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Splitting and lumping: An integrative taxonomic assessment of Malagasy chameleons in the *Calumma guibei* complex results in the new species *C. gehringi* sp. nov.

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Abstract

Calumma guibei (Hillenius, 1959) is a high-altitude chameleon species from the Tsaratanana massif in north Madagascar. Since its description was based on a juvenile holotype, its taxonomic identity is uncertain and little is known about its morphology. A recent molecular study discovered several deep mitochondrial clades in the Tsaratanana region assigned to *C. guibei* and *C. linotum* (Müller, 1924). In this paper we study the taxonomy of these clades and clarify the identity of *C. guibei.* Using an integrative taxonomic approach including pholidosis, morphological measurements, osteology, and molecular genetics we redescribe *C. guibei* and describe the new species *C. gehringi* sp. nov. which comprises two deep mitochondrial lineages. In terms of external morphology the new species differs from *C. guibei* by an elevated rostral crest, the shape of the notch between the occipital lobes (slightly connected vs. completely separated), presence of a dorsal and caudal crest in males (vs. absence), and a longer rostral appendage in the females. Additionally, we analysed skull and hemipenis morphology using micro-X-ray computed tomography (micro-CT) scans and discovered further differences in skull osteology, including a large frontoparietal fenestra, and separated prefrontal fontanelle and naris in *C. guibei*. Furthermore, we provide a comparison of micro-CT scans with traditional radiographs of the skull. The hemipenes have ornaments of two pairs of long pointed cornucula gemina (new term), two pairs of dentulous rotulae, and a pair of three-lobed rotulae, and are similar in both species, but significantly different from other species in the *C. nasutum* group. Geographically, *C. guibei* has been recorded reliably from the higher elevations of the Tsaratanana Massif above 1580 m a.s.l., whereas *C. gehringi* sp. nov. is found at mid-altitude (730–1540 m a.s.l.) in Tsaratanana and the surrounding area.

Key words

Calumma guibei, *Calumma gehringi* sp. nov., Chamaeleonidae, micro-computed tomography, hemipenis morphology, skull structure, Madagascar, diceCT.

Introduction

Chameleons are a characteristic element of the herpetofauna of Madagascar, and show an impressive diversity (Tolley *et al*., 2013). Although the island is only a fraction of the size of the African continent, it hosts nearly the half (86) of the currently recognized 207 chameleon species (Glaw, 2015; Menegon *et al*., 2015; Hughes *et al*., 2017). Although they are charismatic and attractive animals, their species-level taxonomy remains poorly studied. The Madagascar-endemic genus *Calumma* in particular has increased by eight species (24%) over the last decade (Glaw, 2015). Despite this increase, several complexes within this genus remain to be satisfactorily addressed taxonomically (Gehring *et al*., 2011, 2012).

A revision is particularly needed for small *Calumma* species characterised by a soft dermal appendage on the snout tip (in most species), assigned to the *Calumma nas-*

utum group. This group includes nine described species: *C. boettgeri* (Boulenger, 1888), *C. fallax* (Mocquard, 1900), *C. gallus* (Günther, 1877), *C. guibei* (Hillenius, 1959), *C. linotum* (Müller, 1924), *C. nasutum* (Duméril & Bibron, 1836), *C. vohibola* Gehring, Ratsoavina, Vences & Glaw, 2011, *C. peyrierasi* (Brygoo, Blanc & Domergue, 1974), and *C. vatosoa* Andreone, Mattioli, Jesu & Randrianirina, 2001 (Gehring *et al*., 2012; Prötzel *et al*., 2016). However, a recent molecular phylogeny and DNA barcoding data suggest that the *C. nasutum* group is not monophyletic (Nagy *et al*., 2012; Tolley *et al*., 2013). Within the phenetic *C. nasutum* group, three species (*C. boettgeri, C. guibei,* and *C. linotum*) differ from the others by the possession of well-defined occipital lobes, which are either well connected or separated by a distinct notch (Brygoo, 1971).

It is clear from the comprehensive molecular study of Gehring *et al*. (2012) that there are more than just these nine species in the *Calumma nasutum* group; these authors distinguished an impressive 33 deep mitochondrial lineages, considered as operational taxonomic units (OTUs). Seven of these corresponded to nominal species (*C. peyrierasi* and *C. vatosoa* were added to this group after 2012), leaving 26 mitochondrial lineages in need of taxonomic assessment. To investigate the significance of these lineages, an integrative correlation of morphological and genetic data is crucial (Tilbury, 2014). As a first step, we have recently clarified the identity of *Calumma boettgeri* and *C. linotum* (Prötzel *et al*., 2015). In the present work, we focus on the third species with occipital lobes within the *C. nasutum* group, *C. guibei.*

Hillenius (1959) described *Calumma guibei* based on a presumably female juvenile individual (snout-vent length 33 mm) and two juvenile paratypes. The type locality is stated as 'Mount Tsaratanana' at an altitude of 1800 m a.s.l. The species was distinguished from *C. boettgeri* and *C. linotum* by the total separation of the occipital lobes, the lack of a dorsal crest, and the very short rostral appendage (Hillenius, 1959). This author later placed *C. guibei* together with some Madagascan species and the African *Kinyongia tenuis* (Matschie, 1892) and *Rhampholeon spinosus* (Matschie, 1892) in a group of chameleons with flexible rostral appendages (Hillenius, 1963), but this was undone by Brygoo (1971). In the last 20 years, this species has been recorded repeatedly, on the Tsaratanana Massif over an altitudinal range of 1600–2100 m a.s.l. (Raxworthy & Nussbaum, 1996) and on the Tsaratanana Mountain (Maromokotro) from 1975–2250 m a.s.l. (Raxworthy *et al*., 2008). Glaw & Vences (2007) presented a photograph of a male assigned to *C. guibei* with a distinct dorsal crest from Antsahamanara, Tsaratanana. Rabearivony *et al*. (2015, Suppl. Mat. S4) recorded *C. guibei* from the Tsaratanana Massif from 1000–1600 m a.s.l.. Andreone *et al*. (2009) found *C. boettgeri/guibei* in Andampy, Tsaratanana (1000 m a.s.l.), Antsahamanara, and Manongarivo, but they did not distinguish between the two species. However, most of these records cannot be proofed, because no voucher material was mentioned. At present, no records except those of the type collections of this species can be confirmed as belonging to this species.

The identity of *Calumma guibei* may be further complicated by it being a species complex. According to a Bayesian inference analysis of a fragment of the mitochondrial ND2 gene, Gehring *et al*. (2012) found the clade they assigned to *C. guibei* sensu lato to be split into two subclades ('E' and 'F'), containing four deep mitochondrial lineages (EI, EII, FI, FII). Their assignment of clade E to *C. linotum*, and clade F to *C. guibei*, largely followed Raxworthy *et al*. (2008) who used the name *C. linotum* for populations from mid-elevations in the Tsaratanana Massif, and *C. guibei* for populations from higher elevations in the same massif. However, this preliminary assignment was done without naming any morphological criteria or genetic data of the holotypes, which were probably fixed in formalin and have been stored in alcohol for more than 50 years. As the identity of *C. linotum* has been revised recently (assigned to part of clade D; Prötzel *et al*., 2015), the attribution of clade E must also be revised.

In this work, we investigate the identity of *Calumma guibei* based on morphological comparisons of new material with the type specimens, and we describe specimens of clade E (sensu Gehring *et al*., 2012) as new species *Calumma gehringi* sp. nov. on the basis of morphological and molecular datasets. Anticipating our taxonomic conclusions and to improve clarity, we will use the name '*C. gehringi*' within the manuscript already before its formal description.

Material and Methods

We studied 29 specimens of the *C. guibei* complex from the collections of the Muséum National d'Histoire Naturelle de Paris (MNHN) and the Zoologische Staatssammlung München (ZSM) and in addition tissue samples of specimens deposited in the Université d'Antananarivo, Département de Biologie Animale (UADBA). Specimens of *C. gehringi* sp. nov. were collected in the field by surveying at night. They were euthanized by injection of concentrated MS222 or chlorobutanol, fixed in 90% ethanol, and transferred to 70% ethanol for long-term storage. Field numbers refer to Mark D. Scherz (MSZC), Miguel Vences (ZCMV), David R. Vieites (DRV), and Angelica Crottini (ACZC).

Morphological investigation

Terms of morphological measurements taken on these specimens were adapted from previous studies (Gehring *et al.*, 2011; Eckhardt *et al.*, 2012; Prötzel *et al.*, 2015). The following characters (Table 1) were measured with a digital caliper to the nearest of 0.1 mm, counted using a binocular dissecting microscope, evaluated by eye or cal-

Table 1. Morphological measurements (all in mm) and scale counts of *Calumma guibei* and *C. gehringi* sp. nov. Abbreviations: male (m), female (f), juvenile (j), holotype (HT), paratype (PT), further abbreviations see Material & Methods.

species	collection no.	field no.	clade	type status	altitude [m]	locality	sex	SVL	TaL	TL	RTaSV
C. guibei	MNHN 50.354	—		HT	1800	Mt. Tsaratanana	j(f)	33.4	31.8	65.2	0.95
C. guibei	MNHN 57.115	—		PT	1800	Mt. Tsaratanana	j	33.3	33.8	67.1	1.02
C. guibei	MNHN 57.116	—		PT	1800	Mt. Tsaratanana	j	26.9	30.9	57.8	1.15
C. guibei	ZSM 2855/2010	DRV 6140		—	2021	Tsaratanana	m	51.7	60.2	111.9	1.16
C. guibei	ZSM 2853/2010	DRV 6131		—	1589	Tsaratanana	m	53.0	62.8	115.8	1.18
C. guibei	ZSM 2854/2010	ZCMV 12325	FI	—	1589	Tsaratanana	m	53.7	62.1	115.8	1.16
C. guibei	ZSM 2857/2010	DRV 6168	FI	—	2021	Tsaratanana	f	49.1	49.6	98.7	1.01
C. guibei	ZSM 2856/2010	DRV 6167	FI	—	2021	Tsaratanana	f	48.1	45.5	93.6	0.95
C. gehringi	ZSM 2851/2010	ZCMV 12307	EII	HT	1207	Antsahan'i Ledy	m	52.6	63.2	115.8	1.20
C. gehringi	ZSM 1834/2010	ZCMV 12511	EI	PT	1466	Bemanevika	m	52.1	58.0	110.1	1.11
C. gehringi	ZSM 1835/2010	ZCMV 12512	EI	PT	1466	Bemanevika	m	52.1	57.0	109.1	1.09
C. gehringi	ZSM 2841/2010	DRV 6392	EII	PT	1466	Bemanevika	m	44.7	49.9	94.6	1.12
C. gehringi	ZSM 2842/2010	DRV 6393	EI	PT	1466	Bemanevika	m	51.6	61.3	112.9	1.19
C. gehringi	ZSM 2843/2010	DRV 6414		PT	1538	Bemanevika	m	53.7	60.0	113.7	1.12
C. gehringi	ZSM 42/2016	MSZC 0154	EI	PT	1434	Ampotsidy	m	50.1	55.4	105.5	1.11
C. gehringi	ZSM 39/2016	MSZC 0128	EI	PT	1320	Ampotsidy	m	53.3	68.2	121.5	1.28
C. gehringi	ZSM 2840/2010	DRV 6318	EII	PT	1411	Ambodikakazo	m	49.3	58.9	108.2	1.19
C. gehringi	ZSM 43/2016	MSZC 0211	EI	PT	1172	Andranonafindra	m	55.5	68.1	123.6	1.23
C. gehringi	ZSM 38/2016	MSZC 0041	EI	PT	1307	Ampotsidy	f	52.0	52.2	104.2	1.00
C. gehringi	ZSM 40/2016	MSZC 0084	EI	PT	1456	Ampotsidy	f	48.4	47.3	95.7	0.98
C. gehringi	ZSM 41/2016	MSZC 0139	EI	PT	1414	Ampotsidy	f	47.5	45.1	92.6	0.95
C. gehringi	ZSM 2844/2010	DRV 6415	EI	PT	1538	Bemanevika	f	48.3	51.4	99.7	1.06
C. gehringi	ZSM 2852/2010	ZCMV 12308		PT	1207	Antsahan'i Ledy	f	52.1	49.5	101.6	0.95
C. gehringi	ZSM 2847/2010	ZCMV 12244	EI	PT	1361	Analabe Forest	f	52.3	46.5	98.8	0.89
C. gehringi	ZSM 2848/2010	—		PT	1361	Analabe Forest	jf	44.5	45.4	89.9	1.02
C. gehringi	ZSM 2846/2010	—		PT	1361	Analabe Forest	j	32.9	35.0	67.9	1.06
C. gehringi	ZSM 2839/2010	DRV 6316	EII	PT	1411	Ambodikakazo	j	37.5	41.6	79.1	1.11
C. gehringi	ZSM 2850/2010	—		PT	1207	Antsahan'i Ledy	j	34.4	33.2	67.6	0.97
C. sp.	ZSM 2845/2010	DRV 6417	FI		1538	Bemanevika	f	51.8	51.9	103.7	1.00

Table 1 continued.

species	collection no.	LRA	RRASV	DRA	RDRSV	NDRA	RC	LC	TCL	TCR	PC	OL	OLND	RODSV	DSOL
C. guibei	MNHN 50.354	1.3	0.039	1.3	0.039	5	(+)	+	—	—	—	s	1.1	0.033	0.4
C. guibei	MNHN 57.115	1.0	0.030	1.1	0.033	6	(+)	+	—	—	—	s	1.0	0.030	0.5
C. guibei	MNHN 57.116	0.7	0.026	0.8	0.030	5	(+)	+	—	—	—	s	0.7	0.026	0.4
C. guibei	ZSM 2855/2010	4.0	0.077	2.3	0.044	6	(+)	+	—	1	(+)	s	1.5	0.029	0.8
C. guibei	ZSM 2853/2010	4.5	0.085	1.8	0.034	5	(+)	+	—	—	(+)	s	1.2	0.023	1.0
C. guibei	ZSM 2854/2010	4.0	0.074	2.3	0.043	6	(+)	+	—	—	(+)	s	1.5	0.028	0.9
C. guibei	ZSM 2857/2010	2.0	0.041	2.0	0.041	5	(+)	+	—	—	(+)	s	1.9	0.039	1.0
C. guibei	ZSM 2856/2010	1.7	0.035	1.5	0.031	4	(+)	+	—	—	+	s	1.5	0.031	0.9
C. gehringi	ZSM 2851/2010	5.1	0.097	3.2	0.061	6	+	+	1	1	+	c	0.5	0.010	0.9
C. gehringi	ZSM 1834/2010	3.6	0.069	2.5	0.048	6	+	+	—	—	—	c	1.3	0.025	1.0
C. gehringi	ZSM 1835/2010	3.6	0.069	2.5	0.048	5	+	+	—	—	—	c	1.3	0.025	1.3
C. gehringi	ZSM 2841/2010	3.4	0.076	2.1	0.047	5	+	+	—	—	—	c	1.0	0.022	1.0
C. gehringi	ZSM 2842/2010	3.1	0.060	2.3	0.045	5	+	+	1	1	—	c	1.2	0.023	1.0
C. gehringi	ZSM 2843/2010	4.4	0.082	2.8	0.052	5	+	+	1	1	+	c	1.3	0.024	0.8
C. gehringi	ZSM 42/2015	3.4	0.068	2.3	0.046	5	+	+	—	—	+	(c)	1.5	0.030	1
C. gehringi	ZSM 39/2016	3.1	0.058	2.2	0.041	6	+	+	1	1	—	s	1.4	0.026	0.8
C. gehringi	ZSM 2840/2010	5.4	0.110	3.5	0.071	8	+	+	2	1	+	c	0.7	0.014	1.0
C. gehringi	ZSM 43/2016	5.0	0.090	2.6	0.047	5	+	+	1	1	(+)	c	1.0	0.018	0.9
C. gehringi	ZSM 38/2016	3.4	0.065	2.0	0.038	5	+	+	1	1	(+)	s	1.3	0.025	0.8
C. gehringi	ZSM 40/2016	3.3	0.068	2.1	0.043	6	+	+	—	—	(+)	(c)	1.4	0.029	0.9
C. gehringi	ZSM 41/2016	3.2	0.067	2.0	0.042	5	+	+	1	—	(+)	s	1.2	0.025	1
C. gehringi	ZSM 2844/2010	3.3	0.068	2.0	0.041	6	+	+	0	0	(+)	s	1.3	0.027	0.6
C. gehringi	ZSM 2852/2010	3.9	0.075	2.6	0.050	6	+	+	—	—	+	c	0.5	0.010	1.1
C. gehringi	ZSM 2847/2010	4.4	0.084	2.1	0.040	5	+	+	—	1	—	c	0.7	0.013	0.7
C. gehringi	ZSM 2848/2010	4.0	0.090	2.2	0.049	6	+	+	—	—	—	c	1.5	0.034	0.8
C. gehringi	ZSM 2846/2010	2.7	0.082	2.0	0.061	6	+	+	—	—	—	c	0.8	0.024	0.6
C. gehringi	ZSM 2839/2010	3.2	0.085	2.0	0.053	6	+	+	1	—	+	c	1.0	0.027	0.7
C. gehringi	ZSM 2850/2010	3.0	0.087	2.3	0.067	5	+	+	—	—	+	c	0.7	0.020	0.8
C. sp.	ZSM 2845/2010	3.6	0.069	2.8	0.054	5	+	+	1	1	(+)	c	1.4	0.027	0.8

Table 1 continued.

species	collection no.	OLD	RODSV	OLW	ROWSV	DSCT	DC	CaC	DSA	NSA	SL	NSL	NIL	HNC	HNR
C. guibei	MNHN 50.354	3.6	0.108	1.5	0.045	0.5	—	—	0.4	22	het	14	13	—	—
C. guibei	MNHN 57.115	3.9	0.117	1.7	0.051	0.4	—	—	0.4	20	het	15	15	—	—
C. guibei	MNHN 57.116	2.8	0.104	1.2	0.045	0.6	—	—	0.3	18	het	14	12	—	—
C. guibei	ZSM 2855/2010	4.9	0.094	2.5	0.048	0.9	—	—	0.7	16	het	11	11	4	4+2
C. guibei	ZSM 2853/2010	4.1	0.077	2.0	0.038	1.1	—	—	0.6	22	het	12	13	4	4+2
C. guibei	ZSM 2854/2010	4.5	0.084	2.1	0.039	0.8	—	—	0.5	20	het	12	13	4	4+2
C. guibei	ZSM 2857/2010	5.4	0.110	2.6	0.053	1.0	—	—	0.5	17	het	11	12	—	—
C. guibei	ZSM 2856/2010	5.1	0.106	2.6	0.054	0.7	—	—	0.5	19	het	11	11	—	—
C. gehringi	ZSM 2851/2010	5.1	0.097	2.8	0.053	0.7	13	+	0.7	13	het	11	13	4	4 (nfe)
C. gehringi	ZSM 1834/2010	5.5	0.106	3.0	0.058	0.8	8	—	0.5	16	het	13	14	4	4 (nfe)
C. gehringi	ZSM 1835/2010	5.7	0.109	3.1	0.060	0.9	8	+	0.8	15	het	11	13	4	4+2
C. gehringi	ZSM 2841/2010	4.7	0.105	2.9	0.065	0.8	13	+	0.7	13	het	10	13	4	4 (nfe)
C. gehringi	ZSM 2842/2010	5.5	0.107	2.9	0.056	1.0	10	—	0.9	12	het	12	13	4	4+2
C. gehringi	ZSM 2843/2010	6.0	0.112	3.0	0.056	1.0	14	+	0.6	20	het	12	12		4+2
C. gehringi	ZSM 42/2015	5.8	0.116	3.5	0.070	0.9	9	+	0.5	16	het	12	14	4	4+2
C. gehringi	ZSM 39/2016	5.7	0.107	3.3	0.062	1.0	7	+	0.5	15	het	12	12	4	4+2
C. gehringi	ZSM 2840/2010	6.1	0.124	3.5	0.071	1.1	15	+	0.8	15	het	12	13	4	4 (nfe)
C. gehringi	ZSM 43/2016	6.4	0.115	3.0	0.054	1.0	15	+	0.6	21	het	13	12	4	4+2
C. gehringi	ZSM 38/2016	5.3	0.102	3.1	0.060	1.0	—	—	0.6	17	het	12	11	—	—
C. gehringi	ZSM 40/2016	4.8	0.099	2.7	0.056	0.9	—	—	0.6	17	het	12	11	—	—
C. gehringi	ZSM 41/2016	5.1	0.107	2.8	0.059	0.9	—	—	0.6	18	het	11	11	—	—
C. gehringi	ZSM 2844/2010	5.0	0.104	2.2	0.046	0.7	—	—	0.5	23	het	11	12	—	—
C. gehringi	ZSM 2852/2010	5.2	0.100	2.8	0.054	1.0	—	—	0.6	15	het	10	11	—	—
C. gehringi	ZSM 2847/2010	5.3	0.101	2.5	0.048	0.8	—	—	0.5	14	het	12	11	—	—
C. gehringi	ZSM 2848/2010	5.3	0.119	3.3	0.074	0.8	—	—	0.5	15	het	10	13	—	—
C. gehringi	ZSM 2846/2010	3.8	0.116	2.4	0.073	0.6	—	—	0.4	13	het	11	14	—	—
C. gehringi	ZSM 2839/2010	4.3	0.115	2.3	0.061	0.8	—	—	0.4	16	het	13	14	—	—
C. gehringi	ZSM 2850/2010	4.0	0.116	2.0	0.058	0.5	—	—	0.4	17	het	11	13	—	—
C. sp.	ZSM 2845/2010	5.5	0.106	2.8	0.054	0.8	—	—	0.6	17	het	13	14	—	—

Table 2. Osteological measurements (all in mm) and relations of important characters of the skull for differentiation between *Calumma guibei* und *C. gehringi* sp. nov., obtained from micro-CT scans. Abbreviations: snout-vent length (SVL; measured externally), width of frontal between the orbits (FW), length of frontal (FL), ratio of FL and SVL (RFL), lateral diameter of frontoparietal fenestra (FFD), ratio of FFD and SVL (RFD), parietal width (PW), ratio of PW and SVL (RPW), length of parietal (PL), ratio of PL and SVL (RPL), ratio of FW and SVL (RFW), prefrontal fontanelle and naris separated (PNS), squamosal meets parietal (SMP), anterior tip of the frontal exceeds more than the half of the naris (FEN).

species	collection number	clade	type status	SVL	FW	RFW	FL	RFL	FFD	RFD	PW	RPW	PL	RPL	PNS	SMP	FEN
C. guibei	MNHN 50.354		HT	33.4	1.7	0.051	3.7	0.111	2.8	0.085	1.6	0.047	3.1	0.094	—	—	—
C. guibei	MNHN 57.115		PT	33.3	1.3	0.040	3.6	0.107	2.2	0.066	1.2	0.035	3.2	0.097	—	—	—
C. guibei	ZSM 2855/2010	FI		51.7	2.8	0.054	4.6	0.089	2.6	0.050	1.6	0.030	4.8	0.092	—	—	—
C. gehringi	ZSM 2851/2010	EII	HT	52.6	2.5	0.048	6.2	0.118	0.8	0.015	1.4	0.027	6.4	0.122	+	+	+
C. gehringi	ZSM 2840/2010	EII	PT	49.3	3.9	0.080	5.5	0.112	1.1	0.022	1.3	0.026	5.9	0.119	+	+	—
C. gehringi	ZSM 2841/2010	EII	PT	44.7	2.4	0.054	5.8	0.130	0.9	0.020	0.9	0.020	5.3	0.119	+	+	+
C. gehringi	ZSM 2842/2010	EI	PT	51.6	2.9	0.056	6.6	0.128	0.7	0.014	1.0	0.019	6.0	0.115	+	+	+

culated: 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); 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) or right side (TCR); parietal crest (PC) presence (+) or absence (–); occipital lobes (OL) completely separated (s) or at least slightly, connected (c); depth of the dorsal notch in the occipital lobes (OLND); ratio of OLND and SVL (RODSV); diameter of largest scale on OL (DSOL); 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 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); caudal crest (CaC) presence (+) or absence (–); diameter of broadest scale 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 (NSA); scalation on lower arm (SL), heterogeneous (het) or homogeneous (hom); 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. In male specimens additionally hemipenial morphology was investigated, concerning number of cornucula gemina (HNC; new term, see discussion) and number of rotulae (HNR). This was not possible in all specimens, since the hemipenes were not fully everted (nfe).

Micro-CT

For internal morphology, micro-Computed Tomography (micro-CT) scans of the head were prepared for seven specimens of the *Calumma guibei* complex representing three OTUs from the clades EI, EII, and FI in GEHRING *et al.* (2012): ZSM 2851/2010 (clade EII), male from Antsahan'i Ledy; ZSM 2840/2010 (clade EII), male from Ambodikakazo; ZSM 2841/2010 (clade EII) and ZSM 2842/2010 (clade EI), both males from Bemanevika; ZSM 2855/2010 (clade FI), male from Tsaratanana massif and the type material of *C. guibei*: holotype MNHN 50.354 and paratype MNHN 57.115, both from Mount Tsaratanana and presumably juvenile females. For micro-CT scanning, specimens were mounted vertically in a

closed plastic vessel slightly larger than the specimen with the head oriented upwards, and stabilized with ethanol soaked paper. To avoid artefacts, 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 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 software (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. Osteological terminology follows Rieppel & Crumly (1997). Skull measurements were taken in VG Studio Max 2.2 using the following abbreviations (Table 2): width of frontal between the orbitals (FW); ratio of FW and SVL (RFW); length of frontal (FL); ratio of FL and SVL (RFL); diameter of frontoparietal fenestra (FFD), measured laterally at the border of frontal and parietal; ratio of FFD and SVL (RFD); parietal width, measured at the midpoint (PW); ratio of PW and SVL (RPW); length of parietal along the midline (PL); ratio of PL and SVL (RPL); prefrontal fontanelle and naris separated (PNS) by contact of prefrontal with maxilla (+) or fused (−); presence (+) or absence (−) of squamosal-parietal contact (SMP); anterior tip of the frontal exceeding the midpoint of the naris (FEN), (+) or (−). The presence of the frontoparietal fenestra was also checked externally in preserved specimens by gently pushing the top of the head with forceps.

Hemipenes of one *Calumma guibei* (ZSM 2855/2010) and two *C. gehringi* sp. nov. (ZSM 2840/2010, ZSM 2842/2010) were diceCT (diffusible Iodine contrast enhanced micro-CT) scanned. One hemipenis was clipped off from each specimen and immersed in iodine solution (I_2 in 1% ethanol) for two days to increase X-ray absorbance. For scanning, the hemipenes were placed with their apices oriented upwards in a plastic tube immersed in 70% ethanol. Scanning was performed for 30 min at a voltage of 60 kV and a current of 200 µA (2400 projections). 3D data were processed in VG Studio Max 2.2 as described above. Hemipenial terminology follows largely Klaver & Böhme (1986). Due to their incomplete eversion the hemipenes of the holotype of *C. gehringi* sp. nov. (ZSM 2851/2010) were not scanned and investigated externally only. Hemipenes of the remaining males were investigated using a binocular dissecting microscope.

The skulls of all adult male specimens of both species were additionally radiographed using a Faxitron UltraFocus LLC x-ray unit. Morphological terminology and description structure largely follow Prötzel *et al.* (2015).

Genetic analysis

We extracted total genomic DNA from tissue samples using proteinase K digestion (10 mg/mL concentration) followed by a salt extraction protocol (Bruford *et al.*, 1992). We amplified a segment of the mitochondrial gene for NADH dehydrogenase subunit 2 (ND2) using standard PCR protocols with the primers ND2F17 (5'-TGACAAAAAATTGCNCC-3') (Macey *et al.*, 2000) and ALAR2 (5'-AAAATRTCTGRGTTGCATTCAG-3') (Macey *et al.*, 1997). PCR products were purified using ExoSAPIT (USB) and sequenced on an automated DNA sequencer (ABI 3130 XL; Applied Biosystems). The newly determined DNA sequences were checked for sequencing errors with the software CodonCode Aligner (CodonCode Corporation), and submitted to GenBank (accession numbers MF579737–MF579749). ND2 sequences were combined with those of Gehring *et al.* (2012) and aligned manually by amino-acid translation in MEGA 7 (Kumar *et al.*, 2016). We used jModeltest 2 (Darriba *et al.*, 2012) to determine the most appropriate model of evolution under the Bayesian Information Criterion (a TNR + I + G model), and subsequently reconstructed the phylogeny under the maximum likelihood (ML) optimality criterion in MEGA 7, with 1000 bootstrap replicates to test the robustness of nodes. A sequence of *Calumma oshaughnessyi* was used as an outgroup. In our species delimitation rationale, we furthermore rely on concordance of the differentiation in mitochondrial DNA represented by the ND2 gene, with differentiation in the nuclear gene for oocyte maturation factor (CMOS) for which we exclusively used previously published sequences from Gehring *et al.* (2012).

Results

Molecular differentiation of *Calumma nasutum* group species with occipital lobes

The maximum likelihood tree based on the mitochondrial ND2 gene (Fig. 1) agrees with the tree in Gehring *et al.* (2012) in most aspects. At the basal-most nodes, specimens of clade FI (herein considered as *C. guibei)* and FII (a candidate species from Andrevorevo that will be treated elsewhere) split off the tree, whereas the remaining clades DI (*C. boettgeri*), DII/DIII (*C. linotum*), and EI/EII (*C. gehringi*) together form a monophyletic group but with negligible bootstrap support (52%). On the contrary, each of the main lineages receives strong support (94–97%): the sister species (1) *C. linotum* and (2) *C. boettgeri* as defined in Prötzel *et al.* (2015); (3) *C. guibei*; and (4) the new species *C. gehringi* sp. nov. Our tree only contains a representative set of sequences of *C. boettgeri* and *C. linotum*, as the differentiation among and within these species has been discussed before (Prötzel *et al.*, 2015). Uncorrected pairwise distances in the ND2 gene among the four included species of the *C. boettgeri* group ranged from 11.8% (*C. boettgeri* vs. *C. linotum*) to 20.8% (*C. boettgeri* vs. *C. guibei*). Important but consistently lower distances were also found within species: up to 11.4% within *C. gehringi* sp. nov.,

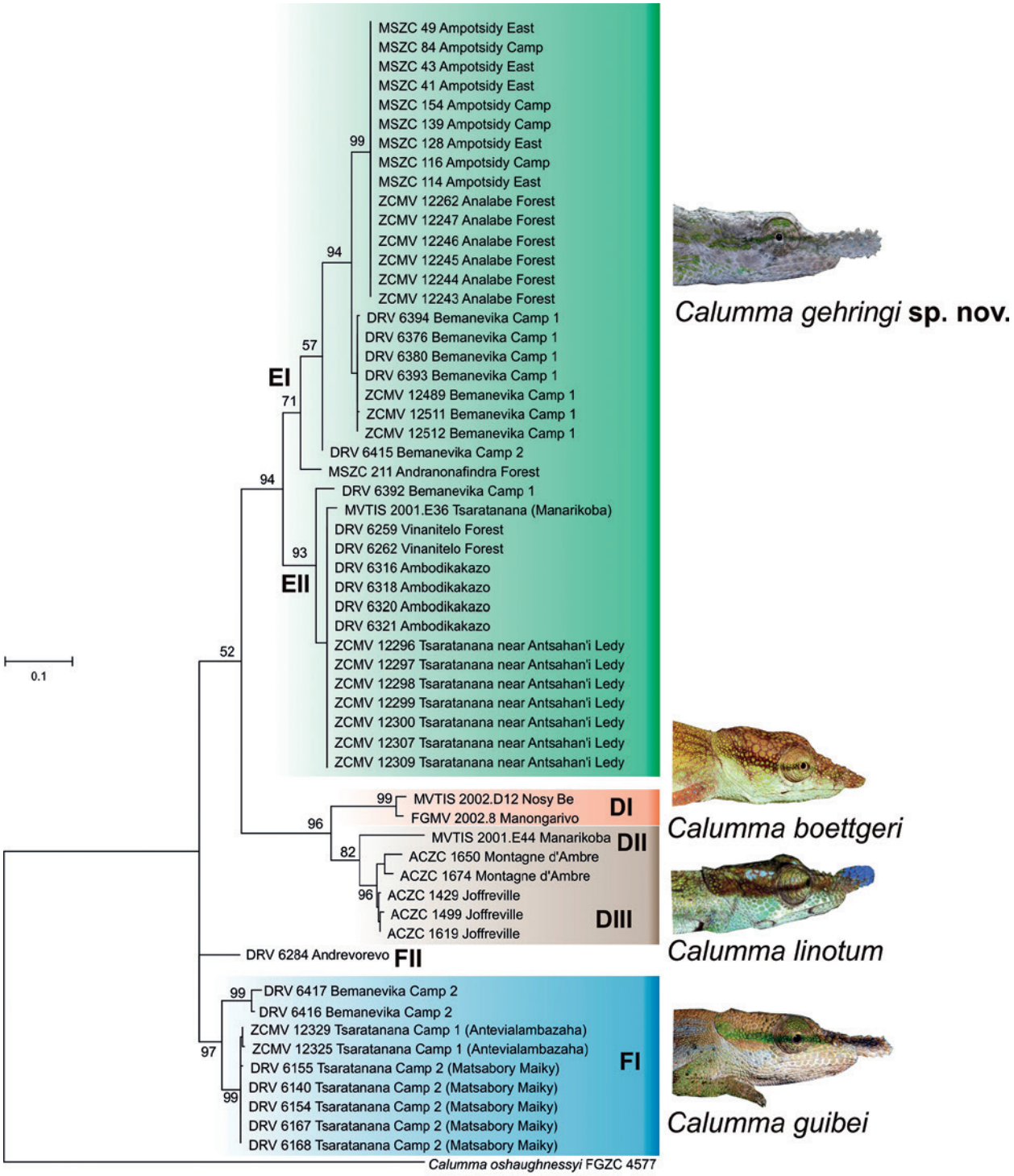

Fig. 1. Maximum likelihood tree based on an alignment of 508 bp DNA sequences of the mitochondrial ND2 gene, depicting phylogenetic relationships among species of the *Calumma nasutum* group with distinct occipital lobes. Numbers at nodes are bootstrap proportions in percent (1000 replicates). EI, EII, DI, DII, DIII, FI, FII are clade numbers according to Gehring *et al*. (2012) as discussed in the text.

10.0% within *C. linotum* (Manarikoba vs. Montagne d' Ambre), 6.9% within *C. guibei,* and 2.3% within *C. boettgeri.* The new species described herein (*C. gehringi* sp. nov.) differed from all other species of the group by a minimum pairwise divergence of 12.3% (to *C. guibei*).

The data for the nuclear CMOS gene as analysed and documented by GEHRING *et al.* (2012) reveal that there is no haplotype sharing between the four species *C. guibei, C. gehringi* sp. nov.*, C. linotum,* and *C. boettgeri* (clade F, clade E, clade DI, clade DII–III, respectively). On the contrary, the two deep mitochondrial clades observed in *C. gehringi* do share nuclear haplotypes (clades EI and EII).

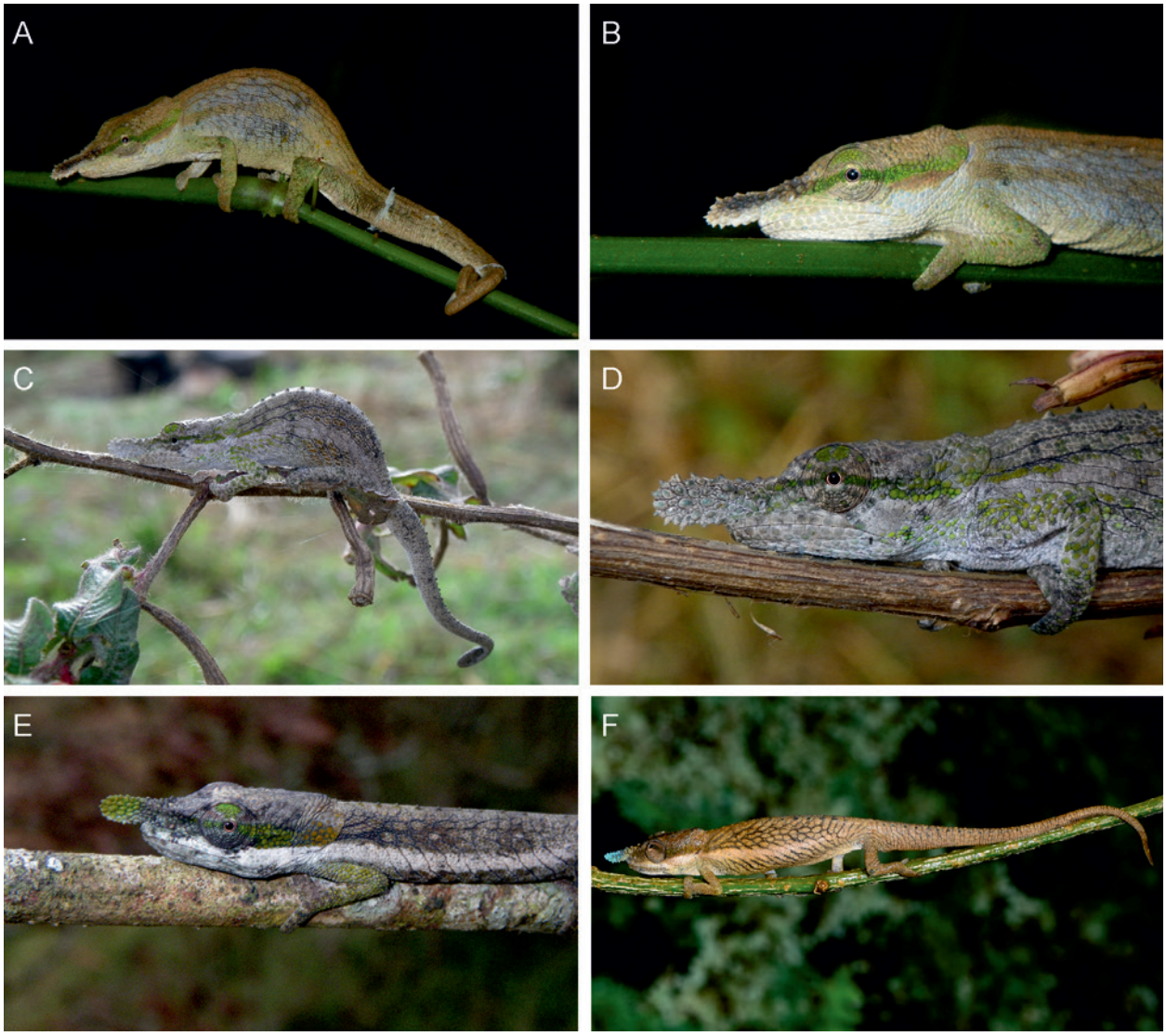

Fig. 2. Chameleon colouration in life: (**A**, **B**) *Calumma guibei*, male ZSM 2854/2010, clade FI; (**C**, **D**) *C. gehringi* sp. nov., male holotype ZSM 2851/2010, clade EII; (**E**) *C. gehringi* sp. nov., male ZSM 2843/2010; (**F**) *C. gehringi* sp. nov., male ZSM 43/2016, clade EI.

Identity and re-description of *Calumma guibei* (Hillenius, 1959)

Due to their immature state, many important characters to delimit the type series of *C. guibei* from other species are weakly developed or even lacking, e.g. several crests, adult size, sex or shape of occipital lobes. However, some characters are conspicuous (Table 1, 2): a very short rostral appendage of 0.7–1.3 mm length (2.6–3.9% of SVL), which is unusual even for juvenile specimens of the *C. nasutum* group (Table 1, Hillenius, 1959; Prötzel, unpublished data); deeply cut notch between the occipital lobes of 0.7–1.1 mm (2.6–3.3% of SVL); no traces of a dorsal crest; heterogeneous scalation of 18–22 enlarged tubercle scales from elbow to manus; a large frontoparietal fenestra; prefrontal fontanelle and naris fused; and absence of dorsal contact between squamosal and parietal, as shown for a female *C. nasutum* in Rieppel & Crumly (1997). These osteological characters might be a result of the juvenile stage of development of the types and change in an adult organism. However, we found similar characters in an adult male specimen (ZSM 2855/2010) of clade FI (Fig. 2A, B; Fig. 3F; Fig. 4B; Fig. 5A), with a distinct frontoparietal fenestra, fused prefrontal fontanelle and naris, and a squamosal not in contact with the parietal. Radiographs taken of all male specimens from this complex confirmed a large frontoparietal fenestra also in ZSM 2853/2010 and ZSM 2854/2010.

The morphological characters that are mentioned above also support the assignment of clade FI (n=5) to *C. guibei*: short rostral appendage in females (1.7–2.0 mm; 3.5–4.1% of SVL; n=2), deeply cut notch completely separating the occipital lobes (1.2–1.9 mm; 2.3–3.9% of SVL; n=5); no dorsal crest; heterogeneous scalation on arms with 16–22 enlarged tubercle scales from elbow to manus.

Fig. 3. Chameleon colouration in life: (**A**) *Calumma gehringi* sp. nov., female ZSM 41/2016, clade EII; (**B**) *C. gehringi* sp. nov., female MSZC 0049, clade EI; (**C**) *C. gehringi* sp. nov., female ZSM 2844/2010, clade EI; (**D**) *C. gehringi* sp. nov., female ZSM 38/2016, clade EI; (**E**) *C. gehringi* sp. nov., female ZSM 2852/2010, note the shape of the notch of the occipital lobes compared to (F); (**F**) *C. guibei*, male ZSM 2854/2010.

Calumma guibei (HILLENIUS, 1959)

Holotype. MNHN 50.354, juvenile, Mount Tsaratanana in the North of Madagascar at 1800 m a.s.l., collected by Paulian on an unknown date.

Paratypes. MNHN 57.115 and MNHN 57.116, both juvenile, collected by Paulian (see above).

Referred material. ZSM 2855/2010 (DRV 6140), adult male, ZSM 2857/2010 (DRV 6168), ZSM 2856/2010 (DRV 6167), both adult females, all three collected in Tsaratanana massif, camp 2 (14.1526°S, 48.9573°E, 2021 m a.s.l.) on 13 June 2010; ZSM 2853/2010 (DRV 6131), ZSM 2854/2010 (ZCMV 12325), both adult males collected in Tsaratanana massif, camp 1 (14.1741°S, 48.9452°E, 1589 m a.s.l.) on 11 and 10 June 2010; collectors are M. Vences, D. Vieites, R.D. Randrianiaina, F. Ratsoavina, S. Rasamison, A. Rakotoarison, E. & T. Rajoafiarison.

Diagnosis. *Calumma guibei* is a member of the phenetic *C. nasutum* group (PRÖTZEL *et al.*, 2016), because of the presence of a soft, dermal, unpaired rostral appendage, absence of gular or ventral crest and heterogeneous scalation at the lower arm, consisting mostly of enlarged tubercles with a diameter of 0.3–0.7 mm. Within the genus it is a small sized, beige to greenish chameleon (SVL 48.1–53.7 mm, TL 93.6–115.8 mm) that is characterized by a long rostral appendage in males (4.0–4.5 mm) and a short rostral appendage in females (1.7–2.0 mm), occipital lobes that are clearly notched in V-form and completely separated, absence of axillary pits, absence of a dorsal crest in both sexes, and a unique skull morphology including a large frontoparietal fenestra (with a width of 5.0–8.5% of SVL).

Calumma guibei 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* and *C. linotum* by the completely separated occipital lobes (vs. not or slightly notched, PRÖTZEL *et al.*, 2015), hemipenis with three pairs of ro-

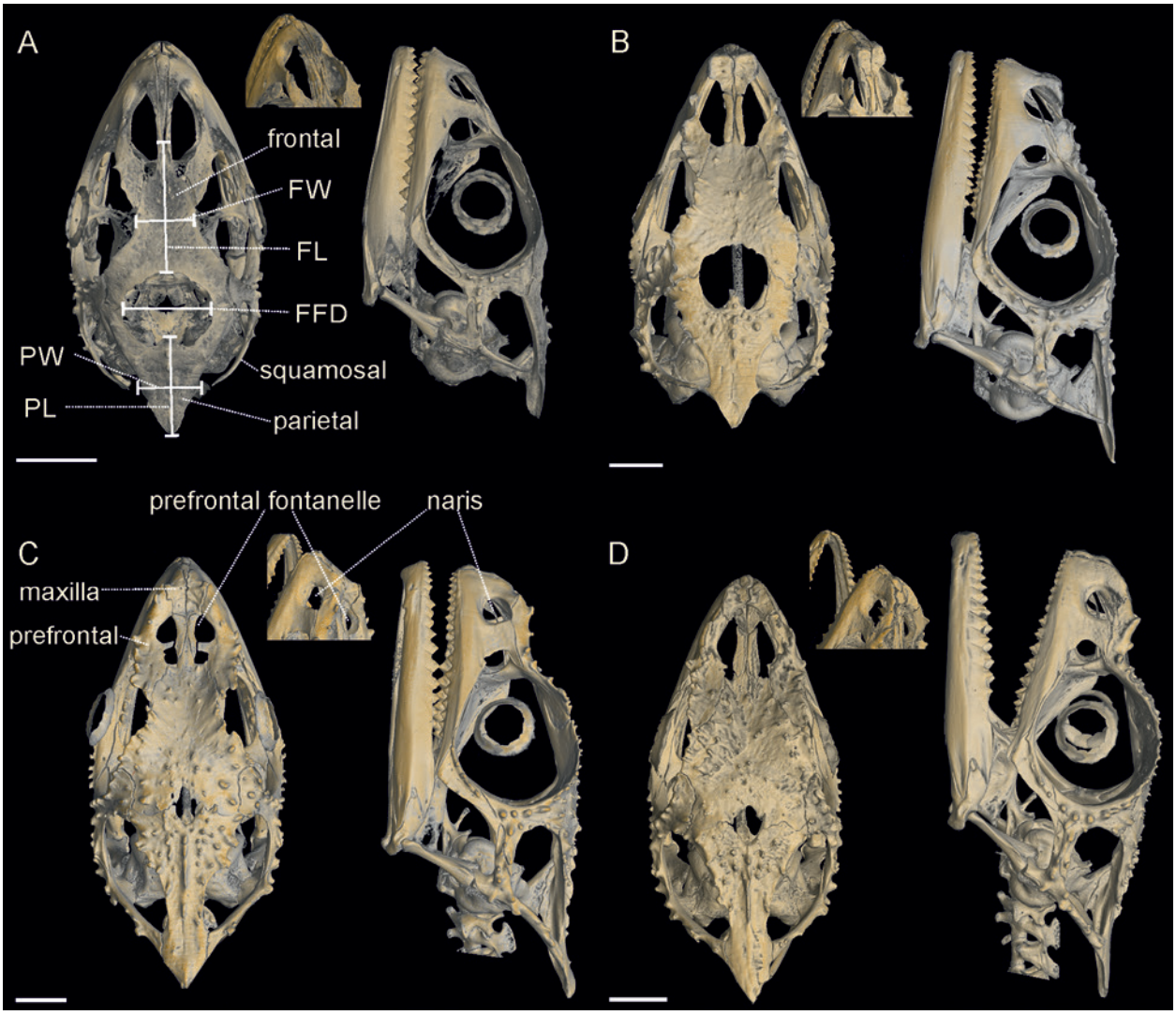

Fig. 4. Micro-CT scans of skulls of *Calumma* in dorsal and lateral view, as well as anterior parts of the skull in dorsolateral view; (**A**) holotype *C. guibei* (MNHN 50.354); (**B**) male *C. guibei* (ZSM 2855/2010); (**C**) male holotype *C. gehringi* sp. nov. (ZSM 2851/2010, clade EII); (**D**) male *C. gehringi* sp. nov. (ZSM 2840/2010, clade EII); scale bar = 2.0 mm. Abbreviations: parietal width (PW), parietal length (PL), diameter of frontoparietal fenestra (FFD), frontal width (FW), frontal length (FL).

tulae (vs. two pairs) and strongly developed cornucula gemina (vs. smaller cornucula gemina, Prötzel *et al.*, 2015), presence of a large frontoparietal fenestra with a width of 5.0–8.5% of SVL (vs. completely closed brain case), fused prefrontal fontanelle and naris in males (vs. separated); additionally from *C. boettgeri* by larger, juxtaposed tubercle scales on the extremities (diameter 0.5–0.9 mm vs. small, 0.2–0.5 mm, and isolated from each other). For the differentiation *Calumma gehringi* sp. nov., see Diagnosis of that species.

Re-description of the holotype (Fig. 6). Juvenile, in a good state of preservation, except body completely slit on the ventral side and on left lateral side behind the occipital lobes; mouth slightly opened; SVL 33.4 mm; tail length 31.8 mm; indistinct rostral ridges that fuse on the anterior snout in a soft, laterally compressed dermal rostral appendage that projects 1.3 mm beyond the upper snout tip, rounded distally; 13 infralabial and 14 supralabial scales; supralabials dorsally serrated (character 'dents de scie' in Angel, 1942); no supra-orbital crest; lateral crest poorly developed and pointing straight posteriorly; no temporal or parietal crests; occipital lobes clearly developed and separated by a notch of 1.1 mm; casque crest from the notch pointing towards the eye; casque not elevated from the head; no traces of gular, ventral or dorsal crest; body laterally compressed with fine homogeneous scalation with the exception of the extremities and head region; legs with small rounded tubercle scales of 0.3 mm diameter; slightly heterogeneous scalation on the head and tubercle scales on rostral appendage; no axillary or inguinal pits. Further morphological measurements are provided in Table 1.

Skull osteology of the holotype (Fig. 4A; Table 2; suppl. Fig. 1). Narrow nasal bones paired and completely separated by the frontal and the premaxilla that meet between them; prefrontal fontanelle and naris fused; smooth fron-

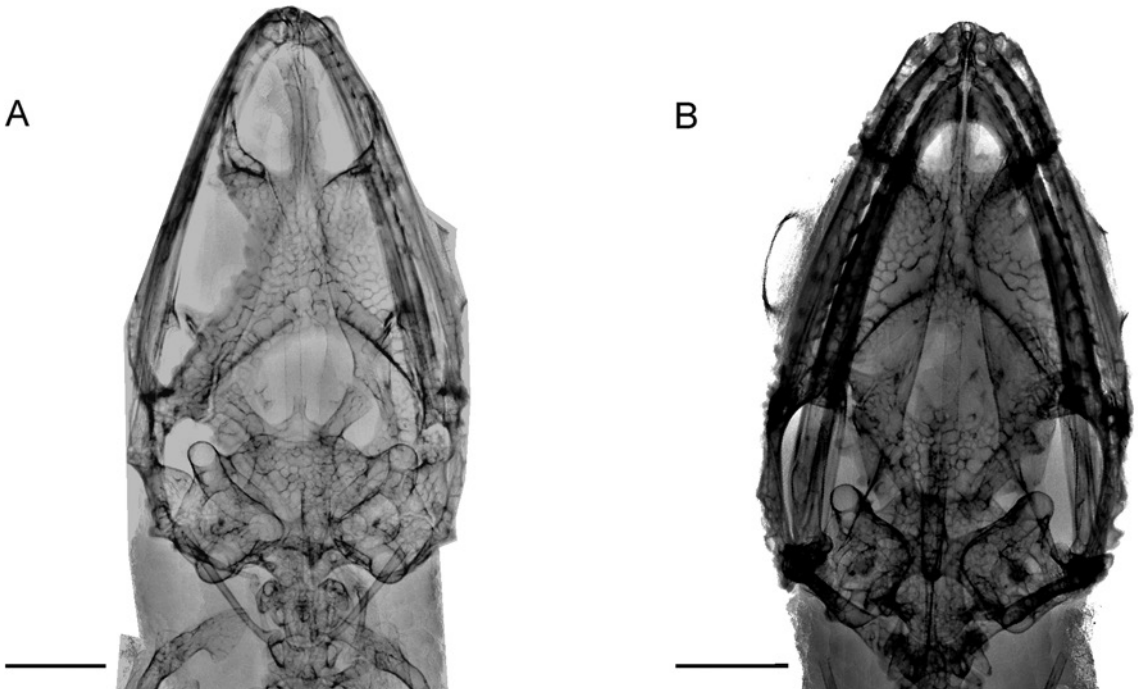

Fig. 5. Radiographs of *Calumma* in dorsal view in comparison to Fig. 3; (**A**) male *C. guibei* (ZSM 2855/2010); (**B**) male holotype *C. gehringi* sp. nov. (ZSM 2851/2010); scale bar = 2.0 mm. Note the frontoparietal fenestra in (A).

tal and parietal with only two tubercles on the parietal; frontal slim with a width of 1.7 mm (5.1% of SVL) between the orbits and a length of 3.7 mm (11.1% of SVL); large frontoparietal fenestra, lateral diameter 2.8 mm (8.5% of SVL); parietal V-shaped with straight lateral margins, tapering posteriorly; parietal 3.1 mm long at the midline (9.4% of SVL), 1.6 mm wide (4.7% of SVL); squamosal not in contact with the parietal.

Colouration of the holotype. The colour of the holotype (in 2016) is almost completely faded after storage in alcohol for more than 50 years. The body is grey-beige in colour without any recognizable pattern. The head and extremities are darkened.

Variation. For measurements of available specimens see Table 1. Within the specimens assigned to *Calumma guibei* there is only little variation: Taking into account their juvenile state, the paratypes (MNHN 57.115, 57.116) with relatively short rostral appendages (3.0 and 2.6% of SVL); male ZSM 2855/2010 is the only specimen with a lateral crest of a single tubercle on the right side; paratype MNHN 57.115 with the most supra- and infralabial scales (15 each). In skull morphology, the width of the frontoparietal fenestra of the adult specimen ZSM 2855/2010 (Fig. 4B; suppl. Fig. 2) is slightly smaller relative to its SVL (5.0%) than in the juvenile type specimens (6.6–8.5%).

Colouration in life. Although it can be assumed that there is variation in the colouration of *Calumma guibei*, we can only provide a description based on photographs of a single male specimen: in relaxed state with beige or light brown body colouration with an indistinct dark, net-like pattern and a beige lateral stripe; rostral appendage of same colouration as the body and with a dark brown lateral stripe that becomes green in colour over the snout, crossing the eyes and ending in the occipital lobes; extremities tending to more greenish in relation to the body and the throat to white-beige; the upper eyelid with a greenish-yellow spot.

Justification for a new species of *Calumma* and taxonomic relevance of its mitochondrial clades

After revising *Calumma boettgeri* and *C. linotum* (Prötzel *et al.*, 2015) and assigning clade FI to *C. guibei* (see previous section), the status of three main lineages of *C. nasutum* group species with distinct occipital lobes remain to be clarified: clades EI, EII, and FII (sensu Gehring *et al*., 212; see Fig. 1). Only a single male specimen is available for clade FII, and it differs by genetics and morphology (Gehring *et al.*, 2012; Prötzel, unpublished data). The identity of this candidate species will be studied elsewhere.

Clades EI and EII together form a monophyletic group in the mitochondrial tree (Fig. 1). Although each is monophyletic as well, they are not very homogeneous groups, and especially EI contains various divergent haplotypes such as one from Bemanevika and a newly determined one from Andranonafindra Forest. Specimens of the two clades also share alleles in the nuclear CMOS gene (Gehring *et al.*, 2012), and we did not observe any consistent morphological differences between them. Key

characters, used to distinguish between *C. gehringi* sp. nov. and *C. guibei* do not allow a differentiation between clade EI and EII (Table 1): males with dorsal crests of 7–15 spines and some with additional spines on the tail in EI and 13–15 spines and caudal crest in EII; distinctly elevated rostral crest and elevated casque in EI and EII; occipital lobes notched, but lobes still slightly connected or separated in EI and slightly connected in EII; in skull morphology (Fig. 4, Table 2), presence of a small frontoparietal fenestra (1.4% of SVL in EI and 1.5–2.2% of SVL in EII); prefrontal fontanelle and naris separated by contact of prefrontal with maxilla in both clades; parietal at its narrowest point (1.9% of SVL and 2.0–2.7%), and length along the midline (11.5% of SVL and 11.9–12.2%). Therefore, the available evidence suggests that these two clades are deep conspecific lineages of a single species, which we herein describe as *C. gehringi* sp. nov.

Specimens of *C. gehringi* sp. nov. differ morphologically from all other species of *Calumma* and also from its close relative *C. guibei* (see chapter 'Diagnosis' below; Table 1). Although it shares the characters of *C. gehringi* sp. nov. (long rostral appendage of 3.6 mm, occipital lobes connected, small frontoparietal fenestra), the female specimen ZSM 2845/2010 (DRV 6417) was genetically assigned to clade FI in our phylogeny of this complex (Fig. 1). This specimen was collected at 1538 m a.s.l., which is slightly lower than all other *C. guibei* (1589–2021 m) but the highest altitude of *C. gehringi* sp. nov. (1172–1538 m). It is not clear if this is a result of mitochondrial introgression in a parapatric hybrid zone or due to contamination or sequencing error, and we therefore consider this specimen putatively as *C.* sp. in need of further investigation.

There are two more nominal species of the *C. nasutum* group with soft rostral appendages and occipital lobes in Madagascar, *C. boettgeri* and *C. linotum*, whose taxonomy has been revised recently (Prötzel *et al.*, 2015). In addition to differences in distribution, these species also have no or only a slight notch between their occipital lobes and a different skull morphology.

Based on the above rationale, we here formally describe *C. gehringi* sp. nov.

Calumma gehringi sp. nov.

Remark. DNA sequences probably belonging to this species based on the tissue sample MVTIS 2001.G56 were published in the phylogeny of Tolley *et al.* (2013) under the name *C. linotum*. Sequences of OTU 10 and '*C. linotum*' of clade E in Gehring *et al.* (2012) are here assigned to *C. gehringi*, as well as the photographs of '*C. guibei*' in Glaw & Vences (2007: 290, 291).

Holotype. ZSM 2851/2010 (ZCMV 12307) adult male, collected in Antsahan'i Ledy in the Tsaratanana Massif (14.2332°S, 48.9800°E, 1207 m a.s.l.), Bealanana District, Sofia Region, Mahajanga Province, North Madagascar, on 9 June 2010 by D.R. Vieites, M. Vences, R.D. Randrianiaina, S. Rasamison, A. Rakotoarison, E. Rajeriarison, and T. Rajoafiarison (Fig. 6).

Paratypes. ZSM 1834/2010 (ZCMV 12511), ZSM 1835/2010 (ZCMV 12512), ZSM 2841/2010 (DRV 6392), ZSM 2842/2010 (DRV 6393), all four adult males, DRV 6376, 6380, 6394, ZCMV 12489 (four uncatalogued specimens in UADBA) collected near Bemanevika (14.4306°S, 48.6018°E, 1466 m a.s.l.) on 27 June 2010; ZSM 2840/2010 (DRV 6318), adult male, ZSM 2839/2010 (DRV 6316), juvenile, DRV 6320, 6321 (both uncatalogued in UADBA), all collected at Ambodikakazo (14.2098°S, 48.8982°E, 1411 m a.s.l.) on 15 June 2010; ZSM 2843/2010 (DRV 6414), adult male, ZSM 2844/2010 (DRV 6415), adult female, both collected near Bemanevika (14.3599°S, 48.5902°E, 1538 m a.s.l.) on 28 June 2010; ZSM 2846/2010 (ZCMV 12243), juvenile, ZSM 2847/2010 (ZCMV 12244), female, ZSM 2848/2010 (ZCMV 12247), subadult female, ZCMV 12245–12247, 12262 (four uncatalogued specimens in UADBA) all collected in Analabe Forest (14.5048°S, 48.8760°E, 1361 m a.s.l.) on 6 June 2010; ZSM 2850/2010 (ZCMV 12297), juvenile, ZSM 2852/2010 (ZCMV 12308), adult female, ZCMV 12296–12300, 12309 (six uncatalogued specimens in UADBA), all collected in Antsahan'i Ledy (14.2332°S, 48.9800°E, 1207 m a.s.l.) on 9 June 2010; DRV 6259, 6262 (two uncatalogued specimens in UADBA) collected in Forest Vinanitelo (14.2097°S, 48.9700°E, 1280 m a.s.l.) on 22 June 2010; collectors of the specimens above are M. Vences, D.R. Vieites, R.D. Randrianiaina, F.M. Ratsoavina, S. Rasamison, A. Rakotoarison, E. Rajeriarison, and T. Rajoafiarison; ZSM 38/2016 (MSZC 0041; 14.4231°S, 48.7189°E, 1325 m a.s.l.) on 20 December 2015, ZSM 40/2016 (MSZC 0084; 14.4163°S, 48.7181°E, 1456 m a.s.l.) on 24 December 2015, and ZSM 41/2016 (MSZC 0139; 14.4171°S, 48.7198°E, 1414 m a.s.l.) on 4 January 2016, all three adult females; MSZC 0128 (ZSM 39/2016; 14.4193°S, 48.7194°E, 1320 m a.s.l.) on 2 January 2016 and MSZC 0154 (ZSM 42/2016; 14.4159°S, 48.7210°E, 1434 m a.s.l.) on 3 January 2016, both adult males; MCSZ 0043, 0049, 0114, 0116 (four uncatalogued specimens in UADBA), all collected on the Ampotsidy Mountains by M.D. Scherz, J. Borrell, L. Ball, T. Starnes, E. Razafimandimby, D. Herizo Nomenjanahary, and J. Rabearivony; ZSM 43/2016 (MSZC 0211), adult male, collected in Andranonafindra Forest (14.7358°S, 48.5480°E, 1172 m a.s.l.) on 14 January 2016, by M.D. Scherz and M. Rakotondratisma.

Diagnosis. *Calumma gehringi* sp. nov. is a member of the phenetic *C. nasutum* group (Prötzel *et al.*, 2016), because of the presence of a soft, dermal, unpaired rostral appendage, absence of gular or ventral crest and heterogeneous scalation at the lower arm, consisting mostly of tubercles of large diameter (0.4–0.9 mm). Within the genus it is a small-sized, grey to greenish chameleon (SVL 44.7–55.5 mm, TL 92.6–123.6 mm) that is characterized by a large rostral appendage of green or blue colour in males and yellow in females when unstressed, occipital lobes that are clearly notched but usually still slightly connected, distinctly elevated rostral crest, absence of axillary pits, presence of a dorsal crest in males, and a unique skull morphology (see below).

Calumma gehringi 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* and *C. linotum* by the completely separated or only slightly connected occipital lobes (vs. not or slightly notched, Prötzel *et al.*, 2015), hemipenis with three pairs of rotulae (vs. two pairs) and strongly developed cornucula gemina (vs. smaller cornucula gemina, Prötzel *et al.*, 2015), presence of a frontoparietal fenestra with a width of 1.4–2.2% of SVL (vs. completely closed brain case), frontal and parietal with many tubercles

Fig. 6. Male holotype of *Calumma gehringi* sp. nov. (ZSM 2851/2010, above) and juvenile holotype of *C. guibei* (MNHN 50.354, below) as preserved specimens. Scale bar = 10 mm.

(vs. smooth or only a few tubercles); additionally from *C. boettgeri* by larger, juxtaposed tubercle scales on the extremities (diameter 0.5–0.9 mm vs. 0.2–0.5 mm, and isolated from each other).

From the most similar taxon, *Calumma guibei*, *C. gehringi* differs most strongly in skull morphology (Fig. 4, Table 2), by possession of a smaller frontoparietal fenestra (width 1.4–2.2% of SVL vs. 5.0–8.5% of SVL); prefrontal fontanelle and naris separated by contact of prefrontal with maxilla (vs. not separated); parietal narrower at its narrowest point (1.9–2.7% of SVL vs. 3.0–4.7%) and longer along the midline (11.5–12.2% of SVL vs. 9.2–9.7%); thick squamosal (vs. thin) in broad dorsal contact with the parietal (vs. not meeting parietal), occipital lobes clearly notched but usually slightly connected (vs. completely separated, Fig. 3E, F), and presence of a dorsal crest with 7–15 tubercles in males (vs. absence). Furthermore, the new species differs from all other members of the *C. nasutum* group with occipital lobes by the possession of a distinctly elevated rostral crest, and a dorsal crest continuing on the tail in most specimens. In addition, *C. gehringi* differs from all other species of the genus *Calumma* by a substantial genetic differentiation (> 12% uncorrected pairwise distance in the mitochondrial ND2 gene; no haplotype sharing in the nuclear CMOS gene).

Description of the holotype. Adult male in a good state of preservation, its left forelimb removed for DNA analysis; mouth slightly opened with tongue between the jaws; both hemipenes incompletely everted (Fig. 6); SVL 52.6 mm; tail length 63.2 mm; distinct and elevated rostral ridges that form a concave cup on the snout and fuse on the anterior snout in a large, laterally compressed dermal rostral appendage that projects straight forward over a length of 5.1 mm and a diameter of 3.2 mm, rounded distally with rough tubercle scales; 13 infralabial and 11 supralabial scales; supralabials dorsally serrated; no supraorbital crest; distinct lateral crest running horizontally; indistinct parietal crest, short temporal crest consisting of two tubercles on the left side and one on the right; occipital lobes clearly developed and deeply notched (0.5 mm), but not completely separated; casque raised; dorsal crest present, starting 1.6 mm from the base of the notch between the occipital lobes and continuing on the tail, consisting of a row of 13 separated conical scales spaced increasingly broadly from 1.4–2.1 mm to the cloaca and several more on the tail decreasing in size toward the tip; no traces of gular or ventral crest. Body laterally compressed with fine homogeneous scalation with the exception of the extremities and head region; limbs with large rounded tubercle scales of maximum 0.7 mm diameter; heterogeneous scalation on the head and large, oval tubercle scales on rostral appendage; no axillary or inguinal pits. Further morphological measurements are provided in Table 1.

Skull osteology of the holotype (Fig. 4C; Table 2; suppl. Fig. 3). Broad paired nasals meeting anteriorly; anterior tip of frontal exceeding more half of the naris; prefrontal fontanelle and naris separated by contact of prefrontal with maxilla; prominent prefrontals that are dorsolaterally raised; frontal and parietal with several tubercles, some forming a parietal crest; frontal with a width of 2.5 mm (4.8% of SVL) between the orbits and a length of 6.2 mm (11.8% of SVL); small frontoparietal fenestra with lat-

eral diameter of 0.8 mm (1.5% of SVL); lateral margin of parietal concave, 1.4 mm (2.7% of SVL) wide at its narrowest point; 6.4 mm (12.2% of SVL) long at the midline; posterodorsally directed parietal platform meets the squamosal laterally; squamosal thick with several tubercles.

Colouration of the holotype (Fig. 2C, D; Fig. 6). The body of the holotype in preservative is of grey-blue colour without any recognizable pattern; internal hind limbs and tail tip beige, neck region and forelimbs also of beige colour and speckled with bluish tubercle scales; rostral appendage of beige-white colour at the tip. In life, the body colouration was bright with an indistinct dark, net-like pattern, and bright green tubercle scales, also on limbs and head region; a beige lateral stripe can occur from snout tip to hip; rostral appendage same colour as the body (Fig. 2C, D); the eyelid is sectioned by a lateral stripe, crossing the eye, and a spot on the upper eyelid, that are both green in colour.

Variation. For measurements of available type specimens see Table 1. Within the clade E there is variation in colouration and morphology, but in most characters the paratypes agree well with the holotype: male ZSM 2840/2010 has the longest rostral appendage (5.4 mm), appendages of the males ZSM 2841/2010, 2842/2010, 39/2016, and 42/2016 significantly shorter (3.1 – 3.4 mm); the appendage of female ZSM 2844/2010 has fine tubercle scales; there is significant variation in the temporal crest, from none to two tubercles, with some individuals even having asymmetrical tubercle numbers (Table 1); in the same way, the parietal crest is absent, indistinct or present within both sexes; notch of occipital lobes in most paratypes deeper than in holotype (0.5 – 1.5 mm) and still slightly connected—only totally separated in ZSM 2844/2010, 38/2016, 39/2016, and 41/2016; dorsal crest present in all males, but number of cones highly variable (7 – 15), indistinct and small cones in ZSM 1834/2010, 39/2016, 42/2016, and 43/2016; all males with caudal crest except ZSM 1834/2010 and 2842/2010, indistinct in ZSM 43/2016; dorsal crest lacking in all females; number of supralabial and infralabial scales from 10 – 14. The male ZSM 43/2016 is geographically isolated and from the lowest elevation of all paratypes, and has the largest body size (55.5 SVL mm and 123.6 mm TL) and a distinct blue rostral appendage in life (Fig. 2F); it is also genetically basal to clade EI, but still strongly supported as a member of clade E, and we therefore consider its deviation from the rest of the specimens to reflect geographic variation in this species, but emphasise that more material from the Bealanana district is needed.

The three micro-CT scanned paratypes ZSM 2840/2010, 2841/2010, and 2842/2010 are more or less identical in skull osteology with the holotype (Table 2), including the prefrontal fontanelle and naris separated from each other, a small frontoparietal fenestra of 0.7 – 1.1 mm diameter and the squamosal meeting the parietal. The shape of the frontals is variable, with lengths of 5.5 to 6.6 mm and widths of 2.4 to 3.9 mm. In ZSM 2840/2010 the anterior tip of the frontal does not exceed more than the half of the naris.

Colouration in life. Both sexes in relaxed state have green, grey, or brown body colouration with an indistinct dark, net-like pattern; a beige-white lateral stripe can occur from snout tip to hip; males usually with a bright green rostral appendage and green-coloured extremities, the eyelid is sectioned by a lateral stripe crossing the eye, and a spot is present on the upper eyelid, both of which can be bright green in colour; additionally the temporal region and the occipital lobes can be of conspicuous green colour. Sexes are generally dichromatic, with females typically bearing a yellow, instead of a green, rostral appendage; that yellow colouration can spread over the eyelids and the temporal region to the occipital lobes. One exception is ZSM 43/2016, which had a strongly blue rostral appendage in life (as did all other males encountered in Andranonafindra; MDS pers. obs.). The extremities are usually of the same colour as the body. One gravid female was almost entirely green, including her rostral appendage.

Stress colouration is significantly darker, with a net-like black pattern of small scales on the lateral body. The rostral appendage typically becomes more distinctly bright in colouration against the darker lateral head colouration.

Hemipenial morphology based on micro-CT scans. The hemipenis of *Calumma gehringi* (Fig. 7B; suppl. Fig. 4) shows large and deep calyces with smooth ridges on the asulcal side of the truncus. The apex is ornamented with two pairs of long pointed cornucula (see discussion) and paired rotulae. The cornucula gemina rise from the sulcal side of the apex, are curved to the asulcal side and are completely everted in the investigated specimen (Fig. 7B). Two pairs of rotulae are placed on the asulcal side and are finely denticulated. Additionally on the asulcal side next to the pair of cornucula, there is a pair of rotulae with three lobes. This ornament is only recognizable when the hemipenis is fully everted.

Available names. Apart from *C. guibei* there is no other valid species or synonym in the *Calumma nasutum* group with deeply notched occipital lobes.

Etymology. We dedicate the new species to Philip-Sebastian Gehring. His comprehensive molecular phylogenetic study on the *Calumma nasutum* group was the basis for the description of the new species, and will be instrumental to the resolution of the rest of this complex. The species epithet '*gehringi*' is a patronym in the Latin genitive form.

Distribution. *Calumma gehringi* has, so far, been collected in Northern Madagascar on the Tsaratanana Massif and south of it (Fig. 8). In contrast to *C. guibei*, whitch covers the higher elevations in Tsaratanana from

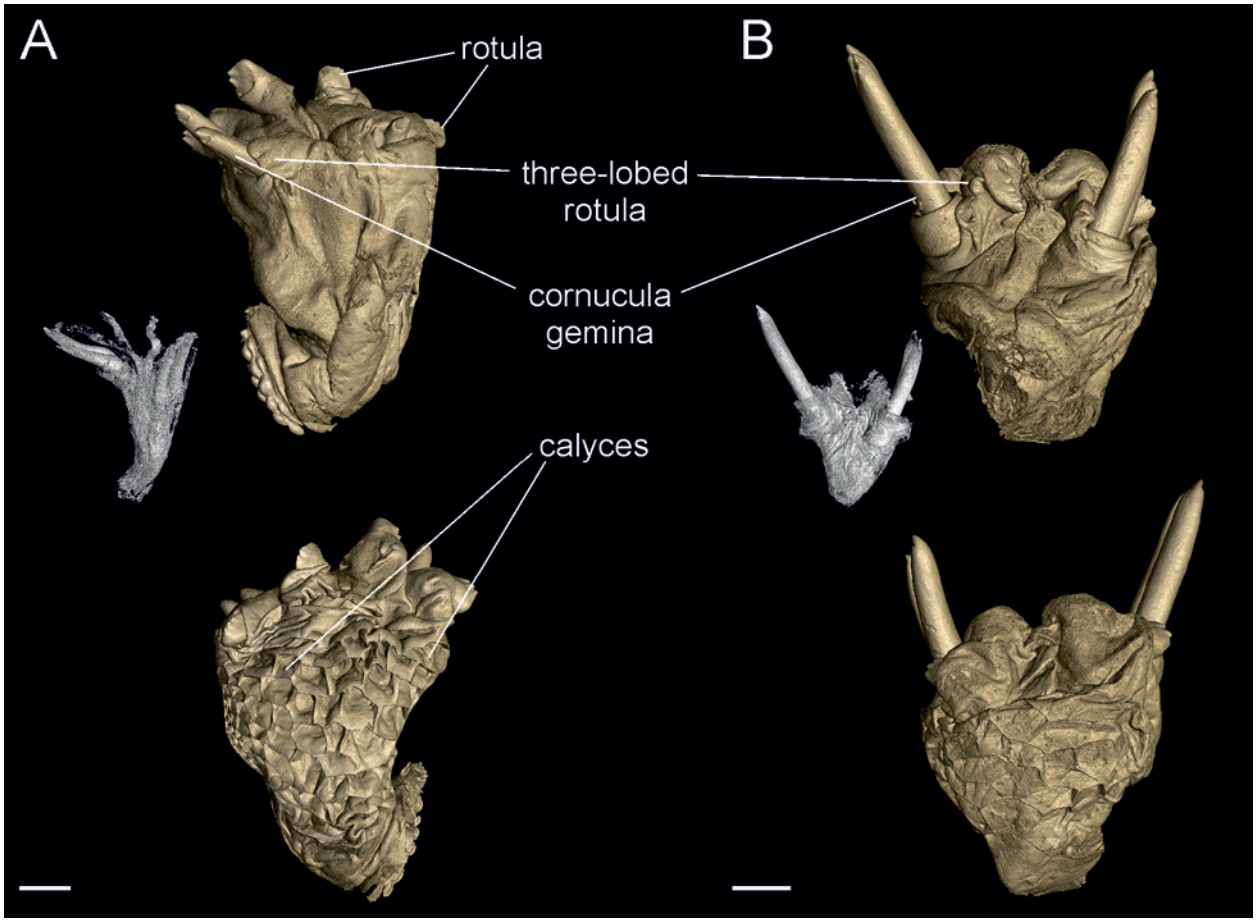

Fig. 7. Micro-CT scans of hemipenes of *Calumma* species in sulcal (above) and asulcal view (below). (**A**) *C. guibei* (ZSM 2855/2010); (**B**) *C. gehringi* sp. nov. (ZSM 2842/2010). Small images show the (everted or retracted) cornucula gemina inside of the hemipenis at a different threshold. Scale bar = 1 mm.

1590–2020 m a.s.l. (according to our data) or even up to 2250 m a.s.l. (Raxworthy *et al.*, 2008), *C. gehringi* lives at mid-altitudinal level from 730–1540 m a.s.l. and is recorded from Ambodikakazo, Ampotsidy, Antsahan'i Ledy, Analabe Forest, Andranonafindra Forest, Bemanevika, Manarikoba (14.0422°S, 48.7616°E, 730 m a.s.l.) and Vinanitelo Forest. The location at 730 m a.s.l. is based on a single (tissue) record; the distribution of most specimens starts from an altitude of 1200 m a.s.l. or higher. For geographic coordinates of the other localities, see chapter 'Paratypes'.

Natural history and ecology. *Calumma gehringi* is an arboreal, diurnal species occurring from 0.5 to at least 4 m above the ground in secondary, degraded primary, and pristine primary rainforest. Specimens were often observed on bushes and low branches of trees near rivers, almost always roosting at night, on leaves or thin branches/twigs. The species can be locally abundant, often occurring in couples a few metres from one another, occasionally forming mixed-sex clusters of up to eight individuals over a few square metres. Heavily gravid females were collected from Ampotsidy in late December 2015 and early January 2016, indicating a mating season coinciding with seasonal rains. An absence of juveniles in this period suggests that these hatch later in the season. At lower altitude, in Andranonafindra Forest (1172 m a.s.l.), hatchlings were encountered in mid-January 2016, indicating that there may be some degree of altitudinal variation in the reproductive cycle or timing of these chameleons. The following females contained well-developed eggs, that were ready to be laid: ZSM 40/2016, four eggs (dimensions from 8.3–9.6 × 5.3–5.7 mm); 41/2016, two eggs (8.1 × 4.0 mm and 7.9 × 4.9 mm); ZSM 38/2016, three eggs (8.9–9.3 × 4.2–4.7 mm); ZSM 2847/2010 (collected in June 2010), two eggs (12.1 x 5.8 mm and 11.5 × 5.9 mm). When disturbed on thin branches and vines during the day, individuals moved their bodies to the opposite side from the observer, and, if the perch was thin enough, were able to keep looking at the observer whilst being difficult to detect, by the lateral position of their eyes.

Discussion

In this work, we have taken another step towards clarifying the systematics of the *Calumma nasutum* group, by revising the identity of *C. guibei* and describing the new

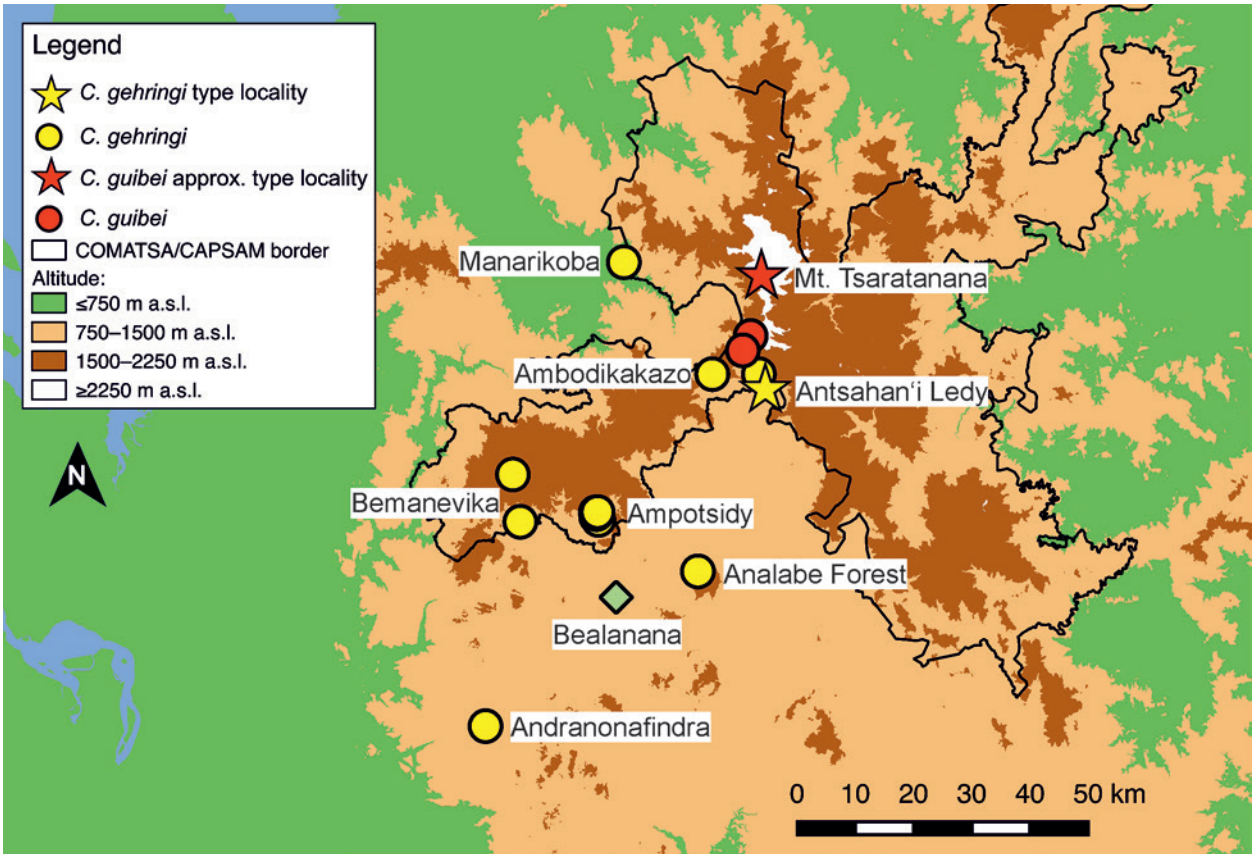

Fig. 8. Map of north-western Madagascar with localities of *Calumma guibei* and *C. gehringi* sp. nov. COMATSA/CAPAM border is that of a series of established and recently proposed protected areas. The green diamond indicates the town of Bealanana.

species *C. gehringi*. One of the several genetic lineages within the *C. guibei* complex (Gehring *et al*., 2012) must represent the true *C. guibei*, but because that species was described based on a juvenile holotype (from which genetic data are not available), assignment is difficult. After examining the holotype, the two paratypes, and specimens of the mitochondrial clades FI, EI and EII of Gehring *et al.* (2012) we have assigned *C. guibei* to clade FI and described the chameleons belonging to the clades EI and EII as a new species. No consistent differences between them in morphology or osteology were recognizable, and as mentioned above, they share haplotypes of the nuclear CMOS gene (400 bp). Additionally, two specimens (ZSM 2841/2010 and ZSM 2842/2010), representing clade EII and EI, occurred sympatrically at the same collection site in Bemanevika (camp 1, Antsirakala, 14.4306°S, 48.6018°E), without differentiation in the nuclear gene studied. Consequently, we merged these two OTUs of Gehring *et al.* (2012) to one new species, *C. gehringi*. This shows the importance of an integrative taxonomic approach to avoid over-splitting of species. However, additional work is needed in the future for better understanding of the differentiation among the various deep conspecific lineages within *C. gehringi* and to fully rule out the possibility that some of these represent cryptic species.

In conclusion, *Calumma guibei* is a species of the phenetic *C. boettgeri* complex with clearly notched and completely separated occipital lobes, a short rostral appendage in females, a unique skull morphology and lacking a dorsal crest—though this has not been a constant character in previous studies (Prötzel *et al.*, 2015). We confirm the characters stated by Hillenius (1959), specifically the separation of the occipital lobes and the lack of a dorsal crest, as diagnostic, except for the short rostral appendage that is of usual length (4.0–4.5 mm) in the males. In contrast, *C. gehringi* has notched, but not totally separated, occipital lobes, a long rostral appendage in females, and a small frontoparietal fenestra. Additionally, the species separate geographically; *C. guibei* occurs at higher elevations, from 1590–2250 m a.s.l. on the Tsaratanana Massif, and *C. gehringi* at mid-altitudes from 730–1540 m a.s.l. from Tsaratanana south and southwest to Bemanevika. Consequently, the specimens mentioned as *C. guibei* in Raxworthy & Nussbaum (1996) and Raxworthy *et al.* (2008) probably were correctly assigned to this species, while the '*C. linotum*' of Raxworthy *et al.* (2008) almost certainly refer to *C. gehringi*. The scalation of the extremities that was used to distinguish between *C. boettgeri* and *C. linotum* (Prötzel *et al.*, 2015) was not as characteristic in the present species, though *C. gehringi* has a more homogenous scalation with fewer scales in a row from elbow to manus (NSA, see Table 1). The size and shape of the rostral appendage is surprisingly variable within both species, decreasing its value as a diagnostic character. However, it is interesting that it tends to show sexual

dichromatism in *C. gehringi*, with males usually having green, and females usually yellow appendages—although some exceptions have been found.

With the aid of the micro-CT technique, we have shown that the presence and size of the frontoparietal fenestra is an informative character in this group, and particularly in the distinction of *C. guibei* from its congeners. Additionally, the squamosal is not connected with the parietal bone in *C. guibei.* These characters are reminiscent of juvenile skull morphology, and it is difficult to derive a biological function from this. Generally, cranial sutures allow small intercranial movements and if they remain open, they might allow micro-movements to dissipate forces acting on the skull (Moazen *et al*., 2009). Rieppel & Crumly (1997) suggest that this is a result of paedomorphosis. In chameleons, adults of small taxa often resemble juveniles of larger ones (Rieppel & Crumly, 1997). Thus, paedomorphosis is a potential explanation, but why the fenestra is so much more strongly developed in *C. guibei* than in closely related, and equally sized chameleons, remains a mystery. The skull of *C. gehringi* is more robust, with only a small frontoparietal fenestra, separated prefrontal fontanelle and naris, a strongly developed squamosal that is connected to the parietal, and differently shaped frontal and parietal bones. Though cheaper and faster in production, traditional radiography appears to be of limited use for identification of skull characters. Due to the flattening of a 3D object onto a 2D image plane, many characters overlap and are difficult to distinguish. However, the frontoparietal fenestra of *C. guibei* was recognizable as a slightly brighter grey contrast.

Although the hemipenis morphology of the two species considered here appears superficially different (Fig. 7A and B), there are in fact no substantial differences, except for the calyces on the asulcal side of the truncus, which are slightly larger in *C. gehringi.* Dice-CT scans enable a detailed view of the structure and the inside of a hemipenis and show that the two pairs of long spines, visible in Fig. 7A, are completely everted and in Fig. 7B largely retracted, but approximately of the same size. This ornament is not homologous to the papillae of e.g. Calumma brevicorne that are defined as 'fleshy and pliable projections' in Klaver & Böhme (1986). Due to its structure that reminds of paired, small horns we propose to name this ornament with the Latin equivalent '*cornuculum geminum*' (plural '*cornucula gemina*'). This ornament also exists in *C. boettgeri* and *C. linotum*, and we revise the description in Prötzel *et al*. (2015) accordingly. The tip of a *cornuculum geminum* is also reminiscent of a hypodermic needle, and raises questions about its function, which may be to do with anchoring inside the cloaca, but further research is necessary. The fact that the *cornucula gemina* are retractable makes it even more important that conclusions from genital morphology are based on fully everted hemipenes.

The differences between clade FI and E listed in Gehring *et al*. (2012, Table 1) concerning the presence of apical sulcal lobes and the size and position of the rotulae could not be substantiated and would require further studies on a larger number of fully everted hemipenies for clarification.

Similar genital morphology also exists in the species pair *C. boettgeri* and *C. linotum* (Prötzel *et al.*, 2015). However, these species differ from *C. gehringi* and *C. guibei* in having ornaments of only two pairs of rotulae, the sulcal pair enlarged, and apparently smaller cornucula gemina. Thus, in these taxa, genital morphology appears to have evolved at a slower rate than other characters, which is counter to typical expectation. According to current knowledge, both species pairs occur either allopatrically (*C. boettgeri* and *C. linotum*) or possibly parapatrically (*C. gehringi* and *C. guibei*), and their speciation may therefore have involved other selective forces than genital ornamentation.

The objective visualisation and the more detailed view of characters like hemipenes or skull structures show once again the value of X-ray micro-CT as a modern tool for integrative taxonomy. Integrating morphology, osteology, and geographic data with genetics led on the one hand to the splitting of the former species *C. guibei*, and on the other, to the lumping of two OTUs to the species *C. gehringi*. As there are still more genetic lineages within the *C. nasutum* group than currently recognised species, its resolution is far from complete, but this approach is certainly the key to unravelling its mysteries.

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Electronic Supplement Files

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File 1: Suppl. Fig. 1_skull_C_guibei_ht_MNHN 50.354.avi
File 2: Suppl. Fig. 2_skull_adult_C_guibei_ZSM_2855_2010.avi
File 3: Suppl. Fig. 3_skull_C_gehringi_ht_ZSM_2851_2010.avi
File 4: Suppl. Fig. 4_hemipenis_C_gehringi_ZSM_2842_2010.avi