Phylogeography of the lacertid lizard, *Psammodromus algirus*, in Iberia and across the Strait of Gibraltar

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ABSTRACT

Aim To determine genetic substructuring within the lacertid lizard *Psammodromus algirus*. To compare levels of variation across a geological barrier, the Strait of Gibraltar, and to compare this against the known age of the barrier using a molecular clock hypothesis. To compare the effect of the barrier within this species with previously published data from other organisms.

Location The Iberian Peninsula and North Africa.

Methods Partial sequences from the mitochondrial cytochrome *b*, 12S rRNA and 16S rRNA genes were obtained from 101 specimens belonging to the subfamily Gallotiinae and used in this study. The data set was aligned using CLUSTALX and phylogenetic trees produced using both maximum-parsimony and maximum-likelihood methods. Maximum likelihood estimates of divergence times for the combined data set (12S + 16S + cytochrome *b*) were obtained after discovery of lineage rate constancy across the tree using a likelihood ratio test.

Results *Psammodromus algirus* contains divergent eastern and western mtDNA clades within the Iberian Peninsula. The western clade has northern and southern lineages in Iberia and one in North Africa. This phylogeographical pattern indicates that the lizard invaded North Africa after the opening of the Strait, presumably by natural rafting.

Main conclusions As in several other species, current patterns of genetic diversity within *P. algirus* are not directly related to the opening of the Strait of Gibraltar. Widespread sampling on both sides of the barrier is necessary to determine its effect on species in this area accurately.

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INTRODUCTION

Psammodromus algirus (Linnaeus, 1758) is a west Mediterranean lacertid lizard belonging to the subfamily Gallotiinae, which also includes three other species of *Psammodromus* and nine species of *Gallotia*, a genus endemic to the Canary Islands. *Psammodromus algirus* is found in habitats characteristic of the Mediterranean region from Tunisia to Morocco and across Portugal and Spain as far as the Laguedoc in France (Carretero *et al.*, 2002). It occurs ubiquitously in a wide variety of situations, from open sandy areas and agricultural fields to maquis and open forests. Despite being one of the most

frequently encountered reptiles across much of its range, little is known about intraspecific variation in *P. algirus*. Subspecies have been recognized in the past but are not now usually considered valid (Schleich *et al.*, 1996). Busack (1986), in the first biogeographical analysis of the herpetofauna occurring across the Strait of Gibraltar, which was based on data produced by protein electrophoresis, found little or no genetic differentiation between populations of *P. algirus* from southern Spain and those from northern Morocco. However, later biogeographical studies of other reptiles in the Iberian Peninsula and North Africa have demonstrated the need for widespread sampling of taxa if their often complicated genetic

differentiation is to be revealed and interpreted in historical terms. This is especially so as the region has had a complex geological history. For example, the Strait of Gibraltar closed about 5.96 Ma causing the Mediterranean Sea to desiccate in the Messinian Salinity Crisis, and then opened again about 5.33 Ma (Hsü *et al.*, 1977; Hsü, 1983; Krijgsman *et al.*, 1999; Duggen *et al.*, 2003). Detailed investigation with adequate sampling has shown that different taxa may have very different histories. For example, the lacertid lizard *Podarcis hispanica sensu lato* was shown to be a species complex that dispersed south from south-west Europe into North Africa at least twice in the past few million years, once probably when the Mediterranean dried up and again some time after the Strait of Gibraltar reopened (Harris *et al.*, 2002; Busack *et al.*, 2005; Pinho *et al.*, 2006). In contrast, other forms have moved north much more recently. These include the chameleon *Chamaeleo chameleon* (Paulo *et al.*, 2002), the false smooth snake *Macroprotodon brevis ibericus* (Carranza *et al.*, 2004a) and a gecko, *Tarentola mauritanica* (Harris *et al.*, 2004b,c).

In this paper we explore the phylogeography of *P. algirus*, determining the varying degrees of divergence between its populations by sequencing part of its mitochondrial genome (mtDNA), using fragments of the cytochrome *b*, 12S rRNA and 16S rRNA genes totalling 1081 base pairs (bp). Employing a molecular clock calibrated within *Gallotia* we assess whether populations of *P. algirus* on each side of the Strait of Gibraltar separated vicariantly when this reopened approximately 5.3 Ma, and whether any transmarine migration across the strait occurred before or after this event.

MATERIALS AND METHODS

Samples and DNA extraction

A total of 101 specimens belonging to the subfamily Gallotiinae were used in this molecular study. These included 88 individuals of *P. algirus*, one *Psammodromus blanci* (Lataste, 1880) two *Psammodromus hispanicus* Fitzinger, 1826 and 10 representatives of species of *Gallotia* that were used to root the *Psammodromus* tree. Specimen data and GenBank accession numbers of the gene fragments sequenced for these are given in Table 1. In total, 34 specimens were sequenced for all three genes, 46 for the 12S rRNA and 16S rRNA genes and 21 for the cytochrome *b* gene only.

Genomic DNA was extracted from tissue samples following standard protocols described elsewhere (Harris *et al.*, 1998; Carranza *et al.*, 1999, 2000). Primers used in both amplification and sequencing were cytochrome *b1* and cytochrome *b2* (Kocher *et al.*, 1989) for the cytochrome *b* (cytb) gene, 12Sa and 12Sb (Kocher *et al.*, 1989) for the 12S rRNA gene, and 16Sar and 16Sbr (Palumbi, 1996) for the 16SrRNA gene. The three gene fragments were amplified by the polymerase chain reaction (PCR) and the resultant DNA was sequenced using the same standard protocols and conditions described by Carranza *et al.* (1999) and Harris *et al.* (1998).

Phylogenetic analyses

DNA sequences were aligned using CLUSTALX (Thompson *et al.*, 1997) with default parameters (gap opening = 10; gap extension = 0.2). All the cytb sequences had the same length and therefore no gaps were postulated. These sequences were translated into amino acids using the vertebrate mitochondrial code and no stop codons were observed, suggesting that they were probably all functional. Although some gaps were postulated in order to resolve length differences in the 12S rRNA and 16S rRNA gene fragments, all positions could be unambiguously aligned and were therefore included in the analyses.

Phylogenetic analyses were carried out using maximum-likelihood (ML) and maximum-parsimony (MP). MODELTEST (Posada & Crandall, 1998) was used to select the most appropriate model of sequence evolution for the ML analyses using the Akaike information criterion. This was the general time reversible (GTR) model, taking into account the shape of the gamma distribution (G) and the number of invariable sites (I) for the data set containing all three genes together (12S, 16S and cytb). For the data set containing both ribosomal genes together (12S and 16S) the model selected was the GTR + G. ML analyses were performed using PHYML (Guindon & Gascuel, 2003) with model parameters fitted to the data by likelihood maximization.

MP analyses were performed in PAUP* v. 4.0b10 (Swofford, 1998) and included heuristic searches involving tree bisection and reconnection (TBR) branch swapping with 100 random stepwise additions of taxa. Gaps were included as a fifth state. Nodal support for both MP and ML trees was assessed using bootstrap analysis (Felsenstein, 1985) involving 1000 bootstrap pseudoreplications.

Topological incongruence among partitions was tested using the incongruence length difference (ILD) test (Michkevich & Farris, 1981; Farris *et al.*, 1994). In this test, 10,000 heuristic searches were carried out after removing all invariable characters from the data set (Cunningham, 1997). To test for incongruence among data sets we also used a reciprocal 70% bootstrap proportion (Mason-Gamer & Kellogg, 1996). Topological conflicts were considered significant if two different relationships for the same set of taxa were supported with bootstrap values ≥ 70% in different partitions.

Molecular clock calibration

ML estimates of divergence times for the combined data set (12S + 16S + cytb) were obtained after the discovery of lineage rate constancy across the tree using a likelihood ratio test (Huelsenbeck & Crandall, 1997). The error associated with finite sample of nucleotides for reconstructing branch lengths was calculated by a three-step nonparametric bootstrap procedure (Efron & Tibshirani, 1993): (1) 100 data matrices were generated using the SEQBOOT program in PHYLIP 3.57 (Felsenstein, 1993), (2) the matrices were imported into PAUP* 4.0b10 and 100 trees with branch lengths were obtained using

Table 1 Details of material and sequences used in the present study. Specimen numbers identify each individual shown in Figs 1–3

Taxa	Specimen number	Locality	GenBank Accession Nos			Code
			cytb	12S	16S	
Gallotia stehlini		Gran Canaria (Canary Islands)	AY151838	AY151917	DQ298688	Gst2
Gallotia atlantica atlantica		Fuerteventrua (Canary Islands)	AY151836	AY151915	DQ298679	Gatat21
Gallotia atlantica mahoratae		Lanzarote (Canary Islands)	AY151837	AY151916	DQ298680	Gatmaj1
Gallotia intermedia		Tenerife (Canary Islands)	AY151844	AY151923	DQ298681	Gint1
Gallotia simonyi machadoi		El Hierro (Canary Islands)	AF101219	AY151924	DQ298682	Gsih16n
Gallotia caesaris gomerae		La Gomera (Canary Islands)	AY151842	AY151921	DQ298683	GagaG1
Gallotia caesaris caesaris		El Hierro (Canary Islands)	AY151843	AY151922	DQ298684	GagaH1
Gallotia galloti palmae		La Palma (Canary Islands)	AY151841	AY151920	DQ298687	GagaP2
Gallotia galloti eisentrauti		N. Tenerife (Canary Islands)	AY151839	AY151918	DQ298685	Gagat1
Gallotia galloti galloti		S. Tenerife (Canary Islands)	AY151840	AY151919	DQ298686	Gagat2
P. blanci		Bou Chebka (Tunisia)	DQ298563	DQ298608	DQ298678	E281140
P. h. hispanicus		Encinasola, Huelva (Spain)	DQ298561	DQ298606	DQ298676	E281143
P. h. edwardsianus		Sierra de Baza, Granada (Spain)	DQ298562	DQ298607	DQ298677	E281145
P. algirus	4	Salinas Cabo de Gata, Almeria (Spain) WF76	DQ298560	DQ298605	DQ298675	E281125
P. algirus	5	Ensenada de Getares, Cadiz (Spain) TE79	DQ298559	DQ298604	DQ298674	E281133
P. algirus	6	Embalse Fuensanta, Albacete (Spain) WH64	DQ298557	DQ298602	DQ298672	E281129
P. algirus	7	Tartareu, Lleida (Spain)	DQ298558	DQ298603	DQ298673	E140118
P. algirus	8	Malaga city, Malaga (Spain)		DQ298653	DQ298733	E281118
P. algirus	9	Malaga city, Malaga (Spain)		DQ298654	DQ298734	E281122
P. algirus	10	Granada city, Granada (Spain)		DQ298651	DQ298731	E281123
P. algirus	11	Virgen de la Cabeza, Jaen (Spain) VH03	DQ298580			E10711
P. algirus	12	Salar, Granada (Spain) VG00	DQ298579			E10716
P. algirus	13	La Calahorra, Granada (Spain) VG90	DQ298578			E1071
P. algirus	14	Pto. Aguilas, Murcia (Spain) XG24	DQ298577			E1074
P. algirus	15	Embalse de Negratin, Granada (Spain)	DQ298576			E1073
P. algirus	16	La Sauceda, Cadiz/Malaga (Spain) TF64	DQ298584			E10712
P. algirus	17	Rio Seco, Granada (Spain) VF37	DQ298583			E10715
P. algirus	18	Embalse de Baico, Albacete (Spain) XH28	DQ298581			E1079
P. algirus	19	Sierra Gador, Almeria (Spain) WF08	DQ298582			E10714
P. algirus	20	Plasencia, Caceres (Spain)		DQ298652	DQ298732	Pa26
P. algirus	21	Cazorla, Jaen (Spain)		DQ298650	DQ298730	Pa23
P. algirus	22	Ain Draham (Tunisia)	DQ298551	DQ298596	DQ298666	E281134
P. algirus	23	Ain Draham (Tunisia)	DQ298552	DQ298597	DQ298667	E281135
P. algirus	24	Ain Draham (Tunisia)	DQ298553	DQ298598	DQ298668	E281136
P. algirus	25	Ain Draham (Tunisia)	DQ298555	DQ298600	DQ298670	E281137
P. algirus	26	Ain Draham (Tunisia)	DQ298554	DQ298599	DQ298669	E281138
P. algirus	27	El Kebir (Tunisia)		DQ298624	DQ298704	Pa49
P. algirus	28	J. Zebla (Tunisia)		DQ298625	DQ298705	Pa46
P. algirus	29	Tabarka (Tunisia)		DQ298626	DQ298706	Pa50
P. algirus	30	J. Zaghouan (Tunisia)		DQ298627	DQ298707	Pa45
P. algirus	31	Bab-Taza (Morocco)		DQ298618	DQ298698	Pa35
P. algirus	32	Jbel Hebri (Morocco)		DQ298620	DQ298700	Pa40
P. algirus	33	Ketama (Morocco)		DQ298636	DQ298716	Pa37
P. algirus	34	Bab-Taza (Morocco)		DQ298630	DQ298710	Pa33
P. algirus	35	Jbel Hebri (Morocco)		DQ298628	DQ298708	Pa39
P. algirus	36	Taza (Morocco)		DQ298629	DQ298709	Pa43
P. algirus	37	Ketama (Morocco)		DQ298631	DQ298711	Pa38
P. algirus	38	Morocco		DQ298619	DQ298699	PaM
P. algirus	39	Chefchaouen (Morocco)	DQ298556	DQ298601	DQ298671	E140115
P. algirus	40	Ued Lau (Morocco)		DQ298621	DQ298701	E28119
P. algirus	41	Bab-Berret (Morocco)		DQ298622	DQ298702	E28113
P. algirus	42	Ras el Ma (Morocco)		DQ298623	DQ298703	E28116
P. algirus	43	Cantera Bercana (Morocco)		DQ298637	DQ298717	E28114
P. algirus	44	15 km E. Tanger (Morocco)		DQ298635	DQ298715	E281110
P. algirus	45	Bab-Berret (Morocco)		DQ298632	DQ298712	E28112

Table 1 *continued*

Taxa	Specimen number	Locality	GenBank Accession Nos			Code
			cytb	12S	16S	
P. algirus	46	Jebala (Morocco)		DQ298633	DQ298713	E28115
P. algirus	47	Morocco		DQ298634	DQ298714	E28118
P. algirus	48	Beni-Mellal (Morocco)	DQ298575			E3113
P. algirus	49	Tlemcen (Algeria)	DQ298574			E3112
P. algirus	50	Sidi Freig (Algeria)	DQ298573			E3111
P. algirus	51	Casares, Malaga (Spain) TF93	DQ298550	DQ298595	DQ298665	E281131
P. algirus	52	Oliva de la Frontera, Badajoz (Spain) PC83	DQ298549	DQ298594	DQ298664	E281126
P. algirus	53	Matalascanyas, Huelva (Spain) QA19	DQ298548	DQ298593	DQ298663	E281128
P. algirus	54	Barbate, Cadiz (Spain) TF30	DQ298541	DQ298586	DQ298656	E140117
P. algirus	55	Bodegones, Huelva (Spain) QB01	DQ298543	DQ298588	DQ298658	E281127
P. algirus	56	Near Cordoba city, Codoba (Spain)	DQ298540	DQ298585	DQ298655	E281114
P. algirus	57	Mairena, Sevilla (Spain) QB63	DQ298542	DQ298587	DQ298657	E140116
P. algirus	58	Linares de la Sierra, Huelva (Spain)	DQ298544	DQ298589	DQ298659	E281115
P. algirus	59	Linares de la Sierra, Huelva (Spain) QB09	DQ298547	DQ298592	DQ298662	E281119
P. algirus	60	Canteras de Gerena, Sevilla (Spain) QB55	DQ298545	DQ298590	DQ298660	E281116
P. algirus	61	Linares de la Sierra, Huelva (Spain)	DQ298546	DQ298591	DQ298661	E281117
P. algirus	62	Marbella, Malaga (Spain) UF24	DQ298572			E10713
P. algirus	63	Marbella. Malaga (Spain) UF24	DQ298571			E10717
P. algirus	64	Genaguacil, Malaga (Spain) UF04	DQ298570			E10710
P. algirus	65	Ayamonte, Huelva (Spain) PB42	DQ298569			E1075
P. algirus	66	Berrocal, Huelva (Spain) QB16	DQ298566			E1076
P. algirus	67	Tentudia, Badajoz (Spain) QC31	DQ298565			E10719
P. algirus	68	Caños de Meca, Cadiz (Spain) QA60	DQ298564			E1078
P. algirus	69	Cañada pajaros, Sevilla (Spain) QB52	DQ298568			E1077
P. algirus	70	Doña Rama, Cordoba (Spain) UH03	DQ298567			E1072
P. algirus	71	Rio Guadiamar, Sevilla (Spain)		DQ298611	DQ298691	E281121
P. algirus	72	Aldea del Cano, Caceres (Spain)		DQ298612	DQ298692	Pa20
P. algirus	73	Almograve (Portugal)		DQ298616	DQ298696	Pa4
P. algirus	74	Almograve (Portugal)		DQ298615	DQ298695	Pa1
P. algirus	75	Olhos de Água (Portugal)		DQ298617	DQ298697	Pa8
P. algirus	76	São Pedro de Moel (Portugal)		DQ298610	DQ298690	Pa11
P. algirus	77	Rompeculos, Huelva (Spain)		DQ298609	DQ298689	Pa28
P. algirus	78	São Pedro de Moel (Portugal)		DQ298613	DQ298693	Pa13
P. algirus	79	São Pedro de Moel (Portugal)		DQ298614	DQ298694	Pa12
P. algirus	80	Almograve (Portugal)		DQ298638	DQ298718	Pa2
P. algirus	81	S. Torpes (Portugal)		DQ298639	DQ298719	Pa15
P. algirus	82	Alberca, Salamanca (Spain)		DQ298647	DQ298727	Pa18
P. algirus	83	Murça (Portugal)		DQ298648	DQ298728	Pa17
P. algirus	84	Plasencia, Caceres (Spain)		DQ298646	DQ298726	Pa27
P. algirus	85	Alberca, Salamanca (Spain)		DQ298645	DQ298725	Pa19
P. algirus	86	Barrocal (Portugal)		DQ298644	DQ298724	Pa5
P. algirus	87	Pendilhe (Portugal)		DQ298640	DQ298720	Pa9
P. algirus	88	Picote (Portugal)		DQ298642	DQ298722	Pa10
P. algirus	89	Barrocal (Portugal)		DQ298643	DQ298723	Pa6
P. algirus	90	Tui, Orense (Spain)		DQ298641	DQ298721	Pa30
P. algirus	91	Sendim (Portugal)		DQ298649	DQ298729	Pa14

the GTR + I + G model of sequence evolution (see above) with the tree of Fig. 1 as a constraint, (3) trees with branch lengths were transformed into trees with node times using TREEEDIT v. 1.0 (http://evolve.zoo.ox.ac.uk). The different values across the 100 trees were used to calculate the average and the standard deviation for the relevant nodes.

To calibrate the phylogenetic tree with relative node times, we used the methods described above and an internal calibration point based on the assumption that divergence between *Gallotia caesaris caesaris* (Lehrs, 1914) (endemic from the island of El Hierro) and *Gallotia caesaris gomerae* (Boettger & Müller, 1914) (endemic from the island of La Gomera)
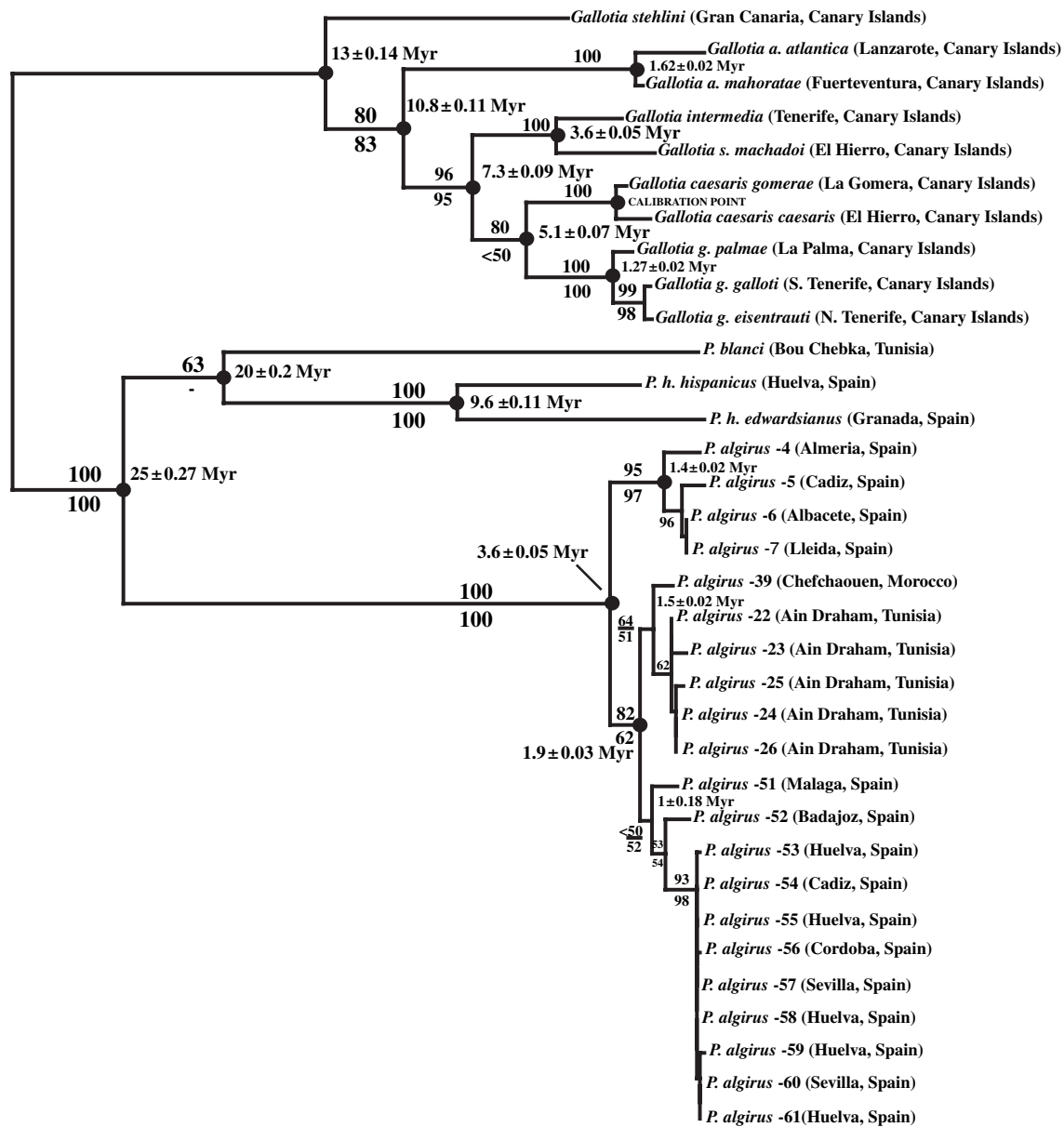
Figure 1 Estimate of relationships of *Psammodromus* and *Gallotia* derived from ML analyses using the model explained in the text, based on partial 12S rRNA, 16S rRNA and cytochrome *b* sequences. Numbers above and below the nodes correspond to bootstrap support from ML and MP analyses, respectively. Estimated ages for major splits are indicated.

initiated approximately 1 Ma, soon after El Hierro was formed and rapid colonization by the ancestor of *G. c. gomerae* occurred from La Gomera. These taxa are suitable for use in calibration as they are sister species and each is monophyletic with low intraspecific variability (Maca-Meyer *et al.*, 2003). Apart from the assumption that El Hierro was colonized rapidly, factors that could affect clock calibrations include stochastic variation at low levels of sequence divergence and the possibility of extinct or unsampled lineages (Emerson *et al.*, 2000a,b; Emerson, 2002), although there is no evidence for any of these occurring in *Gallotia* (González *et al.*, 1996; Barahona *et al.*, 2000; Maca-Meyer *et al.*, 2003).

RESULTS

Phylogenetic relationships

The incongruence length difference (ILD) test (ILD, $P > 0.66$) and the reciprocal 70% bootstrap proportion method showed that the phylogenies derived from the three genes independently were not incongruent, and therefore we decided to carry out a combined analysis including 34 specimens of *Psammodromus* and *Gallotia* that had been sequenced for all three mitochondrial gene regions. In total, the combined data set included 1081 bp (300 bp of cytb, 366 bp of 12S rRNA and
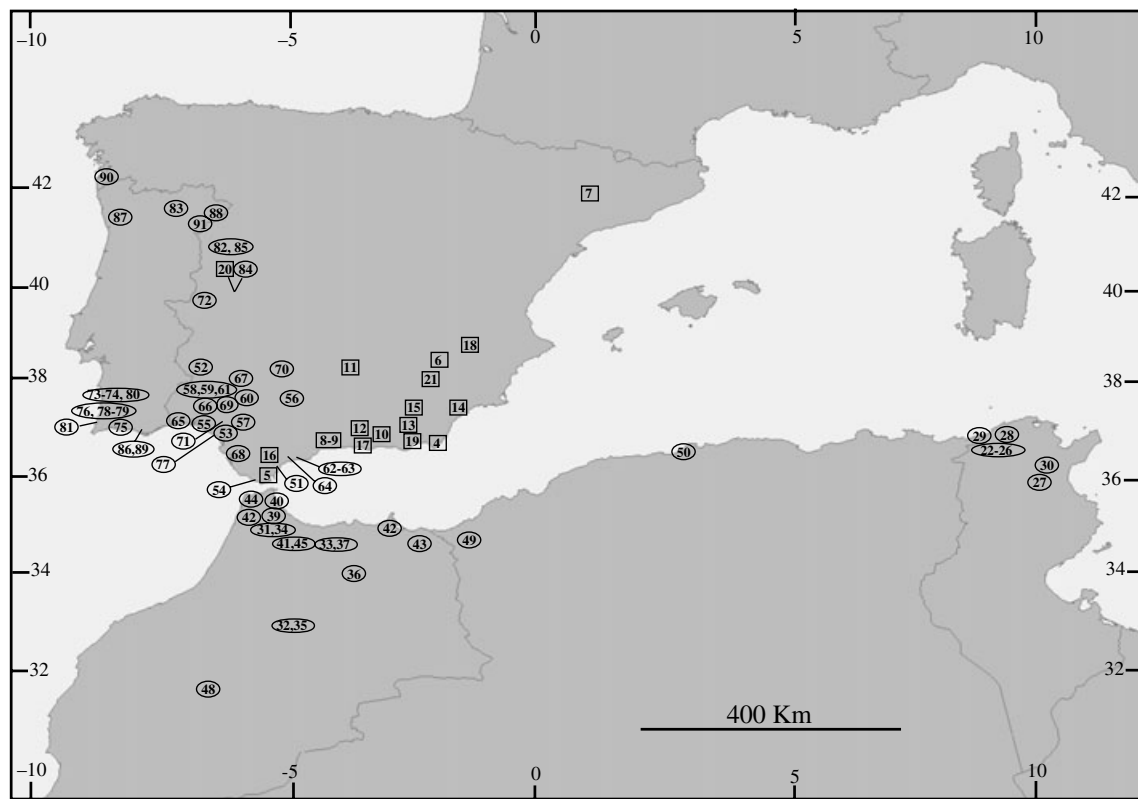
Figure 2 Map showing sampling localities for *Psammodromus algirus*. Numbers correspond to those in Table 1. Ovals mark haplotypes belonging to the 'western clade' and rectangles belong to the 'eastern clade'. The particular genes sequenced for each sample are shown in Table 1.

415 bp of 16S rRNA). Of these, 321 were variable and 265 parsimony-informative. The results of the ML and MP analyses are shown in Fig. 1. Both gave very similar results and only differed in one basal node (see below) and in the degree of resolution of some of the most recent nodes, which were not resolved in the strict consensus of the 21 most parsimonious trees (673 steps). When the log-likelihood value of the ML (GTR + G) tree from Fig. 1 (−4656.0) was compared with the log-likelihood of the same tree constructed under molecular clock assumptions (−4671.6), there was no significant difference between the two (likelihood ratio test statistic, $-2 \log \Delta = 31.07$, which approximates to a χ^2_{32} distribution under the null hypothesis; $P < 0.05$). The sequences could therefore be used for estimating dates.

The results of the combined phylogenetic analyses indicate that *Psammodromus* and *Gallotia* form two separate mono- phyletic groups. According to the clock used here, diversifi- cation in the genus *Psammodromus* might have started in the early Miocene, approximately 12 Myr before speciation started in the genus *Gallotia*.

In the ML tree, the North African *Psammodromus blanci* and Iberian *P. hispanicus* are each other's closest relatives among the species studied here, in agreement with available morphological evidence (Arnold, 1973, 1989). However, the bootstrap support for this clade is very low and it is not recovered in the MP analysis, in which *P. blanci* is sister to all

the remaining representatives of *Psammodromus*. The two subspecies of *P. hispanicus* (*P. h. hispanicus* and *P. h. edwarsianus* Dugès, 1829) form a very well-supported clade that started diverging more than 9 Ma.

All representatives of *P. algirus* included in this analysis form a well-supported clade, which split into two monophyletic groups during the Pliocene, some 3.6 Ma. One of these clades includes specimens from the south-east and north-east of Spain (see Fig. 2; called here the eastern clade) and the other clade includes specimens from the south-west and north-west of Iberia and North Africa (see Fig. 2; called here the western clade). Within this latter assemblage, Iberian and North African specimens form two independent monophyletic groups, albeit with relatively low bootstrap support. According to our clock calibration, they may have split approximately 1.9 Ma, around the beginning of the Pleistocene (see Fig. 1).

In order to explore the phylogeography of *P. algirus* further, another analysis was performed using only 12S rRNA and 16S rRNA mitochondrial DNA sequences but including many additional samples (Fig. 3). The 12S + 16S data set included a total of 771 positions, of which 118 were variable and 103 were parsimony-informative. The results of the ML and MP analyses were very similar and corroborate the existence of both the eastern and western clades within *P. algirus* (see Fig. 3). Relationships within the western clade are not very well supported but three allopatric subclades are discernible: one in
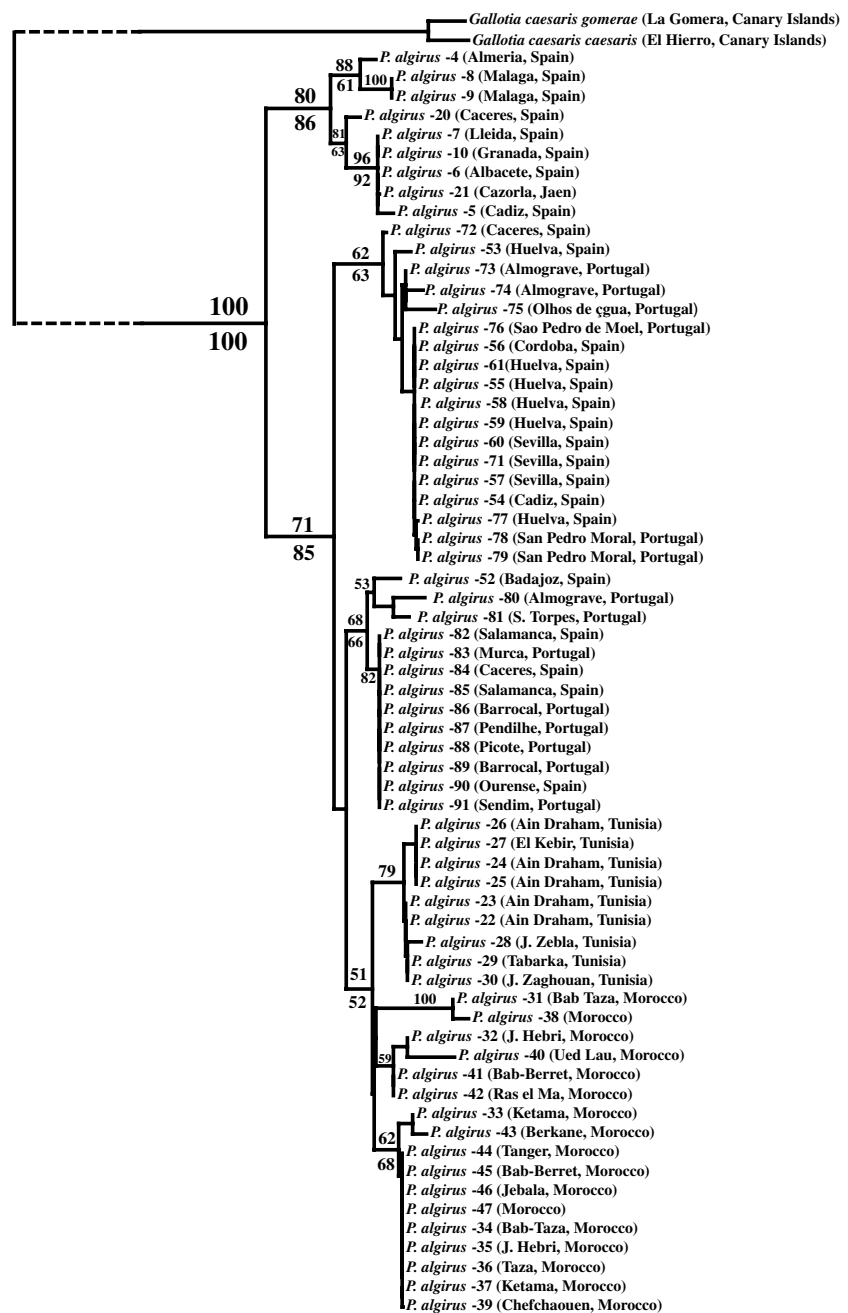
Figure 3 Estimate of relationships of *Psammodromus algirus* derived from ML analyses using the model explained in the text, based on partial 12S rRNA and 16S rRNA sequences. Numbers above and below nodes correspond to bootstrap support from ML and MP analyses respectively. Dashed lines indicating the length of the branches concerned are not proportional to the amount of change.

north-west Iberia, one in the south-west and the last in North Africa. Within the latter there is some structure, with Tunisian animals forming a distinct clade. For an additional set of samples only the short cytochrome *b* fragment was obtained. Analysis of this alone identified the same major groups already discussed (Fig. 2).

DISCUSSION

Psammodromus algirus appears to have had a relatively eventful phylogeographical history. It differentiated into western and eastern clades in the Iberian Peninsula about 3.6 Ma, something that has occurred in several other reptile

and amphibian groups although over a considerable time range. The taxa concerned include *Pleurodeles* newts (Batista *et al.*, 2004; Carranza & Arnold, 2004; Carranza & Wade, 2004), *Discoglossus* frogs (Martínez-Solano, 2004) and perhaps the lacertid *Acanthodactylus erythrurus* (Harris *et al.*, 2004a). Around 1.9 Ma the western clade divided into Iberian and Maghreb sections. As the Strait of Gibraltar was open by this time, parsimony suggests the division is likely to be a result of the transmarine colonization of the Maghreb by *P. algirus*. Some minor divergence subsequently occurred in this last area, Tunisian animals forming a particularly well-supported clade. Divergence also occurred in the Iberian section of the western clade with southern and northern

subclades separating perhaps < 1 Ma. The production of such multiple units within Iberia, beyond a simple west–east split, occurs in other taxa, including fire salamanders (García-París *et al.*, 2003; Escoriza *et al.*, in press), *Alytes* and *Discoglossus* anurans (Martínez-Solano *et al.*, 2004; Martínez-Solano, 2004) and *Podarcis* lizards (Harris *et al.*, 2002; Harris & Sa-Sousa, 2002).

Examining the effects of geological barriers on variation within taxa is a fundamental part of biogeography. The Strait of Gibraltar has been a classical example of such a barrier, given its location separating two continents and its well-known geological history. The Messinian Salinity Crisis, when the strait closed, causing the Mediterranean Sea to dry up, and then opened again, was one of the most profound local geological events during the Neogene and helped shape the flora and fauna of this biodiversity hotspot (Blondel & Aronson, 1999). But there are many examples where this event has had little effect on the history of current populations on each side of the strait. This is true of *P. algirus*, apart from perhaps the opening of the strait slowing its spread into North Africa. As already noted, other groups were similar in also dispersing across the strait subsequent to this event. They include *P. hispanica sensu lato* which moved southwards while the lacertid *A. erythrurus* (Harris *et al.*, 2004a), a gecko, *Tarentola* (Harris *et al.*, 2004b,c), the chameleon, *C. chamaeleon* (Paulo *et al.*, 2002), the tortoise, *Testudo graeca* (Álvarez *et al.*, 2000), the false smooth snake, *M. brevis ibericus* (Carranza *et al.*, 2004b), and a worm lizard, *Blanus* (Vasconcelos *et al.* in press) dispersed to the north.

Like *P. algirus* and some other taxa discussed here, *P. hispanicus* shows west–east differentiation in the Iberian Peninsula with two subspecies, a western *P. h. hispanicus* and an eastern *P. h. edwarsianus*, which were each represented by a single individual in the present analysis. The cytochrome *b* gene fragments used show a 13% divergence between the subspecies, and the molecular clock based on fragments of three genes indicates the separation between them occurred some 9.6 Ma. This genetic divergence is deeper than for many reptile species (Harris, 2002) and is also supported by extensive morphological differences between typical members of each subspecies of *P. hispanicus* (see for instance Boulenger, 1921). This suggests that these deserve to be recognized as separate species. However, much more extensive sampling of *P. hispanicus* will be needed to confirm this.

CONCLUSIONS

Psammodromus algirus appears to be an additional example of a non-volant species that has crossed the Strait of Gibraltar after its formation. It underwent differentiation in Iberia before this event and the two main mtDNA lineages here show a small amount of geographical overlap. It is uncertain if this results from the occurrence of different haplotypes in the same populations, or sympatry between separate taxa. Complementary data from nuclear markers and reassessment of morpho-

logical variation may resolve this issue. The western lineage is the sister taxon to the clade recovered from North Africa. Variation between the lineages of *P. algirus* is considerable, but not as great as that within *P. hispanicus*. The Strait of Gibraltar is a more porous barrier than previously supposed, and extensive sampling around it is needed to understand fully its effect as a geological barrier on terrestrial fauna.

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