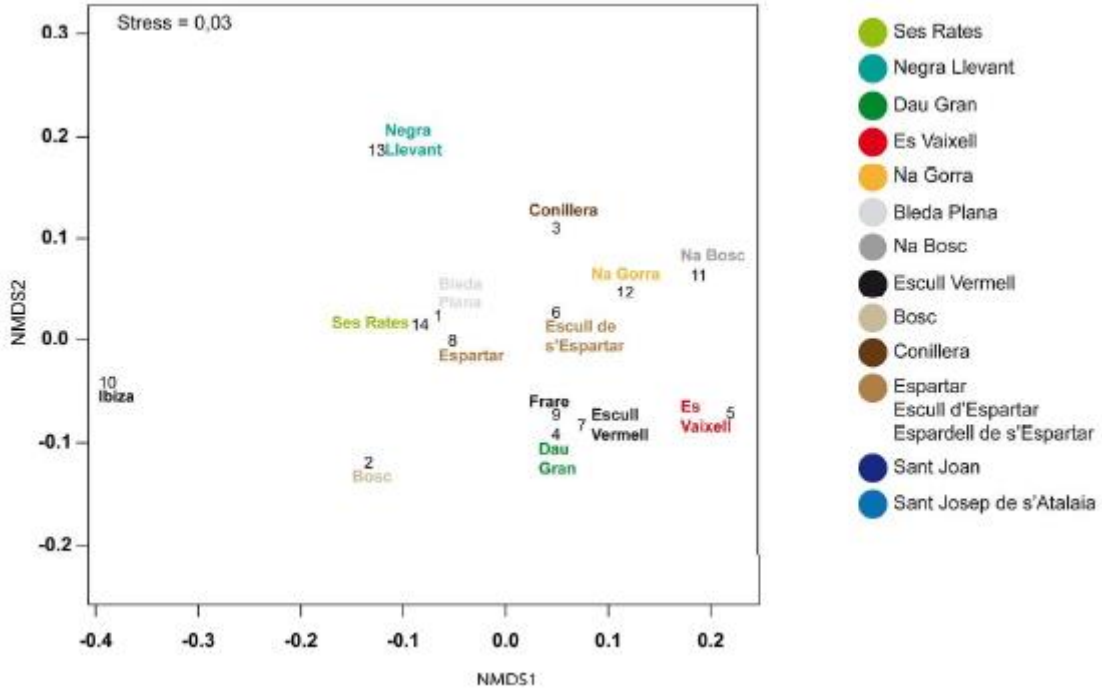
585
586
587 **Figure 6.** Non-Metric multidimensional scaling of adult females
588

589
590
591

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**
		Islands surrounding Ibiza			
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	**Vx**	**Es Vaixell**	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	**Dg**	**Dau Gran**	3	21	34
13	**In**	**Negra Llevant**	3	24	4
		Ibiza Island			
14		11 localities		78	39
14a	E	Sant Joan	5		
14b	At	Sant Josep de s'Atalaia	1-2		
		TOTAL	48	472	402

596

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).

n	Id	Locality	Island area (ha)	Maximum altitude (m)	Number of vascular plants
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	**Vx**	**Es Vaixell**	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	**Dg**	**Dau Gran**	0.02	12	1
13	**In**	**Negra Llevant**	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