Zootaxa 2982: 40–48 (2011)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Deciphering the cryptic species diversity of dull-coloured day geckos *Phelsuma* (Squamata: Gekkonidae) from Madagascar, with description of a new species

ANGELICA CROTTINI[1,2,6,7], PHILIP-SEBASTIAN GEHRING[1], FRANK GLAW[3], D. JAMES HARRIS[4], ALEXANDRA LIMA[4,5] & MIGUEL VENCES[1]

[1]*Technical University of Braunschweig, Zoological Institute, Mendelssohnstr. 4, 38106 Braunschweig, Germany.*
[2]*Università degli Studi di Milano, Dipartimento di Biologia, Sezione di Zoologia e Citologia, Via Celoria 26, 20133 Milano, Italy*
[3] *Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 München, Germany*
[4]*CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, R. Padre Armando Quintas, 4485-661 Vairão, Portugal*
[5]*Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, R. Campo Alegre, 4169-007 Porto, Portugal*
[6]*Corresponding author. E-mail: tiliquait@yahoo.it*
[7]*Current address: CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, R. Padre Armando Quintas, 4485-661 Vairão, Portugal*

Abstract

We describe a new *Phelsuma* species from the relictual forest of Anja Reserve (13 km south from Ambalavao, on the central high plateau of southern Madagascar). *Phelsuma gouldi* **sp. nov.** seems to be an arboreal and possibly rock-dwelling species that has been observed in the private Anja Reserve (949 m a.s.l.), and possibly near Betroka almost 160 km further south-west. The species belongs to the *P. mutabilis* species group and differs from the other three species of the group, *P. mutabilis*, *P. breviceps*, and *P. borai* by a high genetic divergence of more than 10% in the mitochondrial 16S rRNA gene, and by a combination of 7–9 subdigital lamellae under the fourth toe, 6–7 supralabials, one internasal, and numerous details of throat scalation. *Phelsuma mutabilis* comprises three mitochondrial clades with divergences of more than 4% in the 16S rRNA gene, but the lack of distinct morphological differences and absence of a geographical structure among these clades indicate that this pattern is currently best considered as reflecting intraspecific variability.

Key words: Squamata, Gekkonidae, *Phelsuma*, new species, Madagascar, Ambalavao

Introduction

Undescribed cryptic species diversity is widespread in different Malagasy vertebrate groups as shown for example in lemurs (Yoder *et al.* 2000), amphibians (Vieites *et al.* 2009), and chameleons (Townsend *et al.* 2009), and it occurs both in poorly explored and in better studied areas. The accumulation of molecular data sets and the increasing use of integrative taxonomic approaches that combine molecular genetics and comparative morphology allow for a proper delimitation of cryptic but genetically distinct species, as well as for the identification of species complexes hidden under a single scientific name (Vences & Wake 2007; Vieites *et al.* 2009; Padial *et al.* 2010; Miralles *et al.* 2011). Completing the species inventory of Madagascar's highly endemic fauna is a relevant prerequisite for conservation assessments and thus needs to be accelerated in view of the ongoing habitat destruction on the island. With currently 42 recognised species and subspecies, the genus *Phelsuma* Gray represents the most diverse lizard genus of Madagascar. The genus *Phelsuma* probably originated in Madagascar and subsequently dispersed and radiated in the other Indian Ocean archipelagos, with a different colonisation history on each island group (Austin *et al.* 2004; Harmon *et al.* 2008; Rocha *et al.* 2009, 2010). *Phelsuma* are mostly colourful diurnal geckos of great morphological homogeneity. The colouration ranges from bright green, often with red spots and markings in most species, to dull grey or brownish in a few others. Despite extensive works recently published on *Phelsuma* systematics (e.g. Rocha *et al.* 2009, 2010) and the discovery of three new species within the last two years (Berghof &

 Accepted by A. Bauer: 23 May 2011; published: 2 Aug. 2011

Trautmann 2009; Glaw *et al.* 2009, 2010) there are still many uncertainties regarding their taxonomy and systematics. Many taxa, especially subspecies, have been described only based on chromatic characters and in several cases colour transitions/polymorphisms may represent local colour morphs (Glaw & Vences 2007; Rocha *et al.* 2009, 2010). Furthermore, several studies (Boumans *et al.* 2007; Raxworthy *et al.* 2007; Rocha *et al.* 2009, 2010) indicate the existence of problematic species complexes within the genus *Phelsuma*, with low morphological differentiation but high genetic divergences among populations.

The dull-coloured *Phelsuma* species from the southwestern and western arid regions of Madagascar (*P. mutabilis* (Grandidier), *P. breviceps* Boettger, *P. borai* Glaw, Köhler & Vences, and *P. standingi* Methuen & Hewitt) occupy rather basal positions in the phylogeny of this genus (Rocha *et al.* 2010). The *Phelsuma mutabilis* species group (including *P. mutabilis*, *P. breviceps*, and *P. borai*) forms a well supported monophyletic lineage with high genetic divergences observed between all three species (more than 20% uncorrected pairwise sequence divergence in the cytochrome *b* gene and more than 10% divergence in the 16S rRNA gene), indicating a long divergent evolutionary history (Rocha *et al.* 2010). Species in the *P. mutabilis* group share, among other morphological character states, a relatively low number of infralabial scales (5–6), smooth ventrals and subcaudals, the absence of nostril-rostral contact, the absence of bright green colour, and a non-gluing egg laying behaviour (Rocha *et al.* 2010). *Phelsuma mutabilis* is assumed to be one of the most widespread *Phelsuma* species in Madagascar, occupying a distribution range throughout nearly the whole western and southern coastal areas of Madagascar, from Ankarafantsika in the north to the Tolagnaro region in the south-east (Glaw & Vences 2007; Schönecker 2008). In addition, the occurrence of *P. mutabilis* is recorded from several inland localities, e.g. Zombitse forest, Isalo, and Betioky (Glaw & Vences 2007; own observations).

Our aim in this paper is to contribute to a better understanding of the systematics within the *Phelsuma mutabilis* group by (1) providing molecular data for samples of *P. mutabilis* from across its distribution range and (2) describing a new species of the *P. mutabilis* group based on its molecular and morphological distinctness.

Material and methods

The type specimen of the new species described herein was anesthetised and subsequently killed by injection with chlorobutanol, fixed with 90% ethanol, stored in 70% ethanol and subsequently deposited in the collection of the Zoologische Staatssammlung München, Germany (ZSM). Other collection acronyms used in this manuscript: SMF, Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main, Germany; ACZC and ZCMV refer to field numbers of A. Crottini and M. Vences. Tissue samples were taken by tail clipping and stored in 95% ethanol for further genetic analyses. Locality information was recorded using a GPS.

Morphological measurements were taken with digital callipers to the nearest 0.1 mm by P.-S. Gehring and A. Crottini. Definition of measurements and the description scheme of the holotype follows Glaw *et al.* (2009). Abbreviations are as follows: a.s.l.: above sea level; SVL: snout-vent length (measured from snout tip to cloaca); TL: total length (measured from snout tip to tail tip).

Total genomic DNA was extracted from the tissue samples using proteinase K digestion (10 mg/ml concentration) followed by a standard salt-extraction protocol (Bruford *et al.* 1992). A fragment of ca. 360 bp of the 3' terminus of the mitochondrial 16S rRNA gene was sequenced for seven individuals, *Phelsuma mutabilis* from Ankarafantsika (ZSM-DNA 20, tissue sample only, no voucher collected), from Toliara (ZSM 587/2000 and ZSM 945/2003), from a locality between Ampanihy and Tranoroa (ZSM 186/2004), from Tranomaro (ZSM 344/2005), from Ifaty (ACZC 1886), and the holotype of *P. gouldi* **sp. nov.** (ZSM 804/2010) using the primers 16S-Phel-L1 5'-AACCGTGCAAAGGTAGCATAA-3' and 16S-Phel-H1 5'-GAGGTCGTAAACCCCCTTG-3' (Glaw *et al.* 2010). The thermal profile was as follows: initial denaturation at 94 °C for 90 sec, 33 cycles of denaturation at 94 °C for 45 sec, annealing at 50 °C for 45 sec, elongation at 72 °C for 90 sec, followed by 10 minutes of final elongation. PCR products were resolved on an automated sequencer ABI 3130XL (Applied Biosystems). Sequences were blasted in GenBank and chromatographs were checked by eye and edited, when necessary, using CodonCode Aligner (version 3.7.1; Codon Code Corporation). Additional sequences of *P. standingi* from Ifaty (one of the most basal *Phelsuma* species; Rocha *et al.* 2009), *P. borai* from Bemaraha, *P. breviceps* from Toliara, and *P. mutabilis* from Makay, Toliara, Ejeda, and Antsalova were retrieved from GenBank and added to the alignment. The alignment of all sequences required the inclusion of gaps to account for indels in only a few cases in some hypervariable regions. All newly determined sequences have been deposited in GenBank (JF810247-JF810253). Uncorrected

pairwise distances (*p*-distances transformed into percent) within individuals, and between species (averaged across individuals) were computed using MEGA, version 4 (Kumar *et al*. 2008).

We performed maximum parsimony (MP) and Bayesian inference searches. PAUP* 4.0b10 (Swofford 2002) was used to conduct heuristic searches under the MP optimality criterion, with 100 random addition sequence replicates, equal character weighting, tree bisection and reconnection (TBR) branch swapping, and gaps coded as missing data. Nodal support was calculated by bootstrapping, with 2,000 replicates, ten random addition sequence replicates, and TBR branch swapping. Bayesian analyses were performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The GTR+G model was determined by AIC in MrModeltest (Nylander 2004) as the best-fitting model of substitution. We performed two runs of 10 million generations (started on random trees) and four incrementally heated Markov chains (using default heating values), sampling the Markov chains at intervals of 1,000 generations. Stabilisation and convergence of likelihood values occurred after about 1 million generations. The first five million generations were conservatively discarded, and five thousand trees were retained post burn-in and used to generate the majority rule consensus tree.

FIGURE 1. (A) Anja Reserve and surrounding anthropogenically modified areas; (B–C) views of the fragmented forest in Anja Reserve. A and B, photos by AC; C photo by Thomas Althaus.

Results

Phelsuma gouldi sp. nov.

(Figs. 2–3)