



Day geckos (*Phelsuma*) in northern Madagascar: first step to resolve a paradoxical case of mitochondrial parphyly and morphological differentiation

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Abstract. We describe a new subspecies of day gecko, *Phelsuma dorsivittata paradoxa* ssp. n. (Squamata: Gekkonidae) from Nosy Be Island and the Sambirano region in northwestern Madagascar. Due to its colouration and small size, *Phelsuma d. paradoxa* shares at first glance more similarities with *P. parva* or *P. quadriocellata* than with *P. d. dorsivittata*. Yet, phylogenetic analysis of mitochondrial and nuclear-encoded DNA sequences unambiguously shows that *P. d. paradoxa* is related to the allopatrically distributed *P. d. dorsivittata*. Due to the substantial and constant morphological divergence and the geographic separation between the two taxa we concluded that assigning them an initial status of subspecies is adequate, although they are not reciprocally monophyletic in mitochondrial DNA. Subsequently we discuss possible scenarios for eyespot convergence and mitochondrial parphyly within *P. dorsivittata*. Furthermore, the molecular data presented here confirm the separate position of *P. lineata punctulata* within the *P. dorsivittata* complex. In order to resolve the parphyly of the *Phelsuma lineata* complex, we here elevate this taxon to species rank, as *Phelsuma punctulata* MERTENS, 1970.

Key words. Squamata, Gekkonidae, *Phelsuma punctulata*, *Phelsuma dorsivittata paradoxa* new subspecies, molecular genetics, taxonomy, Madagascar.

Introduction

Day geckos of the genus *Phelsuma* are among the most conspicuous lizards of the Malagasy region. Madagascar was most probably the origin of the diversification of *Phelsuma* after the initial colonization of this island by rafting ancestors from Africa. The crown age of the *Phelsuma* diversification was estimated between 43–35 million years (CROTTINI et al. 2012, MOHAN et al. 2020). The genus contains around 51 extant species, of which ca. 34 occur in Madagascar (GLAW & RÖSLER 2015). Despite the extensive published works on *Phelsuma* taxonomy, ecology, biogeography and ethology (e.g. LERNER 2004, RAXWORTHY et al. 2007, HARMON et al. 2008, GLAW et al. 2009, 2010, ROCHA et al. 2010, 2013, GEHRING et al. 2010, 2013, CROTTINI et al. 2011, BAXTER-GILBERT et al. 2021) there are still many important gaps of knowledge and uncertainties, including the alpha-taxonomy of these geckos.

One species assemblage with a poorly resolved taxonomy is the *Phelsuma lineata* species group. According to

molecular data (ROCHA et al. 2009, 2010, NAGY et al. 2012, GEHRING et al. 2013) two major clades can be defined within this species group: (1) *P. quadriocellata* including all its subspecies, as well as *P. parva*, and *P. antanosy* and (2) the *P. lineata* clade containing *P. lineata* (sensu lato), *P. dorsivittata*, *P. pusilla*, *P. kely*, *P. hoeschi*, and *P. comorensis*. Within the latter clade GEHRING et al. (2013) identified a monophyletic group containing all taxa of lined day geckos limited to northern Madagascar (in the following referred to as the *P. dorsivittata* complex).

Within the *P. dorsivittata* complex, the lineage splitting from the most basal node occurs at high elevations in the Marojejy massif and was called *P. cf. dorsivittata* by GEHRING et al. (2013), but will be named *P. sp.* “Marojejy highland” throughout this manuscript for clarity. Furthermore, the nominal taxa *P. dorsivittata* and *P. lineata punctulata* are part of this complex. The sequence divergence between both taxa ranged between 5.8 and 8% in a fragment of the mitochondrial 16S rRNA gene (GEHRING et al. 2013). In its current taxonomic circumscription, *P. l.*

punctulata leaves *P. lineata* paraphyletic since all other *P. lineata* subspecies and populations are unambiguously placed outside of this monophyletic group.

Substantial genetic variation was also detected within *P. dorsivittata*, which was found to contain two lineages sympatrically distributed in the Montagne d'Ambre mountain massif, and a third lineage known from two samples from the area of Iharana (formerly known as Vohemár) in north-eastern Madagascar. Surprisingly, a specimen from another population, collected from the island Nosy Be on the north-western coast, was also nested within *P. dorsivittata* (ROCHA et al. 2010, GEHRING et al. 2013). The phylogenetic position of this form was rather unexpected, as this population from Nosy Be was previously considered to be *P. cf. quadriocellata* or *P. cf. parva* based on its general appearance and colouration (e.g., ANDREONE et al. 2003).

The first record of "*Phelsuma quadriocellata*" from Nosy Be is found in a short report by CHRISTOPHEL in 1976 (see WOHLER 2014a), who unmistakably described the typical colouration, the eyespots behind the forearms and the prefemoral spots of these geckos. 25 years later, BUDZINSKI (2001) reported on the discovery of numerous individuals of "*P. quadriocellata parva*" on coconut trees in a hotel garden at the west coast of Nosy Be and along the adjacent coast. He assumed that this apparently isolated population might have been accidentally introduced by imported wood from northeastern Madagascar, but subsequent studies revealed that this form is more widespread on Nosy Be (as *P. cf. quadriocellata*; ANDREONE et al. 2003), Nosy Komba (HYDE ROBERTS & DALY 2014), in the mainland Sambirano region in northwestern Madagascar (VAN HEYGEN 2004) and on the Sahamalaza peninsular (PENNY et al. 2017).

In summary, the data from the comparison of mtDNA and general morphology are strongly contradictory in this case. Estimating accurate phylogenetic histories is relatively straightforward when the same or similar topologies supported by different types of data and different methodological approaches are observed. However, introgression and rapid diversification events can lead to reticulate evolution and are additional challenges for phylogenetic reconstructions (MALLET 2005, MALLET et al. 2016, ESQUERRÉ et al. 2022). Especially rather young species with incomplete reproductive isolation are likely to experience some gene flow with closely related species. In this case, considerable amounts of introgression can result, with genomes being invaded by heterospecific DNA (ZHANG et al. 2019, ESQUERRÉ et al. 2022).

In this study we provide novel data on the morphology and genetic differentiation within this complex of northern lined day geckos, and take a first step to solve the associated taxonomic problems by elevating *P. l. punctulata* to species rank, and describing *P. cf. dorsivittata* from Nosy Be and the Sambirano region as new subspecies. In order to explain the unexpected phylogenetic position of *P. cf. dorsivittata* we discuss three hypotheses, on (1) convergent evolution of the posthumeral eyespot phenotype, (2) possible mitochondrial introgression from *P. dorsivittata* (into *P. parva*), and (3) on the possibility of mitochondrial

pseudogenes amplified. Furthermore, we discuss the evolution of the posthumeral eyespot phenotype in *Phelsuma*.

Material and methods

Specimens collected in the field were anesthetized with chlorobutanol or lidocaine, killed with an overdose of the same anesthetic, fixed with 90% ethanol and stored in 70% ethanol. Tissue samples were preserved in 100% ethanol. Locality information was recorded with GPS receivers. Acronyms and institutional abbreviations used are: SMF, Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main; UADBA, Université d'Antananarivo, Département de Biologie Animale (now called Mention Zoologie et Biodiversité Animale); ZSM, Zoologische Staatssammlung München. DRV, FGZC, PSG and ZCMV, ZCSH refer to field numbers of D. R. VIEITES, F. GLAW, P.-S. GEHRING, M. VENCES and S. HAUSWALDT, respectively. Morphological measurements and counts (in millimeters or total numbers) were taken by F. GLAW and P.-S. GEHRING with a digital caliper to the nearest 0.1 mm. Abbreviations used are total length (TL) and snout-vent length (SVL). Additional abbreviations are provided in the captions of Table 1.

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. 350 bp of the 3' terminus of the mitochondrial 16S rRNA gene was sequenced for 18 individuals: *P. parva* from Vohibola (PSG 146, PSG 135 and PSG 137), *P. dorsivittata* from Montagne d'Ambre (ZCSH 361, ZCSH 364 and ZCSH 367), *P. lineata elanthana* from Anjzorobe (PSG 2577) and Andreba Gara (PSG 2862), *P. lineata bombetokensis* from Ankarafantsika (ZCMV 3643) and *P. cf. dorsivittata* from Nosy Be (ZSM-DNA 009, ZSM 39/2012; ZSM 57/2011, ZSM 93/2015 and FGZC 4689) and from Nosy Sakatia (ZSM 301/2018), using the primers 16S-Phel-L1 (5'-AACCGTGC AAAGGTAGCATAA-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. Additional 16S sequences of *P. sp.* "Marojejy highland" (ZSM 63/2005); *P. dorsivittata* from Montagne d'Ambre (ZSM 250/2004, ZSM 249/2004 and UADBA 24777) and Iharana (= formerly known as Vohemár) (voucher B1 and voucher B2); *P. cf. lineata* from St. Luce (FGZC 2643) and *P. lineata* from Ambohitantely (ZSM 62/2005) and Fierenana (ZMA 19302), *P. l. punctulata* from Tsaratanana mountain massif (DRV 6134, DRV 6238, DRV 6240, DRV 6243, DRV 6252, DRV 6253, DRV 6408, DRV 6424, DRV 6425, DRV 6426, ZCMV 12362, ZCMV 12363), *P. l. punctulata* from the Makira plateau (DRV 5909) and *P. pusilla pusilla* from Nosy Mangabe (ZCMV 2174); were available from previous studies (ROCHA et al. 2009, 2010, GEHRING et al. 2013) retrieved from GenBank and added to the alignment.

New subspecies of *Phelsuma*

Table 1. Selected scale characters in species and subspecies of day geckos. (*) data taken from ROCHA et al. (2010). Abbreviations: TL, total length (in mm); SVL, snout-vent length (in mm); SL, number of supralabials (min-max); SEC, number of supralabials to the center of eye; IL, number of infralabials (min-max); N, number of nasalia (defined as scales in contact with nostril); NC, contact of nostril only with first supralabial (0) or with rostral and first supralabial (1); NI, number of internasalia; S, scales on snout smooth (s), weakly keeled (wk), keeled (k); I, number of interorbitalia (min-max); IS, interorbitalia smooth (s), weakly keeled (wk), keeled (k); S3S, number of scales vertically between third sublabialia; G, gulars smooth (s) or keeled (k); DL, dorsal and lateral scales smooth (s), weakly keeled (wk), keeled (k); V, ventrals smooth (s), weakly keeled (wk), keeled (k); NSL, number of subdigital lamellae under 4th toe; PFP, number of preanofemoral pores in males; SRW, number of scale rows in whorls; DST, dorsal scales on tail smooth (s), weakly keeled (wk), keeled (k); SC, subcaudalia broadened (b) or not broadened (nb); SS, subcaudalia smooth (s), weakly keeled (wk), keeled (k). Missing data are represented by question marks (?).

| | TL (mm) | SVL (mm) | SL | SEC | IL | N | NC | NI | S | I | IS | S3S | G | DL | V | NSL | PFP | SRW | DST | SC | SS |
|---|-----------------------|-------------|-------|-----|------|---|-----|-----|----|-------|----|------|---|----|---|-------|-------|-----|-----|----|----|
| <i>P. punctulata</i> * | 115 | 57.3 | 7-8 | 6-7 | 6-8 | 3 | 1 | 2 | s | 19 | s | 12 | s | k | k | 13-14 | 26 | 7 | S | nb | s |
| <i>P. p. pusilla</i> * | 85 | 37 | 7-9 | 7-8 | 6-8 | 3 | 1 | 1 | k | 19-27 | k | 7-8 | s | k | k | 12-16 | 21-25 | 6-7 | K | nb | k |
| <i>P. l. lineata</i> * | 145 | 64 | 7-10 | 7 | 6-9 | 3 | 1 | 1-2 | k | 23-25 | k | 7-10 | s | k | k | 12-16 | 20-34 | 8 | K | nb | k |
| <i>P. d. paradoxa</i> ZSM 387/2010 FGZC 4689 (HT) | 69 | 32.4 | 10/10 | 7/7 | 9/9 | 3 | 1 | 2 | wk | 27-29 | k | 8 | s | wk | k | 15-15 | 21 | 7 | wk | nb | k |
| <i>P. d. paradoxa</i> ZSM 128/2019 | 92.3 | 40.96 | 9/9 | 7/7 | 9/8 | 3 | 1 | 1 | wk | 26 | k | 7 | s | k | k | 15 | 27 | 7 | k | nb | k |
| <i>P. d. paradoxa</i> ZSM 129/2019 | 52.7 (tail cut) | 34.6 | 9/9 | 7/7 | 8/8 | 3 | 1 | 2 | wk | 26 | k | 10 | s | k | k | 16 | 26 | 7 | k | nb | k |
| <i>P. d. paradoxa</i> ZSM 57/2011 | 83.5 | 40.6 | 9/10 | 7/7 | 10/8 | 3 | 1 | 1 | wk | 26 | wk | 10 | s | k | k | 15 | 0 | 7 | k | nb | k |
| <i>P. d. paradoxa</i> ZSM 31/2012 | 91.6 | 44.1 | 9/9 | 7/7 | 8/8 | 3 | 1/0 | 1 | wk | 26 | wk | 10 | s | k | k | ? | 0 | 8 | wk | nb | wk |
| <i>P. d. paradoxa</i> ZSM 93/2015 | 87.4 | 39.5 | 9/10 | 7/7 | 8/8 | 3 | 1 | 2 | wk | 25 | k | 7 | s | k | k | 14 | 25 | 7 | k | nb | k |
| <i>P. d. paradoxa</i> ZSM 301/2018 | 59.8 | 27.8 | 8/9 | 7/7 | 8/7 | ? | ? | ? | wk | 27 | k | 11 | s | k | k | 14/13 | 0 | 7 | k | nb | k |
| <i>Phelsuma dorsivittata</i> * | 130 | 57 | 8-9 | 7 | 6-9 | 3 | 1 | 1 | k | 25 | k | 13 | s | k | k | 17-19 | 25-29 | 7-8 | wk | nb | k |
| <i>P. dorsivittata</i> ZSM 249/2004 | 121.4 | 57.2 | 8/8 | 7/7 | 9/9 | 3 | 1 | 1 | wk | 25 | wk | 6-7 | s | k | k | 16/16 | 29 | 8 | K | nb | k |
| <i>P. dorsivittata</i> ZSM 911/2003 | 109.3 | 52.7 | 8/8 | 7/7 | 8/8 | 3 | 1 | 1 | wk | 24 | wk | 7 | s | k | k | 16/16 | 28 | 8 | K | nb | k |
| <i>P. dorsivittata</i> ZSM 250/2004 | 97.1 | 48.2 | 10/10 | 7/7 | 8/8 | 3 | 1 | 1 | wk | 25 | wk | 9 | s | k | k | 16/17 | 0 | 8 | K | nb | k |
| <i>Phelsuma parva</i> * | 85 | 36 | 7-10 | 7-9 | 7-8 | 3 | 1 | 1-3 | k | 21-30 | k | 7 | s | k | k | 12-15 | 20-24 | 6-8 | Wk | nb | k |
| <i>P. parva</i> ZSM 601/2006 | 83.2 | 37.4 | 9/8 | 7/6 | 7/8 | 3 | 1 | 1 | wk | 24 | wk | 8 | s | wk | k | 12/13 | 23 | 7 | K | nb | k |
| <i>P. parva</i> ZSM 190/2006 ZFMK 34481 (PT) | 67.8 | 36.7 | 9/8 | 7/7 | 7/7 | 3 | 1 | 1 | wk | 23 | k | 7 | s | k | k | 12/12 | 0 | 6 | k | nb | k |

A fragment of the nuclear-encoded gene oocyte maturation factor (C-mos) was amplified using the primers CO8 (5'-GCTTGGTGTTC AATAGACTGG-3') and CO9 (5'-TTTGGGAGCATCCAAAGTCTC-3'), following standard protocols (HAN et al. 2004). The obtained sequences were combined with those from ROCHA et al. (2009, 2010) and AUSTIN et al. (2004): *P. dorsivittata* from Montagne d'Ambre (FGMV 2002-937, FGZC 488) and Iharana (voucher B1 and voucher B2); *P. sp.* "Marojejy highland" (ZSM 63/2005 = ZCMV 2029); *P. pusilla pusilla* from Nosy Mangabe (ZCMV 2174) and of *P. parva* (PSG 135

and PSG 137). Because for the C-mos gene fragment only a limited number of sequences were available in GenBank, the datasets of the mitochondrial and nuclear gene fragments differ in the number of included sequences.

Following Exonuclease I and Shrimp Alkaline Phosphatase digestion, PCR products were resolved on automated sequencers ABI 3130XL (Applied Biosystems). Chromatographs were checked by eye and poor-quality stretches removed and read errors corrected, when necessary, using CodonCode Aligner (version 3.7.1; Codon Code Corporation).

All newly determined sequences have been deposited in GenBank (OM885341–OM885349 for C-mos and OM897121–OM897143 for 16S). Uncorrected pairwise distances (p-distances transformed into percent) between individuals and between species (averaged across individuals) were computed using MEGA, version X (KUMAR et al. 2018). We used ASAP (PULLANDRE et al. 2021) as implemented in the iTaxoTools package (VENCES et al. 2021) for an explorative species delimitation analysis based on the 16S alignment.

Our main goal here is not to study phylogenetic relationships within *Phelsuma*, which already have been assessed by ROCHA et al. (2010) and in detail in the *Phelsuma lineata* species group by GEHRING et al. (2013) using more appropriate multigene data sets. Instead we mainly aim at assessing differentiation among populations using two unlinked and thus partly independently evolving markers (mitochondrial and nuclear) and to use arguments of congruence among these markers, and of the molecular and morphological data sets, to delimit independent evolutionary lineages (see PADIAL et al. 2010). We therefore analysed the two DNA fragments independently. We chose a network analysis for the only minimally variable C-mos sequences, and maximum likelihood (ML), maximum parsimony (MP) and Bayesian phylogenetic analysis for the mitochondrial 16S fragment.

In the data set including the outgroup 81 of 322 characters were parsimony informative. 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 using the bootstrap analyses, with 2,000 replicates, ten random addition sequences replicates, and TBR branch swapping. Bayesian analyses were performed in MrBayes 3.1.2 (RONQUIST & HUELSENBECK 2003). The TIMe model was determined by AIC in Modeltest 3.7 (POSADA & CRANDALL 1998) as the best-fitting model of substitution. We performed two runs of 5 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. The two runs had achieved stationarity after the first 5,000 generations (discarded as burnin) as judged by plotting the generation numbers against their log-likelihoods, leaving 2,000 trees from which a majority rule consensus was produced. As a further measure of node support, we performed a bootstrap analysis under the Maximum likelihood criterion with 2000 replicates, using the GTR model in MEGA version X (KUMAR et al. 2018).

For graphically representing the relationship among alleles (haplotypes) of the nuclear-encoded gene, in particular of haplotype sharing or absence thereof, we visualized nuclear gene variation as haplotype networks. We inferred haplotype of the nuclear-encoded gene using the PHASE algorithm (STEPHENS et al. 2001) implemented in the DnaSP software (Version 6; ROZAS et al. 2017) and used the

phased sequences to reconstruct a Maximum Likelihood tree with the Jukes-Cantor substitution model in MEGA X (KUMAR et al. 2018). We then used this tree together with the respective alignments as input for Haploviewer (written by G. B. EWING; <http://www.cibiv.at/~greg/haploviewer>), a software that implements the methodological approach of SALZBURGER et al. (2011). The obtained network was manually redrawn using CorelDraw X3.

To be sure that the *Phelsuma* individuals with post-humeral eyespots do not show a closer relationship with taxa from the *P. quadriocellata* or *P. parva* group, these taxa were also included in all phylogenetic analyses, but for graphical reasons and easier understanding only the part of the tree corresponding to the *P. dorsivittata* complex is shown in the figures. The tree with full taxon coverage is provided in the Supplementary material (Fig. S1). *Phelsuma madagascariensis boehmei* served as an outgroup.

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: [lsid:zoobank.org/pub:AB8725A3-CD19-4CCC-858F-EA29481A18B3](https://zoobank.org/pub:AB8725A3-CD19-4CCC-858F-EA29481A18B3). The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: salamandra-journal.com, zenodo.org.

Results

The phylogenetic tree based on the ML and BI analysis of 322 bp of the mitochondrial 16S gene (Fig. 1 and Fig. S1) resolved the phylogeny of the northern *P. dorsivittata* group in agreement with previous more comprehensive studies (ROCHA et al. 2010, GEHRING et al. 2013) while MP analysis could not resolve several of the deep nodes. BI and ML agreed in recovering a monophyletic group containing *P. dorsivittata*, *P. cf. dorsivittata* (from Nosy Be), *P. sp.* “Marojejy highland” and *P. l. punctulata* with high statistical support. The candidate species *P. sp.* “Marojejy highland” was placed as sister to the clade containing all other *P. dorsivittata* (sensu lato) and *P. l. punctulata* populations in our phylogeny. The uncorrected pairwise distance between *P. sp.* “Marojejy highland” to *P. dorsivittata* (sensu lato) and *P. l. punctulata* ranges between 4.8% and 7.1% in 16S. Within the second clade, the following clearly differentiated lineages can be distinguished: five northern lineages of *P. dorsivittata* (sensu lato) and five to six lineages of *P. l. punctulata*, most of them restricted to only one of the mountain massifs in the area. The uncorrected pairwise distance between *P. dorsivittata* (sensu lato) and *P. l.*

punctulata ranges between 6.5 and 8.9% in 16S. Within the sampled *P. l. punctulata* and *P. dorsivittata* populations, sequence divergence reached up to 3.2% and 5.9% uncorrected pairwise distance respectively. Within *P. l. punctulata* from the Tsaratanana mountain massif three clades are separated by elevation (high elevation > 2400 m a.s.l. vs. ca. 2000 m a.s.l., vs. < 1500 m a.s.l.). An additional deeply separated *P. l. punctulata* lineage was found to occur on the western Makira plateau, and strikingly, at Bemanevika, two additional lineages were found to occur in sympatry (Fig. 1). The uncorrected pairwise distance between the Tsaratanana and Makira lineages reached up to 3.2% in the 16S gene fragment.

An unexpected phylogeographic pattern was also encountered for *P. dorsivittata*. This species was recovered as paraphyletic, with two lineages distributed in the Montagne d'Ambre mountain massif in northern Madagascar, another one in the area of Iharana in northeastern Madagascar and two lineages of the focal candidate species phenotypically similar to *P. parva* from the island of Nosy Be and Nosy Sakatia deeply nested within *P. dorsivittata* (Fig. 1). Uncorrected pairwise distances in 16S for *P. cf. dorsivittata* from Nosy Be reached up to 6.2% to *P. sp.* "Marojejy highland", 8.2% to *P. l. punctulata*, and 6.2% to *P. dorsivittata* from Montagne d'Ambre. The sequence divergence within the different lineages of *P. dorsivittata* reached up to 4.7%.

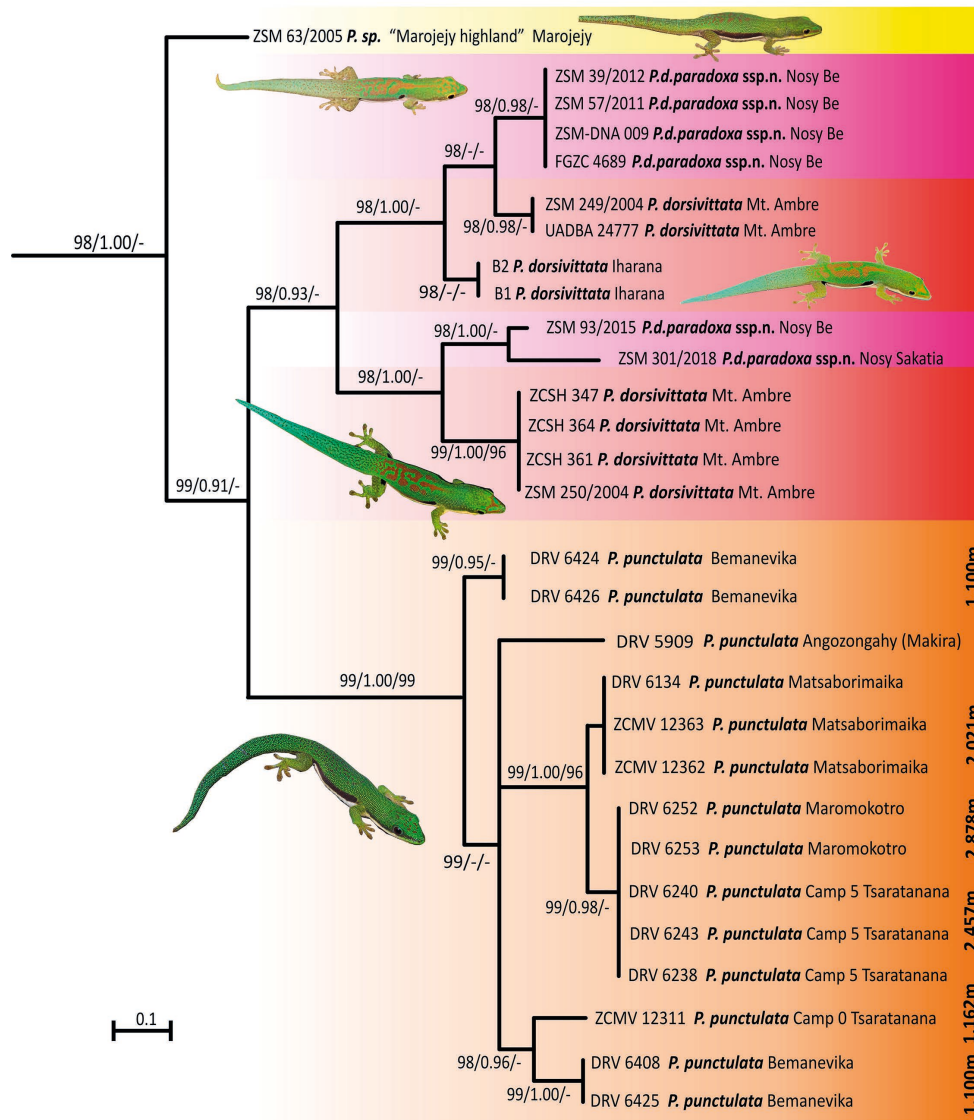


Figure 1. Maximum Likelihood phylogenetic tree of species in the *Phelsuma dorsivittata* species complex, based on 322 bp of the mitochondrial 16S rRNA gene. In the tree, values of Maximum likelihood bootstrap proportions followed by Bayesian posterior probabilities and MP bootstrap proportions (only values above 95% are shown). Additionally, the elevation (m a.s.l.) of sampling localities for *P. punctulata* is given. For the full tree that includes additional taxa of the *P. lineata* group and *P. madagascariensis boehmei* as outgroup (here removed to graphically focus on our target taxa), see Supplementary Figure S1.

Analysis of the 427 bp fragment of the conserved nuclear-encoded C-mos gene (Fig. 2) confirmed the lack of differentiation of *P. cf. dorsivittata* (Nosy Be) to the populations from Iharana and Marojejy, although the *P. sp.* “Marojejy highland” also had a second, distinct haplotype, separated from the other haplotype by three mutational steps (Fig. 2). Unfortunately, no C-mos sequences were available for *P. l. punctulata*. No haplotype sharing between *P. dorsivittata* (from Mt. Ambre) and the *P. parva*-like specimens from Nosy Be was recovered by our analysis, probably due to the small sample size. However, the analyses again show a clear separation of these specimens to true *P. parva* from Madagascar’s East Coast (2–4 mutational steps).

Analysis with ASAP compared ten alternative species partitions recovered from the 16S data set, which received ASAP scores between 4.5 and 9.5. The lowest score of 4.5 corresponded to a preferred partition with the highest amount of splitting, with 12 suggested species in the *P. dorsivittata* complex. The next best score of 5.0 was assigned both to a partition with nine species, and to the most lumping partition, which considered the entire *P. dorsivittata* complex as a single species. In the two partitions with high amount of splitting, the different sympatric mitochondrial lineages of *P. dorsivittata* at Montagne d’Ambre were assigned to distinct species-level lineages, and at least some of the *P. parva*-like samples from Nosy Be were placed as separate species-level unit as well.

Recognition of *P. punctulata* at species level

Our study confirms the deeply nested position of *P. l. punctulata* in a clade of day geckos distributed in northern Madagascar. In the work of ROCHA et al. (2010), *P. dorsivittata* was elevated from subspecies to species rank be-

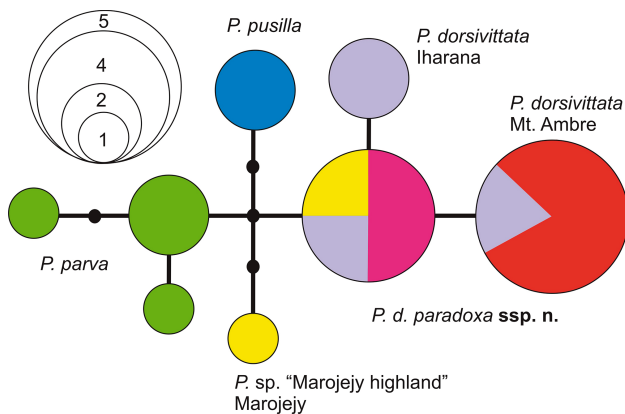


Figure 2. Haplotype network of the nuclear C-mos gene. Colours correspond to taxa and lineages (dark red = *P. dorsivittata* from Mt. Ambre; purple = *P. dorsivittata* from Iharana; pink = *P. d. paradoxa* ssp. n. Nosy Be; yellow = *P. sp.* “Marojejy highland”; blue = *P. pusilla*; grey = *P. parva*); circles are scaled according to the number of samples per haplotype; inferred intermediate steps between haplotypes are represented by small circles. Samples of *P. punctulata* were not included in the analyses.

cause of its clear molecular and morphological differentiation and the fact that the taxon *Phelsuma lineata* was being left paraphyletic with respect to *P. kely*, *P. comorensis* and *P. pusilla* (if *P. lineata* was defined, as previously, including *dorsivittata* as subspecies). Unfortunately, no samples of *P. l. punctulata* were included in those analyses. Three years later, in the comprehensive phylogeographic multi-gene study (four mitochondrial and one nuclear genes) of the *P. lineata* complex by GEHRING et al. (2013), samples of *P. l. punctulata* from the type locality, the Tsaratanana mountain massif and the Makira plateau were included and the phylogenetic position of *P. l. punctulata* was consistently recovered by mitochondrial markers as deeply nested within the northern *P. dorsivittata* clade. However, no formal taxonomic changes have yet been proposed. Our data presented here confirm the separate position of *P. l. punctulata* (Fig. 1). In order to resolve the paraphyly of the *Phelsuma lineata* complex, we here elevate *P. l. punctulata* to species rank. In addition to the genetic evidence *P. punctulata* differs morphologically from all other species in the *P. lineata* complex by smooth snout scales, interorbitalia, subcaudalia and dorsal scales on the tail, although these differences are relatively weak.

In life, *P. punctulata* has a shiny dark green metallic scaling and can appear completely black depending on the angle of view. The head and nape of the neck have fine red dots. From the nose opening a red line runs under the eye to the ear opening. Dorsally the animals show a red, partly also black dotted pattern. The upper side of the tail shows a weak, dark marbling. A white lateral band runs from the tip of the snout to the rear extremities. Above this is another black lateral band, starting at the forelimbs and running to just behind the hindlimbs. A gray lateral line runs below the white lateral band from the tip of the snout to the base of the tail. The underside is coloured white. Upon examination in 2021 the male holotype (SMF 65500, Fig. 3) of *Phelsuma l. punctulata*, shows the general colour pattern as described above, although the green and red colours are faded. Selected morphological characters on the holotype SMF 65500 are as follows (for a detailed description see MERTENS 1970 and RÖSLER & INEICH 2010): SVL 57.3 mm; TL 117.8; 8/7 supralabials on the left and right side of the head; 8/6 infralabials; 1 internasal; nostril in contact with rostral and first supralabial; 25 preanofemoral pores; mental triangle-shaped; dorsals, ventrals, and subcaudals keeled.

Description of a new subspecies *Phelsuma dorsivittata paradoxa* ssp. n.

LSID: zoobank.org:act:4C208294-6391-40D7-8B05-F18DF131B125

Remarks: This species has been mentioned and depicted by the following authors: CHRISTOPHEL (1976) – “Pfauenaugen-Phelsume”, record from Nosy Be; BUDZINSKI (2001) – *Phelsuma quadriocellata parva*, record and photo from Nosy Be; ANDREONE et al. (2003) – *Phelsuma cf. quadriocellata*, record from Lokobe reserve on Nosy Be (no

voucher listed); VAN HEYGEN (2004) – *Phelsuma quadriocellata parva*, records and photo from Ampasindava (site 1); BUDZINSKI (2005) – *P. quadriocellata* cf. *parva*, captive breeding, photo from Nosy Be; BERGHOF (2005: 124) – *Phelsuma quadriocellata* cf. *parva* (2), photo from Manongarivo; HALLMANN et al. (2008) – *Phelsuma quadriocellata parva*, distribution discussed in text; SCHÖNECKER (2008) – *Phelsuma quadriocellata* aff. *parva*, photo from Anki-fy; GEHRING & GLAW (2010) – *Phelsuma* cf. *parva*, photo (of the holotype) from Nosy Be; SCHÖNECKER & SCHÖNECKER (2010) – *Phelsuma* aff. *parva*, photo from Anki-fy; NAGY et al. (2012) – *Phelsuma* aff. *parva*, in phylogenetic tree; GEHRING et al. (2013) – *Phelsuma* cf. *dorsivittata*, in phylogenetic tree; BERGHOF (2014: 172) – *Phelsuma* cf. *dorsivittata*, photo from Nosy Be; HYDE ROBERTS & DALY (2014) – *Phelsuma quadriocellata*; record and photo from Nosy Komba; WOHLER (2014a) – *Phelsuma* cf. *dorsivittata*; photo from Nosy Be; WOHLER (2014b) – *Phelsuma* cf. *dorsivittata*, discovery of record by CHRISTOPHEL (1976); BLUMGART et al. (2017) – *Phelsuma quadriocellata*, record from Nosy Komba; PENNY et al. (2017) – *Phelsuma* sp. aff. *quadriocellata*, record and photo from Ankarafa forest; ESSER (2021) – *Phelsuma* cf. *dorsivittata*, captive breeding.

Holotype. ZSM 387/2010 (field number FGZC 4689), (sub?)adult male with complete original tail, hemipenes not everted, collected at Chanty Beach Hotel (-13.31342°, 48.18406°, 15 m above sea level), western side of Nosy Be Island, Diana region (former Antsiranana province), northern Madagascar, on 14 March 2010 by F. GLAW.

Paratypes. ZSM 128/2019 (FGZC 5543), adult male with complete tail, hemipenes almost not everted, and UADBAR-FGZC5546 (FGZC 5546), adult female with two eggs in its body cavity, both collected in Antsahabe settlement (-14.002833°, 48.18465°, 125 m a.s.l.), ca. 3 km south of Ankarimbe, on 10 April 2019 by F. GLAW, K. GLAW, T. GLAW, A. RAZAFIMANANTSOA and J. H. RAZAFINDRAIBE; ZSM

129/2019 (FGZC 5545), adult male, tail broken, hemipenes everted, collected at the western foot of Maromiandra mountain (-14.01577°, 48.19048°, 113 m a.s.l.), ca. 4.5 km southeast of Ankarimbe, on 10 April 2019 by F. GLAW, K. GLAW, T. GLAW, A. RAZAFIMANANTSOA and J. H. RAZAFINDRAIBE; ZSM 93/2015 (no field number), adult male with broken tail, hemipenes not everted, collected near Lokobe National Park (-13.40127°, 48.34314°, ca. 20 m a.s.l.), southeastern side of Nosy Be Island, on 5 February 2014 by the Frontier Team Nosy Be (donated by S. HYDE ROBERTS); ZSM 301/2018 (no field number), subadult (unsexed) from Nosy Sakatia close to Nosy Be (ca. -13.31417°, 48.15556°, altitude unknown), donated by H.-P. BERGHOF in 2018; ZSM 57/2011 and ZSM 31/2012, two adult females, from near Chanty Beach Hotel (-13.31342°, 48.18406°, 15 m above sea level), Nosy Be Island, donated by R. BUDZINSKI in 2011. All paratypes are from the Sambirano region in northern Madagascar.

Diagnosis. *Phelsuma dorsivittata paradoxa* is a member of the *Phelsuma lineata* species group and characterized by small size, green dorsal colour in life with red markings and a relatively large black posthumeral spot that is dorsally surrounded by light blue colour. It differs from all other species in the genus *Phelsuma* (except *P. quadriocellata* and *P. parva*) by the presence of an oval blackish spot behind the insertion of forelimbs (posthumeral spots), which is dorsally bordered by a light blue ring that does not extend onto the back. It furthermore differs from most other species in the *lineata* group (*P. antanosy*, *P. comorensis*, *P. dorsivittata*, *P. lineata*, *P. punctulata*, and *P. quadriocellata*) by smaller size (max. TL ≤ 92 mm versus ≥ 110 mm; max. SVL ≤ 44 mm versus ≥ 46 mm). *P. d. paradoxa* differs from the most similar species *P. parva* by the presence of a distinct dark prefemoral spot, by less distinct whorls on the tail (compare Fig. 6), and by the pattern of mental scales, which are continuously and gradually decreasing in size in *P. d. paradoxa* (versus irregularly decreasing in *P. parva*). It differs from

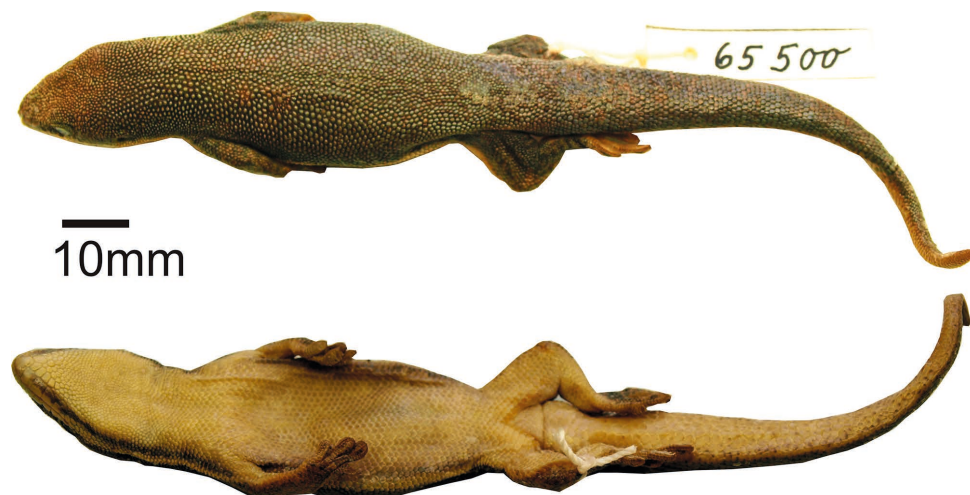


Figure 3. Holotype of *Phelsuma lineata punctulata* (SMF 65500) in dorsal and ventral view.

P. quadriocellata by smaller size (max. TL 92 mm; max. SVL 44 mm versus max. TL 110 mm, max. SVL 63 mm) and usually by larger red markings on the back. *P. d. paradoxa* differs from other *Phelsuma* species by a distinct genetic distance in mitochondrial and nuclear genes.

Phelsuma dorsivittata paradoxa differs from individuals assigned to the nominal subspecies, *P. dorsivittata dorsivittata*, by distinctly smaller size (max. TL 92 mm / max. SVL 44 mm versus max. TL 130 mm / max. SVL 57 mm), presence of blue colour around the posthumeral spot (versus absent), a slightly lower number of preanofemoral pores in males (21–27 versus 25–29), slightly fewer subdigital lamellae under the fourth toe (13–16 versus 16–19), and higher number of interorbitalia (25–29 versus 24–25). Most of these differences also apply to the male holotype of *P. dorsivittata* (SMF 59373) with the following data (according to MERTENS 1964 and ROCHA et al. 2010: 21, respectively): 57 / 55.3 mm SVL, 130 / 124.9 mm TL, no blue ring around posthumeral spot, 27 / 28 preanofemoral pores, 18 subdigital lamellae under the fourth toe, number of interorbitalia not available.

Description of the holotype. Well preserved, left forelimb removed as tissue sample with complete original tail (verified by x-ray photographs, which show distinct tail vertebrae). Hemipenes not everted. Body and head flattened dorsoventrally. Head as wide as neck, about as wide as body. Ear opening rounded. Tail (36.1 mm) longer than snout–vent length (32.1 mm), dorsoventrally flattened in cross section. Tail whorls poorly recognizable in preservative (each whorl with seven scale rows), but clearly visible in life. Digits strongly expanded at tips, first finger and first toe vestigial, comparative finger and toe lengths $1 < 2 < 5 < 3 < 4$. Number of transversely enlarged subdigital lamellae under fourth toe 15/15 (left/right). Rostral scale wider than tall, as wide as mental. No rostral cleft in dorsal process of rostral scale. Two internasal scales. Center of nostril placed behind the suture between rostral and first supralabial. Nostril in contact with four scales (first supralabial, nasal, two small postnasals), but not in contact with rostral. Pupil round. Dorsal and lateral scales of head weakly keeled, becoming increasingly smaller on the posterior regions of the head. Dorsal and lateral scales of body semi-spherical, some of the dorsal scales keeled. Dorsal scales of tail mostly conical and unkeeled. All ventral and subcaudal scales slightly keeled. 78 scales around midbody. The median row of subcaudal scales not enlarged transversely. Mental scale intermediate between triangular and bell-shaped, bordered posteriorly by a pair of enlarged, elongated, irregular hexagonal postmentals. Postmentals contact mental, first infralabial and two gulars. Gulars decrease in size posteriorly behind postmentals and become increasingly roundish. Number of supralabials (left/right) 10/10; number of infralabials (left/right) 9/9, number of well developed preanofemoral pores 12, with additional 9 distinctly smaller and poorly developed pores.

Measurements: Snout–vent length 32.1 mm; tail length 36.1 mm; head width (at widest point) 6.1 mm; snout length

(anterior edge of eye to tip of snout) 4.2 mm; horizontal eye diameter 2.0 mm; horizontal ear opening diameter 0.4 mm; eye–ear distance 2.8 mm; internarial distance 1.5 mm; nostril–eye distance 3.9 mm, axilla–groin distance 12.5 mm; forelimb length (from axilla to tip of longest finger) 8.5 mm; hindlimb length (from groin to tip of longest toe) 10.6 mm.

In life, the holotype was dorsally bright green with an irregular pattern of connected red spots forming a continuous marking that points in anterior direction into a thin mediodorsal red line extending onto the neck (Fig. 4). There was a pattern of partly separated, partly connected red spots on snout and head as well as in the pelvic region. A narrow brown band was running from the snout tip to the anterior border of the eye and a wider, less distinct greyish band from the eye to the insertion of forelimbs. An oval black spot behind the forelimbs was dorsally bordered by a blue ring that did not extend onto the back. Posteriorly to this posthumeral spot a distinct brown lateral band continued until the insertion of the hindlimbs where a small but distinct, black prefemoral spot was recognizable. The dorsal surface of the tail was bluish-green scattered with small reddish dots, partly bordered by dark pigment. A narrow grey band extended laterally on the tail where it faded shortly before the tail tip. The iris was copper-coloured and the eye was surrounded by a conspicuous narrow, yellow ring. The dorsal surfaces of forelimbs and especially of the hindlimbs were marbled green, grey and brown. The ventral colour was almost white, with a lateral narrow grey band that extended between the limbs and anteriorly until the ear opening. The ventral surfaces of the feet were yellowish and the lamellae of fingers were grey. When distressed, the splendid dorsal colouration darkened partly to dirty shades of brown.

After 11 years in alcohol, the colouration has strongly faded. The dorsal ground colour of body and tail are now grey-brown with the black ocelli behind the forelimbs as the only exception. A distinct black spot on the head behind the eyes was not present in life. The red stripes and markings on the back have almost entirely disappeared and do not allow a re-identification of the pattern photographed in life. The dark lateral band is not recognizable anymore. The ventral surfaces are now uniformly whitish except for the greyish tail tip and lamellae of the toes.

Variation. Morphological data of the available type specimens are provided in Table 1 and the colour variation in life is shown in Figs 4–5. All individuals had a yellow ring around the eyes and the posthumeral spot was consistently bordered by a blue ring in unstressed individuals. In general, there seems to be remarkably little colour variation throughout the range of this subspecies.

Distribution and conservation status. The species is widespread in the Sambirano region (Fig. 7), where it is known from the following localities: west coast of Nosy Be (BUDZINSKI 2001, WOHLER 2014a); Nosy Sakatia Island west of Nosy Be (Fig. 5); Lokobe National Park and its surround-

ings in southeastern Nosy Be (ANDREONE et al. 2003 [no voucher specimen listed]; ZSM 93/2015); Nosy Komba (HYDE ROBERTS & DALY 2014; BLUMGART et al. 2017), Ankify (SCHÖNECKER 2008: 114 and photograph of A. HARTIG Fig. 5); Ampasindava Peninsula up to 400 m a.s.l. (Site 1 [0–200 m], Site 2, Site 5; VAN HEYGEN 2004); Manongarivo Special Reserve (BERGHOF 2005: 124); Maherivaratra 50 km north of Ambanja (-13.5000°, 48.6644°, 30 m a.s.l. (BERGHOF 2005: 124); Ankarimbe (-13.97376°, 48.17692°, 140 m a.s.l.; photograph of M. BARTELS); Antsahabe settlement near Ankarimbe (UADBAR-FGZC5546); western foot of Maromandra mountain near Ankarimbe (ZSM 129/2019); and Ankarafa Forest (-14.3800°, 47.7583°, 150–170 m a.s.l.) on the Sahamalaza peninsular (PENNY et al. 2017). Most records are from very low elevation between sea level and 150 m a.s.l., with the highest recorded elevation of ca. 400 m a.s.l.

No *Phelsuma quadriocellata*, *P. parva* or *P. dorsivittata* were recorded in surveys at Manongarivo (RAKOTOMALALA 2002), several localities in the Sambirano region (RASELIMANANA 2008), and the Tsaratanana region (ANDREONE et al. 2009).

This day gecko is presumably endemic to the Sambirano-Region in north-western Madagascar, where there is substantial and continuing decline in the extent and quality of natural habitats. A minimum convex polygon of the known distribution of this subspecies covers an area of ca. 6,000 km², qualifying the subspecies for a status of Vulnerable under the IUCN Red List Criterion B (IUCN 2012). Nevertheless, *Phelsuma d. paradoxa* appears to be relatively tolerant of anthropogenic alteration of habitats and occurs in villages, plantations, and hotel gardens. Additionally, this subspecies is known from three protected areas in north-western Madagascar (Lokobe National Park,

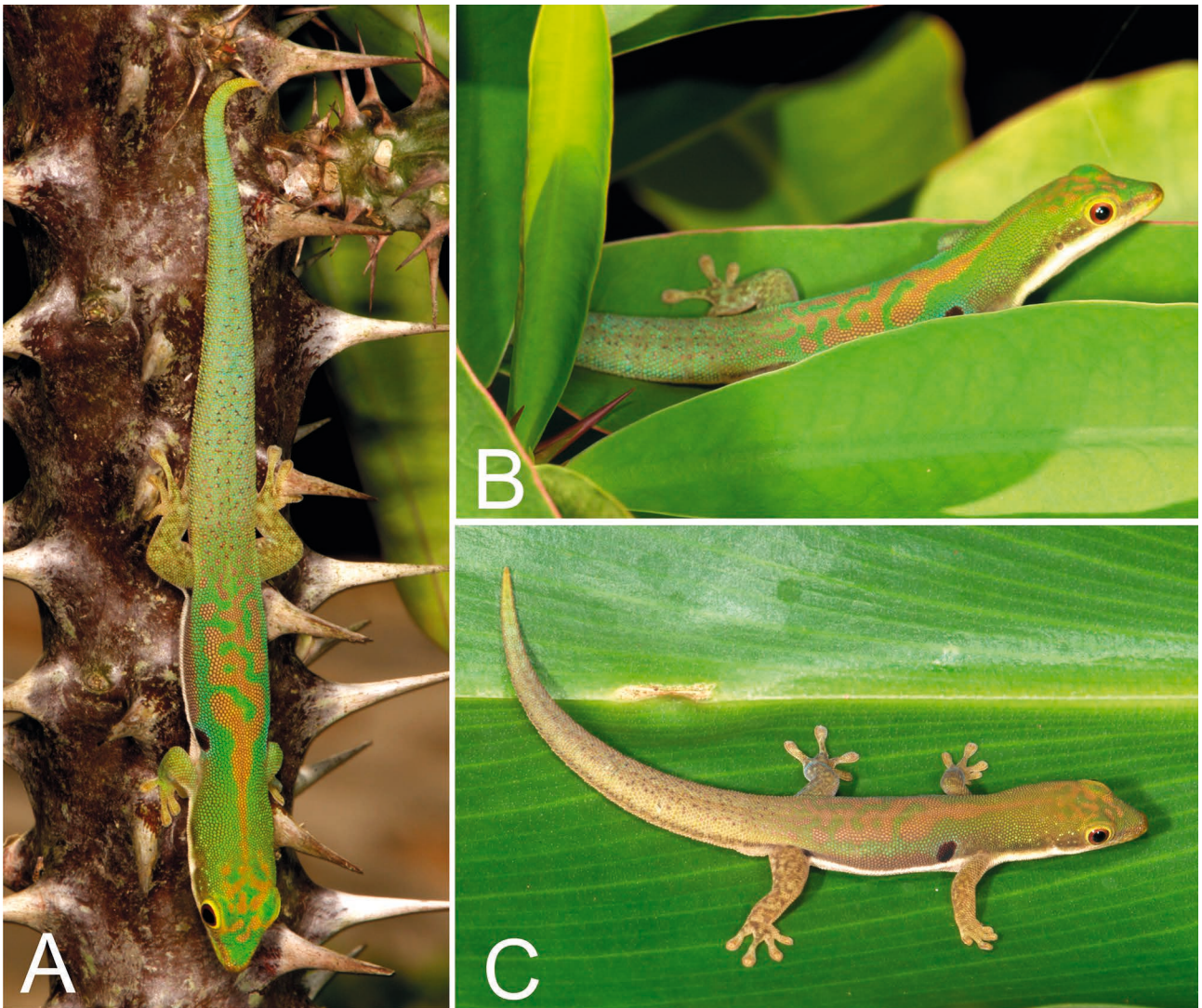


Figure 4. *Phelsuma dorsivittata paradoxa* ssp. n., male holotype from Nosy Be (ZSM 387/2010) in life. (A) dorso-lateral view; (B) lateral view; (C) in stressed state.



Figure 5. Photographs of *Phelsuma dorsivittata paradoxa* ssp. n. in life. (A) Male paratype from Maromiandra (ZSM 129/2019); (B) male paratype from Nosy Sakatia (ZSM 301/2018); (photo H.-P. BERGHOF); (C, D) two individuals from Nosy Be (photo R. BUDZINSKI); (E) individual from Ankify (photo A. HARTIG); (F) individual from Ankarimbe (photo M. BARTELS); (G) individual from Manongarivo (photo H.-P. BERGHOF); (H) individual from Ankarafa forest (photo G. M. ROSA); and (I) individual from the Ampasindava peninsula (photo E. VAN HEYGEN).

Manongarivo Special Reserve, Sahamalaza-Îles Radama National Park).

Habitat and habits. The holotype (ZSM 387/2010) and a few further specimens were observed in and around a hotel garden on palm trees, *Euphorbia* plants and stems of small trees and bushes. The same garden was populated by *Phelsuma abbotti*, *P. dubia*, *P. laticauda* and *P. grandis*. ZSM 128/2019 and UADBAR-FGZC5546 were found in coconut trees near the huts of a small settlement. ZSM 129/2019 was sitting on a screw palm (*Pandanus*). BUDZINSKI (2001) found numerous individuals of *P. d. paradoxa* on coconut trees. VAN HEYGEN (2004) observed this subspecies along the western coast of the Ampasindava peninsula (site 1) in secondary vegetation on trees up to 5 m and many juveniles in a coffee plantation. In addition, he observed one specimen at site 2 at the edge of a bamboo forest at higher elevations and on palms at higher elevations (400 m, a.s.l., site 5). WOHLER (2014a) observed it on a *Ravenala madagascariensis*, on the wall of a bungalow, and on coconut trees. PENNY et al. (2017) observed this subspecies in *Pandanus* screw palms in low density between 150–170 m a.s.l. MARKUS BARTELS (pers. comm.) observed *P. d. paradoxa* on tree trunks and palms in Ankarimbe. All these observations demonstrate that *P. d. paradoxa* is not specialized on bamboo, in contrast to the other *Phelsuma* species endemic or largely endemic to the Sambirano region (*P. klemmeri*,

P. seippi, *P. vanheygeni*). Observations in captivity revealed information on its behaviour and natural history, including the fact that this subspecies does not glue its eggs to the substrate (BUDZINSKI 2005, ESSER 2021). *P. d. paradoxa* is apparently more commonly bred in captivity than the externally very similar *P. parva*, and both taxa can be easily confused.

Etymology. The subspecies epithet ‘*paradoxa*’ is the feminine form of the Latin adjective *paradoxus* (derived from Greek παράδοξος—*parádoxos*) meaning “contrary to expectation” but also “marvellous”. This name was given in reference to the fact that these day geckos appear very similar to *P. quadriocellata* and *P. parva* at first sight, but contrary to this expectation they are actually closely related to the northern *P. dorsivittata* clade.

Available names. Most species-level nomina of *Phelsuma* are either valid species or subspecies, or unambiguously assignable as synonyms to valid species, but two exceptions are evident. *Phelsuma minuthi* was described by BÖRNER (1980), based on a single specimen without locality data. It is currently considered as synonym of *P. lineata* (RAXWORTHY & NUSSBAUM 1994, GLAW et al. 2010). *P. d. paradoxa* can be easily distinguished from the holotype of *P. minuthi* by the presence of a large black posthumeral spot that is surrounded by blue colour in life. Another ocellated day

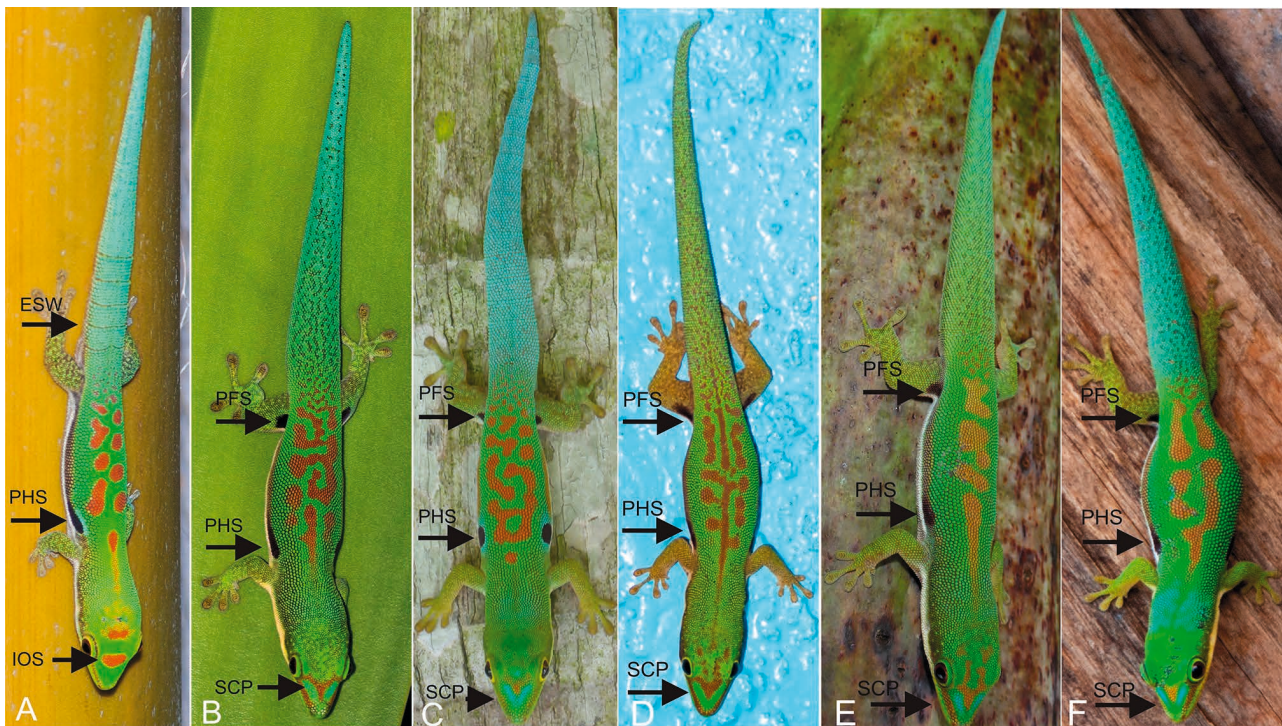


Figure 6. Detailed view of the typical colouration and colour patterns of specimens of (A) *P. parva* (from Lac Rasoamasay; ca. 60 km south of Toamasina), (B) *P. d. dorsivittata* (from Montagne d’Ambre); (C) *P. d. paradoxa* ssp. n. (from Maromandia); (D) *P. d. dorsivittata* (from Iharana); (E) *P. d. dorsivittata* (from Black Lemur Camp); (F) *P. d. dorsivittata* (from Antsahatopy; photo A. HARTIG). ESW = enlarged scales on whorl; IOS = interorbital spot; PFS = prefemoral spot; PHS = posthumeral spot; SCP = snout colouration pattern. Photos A, B, D and F by A. HARTIG.

gecko was described as *Phelsuma quadriocellata leiura* by MEIER (1983). The holotype of this subspecies (115 mm total length) is larger than *P. d. paradoxa* (≤ 92 mm total length) and the type locality “Imotra” is located at high elevation (1300 m a.s.l.) in southern Madagascar (MEIER 1983), further excluding a confusion with *P. d. paradoxa* on the basis of biogeographical arguments. *P. q. leiura* is currently considered as synonym of *P. quadriocellata quadriocellata* (KRÜGER 1996).

Discussion

Evolutionary relationships in the *P. dorsivittata* complex

The study at hand recovered the monophyly of a clade of lined day gecko populations in northern Madagascar (clade A in GEHRING et al. 2013; referred to as *P. dorsivittata* complex in this discussion).

Phelsuma sp. “Marojejy highland” represents the most basal lineage of this clade. Phenotypically, this candidate species is also known from the adjacent Anjanaharibe-Sud

massif and the surroundings of Andapa (GEHRING, pers. obs.). This form differs from all other members of this clade by clear genetic differentiation (uncorrected pairwise distance 4.8–7.1% in 16S). This observation is congruent with the assumed high level of endemism of the Marojejy massif, which might have acted as a mountain refuge and center of speciation (RAXWORTHY & NUSSBAUM 1995, WOLLENBERG et al. 2008, BROWN et al. 2016).

With the recognition of *P. punctulata* at species level, we resolve the problem of the paraphyly within *P. lineata*, but intensive work will still be required to solve all taxonomical difficulties within the *P. lineata* group (GEHRING et al. 2013). We classify the different populations of *P. punctulata* from Tsaratanana (at different elevations) and Makira as deep conspecific lineages (DCL) given that they are monophyletic, differ by only low genetic distances (up to 3.2% uncorrected pairwise distance in 16S), are geographically connected, and show no obvious morphological divergence. These populations are separated by elevation (high elevation > 2000 m a.s.l. vs. < 1500 m a.s.l.) within the Tsaratanana mountain massif. *P. punctulata* was found mainly in more open habitats such as the summit

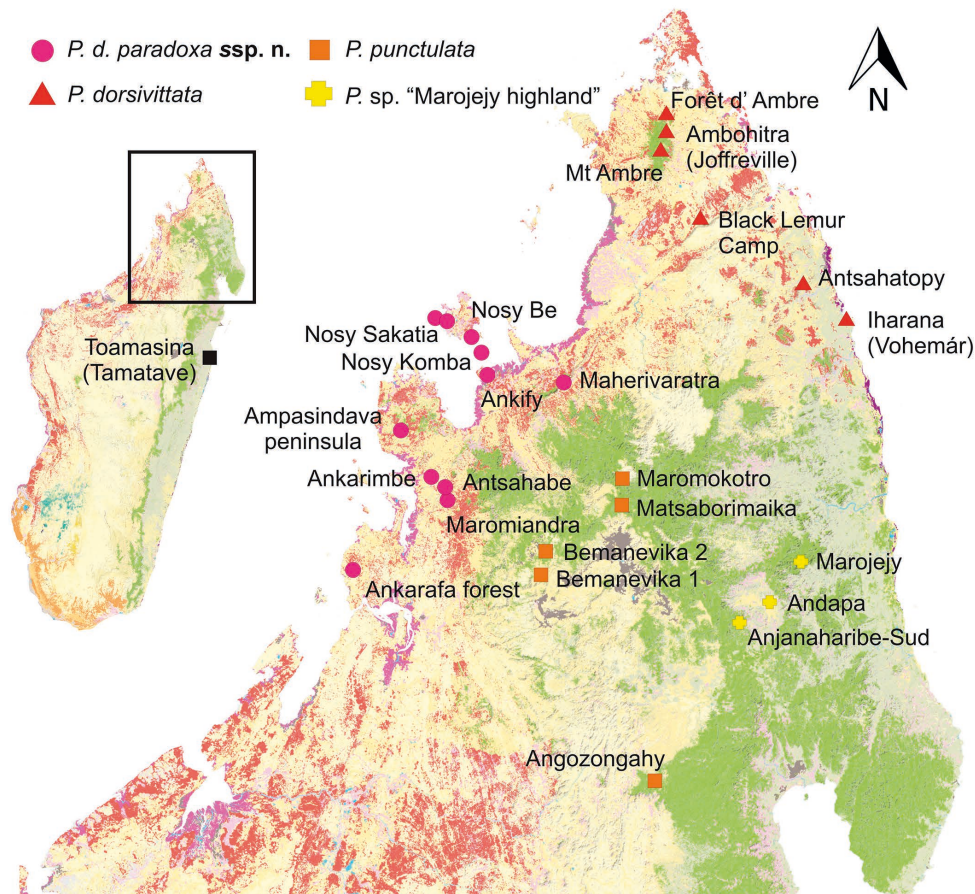


Figure 7. Map of northern Madagascar showing reliable locality records for species of the *P. dorsivittata* complex. For comparison the type locality of *P. parva* (Toamasina) is given in the overview map. Colours in map show remaining primary vegetation following Madagascar Vegetation Mapping Project carried out from 2003–2006 (MOAT & SMITH 2007); green is humid forest (rainforest), red-dish tones are deciduous forest and spiny forest-thicket.

area of Maromokotro or along river courses, but rarely in closed forest. It also is of interest that variation of up to 2.3% uncorrected pairwise distance is found among individuals from Bemanevika, where individuals of different haplotypes were found in close syntopy (DRV 6424, 6425, and 6426). Sequences corresponding to the populations assigned to *P. dorsivittata* are restricted to either the isolated rainforests of Montagne d'Ambre in Madagascar's far north or to the relatively dry lowland stretch that connects as transitional zone the north with the Sambirano region in the west and the eastern rainforest biome.

As emphasized by PULLANDRE et al. (2021), a single locus may not be representative of the evolutionary history of a species because of introgression and incomplete lineage sorting. The use of a single marker with a single method of species delimitation should be avoided, and should only be used for defining primary species hypotheses that will then be tested in integrative approaches. The complex mitochondrial relationships within the *P. dorsivittata* complex, with various sympatric lineages and probable instances of mitochondrial introgression (see next section), also exemplify one of the limits of single-locus species delimitation approaches, even for a program such as ASAP which is less prone to oversplitting than other single-locus species delimitation methods. We found an extreme lumping scenario (the *P. dorsivittata* complex consisting of a single species) only receiving a slightly worse score than an extreme splitting scenario with 12 species. Apparently, the high degree of mitochondrial phylogeographic structure in these geckos in combination with dense sampling as in our study leads to a plethora of separated mitochondrial lineages, without a clear DNA barcoding gap, not allowing the program to distinguish among drastically different species partition hypotheses. As further elaborated below, a robust taxonomic resolution of the *P. dorsivittata* complex will require thorough assessments of genome-wide gene flow among lineages.

Distinctness and relationships of *P. dorsivittata paradoxa*

The presence of day geckos with a phenotype reminiscent of *P. parva* in the Sambirano region of Madagascar has been known for many years (e.g. CHRISTOPHEL 1976, BUDZINSKI 2001, ANDREONE et al. 2003), but their identity has so far not been satisfyingly clarified. Our study adds a considerable amount of new morphological and molecular data and attempts a first taxonomic conclusion describing this form as *P. dorsivittata paradoxa*, although we are aware that multiple questions regarding these gecko populations remain unanswered.

As a first important fact, our data corroborate previous studies (GEHRING et al. 2013) in that *P. d. paradoxa* belongs into the *P. d. dorsivittata* complex and is not related to the phenotypically similar *P. parva*. This is unambiguous in our phylogenetic tree derived from mitochondrial DNA sequences, and it is also supported by our data on the

nuclear-encoded gene *C-mos*: For this marker, there was one shared allele and little sequence difference between *P. sp.* "Marojejy highland", *P. d. dorsivittata* from Iharana, and *P. d. paradoxa*, with the latter taxon clearly separated from *P. parva*. Although only one nuclear-encoded marker has been studied to date, this provides new and important information on these geckos, as it allows to reject with a certain likelihood the hypothesis that they are genuine *P. parva* that captured a mitochondrial genome from *P. dorsivittata* via introgressive hybridization. Instead, pending confirmation from further nuclear-encoded markers, it seems that these geckos may have evolved their *parva*-like phenotype, in particular their small size and posthumeral eyespot, convergently.

As a second novel aspect from our data set, compared to GEHRING et al. (2013) who only included a single sample of *P. d. paradoxa* (FGZC 487), our data revealed that this taxon is not reciprocally monophyletic with *P. d. dorsivittata*, and that there are two highly supported mitochondrial lineages within *P. dorsivittata*, each of which contains samples of *P. d. dorsivittata* from Montagne d'Ambre, and of *P. d. paradoxa* from Nosy Be. GEHRING et al. (2013) documented the two lineages of *P. d. dorsivittata* that apparently co-occur at Montagne d'Ambre in a data set from multiple mitochondrial genes. We can largely exclude that this situation is due to a pseudogene (nuclear mitochondrial DNA, NUMT; e.g., HAZKANI-COVO et al. 2010) amplified in some of the samples, except if we postulate very unlikely scenarios.

In the following sections we will elaborate on possible scenarios for eyespot convergence and mitochondrial paralogy within *P. dorsivittata*.

Evolution of the posthumeral eyespot phenotype in *Phelsuma*

Our results confirm that *P. d. paradoxa* is nested in the *P. dorsivittata* complex – and thus phylogenetically distant from *P. parva* and *P. quadriocellata*, the other *Phelsuma* with oval black spot behind the forelimbs usually bordered by a distinct blue ring. The function of this peculiar pattern has so far not been experimentally assessed in *Phelsuma*. Eyespots are common in many terrestrial animals and often play a role in intraspecific communication or reproductive signalling (ROBERTSON & MONTEIRO 2005). For example, in ocellated lizards *Timon lepidus* prominent eyespots and blue outer ventral scales are strongly correlated with sexual selection (FONT et al. 2009). Laboratory studies on butterflies have shown that the processes that explain the development of eyespot patterns are relatively simple, and that it requires only one, or at least very few changes in the regulatory genes to change the position, colour and number of eyespots (BRAKEFIELD et al. 1996). Although these processes could be more complex in vertebrates, this nevertheless suggests that the evolution of eyespots can occur relatively rapidly without major genetic alterations. In the green anole (*Anolis carolinensis*) field studies indicated that body colour is most typically affected

by social activities (JENSSEN et al. 1995). When stressed, the colouration of *A. carolinensis* shifts from green to brown and a small “eyespot” just behind the eye appears (GREENBERG 2002). There is, however, evidence that the eyespot may function as a social signal to limit aggressive interaction. When KORZAN et al. (2000) covered a territorial male’s stress-evoked eyespot, its aggressive behaviour directed toward a mirror was more intense than when the eyespot was not covered or artificially provided. In other words, the perception of an eyespot appeared to suppress aggression. The same intraspecific function of the eyespot may also apply to *P. d. paradoxa* in a habitat with high interspecific competition, although empirical evidence for this hypothesis is lacking.

If our phylogenetic hypothesis is correct, then it is plausible to assume that posthumeral eyespots evolved at least two times in *Phelsuma*: (1) in the *P. quadriocellata* / *P. parva* clade, and (2) in *P. d. paradoxa*. Rapid and recurrent evolution of this pattern is not unlikely since body coloration plays a crucial role for *Phelsuma* in both interspecific recognition and intraspecific communication (e.g., HALLMANN et al. 2008, GEHRING et al. 2010) and is therefore subject to high natural and sexual selection pressure. An increased pressure for interspecific recognition associated with high species richness in situ may result in an increased necessity and selection for visual signal complexity. This scenario would perfectly apply to *P. d. paradoxa*, the distributional range of which (more or less limited to the Sambirano region) is one of the richest in *Phelsuma* species in Madagascar (GLAW & VENCES 2007, BROWN et al. 2014) with eight nominal taxa currently known from this area (*P. abbotti chekei*, *P. dorsivittata paradoxa*, *P. dubia*, *P. grandis*, *P. klemmeri*, *P. laticauda*, *P. seippi* and *P. vanheygeni*). Accordingly, bird species living in sympatry with more confamilial species exhibit more elaborate visual signals, probably as a result of selection against hybridization (DOUTRELANT et al. 2016).

In another line of argument, the importance of eye spots as an anti-predator strategy is emphasized. Eyespots can help animals survive predator attacks as they are regarded as mimics of vertebrate eyes and thought to intimidate predators (STEVENS 2005). Such eyespots can reduce the propensity of attack or induce a longer attack latency (KODANDARAMAIAH et al. 2009). It has also been argued that large contrasting eyespots function simply due to innate cautiousness of predators to contrasting conspicuous signals (STEVENS et al. 2008).

Which of the hypotheses is the driving force behind the evolution of eyespots in *Phelsuma* would have to be empirically tested in behavioral studies.

Dual paraphyly of the two subspecies of *P. dorsivittata*

Because the two main mitochondrial lineages within *P. dorsivittata* occur in sympatry both at Montagne d’Ambre (in the massif’s populations of *P. d. dorsivittata*)

and Nosy Be (in the island’s populations of *P. d. paradoxa*), we do not consider it likely that this section of the mitochondrial gene tree represents the species tree. Instead, it is likely that the observed pattern was caused by a complex combination of multiple events of divergence and subsequent admixture, as well as mitochondrial capture.

Hybridization and the following permanent incorporation of genes from one set of differentiated populations into another has been found to be more widespread in animals than previously assumed; it usually occurs between closely related, broadly syntopic species or is confined to particular contact zones (JIGGINS & MALLETT 2000, MALLETT 2005). Observations in captivity have shown that closely related *Phelsuma* species interbreed (e.g. *P. masohoala* x *P. abbotti chekei*; *P. madagascariensis boehmei* x *P. m. madagascariensis*; HALLMANN et al. 2008), and molecular data from *Phelsuma* from the Seychelles pointed to the possibility of gene flow between the subspecies *P. s. sundbergi* and *P. s. longinsulae* (ROCHA et al. 2013). While the extent of its evolutionary significance remains controversial (SEEHAUSEN 2004), it is increasingly seen as an important process for generating biotic diversity (ARNOLD 1997, FERGUSON & SANG 2001, RIESEBERG et al. 2003) and for rapid adaptation (GRANT & GRANT 1996, ELLSTRAND & SCHIERENBECK 2000). Mitochondrial DNA (mtDNA) is more prone to introgression than the nuclear genome, may spread over significant geographic distances, and may completely replace the native mtDNA of a species, even in the presence of little or no nuclear introgression (e.g. BABIK et al. 2005, TOEWS et al. 2012, ZIELIŃSKI et al. 2013, JEZKOVA et al. 2013, MASTRANTONIO et al. 2016). In zones of sympatry or parapatry, the foreign mtDNA is transferred by fertile or partially fertile female hybrids that backcross with males of the paternal species (PLÖTNER et al. 2008). Furthermore, mtDNA introgression is often asymmetric between populations (i.e. from one lineage to another) and mostly occurring from local to colonizing species (CANESTRELLI et al. 2014, HARRISON & LARSON 2014).

In the case of *P. dorsivittata*, one possible, admittedly speculative scenario would postulate an initial divergence of populations of the ancestral *P. dorsivittata* in rainforest fragments of northern Madagascar. This is plausible as high mitochondrial variation is also observed in *P. punctulata*, and northern Madagascar with its highly heterogeneous landscape, is known to be a cradle of species formation in many groups of reptiles and amphibians (e.g., RAXWORTHY & NUSSBAUM 1995, WOLLENBERG et al. 2008, BROWN et al. 2016). It is appealing to link this initial divergence to a period of changing climate with associated contraction of forest areas perhaps isolating populations in forest refugia on mountain massifs. *P. dorsivittata* seems to be rather bound to moderately humid habitats, since most surveys in deciduous dry forests north and south of the Montagne d’Ambre did not yield any records of this day gecko (e.g., D’CRUZE et al. 2006, 2007, MEGSON et al. 2009), although the species was observed in the dry forest areas around the Black Lemur Camp east of Ankarana (Fig. 6E). Because other lineages of the *P. lineata* group lack posthumeral eyespots, it is

further plausible that these were also absent in the ancestral *P. dorsivittata*.

We hypothesize that after this allopatric differentiation, the two diverged populations came into secondary contact and admixed, retaining the two deeply divergent mitochondrial genome variants. Two alternative scenarios can explain the presence of both mitochondrial variants in *P. d. paradoxa*: both mt-genomic variants were retained after its split from *P. dorsivittata*, without lineage sorting taking place; or through repeated events of introgressive hybridization, mitochondrial genomes were moved from one taxon to the other. Testing these fascinating hypotheses with phylogenomic datasets emerges as a highly attractive perspective for future research projects.

Rationale for naming *P. d. paradoxa* as a subspecies

As elaborated above, numerous aspects of the evolutionary history of the eyespot-bearing populations of the *P. dorsivittata* complex still remain to be clarified. In such a situation, one could argue for postponing taxonomic decisions until more complete data sets become available. However, given the high attention that *Phelsuma* day geckos receive in the scientific and conservation community, and beyond, it is of importance to be able to refer to these geckos with a scientific name.

In publications reporting geckos now considered as *P. d. paradoxa*, this form has been almost exclusively attributed to *P. parva* or *P. quadriocellata* (e.g. BUDZINSKI 2001, ANDREONE et al. 2003, VAN HEYGEN 2004). Even after genetic assignment to the *P. dorsivittata* complex by GEHRING et al. (2013), findings of *P. d. paradoxa* in the Sambirano area have been referred to as *P. sp. aff. quadriocellata* (PENNY et al. 2017) and discussed in light of a disjunct range of *P. quadriocellata*. For any species distribution model or other biogeographic inference, a taxonomy highlighting the true relationships of *P. d. paradoxa* is imperative.

In addition, the systematic naming of *P. d. paradoxa* plays a special role in species conservation, as these geckos enter the trade declared as *P. quadriocellata* or *P. parva*. In 2007–2012, export quotas of *P. parva* were set to 2000 live specimens (www.cites.org) and also at 2000 live specimens of *P. quadriocellata* in 2007–2021 (www.cites.org). Although the subspecies does not appear to be at particularly high risk of extinction, as it occurs in its range even in habitats that have been heavily anthropogenically modified, it is important to distinguish this species from other *Phelsuma* taxa as soon as possible in the Malagasy quota system, which regulates the number of exported individuals of each traded species listed by CITES per year since day geckos are of high demand in the international pet trade (JENKINS et al. 2014).

Furthermore, a correct taxonomic identification is also important for *Phelsuma* breeders to avoid unintentional and/or unsuccessful breeding attempts with individuals of different species that share a similar morphology and

colouration. This problem might have happened in the past when, for example, several genetically different but morphologically similar species were all traded under the name '*Phelsuma parva*'.

Although we cannot exclude that *P. d. dorsivittata* and *P. d. paradoxa* have reached a status of different species, given their substantial and constant morphological divergence, the convoluted and paradoxical mitochondrial relationships among these species call for caution. Since the two taxa are also geographically separated, we feel that assigning them an initial status of subspecies is adequate. In this we adopt the view of HAWLITSCHKE et al. (2012) and the conceptual suggestions of HILLIS (2020) and DE QUEIROZ (2020), seeing the subspecies category as appropriate for such lineages that can be defined by genetic or morphological means but have not yet reached evolutionary independence.

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Supplementary data

The following data are available online:

Supplementary Figure S1. Bayesian inference phylogenetic tree of several species in the *Phelsuma lineata* species group, based on 322 bp of the mitochondrial 16S rRNA gene.