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Systematic revision of the Malagasy chameleons *Calumma boettgeri* and *C. linotum* (Squamata: Chamaeleonidae)

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Abstract

We revise the taxonomic status of two species of Madagascan chameleons in light of a recent molecular phylogenetic study on the *Calumma nasutum* group. The investigation of morphological and osteological characters led to a clear delineation between two species within the *C. boettgeri* complex, *C. boettgeri* and *C. linotum. Calumma linotum* has been considered either a synonym of *C. boettgeri* or a dubious, poorly defined taxon. So far it has only been known from the male holotype with the imprecise locality 'Madagascar'. Based on pholidosis, morphological measurements and characters of the skull that were analyzed using micro-X-ray computed tomography (micro-CT) scans, we ascribe the population of chameleons from Montagne d'Ambre, formerly assigned to *C. boettgeri,* to *C. linotum*. *Calumma linotum* differs from *C. boettgeri* in the larger size of tubercle scales on the extremities and rostral appendage, the larger diameter of the extremities relative to the body size, the presence of a parietal crest as well as the form of the nasal bones and the anterior tip of the frontal. The life colouration of the males is also characteristic, with a blue rostral appendage and greenish turquoise extremities. The body and rostral appendage of *C. boettgeri* in contrast are inconspicuously yellowish brown coloured. All confirmed distribution records of *C. boettgeri* are confined to the biogeographic Sambirano region whereas *C. linotum* is only known from Montagne d'Ambre and a locality at the base of the Tsaratanana massif. Additional literature records of *C. boettgeri* and *C. linotum* from northeastern Madagascar are in need of confirmation. We also confirm the synonymy of *Chamaeleo macrorhinus* (described from a female holotype with an unknown locality) with *Calumma boettgeri*. The use of micro-CT exposed further characteristics for species delimitation in an integrative taxonomic approach. In addition to the skull, we also micro-CT scanned the hemipenes of *C. boettgeri* and *C. linotum*, using an iodine-based tissue stain, and provide 3D PDF models of these organs. This method enables detailed illustration and the detection of variation in particular characters, and might be an important tool in further taxonomic studies on the *C. nasutum* group and other squamate reptiles.

Key words: *Calumma boettgeri, Calumma linotum*, Chamaeleonidae, micro-computed tomography, hemipenis morphology, skull structure, Madagascar

Introduction

The island of Madagascar is one of the most valuable natural resources on the planet. In addition to an impressive number of animal and plant species, endemism reaches 85% for vascular plants, 84% for land vertebrates (Goodman & Benstead 2005), 92% for non-marine reptiles (Glaw & Vences 2007), and 100% for species in the chameleon genus *Calumma* (Townsend *et al.* 2011). Description of the island's species level diversity is far from complete, and modern molecular methods have contributed to an increase of species descriptions in recent years. Molecular analyses enable the discovery of cryptic species that show few or no external morphological differences (Bickford *et al*. 2007). Among Malagasy reptiles, cryptic diversity and microendemism is presumed to be widespread, but the level of these phenomena has not been rigorously explored (Gehring *et al*. 2012; Glaw *et al.* 2012).

The chameleon genus *Calumma* currently comprises 33 species, many of them described in the last 10 to 15 years (Tilbury 2014; Glaw 2015). Small *Calumma* species with a soft dermal appendage on the snout tip are clustered into the *Calumma nasutum* group, which includes the seven described species *C. boettgeri, C. fallax, C.*

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gallus, C. guibei, C. linotum, C. nasutum and *C. vohibola* (Gehring *et al*. 2012). Within the *C. nasutum* group, three species (*C. boettgeri, C. guibei* and *C. linotum*) differ from the other species by the possession of well-defined occipital lobes, and this character has provided support for the phenetic *C. boettgeri* complex (Gehring *et al.* 2012). In contrast, molecular phylogenetic evidence suggests that neither the *C. nasutum* group nor the *C. boettgeri* complex are monophyletic (Gehring *et al*. 2012; Nagy *et al.* 2012; Tolley *et al.* 2013). Therefore, a comprehensive assessment within a phylogenetic framework is needed to fully resolve the taxonomy of this species complex.

The first species of the *C. nasutum* group with occipital lobes was described by Boulenger (1888) as '*Chamaeleon Boettgeri*', based on two males and one female from Nosy Be. Fifteen years later Barbour (1903) described '*Chamaeleo macrorhinus*' on the basis of an adult female from the imprecise type locality 'Madagascar' without naming any delimiting characters from *Ch. boettgeri*. *Chamaeleo macrorhinus* was synonymized with *Ch. boettgeri* by Mocquard (1909). In 1924, Müller described '*Chamaeleo linotus*' based on one adult male from the collection of the Zoologische Staatssammlung München (ZSM), again with the vague type locality 'Madagascar'. According to the ZSM catalogue, this specimen was received in an exchanged with the Natural History Museum in Stuttgart (SMNS) in 1923, but no additional data is available on this specimen, and the catalogue of the SMNS collection was destroyed during WWII (A. Kupfer, pers. comm., 2014). Müller (1924) distinguished this species from *Ch. boettgeri* by the absence of a dorsal crest, the broader scales on the extremities, which also stand closer together, and the less distinct and less regular gular folds. Mertens (1933) assigned four female chameleons from northeast Madagascar to *Ch. linotus*, though these females lack rostral appendages. According to Angel (1942) *Ch. linotus* differs from *Ch. boettgeri* in the absence of a dorsal crest, larger scales on the legs and absence of a rostral appendage in females. Hillenius (1959) claimed the only important distinguishing characters of *Ch. linotus* compared to *Ch. boettgeri* are the lack of a dorsal crest and the lack of a rostral appendage in females. Brygoo (1971) characterised *Ch. linotus* as having a total length between 124 mm (males) and 109 mm (females), and a rostral appendage length of more than 3 mm (males), with un-notched occipital lobes and without a dorsal crest, claiming the latter to be the only difference between it and *Ch. boettgeri*. Based on their phylogenetic analysis, Klaver & Böhme (1986) transferred numerous Malagasy chameleons, including all species of the *Ch. nasutus* group, into the genus *Calumma*. Since these authors assumed that *Calumma* would be of feminine gender, they changed names accordingly; *Chamaeleo linotus* became *Calumma linota*. Lutzmann & Lutzmann (2004) and Walbröl & Walbröl (2004) argued *Calumma* to be of neutral gender and changed the species epithet to *C. linotum*.

The identity of *Calumma linotum,* which has only been reliably known from the holotype, is disputed and considered as poorly defined, and is sometimes considered a synonym of *C. boettgeri* (Gehring *et al.* 2011). Photographs shown in Nečas (2004), titled as an undescribed species of the *C. nasutum* complex, correspond well with *C. linotum* as it is herein re-defined. Raxworthy *et al*. (2008) applied the name "*Calumma* cf. *linota*" to a population from the Tsaratanana massif but without description or justification for this assignment. Gehring *et al*. (2012) tentatively adopted this assignment, emphasizing that this definition requires confirmation. Since the holotype of *C. linotum* is too old for successful DNA extraction and was probably fixed in formalin, it was not included in their phylogenetic analyses.

Species identification within the *Calumma nasutum* group is difficult due to high morphological variation, e.g. absence or presence of dorsal crests, heterogeneity in scalation or colour and shape of the rostral appendage. Similar to the *Calumma brevicorne* complex (Raxworthy & Nussbaum 2006), the broad distribution of *C. nasutum* along Madagascar's eastern rainforest chain raises expectations that it actually consists of a complex of cryptic species (Glaw & Vences 2007). The molecular phylogenetic study of Gehring *et al*. (2012) for the *C. nastutum* group based on the mitochondrial gene *ND2* suggested there may be as many as 33 operational taxonomical units (OTUs), despite only seven species being currently recognized (see above). That study included 215 individuals from 60 localities, of which 82 individuals were from the *C. boettgeri* complex (*C. boettgeri*, *C. guibei* and *C. linotum*). Eleven clades (A–K) were recovered, with at least 8% sequence divergence between them. Based on morphological features, clade D corresponded to *C. boettgeri*, clade E to *C. linotum*, and clade F to *C. guibei.* The classification of *C. linotum* by Gehring *et al*. (2012) remained uncertain because the holotype was not studied. In addition, clade D (*C. boettgeri*) showed geographic variation, with populations from Nosy Be and Manongarivo (D I), Montagne d'Ambre (D III), and Tsaratanana (D II). To define OTUs, Gehring *et al*. (2012) chose an uncorrected pairwise distance of > 6% as threshold, supported by additional Bayesian species delimitation algorithms. According to this threshold, genital morphology and pholidosis, *C. boettgeri* from Montagne d'Ambre forms a confirmed candidate species (CCS).

Although the information to date suggests that the *C. nasutum* group is in need of revision, molecular phylogenetics or barcoding approaches based on single markers alone are insufficient for taxonomic decisions and the choice of an inappropriate gene can result in incorrect tree topologies, making additional lines of evidence important for validation. With modern methods of imaging and three-dimensional computed tomography, the anatomical features of chameleons can be much better appreciated than with traditional methods, and this may potentially provide a wealth of new structural apomorphies (Tilbury 2014). In this work, the taxonomy of *Calumma boettgeri* and *C. linotum* is revised with an integrative taxonomic approach. In addition to the widely used morphological measurements and pholidosis, we investigated internal morphology using the example of sutures of the skull and, for the first time, fine details of the hemipenes, with the aid of X-ray micro-Computed Tomography (micro-CT).

Material and methods

Thirty-eight specimens of *C. boettgeri* sensu lato were obtained from the collections of the Museum of Comparative Zoology of Harvard University, Cambridge, Massachusetts (MCZ), the Senckenberg Museum, Frankfurt am Main (SMF), the Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK) and the Zoologische Staatssammlung München (ZSM).

Terms of morphological measurements taken on these specimens were adapted from previous studies (Gehring *et al*. 2011; Eckhardt *et al*. 2012). Measurements (Fig. 1) were taken with a digital caliper to the nearest 0.1 mm through a binocular dissecting microscope: Snout-vent length (SVL) from the snout tip (not including the rostral appendage) to the cloaca; tail length (TaL) from the cloaca to tail tip; total length (TL) as a sum of SVL + TaL; the ratio of TaL to snout-vent length (RST); length of the rostral appendage (LRA) from the upper snout tip; ratio of length of rostral appendage and snout-vent length (RRS); number of peripheral scales on the rostral appendage (RAPSC); RAPSC divided by the length of the rostral appendage (NPSCM); rostral crest (RC) present (+) or absent (-); number of supralabials (NSL); number of infralabials (NIL); lateral diameter of the occipital lobes (OLD); depth of the dorsal notch in the occipital lobes (OLN); parietal crest (PC) absent (-) or represented by a number of scales; likewise the dorsal crest (DC), dorsal cones counted when visible with the naked eye without the use of a binocular microscope according to Eckhardt *et al*. (2012); axillary pits (AP) of the upper extremities present (+) or absent (-); diameter of the broadest scale (DSC) of the upper arm (defined as the area from the armpits to the elbow in lateral view, Fig. 1); relation of DSC to the snout-vent length (RSB); number of large scales (diameter $\geq$ 0.2 mm) of the heterogeneous scalation of the upper arm (NSC); upper arm diameter at the level of the armpit (UAD); and the ratio of UAD to snout-vent length (RAS). Figures 1–3 were taken with digital and analogous cameras.

For internal morphology micro-CT scans of the head were prepared for ten specimens *C. boettgeri* sensu lato representing both major localities (5 of each sex): ZSM 440/2000 and ZSM 444/2000, males from Nosy Be; ZSM 441/2000 and ZSM 227/2002, females from Nosy Be; ZSM 2072/2007 and ZSM 2073/2007, males from Montagne d'Ambre; ZSM 551/2001, female from Andampy; ZSM 873/1920/2, female with unknown locality, but morphologically identical with the Montagne d'Ambre form; ZSM 21/1923, male holotype of *C. linotum*, type locality 'Madagascar'; MCZ 5988, female holotype of *C. macrorhinus*, type locality 'Madagascar'. For micro-CT scanning, specimens were placed in a closed plastic vessel slightly larger than the specimen with the head oriented upwards, and stabilized with ethanol soaked paper. To avoid disturbances during scanning, it was ensured that the paper did not cover the head region. Micro-CT scanning was performed with a phoenix nanotom m (GE Measurement & Control, phoenix|x-ray, Wunstorf, Germany) 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. Osteological terminology follows Rieppel & Crumly (1997). Measurements were taken with VG Studio Max 2.2 using the following abbreviations (Fig. 4): NL, nasal length; NW, nasal width; PS, parietal at the smallest diameter; PL, parietal at the largest diameter; PC, parietal crest absent (-) or number of tubercles.

Hemipenes of three male specimens from Montagne d'Ambre (ZSM 1683/2012, ZSM 2072/2007 and ZSM 2073/2007) and two males from Nosy Be (ZSM 440/2000 and ZSM 444/2000) were micro-CT scanned (Fig. 5). 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 or 60 min at a voltage of 60 kV and a current of 300 or 240 µA (2400/3600 projections), respectively. 3D data were processed with VG Studio Max 2.2 as described above. 3D models were prepared with the software Amira (version 5.4.5, VSG, Hillsboro, OR) essentially following Ruthensteiner & Heß (2008). Hemipenial terminology follows Klaver & Böhme (1986). The distribution map was modified from vegmad.org (Fig. 6).

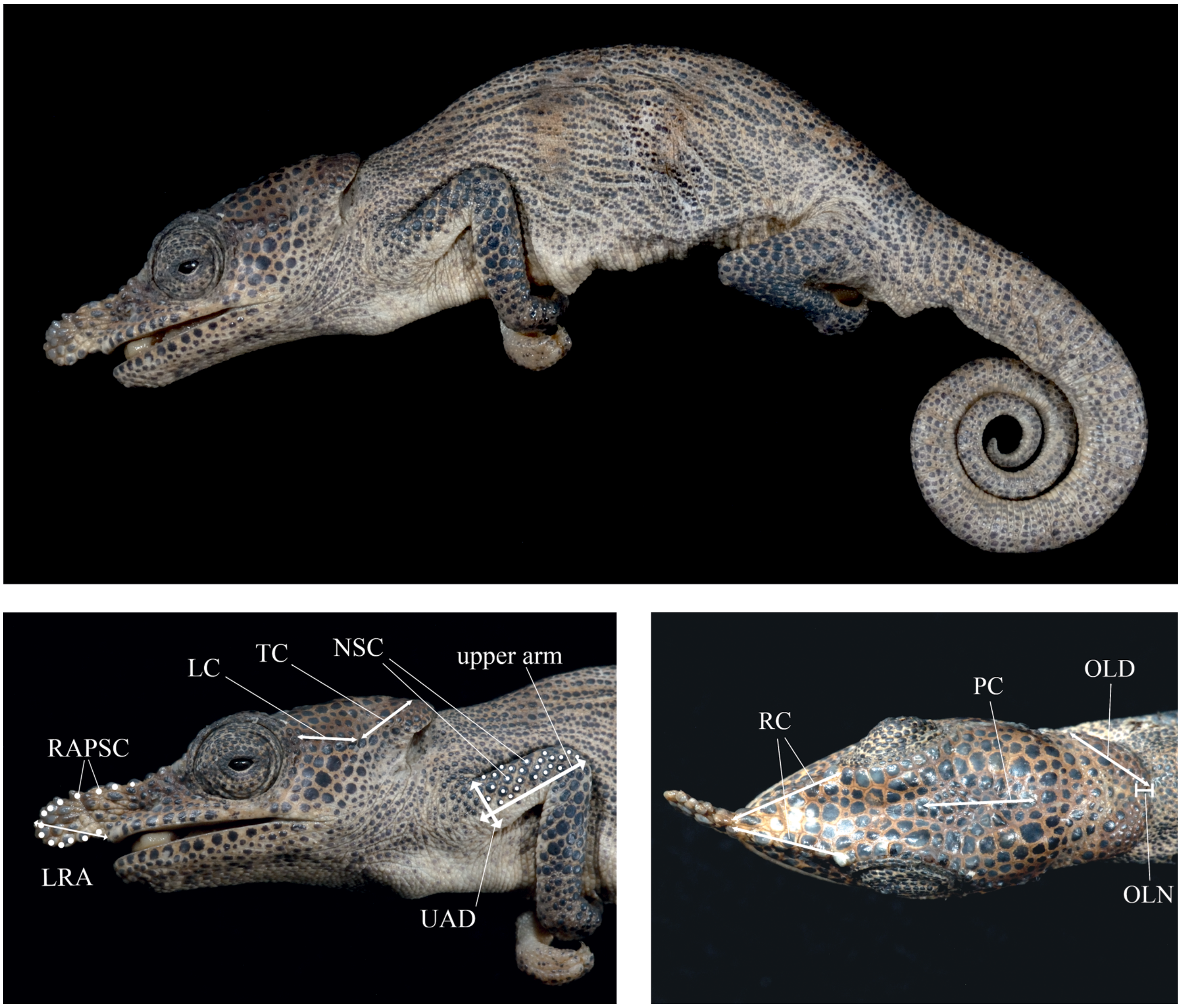

FIGURE 1. Landmarks for morphometric measurements and pholidosis, shown in lateral and dorsal view of a male *C. linotum* (ZSM 873/1920/3) with unknown locality. Notes: LRA, length of rostral appendage from snout tip; RAPSC, number of peripheral scales on rostral appendage; RC, rostral crest; PC, parietal crest; LC, lateral crest; TC, temporal crest; OLD, lateral diameter of the occipital lobe; OLN, occipital lobe dorsally notched; UAD, upper arm diameter; NSC, number of big scales on upper arm from lateral view.

Results

Morphology of *Calumma boettgeri* sensu lato

External morphology. Measurements of important morphological parameters were taken from 23 specimens (11 males, 12 females) from Nosy Be, six specimens (five males, one female) from Montagne d'Ambre, and another nine specimens without exact locality data (Table 1). Because there was only one female from Montagne d'Ambre available, only males from both species were considered for comparison of body size, extremities and appendages. The specimens without localities were not included in mean value calculations but could be assigned according to their morphology to the Nosy Be morphotype (four specimens) and the Montagne d'Ambre morphotype (five specimens), respectively.

TABLE 1. Morphological measurements of *Calumma boettgeri* and *C. linotum.* Abbreviations: m, male; f, female; SVL, snout-vent length; TaL, tail length; TL, total length; RST, ratio of tail to snout-vent length; LRA, length of rostral appendage from snout tip; RRS, ratio of length of rostral appendage and snout-vent length; RAPSC, number of peripheral scales on rostral appendage; NPSCM, number of peripheral scales per mm on rostral appendage; RC, rostral crest present (+) or absent (-); NSL, number of supralabials; NIL, number of infralabials; OLD, lateral diameter of the occipital lobe; OLN, depth of the dorsal notch in occipital lobe; PC, parietal crest absent (-) or number of parietal cones; DC, dorsal crest absent (-) or number of dorsal cones; AP, axillary pits present (+) or absent (-); DSC, diameter of broadest scale on upper arm; RSB, ratio of broadest scale to snout-vent length; NSC, number of big scales on upper arm from lateral view; UAD, upper arm diameter; RAS, ratio of arm diameter to snout-vent length; all measurements in mm.

collection no.	species	locality	sex	SVL	TaL	TL	RST	LRA	RRS	RAPSC	NPSCM	RC	NSL
ZFMK 51389	*C. boettgeri*	Nosy Be	m	51.5	51.8	103.4	0.99	4.0	0.077	26	6.5	+	12
ZFMK 51520	*C. boettgeri*	Nosy Be	m	50.7	54.2	104.9	0.94	3.0	0.060	21	7.0	+	13
ZFMK 45987	*C. boettgeri*	Nosy Be	m	51.6	52.8	104.4	0.98	2.6	0.051	13	4.9	+	11
ZFMK 45988	*C. boettgeri*	Nosy Be	m	49.9	54.4	104.3	0.92	3.1	0.062	18	5.8	+	12
ZFMK 51518	*C. boettgeri*	Nosy Be	m	46.0	45.5	91.4	1.01	2.6	0.057	17	6.5	+	12
ZFMK 51519	*C. boettgeri*	Nosy Be	m	50.7	50.2	100.9	1.01	3.2	0.062	26	8.3	+	12
ZFMK 51521	*C. boettgeri*	Nosy Be	m	41.1	45.8	86.9	0.90	4.1	0.100	22	5.4	+	12
ZSM 36/1913	*C. boettgeri*	Nosy Be	m	41.4	44.0	85.4	0.94	2.5	0.059	15	6.1	+	12
ZSM 440/2000	*C. boettgeri*	Nosy Be	m	50.1	54.1	104.2	0.93	3.0	0.060	14	4.6	+	12
ZSM 444/2000	*C. boettgeri*	Nosy Be	m	51.9	55.0	106.9	0.94	2.9	0.057	17	5.8	+	12
SMF 16471	*C. boettgeri*	Nosy Be	m	50.3	54.3	104.6	0.93	3.6	0.072	15	3.6	+	13
ZFMK 51514	*C. boettgeri*	-	m	49.4	51.2	100.6	0.96	3.5	0.072	17	4.8	+	13
ZSM 866/1920	*C. boettgeri*	-	m	54.6	55.7	110.3	0.98	3.6	0.065	17	4.8	+	16
ZFMK 45985	*C. boettgeri*	Nosy Be	f	48.9	46.7	95.6	1.05	3.8	0.077	20	5.3	+	12
ZFMK 51516	*C. boettgeri*	Nosy Be	f	51.4	51.2	102.6	1.00	2.2	0.042	20	9.2	+	11
ZFMK 51517	*C. boettgeri*	Nosy Be	f	50.5	47.6	98.1	1.06	3.1	0.062	18	5.8	+	11
ZFMK 50615	*C. boettgeri*	Nosy Be	f	42.0	41.8	83.8	1.00	3.0	0.072	18	5.9	+	11
ZFMK 45983	*C. boettgeri*	Nosy Be	f	50.8	48.6	99.4	1.05	4.1	0.081	-	-	+	12
ZFMK 45986	*C. boettgeri*	Nosy Be	f	45.0	45.2	90.3	1.00	-	-	-	-	+	12
ZFMK 48226	*C. boettgeri*	Nosy Be	f	47.6	47.3	94.9	1.01	2.5	0.052	14	5.7	+	15

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TABLE 1. (Continued)

collection no.	species	locality	sex	SVL	TaL	TL	RST	LRA	RRS	RAPSC	NPSCM	RC	NSL
ZFMK 45984	*C. boettgeri*	Nosy Be	f	55.5	52.5	108.0	1.06	1.2	0.022	10	8.1	+	12
ZSM 227/2002	*C. boettgeri*	Nosy Be	f	48.8	52.8	101.6	0.92	3.2	0.065	15	4.7	+	12
ZSM 441/2000	*C. boettgeri*	Nosy Be	f	45.5	43.4	88.9	1.05	2.7	0.060	16	5.9	+	12
SMF 16471	*C. boettgeri*	Nosy Be	f	50.0	46.7	96.7	1.07	2.8	0.056	14	5.0	+	13
SMF 16472	*C. boettgeri*	Nosy Be	f	46.0	45.7	91.7	1.01	3.0	0.065	14	4.7	+	12
ZSM 865/1920	*C. boettgeri*	-	f	49.4	46.7	96.1	1.06	3.5	0.070	20	5.8	+	15
ZFMK 51515	*C. boettgeri*	-	f	51.3	48.2	99.5	1.06	2.9	0.057	17	5.8	+	13
MCZ 5988	*Ch. macrorhinus*	'Madagascar'	f	48.1	44.9	93.1	1.07	2.8	0.058	17	6.1	+	17
ZSM 236/2004	*C. linotum*	M. d'Ambre	m	55.4	63.0	118.4	0.88	4.3	0.077	16	3.7	+	13
ZSM 1683/2012	*C. linotum*	M. d'Ambre	m	53.0	56.3	109.3	0.94	4.7	0.089	cut	-	+	13
ZSM 2073/2007	*C. linotum*	M. d'Ambre	m	59.6	64.8	124.4	0.92	4.5	0.075	18	4.0	+	-
ZSM 2072/2007	*C. linotum*	M. d'Ambre	m	53.7	59.2	112.8	0.91	4.3	0.080	13	3.0	+	13
ZFMK 52308	*C. linotum*	Joffreville	m	36.2	37.9	74.1	0.96	2.5	0.068	15	6.1	+	-
ZSM 873/1920/3	*C. linotum*	-	m	54.6	58.1	112.6	0.94	4.4	0.081	14	3.2	+	12
ZSM 873/1920/1	*C. linotum*	-	m	52.4	cut	52.4	-	4.3	0.081	16	3.8	+	12
ZFMK 36630	*C. linotum*	-	m	55.6	61.9	117.5	-	4.7	0.085	15	3.2	+	12
ZSM 551/2001	*C. linotum*	Andampy	f	50.6	50.7	101.3	1.00	2.0	0.040	9	4.5	+	13
ZSM 622/1999	*C. linotum*	-	f	54.5	54.9	109.4	0.99	4.1	0.075	15	3.7	+	13
ZSM 873/1920/2	*C. linotum*	-	f	42.7	47.4	90.1	0.90	3.1	0.072	15	4.9	+	12
ZSM 21/1923	*C. linotum*	'Madagascar'	m	56.1	70.0	126.1	0.80	4.3	0.077	15	3.5	+	14

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Continued.

collection no.	species	locality	sex	NIL	OLD	OLN	PC	DC	AP	DSC	RSB	NSC	UAD	RAS
ZFMK 51389	*C. boettgeri*	Nosy Be	m	12	3.7	0.3	-	19	-	0.4	0.008	7	2.0	0.038
ZFMK 51520	*C. boettgeri*	Nosy Be	m	13	3.1	0.3	-	2	-	0.4	0.007	10	2.2	0.044
ZFMK 45987	*C. boettgeri*	Nosy Be	m	11	4.7	0.4	-	0	-	0.5	0.010	9	2.1	0.040
ZFMK 45988	*C. boettgeri*	Nosy Be	m	11	3.0	0.4	-	12	-	0.2	0.004	10	2.0	0.041
ZFMK 51518	*C. boettgeri*	Nosy Be	m	13	3.4	0.3	-	0	-	0.4	0.008	8	2.2	0.048
ZFMK 51519	*C. boettgeri*	Nosy Be	m	11	4.3	0.6	-	18	-	0.3	0.006	9	2.0	0.039
ZFMK 51521	*C. boettgeri*	Nosy Be	m	11	3.8	0.6	-	5	-	0.3	0.007	9	1.5	0.036
ZSM 36/1913	*C. boettgeri*	Nosy Be	m	12	3.2	0.0	-	0	-	0.4	0.008	12	1.8	0.043
ZSM 440/2000	*C. boettgeri*	Nosy Be	m	12	4.8	0.3	-	28	-	0.4	0.008	14	2.5	0.050
ZSM 444/2000	*C. boettgeri*	Nosy Be	m	12	3.7	0.5	-	15	-	0.4	0.008	11	2.3	0.045
SMF 16471	*C. boettgeri*	Nosy Be	m	13	4.0	0.0	-	11	-	0.4	0.009	9	2.0	0.040
ZFMK 51514	*C. boettgeri*	-	m	13	3.7	0.3	-	14	-	0.5	0.009	11	2.2	0.044
ZSM 866/1920	*C. boettgeri*	-	m	15	3.7	0.3	-	20	-	0.4	0.008	8	2.0	0.036
ZFMK 45985	*C. boettgeri*	Nosy Be	f	13	4.6	0.5	-	0	-	0.4	0.007	10	1.8	0.036
ZFMK 51516	*C. boettgeri*	Nosy Be	f	11	3.8	0.5	-	0	-	0.3	0.007	12	2.2	0.042
ZFMK 51517	*C. boettgeri*	Nosy Be	f	11	2.7	0.5	-	0	-	0.4	0.007	14	2.2	0.044
ZFMK 50615	*C. boettgeri*	Nosy Be	f	12	3.4	0.4	-	0	-	0.4	0.008	8	1.5	0.036
ZFMK 45983	*C. boettgeri*	Nosy Be	f	12	3.5	0.0	-	0	-	0.3	0.006	11	2.1	0.040
ZFMK 45986	*C. boettgeri*	Nosy Be	f	11	3.0	0.5	-	3	-	0.3	0.007	9	2.0	0.044
ZFMK 48226	*C. boettgeri*	Nosy Be	f	14	2.7	0.3	-	0	-	0.2	0.004	-	1.9	0.039
ZFMK 45984	*C. boettgeri*	Nosy Be	f	11	2.8	0.4	-	0	-	0.3	0.005	11	2.0	0.036
ZSM 227/2002	*C. boettgeri*	Nosy Be	f	12	3.3	0.1	-	0	-	0.3	0.006	11	2.0	0.041
ZSM 441/2000	*C. boettgeri*	Nosy Be	f	12	3.9	0.5	-	0	-	0.4	0.008	9	2.2	0.048

……continued on the next page

TABLE 1. (Continued)

collection no.	species	locality	sex	NIL	OLD	OLN	PC	DC	AP	DSC	RSB	NSC	UAD	RAS
SMF 16471	*C. boettgeri*	Nosy Be	f	10	4.2	0.0	-	0	-	0.4	0.007	9	2.2	0.044
SMF 16472	*C. boettgeri*	Nosy Be	f	13	3.5	0.1	-	11	-	0.4	0.009	10	1.8	0.039
ZSM 865/1920	*C. boettgeri*	-	f	13	2.8	0.1	-	0	-	0.3	0.006	9	1.9	0.038
ZFMK 51515	*C. boettgeri*	-	f	13	3.3	0.7	-	0	-	0.3	0.006	12	2.1	0.041
MCZ 5988	*Ch. macrorhinus*	‘Madagascar’	f	6.1	3.2	0.1	-	0	-	0.3	0.007	8	2.3	0.047
ZSM 236/2004	*C. linotum*	M. d'Ambre	m	13	4.5	0.0	4	10	-	0.7	0.012	18	2.9	0.052
ZSM 1683/2012	*C. linotum*	M. d'Ambre	m	13	4.7	0.1	3	8	-	0.7	0.014	20	2.9	0.055
ZSM 2073/2007	*C. linotum*	M. d'Ambre	m	12	4.2	0.0	3	13	-	0.6	0.010	22	3.1	0.052
ZSM 2072/2007	*C. linotum*	M. d'Ambre	m	12	3.8	0.2	4	12	-	0.8	0.015	21	3.1	0.058
ZFMK 52308	*C. linotum*	Joffreville	m	-	2.8	0.0	3	9	-	0.4	0.011	20	1.7	0.048
ZSM 873/1920/3	*C. linotum*	-	m	14	4.1	0.2	6	5	-	0.7	0.013	19	3.0	0.054
ZSM 873/1920/1	*C. linotum*	-	m	12	4.8	0.1	5	4	-	0.8	0.015	19	3.0	0.058
ZFMK 36630	*C. linotum*	-	m	12	3.7	0.3	4	0	-	0.7	0.012	23	3.0	0.054
ZSM 551/2001	*C. linotum*	Andampy	f	13	5.1	0.0	(5)	6	-	0.6	0.011	16	2.8	0.055
ZSM 622/1999	*C. linotum*	-	f	13	4.1	0.2	(4)	0	-	0.6	0.011	22	2.9	0.054
ZSM 873/1920/2	*C. linotum*	-	f	12	3.9	0.0	6	0	-	0.5	0.013	17	2.8	0.065
ZSM 21/1923	*C. linotum*	‘Madagascar’	m	14	4.7	0.1	(5)	0	-	0.7	0.013	22	3.0	0.054

Individuals from Montagne d'Ambre show clear morphological differences from Nosy Be specimens (Fig. 7). The adult males examined from Montagne d'Ambre are larger than those from Nosy Be (mean values of TL 107.8 mm in Montagne d'Ambre vs. 99.3 mm in Nosy Be, Table 2), their rostral appendage is longer related to the snout-vent length (RRS 0.078 vs. 0.065) and the ratio of arm diameter to snout-vent length is larger (UAD/SVL 0.053 vs. 0.042).

TABLE 2. Mean values and standard deviations (SD) of morphological measurements of *Calumma boettgeri*, n (males) = 11, n (females) = 12 and *C. linotum* males (n =5); all measurements in mm. For abbreviations see Table 1.

species	*C. boettgeri*		*C. boettgeri*		*C. linotum*	
locality	Nosy Be		Nosy Be		M. d'Ambre	
sex	m		f		m	
	mean value	SD	mean value	SD	mean value	SD
SVL	48.5	4.2	48.6	3.8	51.6	9.0
TaL	50.8	4.2	47.7	3.7	56.2	10.8
TL	99.3	8.1	96.3	7.2	107.8	19.7
RST	0.96	0.04	1.02	0.04	0.92	0.03
LRA	3.1	0.5	2.9	0.9	4.1	0.9
RRS	0.065	0.014	0.059	0.018	0.078	0.008
RAPSC	18.9	4.7	16.4	3.4	15.5	2.1
NPSCM	6.1	1.0	6.3	1.5	4.2	1.3
NSL	12	0.5	12	1	13	0
NIL	11.8	0.8	11.9	0.9	12.5	0.6
OLD	3.8	0.6	3.7	0.4	4.0	0.7
DSC	0.36	0.08	0.32	0.05	0.64	0.15
RSB	0.0075	0.0015	0.0066	0.0012	0.0124	0.0019
NSC	9.9	2.0	10.6	1.8	20.2	1.5
UAD	2.1	0.3	2.0	0.2	2.7	0.6
RAS	0.042	0.005	0.041	0.004	0.053	0.004

Both populations show heterogeneous scalation, especially at the extremities and the rostral appendage. The enlarged rounded tubercles on the limbs are distinctly larger in animals from Montagne d'Ambre; the mean diameter of the largest tubercle of the upper arm is 0.64 mm compared to 0.36 mm (Nosy Be). Additionally the number of the enlarged tubercles on the upper arm of males from Montagne d'Ambre is approximately twice that of males from Nosy Be, with a mean of 20.2 compared to 9.9, respectively, and the tubercles are not bordering each other on Nosy Be individuals. This character is also confirmed from a juvenile of SVL 26.9 mm (ZFMK 48227, Nosy Be) and a juvenile of SVL 36.2 mm (ZFMK 52308, Joffreville). Another morphological difference between both populations can be found in the pholidosis of the rostral appendage. Although the rostral appendage is significantly smaller in males from Nosy Be (see above), they have more peripheral scales on it, with a mean of 18.9 compared to 15.5 in Montagne d'Ambre males. In relation to the size of the appendage, this means 6.1 scales per mm compared with 4.1 scales per mm in Montagne d'Ambre. In summary, specimens from Montagne d'Ambre show a more heterogeneous scalation with broader tubercles on extremities and the rostral appendage. This is true of females as well (note that only one female with locality data was examined). Montagne d'Ambre individuals differ also in both sexes from Nosy Be specimens in the presence of a parietal crest which is best visible in the micro-CT scan (see below).

The other morphological features either were highly variable or did not differ between the populations. For example, the number of dorsal cones was 0–28 in Nosy Be, and 9–13 in Montagne d'Ambre. Likewise the edges of the occipital lobes vary between the specimens. They range between un-notched and clearly notched (up to 0.7 mm) in Nosy Be, and are either not or only slightly notched (up to 0.3 mm) in Montagne d'Ambre. All specimens have a rostral crest and none have axillary pits.

Colouration. Males also show great differences between the populations in colouration (see Fig. 2A, B). Males from Montagne d'Ambre were more colourful, with a true blue rostral appendage and greenish turquoise extremities (Fig. 2A). The colour of the legs is induced only by the coloured tubercle scales. The body is pale green or light brown with two dark brown spots and (occasionally) a beige lateral stripe on each side that stops at the base of the tail. The tail is the same colour as the body and (in stress colouration) possesses black annulations. The head is also greenish or brown with a dark stripe from the snout crossing the eyes to the occipital lobes. The skin around the mouth and the throat can be white. The colour description is based on a total of seven pictures of the Montagne d'Ambre form, referred to as *C. boettgeri* in Schmidt *et al.* (2010), *C. boettgeri* (picture 1c) in Glaw & Vences (2007) *Calumma* sp. in Nečas (2004) and *C. boettgeri* in Garbutt *et al.* (2001). The body and head of females is brown; the rostral appendage can be coloured bright blue, see picture of *C. boettgeri* in Schmidt *et al.* (2010).

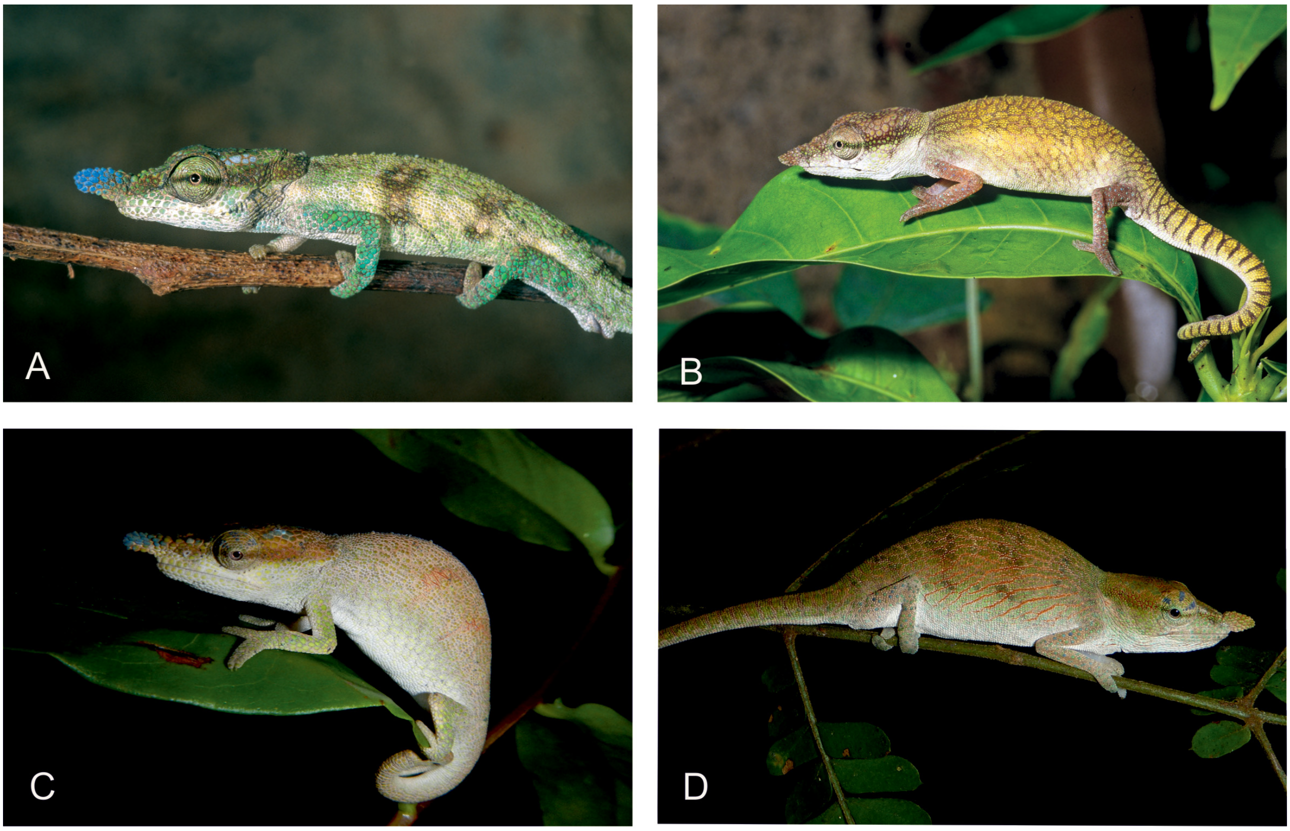

FIGURE 2. (A) Baseline colouration in life of male *Calumma linotum* from Montagne d'Ambre during day; (B) Baseline colouration in life of male *C. boettgeri* from Nosy Be during day; (C) female *C. linotum* from Montagne d'Ambre at night; (D) female *C. boettgeri* from Nosy Be at night. Photos taken by FG.

The body colouration of males from Nosy Be in contrast is yellowish or greenish brown with little dark brown rosettes, when stressed (Fig. 2B). The legs are brown with little blue or green spots resulting from the tubercles. The colouration of the head is similar to the body colouration. The rostral appendage differs clearly from Montagne d'Ambre with the absence of any striking colour and is the same brown colour as the casque. Females are uniformly light or greenish brown coloured. Compare also a total of seven pictures of *Calumma boettgeri* in Hyde Roberts & Daly (2014), in Glaw & Vences (2007: 191) picture 1a and 1b, in Nečas (2004) and in Henkel & Schmidt (1995).

Osteology of the skull based on micro-CT scans. Micro-CT scans of heads of two males and two females from Nosy Be and from Montagne d'Ambre exposed additional differences between the two forms. Specimens from Montagne d'Ambre (Fig. 4, D and E) bear tubercles on the parietal in both sexes. These form a little parietal crest in the middle with three to four tubercles, laterally followed by two tubercles on each side. The frontal is also irregularly spotted with tubercles. The parietal and frontal of animals from Nosy Be in contrast are smooth (Fig. 4, A and B).

As in all species of the genus *Calumma*, the nasal bones are paired (Rieppel & Crumly 1997). These are broader in our specimens from Montagne d'Ambre (mean NW 0.35 mm vs. 0.24 mm in Nosy Be; mean NW/NL

0.18 vs. 0.14, Table 3, Fig. 4) and the anterior tip of the frontal bone does not exceed more than a half of the naris. In skulls from Nosy Be it does exceed this point, and the frontal meets the premaxilla, as described for *C. nasutum* (Rieppel & Crumly 1997). The parietal also varies between the two localities. In Nosy Be samples, the parietal tapers more tightly. Its diameter is at the tightest area on average 0.61 mm (vs. 1.06 mm) and 11% of the largest diameter of the parietal (vs. 22%, Table 3, Fig. 4). The parietal in Montagne d'Ambre samples appears wider and more compact. However, the form of the parietal is variable within localities and cannot be used as a diagnostic character. Although chameleons are sexually dimorphic animals, differences between sexes in skull structure were not proven (Table 3).

TABLE 3. Osteological measurements of important characters of the skull for differentiation between *Calumma boettgeri* und *C. linotum.*
Notes: m, male; f, female; NL, nasal length; NW, nasal width; RNWL, ratio of nasal width to length; PL, largest diameter; PS, parietal smallest diameter; RPSL, ratio of parietal smallest to largest diameter; PC, parietal crest absent (-) or number of tubercles.

collection no.	species	locality	sex	NL	NW	RNWL	PL	PS	RPSL	PC
ZSM 440/2000	*C. boettgeri*	Nosy Be	m	2.1	0.3	0.14	4.7	0.5	0.11	-
ZSM 441/2000	*C. boettgeri*	Nosy Be	f	2.2	0.2	0.09	3.9	0.5	0.13	-
ZSM 444/2000	*C. boettgeri*	Nosy Be	m	1.8	0.3	0.17	4.7	0.3	0.07	-
ZSM 227/2002	*C. boettgeri*	Nosy Be	f	1.3	0.2	0.15	4.1	0.6	0.15	-
mean value				1.85	0.25	0.14	4.35	0.49	0.11	
SD				0.40	0.06	0.03	0.41	0.11	0.03	
MCZ 5988	*Ch. macrorhinus*	'Madagascar'	f	2.0	0.2	0.10	3.9	1.1	0.28	1
ZSM 2072/2007	*C. linotum*	M. d'Ambre	m	2.5	0.4	0.16	4.5	1.5	0.33	4
ZSM 2073/2007	*C. linotum*	M. d'Ambre	m	2.1	0.4	0.19	4.6	0.6	0.13	3
ZSM 873/1920/2	*C. linotum*	M. d'Ambre	f	2.3	0.3	0.15	4.2	0.9	0.21	4
ZSM 551/2001	*C. linotum*	Andampy	f	1.4	0.3	0.23	4.3	0.9	0.21	3
mean value				2.07	0.36	0.18	4.40	0.97	0.22	3.50
SD				0.49	0.04	0.04	0.18	0.38	0.08	0.58
ZSM 21/1923	*C. linotum*	'Madagascar'	m	2.7	0.3	0.11	4.6	1.4	0.30	4

Hemipenial morphology based on micro-CT scans. The scans of hemipenes of specimens from each population enable a detailed view of their structure. The hemipenes are illustrated in sulcal and asulcal view with the apex on top (Fig. 5). Both populations show large and deep calyces with smooth ridges on the asulcal side of the truncus. The apex is ornamented with two pairs of long pointed papillae and two pairs of rotulae. The papillae rise from the sulcal side of the apex and are curved to the asulcal side. They can be completely everted (Fig. 5, C) or retracted in the apex (Fig. 5, D). One pair of rotulae is placed on the asulcal side (the smaller one) and one pair on the sulcal side. Here some differences between the populations are recognizable; in Nosy Be (n = 2) the rotulae are slightly more denticulated, with 6–11 tips on asulcal side and 14–16 tips on sulcal side, compared to Montagne d'Ambre (n = 3) with 6–8 tips on the rotulae of the asulcal side and 11–14 tips on both rotulae on the sulcal side.

Re-description of the holotype of *Calumma linotum* (Müller, 1924)

Holotype. ZSM 21/1923, adult male, hemipenes not everted, location unknown ('Madagascar'), collected by an unknown individual on an unknown date; in a good state of preservation, except a slit on the ventral side of the body; mouth open with tip of the tongue between the jaws.

FIGURE 3. Preserved specimens with detail view on the different scalation of the upper arm; (A) male holotype of *Calumma linotum* (ZSM 21/1923, Madagascar), note the bluish colour of the limb scales and the two large grey markings on back and flanks; (B) female holotype of *Chamaeleo macrorhinus* (MCZ 5988, Madagascar). Scale bar = 10 mm.

Morphology. SVL 56.1 mm; tail length 70.0 mm; distinct rostral ridges that fuse on the anterior snout in a soft, laterally compressed dermal rostral appendage that projects 4.3 mm beyond the upper snout tip, rounded distally; supra-orbital crest rounded in lateral view and formed by a single, rather smooth row of tubercles; lateral crest poorly developed and pointing straight posteriorly, fusing to form the poorly developed temporal crest that curves upwards to the highest point of the casque; distinct parietal crest; occipital lobes clearly developed and slightly notched (0.1 mm); no traces of gular and ventral crest; dorsal crest in the form of enlarged tubercles present, starting 2.7 mm behind the occipital lobes without a defined ending, consisting of seven tubercles that are each separated by ~2.5 mm.

Body laterally compressed with fine homogeneous scalation, with the exception of the extremities and head region; legs with enlarged rounded tubercle scales bordering each other; heterogeneous scalation on the head and more strongly raised tuberclular scales on the rostral crest and rostral appendage; no axillary pits. Full morphological measurements are provided in Table 1.

Skull osteology of the holotype (Fig. 4F; Table 3). Broad nasal bones (NW: 0.3 mm, NL: 2.7 mm) are paired and meet anteriorly; the anterior tip of the frontal bone does not exceed more than half of the naris; the frontal is irregularly spotted with tubercles; four tubercles form a parietal crest in the middle of the parietal, laterally followed by two tubercles on each side. The parietal tapers posteriorly from 4.6 mm (largest diameter) to 1.4 mm (smallest diameter).

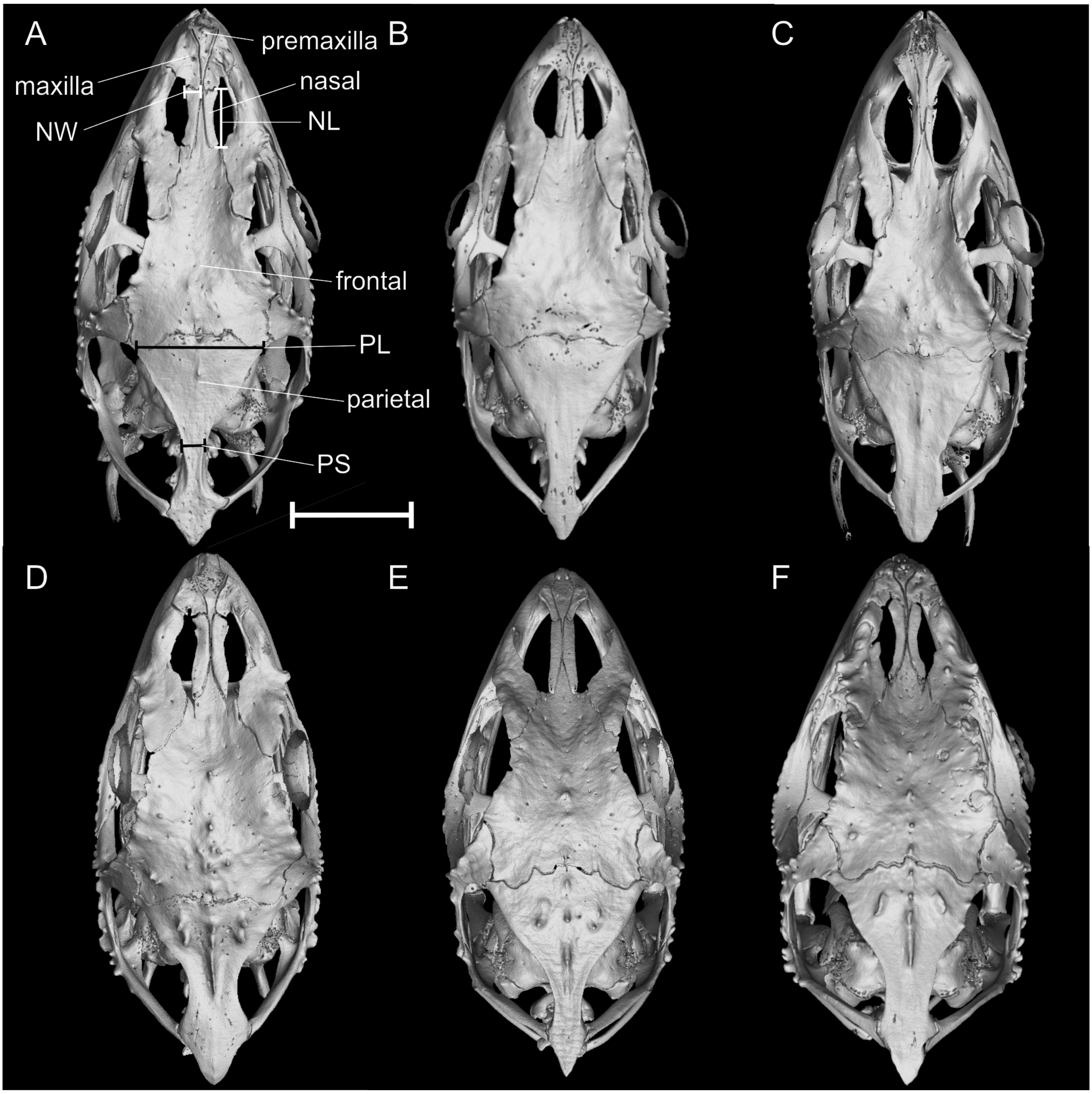

FIGURE 4. Micro-CT scans of skulls of *Calumma* in dorsal view; (A) male *C. boettgeri* (ZSM 440/2000, Nosy Be); (B) female *C. boettgeri* (ZSM 227/2002, Nosy Be); (C) female holotype of *Chamaeleo macrorhinus* (MCZ 5988, Madagascar); (D) male *Calumma linotum* (ZSM 2072/2007, Montagne d'Ambre); (E) female *C. linotum* (ZSM 873/1920/2, unknown locality); (F) male holotype of *C. linotum* (ZSM 21/1923, Madagascar). Scale bar = 5 mm. See Material & Methods for abbreviations.

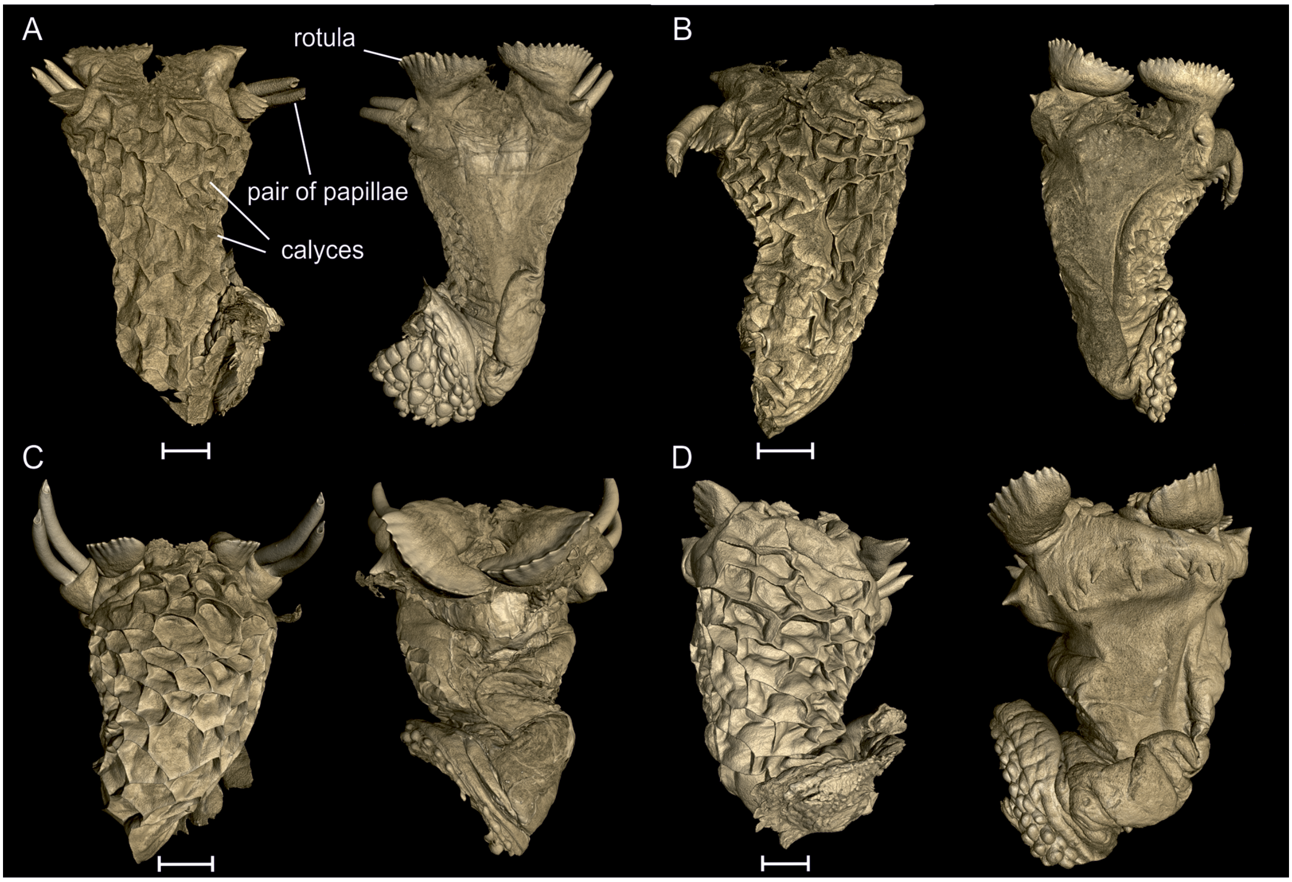

FIGURE 5. Micro-CT scans of hemipenes of *Calumma* species in asulcal and sulcal view. (A) *C. boettgeri* (ZSM 444/2000, Nosy Be); (B) *C. boettgeri* (ZSM 440/2000, Nosy Be); (C) *C. linotum* (ZSM 1683/2012, Montagne d'Ambre); (D) *C. linotum* (ZSM 2073/2007, Montagne d'Ambre). Scale bar = 1 mm.

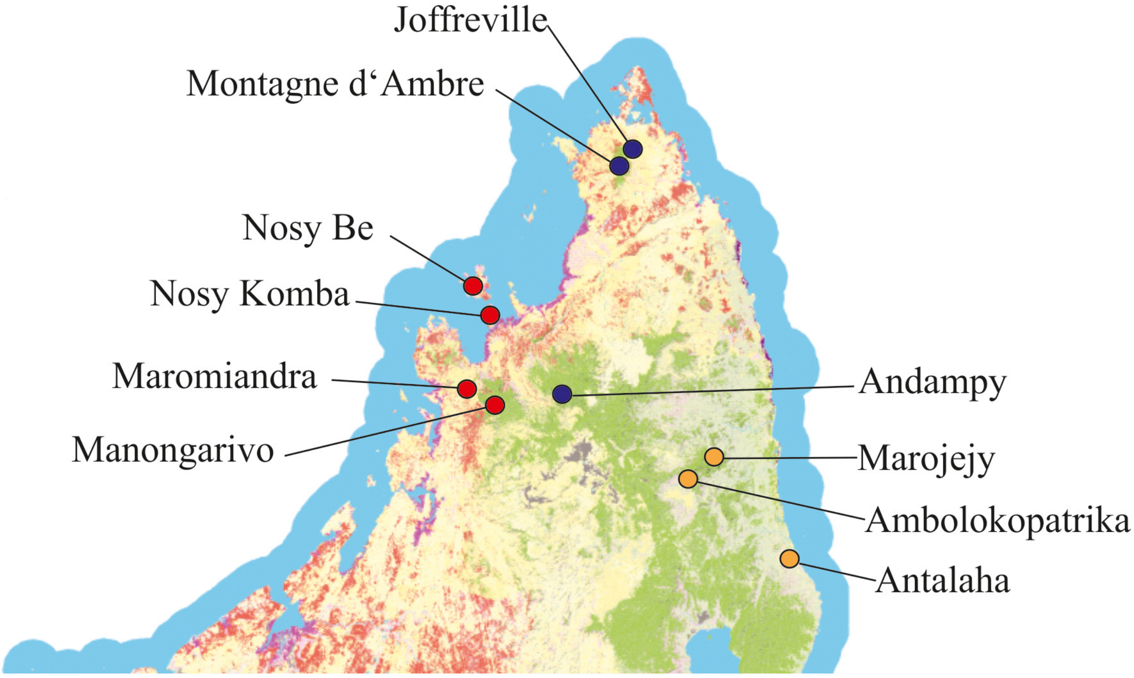

FIGURE 6. Map of Northern Madagascar with confirmed localities of *Calumma boettgeri* (red points) and *C. linotum* (purple points) and localities of *C. boettgeri* from the literature that are in need of confirmation (orange points). (www.vegmad.org)

Colouration of the holotype (Fig. 3A). The colour of the holotype is faded after storage in alcohol for almost one hundred years. Nonetheless, light blue colour of the extremities and the tip of the rostral appendage, as well as two dark spots on each side of the body and a slightly annulated tail are still recognizable.

Re-description of the holotype of *Chamaeleo macrorhinus* Barbour, 1903

Holotype. MCZ 5988, adult female, exact location unknown ('Madagascar'), collected by an unknown individual on an unknown date; in a good state of preservation, except body completely slit on the ventral side; mouth slightly opened.

Morphology. SVL 48.1 mm; tail length 44.9 mm; distinct rostral ridges that fuse on the anterior snout in a soft, laterally compressed dermal rostral appendage that projects 2.8 mm beyond the upper snout tip, rounded distally; no supra-orbital crest; lateral crest poorly developed and pointing straight posterior over a length of 3.0 mm; no temporal or parietal crests; occipital lobes clearly developed and slightly notched; 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 that are isolated from each other; heterogeneous scalation on the head and smooth tubercle scales on the rostral crest and rostral appendage; no axillary pits. Further morphological measurements are provided in Table 1.

Skull osteology of the holotype (Fig. 4C; Table 3). Narrow nasal bones (NW: 0.2 mm, NL: 2.0 mm) are paired and separated by the frontal and the premaxilla that meet between the nasal; the anterior tip of the frontal bone exceeds half of the naris; the frontal and parietal are smooth only with single tubercles. The parietal tapers strongly from 3.9 mm (largest diameter) to 1.1 mm (smallest diameter).

Colouration of the holotype (Fig. 3B). The colour of the holotype is almost completely faded after storage in alcohol for more than one hundred years. The light blue colour of tubercle scales on the extremities is still recognizable.

Identity and re-description of *Calumma linotum* (Müller, 1924)

Morphology, pholidosis, skull osteology, and colouration of the holotype of *Calumma linotum* allow us to assign it to the blue-nosed chameleons of Montagne d'Ambre, which were formerly considered *C. boettgeri* (Table 1–3, Figs. 2–4). The mean values of morphological measurements of male individuals from Montagne d'Ambre match those of the holotype (Table 1, 2): mean size of the broadest tubercle scale at the upper arm with 0.74 mm (vs. a mean of Montagne d'Ambre of 0.64); number of enlarged tubercles on the upper arm of 22 (vs. 20.20, SD 1.5); the diameter of the upper arm relative to the body size with 0.054 (vs. 0.053, SD 0.0038); the length of the rostral appendage, 4.3 mm (vs. 4.1 mm, SD 0.9) and the number of peripheral enlarged scales in relation to the length of the appendage, 3.5 per mm (vs. 4.1 per mm, SD 1.3).

Diagnosis. A small-sized chameleon (SVL 50.6–59.6 mm, TL 101.3–126.1 mm; TL up to 130 mm according to Mocquard 1895) that is characterised by a soft dermal, distally rounded, typically blue-coloured rostral appendage, slightly notched occipital lobes, a small parietal crest, a low casque, the absence of axillary pits, large rounded tubercles on the extremities bordering each other, presence or absence of a dorsal crest in males, low casque, and absence of gular and ventral crests. The three species *C. boettgeri, C. guibei,* and *C. linotum* differ from the other species of this group by the presence of occipital lobes. In *C. guibei* the occipital lobes are completely notched (vs. not or slightly notched in *C. linotum*). *Calumma linotum* differs from *C. boettgeri* in the larger rostral appendage of males related to the snout-vent length (RRS 0.078 vs. 0.065), the larger ratio of arm diameter to snout-vent length (UAD/SVL 0.053 vs. 0.042), the more heterogeneous scalation of the extremities (mean diameter of the largest tubercle of the upper arm 0.64 mm vs. 0.36 mm, mean number of enlarged tubercles on the upper arm 20.2 vs. 9.9), in skull structure (presence of parietal crest vs. absence, frontal bone not meeting premaxilla vs. meeting premaxilla, broad vs. narrow nasals), larger maximum total length, and in colouration (blue rostral appendage vs. brown; bright green extremities vs. brown). The karyotype of the species is described in Bourgat (1973) under the name of *C. boettgeri* from Montagne d'Ambre.

Colouration in life. The body of males in relaxed state is pale green or yellowish or light brown with two dark brown spots and (occasionally) a beige lateral stripe on each side that stops at the base of the tail. Under certain conditions (e.g. during stress or conspecific encounters), dark colour patterns can become prominent and the tail becomes annulated. The head is also greenish or brown with a dark line running from the snout crossing the eyes to the occipital lobes. The skin around the mouth and the throat can be white. A noticeable characteristic of the species is the true blue rostral appendage and the greenish turquoise extremities (Fig. 2A). The colour of the legs is induced only by the coloured tubercle scales.

The colouration of the females can vary from beige to a reddish or greenish brown ground colouration without any obvious colour patterns in a relaxed state, except for two inconspicuous brown spots on each flank. The rostral appendage is bright blue and the extremities are less striking green than in the males. In a stressed state, three parallel bright blue spots appear on the upper half of the eyelids and one blue spot on each side of the casque and on the frontal (Fig. 2C).

Distribution. Most samples of *C. linotum* are from Montagne d'Ambre—a well protected area in the North of Madagascar. Further localities are Joffreville north of Montagne d'Ambre (ZFMK 52308, see table 1) and Andampy (Manarikoba forest) in the Tsaratanana massif (ZSM 551/2001, see table 1). We also consider the *C. boettgeri* records of Mocquard (1895) from the surroundings of Diégo-Suarez (today Antsiranana) and Montagne d'Ambre as belonging to *C. linotum*. All studied individuals were found at an altitude between 730 m and 1050 m a.s.l. (Fig. 6). However, Raxworthy & Nussbaum (1994) found this species (as *C. boettgeri*) at Montagne d'Ambre between 650–1250 m a.s.l., and the maximum altitudinal distribution was recorded at 1306 m a.s.l. (Gehring *et al.* 2012 suppl. Table 1). The females (SMF 26357–26359) from Col Pierre Radama (=Ambatond'Radama, 35–40 km northeast of Maroantsetra) mentioned in Mertens (1933) neither belong to *C. linotum* nor to *C. boettgeri.* Their identity will be discussed in a subsequent publication.

Identity of *Calumma boettgeri* (Boulenger, 1888)

Morphological measurements and pholidosis of the holotype require the assignment of *Chamaeleo macrorhinus* to the Nosy Be form of *Calumma boettgeri.* The measurements are similar to the mean values of the females from Nosy Be (Tables 1–2): diameter of the broadest tubercle on the upper arm 0.34 mm (vs. mean of Nosy Be females of 0.32 mm, SD 0.048), number of enlarged tubercles on the upper arm 8 (vs. 10.6, SD 1.8); ratio of the upper arm diameter to the body size, 0.046 (vs. 0.041, SD 0.0038), total length, 93.1 mm (vs. 96.3 mm, SD 8.1 mm) and length of the rostral appendage, 2.8 mm (vs. 2.7 mm, SD 0.5 mm).

Diagnosis. A small-sized chameleon (SVL 41.1–55.5 mm, TL 83.8–108.0 mm) that is characterised by a soft dermal, distally rounded, typically brown rostral appendage, slightly notched occipital lobes, the absence of a parietal crest, a low casque, the absence of axillary pits, small rounded tubercles not bordering each other on the extremities, presence or absence of a dorsal crest in males, low casque, and absence of gular and ventral crests. It differs from *C. guibei* by unnotched or only slightly (max. 0.7 mm) notched versus completely notched occipital lobes. For a distinction from *C. linotum*, see above.

Colouration in life. The body and head colouration of males in relaxed state ranges from light brown to yellow without any obvious colour patterns. When stressed, dark colour patterns become prominent and the tail becomes annulated. A dark line runs from the snout tip across the eyes to the occipital lobes. The skin around the mouth and the throat can be white. Remarkable is the inconspicuous brown colour of the rostral appendage. The extremities appear brown also, except for a few green or blue coloured tubercle scales (Fig. 2B).

The colouration of the females can vary from beige to a reddish or greenish brown ground colouration in a relaxed state. The rostral appendage and the extremities show the same colour as the body, except for a few green tubercle scales on the legs. In a stressed state, three parallel bright blue spots appear on the upper half of the eyelids.

Distribution. All confirmed distribution records of *C. boettgeri* are confined to the biogeographic Sambirano region in northwest Madagascar. It was found in both primary rainforest (Lokobe) and secondary forests (near Andoany) of Nosy Be (Andreone *et al.* 2003), in Manongarivo (Rakotomalala 2002; Gehring *et al.* 2012), and on Nosy Komba (Hyde Roberts & Daly 2014). Additionally Nagy *et al.* (2012, suppl. Fig. 2) identified a population with similar gene sequences to *C. boettgeri* from a forest fragment locally known as Maromiandra (13°99'65"S, 48°21'77"E, 283 m). According to molecular phylogenetic data, *C. boettgeri* from Nosy Be and one individual from Manongarivo (FGMV 2002-813, 13°58’62”S, 48°25’32”E, 751 m a.s.l.) form their own clade (Gehring *et al.* 2012). The elevations of all these localities range from 0 to 751 m a.s.l. (Fig. 6).

We consider all additional records of *C. boettgeri*, all located in northeastern Madagascar, as in need of confirmation: Ambolokopatrika, 810–860 m a.s.l. (Andreone *et al.* 2000), Antalaha (Brygoo 1971), Andrakaraka forest station ca. 10 km from Antalaha (Ramanantsoa 1974), and Marojejy, 1100–1200 m a.s.l. (Raselimanana *et al.* 2000).

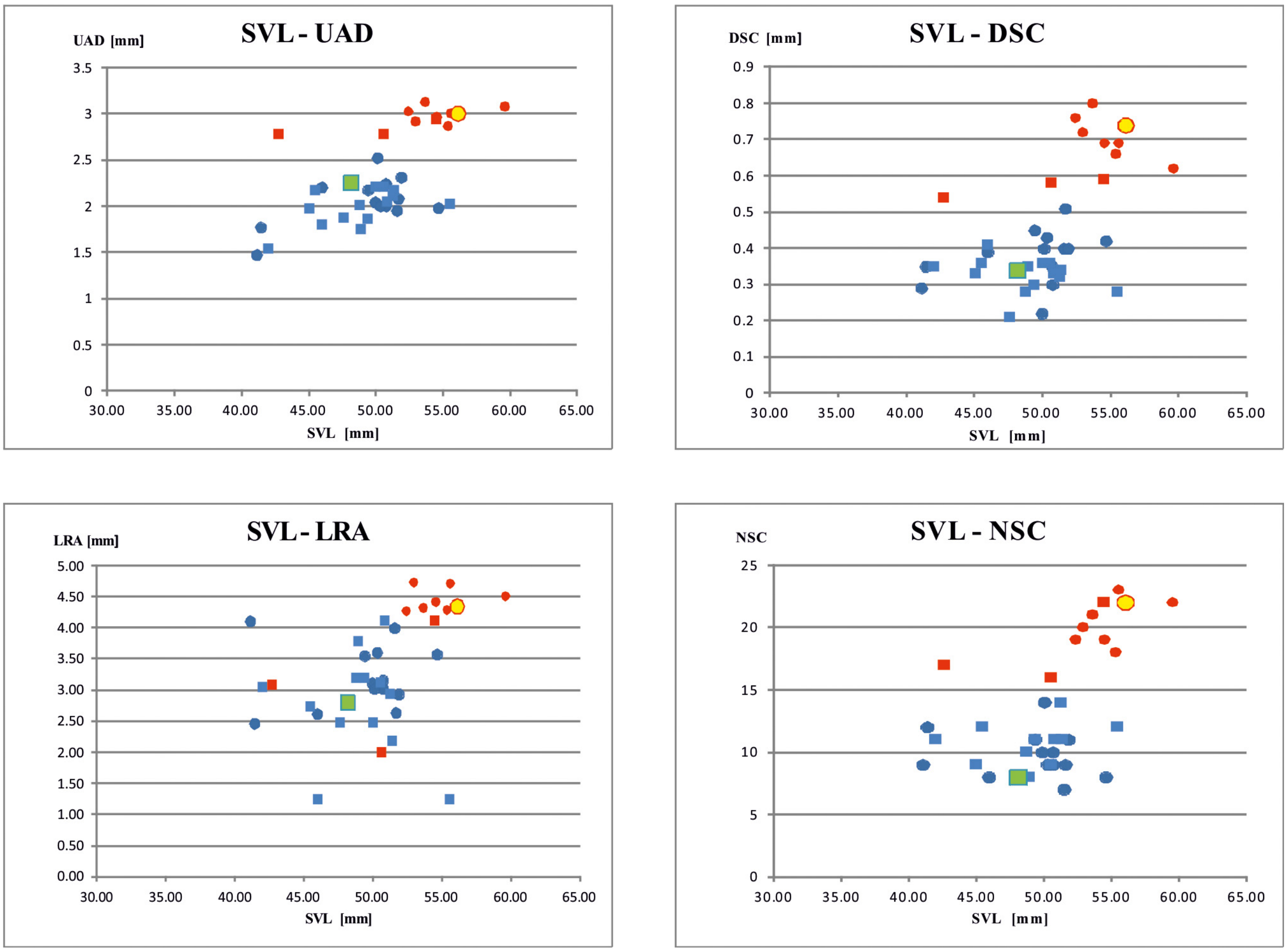

FIGURE 7. Important characters for the distinction of *C. boettgeri* (n = 27; blue symbols) and *C. linotum* (n = 11; red symbols) and the assignment of the holotypes of *C. linotum* (ZSM 21/1923; yellow symbol) and *Ch. macrorhinus* (MCZ 5988; green symbol) relative to the snout-vent length. Circles indicate males, while squares indicate females. Abbreviations: SVL, snout-vent length; UAD, upper arm diameter; DSC, diameter of broadest scale on upper arm; LRA, length of rostral appendage from snout tip; NSC, number of big scales on upper arm from lateral view.

Discussion

Using the approach of integrative taxonomy, the systematics of *C. linotum* is clarified in this work. Morphological characteristics could be assigned to two phylogenetic lineages of Gehring *et al.* (2012) within the *C. boettgeri* complex. The internal and external morphology of the holotype of *C. linotum* is in line with the individuals from Montagne d’Ambre and allows their assignment to *C. linotum.* Indeed, it is quite likely that the holotype was actually collected in Montagne d’Ambre, because the museum in Stuttgart harbours other reptile specimens without locality data which obviously originate from northern Madagascar, including the holotype of the typhlopid snake *Madatyphlops microcephalus*, a species which is mainly known from Montagne d’Ambre (Glaw & Vences 2007). Already Brygoo (1978) mentioned that a specimen from Montagne d’Ambre differs from specimens from Nosy Be.

Morphological measurements showed that *C. linotum* from Montagne d'Ambre is larger and more robustly built than *C. boettgeri*. The scalation of the extremities clearly differs between the species. In *C. linotum,* scales are heterogeneous with more tubercles, which are also larger. In contradiction of Brygoo (1971), the presence of a dorsal crest is not a characteristic for *C. linotum*, as it can be absent, present, or strongly developed in both species. Neither are the gular folds a distinctive character from *C. boettgeri* as mentioned in Müller (1924). According to Eckhardt *et al.* (2012), male *C. boettgeri* have significantly more dorsal spines than females; this is true for *C. linotum* as well, though in both sexes there are also specimens lacking a dorsal crest completely. Also variable is the shape of the notch between the occipital lobes. Both species possess rostral appendages in both sexes that are generally larger in males and possibly related to mate recognition and driven by sexual selection (Gehring *et al.* 2011). In *C. linotum* the rostral appendage is conspicuously blue coloured in both sexes and possibly reflecting in the ultraviolet spectrum. In the diffuse light of the rainforest in Montagne d'Ambre the coloured rostral appendage can play an important role in finding mates, as the experiments of Parcher (1974) have already shown in *C. nasutum*. The rostral appendage of *C. boettgeri* is of inconspicuous colour.

Calumma linotum also differs from *C. boettgeri* in the presence of a parietal crest. Micro-CT scans of the skull exposed this characteristic that is barely noticeable externally. Furthermore, differences in the form of the nasal, frontal, and parietal bone were identified. Some chameleons are known to show strong sexual dimorphism in terms of body size, colour or ornamentation (Stuart-Fox 2014). The micro-CT scans of several individuals of the same species showed remarkably little variation in characteristics like skull sutures between sexes within the *C. boettgeri* complex. Skeletal morphology has the potential to play an important role in future chameleon taxonomy. Unlike the method of clearing and staining (Rieppel & Crumly 1997), with micro-CT osteological data are more easily accessible and demonstrative to illustrate. This method is not invasive and the preserved samples are not destroyed, which is especially important when working with type specimens (Faulwetter *et al.* 2013).

The third species, *C. guibei*, which was not included in this study, differs morphologically from *C. boettgeri* and *C. linotum* in having largely separated occipital lobes (Hillenius 1959) and in the hemipenial structure, and represents its own phylogenetic lineage (clade F) as well, which contains two OTUs (Gehring *et al.* 2012). Further studies are necessary to refine the taxonomy of these OTUs as well as that of clade E (with two OTUs), which occur around the Tsaratanana massif at elevations from 1300–1550 m a.s.l (Gehring *et al.* 2012). For conservation issues clear species assignments with morphological characteristics are urgently needed. Due to its uncertain identity, *Calumma linotum* has not been assessed for the IUCN Red list of Threatened Species; it is commented within *C. boettgeri;* 'the identity of *C. linotum* is particularly unclear' (Jenkins *et al.* 2011a). *C. boettgeri* and *C. guibei* are listed as Least Concern and Near Threatened, respectively (Jenkins *et al.* 2011a, b). A new evaluation of the conservation status of *C. linotum* and *C. boettgeri* is in progress, in light of the revised taxonomy of this complex and our new understanding of their distributions. Species of the genus *Calumma* are also exported as pets, although the genus *Calumma* constitutes only a small part (1.7%) of chameleon exports (Jenkins *et al.* 2014). Clearly delimited species are the basis for correct export lists and also the protection of species with small distribution patterns.

In this study, micro-CT scans of hemipenes are presented for the first time for chameleons or even for squamate reptiles. Generally the hemipenis of *C. boettgeri* have rotulae that are slightly more denticulated than those of *C. linotum*, but no great differences are apparent. In cases of allopatric speciation, no pre-zygotic isolation is necessary to reinforce speciation. The hemipenis scans provide a comparatively objective and much more detailed view of the hemipenis structure than traditional illustrations; compare 3D PDF model (supplementary file) with illustrations of hemipenis from *C. boettgeri* in Gehring *et al.* (2012). By investigating more than one hemipenis per species, it became clear that there is intraspecific variation even in genital morphology. Furthermore, the ability to evert or retract the papillae can lead to an incorrect description. The risk of misinterpretation is much greater using classical techniques such as light microscopy and illustration than by 3D visualization and digitalization using X-ray micro-CT, and this technique should become the standard approach for the examination and description of this and similar important anatomical features. This further underlines the value and potential of micro-CT on its own and in combination with molecular analyses and classical morphological measurements in questions of taxonomy and systematics.

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Supporting information

Additional Supporting Information may be found online from:
http://www.mapress.com/zootaxa/2015/data/4048p211-231SupplementaryFile.pdf

Fig. S1. 3D model of hemipenis of *Calumma linotum* (ZSM 1683/2012).