

**ADDITION OF A NEW LIVING GIANT LIZARD FROM LA GOMERA ISLAND TO THE PHYLOGENY OF THE ENDEMIC GENUS *GALLOTIA* (CANARIAN ARCHIPELAGO)**

MARIANO HERNÁNDEZ<sup>1</sup>, NICOLE MACAMEYER<sup>1</sup>, J. CARLOS RANDO<sup>2</sup>, ALFREDO VALIDO<sup>2</sup> AND MANUEL NOGALES<sup>2</sup>

<sup>1</sup>Department of Genetics and <sup>2</sup>Department of Zoology, University of La Laguna, Tenerife, Canary Islands, Spain

*Key words:* Phylogeny, *Gallotia*, La Gomera, Canary Islands

The lacertid lizards of the endemic genus *Gallotia* (Arnold, 1973) from the Canary Islands represent one of the most important and best studied examples of island reptile radiation and evolution (Klemmer, 1976). Although there have been several attempts to reconstruct their phylogeny and evolution using molecular techniques (Thorpe, McGregor & Cumming, 1993a,b; Thorpe *et al.*, 1994), only the most recent (González *et al.*, 1996; Rando *et al.*, 1997) included all known extant species of the group and could therefore be used to understand the magnitude of this island-lizard radiation. These phylogenies suggested that the ancestor of *Gallotia* colonized the eastern islands of Lanzarote and Fuerteventura first, moving later to the islands of Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro, following an eastern-western geographic transect (González *et al.*, 1996). Rando *et al.* (1997) positioned the recently discovered species *G. intermedia* from Tenerife (Hernández, Nogales & Martín, 2000) in the phylogeny of *Gallotia* (González *et al.*, 1996) and demonstrated that it was sister to the rediscovered *G. simonyi machadoi* from El Hierro (Böhme & Bings, 1975; Machado, 1985; Carranza *et al.*, 1999). In total, Rando *et al.* (1997) recognized five species of *Gallotia* at the molecular level: *G. atlantica* from the eastern islands of Lanzarote and Fuerteventura, *G. stehlini* from the central island of Gran Canaria, *G. simonyi* from the westernmost island of El Hierro, *G. intermedia* from Tenerife and *G. galloti* from Tenerife, La Palma, La Gomera and El Hierro. However, experiments – based on mating, viability of hybrid offspring and allozyme distances – on this last species suggested that the lizards present in La Gomera and El Hierro should be considered as a different species (*G. caesaris*) (López-Jurado, Mateo & Guillaume, 1997). Genetic distances between *G. simonyi machadoi* and *G. intermedia* (belonging to the ‘*simonyi* group’, which includes all giant lizards from the western islands) were very similar to those between *G. galloti*

and *G. caesaris* (‘*galloti-caesaris* group’), suggesting that colonization of the western Canary Islands by each lineage was probably simultaneous.

The casual discovery of this new lizard in Tenerife led to the possibility that other giant lizards could still survive in some remote areas of La Gomera and La Palma islands. Therefore, in June 1999, we started a systematic search mainly focused on the most coastal areas of La Gomera, and fortunately, a new giant lizard was found still living in the westernmost part (Valle Gran Rey) (Valido *et al.*, 2000).

Hutterer (1985), based on the analysis of subfossil material from La Gomera, described two new subspecies of giant lizards, *G. goliath bravoana* and *G. simonyi gomerana*. Morphological studies (Nogales *et al.* 2001) indicate that this new extant lizard belongs to the ‘*simonyi* group’ and could correspond with the form described as *G. simonyi gomerana*, but with enough differences as to be treated as a full species (*G. gomerana*).

This finding provides an opportunity for further insight into the evolution and radiation of the genus *Gallotia* in the western Canary Islands. Therefore, in order to establish the phylogenetic position of this new lizard at the molecular level, we amplified and sequenced two enlarged segments of the previously analysed mtDNA gene fragments (500 bp of the 12S ribosomal RNA (rRNA) and 405 bp of the cytochrome *b* (*cytb*) using the same methods and conditions as in González *et al.* (1996) and Rando *et al.* (1997). Representatives of the *Gallotia* genus at specific and subspecific levels – as well as the six specimens of the new lizard captured in La Gomera – were analysed. For this, new primers were designed: L14724 (5’ TGAAGTGAAGAACCACCGTTG 3’) and H15149 (5’ AAAGTGCAGCCCTCAGAATGATATTTGTCCTCA 3’) for *cytb* and L1064 (5’ TTGACCACACGAAAGCTTAGAA 3’) and H1565 (5’ TTCCGGTACGCTTACCATGT 3’) for 12S rRNA fragments. The new lizard sequences were deposited in the GenBank/EMBL with accession numbers AJ272395 and AJ272396 for 12S rRNA and for *cytb*, respectively.

Out of the 405 bp analysed for the *cytb*, 144 were variable and 108 parsimony-informative. For the 12S rRNA fragment, 122 out of 500 bp were variable and 69 parsimony-informative. Parsimony analyses were performed using PAUP, version 3.1.1 (Swofford, 1993). Confidence in the nodes was determined by 1000 bootstrap replications using Branch-and-Bound searches. Phylogenetic relationships were also determined with the neighbour-joining algorithm as implemented in MEGA version 1.01 (Kumar, Tamura & Nei, 1993) using the Kimura 2N-parameters distance (Kimura, 1980). Since both methods gave similar topologies, only the results from the parsimony analysis are reported.

Phylogenetic relationships among the different representatives of the genus *Gallotia* based on the *cytb* and 12S rRNA sequences are shown in Figs. 1A and 1B respectively. Discrepancies at the specific level between both phylogenies commented upon in our previous arti-

*Correspondence:* M. Hernández, Department of Genetics, Faculty of Biology, University of La Laguna, E-38271 Tenerife, Canary Islands, Spain. E-mail: mnhdez@ull.es

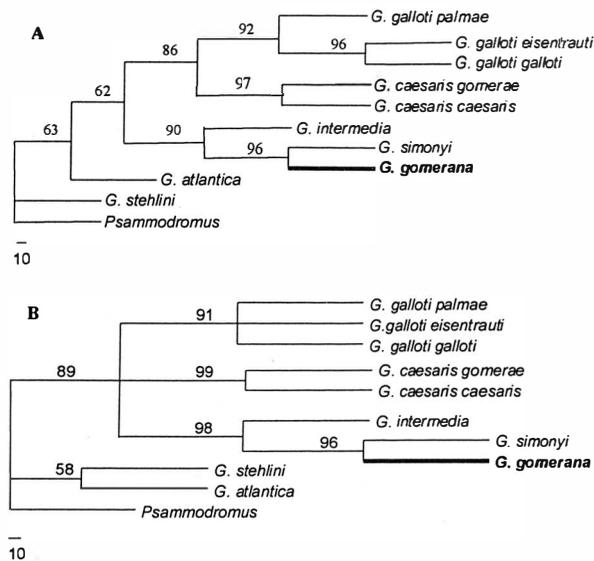


FIG. 1. Phylogenetic relationships among the Canarian endemic lizard genus *Gallotia* and one outgroup, based on cytochrome *b* (A) and 12S rRNA (B) partial sequences. Numbers on branches indicate the percentage of bootstrap support. The new branch is highlighted with bold lines. *Psammodromus* sequences for *cytb* and 12S rRNA have been taken from GenBank (Accession numbers: AF206535 and AF206588, respectively) (Fu, 2000).

cles (González *et al.*, 1996; Rando *et al.*, 1997) persist. *G. stehlini* is basal to the rest of the *Gallotia* species from the Canary Islands on the *cytb* tree, but it is sister to *G. atlantica* on the 12S rRNA one. In González *et al.* (1996) we propose that *cytb*, due to its faster divergence rate, would be a good marker only at subspecific level. A similar situation has been found by Graybeal (1993) in bufonid frogs, evidencing that the *cytb* gene has little phylogenetic signal for solving deep nodes. In spite of the relatively poor performance of the *cytb* gene, there seems to be no controversy in the position of the new giant lizard from La Gomera (Figs. 1A and 1B). In both cases it is related to other taxa of the 'simonyi group' and more closely to *G. simonyi* from El Hierro (1.5% and 0.2% divergence for *cytb* and 12S rRNA, respectively) than to *G. intermedia* from Tenerife (5.2% and 1.9%). Curiously the relationship among the giant lizards of Tenerife, La Gomera and El Hierro runs parallel to that of the 'galloti-caesaris group', with *G. caesaris caesaris* from El Hierro closer to *G. caesaris gomerae* from La Gomera than to *G. galloti galloti* from Tenerife. This supports our hypothesis that both groups followed the same colonization pattern on the western islands: stepwise sequence from the oldest (La Gomera) to the youngest (El Hierro) (Rando *et al.*, 1997). This is in agreement with the stepping-stone model proposed for several taxa from the Canary Islands (Juan *et al.*, 2000). Nucleotide divergence, measured as the maximum number of differences between taxa within the 'simonyi group' (20 and 9 substitutions for *cytb* and 12S rRNA, respectively) or by the Kimura 2N-parameters distance (Kimura, 1980) ( $0.052 \pm 0.011$  and  $0.023 \pm 0.006$  for *cytb* and

12S rRNA, respectively), is always lower than that found among subspecies of the 'galloti-caesaris group' (33 and 25 substitutions;  $0.092 \pm 0.017$  and  $0.053 \pm 0.011$ , for *cytb* and 12S rRNA, respectively), suggesting that the 'galloti-caesaris radiation' could predate that of *simonyi*. Nevertheless, the existence of a common ancestor of the 'simonyi and galloti-caesaris groups', suggested by Rando *et al.* (1997), is supported by the fact that both conform to a monophyletic group (Fig. 1B).

It is also interesting to note that *G. intermedia* was considered morphologically as a different species from *G. simonyi* (Hernández *et al.*, 2000), but the Kimura 2N-parameters distances are only 0.023 for 12S rRNA and 0.046 for *cytb*. In the case of the 'galloti-caesaris group' the two lineages (Tenerife-La Palma and La Gomera-El Hierro) should also be treated as two different species as proposed by López-Jurado *et al.* (1997) on the basis of interspecific-cross incompatibilities, which is reflected in the higher genetic distances ( $0.048 \pm 0.004$  and  $0.076 \pm 0.014$  for 12S rRNA and *cytb*, respectively).

Finally, the null variation observed for both fragments among the only six individuals of *G. gomerana* captured after four months of intensive trapping, seems to indicate that this lizard is in danger of extinction. In the last twenty-five years, living giant lizards from the 'simonyi group' have been found in El Hierro, Tenerife and La Gomera; La Palma is currently the only western Canary Island where their presence is unknown. In this island giant fossil bones have been recorded (see Bischoff, 1998) but, after prospecting 57 suitable localities for its presence, we have not yet succeeded in finding living animals.

**Acknowledgements.** We would like to thank G. M. Hewitt for his critical reading of the manuscript and our colleagues from the Departments of Genetics and Zoology, particularly V. M. Cabrera and A. Martín. We also thank an anonymous referee for his useful comments and suggestions.

## REFERENCES

- Arnold, E. N. (1973). Relationships of the palearctic lizards assigned to the genera *Lacerta*, *Algyroides* and *Psammodromus* (Reptilia: Lacertidae). *Bull. Br. Mus. Nat. Hist. (Zool.)* **25**, 1-366.
- Bischoff, W. (1998). Bemerkungen zu den fossilen Rieseneidechsen der Kanarischen Inseln. In *Handbuch der Reptilien und Amphibien Europas*. 6. Die Reptilien der Kanarischen Inseln, der Selvagens-Inseln und des Madeira-Archipels, 387-407. W. Bischoff (Ed). Bonn: Aula-Verlag Wiesbaden.
- Böhme, W. & Bings, W. (1975). Zur Frage des Überlebens von *Lacerta s. simonyi* Steindachner (Sauria: Lacertidae). *Salamandra* **11**, 39-46.
- Carranza, S., Arnold, E. N., Thomas, R. H., Mateo, J. A. & López-Jurado, L. F. (1999). Status of the extinct giant lacertid lizard *Gallotia simonyi simonyi* (Reptilia: Lacertidae) assessed using mtDNA sequences from museum specimens. *Herpetol. J.* **9**, 83-86.

- Fu, J. (2000). Toward the phylogeny of the family Lacertidae - Why 4708 base pairs of mtDNA sequences cannot draw the picture. *Biol. J. Linn. Soc.* **71**, 203-217.
- González, P., Pinto, F., Nogales, M., Jiménez-Asensio, J. J., Hernández, M. & Cabrera, V. M. (1996). Phylogenetic relationships of the Canary Islands endemic lizard genus *Gallotia* (Sauria: Lacertidae), inferred from mitochondrial DNA sequences. *Mol. Phyl. Evol.* **6**, 63-71.
- Graybeal, A. (1993). The phylogenetic utility of cytochrome b: Lessons from bufonid frogs. *Mol. Phyl. Evol.* **2**, 256-269.
- Hernández, E., Nogales, M. & Martín, A. (2000). Discovery of a new lizard in the Canary Islands, with a multivariate analysis of *Gallotia* (Reptilia: Lacertidae). *Herpetologica* **56**, 63-76.
- Hutterer, R. (1985). Neue Funde von Reisenidechsen (Lacertidae) auf der Insel Gomera. *Bonn. zool. Beitr.* **36**, 365-394.
- Juan, C., Emerson, B. C., Oromí, P. & Hewitt, G. M. (2000). Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. *Tree* **15**, 104-109.
- Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**, 111-120.
- Klemmer, K. (1976). The Amphibia and Reptilia of the Canary Islands. In: *Biogeography and Ecology in the Canary Islands. Monographia Biologica* **30**, 433-456. Kunkel, G. (Ed). Junk, The Hague.
- Kumar, S., Tamura, K. & Nei, M. (1993). MEGA: *Molecular Evolutionary Genetics Analysis*, version 1.01. Pennsylvania State University, University Park, PA. 16802.
- López-Jurado, L. F., Mateo, J. A. & Guillaume, C. P. (1997). El complejo *Gallotia galloti* (Oudart, 1839) (Sauria: Lacertidae) de las Islas Canarias: nuevos datos para la interpretación del proceso evolutivo del grupo. *Rev. Esp. Herp.* **11**, 35-46.
- Machado, A. (1985). New data concerning the Hierro Giant lizard and the lizard of Salmor (Canary Islands). *Bonn. zool. Beitr.* **36**, 429-470.
- Nogales, M., Rando, J. C., Valido, A. & Martín, A. (2001). Discovery of a living giant lizard, genus *Gallotia* (Reptilia: Lacertidae), from La Gomera, Canary Islands. *Herpetologica* **57**, 169-179.
- Rando, J. C., Hernández, E., López, M. & González, A. M. (1997). Phylogenetic relationships of the Canary Islands endemic lizard genus *Gallotia* inferred from mitochondrial DNA sequences: incorporation of a new subspecies. *Mol. Phyl. Evol.* **8**, 114-116.
- Swofford, D.L. (1993). *Phylogenetic analysis using parsimony*. Version 3.1.1. Washington, D.C.: Smithsonian Institution.
- Thorpe, R. S., McGregor, D. P. M. & Cumming, A. M. (1993a). Molecular phylogeny of the Canary Island lacertids (*Gallotia*): Mitochondrial DNA restriction fragment divergence in relation to sequence divergence and geological time. *J. Evol. Biol.* **6**, 725-735.
- Thorpe, R. S., McGregor, D. P. M. & Cumming, A. M. (1993b). Population evolution of western Canary Island lizards (*Gallotia galloti*): 4-base endonuclease restriction fragment length polymorphisms of mitochondrial DNA. *Biol. J. Linn. Soc.* **49**, 219-227.
- Thorpe, R. S., McGregor, D. P. M., Cumming, A. M. & Jordan, W. C. (1994). DNA evolution and colonization sequence of island lizards in relation to geological history: mtDNA RFLP, cytochrome *b*, cytochrome oxidase, 12s rRNA sequence, and nuclear RAPD analysis. *Evolution* **48**, 230-240.
- Valido, A., Rando, J. C., Nogales, M. & Martín, A. (2000). 'Fossil' lizard found alive in the Canary Islands. *Oryx* **34**, 75-76.

Accepted: 6.7.01

## ERRATUM

Hernández, M., Maca-Meyer, N., Rando, J. C., Valido, A. & Nogales, M. (2001). Addition of a new giant lizard from La Gomera Island to the phylogeny of the endemic genus *Gallotia* (Canarian archipelago). *Herpetological Journal* **11**, 171-173:

Page 171, left column, line 23: Rando *et al.* ( 1997) recognised four species and not five.

Page 171, left column, lines 35-36: *Gallotia simonyi* not *Gallotia simonyi machadoi*.