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A new leaf-tailed gecko of the *Uroplatus ebenaui* group (Squamata: Gekkonidae) from Madagascar's central eastern rainforests

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Abstract

We describe a new leaf-tailed gecko species of the *Uroplatus ebenaui* group from the eastern central rainforests of Madagascar, which had previously been considered as a confirmed candidate species. Our description of *Uroplatus fiera* **sp. nov**. relies on integrating evidence from molecular and morphological characters and is based on newly collected material from two localities. A phylogenetic analysis based on multiple mitochondrial DNA fragments places the new species as sister to a lineage of uncertain status (*Uroplatus ebenaui* [Ca8]), and the clade consisting of these two lineages is sister to a further undescribed candidate species (*U. ebenaui* [Ca1]). This entire clade is sister to *U. phantasticus* plus another candidate species. The new species differs from these close relatives, and all other congenerics, by strong differences in DNA sequences of mitochondrial genes (>8.5% uncorrected *p*-distance in 16S rDNA to all nominal species of the genus) and lacks shared alleles with any of the nominal species in the nuclear CMOS gene. From its closest relatives the new species further differs in its much smaller tail size (relative to *U. phantasticus*), and a narrower tail, fewer supralabials, and more toe lamellae (relative to *U. ebenaui* [Ca1]). Morphologically the new species is most similar to *U. ebenaui* but differs in its larger body size and unpigmented oral mucosa. Given its distribution in central eastern Madagascar, with records from near Fierenana and Ambatovy, its range overlaps with that of *U. phantasticus*. Based on examination of the *U. phantasticus* holotype, we confirm that this latter has a blackish pigmented oral mucosa as do those specimens typically attributed to this nomen, thereby confirming its distinctness from *U. fiera* **sp. nov.**, in which the mucosa is unpigmented.

Key words: *Uroplatus fiera* **sp. nov.**, *Uroplatus ebenaui* group, Taxonomy, Phylogeny, Gekkonidae, Fierenana, Madagascar

Introduction

As indicated in numerous recent species descriptions, the knowledge of Madagascar's vertebrate fauna is far from being complete (Yoder & Nowak 2006). For the island's herpetofauna, increased field exploration coupled with routine application of molecular techniques, have led to an unprecedented rate of discovery of new, genetically and morphologically distinctive lineages of amphibians and reptiles (Vieites *et al.* 2009; Nagy *et al.* 2012; Perl *et al.* 2014) which in many cases might correspond to evolutionarily independent entities meriting recognition as separate species. Although these candidate species are usually characterized by substantial genetic divergence, available data on their morphology, ecology and distribution are often deemed insufficient for a formal taxonomic recognition, usually necessitating additional field explorations to gather information needed to characterize them in a biologically meaningful way (e.g. Vences *et al.* 2012).

The central eastern rainforests of Madagascar include some of the biologically best-studied primary habitats of the island. One of these areas is located around the village Andasibe (previously Perinet) including the Mantadia-Analamazaotra National Park and several smaller protected forests (Lees 1996; Lees *et al.* 1999), belonging to the

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Northern Central East region (Boumans *et al.* 2007). Similar to other parts of Madagascar (Wilmé *et al.* 2006), this area also harbors microendemic species, which are restricted to surprisingly small ranges (Glaw & Vences 2007), such as the prominent Golden Mantella frog, *Mantella aurantiaca*. The Northern Central East region also harbors at least three enigmatic lineages of leaf-tailed geckos (*Uroplatus*), about which only sparse knowledge is available and which have been provisionally identified as candidate species (*Uroplatus ebenaui* [Ca7], *U. ebenaui* [Ca8], and *U. phantasticus* [Ca10]) (Ratsoavina *et al.* 2012, 2013).

There are 14 species of *Uroplatus* recognized, of which some rank among the most emblematic reptiles of Madagascar (Ratsoavina *et al.* 2013). Previous studies (Glaw *et al.* 2006; Greenbaum *et al.* 2007; Raxworthy *et al.* 2008, Ratsoavina *et al.* 2011, 2012, 2013) employed DNA sequences to clarify phylogenetic relationships within the genus, and have revealed a striking diversity of candidate species requiring further taxonomic revision. Often, these belong to the clade of small-sized *Uroplatus* species named the *U. ebenaui* species group (Ratsoavina *et al.* 2013). The systematic revision of the genus by Bauer & Russell (1989) recognized only a single species in this group, *U. ebenaui* Boettger, 1879. Subsequently, Böhme & Henkel (1995) noted that the name *U. ebenaui* should be restricted to a short-tailed species from north-western Madagascar, whereas the long-tailed species occupying most of the eastern rainforest band corresponded to *U. phantasticus* Boulenger, 1888. Two additional species assignable to this group were subsequently described (*U. malama* Nussbaum & Raxworthy, 1995 and *U. finiavana* Ratsoavina, Louis, Crottini, Randrianirina, Glaw & Vences, 2011), and numerous candidate species have been identified (Greenbaum *et al.* 2007; Raxworthy *et al.* 2008; Ratsoavina *et al.* 2012, 2013). These findings lead to a current inventory of four nominal species, five confirmed candidate species, and five unconfirmed candidate species in this species group (Ratsoavina *et al.* 2013).

For several years, the commercial pet trade has provided short-tailed specimens under the name of *U. ebenaui* that were purportedly collected in the Northern Central East area of Madagascar. Ratsoavina *et al.* (2012) presented the first molecular evidence based on one of these specimens, supplied by local collectors from Fierenana, and provisionally named it *Uroplatus ebenaui* [Ca7]. After intensive fieldwork in this region, we recently obtained several additional wild-caught specimens of this taxon. Based on detailed morphological and molecular analysis, we here provide an updated hypothesis on the phylogenetic relationships of this lineage, conclude that these specimens belong to a new species, and provide its formal taxonomic description.

Materials and methods

Sampling and terminology. Specimens were collected during night walks along existing small forest trails at the study sites, using flashlights or headlamps to spot animals on the branches of shrubs and trees. Representative individuals were collected, euthanized by ketamin injection, fixed in 90% ethanol and stored in 70% ethanol. Specimens were deposited in the Université d'Antananarivo, Département de Biologie Animale, Madagascar (UADBA) and in the Zoologische Staatssammlung München, Germany (ZSM). Other acronyms used are BMNH (The Natural History Museum, London, UK), and FRC, FGZC and ZCMV (field numbers of F. M. Ratsoavina, F. Glaw and M. Vences, respectively). In a previously published data set of mitochondrial DNA sequences (re-analyzed along with new data and represented in Fig. 1) we furthermore used the acronyms MPFC, AC, ACZC and APR (field numbers of M. Pabijan A. Crottini and A.P. Raselimanana), FGMV (field numbers of F. Glaw and M. Vences), DRV (field number of D.R. Vieites), KAF, ZAH and RATF (lab sample numbers of F. M. Ratsoavina).
Naming of candidate species follows Padial *et al.* (2010), with the name of the most similar nominal species followed in square brackets by Ca for candidate species and a consecutive number. The naming of biogeographical regions of Madagascar follows the convention of Boumans *et al.* (2007).

In the following, we anticipate our taxonomic conclusion by using consistently the name *Uroplatus fiera* to the lineage described herein. While we are aware that this conclusion is formally only warranted after presenting the respective evidence, we feel that this consistent use of the name makes our study more easily comprehensible and avoids confusion that could arise e.g. by inconsistent naming of the lineage among figures, or between figures and text.

Morphology and morphometrics. Morphometric measurements follow those employed by Ratsoavina *et al.* (2011) and were gathered for all nominal species of the *U. ebenaui* group, for the new species *U. fiera* described herein, and for the candidate species *U. ebenaui* [Ca1] which turned out to be closely related to *U. fiera* in the molecular analysis. Specimens of another candidate species which is even more closely related to the new species, *U. ebenaui* [Ca8], were not available for morphological examination.

Calipers were used by the first author 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: Fig. 1); head length, measured from snout tip to the point corresponding to posterior edge of occipital condyle (HL2), maximum head width (HW), forelimb length, measured from forelimb insertion (corresponding to proximal end of the humerus) to the distal tip of the longest finger (FORL), hindlimb length, measured from hindlimb insertion (corresponding to the proximal end of the femur) to the distal tip of the longest toe (HIL), length of medial posterior projection of the interorbital ridge (IRPL), neck triangle length, measured from the posterior edge of a small central head prominence (which is present in the *U. phantasticus* group species behind the interorbital ridge) to the point where the two lateral skin folds of the posterior head meet centrally on the neck (NTL).

For the goal of an upcoming more comprehensive revision of the *Uroplatus ebenaui* group, we further implemented several counts of scales, tubercles and lamellae of which we here only report those that are important for diagnosis of the new species. This includes the number of supralabials (SUPL) counted from the tip of snout to the corner of the mouth, including numerous small scales next to the corner; and the number of adhesive lamellae under the third toe (LAM3T). Bilateral measurements or counts were taken on the right side of the body. Morphometric and meristic data were analyzed using Statistica 7 software (Statsoft).

DNA sequencing and analysis of sequences. Tissue samples were collected either as tail tips (from specimens subsequently released) or as muscle samples obtained from the femoral region (from preserved voucher specimens). Samples were preserved in ethanol (96%) and total DNA extracted following standard salt extraction protocol using proteinase K digestion in a concentration of 10 mg/ml (Bruford *et al.* 1992).

We used the primers and PCR protocols employed in previous studies with the goal of adding the DNA sequences of new specimens to previous alignments; see Ratsoavina *et al.* (2011, 2012) for primer references and detailed protocols. We targeted fragments of four mitochondrial genes and one nuclear gene using the following primers: NADH dehydrogenase subunit 4 (ND4), using primers ND4 5’-CACCTATGACTACCAAAAGCTCATG TAGAAGC-3’ and LeutRNA 5’-CATTACTTTTACTTGGATTTGCACC-3’, cytochrome oxidase I (COXI) using primers CO1vertF2 5’-TCAACCAACCACAAAGACATTGGCAC-3’ and CO1vertR1 5’-TAGACTTCTGGGTG GCCAAAGAATCA-3’; 16S ribosomal RNA (16S rRNA) using primers 16SAL5’-CGCCTGTTTATCAAAAACA T-3’ and 16SBH 5’-CCGGTCTGAACTCAGATCACGT-3’, 12S ribosomal RNA (12SrRNA) using primers12SAL 5’-AAACTGGGATTAGATACCCCACTAT-3’ and 16SHBnew 5’-CCTGGATTACTCCGGTCTGA-3’, Oocyte maturation factor Mos (CMOS) using the primers CO8 5’-GCTTGGTGTTCAATAGACTGG-3’ and CO9 5’-TTTGGGAGCATCCAAAGTCTC-3’.

PCR products were sequenced directly using an automated DNA sequencer (ABI 3130 XL, Applied Biosystems). Quality control of sequences was carried out using CodonCode Aligner (Codon Code Corporation). For sequence alignment, as well as calculation of uncorrected *p*-distances between sequences, we used MEGA 5 (Tamura *et al.* 2011) where the new sequences were manually added to the alignments of Ratsoavina *et al.* (2012) for the four mitochondrial gene fragments, and of Ratsoavina *et al.* (2011) for the nuclear marker CMOS. Newly determined sequences were deposited in GenBank with the following accession numbers: KP792409–KP792432.

The concatenated alignment of the four mitochondrial gene fragments, was subjected to Bayesian phylogenetic inference (BI) searches using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), under the same specifications and models of nucleotide substitution (previously selected by the Akaike Information Criterion in MrModeltest v2.2; Nylander 2002) as in Ratsoavina *et al.* (2012). Sequences of *Uroplatus alluaudi,* employed as a representative of the *U. alluaudi* species group, were used as the outgroup.

The nuclear sequences (CMOS gene) were analyzed separately because our primary objective was to obtain evidence from unlinked loci (mitochondrial *versus* nuclear) for genetic differentiation of lineages which by such differentiation would be further supported as distinct species. We assembled a CMOS data set containing sequences from all described species of the *U. ebenaui* group, the lineage herein described as new species *U. fiera,* plus *U. ebenaui* [Ca1] and *U. ebenaui* [Ca8] which are the closest relatives of the new species. Haplotypes of the CMOS fragment were inferred using the PHASE algorithm (Stephens *et al.* 2001) implemented in DnaSP software (Version 5.10.3; Librado & Rozas 2009). The alignment of phased haplotypes was used to construct a medium joining network (Bandelt *et al.* 1999) using the software Network 4.612 (www.fluxus-engineering.com).

Results

Molecular phylogenetics. During phylogenetic analysis of mitochondrial DNA sequences, we noted that the previously published COX1 sequence (Genbank accession number JX205405) of *Uroplatus fiera* (under the name *U. ebenaui* [Ca7]) is probably erroneous. We decided to exclude this sequence from further analysis, and we hereby provide the rationale for this decision.

For the multigene analysis of Ratsoavina *et al.* (2012), only one sample of *U. fiera* (as *U. ebenaui* [Ca7]) was available, and sequences of 12S, 16S and COX1 of this individual were included in the analysis (ND4 sequences not available). The analysis in that paper placed this specimen as the sister clade of all *U. ebenaui* group lineages except for *U. malama* and *U. ebenaui*, which sequentially diverged from the two basalmost nodes in the tree. From the next upper node of the tree, a clade of several individuals from northern Madagascar assigned to *U. ebenaui* [Ca1] split off and thus was the sister clade to the remaining lineages of the group. In contrast, the 12S single gene tree of Ratsoavina *et al.* (2013) showed *U. fiera* (as *U. ebenaui* [Ca7]) and *U. ebenaui* [Ca1] as sister clades, with a maximum posterior probabilities of 1.0.

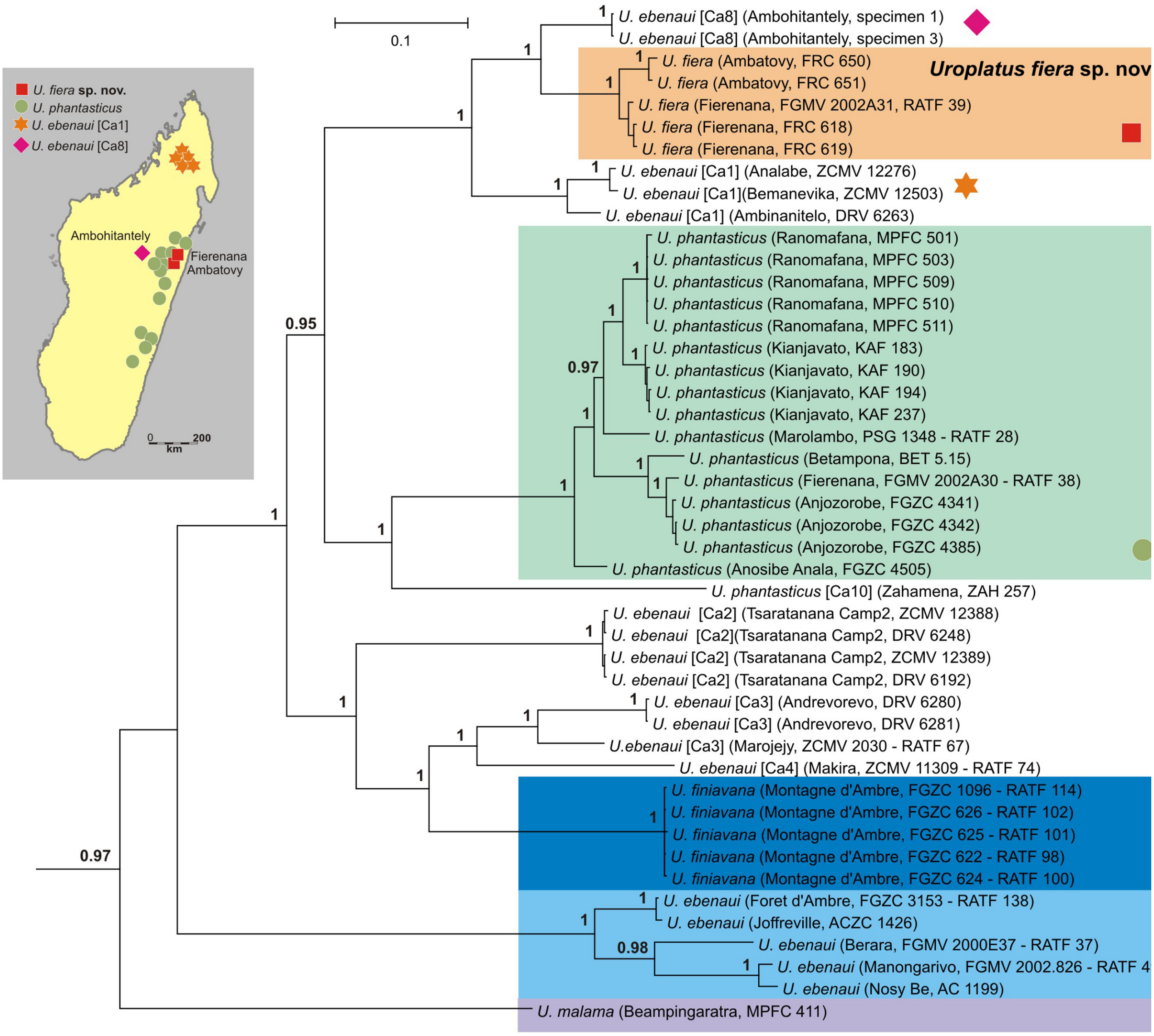

FIGURE 1. Bayesian inference tree of the *Uroplatus ebenaui* group based on a 2313 bp fragment of four concatenated mitochondrial gene fragments (ND4, 12SrRNA, 16SrRNA, COX1). Only posterior probability values of 0.95 or greater are displayed at nodes. Shaded boxes indicate nominal species, undescribed candidate species reside outside such box. An outgroup (*U. alluaudi*) was used for rooting but omitted from the tree for graphical reasons. The inset map shows distribution of the new species *U. fiera* and related species; for more detailed distribution maps of all species of the *U. ebenaui* group refer to Ratsoavina *et al.* (2013).

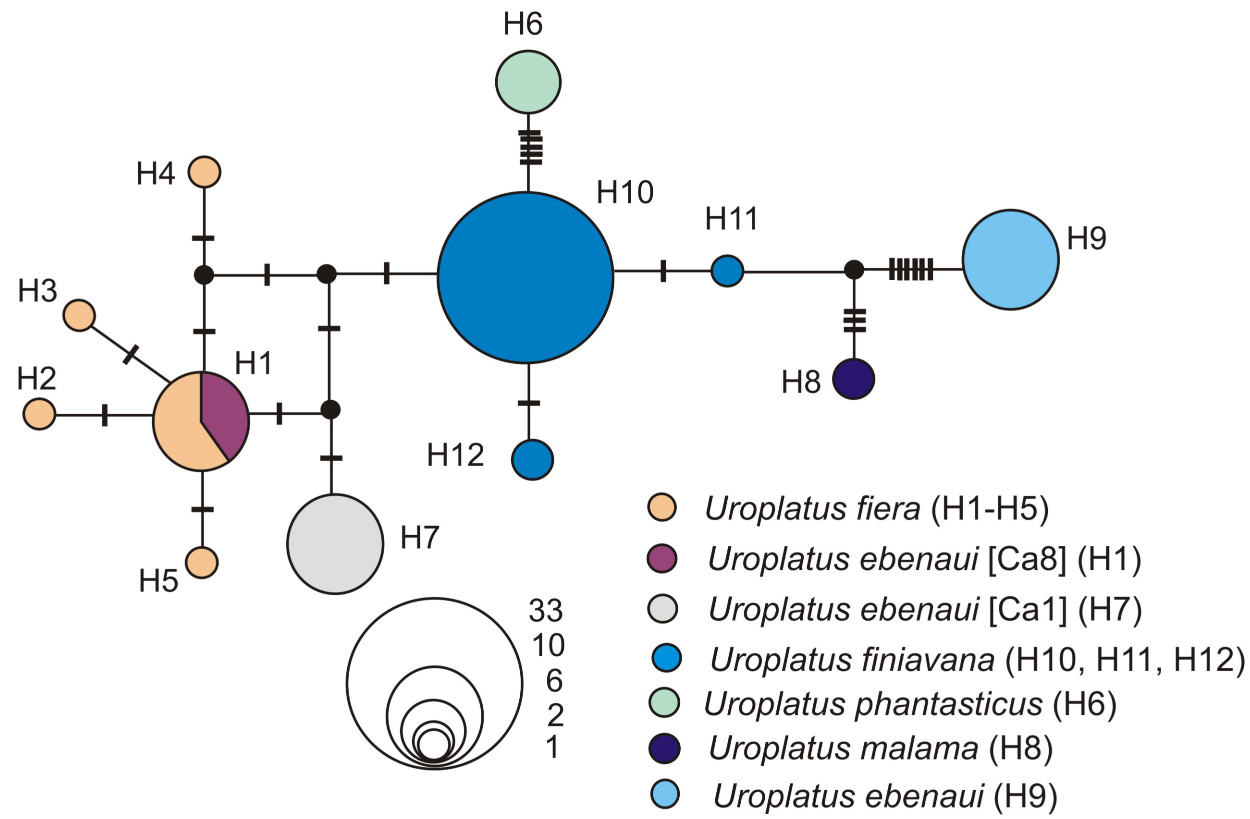

FIGURE 2. Median joining network reconstructed from haplotypes of a 400 bp fragment of the nuclear CMOS gene for the described species of the *Uroplatus ebenaui* group, plus the newly described *U. fiera* and its closest relatives, the candidate species *U. ebenaui* [Ca1] and [Ca8]. Haplotypes were inferred using the PHASE algorithm. After each species name, the haplotypes assigned to this species are listed. Bars and small black dots represent mutational steps and circle size refers to number of haplotype sequences involved and summarized at the bottom of the network diagram.

To better understand the cause of this conflict, we performed exploratory single gene analyses of the previously published data. These indicated that the 12S and 16S sequences of Ratsoavina *et al.* (2011) were unanimous in supporting close relationships between *U. fiera* (as *U. ebenaui* [Ca7]) and *U. ebenaui* [Ca1] whereas in the tree based on COX1 the sample of *U. fiera* was deviant, being segregated from all other *U. ebenaui* group specimens. After adding the data of the newly collected samples to the single-gene alignments, we found that (i) for 12S and 16S, the new samples tentatively assigned to *U. fiera* were indeed very similar or identical to the previous sequences assigned to this lineage in Ratsoavina *et al.* (2012), and the *U. fiera* clade persisted as the sister of *U. ebenaui* [Ca1]; (ii) for ND4, the two newly obtained sequences for the new species (no sequence available previously) were also placed sister to *U. ebenaui* [Ca1]; (iii) for COX1, the newly obtained sequences were placed sister to *U. ebenaui* [Ca1] whereas the old sequence (from Ratsoavina *et al.* 2012) continued to be placed outside the *U. ebenaui* group. We therefore concluded that the previously determined COX1 sequence is erroneous, either due to contamination or, more likely, because it represents a nuclear pseudocopy (NUMT).

Phylogenetic analysis of the modified mitochondrial data set (after exclusion of the putatively erroneous COX1 sequence and addition of sequences from the new specimens) generated a tree largely in agreement with that of Ratsoavina *et al.* (2012) except for the basal nodes (Fig. 1). *U. malama* and *U. ebenaui* persisted in being the two basalmost nodes of the tree, but *U. fiera* now formed a strongly supported clade with *U. ebenaui* [Ca8] specimens from Ambohitantely (posterior probability 1.0). The clade of the new species and *U. ebenaui* [Ca8] formed with strong support the sister group of populations from the Tsaratanana Massif (northern Madagascar) assigned to *U. ebenaui* [Ca1] (posterior probability 1.0). The clade of these three lineages was placed with moderately strong support (posterior probability 0.95) as sister to the clade containing *U. phantasticus* and *U. phantasticus* [Ca10] from Zahamena. All specimens of the new species *U. fiera* (the new ones plus the old sample) formed a monophyletic group.

Uncorrected pairwise distances between *Uroplatus fiera* and other nominal species of the *U. ebenaui* group for the 16S rRNA gene fragment were between 8.8% (*U. phantasticus*) and 19.6% (*U. ebenaui*). Pairwise distances to *U. ebenaui* [Ca8] were 3.6–4.5% and to *U. ebenaui* [Ca1] were 4.9–9.9%. Intraspecific pairwise distances between the localities around Ambatovy and Fierenana were 1.9–2.0%. The CMOS haplotype network (Fig. 2) largely confirmed the results of Ratsoavina *et al.* (2011) with no haplotype sharing between the four nominal species (*U. ebenaui, U. finiavana, U. malama, U. phantasticus*). Also, the newly added sequences of *U. fiera* differed by a minimum of six mutational steps from the most similar haplotypes of *U. finiavana*, and by a minimum of four steps from the likewise included haplotype of *U. ebenaui* [Ca1]. Haplotype sharing was however observed between *U. fiera* and *U. ebenaui* [Ca8] from Ambohitantely.

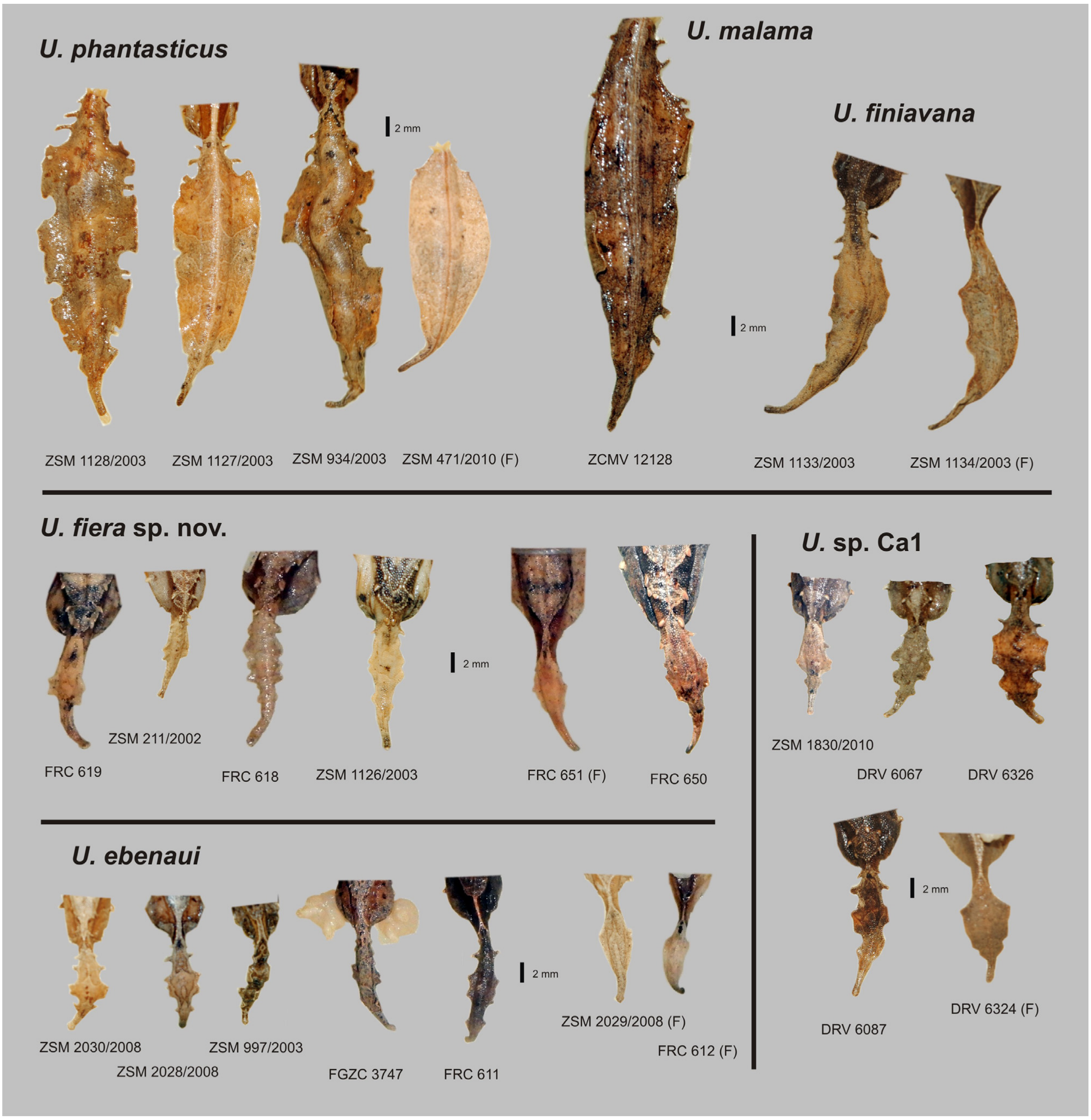

FIGURE 3. Tails of specimens of all described species of the *Uroplatus ebenaui* group, plus the newly described *U. fiera* and the candidate species *U. ebenaui* [Ca1] (no voucher specimens or photos could be examined for *U. ebenaui* [Ca8] which is phylogenetically the closest lineage to *U. fiera*). All tails sized to scale (scale bar 2 mm). Female specimens are labelled with F in parentheses. All other specimens are males.

Morphological differentiation and the identity of *U. ebenaui* [Ca1]. As established by Böhme & Henkel (1995) and Ratsoavina *et al.* (2011), the size and shape of the tail provides one of the most useful morphological characters for distinguishing species in the *U. ebenaui* group. A visual comparison of the tails of *U. fiera* with those of the four nominal species in the *U. ebenaui* group, plus *U. ebenaui* [Ca1], suggests clear and constant differences in general tail size of the new species from all species except *U. ebenaui* (Fig. 3), regardless of sex. The ratio TAL/SVL (Fig. 4; original data in Table 1) yields similar values for *U. fiera, U. ebenaui* [Ca1] and *U. ebenaui,* but tails are relatively much longer in *U. phantasticus, U. finiavana* and *U. malama*. Where sample size is large enough for a statistical testing (*U. fiera* versus *U. phantasticus*) this difference is significant (Mann-Whitney U test; P=0.002). Another important character of the *U. ebenaui* group is the pigmentation of the oral mucosa: it is black in *U. phantasticus, U. ebenaui,* and *U. malama,* but unpigmented in *U. finiavana.* We confirmed that all studied specimens of *U. fiera* and *U. ebenaui* [Ca1] (those listed in Table 1 plus additional specimens seen in collections and in the field) have an unpigmented oral mucosa, thus clearly differing from especially *U. ebenaui*, which by tail size and shape cannot be reliably distinguished from these lineages. A further difference between the new species *U. fiera* and *U. ebenaui* is observed in the number of lamellae under the third toe (Figs. 4–5), with a greater number in *U. fiera* (7–8 versus 5–6).

The mitochondrial tree (Fig. 1) reveals *U. fiera* being a distinct lineage, but related to *U. ebenaui* [Ca8] from Ambohitantely (16S uncorrected *p*-distance 4.5%). The identity of this latter candidate species could not be further clarified at present due to the lack of morphological data (no vouchers were collected for the samples sequenced herein).

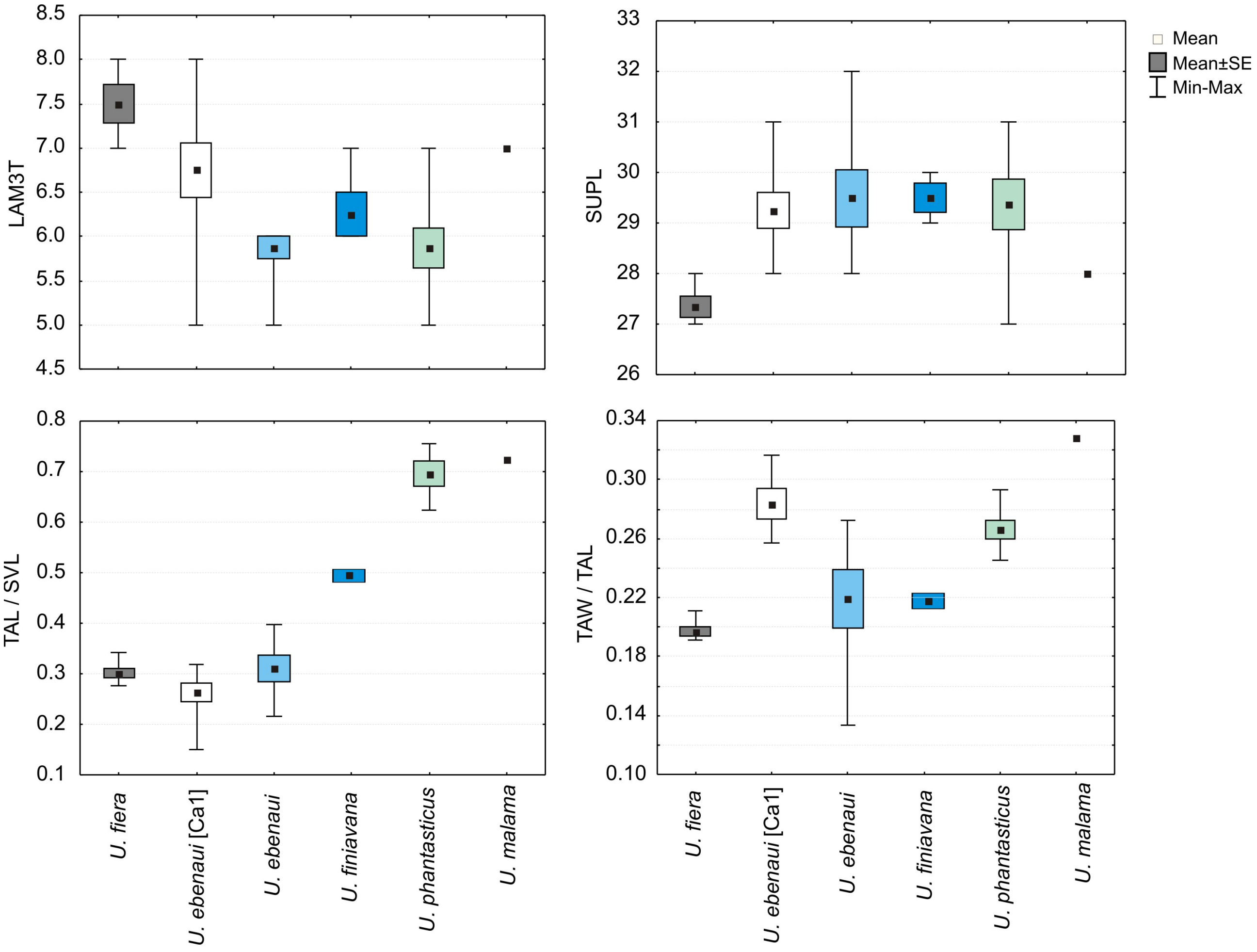

FIGURE 4. Boxplots showing variation in two meristic counts and two morphometric ratios in the specimens studied herein of all described species of the *Uroplatus ebenaui* group, plus the newly described *U. fiera* and the candidate species *U. ebenaui* [Ca1]. Information on its phylogenetically closest relative *U. ebenaui* [Ca8] is not available. SE = Standard error. For additional abbreviations see Materials and Methods.

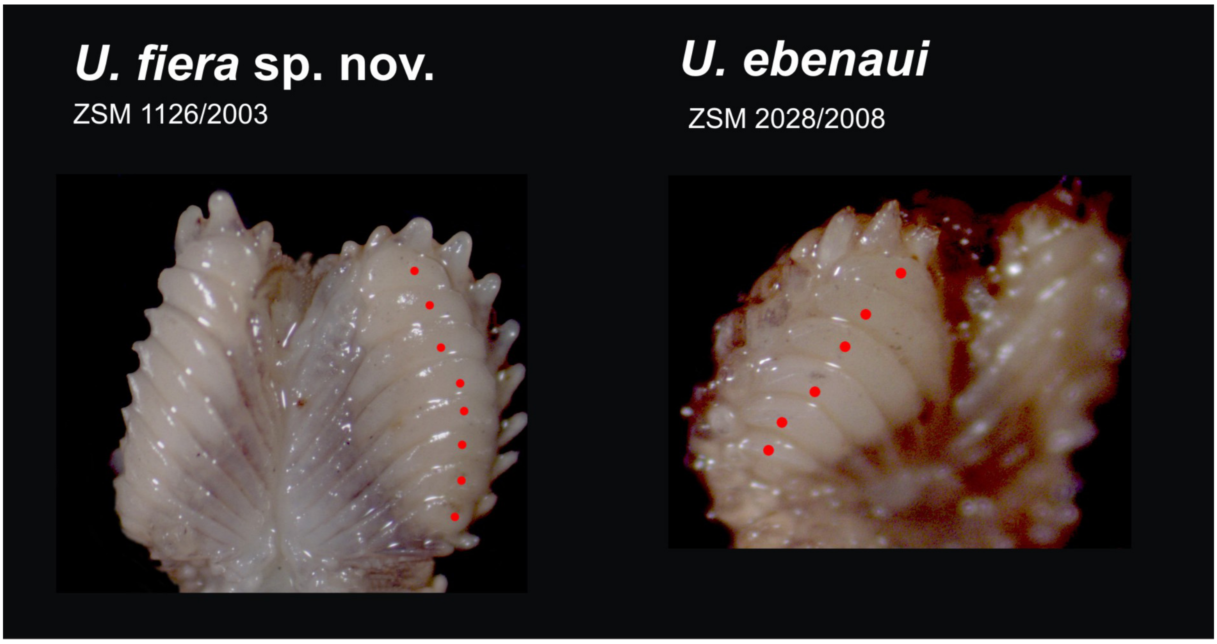

FIGURE 5. Number of adhesive lamellae under the third toe (LAM3T) in *U. fiera* **sp. nov.** and *U. ebenaui,* showing smaller numbers of lamellae in the latter.

Uroplatus fiera and *U. ebenaui* [Ca8] together form the sister group to the clade containing *U. ebenaui* [Ca1] from Tsaratanana (see Fig. 1). This allopatric form has tail of roughly similar length as the new species *U. fiera* (Fig. 4), and its mitochondrial differentiation is not much larger than that between *U. fiera* populations (lowest 16S distance to *U. fiera* 4.9%, versus 1.9–2.0%. between populations of *U. fiera*). Therefore the question arises whether populations of *U. ebenaui* [Ca1] should be considered to be a deep conspecific lineage of *U. fiera*. This is contradicted by two morphological differences. On one hand, the (short) tails are wider in *U. ebenaui* [Ca1] when compared to those of *U. fiera* (Fig. 4; U test of TAW/TAL, P=0.001). In addition, there are fewer supralabials in *U. fiera* than in all other *U. ebenaui* group species (Fig. 4), and also fewer than in *U. ebenaui* [Ca1] (U test, P=0.003). Furthermore, although haplotypes of the nuclear gene CMOS are relatively similar in *U. ebenaui* [Ca1] and *U. fiera*, in our samples we found no instance of haplotype sharing (Fig. 2). Samples from the rather considerable geographical gap between the known localities of *U. fiera* and *U. ebenaui* [Ca1] might help to resolve this question more reliably.

Available names and the identity of described taxa. Based on arguments and data on the respective types and type localities presented by Ratsoavina *et al.* (2011), the identity of the described species, *Uroplatus ebenaui*, *U. malama* and *U. finiavana*, is well established. The same is true for the nomina *Uroplatus boettgeri* Fischer, 1884 (junior synonym of *U. ebenaui*, based on its morphology and provenance from Nosy Be), and *U. schneideri* Lamberton, 1913 (junior synonym of *U. phantasticus* based on its long tail and provenance from the Northern Central East of Madagascar). However, the identity of *U. phantasticus* relative to the new species described herein requires discussion. The holotype of *U. phantasticus* is a female specimen that lacks clear locality data, and that has an autotomized (and therefore missing) tail. Böhme & Henkel (1995) assigned this nomen to the widespread long-tailed form occurring over much of eastern Madagascar, and this view was endorsed by Ratsoavina *et al.* (2011) on the basis of the travel routes of the collector of the holotype. However, morphological data were based only on the original description (Boulenger 1888) and a photo of the preserved type specimen figured by Böhme & Henkel (1995). We recently re-examined the holotype of *U. phantasticus* (BMNH 1946.8.26.64) and provide our own measurements of this specimen (Table 1). Based on our re-examination, we confirm that the holotype (Fig. 6) conforms more closely to the long- and wide-tailed form from eastern Madagascar, to which it is usually assigned. Ratsoavina *et al.* (2011) assigned the type specimen to this form based, among other arguments, on its rather short head, but this argument is only valid in comparison with *U. finiavana*, which has a relatively long head. Specimens of *U. fiera* also have slightly longer heads (ratio HL1/SVL 0.28–0.32, mean 0.30) than the long-tailed form considered to represent *U. phantasticus* 0.27–0.30, mean 0.29, *U. phantasticus* holotype not included), but this

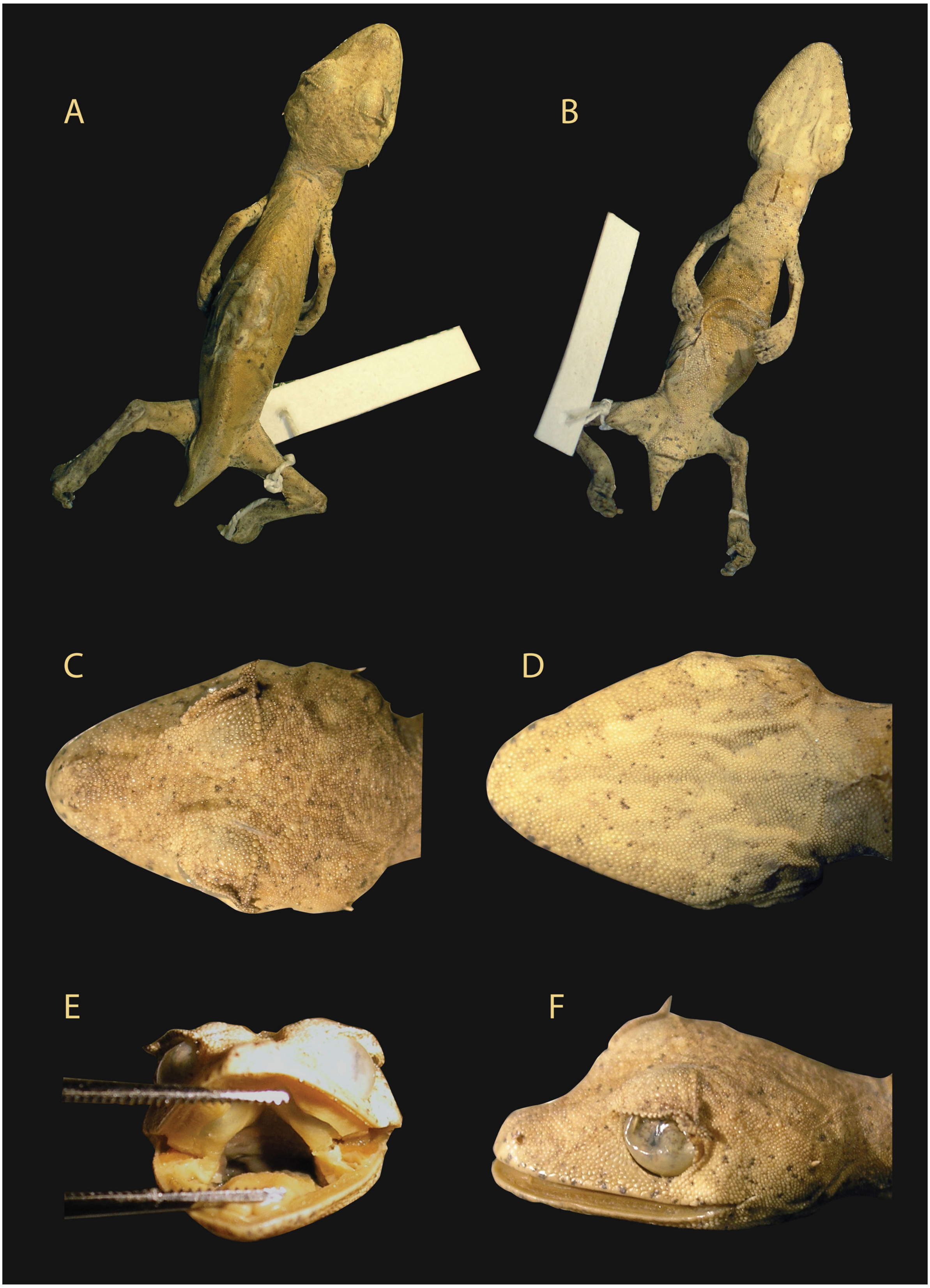

FIGURE 6. Holotype of *Uroplatus phantasticus* (BMNH 1946.8.26.64) photographed in 2011 in various views; A–B: overview of the dorsal and ventral sides, C–D: dorsal and ventral view of the head, E–F: open mouth for the oral mucosa observation and lateral view of the head.

TABLE 1. Measurements in mm, and two scale counts (SUPL and LAM3T) taken from all *Uroplatus* specimens included in this study. HT, holotype; PT, paratype; NA, not available, NM, not measured. See materials and methods for other abbreviations. All measurements taken by the first author except those marked by an asterisk.

Locality	Sex	Museum number	Field number	SVL	TAL	TAW	HL1	HL2	HW	FORL	HIL	NTL	IRPL	SUPL	LAM3T
U. phantasticus															
Fierenana region	male	ZSM 1127/2003	FGMV 002.3100	51.7	39.1	9.6	15.7	14.8	11.7	21.3	29.9	7.1	1.2	30	5
Fierenana region	male	ZSM 1128/2003	FGMV 002.3103	58.8	44.4	11.4	17.6	17.1	12.6	24.5	34.9	10.1	2.3	30	6
Fierenana region	male	ZSM 212/2002	MV 2001.1402	55.3	40.0	10.8	16.6	16.1	13.1	24.9	34.7	14.1	2.1	31	6
Anjozorobe	female	ZSM 471/2010	FGZC 4341	55.1	34.48	10.1	15.7	14.7	12.2	21.8	26.1	11.4	1.7	28	6
Anjozorobe	male	ZSM 472/2010	FGZC 4385	58.3	NA	NA	16.7	15.7	11.6	24.6	33.2	4.1	NM	27	5
Tarzanville	male	ZSM 473/2010	FGZC 4505	66.3	46.0	12.4	18.9	18.2	13.8	26.8	36.6	12.1	2.9	31	7
Ranomafana	male	ZSM 934/2003	FGMV 002.1452	60.4	37.6	9.8	17.8	17.2	13.0	25.5	30.1	8.4	2.7	29	6
Ranomafana	female	ZSM 935/2003	FGMV 002.1453	76.1	NA	NA	20.8	19.8	15.7	29.9	39.9	16.5	2.9	29	6
Imerina	female	*BMNH 1946.8.26.64 (HT)		56.8	NA	NA	16.8	NM	11.6	21.4	31.1	NM	NM	NM	NM
***U. ebenaui* [Ca1]**															
Tsaratanana (Analabe)	male	ZSM 639/2014	DRV 6067	59.2	16.9	4.4	17.8	16.1	13.4	23.6	23.5	9.3	2.7	29	7
Tsaratanana (Ambodikakazo)	male	ZSM 646/2014	DRV 6326	68.0	21.6	6.7	20.7	18.9	14.9	27.2	33.6	7.8	NM	31	8
Tsaratanana (Ambinanitelo)	female	ZSM 648/2014	DRV 6263	70.7	10.7	NA	19.7	17.8	14.6	26.1	35.8	11.5	2.9	30	7
Bemanevika	female	ZSM 647/2014	DRV 6409	57.2	14.5	4.5	17.4	15.8	13.4	23.0	30.6	11.2	2.3	28	7
Tsaratanana (Ambodikakazo)	female	ZSM 645/2014	DRV 6324	70.2	18.0	5.7	19.5	18.2	15.2	27.7	36.4	12.2	2.6	29	7
Tsaratanana (Analabe)	female	ZSM 1829/2010	ZCMV 12277	68.8	19.2	5.0	19.5	18.9	14.6	26.0	33.3	12.8	1.8	28	5
Tsaratanana (Analabe)	male	ZSM 1830/2010	ZCMV 12279	56.0	16.2	4.2	18.1	16.3	13.6	25.2	30.7	8.3	1.9	29	7
Tsaratanana (Analabe)	male	ZSM 1831/2010	ZCMV 12280	67.3	18.3	4.9	19.1	18.1	15.5	25.8	38.2	10.3	3.1	30	6
***U. fiera* sp. nov.**															
Fierenana region	male	ZSM 1126/2003 (PT)	FGMV 002.3097	63.6	18.1	3.6	20.1	17.7	14.2	20.6	25.9	8.1	2.8	27	8
Fierenana region	male	ZSM 211/2002 (PT)	MV 2001.1403	55.9	19.1	3.7	17.7	17.0	13.9	23.9	33.8	10.1	2.0	27	7
Ambatovy region	female	UADBA-R 70850 (PT)	FRC 651	67.2	18.5	3.9	20.3	18.5	15.3	28.1	35.5	11.3	3.2	27	7
Fierenana	male	ZSM 212/2014 (PT)	FRC 618	60.4	17.5	3.4	17.6	16.4	13.8	28.6	34.2	6.6	2.2	28	8
Fierenana	male	ZSM 213/2014 (HT)	FRC 619	66.2	19.9	3.8	19.6	19.2	15.3	29.1	36.3	9.7	3.1	27	7
Ambatovy region	male	UADBA-R 70849 (PT)	FRC 650	78.0	24.4	4.7	21.8	20.3	16.6	30.1	41.1	8.3	3.2	28	8

... continued next page

Table 1. continued

U. finiavana															
Montagne d'Ambre	male	ZSM 1135/2003 (PT)	FGMV 2002.3083	58.0	NA	NA	18.8	17.2	13.1	26.1	34.6	10.7	2.7	30	6
Montagne d'Ambre	female	ZSM 1134/2003 (PT)	FGMV 2002.2390	52.0	24.8	5.6	15.2	15.1	11.4	22.4	30.1	9.8	2.8	29	6
Montagne d'Ambre	male	ZSM 1133/2003 (PT)	FGMV 2002.2388	63.5	32.8	6.8	18.0	17.5	13.3	25.0	34.5	NA	NA	29	6
Montagne d'Ambre	male	ZSM 1132/2003 (PT)	FGMV 2002.2387	61.0	NA	NA	18.8	17.8	13.2	27.0	35.8	10.0	1.8	30	7
U. ebenaui															
Foret d'Ambre	male	ZSM 2028/2008	FGZC 1876	49.6	18.6	4.2	15.4	14.7	11.8	19.2	27.2	3.4	0.9	32	6
Foret d'Ambre	female	ZSM 2029/2008	FGZC 3116	59.8	12.9	3.5	17.7	16.7	13.3	22.5	30	2.2	0	32	5
Foret d'Ambre	male	ZSM 2030/2008	FGZC 3153	45.6	15.4	4	15.1	13.5	11.6	18.3	27.4	1.7	1.1	29	6
Nosy Be	female	ZSM 2498/2009	ZCMV 13013	63.1	16.1	4.1	19.1	17.2	14.4	21	32.1	2.5	0.2	28	6
Manongarivo	male	ZSM 856/2003	FGMV 2002.826	56.5	22.5	3	16.9	16.6	13.4	23.8	34.2	1.8	0.6	29	6
Manongarivo	male	ZSM 997/2003	FGMV 2002.2403	51.4	16.4	2.5	15.8	14.5	11.6	19.2	30	3	0	29	6
Nosy Be	male	ZSM uncatalogued	FGZC 3747	55.5	12.1	3.3	17.3	11.1	13.1	23.6	31.1	4.3	0	29	6
Fanambana	male	UADBA-R 70851	FRC 611	54.1	19.7	3.6	20.6	15.3	12.3	20.1	30.7	1.1	0.0	28	6
U. malama															
Beampingaratra/Andreoky	male	ZSM 2860/2010	ZCMV 12128	77.5	56.1	18.4	21.3	20.6	15.4	31.2	41.9	4.2	1.5	28	7

difference is not statistically significant and the values largely overlap (U-test, P=0.24; original data in Table 1). Still it should be mentioned that the value for the *phantasticus* holotype according to our own new measurements (0.296) is closer to the mean of the long-tailed form. Most importantly, however, the *phantasticus* holotype has clearly recognizable remains of dark pigmentation on the posterior part of the oral mucosa, although the coloration of the specimen lacks contrast and has partly faded after more than 125 years in preservative. Therefore, we confirm the current taxonomy and continue to apply the name *U. phantasticus* to the long-tailed form from eastern Madagascar rather than to the partly sympatric short-tailed species that has an unpigmented oral mucosa and which, due to its concordant molecular and morphological differentiation from all other *Uroplatus*, is hereafter described as a new species.

Uroplatus fiera **sp. nov.**

(Figures 7–8)

Remark. This new species was previously referred to as *Uroplatus ebenaui* [Ca7] by Ratsoavina *et al.* (2012, 2013), and samples from the locality Fierenana preliminarily as *Uroplatus* sp. 1 (Ratsoavina *et al.* 2011). Ratsoavina *et al.* (2013) assigned one additional specimen that lacks precise locality to their *U. ebenaui* [Ca7] (specimen RAX 4012, named *Uroplatus* sp. F by Raxworthy *et al.* 2008).

Holotype. ZSM 213/2014 (field number FRC 619), adult male with everted hemipenes, collected in the Marohonkona forest of the Fierenana region, 18°27' 40.1" S, 48° 27' 15.1" E, 1041 m above sea level, on the night of 2 May 2013, by F. M. Ratsoavina, F. A. Ranjanaharisoa, and A. P. Raselimanana.

Paratypes. ZSM 212/2014 (FRC 618), adult male, with same collecting data as the holotype; ZSM 1126/2003 (FGMV 2002.3097), adult male, collected by local collectors in the Fierenana region (no precise locality) in February 2003; ZSM 211/2002 (MV 2001-1403), male, collected by local collectors in the Fierenana region (no precise locality) in December 2001; UADBA-R 70849 (FRC 650), adult male, and UADBA-R 70850 (FRC 651), adult female, collected by F. A. Ranjanaharisoa in 2012 at a site in the forest corridor between Fierenana and Ambatovy, 18° 47' 48.7" S, 48° 22' 16.2" E, 962 m above sea level.

Diagnosis. *Uroplatus fiera* **sp. nov.** is included in the *Uroplatus ebenaui* group of small-sized leaf-tailed geckos due to its triangular head with supraocular spines, laterally compressed body, and short tail. It differs from all members of the *U. fimbriatus* group (*U. fimbriatus*, *U. giganteus*, *U. henkeli*, *U. sikorae* and *U. sameiti*) and *U. lineatus* by its much smaller size (adult SVL 55.9–67.2 mm versus at least 85 mm), lack of lateral integumentary fringing on any part of the body, and lateral compression of the body (versus depressed body shape with lateral integumentary fringes), and a relatively much shorter tail. The evident triangular head, rather smooth skin and short tail distinguish *U. fiera* from *U. alluaudi, U. guentheri, U. pietschmanni,* and *U. malahelo.*

Within the *U. ebenaui* group, the new species differs from *U. malama*, *U. phantasticus* and *U. finiavana* by a shorter (adult TAL/SVL 0.28–0.34 versus 0.72 in *U. malama*, 0.62–0.76 in *U. phantasticus,* 0.42 in *U. finiavana*) and narrower tail (TAW/SVL 0.06–0.07 versus 0.24 in *U. malama*, 0.16–0.20 in *U. phantasticus,* and 0.14–0.16 in *U. finiavana*). It is further distinguished from *U. malama, U. phantasticus* and *U. ebenaui* by its unpigmented oral mucosa (versus blackish pigmented oral mucosa). Furthermore, *U. fiera* **sp. nov.** differs from *U. ebenaui* by having a greater number of lamellae under the third toe (7–8 versus 5–6), and apparently by larger body size (SVL 56–78 mm versus 50–63 mm). Additionally, *U. fiera* **sp. nov.** differs from all the other nominal species in the *U. ebenaui* group by its strong genetic divergence (*p*-distances of 16S rDNA>8.5%).

Description of the holotype. Adult male in good condition with intact tail and everted hemipenes. SVL 66.2 mm, tail length 19.9 mm, maximum tail width 3.8 mm, for further measurements see Table 1. Head triangular in dorsal view, postorbital region 6.8 mm; snout length 7.3 mm; canthus rostralis indistinct; snout sloping strongly and continuously downward anteriorly; snout depressed, short (1.2 times longer than eye diameter); eyes large (eye diameter 6.1 mm), bulging slightly above dorsal surface of cranium, directed laterally, pupil vertical with crenate borders; ear opening very small (horizontal diameter 1.0–1.1 mm), its opening facing posterolaterally, but also posteroventrally (ear opening clearly visible in ventral view but not in dorsal view); nostrils laterally oriented; body somewhat laterally compressed, without lateral fringes; limbs well developed, without fringes, forelimb reaches beyond tip of snout when adpressed forward and almost to the groin when adpressed backwards along body (forelimb length/axilla-groin distance 29.1/25.7 mm =113%), hind limb reaches beyond axilla when adpressed forward along body (hind limb length/axilla-groin distance 36.3/34.9 mm= 104%); tail length 30% of snout-vent length, membranous borders of the tail narrow and completely absent from the distal tip of the tail, tail with 6 lateral spine-like indentations. Nares separated from each other by at least six small granular scales, from the first supralabial by one scale, and from the rostral scale by one scale; first supralabial taller than the others; rostral entire, much wider than tall; mental scale very small, not differentiated from infralabial scales (total series of infralabials on one side of the head, without mental scale and counting tiny scales at the jaw commissure, equals 28; supralabials yield the same count of 28 on one side); 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 for the irregular lines on the head and body which consist of series of slightly enlarged scales. Two curved lines (rows of slightly enlarged scales) extending from the posterolateral parts of the head (nuchal region) converge on the neck forming a V-shaped pattern (neck triangular line). A similar, curved, moderately distinct and posteriorly directed line (also formed by a row of slightly enlarged scales) is present between the eyes and connects the supraciliary spines. Three additional transverse lines, consisting of slightly enlarged scales, are recognizable on the frontal region of the head. Several spines on the posterior part of the head (ca. 16), on hind limbs (ca. 11 per limb), two spines on knee, spines absent from elbow and forelimb; a prominent pointed flap on the posterior portion of each upper eyelid; upper eyelid becomes broader as it approaches the parietal region of the head.

FIGURE 7. Photos of the male holotype ZSM 213/2014 (FRC 619) of *Uroplatus fiera* **sp. nov.** in life from Marohonkona forest near Fierenana, taken in the night of May 2nd, 2013.

Coloration (Fig. 8). After one year of preservation in 70% ethanol the colour pattern remains the same as in the living animal (Fig. 7) but its vivacity and intensity have faded. All dorsal surfaces are beige to light brown except on the dorsal sides of the head, neck, forelimbs, in the cloacal region, and on the hind limbs, where the colour is darker, showing an irregular light pattern. The tail is uniformly light brown mottled with few dark spots. The mottling of the body is configured as a series of fine posteriorly directed markings along the dorsum. Two whitish spots are present below each eye. The chin is darker than the throat, which is beige with a distinct blackish wide V-marking, the arms of which merge to form an indistinct dark longitudinal line that fades on the throat. The venter is light brown with irregular dots, which become more numerous towards the posterior half of the body,

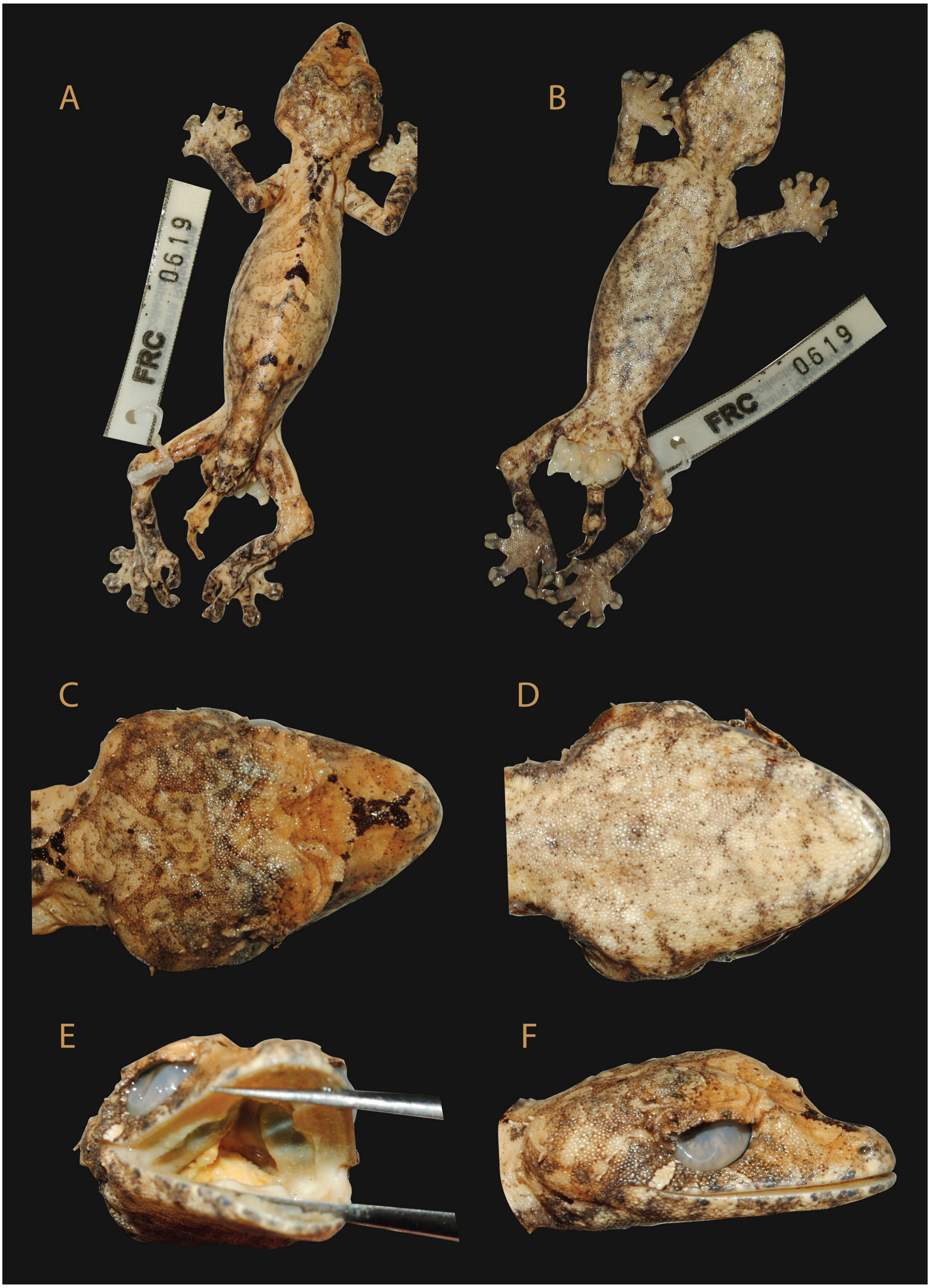

FIGURE 8. Preserved holotype (ZSM 213/2014 - FRC 619) of *Uroplatus fiera* **sp. nov.**

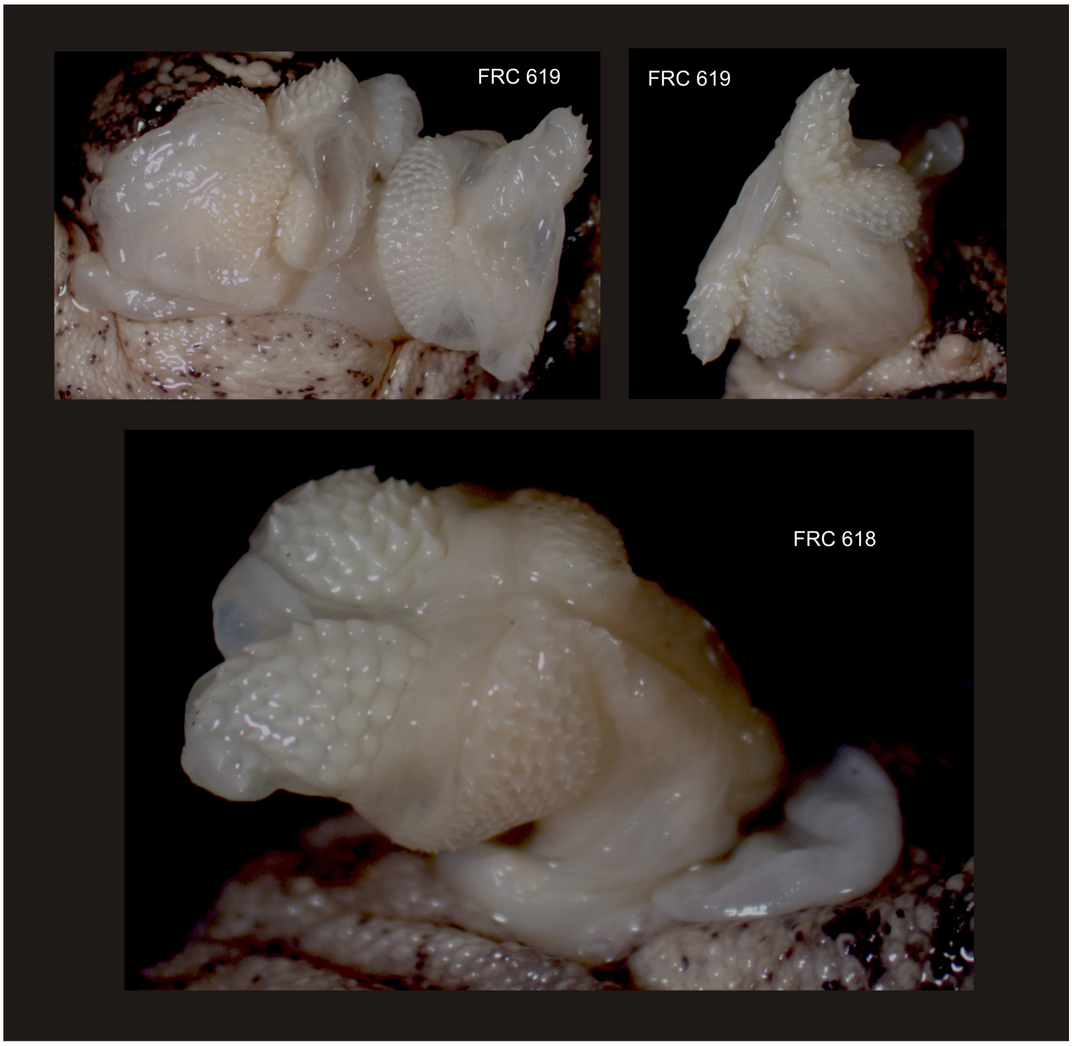

FIGURE 9. Photos of everted hemipenes of two males of *Uroplatus fiera*: holotype ZSM 213/2014 (FRC 619) and paratype ZSM 212/2014 (FRC 618). Not to scale.

where a distinct dark line delimits the ventral and the dorsal side of the body. The lower hind limbs and feet are slightly darker than other ventral surfaces. The postpygal portion of the tail is marked with a whitish spot. A dorsolateral dark line runs from each forelimb insertion backwards, both lines joining at the tail insertion; just above this dark line four obvious spines are visible on each side of the body. The oral mucosa is unpigmented (whitish in preservative, pinkish in life).

Variation. In general, the paratypes agree well with the holotype in morphology. For measurements, see Table 1. The number of head spines varies from 4 (FRC 651) to 25 (ZSM 211/2002). FRC 650 also has spines on elbow (n=1) and forelimb (n=4). The oral mucosa is unpigmented in all specimens. The number of recognizable transverse lines across the head (in addition to the one between supraciliaries) varies from zero (ZSM 211/2002) to three (in various specimens). FRC 650 is the largest specimens (SVL 78.0 mm; Table 1). The colour pattern is highly variable. FRC 650 is rather strongly patterned including an isolated small dark brown patch on the neck and a second one on the dorsum. Other specimens such as ZSM 212/2014 are dorsally almost uniform pale brown and ventrally with very limited mottling.

Hemipenis structure. The everted hemipenes of the holotype (ZSM 213/2014) and one paratype (ZSM 212/2014) were examined (Fig. 9). In both specimens, and as in other species of the *U. ebenaui* group, each hemipenis has two lobes that become more obvious towards the apical region. The calyx displays a protuberance with a honeycomb appearance, especially on the asulcal side. The area of the sulcus spermaticus is smooth. Each lobe has a dense field of pointed papillae at its apex.

Etymology. The species name *fiera* is used as noun in apposition to the generic name. It was chosen because it alludes, on one hand, to an euphonious abbreviation of the species' type locality Fierenana, and on the other hand it corresponds to the Spanish noun fiera = beast, thus making allusion to the devil-like appearance of this species and other small *Uroplatus* in frontal view.

Distribution. Known from the Fierenana and Ambatovy regions, of Northern Central East of Madagascar.

Habitat and habits. The holotype was collected in the Fierenana region in a primary forest called Marohonkona. Part of the Northern Central East of Madagascar, the type locality has a humid tropical climate, and its vegetation at is dominated by moist evergreen forest. The holotype was found active during a night search, perched on a fern tree at 2 m above ground.

Discussion

With *Uroplatus fiera,* we describe a distinctive new species of the *U. ebenaui* group and show that upon close inspection, the confusing undescribed diversity of geckos in this group can be separated into well-differentiated evolutionary entities. The two entities so far studied in detail were relatively easily diagnosable by a few morphological characters and could thus be characterized as new species (i.e., *U. fiera* and *U. finiavana*). Except for the comparison of *U. fiera* to *U. ebenaui,* our data suggest that probably all species and candidate species of *Uroplatus* included in the present study can be distinguished by a comparison of relative tail length and relative tail width. In contrast, we could not find any obvious differences between male and female tail size, although females on average might have smoother, less spiny tails (compare tails of the female specimens ZSM 2029/2008 and FRC 612 with those of other specimens of *U. ebenaui*: Fig. 3). Given these apparent species-specific tail sizes, and possible sex-specific tail shapes, it is worth exploring to what degree these differences are due to ecological adaptation or sexual selection. A similar question surrounds the differences in oral mucosa pigmentation, which become visible when captured specimens open their mouth in threat behavior. It is unknown whether these geckos also display such behavior in intraspecific interactions, in which case sexual selection for oral mucosa pigmentation may occur.

In this paper, we opted to continue a stepwise procedure of revising the genus *Uroplatus*, and in particular the *U. ebenaui* group. After describing one new species (Ratsoavina *et al.* 2011), discussing phylogeny and phylogeography (Ratsoavina *et al.* 2012), and merging molecular data sets of other authors to define the distribution of all species and candidate species (Ratsoavina *et al.* 2013), the main task is now to find out which of the candidate species are also consistently characterized by morphological characters or can otherwise be clearly delimited as entities at the species level. Although we will soon be able to provide such evidence for several of the candidate species, we decided here to avoid any premature descriptions.

As an example of the need of caution, the analysis of newly determined sequences now verified close relationships between *U. fiera* and *U. ebenaui* [Ca8] from Ambohitantely. This relationship had not been detected previously because only a single sequence for one mitochondrial gene (ND4) was available for *U. ebenaui* [Ca8], and this gene had not been sequenced for *U. fiera* (Ratsoavina *et al.* 2012, 2013). The ND4 sequences of the two new samples of *U. ebenaui* [Ca8] obtained were identical to the previously sequenced specimen APR 7667 (Ratsoavina *et al.* 2013). However the two new specimens were not collected as vouchers and the morphological differentiation of this lineage therefore remains unstudied. Hence, *U. ebenaui* [Ca8] could be yet another new species of the *U. ebenaui* group, but we rather hypothesize that it will turn out to represent a deep conspecific lineage of *U. fiera.*

The new species *Uroplatus fiera* has been described from a type locality (a forest near Fierenana) that is currently unprotected. The other locality (Ambatovy region) has not been reported in detail. Thus a reliable calculation of the extent of occurrence of this species is currently not possible and it is uncertain whether the samples might have been collected from one of the protected nearby areas. In any case, we hypothesize that it is likely that the species occurs in the new protected area "Corridor Ankeniheny-Zahamena (CAZ)", managed by Conservation International Madagascar, and might also be present in the Analamazaotra-Mantadia National Park. However the species is apparently not very common as, in the past, it has only rarely been collected, and its known range lies in an area subject to strong anthropogenic pressure and deforestation, even within officially protected habitats. We therefore propose a Red List status of Vulnerable according to IUCN criteria B1a-b for this species (IUCN 2001), but emphasize the need for further field studies to determine its actual range. Also its population sizes and area of occupancy should be clarified, as a basis to define reasonable and sustainable quotas for the international pet trade.

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