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Towards a revision of the Malagasy chameleons of the *Calumma gallus* complex: Redefinition of *Calumma nasutum* based on a museomics approach and descriptions of two new species

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Abstract. *Calumma gallus* (Günther, 1877) is a small chameleon from eastern Madagascar which remains poorly known. Fieldwork and molecular phylogenetic studies in the last decade have revealed that *C. gallus*, as currently understood, is a species complex, but available data is still insufficient to comprehensively resolve its taxonomy. In this study, we combine mitochondrial DNA sequences and morphological data to draw first taxonomic conclusions. Based on a museomics approach, i.e., archival DNA sequencing of the historical lectotype of *C. nasutum* we re-define *C. nasutum* as a species of the *C. gallus* complex, although it is lacking the elongated rostral appendage usually considered to be diagnostic for the complex. We furthermore describe the populations previously considered under the name *C. nasutum* as a new species, *Calumma hofreiteri* sp. n. By analyzing an extended mitochondrial data set of the *C. gallus* complex, we clarify the genetic and phylogeographic variation of these chameleons, with genetic distances of 7.7–14.0% in ND2 and up to 4.1% in 16S rRNA between mitochondrial clades within the complex. We assign the name *C. gallus* sensu stricto to a mitochondrial clade containing specimens with distinctly elongated and serrated rostral appendages and describe the northernmost and phylogenetically most divergent populations of the complex as a new species, *Calumma pinocchio* sp. n., based on their genetic divergence and comparatively smooth-edged elongated rostral appendage. We discuss the rapid evolution of rostral appendages in this species complex and highlight the need for expanded collection and in-depth phylogenomic analysis to fully clarify species limits and evolution of these chameleons.

Key words. Squamata, Chamaeleonidae, *Calumma gallus, Calumma nasutum, Calumma pinocchio* sp. n., *Calumma hofreiteri* sp. n., museomics, archival DNA, taxonomy.

Introduction

Madagascar is a model region of species diversification (Vences et al. 2009) and center of chameleon species richness, with 98 of 234 species in the family being endemic to the island (Jenkins et al. 2014, Uetz 2025). With 42 species (Uetz 2025) subdivided into four informal species groups (see Hillenius 1959; still included in the genus *Chamaeleo*), the genus *Calumma* contains the majority of rainforest-dwelling arboreal chameleons on Madagascar. One of these species groups, the *Calumma nasutum* group, historically comprised only five species (*C. boettgeri, C. fallax, C. gallus, C. guibei,* and *C. nasutum*), all characterized by a rostral appendage present in both sexes but often sexually dimorphic and typically smaller and less conspicuous in females (Brygoo 1971, Glaw & Vences 2007).

Since the molecular study of Gehring et al. (2012) it has become obvious that species diversity in the Malagasy chameleons of the *C. nasutum* group has been greatly underestimated. Based on mitochondrial DNA sequence data, these authors identified no less than 33 operational taxonomic units (OTUs), highlighting the need for a comprehensive revision of the group. Intensive studies in recent years resulted in the re-definition of several taxa (e.g., Prötzel et al. 2015) and the description of nine new species in this group: *Calumma vohibola, C. gehringi, C. juliae, C. lefona, C. uetzi, C. roaloko, C. emelinae, C. ratnasariae,* and *C. tjiasmantoi* (Gehring et al. 2011, Prötzel et al. 2017, 2018a, 2018b, 2020). Despite the enormous progress in our understanding of species richness in the *C. nasutum* group, several taxonomic questions still await an in-depth study. On the one hand, *C. nasutum*, originally described from Madagascar without specific locality information, was re-defined by Prötzel et al. (2020) to correspond to the mitochondrial clade "K" with populations from the Andasibe region in the Northern Central East, and Sorata in the North East of Madagascar based on morphological comparisons, but without decisive confirmation from molecular data. On the other hand, taxonomic uncertainty surrounds the populations currently included in *C. gallus* (clade A in Gehring et al. 2012 and Prötzel et al. 2020), a conspicuous species characterized by a very long rostral appendage with a pointed tip. According to Prötzel et al. (2020: Table 3) *Calumma gallus* exhibits the greatest intraspecific genetic divergence in the mitochondrial ND2 gene (8.5%) among all of the 15 genetically studied species of the *Calumma nasutum* group, only slightly below the interspecific distance observed between *C. guibei* and *C. lefona* (9.1%) and above most interspecific distances among species of the continental African genus *Bradypodion* (e.g., Tolley et al. 2022). Phylogenetics within the *C. gallus* complex are furthermore geographically structured: the mitochondrial ND2 trees of Gehring et al. (2012: suppl. material) and Prötzel et al. (2020) revealed a northern clade in central eastern Madagascar being the sister group to all remaining clades in the complex (see also the full multigene tree of Tolley et al. 2013). This supports characterizing *C. gallus* as a species complex that may contain more than one species.

The substantial sexual dimorphism in *C. gallus* and the lack of data from much of its range has impeded the ability to fully understand its morphological variation. Hillenius (1959) provided only a limited amount of information, highlighting the pointed rostral appendix as a difference to *C. nasutum* (a species that at the time was very broadly defined). In his monograph of Malagasy *Chamaeleo* species, Brygoo (1971) summarized the existing knowledge and illustrated a male specimen examined from Ambavaniasy characterized by a very long and smooth-edged rostral appendage. Brygoo (1978) reported on several newly examined specimens, among them a male and a female from Mahanoro (the type locality of the species), and Rieppel & Crumly (1997) provided osteological data of a single skull of *C. gallus*. In a popular contribution, Glaw & Vences (2001) provided photographs in life of males and supposed females of this species, the latter being distinguishable by a non-pointed, shorter rostral appendage of reddish color. Subsequent DNA sequence analysis (e.g., Gehring et al. 2012, Prötzel et al. 2020) confirmed these red-nosed females to represent *C. gallus*. The most recent point distribution map of the species was published by Glaw & Vences (2007), mostly based on the localities listed in Brygoo (1971) and thus mostly not confirmed by genetic data. In 2011, *Calumma gallus* was assessed by the IUCN Red List and a distribution map was created. However, the IUCN range map does not include substantial parts of the species' distribution range as given in Brygoo (1971) and Glaw & Vences (2007).

A preliminary discussion of the genetic and morphological variation within *C. gallus* was published by Gehring et al. (2010). These authors discovered that two different morphological and genetic lineages occur north and south of the Mangoro River, which they considered as *C. gallus* (north of the Mangoro River, recorded at Sahafina and Mahanoro) and as a probably undescribed species, *C.* sp. aff. *gallus* "south" (south of the Mangoro River, recorded at Ambodiharina and in the Marolambo region). The rostral appendage of the males observed south of the Mangoro was not as elongated as in *C. gallus*, and its tip was more-or-less rounded and not as pointed as in male *C. gallus* (Gehring et al. 2010). Moreover, there were upright spine-like scales present on the outer edge of the appendage, so the rostral appendage resembled the blade of a chain saw (see Fig. 2; Clade A5 in Gehring et al. 2012). These spine-like scales were also present in the smaller rostral appendage of the female (Gehring et al. 2010). However, these authors did not propose any taxonomic changes given the incomplete data and scarcity of voucher specimens available for morphological comparison.

The goal of the present study is to provide an initial taxonomic resolution of the *C. gallus* complex. For this purpose, we combine various data sets: First, we present the results of archival DNA sequencing of the historical lectotype of *C. nasutum* which surprisingly assigned this specimen to the *C. gallus* complex, thus requiring the re-definition of *C. nasutum* and description of the populations previously considered under this name as new species. Second, we extend the previous molecular assessments of Gehring et al. (2012) and Prötzel et al. (2020) to include mitochondrial sequences of all samples of *C. gallus* available to us. Third, we provide provisional morphological comparisons limited to a rather small number of voucher specimens that could be genotyped. Although hampered by the inconclusiveness of nuclear-encoded DNA analysis and by the scarcity of material from some mitochondrial clades, our study conclusively assigns the nomina *C. nasutum* and *C. gallus* to mitochondrial clades and allows for the description of two new species, thereby setting the stage for a future comprehensive phylogenomic revision of these chameleons.

Materials and methods

Fieldwork, comparative material and morphological analysis

Specimens of the *C. gallus* complex were collected during the period 1996–2016 by opportunistic searches during both day and night. They were euthanized, fixed in 90% ethanol and then transferred to 70% ethanol for long-term storage, and subsequently deposited in the collections of the Zoologische Staatssammlung München, Germany (ZSM), the Université d'Antananarivo, Mention Zoologie et Biodiversité Animale, Antananarivo, Madagascar (UADBA), and the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK). Additional specimens from the Muséum National d'Histoire Naturelle, Paris, France (MNHN), the Senckenberg Naturmuseum, Frankfurt, Germany (SMF), and the Natural History Museum, London, UK (BMNH, now NHMUK) were also included in this study. Field numbers of preserved specimens and tissue samples refer to the collections of A. Crottini (ACZC), P.-S. Gehring (PSG), M. Pabijan (MPFC), F. Glaw (FGMV, FGZC) and M. Vences (FGMV, MV, ZCMV). Geographical coordinates were obtained with GPS receivers set to WGS84 datum. Biogeographic regions of Madagascar are named following the scheme of Boumans et al. (2007) and Brown et al. (2016).

The following morphological measurements were taken with a digital caliper to the nearest 0.1 mm, and meristic variables were counted using a binocular dissecting microscope (see also Table 1), evaluated by eye or calculated by the same person (D.P.) following the methods in Prötzel et al. (2020): 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 and TaL; ratio of TaL to SVL (TaL/SVL); length of the rostral appendage (LRA) from the upper snout tip; ratio of LRA to SVL (LRA/SVL); casque height (CH), measured from the peak of the casque to the beginning of the dorsal ridge of the torso; diameter of largest scale on temporal region (DSCT), measured on the right side; rostral scale integrated in rostral appendage (RSI) present (+) or absent (–); distinct rostral crest (RC) present (+) or absent (–); lateral crest (LC), running from the posterior of the eye horizontally, present (+) or absent (–); temporal crest (TC), running dorsally to the LC, curving toward the midline, present (+) or absent (–); cranial crest (CC), defined by the lateral ridges of the parietal bone that give an edge to the casque, present (+) or absent (–); parietal crest (PC) present (+) or absent (–); dorsal crest (DC) absent (–) or number of dorsal cones visible to the naked eye without the use of a binocular microscope according to Eckhardt et al. (2012); number of supralabial scales (SUPL), 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 (i.e. excluding the small granular scales bordering the rictus); and number of infralabial scales (INFL), analogous to the definition of NSL above, on the right side; axillary pits (AP) present (+) or absent (–).

Museomics

In an approach to phylogenetically place the wet-preserved *Chamaeleon nasutus* Duméril & Bibron, 1836 lectotype in a mitochondrial DNA-based phylogeny, we minimally invasively sampled specimen MNHN-RA-1994.610 in 2024 for a small piece of skin with underlying tissue taken from the left side of the abdomen. The sample was stored in a vial with pure ethanol and then processed along with a batch of other (non-chameleon) samples. The sample was first weighed and then incubated in a guanidine thiocyanate (GuSCN) based extraction buffer solution at 37 °C overnight. The next day, we extracted a total volume of 25 µl genomic DNA following the protocol of Rohland et al. (2004), in several consecutive steps as described in Straube et al. (2021). The yield of DNA was quantified based on 1 µl DNA extract using the Qubit dsDNA HS Assay Kit 0.2–100 ng/µl (Life Technologies, Carlsbad, California, US) according to the instructions of the manufacturer. We used <0.5 ng DNA as input for single-stranded library preparation according to the protocol of Gansauge et al. (2017). All lab work prior to qPCR was conducted in a dedicated DNA facility at the University of Potsdam, Germany, which meets all requirements to work with historical samples (see Fulton & Shapiro 2019). Extraction and library blanks were run alongside the sample batch to check for cross-contamination. Final library concentrations and fragment length distributions were assessed using a 2200 TapeStation (Agilent Technologies) assay. The library was then shotgun-sequenced for approximately five million 75-bp single-end reads on an Illumina Nextseq 500/550 sequencing platform at the University of Potsdam, following the procedure described in Paijmans et al. (2017). This initial sequencing was performed to check for the presence and estimate the amount of endogenous DNA. The quality of the obtained reads was visualized twice using FastQC (https://www.bioinformatics.babraham.ac.uk), both before and after trimming of Illumina adapter sequences and discarding reads shorter than 30 bp with cutadapt v2.10 (Martin 2011). After confirmation of endogenous DNA, target capture was performed using the customized mixed RNA bait set as described in Agne et al. (2022). This myBaits® kit (Arbor Biosciences, Ann Arbor, Michigan, USA) was designed to contain RNA baits covering multiple different markers relevant for the phylogenetic placement of diverse animals. For capturing relevant mitochondrial sequences of the *C. nasutus* lectotype, these RNA baits were designed to include sequence information of two mitochondrial (16S rRNA gene (16S) and the NADH Dehydrogenase gene, Subunit 2 (ND2)) markers using published *Calumma amber* sequences for bait design-GenBank numbers HF570477 (16S) and HF570414 (ND2) (Agne et al. 2022). The DNA libraries were captured twice to maximize capture success (e.g., Li et al. 2013, 2015, Paijmans et al. 2016) and underwent quality measures and sequencing as described for the initial shotgun sequencing step.

Table 1. Morphometric and meristic characters in examined voucher specimens of the target species of this study (*Calumma gallus, C. pinocchio* sp. n., *C. nasutum, C. hofreiteri* sp. n.). All morphometric measurements in mm, all ratios in percent. See Materials and methods for abbreviations of characters; additional abbreviations: m, male; f, female; HT, holotype; PT, paratype; LT, lectotype; PLT, paralectotype. Genetic lineage is given according to the mitochondrial phylogeny (see Fig. 1).

Catalogue number	Field number	Type status	Locality	Line-age	Sex	SVL	TaL	TL	TaL/ SVL	LRA	LRA/ SVL	CH	DSCT	RSI	RC	LC	TC	CC	PC	DC	SUPL	INFL	AP
C. nasutum																							
ZSM 622/2009	ZCMV 8642		Ranomafana-Samalaotra	A1	m	47.3	45.3	92.6	96%	2.8	5.9%	1.5	1.3	–	+	+	–	+	–	0	12	13	+
ZSM 793/2003	FGMV 2002.642		Ambohitsara	A1	m	45.7	43.3	89.0	95%	4.1	9.0%	2.3	1.1	–	+	+	–	+	–	0	13	12	+
MNHN-RA-1994.610	MNHN-RA-6643C	LT	Madagascar	A1	m	49.0	51.8	100.8	106%	2.6	5.3%	2.0	0.9	–	+	+	+	+	–	0	15	15	+
MNHN-RA-1994.609	MNHN-RA-6643B	PLT	Madagascar		m	46.9	43.1	90.0	92%	cut		1.7	0.9	–	+	+	+	+	–	0	14	13	+
ZSM 623/2009	ZCMV 8643		Ranomafana, Samalaotra	A1	f	43.9	42.0	85.9	96%	1.2	2.7%	0.8	1.0	–	+	+	–	+	–	0	12	13	+
MNHN-RA-6643		PLT	Madagascar		f	49.4	45.7	95.1	93%	1.5	3.0%	0.7	0.9	–	+	+	+	+	–	0	14	15	+
MNHN-RA-1994.608	MNHN-RA-6643A	PLT	Madagascar		f	43.0	37.7	80.7	88%	1.2	2.8%	1.0	0.8	–	+	+	+	+	–	0	15	16	+
C. gallus																							
ZSM 456/2010	FGZC 4508		Tarzanville	A2	m	55.1	54.2	109.3	98%	8.5	15.4%	0.8	1.2	–	+	+	–	–	–	0	13	13	+
C. hofreiteri sp. n.																							
ZSM 454/2010	FGZC 4506	HT	Anosibe An'Ala	K	m	44.2	46.3	90.5	105%	2.0	4.5%	1.5	1.2	–	+	+	+	+	+	12	14	14	–
ZSM 924/2003	FGMV 2002.984	PT	Andasibe	K	m	43.7	45.3	89.0	104%	2.2	5.0%	1.7	1.6	–	+	+	+	–	+	8	12	13	–
ZSM 1699/2012	FGZC 3711		Sorata	K	f	47.3	45.4	92.7	96%	1.5	3.2%	0.9	1.2	–	+	+	+	+	+	0	14	13	–
ZSM 1700/2012	FGZC 3744		Sorata	K	f	45.8	44.3	90.1	97%	1.4	3.1%	0.7	1.0	–	+	+	+	–	+	0	14	15	–
C. pinocchio sp. n.																							
ZSM 137/2016	FGZC 5050	HT	Vohimana	A4	m	49.0	43.6	92.6	89%	7.2	14.7%	1.3	1.1	–	+	+	–	+	–	0	16	15	+
ZSM 550/2001	FGMV 2001.247	PT	Vohidrazana	A4	m	43.0	40.0	83.0	93%	7.1	16.5%	1.1	0.8	–	+	+	–	+	–	0	14	13	+
ZSM 321/2000		PT	Vohidrazana	A4	m	44.1	44.2	88.3	100%	8.7	19.7%	1.1	0.8	–	+	+	–	–	–		14	13	+
ZSM 139/2016	FGZC 5151		Analalava	A4	m	44.7	38.0	82.7	85%	6.9	15.4%	0.5	0.8	–	+	–	–	+	–	0	12	12	–
ZSM 319/2000		PT	Vohidrazana	A4	f	42.7	43.4	86.1	102%	3.1	7.3%	0.9	0.8	–	+	+	–	+	–	0	15	13	+
ZSM 322/2000		PT	Vohidrazana	A4	f	50.2	43.0	93.2	86%	2.6	5.2%	0.9	0.7	–	+	+	–	+	–	0	14	13	+
ZSM 138/2016	FGZC 5075	PT	Vohimana	A4	f	47.7	45.9	93.6	96%	2.3	4.8%	0.9		–	+	–	–	+	–	0	13	12	+

To assemble the sequences of the two target loci ND2 and 16S for the *C. nasutum* lectotype from the target captured DNA libraries fully avoiding reference bias, we applied a procedure consisting of multiple steps: We used local Blast (Blast+; Camacho et al. 2009) against a library of sequences of 16S and ND2 from representatives of the *C. nasutum* species group (*C. boettgeri, C. gallus*, and the mitochondrial lineage K considered as *C. nasutum* by Prötzel et al. 2020), transformed the sequences into a Blast reference database, blast-searched the *C. nasutum* lectotype reads against it, and collected all matching reads with >90% sequence identity to any of the reference sequences. All matching reads were collected in a FASTA file and CodonCode Aligner v 3.7.1 (CodonCode Corporation) was used to map the matching reads to 16S and ND2 reference sequences (option: "align to reference"). We verified that the resulting assemblies were congruent (thus, no reference bias was introduced) by running exploratory phylogenetic analyses, and eventually used the consensus sequence obtained by aligning all reads matching to one of the references for downstream analysis, with missing sections in-between contigs coded by the letter "N". The 16S sequence has been deposited in GenBank (accession number PX376991) while the (very short) ND2 sequence is made available via the Zenodo repository (https://doi.org/10.5281/zenodo.17145003).

Sanger sequencing and molecular phylogenetics

Our molecular genetic study integrated ND2 sequences from previous work (Prötzel et al. 2020) with new ND2 and 16S sequences generated for this study. DNA was extracted from these tissue samples stored in 96% ethanol following a standard salt-extraction protocol (Bruford et al. 1992). The ND2 fragment was PCR-amplified with primers ND2F17 (5'-TGACAAAAAAT TGCNCC-3') (Macey et al. 2000) and ALAR2 (5'-AAAATRTCTGRGTTGCATTCAG-3') (Macey et al. 1997), and the following protocol: initial denaturation at 95 °C for 180 s, 41 cycles of denaturation at 95 °C for 60 s, annealing at 48 °C for 60 s, elongation at 72 °C for 90 s, followed by 10 minutes of final elongation at 72 °C. The fragment of the 3' terminus of the 16S rRNA gene was amplified with primers 16SAL (CGC CTG TTT ATC AAA AAC AT) and 16SBH-new (CCT GGA TTA CTC CGG TCT GA), modified from Palumbi et al. (1991), with the following cycling protocol: initial denaturation at 94 °C for 90 s, 33 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, elongation at 72 °C for 90 s, and 300 s of final elongation at 72 °C. Reaction mixes contained 1 µl template DNA, 0.25 µl of 10 µM dNTPs, 0.3 µl of each 10 µM Primer, 2.5 µl Colorless 5x GoTaq Reaction Buffer, and 0.1 µl GoTaq G2 DNA Polymerase (5 U/µl) in a total volume of 12.5 µl. Nucleotide debris was removed by adding 2.4 µl ExoSAP to 8 µl PCR. Sequencing of purified PCR products was conducted on capillary sequencers by LGC Biosearch Technologies in Berlin, Germany. CodonCode Aligner 6.0.2 (CodonCode Corporation) was utilized to verify sequence quality of chromatograms and stretches of poor read quality were removed. New sequences were submitted to GenBank (accession numbers PX377524–PX377534 and PX379538–PX379567), and complemented with sequences from Prötzel et al. (2020) available from GenBank and with the museomics sequences of the *C. nasutum* type. A table with all sequences used and their accession numbers, as well as the tree files and alignments, are available from the Zenodo repository (https://doi.org/10.5281/zenodo.17145003). Our sampling includes all available ND2 sequences, plus complementary new sequences, for the *Calumma gallus* complex sensu Gehring et al. (2012) and lineage K (considered to represent *C. nasutum* by Prötzel et al. 2020), as well as 2–3 representative samples per species of all other species in the *C. nasutum* group. We aligned DNA sequences using the G-INS-i option in MAFFT (Katoh & Standley 2013) as implemented in the program Concatenator (Vences et al. 2022) and used the same program to concatenate the two mitochondrial gene fragments (ND2 and 16S) for analysis. From the concatenated ND2 + 16S alignment we then reconstructed a Maximum Likelihood tree in RAxML (Stamatakis 2014) using raxmlGUI v. 2.0 (Edler et al. 2020), under a General Time Reversible model (GTR+G) based on the Bayesian Information Criterion from a model testing analysis performed in MEGA7 (Kumar et al. 2016), and testing node support with 500 thorough bootstrap replicates. Sequences of *Calumma gastrotaenia* were used as the outgroup. Uncorrected pairwise genetic distances were calculated from the 16S and ND2 sequences using MEGA7. To calculate ND2 distances, a trimmed alignment of 378 bp and 74 ingroup sequences was used, without missing data in any sequence, while 16S distances were calculated from an alignment of 481 bp and 20 ingroup sequences, with a maximum of 68 missing nucleotides (for *C. guibei*). The names of the mitochondrial clades used throughout this paper follow Gehring et al. (2012).

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new name contained herein is available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: urn:lsid:zoobank.org:pub:8E502EC8-75EE-4CBF-A8EE-3E16C5BB963A. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: zenodo.org, salamandra-journal.com.

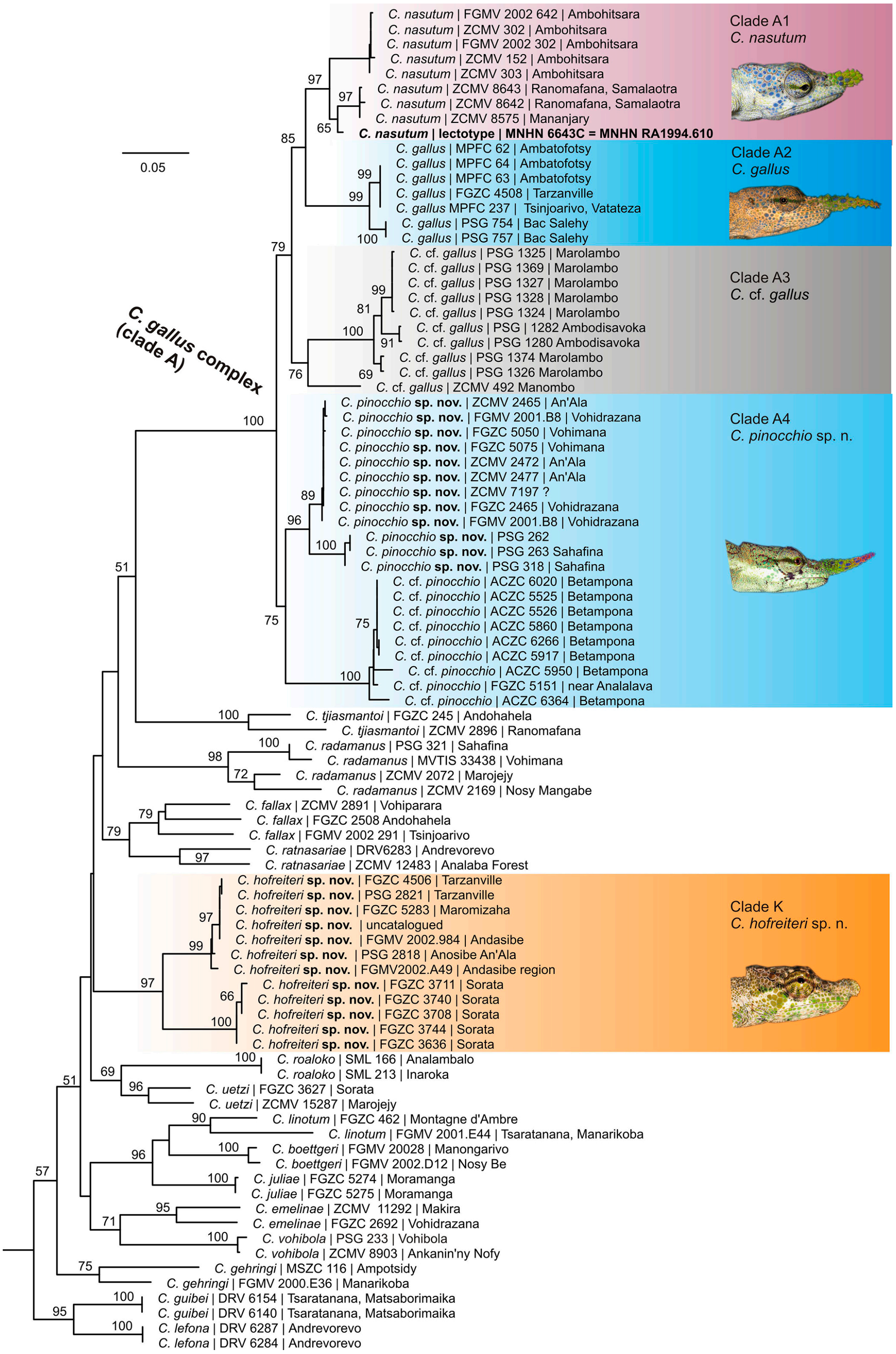

Figure 1. Maximum Likelihood phylogenetic tree of the *Calumma nasutum* group, based on concatenated DNA sequences of the mitochondrial ND2 and 16S genes (1464 bp). The tree includes sequences of 1–2 specimens per species, plus all available samples of the target taxa, i.e., *C. nasutum, C. hofreiteri* sp. n., and the *C. gallus* complex. Numbers at nodes are support values in percent from a bootstrap analysis (500 replicates; not shown if <50%). The tree was rooted with *C. gastrotaenia* (removed a posteriori from graph to better illustrate branch lengths within the *C. nasutum* group). Inset photos show lateral views of heads of adult males.

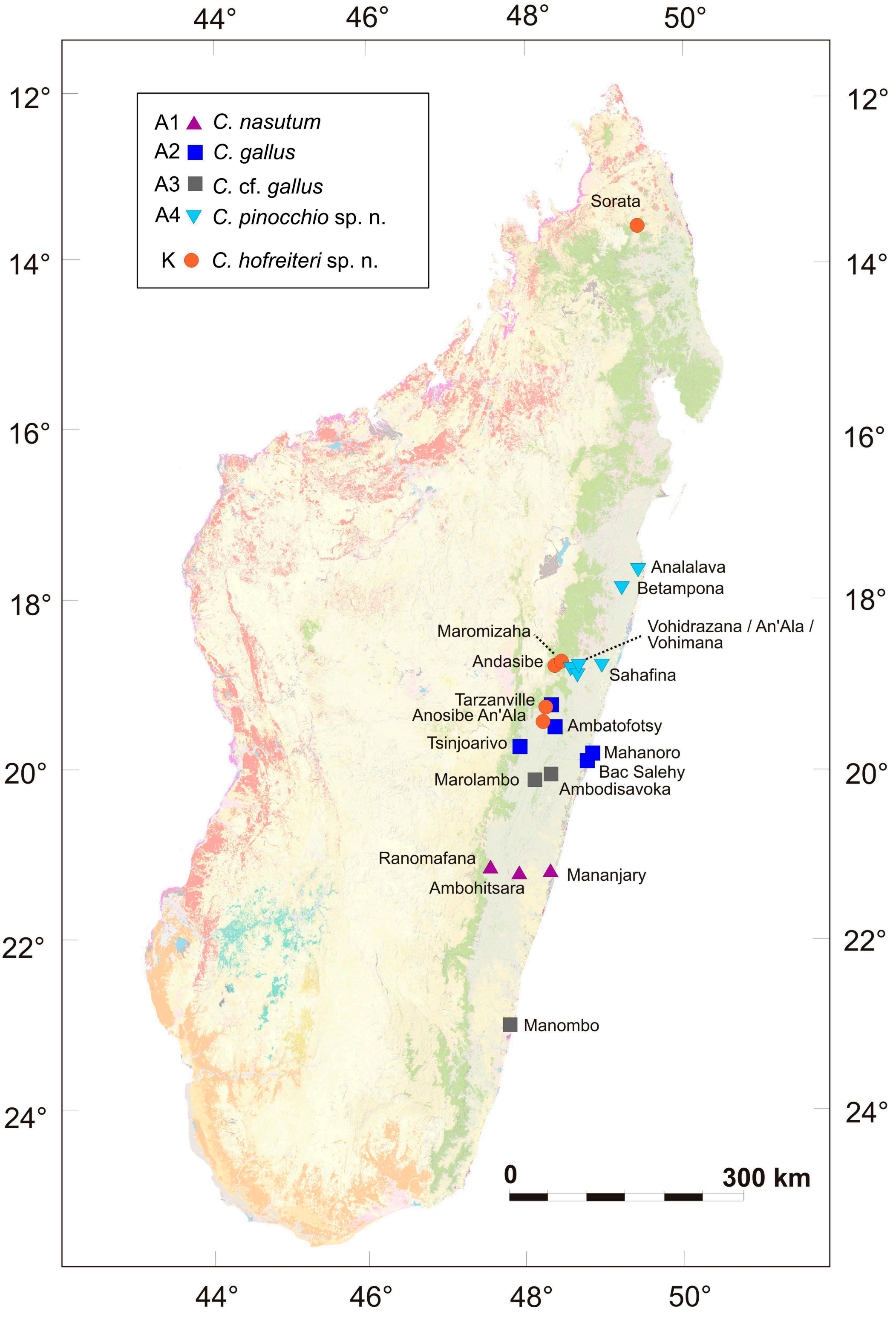

Figure 2. Distribution map of *C. hofreiteri* sp. n. and the *Calumma gallus* complex. A1–A4 and K in the figure legend refer to mitochondrial clades as in Fig. 1 and Prötzel et al. (2020). Only locations confirmed by genetic data, plus the type locality of *C. gallus* (Mahanoro; specimens not genotyped), are shown. The base map shows vegetation across Madagascar from the CEPF Madagascar Vegetation Mapping Project (Moat & Smith 2007; https://web.archive.org/web/20170615094352/http://vegmad.org/). Vegetation is colored as follows: green, humid forest (rainforest); red, western dry deciduous forest; bluish, western subhumid forest; orange, south western dry spiny forest-thicket; yellow, tapia forest; pink, mangroves.

Results

Molecular phylogeny and genetic divergences

The Maximum Likelihood tree inferred from the concatenated alignment of 1464 bp of the mitochondrial 16S and ND2 fragments (Fig. 1) largely agrees with the tree of Prötzel et al. (2020) which was based on ND2 only but has slightly stronger bootstrap support (BS) values in several of the deeper nodes. Conspecific samples of established species in the *C. nasutum* group in all cases clustered together with >70% bootstrap support. The *C. gallus* complex (the target of the present study, as defined by Prötzel et al. 2020) received maximum support (BS = 100%) and included sequences of the lectotype of *Chamaeleon nasutus* (see next section). This complex (corresponding to clade A of Prötzel et al. 2020) contained samples from numerous sites in the Northern Central East and Southern Central East of Madagascar, confirming that the *C. gallus* complex has a wide distribution in these geographical regions, reaching southwards to Manombo (Fig. 2). Within the complex, several clearly defined mitochondrial clades with BS >70% were recognizable, here named A1 to A4. In brief, A1 included male specimens with a non-elongated, laterally compressed rostral appendage as well as sequences of the lectotype of *Chamaeleon nasutus*, A2 contained males with an elongated and serrated rostral appendage pointing downward, here considered to conform with the type material of *C. gallus*, A3 contained samples from a series of lo-

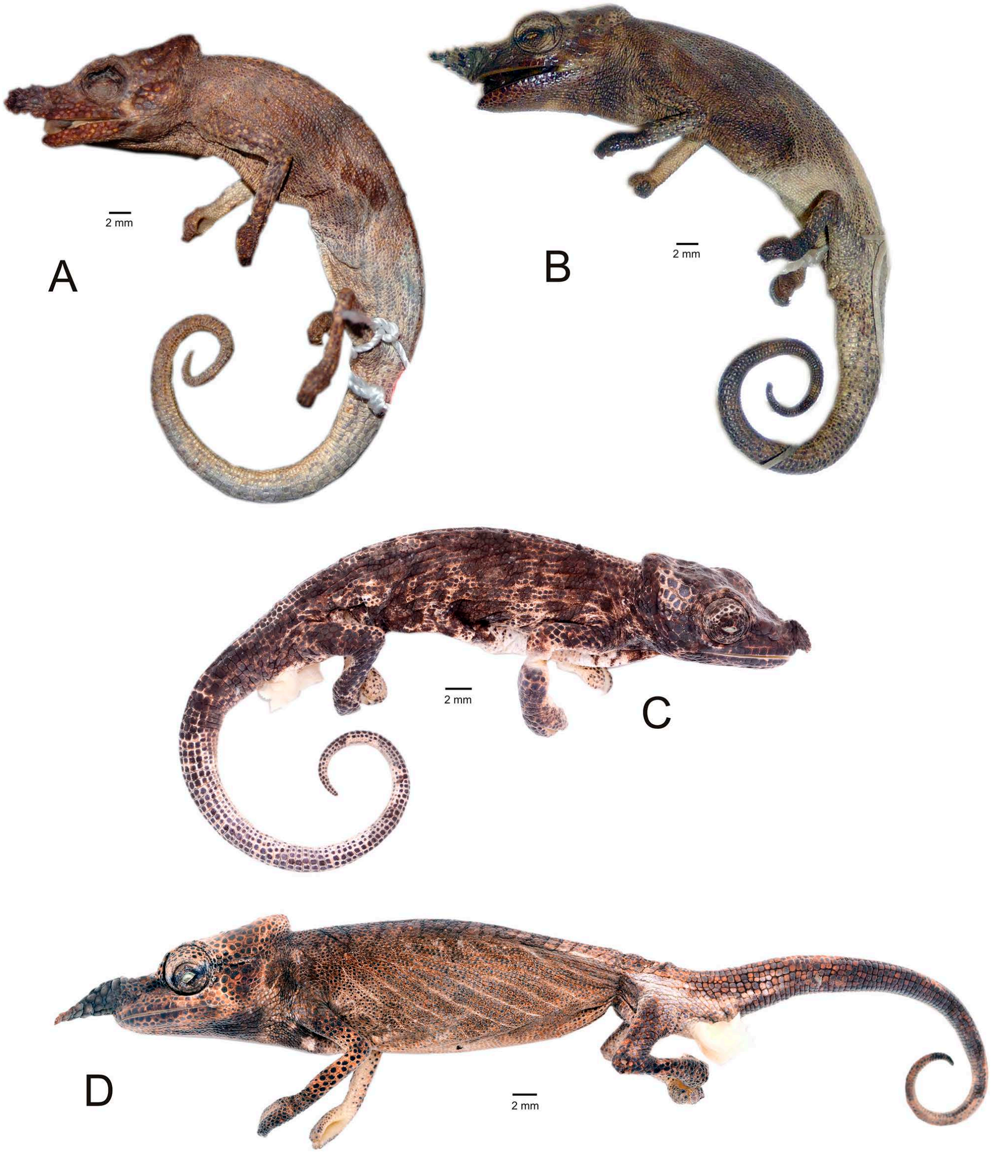

Figure 3. Preserved name-bearing types of species in the *Calumma nasutum* group targeted in this study in lateral views: (A) male lectotype of *C. nasutum* (MNHN-RA-6643C); (B) male holotype of *C. gallus* (NHMUK 1946.8.21.55); (C) male holotype of *C. hofreiteri* sp. n. (ZSM 454/2010); (D) male holotype of *C. pinocchio* sp. n. (ZSM 137/2016).

calities in central east and south east with only limited morphological information available, and A4 contained males from the Northern Central East with elongated and non-serrated rostral appendage pointing upward. A3 contained a genetically divergent subclade only represented by one individual from Manombo, and A4 was partitioned in two subclades, one with samples from Betampona and Analalava and one with samples from the remaining locations.

Using the ND2 alignment for distance calculations (see Methods), uncorrected pairwise distances between established species (as in Prötzel et al. 2020) ranged from 10.8% (*C. emelinae* vs. *C. uetzi*) to 20.9% (*C. boettgeri* vs. a sequence of the *C. gallus* complex from Betampona). Distances between main clades in the *C. gallus* complex ranged from 7.7% (A1 vs. A4) to 14.0% (A3 vs. A4), thus overlapping with distance values between established species. The inclusion of 16S sequences also allowed for an assessment of genetic distances in this fragment which has been routinely used for DNA barcoding of Madagascar's amphibians (e.g., Vieites et al. 2009) and also has been widely used for reptiles, thus allowing comparisons with other taxa. Within the *C. nasutum* group, established taxa had 16S distances (uncorrected pairwise distances) between 4.5% (*C. boettgeri* vs. *C. linotum*) and 12.0% (*C. radamanus* vs. *C. tjiasmantoi*). Within the *C. gallus* complex, not all subclades were represented by 16S sequences but recorded distances ranged from 3.0% (A3 to A4) to 4.1% (A1 to A4).

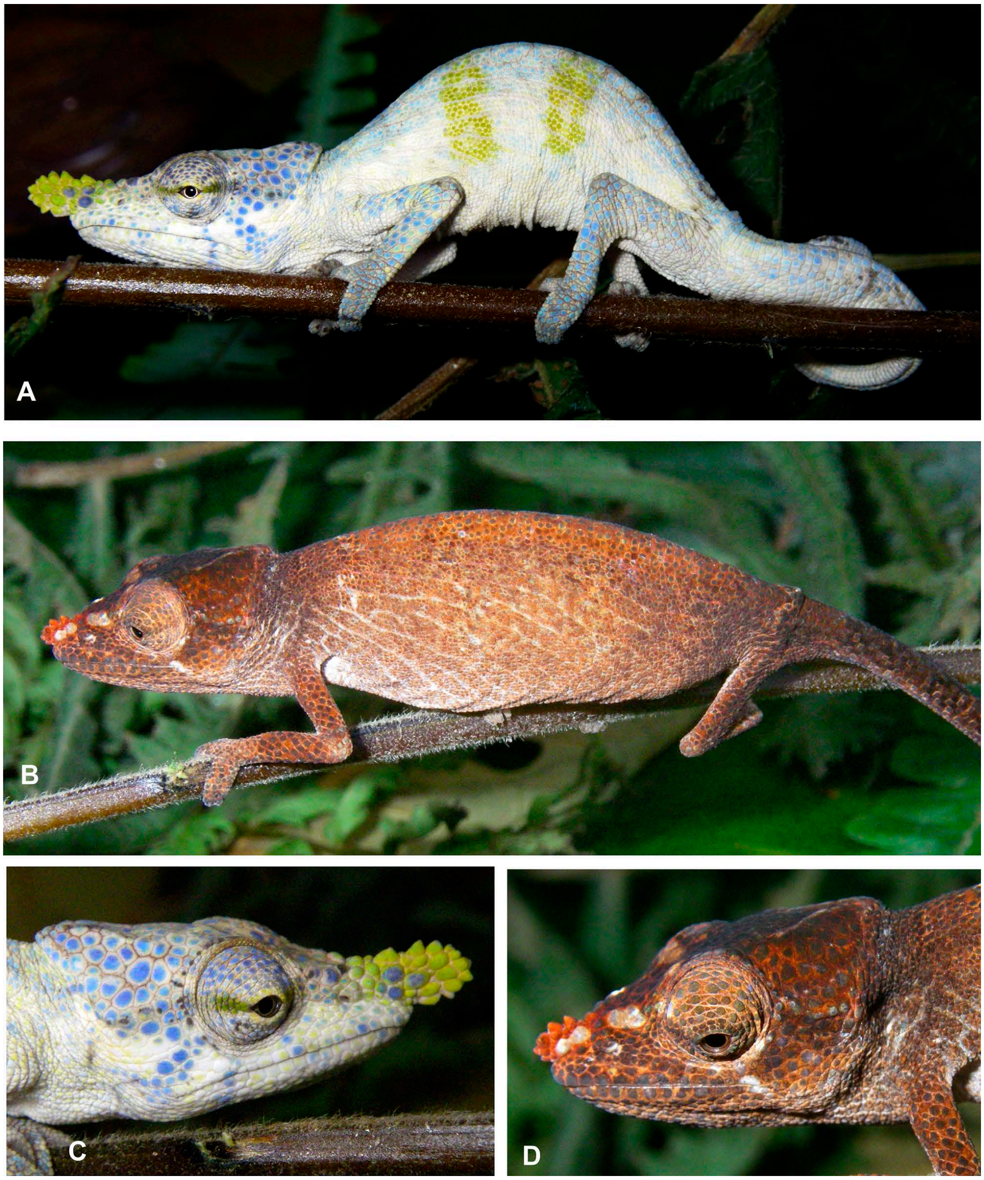

Figure 4. Specimens of *Calumma nasutum* from a bamboo forest site locally called Samalaotra in Ranomafana National Park. (A, C) male ZSM 622/2009 (ZCMV 8642); (B, D) female ZSM 623/2009 (ZCMV 8643).

Molecular and morphological identity of *Calumma nasutum*

The nomen *Chamaeleon nasutus* Duméril & Bibron, 1836 was originally based on two adult females, MNHN-RA-6643 and MNHN-RA-1994.608 (originally 6643A), and two adult males, MNHN-RA-1994.609 (originally 6643B) and MNHN-RA-1994.610 (originally 6643C), all collected by Alphonse Charles Bernier from the unspecific type locality "Madagascar" (see Duméril & Bibron 1836, Klaver & Böhme 1997, Gehring et al. 2011, Prötzel et al. 2020). Of this type series, Prötzel et al. (2020) designated the male MNHN-RA-1994.610 (6643C) (Fig. 3) as the lectotype, and provided a detailed redescription. In the current study, we sequenced archival DNA from the historical lectotype to clarify the identity of this nomen by checking its phylogenetic placement in a mitochondrial sequence-based phylogeny including all currently available data of ND2 and 16S for this group.

As in previous DNA barcode fishing studies (e.g., Rancilhac et al. 2020) where mitochondrial protein-coding and ribosomal RNA genes were targeted, 16S fragments were overrepresented compared to ND2 reads confirming previous findings in archival DNA studies (Straube et al. 2021). In the BLAST searches of the *C. nasutum* lectotype against sequences of different species of the *C. nasutum* group, maximally 23175 matching reads were found for 16S, and 1684 matching reads for ND2. Assemblies resulted in a contig of 381 bp for 16S (including two stretches of missing data, one of ca. 76 bp and one of 3 bp), and in a short contig of 77 bp for ND2. Visual inspection of the assemblies revealed a large homogeneity and concordance of overlapping reads, thus confirming the validity of the contig sequences. Exploratory single-gene phylogenetic analyses (not shown) were concordant and unambiguous in placing the lectotype of *Chamaeleon nasutus* into the *C. gallus* complex, and more specifically into clade A1 containing samples from Samalaotra in Ranomafana National Park, the nearby site Ambohitsara, and Mananjary. This relationship was strongly supported by the analysis of the concatenated data (BS = 97%; Fig. 1) which furthermore suggested that the lectotype probably is genetically closest to the specimens from Samalaotra and Mananjary (BS = 65%).

Three comparative specimens included in clade A1 in our molecular tree (Fig. 1) were available for morphological examination: ZSM 793/2003 (FGMV 2002.642), an adult male from Ambohitsara, collected on 24 January 2003 by F. Glaw, M. Puente, L. Raharivololoniaina, M. Thomas, and D. R. Vieites; ZSM 622/3009 (ZCMV 8642; adult male) and ZSM 623/2009 (ZCMV 8643; adult female), both from Samalaotra, collected on 22 February 2009 by M. Vences, L. Raharivololoniaina, S. Ndriantsoa, T. Rajoafiarison, and E. Rajeriarison. The two males are characterized by an only moderately elongated rostral appendage, which is rounded at its tip in lateral view (see Fig. 4 for the Samalaotra specimen) that strongly differs from the pointed and elongated appendage characteristic for specimens usually assigned to *C. gallus*, but is fully concordant with the character state in the lectotype of *C. nasutum* (Fig. 3). Furthermore, the female specimen ZSM 623/2009 is characterized by a remarkably short rostral appendage (Fig. 4) that fully agrees with the two female paralectotypes, both of which have rostral appendages of about 1 mm only in length (see figure in Gehring et al. 2011).

This conclusive and concordant morphological and molecular assignment of the *C. nasutum* lectotype to clade A1, leads to two main taxonomic conclusions:

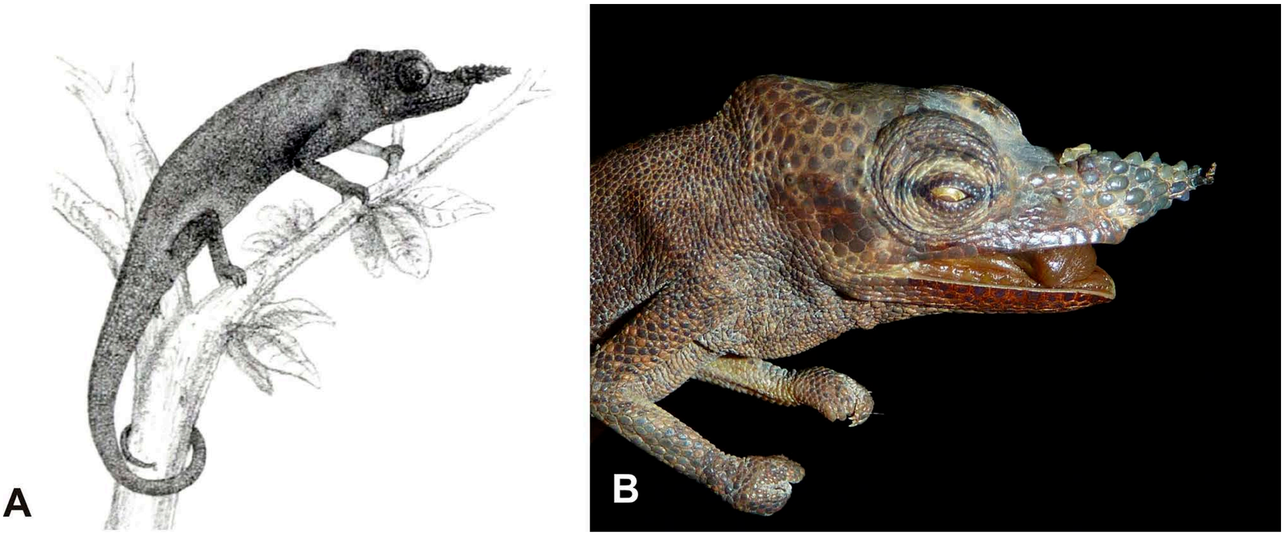

Figure 5. Male holotype of *Calumma gallus*. (A) Illustration reprinted from Günther (1877: plate XVI), note the tip of the rostral appendage, which is serrated and terminates in two tips. (B) Photograph of preserved holotype NHMUK 1946.8.21.55 (the terminal part of the dorsal appendage is bent away). Note the tubercular and serrated structure of the visible portion of the rostral appendage.

(1) Since *Chamaeleon nasutus* Duméril & Bibron, 1836 is the earliest nomen in the *C. nasutum* group, it definitely is a valid name and should be applied to specimens from clade A1, rather than to specimens from clade K as inferred by Prötzel et al. (2020).

(2) Consequently, no scientific name is available for chameleons in the mitochondrial clade K which are genetically (both in mitochondrial and nuclear-encoded DNA) and morphologically highly distinct from all other species in the *C. nasutum* group (see Prötzel et al. 2020). Therefore, lineage K specimens are named and described herein as new species *Calumma hofreiteri* sp. n. (see account below).

Identity and morphological characterization of *Calumma gallus*

Calumma gallus was originally described as *Chamaeleon gallus* Günther, 1877, based on the male holotype BMNH 1946.8.21.55 (e.g., Klaver & Böhme 1997, Glaw 2015), but recently given as "NHMUK 1946.8.21.55 (previously NHMUK 1876.10.3.7)" by Campbell & Denzer (2019) who also state the original type locality as "Mahanova", probably based on the catalogue entry. The type locality in the original description (Günther 1877) was given as "Mahanoro", with the additional information (in the introductory notes of the paper) that this locality is "a short

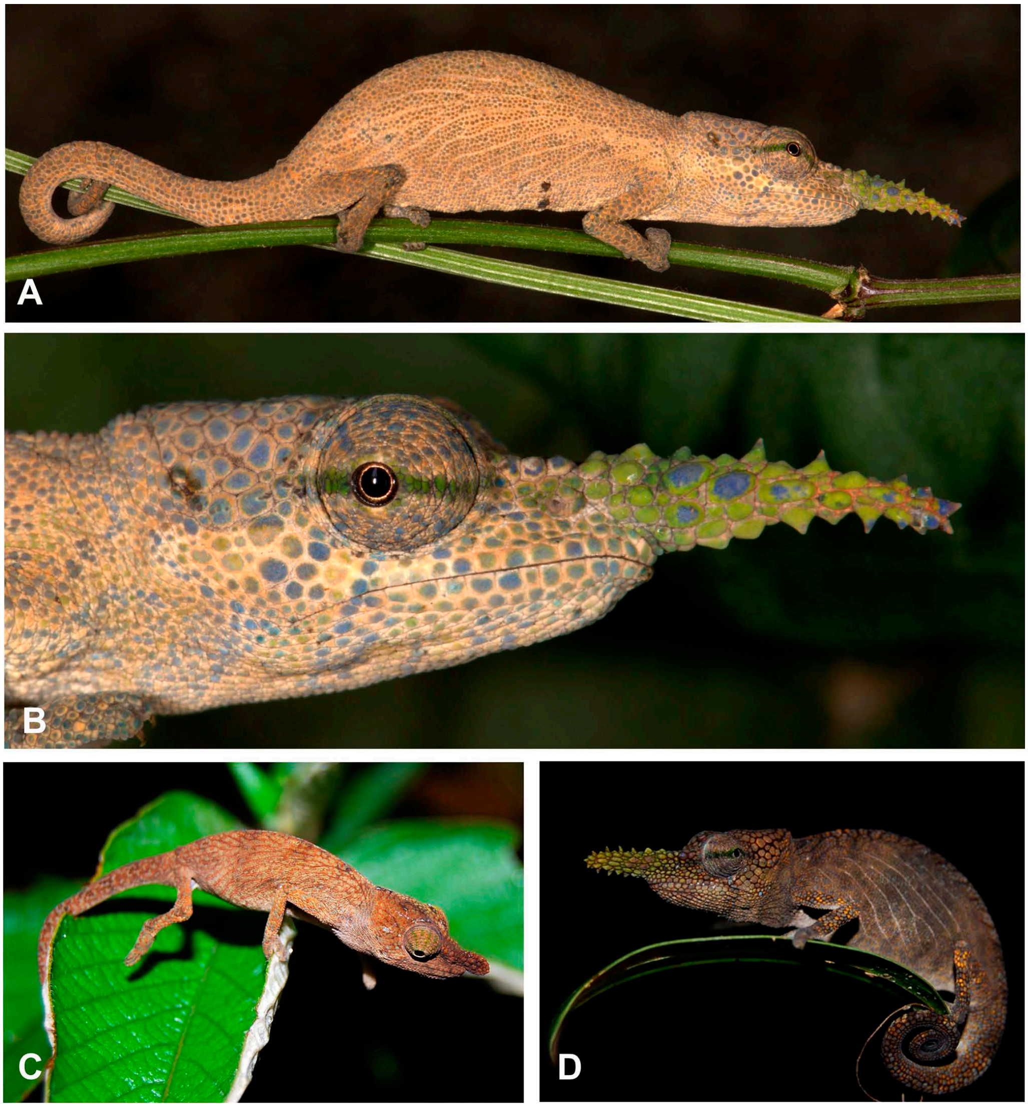

Figure 6. Specimens assigned to *Calumma gallus* (mitochondrial clade A2) and *C.* cf. *gallus* (clade A3) in life. (A, B) Male of *C. gallus* sensu stricto from Tarzanville, north of Anosibe An'Ala, ZSM 456/2010 (FGZC 4508). Note the serrated rostral appendage terminating in two tips as in the holotype (Fig. 5). (C, D) Specimens of the *C. gallus* complex sharing a serrated rostral appendage in males but belonging to a distinct mitochondrial lineage (Fig. 1): female PSG 1281 from Ambinanindrano (= Ambodisavoka, close to Marolambo) (C); male PSG 1282 from Ambinanindrano (= Ambodisavoka, close to Marolambo) (D). Note also the predominantly greenish color of the rostral appendage of the males, without colorful terminal tip.

distance south of Tamatave" (Tamatave being the French name for the town of Toamasina). In fact, Mahanoro is a rural municipality located about 9 km north of the mouth of the Mangoro River along Madagascar's eastern coast, at geographical coordinates 19.8958° S, 48.8063° E (Fig. 2).

Günther (1877) described *C. gallus*, along with several other amphibian and reptile species (*Paracontias holomelas*, *Madascincus melanurus*, *M. melanopleura*, *Aglyptodactylus inguinalis* and *Plethodontohyla notostica*) from the same type locality (Mahanoro) or from "Anzahamaru", which possibly refers to Anjahamaro, a locality that was located by Blommers-Schlösser & Blanc (1991) further north than Mahanoro, close to Toamasina. No data were given about the collector(s) of these specimens, but Günther (1877) apparently asked "M. Grandidier" (the "M" probably meaning "Monsieur", and referring to Alfred Grandidier) for advice concerning the position of the two localities. Given these uncertainties and the fact that historical localities were usually not given very precisely and often referred to the further surroundings of the nearest village, it remains unclear if the holotype of *C. gallus* has actually been collected north or south of the Mangoro River, but we here assume the collecting locality indeed coincides closely with present-day Mahanoro and thus north of the Mangoro River.

The holotype of *C. gallus*, as depicted by Günther (1877) and upon our own examination, is characterized by a pointed rostral appendage with some scales being spiny, thus giving the appendage a somewhat serrated appearance (Figs 3, 5); the specimen's size, according to the original description, is 93 mm in total length, of which 7 mm correspond to the rostral appendage and 45 mm to the tail. Its rostral appendage's shape is typical for specimens observed in the region of Marolambo, for example a male from Tarzanville (ZSM 456/2010, FGZC 4508; Fig. 6) which was placed in clade A2 in our molecular tree. Notably, clade A2 also contains samples from the coastal locality Bac Salehy (19.9861° S, 48.7856° E; see Gehring et al. 2010), which is about 10 km south of Mahanoro, but still on the northern side of the Mangoro River. Because an A2 locality is directly neighboring the *C. gallus* type locality (Fig. 2), and an A2 specimen morphologically agrees in rostral appendage shape and in general morphology with the *C. gallus* holotype, we conclude that the nomen *C. gallus* should be assigned to the genetic clade A2. Although we did not sequence archival DNA from the holotype, this assignment is robust enough to serve as basis for taxonomic conclusions:

(1) Clade A2 (*C. gallus* sensu stricto) is sister to clade A1 (*C. nasutum*). Specimens from these two lineages differ strongly in their morphology, in particular in the shape of male rostral appendages (compare Figs 4 and 6). Despite a comparatively low genetic divergence, with uncorrected pairwise distance of 8.7–9.5% in ND2 (no 16S sequences available for A2), it is highly unlikely that A1 and A2 are conspecific, especially taking into account that the sexually dimorphic rostral appendages most likely play an important role in sexual selection and thus reproductive isolation in these chameleons. Studies on continental African chameleons have delimited species of low genetic distances based on in-depth analysis of ecological differentiation, and indeed, in the African genus *Bradypodion*, ND2-distances among the majority of species are in the range between 6–10% (e.g., Tolley et al. 2022). We therefore conclude that it is plausible that chameleon species of similar levels of divergence may also exist in Madagascar, and hypothesize that A2 and A1 represent two different species that can be assigned to the nomina *C. gallus* and *C. nasutum*, respectively.

(2) Assuming that the mitochondrial gene tree (Fig. 1) corresponds to the species tree, assigning populations of clades A3 and A4 to *C. gallus* (defined as corresponding to A2) would result in paraphyly of this species. While monophyly is not a necessary criterion for a species (see Vences et al. 2024 for detailed explanation), it is of relevance that the most divergent clade A4 (sister to the clade containing A1, A2, and A3) also is morphologically distinct: the male rostral appendage is very elongated, not serrated and pointing upward (Figs 8–9) and males have a light lateral band that is indistinct or less obvious in specimens of the other clades. Given the morphological divergence (non-serrated elongated rostral appendage pointing upward) and phylogenetic position of A4 specimens, we conclude that they most likely belong to a different species. Therefore, A4 is herein formally named and described as *Calumma pinocchio* sp. n. (see taxonomic accounts below).

(3) After assigning names to mitochondrial clades A1, A2 and A4, the taxonomy of the *C. gallus* complex remains complicated due to the occurrence of A3 (from Ambodisavoka, Manombo and Marolambo) which also bears serrated rostral appendages (similar to *C. gallus*; see Fig. 6D) but is sister to the *C. gallus* + *C. nasutum* clade with limited support. Due to a very limited amount of voucher specimens available for A3, we here refrain from further taxonomic conclusions and have in the tree referred to these specimens as *C.* cf. *gallus*. We emphasize that the taxonomy of the entire complex, and our hypothesis of *C. gallus*, *C. nasutum* and *C. pinocchio* sp. n. representing distinct species, will require additional scrutiny in the future. In particular, denser sampling and contact zone analysis using genomic approaches is needed, as well as a comparative morphological analysis based on more adult specimens across the range of the *C. gallus* complex.

Taxonomy

***Calumma hofreiteri* sp. n.**

ZooBank LSID: urn:lsid:zoobank.org:act:
574EE195-8D63-4254-A434-7B3F901D1F79

Remark: This species corresponds to the mitochondrial lineage K sensu Prötzel et al. (2020), which by those authors was assigned to *C. nasutum* based on morphological similarity of lineage K specimens with the *C. nasutum* lectotype.

Holotype: ZSM 454/2010 (FGZC 4506), adult male, from a forest near Tarzanville (19.32435° S, 48.21988° E, 881 m above sea level), ca. 12 km north of Anosibe An'Ala, Northern Central East of Madagascar, collected on 13 April 2010 by F. Glaw, J. Köhler, P.-S. Gehring, K. Mebert, E. Rajeriarison, and F. M. Ratsoavina.

Paratypes: ZSM 256/2016 (FGZC 5283), adult male, from near Maromizaha (18.9555° S, 48.4658° E), Northern Central East of Madagascar, collected on 2 August 2016 by F. Glaw, D. Prötzel, J. Forster, and N. Raharinoro; ZSM 924/2003 (FGMV 2002.984), adult male, from near Andasibe (18.9229° S, 48.4186° E), Northern Central East of Madagascar, collected on 18 February 2003 by G. Aprea and collaborators.

Referred material: Several additional specimens from Sorata in northern Madagascar are here assigned to *C. hofreiteri* but explicitly not included in the paratype series due to their substantial genetic divergence from the other specimens. ZSM 1699/2012 (FGZC 3711), adult female, from the Sorata Massif (ca. 13.685° S, 49.44° E, ca. 1060 to 1485 m a.s.l), collected on 29 November 2012 by F. Glaw, O. Hawlitschek, T. Rajoafiarison, A. Rakotoarison, F. M. Ratsoavina, and A. Razafimanantsoa; ZSM 1700/2012 (FGZC 3744), adult female from the So-

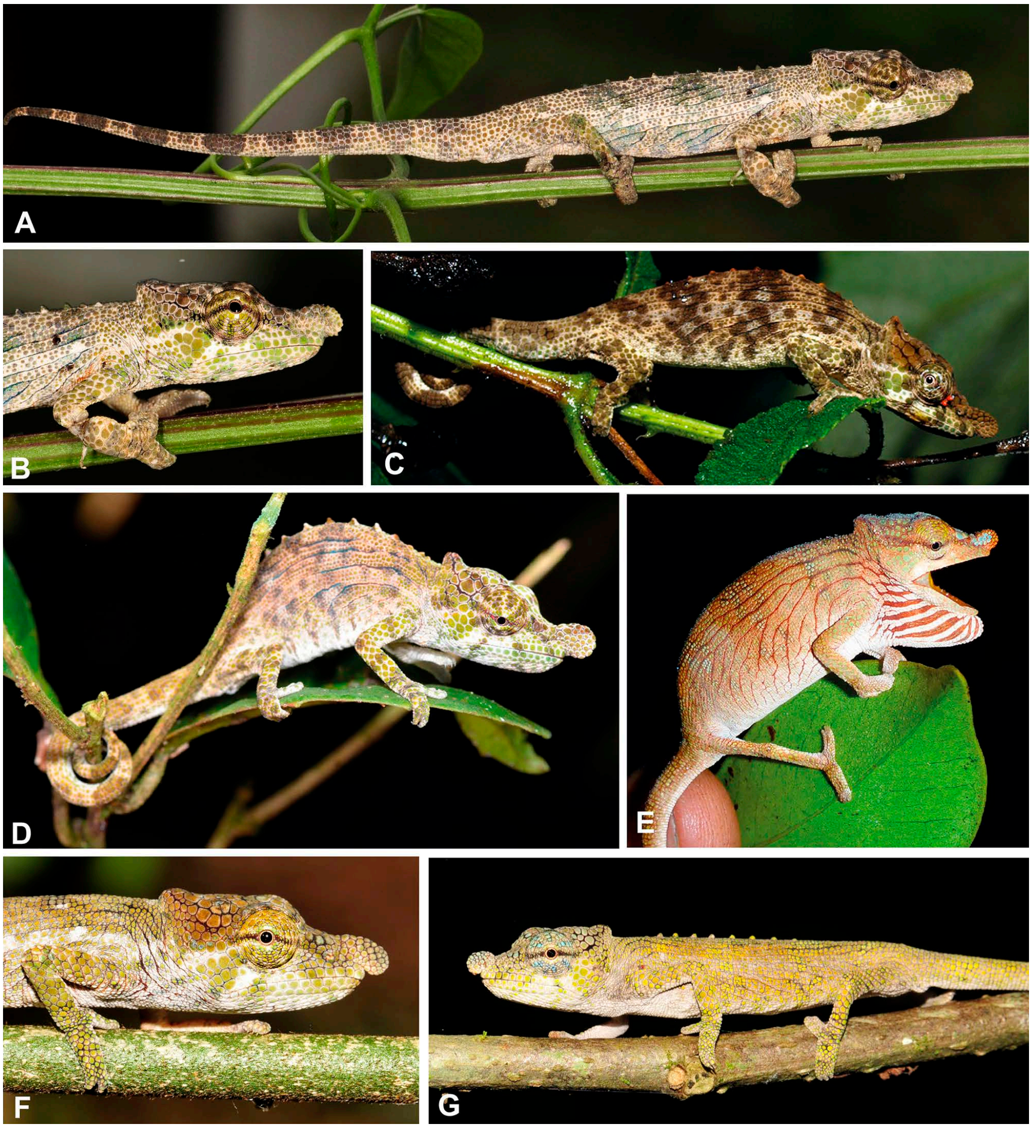

Figure 7. Specimens of *Calumma hofreiteri* sp. n. in life. These specimens correspond to the mitochondrial lineage "K", which has previously (Prötzel et al. 2020) been considered as *C. nasutum*. (A, B) Male holotype (ZSM 454/2010) from Tarzanville (12 km north of Anosibe An'Ala); (C) adult male from Andasibe (not collected) in stressed coloration; (D) subadult male from Maromizaha (ZSM 256/2016); (E) female from Maromizaha in stressed coloration (not collected); (F) female from Sorata (not reliably assignable to a voucher specimen); (G) subadult male from Sorata (UADBA-FGCZ 3636).

rata Massif, along a creek above the campsite (13.6772° S, 49.4413° E, 1394 m a.s.l.), collected on 30 November 2012 by F. Glaw, O. Hawlitschek, T. Rajoafiarison, A. Rakotoarison, F. M. Ratsoavina, and A. Razafimanantsoa. Three further specimens (not examined morphologically and thus unsexed): UADBA-FGZC 3636, from Sorata (13.6944° S, 49.4441° E, 1100 m a.s.l.), collected on 27 November 2012 by F. Glaw, O. Hawlitschek, T. Rajoafiarison, A. Rakotoarison, F. M. Ratsoavina, and A. Razafimanantsoa; UADBA-FGZC 3708, from an unspecified site in the Sorata Massif, collected on 29 November 2012 by F. Glaw, O. Hawlitschek, T. Rajoafiarison, A. Rakotoarison, F. M. Ratsoavina, and A. Razafimanantsoa; UADBA-FGZC 3740, from the Sorata Massif, (13.6772° S, 49.4413° E, 1394 m a.s.l.), collected on 30 November 2012 by F. Glaw, O. Hawlitschek, T. Rajoafiarison, A. Rakotoarison, F. M. Ratsoavina, and A. Razafimanantsoa.

Definition: Assigned to the *C. nasutum* species group based on small body size (SVL <50 mm), presence of soft rostral appendages in both sexes, arboreal habits and molecular phylogenetic relationships. Within the *C. nasutum* group, distinguishable by a combination of (1) absence of occipital lobes, (2) presence of a laterally compressed rostral appendage, which is rounded at the tip in lateral view (3) rostral scale not integrated in rostral appendage, (4) rostral appendage in males longer than 1.5 mm (its length 4.5–5.0% of SVL), (5) casque height in adult males 1.5 mm or more, (6) males with a distinct dorsal crest consisting of cones, (7) parietal crest present, (8) axillary pits absent.

Diagnosis: The new species can be distinguished from *C. boettgeri, C. gehringi, C. guibei, C. juliae, C. lefona, C. linotum, C. roaloko,* and *C. uetzi* by the absence of occipital lobes (vs. presence); from males of *C. gallus* and *C. pinocchio* sp. n. (described below) by a rounded tip of the rostral appendage (vs. spear-like), from *C. radamanus* by rostral scale not integrated in rostral appendage (vs. integrated), rostral appendage straight (vs. oriented downwards), and parietal crest present (vs. absent); from *C. vohibola* and *C. vatosoa* by male rostral appendage >1.5 mm (vs. <1.5 mm or completely lacking); from *C. emelinae, C. ratnasariae*, and *C. tjiasmantoi* by a casque higher than 1.5 mm in adult males (vs. lower), and from *C. emelinae* furthermore by a dorsal crest consisting of cones (vs. spines) in males. The new species is morphologically very similar to *C. nasutum* (with which it has been confused previously; Prötzel et al. 2020) and *C. fallax.* It can be distinguished from *C. nasutum* by a dorsal crest consisting of distinct cones in males (vs. absence) and a shorter rostral appendage making up 4.5–5.0% of SVL in males (vs. 5.3–9.0%). From *C. fallax*, it can be distinguished by a relatively shorter tail (Tal/SVL 104–105% vs. 102–124% in males, with only one out of seven *C. fallax* having a value <105%; Prötzel et al. 2020), and a relatively shorter rostral appendage in males (4.5–5.0% of SVL vs. 3.6–8.5%, with only one out of seven males having a value <5.5%; Prötzel et al. 2020), and frontoparietal fenestra absent (vs. present). Females of *C. hofreiteri*, which have no dorsal crest, cannot be reliably distinguished morphologically from females of the sympatric *C. emelinae* and other similar species according to current knowledge. Many photographs available on iNaturalist (accessed on 1 June 2025) under the names *C. nasutum* and *C. emelinae* from the Andasibe region show females without dorsal crests, making a reliable identification of many of these observations difficult.

Description of the holotype: Adult male with everted hemipenes in good state of preservation; mouth closed; SVL 43.7 mm, tail length 43.3 mm, total length 87.0 mm, ratio of TaL to SVL 0.991; for other measurements, see Table 1; rostral ridges running from the anterior edge of the eye to the snout tip where they fuse to a laterally compressed dermal rostral appendage, surpassing the upper snout tip by 2.0 mm. Rostral appendage broad at its base, not including the rostral scale and tapering to a point. Rostral appendage covered with large oval tubercle scales and smaller scales of irregular shape in the anterior half. Rostral appendage dorsally, ventrally and laterally rather smooth and flat and not serrated from any view. 15 (left) and 14 (right) large infralabials followed by ca. 5 distinctly smaller scales to the corner of the mouth; 14 (left) and 15 (right) large supralabial scales, followed by 5–6 distinctly smaller scales until the corner of mouth; supralabials with a smooth (not serrated) dorsal margin; distinct lateral crest running horizontally; temporal crest indistinct, consisting of one tubercle per side; distinct cranial crest; no parietal crest; no occipital lobes; medium sized (1.5 mm height above the dorsal line in the neck) and rounded casque; no trace of a dorsal, gular or ventral crest. Body laterally compressed with fine homogeneous scalation and larger scales on extremities, head region and on the tail, largest scale in temporal region with maximum diameter of 0.8 mm and in cheek region of 1.1 mm; no axillary or inguinal pits. Scales on the tail arranged in distinct scale rows.

Variation: The male paratype ZSM 924/2003 agrees well with the holotype (see measurements in Table 1). For variation in coloration, see Figure 7. Although the species occurs around Andasibe and therefore in one of the best-surveyed sites in Madagascar, only few reliably genotyped specimens are available at present. Future research effort is needed to assess more widely the species' morphological variation, and in particular, the morphology of topotypical females.

Etymology: The species epithet is an eponym dedicated to Michael "Michi" Hofreiter, as a recognition to his substantial contribution to the field of palaeogenomics and of his assistance with archival DNA analysis of the *C. nasutum* type, as well as numerous other name-bearing types of Madagascar's amphibians and reptiles.

Suggested common names: Hofreiter's chameleon (English), Hofreiters Chamäleon (German).

Natural history: Poorly known because many observations published under the name *C. nasutum* may subsume information on *C. hofreiteri* and other, morphologically similar species. In Andasibe, specimens probably assignable to this species can be found at night in forest edge habitats and secondary vegetation, often sleeping on leaves, at perch heights between 1–4 m. Behavioral experiments of Parcher (1974) under the name *C. nasutum* probably refer to this species or to *C. emelinae*, which occur sympatrically at Andasibe (Prötzel et al. 2020). A photograph on the internet, taken on 1 February 2023 by Frank Deschandol and Philippe Sabine (https://www.alamy.de/image553001476.html), shows a dead juvenile probably of *C. hofreiteri* or *C. emelinae* from Analamazaotra sticking on a branch of the plant *Desmodium uncinatum*. The stems of this invasive plant are covered with short hooked hairs that can entrap and kill small animals including chameleons, frogs, bats and insects (Nash & Adriamihaya 2024).

Distribution: Based on DNA barcoded samples and specimens (Figs 1–2), the species is reliably known from five locations: (1) Andasibe (including Analamazaotra-Mantadia National Park), (2) the nearby Maromizaha Reserve, (3) forest fragment near Anosibe An'Ala and (4) forest fragment near Tarzanville, as well as (5) the Sorata Massif (whose population is however genetically quite divergent: Fig. 1). The known elevational range extends from 881 m (type locality near Tarzanville) to 1394 m a.s.l. (Sorata Massif).

Calumma pinocchio sp. n.

ZooBank LSID: urn:lsid:zoobank.org:act:
E85DBDD9-165C-48AE-8925-A82886A3DE26

Remarks: This new species as defined here encompasses specimens from the northernmost populations previously referred to *C. gallus* and characterized by a non-serrated rostral appendage. Consequently, the largest portion of published records of *C. gallus* actually refer to *C. pinocchio*, including many locality records (see Fig. 2 for details) and almost all published photographs (e.g., Glaw & Vences 1994, LeBerre 1995, Henkel & Schmidt 1995, Glaw & Vences 2001). However, the image published in Oberle (1981) may not show *C. pinocchio* as the pictured specimen has much smaller scales on the lower forelimbs, a relatively short rostral appendage and no elongated scales in the rostral appendage.

Holotype: ZSM 137/2016 (FGZC 5050), adult male, from Vohimana (ca. 18.92° S, 48.50° E, ca. 800 m above sea level), Northern Central East of Madagascar, captured by a local guide and collected on 27 December 2015 by F. Glaw, D. Prötzel and L. Randriamanana.

Paratypes: ZSM 319/2000 (female), 321/2000 (male), 322/2000 (female), three adult specimens (without field numbers and tissue samples) from Vohidrazana (18.9658° S, 48.5103° E, 731 m a.s.l.), collected on 10 April 2000 by F. Glaw; ZSM 550/2001 (MV 2001-247), adult male, from Vohidrazana (18.9661° S, 48.5097° E, 810 m a.s.l.), collected on 17 February 2001 by M. Vences and D. R. Vieites; ZSM 311/2006 (ZVMV 2477), adult female, from An'Ala (forest camp) (18.91926° S, 48.48796° E, 889 m a.s.l.), collected by D. R. Vieites, M. Vences, F. Rabemananjara, P. Bora, C. Weldon and J. Patton; ZSM 138/2016 (FGZC 5075), adult female, from Vohimana near "Relais de Naturaliste" (18.9203° S, 48.5160° E, 786 m a.s.l.), collected on 28 December 2015 by F. Glaw, D. Prötzel and L. Randriamanana; ZFMK 62319, adult male with everted hemipenes, from Ambavaniasy near Vohidrazana (no coordinates available), collected in 1996 by F. Glaw; UADBA-FGZC 5074, adult male, same data as holotype (not sequenced).

Referred material: ZSM 139/2016 (FGZC 5151, adult male) and UADBA-FGZC 5099 (adult female) from near Analalava (ca. 17.7071° S, ca. 49.4599° E, ca. 30 m a.s.l), collected on 1 January 2016 by F. Glaw, D. Prötzel, and L. Randriamanana, as well as several specimens from Betampona (see Fig. 1). These specimens were not included in the type series as they belong to a genetically divergent subclade.

Definition: Assigned to the *C. nasutum* species group based on small body size (SVL ≤50 mm), presence of soft rostral appendages in both sexes, arboreal habits and molecular phylogenetic relationships. Within the *C. nasutum* group, distinguishable by a combination of (1) absence of occipital lobes, (2) spear-like and not serrated rostral appendage, (3) rostral scale not integrated in rostral appendage, (4) rostral appendage in males longer than 6 mm (its length ≥14% of SVL), (5) casque height in adult males less than 1.5 mm; (6) males without dorsal crest; (7) parietal crest absent; (8) axillary pits present.

Diagnosis: Adult males of *C. pinocchio* can be easily distinguished from all other species in the *C. nasutum* group, except *C. gallus*, by the long and spear-like rostral appendage with a length of more than 6 mm and making up more than 14% of SVL. From *C. gallus* as re-defined herein, the males of the new species can be distinguished by the non-serrated edge of the rostral appendage, with flat scales and without distinct pointed tubercles (vs. serrated shape caused by pointed tubercular scales); possibly by the rostral appendage pointing upward and probably by a smaller body size (male SVL 44–49 mm vs. 55 mm; Table 1).

Adult females of *C. pinocchio* can be easily distinguished from females of all other species in the *C. nasutum* complex except *C. gallus* and *C. nasutum* by the orange-red color of the rostral appendage (vs. not red or orange) and the reticulated pattern along the flanks (vs. usually not reticulated). They can be distinguished from *C. nasutum* females by a longer rostral appendage (4.8–7.3% vs. 2.7–3.0% of SVL). Differences to females of *C. gallus* cannot be reliably assessed at present due to the scarcity of reliably identified voucher specimens of that species.

Description of the holotype: Adult male with everted hemipenes in good state of preservation; mouth closed; SVL 47.2 mm, tail length 43.3 mm, total length 90.5 mm, ratio of TaL to SVL 0.917; for other measurements, see Table 1; rostral ridges running from the anterior edge of the eye to the snout tip where they fuse to a spear-shaped and long laterally compressed dermal rostral appendage pointing upward, surpassing the upper snout tip by 7.2 mm. Rostral appendage broad at its base, not including the rostral scale and tapering to a point. Rostral appendage covered

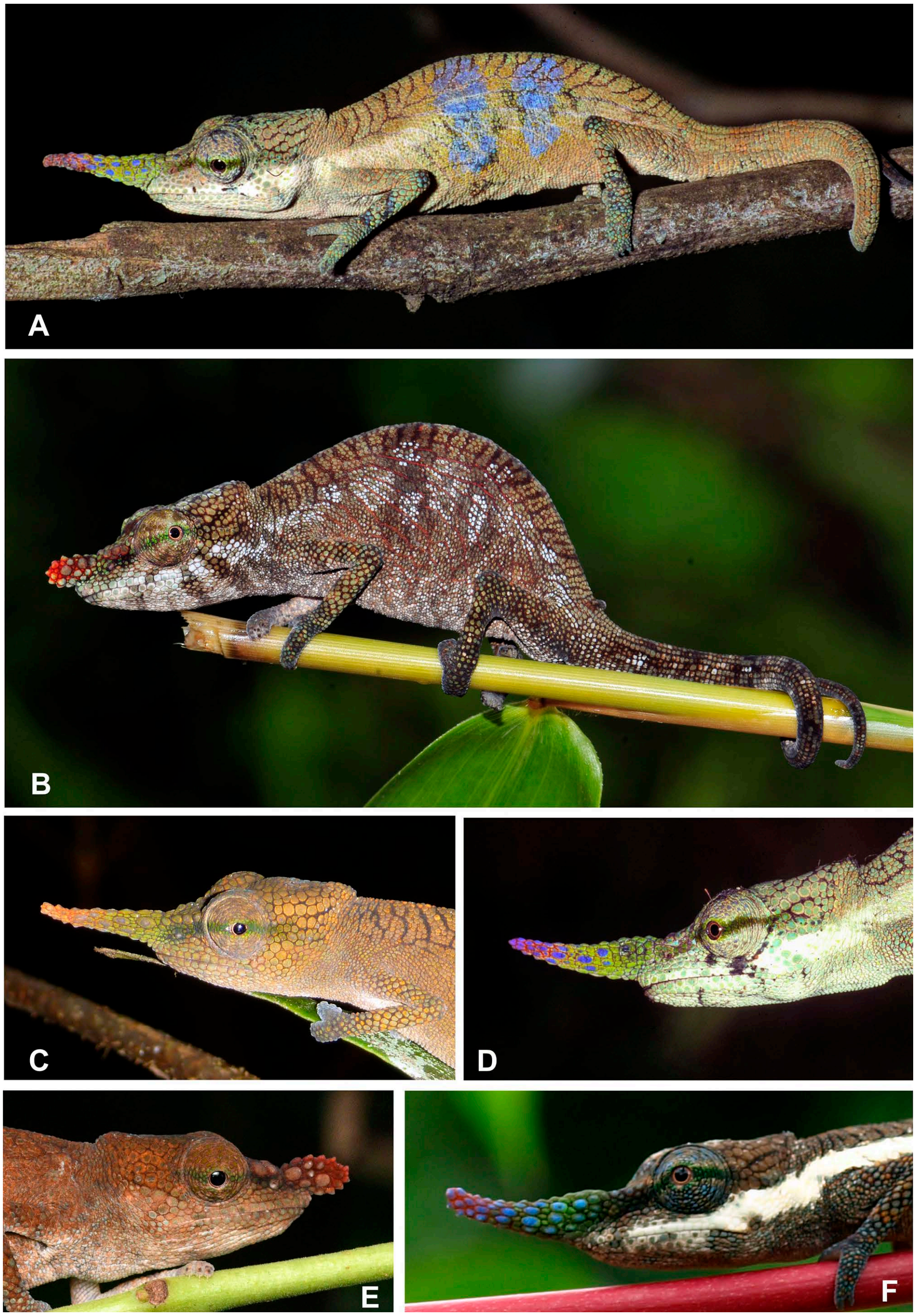

Figure 8. Specimens of *Calumma pinocchio* sp. n. in life: (A) adult male from Vohidrazana, photographed in 2000; (B) adult female from Vohimana (ZSM 138/2016), photographed December 2015; (C) adult male from Vohimana; (D) adult male from near Ambavaniasy (next to Vohidrazana), photographed 1996; (E) female from Vohimana, photographed 2022; (F) adult male from Sahafina, photographed April 2009.

with large oval tubercle scales and smaller scales of irregular shape in the anterior half. Rostral appendage dorsally, ventrally and laterally rather smooth and flat and not serrated from any view. 15 (left) and 14 (right) large infralabials followed by ca. 5 distinctly smaller scales to the corner of the mouth; 14 (left) and 15 (right) large supralabial scales, followed by 5–6 distinctly smaller scales until the corner of mouth; supralabials with a smooth (not serrated) dorsal margin; distinct lateral crest running horizontally; temporal crest absent; distinct cranial crest; no parietal crest; no occipital lobes; medium sized (1.5 mm height above the dorsal line in the neck) and rounded casque; no trace of a dorsal or gular or ventral crest. Body laterally compressed with fine homogeneous scalation and larger scales

Figure 9. Males and females from populations assigned to *Calumma pinocchio* sp. n., but belonging to a divergent mitochondrial lineage, in life. (A, D) Adult males from Betampona; (B, C) adult females from Betampona; (E) adult male from (near) Analalava. Note the lack of distinct serrations in the male rostral appendages, typical for *C. pinocchio* sp. n., the lack of colorful appendage tips (differing from topotypical specimens), and the rostral appendage of males pointing upward.

on extremities, head region and on the tail, largest scale in temporal region with maximum diameter of 1.1 mm; axillary pits present. Scales on the tail arranged in distinct scale rows. When illuminated with UV light, the holotype showed 6–7 fluorescent tubercles at the posterior edge of the eye and 5 fluorescent tubercles along the lateral crest.

Variation: Specimens examined from Vohimana and Vohidrazana agree in morphology (Table 1). In life during the day, males can easily be recognized by the red, green and blue color at the tip of the rostral appendage (Fig. 8), and some red color is usually also visible in sleeping individuals at night (Fig. 8C). This striking color is not known from the genetically divergent specimens from Analalava and Betampona (Fig. 9), although the available images are all from sleeping or stressed individuals and thus may not reflect the regular diurnal color. Males from Vohidrazana/Vohimana and Analalava/Betampona agree however in the non-serrated shape and relative length of the rostral appendage (Figs 8–9; Table 1). Total lengths of specimens measured here range between 83–93 mm in males and 86–94 mm in females (Table 1). Size records of up to 17 cm total length (e.g., Schmidt et al. 2010) for this or any other species in the *C. gallus* complex are doubtful.

Etymology: The species epithet is a noun in apposition, derived from the fictional character and protagonist of the children's novel "The Adventures of Pinocchio", written by the Italian writer Carlo Lorenzini, better known by the pseudonym Carlo Collodi, in 1883. The elongated and pointed rostral appendage of this chameleon is reminiscent of Pinocchio's long nose which grows when he lies, although in *C. pinocchio* the appendage may well be an honest signal of communication reflecting male fitness.

Suggested common names: Pinocchio chameleon (English), Pinocchio-Chamäleon (German).

Natural history: In general, knowledge of the habitats and life history of *C. pinocchio* is limited. The species has been found inside degraded primary forest and in secondary bushes and even in high grass at the border of meadows at Vohimana, more often found in secondary vegetation in forest edge situations than in dense primary forest. In contrast to several other species in the *C. nasutum* group (e.g., *C. juliae*; Prötzel et al. 2018b), adult males are encountered frequently and are not obviously rarer or more difficult to see than females. At the end of November, we found pairs of one male and one female close to each other, sleeping on roosts 10–20 cm apart. The species occurs in sympatry with other species of the *C. nasutum* group, including *C. radamanus* in Vohidrazana (Prötzel et al. 2020) and Betampona. Captive breeding most likely referring to *C. pinocchio* was described by Schmidt et al. (2010): During the courtship the male approaches the female with rhythmic head nodding. Copulation takes up to 10 minutes, and 40–45 days after mating 2–4 eggs are buried in the ground in a humid place. Hatching of juveniles occurs after approximately 130 days of incubation at 20–24 °C.

The population density studies of *C. gallus* by Andriantsimanarilafy et al. (2022) in the evergreen forests of Ambatofotsy (19°32'24.9" S, 48°18'37.5" E, 790–1140 m a.s.l.), Ankorabe (19°38'45.21" S, 48°02'02.23" E, 700–790 m a.s.l.; 94.4 individuals per hectar) and Tarzanville (19°19'38.72" S, 48°13'31.61" E, 810–960 m a.s.l.; 65.3 individuals per hectare) all refer to the true *C. gallus* (clade A2, see Figs 1–2) and not to *C. pinocchio*.

Distribution: Genetically confirmed records of *C. pinocchio* (Figs 1–2) are from (1) Vohimana (2) Vohidrazana, (3) An'Ala, (4) Sahafina, and (genetically divergent subclade) from (5) Betampona and (6) Analalava. For other localities recorded for *C. gallus* in the literature, see Discussion below. The reliably known elevational range of the species is between 30 m (Analalava) and 889 m (An'Ala) above sea level.

Discussion

Species delimitation and reliance on mtDNA and morphology

Besides sequences of the mitochondrial ND2 gene, our previous studies of the *C. nasutum* group also analyzed a nuclear-encoded DNA fragment of the c-mos gene (e.g., Gehring et al. 2012, Prötzel et al. 2020). For the *C. gallus* complex, sequences of c-mos included in the haplotype network of Prötzel et al. (2020) formed a phylogroup of six haplotypes that differed by a minimum of five mutational steps from other species, and Gehring et al. (2011) included a haplotype network in which a specimen from Ambohitsara (i.e., *C. nasutum* as redefined herein) was in the *C. gallus* complex phylogroup but differing by four mutational steps. For the present study, we attempted to obtain additional nuclear gene data, i.e., from more specimens and more gene fragments. The results were, however, inconclusive and in some cases could not be replicated by repeated sequencing which we attempted multiple times. This suggests the possibility of PCR cross-contamination caused by poor DNA quality of some templates that were obtained from tiny pieces of tissue, blood or saliva swabs without voucher collection. Although we support publication of inconclusive data sets (e.g., Wüster et al. 2024) this obviously does not apply to data sets suspected to be flawed by technical error as in this case. Future revisions of the *C. gallus* complex will require new sampling of fresh material of all main lineages for obtaining reliable nuclear-encoded DNA sequences, and ideally phylogenomic approaches, to fully clarify species limits.

Distribution of the *C. gallus* complex

According to our mitochondrial tree (Fig. 1), *C. nasutum* is part of the major clade A of Gehring et al. (2012) and subsequent molecular studies, which in Prötzel et al. (2020) has been named the *C. gallus* complex. For the sake of consistency, we here continue with this informal terminology, although historically speaking the nomen *C. nasutum* precedes *C. gallus*. The molecular data provided herein confirmed 17 localities for the *C. gallus* complex (including our redefined *C. nasutum*), and furthermore we included Mahanoro, the type locality of *C. gallus,* in our map (Fig. 2). Numerous other locality records of *C. gallus* (i.e., the *C. gallus* complex as defined here) have been published in the past, but mostly without mentioning specific voucher specimens or providing illustrations. Brygoo (1971) listed the following records from the literature: Ampasimbe according to Werner (1902), Ile aux Prunes according to Boettger (1913), Karianga (forest) according to Angel (1930, 1942), Nosy Be according to Mertens (1933), Ambavaniasy according to Brygoo (1963), and Andapa according to newly examined specimens. Furthermore, Brygoo (1978) reported on specimens collected by G. Ramanantsoa at Lokomby near Manakara; Brady & Griffith (1999) and Rakotondravony (2004) studied this species at two localities in the Mantadia region (Sity forest and Vohidrazana); Glaw & Vences (2007) listed Andekaleka, Mahanoro, Manombo, and Vohidrazana; Jenkins et al. (2011) listed the locality Zahamena without providing a reference; and the tree of Prötzel et al. (2020) contains sequences purportedly from specimens in Ambatoroma and Vohibola. Of these records, Mahanoro is the type locality of *C. gallus;* Manombo and Vohidrazana are confirmed by molecular data herein, Ambavaniasy is a village very close to two sites confirmed by molecular data, Vohidrazana and Vohimana; and Andekaleka is based on a photographic record of a male provided in Glaw & Vences (1994: color photo 204, taken by François LeBerre) most likely assignable to *C. pinocchio*. The occurrence in Zahamena National Park remains without reference, but is plausible and might be based on personal communication. However, several of the remaining localities require more discussion and scrutiny, as in the following.

Brady & Griffith (1999) and Rakotondravony (2004) studied *C. gallus* at two localities in the Mantadia region, named Sity forest (18°55' S, 48°29' E, 865 m asl) and Vohidrazana (18°58' S, 48°30' E, 875 m asl). However, Vohidrazana is outside of the Mantadia National Park, and the coordinates of the Sity forest correspond to the forest of An'Ala, which is likewise outside of Mantadia.

The record from Ile aux Prunes, a small island north of Toamasina, is based on two putative females with rudimentary rostral appendages (ZMB 18999 and SMF 16456) which were found to belong to *Calumma vohibola* by Gehring et al. (2011).

Another disputable locality is Nosy Be, located in the Sambirano Region in northern Madagascar. This record is particularly dubious and requires clarification; according to Brygoo (1971) it is based on a specimen numbered "13352" from the "Musée de Hambourg" examined by Mertens (1933). In fact, the record is supported by two males occurring in the natural history collections of Hamburg (ZMH 13352; Mertens 1933) and München (ZSM 868/1920) which apparently were both obtained from the same collector ("Schneider in 1884"), as was a specimen in the museum of Strasbourg without locality data (Angel 1950, cited after Brygoo 1971). The two males purportedly from Nosy Be strongly resemble *C. pinocchio* concerning the morphology of the rostral appendage but intensive field work on Nosy Be (Andreone et al. 2003 and many subsequent surveys) never confirmed the existence of *C. gallus*, which should be a species relatively easy to record on the island. In addition, no photographs of this species from Nosy Be are available on iNaturalist (as of 31 May 2025) and its occurrence on Nosy Be would be highly unexpected given the large distance from its known distribution range (see Fig. 2). We therefore consider this locality as unreliable.

A further locality from the above list located in northern Madagascar is "cuvette d'Andapa", a record based on one male specimen with the number "512/C" examined and depicted by Brygoo (1971). Andapa is about 350 km north of the northernmost genetically confirmed site, Analalava (Fig. 2). The digital catalogue of the Muséum National d'Histoire Naturelle of Paris, consulted in 2024, does not contain any *C. gallus* from Andapa, and we therefore suspect that Brygoo did not catalogue this specimen in the MNHN. So far, no other reliable record of *C. gallus* from northern Madagascar has become available. The specimen illustrated by Brygoo (1971) has a pointed, non-serrated and relatively short rostral appendage and apparently no elevated casque. In comparison, the specimen is reminiscent of *C. pinocchio* but its shorter appendage (made up by much fewer scales) and less elevated casque may indicate it is another (still unidentified and perhaps still unknown) species of the *C. nasutum* group. For the time being, we consider the occurrence of the *C. gallus* complex in Andapa and in the whole of northern Madagascar as in need of confirmation (although not necessarily in error).

The tree of Prötzel et al. (2020) based on ND2 sequences contains a sample from Ambatoroma. This name refers to a campsite (precise geographical coordinates not recorded) in the Manompana/Befanjana forest about 150 km north of Toamasina. The female specimen (ZSM 691/2009, ZCMV 7197) was included twice in the original tree of Prötzel et al. (2020) and its ND2 sequence was identical to that of specimens of *C. pinocchio* from Vohidrazana and An'Ala. Given that sequences from Betampona and Analalava included herein are distinctly different from those from Vohidrazana and An'Ala, we consider it as phylogeographically highly unlikely that the same sequence would reappear much further north, although we cannot fully exclude the possibility that both lineages represent different species that occur sympatrically. We thus hypothesize a sample or DNA template confusion or mislabeling and the record as in need of confirmation. We have therefore excluded this sequence from our final phylogenetic analysis (Fig. 1).

Finally, the locality Vohibola (sample PSG 262 in the tree of Prötzel et al. 2020) also requires comments. In the analysis of these authors, the sequence corresponding to this sample was almost identical to a sequence of sample PSG

318 from Sahafina (both in clade A4, thus corresponding to *C. pinocchio*). We here added one further Sahafina sample to the analysis (PSG 263) which turned out to be fully identical in its ND2 sequence to PSG 262. We here hypothesize that the Vohibola record is incorrect and likely due to mislabelling of a sample from Sahafina because (i) PSG 262 and 263 are immediately consecutive sampling from an expedition that first visited Sahafina and then Vohibola, (ii) the two samples yielded identical ND2 sequences which is unlikely for samples occurring in these two separate forest fragments, (iii) no other *C. gallus* complex records from Vohibola are available and the species was not reported for this site in the specific expedition report of Gehring et al. (2010).

Conservation and Red List status

Proposing a Red List status (IUCN 2020) for the species discussed herein (*C. hofreiteri, C. nasutum, C. gallus, C. pinocchio*) is hindered by several remaining uncertainties about their distribution and status.

Calumma hofreiteri is known from a wide distribution range if the genetically divergent population from Sorata in northern Madagascar is considered as conspecific, but many of the five known locations are small forest fragments under strong pressure by slash-and-burn agriculture. In the national network of protected areas managed by Madagascar National Parks, the species is only known to occur in Analamazaotra-Mantadia National Park. It may be assigned a status of Vulnerable according to IUCN criteria B1a,biii, i.e., an extent of occurrence <20,000 km^2, less than 10 known threat-defined locations, and continuing decline of the available habitat (IUCN 2012).

Calumma nasutum as redefined in this paper is known from only three sites and a small range probably not exceeding 5000 km^2. It occurs in Ranomafana National Park but the other two known locations are unprotected and under heavy anthropogenic pressure. It therefore may be appropriate to assign a status of Endangered to this species based on criteria B1a,biii, i.e., an extent of occurrence < 5,000 km^2, ≤ five known threat-defined locations, and continuing decline in extent and quality of the habitat (IUCN 2012).

Calumma gallus as redefined in this paper and excluding *C.* cf. *gallus* is only known from five localities at maximum distances of about 100 km from each other, which are mostly tiny and unprotected forest fragments under heavy anthropogenic pressure, suggesting a status of Endangered based on criteria B1a,biii, i.e., an extent of occurrence <5,000 km^2, ≤ five known threat-defined locations, and continuing decline in extent and quality of the habitat (IUCN 2012). The application of a wider species concept including *C.* cf. *gallus* would result in the status of Vulnerable (extent of occurrence < 5,000 km^2, ≤ 10 known threat-defined locations, and continuing decline of the extent and quality of the habitat.

Calumma pinocchio as defined herein is known from an extent of occurrence of < 5,000 km^2. Including the genetically divergent populations from Analalava and Betampona, its known distribution extends at least over a stretch of 170 km in the low to mid elevational rainforests of the Northern Central East of Madagascar. In this area, rainforests at lower elevations are highly fragmented and the remaining fragments are under constant pressure from slash-and-burn agriculture. Most of the known sites receive some kind of protection, and Betampona is a Strict Nature Reserve managed by Madagascar National Parks. Although *C. pinocchio* prefers open forest edge habitats, it has not been found in fully deforested areas. Considering this situation, it makes sense to also apply to *C. pinocchio* the current IUCN status of Endangered of *C. gallus* (Jenkins et al. 2011) given that this evaluation was largely based on records of *C. pinocchio*.

Function and evolution of rostral appendages in the *Calumma nasutum* group

Why has *C. pinocchio* evolved such a long and colorful rostral appendage? External body ornaments such as rostral appendages are a striking and highly diversified feature of many chameleon species and several other lizard species (e.g., Tilbury 2018, Ineich et al. 2022, Scherz et al. 2022). A variety of functions could a priori be invoked for such ornaments: they could serve a function of crypsis, helping the animal to blend in the environment of twigs and leaves they live in; they could be used in aggressive interactions, e.g., as physical weapon in intraspecific combat or interspecific predator deterrence; or they could be used in intraspecific signaling, e.g., for the purpose of courtship or aggressive display.

Ornamentation in chameleons is known as being correlated to fighting ability, via emphasis during displays and partly in direct male–male contests (Stuart-Fox 2014). Parcher (1974) studied three chameleons with rigid rostral appendages (*Calumma brevicorne, C. parsonii,* and *Furcifer willsii*) and found that the appendages were used in agonistic encounters in those species. Van Kleeck-Hann & Wiens (2023) consequently referred to rostral appendages and other ornaments as sexually selected "weapons" due to their sexual dimorphism. However, in the *C. nasutum* group, the appendage is not rigid but rather a flexible lobe consisting mostly of skin that bends when it comes into contact, even gently, with other objects. Parcher (1974) also studied a species of the *C. nasutum* group (most likely *C. hofreiteri*) and found that removal of the appendage in females reduces the ability of males to recognize the female as conspecific. In another species with rigid rostral appendages, *Furcifer labordi,* Karsten et al. (2009) found that the appendage was only used during courtship but not in male–male combat. These authors hypothesized that chameleons with rigid rostral appendages have evolved them for male–male combat via intrasexual selection, whereas flexible rostral appendages are used in courtship behavior and evolved via intersexual selection. Many aspects of the displays of male chameleons and males of other lizard species serve to enhance their apparent size, such as ventrolateral flattening of the body or throat engorgement (e.g., Stuart-Fox 2014, Ineich et al. 2022). Larger males may possess an advantage in male–male combat, therefore any characteristic that makes one male appear larger than another could be beneficial. Additionally, females may be more attracted by males with an extended appendage, potentially because it makes those males appear larger or for other reasons. However, according to Stuart-Fox & Ord (2004), larger males do not always have advantages. This entire hypothesis could be experimentally tested in captive animals using, for example, artificial proboscises of different lengths and/or colors placed on the snout of males and females (Ineich et al. 2022).

The rostral appendages of male *C. gallus* and *C. pinocchio* are relatively longer than in any other species of the *C. nasutum* group, with 15–20% of SVL. In contrast, male appendage length in other species range to a maximum of 9.0% in *C. nasutum* (as redefined herein) and 8.5% in *C. fallax* (see measurements in Prötzel et al. 2020 and herein). The shortest appendages are found in *C. vohibola* which almost completely lacks rostral appendages in both sexes. The poorly known *C. vatosoa* has no rostral appendage at all, but so far, its phylogenetic assignment to either the *C. gastrotaenia* group or the *C. nasutum* group is tentative, since no genetic data are yet available for this species (Andreone et al. 2001, Prötzel et al. 2016, 2020). The *C. gallus* complex is also exceptional for two other axes of rostral appendage variation: firstly, within the complex, there is a high variation in rostral appendage length and shape, and apparently evolutionarily fast transitions occurred between rounded and moderate-sized appendages such as in *C. nasutum,* and long and pointed appendages as in *C. gallus.* Secondly, sexual dimorphism in rostral appendage length and shape is extreme, with very short vs. moderate-sized appendages in females vs. males of *C. nasutum,* and moderate-sized vs. very long appendages in females vs. males of *C. pinocchio*.

A further remarkable feature of the rostral appendages in the *C. gallus* complex is their coloration. In males, it can vary from greenish with blue elements as in *C. nasutum* and *C. gallus* (Figs 4, 6), green-brownish uniform or with reddish color on the tip (*C. pinocchio,* especially from Betampona; Figs 8C, 9) or greenish with some blue elements and a pink-purple tip (*C. pinocchio*; Fig. 8A, D, F). Female appendages appear to consistently lack green and blue elements and instead are usually red or red-brown, entirely or in their proximal half. Such red appendages are not found in any other species of the *C. nasutum* group and therefore in most cases allow to identify a female as belonging to the *C. gallus* complex. Bright color can also be found on the rostral appendages of other species, e.g., *C. fallax, C. linotum, C. radamanus* (blue), *C. emelinae* (reddish brown) or *C. gehringi* (bright green) (Glaw & Vences 2007, Prötzel et al. 2017, 2020), the combination of several of these elements especially in *C. pinocchio* makes appendage color pattern more complex in this species compared to all other species of the group, and is exceptional even among all chameleons.

Taken together, the available data support that in the *C. nasutum* group, the rostral appendage has a primary function in intraspecific communication. Based on Parcher's (1974) experiments it is likely that these appendages are of importance for the animals to recognize each other as conspecifics, and along with Karsten et al. (2009) and Ineich et al. (2022) we hypothesize that they play a role in courtship and are influenced by intersexual selection. This also supports the value of rostral appendage length, shape and color for species delimitation as applied herein. However, it remains unanswered why such an exceptional expression and variation in length, sexual dimorphism and color occurs in the *C. gallus* complex in comparison to other *Calumma* groups. According to the available data, *C. gallus* and *C. pinocchio* are among the smallest *Calumma* species, together with the recently described *C. roaloko* which does not belong to this complex (Prötzel et al. 2018a). Given the ease of behavioral experiments in chameleons where responses of specimens to color or morphology of other specimens are recorded (e.g., Parcher 1974, Stuart-Fox et al. 2006, Dollion et al. 2020, Keren-Rotem et al. 2024), it should be possible to design experimental setups where the importance of length, shape and color of rostral appendages for intraspecific communication and mate choice is elucidated. We flag the *Calumma gallus* complex as one of the taxa where such experimental studies could be particularly insightful.

Convergent evolution of lobe-like rostral appendages in chameleons

Arboreal chameleons have different kinds of rostral appendages, including paired or unpaired rigid structures with underlying bones and covered by scaly skin, keratinized horns not covered by skin or scales, and flexible lobe-like structures such as those found in the *C. nasutum* group. Van Kleeck-Hann & Wiens (2023) found that in general, ornaments of potential function as weapons have evolved multiple times in chameleons, and weapon innovations in their analysis were generally more frequent than their losses, but equally common in rostral appendages (13 gains and 13 losses). However, their analysis did not differentiate between the various distinct types of rostral appendages despite some of them, like the flexible lobe-like appendages of the *C. nasutum* group, probably not qualifying as true weapons (Parcher 1974, Karsten et al. 2009; see previous section). Lobe-like rostral appendages are not found in other chameleon species in Madagascar besides the *C. nasutum* group, but can be found in species of *Rhampholeon* such as the *R. uluguruensis/moyeri* complex (Menegon et al. 2022) and perhaps most obviously in *R. acuminatus* (Mariaux & Tilbury 2006), whereas the Seychellean *Archaius tigris* has a lobe-like projection on the chin which projects forward (similar but smaller and not forward-projecting chin flaps are also found in *Rieppeleon*; see Townsend et al. 2011). Interestingly, these are all relatively small-sized chameleons, with maximum SVL up to

55 mm in the *C. nasutum* group (Prötzel et al. 2020 and herein), up to 57 mm in *Rhampholeon acuminatus* (Mariaux & Tilbury 2006), up to 53 mm in the *Rhampholeon uluguruensis/moyeri* complex (Menegon et al. 2022), and up to 82 mm in *A. tigris* (Bourgat & Domergue 1971). A more comprehensive analysis of chameleon ornaments in relation to life history and natural history traits is necessary to fully comprehend the sexual and natural selection mechanisms driving the evolution of these structures.

The importance of collecting biological specimens

Our partial revision of the *C. gallus* complex exemplifies the difficulties in taxonomically assessing taxa with incomplete sampling. This affected our current study at various levels. First, these chameleons occur in an area of relatively low-elevation rainforest along Madagascar's east coast where primary habitat is extremely fragmented, and it might be impossible by now to fully reconstruct the original ranges and sample the geographical contact zones of all lineages identified. Secondly, in some of our field campaigns we minimized the numbers of collected voucher specimens and instead only took tiny tissue or blood samples, or saliva swabs, of the encountered chameleons which were subsequently released. Consequently, we could analyze more samples for genetics than for morphology, and simply do not have information on adult morphology from some sites. Lastly, the problems in obtaining reliable DNA sequences of nuclear-encoded markers, exacerbated by limited quantity and poor DNA quality of several samples, impacted our ability to verify species delimitation with unlinked markers.

The *C. gallus* complex is therefore a prime example illustrating why collecting biological specimens (i.e., voucher specimens, even if only a limited number of individuals per site) is still important (Rocha et al. 2014, Clause et al. 2016) for integrative taxonomy (Padial et al. 2010), which in turn establishes the baseline for conservation management. Future work should target several of the rainforest fragments where the mitochondrial lineages A1, A2 and A3 (i.e., C. *nasutum, C. gallus, C.* cf. *gallus*) are found, as well as additional interspersed fragments since these small chameleons are able to survive even in small patches of substantially degraded vegetation. To be of maximal value for taxonomic work, each voucher should be of an adult specimen, ideally including specimens of both sexes, and accompanied by metadata as detailed, i.e., precise geographical coordinates, habitat, natural history, color photos in life made at night and during the day, as well as by appropriately preserved tissue samples for DNA and RNA sequencing.

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