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High diversity of deep mitochondrial lineages meets low morphological distinctiveness – insights into the complex phylogeography of the Malagasy leaf-tailed geckos *Uroplatus sikorae* and *U. sameiti*

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Abstract. Based on sequences of three mitochondrial and two nuclear-encoded genes, we examine genetic variation in the leaf tailed geckos *Uroplatus sameiti* and *U. sikorae*, and morphological and chromatic characteristics of the genetic clusters identified. The mitochondrial phylogeny reveals a puzzling diversity of 16 deep lineages (4 in *U. sameiti* and 12 in *U. sikorae*) differing by 2.9–9.9% uncorrected pairwise distance in a fragment of the 16S rRNA gene. Populations from Analalava in the North and Zahamena in the Northern Central East form two mitochondrial lineages clustering with *U. sameiti* but being deeply divergent (>8% 16S distance); however, no information on their morphology is available. In *U. sikorae*, the mitochondrial lineages identified form several major geographic clades, two of which (from the northernmost and southernmost populations, respectively) received substantial support in the phylogenetic analysis. No instance of sympatry of two or more mitochondrial lineages was observed, precluding an unambiguous assessment of species status under the biological species criterion without experimental approaches or detailed hybrid zone analyses. In the fragment of the nuclear encoded gene SACS we observed haplotype sharing between species and mitochondrial lineages, while in the fragment of KIAA1239 no haplotype sharing was detected although neither species nor mitochondrial lineages formed coherent phylogroups in the respective network. A screening of colour patterns from live photos, partly of the genotyped individuals, confirmed a large variation within species and populations, with a possible sexual dichromatism where a longitudinally striped phenotype is restricted to males. All individuals from populations of the *U. sikorae* clade from the Southern Central East and South East had an unpigmented oral mucosa just like *U. sameiti*, while all other *U. sikorae* populations are characterized by a black oral mucosa pigmentation. The extremely strong phylogeographic structure in the *U. sikorae* complex without obvious species-level divergences is unprecedented for large-sized squamates in Madagascar and calls for further taxonomic scrutiny using phylogenomic approaches; and it exemplifies how the loss of any major block of the remaining rainforests in Madagascar will inevitably lead to a substantial loss of genetic diversity – even if often intraspecific – in rainforest-specialized species.

Key words. Squamata, Gekkonidae, *Uroplatus*, leaf-tailed geckos, phylogeny, biogeography, Madagascar.

Introduction

Leaf-tailed geckos of the genus *Uroplatus* Duméril, 1806 are among the most prominent reptiles of Madagascar due to their bizarre appearance (Glaw & Vences 2007, Wollenberg et al. 2011). The first published reports of these strange creatures date back to 1658 (Flacourt 1658), although the first species of the genus was only scientifically named 141 years later (Schneider, 1792). Curiously however, their taxonomy and geographic variation has only been intensively and comprehensively studied during the last twenty years, revealing that the genus is characterized by a much higher species diversity than previously assumed, with a high number of range-restricted species.

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In the period between the first description in 1792 and 1990, the species *U. fimbriatus* Schneider, 1792, *U. lineatus* Duméril & Bibron, 1836, *U. ebenaui* Boettger, 1879, *U. alluaudi* Mocquard, 1884, *U. phantasticus* Boulenger, 1888, *U. guentheri* Mocquard, 1908, and *U. sikorae* Boettger, 1913 were scientifically named. Five species, *U. henkeli*, *U. malahelo, U. malama, U. pietschmanni*, and *U. sameiti,* were then discovered and scientifically named between 1990–2004 (Böhme & Ibisch 1990, Nussbaum & Raxworthy 1994, 1995, Böhle & Schönecker 2003), followed by first molecular assessments that revealed widespread cryptic diversity and led to the definition of numerous candidate species (Glaw et al. 2006, Greenbaum et al. 2007, Raxworthy et al. 2008, Ratsoavina et al. 2013). Subsequently, eight new species of leaf-tailed geckos were formally described and named within this genus (*U. finiavana* Ratsoavina et al. 2011a, *U. fiera* Ratsoavina et al. 2015, *U. fotsivava* Ratsoavina et al. 2017, *U. kelirambo* Ratsoavina et al. 2017, *U. finaritra* Ratsoavina et al. 2019a, *U. fetsy* Ratsoavina et al. 2019b, *U. fangorn* Ratsoavina et al. 2020a and *U. fivehy* Ratsoavina et al. 2020a).

Taking into account the latest species descriptions (Ratsoavina et al. 2020a), the genus *Uroplatus* currently contains 21 nominal species which can be grouped into five morphologically distinct species groups (Ratsoavina et al. 2013). Over the last decade, taxonomic research focus has been on deciphering the species diversity within the small-sized, leaf-mimicking forms in the *U. ebenaui* group, although the second most species-rich group, the *U. fimbriatus* group, is also known to contain numerous genetic lineages of uncertain taxonomic status (Ratsoavina et al. 2013). Based on morphological similarity and phylogenetic relationships, the *U. fimbriatus* group can be subdivided into the *U. fimbriatus* complex (*U. fimbriatus, U. giganteus*), the *U. henkeli* complex (*U. henkeli* and the candidate species *U. henkeli* [Ca11] – currently in the process of being described), and the *U. sikorae* complex (*U. sameiti, U. sikorae*). Of these taxa, the genetic variation in the *U. fimbriatus* complex has been analyzed by Gehring et al. (2018), and the taxonomic revision of the *U. henkeli* complex is being completed as well (Glaw et al. submitted). Here, we focus on the *U. sikorae* complex where Ratsoavina et al. (2013) documented, both within *U. sameiti* and *U. sikorae,* a large number of deep mitochondrial lineages whose morphology and relationships are largely unknown.

Previous studies (Greenbaum et al. 2007, Raxworthy et al. 2008, Ratsoavina et al. 2013) based on multiple molecular markers have provided clear evidence for the monophyly of the *U. sikorae* complex, with two major clades that correspond to *U. sikorae* and *U. sameiti*. The morphological diagnosis of these species, however, turned out to be contentious. *Uroplatus sikorae* was scientifically named and described by Boettger (1913), on the basis of its purported smaller size compared to *U. fimbriatus*, as well as some scalation features which however were not considered to be diagnostic by subsequent authors. Prior to 1989, it was thus disputed whether *U. sikorae* represents a species separate from *U. fimbriatus*. The systematic revision of *Uroplatus* by Bauer & Russell (1989) resurrected *U. sikorae* and considered it a distinct species based on morphological characters, i.e., differences in dermal flaps and coloration, and sympatric occurrence with *U. fimbriatus*. Subsequently, Böhme & Ibisch (1990) found evidence for two separate taxa they considered as subspecies: *U. sikorae sikorae* (type locality near Andrangoloaka) represented by the population from "Périnet" (=Andasibe, close to Mantadia-Analamazaotra National Park, geographical coordinates: -18.9333, 48.4167) and other localities mainly at mid-elevations (roughly between 600 and 1,500 m a.s.l.), and *U. sikorae sameiti*, with the type locality Nosy Boraha (= Sainte Marie, -16.9173, 49.87399) from mainly lowland localities. These two taxa were diagnosed mainly by the pigmentation of their oral mucosa: black in *U. sikorae* and unpigmented (pinkish in colour) in *U. sameiti*. Raxworthy et al. (2008) found substantial molecular divergence between these taxa and elevated them to species level, but Ratsoavina et al. (2013) found evidence for a more complex situation, with numerous deeply divergent mitochondrial lineages both within *sikorae* and *sameiti*, and numerous populations in the genetic *sikorae* lineage lacking a black oral mucosa.

In this study we aim to (i) more comprehensively assess the genetic diversity within the *U. sikorae* complex based on mitochondrial DNA, (ii) document the geographical distribution of the main mitochondrial lineages identified, as well as (iii) screen for possible chromatic and morphological characters that would allow a distinction of the genetic groups. Our goal is to provide a detailed review of the *U. sikorae* complex as a baseline for future studies on their biogeography and systematics, and for improved conservation management.

Materials and methods

Sampling for molecular analysis

For molecular phylogenetics, we focused on amplifying and sequencing three mitochondrial gene fragments: 12S ribosomal RNA (12S rRNA or 12S), 16S ribosomal RNA (16S rRNA or 16S), and NADH dehydrogenase subunit 4 (ND4) including partial tRNA stretches. We used sequences compiled by Ratsoavina et al. (2013) and complemented these with new sequences available from GenBank, and further new sequences generated for this study. Since the 12S fragment was already available for many samples (Ratsoavina et al. 2013) we attempted to complement the 12S alignment for as many further samples as possible. As a specific challenge for this study, *U. sikorae* and *U. sameiti* contain a large number of deeply divergent mitochondrial lineages, and neither the short 12S fragment nor the ND4 fragment alone were able to reliably reconstruct their relationships (see Ratsoavina et al. 2013); furthermore, many samples of *U. sameiti/sikorae* sequenced by other studies (e.g., Raxworthy et al. 2008, Ratsoavina et al. 2013) were sequenced (and uploaded to GenBank) only for either 12S or ND4, and the respective tissue samples in many cases were not available to us. This unbalanced availabil-

ity of sequences led to numerous phylogenetic artefacts in exploratory analyses of the combined multigene data set, since representatives of some regional clades were present for only one inference of relationships. New sequencing was therefore directed at obtaining sequences of all three gene fragments for at least one sample per main mitochondrial lineage.

Molecular and phylogenetic methodology

Total genomic DNA was extracted following a standard salt extraction protocol after proteinase K digestion (Bruford et al. 1992). Polymerase chain reaction with standard cycling protocols (Bauer et al. 2011) was carried out using the following primers: a fragment of the 12S ribosomal RNA gene (12S) was amplified with 12SAr-L 5'-AAACTGGGATTAGATACCCCACTAT-3' and 12SBr-H 5'-GAGGGTGACGGGCGGTGTGT-3' (Palumbi et al. 1991), a fragment of the 16S ribosomal RNA gene (16S) with 16SAr-L 5'-CGCCTGTTTATCAAAAACAT-3' and 16SBr-H 5'-CCGGTCTGAACTCAGATCACGT-3' (Palumbi et al. 1991), and a fragment of the mitochondrial gene for NADH dehydrogenase subunit 4 and following tRNAs (ND4) with ND4 5'-CACCTATGACTACCAAAAGCTCATGTAGAAGC-3' and LeutRNA 5'-CATTACTTTTACTTGGATTTGCACC-3'(Arévalo et al. 1994). In addition, we sequenced fragments of the nuclear-encoded genes for sacsin (SACS) and Leucine-rich repeat and WD repeat-containing protein (KIAA1239) following primers and nested PCR approach of Shen et al. (2012).

PCR products were purified with Exonuclease I and Shrimp Alkaline Phosphatase digestion, and the purified products along with sequencing primers were shipped to LGC Genomics (Berlin) for sequencing on automated capillary sequencing instruments, with a select number of samples processed and sequenced in-house at Omaha's Henry Doorly Zoo and Aquarium. Sequences were quality-checked and poor-quality stretches manually trimmed in CodonCode Aligner (Codon Code Corporation). We used MEGA7 (Kumar et al. 2016) for initial sequence alignment, exploratory phylogenetic inference, and for calculating uncorrected p-distances between sequences. Newly generated sequences were deposited in GenBank under the following accession numbers: OQ302235-OQ302269, OQ303766-OQ303827, and OQ318161-OQ318166.

Sequences and associated metadata were curated in a Microsoft Excel spreadsheet, exported as tab-delimited text, and used as input for Concatenator (Vences et al. 2022a) which is part of the iTaxoTools project (Vences et al. 2021) where each gene fragment was aligned with MAFFT (Katoh & Standley 2013) and concatenated into a Nexus-formatted file. For phylogenetic inference we performed a partitioned Bayesian analysis of the concatenated mitochondrial fragments (2,225 bp) with MrBayes 3.2 (Ronquist et al. 2012), defining three partitions (12S + 16S, with a GTR+G model; ND4 third codon positions with a GTR+G model; and ND4 first and second codon positions with a K2P+G model, based on model testing under the Bayesian Information Criterion in MEGA7), running 20 million generations, sampling every 1,000 trees, and discarding the first 25% of sampled trees as burn-in.

We inferred haplotypes of each of the nuclear DNA fragments with the PHASE algorithm (Stephens et al. 2001) using the software DnaSP (Version 5.10.3; Librado & Rozas 2009). We reconstructed a Maximum Likelihood tree with Jukes-Cantor substitution model (chosen to avoid overparametrisation) in MEGA7 and then used this tree, separately for each fragment, to build a haplotype network by entering the tree together with the alignment in the software Haploviewer, written by G. B. Ewing (http://www.cibiv.at/~greg/haploviewer), which implements the methodological approach of Salzburger et al. (2011).

Morphological analyses

For this study, we focused on specimens housed at the Zoologische Staatssammlung München (ZSM), collected over the last 20 years by ourselves and other colleagues on numerous expeditions. Field number abbreviations of these specimens and all others used in our phylogenetic trees refer to tissue or specimen numbers of P.-S. Gehring (PSG), R. A. Nussbaum (RAN), M. Pabijan (MPFC), A. Rakotoarison (AND), A. P. Raselimanana (APR), F. M. Ratsoavina (RATF, KAF, KIAN, M, URANO, ZAH, FRT), C. J. Raxworthy (RAX), M. D. Scherz (MSZC), M. Vences and/or F. Glaw (MVTIS, FG/MV, ZCMV, FGZC), and D. R. Vieites (DRV).

For a selected number of specimens in the ZSM collection we undertook a screening of possibly diagnostic morphological characters, including morphometric measurements, scale counts, and counts of the serrations in the lateral skin flaps. We focused on analyzing only those specimens in relatively good state of preservation, largely adults, which also had been included in the molecular analysis or from the same sites from which we had molecular data available. Unfortunately, this meant that only a limited number of voucher specimens of each genetic lineage was available for examination. We also qualitatively screened all individuals for additional characters such as skin texture, tubercles, and additional scale counts, but did not further report on these given that no consistent differences were observed. The selected measurements and counts were taken by student assistants, where each measurement was taken by the same person but in a haphazard order of individuals. For the measurements and counts taken, see Table 1.

Analyses of colour variation

To assess possible chromatic differences between individuals or populations assigned to genealogical groups (identified above through phylogeographic analyses), especially between mitochondrial lineages of *U. sikorae* and

Table 1. Morphometric and meristic data for a selected subset of specimens of the *Uroplatus sikorae* complex from the ZSM collection, to a large part corresponding to the specimens included in the molecular analysis. Assignment to main (mitochondrial) lineage is abbreviated as in Figs 2–3: SAM, *U. sameiti*; N, *U. sikorae* N; NCE/NE; *U. sikorae* NCE/NE; SCE/SE, *U. sikorae* SCE/SE, with lineage numbers as in Figure 1. Oral mucosa was scored as (partially) black or unpigmented (unpig.). Sex is abbreviated as F, female, M, male, J, juvenile, SA, subadult. NA, not available/applicable. All morphometric measurements in mm: SVL, snout–vent length; TaL, tail length; TaW, tail width; HW, maximum head width; HL, head length; ForL, forelimb length; HiL, hindlimb length. Scale counts and dermal fringe serration counts are given for the left and right body side, where possible; IL, infralabial scales; SL, supralabial scales; F3L, scansor lamellae on third finger; VTS, transversal count of ventral scales at midbody, not counting scales on dermal fringes; FringFl, FringFor, FringHi, FringH: number of serration elements on dermal fringes along flanks, forelimb, hindlimb, and head. Asterisks mark specimens included in the molecular analysis.

Catalogue Nr	Fieldnumber	Clade	Locality	Oral mucosa	Sex	SVL	TaL	TaW	HW	HL	ForL	HiL	IL	SL	F3L	VTS	FringFl	FringFor	FringHi	FringH
U. sameiti																				
ZSM 1577/2009	NA	sam13	Nosy Mangabe	unpig.	F	105.0			22.0	30.0	42.6	56.5	28/22	27/29	8	88	34/34	3/7	15	70
ZSM 1578/2009	NA	sam13	Nosy Mangabe	unpig.	F?	103.0	29.0	13.3	22.8	29.5	41.0	53.0	22	25/27	9	73	35/40	9/9	29/23	68
ZSM 1579/2009	NA	sam13	Nosy Mangabe	unpig.	J	61.5			14.0	18.5	25.8	29.6	NA		9		41/40	10/12	27	56
*ZSM 192/2016	FGZC 5068	sam13	Vohimana	unpig.	F	110.0	53.0	19.0	22.0	30.5	44.5	57.0	26/29	30/33	9	92	43/52	16/16	40/39	72
U. sikorae																				
ZSM 32/2018	MSZC 458	N - sik9	M. d'Ambre	dark	F	112.0	50.0	19.4	24.0	34.0	44.5	57.0	29/25	39/39	10	84	39/39	19/20	34/35	83
ZSM 33/2018	MSZC 492	N - sik9	M. d'Ambre	dark	M	106.0	55.5	17.0	23.0	31.5	42.3	56.5	33/32	38/32	9	87	41/41	15/17	35/28	70
ZSM 34/2018	MSZC 743	N - sik9	M. d'Ambre	dark	M	96.7	51.0	16.0	20.0	29.5	39.0	48.8	30/31	36/37	8	75	33/33	11/11	18/17	79
*ZSM 264/2004	FGZC 508	N - sik9	M. d'Ambre	dark	F	110.4	51.9	14.0	24.6	31.5	43.8	56.4	28/26	34/33	9	88	35/39	14/16	20/21	62
ZSM 1129/2003	FGMV 2002.2393	N - sik9	M. d'Ambre	dark	F	116.0	47.0	25.0	25.9	34.5	41.3	55.5	30/28	35/33	8	89	31/31	11/11	16/18	60
ZSM 1130/2003	FGMV 2002.2396	N - sik9	M. d'Ambre	dark	F	111.0	NA	1.7	24.0	33.0	46.3	54.2	28/28	36/36	9	79	36/40	12/19	25/26	70
*ZSM 265/2004	FGZC 509	N - sik9	M. d'Ambre	dark	M	104.0	35.4	15.2	22.5	29.3	37.5	62.5	NA	NA	9	84	41/33	13/14	23/25	65
ZSM 268/2004	FGZC 515	N - sik9	M. d'Ambre	dark	M	107.5	NA	18.0	22.5	30.1	43.6	57.9	32/31	37/38	10	79	37/33	12/14	37/36	63
*ZSM 2105/2007	FGZC 1098	N - sik9	M. d'Ambre	dark	M	102.4	47.0	16.9	20.5	28.4	41.5	53.0	NA	NA	9	85	23/28	12/10	19/20	62
*ZSM 1725/2012	FGZC 3616	N - sik10	Sorata	dark	M	120.0	65.0	25.5	25.8	31.8	46.0	56.4	NA	NA	10	92	NA	NA	NA	NA
*ZSM 1724/2012	FGZC 3835	N - sik12	Andrafainkona	dark	M	108.0	NA	NA	23.6	36.0	49.4	62.0	32/31	39/38	10	82	28/37	15/12	26/24	58
*ZSM 635/2009	ZCMV 11310	NCE/NE - sik5	Makira	dark	M	103.0	NA	NA	23.0	27.5	44.0	60.0	NA	32	9	69	24/26	13/14	18	54
*ZSM 5045/2005	ZCMV 2037	NCE/NE - sik5	Marojejy	dark	F (SA)	67.3	30.0	9.0	15.0	20.7	29.1	35.3	NA	NA	8	95	22/25	6/5	22/22	41
*ZSM 50/2016	MSZC 117	NCE/NE - sik6	Ampotsidy	dark	F	106.0	NA	NA	22.5	29.3	44.3	53.1	26/26	28/33	9	75	33/35	12/17	31/28	58
*ZSM 474/2010	FGZC 4351	NCE/NE - sik7	Anjozorobe	dark	F	93.4	NA	NA	21.4	26.8	44.2	46.0	28/25	34/35	9	74	28/25	11/13	20/25	66
*ZSM 920/2003	FGMV 2002.951	NCE/NE - sik7	Andasibe	dark	M	105.0	52.0	16.7	22.0	27.5	45.0	55.0	27/27	32/29	10	76	38/36	14/17	21/24	59
*ZSM 921/2003	FGMV 2002.952	NCE/NE - sik7	Andasibe	dark	F	107.5	50.7	17.0	24.0	29.0	41.2	54.8	NA	NA	9	77	26/23	13/10	16/18	44
*ZSM 690/2003	FGMV 2002.311	SCE/SE - sik1	Ranomafana	unpig.	M	109.5	54.0	17.0	21.3	30.5	38.5	55.2	NA	NA	10	74	45/42	12/11	36/32	53

U. sameiti, we compared colour patterns of the body, eye and buccal mucosa based on photographs of specimens from the entire distribution range of the two species, in order to follow an integrative taxonomic approach (Padial et al. 2010). All photographs had been taken of live geckos in the field during day and/or night. Our data did not allow to systematically identify possible differences between day and night coloration, and we therefore do not further elaborate on these (except for iris coloration; see below); however, in general, no fundamental differences between day and night coloration were detected. Photographs were taken in localities that comprised intact or slightly to moderately disturbed rainforest and ranged in elevation from about 0–1,550 m a.s.l. (Supplementary Table S1).

Results

Molecular differentiation

The molecular dataset of concatenated mitochondrial genes consists of 192 ingroup sequences for the three targeted mitochondrial DNA fragments (12S: 80 sequences; 16S: 35 sequences; ND4: 77 sequences) for a total of 120 individual ingroup samples, including 84 sequences newly determined for this study. The phylogenetic tree calculated from the 2,225 bp of concatenated sequences (Fig. 1) divides the *U. sikorae* complex into two main monophyletic groups corresponding to *U. sameiti* and *U. sikorae* as currently understood, but found no significant support for these clades (Bayesian posterior probability PP = 0.60 for *U. sikorae* and < 0.5 for *U. sameiti*). The tree also reveals a puzzling diversity of deep mitochondrial lineages within these two taxa, especially within *U. sikorae* (lineages sik1–sik12; Fig. 1) but also in *U. sameiti* (sam13–sam16; Fig. 1). All of these mitochondrial lineages are allopatrically distributed (Fig. 2). Although various deeper nodes were poorly supported, some geographical groupings received high support: (i) Lineages sik1–sik4, here together named as *U. sikorae* SCE/SE (PP = 0.96) encompassed all populations from the Southern Central East and South East, ranging from Beampingaratra in the south-eastern Anosy Chain to Ranomafana National Park, and encompassing both low-elevation sites near sea level (e.g., Manombo) and mid-elevation sites (e.g., Ranomafana National Park). (ii) Lineages sik5–sik8 were placed in an unsupported group here called *U. sikorae* NCE/NE (PP < 0.5) and included populations from mid-elevation localities, ranging from the Andasibe region in the Northern Central East to the Marojejy Massif in the North East, and reaching the limits of the Sambirano region at Ampotsidy. (iii) Lineages sik9–sik12 formed a highly supported group here called *U. sikorae* N (PP = 1.0), encompassing populations from the northern massifs of Tsaratanana, Manongarivo, Sorata, and Montagne d'Ambre. (iv) Within *U. sameiti*, lineages sam13 and sam14 (grouped together with PP = 1.0), including specimens from the type locality Nosy Boraha, were found in primarily low-elevation sites along Madagascar's east coast, with the two clades encompassing populations from Marolambo to Betampona, and from Tampolo to Nosy Mangabe, respectively. (v) Finally, the enigmatic lineages sam15 and sam16 show a very deep molecular differentiation compared to typical *U. sameiti*.

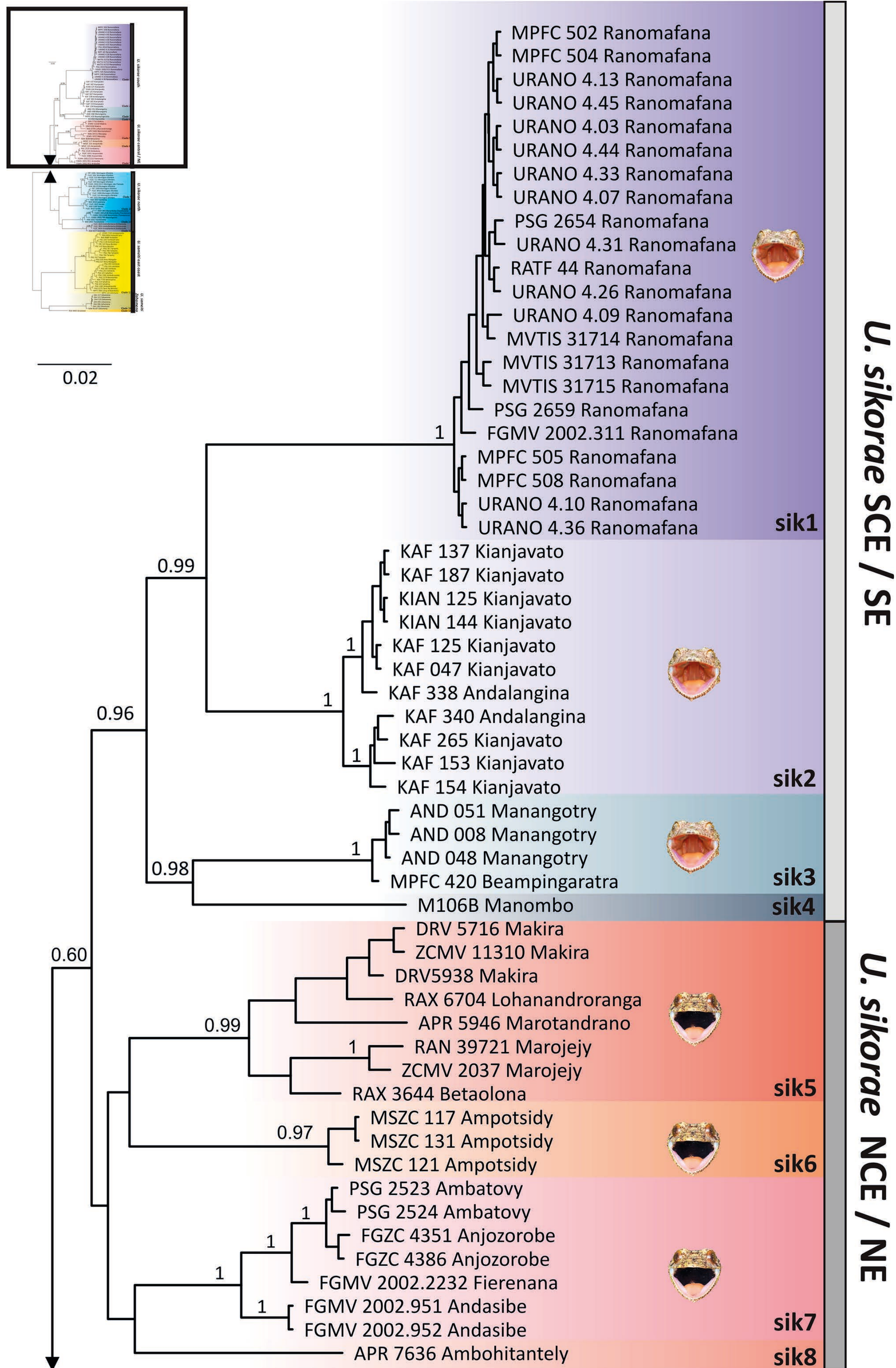
A comparison of elevational distribution of the lineages identified (Supplementary Table S1) revealed for localities confirmed by molecular data an elevational range of *U. sameiti* from 4–774 m above sea level, and for *U. sikorae* from 279–1,550 m a.s.l., with the low elevation *U. sikorae* sites below 600 m all referring to the SCE/SE clade, outside of the latitudinal range of *U. sameiti;* all other localities of *U. sikorae* are at least at an elevation of 600 m, and mostly > 900 m a.s.l.

Mitochondrial sequence divergences between lineages were high. In the 16S gene, uncorrected pairwise distances amounted to 6.3–11.4% between *U. sameiti* and *U. sikorae;* 2.9–6.9% among lineages within *U. sikorae* SCE/SE, 4.5–8.4% among lineages within *U. sikorae* N, 3.3–7.9% among lineages within *U. sikorae* NCE/NE, and 4.3–9.6% among the three main geographical clades. The Zahamena lineage of *U. sameiti* differed by 8.6–9.9% from other *U. sameiti*.

Sequences of the nuclear-encoded SACS (1068 bp) and KIAA1239 (872 bp) fragments were available each for 25 individuals of the *U. sikorae* complex, covering two mitochondrial lineages of *U. sameiti* (sam13 and sam14) and seven lineages of *U. sikorae* (sik1, sik5, sik7, sik9–sik12) that represent the main *U. sikorae* groups N, NCE/NE, and SCE/SE. Although alleles (haplotypes) of the various mitochondrial lineages showed some separation also in the nuclear gene haplotype networks (Fig. 3), several instances of allele sharing were apparent. Notably, in the SACS network, allele sharing was observed between *U. sameiti* from the more southern sam14 with an *U. sikorae* individual from sik7 (from Fierenana), and also other alleles from *U. sikorae* were placed in a separate phylogroup with *U. sameiti* individuals, separated by a minimum of 5 mutational steps from other *U. sikorae.* In KIAA1239, no haplotype sharing between any mitochondrial lineage was observed, but neither the two species nor any of the geographical clades formed coherent, separate phylogroups, and quite a large number of alleles were observed within lineages that differed by numerous mutational steps (up to 26 steps in sik12).

Morphological characteristics of *U. sameiti* and *U. sikorae*

Morphological data for representative individuals of *U. sameiti* and *U. sikorae* are given in Table 1. In the following account we compare these with general information summarized from the literature (Böhme & Ibisch 1990, Glaw & Vences 2007, Gehring 2020). Snout–vent length (SVL) in *U. sameiti* is 103–110 mm in the three presumably adult females available to us, but is known to reach 110–130 mm in this species. In *U. sikorae*, SVL ranges from 97–120 mm in males and 93–116 mm in females in our samples, but is known to reach up to 123 mm. Counting supralabial and infralabial scales in these geckos is not easy be-


0.02
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MPFC 420 Beampingaratra
sik3
M106B Manombo
sik4
DRV 5716 Makira
ZCMV 11310 Makira
DRV5938 Makira
RAX 6704 Lohanandroranga
APR 5946 Marotandrano
RAN 39721 Marojejy
ZCMV 2037 Marojejy
RAX 3644 Betaolona
sik5
MSZC 117 Ampotsidy
MSZC 131 Ampotsidy
MSZC 121 Ampotsidy
sik6
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1
U. sikorae SCE / SE
U. sikorae NCE / NE

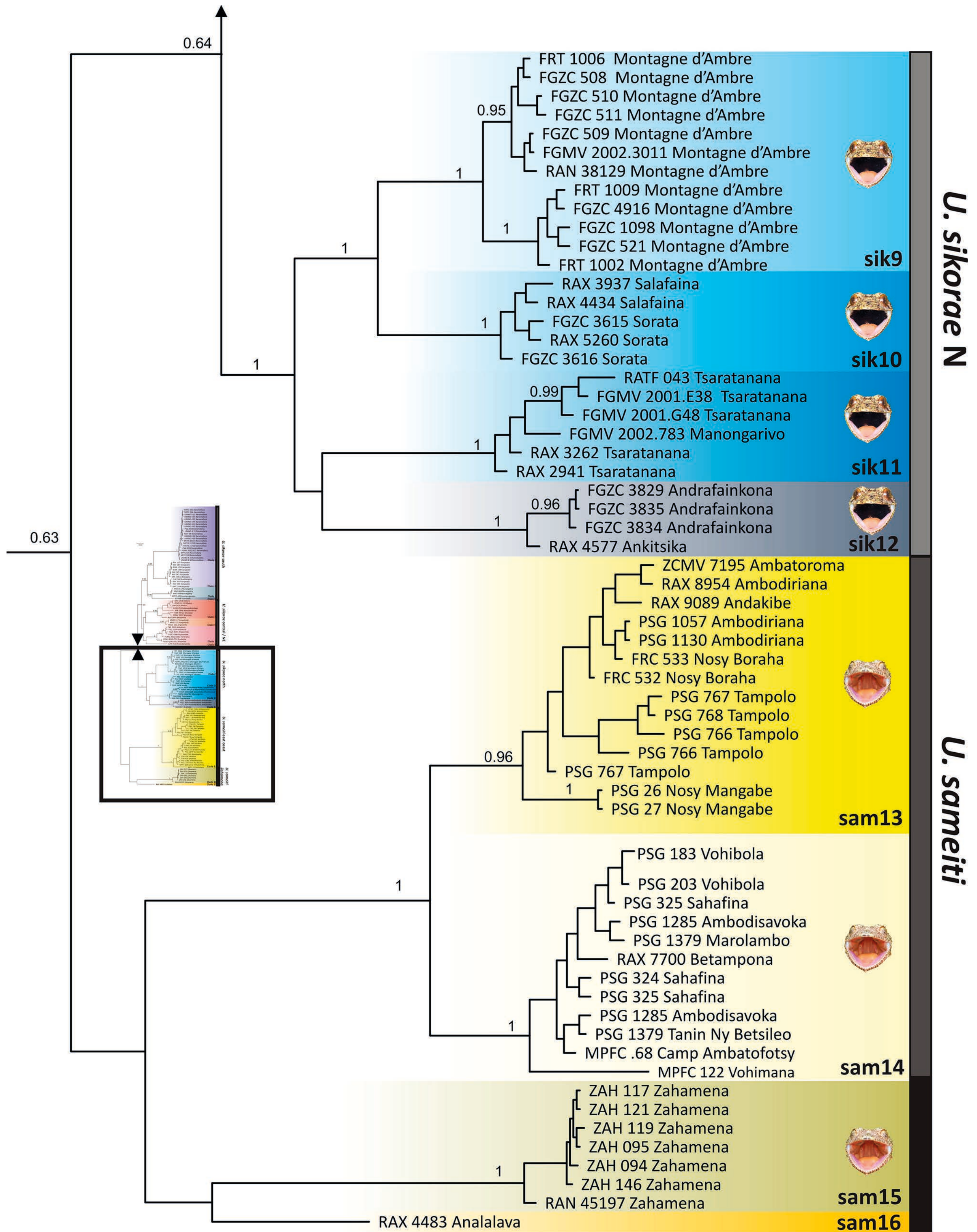

Figure 1. Bayesian phylogenetic tree (50% majority-rule consensus with all compatible bifurcations shown) based on DNA sequences of concatenated fragments of the mitochondrial genes for 12S rRNA, 16S rRNA, and ND4 of samples of the *Uroplatus sikorae* complex. Values at nodes indicate posterior probabilities (PP) > 0.95.

cause they are small, and become tiny towards the rictus. We here counted all scales around the upper and lower lip, including the very small ones in the corner of the mouth, and thus possibly obtained slightly higher counts than reported elsewhere in the literature. We observed 22–29 infralabials and 25–33 supralabials in *U. sameiti*, and 25–33 infralabials and 29–39 supralabials in *U. sikorae* (Table 1). Overall, this might indicate a trend of more labial scales in *U. sikorae,* and within *U. sikorae* the highest values occurring in the northern mitochondrial lineages (Table 1). However, variation of this character was high across the specimens examined, and due to low sample sizes for most lineages we refrained from any statistical analysis.

In individuals of both species, the body is dorsally flattened and the animals possess dermal fringes along their flanks, under their chin and on limbs, which clearly identifies them as members of the *U. fimbriatus* group. We report counts of the number of serrations of these fringes (Table 1) along head, flanks, and limbs, but did not encounter any clear and consistent differences between species or mitochondrial lineages. The head is also strongly flattened and ends in an elongated snout. The rostral is not split. Scalation of the dorsal body surface is heterogenous and consists of more or less round granular scales that are interrupted by enlarged tubercle scales at irregular intervals, which are pointed or cone-like, and distinctly higher than

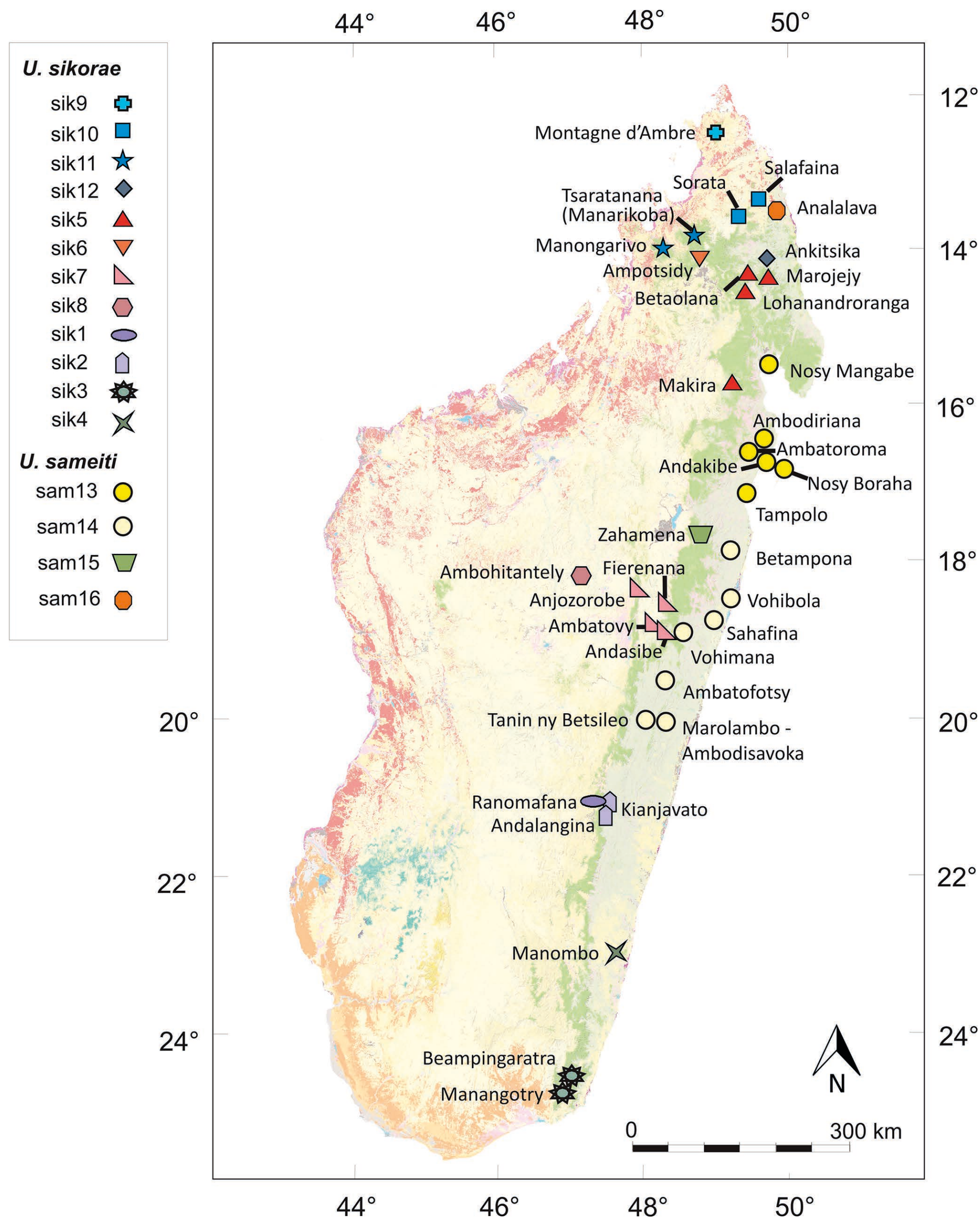

Figure 2. Geographical distribution of species and mitochondrial lineages in the *Uroplatus sikorae* complex, as assessed in the present work. Colours are according to the mitochondrial lineages in the phylogenetic tree (Fig. 1).

granular scales. The belly scales are granular, smooth, and relatively large, with a trend of being larger in the center of the belly. Transverse counts of belly scales at midbody ranged from 69–95, without a clear trend of differences between species or mitochondrial lineages (Table 1). On the upper eyelid, there are 3–5 superciliary lobes. The third finger bears 8–10 scansorial lamellae in both species (Table 1). At the tip of the tail there is no distinct notch and there are 1–2 dermal spines at the root of the tail. The tail is elongated, dorsally flattened and two lateral dermal fringes run towards the rounded tip of the tail.

Genital morphology was not assessed by us, but we here review available data from the literature. Hemipenis descriptions are available for both taxa in Böhme (1988) and Böhme & Ibisch (1990) from Andasibe and Nosy Boraha, respectively. From these descriptions, hemipenes both of *U. sameiti* and *U. sikorae* appear to be characterized by a shield-like bulge of the upper border of the asulcal side of the hemipenis that exceeds the serrated apical lobes. This bulge is covered by deep, honeycomb-like calyces on the asulcal side and embraces the truncus left and right in the upper third of the sulcal side. The presence of this bulge differentiates the male genitals of the *U. sikorae* complex from those of *U. fimbriatus* and *U. henkeli*, but no differences between *U. sameiti* and *U. sikorae* were noted (Böhme & Ibisch 1990).

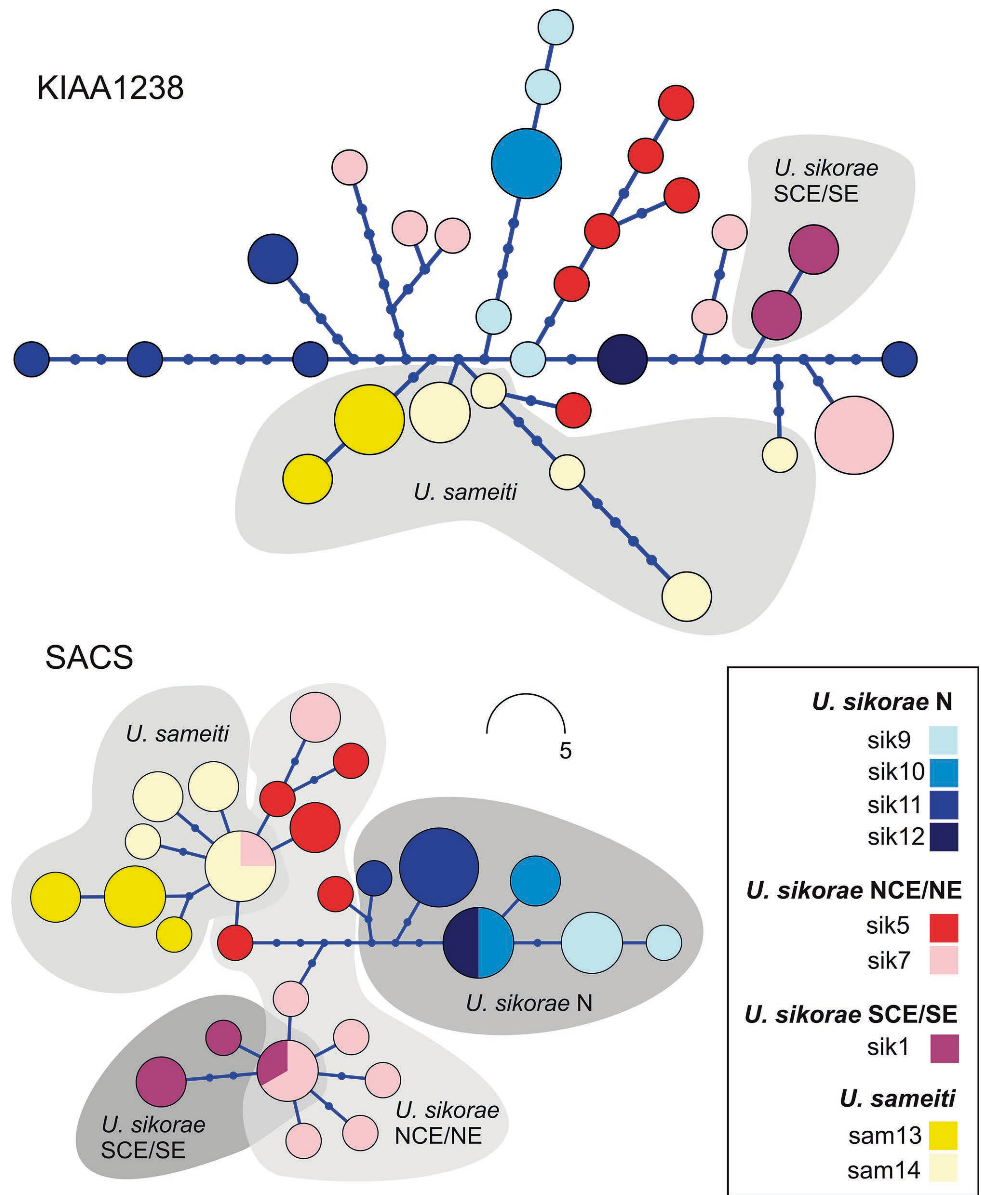

Figure 3. Haplotype networks reconstructed from phased alleles of fragments of the nuclear-encoded SACS (1068 bp) and KIAA1239 (872 bp) genes for 25 individuals of the *Uroplatus sikorae* complex (thus, each individual is represented with two sequences in the analysis of each gene). Haplotype colours and names correspond to mitochondrial lineages in Figure 1.

Comparison of colour patterns

Photographs of 73 living individuals of *U. sikorae* (44 females, 19 males, 10 unknown sex) from Andrafainkoa, Ambolopatrika, Ampotsidy, Anjozorobe, Andasibe (Analamazaotra), Beampingarata, Kianjavato, Makira, Marojejy, Marolambo, Maromizaha, Montagne d'Ambre, Ranomafana, Sorata and Tsararano were available for analysis. Thus, all mitochondrial lineages of *U. sikorae* are photographically covered by representative individuals, and many of these corresponded to the specimens identified via molecular data. For *U. sameiti* photographs of 26 individuals (14 females, 14 males and 2 of unknown sex) from Ambodiriana, Betampona, Masoala, Nosy Boraha, Nosy Mangabe, Sahafina, Tampolo, Vohibola and Vohimana were analyzed. All photographed individuals belonged to lineages sam13 and sam14, unfortunately no pictures of animals from Zahamena (lineage sam15) or Analalava (sam16) were available to us. Many of the photographed individuals corresponded to the specimens identified via molecular data. Additional photographs of *U. sameiti* and *U. sikorae*, which in general comply with the pattern described here, can be found in the publications of Svatek & van Duin (2002), Schönecker (2008) and Gehring (2020) as well as on the internet (e.g. iNaturalist).

Specimens of *U. sikorae* exhibit the following dorsal coloration (an exemplified overview is given in Figs 4–8): the dorsal coloration is highly variable across individuals and mitochondrial lineages; it generally mimics moss- and lichen-covered trunks of rainforest trees. There are mossy-green, lichen- or bark-coloured, reddish or almost completely white specimens. There can be blotches, spots or stripes present as patterns. Longitudinal stripes on the back, neck and head have only been observed in males which may represent a difference between sexes; however, males can also be without a striped pattern (e.g. Figs 5C, 5E). We were unable to detect any consistent differences in dorsal coloration between the mitochondrial lineages of *U. sikorae* that would allow unambiguous assignment. In animals from the northern populations (Montagne d'Ambre, Tsaratanana; lineages sik9 and sik11) a fine reticulation is common that we have not so far observed in individuals of the populations from the southeast coast or the central highlands (e.g. Figs 8A, 8E, 8I, 8J).

The ventral surface of all populations is light grey to white, and single, irregularly dispersed dark spots can be found from the tip of the snout to the tip of the tail. Ventral dermal fringes of the tail are densely covered with dark spots, so they form larger blotches.

As already shown in Ratsoavina et al. (2013), the coloration of the oral mucosa is not constant within *U. sikorae* and differs between main geographical clades. In all southern populations (lineages sik1–sik4; Fig. 1a), the oral mucosa is unpigmented (Figs 4 and 5). In the clades from the central highlands and northern Madagascar (lineages sik5–sik12; Fig. 1a and 1b), the oral mucosa is darkly pigmented (Figs 6–8). The tongue is strikingly yellow coloured in two photographed individuals from Ampotsidy (sik6; Fig. 6C).

According to the photographs examined, *U. sameiti* exhibits the following dorsal coloration (Fig. 9): the dorsal coloration is highly variable and generally mimics tree trunks of rainforests covered in mosses and lichens. There are mossy-green, lichen- or bark-coloured, greyish or almost completely white-coloured specimens. Individuals show a variety of different patterns such as a fine reticulation, dots or even stripes. Also in this species, a longitudinally striped pattern was only observed in males. The ventral surface of all populations is light grey to white and single, irregularly dispersed dark spots can be found from the tip of the snout to the tip of the tail. Ventral dermal fringes of the tail are densely covered with dark spots, so they form larger blotches. No clade-diagnostic colour patterns were observed between lineages sam13 and sam14. The oral mucosa is constantly unpigmented in all populations of *U. sameiti*.

In both species, the eyeballs are encircled by a bright yellow dermal ring that becomes visible when the animals perform their defensive behaviour and protrude their eyes from the sockets. The outer iris area is yellow to light grey or reddish, sometimes with a bluish shade, by day, while at night it is yellow at the outer border and reticulated with an irregular network of fine veins that are condensed towards the pupil, giving the iris a brown colour.

To summarize, an assignment to genetically defined species or mitochondrial lineages in the *U. sikorae* complex is not possible based on coloration. The distinction between *U. sameiti* and *U. sikorae* based on the pigmentation of the oral mucosa is not unambiguous since an unpigmented oral mucosa is also found in the SCE/SE mitochondrial lineages of *U. sikorae.* Northern and southern *U. sikorae* lineages can thus be distinguished based on the coloration of the oral mucosa (black in the north, pink in the south). Note, however, that support for these clades is only tentative, and this feature may not therefore be valuable as a trait for species delimitation. An ungenotyped specimen with a pink oral mucosa cannot be reliably assigned to either the southern *U. sikorae* or *U. sameiti*.

Discussion

In this study we corroborate previous, preliminary data (e.g., Ratsoavina et al. 2013) of strong phylogeographic structure in the *Uroplatus sikorae* complex. Because our molecular sampling of the three mitochondrial gene fragments analyzed (12S, 16S, ND4) were unevenly distributed despite additional sequencing efforts, our data set was unsuitable for an objective lineage-delimiting tool such as ASAP (Puillandre et al. 2021); we are, however, confident that the majority of ad-hoc mitochondrial lineages identified (with 16S distances of 2.9–9.9% among them) would also be identified by objective criteria as putative species-level units.

Recent phylogeographic studies have uncovered high genetic variation in numerous widespread reptile species of Madagascar (Boumans et al. 2007, Florio et al.

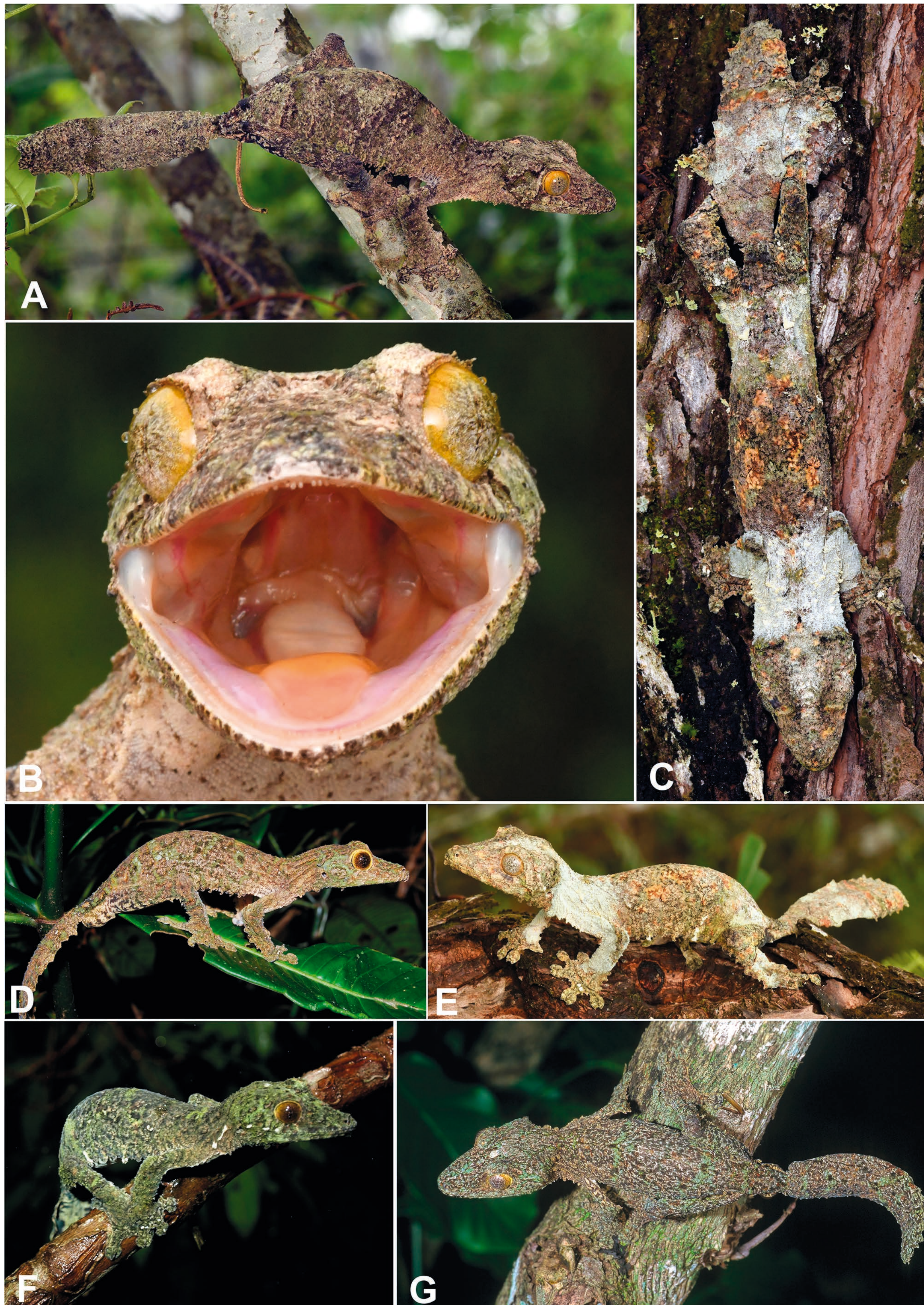

Figure 4. Representative specimens and colour variation of *U. sikorae* of the Ranomafana population (geographical clade *U. sikorae* SCE/SE; mitochondrial lineage sik1). (F) corresponds to sample PSG 2654. All photos by the authors.

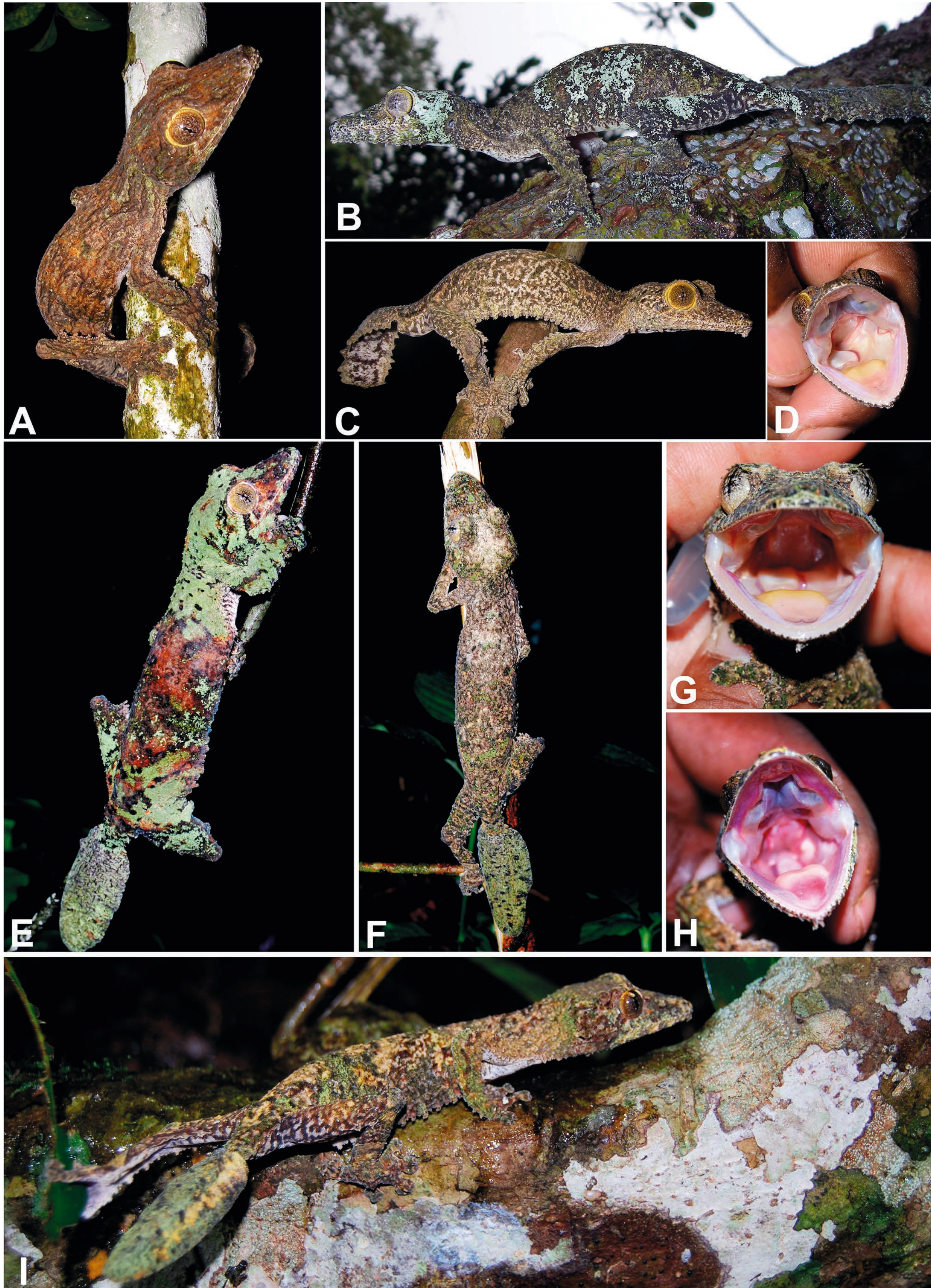

Figure 5. Representative specimens and colour variation of *Uroplatus sikorae* (clade *U. sikorae* SCE/SE) of the populations of (A–D) Kianjavato (mitochondrial lineage sik2), (E–G) Beampingarata (lineage sik3), and (H–I) Andohahela populations. (F) corresponds to sample MPFC 420 in the phylogenetic tree. All photos by the authors.

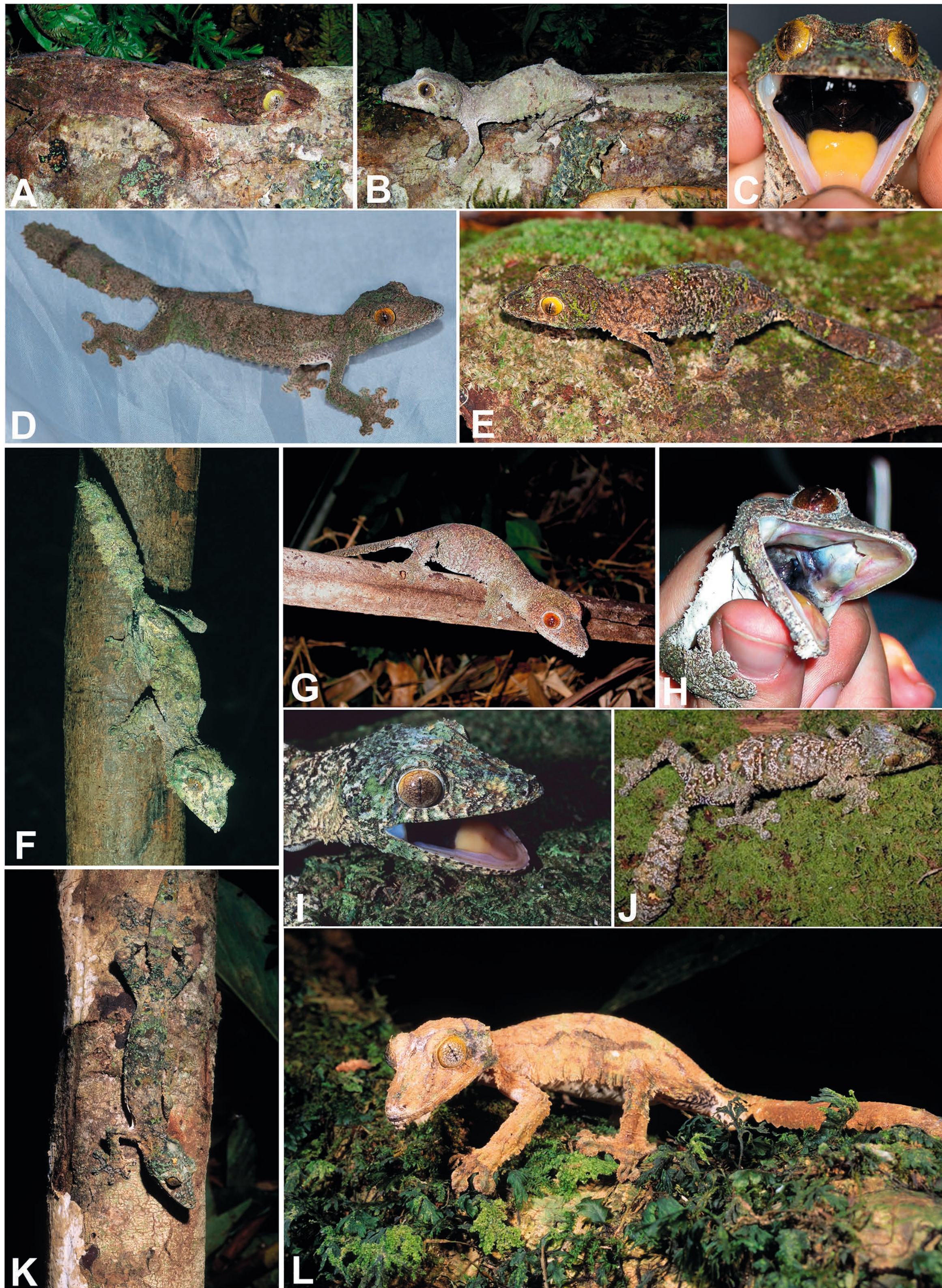

Figure 6. Representative specimens and colour variation of *Uroplatus sikorae* of the mitochondrial clade *U. sikorae* NCE/NE. (A, B) Individuals of the Makira population (mitochondrial lineage sik5); (C–E) individuals from Ampotsidy (sik6); (F–H) individuals of the Marojejy population (sik5). (I, J) individual from Tsararano; (K, L) individuals of the Ambolopatrika population. Tsararano and Ambolopatrika are located between the Anjanaharibe-Sud and Marojejy massifs. Photos I–L: F. Andreone; other photos by the authors.

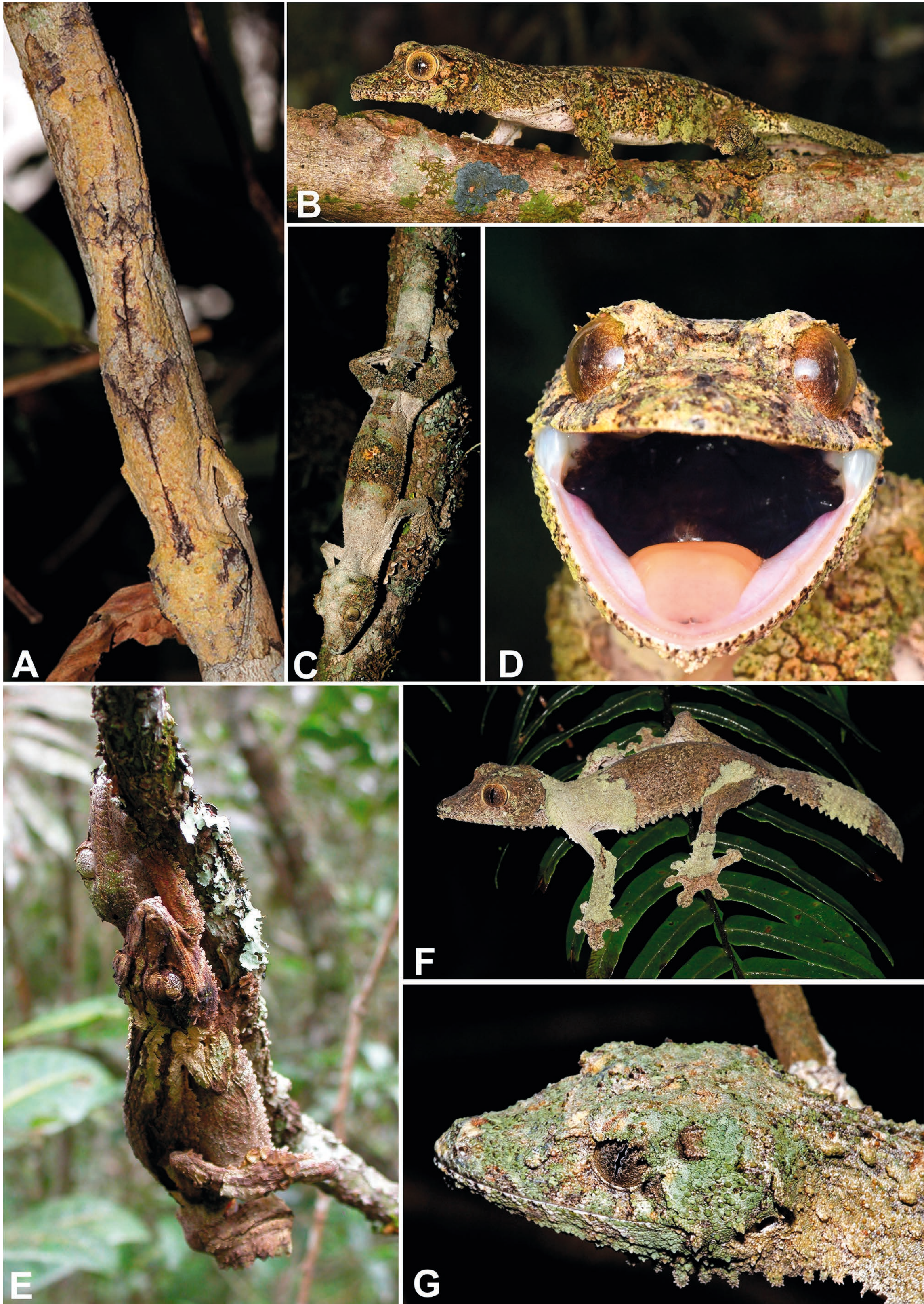

Figure 7. Representative specimens and colour variation of *Uroplatus sikorae* of the mitochondrial clade *U. sikorae* NCE/NE, lineage sik7. (A, B, D, E, G) Individuals of the Andasibe–Analamazaotra population; (C) female from Anjozorobe; (F) female of the Maromizaha population, a rainforest fragment close to Andasibe. Photos A, G: A. Hartig; F: F. Andreone; other photos by the authors.

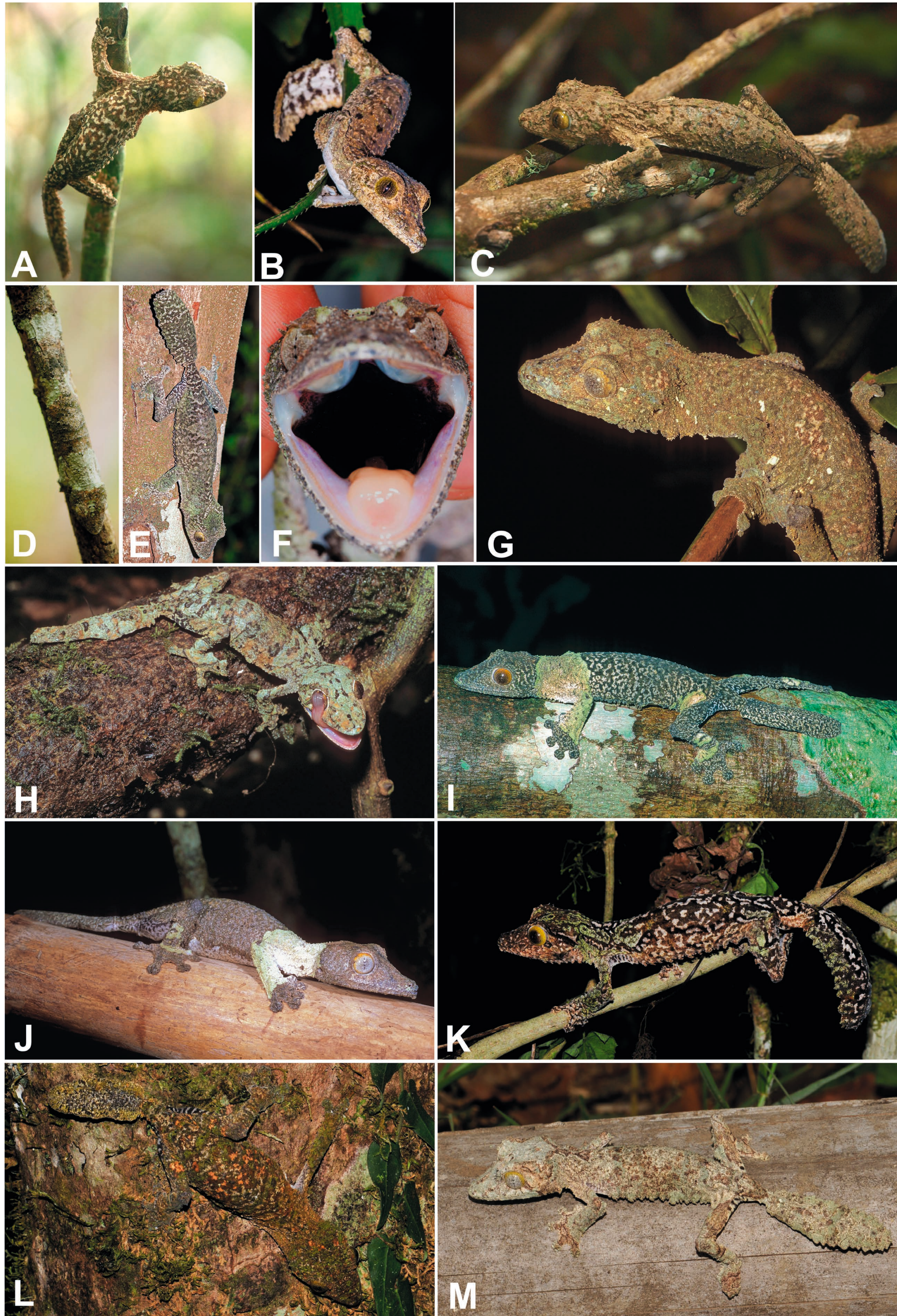

Figure 8. Representative specimens and colour variation of *Uroplatus sikorae* of the mitochondrial clade *U. sikorae* N. (A–G) Individuals of the Montagne d'Ambre population (sik9); (H–J) individuals of the Tsaratanana population (sik11); (K, L) individuals from Sorata; (M) individual from Andrafainkona. Photos B, E: A. Hartig; C: T. Althaus; J: F. Andreone; other photos by the authors.

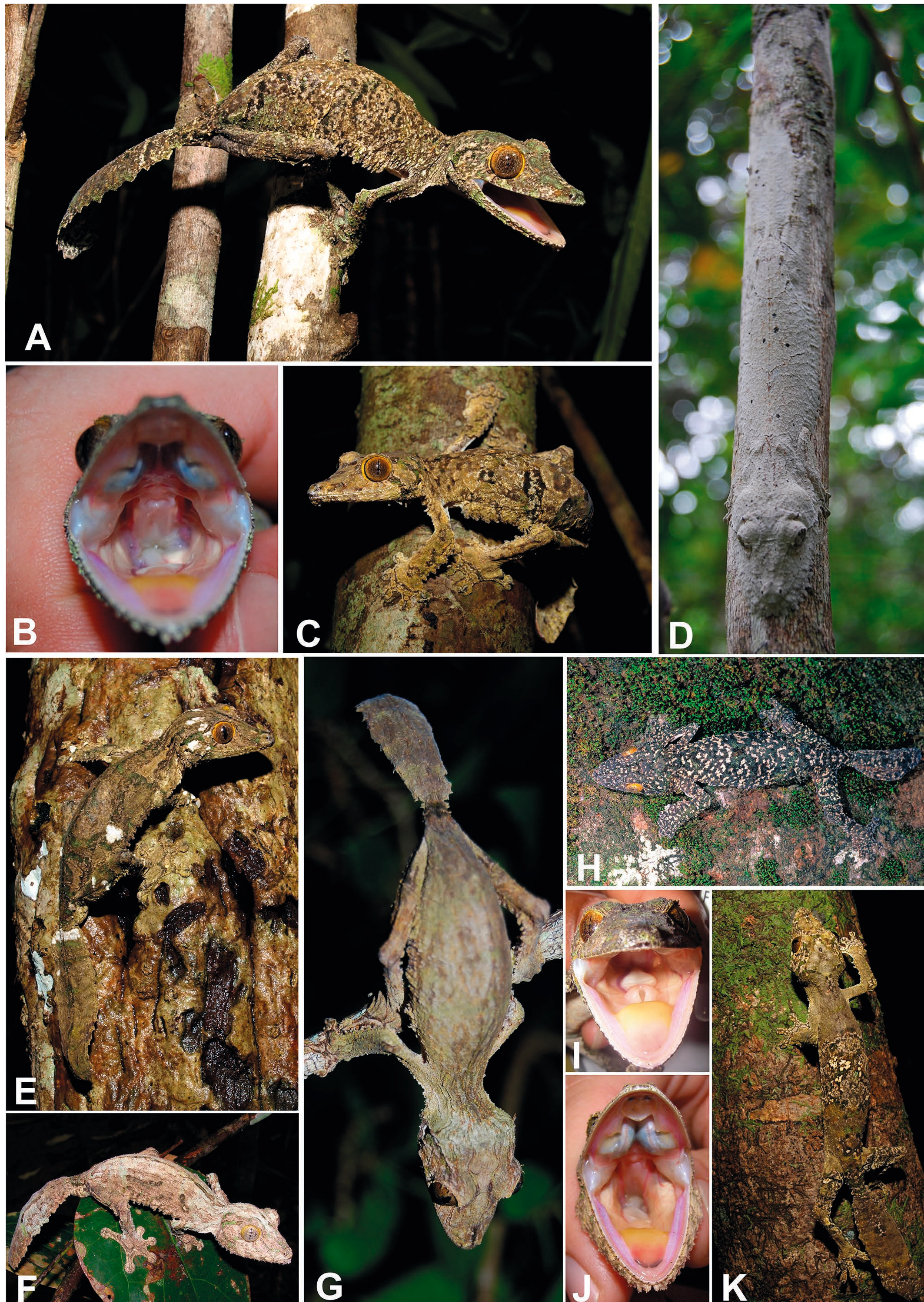

Figure 9. Representative specimens and colour variation of *Uroplatus sameiti*. (A, B, D) Individuals from Vohibola; (C, E) individuals of the Sahafina population (mitochondrial lineage sam14); (F, J) individual from Nosy Mangabe; (G–I) individuals of the population from the type locality Nosy Boraha (sam13); (K) individual from Ambodiriana (sam13). All photos by the authors.

2012, Vences et al. 2014, Grbic et al. 2015). In *Uroplatus*, the majority of recent molecular studies dealt with small-sized species which often are range-restricted; however, the large-sized *U. giganteus* and, to a lesser degree, *U. fimbriatus* do show substantial mitochondrial differentiation across their range (Gehring et al. 2018). The two widespread species of small-sized leaf-tailed geckos (*U. ebenaui* and *U. phantasticus*) are equally subdivided into various deeply divergent lineages, at least from a mitochondrial perspective (Ratsoavina et al. 2012, 2019b). Still, the number of 12 deep mitochondrial lineages in *U. sikorae* represents an exceptional amount of genetic variation even in Madagascar's herpetofauna, and it is not straightforward to find an explanation for this pattern. Among possible factors, it would be worthwhile to investigate in depth (i) the detailed pattern of exceptionally high mitochondrial substitution rates in geckos, and in particular in *Uroplatus*, i.e., by quantifying these substitution rates at different genes and codon positions as well as saturation levels of mitochondrial divergences in comparison to other animals; (ii) the possibility of low effective population sizes in *Uroplatus*, which in combination with repeated bottlenecks could lead to high substitution rates and high phylogeographic fragmentation; (iii) the possibility of small individual home ranges and high site fidelity of *Uroplatus* which also could contribute to phylogeographic fragmentation; and (iv) possible range fragmentations and habitat contractions of *Uroplatus* during past episodes of climate change, given that these geckos are strictly dependent on rainforest which may have experienced phases of contraction over the Pleistocene (e.g., Wilmé et al. 2006).

Range sizes in Madagascar's reptiles decrease with body size (Brown et al. 2016), and the microendemism observed in the *Uroplatus ebenaui* group has been hypothesized to be related to their smaller size (Ratsoavina et al. 2020a). Such an explanation cannot be put forward for the *U. sikorae* complex, which is composed of relatively large-sized geckos, and alternative explanations are therefore required to understand their strong phylogeographic structure. One key might be their elevational occurrence. In fact, from a visual exploration of the distributional information, it appears that the lowland lineages sam13 and sam14, belonging to *U. sameiti,* have colonized a wider area of Madagascar's eastern coast than any of the *U. sikorae* lineages that all occur at higher elevation. Also the two species of the *U. fimbriatus* complex, with a less pronounced phylogeographic structure (Raxworthy et al. 2008, Gehring et al. 2018), occur mostly at lower elevation. If such a trend would be confirmed by a meta-analysis relating range size to elevation in Madagascar's rainforest biota, it could be an indication that low-elevation rainforests would have been less impacted by long-term fragmentation during past climate fluctuations than those at mid- and high-elevations – despite such a scenario being counter-intuitive in light of the riverine barrier mechanism which postulates that rivers constitute stronger barriers to gene flow in the lowlands where they are wider (e.g., Gehring et al. 2012).

The mitochondrial phylogenetic analysis itself is hampered by the large inventory of distinct lineages in the *U. sikorae* complex (which might not even be complete yet, as several areas have not been intensively sampled). Therefore, it is challenging to subdivide the puzzling diversity of lineages into larger clades and infer their evolutionary and biogeographic history. Our efforts to complement the 12S and ND4 sampling of Ratsoavina et al. (2013) with a third gene fragment (16S) for representatives of almost all lineages led to an improvement of phylogenetic resolution, suggesting that an extended gene sampling might be able to fully resolve the phylogenetic tree of these geckos – at least from a mitochondrial perspective. Our tree (Fig. 1) resolves two main clades of *U. sikorae* with substantial support, one that groups the southernmost lineages (the SCE/SE clade; with unpigmented oral mucosa) and one that groups the northernmost lineages (the N clade), supporting a pattern of geographical differentiation. However, the third *U. sikorae* clade (NCE/NE) is not adequately supported in our analysis. It is obvious that the available data are insufficient to conclusively fully resolve the relationships among lineages in the *U. sikorae* complex. Furthermore it is uncertain to which extent the mitochondrial signal may be blurred by introgressive hybridization or the presence of "ghost lineages" differentiated in their mitochondrial but not nuclear genome. Phylogenomic and population genomic data set will be necessary both for improved phylogenetic resolution and species delimitation, especially by targeted sampling of contact/hybrid zones among lineages to understand the extent of genome-wide admixture among them (e.g., Dufresnes et al. 2021).

Do any of the lineages or major geographical clades of *U. sikorae* or *U. sameiti* represent distinct, scientifically unnamed species? Our data do not provide unambiguous support for this hypothesis. In no case did we find evidence for two mitochondrial lineages occurring syntopically which – in combination with lack of admixture in nuclear-encoded genes or morphological differences – could provide strong evidence for the existence of at least two separated evolutionary lineages using the biological species criterion. The two nuclear genes analyzed, furthermore, did not provide a fully concordant signal with the mitochondrial tree, and neither the two currently accepted species (*U. sameiti* and *U. sikorae*) nor any of the major geographical clades belonged to separate phylogroups without haplotype sharing in the SACS network (Fig. 2). In the KIAA1239 network, no haplotype sharing among lineages was observed, but again the haplotypes did not form coherent phylogroups and the number of individuals sequenced per lineage was relatively low. In addition, we did not find any morphological character unambiguously diagnostic for any species, geographical clade, or lineage; the sole informative character identified, black pigmentation of the oral mucosa, characterizes specimens of *U. sikorae* from the NCE/NE and N clades which may not be each other's closest relatives according to the mitochondrial tree; and the absence of pigmentation is shared by *U. sameiti* and *U. sikorae* SCE/SE.

Our data are of importance to guide future studies of morphological differentiation in the *U. sikorae* complex which should focus on identifying combinations of morphological and perhaps osteological characters to differentiate the main genetic lineages. Importantly, individuals from different geographical areas should not be uncritically combined in any comparison, and a reference to the mitochondrial clades identified herein should be included. From our screening and the data presented by Böhme & Ibisch (1990), it is unlikely that diagnostic characters in external colour pattern exist, and the probable sexual dimorphism in the occurrence of longitudinal stripes is probably a common pattern of various large-sized leaf-tailed geckos. This has also been documented in *U. henkeli* where this pattern occurred in males only (Foley & Pfaff 2005). Considering our experience with other morphologically cryptic geckos in Madagascar (e.g., *Lygodactylus*; Vences et al. 2022b), counts of the tubercular scales along the body axis (dorsal and ventral) may be informative to distinguish between species, but their counting is very time-consuming. Large-sized *Uroplatus* are the squamates – and possibly the amniotes – with the largest number of teeth (Bauer & Russell 1989), and we anticipate that tooth number might be a taxonomically valuable character also within the *U. sikorae* complex. Of particular interest is the hemipenial structure which – providing information on sexual selection – is a prime taxonomic character in squamates (Böhme 1988, Padial et al. 2010). A more comprehensive analysis of sexually mature male specimens of the *U. sikorae* complex, of reliably known geographical provenance genetically assigned to lineages, and with fully everted hemipenes (currently not available to us), is needed to assess whether differences in genital structures – even if subtle – may exist among lineages and species of this complex.

Two mitochondrial lineages within *U. sameiti* are particularly enigmatic and worthy of future taxonomic revision: sam15 and sam16. Only a single sample of sam16 from Analalava was sequenced by Raxworthy et al. (2008) for which neither 16S or ND4 sequences nor any morphological information is available. For sam15, four samples from Zahamena were sequenced for ND4 by Ratsoavina et al. (2013) and, independently, one sample from the same locality for 12S and other genes by Raxworthy et al. (2008). We here added ND4 for two additional samples, and 12S and 16S sequences for several of our Zahamena samples, which allowed us to confirm that the sequences of Raxworthy et al. (2008) and Ratsoavina et al. (2013) refer to the same mitochondrial lineage. This population is genetically highly divergent (16S distance >8% to all other samples in the *U. sikorae* complex) and its placement sister to *U. sameiti* sam13/sam14 received no statistical support. This deep lineage (possibly along with the one from Analalava in the north) may well represent a new species of leaf-tailed gecko, but since we had neither life photos nor voucher specimens available for examination, its status cannot be reliably assessed. Strikingly, also in the *U. ebenaui* group of small-sized leaf tail geckos, Zahamena harbors an apparently microendemic and genetically highly distinct candidate species (*U. ebenaui* [Ca10]). In general, the herpetofauna of Zahamena National Park (Goodman et al. 2018) is among the taxonomically least explored amphibian and reptile communities of Madagascar and definitely requires future inventory work, of leaf tail geckos as well as other taxa.

It is striking how poorly known the taxonomy as well as the natural history of the large-sized species of leaf tailed geckos is, considering the high value of these animals as flagship species for ecotourism and nature conservation in Madagascar (e.g., Wollenberg et al. 2011): Most data on the natural history and reproduction of *Uroplatus* available to date derive from captive observations (as summarized by Gehring 2020), and to our knowledge no dedicated long term field study focusing on these aspects has been carried out on any *Uroplatus*. Despite the past and present importance of *Uroplatus* in the pet trade (e.g., Raxworthy & Nussbaum 2000, Todd 2011), not even thorough population density data based on mark-recapture experiments have been published for most species (except transect density estimates for *U. giganteus*: Ingady 2011).

Due to this complex taxonomic situation within the *U. sikorae* complex, information regarding the conservation status of *U. sameiti* and *U. sikorae* must be considered preliminary and should be urgently re-assessed within the IUCN Red List framework. The current distribution range for *U. sameiti* for instance is given with an area of 52,955 km² of lowland areas along Madagascar's east coast, therefore, the species is currently listed as "Least Concern" by the IUCN (Ratsoavina et al. 2020b). However, the assessment also lists all distribution points of the *U. sikorae* SCE/S clades, since genetic information was not yet available at the time of the assessment and all populations with an unpigmented oral mucosa were identified as *U. sameiti.* Based on our new findings, it must be assumed that the actual size of the assumed distribution range for *U. sameiti* is therefore only about half as large, and especially the lowland and coastal forests of Madagascar's east coast that are under immense pressure of deforestation and fragmentation of habitat. Nevertheless, *U. sameiti* still occurs in numerous protected areas (e.g. Vohimana, Betampona, Nosy Mangabe, Ambodiriana, Masoala), so it can be assumed that there is no immediate threat of extinction to the species.

Currently, a distribution area of 77,103 km² is given for *U. sikorae* in the Red List database and the species is therefore considered as "Least Concern" by the IUCN (Ratsoavina et al. 2011b). Recorded localities of the species as currently defined include some protected areas such as Analamazaotra, Mantadia, Maromizaha and Anjozorobe. Again, the problem is that the assessment arose before the remarkable genetic diversity in the complex was recognized, and it is currently still questionable whether the genetic differentiation will lead to further splitting of the species after further investigation. If, after thorough taxonomic reassessment, *U. sikorae sensu lato* is split into multiple species, these will necessarily have smaller ranges than the complex as a whole, and may therefore be threatened under the range-related Criterion B of the IUCN Red

List Categories and Criteria (IUCN, 2012). Treatment of a species complex with multiple, known potential species-level lineages as Least Concern could result in extinction of species going unnoticed, and on this grounds it has been argued that such species complexes should be assessed as Data Deficient, with emphasis on the need for a comprehensive taxonomic revision before an accurate threat status assessment can be made (Scherz et al. 2019). However, due to the puzzling taxonomic situation of *U. sikorae* despite the extensive data presented in this paper, we here refrain from an IUCN threat status suggestion.

The correct identification of species to be exported by the authorities' officials for the pet trade is another problem. Between 2005 and 2019, annually 1,500–2,000 individuals of *U. sikorae* were released for export (UNEP-WCM 2015) and 1,391 individuals were exported between 2019 and 2022 (October) (CITES Trade Database 2022). In the years 2014 and 2015, 500 individuals of *U. sameiti* were respectively released for export by Malagasy authorities (UNEP-WCM 2015). Between 2019 and 2022 (October) 498 individuals were exported (CITES Trade Database 2022). In many cases it is hardly possible for the authorities and breeders to discriminate between *U. sikorae* and its sister taxon *U. sameiti*, and differentiating among genetic lineages is completely impossible without precise information on collection site; avoiding unintentional cross-breeding among these lineages is therefore a big challenge.

According to the data presented herein, populations of the *U. sikorae* complex from almost every remaining rainforest block in Madagascar show substantial genetic divergence. Even if this may not be indicative of cryptic taxonomically-relevant diversity, it still exemplifies how further loss of primary rainforest habitat in Madagascar will inevitably lead to a loss of hitherto unknown and undescribed species and intraspecific genetic diversity, and calls for caution when assembling captive populations for conservation breeding from specimens of unknown provenance.

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Supplementary data

The following data are available online:

Supplementary Table S1. Summary of locality information for samples of the *Uroplatus sikorae* complex confirmed by molecular data.