



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

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

<sup>1</sup>Technical University of Braunschweig, Zoological Institute, Mendelssohnstr. 4, 38106 Braunschweig, Germany.

<sup>2</sup>Università degli Studi di Milano, Dipartimento di Biologia, Sezione di Zoologia e Citologia, Via Celoria 26, 20133 Milano, Italy

<sup>3</sup>Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 München, Germany

<sup>4</sup>CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, R. Padre Armando Quintas, 4485-661 Vairão, Portugal

<sup>5</sup>Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, R. Campo Alegre, 4169-007 Porto, Portugal

<sup>6</sup>Corresponding author. E-mail: tiliquait@yahoo.it

<sup>7</sup>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; Padiál *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 &

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 Betsioky (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 anaesthetised 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'-GAGGTCGTAAACCCCTTG-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)

**Holotype.** ZSM 804/2010 (ZCMV 13056), adult female, collected in Anja Reserve (Fig. 1), 13 km south of Ambalavao 21°51'2.64" S, 46°50'33.80" E, 949 m a.s.l., Haute Matsiatra Region, Fianarantsoa province, southern central Madagascar, on 9<sup>th</sup> December 2009 by Angelica Crottini, D. James Harris, Iker A. Irisarri, Alexandra Lima, Solohery Rasamison and Emile Rajeriarison.

**Paratypes.** None.

**Diagnosis.** A presumably medium-sized, greyish-brown *Phelsuma* (SVL 45.1 mm, TL 81.5 mm) with an irregular pattern of brown to black lines and spots (Fig. 2).



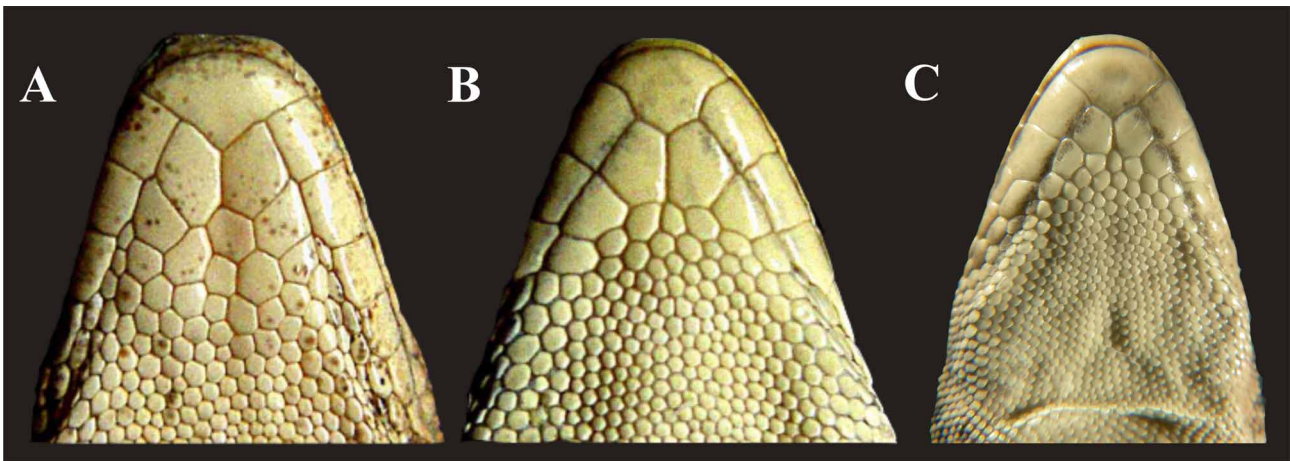
**FIGURE 2.** *Phelsuma gouldi* sp. nov., female holotype (ZSM 804/2010) in (A) dorsal and (B) ventral view.

It differs from all other *Phelsuma* species, except for *P. borai*, *P. mutabilis*, *P. breviceps*, *P. standingi*, and *P. masohoala* Raxworthy & Nussbaum by its cryptic life colouration without traces of green.

*Phelsuma gouldi* differs from *P. standingi* by its smaller total length (TL 81 mm vs. up to 279 mm), number of supralabials (7/6 vs. 9–12), number of infralabials (6 vs. 7–8) and shows a distinct life colouration (brown-greyish dorsal colouration with irregular blackish stripes and dots vs. grey or bluish-green with dark reticulation and with greenish head and bluish tail). It differs from *P. masohoala* by the number of supralabials (7/6 vs. 8–9), number of infralabials (6 vs. 7), smooth dorsal scales on body and tail vs. keeled dorsal scales, a distinct life colouration (brown-greyish dorsal colouration with irregular blackish stripes and dots vs. a white and black pigmentation), and by a single V-shaped chevron along the lower suture of infralabials vs. up to three V-shaped chevrons on the throat. *Phelsuma gouldi* differs from *P. breviceps* by a slender snout vs. a very stout snout, by the number of transversely enlarged subdigital lamellae under the fourth toe of left/right foot (7/9 vs. 11/10), by the presence of a single V-shaped chevron along the lower suture of infralabials vs. absence, and by a set of throat scalation characteristics (for comparison see Fig. 3 of this study and Fig. 3 in Glaw *et al.* 2009): a triangular-shaped mental in *P. gouldi* (vs. a bell-shaped mental in *P. breviceps*), two postmental scales (vs. one postmental scale), four rows of enlarged postmentals (vs. one), the minimum number of scales needed to connect the suture between the second and third infralabial from the left to the right is 10 in *P. gouldi* vs. 14 in *P. breviceps*.

The most similar species to *P. gouldi* are *P. borai* and *P. mutabilis*. *Phelsuma gouldi* differs from *P. borai* by a lower number of transversely enlarged subdigital lamellae under the fourth toe (7/9 vs. -/11), a lower number of

supralabials (7/6 vs. 10/9), one internasal scale (vs. three), the absence of a distinct concave groove between the nasals (vs. presence), by the presence of a V-shaped chevron along the lower suture of infralabials (vs. absence), and by a different configuration of the throat scalation (see Fig. 3, comparison A with C): two postmental scales bordering the mental scale for about one-third in *P. gouldi* vs. two postmental scales border about one half of the mental scale in *P. borai*, the minimum number of scales needed to connect the suture between the second and third infralabial from the left to the right is 10 vs. 5, presence of 4 rows of enlarged postmentals vs. 5, and by a horizontally divided third infralabial vs. undivided. *Phelsuma gouldi* differs from the *P. mutabilis* specimens examined in Glaw *et al.* (2009) comprising thirteen specimens from Toliara (ZSM 945/2003, ZSM 948/2003), Toliara-Arboretum (ZSM 587/2000, ZSM 588/2000), Ampanihy-Tranoroa (ZSM 186/2004), unknown localities (MNHN 1895.152, MNHN 1895.154), Androy Nord (MNHN 1901.150, MNHN 1901.151) and Menabe (SMF 9470-9473) by a lower number of transversely enlarged subdigital lamellae under the fourth toe (7/9 vs. min. 9/10 to max. 11/11) and by a different configuration of the throat scalation (see Fig. 3, comparison A with B): triangular-shaped mental scale vs. bell-shaped, the minimum number of scales needed to connect the suture between the second and third infralabial from the left to the right is 10 in *P. gouldi* vs. 5 in *P. mutabilis*, 4 rows of enlarged postmentals vs. 3 rows, and by a horizontally divided third infralabial vs. undivided. Furthermore, *P. gouldi* differs from *P. mutabilis*, *P. borai*, and *P. breviceps*, and all other *Phelsuma* species for which molecular data are available, by a substantial genetic differentiation (see Fig. 4).

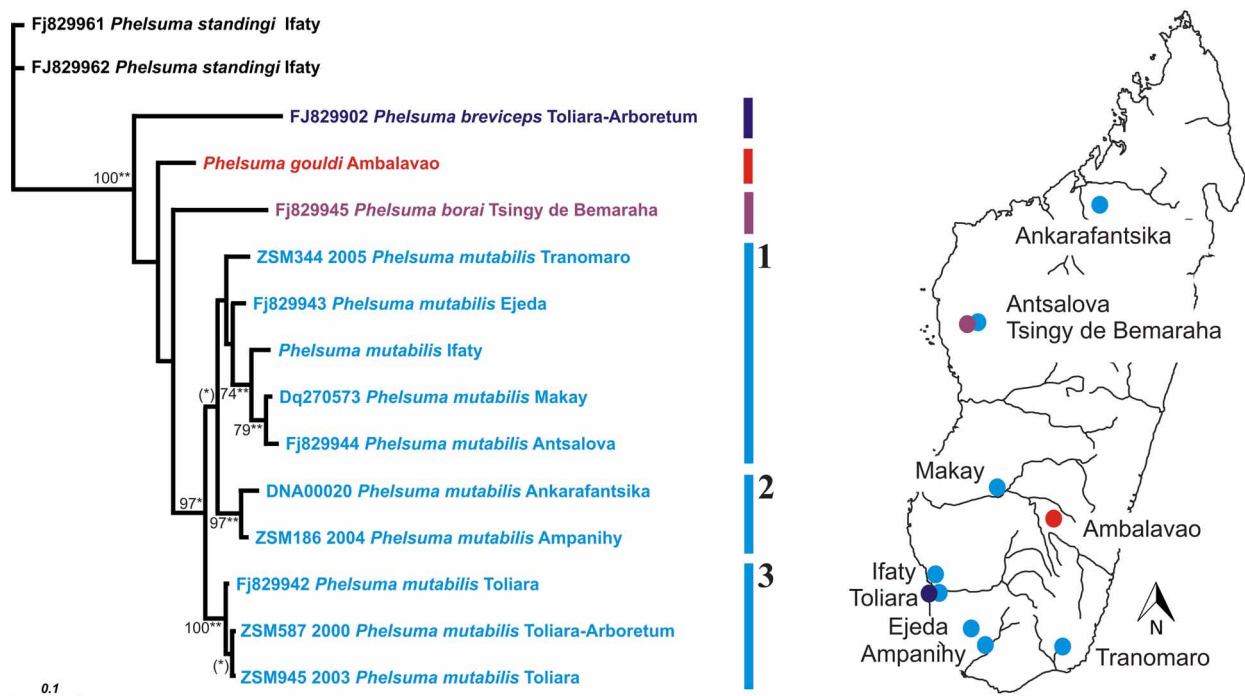


**FIGURE 3.** Mental region of preserved specimens, showing differences in scalation: (A) *Phelsuma borai* holotype (ZSM 103/2006); (B) *Phelsuma mutabilis* (ZSM 588/2000); (C) *Phelsuma gouldi* **sp. nov.**, holotype (ZSM 804/2010). Pictures (A) and (B) were retrieved from Glaw *et al.* (2009).

**Description of the holotype.** Well preserved, with regenerated tail autotomized after the first half. Autotomized tail part preserved separately. The tail tip (ca. 3 mm) was removed as a tissue sample. Body and head flattened dorsoventrally. Head slightly wider than neck, about as wide as body. Ear opening roundish. Tail shorter than snout-vent length, dorsoventrally flattened in cross section. No distinct tail whorls recognisable. Digits strongly expanded at tips, first finger and first toe vestigial, comparative finger and toe length  $1 < 2 < 5 < 3 < 4$ . Number of (transversely enlarged, left/right) subdigital lamellae under fourth toe 7/9. Rostral scale wider than tall and less wide than mental scale. Presence of distinct rostral cleft in dorsal process of rostral scale. One internasal scale. Nostril in contact with four scales, the first supralabial, the nasal and two small postnasals, but no contact with rostral. Pupil round. Dorsal and lateral scales of head smooth, nearly flat, becoming increasingly smaller on the posterior regions of the head. Dorsal and lateral scales of body have approximately uniform size and almost round shape. From the tail base on, enlarged almost rectangular smooth dorsal scales on the tail. All ventral and subcaudal scales smooth. The median row of subcaudal scales irregularly enlarged transversely. Mental scale largely triangular, bordered posteriorly by a pair of elongate, irregular hexagonal to pentagonal postmentals. Postmentals contact mental, first infralabial and three gulars. Gulars decrease gradually in size posteriorly. Number of supralabials (left/right) 7/6; number of infralabials (left/right) 6/6. The fifth supralabial on the left seems to be horizontally divided into two enlarged rectangular scales.

*Measurements:* SVL 45.1 mm; tail length 36.4 mm (plus ca. 3 mm that were taken as tissue sample); head

width (at widest point) 8.6 mm; snout length (anterior edge of eye to tip of snout) 5.4 mm; horizontal eye diameter 2.1 mm; ear opening diameter 0.7 mm; eye-ear distance 3.1 mm; internarial distance 1.5 mm; nostril-eye distance 5.3 mm, axilla-groin distance 20.5 mm; forelimb length (from axilla to tip of longest finger) 14.6 mm; hindlimb length (from groin to tip of longest toe) 18.9 mm.



**FIGURE 4.** Bayesian inference tree based on 358 bp of the mitochondrial 16S rRNA gene. *Phelsuma standingi* was used as an outgroup. Values at nodes are bootstrap values in percent from MP analyses; values below 70% not shown. Asterisks denote Bayesian posterior probabilities values: (\*), 90–94%; \*, 95–98%; \*\*, 99–100%. Numbers 1, 2 and 3 denote the three major clades identified within the analysed samples of *P. mutabilis*.

**Colouration:** Colour after nearly one year in alcohol similar to that shortly after being euthanised (Fig. 2). Unfortunately no pictures of the holotype in life are available, but a photograph provided by H.-P. Berghof resembling the holotype and possibly showing a *P. gouldi* from near Betroka (Fig. 5) suggests that colouration in life and in preservative are probably similar as is also the case in related species. Ground colour of head, body, tail and dorsal parts of limbs dorsally and laterally brown-grey with irregular blackish stripes and dots. Several dark framed light brown spots dorsally on neck, body and limbs. A thin, dark medio-dorsal line extending from mid-body towards neck, where it divides into two thin dark lines extending in direction of snout tip converging between eye socket and nostril. A black band from nostril to anterior eye, continuing from posterior eye above ear opening, fading out as single thin dark line shortly behind neck. Another dark band between mouth corner and ear opening. A distinct dark lateral band starting from neck extending posteriorly until the tailbase, shortly behind the groin which marks the colour border between dorsal and ventral part of animal. Between axilla and groin and underneath the lateral band an irregular line of single spots, which continues on the tail where it fades out in posterior half.

Supralabials and infralabials greyish-white with single dark pigmentations. Thin dark line along first two infralabials extending in posterior direction on gular scales, curving in direction to ear opening, fading shortly before that. Throat, chest, venter, and ventral parts of forelimbs and hindlimbs greyish-white. Toe tips on hand and feet dark grey-coloured.

**Distribution, conservation and IUCN Red List status.** The new species is currently known only from the type locality within the Anja Reserve (Fig. 1), but further investigations are required to understand its actual distribution. It is possible that records of *P. mutabilis* from the central areas of Madagascar actually refer to *P. gouldi*. For instance, Hallmann *et al.* (2008) cite an observation by H.-P. Berghof who found *P. mutabilis* between the Horombe Plateau and the Andringitra massif at an altitude above 1000 m a. s. l. On request H.-P. Berghof (pers. comm.) was able to define this locality more precisely as near Betroka which is some 160 km south-west of the type locality, suggesting that *P. gouldi* might not be a local endemic of the Ambalavao region.



FIGURE 5. A possible individual of *Phelsuma gouldi* from near Betroka in life, photo by Hans-Peter Berghof.

In the area of the type locality there was no evidence of mineral or precious stone extraction or collecting for the pet-trade, but deforestation for agriculture, logging and cattle grazing was observed in all surrounding areas. It is likely that *P. gouldi* will qualify for inclusion in one of the threatened categories, but due to the currently restricted knowledge on this species we suggest to consider its conservation status as “Data Deficient” according to IUCN criteria (IUCN 2001).

**Habitat and habits.** The holotype of *P. gouldi* was found around 4 p.m. on a tree trunk at a roosting height of about 2.5 meters. The tree was in an open and sunny spot inside a forest fragment. However, unpublished records report the sighting of *P. mutabilis*-like specimens on granitic rocks in Anja (Böhle, pers. comm.), and this record most probably refers to *P. gouldi*. No other *Phelsuma* species were found around the type locality.

**Etymology.** We name this new species in honour of Stephen Jay Gould, a paleontologist, evolutionary biologist, historian of science and supreme writer of popular science who also provided invaluable contributions to the public appreciation of natural history and of science in general.

**Available names.** Two junior synonyms are currently recognised for *Phelsuma mutabilis* and need to be considered as possible earlier available names for *P. gouldi* (see Hallmann *et al.* 2008; Glaw *et al.* 2009): *Phelsuma androyensis* Mocquard (originally described as *Phelsuma androyense*) and *Phelsuma micropholis* Boettger. The synonymy with *P. mutabilis* of both taxa was discussed in detail in Glaw *et al.* (2009). Based on comparisons of throat scalation and the number of transversely enlarged subdigital lamellae under fourth toe (11/10 and 11/11 in *P. androyensis* and 10/11, 11/10, 10/10, 11/11 in *P. micropholis*; vs. 7/9 in *P. gouldi*) it is evident that these two nomina are not conspecific with *Phelsuma gouldi* (see Table 1 in Glaw *et al.* 2009 for additional details).

**Mitochondrial DNA variation, differentiation and phylogenetic relationships.** The phylogenetic analyses resulted in a tree with largely unresolved basal relationships (Fig. 4) but with good support for the monophyly of *P. mutabilis* samples from across its range and clearly indicating the distinctness of *P. gouldi* from all the other three currently recognised species of the *P. mutabilis* group. The uncorrected genetic distance of *P. gouldi* to the three other *Phelsuma* species is: 8.1% to *P. mutabilis*, 12.8% to *P. breviceps*, and 8.5% to *P. borai*. The phylogenetic analyses identified three major mitochondrial clades within *P. mutabilis*. Clade 1, although not resolved, encom-

passes samples of *P. mutabilis* from the south east (Tranomaro), south west (Ifaty and Ejeda), and west (Makay and Antsalova); clade 2 includes the samples of *P. mutabilis* from the north west (Ankarafantsika) and from a locality between Tranoroa and Ampaniny (south west); finally, only samples coming from Toliara in the south west belong to the clade 3 (see Fig. 4). Genetic distances within *P. mutabilis* amount to 4.7% between the clades and range between 0.2% to 4.5% within clades.

## Discussion

The description of *P. gouldi* **sp. nov.** adds a distinctive new species to the *Phelsuma mutabilis* group that formerly contained *P. borai*, *P. breviceps*, and *P. mutabilis*. These three species inhabit lowland shrubs and dry forest areas of southwestern (*P. breviceps*, *P. mutabilis*) and western Madagascar (*P. mutabilis*, *P. borai*). On the contrary *P. gouldi* inhabits a dry area of the central high plateau of Madagascar, at an elevation of ca. 950 m a.s.l.

The Anja Reserve, where the holotype was collected, extends for about 30 hectares 13 km south of the district capital of Ambalavao and has been designed as a protected area only in 1999. This area is managed by the local community and it is currently the touristically most visited community managed forest in Madagascar. It is noteworthy that this little reserve seems to host several potential local endemics and probably still undescribed reptile species (e.g. *Brookesia* sp. aff. *brygooi*, *Paragehyra* sp. aff. *petiti*; own unpublished data) and is therefore of great importance for conservation efforts in this highly deforested area of central Madagascar.

The habitat inside of Anja Reserve is well preserved and the geological structure of the area, characterised by huge granitic boulders, provides a certain degree of natural protection for the vegetation among these boulders. In addition, the presence of these large rocks creates a sun-exposed habitat, suitable for this *Phelsuma* species that so far has been observed on tree trunks and rocks, assuming that all observations reported here (Berghof per. comm., Böhle pers. comm., own observation) are correctly attributed to this species, but not on the walls of houses in the surrounding areas. However, the fact that we encountered only a single specimen during intensive searches for one day and a half in the area suggests that *P. gouldi* might be rare or very secretive.

Based on its morphological characteristics, and especially the throat scalation that proved to be a useful character in the species recognition of the *P. mutabilis* group (Glaw *et al.* 2009), *P. gouldi* shows a mosaic-like distribution of characters. It shares with *P. borai* the presence of a triangular mental scale (Fig. 3; A, C) whereas the postmental scales are more similar in shape and dimension to those of *P. mutabilis* (Fig. 3; B, C), and the presence of a divided third infralabial seems to be unique for this taxon.

The comparatively high genetic variability within *P. mutabilis* observed in our study could be interpreted as reflecting the existence of a species complex, and we do not exclude that other records attributed to *P. mutabilis* actually correspond to yet undescribed species. However, the lack of obvious morphological differences among populations of different molecular clades, and the absence of a geographic pattern in the distribution of genetic differentiation suggest that the observed divergence within *P. mutabilis* is better considered as intraspecific variability. Furthermore, since we confirm the presence of *P. mutabilis* at Ankarafantsika where records of this species have been hypothesized to be in need of confirmation (Glaw *et al.* 2009), it appears possible that both *P. mutabilis* and *P. borai* occur sympatrically in this northwestern dry forest.

## Acknowledgements

We are grateful to the local staff of Anja Reserve for the permit to collect a limited number of reptiles from this site, thereby allowing the discovery of remarkable reptiles. For help in the field, we are indebted to our Malagasy assistant Emile Rajeriarison, and to our colleagues Iker A. Irisarri and Solohery Rasamison. Furthermore, we are grateful to Arne Hartig for providing an important tissue sample, to Hans-Peter Berghof and Andreas Böhle for sharing personal observations in the habitat of the new species, and to Thomas Althaus and Hans-Peter Berghof for providing pictures of the Anja Reserve and of a possible *P. gouldi* in life, respectively. This work was carried out in collaboration with the Département de Biologie Animale, Université d'Antananarivo (UADBA). We are grateful to the Malagasy authorities, in particular the Ministère de l'Environnement et des Forêts, for issuing research and export permits (Nos. 1195/09/MEF/SG/DGEF/DSAP/SLRSE) and to the community managing the Anja Reserve.

Fieldwork was partially funded by the Portuguese “Fundação para a Ciência e Tecnologia” (FCT) (PTDC/BIA-BDE/65745/2006). AL holds a PhD grant (SFRH/BF/47438/2008) funded by FCT. The work of PSG was supported by a project of the evolutionary biology funding initiative of the Volkswagen Foundation and the work of AC by a grant (Borsa di peregrinazione all' estero) of the Università degli Studi di Milano and of the FCT (SFRH/BPD/72908/2010).

## References

- Austin, J.J., Arnold, E.N. & Jones, C.G. (2004) Reconstructing an island radiation using ancient and recent DNA: the extinct and living day geckos (*Phelsuma*) of the Mascarene islands. *Molecular Phylogenetics and Evolution*, 31, 102–109.
- Berghof, H.-P. & Trautmann, G. (2009) Eine neue Art der Gattung *Phelsuma* Gray, 1825 (Sauria: Gekkonidae) von der Ostküste Madagaskars. *Sauria*, 31, 1, 5–14.
- Boumans, L., Vieites, D.R., Glaw, F. & Vences, M. (2007) Geographical patterns of deep mitochondrial differentiation in wide-spread Malagasy reptiles. *Molecular Phylogenetics and Evolution*, 45, 822–839.
- Bruford, M.W., Hanotte, O., Brookfield, J.F.Y. & Burke, T. (1992) Singlelocus and multilocus DNA fingerprint. In: Hoelzel, A.R. (Ed.), *Molecular genetic analysis of populations: a practical approach*. IRL Press, Oxford, United Kingdom, pp. 225–270.
- Glaw, F., Gehring, P.-S., Köhler, J., Franzen, M. & Vences, M. (2010) A new dwarf species of day gecko, genus *Phelsuma*, from the Ankarana pinnacle karst in northern Madagascar. *Salamandra*, 46, 83–92.
- Glaw, F., Köhler, J. & Vences, M. (2009) A new species of cryptically coloured day gecko (*Phelsuma*) from the Tsingy de Bemaraha National Park in western Madagascar. *Zootaxa*, 2195, 61–68.
- Glaw, F. & Vences, M. (2007) *A Field Guide to the Amphibians and Reptiles of Madagascar*. Third Edition. Vences & Glaw Verlag, Köln (Cologne), Germany, 496 pp.
- Hallmann, G., Krüger, J. & Trautmann, G. (2008) *Faszinierende Taggeckos. Die Gattung Phelsuma*. Second Edition. Natur und Tier-Verlag, Münster, Germany, 253 pp.
- Harmon, L.J., Melville, J., Larson, A. & Losos, J.B. (2008) The role of geography and ecological opportunity in the diversification of Day Geckos (*Phelsuma*). *Systematic Biology*, 57, 562–573.
- IUCN (2001) *IUCN red list categories and criteria*. Version 3.1. IUCN Species Survival Commission, IUCN, Gland, Switzerland and Cambridge, United Kingdom, 30 pp.
- Kumar, S., Dudley, J., Nei, M. & Tamura, K. (2008) MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics*, 9, 299–306.
- Miralles, A., Vasconcelos, R., Perera, A., Harris, D.J., & Carranza, S. (2011) An integrative taxonomic revision of the Cape Verdean skinks (Squamata, Scincidae). *Zoologica Scripta*, 40, 16–44.
- Nylander, J.A.A. (2004) *MrModeltest v2*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Padial, J.M., Miralles, A., de la Riva, I. & Vences, M. (2010) The integrative future of taxonomy. *Frontiers in Zoology*, 7, 16.
- Raxworthy, C.J., Ingram, C.M., Rabibisoa, N. & Pearson, R.G. (2007) Applications of ecological niche modeling for species delimitation: a review and empirical evaluation using Day Geckos (*Phelsuma*) from Madagascar. *Systematic Biology*, 56, 907–923.
- Rocha, S., Rösler, H., Gehring, P.-S., Glaw, F., Posada, D., Harris, D.J. & Vences, M. (2010) Phylogenetic systematics of day geckos, genus *Phelsuma*, based on molecular and morphological data (Squamata: Gekkonidae). *Zootaxa*, 2429, 1–28.
- Rocha, S., Vences, M., Glaw, F., Posada, D. & Harris, D.J. (2009) Multigene phylogeny of Malagasy day geckos of the genus *Phelsuma*. *Molecular Phylogenetics and Evolution*, 52, 530–537.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
- Schönecker, P. (2008) *Geckos of Madagascar, the Seychelles, Comoros and Mascarene Islands*. Edition Chimaira, Terralog 12, 144 pp.
- Swofford, D.L. (2002) *PAUP\*. Phylogenetic Analysis Using Parsimony (\*and Other Methods)*. Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts, USA.
- Townsend, T.M., Vieites, D.R., Glaw, F. & Vences, M. (2009) Testing species-level diversification hypotheses in Madagascar: the case of microendemic *Brookesia* leaf chameleons. *Systematic Biology*, 58, 461–656.
- Vences, M. & Wake, D.B. (2007) Speciation, species boundaries and phylogeography of amphibians. In: Heatwole, H.H. & Tyler, M. (Eds.), *Amphibian Biology*. Vol. 7, Systematics. Surrey Beatty & Sons, Chipping Norton, Australia, pp. 2613–2669.
- Vieites, D.R., Wollenberg, K.C., Andreone, F., Köhler, J., Glaw, F. & Vences, M. (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 8267–8272.
- Yoder, A.D., Rasoloarison, R.M., Goodman, S.M., Irwin, J.A., Atsalis, S., Ravosa, M.J. & Ganzhorn, J.U. (2000) Remarkable species diversity in Malagasy mouse lemurs (primates, *Microcebus*). *Proceedings of the National Academy of Sciences of the United States of America*, 97, 11325–11330.