



## A new species of *Uroplatus* (Gekkonidae) from Ankarana National Park, Madagascar, of remarkably high genetic divergence

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### Abstract

Previous studies on leaf-tailed geckos of the genus *Uroplatus* identified a lineage from the Ankarana karst massif in northern Madagascar as genetically highly divergent, but only fragmentary information was available on these geckos. Here, we provide an integrative analysis based on molecular and morphological data, including a newly collected specimen from this locality. Phylogenetic analysis placed the Ankarana lineage sister to *U. ebenau*, but with a surprisingly high genetic divergence of over 19% uncorrected pairwise distance in the mitochondrial 16S rRNA gene. We formally describe the Ankarana lineage as *Uroplatus fetsy* **sp. nov.** and provide new information on the distribution and genetic differentiation of its sister species, *Uroplatus ebenau*. Based on DNA sequences of newly examined samples this latter species is confirmed as widespread, ranging from low-elevation dry forests from Beanka in western Madagascar to Forêt d'Ambre and Analalava/Fanambana in northern Madagascar, and thus far has not been found in the Ankarana Massif, where *U. fetsy* occurs.

**Key words:** Squamata, Gekkonidae, *Uroplatus ebenau*, *Uroplatus fetsy* **sp. nov.**

### Introduction

The nocturnal gecko genus *Uroplatus* contains 18 described species (Ratsoavina *et al.* 2019), and several undescribed candidate species awaiting taxonomic assessment (Greenbaum *et al.* 2007; Raxworthy *et al.* 2008; Ratsoavina *et al.* 2013). Most species can be grossly divided into two morphotypes, namely leaf-mimicking and bark-mimicking forms, which also correspond to the species' habits during the day, with bark-mimics resting flat on tree trunks and branches, and leaf-mimics hiding among leaves (or in leaf axils) and resting with a bent body posture. The leaf-mimicking forms belong to a single species-rich clade, the *U. ebenau* species group. Recent taxonomic work on this clade has resulted in the description of several new species and in the identification of additional candidate species (Ratsoavina *et al.* 2011, 2012, 2013, 2015, 2017, 2019).

Among these candidate species was one from the karstic Ankarana massif assigned to the candidate number *Uroplatus ebenau* [Ca5] (Ratsoavina *et al.* 2013). We originally considered this lineage an unconfirmed candidate species (Ratsoavina *et al.* 2013) as it was represented by a limited amount of genetic data (a fragment of the 12S rRNA gene) from a single individual, and an overall morphology strongly similar to *U. ebenau*, although genetically it was highly divergent (25.7% distance from *U. ebenau* in a segment of the 12S rRNA gene). Given this situation we excluded this species from subsequent accounts (Ratsoavina *et al.* 2015, 2017, 2019), awaiting confirmation of the genetic results. For the present study, we disposed of a further specimen and an additional tissue sample, and we included several new samples of its putative closest relative, *U. ebenau*. This material was included in new molecu-

lar and morphological analyses, supporting the hypothesis that the genetically divergent lineage is a new species, which we here describe. This species appears to be microendemic to Ankarana in northern Madagascar.

## Materials and methods

Specimens analyzed in this study were collected at night by searching along paths in the forest, aided by torchlight. Representative specimens were anaesthetized with MS222 and subsequently euthanized with an overdose of MS222, then fixed in 90% ethanol or in formalin, and eventually transferred to 70% ethanol after rinsing with water, for long-term storage. Before fixation, tissue samples (typically leg muscle) were preserved in either ethanol (99%) or EDTA buffer. Vouchers were deposited in the Zoologische Staatssammlung München (ZSM) and the reptile collections of the Mention Zoologie et Biodiversité Animale of the University of Antananarivo (UADBA-R). Field numbers of newly studied specimens refer to the zoological collections of Frank Glaw (FGZC), Achille P. Raselimanana (APR), Fanomezana M. Ratsoavina (FRC, KAF, RATF), Mark D. Scherz (MSZC), and Miguel Vences (ZCMV). One specimen, ZCMV 12147, was captured, photographed, had a tissue sample taken (tail clipping), and subsequently released. Fieldwork was conducted under permits 238-MINENVEF/SG/DGEF/DPB/SCBLF (2004) and N°191/17/MEEF/SG/DGF/DSAP/SCB.Re (2017–2018).

*Uroplatus* specimens were measured using the same methods as described in Ratsoavina *et al.* (2011). A digital caliper was used to measure the following variables to the nearest 0.1 mm: snout–vent length (SVL); tail length (TAL); maximum tail width (TAW); head length, measured from tip of snout to posterior end of the head (HL1, see Ratsoavina *et al.* 2011); head length, measured from snout tip to the point corresponding to the posterior edge of the occipital condyle (HL2; see Ratsoavina *et al.* 2011); maximum head width (HW); axilla-groin distance (AGD); eye diameter (ED); ear diameter (EARD); snout length (SL); forelimb length (FORL), measured from the forelimb insertion (corresponding to proximal end of the humerus) to the distal tip of the longest finger; hind limb length (HIL), measured from the hip joint to the distal tip of the longest toe; neck triangle length (NTL), measured from the posterior edge of a small central head prominence (which is present in *the U. ebenau* group species behind the interorbital ridge) to the point where the two lateral skin folds of the posterior head meet centrally on the neck. Scale counts include the number of supralabials (SUPL) counted from the rostral scale (not included) to the left and right corner of the mouth, not including the small scales in the commissure, the number of infralabials (INFL) counted from the mental scale (not included) to the right and to the left, the number of internarials between the nares (INTN), and the number of adhesive lamellae under the third toe (LAM3T) and the third finger (LAM3F).

Total genomic DNA was extracted following a standard salt extraction protocol using proteinase K digestion in a concentration of 10 mg/ml (Bruford *et al.* 1992). For molecular analysis, we complemented DNA sequences from previous studies (Ratsoavina *et al.* 2011, 2012, 2013, 2015, 2017, 2019) with new sequences for the new species described herein, and for *U. ebenau* and *U. malama*.

Our analysis is based on concatenated alignments of four mitochondrial genes, NADH dehydrogenase subunit 4 (ND4), cytochrome oxidase I (COI), 16S ribosomal RNA (16S rRNA) and 12S ribosomal RNA (12S rRNA), plus the nuclear locus oocyte maturation factor Mos (CMOS). For primers and PCR amplification protocols see Ratsoavina *et al.* (2011) for ND4 and CMOS, and Ratsoavina *et al.* (2012) for 12S, 16S, and COI. PCR products were sequenced directly using an automated DNA sequencer (ABI 3130 XL, Applied Biosystems) and quality-checked in CodonCode Aligner (Codon Code Corporation). We used MEGA7 (Kumar *et al.* 2016) for sequence alignment and for calculating uncorrected p-distances between sequences. Newly generated sequences were deposited in GenBank with the following accession numbers: MN087646–MN087658, MN089641–MN089645. Because of the very high genetic divergence of the individuals described as new species herein in both mitochondrial and nuclear DNA (reported separately in the Results below, to confirm genealogical concordance among mitochondrial and nuclear genes), all fragments were combined into a single dataset to produce a more comprehensive assessment of the phylogenetic relationships in the *U. ebenau* species group.

The concatenated alignment of the five gene fragments (in total 2686 bp), was used for partitioned Bayesian phylogenetic inference (BI) searches in MrBayes 3.2 (Ronquist *et al.* 2012), which ran for 10 million generations, sampling every 100 trees, and discarding the first 25% of sampled trees as burn-in. Following the preferred approach that we also used in Ratsoavina *et al.* (2012), our analyses had each gene fragment defined as a partition and models of nucleotide substitution selected by the Akaike Information Criterion in MrModeltest v2.2 (Nylander

2002): (1) CMOS, HKY model; (2) ND4, GTR+I+G; (3) COI, GTR+I+G; (4) 12S and 16S, GTR+I+G. In addition, we also ran Partition Finder 2.1 (Lanfear *et al.* 2016) with each codon of each protein coding gene as separate character set to estimate a nine-partition model as best fitting the data: (1) COI first codon position, SYM+G model; (2) COI second position, GTR+G; (3) COI third position, GTR+I+G; (4) CMOS first and second positions, K80+I; (5) CMOS third position, HKY; (6) ND4 first position, GTR+G; (7) ND4 second position, GTR+G; (8) ND4 third position, GTR+G; (9) 12S and 16S rRNA, GTR+I+G. Analyses were run at least three times with each of the partition settings, yielding virtually identical trees in both four- and nine-partition cases. Sequences of *Uroplatus alluaudi* were used as the outgroup. The NEXUS-format file used for analysis and the treefile have been deposited in Figshare (10.6084/m9.figshare.8295809 and 10.6084/m9.figshare.8295815).

We inferred haplotypes of the CMOS fragment using the PHASE algorithm (Stephens *et al.* 2001) implemented in DnaSP software (Version 5.10.3; Librado & Rozas 2009), and used the phased CMOS sequences to reconstruct a Maximum Likelihood tree with the Jukes-Cantor substitution model in MEGA7 (Kumar *et al.* 2016). To reconstruct a haplotype network, we used this tree together with the CMOS alignment 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).

## Results

The Bayesian inference tree (Fig. 1) depicts relationships among most taxa in agreement with previous analyses (e.g. Ratsoavina *et al.* 2019), and these are therefore not described in detail here. The focal population from Ankarana (candidate species *Uroplatus ebenau*i [Ca5] – in the following called Ca5) is here represented by three samples, all sequenced for 12S and 16S, one each for COI and CMOS, and none for ND4 due to PCR failure. At the 12S and 16S gene fragments, the three individuals here proposed to represent a new species were identical. The tree confirms that Ca5 is a separate lineage that is deeply divergent from all other species in the *U. ebenau*i group. The combination of all available DNA fragments grouped Ca5 in a clade with *U. ebenau*i, albeit with low support (PP = 0.74). The analysis confirmed *U. malama* as sister group of all other ingroup species, and the poorly resolved (*U. ebenau*i + Ca5) clade splitting next, being sister to the remaining lineages.

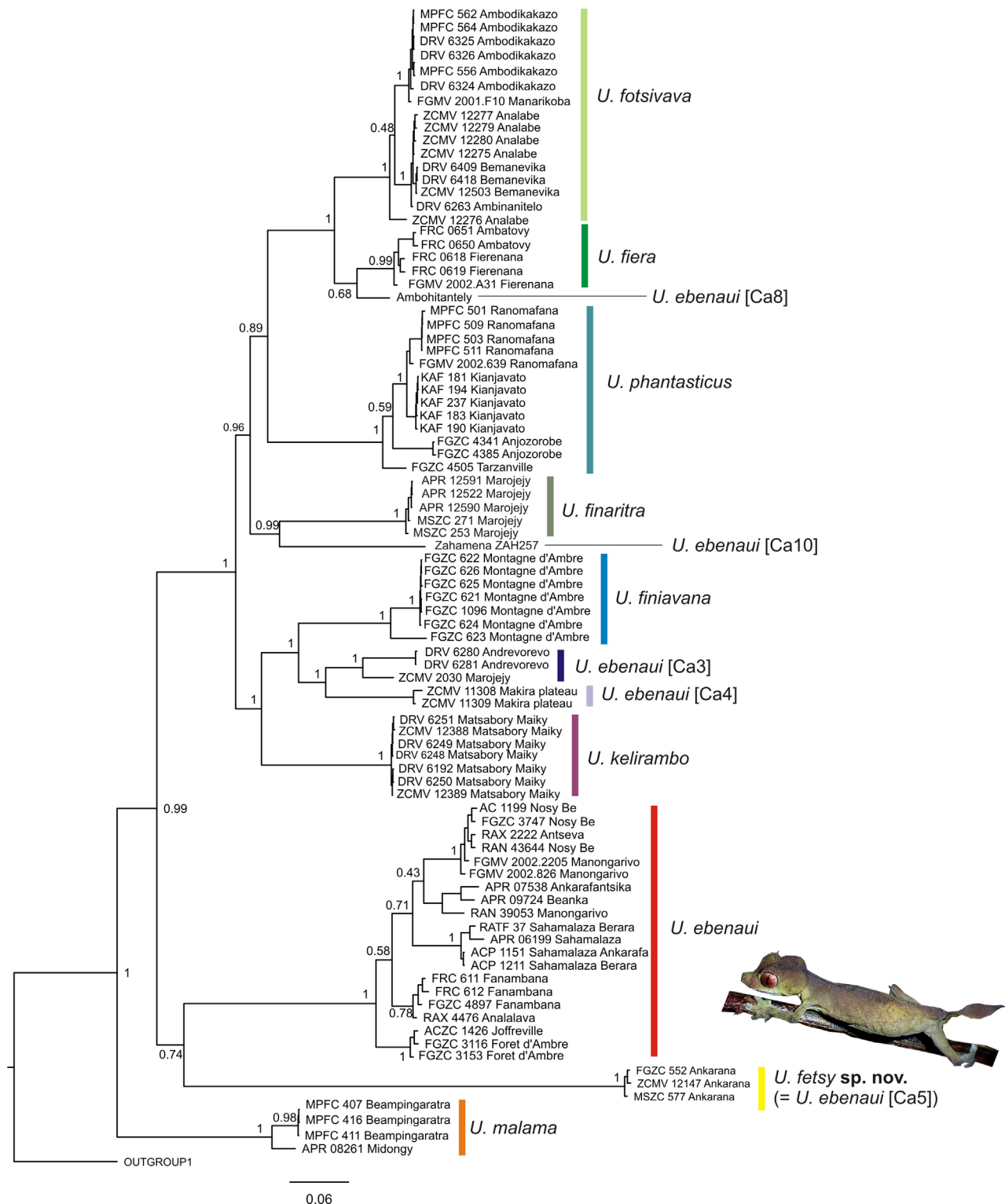
Ca5 is characterized by an extremely high genetic divergence to all other *Uroplatus*, even considering the fact that divergences among and within *Uroplatus* species are overall comparatively high. Uncorrected p-distances of Ca5 in the 16S gene are 19.4–21.3% to *U. ebenau*i, and 18.7–22.4% to all other species in the *U. ebenau*i species group. In COI, p-distances are 23.4–25.6% to *U. ebenau*i, and 23.0–27.9% to all other species. For the 360 bp of the nuclear CMOS gene, in which allele sharing among several other species of *Uroplatus* occurs, *U. ebenau*i and Ca5 are distinguished by 12 mutations — an astonishingly high number if compared with the few mutations separating other well-established species in this group of geckos (Fig. 2).

Our analysis also included one additional sample of *U. malama*, and several new samples of *U. ebenau*i, in order to better understand the divergence of these taxa that all split from basal nodes in the tree of the *U. ebenau*i group. The new sequence of *U. malama* from Midongy du Sud formed a clade with other samples of this species despite some genetic divergence (3.7% uncorrected pairwise distance in 16S). The specimens of *U. ebenau*i formed a clade with several geographically structured sub-lineages. Because only single genes could be sequenced for some samples, the relationships among these sub-lineages did not receive significant support in the phylogeny. Uncorrected pairwise 16S divergences were between 0.4 and 8.2% among sequences assigned to *U. ebenau*i, suggesting that these samples belong to this species, including populations from western Madagascar (Fig. 3) that had previously not been included in molecular analyses.

Morphological examination of the few available specimens and photographs from Ankarana suggested a high morphological similarity of Ca5 to *Uroplatus ebenau*i (comparative morphological data from Ratsoavina *et al.* 2011, 2015), with overlapping values in most measurements and scale counts (Table 1). A possible difference is a slightly longer tail and smoother dorsal skin in Ca5 (see Diagnosis below). Both *U. ebenau*i and Ca5 share black pigmentation of the oral mucosa, but apparently the pigmentation pattern is different: in Ca5, the black pigment extends onto the roof of the mouth and base of the tongue, while reddish color is seen laterally, whereas in *U. ebenau*i, the mucosa is black all over except the pink tongue (Figs. 4–5).

Together, the consistent, albeit weak, morphological differentiation and the extremely high genetic divergences

of Ca5, concordant in mitochondrial and nuclear DNA, leave no doubt that it represents a biologically distinct and independent evolutionary lineage that merits recognition at the species level. We therefore describe it as a new species.



**FIGURE 1.** Bayesian inference phylogenetic tree (50% majority-rule consensus tree) of the *Uroplatus ebenai* group based on a 2686 bp alignment of five concatenated mitochondrial and nuclear gene fragments (ND4, 12S rRNA, 16S rRNA, COI, CMOS). Posterior probability values are shown on the nodes (omitted for most shallow nodes). *Uroplatus alluaudi* was used as the outgroup. The inset photo depicts a specimen of the new species, *Uroplatus fetsy*.

**TABLE 1.** Morphological measurements of *Uroplatus fetsy* **sp. nov.** For abbreviations used, see Materials and methods; additional abbreviations: F, female. All measurements in mm, except for the last five rows which represent scale counts.

	ZSM 288/2004 (FGZC 552)	UADBA-R 70849 (MSZC 577)
	Holotype	Paratype
Sex	F	F
SVL	56.1	55.2
TAL	22.7	22.5
TAW	3.3	6.1
HL1	17.7	16.3
HL2	15.3	15.1
HW	13.2	13.9
AGD	29.9	31.5
FORL	22.8	21.5
HIL	32.1	32.7
NTL	1.3	1.7
ED	4.4	5.1
EARD	0.9	1.1
SL	7.0	6.3
INTN	10	9
INFL (left/right)	24/24	23/22
SUPL (left/right)	26/24	24/26
LAM3F	5	6
LAM3T	5	6

### *Uroplatus fetsy* **sp. nov.**

**Remarks.** This species was reported as *Uroplatus ebenau* [Ca5] by Ratsoaivina *et al.* (2013). The earliest records of geckos of the *U. ebenau* group in Ankarana, probably referring to this species, were provided by Bloxam & Barlow (1987) and Hawkins *et al.* (1990). A photograph of two specimens from Ankarana (reproduced here in Figs. 4f–g) is shown in Böhme & Henkel (1995).

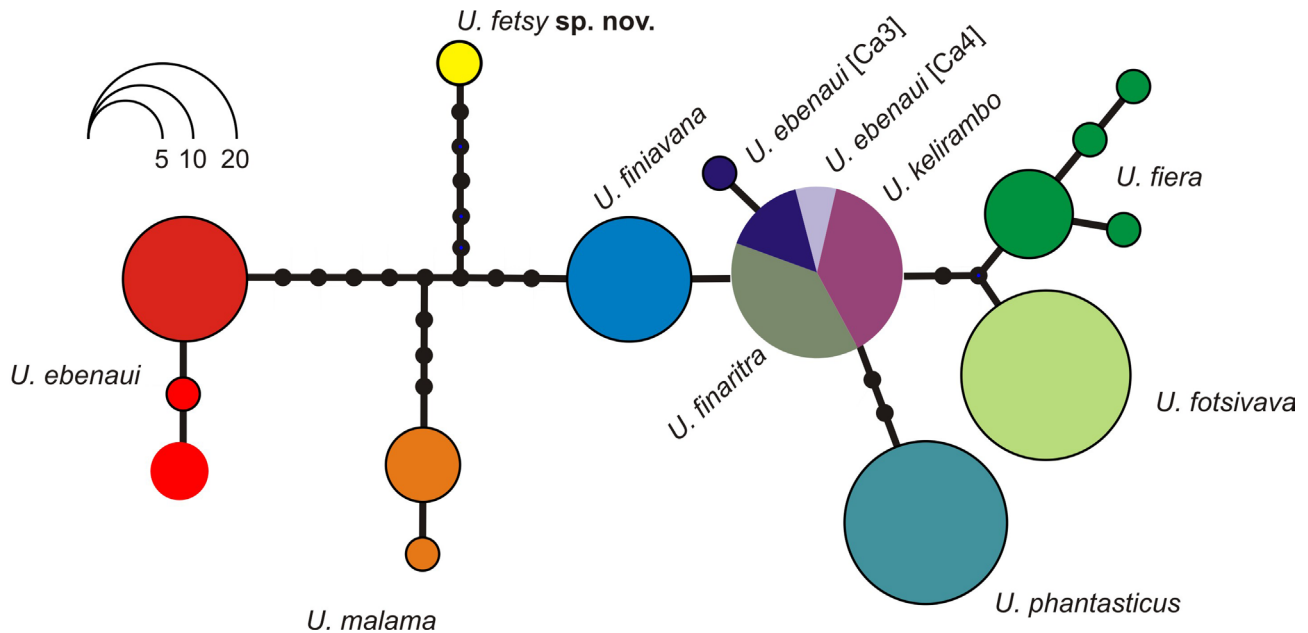
**Holotype.** ZSM 288/2004 (field number FGZC 0552), an adult female collected near Mahamasina in the Ankarana National Park forest, Région Diana, northern Madagascar with the GPS coordinates 12.9683°S, 49.1392°E, ca. 100 m a.s.l. in the evening of 25 February 2004, by F. Glaw, M. Puente, and R. Randrianiaina.

**Paratype.** UADBA-R 70849 (field number MSZC 0577), an adult female collected along the road in the Ankarana National Park forest, Région Diana, northern Madagascar with the GPS coordinates 12.96217°S, 049.13316°E, 133 m a.s.l. at 21h52 on 10 January 2018, by M.D. Scherz, J.H. Razafindraibe, and A. Razafimanantsoa.

**Diagnosis.** *Uroplatus fetsy* **sp. nov.** is assigned to the *Uroplatus ebenau* group based on its relatively small size, leaf-like, laterally-compressed body shape, short tail, and triangular head with supraocular spines. The new species is characterized by the following unique combination of characters: (1) adult female body size of 55.2–56.1 mm ( $n = 2$ ), (2) short (TAL/SVL 0.40–0.41) fairly slender (TAW/SVL 0.15–0.27) tail, (3) oral mucosa black on the roof of the mouth and base of the tongue and reddish laterally with a pink tongue, and (4) relatively smooth skin with few spines. Additionally, it is distinguished by uncorrected pairwise distances of >18% in the 16S gene to all other nominal and candidate new species of *Uroplatus*.

By its leaf-like overall appearance and short tail, the species can easily be distinguished from all members of the *U. fimbriatus* species group, from *U. lineatus*, and from the phenetic *U. alluaudi* species group. Within the *U. ebenau* species group, *U. fetsy* **sp. nov.** may be distinguished from *U. finaritra* and *U. malama* by much smaller body size (SVL 55.2–56.1 mm vs. 77.5–95.3 mm); from *U. malama*, *U. phantasticus*, *U. finivana*, and *U. finaritra*

by shorter tail (TAL/SVL 0.40–0.41 vs. 0.48–0.76); from *U. fiera*, *U. fotsivava* and *U. finiavana* by the color of the oral mucosa (black and red components vs. pale pinkish all over); From *U. finaritra* by the presence of black in the oral mucosa (vs. dark red all over); from *U. fotsivava*, *U. ebenau*, and *U. finiavana* by the possession of fewer spines on the limbs and on the back of the head; From *U. kelirambo* by more rounded tail edges (vs. strongly spear-shaped); and from *U. ebenau*, its putative closest relative, by a differently colored oral mucosa (black on the roof of the mouth and base of the tongue and reddish laterally vs. black all over except the pink tongue; Fig. 5). The new species might differ from *U. ebenau* by a slightly longer tail, despite overlapping values of relative tail length (TAL/SVL 0.40–0.41 vs. 0.22–0.40).

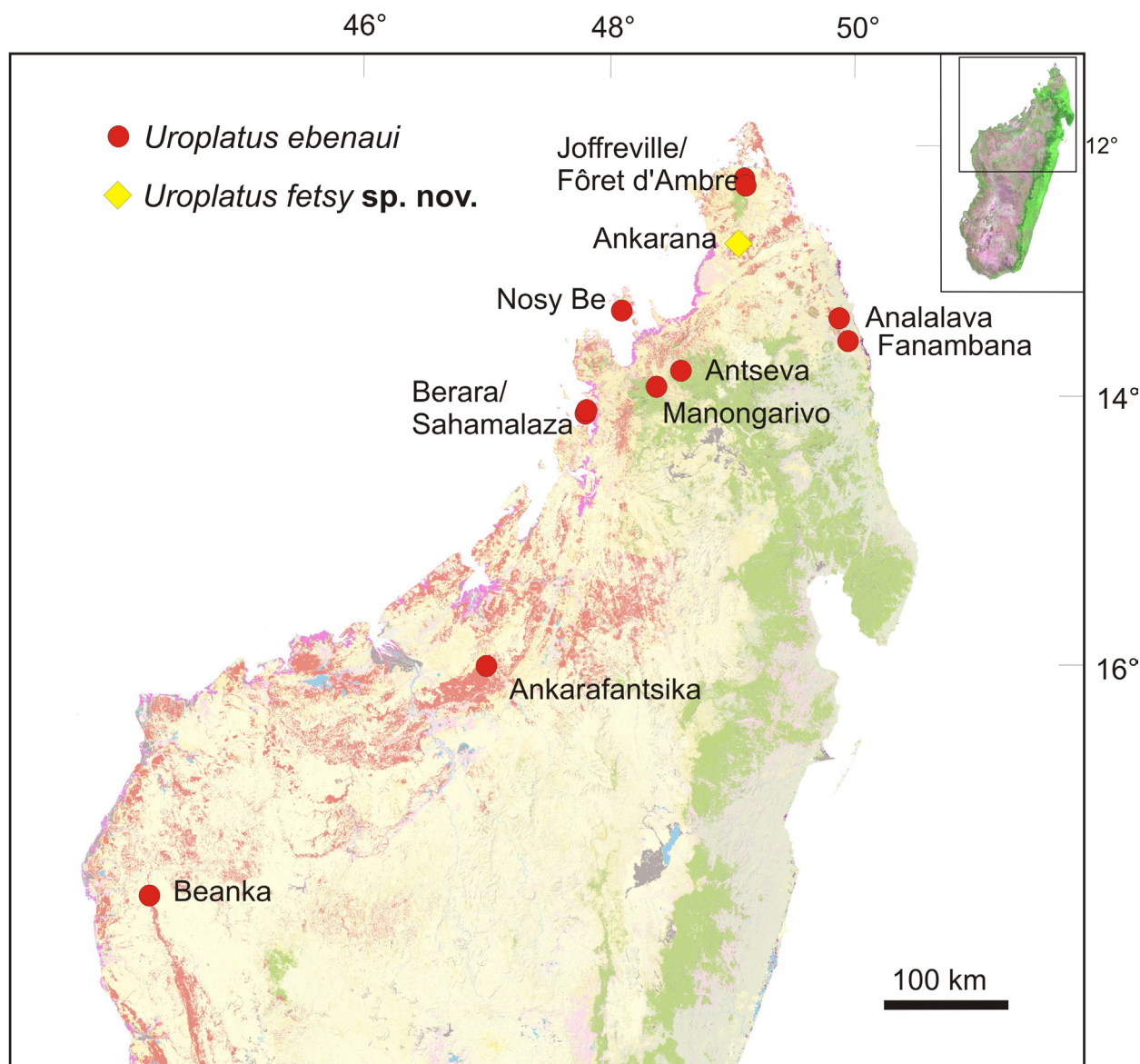


**FIGURE 2.** Haplotype network calculated from a 360 bp segment of the nuclear gene CMOS for the species in the *Uroplatus ebenau* group.

**Description of the holotype.** Adult female in moderately good condition (Fig. 6) though quite soft in the abdomen; the tail has come off in preservative but is stored alongside the specimen; a tissue sample was taken from the tip of the tongue. SVL 56.1 mm, tail length 22.7 mm, maximum tail width 3.3 mm, for further measurements see Table 1. Head triangular in dorsal view; canthus rostralis distinct and concave; snout sloping moderately strongly downwards anteriorly; snout weakly depressed, short (6.7 mm, 1.4 times longer than eye diameter); eyes large (eye diameter 4.7 mm), bulging slightly above the dorsal surface of the cranium, directed laterally, pupil vertical with crenate borders; ear opening very small (horizontal diameter 0.8 mm), ear opening facing posteroventrolaterally (clearly visible in ventral view but not in dorsal view); nostrils laterally oriented; body comparatively round, without lateral dermal fringes; limbs without fringes or spines except on a small flap on the knee and one on the elbow; very distinct round depressed axillary pits present; forelimb almost reaches the nostril when adpressed forward along body and midbody when adpressed backwards along body (forelimb length/axilla–groin distance 22.8/27.9 mm = 82%), hindlimbs cannot be adpressed forward because they are too strongly fixed, but estimated to reach the axilla; original tail length 40% of snout–vent length, membranous borders of the tail symmetrical, without any emarginations, small and leaf-shaped, with a thin, borderless spatulate tip. Toes bear small claws, with the distal phalange not much wider than the rest of the digit; the third finger bears 5 lamellae, the third toe 5 lamellae.

Nares separated from each other by at least 10 small granular scales, from the first supralabial by 2 scales, and from the rostral scale by 3 scales; first supralabial slightly taller than the others; rostral entire, much wider than tall; mental scale very small, not differentiated from infralabial scales; 24/26 (right/left) supralabials and 24/24 infralabials as defined in methods, above; no enlarged postmental scales or chin shields; dorsal and ventral scales of head, neck, body, limbs, and tail small, granular, juxtaposed and largely of uniform size, except on the posterior ventral abdomen, where they are slightly larger than on the rest of the body, and arranged almost uniformly. Two weakly distinct curved lines of only marginally enlarged scales extend from the posterolateral parts of the head (nuchal region) to converge on the neck and form an indistinct V-shaped pattern (neck triangular line). A similar, more distinct,

fairly straight line (also formed by a row of marginally enlarged scales) is present between the eyes and connects the supraocular spines. The body possesses very few dermal spines: a prominent pointed supraocular spine, two diminutive spines at the posterior angle of the head, and one very small spine either side of the vent visible in dorsal but not ventral aspect.



**FIGURE 3.** Map of northern Madagascar showing reliable locality records of *Uroplatus ebenai* and *U. fetsy* **sp. nov.**, based on molecular data.

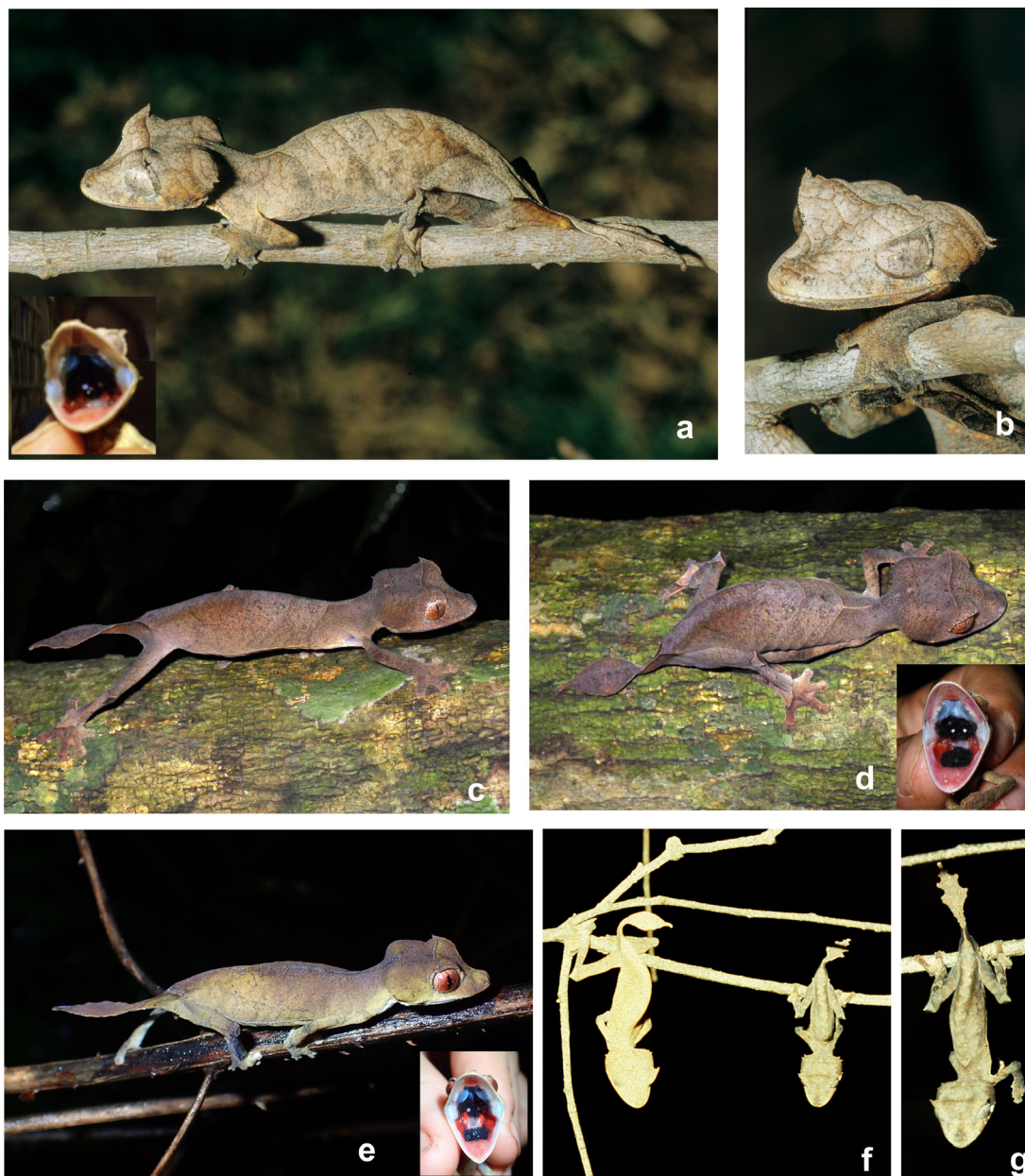
**Coloration of the holotype.** After fourteen years in 70% ethanol, the color is faded. The dorsal coloration of the specimen is a mixture of beige and tan, with more tan on the dorsal head and midbody, and more beige on the anterior dorsum, lateral arms, and dorsal legs. A network of fine lines of contrasting color is weakly present on the dorsum, with the most distinct of these lines running between the supraocular spines. Either side of the base of the tail, dark brown markings are present, but the tail itself is the same beige color of the light areas of the dorsum, without a medial line. The outer surfaces of the legs and arms are darker in color than the thighs and upper arms. The triangular marking at the posterior of the head formed by dark lines converging from the angle of the jaw onto the vertebral line is indistinct. The ventral body is cream, with scales of gray to brown forming maculations on the abdomen, as well as a median line on the throat, and a series of curved lines on the chin. The ventral tail is unremarkable, being light brown without patterns. The tongue in preservative is cream, and the oral mucosa is visibly dark, but the jaw is too stiff to assess the state without damaging the specimen.

In life, the coloration was much as it is in preservative. Areas at the base of the tail were more distinctly brown

than they have become, but otherwise the coloration remains largely unchanged, although faded. The oral mucosa was black at the back of the roof of the mouth and reddish either side of the base of the tongue; the coloration of the base of the tongue was not captured in our photographs (Fig. 4a inset). The iris was silver.

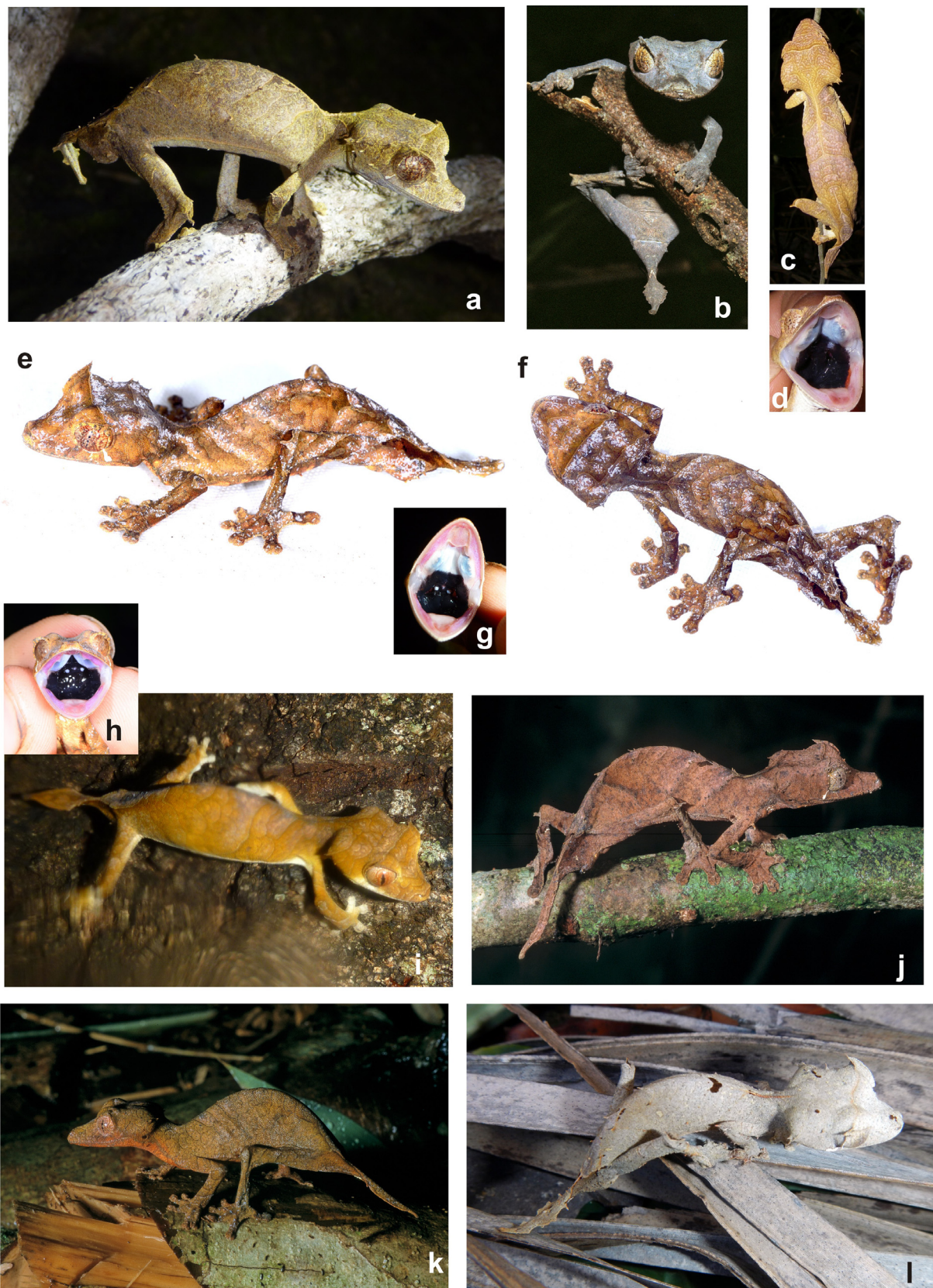
**Variation.** The two type specimens and the tissue voucher ZCMV 12147 (specimen not collected) were all females. So far, a single male, probably assignable to this species, is known from photographs by J. Ganzhorn, reproduced in Fig. 4f–g. The identity of this specimen is not verifiable, but it is probable that it is *U. fetsy* based on the location. If so, *U. fetsy* conforms to the other members of the *U. ebenaui* complex in the sexual dimorphism of tail edges, with females having complete tail edges and males having emarginated edges.

The female paratype UADBA-R 70849 (MSZC 0577) strongly resembles the holotype (Fig. 4). The number of scales between the nares is 9–10, from the naris to the first supralabial is 1–2, and from the naris to the rostral scale is 2–3. It has slightly fewer infralabials (23/22 left/right) than the holotype (24/24). Both type specimens as well as the other photographed individuals have rather few dermal spines.



**FIGURE 4.** Specimens of *Uroplatus fetsy* **sp. nov.** (a–b), female holotype ZSM 288/2004 (FGZC 552), collected in 2004; (c–d) female ZCMV 12147, caught in 2011 (tissue sample only; specimen not collected); (e) female paratype UADBA-R 70849 (MSZC 577), collected in 2018. Inset pictures show coloration of oral mucosa of the respective specimens. (f–g) specimens probably assignable to *U. fetsy* photographed by J. Ganzhorn at Ankarana; the smaller specimen shown in g and on the right in f is most likely a male specimen, recognizable by the enlarged tail base and indentations in the tail.





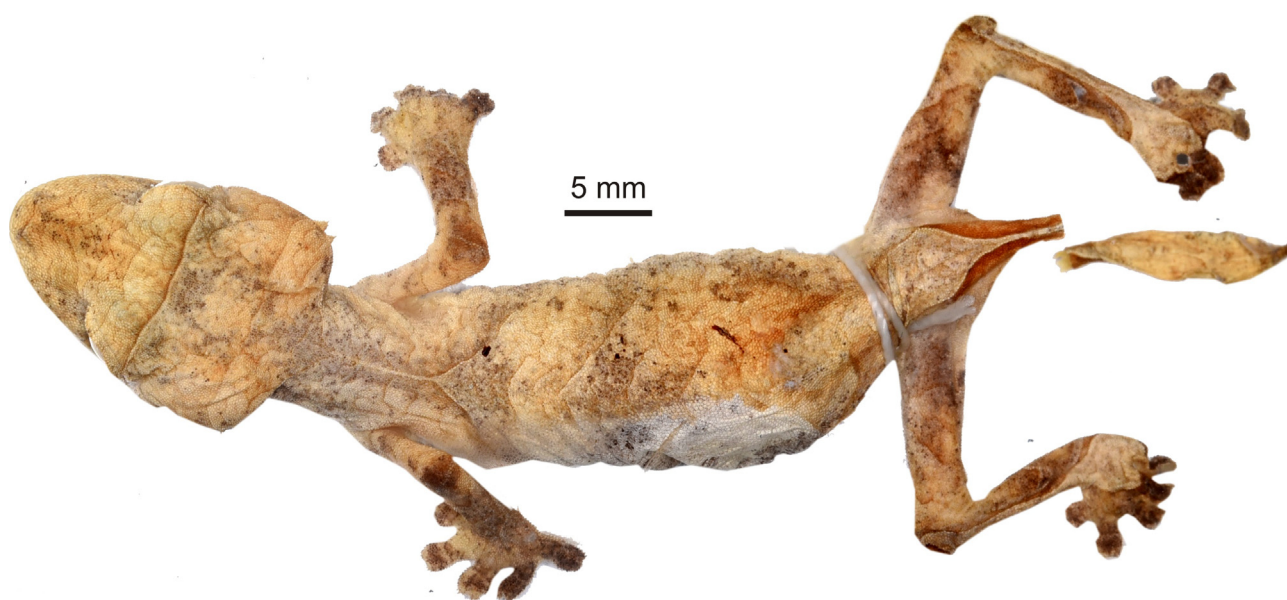
**FIGURE 5.** Photos in life of comparative specimens of *Uroplatus ebenauui* and views of their oral mucosa coloration. (a–d) specimens from Nosy Be (a, adult male, photographed in 2012; b, female, photographed in 1992; c–d, female specimen, photographed in 2009). (e–g) adult male from Forêt d'Ambre (MSZC 794), photographed 2017. (h–i) female from Forêt d'Ambre, photographed 2014; (j) female from Manongarivo, photographed 2003; (k) specimen from Berara (Sahamalaza Peninsula), photographed 2001; (l) male from Fanambana forest, photographed in 2012.

In coloration, the other photographed individuals differ from the holotype in being darker brown and more uniform in color, and also in having more reddish iris coloration (see Fig. 4). Photos made of the mouths of specimens ZCMV 12147 and UADBA-R 70849 (MSZC 0577) clearly show black coloration on the posterior palate and base of the tongue, red coloration on either sides toward the back of the mouth, and a cream or whitish area near the throat (insets of Fig. 4d and Fig. 4e).

**Distribution and conservation status.** This species is only known from Ankarana National Park, at elevations of ca. 100–140 m a.s.l. The park encloses an area of 182 km<sup>2</sup>. Due to the potential rapid decrease in quality of the forests and the area around it due to illicit deforestation, anthropogenic fire, sapphire mining and free-ranging grazing of livestock (Hawkins *et al.* 1990), we propose that *Uroplatus fetsy* should be listed as either Near Threatened or Endangered under the IUCN criteria (IUCN 2012), similar to other Ankarana endemics.

**Natural History.** Very little is known of the ecology of the new species. Specimens were encountered active at night on thin branches and vines less than two meters above the ground. The species is apparently rare, especially in contrast to the syntopic *U. henkeli* [Ca11]—over seven visits to Ankarana between 2003 and 2016, we saw only four specimens. Separate visits to the park by A. Razafimanantsoa also yielded only a small number of sightings (personal communication to the authors).

**Etymology.** Fetsy is a Malagasy word meaning ‘sly’, in reference to the rarity with which this species is encountered, evading researchers for a long time despite the substantial number of research expeditions conducted in Ankarana National Park, as well as its sly ‘smile’. The species epithet is used as an invariable noun in apposition.



**FIGURE 6.** Preserved holotype of *Uroplatus fetsy* sp. nov. (ZSM 288/2004, field number FGZC 552).

## Discussion

*Uroplatus fetsy* shows exceptionally high genetic divergence to all *Uroplatus ebenau*i populations, and indeed to all other members of the *U. ebenau*i group. Morphological differences are quite subtle compared to the degree of the genetic differentiation, but nonetheless strongly argue for its specific distinctness. At present, this species is only known from a small area within Ankarana National Park. Its abundance is apparently very low, because for several consecutive years we have implemented targeted surveys to find the species and have only succeeded in finding few individuals. No other small-sized *Uroplatus* forms have been detected in sympatry with *U. fetsy* in Ankarana National Park, and it will be important to establish if there is a contact zone between *U. fetsy* and *U. ebenau*i here or in surrounding forest fragments.

This study adds an additional species to the exceptionally long list of micro-endemic amphibian and reptile species of the karstic ecosystem of Ankarana, such as *Alluaudina mocquardi*, *Tsingymantis antitra*, *Stumpffia be*, *S. larinki*, *Phelsuma roesleri*, *Brookesia confidens*, *Blaesodactylus microtuberculatus*, *Madagascarophis lolo*, *Gecko-lepis megalepis*, and *Paroedura homalorhina* (Lanza 1990; Glaw *et al.* 2006, 2010, 2012, 2018; Köhler *et al.* 2010;

Jono *et al.* 2015; Ruane *et al.* 2016; Rakotoarison *et al.* 2017; Scherz *et al.* 2017). In several cases, these lineages are very strongly differentiated from others in Madagascar. *Tsingymantis antitra*, for example, is the sole member of a highly divergent genus within the family Mantellidae. While *U. fetsy* is obviously not as divergent as *T. antitra*, its strong genetic differentiation from all other *Uroplatus* is surprising and may result from processes similar to those that have led to the formation of other species within limestone karsts. Elucidating what exactly those processes are is beyond the scope of the present study, but presumably the maze of canyons in karstic formations results in increased genetic structure, strong isolation from external populations, and possibly decreased effective population size. Furthermore, the karstic formations might provide retreats in periods of drought and climate change.

*Uroplatus fetsy* is among the reptile species that are endemic to Ankarana and our analysis suggested a high morphological similarity and possible relationships to sister species of considerably broader distribution, even if the respective clade received only negligible support and requires further confirmation: its hypothetical relative *U. ebenau* is found in the low-elevation forests at the foot of Montagne d'Ambre to the north, and over a vast area to the south of Ankarana (see below). Several similar cases exist, including *Alluaudina mocquardi* (Ankarana endemic) vs. *A. bellyi* (widespread in northern and western Madagascar), *Geckolepis megalepis* (Ankarana endemic) vs. *G. maculata sensu lato* (the closest lineage is more widespread in northern Madagascar; Lemme *et al.* 2013; Scherz *et al.* 2017), and *Stumpffia larinki* (Ankarana endemic) vs. *S. sorata* (found in Sorata and Marojejy; Rakotoarison *et al.* 2017, 2019). These patterns lend further weight to the role of limestone karsts in isolating lineages and producing endemics (Glaw *et al.* 2018).

Aside from describing the new species *U. fetsy*, our data confirm that *Uroplatus ebenau* populations are widely distributed in the western lowland forest of Madagascar, with the northernmost record in Forêt d'Ambre in the far north of Madagascar and the southernmost record at Beanka close to the Tsingy de Bemaraha in central western Madagascar. The genetically confirmed presence of *U. ebenau* at Beanka suggests that this species also occurs in the adjacent Bemaraha massif, which is the southernmost locality where it was recorded previously (Bora *et al.* 2010). Over this range, they have a moderately high degree of genetic variation, with uncorrected pairwise distances of up to 8.2% in the mitochondrial 16S gene. The phylogeography of this species is potentially interesting, but more dense sampling in western Madagascar and larger genetic datasets will be required to understand patterns in its distribution.

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