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Molecular phylogeography of a widespread Malagasy leaf chameleon species, *Brookesia superciliaris*

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Chameleons of the Madagascan endemic genus *Brookesia* Gray are small to extremely small reptiles with a mostly terrestrial lifestyle, and due to their low mobility and their camouflage they are nearly invisible in the leaf litter of Madagascar's forests. The genus *Brookesia* is widely distributed (except in the arid south and south-west) but many *Brookesia* species are restricted to a relatively small area of the island (Glaw & Vences 2007). Speciation has been most prolific in the northern parts of Madagascar, an area that currently host about two-thirds of the 27 nominal *Brookesia* species (Raxworthy & Nussbaum 1995; Glaw & Vences 2007; Townsend *et al.* 2009).

Brookesia superciliaris (Kuhl) is distributed along the rainforest belt of Madagascar's east coast and can be found from lowland to mid-elevations up to about 1000 m above sea level, and it exhibits one of the largest distribution areas in this genus. This species is one of the largest leaf chameleons with a maximum total length of 120 mm (Nečas & Schmidt 2004; Glaw & Vences 2007). Nečas & Schmidt (2004) report that populations of *Brookesia superciliaris* vary in respect of total length and appearance. Glaw & Vences (2007) and Lutzmann (2007) mention that the supraocular cone differs in size between populations. Lutzmann (2007) also describes a reddish colour pattern on the eyelid, and reports the occurrence of two spines on the snout in specimens from the Masoala peninsula.

In the field it is difficult to distinguish *Brookesia superciliaris* from the sympatrically living *Brookesia therezieni*, although this species usually displays a series of dorsolateral pointed tubercles that extend onto the tail, which are absent in *Brookesia superciliaris*.

In the present study we analysed mitochondrial and nuclear DNA sequences of *Brookesia superciliaris* to reveal the genetic variability across the wide distributional range of this leaf chameleon species.

Tissue samples were collected by tail-clipping of all encountered individuals, most of which were subsequently released. Representative voucher specimens were preserved and deposited in the collections of the University of Antananarivo and the Zoologische Staatsammlung München.

After salt extraction of total genomic DNA, fragments of the mitochondrial 12S rRNA gene and of the nuclear Phosducin gene were PCR-amplified using the primers 12SAL (Kocher *et al.* 1989) and 16SR3 (Vences *et al.* 2003), and PHOF2 and PHOR1 (Bauer *et al.* 2007), using standard protocols.

After excluding parts of poor quality sequences, 19 12S rRNA gene sequences of 460 bp length, and 23 Phosducin gene sequences of 220 bp length of *Brookesia superciliaris* were used, together with outgroup sequences of *B. therezieni* from Makira forest. Sequences obtained for this study have been deposited in GenBank under the Accession Nos. GQ921665-GQ921716. Modeltest v3.7 (Posada & Crandall 1998) was used to search for the best nucleotide model of evolution, selecting the model suggested by the Akaike Information Criterion (Akaike 1974). Maximum likelihood (ML) analyses of the 12S rRNA gene dataset were performed in PAUP version 4.0b10 (Swofford 2002), using heuristic searches and nucleotides as equally weighted, and performing ten thousand bootstrap replicates to assess node support. Bayesian inference (BI) was computed with MrBayes v3.0b4 (Ronquist & Huelsenbeck 2003) using Markov Chain Monte Carlo (MCMC) sets for 10×10^6 generations and sampled every 100 generations after an initial burn-in of 20,000 trees. Prior to the Bayesian analysis, the same procedure of the optimal model of sequence evolution testing was conducted using MrModeltest v2.2 (Nylander 2004).

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