Biological Journal of the Linnean Society, 2003, **80**, 659–670. With 3 figures

Status and relationships of the extinct giant Canary Island lizard *Gallotia goliath* (Reptilia: Lacertidae), assessed using ancient mtDNA from its mummified remains

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Received 13 November 2002; accepted for publication 22 May 2003

Ancient mitochondrial DNA sequences (378 base pairs of cytochrome *b* and 368 of 12S rRNA) extracted from a mummified extinct giant lizard, *Gallotia goliath*, from eastern Tenerife, Canary Islands, were used to assess the species status and relationship of this form within the genus. *G. goliath* is clearly a member of the *G. simonyi* group of the western Canary islands (Tenerife, La Gomera, El Hierro and La Palma) and is not closely related to the giant *G. stehlini* of Gran Canaria. Contrary to recent opinion, it is phylogenetically distinct, within the *G. simonyi* group, from the extant *G. simonyi* of El Hierro and also from the recently discovered live *G. gomerana* on La Gomera and from *G. intermedia* in north-western Tenerife. It may be the sister taxon of either all the other members of the *G. simonyi* group or of *G. intermedia*. The phylogenetic distinctness of *G. goliath* makes Tenerife unique among oceanic islands in having had one giant and two medium-sized lizard species that were probably substantially herbivorous, the others being *G. intermedia* and *G. galloti*. *Gallotia* shows great community differences on other islands in the Canaries, two having a single small species, one a single giant, and three a giant and a medium-sized form.

ADDITIONAL KEYWORDS: 12S rRNA – ancient DNA – cytochrome *b* – evolution – phylogeny.

INTRODUCTION

Lacertid lizards of the endemic genus *Gallotia* Boulenger, 1916, together with the geckos *Tarentola* (Carranza *et al*. 2000, 2002; Nogales *et al*., 1998), and the skinks *Chalcides* (Brown & Pestano, 1998), are the only reptile groups to have successfully colonized the Canary Islands and radiated within them. Currently, seven living species of *Gallotia* are recognized, based on both morphological and molecular data (González *et al*., 1996; López-Jurado, Mateo & Guillaume, 1997; Rando *et al*., 1997; Bischoff, 1998; Hernández, Nogales & Martín, 2000; Nogales *et al*., 2001): *G. atlantica* on the eastern islands of Fuerteventura and Lanzarote, the giant *G. stehlini* on Gran Canaria, *G. galloti* on Tenerife and La Palma, *G. caesaris* on La Gomera and El Hierro, and the three giant lizards of the western Canary Islands, namely, *G. simonyi* on El Hierro, *G. intermedia* on Tenerife, and *G. gomerana* on La Gomera (Fig. 1). *G. intermedia* was only discovered in 1996 while *G. gomerana*, originally described from fossils, was found alive in 2000 (Hernández *et al*., 2000; Nogales *et al*., 2001). Phylogenetic analyses of the Lacertidae using morphology (Arnold, 1973, 1989) and molecules (Fu, 1998, 2000; Harris, Arnold & Thomas, 1998) shows that *Gallotia* is monophyletic and most probably related to *Psammodromus* of south-west Europe and north-west Africa. Further molecular analysis including fewer outgroups but almost all extant *Gallotia* species also supports this view and suggests that the ancestor of *Gallotia* first colonized the older eastern islands of Lanzarote, Fuerteventura or Gran Canaria, moving later to the western islands of Ten-

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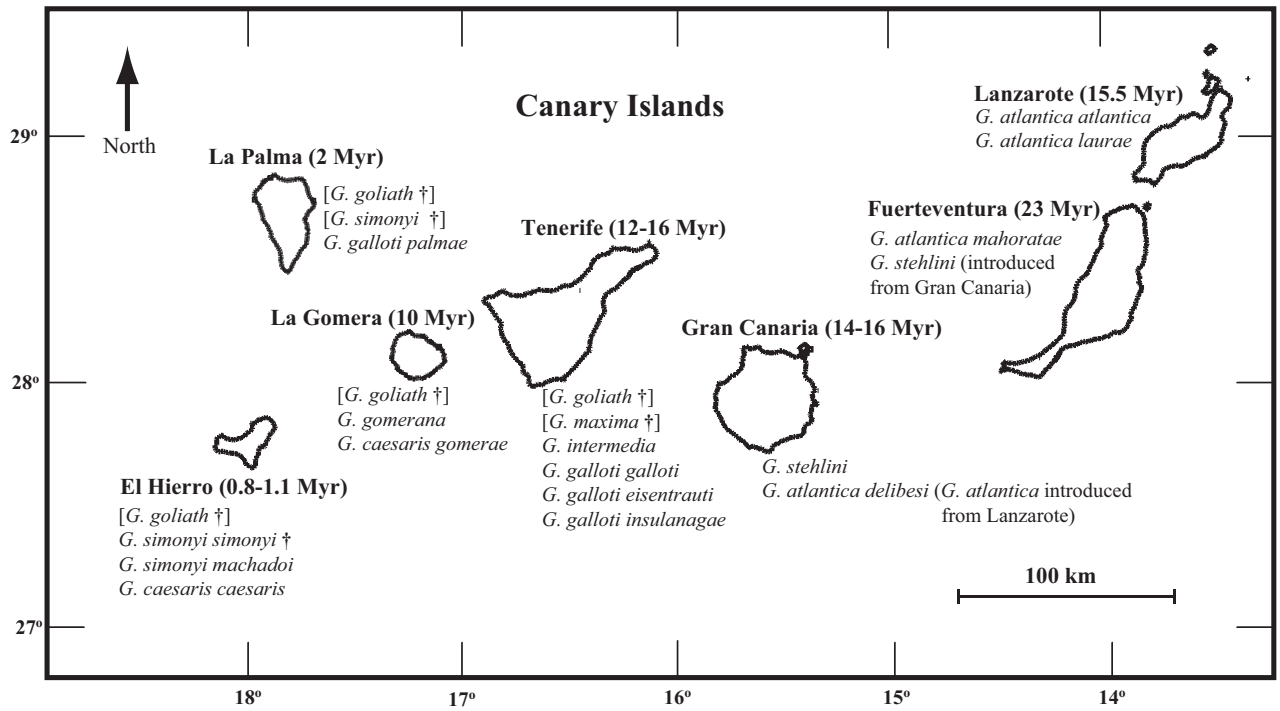


Figure 1. Map of the Canary Islands showing the recorded distribution of species and subspecies of *Gallotia* and the approximate ages of the islands. † indicates extinct forms; square brackets indicate that the taxonomic status of material concerned is uncertain.

erife, La Gomera, La Palma and El Hierro, following an east–west stepping-stone model of colonization (González *et al*., 1996; Rando *et al*., 1997). Molecular analysis also shows that *G. simonyi*, *G. intermedia* and *G. gomerana* are members of an exclusive clade, hereafter referred to as the *G. simonyi* group.

Apart from the living species of *Gallotia*, extinct forms have been described from abundant subfossil material in the western islands. Some of the individuals on which these descriptions were based were much bigger than the largest members of surviving populations but such huge animals disappeared soon after the arrival of people about 2000 years ago (Mateo & López-Jurado, 1992). Some of these giant lizards have been named *G. goliath* (Mertens, 1942) and *G. maxima* (Bravo, 1953), both taxa based on material from Tenerife. The name *G. goliath* has also been applied to the remains of very large lizards from La Palma (Mertens, 1942), La Gomera (*G. g. bravoana* (Hutterer, 1985)) and El Hierro (Izquierdo**,** Medina & Hernández, 1989), and *G. simonyi* has been used for rather smaller remains. A recent investigation of subfossil remains of large lizards from the western Canary Islands that have been assigned to *G. goliath*, *G. maxima* and *G. simonyi* concluded that they are all conspecific with living *G. simony*i of El Hierro (Barahona *et al*., 2000). Some characters previously used to distinguish the extinct taxa fall within the range of intraspecific variation found among living *G. simonyi*, while others probably result from allometric and other ontogenetic changes associated with increased size (Barahona *et al*., 2000). The relatively small size of living members of the *G. simonyi* group may have resulted from predation pressure and degradation of the ecosystem in which they live by people and their associated domestic animals (Pregill, 1986; Nogales *et al*., 1988; Nogales & Medina, 1996; García-Márquez, López-Jurado & Mateo, 1997). In spite of current opinion, there are clear indications that members of the *G. simonyi* group do in fact show some differentiation. Apart from differences in mitochondrial DNA (mtDNA) sequences between living *G. intermedia*, *G. gomerana* and *G. simonyi* (Hernández *et al*., 2001), these forms also differ in external features, such as colouring and aspects of scaling (Hernández *et al*., 2000; Nogales *et al*., 2001; Arnold, 2002). Mummified remains of *G. goliath* also show distinctive features of head scaling (Castillo, Rando & Zamora, 1994) and subfossil populations of the *G. simonyi* group from different islands exhibit differences in tooth number, especially in large animals (Barahona *et al*., 2000). Given this, it would not be unexpected for other, completely extinct populations of giant lizards from the western Canary Islands to show differences in their mtDNA.

Molecular techniques have already been used to assess the taxonomic status of some giant extinct lizards in the Canary Islands (Carranza *et al*., 1999). *Gallotia simonyi simonyi*, known only from El Roque Chico de Salmor, off El Hierro island where it has been extinct since the 1940s, was compared with *G. s. machadoi*, once widespread on El Hierro but now surviving only on an almost inaccessible cliff, La Fuga de Gorreta (Machado, 1985; Pérez-Mellado *et al*., 1997). The study showed that both subspecies were identical in the 1725 base pairs (bp) of mtDNA compared, suggesting that they were part of the same basic population until quite recently.

In this study we used techniques appropriate to ancient DNA to obtain partial sequences of two mitochondrial genes from mummified remains of *G. goliath* from eastern Tenerife to clarify its taxonomic status and assess its relationships to other *Gallotia* species.

MATERIAL AND METHODS

Material

The 25 *Gallotia* specimens used in the phylogenetic analysis are listed in Table 1. They comprise representatives of nearly all extant species and subspecies of *Gallotia* including *G. goliath*. *Lacerta dugesii* and *L. lepida* were used as outgroups. No representatives of the genus *Psammodromus* (the closest outgroup of *Gallotia*) could be sequenced for the 5′ upstream region of the cytocrome *b* (cyt *b*) gene (between primers GLUD-5′-and CB1-5; Palumbi, 1996) and therefore none were included in the analysis.

Extant material

DNA extraction followed standard proteinase K protocols described elsewhere (Carranza *et al*., 1999). Segments of two mtDNA genes (cyt *b* and 12S rRNA)

Table 1. Samples used in this study

Species	Locality	GenBank accession number 12S rRNA/cytochrome *b*
Outgroups		
Lacerta dugesii	Madeira	Z48041/Z48037
Lacerta lepida	Spain	*AY151979*/*AY151899*
Canary Islands		
Gallotia atlantica mahoratae-1	Tindaya (Fuerteventura)	*AY154905*/*AY154896*
Gallotia atlantica mahoratae-2	Island of Lobos (Fuerteventura)	*AF439945*/*AF439950*
Gallotia atlantica laurae	Malpaís de la Corona (Lanzarote)	*AY154906*/*AY154897*
Gallotia atlantica atlantica	La Santa (Lanzarote)	*AY154907*/*AY154899*
Gallotia stehlini-1	Juncalillo (Gran Canaria)	Z48039/Z48036
Gallotia stehlini-2	Maspalomas (Gran Canaria)	*AF439944*/*AF*439949
Gallotia stehlini-3	Tauro (Gran Canaria)	*AY151917*/*AY151838*
Gallotia stehlini-4	Galdar (Gran Canaria)	*AY154908*/*AY154899*
Gallotia goliath	Barranco de las Moraditas (Tenerife)	*AF306568*/*AF306569*
Gallotia intermedia-1	Los Gigantes (Tenerife)	*AY151923*/*AY151844*
Gallotia intermedia-2	Los Gigantes (Tenerife)	*AY154913*/*AY154904*
Gallotia simonyi machadoi	Risco de Tibataje (El Hierro)	*AY151924*/*AF101219*
Gallotia gomerana	Valle Gran Rey (La Gomera)	AJ272395/AJ272396
Gallotia caesaris gomerae-1	Las Rosas (La Gomera)	*AY154910*/*AY154901*
Gallotia caesaris gomerae-2	Playa de Santiago (La Gomera)	*AY154911*/*AY154902*
Gallotia caesaris caesaris-1	El Julan (El Hierro)	*AF439943*/*AF439948*
Gallotia caesaris caesaris-2	Los Llanillos (El Hierro)	*AY151922*/*AY151843*
Gallotia caesaris caesaris-3	Tamaduste (El Hierro)	*AY154912*/*AY154903*
Gallotia galloti palmae-1	Fuencaliente (La Palma)	*AF439941*/*AF439946*
Gallotia galloti palmae-2	Las Caletas (La Palma)	*AY151920*/*AY151841*
Gallotia galloti palmae-3	Puerto Espindola (La Palma)	*AY154909*/*AY154900*
Gallotia galloti eisentrauti-1	El Sauzal (NE Tenerife)	*AF439942*/*AF439947*
Gallotia galloti eisentrauti-2	San Vicente (NE Tenerife)	*AY151918*/*AY151839*
Gallotia galloti galloti-1	El Palmar (Tenerife)	Z48038/Z48034
Gallotia galloti galloti-2	Garachico (Tenerife)	*AY151919*/*AY151840*

Accession numbers of new sequences obtained for this study are in italics.

were amplified via PCR using the following primers: GLUD-5′ (Martin, Naylor & Palumbi, 1992; Palumbi, 1996) and cytochrome *b*2 (Kocher *et al*., 1989) for the cyt *b* gene and 12Sa and 12Sb for the 12S rRNA gene (Kocher *et al*., 1989). Thermocycling consisted of an initial step of 90 s at 94°C followed by 35 cycles of 30 s at 94°C, 45 s at 45°C and 1 min at 72°C. Amplified PCR bands were purified using a silica-based method (Boyle & Lew, 1995). The PCR products were sequenced using an ABI 377 automated sequencer following the manufacturer's protocols.

Mummified specimen of *Gallotia goliath*

The material of *G. goliath* consisted of fragmented mummified remains found in Barranco de las Moraditas in Tenerife, which have already been described in detail (Castillo *et al.*, 1994). This material was found among the otherwise inorganic fill of a small cavity in basaltic rock of the volcanic Series III dating from the Quaternary (Ancochea *et al*., 1990). Ancient DNA was extracted from four vertebrae and the distal portion of a partial left forelimb comprising toe bones, claws and skin. To avoid contamination with DNA from contemporary *Gallotia* material, extractions were performed by only one person (N. M.-M.) who was never involved in handling samples from extant taxa. In addition, this work was carried out in a laboratory dedicated exclusively to the manipulation of ancient DNA that was physically separated from the main laboratory where other DNA work was carried out. To prevent human contamination from previous handling, approximately 0.2 g of the bone/skin sample was dipped in a 10% ClH solution, washed several times in sterile distilled water and dried under a 254-nm UV lamp. The material was then crushed in a sterilized mortar with liquid N_2 and transferred to 1 mL guanidinium thiocyanate (GuSCN) extraction solution following the GuSCn-silica method (Boom *et al*., 1990). To eliminate any remaining contamination with alien DNA, all aliquoted solutions were first treated with UV light (Sarkar & Sommer, 1990) and then with 8-methoxypsoralen (Jinno, Yoshiura & Niikawa, 1990). Three independent extraction controls, in which the sample was substituted by the same weight of sterilized filter paper, were processed in parallel with each extraction. The ancient DNA and controls were released from the silica pellet in which they were enclosed by dissolving these in 50 μL TE buffer (10 mM TrisCl, 1 mM EDTA, pH = 8). Except when being processed, samples were kept at –70°C. The ancient DNA was PCR amplified following the same thermocycling conditions as for the extant specimens (see above). As might be expected, the DNA extracted from the mummified specimen was very degraded and so five overlapping subfragments for the 12S rRNA and four for the cyt *b* gene had to be amplified and sequenced using the set of primers listed in Table 2. To avoid false positives during PCR, strict conditions proposed by Kwok & Higuchi (1989) were followed whenever possible, and all the aliquoted PCR mixes were treated with UV light (Sarkar & Sommer, 1990) before the addition of 5 μL of ancient DNA template. Three negative PCR controls were included with each set of

Table 2. Primers used in this study

Primer	Gene	Position*	Sequence (5′–3′)
L14724	Cyt *b*	14704–14724	TGACTTGAAGAACCACCGTTG
H14929	Cyt *b*	14954–14929	GGATGTGGGCGATGGATGAGAATGCG
L14841	Cyt *b*	14815–14841	AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA
L14921	Cyt *b*	14896–14921	CCTAGCCATGCACTACTCACCAGAC
H15035	Cyt *b*	15060–15035	CCGTAGTACAGGCCACGTCCGATGTG
L15031	Cyt *b*	15006–15031	CCTCACTATTTTTTATCTGCATCTAC
H15149	Cyt *b*	15174–15149	AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA
L1064	12S	1043–1064	TTGACCACACGAAAGCTTAGAA
H1179	12S	1197–1179	TAGGTCGAATGTGGGACACC
L1149	12S	1128–1149	CTTTCCGCCAGAGAACTACAAG
H1256	12S	1275–1256	CGACGGCGGTATATAGGCTG
L1235	12S	1217–1235	CGATACTCCCCGCTCTACC
H1357	12S	1378–1357	AAAAATGTAGCCAATCTCTGCC
L1343	12S	1323–1343	CTAACACGTCAGGTCAAGGTG
H1467	12S	1485–1467	GTGTGTACGCGTCCCAGAG
L1342	12S	1412–1432	CAGCATGAAGGCGAATTTAGT
H1565	12S	1584–1565	TTCCGGTACGCTTACCATGT

*Positions refer to the complete human mitochondrial genome sequence (Anderson *et al*., 1981).

amplifications. The sequencing protocol for the PCR products was the same as for the extant samples.

Phylogenetic analysis

DNA sequences were visualized using an alignment editor (GDE, Smith *et al*., 1994). No gaps had to be postulated to align the cyt *b* sequences, which were translated into amino acids using the vertebrate mitochondrial code. No stop codons were observed in the cyt *b* sequences suggesting that they are probably functional. The 12S rRNA sequences were aligned with reference to the published secondary structure of this gene (Hickson *et al*., 1996) and gaps were inserted to resolve length differences between sequences. Two hypervariable regions of approximately 14 bp (between stems 36 and 38 of Hickson *et al*. (1996) and 8 bp (between stems 42 and 42′ of Hickson *et al*. (1996)) could not be aligned unambiguously and they were therefore excluded from further analyses.

A saturation analysis was carried out in which the observed proportions of transitions (ts) and transversions (tv) were plotted against the uncorrected genetic distances, the 12S rRNA and the highly variable 3rd codon positions of the cyt *b* gene being analysed independently. The results showed no evidence of saturation in the 12S rRNA ts and tv, or in the cyt *b* 3rd codon tv, but the cyt *b* 3rd codon ts show a small degree of saturation for all comparisons between *Gallotia* species and the outgroups (results not shown). Because of this, cyt *b* 3rd codon ts were given a weight of 0 in one of the parsimony analyses. The two gene fragments were tested for incongruence using the incongruence length difference test (ILD) (Mickevich & Farris, 1981; Farris *et al*., 1994); 10 000 heuristic replicates were used, and the invariable characters were removed before starting the analysis (Cunningham, 1997). The result of the ILD test (ILD $P > 0.172$) indicated both genes to be congruent and therefore they could be combined in a total evidence analysis.

Three different methods of phylogenetic analysis were employed and the results compared. These were maximum likelihood (ML), maximum parsimony (MP) and neighbour-joining (NJ). Modeltest version 3.06 (Posada & Crandall, 1998) was used to select the most appropriate model of sequence evolution for the NJ and ML analyses. This was the general time reversible model (GTR) taking into account the number of invariable sites (I) and the shape of the gamma distribution (G) (i.e. GTR + I + G). A simpler analysis was also carried out using the Kimura's two-parameter (K2P) model (Kimura, 1980), which takes into account the differences between the number of ts and tv (ts/tv ratio) but assumes equal frequencies for the four types of nucleotide. This additional analysis was undertaken because, although it has been demonstrated that ML performs better with the most correct model of sequence evolution rather than with a simpler, more incorrect model (Yang, 1996; Rosenberg & Kumar, 2001), it has also been found that with a complex model ML does not work well when the true evolutionary pattern is simple (Yang, 1996).

The MP and ML analyses were heuristic searches involving tree bisection and reconnection (TBR) branch swapping with 1000 and 10 random stepwise additions of taxa, respectively. In the MP analyses gaps were considered as a fifth state. The weight given to tv was varied relative to that of ts (tv was allocated the same, two times, four times, six times and ten times the weight of ts). The weight of the gaps was always equal to the maximum weight assigned to either ts or tv. The reason for using these weights was because all fell around the estimated ts/tv ratio for our dataset (ts/tv = 5.6). As noted, the cyt *b* 3rd codon ts were given a weight of 0 in some analyses.

In total, 11 different kinds of phylogenetic analyses were used: (1) MP (ts = tv); (2) MP (ts = 1, tv = 2); (3) MP (ts = 1, tv = 4); (4) MP (ts = 1, tv = 6); (5) MP (ts = 1, tv = 10); (6) MP (cyt *b* 3rd codon ts = 0); (7) NJ (GTR + I + G); (8) NJ (K2P); (9) ML (GTR + I + G); (10) ML (K2P); (11) ML (enforcing molecular clock) (GTR + I + G). All analyses were performed in PAUP* version 4.0b10 (Swofford, 1998) except where stated. Robustness of the trees was assessed by bootstrap analysis (Felsenstein, 1985) and involved 500 pseudoreplications for the ML (GTR + I + G) tree and 1000 for all the other analyses.

Where appropriate, topological constraints were generated using MacClade version 4.0 (Maddison & Maddison, 1992) and they were compared with our optimal topologies using the Kishino-Hasegawa (1989) and Shimodaira-Hasegawa (1999) tests employing RELL bootstrap with 1000 bootstrap replicates. In order to assess the age of speciation events, molecular clock assumptions were incorporated. The likelihood ratio test (Huelsenbeck & Crandall, 1997) was used for testing the statistical significance of the difference between the log likelihood of the trees calculated with and without the clock assumptions.

RESULTS

Phylogenetic relationships of *Gallotia goliath* and its independent status

The sequences obtained from the mummified remains of *G. goliath* differed from all other species and subspecies of *Gallotia* for which these gene fragments have been studied, increasing our confidence that the sequences belonged to *G. goliath* itself. After excluding all the regions that could not be unambiguously aligned, the dataset contained 746 characters (378 bp

of cyt *b* and 368 bp of 12S rRNA) of which 221 were variable and 180 parsimony-informative. The strict consensus tree of all 26 trees obtained from the 11 different analyses employed is shown in Figure 2. Additional information about the ML and MP analyses is given in Table 3. The consensus tree clearly showed that *G. goliath* belongs to the *G. simonyi* group, together with *G. simonyi*, *G. gomerana* and *G. intermedia*. Within this grouping, *G. s. machadoi* and *G. gomerana* formed a monophyletic group with a bootstrap support of 100 in all the independent analyses with the exception of MP (cyt *b* 3rd ts = 0), which had a bootstrap value of only 69. This underlines the role of the highly variable 3rd codon ts of the cyt *b* gene in resolving recent cladogenetic events. Other relationships within the *G. simonyi* group were not consistently resolved. In the NJ trees and the two ML trees, in which the molecular clock was not enforced, *G. goliath* was sister to all the other lizards of the *G. simonyi* group but with low bootstrap support. All MP analyses except that in which the cyt *b* 3rd codon ts were given a null value (MP (cyt *b* 3rd ts = 0)), produced an alternative topology of exactly the same number of steps, in which *G. goliath* was sister to *G. intermedia* (Fig. 2B). The ML tree enforcing the molecular clock and the tree from the MP (cyt *b* 3rd ts = 0) analysis also supported the hypothesis that *G. goliath* is sister to *G. intermedia*. To investigate these relationships further, the ML (GTR + I + G) topology in which the molecular clock was not enforced (*G. goliath* sister to all other lizards of the *G. simonyi* group) was compared with a tree constrained so that *G. goliath* was sister to *G. intermedia*. The results are presented in Table 4; the difference between the two trees was not significant. Moreover, both trees presented the same number of steps (379). Genetic distances between all the members of the *G. simonyi* group indicated *G. goliath* is genetically well differentiated from all the rest, the average divergence ranging between 2.71% and 4.20% (Table 5). This range is higher than the genetic distances between *G. gomerana* and *G. simonyi* (0.94%) and at the same general level as the genetic distances between these and *G. intermedia* (3.46%–3.89%).

Timing of events in the history of the *Gallotia simonyi* group

Inferring the ages of molecular divergence events and island colonization often requires the use of a molecular clock calibration. Several sources of error may affect this calibration and should be taken into account when drawing any conclusions from the results. Factors that can affect clock calibrations on islands include increased stochastic variation at low

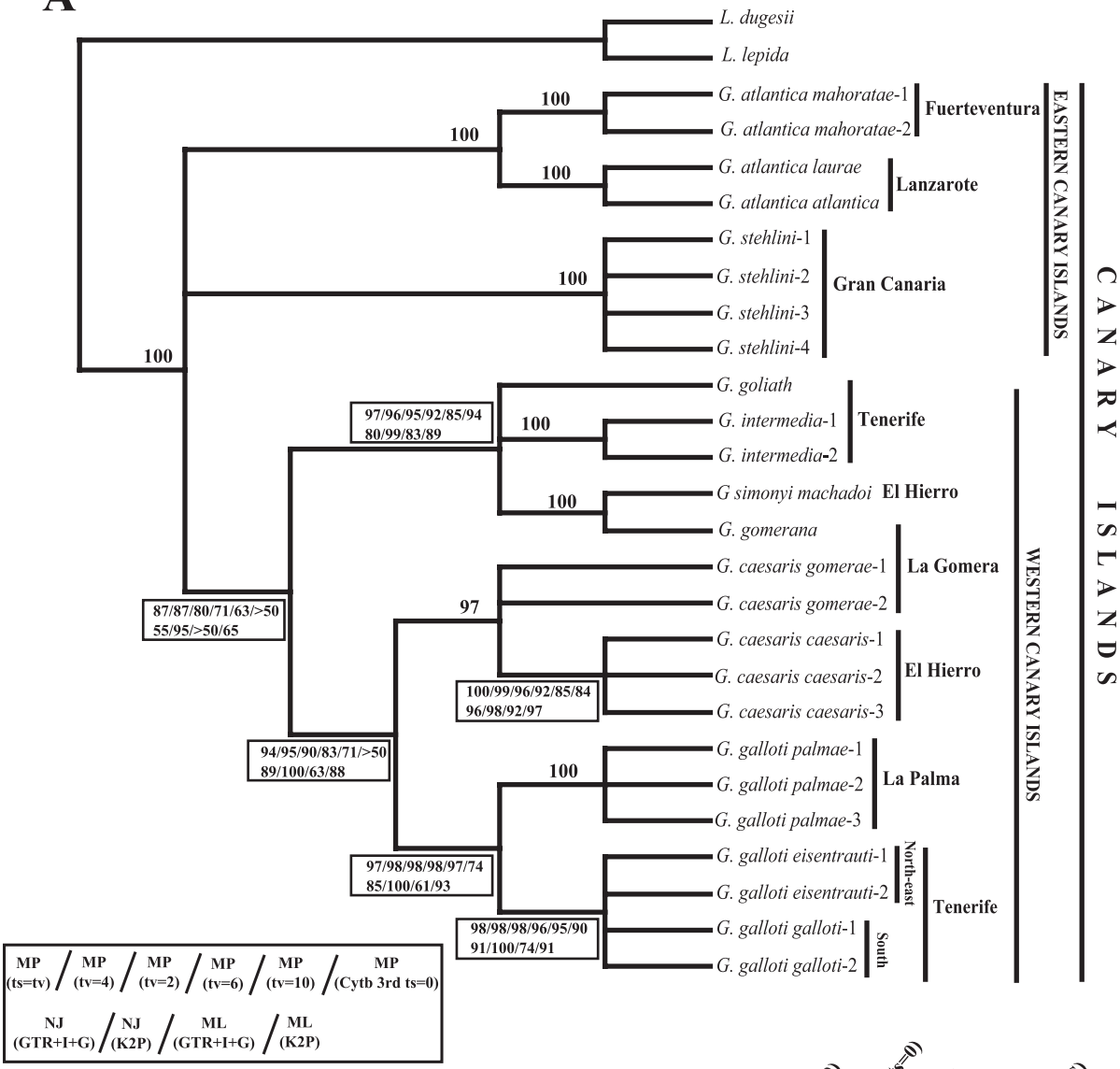
Table 3. Additional data for the maximum parsimony (MP) and maximum likelihood (ML) trees

Type of analysis	Trees (*N*)	Steps (*N*)	CI	RI	–log likelihood
MP (ts = tv)	4	379	0.628	0.844	–
MP (ts = 1; tv = 2)	4	477	0.652	0.850	–
MP (ts = 1; tv = 4)	4	673	0.679	0.857	–
MP (ts = 1; tv = 6)	4	869	0.694	0.860	–
MP (ts = 1; tv = 10)	4	1261	0.710	0.865	–
MP (cyt *b* 3rd ts = 0)	2	220	0.668	0.849	–
ML (GTR + I + G)	1	–	–	–	2965.89073
ML (K2P)	1	–	–	–	3207.86958
ML (GTR + I + G) enforcing molecular clock	1	–	–	–	2984.43213

MP values are after excluding uninformative sites. CI, consistency index; GTR + I + G, general time reversible model taking into account the number of invariable sites and the shape of the gamma distribution; K2P, Kimura's two-parameter model (Kimura, 1980); RI, retention index; ts, transitions; tv, transversions.

Figure 2. (A) Strict consensus of the 26 trees obtained from the combined analysis of the cyt *b* and 12S rRNA genes using 11 different approaches (see Material and Methods and Table 3 for details). Where bootstrap percentages vary, numbers by nodes indicate support for the individual methods and parameters used, given in the order indicated in the box below the tree. When differences between bootstrap figures are <10% only the average value is shown. (B) Support for alternative phylogenetic hypotheses at unresolved nodes in the strict consensus tree (A) for each of the 11 methods used. Dark boxes indicate all trees of a particular analysis support a hypothesis; grey boxes indicate support by some of the equally parsimonious trees but not all. Figures indicate bootstrap values and a question mark that no bootstrap assessment was made.

A



B

	MP(ts=tv)	MP(ts=1;tv=2)	MP(ts=1;tv=4)	MP(ts=1;tv=6)	MP(ts=1;tv=10)	MP(Cytb 3rd ts=0)	NJ(GTR+I+G)	NJ(K2P)	ML(GTR+I+G)	ML(K2P)	ML GTR+I+G Mol. Clock
G. stehlini sister to the rest of *Gallotia*	62	59	54	>50	>50	>50		>50			
G. atlantica sister to the rest of *Gallotia*									>50	>50	?
G. stehlini and *G. atlantica* sister groups							>50				
G. goliath sister to the rest of the *G. simonyi* group							70	72	66	55	
G. goliath sister to *G. intermedia*						>50					?
G. caesaris gomerae monophyletic								100			?
G. galloti eisentrauti monophyletic	86	86	86	86	85		82	86	83	85	?
G. galloti galloti monophyletic	63	62	61	60	63		65	70	54	60	?

Table 4. Statistical support for alternative hypotheses on *Gallotia* phylogeny

Tree	–log likelihood	Δ–log likelihood	SH *P*	KH *P*
Unconstrained ML (GTR + I + G) tree (Fig. 2). *G. goliath* sister to all other lizards of the *G. simonyi* group.	2965.89073	(best)		
Constrained so *G. goliath* is sister to *G. intermedia*.	2968.67547	2.78474	0.208	0.4364

$P > 0.05$ suggests that the constrained and unconstrained solutions are not significantly different. GTR + I + G, general time reversible model taking into account the number of invariable sites and the shape of the gamma distribution; KH, Kishino-Hasegawa (1989) test; SH, Shimodaira-Hasegawa (1999) test; ML, maximum likelihood.

Table 5. Genetic divergence of cyt *b* and 12S rRNA genes between all members of the *Gallotia simonyi* group calculated using the Kimura 2-parameter correction

Comparison	Maximum genetic variability (%)	Minimum genetic variability (%)	Average (%)
G. goliath vs. *G. intermedia*	2.75	2.47	2.61
G. goliath vs. *G. simonyi*	3.70	3.70	3.70
G. goliath vs. *G. gomerana*	4.20	4.20	4.20
G. intermedia vs. *G. simonyi*	3.46	3.17	3.31
G. intermedia vs. *G. gomerana*	3.89	3.60	3.75
G. gomerana vs. *G. simonyi*	0.94	0.94	0.94

levels of sequence divergence, possible extinct or unsampled lineages, and the assumption that islands are colonized immediately after their appearance (Emerson, Oromi & Hewitt, 2000a,b; Emerson, 2002 and references therein).

The –log likelihood value of the ML tree (GTR + I + G) (2965.89073) was compared with that of the same tree constructed under molecular clock assumptions (2984.43213). The results showed that no significant difference between the likelihoods of the two trees (likelihood ratio test statistic ($-2\log\Delta$) = 37.0828, which approximates to X^2_{25} distribution under the null hypothesis; $P > 0.05$). So the sequences could be used for estimating dates. The clock was calibrated using the ML (GTR + I + G) genetic distances and two approximate ages for the island of El Hierro: 0.8 Myr (Abdel-Monem, Watkins & Gast, 1972; Fuster *et al*., 1993) and 1.1 Myr (Guillou *et al*., 1996)). El Hierro is quite close to the much older island of La Gomera and presumably received its lizard colonists from there when they rafted on the prevailing ocean currents which run in an appropriate direction. This would apply both to *G. caesaris caesaris* and *G. simonyi*, the colonization events being indicated on the phylogeny by the nodes marking the separation of these taxa from their sister lineages on La Gomera, *G. caesaris gomerae* and *G. gomerana*, respectively. The degree of genetic differentiation between *G. c. caesaris* and *G. c. gomerae* was greater than that between *G. gomerana* and *G. simonyi*, suggesting that *G. c. caesaris* colonized El Hierro first. The maximum age of this event would be the age of the island itself. Using this as a basis for calibration, the evolutionary rate of *Gallotia* for the combination of gene fragments used in this investigation would range between 2% and 1.48% per Myr, depending on which estimate of the age of El Hierro was used (0.8 Myr or 1.1 Myr, respectively). Dates derived from this calibration are shown in Figure 3. They suggest that the *G. simonyi* group separated from the *G. galloti–G. caesaris* clade about 5–7 Mya and that its currently recognized lineages began to diverge 2.1–2.8 Mya. Whether *G. goliath* is sister to the rest of the *G. simonyi* group or to *G. intermedia*, the age of its exclusive lineage is much greater than those of *G. simonyi* and *G. gomerana*.

DISCUSSION

Gallotia goliath is clearly a member of the *G. simonyi* group of very large lacertid lizards in the western Canary islands (Tenerife, La Gomera, El Hierro and La Palma) and is not closely related to the quite similar giant *G. stehlini* of Gran Canaria. *Gallotia goliath* is one of several distinct forms in the *G. simonyi* group and is separable on the basis of morphology and mtDNA sequence from the extant *G. simonyi* of El Hierro, from the recently discovered living *G. gomerana* on La Gomera, and from *G. intermedia* in north-western Tenerife. Within the *G. simonyi* group,

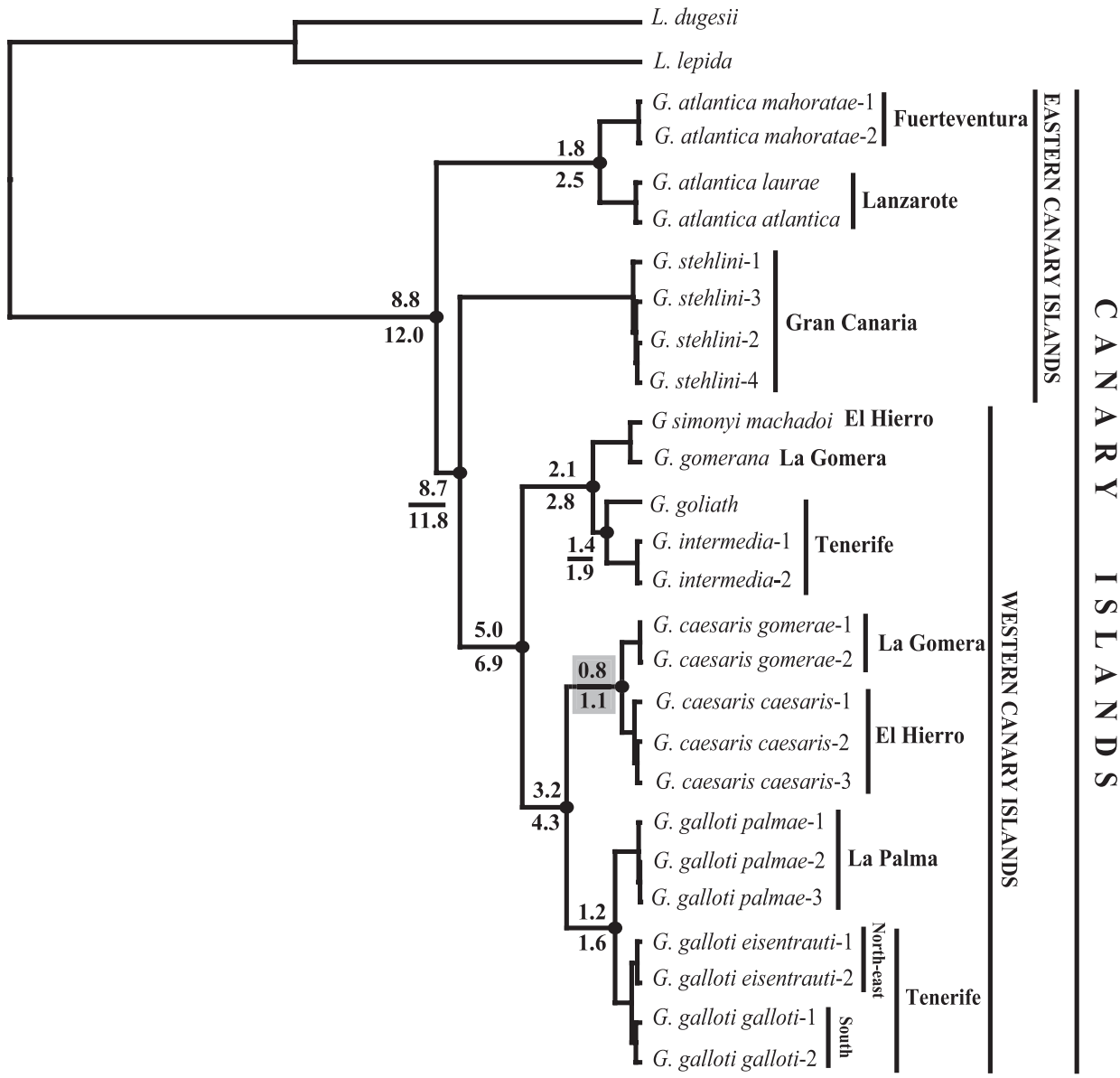


Figure 3. Maximum likelihood (GTR + I + G) tree enforcing the molecular clock. Numbers at nodes indicate millions of years since the cladogenetic event represented. Upper numbers assume an age for El Hierro of 0.8 Myr, lower numbers one of 1.1 Myr. The calibration point is highlighted with a grey box.

G. goliath may be sister either to all the other members, or to *G. intermedia*.

The distinctness of *G. goliath* makes Tenerife unique among oceanic islands in having had one giant and two medium-sized lizard species that were probably substantially herbivorous, the others being *G. intermedia* and *G. galloti*. *Gallotia* shows great community differences on other islands in the Canaries, two having a single small species, one a single giant, and three a giant and a medium-sized form. This irregularity is similar to that found in *Phelsuma* geckos on the Mascarene islands in the south-west Indian Ocean (Arnold, 2000) but contrasts with the uniform patterns of radiation seen in some *Anolis* (Iguanidae) clades in the Greater Antilles (Losos *et al*., 1998).

ACKNOWLEDGEMENTS

We are very grateful to the 'Viceconsejería de Medio Ambiente del Gobierno de Canarias' for their help. This work was partially supported by grants form the Natural Environment Research Council (GR9/03327) and the Royal Society (European Science Exchange

Programme, 1999–2001). S. Carranza was supported by a Marie Curie Individual Fellowship under the European Union Human Potential Programme (MCFI-1999-00523).

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