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journal homepage: www.elsevier.com/locate/ympevDivergence times and colonization of the Canary Islands by *Gallotia* lizardsSiobhan C. Cox^a, Salvador Carranza^b, Richard P. Brown^{a,*}^aSchool of Natural Sciences & Psychology, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK^bInstitute of Evolutionary Biology (CSIC-UPF), CMIMA, Passeig Marítim de la Barceloneta, 37-49, E-08003 Barcelona, Spain

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ABSTRACT

The Canary Islands have become a model region for evolutionary studies. We obtained 1.8 Kbp of mtDNA sequence from all known island forms of the endemic lizard genus *Gallotia* and from its sister taxon *Psammodromus* in order to reanalyze phylogenetic relationships within the archipelago, estimate lineage divergence times, and reconstruct the colonization history of this group. Well-supported phylogenies were obtained using maximum parsimony and Bayesian inference. Previous studies have been unable to establish the branching pattern at the base of the tree. We found evidence that *G. stehlini* (Gran Canaria) originated from the most basal *Gallotia* node and *G. atlantica* from the subsequent node. Divergence times were estimated under a global clock using Bayesian Markov Chain Monte Carlo methods implemented by three different programs: BEAST, MCMCTREE, MULTIDIVTIME. Node constraints were derived from subaerial island appearance data and were incorporated into the analyses as soft or hard maximal bounds. Posterior node ages differed slightly between programs, possibly due to different priors on divergence times. The most eastern Canary Islands first emerged just over 20 mya and their colonization appears to have taken place relatively quickly, around 17–20 mya. The subsequent node is consistent with cladogenesis due to colonization of Gran Canaria from the eastern islands about 11–13 mya. The western islands appear to have been colonized by a dispersal event from Lanzarote/Fuerteventura in the east to either La Gomera or one of the ancient edifices that subsequently formed Tenerife in the west, about 9–10 mya. Within the western islands, the most recent node that is ancestral to both the *G. intermedia*/*G. gomerana*/*G. simonyi* and the *G. galloti*/*G. caesaris* clades is dated at about 5–6 mya. Subsequent dispersal events between ancient Tenerife islands and La Gomera are dated at around 3 mya in both clades, although the direction of dispersal cannot be determined. Finally, we show that *G. galloti* is likely to have colonized La Palma more than 0.5 Ma after emergence of the island 1.77 mya, while *G. caesaris* from the same clade may have colonized El Hierro very soon after it emerged 1.12 mya. There are tentative indications that the large-bodied endangered *G. simonyi* colonized El Hierro around the same time or even later than the smaller-bodied *G. caesaris*. This study demonstrates the effectiveness of Bayesian dating of a phylogeny in helping reconstruct the historical pattern of dispersal across an oceanic archipelago.

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1. Introduction

The Canary Islands have become a focal point for evolutionary studies during the past 20 years, largely due to their high rates of endemism (e.g., Juan et al., 2000; Emerson, 2002). They also have a well-known geological history, with times of subaerial appearances established for most islands. This offers a temporal framework within which to examine biological diversification. New Bayesian methods provide a robust statistical framework for such an analysis (Thorne et al., 1998; Yang and Rannala, 2006; Drummond and Rambaut, 2007).

Bayesian estimation of lineage divergence times is superior to current maximum likelihood approaches because maximum and/

or minimum limits on the nodes, and the uncertainties associated with them, are correctly incorporated into the analysis through the prior (Yang, 2006, pp. 245–258). Here, island emergence determines the earliest possible time of island colonization allowing specification of an upper limit to the time of between-island divergence. Poor date estimation may occur if a maximal date is unreliable (Ho and Phillips, 2009), which may be difficult to detect when only one node is calibrated. However, incorrect constraints may be detected by comparison of posterior with prior node distributions when multiple calibrations are used (Sanders and Lee, 2007). Successful dating also requires minimal bounds, which may be lacking if only island emergence is used. This problem is avoided for recently-colonized young islands because the ancestral node age will be tightly constrained between zero and the (recent) date of subaerial emergence. Dated phylogenies therefore provide the best method for inferring the historical pattern of dispersal across an

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archipelago, even though this may require several assumptions, particularly in the absence of a fossil record.

The subfamily Gallotiinae within the family Lacertidae comprises two sister lineages that have been assigned to the genera *Gallotia* and *Psammadromus*. The former are endemic to the Canary Islands, while the latter are found in southern Europe and north-west Africa. *Gallotia* originated from a Miocene colonization of the Canaries by an ancestor from southern Europe or northwest Africa (Arnold et al., 2007). Subsequent diversification within the islands has occurred, and most authors recognize seven extant species, each comprising several subspecies (Fig. 1). Previous studies have examined phylogenetic relationships within *Gallotia* using morphology (Thorpe, 1985) or molecular approaches based on RFLPs (Thorpe et al., 1993, 1994), DNA sequence (Thorpe et al., 1994; Gonzalez et al., 1996; Rando et al., 1997; Maca-Meyer et al., 2003; Arnold et al., 2007) or microsatellite DNA (Richard and Thorpe, 2001). There is clear support for two western sister clades with overlapping distributions: the *G. galloti*/*G. caesaris* clade from the islands of La Palma, La Gomera, El Hierro and Tenerife, and the clade containing the larger-bodied endangered lizards *G. simonyi* (El Hierro), *G. gomerana* (called *G. bravoana* by some authors) (La Gomera) and *G. intermedia* (Tenerife) (Rando et al., 1997; Maca-Meyer et al., 2003; Arnold et al., 2007). The latter clade is of considerable interest because it was considered extinct until *G. simonyi* was rediscovered on the island of El Hierro during the 1970s (Böhme and Bings, 1975). This was followed by the relatively recent rediscovery of the Tenerife and La Gomera lizards (Hernandez et al., 2000; Nogales et al., 2001). The existence of subfossils shows that this clade was also found on La Palma (Barahona et al., 2000) but as yet there is no hard evidence to demonstrate its continued survival there. The between-island relationships within the two western clades are well-established and show similarities. In both cases, the Tenerife species (together with La Palma for *G. galloti*/*G. caesaris*) outgroups the (La Gomera, El Hierro) clade (Thorpe et al., 1993, 1994; Rando et al., 1997; Arnold et al., 2007). The two remaining lineages within the *Gallotia* phylogeny are the small-bodied *G. atlantica* from the eastern islands of Fuerteventura and Lanzarote and the large-bodied *G. stehlini* from the central island of Gran Canaria. Previous studies show that these lineages originate from the most basal nodes within the *Gallotia* phylogeny, but their branching order has proved difficult to resolve.

All seven major islands have independent origins and tend to be older in the east and relatively recent in the west (Fig. 1). The oldest islands are Fuerteventura and Lanzarote, with subaerial rocks being dated at 20.4–20.6 million years (Ma) (Coello et al., 1992; Carracedo et al., 1998). The central island of Gran Canaria then emerged 14.5 million years ago (mya) (Carracedo et al., 1998). In the west, the Roque del Conde edifice within the current island

of Tenerife and the island of La Gomera emerged about 11.6 mya (Ancochea et al., 1990; Guillou et al., 2004) and 10.5 mya (Ancochea et al., 2006), respectively. Tenerife is also composed of two additional formerly independent islands that appeared after Roque del Conde: Anaga (6.5 mya) and Teno (7.4 mya) (Guillou et al., 2004). The two most recently emerged islands are in the western extreme of the archipelago. La Palma appeared 1.77 mya (Guillou et al., 2001) while the oldest subaerial rocks on El Hierro have been dated at 1.12 Ma (Guillou et al., 1996). Previous phylogenies all suggest a general east–west pattern of colonization as might be predicted from these ages. La Palma and El Hierro were the last islands to be colonized by *Gallotia*. Other lizards (Brown and Pestano, 1998; Carranza et al., 2002) and bats (Pestano et al., 2003) also appear to have colonized these latter islands soon after their appearance.

There have been previous attempts to estimate divergence times in this group. Most recently, Arnold et al. (2007) used maximum likelihood to estimate divergence times in the Lacertini, including the Gallotiinae, based on 620 bp of mtDNA sequence and a single calibration point. This analysis was unable to fully resolve the branching order for *G. atlantica* and *G. stehlini* but suggested an initial colonization of the Canaries 12.8 mya, and separation of the two western island clades about 6.9 mya. Here, we aim to provide the most rigorous analysis to date using: (1) Bayesian methods which correctly incorporate constraints derived from multiple island appearance data, (2) all available geological data on island appearance, (3) more DNA sequence, (4) a large number of taxa, including *Psammadromus* to allow estimation of the time of origin of *Gallotia*. In addition, we used the divergence times and the phylogeny to infer the historical pattern of island colonization by *Gallotia*.

2. Materials and methods

2.1. Samples and sequences

Sequences were obtained from 76 *Gallotia* and 13 *Psammadromus* specimens covering all species in these genera (Appendix A). All known island forms of *Gallotia* were included with several specimens available for some islands. Whole genomic DNA was extracted from digested blood and tail-tip samples using phenol/chloroform or spin column (DNeasy tissue kit, QIAGEN) extraction methods. Sequences from four mitochondrial genes were amplified using PCR: cytochrome *b* (*cytb*), cytochrome oxidase subunit 1 (*COI*), 12S rRNA, and 16S rRNA (primers are detailed in Appendix B). All sequences were examined carefully to ensure that amplified fragments represented authentic mtDNA rather than numts. Sequences were aligned using ClustalW and manual adjustments made by eye, taking into account 16S rRNA (Brown, 2005) and

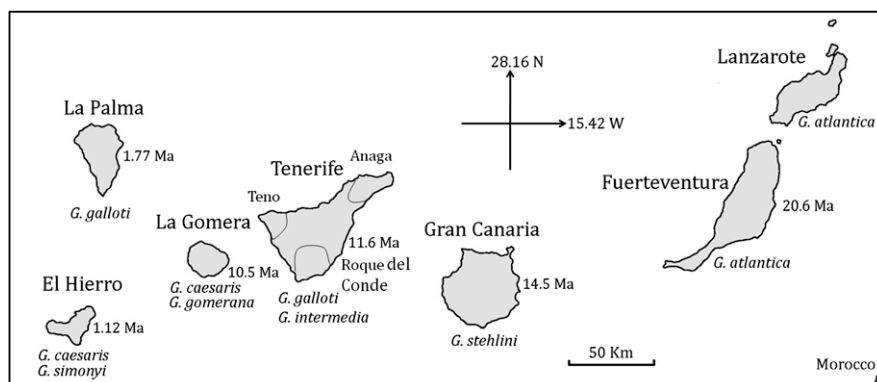


Fig. 1. Canary Island ages and *Gallotia* distribution, by island.

12S rRNA secondary structures (Wuyts et al., 2004). The nuclear *C-mos* gene was also sequenced but was not phylogenetically informative and so the results are not reported.

2.2. Phylogenetic inference

MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist, 2001) was used for Bayesian inference (BI) of the phylogeny. A *Timon lepidus* (= *Lacerta lepida*) sequence from a previous study (AF206595) was selected as an outgroup to the Gallotinae clade. All mtDNA sequence corresponded to either protein-coding or rRNA genes. Strong selective constraints on proteins and rRNA structures cause major differences in substitution rates and other evolutionary features among sites within genes, suggesting that partitioning by function is likely to be more appropriate than partitioning by gene. Empirical analyses support this approach (Brandley et al., 2005). Five functional partitions were therefore used to construct the BI phylogeny, corresponding to the three codon positions from protein-coding sequences and to stem or loop regions from rRNA sequences. The latter were determined using published lizard secondary structures (Brown, 2005; Wuyts et al., 2004). The same GTR+G model was applied to all partitions independently (unlinked) because it incorporates all main features of the evolutionary process. We also ran analyses on the unpartitioned data using (i) the GTR+G model and (ii) the much simpler K80 model. This allowed assessment of the sensitivity of the inferred topology to the substitution model and the partitioning strategy. An approximate method was used to compare among the three models: Bayes factors were calculated using the harmonic means of the likelihoods of trees sampled from the stationary posterior distributions (Nylander et al., 2004). Diffuse Dirichlet priors were used for all parameters (default values in MrBayes). Each analysis was run with four MCMC chains of 4 million generations, with a sampling frequency of 100, and then repeated. Convergence of the resultant eight chains was examined in detail using AWTY (Nylander et al., 2008). A 50% majority-rule consensus tree was used to summarize trees sampled from the stationary posterior.

The phylogeny was also inferred under the maximum parsimony (MP) criterion using the program TNT (Goloboff et al., 2008) in order to examine whether the inferred topology was robust to the method of inference. Heuristic tree searches were performed using 10 random addition sequences. Indels were excluded. This was done for 1000 bootstrap samples obtained from the concatenated sequence (gaps excluded; unweighted) and the trees were summarized using a 50% majority-rule consensus tree.

2.3. Estimation of divergence times

Closely-related haplotypes were excluded from analyses of divergence times to enable more efficient computation, although examples of all island forms were included. *Psammadromus hispanicus edwardsianus*, *P.h. hispanicus*, and *P. blanci* were also excluded because of missing base positions for the COI fragment. This appeared justified because: (i) the aim was to estimate divergence times within *Gallotia* not *Psammadromus*, (ii) longer sequence lengths may reduce the relative influence of the prior on the posterior in divergence times estimation (Brown and Yang, 2010). A single topology representing the inferred phylogeny and containing 20 *Gallotia* and three *Psammadromus* was used in all divergence time analyses. Sequences were partitioned using the five functional partitions described earlier.

The HKY+G model was used because it was the most complex model of DNA substitution that was available in BEAST, MCMCTREE and MULTIDIVTIME. We tested for violation of the molecular clock using a likelihood ratio test (Felsenstein, 1981). HKY+G likelihoods were compared between rooted clock-like and unrooted

nonclock trees using BASEML in PAML 4 (Yang, 2007). The likelihoods of the two trees did not differ significantly ($2\Delta l = 28.55$, $P = 0.13$ [from a χ^2 distribution with 21 d.f.]) and so Bayesian MCMC analyses were computed using a global clock.

Different programs implement slightly different Bayesian MCMC analyses of divergence times. Some of the differences between MULTIDIVTIME (Thorne et al., 1998) and MCMCTREE (Yang, 2007) have been described previously (e.g., Brown and Yang, 2010; Inoue et al., 2010). We used these two programs and BEAST ver. 1.4.8 (Drummond and Rambaut, 2007) on a single topology to examine the sensitivity of the results to different analyses.

All analyses used calibrations from the island ages described in the Section 1 (see Fig. 1). These represented times of earliest possible colonizations of the islands and so were specified as maximal node age constraints. The constraints were applied using island ages as follows. The oldest island within each of the two daughter clades was determined for each node, starting with the root. The maximal age of the node was then constrained to the time of emergence of the younger of these two islands, corresponding to the earliest possible time of between-island dispersal. This could lead to consecutive nodes having the same maximal constraints, in which case constraints were only specified for the oldest node in the sequence. Minimal node ages of 0.5 mya were also used to constrain the (Tenerife, La Palma) and the two (El Hierro, La Gomera) nodes. Previous studies indicate that *Gallotia* have been present on the two most recent islands of La Palma and El Hierro for substantial proportions of their post-emergence periods (e.g., Thorpe et al., 1994). Application of these rather arbitrary minimal constraints therefore avoided proposal states with unrealistically recent node ages.

Unsuitable priors on divergence times can lead to bias in the posterior distributions of specific node ages (see Brown and Yang, 2010). All priors on divergence times specified by MCMCTREE, BEAST and MULTIDIVTIME were therefore assessed by running chains without data, and then examining prior intervals in relation to expected divergence times. One time unit was set equal to 10 Ma in all analyses. Main differences between the analyses are described here. Node ages were constrained by hard bounds in MULTIDIVTIME and BEAST. In MULTIDIVTIME, the prior on times was specified by a Dirichlet distribution with $\text{Minab} = 0.3$ (which seems to provide quite flexible priors on divergence times: Brown and Yang, 2010), while the means and standard deviations of gamma distributions were $\text{rttm} = 2$ and $\text{rtmsd} = 0.5$ for the prior on root age, and $\text{rtrate} = 0.1$ and $\text{rtratesd} = 0.3162$ for the prior on the rate. In MCMCTREE, the age constraints were soft and specified by uniform distributions between the maximum/minimum time constraints with 2.5% tail probabilities above/below these limits (Yang and Rannala, 2006). MCMCTREE uses a birth (λ), death (μ), sampling (ρ) prior on times, which we specified as $\lambda = 5$, $\mu = 5$, $\rho = 0.1$. The transition: transversion rate ratio (kappa_gamma), the shape parameter for rate heterogeneity between sites (alpha_gamma), and the prior on rates (rgene_gamma) were all specified by gamma distributions. Respective means and standard deviations were (7.5, 3.35) for kappa_gamma , (1, 1) for alpha_gamma , and (0.1, 0.3162) for rgene_gamma (as for MULTIDIVTIME). The same gamma distributions were used for these priors in BEAST (unlinked across partitions). We found that Yule and Birth–Death priors on divergence times in BEAST specified rather unsuitable prior distributions for several nodes and so we used a constant population size coalescent prior with the constant. popSize parameter being specified from a Jeffreys prior.

All programs were run three or more times from different starting positions in order to ensure convergence. We analysed 9000 posterior samples from MCMCTREE and MULTIDIVTIME (500,000 generations, sample frequency 50, burnin 50,000), and 180,000

posterior samples from BEAST (2,000,000 generations, sample frequency 100, burnin 2,000,000).

3. Results

3.1. Sequences and alignment

The *Gallotia* specimens provided 53 haplotypes (Appendix A; TreeBASE Accession S2638-M5067). All 13 *Psammodromus* specimens were represented as individual haplotypes. The *Gallotia*, *Psammodromus*, and outgroup sequence alignment consisted of 1786 bp, including 37 indels within the rRNA sequences: 715 bp of cytochrome b, 261 bp COI, 414 bp 16S rRNA, 396 bp 12S rRNA. There was no evidence of numt amplification in any of the sequences. COI sequence from the three *Psammodromus* species/subspecies: *P. blancii*, *P. h. hispanicus*, *P. h. edwardsianus* showed only partial overlap with COI sequence from other specimens, which reduced the length of the sequence used for phylogenetic inference to 1544 bp. Exclusion of these *Psammodromus*, some *Gallotia* haplotypes (see Materials and Methods) and the *T. lepidus* outgroup meant that 1751 bp sequence (excluding indels) were available for analysis of divergence times.

3.2. Phylogeny

The GTR+G partitioned model was strongly favoured over the GTR+G unpartitioned model ($2\text{LnB} = 950$, where Bayes factor is denoted as B) (Nylander et al., 2004). The GTR+G unpartitioned model was similarly favoured over the simple K80 unpartitioned model ($2\text{LnB} = 1896$). Values of $2\text{LnB} > 10$ indicate a very strong difference between models (Kass and Raftery, 1995). Nevertheless, the topologies of the consensus trees (20,000 posterior trees) were insensitive to the different models apart from one minor difference within *G. atlantica*. From this point forward we will refer only to the consensus tree obtained using the GTR+G partitioned model (Fig. 2). All major nodes were supported by clade credibility values of 1.00. We obtained identical relationships among major nodes under the MP criterion with generally high bootstrap support (83–100%), so results are not shown.

The most basal node within *Gallotia* represents the divergence of *G. stehlini* (Gran Canaria) from the remaining *Gallotia* situated on islands to the east and west. The subsequent node represents the divergence of the eastern *G. atlantica* from other *Gallotia* and has a clade credibility value of 1.00. Bootstrap support was also significant (83%) despite it being the most weakly supported major node in the MP analysis. Divergence of the smaller-bodied *G. galloti*/*G. caesaris* clade and the larger-bodied *G. intermedia*/*G. gomerana*/*G. simonyi* clade (both from the western islands) occurs at the subsequent node. Recent between-island divergences are found between La Gomera-El Hierro for both of these clades, Tenerife-La Palma for *G. galloti*, and Fuerteventura-Lanzarote for *G. atlantica* clade. Well-supported within-island divergence is found within the island of Tenerife for *G. galloti*.

3.3. Timing of mtDNA divergence

Substitution rates differed considerably between partitions. For brevity we give only the MCMCTREE posterior mean rates here (95% intervals in parentheses, units are substitutions/site/Ma): 1st codon position, 0.00399 (0.00276–0.00562); 2nd codon position, 0.00073 (0.00040–0.00118); 3rd codon position 0.04020 (0.03393–0.05280); rRNA stems, 0.00286 (0.00200–0.00401); rRNA loops 0.00659 (0.00499–0.00870).

A chronogram based on posterior means from MCMCTREE is shown in Fig. 3. Full details of BEAST, MCMCTREE and MULTIDIV-

TIME prior and posterior intervals are given in Table 1. In this section, we draw attention to the most important results. The 95% posterior intervals for the time of the *Psammodromus*-*Gallotia* divergence are most recent in MULTIDIVTIME (13.4–20.4 Ma), intermediate in BEAST (14.0–20.6 Ma) and most ancient in MCMCTREE (16.3–21.9 Ma). Differences between programs for other nodes were relatively small, with MULTIDIVTIME and BEAST generally providing the most similar posteriors. There was considerable overlap in divergence times between the most basal and the subsequent *Gallotia* nodes in all programs, e.g., 8.0–13.4 mya and 6.6–11.1 mya, respectively, in BEAST. This suggests that the cladogenesis events leading to the *G. stehlini* and *G. atlantica* lineages were quite close in time. The ancestral node to the large- and small-bodied western island clades appear to correspond to the end of the Miocene (posterior intervals were broadly 4/5–7 mya for all programs). The posterior divergence times between *G. intermedia* from Tenerife and the sister (La Gomera, El Hierro) lineage from the larger-bodied endangered clade (e.g., BEAST posterior interval: 1.9–3.5 Ma) overlap with the parallel (Tenerife, (La Gomera, El Hierro)) node in the smaller-bodied *G. galloti*/*G. caesaris* clade (BEAST: 2.3–4.2 Ma). The (La Gomera, El Hierro) divergence times within these respective clades are constrained by the recent appearance of El Hierro, causing considerable overlap between them. However, the shapes of the posteriors (not shown) and the upper 97.5% posterior limits provide a tentative indication that the small-bodied *G. galloti*/*G. caesaris* clade may have colonized El Hierro slightly before the large-bodied clade. Divergence of La Palma *G. galloti* occurred around the same time, despite the subaerial emergence of La Palma predating El Hierro by 0.5 Ma.

4. Discussion

4.1. Phylogeny

Our mtDNA phylogeny uses the most extensive sampling of *Gallotia* and *Psammodromus* to date and confirms most relationships previously inferred using mtDNA and microsatellites (Thorpe et al., 1994; Rando et al., 1997; Richard and Thorpe, 2001; Maca-Meyer et al., 2003; Arnold et al., 2007). Unlike previous studies, we find strong support for the hypothesis that the *G. stehlini* lineage from Gran Canaria originates from the most basal *Gallotia* node and *G. atlantica* from the subsequent node. This does not necessarily mean that Gran Canaria was colonized first (see later), but it does indicate that the western *Gallotia* lineages originated directly from *G. atlantica* in the east, rather than via this central island. We also show the interesting parallel relationships within the large-bodied endangered clade and the smaller-bodied *G. galloti*/*G. caesaris* clade from the western islands, i.e., (Tenerife, (La Gomera, El Hierro)). Relationships within *Psammodromus* are the same as those described previously (Carranza et al., 2006).

4.2. Estimation of divergence times

MULTIDIVTIME and BEAST analyses provided the most similar divergence times, while those in MCMCTREE were often slightly higher. The only priors on node ages that were potentially inappropriate were specified by BEAST: prior intervals on nodes 3, 5 and 8 were quite deviant from the posteriors. Poor priors can lead to significant bias in posterior divergence times in relaxed clock analyses (Brown and Yang, 2010). This does not appear to have caused poor estimation here because the posteriors on these nodes were quite similar to those for MULTIDIVTIME (where prior intervals appeared more appropriate). Instead, other components of the analyses were probably more influential. The impact of using soft bounds was examined by recompiling MCMCTREE with upper and lower tail

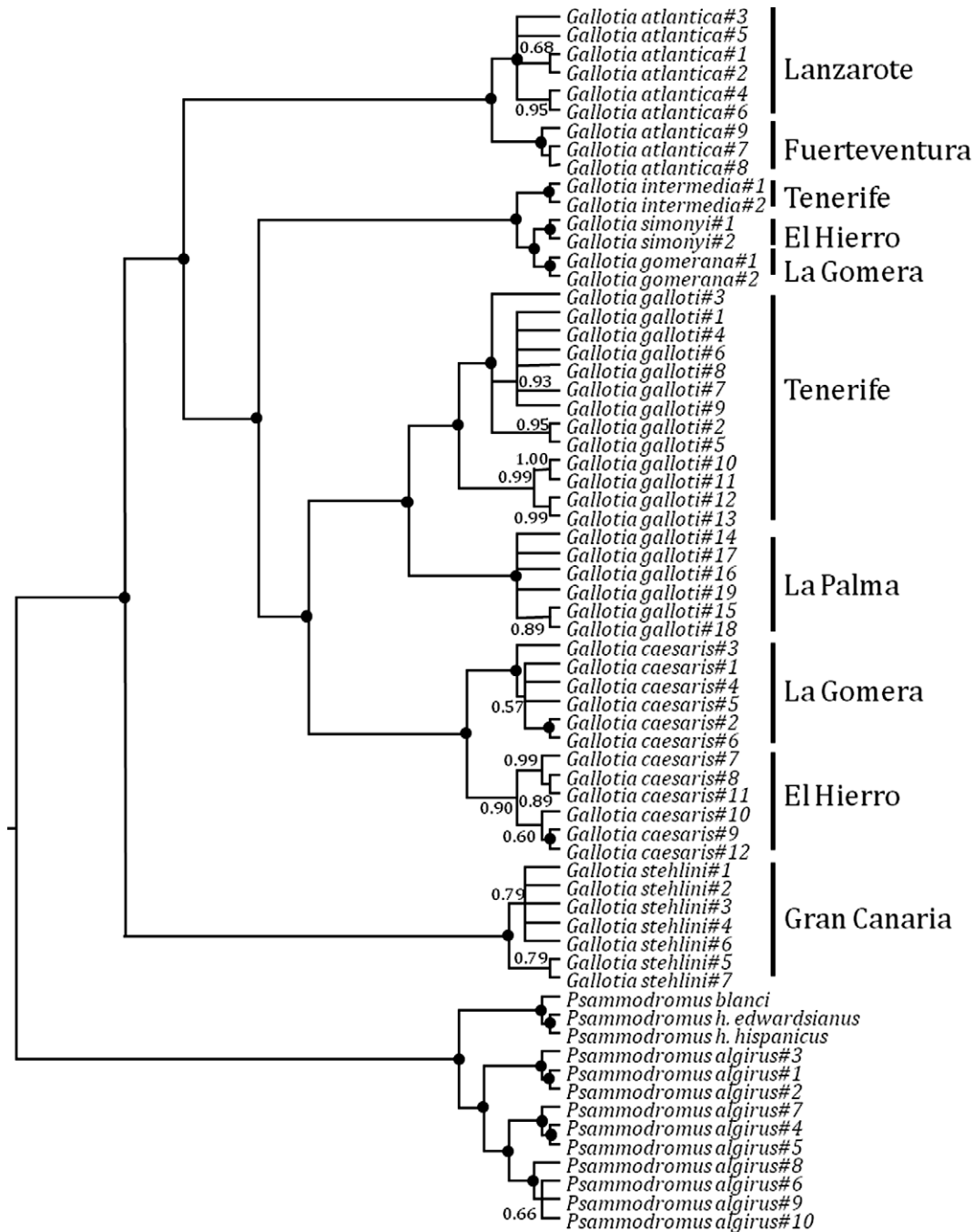


Fig. 2. Bayesian phylogeny showing relationships among ingroup haplotypes. Bayesian clade credibility values of 1.00 are shown as a filled circle on the node. Other credibility values are given as proportions.

probabilities set to 10^{-300} to emulate hard bounds. This led to a decrease in the mean of the root from 19.7 to 19.2 Ma, with a similar effect for more recent nodes. Nevertheless, posterior means in this hard bound analysis were still generally greater than those estimated by MULTIDIVTIME and BEAST indicating that use of soft bounds is not the only explanation. One potential cause of the difference was the widths of the prior intervals for many nodes ages in BEAST and MULTIDIVTIME which generally extended down to more recent times than for MCMCTREE. This could have led to lower posterior intervals.

Anderson et al. (2009) have recently discussed divergence times of the Canary Island flora and criticized the use of maximal island ages in Bayesian dating. Use of a single (incorrect) calibra-

tion could lead to incorrect times on all nodes. However, use of multiple constraints should allow detection of this error, particularly under the global clock. A conflict between correct and incorrect calibrations on the same tree should be manifest in the posterior distributions of divergence times when hard bounds are used. For example, it could be contended that the root age was underestimated here either because: (1) geological dating of the eastern islands underestimated their true age, or (2) colonization occurred via an older, now-submerged island. However, conflict with more recent constraints would lead to unusual posterior distributions, such as a posterior compressed around the maximal root constraint (assuming that maximal constraints on other islands did not overestimate other island emergence in the same

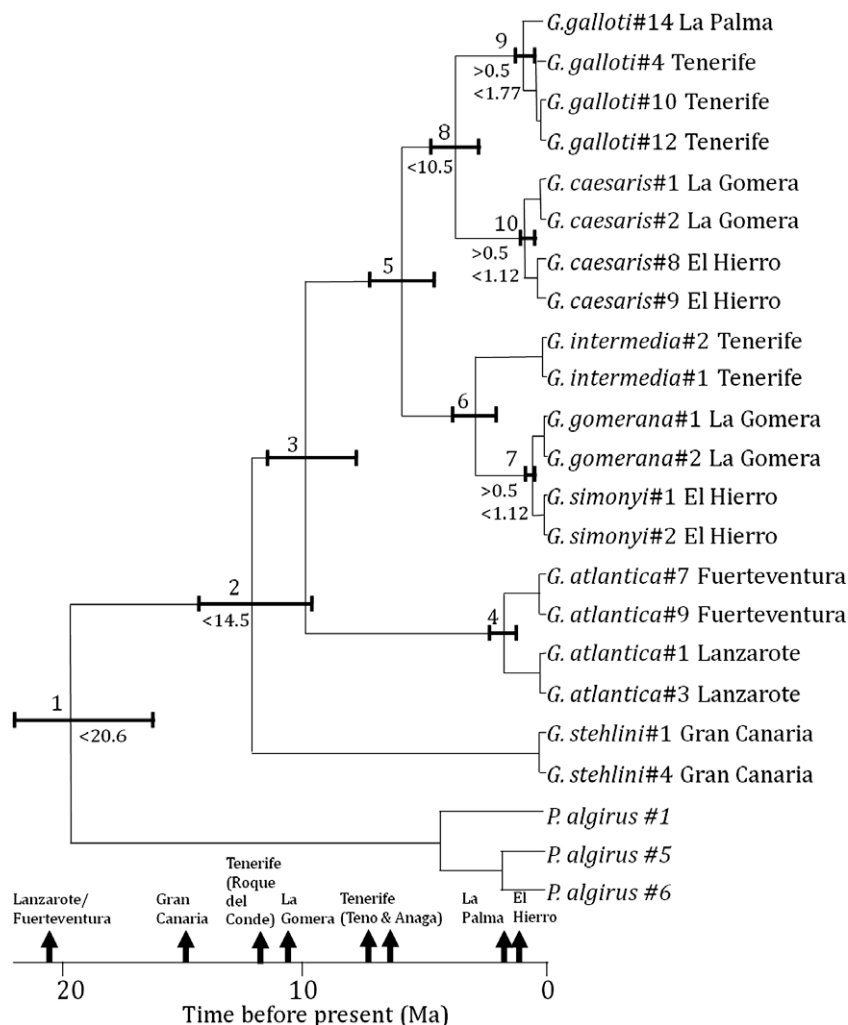


Fig. 3. Chronogram showing results from MCMCTREE in relation to dates of island emergence. Divergence times are posterior means, with 95% posterior intervals represented as bars on nodes. Node numbers are those used in Table 1. Maximal (<) and minimal (>) node constraints are given in Ma.

Table 1

Bayesian estimation of divergence times. Posterior means and 95% intervals (in parentheses) are given in mya. Node numbers are shown in Fig. 3.

Node	BEAST		MCMCTREE		MULTIDIVTIME	
	Prior	Posterior	Prior	Posterior	Prior	Posterior
1 (Root)	10.98 (3.57, 19.73)	17.72 (13.98, 20.60)	16.14 (9.00, 21.02)	19.66 (16.29, 21.92)	14.51 (8.92, 20.00)	17.50 (13.43, 20.38)
2 (<i>G. stehlini</i> , W & E <i>Gallotia</i>)	6.03 (1.68, 11.81)	10.72 (8.00, 13.38)	11.04 (5.47, 14.67)	12.17 (9.78, 14.29)	8.45 (1.48, 14.13)	12.61 (9.51, 14.42)
3 (<i>G. atlantica</i> , W <i>Gallotia</i>)	3.86 (1.11, 7.57)	8.85 (6.62, 11.07)	8.06 (3.31, 11.65)	9.95 (7.95, 11.61)	5.11 (0.98, 11.18)	9.07 (6.61, 11.29)
4 <i>G. atlantica</i> : (Lanzarote,Fuerteventura)	1.52 (0.03, 3.90)	1.51 (0.99, 2.04)	3.67 (0.36, 10.27)	1.74 (1.24, 2.34)	3.48 (0.04, 10.39)	1.34 (0.83, 2.16)
5 (Small W <i>Gallotia</i> , large W <i>Gallotia</i>)	2.54 (0.84, 4.89)	5.25 (3.85, 6.65)	6.78 (2.56, 10.77)	5.96 (4.71, 7.29)	2.49 (0.72, 9.21)	5.61 (4.11, 7.24)
6 (<i>G. intermedia</i> ,(<i>G. simonyi</i> + <i>G. gomerana</i>))	1.43 (0.52, 2.80)	2.67 (1.86, 3.51)	4.36 (0.87, 9.57)	2.93 (2.15, 3.86)	1.66 (0.06, 6.93)	2.60 (1.75, 3.60)
7(<i>G. simonyi</i> , <i>G. gomerana</i>)	0.75 (0.50, 1.07)	0.61 (0.50, 0.80)	0.80 (0.50, 1.12)	0.57 (0.47, 0.79)	0.80 (0.52, 1.10)	0.61 (0.50, 0.85)
8 (<i>G. galloti</i> , <i>G. caesaris</i>)	1.55 (0.58, 2.88)	3.27 (2.32, 6.65)	4.51 (1.17, 9.59)	3.73 (2.83, 4.73)	1.34 (0.60, 4.39)	3.14 (2.19, 4.28)
9 <i>G. galloti</i> : (La Palma, Tenerife)	0.91 (0.50, 1.55)	0.79 (0.50, 1.06)	1.11 (0.50, 1.77)	0.94 (0.61, 1.33)	0.97 (0.53, 1.68)	0.81 (0.52, 1.24)
10 <i>G. caesaris</i> : (El Hierro, La Gomera)	0.77 (0.50, 1.07)	0.77 (0.50, 1.02)	0.81 (0.50, 1.12)	0.89 (0.59, 1.12)	0.81 (0.52, 1.10)	0.68 (0.50, 1.00)

way). This pattern was not found in the analyses we present here. Further evidence that the constraints were broadly correct was provided by the similarities between soft- and hard-bound analyses (Yang and Rannala, 2006).

4.3. Island colonization

Use of divergence dates to infer timing of individual island colonization requires knowledge of the geographic pattern of dispersal. To achieve this, internal nodes on the tree need to be assigned to geographical locations. This is only reliable when external evidence is available (Brown and Pestano, 1998). Fossil data are often lacking but data on island emergence times may provide the information required. Assuming no undocumented extinctions, the island associated with an ancestral node will also be one of the descendant tip islands, and represent cladogenesis due to a dispersal event between two of them. Tip islands in which subaerial emergence postdates the estimated internal node age can be ruled out, reducing the number of putative ancestral islands. When single islands are determined for consecutive nodes, then colonization events can be inferred unambiguously. We used this general procedure to infer patterns of colonization. We also imposed an additional rule when more than one colonization event was possible: colonization of uninhabited islands was preferred over back-colonization. The key features of the inferred historical dispersal that we describe below are shown in Fig. 4.

Timing of colonization of the Canaries was previously estimated at 12.8 mya using 620 bp of mtDNA (Arnold et al., 2007). Here,

none of the analyses provided 95% posterior intervals on the root that overlapped with this value. Posterior means suggest that the most likely time of colonization was 17–20 mya.

Which was the first island to be colonized? If the MCMCTREE analysis is correct then the eastern islands would have been colonized first because the posterior interval on the root node predates the appearance of all other islands (Fig. 3) (in fact even MULTIDIVTIME and BEAST root age posteriors also show only limited overlap with the emergence time of Gran Canaria). Under this scenario, the subsequent node represents the colonization of Gran Canaria around 12 mya, giving rise to the *G. stehlini* lineage. The western islands were then colonized from Fuerteventura/Lanzarote in the east, possibly around 9 or 10 mya although posterior intervals on this node were quite wide (e.g., 8.0–11.6 mya in MULTIDIVTIME). The only emerged western islands that were available for colonization would have been La Gomera and Roque del Conde (one of the three ancient islands that subsequently became Tenerife), indicating that these provided the centers of origin for the two western clades.

Gallotia underwent considerable diversification after it had reached the western islands. The genesis of the larger-bodied *G. simonyi*/*G. gomerana*/*G. intermedia* and the smaller *G. gallotia*/*G. caesaris* lineages is dated at around 6 mya. The posterior intervals for this node indicate that it represents dispersal between two of the four western islands that existed at that time, namely, Anaga, Teno, Roque del Conde and La Gomera.

The large-bodied western clade evolved from the ancestor of all western island *Gallotia*. The most basal node in this group repre-

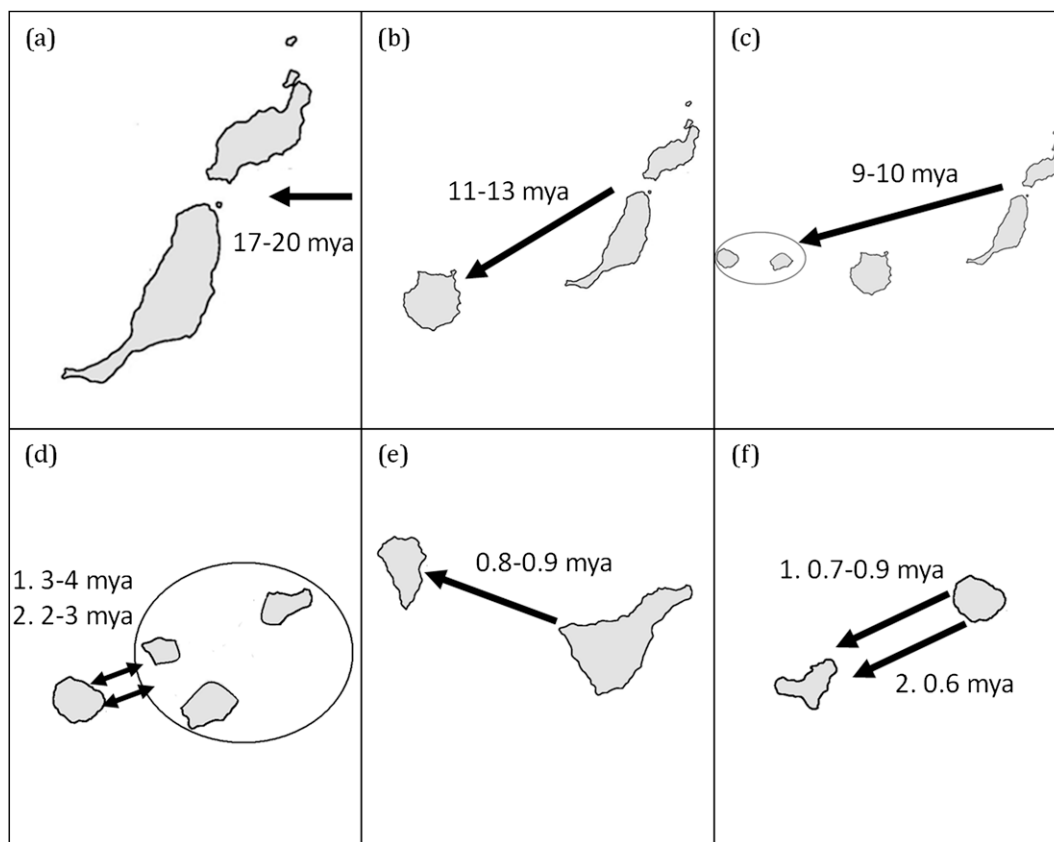


Fig. 4. Main components of the inferred colonization of the Canary Islands by *Gallotia*: (a) Colonization of the Eastern Islands, (b) colonization of Gran Canaria from the Eastern Islands, (c) Colonization of the early Western Islands from the Eastern Islands, (d) Dispersal between La Gomera and ancient Tenerife islands by ancestors of *G. galloti/caesaris* (1) and *G. intermedia/gomerensis* (2), (e) Colonization of La Palma from Tenerife by *G. galloti* (although there is clear evidence that La Palma was also colonized by a member of the *G. intermedia/gomerana/simonyi* clade, no inferences on timing/direction of dispersal can be made due to lack of DNA evidence), and (f) colonization of El Hierro from La Gomera by *G. caesaris* (1) and by the ancestor of *G. gomerana* (2). Dates are based on posterior means from the three dating analyses.

sents a colonization event between two of the older western islands, one of which would be La Gomera, close to 3 mya. This gave rise to the currently endangered Tenerife species *G. intermedia*. The final clade in this group comprises the endangered *G. simonyi* and *G. gomerana* and is due to the colonization of El Hierro from La Gomera. Upper 95% limits on the posterior distributions indicate that this was less than 0.85 mya (in all analyses).

The smaller-bodied *G. galloti*/*G. caesaris* evolved in parallel to the large-bodied clade. First, the most basal node also appears to have originated by dispersal between two of the ancient western islands (one of which would be La Gomera). This may have occurred earlier than in the larger-bodied clade, i.e., more than 3 mya, although posterior intervals show considerable overlap. There is tentative evidence that dispersal between La Gomera and El Hierro also occurred slightly earlier than the parallel event in the large-bodied lizards: the posteriors allow for almost immediate colonization of El Hierro after it appeared 1.12 mya. *G. galloti* seems to have colonized La Palma at least 0.5 Ma after its subaerial emergence, i.e., around the time that members of this clade colonized El Hierro from La Gomera. We cannot compare colonization of La Palma between this clade and the larger-bodied clade because no sequence data are available from the latter. This may be resolved in the future by DNA extraction from suitable ancient remains, or the discovery of an extant population.

The patterns of diversification in *Gallotia* show some parallels with *Chalcides* skinks from the Canary Islands (Brown and Pestano, 1998; Carranza et al., 2008). *Chalcides* also colonized El Hierro from La Gomera soon after its appearance. The other Canary reptile, *Tarentola* geckos, show greater dispersal capacities than *Gallotia* or *Chalcides* which seems to have provided a different colonization history. For example, one *Tarentola* lineage seems to have colonized El Hierro from the Selvagens, 165 km to the north of the Canaries (Carranza et al., 2002). There is evidence that other groups, such as *Pipistrellus* bats and *Tarphius* beetles also dispersed from La Gomera to El Hierro about 1 mya or less (Pestano et al., 2003; Emerson and Oromi, 2005), although *Pimelia* beetles colonized El Hierro from the island of La Palma (Juan et al., 1995).

While the inferred pattern of colonization appears consistent with island emergence, we cannot ignore the possible influence of factors such as colonization of currently submerged islands. Several seamounts exist around the Canaries that may have been exposed during periods of low sea levels. For example, the old Dacia seamount to the North of Lanzarote is currently only 120 m below sea level (Geldmacher et al., 2005). However, this seamount is further away than the Selvagens, which have no *Gallotia* on them. *Gallotia* are not present on any present-day islands outside the archipelago which suggests that their diversification has also taken place within this small geographical area.

5. Conclusion

We show that the *Gallotia* ancestor colonized the Canary Islands in the early Miocene, soon after the appearance of the most eastern islands. The next detectable cladogenesis event was some 5 Ma later, and can probably be attributed to colonization of the central island of Gran Canaria. The genus reached the western islands from the most eastern islands around 9–10 mya and subsequently showed major diversification as more islands emerged. Ten of the eleven individual island lineages have originated since this time (not including within-island cladogenesis). Finally, the divergence times and pattern of colonization that we describe could be refined in the future by (i) extensive studies of fossil material from volcanic deposits, both from the Canary Islands and currently submerged seamounts in the region (in addition to DNA analyses of the large-bodied lizard from La Palma), (ii) accounting for ancestral polymorphism within islands.

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Appendix A

Specimens used.

Species	Haplo-type number	Location	Latitude/ Longitude
<i>Gallotia atlantica</i>	1	Arrecife, Lanzarote	28.981232/ –13.526589
<i>G. atlantica</i>	1	Montaña Baja, Lanzarote	28.861275/ –13.855605
<i>G. atlantica</i>	2	Atlante del Sol, Lanzarote	28.891480/ –13.871861
<i>G. atlantica</i>	3	Malpaís de la Corona, Lanzarote	29.215697/ –13.468151
<i>G. atlantica</i>	4	Malpaís de la Corona, Lanzarote	29.215697/ –13.468151
<i>G. atlantica</i>	5	Malpaís de la Corona, Lanzarote	29.215697/ –13.468151
<i>G. atlantica</i>	6	Malpaís de la Corona, Lanzarote	29.215697/ –13.468151
<i>G. atlantica</i>	7	Tindaya, Fuerteventura	28.587873/ –13.980391
<i>G. atlantica</i>	8	Pájara, Fuerteventura	28.350592/ –14.107613
<i>G. atlantica</i>	9	Tuineje, Fuerteventura	28.324174/ –14.047583
<i>G. stehlini</i>	1	Barranco del Tauro, Gran Canaria	27.807587/ –15.725890
<i>G. stehlini</i>	2	Galdar, Gran Canaria	28.144045/ –15.650355
<i>G. stehlini</i>	3	Galdar, Gran Canaria	28.144045/ –15.650355
<i>G. stehlini</i>	4	Galdar, Gran Canaria	28.144045/ –15.650355
<i>G. stehlini</i>	4	Galdar, Gran Canaria	28.144045/ –15.650355
<i>G. stehlini</i>	5	Tamaraceite, Gran Canaria	28.098765/ –15.472596
<i>G. stehlini</i>	6	Tamaraceite, Gran Canaria	28.098765/ –15.472596
<i>G. stehlini</i>	7	Tamaraceite, Gran Canaria	28.098765/ –15.472596
<i>G. simonyi</i>	1	El Risco de Tibataje, El Hierro (from breeding center).	Captive
<i>G. simonyi</i>	1	El Risco de Tibataje, El Hierro (from breeding center).	Captive
<i>G. simonyi</i>	1	El Risco de Tibataje, El Hierro (from breeding center).	Captive

Appendix A (continued)

Species	Haplo-type number	Location	Latitude/Longitude	Species	Haplo-type number	Location	Latitude/Longitude
<i>G. simonyi</i>	1	El Risco de Tibataje, El Hierro (from breeding center).	Captive	<i>G. galloti</i>	3	Las Cañadas del Teide, Tenerife	Exact locality unknown
<i>G. simonyi</i>	1	El Risco de Tibataje, El Hierro (from breeding center).	Captive	<i>G. galloti</i>	5	Las Caletillas, Tenerife	28.350296/ –16.373020
<i>G. simonyi</i>	1	El Risco de Tibataje, El Hierro (from breeding center).	Captive	<i>G. galloti</i>	6	Aguerche, Tenerife	28.253620/ –16.443270
<i>G. simonyi</i>	1	El Risco de Tibataje, El Hierro (from breeding center).	Captive	<i>G. galloti</i>	7	Adeje, Tenerife	28.122516/ –16.722672
<i>G. simonyi</i>	1	El Risco de Tibataje, El Hierro (from breeding center).	Captive	<i>G. galloti</i>	7	El Fralle, Tenerife	28.011212/ –16.673655
<i>G. simonyi</i>	1	El Risco de Tibataje, El Hierro (from breeding center).	Captive	<i>G. galloti</i>	7	Tijoco Alto, Tenerife	28.167142/ –16.734572
<i>G. simonyi</i>	2	El Risco de Tibataje, El Hierro (from breeding center).	Captive	<i>G. galloti</i>	8	Tijoco Alto, Tenerife	28.167142/ –16.734572
<i>G. simonyi</i>	2	El Risco de Tibataje, El Hierro (from breeding center).	Captive	<i>G. galloti</i>	7	Las Manchas, Tenerife	28.284040/ –16.799398
<i>G. simonyi</i>	2	El Risco de Tibataje, El Hierro (from breeding center).	Captive	<i>G. galloti</i>	9	Los Carrizales, Tenerife	28.319298/ –16.854083
<i>G. simonyi</i>	2	El Risco de Tibataje, El Hierro (from breeding center).	Captive	<i>G. galloti</i>	10	Aguas, Tenerife	Exact locality unknown
<i>G. intermedia</i>	1	Acantilado de los Gigantes, Tenerife	Exact locality unknown	<i>G. galloti</i>	10	Anaga, Tenerife	28.516994/ –16.207907
<i>G. intermedia</i>	1	Acantilado de los Gigantes, Tenerife	Exact locality unknown	<i>G. galloti</i>	11	Anaga, Tenerife	28.516994/ –16.207907
<i>G. intermedia</i>	1	Acantilado de los Gigantes, Tenerife	Exact locality unknown	<i>G. galloti</i>	12	La Laguna, Tenerife	28.487016/ –16.318979
<i>G. intermedia</i>	2	Acantilado de los Gigantes, Tenerife	Exact locality unknown	<i>G. galloti</i>	13	Anaga, Tenerife	28.516994/ –16.207907
<i>G. intermedia</i>	2	Acantilado de los Gigantes, Tenerife	Exact locality unknown	<i>G. galloti</i>	14	Los Canarios, La Palma	28.499633/ –17.824883
<i>G. intermedia</i>	2	Acantilado de los Gigantes, Tenerife	Exact locality unknown	<i>G. galloti</i>	15	Fuencaliente de La Palma	28.508131/ –17.840037
<i>G. intermedia</i>	2	Acantilado de los Gigantes, Tenerife	Exact locality unknown	<i>G. galloti</i>	16	Malpaises, La Palma	28.573022/ –17.772792
<i>G. gomerana</i>	1	Risco de las Américas, La Gomera (from breeding center).	Captive	<i>G. galloti</i>	14	Playa los Nogales, La Palma	28.739896/ –17.752499
<i>G. gomerana</i>	2	Risco de las Américas, La Gomera (from breeding center).	Captive	<i>G. galloti</i>	17	Ramírez, La Palma	28.812163/ –17.768323
<i>G. gomerana</i>	1	Risco de las Américas, La Gomera (from breeding center).	Captive	<i>G. galloti</i>	18	Gallegos, La Palma	28.831835/ –17.837887
<i>G. gomerana</i>	2	Risco de las Américas, La Gomera (from breeding center).	Captive	<i>G. galloti</i>	19	Puntagorda, La Palma	28.757765/ –17.960321
<i>G. gomerana</i>	1	Risco de las Américas, La Gomera (from breeding center).	Captive	<i>G. galloti</i>	19	Tazacorte, La Palma	28.649101/ –17.890497
<i>G. gomerana</i>	2	Risco de las Américas, La Gomera (from breeding center).	Captive	<i>G. caesaris</i>	1	Playa de La Hermigua, La Gomera	28.180552/ –17.185069
<i>G. galloti</i>	1	Cruz Grande, Tenerife	28.355614/ –16.784512	<i>G. caesaris</i>	2	Playa Santiago, La Gomera	28.030704/ –17.200091
<i>G. galloti</i>	2	Close to Fuente del Bardo, Tenerife	28.362539/ –16.623222	<i>G. caesaris</i>	3	La Dama, La Gomera	28.051529/ –17.299888
<i>G. galloti</i>	3	Vilafior, Tenerife	28.178085/ –16.634714	<i>G. caesaris</i>	4	Tazo, La Gomera	28.175699/ –17.321534
<i>G. galloti</i>	4	Puerto de Güimar, Tenerife	28.299286/ –16.373091	<i>G. caesaris</i>	5	Tazo, La Gomera	28.175699/ –17.321534
				<i>G. caesaris</i>	6	Playa Santiago, La Gomera	28.030704/ –17.200091

(continued on next page)

Appendix A (continued)

Species	Haplo-type number	Location	Latitude/Longitude
<i>G. caesaris</i>	7	Los Llanillos, El Hierro	27.752340/ –18.035900
<i>G. caesaris</i>	8	Tamaduste, El Hierro	27.824452/ –17.896616
<i>G. caesaris</i>	9	Los Llanillos, El Hierro	27.752340/ –18.035900
<i>G. caesaris</i>	10	Los Llanillos, El Hierro	27.752340/ –18.035900
<i>G. caesaris</i>	11	Tamaduste, El Hierro	27.824452/ –17.896616
<i>G. caesaris</i>	12	Tamaduste, El Hierro	27.824452/ –17.896616
<i>Psammodromus blanci</i>		Bou Chebka (Tunisia)	35.173325/ 8.438130
<i>P. hispanicus edwardsianus</i>		Sierra de Baza, Granda (Spain)	37.251750/ –2.756211
<i>P.h. hispanicus</i>		Cabeza de Buey, Badajoz (Spain)	Exact locality unknown
<i>P. algirus</i>	1	Salinas Cabo de Gata, Almería (Spain)	36.761443/ –2.224257
<i>P. algirus</i>	2	Tartareu, Lleida (NE Spain)	41.921560/ 0.718136
<i>P. algirus</i>	3	Rio Seco, Granada (SE Spain)	Exact locality unknown
<i>P. algirus</i>	4	Ain Draham (Tunisia)	36.781468/ 8.684154
<i>P. algirus</i>	5	Sidi Freig (Algeria)	Exact locality unknown
<i>P. algirus</i>	6	Matalascañas, Huelva (SW Spain)	37.013583/ –6.562107
<i>P. algirus</i>	7	Chefchaouen (Morocco)	35.181507/ –5.283184
<i>P. algirus</i>	8	Ayamonte, Huelva (SW Spain)	37.247559/ –7.390702
<i>P. algirus</i>	9	Cañada de los Pájaros, Sevilla (SW Spain)	37.259724/ –6.062965
<i>P. algirus</i>	10	Doña Rama, Córdoba (SW Spain)	Exact locality unknown

Appendix B

Primers used for PCR amplification and cycle sequencing reactions.

Name/direction	Source	Gene
CB1 (F)	Kocher et al. (1989)	Cytb
CGSim (R)	Carranza et al. (1999)	Cytb
L15369 (F)	Fu et al. (2000)	Cytb
H15915 (R)	Fu et al. (2000)	Cytb
COI (F)	Palumbi (1996)	COI
COI (R)	Palumbi (1996)	COI
12L (F)	Kocher et al. (1989)	12S
12H (R)	Kocher et al. (1989)	12S
16SL1 (F)	Carranza et al. (1999)	16S
16SH1 (R)	Carranza et al. (1999)	16S

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