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ENDEMIC *PODARCIS* LIZARDS IN THE BALEARIC ARCHIPELAGO STUDIED BY MEANS OF mtDNA AND ALLOZYME VARIATION

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Abstract: Two endemic species of *Podarcis* inhabit the Balearic archipelago: *Podarcis lilfordi* in the Gymnesies islands (Mallorca, Menorca, Cabrera and associated islets) and *Podarcis pityusensis* in the Pityuses islands (Ibiza, Formentera and surrounding islets). Predation by introduced weasels, hedgekogs, feral cats, rats and snakes probably eliminated these species from the main islands of Menorca and Mallorca. A high number of subspecies have been described by means of morphological data, but they have not been confirmed by molecular data. We have studied mtDNA sequence from part of the cytochrome *b* gene from specimens from different islands and islets. A clear separation between both species is found, although the genetic distance is lower than those between mainland species of *Podarcis,* indicating a shorter divergence time. These results were positively correlated with those obtained by means of enzymatic polymorphisms. A F_{ST} mean value (0.868) was found for all samples indicating strong structuring of the populations, higher than values obtained in other populations of lizards. The differentiation between *P. lilfordi* samples, estimated by F_{ST} and genetic distances, does not confirm the monophyly of the two presently accepted subspecies. A high migration rate detected among *P. pityusensis* samples does not support the numerous subspecies described in Ibiza and Formentera area, confirming the systematic pattern proposed by Salvador (1984). The divergence time estimated are in accordance with geological data, and suggest a similar rate of divergence, i.e., 2% per my, postulated for this gene region by other studies.

Keywords: Cytochrome *b* sequences, mtDNA, allozymes, *Podarcis lilfordi*, *Podarcis pityusensis*, Balearic Archipelago.

Resumen: Estudio del ADN mitocondrial y la variación aloenzimática de las lagartijas endémicas del género *Podarcis* en el archipiélago Balear. Dos especies endémicas del género *Podarcis* habitan en el archipiélago balear: *Podarcis lilfordi* en las islas Gimnésicas (Mallorca, Menorca, Cabrera e islotes asociados) y *Podarcis pityusensis* en las islas Pitiusas (Ibiza, Formentera e islotes circundantes). Probablemente estas especies fueron eliminadas en Mallorca y Menorca por la predación de especies introducidas como erizos, comadrejas, gatos silvestres, ratas y serpientes. Se han descrito un gran número de subespecies basándose en datos morfológicos, pero no han sido confirmadas mediante datos moleculares. Se ha estudiado la secuencia parcial del gen del citocromo *b* a partir de especímenes procedentes de difer-

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entes islas e islotes. Hemos detectado una clara separación entre ambas especies, aunque con una distancia genética menor que la existente entre las especies continentales de *Podarcis*, lo que nos indica un menor tiempo de divergencia. Estos resultados presentan una correlación positiva con los obtenidos mediante el estudio de polimorfismos enzimáticos. El valor de F_{ST} medio (0.868) estimado entre todas los muestras indicó una fuerte estructuración de las poblaciones, mayor a los valores obtenidos en otras poblaciones de lagartijas. La diferenciación entre las muestras de *P. lilfordi*, estimada mediante distancias genéticas y F_{ST}, no confirmó la monofilia de las dos subespecies aceptadas. Una alta tasa de migración, se detectó entre las muestras de *P. pityusensis*, que no apoya a la existencia de las numerosas subespecies descritas en el área de Ibiza y Formentera y que confirmaría el patrón sistemático propuesto por Salvador (1984). El tiempo de divergencia estimado concuerda con los datos geológicos y sugieren una tasa de divergencia (2%) similar a la propuesta para esta región génica en otros estudios.

Palabras clave: Secuencias del Citocromo *b*, ADN mitocondrial, alozimas, *Podarcis lilfordi*, *Podarcis pityusensis*, Archipiélago Balear.

Resum: Estudi del ADN mitocondrial i la variació aloenzymàtica de les sargantanes endèmiques del gènere *Podarcis* a l'arxipèlag Balear.-Dues espècies endèmiques del gènere *Podarcis*, habiten a l'arxipèlag balear, *Podarcis lilfordi* en les illes Gimnèsies (Mallorca, Menorca, Cabrera i els illots associats) i *Podarcis pityusensis* en les Illes Pitiüses (Eivissa, Formentera i illots circumdants). Probablement aquestes espècies foren eliminades de Mallorca i Menorca degut a la predació d'espècies introduïdes com els eriçons, les mosteles, els gats silvestres, les rates i les serps. S'han descrit un gran nombre de subspècies en base a dades morfològiques, però aquestes no han estat confirmades mitjançant dades moleculars. S'ha estudiat la seqüència parcial del gen del citocrom b a partir d' espècimens procedents de diferents illes e illots. Hem detectat una clara separació entre ambdues espècies, encara que amb una distància genètica menor a l'existent entre les espècies continentals de *Podarcis*, el que ens indicaria un menor temps de divergència. Aquests resultats presenten una correlació positiva amb les dades obtingudes mitjançant l'estudi dels polimorfismes enzimàtics. El valor mitjà de F_{ST} (0.868) estimat entre totes les mostres ens va indicar una forta estructuració de les poblacions, major als valors obtinguts en altres poblacions de sargantanes. La diferenciació entre les mostres de *P. lilfordi*, estimada mitjançant distancies genètiques i F_{ST}, no va confirmar la monofília de les dues subespècies acceptades. Una alta taxa de migració, es va detectar entre les mostres de *P. pityusensis*, el que no dóna suport a l'existència de les nombroses subspècies descrites dins l'àrea d'Eivissa i Formentera, que a més confirmaria el patró sistemàtic proposat per Salvador (1984). El temps de divergència estimat concorda amb les dades geològiques i suggereix una taxa de divergència (2%) similar a la proposta per aquesta regió gènica en altres estudis.

Paraules clau: Seqüències del citocromo *b*, ADN mitocondrial, al. loenzims, *Podarcis lilfordi*, *Podarcis pityusensis*, Arxipèlag balear.

INTRODUCTION

Lacertid lizards are a monophyletic group of approximately 250 species, distributed throughout Africa and most of Eurasia (ESTES *et al.*, 1989). They constitute the dominant reptile group in Europe, and considerable research has

been carried out based on morphology, ecology and behavior, and mtDNA in an attempt to establish their phylogenetic structure (ARNOLD, 1989, 1990; HARRIS *et al.*, 1998b, FU *et al.*, 1998).

The lacertids are represented in the Balearic Archipelago (Western Mediterranean Sea) by two endemic species of the genera *Podarcis* (ARNOLD, 1973): *Podarcis lilfordi* in the Gymnesies Islands (Mallorca, Menorca, Cabrera and associated islets) and *Podarcis pityusensis* in the Pityuses Islands (Ibiza, Formentera and their surrounding islets). These have already been shown to be sister taxa in phylogenetic analyses of *Podarcis* (HARRIS & ARNOLD, 1999; OLIVERIO *et al.*, 2000).

Paleontological data suggested that the genera *Podarcis* arrived in the Balearic Archipelago during the Mediterranean Messalinity crisis in the Miocene (6 Myr). During this period the Balearic Islands must then have been linked together and attached to the Iberian Peninsula, forming a vast promontory (RANGHEARD, 1984). From the upper Miocene considerable dislocations took place in the Balearic area. One shift occurred between Cap de la Nau (eastern coast of mainland Spain) and Ibiza, and the same happened between Ibiza and Majorca, between Majorca and Minorca and to the east of Minorca. These vertical faults and shifts-faults caused the insularity of the Balearic Archipelago, permitting the divergent evolution from the ancestral form and subsequent separation within the Balearic Archipelago (*Podarcis lilfordi* and *Podarcis pityusensis*) (COLOM, 1978). Later during the two last Pleistocene glaciations (Riss and Würm) the sea descended to some 100 m below its present level. This created connections between the Islands, thus the archipelago was comprised of only two large islands, permitting a greater dispersion of its lacertids. Nonetheless the Gymnesies were never united with the Pityusic Islands at any time during the Quaternary Era (CUERDA, 1993).

The two species of *Podarcis* are currently present in almost all islands and islets, but are absent in the largest islands of Mallorca and Menorca. The lizards probably become extinct in these islands as a result of predation from species introduced by man, such as weasels (ALCOVER *et al.*, 1981), hedgehogs, feral cats (KOTSAKIS, 1981), and snakes (MERTENS, 1957).

The different populations of the genera *Podarcis* that are living now form a polytypic taxa, compounded by a large number of subspecies or local races. Substantial morphological variation has led to the recognition of one subspecies for each island/islet population, although other different taxonomic patterns have also been suggested (MÜLLER, 1927, 1928; EISENTRAUT, 1928, 1950; SALVADOR, 1984; PÉREZ-MELLADO 1997, 1998; CIRER, 1987). Genetics studies investigated variation by means of enzymatic polymorphisms: *P. pityusensis* (CIRER & GUILLAUME, 1986; GUILLAUME & CIRER, 1985) and *P. lilfordi* (RAMON *et al.*, 1986, 1991; PETITPIERRE *et al.*, 1987), showed a high level of intrapopulational diversity but low variation between populations. Thus the high number of subspecies described by means of morphological data was not confirmed at the enzymatic level.

Recently, DNA sequencing has becoming the commonest method for analysis of population structure (CLARK *et al.*, 1999). The cytochrome *b* gene has been recognized as particularly suitable for recovering phylogenetic relationships, especially in lacertids (THORPE *et al.*, 1994; GONZÁLEZ *et al.*,1996; FU *et al.*, 1997; HARRIS *et al.*, 1998a). In some studies, the mtDNA variation was compared with morphological data, being both complementary and without significant discrepancies (HARRIS *et al.*, 1998b).

The purpose of the present work was to determine the variability in the cytochrome *b* gene in some populations of the endemic Balearic species of lizards, and to compare their differentiation with the enzymatic phylogeny suggested for this group.

MATERIAL AND METHODS

The specimens of *Podarcis lilfordi* and *P. pityusensis* were captured in some islands and islets of the Balearic Archipelago, whose location is indicated in Figure 1. Three to five individuals were analyzed for each locality. The number of specimens and subspecies are indicated in Table 1. A tail tip of each individual was clipped off, and put in ice during the transport to the laboratory, where it was stored at -20ºC. The live animals were released again in the same capture place. Total genomic DNA was extracted following the methodology of GONZÁLEZ *et al.* (1996) with minor modifications: the DNA was precipited by ethanol, vacuum-dried an resuspended in H_2O for a 80 ng/ml. A 306 bp fragment of the mitochondrial

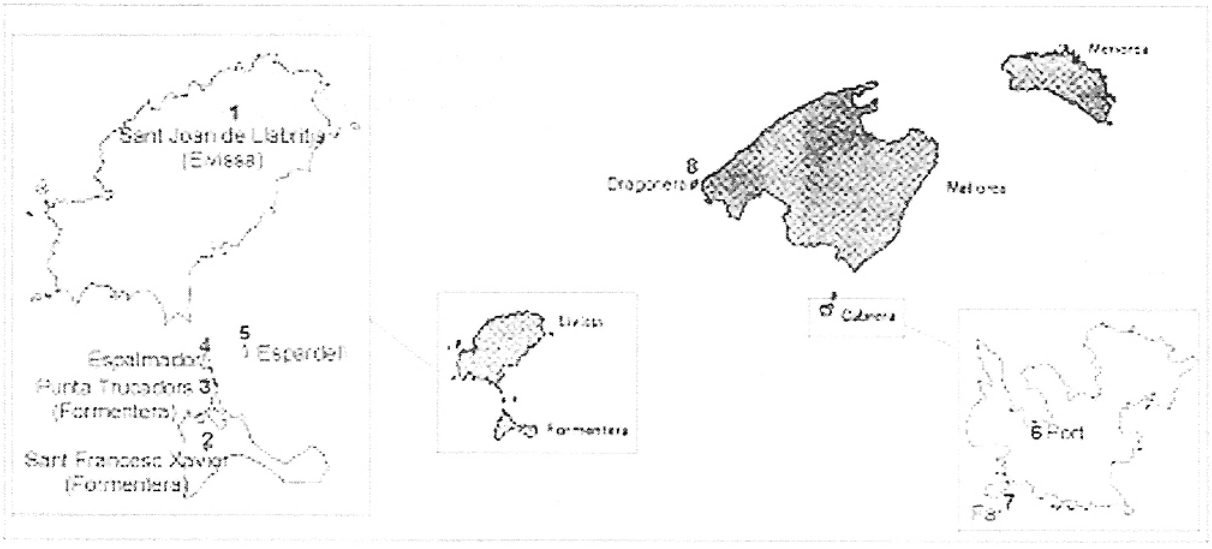

Figure 1. Geographic location of populations included in this study

Species	Subspecies	Locality	Samples
Podarcis pityusensis			
	P. p. pityusensis (Boscá, 1882)	1	PpE (4)
	P. p. formenterae (Eisentraut, 1928)	2	PpFx (5)
	P. p. grueni (Müller, 1928) *P. p. formenterae* (Eisentraut, 1928)	3	PpFt (5)
	P. p. espalmadoris (Müller, 1928) *P. p. formenterae* (Eisentraut, 1928)	4	PpEr (3)
	P. p. formenterae (Eisentraut, 1928)	5	PpEl (3)
Podarcis lilfordi			
	P. l. kuligae (Müller, 1927)	6	PlCp (4)
	P. l. kuligae (Müller, 1927) *P. l. muelleri* (Eisentraut, 1928)	7	PlCf (4)
	P. l. giglioli (Bedriaga, 1879)	8	PlD (4)

a- The numbers correspond with those of Fig. 1
b- Number of individuals in brackets

Table 1. The different populations of the subspecies sampled from *P. lilfordi* and *P. pityusensis*

cytochrome b gene was amplified using the primers L14841 and H15149 (KOCHER *et al*, 1989). Thermocycling consisted of 36 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1 min. Both strands of the amplified DNA were sequenced on an automated ABI 310 sequencer using Taq DyeDeoxy' Terminator Cycle sequencing kit (Perkin Elmer). Sequences were submitted to Genbank (accession numbers AY046281 to AY0463312).

Sequence analyses

Cytochrome *b* nucleotide sequences were aligned by CLUSTAL IV using the MegAlign 1.05 and EditSeq 3.75 programs (cDNASTAR Inc, 1993). Single sequences of *Podarcis muralis*, *P. hispanica* (CASTILLA *et al.*, 1998), and *Lacerta agilis* (HARRIS *et al.*, 1998b) were used as outgroups, sequences of one individual from each species were included from Genbank. Sequence divergence between taxa was calculated by the Kimura two parameters model (KIMURA, 1980) using the MEGA 1.02 program (KUMAR *et al.*, 1993). Phylogenies were constructed assuming constant (UPGMA, SNEATH & SOKAL, 1973) evolutionary rates.

WRIGHT'S (1965) F_{ST} values were calculated to estimate genetic divergence

among populations, and their significance was testing using a nonparametric permutation approach (significance level $P<0.05$) using the Analysis of Molecular Variance procedure (EXCOFFIER *et al.*, 1992) in the AMOVA option of ARLEQUIN v. 2.0 package (SCHNEADER *et al.*, 2000).

Enzymatic data

The genetic distances based in enzymatic polymorphisms (NEI, 1972) were estimated using previously published electrophoretic data from LDH-1, LDH-2, MDH-1, PGI, PGM, GOT-1 and GOT-2 (RAMON *et al.*, 1986; GUILLAUME & CIRER, 1985) using BIOSYS-2 program (SWOFFORD *et al.*, 1997). The cophenetic relationships were estimated using UPGMA clustering by means of PHYLIP version 3.5c (FELSENSTEIN, 1993).

To test for correlation between genetic distance matrices obtained from allozymes and cytochrome *b* gene sequences, a Mantel nonparametric test was used with 1000 permutations. Computations were made using the matrix of Euclidian square distances used for the AMOVA option (EXCOFFIER *et al.*, 1992) in ARLEQUIN v. 2.0 package (SCHNEADER *et al.*, 2000).

RESULTS

Sequences of the cytochrome b fragment studied (306 bp) were obtained for 32 samples of *Podarcis*. No indels were observed. There were 48 variable positions (15.7%), 46 of which were phylogenetically informative sites under parsimony criterion. 44 of these were variable in *P. lilfordi* and only 6 sites in *P. pityusensis*. In most cases (83.3%) the substitutions occurred in the third codon position, only 6 substitutions gave aminoacids replacements, five of them were found in *P. lilfordi* and one in *P. pityusensis*.

Table 2 shows the genetic distances based on sequence divergence estimated using the Kimura two parameters model. The distance values ranged from 0.027 to 0.048 within *P. lilfordi* samples with a mean of 0.037, which is 6 times higher than the distance between localities sampled in *P. pityusensis* (ranged from 0 to 0.013 with a mean of 0.006). The average distance between the two species studied was 0.097. This is similar to other insular species (e.g. CARRANZA *et al.*, 2000, 2001) and can be compared to the average congeneric distance for this gene within reptiles of 0.12, and is much higher than is typically found within reptile species (HARRIS, 2002).

The phylogenetic analysis gave a tree indicated in Figure 2. Two clear groupings are discernible. The first grouping includes *P. pityusensis*, within which it is possible to recognise two clusters, one with the Ibizan samples and other with the most of the samples from Formentera Island and neighbouring islets. The second grouping includes *P. lilfordi*. It is clear that genetic variation within *P. lilfordi* is much higher than within *P. pityusensis*. The level of nucleotide diversity (π)

	PpE	PpEr	PpFt	PpFx	PpEl	PlD	PlCf	PlCp	Ph	Pm	La
PpE	-	0.007	0.009	0.010	0.009	0.092	0.094	0.108	0.190	0.180	0.207
PpEr		-	0.008	0.010	0.008	0.094	0.097	0.111	0.196	0.180	0.213
PpFt			-	0.001	0.000	0.093	0.095	0.107	0.197	0.179	0.214
PpFx				-	0.001	0.093	0.095	0.106	0.197	0.179	0.214
PpEl					-	0.093	0.095	0.107	0.197	0.179	0.214
PlD						-	0.037	0.048	0.172	0.158	0.198
PlCf							-	0.027	0.170	0.170	0.199
PlCp								-	0.176	0.180	0.191
Ph									-	0.170	0.201
Pm										-	0.179

Table 2. Genetic distances (Kimura 2P) between localities sampled in the two Balearic species of *Podarcis* and data from *Podarcis hispanica* (Ph), *Podarcis muralis* (Pm) and *Lacerta agilis* (La)

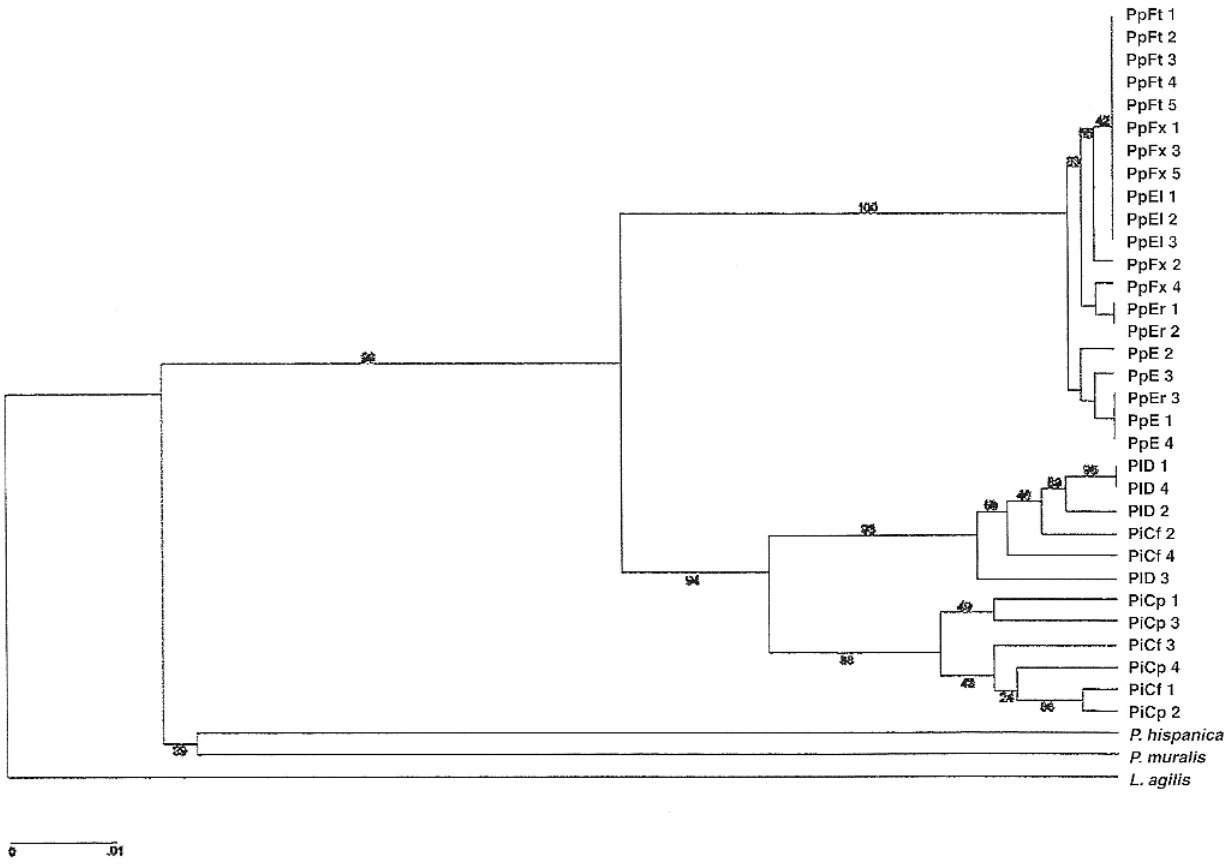

Figure 2. UPGMA tree based on mtDNA sequences. Bootstrap values are indicated

based on the Kimura 2P are indicated in Table 3. The values of diversity in *P. lilfordi* are around 10 times higher than in *P. pityusensis*. In Table 3 the F_{ST} pairwise values obtained are also shown.

A high F_{ST} mean value (0.868) was found when all the samples of *Podarcis pityusensis* and *P. lilfordi* were analyzed. The migratory flow (Nm) detected is indicated below the diagonal (Table 3)

	PpE	PpEr	PpFt	PpFx	PpEl	PlD	PlCf	PlCp
π	0.0038 (0.0036)	0.0066 (0.0062)	0.0026 (0.0026)	0	0	0.0155 (0.0114)	0.0373 (0.0257)	0.0271 (0.0190)
PpE	-	0.266^{ns}	0.815	0.651	0.751	0.892	0.786	0.856
PpEr	1.379	-	0.690	0.461	0.571 ^{ns}	0.870	0.753	0.831
PpFt	0.113	0.225	-	0.000 ^{ns}	0.000 ^{ns}	0.925	0.833	0.890
PpFx	0.267	0.584	inf	-	0.132 ^{ns}	0.909	0.817	0.875
PpEl	0.165	0.376	inf	inf	-	0.897	0.775	0.849
PlD	0.061	0.074	0.040	0.050	0.057	-	0.348	0.704
PlCf	0.136	0.164	0.100	0.112	0.145	0.934	-	0.254 ^{ns}
PlCp	0.084	0.101	0.061	0.071	0.089	0.210	1.463	-

^{ns} not significant inf = infinite

Table 3. Nucleotide diversity (π) based on the Kimura 2P distances (errors in parentheses). F_{ST} are indicated above the diagonal values. Below diagonal, estimates of effective numbers of migrants (Nm) per generation between the eight *Podarcis* populations. The name of populations corresponds with those of Table 1

and the values are higher than 1 migrant per generation between the samples of Ibiza and Espardell, Cabrera Port and Cabrera Far. Between the two samples from Formentera and Espardell the number of migrants are infinite. The remaining pairwise comparisons indicate a non-significant gene flow between the samples.

The frequencies of seven enzymatic polymorphisms (Appendix) were used to estimate the genetic distances between the samples of *P. lilfordi* and *P. pituysensis*. Figure 3 showed the

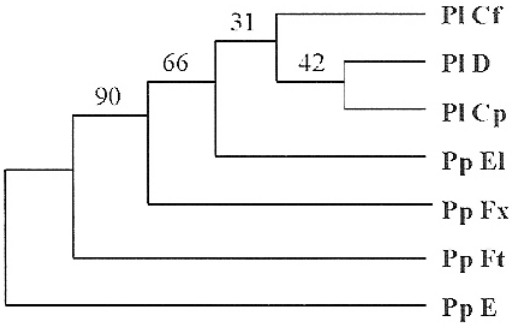

Figure 3. UPGMA tree based on enzymatic polymorphisms. Bootstrap values are indicated

dendrogram obtained from them. The agreement between the differentiation patterns obtained from allozyme and from cytochrome *b* sequence data was supported significantly by a Mantel test, showing a significant (P=0.005) correlation between Nei's genetic distances and sequence divergence among populations (r = 0.467).

DISCUSSION

Cytochrome *b* mitochondrial sequences have been widely used in phylogenetic and conservation studies of reptiles (e.g. CLARK *et al.*, 1999), showing that this gene is very useful in differentiating populations. The pattern of nucleotide substitution for cytochrome *b* sequences has been well documented and our results agree with the postulated pattern: synonymous differences are more numerous than non-synonymous, the variation is highest in the third position of each codon; and transitions exceed transversions. In particular C-T transitions outnumber transversions by almost 10 to 1.

The transitions bias found in most of the previous published studies can also be observed in the present results: within *P. lilfordi* the overall ts/tv ratio was 7.5 and in *P. pityusensis* was 10.8, higher values than those found in other species of island lizards (GONZÁLEZ *et al.*, 1996). As expected transition-transversion ratio declines the sequence divergences increase.

The average sequence divergence detected between the two *Podarcis* species was approximately 9.7%, which is in agreement with *Podarcis* from Columbretes islands (CASTILLA *et al.*, 1998), and Iberian cryptic forms (HARRIS & SÁ SOUSA, 2002) but slightly lower than the average observed between sister species of other reptiles (HARRIS, 2002). The divergence detected between the Balearic and mainland species was 19%.

For a more detailed analysis of the variability distribution, an ANOVA was made, grouping the populations in two units: *P. lilfordi* and *P. pityusensis*. Only 12.2% of the variation occurred within the populations, 11.2% among populations within groups and 76.6% of the variation was between the groups, confirming that *P. lilfordi* and *P. pityusensis* are genetically distinct species with respect to mtDNA. This is important as their species status has previously been questioned (CAPULA, 1997). When the samples were structured in four units according to geographical criteria: Dragonera, Cabrera, Ibiza and Formentera with their surroundings islets, only 4.74% of the variation among populations within groups was detected. The degree (80.1%) of among island genetic differentiation observed in the Balearic archipelago was high, in comparison to that observed in other *Podarcis* lizard populations. CASTILLA *et al.* (1998) found 73% of the variation occurred between populations in the Columbretes lizards, and CAPULA (1996) 46% for *Podarcis tiliguerta*. The geographic distances among the different islands and the sea cliffs, particularly in the case of *P. lilfordi*

(Dragonera and Cabrera) could explain this level of differentiation.

To assess the different subspecies in accordance with the hypothesis based in morphological parameters of one subspecies for each island or islet, the F_{ST} pairwise values and phylogenetic trees were compared. In *P. lilfordi* the two samples of Cabrera island are homogeneous (Nm=1.46). Eisentraut (1928) described two different subspecies in different localities of Cabrera, but this was never confirmed by others authors and it is not supported by these analyses, although more sampling would be needed to be certain of this. The differentiation between Cabrera and Dragonera is not clear, because although the sample of Cabrera Port is well differentiated from Dragonera, between Cabrera Far and Dragonera (with F_{ST} not significant) the migratory flow is high (0.934). In the dendrograms we can observed two clusters: Cabrera and Dragonera but with the inclusion of two specimens from Cabrera Far in the cluster of Dragonera specimens. From a morphological perspective, the subspecies *P. l. giglioli* is found in Dragonera, without discrepancies in the literature. The analyses of cytochrome *b* sequences made with the three samples (Cabrera Far, Cabrera Port and Dragonera) do not confirm the existence of two subspecies. However, it is important to assess both ecological and genetic variability when defining subspecies or evolutionary significant units (CRANDALL *et al.*, 2000). We think that it is necessary to analyse more individuals from these islands before speculating about the different possibilities that could generate these results, or their taxonomic implications.

The situation for *P. pityusensis* is slightly different, because the islands and islets are geographically closer and their coasts are mainly low and sandy, which should facilitate migration of lizards. The F_{ST} values between the two samples of Formentera and the Espardell islet indicated the existence of a single area of distribution for the lizards (Nm=inf), and even between Ibiza and Espalmador islet there is significant gene flow (Nm>1). The different subspecies for *Podarcis pityusensis* in the Formentera area (Table 1), postulated by BUCHHOLZ (1954) and confirmed by RODRÍGUEZ-RUIZ (1977), were: *P. p. formenterae* in Formentera, *P. p. grueni* in Punta Trocadors, *P. p. espalmadoris* in Espalmador islet, and *P. p. espardalensis* in the Espardell islet. SALVADOR (1984) recognised only one subspecies, *P. p. formenterae* in Formentera and their islets, although the author described a north-south clinal morphological variation. This result is more in accordance with the mitochondrial data obtained in the present study.

In the phylogenetic trees based on the cytochrome *b* sequences, the Balearic endemic species of *Podarcis* form a monophyletic group. This was also found in other analyses including more *Podarcis* species (HARRIS & ARNOLD, 1999; OLIVERIO *et al.*, 2000). Assuming an overall rate of change for cytochrome *b* of 2% per million of year (CARRANZA *et al.*, 2000), the nucleotide divergence of 9.7 between *P. lilfordi* and *P. pityusensis*, could correspond to a

divergence time of 4.95 millions of year. The separation time between Gymnesies and Pityuses archipelagos according to geological data is around 5 million of years. Thus our results suggest that the ancestors of the two present forms became isolated during this event.

Finally, to point out the need to continue with the studies in the archipelago by increasing the number of samples (other islands and/or islets) and by using different genes of the mitochondrial and nuclear genome to better understand the genetic structure of the populations. Moreover, this knowledge will be very important to develop an appropriate conservation program for these endemic *Podarcis* lizards.

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Appendix. Enzymatic allele frequencies in Balearic populations of genus *Podarcis*. The name of populations corresponds with those of Table 1.

	Population						
Locus	PpE	PpFx	PpFt	PpEl	PlD	PlCp	PlCf
LDH-1							
(N)	31	5	5	5	40	28	12
A	1	1	1	1	1	0.964	1
B	0	0	0	0	0	0.036	0
LDH-2							
(N)	31	5	5	5	40	28	12
A	1	1	1	1	1	0.964	1
B	0	0	0	0	0	0.036	0
MDH-1							
(N)	31	5	5	5	40	28	12
A	0.758	0.400	1	1	0..813	0.571	0
B	0.242	0.600	0	0	0.188	0.426	1
PGI							
(N)	31	5	5	5	40	28	12
A	0	0	0	1	0.087	0	0
B	0	0	0	0	0.300	.446	1
PGM							
(N)	31	5	5	5	40	28	12
A	0.935	1	1	0.500	0.850	0.964	0.875
B	0.065	0	0	0.500	0.150	0.036	0.127
GOT-1							
(N)	31	5	5	5	40	28	12
A	0	0	0	0	0.962	0.625	0.417
B	1	0.800	1	1	0.038	0.375	0.583
C	0	0.200	0	0	0	0	0
GOT-2							
(N)	31	5	5	5	40	28	12
A	0.113	0	0.200	0	0	0	0
B	0.887	0.600	0.800	1	1	1	1
C	0	0.400	0	0	0	0	0

N = Number of individuals