

## SHORT NOTE

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**STATUS OF THE EXTINCT GIANT  
LACERTID LIZARD *GALLOTIA  
SIMONYI SIMONYI* (REPTILIA:  
LACERTIDAE) ASSESSED USING  
mtDNA SEQUENCES FROM MUSEUM  
SPECIMENS**

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*Gallotia simonyi* (Steindachner, 1889) is a member of an endemic Canary Island genus and is among the largest of the approximately 250 recent species of lacertid lizards. Although Steindachner gave the locality of the types as the Roques de Salmor, off El Hierro island, previous workers (Urusaustegui, 1983; Manrique & Saavedra, 1873; Viera & Clavijo, 1983) and subsequent ones (Machado, 1985) regard the more western of the two rocks in the group, the Roque Chico de Salmor, as the actual source of material. The Roque Chico is 37 m high with a surface area of less than 10,000 m<sup>2</sup> and lies 830 m from the northern coast of El Hierro (Machado, 1985). Several specimens were collected from the rock after *G. simonyi* was described but none were encountered later than about 1940 (Salvador, 1971; Machado, 1985). The species was consequently considered extinct by the scientific community. In fact, residents on El Hierro were aware of a surviving population on an almost inaccessible cliff, the Fuga de Gorreta, on El Hierro itself (Salvador, 1971). This was formally reported in 1975 (Böhme & Bings, 1975; see also 1977; Böhme *et al.*, 1981). Some morphological differences from the extinct population of *Gallotia simonyi* from the Roque Chico de Salmor, were noted by Machado (1985), who referred to the El Hierro population as *Gallotia affinis simonyi*. This population was subsequently given formal subspecies status as *Gallotia simonyi machadoi* López-Jurado, 1989. Morphological differences from the Roques de Salmor subspecies, *G. s. simonyi*, are said to include: smaller size, less robust build, a less triangular and more oval pileus, a less depressed head, and smaller more numerous temporal scales surrounding a clearly defined enlarged masseteric scale (Machado, 1985;

López-Jurado, 1989). The temporal and masseteric characters have been confirmed by Rodríguez-Domínguez *et al.* (1998), who also note that *G. s. machadoi* has relatively shorter hind legs than *G. s. simonyi*. It should, however, be borne in mind that the samples available of *G. s. simonyi* were small and that those of *G. s. machadoi* came from a captive population derived from few individuals (Pérez-Mellado *et al.*, 1997).

It is possible that the differences between the two populations of *G. simonyi*, one living and one extinct, are merely part of an original pattern of complex minor geographical variation which has been accentuated by the restriction of a once widespread species to two very small and different localities. *G. simonyi* was certainly once far more widely distributed and recent fossil remains are known from many localities on El Hierro (Böhme *et al.*, 1981; Bings, 1985; Izquierdo *et al.*, 1989; López-Jurado *et al.*, 1998; Fabiola Barahona *pers. com.*). Alternatively, given the apparent degree of morphological difference between them, the two populations could represent lineages that have been separate for a long time. We distinguished between these two hypotheses by examining mitochondrial DNA gene sequences.

Although suitable fresh tissue samples were available for *G. s. machadoi*, they were inevitably lacking for *G. s. simonyi*, which is now only known from about ten specimens in museum collections, mainly preserved in alcohol. Those most easily available to us, in the Natural History Museum, London (formerly the British Museum (Natural History)), are kept in industrial methylated spirit (IMS), a commercial preparation containing ethanol but also methanol and a wide range of other chemicals, some of which are known to be damaging to DNA. As might be expected, the DNA extracted from these specimens, which are 68 years old, was very degraded, the largest fragments consisting of about 500 bp. Nevertheless, it has been possible to compare it with homologous regions of sequence from *Gallotia simonyi machadoi*.

Data for the animals used are as follows.

*Gallotia s. simonyi*, BMNH 1967.1736-1737, male, female; labelled 'Roques Zalmor'; collected by H. B. Cott in 1931.

*Gallotia s. machadoi*. Six captive animals from the "Centro de Reproducción e Investigación del Lagarto Gigante de El Hierro 38911, Frontera"

To reduce the possibility of contamination, all the molecular work on the *Gallotia s. simonyi* tissue was completed before the six *Gallotia s. machadoi* samples were received. Total genomic DNA was extracted either from 0.5-1 cm<sup>3</sup> of thigh muscle (*G. s. simonyi*) or 2-3 mm<sup>3</sup> of tail tissue (*G. s. machadoi*). The material was air dried for two minutes, finely diced with a sterile scalpel blade and transferred to a tube containing 800 µl of proteinase K digestion solution (100mM Tris-HCL pH8; 100mM NaCl; 10 mM EDTA pH8; 0.5%

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TABLE 1. List of the primers used to amplify the three mitochondrial genes. \* represents the position of the 3' end of the primer in reference to the human mitochondrial complete genome (Anderson *et al.* 1981). # it is not possible to homologise these positions with the human reference but both are internal with respect to 16SL(F) and 16SH1(R).

Primer	Gene	Sequence (5'-3')	Position*	Reference
GLUD-5'(F)	Cyt b	TGA TAT GAA AAA CCA TCG TTG	14724	Martin <i>et al.</i> (1992)
CB107(F)	Cyt b	CAC ATY CAY CGT GAY GTY CAA	14968	This study
CB144(R)	Cyt b	CGR ATT ART CAR CCR TGT TG	14966	This study
CB1(F)	Cyt b	CCA TCC AAC ATC TCA GCA TGA TGA AA	14841	Kocher <i>et al.</i> (1989)
CB2(R)	Cyt b	CCC TCA GAA TGA TAT TTG TCC TCA	15149	Kocher <i>et al.</i> (1989)
CB2(F)	Cyt b	TGA GGA CAA ATA TCA TTC TGA GGG	15172	This study
CBSim(F)	Cyt b	CCT TCT ACC ATT TAT AAT YTT AGG YAC	15321	This study
CBSim(R)	Cyt b	GTC TGA GTT TGA GTT YAR TCC GGT T	15369	This study
CB3(R)	Cyt b	GGC AAA TAG GAA RTA TCA TTC	15556	Martin <i>et al.</i> (1992)
COI(F)	COI	CCT GCA GGA GGA GGA GAY CC	6586	Palumbi (1996)
COI2(F)	COI	TGA CTT GCA ACR CTT CAC GGA GG	6892	This study
COI2(R)	COI	CCT CCG TGA AGY GTT GCA AGT CA	6870	This study
COI(R)	COI	CCA GAG ATT AGA GGG AAT CAG TG	7086	Palumbi (1996)
16SL1(F)	16S	CCG TGC AAA GGT AGC ATA ATC AC	2605	This study
16SSI(F)	16S	TAG TTG GGG CGA CTT CGG AGY A	#####	This study
16SSI(R)	16S	GTT GGC CRT GAT ATG GTA GGG	#####	This study
16SH1(R)	16S	CCG GTC TGA ACT CAG ATC ACG T	3059	This study

SDS and 0.75 mg/ml of proteinase K); the tube was then incubated at 37°C overnight in an orbital shaker. Purification was by phenol/chloroform extraction (Sambrook *et al.*, 1989), followed by centrifugal dialysis though a Centricon 30000 MW membrane (Amicon). The Polymerase Chain Reaction (PCR) technique was used to amplify and directly sequence 813 bp of the cytochrome-*b* (*cyt-b*), 501 bp of the cytochrome oxidase I (COI) and 411 bp of the 16S rDNA mitochondrial genes from the eight *Gallotia simonyi*. The primers used in both the amplification and the sequencing of these three mitochondrial fragments are listed in Table 1. Thermocycling consisted of an initial 90 s at 94°C followed by 35 cycles of 94°C for 30 s, 45°C for 45 s, and 72°C for 1 min, and then by a single cycle at 72°C for 10 min. Successful PCR bands were cut out and purified using a silica-based method (Boyle & Lew, 1995). The PCR products was sequenced using an ABI 377 automated sequencer, following the manufacturer's protocols.

Fragments of three different mitochondrial genes (*cyt-b*, COI and 16S rDNA) comprising a total of 1725 bp were sequenced for two specimens of the extinct *G. s. simonyi* and six of the extant *G. s. machadoi*. Results showed that the sequences are identical at all sites, suggesting that the two subspecies are more similar than previously thought and represent an example of morphological variation without molecular divergence, at least in the studied sequences. The eight *cyt-b* sequences analysed here differ in three positions (positions 15142, 15144-45 of the human mitochondrial genome; Anderson *et al.*, 1981) from the three *G. s. machadoi* *cyt-b* partial sequences cited by González *et al.* (1996). These apparent nucleotide differences are

situated in a short region spanning the last seven nucleotides of the sequence analysed by González *et al.* (1996) and, if real, would cause an amino acid substitution. The amino acid concerned is a leucine encoded by nucleotides 15143-45 of the human mitochondrial genome (Anderson *et al.*, 1981) which is conserved in insects, mammals and reptiles (including the *Gallotia simonyi* material investigated here and all the other species of *Gallotia*). In contrast, it would be replaced by a proline in the *G. s. machadoi* sequence of González *et al.* (1996). A comparison of all available *Gallotia* sequences point towards a mistakenly inserted G at position 15142 of the González *et al.* (1996) *G. simonyi* sequences, causing a displacement of the reading frame and, consequently, the other two nucleotide changes.

The identity of 1725 bp of their mitochondrial DNA sequence indicates that *G. s. simonyi* and *G. s. machadoi* are likely to have been part of the same basic population until quite recently. When the *cyt-b* sequences of *G. simonyi* are compared with those of other species of *Gallotia*, the lack of genetic variability between the two subspecies of *G. simonyi* contrasts strongly with both the mean *cyt-b* genetic divergence for other subspecies (5.5%), and the within subspecies mean variability (0.6%) (González *et al.*, 1996). These results do not necessarily indicate that *G. s. machadoi* and *G. s. simonyi* are genetically identical overall, and faster evolving genetic material, such as micro satellites, may possibly still show differences between them. Nevertheless, any such differences are likely to have developed over a very short time span, given that the total of 438 third codon positions (*cyt-b* and COI genes), in principle selectively near-neutral and free to vary, show no differentiation at all between the two

populations. Similar insular studies with the lizard *Podarcis atrata* (Castilla *et al.*, 1998*a,b*) using 306 bp of the *cyt-b* (106 third codon positions) exhibit a medium to high degree of genetic variability between different island populations, again contrasting with the genetic identity of *G. s. simonyi* and *G. s. machadoi*.

Disparity in amount of difference within morphological and molecular data sets for the same taxa is quite common. Thus, molecular differences may sometimes be more marked than morphological ones, for example in the Pacific scincid lizard genus, *Emoia*. (Bruna *et al.*, 1996), *Thropheus* fishes of Lake Tanganyika (Sturmbauer & Meyer, 1992), and the "living fossil" horseshoe crabs, *Limulus*, in which morphology has remained essentially unchanged for many millions of years, even though they exhibit normal levels of molecular evolution (Selander *et al.*, 1970). On the other hand, the reverse is true in the species flocks of cichlid fishes in the rift lakes of East Africa, that originated extremely rapidly through adaptative radiation and are genetically exceptionally similar (Meyer *et al.*, 1990). In the scincid lizard *Gongylomorphus bojeri* of Mauritius, some small-island populations are virtually identical in mtDNA but differ markedly in morphology (J. Austin, *pers. com.*), a situation paralleling that in *Gallotia simonyi*. In both the latter cases identity, or near-identity of DNA sequence, together with the fact that the islands concerned probably only became separated a few thousand years ago as a result of sea-level rise at the end of the Pleistocene, suggests that morphological differentiation was rapid. This being so, it is possible that, if examples of *G. s. machadoi* were placed on the Roques de Salmor, the introduced population would soon develop the morphological characteristics of the original inhabitants. A recovery plan for *G. simonyi* is already in progress (Pérez-Mellado *et al.*, 1997).

The nucleotide sequences in this paper are available from GenBank: *G. s. simonyi* (BMH 1967-1736) AF101217, AF101209 and AF101201; *G. s. simonyi* (BMH 1967-1737) AF101218, AF101210 and AF101202; *G. s. machadoi* (number 16) AF101219, AF101211 and AF101203; *G. s. machadoi* (number 25) AF101220, AF101212 and AF101204; *G. s. machadoi* (number 26) AF101221, AF101213 and AF101205; *G. s. machadoi* (number 46) AF101222, AF101214 and AF101206; *G. s. machadoi* (number 160) AF101223, AF101215 and AF101207; *G. s. machadoi* (number 161) AF101224, AF101216, AF101208; for *cyt-b*, COI and 16S rDNA respectively.

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