Zoosyst. Evol. 94 (2) 2018, 409–423 | DOI 10.3897/zse.94.27305

The smallest 'true chameleon' from Madagascar: a new, distinctly colored species of the *Calumma boettgeri* complex (Squamata, Chamaeleonidae)

David Prötzel[1], Shea M. Lambert[2], Ginah Tsiorisoa Andrianasolo[3], Carl R. Hutter[4], Kerry A. Cobb[5], Mark D. Scherz[1,6], Frank Glaw[1]

1 *Zoologische Staatssammlung München (ZSM-SNSB), Münchhausenstr. 21, 81247 München, Germany*
2 *Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA*
3 *Mention Zoologie et Biodiversité Animale, Université d'Antananarivo, BP 906, Antananarivo 101, Madagascar*
4 *Biodiversity Institute and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045–7561, USA*
5 *Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA*
6 *Zoologisches Institut, Technische Universität Braunschweig, Mendelssohnstr. 4, 38106 Braunschweig, Germany*

http://zoobank.org/2433A9DD-8AC1-4139-A639-E24053D5C33F

Corresponding author: *David Prötzel* (david.proetzel@mail.de)

Abstract

Received 8 June 2018
Accepted 10 August 2018
Published 19 October 2018

Academic editor:
Johannes Penner

On a recent expedition to eastern Madagascar, we discovered a distinct new species of the genus *Calumma* that we describe here using an integrative approach combining morphology, coloration, osteology and molecular genetics. *Calumma roaloko* **sp. n.** has a dermal rostral appendage and occipital lobes, and belongs to the *C. boettgeri* complex, within the Madagascar-endemic phenetic *C. nasutum* species group. It is readily distinguished from other species of the *C. boettgeri* complex by a characteristic two-toned body coloration and small body size with a snout-vent length of 45.6 mm in an adult male. The osteology of the skull, with a prominent maxilla and broad parietal, is similar to the closest related species, *C. uetzi*. Analysis of uncorrected genetic distances within the *C. nasutum* group using the mitochondrial gene ND2 shows a minimum pairwise distance of 11.98% to *C. uetzi* from the Sorata massif and Marojejy National Park >500 km north of the type locality of *C. roaloko* **sp. n.**. Given an apparently small range (potentially <300 km^2), located entirely outside of any nationally-protected areas, we recommend this new species be classified as Endangered under criterion B1ab(iii) of the IUCN Red List. The discovery of clearly distinct species like *C. roaloko* **sp. n.** in an area of Madagascar that is comparatively thoroughly surveyed highlights the critical role of continued field surveys for understanding the true extent of Madagascar's spectacular biodiversity.

Key Words

Calumma roaloko sp. n.
Integrative taxonomy
Micro-computed tomography
Osteology
Calumma nasutum group

Introduction

The biota of Madagascar is recognized as exceptional, both in terms of endemism and density of species (Myers et al. 2000). In recent years, revised estimates of species richness for the island have revealed a significant underestimation of animal species richness by current taxonomy, e.g., in primates (Yoder et al. 2000), anurans (Vieites et al. 2009) and squamates (Nagy et al. 2012). In addition to the recognition of many morphologically 'cryptic' species (Bickford et al. 2006), often identified by the application of integrative taxonomy (Dayrat 2005, Padial et al. 2010), biodiversity field surveys in Madagascar continue to reveal morphologically distinct and often deeply divergent species, frequently characterized by restricted ranges and/or highly secretive habits (e.g., among herpetofauna, Nussbaum and Raxworthy 1994, Glaw et al. 1998, 2006, Vieites et al. 2010, Gehring et al. 2011, Rosa et al. 2014, Scherz et al. 2015, 2017, Lambert et al. 2017).

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With currently 90 endemic species (Glaw 2015, Prötzel et al. 2017, 2018) chameleons are among the most diverse squamate families on Madagascar. The application of widespread genetic sampling and species delimitation methods (Gehring et al. 2012) confirmed long-standing suspicions that *Calumma nasutum* and other species are actually complexes of species (e.g., Hillenius 1959, Brygoo 1971, Glaw and Vences 2007). As many as 33 potential species (OTUs) were identified by Gehring et al. (2012) in the *C. nasutum* species group, but a higher taxonomic resolution awaits the completion of ongoing detailed morphological and genetic analyses (Prötzel et al. 2015, 2016, 2017, 2018).

The small-bodied chameleons of the *Calumma nasutum* group, usually characterized by their dermal rostral appendages, are distributed across the forests of eastern and northern Madagascar. Within this group, the species *C. boettgeri* (Boulenger, 1888), *C. guibei* (Hillenius, 1959) and *C. linotum* (Müller, 1924) differ from the others by the possession of well-defined occipital lobes and are referred to as the *C. boettgeri* complex (Gehring et al. 2012). Recently the number of species in the *C. boettgeri* complex has more than doubled with the description of *C. gehringi* Prötzel et al., 2017, *C. juliae* Prötzel et al., 2018, *C. lefona* Prötzel et al., 2018, and *C. uetzi* Prötzel et al., 2018 due to discoveries on recent expeditions. So far, *C. juliae* has been the only member of the *C. boettgeri* complex that occurs in eastern Madagascar; the other species are from northern Madagascar.

During fieldwork in a forest fragment within the Réserve de Ressources Naturelles du Corridor Ankeniheny-Zahamena just south of Andasibe-Mantadia National Park in 2015/2016, we discovered a small-bodied chameleon with distinct coloration belonging to the *Calumma nasutum* group. Integrating morphological, molecular, and osteological data, we describe this new species of the *C. boettgeri* complex.

Materials and methods

Specimen collection

We located specimens at night using targeted searches of arboreal habitats during the rainy season, using flashlights to locate sleeping individuals. Following euthanasia, we removed a portion of liver tissue and transferred it immediately into 95% ethanol for use in DNA extractions for genetic analyses. Specimens were fixed in 10% formalin (buffered to pH 7.0 with sodium phosphate), and transferred to 75% ethanol for long-term storage after approximately two weeks. We deposited the holotype and four paratypes at the University of Kansas Biodiversity Institute, Lawrence, KS (KU). Two of the paratypes will be repatriated to the Université d'Antananarivo, Mention de Zoologie et Biologie Animale (UADBA) probably during 2018 and one paratype was exchanged with the Zoologische Staatssammlung München (ZSM). All type specimens will maintain their original KU museum number so that they can more easily be referenced in the future. SML refers to field numbers of S. M. Lambert.

Morphological investigation

Terms of morphological measurements taken on these specimens were adapted from previous studies (Prötzel et al. 2015, 2017). The following characters (Table 1) were measured with a digital caliper to the nearest 0.1 mm, counted using a binocular dissecting microscope, evaluated by eye or calculated: snout-vent length (SVL) from the snout tip (not including the rostral appendage) to the cloaca; tail length (TaL) from the cloaca to the tail tip; total length (TL) as a sum of SVL + TaL; ratio of TaL and SVL (RTaSV); length of the rostral appendage (LRA) from the upper snout tip; ratio of LRA and SVL (RRASV); diameter of rostral appendage (DRA), measured dorsoventrally at the widest point; ratio of DRA and SVL (RDRSV); number of scales across DRA (NDRA); number of tubercle scales (diameter >0.3 mm) on rostral appendage, counted on the right side (NSRA); ratio of NSRA and LRA (RNLRA); distinct rostral crest (RC) presence (+) or absence (–); lateral crest (LC), running from the posterior of the eye horizontally, presence (+) or absence (–); temporal crest, running dorsally to the LC, curving toward the midline, absence (–) or number of tubercles on left side (TCL) and right side (TCR); parietal crest (PC) presence (+) or absence (–); occipital lobes (OL) completely separated (s) or still, at least slightly, connected (c); depth of the dorsal notch in the occipital lobes (OLND); ratio of OLND and SVL (ROLSV); lateral diameter of OL (OLD); ratio of OLD and SVL (RODSV); width of OL measured at the broadest point (OLW); ratio of OLW and SVL (ROWSV); diameter of largest scale on OL (DSOL); diameter of largest scale on temporal region (DSCT), measured on the right side; dorsal crest (DC) absence (–) or number of dorsal cones visible to the naked eye without the use of a binocular microscope according to Eckhardt et al. (2012); diameter of broadest scales on the lower arm (DSA), defined as the area from the elbow to the manus in lateral view on the right side; number of scales on lower arm in a line from elbow to manus on the right side (NSA); number of supralabial scales (NSL), counted from the first scale next to the rostral to the last scale that borders directly and entirely (with one complete side) to the mouth slit of the upper jaw on the right side; and number of infralabial scales (NIL), analogous to the definition of NSL above, on the right side. Terminology of hemipenial structures follows Klaver and Böhme (1986) and Prötzel et al. (2017). For the "diagnosis", only adult specimens were considered. "Adult" is defined for specimens with 90–100% SVL of the largest specimen and additionally for males with completely developed hemipenes; "subadult" (subad.) refers to specimens with 70–90% of SVL and already developed hemipenes; "juvenile" is defined as <70% of SVL without any distinct sexual characteristics.

Table 1. Morphological measurements of *Calumma roaloko* sp. n. All measurements in mm. For abbreviations, see Materials and methods.

Final museum no.	**KU 343178**	**KU 343168**	**ZSM 244/2018**	**UADBA-R (uncatalogued)**	**UADBA-R (uncatalogued)**
original museum no.	**KU 343178**	**KU 343168**	**KU 343177**	**KU 343176**	**KU 343167**
field no.	**SML 213**	**SML 177**	**SML 210**	**SML 178**	**SML 166**
sex	**adult male**	**adult female**	**subad. male**	**subad. male**	**subad. female**
type status	**holotype**	**paratype**	**paratype**	**paratype**	**paratype**
altitude [m]	1100	1100	1100	1100	1100
SVL	45.6	44.5	37.6	38.6	40.0
TaL	48.1	41.0	44.3	42.3	34.8
TL	93.7	85.5	81.9	80.9	74.8
RTaSV	105%	92%	118%	110%	87%
LRA	5.2	2.3	4.6	3.9	2.7
RRASV	11.4%	5.2%	12.2%	10.1%	6.8%
DRA	2.6	1.9	2.3	2.7	1.6
RDRSV	5.7%	4.3%	6.1%	7.0%	4.0%
NDRA	5	6	4	5	7
NSRA	16	29	28	33	31
RNLRA	3.1	12.6	6.1	8.5	11.5
RC	+	+	+	+	+
LC	+	+	+	+	+
TCL	–	–	–	–	–
TCR	–	–	–	–	–
PC	+	–	–	–	–
OL	c	c	c	c	c
OLND	0.4	0.2	0.3	0.2	0.3
RODSV	8.8%	10.1%	10.4%	10.6%	10.3%
OLD	4.0	4.5	3.9	4.1	4.1
ROLSV	4.6%	4.3%	5.1%	5.2%	4.5%
OLW	2.1	1.9	1.9	2.0	1.8
ROWSV	0.9%	0.4%	0.8%	0.5%	0.8%
DSOL	0.7	0.5	0.7	0.6	0.7
DSCT	0.7	0.6	0.7	0.6	0.6
DC	2	0	1	0	0
DSA	0.7	0.5	0.5	0.4	0.4
NSA	11	14	13	15	15
NSL	13	13	13	13	13
NIL	13	14	13	13	12

Micro-CT

For internal morphology, micro-Computed Tomography (micro-CT) scans of the head were prepared for the male holotype KU 343178 and the female paratype KU 343168. For micro-CT scanning, specimens were placed in a closed plastic vessel slightly larger than the specimen with the head oriented upwards and stabilized with ethanol-soaked paper. To avoid artifacts, it was ensured that the paper did not cover the head region. Micro-CT scanning was performed with a phoenix|x nanotom m (GE Measurement & Control, Wunstorf, Germany) using a tungsten or diamond target at a voltage of 130 kV and a current of 80 µA for 29 minutes (1800 projections). 3D data sets were processed with VG Studio Max 2.2 (Visual Graphics GmbH, Heidelberg, Germany); the data were visualized using the Phong volume renderer to show the surface of the skull and reflect a variety of different levels of x-ray absorption following recommendations of Scherz et al. (2017). Osteological terminology follows Rieppel and Crumly (1997). Measurements were taken in VG Studio Max 2.2 using the caliper tool, given the following abbreviations (Table 2, Fig. 1): nasal length (NaL); frontal width measured at prefrontal border (FWPf); frontal width measured at anterior border to postorbitofrontal (FWPo); frontal width measured at frontal-parietal-border (FWPa); parietal width measured at posterior border to postorbitofrontal (PWPo); parietal width at midpoint (PWm); parietal length (PL); frontal length (FL); snout-casque length, measured from tip of upper jaw to posterior end of parietal (SCL); skull length, measured from tip of upper jaw to skull capsule (SkL); the respective ratios, divided by SkL, are indicated with an 'R' in front of the character-abbreviations.

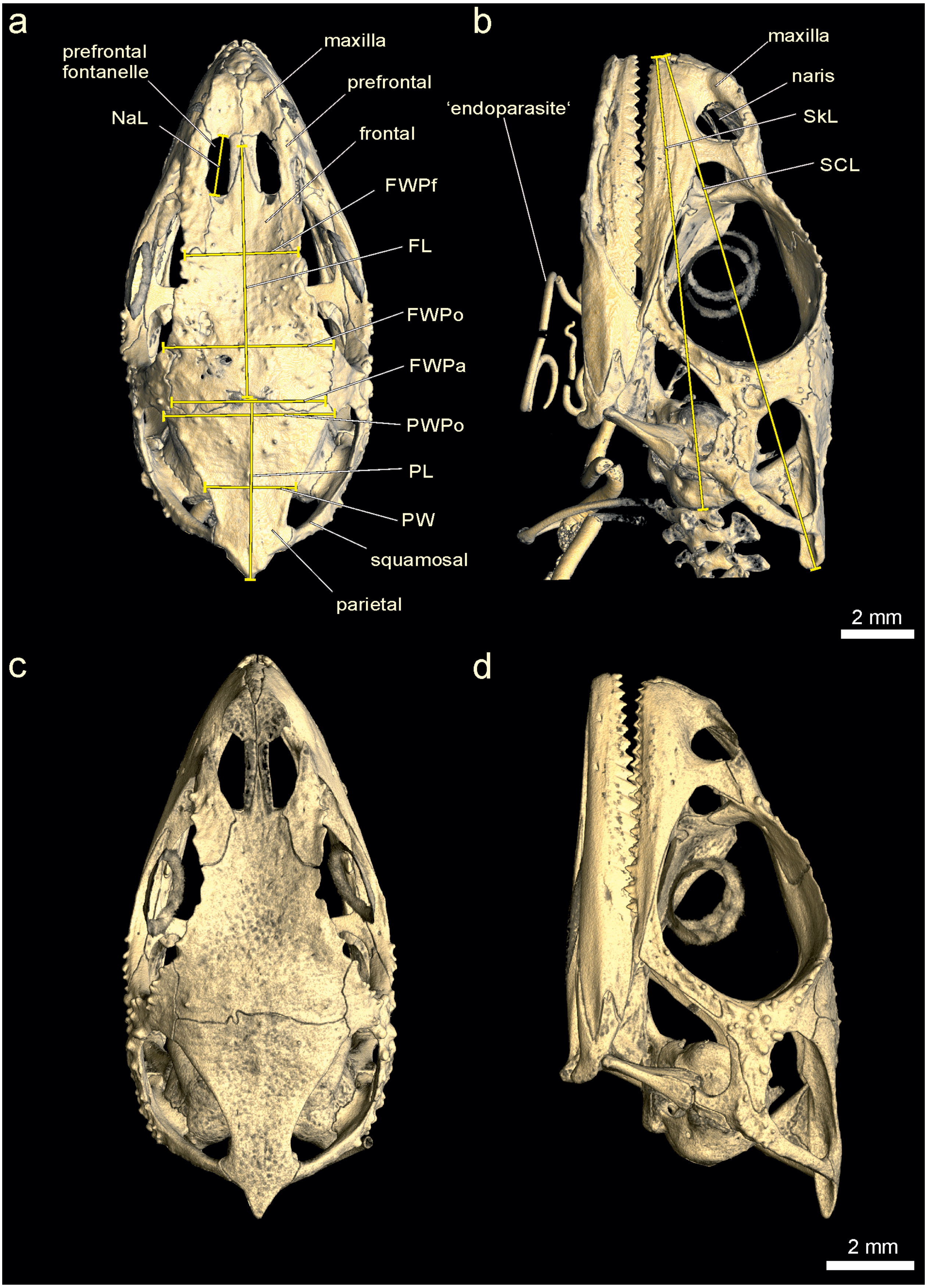

Figure 1. Micro-CT scans of skulls of *Calumma roaloko* sp. n. Male holotype KU 343178 in dorsal view (**a**) and lateral view (**b**), note the worm-like structure (presumably an endoparasite) in the throat of the holotype; female KU 343168 in dorsal view (**c**) and lateral view (**d**). See Materials and methods for abbreviations. See also Suppl. material 3 and 4 for a 360° movie of the skull.

Table 2. Osteological measurements based on micro–CT scans of the skulls of the male holotype and an adult female of *Calumma roaloko* sp. n. All measurements in mm. For abbreviations, see Materials and methods.

Collection no.	KU 343178	KU 343168
field no.	**SML 213**	**SML 177**
sex	male	female
type status	holotype	paratype
NaL	1.4	1.8
RNaL	11.6%	15.7%
FWPf	3.2	2.8
RFWPf	26.4%	24.3%
FWPo	4.5	4.1
RFWPo	37.2%	35.7%
FWPa	4.1	3.9
RFWPa	33.9%	33.9%
PWPo	4.7	4.0
RPWPo	38.8%	34.8%
PWm	2.7	2.2
RPWm	22.3%	19.1%
PL	5.1	4.9
RPL	42.1%	42.6%
FL	7.0	6.0
RFL	57.9%	52.2%
SCL	14.5	13.5
RSCL	119.8%	117.4%
SkL	12.1	11.5

DNA sequencing and phylogenetic analysis

We extracted genomic DNA from tissue samples at the KU Biodiversity Institute using a phenol-chloroform protocol. We amplified two mitochondrial gene fragments, COI and ND2, using standard protocols. Primers and protocols used for ND2 are described in Gehring et al. (2011) for ND2 and in Nagy et al. (2012) for COI. For ND2 alignments, we used previously published sequences from Gehring et al. (2012) and Prötzel et al. (2018), supplemented by sequences of the new species described herein (Fig. 2). For COI alignments, we downloaded all available sequences for the *C. nasutum* group taxa from GenBank. All newly generated sequences were submitted to GenBank (accession numbers MH668289–MH668297). We aligned sequences using MUSCLE (Edgar 2004) in Geneious version 6 (Kearse et al. 2012), under default settings. We manually inspected alignments for accuracy and open reading frames, but no changes were necessary. We calculated uncorrected pairwise genetic distances from our alignments in R v3.3.2 (R Development Core Team 2017), using the dist.dna function of the ape package (Paradis et al. 2004), with deletion of non-shared sites for each pairwise comparison. We used *C. oshaughnessyi* (FGZC 4577) as an outgroup.

Prior to phylogenetic analysis of the ND2 gene, conducted using maximum-likelihood in RAxML 8.2.6 (Stamatakis 2014), we used PartitionFinder2 (Lanfear et al. 2012) to select an optimal partitioning scheme. We provided the first, second, and third positions as initial partitions. As RAxML can only use a single model across all partitions, we evaluated only the 'GTR+G' model of sequence evolution. We then used the '-f a' option in RAxML to run 1000 rapid bootstraps and searched for the best-scoring maximum-likelihood tree, providing the optimal partitioning scheme identified by PartitionFinder2 using the '-q' option.

Registration of nomenclature

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:2433A9DD-8AC1-4139-A639-E24053D5C33F. The online version of this work will be archived and made available from the following digital repositories: CLOCKSS and Zenodo.

Results

Genetic differentiation in the *Calumma boettgeri* complex

The ND2 alignment contained 513 sites and a total of 235 variable sites, of which 177 were parsimony informative. The genetic analysis of the ND2 gene fragment (Fig. 2) revealed strong differences of the newly discovered form to all other species of the *C. boettgeri* complex including the three recently described species. Comparisons of genetic distance using mitochondrial genes show minimum distances of 11.98% in ND2 to *C. uetzi,* a species from Sorata and Marojejy (>500 km north from the type locality of our novel species) that was described only recently (Prötzel et al. 2018), and maximum distances to *C. boettgeri* and *C. gehringi* (>17%, Suppl. material 1). The intraspecific variation is small at 0.00–0.19%. In the COI sequences there is at least 12.20% distance between the collected specimens and all sequences available for the *C. nasutum* group, however *C. uetzi* is not included in this dataset (Suppl. material 2). According to the ND2 phylogeny (Fig. 2) *C. uetzi* is the sister taxon to the new species and together they form a clade which is sister to a clade including *C. boettgeri*, *C. linotum* and *C. juliae*. However, bootstrap values are relatively low, possibly due to the limited number of informative base-pairs. Supported by the genetic data provided we describe the following new species:

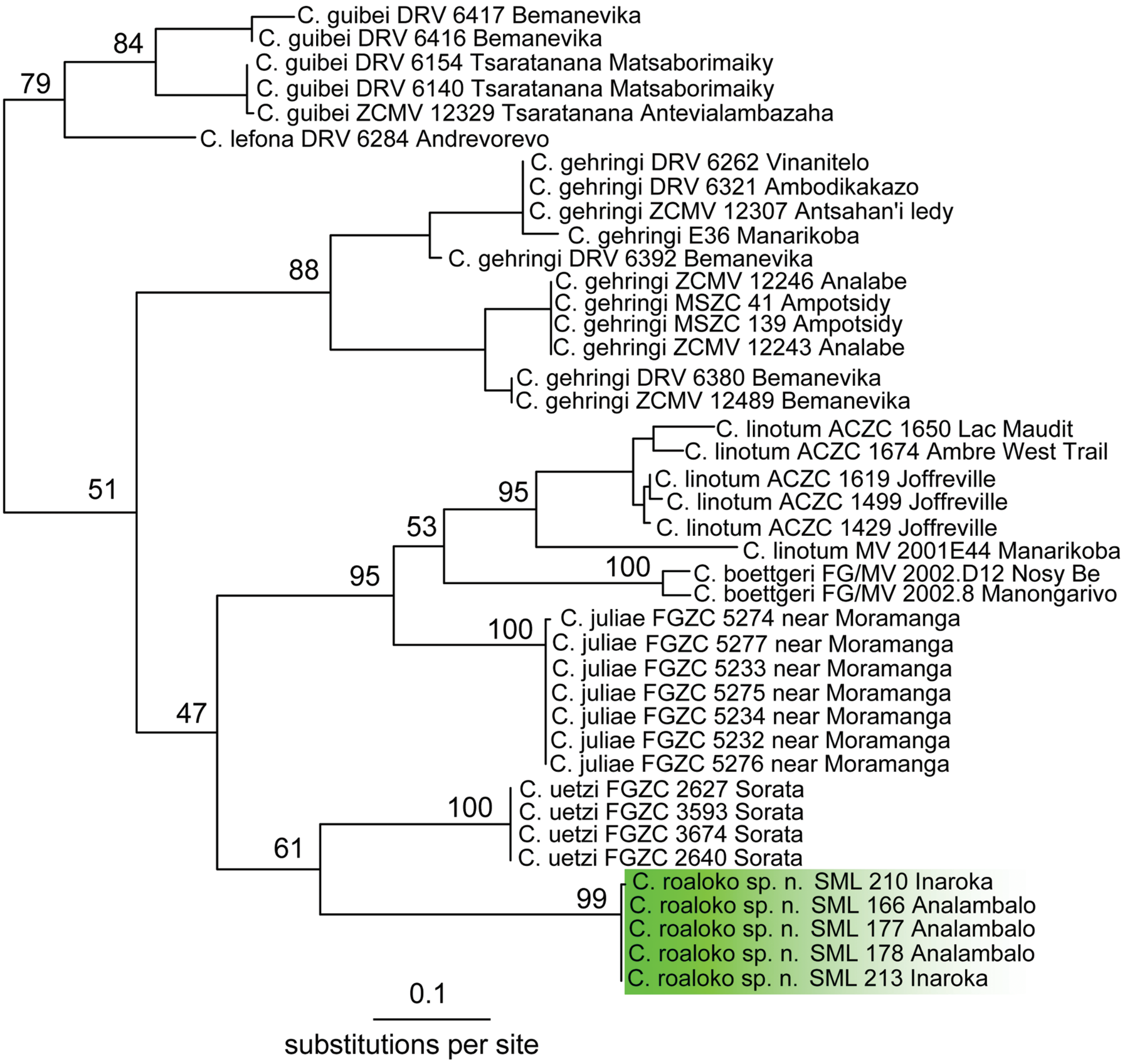

Figure 2. Maximum-likelihood tree of the *Calumma boettgeri* complex, based on 513 base-pairs of the mitochondrial ND2 gene. Nodal support values indicate the proportional support from 1000 rapid bootstrap replicates. Support values for intra-specific relationships are not shown. Outgroup (*C. oshaughnessyi* FGZC 4577) not shown for graphical reasons.

Calumma roaloko sp. n.

http://zoobank.org/B2018AA8-8C9A-4F1C-8B18-FA49ADA627EA

Suggested common English name: The two-toned soft-nosed chameleon
Suggested common Malagasy name: Tanalahy roa loko

Holotype. KU 343178 (field number SML 213), adult male in a good state of preservation with incompletely everted hemipenes (Fig. 3), collected on January 12th, 2016 by Shea M. Lambert, Carl R. Hutter, Kerry A. Cobb and Ginah Tsiorisoa Andrianasolo in mid-altitude rainforest, locally known as Inaroka (ca. 19.0050°S, 48.4613°E, ca. 1100 m a.s.l., Fig. 4) near Vohidrazana, Alaotra-Mangoro Region, in central-eastern Madagascar.

Paratypes. ZSM 244/2018 (KU 343177, field number SML 210), subadult male, same locality and collectors as holotype; KU 343168 (field number SML 177), adult female, UADBA-R uncatalogued (KU 343176, field number SML 178), subadult male, and UADBA-R uncatalogued (KU 343167, field number SML 166), subadult female, all three collected on December 28th, 2015 (SML 166) and December 29th, 2015 (SML 177, 178) by Shea M. Lambert, Carl R. Hutter, Kerry A. Cobb and Ginah Tsiorisoa Andrianasolo in mid-elevation rainforest, locally known as Analambalo (ca. 18.9659°S, 48.4888°E, ca. 1100 m a.s.l., Figs 4–6) near Vohidrazana, Alaotra-Mangoro Region, in central-eastern Madagascar.

Diagnosis. *Calumma roaloko* sp. n. is a member of the phenetic *C. nasutum* species group (Prötzel et al. 2016), on the basis of the presence of a soft, dermal unpaired rostral appendage, absence of gular and ventral crests, and heterogeneous scalation on the lower

Figure 3. Preserved holotype (KU 343178) of *Calumma roaloko* sp. n. Scale bar = 10 mm.

arm, consisting mostly of tubercles of 0.4–0.7 mm diameter. With 44.5–45.6 mm SVL and 85.5–93.7 mm total length in adult specimens it is currently the smallest known species in the genus *Calumma*. The body of the chameleon is uniquely two-colored with beige/white on the ventral and bright green on the dorsal half. Furthermore, it is characterized by a prominent and distally rounded rostral appendage, occipital lobes that are slightly notched, a distinctly elevated rostral crest, absence of a dorsal crest (or presence of at most two cones) in both sexes, absence of axillary pits, and a unique skull morphology.

Calumma roaloko sp. n. differs from *C. fallax*, *C. gallus*, *C. nasutum*, *C. peyrierasi*, *C. vatosoa* and *C. vohibola* of the *C. nasutum* group by the presence of occipital lobes; from *C. boettgeri*, *C. gehringi*, *C. guibei*, *C. lefona*, *C. linotum* and *C. juliae* in the generally smaller body size with a maximum SVL of 45.6 mm and a maximum TL of 93.7 mm (vs. a range of SVL maxima in the former species of 49.1–59.6 mm and TL maxima of 98.7–126.1 mm), and a straight-lined dorsal margin of the supralabial scales vs. serrated (character 'en dents de scie' in Angel 1942); additionally from *C. gehringi*, *C. guibei*, and *C. lefona* in the slightly notched occipital lobes of 0.2–0.4 mm (vs. clearly notched with 0.5–1.8 mm) and in the absence of frontoparietal fenestra; from *C. boettgeri* by the large juxtaposed tubercle scales on the extremities (vs. isolated from each other).

From the most similar taxon *Calumma uetzi*, *C. roaloko* sp. n. differs in the absence of a dorsal crest or presence of at most two cones (vs. presence of 5–14 cones), absence of a temporal crest (vs. presence of 1–2 temporal tubercles), greater number of supralabial scales (13 vs. 10–12) and infralabial scales (12–14 vs. 11–12), a longer rostral appendage in adult males of 5.2 mm with large tubercle scales (vs. 3.8 mm, small and smooth tubercle scales; note: n = 1 each), and less heterogeneous scalation on the head with diameter of largest scale in temporal region of 0.6–0.7 mm (vs. 1.0–1.3 mm). The osteology of the skull is similar in both species; *C. roaloko* sp. n. differs from *C. uetzi* only in the absence of elevated protuberances at the anterior end of the maxilla that characterize the skull of male *C. uetzi*. *Calumma roaloko* sp. n. furthermore differs from all other species by distinct differences in the mitochondrial genes ND2 and COI and a unique two-colored life-coloration.

Description of the holotype. Adult male (Figs 3, 5b) in a good state of preservation; mouth slightly open; both hemipenes incompletely everted; SVL 45.6 mm, tail length 48.1 mm, for further measurements see Table 1; distinct and elevated rostral ridges that form a concave cup on the snout and fuse on the anterior snout at the base of a tapering, laterally compressed dermal rostral appendage that projects straight forward over a length of 5.2 mm with a diameter of 2.6 mm, rounded distally; 13 infralabi-

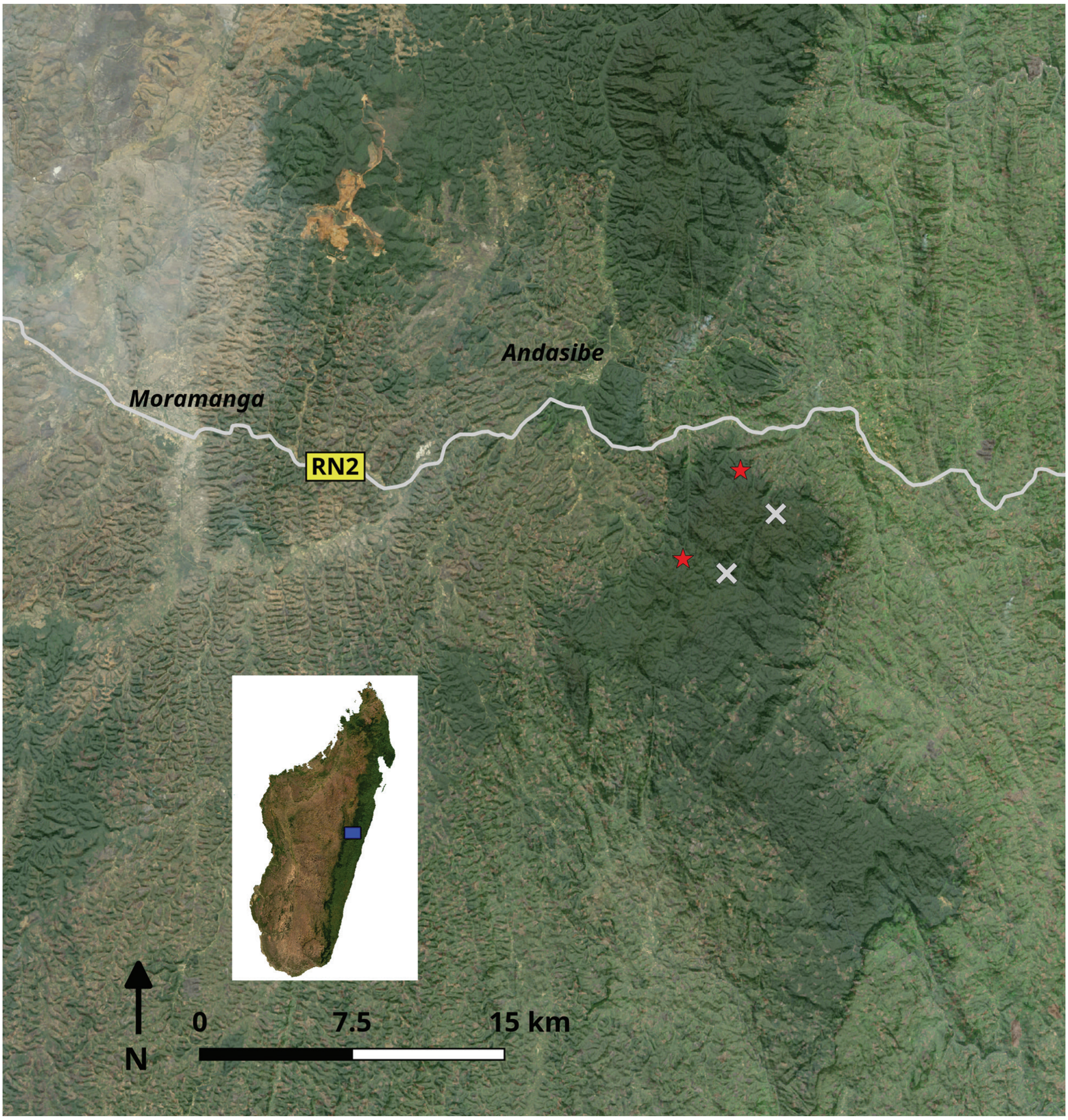

Figure 4. Map showing the location of the known range of *Calumma roaloko* sp. n. in central-eastern Madagascar. Red stars indicate localities where *C. roaloko* sp. n. was found, gray "X" indicate localities surveyed but with no detection of the species. The map is a composite of Landsat 7 and SRTM (Shuttle Radar Topographic Mission; Farr and Kobrick 2000) digital elevation data (U.S. Geological Survey (USGS) Earth Resources Observation and Science (EROS) Center) created in QGIS v2.18.

al and 13 supralabial scales; supralabials with a straight dorsal margin; no supra-orbital crest; distinct lateral crest running horizontally; no temporal crest; indistinct parietal crest; occipital lobes clearly developed and slightly notched (0.4 mm); casque raised; dorsal crest absent, only two single cones 0.7 and 1.1 mm from the base of the notch between the occipital lobes; no caudal crest; no traces of gular or ventral crest. Body laterally compressed with fine homogeneous scalation, slightly more heterogeneous on the extremities and head region; limbs with rounded tubercle scales with maximum of 0.7 mm diameter; heterogeneous scalation on the head with largest scale on temporal region with diameter of 0.7 mm; 16 large, oval tubercle scales (diameter >0.3 mm) on the right side of the rostral appendage; no axillary or inguinal pits.

Skull osteology of the holotype. Description based on a micro-CT scan (Fig. 1a, b). Skull length 12.1 mm; snout-casque length 14.5 mm; maxillae dorsolaterally forming ridges—externally seen as rostral crest; narrow paired nasals tightly bordering anteriorly and separating frontal from maxillae; anterior tip of frontal exceeding more than

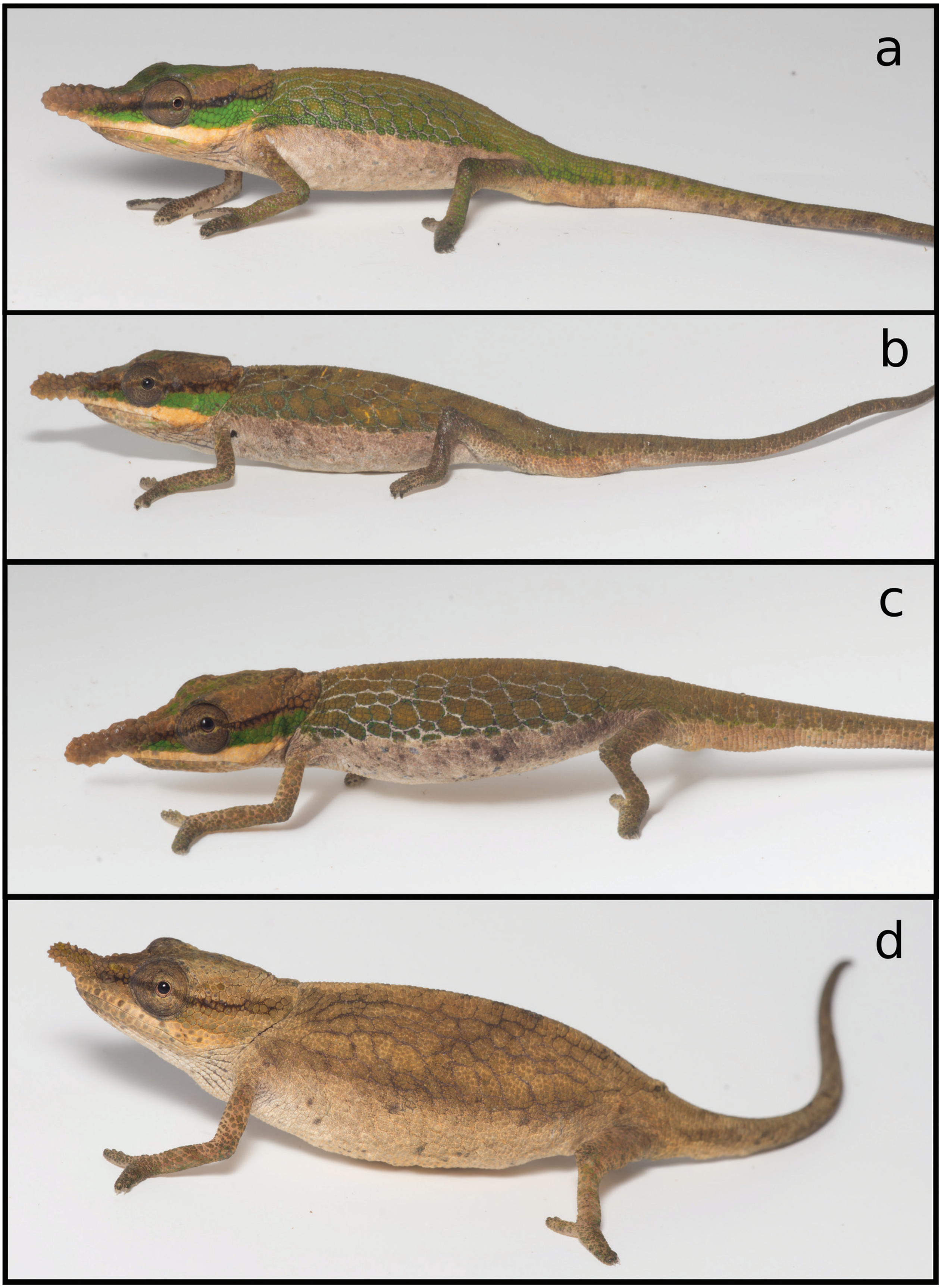

Figure 5. In-life photos of four specimens of *Calumma roaloko* sp. n.; (**a**) subadult male (ZSM 244/2018, KU 343177); (**b**) the holotype, adult male (KU 343178); (**c**) subadult male (UADBA-R, KU 343167); (**d**) adult female (KU 343168).

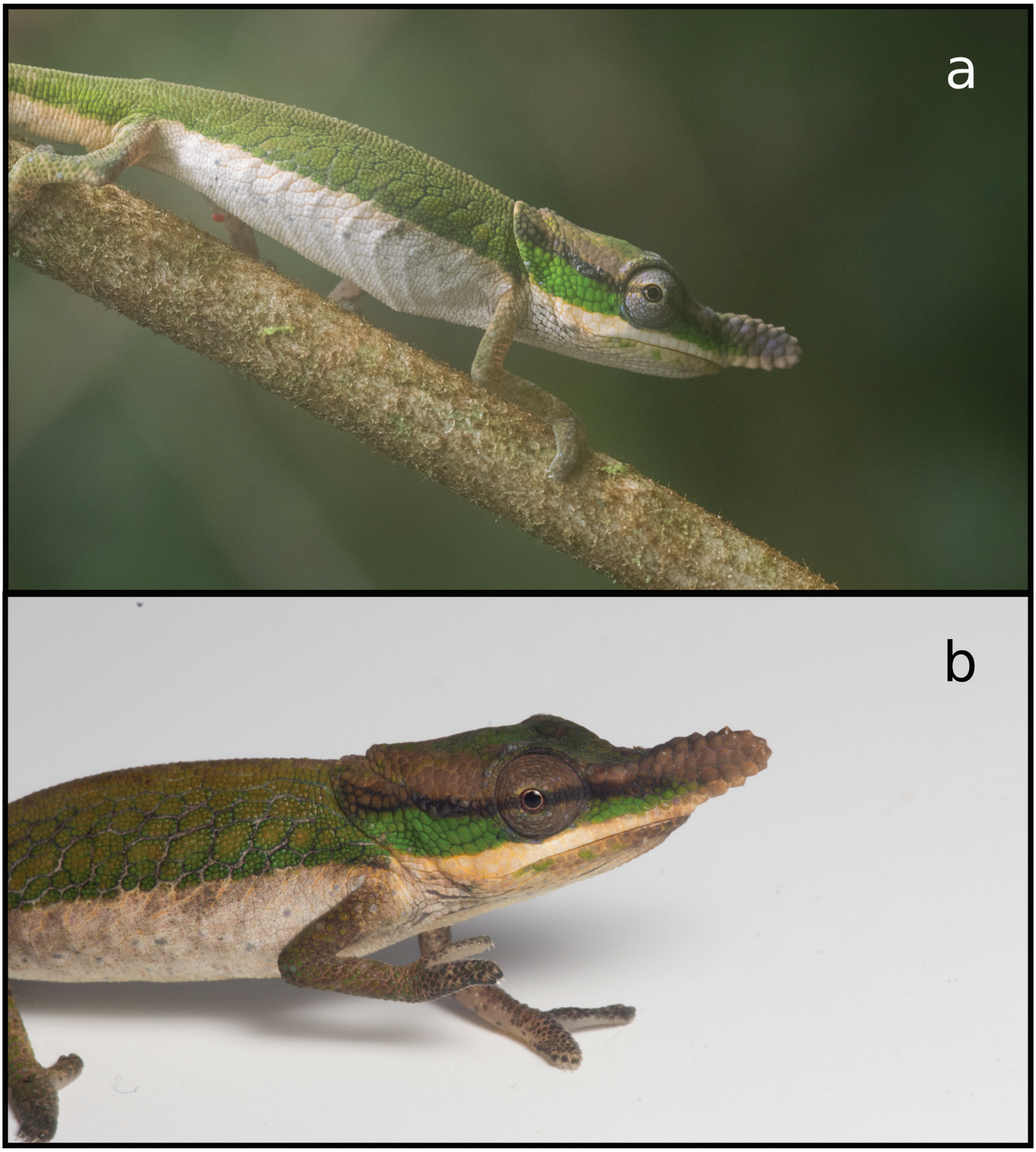

Figure 6. Posed photos of a subadult male specimen of *Calumma roaloko* sp. n. (ZSM 244/2018, KU 343177); (**a**) Indigo coloration on the rostral appendage and head scalation is apparent; (**b**) portrait of the same specimen.

half of the naris; prefrontal fontanelle and naris separated by contact of prefrontal with maxilla; frontal and parietal smooth with few tubercles; frontal with a width of 3.2 mm (26.4% of skull length) at border to prefrontal, extending to 4.5 mm (37.2%) at border to postorbitofrontal; broad parietal tapering more or less constantly from a width of 4.7 mm (38.8%) at the border to frontal and still broad at midpoint at 2.7 mm (22.3%) until it meets the squamosals, then narrowing to a tip; posterodorsally directed parietal in broad lateral contact with the squamosal; squamosal thick with a few tubercles. For further measurements, see Table 2 and also Suppl. material 3 for a 360° video of the skull. The skull of *Calumma roaloko* sp. n. shows notable similarity to *C. uetzi* except for the shape of the maxilla.

The micro-CT scan uncovered a worm-like structure that lies curled and fractured in the throat and proceeds posteriorly into the chameleon’s body presumably via the esophagus. We suppose that this shows an endoparasite trying to leave the dying chameleon after the processing, but note that it is remarkably strongly mineralized.

Coloration of the holotype in preservative. The body of the holotype in preservative (Fig. 3) is of gray and blue/violet color; the rostral appendage and the head are dark brown with a beige stripe from the snout tip via the supralabial scales to the ventral margin of the occipital lobes and a dark blue temporal region; broad lateral stripe on the body violet with a diffuse net-like pattern, tubercles on extremities also violet; ventral half of the body and inner side of extremities beige-white, dorsal margin of the body and tail of same dark brown color as the head.

Variation. The four paratypes agree well with the holotype in most characters of morphology and osteology. However, all four paratypes have more tubercle scales on rostral appendage on right side (28–33 vs. 16) and all paratypes lack a parietal crest (vs. indistinctly present); dorsal crest absent in UADBA-R (KU 343167), KU 343168, and UADBA-R (KU 343176). In osteology of the skull the only other micro-CT scanned specimen (the female KU 343168; Fig. 1c, d, Suppl. material 4) differs by the fused prefrontal fontanelle and naris, and the slightly narrower parietal with 34.8% of skull length at postorbitofrontal border (vs. 38.8%) and 19.1% of skull length at midpoint (vs. 22.3%). Both osteological characters can be attributed to sexual dimorphism or intraspecific variation.

Coloration in life. Based on observations and photographs of the type specimens (Figs 5–7) the species is sexually dichromatic, with males showing a body coloration with an olive green to bright green dorsal half of the body and beige to white ventral half that is continuing on the tail. Females are generally brown and tan or cream ventrally. Both sexes can display a netlike pattern caused by skin between scales in dark brown or beige. Extremities indistinct brown or beige; throat and upper labial scales beige in both sexes; rostral appendage of same brown color as the upper head region, can turn violet in males (Fig. 6a), as well as the eyes, with a beige line on ventral side; in females the appendage can turn yellowish (Fig. 7); dark lateral stripe from the base of the appendage crossing the eyes and ending at the occipital lobes; cheek region highlighted in bright green in the males, continued anteriorly to the base of the appendage.

Hemipenial morphology. The hemipenes of the three male specimens (the holotype KU 343178, UADBA-R (KU 343176), and ZSM 244/2018) are not completely everted and consequently we can only provide a preliminary and possibly incomplete description. On the asulcal side of the truncus the hemipenis shows large calyces with smooth ridges. The apex is ornamented with two pairs of rotulae, which are larger on the sulcal side (with 12–14 tips) and with 8–10 tips on asulcal side. In the holotype KU 343178 and UADBA-R (KU 343176) there is a small peak between the lobes on the posterior side that might be the tip of a cornuculum (Prötzel et al. 2017), but this interpretation is in need of confirmation due to the incomplete eversion of the hemipenes. The top of the apex has a papillary field of several fleshy papillae.

Available names. There are no available names that could be attributed to a species of the *C. nasutum* group with occipital lobes.

Etymology. The specific epithet "roaloko" is a combination of the Malagasy words "roa" meaning "two" and "loko" meaning "color", in reference to the characteristic two-toned body colorations of males (green and white) and females (brown and tan) of this species. The epithet is to be treated as an invariable noun in apposition.

Natural history. The specific natural history of *C. roaloko* sp. n. is little-known, but assumed to be similar to other small-bodied *Calumma*. As with other *C. nasutum* group species, individuals of *C. roaloko* sp. n. were encountered sleeping at night on leaves (Fig. 7) or small branches, and most often spotted ~2–5 m above the ground. *Calumma roaloko* sp. n. may be restricted to higher-elevation habitats, as it has only been found at ca. 1100 m a.s.l., although this is difficult to determine with certainty as most forests below ~1000 m a.s.l. in the area have been cleared. Interestingly, it is known from only two sites, both on the periphery of the forest fragment, and characterized by qualitatively more degraded habitat and/or secondary forest growth as compared to two sites located with more intact primary forest, where it was not encountered (Fig. 4). In summary, either *C. roaloko* sp. n. may have a higher detection probability in disturbed habitats, and/or may be out-competed in primary forest by close relatives (e.g., *C. nasutum* complex species that we found in all four sites). Several specimens were observed to have small red acarid ectoparasites (visible on the hindlimb in Fig. 6a).

Distribution. Given current evidence, the distribution of *C. roaloko* sp. n. is potentially restricted to a small fragment (~300 km^2) of mid-elevation rainforest that lies outside of nearby Analamazaotra Special Reserve and Andasibe-Mantadia National Park in central-eastern Madagascar (Fig. 4), but within the Réserve de Ressources Naturelles du Corridor Ankeniheny-Zahamena newly protected area. However, we believe that *C. roaloko* sp. n. may still be discovered in nearby areas, including Andasibe-Mantadia National Park, although it has never been found over dozens of surveys in nearby protected areas over the last century, including our own surveys (Hutter, Lambert, Scherz, Prötzel, Glaw, etc. unpubl. data). It is also possible that *C. roaloko* sp. n. could be found in other smaller and more fragmented forests located to the west of the type locality of *C. roaloko* sp. n., south of the city of Moramanga, but recent work in one remnant forest fragment in that area discovered *C. juliae* there, and no specimens of *C. roaloko* sp. n. were found (Prötzel et al. 2018).

Figure 7. In-situ photograph of an uncollected (in sleeping position) female of *Calumma roaloko* **sp. n.**, from the same locality as KU 343168.

Suggested Conservation Status. The ~300 km^2 fragment of mid-elevation rainforest from which *C. roaloko* sp. n. is known is managed by several local government councils, and has recently been established as a new protected area (Réserve de Ressources Naturelles du Corridor Ankeniheny-Zahamena) within the scope of the expansion of Madagascar’s national parks (Gardner et al. 2018). Forest in this area is dramatically fragmented and its area is decreasing. We suggest to evaluate the species as Endangered under the IUCN Red List criterion B1 (Extent of occurrence <5000 km^2) subcriteria a (severely fragmented or known from fewer than five threat-defined locations) and b(iii) (continuing decline in the area, extent, and/or quality of habitat). However, potentially suitable habitat for *C. roaloko* sp. n. also exists in other nearby protected areas (Andasibe-Mantadia, Analamazaotra) and private reserves (Vohimana). Although field surveys to these areas have not yet uncovered *C. roaloko* sp. n., they have revealed the presence of several other undescribed species of amphibians and reptiles, found originally in the same forest fragment as *C. roaloko* sp. n. (Hutter, unpubl. data). Furthermore, current evidence suggests that *C. roaloko* sp. n. is amenable to disturbed habitat (see Natural History). As such, the conservation status of *C. roaloko* sp. n. as suggested herein may need revision pending future survey work, particularly in nearby protected areas.

Discussion

The discovery of *C. roaloko* adds to a growing understanding of the diversity of small-bodied *Calumma* in Madagascar (Gehring et al. 2011, 2012, Prötzel et al. 2015, 2016, 2017, 2018). The *C. nasutum* group has grown significantly over the past few years, and is likely to continue to grow as taxonomic revision on it continues, and given the number of OTUs identified for the group by Gehring et al. (2012). Yet with this contribution and those of Prötzel et al. (2017, 2018), the *C. boettgeri* complex has expanded from three known species (Prötzel et al. 2015) to eight.

Biogeographically the pattern of diversity in the *C. nasutum* group currently suggests a complex history, possibly involving several major dispersal events, especially within the *C. boettgeri* complex, with *C. roaloko* being sister to *C. uetzi*, a species found >500 km to the north in the Sorata massif and Marojejy NP (Prötzel et al. 2018), and a similar situation in the recently described *C. juliae*, whose sister species are *C. boettgeri* and *C. linotum*, separated also by over 500 km. These distributions highlight the north central east of Madagascar as an important biogeographic gap in the *C. boettgeri* complex that may yield intermediate members connecting these species over their long sister-pair distances.

With a total length of 93.7 mm and a body size of 45.6 mm in the largest specimen (the male holotype), *C. roaloko* represents the smallest member of the "true" chameleons, subfamily Chamaeleoninae, (excluding the small, mostly ground dwelling species of the subfamily Brookesiinae) on Madagascar, and one of the smallest members of the Chamaeleoninae in the world. Within the *C. nasutum* group *C. uetzi* (with maximum TL of 101.2 mm and maximum SVL of 45.7 mm) and *C. vohibola* (with maximum TL of 90.5 mm – resulting from a measuring error due to a cut-off tail for DNA analysis – and maximum SVL of 49.8 mm, Gehring et al. 2011) are only slightly larger. Other small species are *C. guillaumeti* and *C. peyrierasi* with below 110 mm (TL), but all of these data are based on relatively small sample sizes (Prötzel et al. 2016).

Interestingly, *Calumma roaloko* and its sister taxon *C. uetzi* are among the only species within the *C. nasutum* group with strong sexual dichromatism. Males of both species differ clearly from the females by a conspicuous display coloration that contrasts well from the green and brown overall background of their habitat as shown for some chameleon species of the genus *Bradypodion* (Stuart-Fox et al. 2007). Displaying male *C. roaloko* are still well-camouflaged however when seen from above due to the green color on the dorsal part of their bodies. Laterally, from a conspecific's eye perspective, they may signal with the white ventral body part and the violet rostral appendage—a strategy employed still more strongly in several other lizards, e.g. *Algyroides*, *Sceloporus*, *Uta* (Ossip-Drahos et al. 2016).

The increase in species richness in chameleons may come not only from the splitting of currently recognized and often widespread species (e.g., in the African chameleon genus *Kinyongia*, Hughes et al. 2017), but from the continued discovery of clearly distinct, previously unknown species, often with geographically or elevationally restricted ranges, and/or low detection probabilities, such as *C. roaloko* (see also Gehring et al. 2011 and Prötzel et al. 2018). Such discoveries highlight the unabated importance of field research for phylogenetic systematics. Indeed, far more examples of "unexpected" species discoveries, also revealed by recent field surveys, are found in other squamate lineages and tropical regions (Welton et al. 2010, Mahler et al. 2016), but also occur in other Malagasy herpetofauna (e.g. Glaw et al. 2006), as well as in other tropical vertebrate clades (e.g., in mammals, Helgen et al. 2013, Hrbek et al. 2014). Clearly, if we are to understand the evolutionary extent of Madagascar's many endemic radiations, and of biodiversity in the tropics generally, a great deal of basic field survey work yet remains.

Acknowledgements

We thank the Malagasy authorities for granting permits used to conduct field research and collecting efforts (Research conducted under research permit Nº329/15/MEEMF/SG/DGF/DAPT/SCBT, specimens exported under CITES permit Nº065C-EA01/MG16, 26/01/2016). Field research was supported by Global Wildlife Conservation through Grant 5019-0096 to CRH and SML. Landsat data were made available from the U.S. Geological Survey. We are grateful to Miguel Vences for providing DNA sequences and to the organization MICET for logistical support. We also thank the Vondron'Olona Ifotony (V.O.I.) of Iaroka, Maroala, and Fandrefiala for granting permission to work in their forests. We thank all of our Malagasy guides, cooks and porters, with special thanks to guides Jean Aime Rajaonarivelo ('Gagah') and Regis. We are also grateful to Asa Conover, Devin Edmonds and Vincent Premel for their assistance in the field.

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Supplementary material 1

Genetic distances of ND2

Authors: David Prötzel, Shea M. Lambert, Ginah Tsiorisoa Andrianasolo, Carl R. Hutter, Kerry A. Cobb, Mark D. Scherz, Frank Glaw

Data type: .ods spreadsheet

Explanation note: Uncorrected genetic distances for all pairwise comparisons of ND2.

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Link: https://doi.org/10.3897/zse.94.27305.suppl1

Supplementary material 2

Genetic distances of COI

Authors: David Prötzel, Shea M. Lambert, Ginah Tsiorisoa Andrianasolo, Carl R. Hutter, Kerry A. Cobb, Mark D. Scherz, Frank Glaw

Data type: .ods spreadsheet

Explanation note: Uncorrected genetic distances for all pairwise comparisons of COI.

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Link: https://doi.org/10.3897/zse.94.27305.suppl2

Supplementary material 3

Movie of 3D model of the skull

Authors: David Prötzel, Shea M. Lambert, Ginah Tsiorisoa Andrianasolo, Carl R. Hutter, Kerry A. Cobb, Mark D. Scherz, Frank Glaw

Data type: .avi video file

Explanation note: Movie of micro-CT scan of the skull of the male holotype of *Calumma roaloko* (KU 343178).

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Link: https://doi.org/10.3897/zse.94.27305.suppl3

Supplementary material 4

Movie of 3D model of the skull

Authors: David Prötzel, Shea M. Lambert, Ginah Tsiorisoa Andrianasolo, Carl R. Hutter, Kerry A. Cobb, Mark D. Scherz, Frank Glaw

Data type: .avi video file

Explanation note: Movie of micro-CT scan of the skull of the female *Calumma roaloko* (KU 343168).

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