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# Biochemical Systematics and Ecology

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## Phylogenetic relationships of the *Chalcides* skink species from the Chafarinas Islands with those from mainland North Africa



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### ARTICLE INFO

#### Article history:

Received 19 November 2016

Received in revised form 15 February 2017

Accepted 17 February 2017

#### Keywords:

Skinks

*Chalcides*

Phylogeny

Chafarinas Islands

Mitochondrial DNA

North Africa

### ABSTRACT

Species whose geographical distribution encompasses both mainland and island populations provide ideal systems for examining potential isolation and genetic divergence. This has also interest from a conservationist point of view, as it is important to protect “evolutionarily significant units”. We report a phylogenetic mitochondrial DNA analysis comparing the populations of the three *Chalcides* skink species from the Chafarinas Islands (NW Africa) with specimens of the same species from the nearest mainland. We tested for the potential genetic distinctiveness of the skink island populations. However, the results of the comparison of the genetic variability of the mitochondrial coding gene *cytb* were conclusive showing that the genetic divergence between continental and island *Chalcides* species was either non-existent or extremely low. We discuss how genetic divergence may be lower than expected if separation time of the islands with the mainland has not been long enough or if the island skink populations were currently communicated via ocean rafting with individuals coming from the mainland ones.

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### 1. Introduction

Islands provide a good opportunity to understand how biodiversity is generated and maintained. This is because islands are discrete geographical entities within defined oceanic boundaries and because gene flow with the mainland is reduced by oceanic barriers (Whittaker and Palacios, 2007). Therefore, it is interesting to study the evolutionary history of species with both mainland and island populations, as they provide an ideal system for examining potential isolation and genetic divergence. This also has interest from a conservationist point of view, as it is important to protect “evolutionarily significant units” (Crandall et al., 2000; Fraser and Bernatchez, 2001).

The Chafarinas Islands is a small archipelago of volcanic origin (aprox. 8–9 Ma) (Barrera and Pineda, 2007) located in NW Africa in the southwestern Mediterranean Sea (35°11'N, 2°25'W), 3.4 km off to the northern mainland Moroccan coast (Ras el Ma, Morocco). It consists of three small islands with a total surface of 0.75 Km<sup>2</sup>: Congreso, Isabel II (the only inhabited island), and Rey Francisco. The Chafarinas Islands have been entirely isolated from the mainland since the rise in sea level during the Flandrian transgression, with the last possible connexion by a narrow sand dome between the coast and Congreso Island, but not the other islands, being probably lost at least 6500 years ago (Barrera and Pineda, 2007). There are ten reptile species in

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the Chafarinas Islands (García-Roa et al., 2014), but their biogeography and evolutionary relationships with their African mainland relatives are unknown. The Chafarinas Islands are strictly protected as a nature reserve and, therefore, it is important to know whether these populations form “evolutionarily significant units” worth to be protected.

Among the most characteristic reptile species of North Africa, there are around 24 skink species belonging to the genus *Chalcides* (Squamata, Scincidae), most of which occur in Morocco and surrounding areas (Mateo et al., 1995; Bons and Geniez, 1996; Carranza et al., 2008). A few of these *Chalcides* species are common and widespread, but many others have restricted distributions and are little known and threatened (Bons and Geniez, 1996). Three species of *Chalcides* skinks can be found in Chafarinas Islands (García-Roa et al., 2014; Martín et al., 2015) and in the nearest mainland (Mateo et al., 1995; Bons and Geniez, 1996; Mediani et al., 2015). The Ocellated skink, *Chalcides ocellatus*, is a widespread species in North Africa (Mateo et al., 1995; Kornilios et al., 2010; Mediani et al., 2015) that is also found in all of the three Chafarinas Islands (García-Roa et al., 2014). The Chafarinas skink, *Chalcides parallelus*, is a poorly known threatened species, which distribution is limited to the Rey Island in the Chafarinas Archipelago, where it is abundant (Civantos et al., 2013; García-Roa et al., 2014), and to a very narrow mainland coastal strip in North Africa where it is found sporadically (Mateo et al., 1995; Bons and Geniez, 1996; Mediani et al., 2015). The third skink species is the two-fingered skink, *Chalcides mauritanicus*, a poorly known fossorial species, whose distribution is limited to Congreso Island in Chafarinas (Martín et al., 2015) and a very small sandy coastal mainland area in North Africa (Mateo et al., 1995; Bons and Geniez, 1996; Mediani et al., 2015).

Here, we report a phylogenetic mitochondrial DNA analysis of the three *Chalcides* skink species from the Chafarinas Islands in comparison with specimens of the same species from the nearest mainland. We tested for the potential genetic distinctiveness of the Chafarinas Islands populations. We hypothesized that because the island populations should be isolated from the mainland ones, they might have evolved separately leading to genetic divergence and forming “evolutionarily significant units”. Alternatively, it is possible that genetic divergence may be low if separation time of the islands with the mainland has not been long enough or if there was gene flow via ocean rafting of individuals between continental and island populations (Carranza et al., 2000; Carranza and Arnold, 2003; De Queiroz, 2005).

## 2. Materials and methods

### 2.1. Sampling procedures

We conducted field work during two weeks in March 2014 (spring) at the Chafarinas Islands. Skinks were found and captured by hand under stones or burrowed in sandy areas. A small piece of tail (less than 5%) was sampled and preserved for DNA analysis in 96% ethanol. Skinks were released at the capture sites in less than 5 min after ensuring they were in good condition.

Samples from a total of nine skinks from the Chafarinas Islands, including two *C. mauritanicus*, four *C. parallelus*, and three *C. ocellatus*, were included in the phylogenetic analysis. To test the genetic distinctiveness of the skinks from Chafarinas, these were put into a broader context of related mainland taxa from different localities in North Africa and especially with specimens of the same species from the nearest mainland (Ras el Ma and Moulouya, Morocco), which had been collected for a previous study (Carranza et al., 2008) (Fig. 1). We assumed that these closest mainland populations are the most probable founding populations and the origin of possible migrants to the islets. A specimen of the skink *Scincopus fasciatus* was used to root the tree (Carranza et al., 2008). A list of the specimens included in the phylogenetic analysis with their corresponding geographical distribution, sample codes and GenBank accession numbers for the sequenced gene analyzed can be found as Supplementary Material.

### 2.2. Genetic analyses

Genomic DNA was extracted from alcohol preserved tissue samples using the Speedtools Tissue DNA Extraction Kit (Biotools BandM Labs S.A.). All samples were amplified for the cytochrome *b* mitochondrial gene (*cytb*) using the same primers and PCR conditions as in Carranza et al. (2008). The purification and sequencing of PCR products was carried out by MacroGen Inc., Amsterdam. The chromatographs were edited and assembled using Geneious v.R6 (Biomatters Ltd.). Sequences were also translated into amino acids and no stop codons were observed. DNA sequences were aligned using MAFFT v.6 (Katoh and Toh, 2008) applying parameters by default (i.e. Auto strategy, Gap opening penalty: 1.53, Offset value: 0.0).

To estimate the level of genetic divergence between samples, uncorrected (*p*-distance) mean genetic distances for the *cytb* gene fragment (307 bp analyzed) were calculated with MEGA 5 (Tamura et al., 2011). Best-fitting model of sequence evolution for the *cytb* gene was inferred using jModeltest v.0.1.1 (Posada, 2008) under the Akaike information criterion (AIC) (Akaike, 1973), being the GTR + G the model selected. The phylogenetic analysis was performed using Maximum Likelihood (ML) implemented in the software RAxML v.7.0.3 (Stamatakis, 2006). Reliability of the ML tree was assessed by bootstrap analysis (Felsenstein, 1985) including 1000 replications. Nodes were considered strongly supported if they received ML bootstraps values  $\geq 70\%$  (Wilcox et al., 2002).

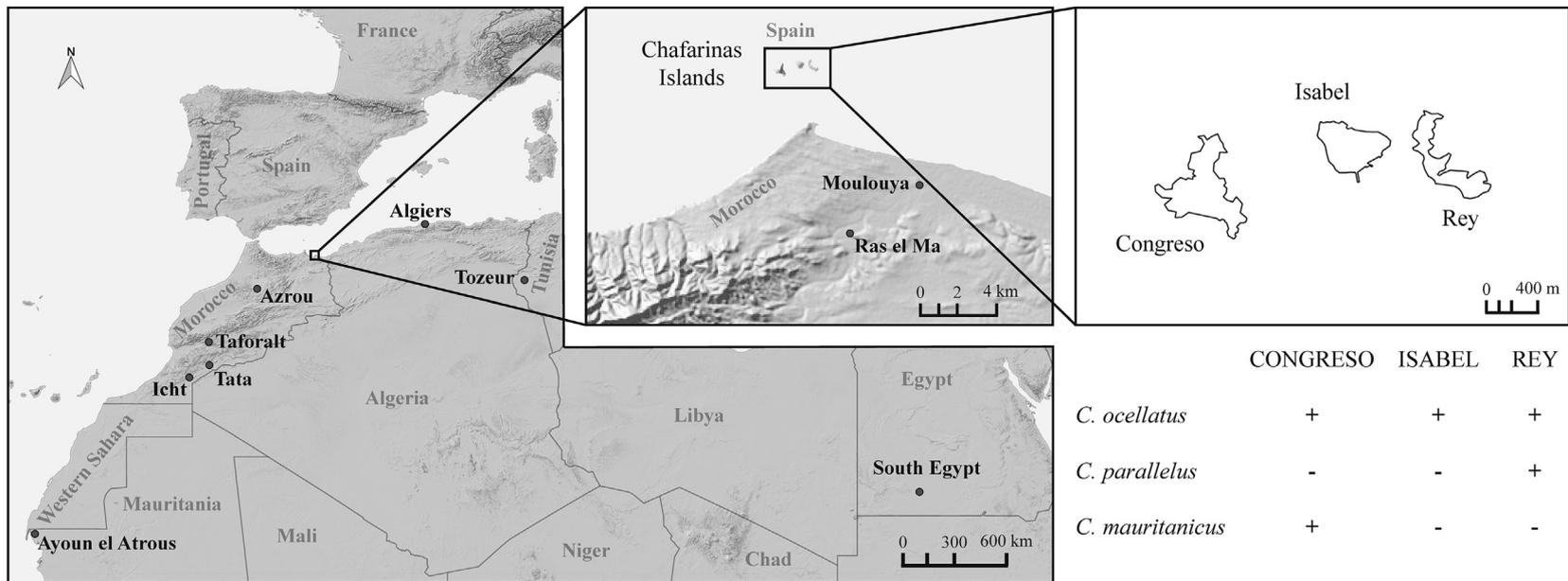


Fig. 1. Geographic location of the Chafarinas Islands, indicating the *Chalcides* skink species found at each islet (+), and mainland localities of the North African skinks used in the phylogenetic analyses.



In fact, rafting of terrestrial organisms dispersed while being confined to floating items has been reported from all oceans (De Queiroz, 2005; Thiel and Gutow, 2005). In particular, many reptiles can “travel” between islands even at long distances. For example, *Anolis* lizards and green iguanas are thought to be frequently dispersed between Caribbean Islands via rafting (Censky et al., 1998; Calsbeek and Smith, 2003). Also, the evolutionary history of *Tarentola* geckos from the Canary Islands has involved trans-oceanic dispersal from Africa, between the Canary and the Cape Verde islands, as well as between islands (Carranza et al., 2000). Moreover, two independent long range crosses between Africa and America have been suggested for skinks (Carranza and Arnold, 2003).

The Chafarinas Islands are not far from the coast, and after episodes of heavy storms, floods from the mainland Moulouya river (only 11 km away) result in a current of sediments and floating vegetal materials that can be observed on the sea heading towards the Chafarinas Islands. As a result, tree trunks and other materials often arrive to the island shores, which very likely could be the source of mainland skinks arriving to the islands. However, to assess whether there is current gene flow further genetic studies using nuclear markers would be needed.

An additional potential explanation might be that some island skinks could have been translocated by humans (Kornilios et al., 2010). However, this possibility is unlikely because access of visitors to the two uninhabited islands (Rey and Congreso islands) is highly restricted.

In spite of the low genetic distinctiveness of the populations of the skink species from the Chafarinas Islands, the conservation interest of the Chafarinas populations of skinks is still high. This is because this is a protected area that holds well-preserved high density populations (much higher than in the mainland) of these threatened skinks, which in North African mainland regions suffer a human-induced, progressive quick loss of the quality of their habitat (Mediani et al., 2015). The results of this study will contribute to the design of conservation plans for these skinks.

## Acknowledgements

The field station of the “Refugio Nacional De Caza de las Islas Chafarinas” provided logistical support. We thank Juan Ignacio Montoya, Javier Díaz, Gonzalo Martínez, Angel Sanz, Francisco López, Alfredo Ruiz and Javier Zapata for friendship and help in the Islands, and Meritxell Txipell for drawing the map. R. García-Roa benefited from a FPI grant, J. Ortega from a CSIC JAE-pre grant. Legal authorization and support for the study was provided by the Organismo Autónomo de Parques Nacionales (Spain), with additional financial support from the Ministerio de Economía e Innovación research project CGL2014-53523-P.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.bse.2017.02.007>.

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