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ORIGINAL PAPER

Computational molecular species delimitation and taxonomic revision of the gecko genus *Ebenavia* Boettger, 1878

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Abstract

Cryptic species have been detected in many groups of organisms and must be assumed to make up a significant portion of global biodiversity. We study geckos of the *Ebenavia inunguis* complex from Madagascar and surrounding islands and use species delimitation algorithms (GMYC, BOLD, BPP), COI barcode divergence, diagnostic codon indels in the nuclear marker PRLR, diagnostic categorical morphological characters, and significant differences in continuous morphological characters for its taxonomic revision. BPP yielded ≥ 10 operational taxonomic units, whereas GMYC (≥ 27) and BOLD (26) suggested substantial oversplitting. In consequnce, we resurrect *Ebenavia boettgeri* Boulenger 1885 and describe *Ebenavia tuelinae* sp. nov., *Ebenavia safari* sp. nov., and *Ebenavia robusta* sp. nov., increasing the number of recognised species in *Ebenavia* from two to six. Further lineages of *Ebenavia* retrieved by BPP may warrant species or subspecies status, but further taxonomic conclusions are postponed until more data become available. Finally, we present an identification key to the genus *Ebenavia*, provide an updated distribution map, and discuss the diagnostic values of computational species delimitation as well as morphological and molecular diagnostic characters.

Keywords BOLD · Operational Taxonomic Unit · Madagascar clawless gecko · Integrative taxonomy · Taxonomic inflation · Species complex

Introduction

Cryptic species is a term for lineages that are considered conspecific with a previously recognised species but should actually be regarded as distinct species. Typically, species remain cryptic for a long time or 'withstand' taxonomic revision not only because they belong to morphologically conservative taxa (Bickford et al. 2007) or because new and previously undetected diagnostic characters become available, e.g. at the karyological level (Marotta et al. 2014), but also because it might be difficult to assign a taxonomic name to one or more populations (Scherz et al. 2017; Bellati et al. 2018). However, cryptic species are probably very widespread across all groups of organisms (Pfenninger and Schwenk 2007) and may make up a considerable portion of global biodiversity (Adams et al. 2014), although they are probably unevenly distributed both geographically and among different groups of organisms (Giam et al. 2012).

The advent of molecular methods in systematic biology triggered a leap forward in the detection of cryptic species (e.g. Miralles et al. 2016). In many cases, the pre-sorting of specimens according to molecular lineages facilitated the detection of diagnostic morphological characters that had previously been neglected (Hebert et al. 2004). In particular, DNA barcoding (Hebert et al. 2003) with its employment of standard markers for large groups of organisms (e.g. cytochrome C oxidase I (COI) for animals) provided the framework for large national or even global sampling campaigns (Ratnasingham and Hebert 2007). These led to the detection of some spectacular cases of 'hyper-cryptic' species (Adams et al. 2014).

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The growing bulk of data generated by molecular phylogenetics and DNA barcoding makes manual detection and delineation of potential cryptic species difficult, and so the employment of algorithms for this task has increased. Out of a multitude of approaches, GMYC (Generalised Mixed Yule-Coalescent model) soon rose to prominence because it was specifically developed for use with single loci (Pons et al. 2006; Talavera et al. 2013). BOLD (Barcode Of Life Data systems), the main global repository for DNA barcoding data, started employing their own algorithm (Ratnasingham and Hebert 2013) which automatically, and conveniently for the user, assigns all barcodes to a Barcode Index Number (BIN) representing an operational taxonomic unit (OTU) or 'barcoding species'.

The BIN algorithm has been found to produce results superior to GMYC and other algorithms when compared to existing taxonomy in well-studied groups (Ratnasingham and Hebert 2013). However, like GMYC, it suffers from its otherwise advantageous property of analysing only a single molecular marker, normally the standard marker used in BOLD. Furthermore, most DNA barcoding markers represent mitochondrial loci, which are prone to provide misleading signals due to mitochondrial introgression and other effects (Hazkani-Covo et al. 2010). Therefore, advanced algorithms that enable the use of several markers, such as Bayesian species delimitation in BPP (Yang and Rannala 2010; Yang 2015), have been advised for use whenever adequate data are available.

Cryptic diversity in squamate reptiles is not uncommon (e.g. 14 operational taxonomic units or OTUs were detected in the gecko *Pristurus rupestris rupestris*; Garcia-Porta et al. 2017), especially in tropical countries like Madagascar (Gehring et al. 2012; Lemme et al. 2013; Ratsoavina et al. 2013; Miralles and Vences 2013; Vences et al. 2014). A recent phylogenetic and biogeographic study by Hawlitschek et al. (2017b) detected old and deeply divergent lineages in a molecular dataset of the Malagasy gecko *Ebenavia inunguis* Boettger, 1878. This lizard is widespread across the north and the east coast of Madagascar and on other islands of the Western Indian Ocean region, although it is relatively seldom seen in most areas. It can be locally difficult to collect and is relatively scarce in museum collections, preventing an in-depth taxonomic revision. A second species, *Ebenavia boettgeri* Boulenger, 1885, was erected several years after the original description of *E. inunguis*, but was poorly diagnosed and considered to be a synonym by Boulenger and all subsequent authors after the former examined the type of *E. inunguis* himself (Boulenger 1887). More than a hundred years later, *Ebenavia maintimainty* Nussbaum and Raxworthy, 1998 was discovered in Southwestern Madagascar and described as a morphologically clearly distinct species.

The study by Hawlitschek et al. (2017b) discussed five genetically divergent clades corresponding to (1) the Comoros Islands (excluding Mayotte Island), (2) North Madagascar + Mayotte Island + Pemba Island, (3) the eastern slopes of the central eastern highlands of Madagascar, (4) Nosy Be + nearby mainland areas of Madagascar, and (5) the eastern lowlands of Madagascar. In the present study, we use computational species delimitation to pin down cryptic lineages within the *E. inunguis* complex. We then revise the complex using a new set of voucher specimens from across the known range of *E. inunguis*. Finally, we compare the results of different species delimitation approaches and discuss them in the light of our morphological findings.

Material and methods

Sampling and sequencing

All sequence data analysed, consisting of alignments of three mitochondrial markers (12S, COI, CYTB) with 1329 bp and two nuclear markers (PRLR, RAG2) with 922 bp, was from Hawlitschek et al. (2017b), deposited under BOLD process IDs EBINU001–15 to 064–15 and GenBank accession numbers LT591928LT592132. Sampling and sequencing procedures were described in Hawlitschek et al. (2017b), and Table S1 in Appendix S2 of that work lists all metadata of the samples used.

Computational species delimitation

We conducted molecular species delimitation based on COI only using the GMYC (Pons et al. 2006) and BIN (Ratnasingham and Hebert 2013) methods, and based on the available nuclear markers (PRLR and RAG2) using Bayesian species delimitation in BPP 3.1 (Yang and Rannala 2010; Yang 2015). GMYC was run in R 3.1 and higher (R Core Team 2014) using the ultrametric tree reconstructed in BEAST by Hawlitschek et al. (2017b; shown in Fig. 1 of this paper) under single- and multiple-threshold models. The BIN algorithm was automatically employed after uploading COI data of *Ebenavia* to the BOLD database (Ratnasingham and Hebert 2007), assigning BINs to OTUs. We then compared the results of these methods and used the most conservative outcome, i.e. the lowest number of OTUs, as a priori species for input in BPP.

BPP 3.1 requires a fully resolved guide tree which is often generated using coalescent species tree methods, e.g. in *BEAST (Drummond et al. 2012). We attempted this, but *BEAST failed to produce a well-resolved tree even after unusually high numbers of generations (> 100 million). This mirrored the situation of the gene tree presented in Fig. 1 of Hawlitschek et al. (2017b), in which the topology of the clades (North + (Highland + (Nosy Be + East Coast))) was retrieved with high posterior probability in BEAST, but not in MrBayes

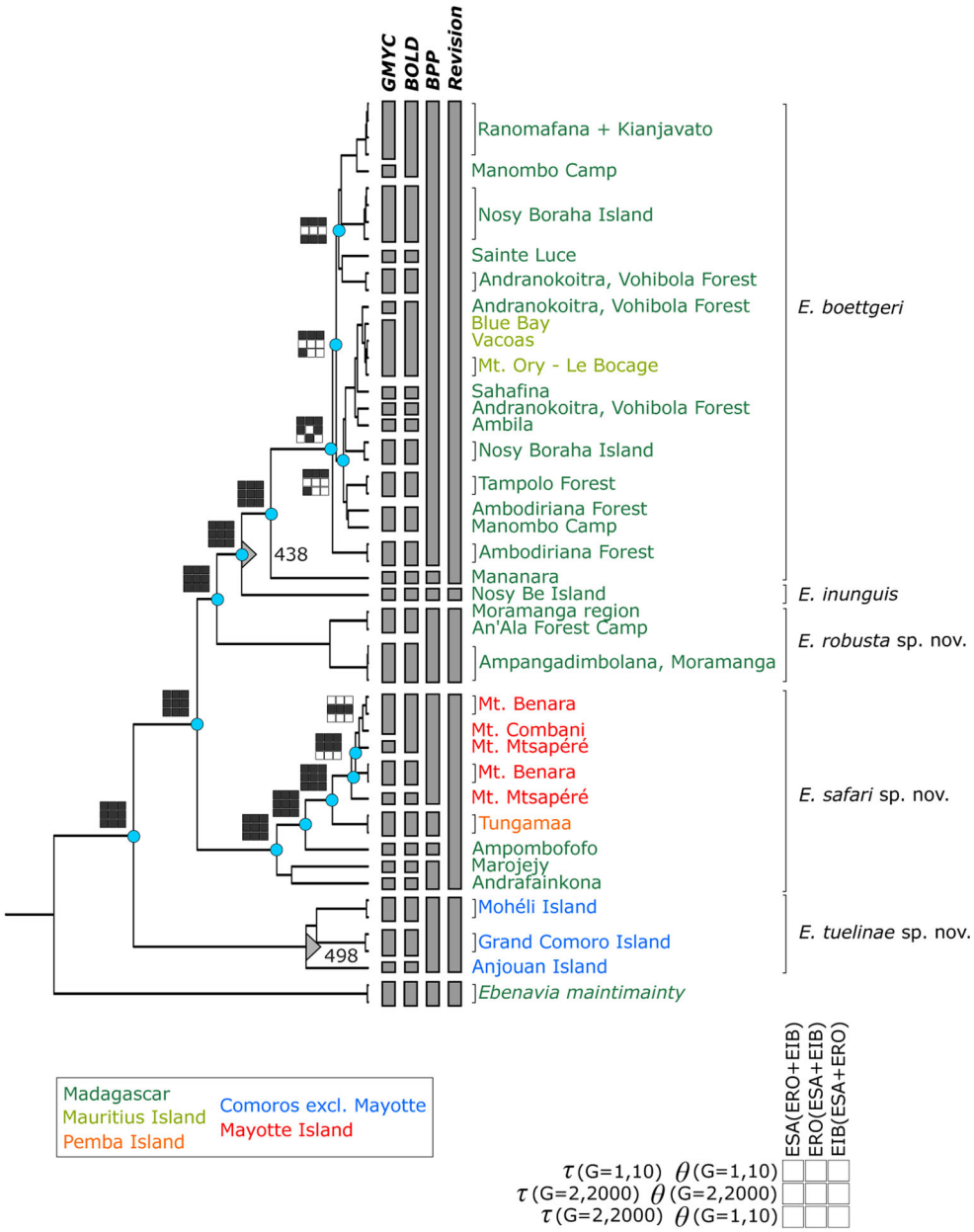

Fig. 1 A comparison of computational species delimitation results. The tree from Hawlitschek et al. (2017b) lists sample localities and compares species delimitation in GMYC, BOLD, and BPP (giving the most conservative results of GMYC and BPP) with the results of our taxonomic revision. Cyan circles mark the nodes that were supported by at least one BPP run. The boxes represent support BPP values and are filled if support was ≥ 0.95. Support values are given for different τ, θ, and starting tree topologies as explained in the bottom right of the figure. The grey triangles mark the amino acid insertions at positions 438 and 498 of PRLR. Geography is colour coded as explained in the bottom left of the figure

and RAxML. We therefore resort to manually generating an input species tree for BPP by removing the branch lengths of the original gene tree and pruning all tips to leave only one per input species. Then, we created two additional species trees by manually swapping the topologies of the North, Highland, and (Nosy Be + East Coast) clades to represent all three possible combinations. We then assigned the phased sequences of the nuclear markers to input species.

Species delimitation in BPP requires the a priori estimation of two evolutionary significant parameters: ancestral population size (θ) and degree of divergence among species (τ). We followed the procedure suggested in Yang (2015) in conducting runs with the following combinations: large ancestral population size ($\theta = G1, 10$) and deep divergences among species ($\tau = G1, 10$), small θ (G = 2, 2000) and shallow τ (G = 2, 2000), and large θ (G = 1, 10) but shallow τ (G = 2, 2000). We ran BPP under these three patterns for 500,000 generations for all three alternative topologies. For verification, we repeated the runs with a reduced guide tree, pruning all tips except those that were supported as species in the first BPP run with the same parameters. The results of these runs were considered final.

Divergence and diagnostic molecular characters

We used MEGA 7.0 (Kumar et al. 2016) to calculate genetic divergence (given as p-distances) between individuals, within clades, and between clades. We also inspected alignments of

nuclear markers for indels. Deletions corresponding to full amino acids according to the detected reading frame of nuclear genetic markers were considered diagnostic molecular characters. We follow Renner (2016) in considering such molecular diagnostic characters for formal species descriptions.

Morphological and statistical analyses

All morphological examinations were conducted by the same person (OH) on specimens of the following natural history collections: Zoologische Staatssammlung München, Germany (ZSM); Senckenberg-Museum, Frankfurt, Germany (SMF); Natural History Museum, London, United Kingdom (BMNH); Zoologisches Forschungsmuseum A. Koenig, Bonn, Germany (ZFMK); Museum für Naturkunde, Berlin (ZMB). Additional acronym used: American Museum of Natural History, New York (AMNH). No experiments were conducted on living animals, and no specimens were newly collected for this study; see Hawlitschek et al. (2011, 2013, 2017b) for information on the collection of the more recent samples. Based on the genetic analysis of Hawlitschek et al. (2017b), our new species delimitation analyses, and numbers of specimens available, we studied the morphological differences among five lineages of *Ebenavia*.

We examined 13 external morphometric (with 15 derived ratios) and 13 meristic characters (11 continuous, two categorical) in 66 specimens, taking all measurements with a digital calliper (0.01 mm precision) to the nearest 0.1 mm. We used a stereomicroscope to study meristic characters. Counts of bilateral meristic characters are given as 'left/right (mean)'. We studied the following morphometric characters: SVL = snout-vent length, TL = tail length (from cloaca to tip of tail, only given for original tails), BW = body width (at widest point), HL = head length (from snout tip to posterior end of cranium), HH = head height (measured at the level of the eyes), ED = horizontal eye diameter, SN = snout length (from snout tip to anterior margin of the eye), EE = distance between eye (posterior margin) and ear opening, IO = interorbital distance or distance between eyes (measured at anterior margin), EO = distance between ear openings, FORL = forelimb length (measured from the point where the limb attaches to the axilla to the tip of the longest digit), HINL = hindlimb length (measured from the point where the limb attaches to the groin to the tip of the longest digit), and AGD = axilla-groin distance (distance between limbs). We studied the following continuous meristic characters: SLAB = total number of supralabial scales on one side of the head not including the rostral scale, ILAB = total number of infralabial scales on one side of the head not including the mental scale, SCIL = number of superciliary scales, CANS = number of scales along the canthus rostralis, SDMiv = subdigital lamellae of fourth finger of hand (manus), SDPiv = subdigital lamellae of fourth finger of foot (pes), IOS = number of interocular scales (counted between anterior margin of eyes), PROS = number of postrostral scales, PMEN = number of postmental scales, DTAP = number of dorsal tubercles in a single row from anterior to posterior, and DTLR = number of rows of dorsal tubercles from left to right. We studied the following categorical meristic characters: TUB = presence of tubercular scales on the hindlimbs (yes/no) and RNO = rostral scale in contact with nostril (yes/no).

We conducted one-way ANOVA for all characters that were found to be informative in preliminary exploratory analyses (not shown) to test for significant differences between the clades. We also compared morphological characters among sublineages of the *Ebenavia* clade from North Madagascar + Mayotte + Pemba, but did not run any statistical analyses due to insufficient sample sizes.

To study osteology, we used X-ray photographs and X-ray micro-computed tomography (micro-CT). X-ray photographs of 14 specimens representing all major lineages were taken on a Faxitron Bioptics LLC Ultrafocus radiograph (ZSM 442/2000, ZSM 716/2000, ZSM 64–68/2010, ZSM 296–297/2010, ZSM 2724–2726/2010, ZSM 28–29/2012). We used these photographs to count presacral vertebrae and phalanges in manus and pes. Scans using micro-CT were made of the skulls of nine representative specimens (ZSM 29/2012, ZSM 68/2010, ZSM 442/2000, ZSM 1533/2012, ZSM 66/2010, SMF 8318, SMF 8319, ZSM 249/2013, ZSM 296/2010) to study characters of skull osteology. X-ray micro-CT scans were produced using a phoenix|x nanotom m (GE Measurement & Control, Wunstorf, Germany). Specimens were scanned at a voltage of 120 kV and a current of 80 μA for 2440 projections over 30 min. The 3D volumes (voxel size ranging from 4.7 to 6.8 μm) were converted to 8-bit and exported using VG Studio Max 2.2 (Volume Graphics GmbH, Heidelberg, Germany).

Species concept and taxonomical approach

We follow the General Lineage Concept of de Queiroz (1998) and use the following lines of evidence (Padial et al. 2010; Miralles et al. 2011) in an integrative approach to recognise species within the *Ebenavia inunguis* complex: (1) Support of ≥ 0.95 of the lineage in BPP. (2) Average COI divergence from other lineages of the complex ≥ 13.3%, referring to the average value identified in Nagy et al. (2012) between sister pairs of Malagasy gekkonid lizards. (3) Insertion or deletion of entire codons corresponding to amino acids in any of the nuclear markers studied, if diagnostic for a lineage. (4) Diagnostic categorical characters in external morphology or osteology. (5) Significant divergence in continuous characters in external morphology. We consider meeting the criteria for at least two of these lines of evidence sufficient for recognising a lineage as a species. We abstain from erecting paraphyletic taxa by describing species or subspecies nested in unresolved groups. Throughout this paper, we use the newly created names in anticipation of our taxonomic results for ease of understanding. The new taxonomic names established herein are available under the International Code of Zoological

Nomenclature from the appearance of the electronic edition. This work has been registered in ZooBank with the LSID (Life Science Identifier) urn:lsid:zoobank.org:pub:9BD9CD26-5D27-4CA4-A73E-F5DE031F7F53. The electronic edition of Science of Nature has an ISSN, and the work is archived and will be available from the digital repository of Zenodo.org.

Results

Computational species delimitation

Species delimitation of the *Ebenavia inunguis* complex in GMYC resulted in 27 OTUs in the multiple-threshold model and 29 in the single-threshold model. BOLD delimited 26 BINs (OTUs), which we used as a priori input for BPP. The BPP analyses yielded 10 to 16 OTUs. BPP retrieved more (max. 15) species under the assumptions of shallow divergence and small ancestral population sizes τ (G = 1, 10), small θ (G = 2, 2000), constant for all topologies. Generally, different topologies were equally supported under identical assumptions of τ and θ, but the topology with the sister group relationship of (Highland + (Nosy Be + East Coast) clades yielded one more OTU under τ (G = 1, 10) and θ (G = 2, 2000) than other topologies. BPP results based on different priors only differed for shallower nodes (average node ages < 2.4 ma as retrieved by Hawlitschek et al. 2017b). The results of different runs under identical prior assumptions did not differ significantly. A visual representation of the results of the species delimitation analyses is given in Fig. 1.

Genetic divergence and diagnostic molecular characters

Divergences in COI within and between lineages are given in Table 1. The divergences among all five major lineages of *Ebenavia inunguis* are similar to, or higher than, the value of 13.3% detected as average COI divergence between sister species of Malagasy gekkonid lizards by Nagy et al. (2012), and the maximum divergences between specimens of different lineages always exceed 13.3%. Divergences within lineages are also high, particularly within the Comoros, North, and East Coast lineages (see Table 1 for more details).

The visual inspection of the PRLR alignment detected two diagnostic codon insertions within the *E. inunguis* complex (highlighted in Fig. 1): One insertion of AGG (Glutamine) at position 498 from the 5′ primer binding site of PRLR was exclusively found in all individuals of the OTU assigned to *Ebenavia tuelinae* sp. nov. below; one insertion of CGA (Proline) at position 438 was exclusively found in all individuals assigned to the OTUs below assigned to *E. inunguis* + *E. boettgeri* bona species (resurrected below).

Morphology

A summary of diagnostic characters is given in Table 2. Results for all specimens studied, as well as the ANOVA results, are given in ESM 1. Figure 2 displays some external diagnostic characters, Fig. 3 and ESM 2 the osteology based on X-ray photographs and micro-CT scans, and Fig. 4 the live photographs of *Ebenavia inunguis* (Fig. 4a), *E. boettgeri* (Fig. 4b), *E. tuelinae* sp. nov. (Fig. 4c), *Ebenavia safari* sp. nov. (Fig. 4d), and *Ebenavia robusta* sp. nov. (Fig. 4e). We provide detailed results of our morphological analyses in the taxonomic accounts below.

Taxonomic acts

The following section includes diagnoses for *Ebenavia inunguis* and *E. boettgeri* and the descriptions of three new species in the *E. inunguis* complex. The name *E. inunguis* Boettger, 1878 is assigned to specimens forming a clade including all individuals

Table 1 Genetic divergence between *Ebenavia* species, given as p-distances of the cytochrome C oxidase I gene. All values are given as mean ± standard deviation (minimum–maximum)

	E. maintimainty (*N* = 2)	*E. tuelinae* sp. nov. (*N* = 4)	*E. safari* sp. nov. (*N* = 14)	*E. robusta* sp. nov. (*N* = 9)	*E. inunguis* (*N* = 5)	*E. boettgeri* (*N* = 39)
E. maintimainty	0 ± 0 (0–0)					
E. tuelinae sp. nov.	18.6 ± 1.5 (17.1–20.6)	8.3 ± 4.0 (0.3–10.6)				
E. safari sp. nov.	17.5 ± 0.8 (16.3–18.6)	17.0 ± 1.0 (14.0–18.9)	7.1 ± 4.0 (0–13.1)			
E. robusta sp. nov.	20.3 ± 0.4 (20.0–20.9)	18.0 ± 1.4 (15.4–19.7)	16.3 ± 1.0 (14.3–18.9)	4.8 ± 3.6 (0–8.0)		
E. inunguis	18.7 ± 0.6 (18.0–19.4)	16.7 ± 0.8 (15.1–18.0)	13.1 ± 0.7 (10.9–14.3)	16.3 ± 0.9 (15.1–18.6)	7.7 ± 4.0 (0.6–11.1)	
E. boettgeri	18.5 ± 0.8 (17.1–19.7)	16.1 ± 1.1 (14.0–18.0)	12.8 ± 1.0 (10.3–15.4)	17.0 ± 1.1 (13.7–19.1)	12.2 ± 1.7 (8.3–14.3)	4.0 ± 2.2 (0–12.3)

Table 2 Morphological differences between species of the *Ebenavia inunguis* complex. All values are given as mean ± standard deviation (minimum–maximum). Characters measured specifically in females are marked with an 'f'. N is given for all adult specimens studied, with N of females in brackets. Asterisks (*) mark characters with significant support or diagnostic value to distinguish a species. See the "Material and methods" section for abbreviations of characters. Individual values of the holotypes of *E. inunguis* and *E. boettgeri* are given in separate columns

	E. tuelinae sp. nov.	*E. safari* sp. nov.	*E. robusta* sp. nov.	*E. inunguis*	*E. inunguis* SMF 8318	*E. boettgeri*	*E. boettgeri* BMNH 81.7.8.17
N	6 (1)	17 (11)	5 (4)	7 (5)	1	26 (12)	1
SVL	33.78 ± 0.83 (32.90–35.20)	34.58 ± 2.63 (31.00–39.50)	38.80 ± 3.11 (34.40–42.60)*	35.00 ± 1.36 (32.80–37.00)	32.8	34.04 ± 2.52 (28.90–37.80)	34.2
SVL f	34.2	35.13 ± 3.09 (31.00–39.50)	39.00 ± 3.56 (34.40–42.60)*	35.43 ± 1.16 (33.80–37.00)	n/a	34.58 ± 2.24 (28.90–37.50)	34.2
BW/SVL	0.15 ± 0.01 (0.13–0.17)	0.16 ± 0.02 (0.13–0.20)	0.18 ± 0.03 (0.15–0.22)*	0.16 ± 0.02 (0.12–0.18)	0.15	0.16 ± 0.02 (0.11–0.20)	0.16
BW/SVL f	0.16	0.16 ± 0.02 (0.13–0.20)	0.18 ± 0.03 (0.15–0.22)	0.17 ± 0.02 (0.15–0.18)	n/a	0.17 ± 0.02 (0.13–0.20)	0.16
HL/SVL	0.27 ± 0.02 (0.24–0.30)*	0.25 ± 0.02 (0.22–0.29)	0.25 ± 0.02 (0.22–0.29)	0.24 ± 0.01 (0.23–0.26)*	0.25	0.24 ± 0.02 (0.22–0.29)	0.27
EE/HL	0.30 ± 0.03 (0.27–0.36)*	0.35 ± 0.03 (0.29–0.42)	0.35 ± 0.03 (0.32–0.39)	0.35 ± 0.02 (0.33–0.39)	0.34	0.35 ± 0.03 (0.29–0.41)	0.32
SN/EE	1.51 ± 0.17 (1.29–1.72)*	1.32 ± 0.12 (1.09–1.57)	1.30 ± 0.14 (1.14–1.52)	1.35 ± 0.12 (1.13–1.46)	1.46	1.34 ± 0.12 (1.07–1.56)	1.37
HNL/AGD	0.76 ± 0.07 (0.67–0.86)*	0.63 ± 0.07 (0.55–0.80)	0.71 ± 0.11 (0.62–0.89)	0.65 ± 0.07 (0.58–0.75)	0.75	0.69 ± 0.08 (0.57–0.94)	0.69
SLAB mean	9.42 ± 0.49 (9–10)	9.33 ± 0.58 (8–11)	9.60 ± 0.55 (9–10)	9.43 ± 0.45 (9–10)	10.0	9.20 ± 0.50 (8–10)	8.0
ILAB mean	9.67 ± 0.41 (9–10)	9.63 ± 0.68 (9–11)	10.60 ± 0.42 (10–11)*	9.50 ± 0.65 (8–10)	10.0	9.45 ± 0.60 (8–11)	8.0
IOS	19.83 ± 1.47 (18–21)	19.75 ± 1.66 (17–22)	22.00 ± 1.22 (20–23)*	18.86 ± 1.68 (16–20)	20	18.64 ± 1.33 (15–21)	15
DTAP	57.00 ± 4.24 (52–62)*	44.58 ± 5.76 (38–54)*	48.40 ± 1.82 (46–51)*	38.00 ± 3.32 (33–43)*	40	38.90 ± 4.48 (33–49)*	39
DTLR	11.00 ± 0.63 (10–12)*	12.08 ± 1.88 (10–16)	12.40 ± 2.41 (10–15)	12.29 ± 0.76 (11–13)	13	12.24 ± 1.41 (9–15)	10
RNO	Yes*	Yes*	No*	Yes*	Yes	No*	No
TUB	No	No	Yes*	No	No	No	No

from Nosy Be based on the original type locality 'Nossibé' [Nosy Be]. The name *E. boettgeri* Boulenger, 1885 (type locality 'Madagascar') is assigned to the East Coast clade of the *E. inunguis* complex based on morphological characters of the type specimen and comparison with recently collected material.

Taxon treatments

Genus *Ebenavia* Boettger, 1878

Type species: *Ebenavia inunguis* Boettger, 1878, by monotypy.

Gender of the genus: As explicitly stated in the original description (Boettger 1878:10), the genus *Ebenavia* was named after the collector Carl (sometimes written as Karl) Ebenau and is therefore formed neither from a Latin nor a Greek word. The fact that the name is consistently written as *Ebenavia* (as opposed to 'Ebenauia') throughout the paper suggests that the 'u' was intentionally changed to 'v'. The reason for this change was not given by Boettger, but it may have been a latinisation of the name, or for greater euphony, or both. The gender of the genus was not mentioned by Boettger (1878) and can neither be derived from the species name *inunguis* nor from the later described taxa *boettgeri* (named after Oskar Boettger) and *maintimainty* (Malagasy adjective). Based on Article 30 ('Gender of genus-group names') of the International Code of Zoological Nomenclature (1999) and more specifically Article 30.2.4. ('If no gender was specified or indicated, the name is to be treated as masculine, except that, if the name ends in *-/a/* the gender is feminine, and if it ends in *-/um/*, *-/on/*, or *-/u/* the gender is neuter.'). The gender of *Ebenavia* is feminine.

Diagnosis and description: A comprehensive diagnosis and description of the genus *Ebenavia* are provided in Nussbaum and Raxworthy (1998). Our data on the genus leads us to agree with the findings of that study, except that we were not able to confirm the presence of claws on the pes of any of the females we examined, as reported by these authors, in agreement with Boettger (1878) and many subsequent authors. We did not examine any specimens of *E. maintimainty.*

Content: *Ebenavia inunguis* Boettger, 1878; *E. boettgeri* Boulenger, 1885; *E. maintimainty* Nussbaum & Raxworthy, 1998; and three new species described below.

Distribution: Madagascar except most of the central and western areas; all major island areas of the Comoros Archipelago; Pemba Island (Tanzania); Mauritius.

Ebenavia inunguis Boettger, 1878

Remark: This species has been referred to as '*Ebenavia inunguis* complex Clade Cb' or 'Nosy Be Clade' (Hawlitschek et al. 2017b). Most historical records of *E. inunguis* refer to populations that are here assigned to other species. Based on our refined definition of *E. inunguis*, only records from northwestern Madagascar (see below) are

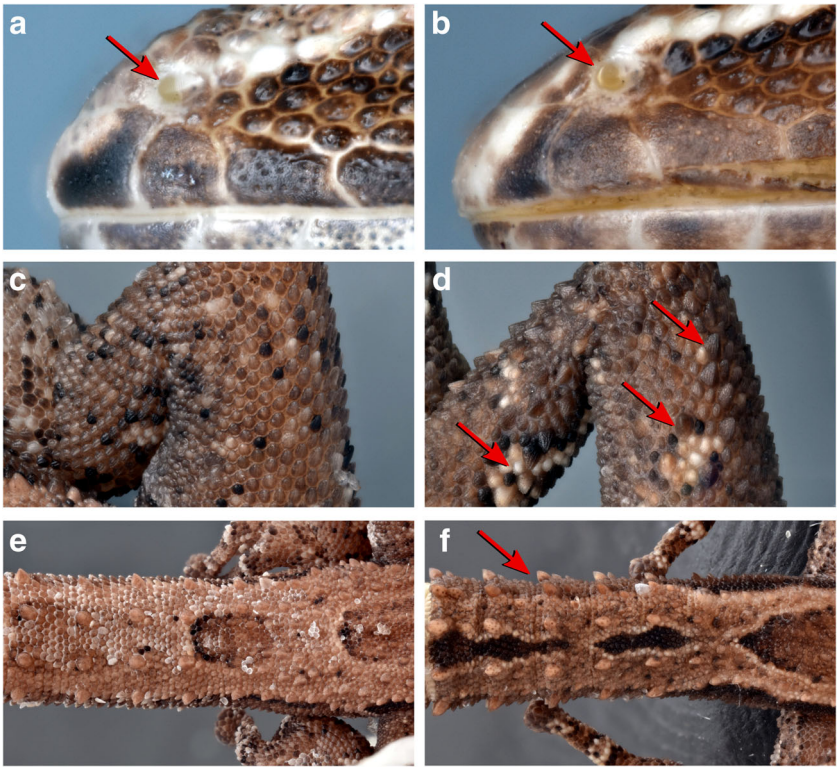

Fig. 2 Photographs of morphological characters that are diagnostic in the *Ebenavia inunguis* complex, exemplified by *E. tuelinae* sp. nov., ZSM 68/2010 (**a**, **c**, **e**) and *E. robusta* sp. nov., ZSM 296/2010 (**b**, **d**, **f**). Top: the arrangement of scales around the nostril (indicated with a red arrow), with the rostral scale, visible as the leftmost scale in each photograph, in contact (**a**) or not in contact (**b**) with the nostril. Middle: Hindlimbs only with granular scales of the same size class (**c**) or with distinct tubercles clearly larger than the surrounding granular scales (**d**; some enlarged tubercles highlighted by red arrows). Bottom: Original tail with regular whorls of small, spiny tubercles (**e**) or whorls of enlarged spiny tubercles interspersed with smaller tubercles (**f**)

assignable to this species. See the accounts of other members of this genus below for the assignment of other records.

Holotype: SMF 8318, originally 4096a, adult male, collected in 1878 in 'Nossibé' [Nosy Be], Madagascar by C. Ebenau. Boettger (1878:10) explicitly stated that his description was based on a single individual. Boettger (1893:26) indicated that 4096a consisted of four individuals. Mertens (1967) selected the original holotype as the lectotype of a series that also comprised three adult females SMF 8332–8334, which were originally joined with SMF 8318 under the number 4096a.

Further material examined: BMNH 86.2.25.2, adult male, collected on Nosy Be, Madagascar; ZSM 442/2000 (FG/MV 2000-198), adult female, collected 11 March 2000, East of Lokobe, Nosy Be, Diana Region, former Prov. Antsiranana, Madagascar (13.4133° S, 48.3342° E, 110 m a.s.l.), by F. Glaw, K. Schmidt, and M. Vences; ZSM 92/2015 (no field number), adult female, collected 18 November 2013 on Nosy Be, Diana Region, former Prov. Antsiranana, Madagascar (13.4068° S, 48.3407° E, 19 m a.s.l.), by Frontier Team Nosy Be.

Synonyms: *Ebenavia boettgeri* Boulenger, 1885 was considered a synonym by Boulenger (1887) and subsequent authors but is resurrected as a valid species by us below.

Rationale for taxon assignment: Nosy Be is the type locality of *Ebenavia inunguis* Boettger, 1878, and the holotype is morphologically indistinguishable from specimens of the Nosy Be Clade (Clade Cb) of Hawlitschek et al. (2017b). The name *Ebenavia inunguis* Boettger, 1878 is therefore assigned to this lineage.

Diagnosis: Morphological data is given in Table 2 and ESM 1, the photograph of a living specimen is available in Fig. 4a. Distinguished from *Ebenavia maintimainty*, *E. boettgeri*, and *E. robusta* sp. nov. by rostral scale in contact with nostril (RNO = yes); from *E. maintimainty* by larger SVL (32.8–37.0 vs. ≤ 24 mm), rostral scale bordered by postrostrals distinct from posterior head scales, absence of prenasal scale between rostral and nostril, keeled abdominal scales, and lighter colour; from *E. robusta* sp. nov. by absence of distinct tubercles on hindlimbs (TNO = no), smaller SVL (32.8–37.0 vs. 34.4–42.6 mm), smaller ratio of BW/SVL (0.12–0.18 vs. 0.15–0.22), fewer ILAB (8–10 vs. 10–11), IOS (16–20 vs. 20–23), and DTAP (33–43 vs. 46–51); from *E. tuelinae* sp. nov. by ratio EE/HL (0.33–0.39 vs. 0.27–0.36), HINL/AGD (0.58–0.75 vs. 0.67–0.86), and lower DTAP (33–43 vs. 52–62); from *E. safari* sp. nov. by generally lower DTAP (33–43 vs. 38–54). Furthermore, distinguished from *E. boettgeri* and *E. safari* sp. nov. by short nasal process of prevomer; from *E. tuelinae* sp. nov. and *E. robusta* sp. nov. by long premaxillary lappets of nasals; from *E. safari* sp. nov. by recognisable basal tubercle on basisphenoid; and from all other analysed *Ebenavia* except *E. boettgeri* by a codon insertion of CGA (Proline) at position 438 from the 5′ primer binding site of the PRLR fragment.

Osteological description of the holotype: Endolymphatic sacs in the neck not calcified. Premaxilla with ca. 8 teeth, maxilla with ca. 42 teeth. Nasals fused. Nasal process of premaxilla short and sharply tapering. Premaxillary lappet of nasal long and tapering. Parietals paired, not fused.

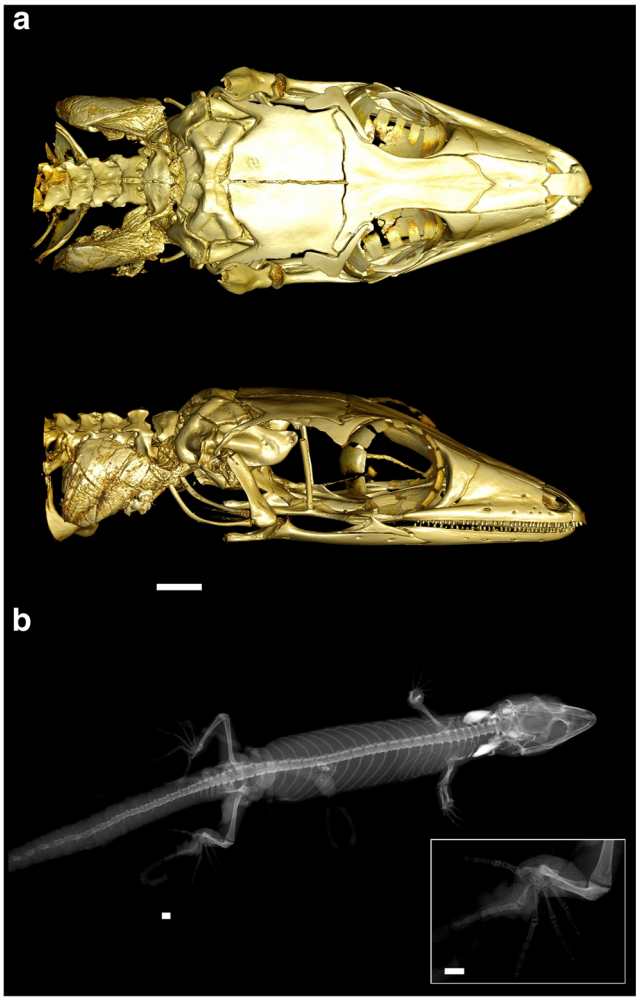

Fig. 3 The osteology of *Ebenavia*, exemplified by *E. robusta* sp. nov. (ZSM 296/2010). Dorsal and lateral views of micro-CT scans of the head (**a**) and a full-body X-ray photograph (**b**), with an inset magnified image of the pes. Note the white calcified endolymphatic sacs in the neck in **b**. Length of scale bars = 1 mm

Maxilloprefrontal suture connects with nasals anterior to contact point of frontonasal suture with prefrontals. Frontonasal suture with shape similar to brace or curly bracket, with tip in anterior direction. Basal tubercle on basisphenoid present. Presacral vertebrae 26, including 8 cervical vertebrae and 3 vertebrae connected to sternal ribs. Phalangeal formulae 2-3-4-5-3 in manus and 2-3-4-5-4 in pes.

Variation: No remarkable variation detected.

Species delimitation rationale: *Ebenavia inunguis* was the first species described in the genus *Ebenavia* and is a valid species. The genetic lineage assigned to this name is delimited by BPP support of ≥ 0.95, by COI divergence of at least 13.3% from *E. tuelinae* sp. nov. and *E. robusta* sp. nov., by the insertion of a codon in PRLR from all other members of the *E. inunguis* complex except *E. boettgeri*, by diagnostic categorical morphological characters from *E. boettgeri* and *E. robusta* sp. nov., and by significant divergence in continuous morphological characters from *E. tuelinae* sp. nov. and *E. robusta* sp. nov.

Distribution, natural history, and conservation: See Fig. 5 for a distribution map. All specimens studied morphologically are from Nosy Be Island. Almost certainly, but genetically not yet confirmed, *Ebenavia inunguis* also occurs on the island of Nosy Komba very close to Nosy Be: Hyde-Roberts and Daly (2014) recorded one individual in degraded habitat and provided a colour photo from Nosy Komba. Blumgart et al. (2017) recorded the species in the three habitat types 'closed canopy forest', 'disturbed canopy forest', and in 'shade-grown coffee plantations', but not in 'open plantations'. Hawlitschek et al. (2017b) and Penny et al. (2018) found additional COI sequences from mainland Madagascar clustering with the Nosy Be clade. These sequences are from the Ankarafa Forest (14.3803° S, 47.7577° E, 154 m a.s.l.) in the Sahamalaza Peninsula, on the Northwest Coast around 100 km south of Nosy Be (Fig. 5). In Nosy Be, the species is found in many areas and habitats, including forest in the reserve of Lokobe (Andreone et al. 2003). As the forest of Lokobe is well protected, and *Ebenavia inunguis* is also found in modified habitats, the species is probably not severely threatened in Nosy Be. On the contrary, the mainland population of Sahamalaza Peninsula is probably severely threatened (Penny et al. 2014, 2018; Seiler et al. 2017). The forests in this area are extremely fragmented, and fragments are more or less degraded (Schwitzer et al. 2007). Steep increases in the rates of habitat destruction over the recent years will potentially lead to the total deforestation of the area within one or two decades (Penny et al. 2016), as was recently observed in a forest fragment in Analavory (Volampeno 2009). One individual of *E. inunguis* was found at Ankarafa (14.3789° S, 47.7622° E, 164 m a.s.l.) perching at around 1 m on the side of a shrub within a swampy flooded grassy clearing in a secondary forest fragment on 10 January 2012, 21:01 (S. Penny, personal observation). Another genetically confirmed record of *E. inunguis* is AMNH R152971 from Antsahabe (Raxworthy et al. 2008), with GenBank accession numbers EU596608 (12S) and EU596692 (CYTB), most likely located at ca. 14.8000° S, 48.3833° E, ca. 160 km south of Nosy Be.

In summary, *E. inunguis* suffers from habitat degradation and fragmentation, but not throughout its entire range. The minimum convex polygon derived from the three localities Nosy Be–Sahamalaza–Antsahabe covers 4640 km^2 (including ocean area).

Ebenavia boettgeri Boulenger, 1885, bona species

Remark: This taxon was previously considered a synonym of *Ebenavia inunguis* Boettger, 1878 and is hereby resurrected. *E. boettgeri* has been referred to as '*Ebenavia inunguis* complex Clade Cc' or 'East Coast Clade' (Hawlitschek et al. 2017b) and has been treated under the name *E. inunguis* in all other publications since its synonymisation (Boulenger 1887).

Holotype: BMNH 81.7.8.17, adult female; collected in 'Madagascar' by an unknown collector on an unknown date.

Fig. 4 Photographs of live individuals of the *Ebenavia inunguis* complex. **a** *E. inunguis*, Nosy Be. **b** *E. boettgeri*, Betampona. **c** Holotype of *E. tuelinae* sp. nov., ZSM 68/2010. **d** Holotype of *E. safari* sp. nov., ZSM 2725/2010. **e** *E. robusta* sp. nov., specimen from same captive breeding stock, as sample Ei_F2 from Hawlitschek et al. (2017b). **f** Hatchling of *E. safari* sp. nov., ZSM 1698/2008. **g** Hatchling of *E. robusta* sp. nov., same breeding stock as **e**. Photographs not to scale; **b** by G. Rosa; **e**, **g** by K. Glaw; all other photographs by the authors

Further material examined: BMNH 92.3.7.1–5, two adult males, two adult females, and one juvenile, collected at Sahambendrana, Betampona, Antsinana Region, former Prov. Toamasina, Madagascar; BMNH 93.8.5.1–2, adult female and

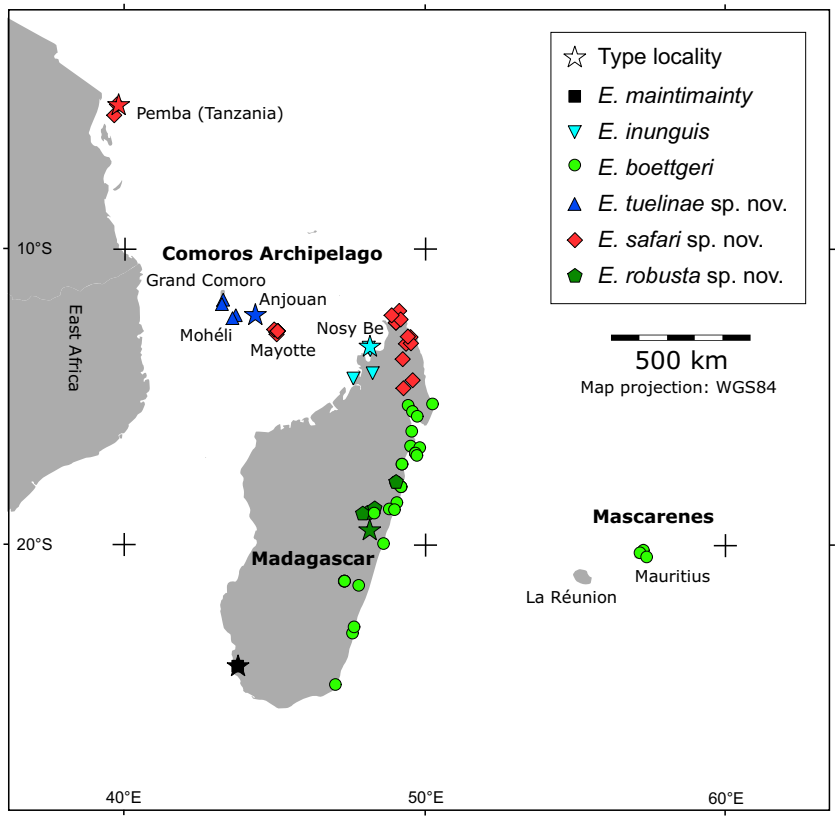

Fig. 5 Map of the distribution of all known species of the genus *Ebenavia*. The type locality of *E. boettgeri* is not shown because it is not precisely known

juvenile, collected at Farafangana, Atsimo-Atsinanana Region, former Prov. Fianarantsoa, Madagascar; SMF 8319, adult female, collected at Anevoka, Alaotra-Mangoro Region, former Prov. Toamasina, Madagascar; SMF 8320–8322 and 8329–8330, three adult males and two adult females, collected 1905 at Nosy Boraha (Île Sainte-Marie), Analanjirofo Region, former Prov. Toamasina, Madagascar; SMF 8331, adult male, collected 1905 at 'Toliara' (see note below), Madagascar; SMF 22538, adult female, collected 1931 at Nosy Mangabe, Analanjirofo Region, former Prov. Toamasina, Madagascar; SMF 57296, adult male, collected 1953 at Tolagnaro (Fort Dauphin), Anosy Region, former Prov. Toliara, Madagascar; ZMB 19007-1 and 3–4, adult males, collected at Nosy Boraha (Île Sainte-Marie), Analanjirofo Region, former Prov. Toamasina, Madagascar; ZSM 692/2003 (FG/MV 2002-0315), adult male, collected 20 January 2003 at Hotel Manja, Ranomanjana, Vatovavy-Fitovinany Region, former Prov. Fianarantsoa, Madagascar, by F. Glaw, M. Puente, L. Raharivololoniaina, M. Thomas, and D.R. Vieites; ZSM 141/2005 (FGZC 2636), adult female, collected 04 February 2005 at Sainte Luce, Anosy Region, former Prov. Toliara, Madagascar (24.7750° S, 47.1500° E, 22 m a.s.l.), by F. Glaw and P. Bora; ZSM 1920/2008 (no field number), adult male, collected prior to 2008 at Ambohitralanana, Sava Region, former Prov. Antsiranana, Madagascar; ZSM 294/2010 (FGZC 4291), adult male, collected 01–04 March 2010 at Ambodivoahangy, close to Makira reserve, Analanjirofo Region, former Prov. Toamasina, Madagascar (15.2899° S, 49.6203° E, ca. 100 m a.s.l.), by F. Glaw, J. Köhler, P.-S. Gehring, M. Pabijan, and F.M. Ratsoavina; ZSM 24/2017 (ACZCV 0014), adult male, collected on 03 November 2013 at Rendryrendry, Betampona, Antsinana Region, former Prov. Toamasina, Madagascar (17.9312° S, 49.2039° E, 299 m a.s.l.), by A. Crottini, D. Salvi, E. Scanarini, George, G.M. Rosa, D.J. Harris, M. Randriamialisoa, and H. Lava; ZSM 25/2017 (ACZCV 0153), juvenile, collected on 22 November 2013 at Maintimbato, Betampona, Antsinana Region, former Prov. Toamasina, Madagascar (17.8988° S, 49.2290° E, 425 m a.s.l.), by G.M. Rosa, D.J. Harris, M. Randriamialisoa, and H. Lava; ZSM 26–27/2017 (ACZCV 185/189), adult females, collected on 27 November 2013 at Ivoloina Park, Tamatave, Antsinanana Region, former Prov. Toamasina, Madagascar (18.0828° S, 49.3483° E), by A. Crottini; ZSM 28/2017 (FAZC 15445), sex not determined, collected in 2010 at Rendryrendry, Betampona, Antsinana Region, former Prov. Toamasina, Madagascar (17.9312° S, 49.2039° E, 299 m a.s.l.), by F. Andreone and A. Crottini; ZSM 263/2016 (FGZC 5435), adult female, collected 11 August 2016 near 'Eco-Lodge chez Arol', Masoala, Analanjirofo Region, former Prov. Toamasina, Madagascar (15.7122° S, 49.9640° E, ca. 21 m a.s.l.), by F. Glaw, D. Prötzel, J. Forster, K. Glaw, and T. Glaw.

Rationale for taxon assignment: The type locality of *Ebenavia boettgeri* Boulenger, 1885 is imprecisely given as 'Madagascar'. The holotype is distinguished from *E. inunguis* and *E. safari* sp. nov. by the nostril in contact with the rostral and from *E. robusta* sp. nov. by the absence of distinctly enlarged tubercles on the hindlimbs. The name *Ebenavia boettgeri* Boulenger, 1885 is therefore assigned to the East Coast Clade (Cc) of Hawlitschek et al. (2017b), with which the holotype agrees in all characters.

Diagnosis: Morphological data is given in Table 2 and ESM 1, a photograph of a living specimen is available in Fig. 4b. Distinguished from *Ebenavia maintimainty* by larger SVL (28.9–37.8 vs. ≤24 mm), rostral scale bordered by postrostrals distinct from posterior head scales, absence of prenasal scale between rostral and nostril, keeled abdominal scales, and lighter colour; from *E. robusta* sp. nov. by absence of distinctly enlarged tubercles on hindlimbs (TUB = no), smaller SVL (28.9–37.8 vs. 34.4–42.6 mm), smaller ratio of BW/SVL (0.11–0.20 vs. 0.15–0.22), lower ILAB (8–11 vs. 10–11), IOS (15–21 vs. 20–23), and DTAP (33–49 vs. 46–51); from *E. inunguis*, *E. tuelinae* sp. nov., and *E. safari* sp. nov. by rostral scale not in contact with nostril (RNO = no); from *E. tuelinae* sp. nov. by ratio EE/HL (0.29–0.41 vs. 0.27–0.36), and lower DTAP (33–49 vs. 52–62); from *E. safari* sp. nov. by generally lower DTAP (33–43 vs. 38–54). Furthermore, distinguished from all other analysed *Ebenavia* except *E. safari* sp. nov. by long tapering nasal process of premaxilla; from *E. tuelinae* sp. nov. and *E. robusta* sp. nov. by long premaxillary lappets of nasals; from *E. safari* sp. nov. by recognisable basal tubercle on basisphenoid; and from all other analysed *Ebenavia* except *E. inunguis* by a codon insertion of CGA (Proline) at position 438 from the 5′ primer binding site of the PRLR fragment.

Osteological description: SMF 8319: Calcified endolymphatic sacs in the neck present. Premaxilla with ca. 8 teeth, maxilla with ca. 42 teeth. Nasals fused. Nasal process of premaxilla extended, tapering. Premaxillary lappet of nasal long and tapering. Parietals paired, not fused. Maxilloprefrontal suture connects with nasals anterior to contact point of frontonasal suture with prefrontals. Frontonasal suture with shape similar to brace or curly bracket ('}'), with tip in anterior direction. Basal tubercle on basisphenoid present. Presacral vertebrae 26, including 8 cervical vertebrae and 3 vertebrae connected to sternal ribs. Phalangeal formulae 2-3-4-5-3 in manus and 2-3-4-5-4 in pes.

Variation: ZSM 1920/2008: rostral scale in contact with nostril. This specimen from Ambohitralanana agrees with *Ebenavia boettgeri* in all other characters studied except the ratio of FORL/HINL, which is closer to *E. safari* sp. nov. It was originally assigned to the clade based on genetic data in Hawlitschek et al. (2017b). However, its position in the phylogenetic tree was the sister group to all other *E. boettgeri*, and due to poor node support a closer relationship with *E. inunguis* cannot be ultimately ruled out. Morphological data also cannot exclude placement in *E. inunguis*. The specimen was not

included in the BPP analysis because only COI data was available. We tentatively maintain the assignment of this individual to *E. boettgeri*, but highlight the uncertainty of this placement and remark that it might be found to represent a species distinct from *E. inunguis* and *E. boettgeri*.

Species delimitation rationale: *Ebenavia boettgeri* is delimited by BPP support of ≥ 0.95, by COI divergence of at least 13.3% from *E. tuelinae* sp. nov. and *E. robusta* sp. nov., by the insertion of a codon in the analysed PRLR fragment from all other members of the *E. inunguis* complex except *E. inunguis*, by diagnostic categorical morphological characters from all other members of the *E. inunguis* complex, and by significant divergence in continuous morphological characters from all other members of the *E. inunguis* complex except *E. safari* sp. nov.

Distribution, natural history, and conservation: See Fig. 5 for a distribution map. *Ebenavia boettgeri* is the most widespread species of the complex, inhabiting the lowlands of the eastern rainforest belt from Sainte Luce in the southeast to Ambodivoahangy and possibly Ambohitralanana on the Masoala Peninsula in the northeast (Fig. 5). The genetically identified localities are listed in Hawlitschek et al. (2017b). Numerous additional *Ebenavia* localities are known from the East Coast (e.g. Glaw and Vences 2007), but for most of them it is uncertain if they refer to *E. boettgeri* or to *E. robusta* sp. nov. The locality of SMF 8331 is given as 'Tulear'; we agree with Nussbaum and Raxworthy (1998) who argued, while erroneously listing the Tulear specimen as SMF 57296 (true locality Tolagnaro), that this locality is unusual regarding the known distribution and ecology of *E. boettgeri* and probably represents either a mistaken record, an accidental introduction, or refers to the province in which the collecting site is situated (Tolagnaro was in the province of Tulear until Madagascar's provincial system was dissolved in 2009). There is also a population on Mauritius, most likely unintentionally introduced via shipping traffic from Toamasina to Port Louis (Hawlitschek et al. 2017b). *Ebenavia boettgeri* has been recorded at elevations from sea level to 425 m a.s.l. (specimen from Maintimbato in Betampona Strict Nature Reserve, Madagascar, 17.8988° S, 49.2290° E, Hawlitschek et al. 2017b). Its range apparently does not overlap with *E. safari* sp. nov. in the north. However, it is found in sympatry with *E. robusta* sp. nov. at Betampona, where the only individual found thus far occurred at slightly higher elevation (493 m a.s.l.; sample ACP 1999 in Hawlitschek et al. 2017b).

We estimate the extent of occurrence as ca. 29,260 km^2, although its exact western borders are uncertain due to its confusion with *E. robusta* sp. nov. There is certainly substantial decline in the extent and quality of its habitats, but due to its wide distribution range, *E. boettgeri* does not fall in any of the threatened categories of the IUCN Red List.

Ebenavia tuelinae sp. nov.

LSID for this species: urn:lsid:zoobank.org:act:49936D82-DBF6-4CCF-BCE2-6F2876DF651E

Available names: None.

Remark: This species has been referred to as *Ebenavia* cf. *inunguis* (Hawlitschek et al. 2017a), '*Ebenavia inunguis* complex Clade A' or 'Comoros Clade' (Hawlitschek et al. 2017b), and as *Ebenavia inunguis* (Blanc 1971, 1972, partim; Nussbaum and Raxworthy 1998, partim; Meirte 1999, partim; Meirte 2004; Hawlitschek et al. 2011; Hawlitschek et al. 2013).

Holotype: ZSM 68/2010 (FGZC 1592), adult male, collected 21 March 2010, at Dzialandée, Anjouan, Comoros (12.2277° S, 44.4320° E, 922 m a.s.l.), by O. Hawlitschek, J. Berger, B. Brückmann, and F. Glaw.

Paratypes: ZSM 716/2000 (FG/MV 2000–816), adult male, collected 05 March 2000, on the slope of Mt. Karthala, Grand Comoro, Comoros (11.7161° S, 43.2886° E, 640 m a.s.l.), by F. Glaw and K. Schmidt; ZSM 64–65/2010 (FGZC 1529–1530), adult males, and ZSM 66/2010 (FGZC 1531), adult female, collected 01 March 2010, at Chalêt St. Antoine, Mohéli, Comoros (12.2884° S, 43.6642° E, 702 m a.s.l.), by O. Hawlitschek, J. Berger, and B. Brückmann; ZSM 67/2010 (FGZC 4027), adult male, collected 04 April 2010, at Singani, Grand Comoro, Comoros (11.8503° S, 43.2980° E, 22 m a.s.l.), by O. Hawlitschek, J. Berger, and B. Brückmann.

Diagnosis: Morphological data is given in Table 2 and ESM 1, a photograph of a living specimen is available in Fig. 4c. Distinguished from *Ebenavia maintimainty*, *E. boettgeri*, and *E. robusta* sp. nov. by rostral scale in contact with nostril (RNO = yes); from *E. maintimainty* by larger SVL (32.9–35.2 vs. ≤ 24 mm), rostral scale bordered by postrostrals distinct from posterior head scales, absence of prenasal scale between rostral and nostril, keeled abdominal scales, and lighter colour; from *E. inunguis* by ratio HL/SVL (0.24–0.30 vs. 0.23–0.26), EE/HL (0.27–0.36 vs. 0.33–0.39), and higher DTAP (52–62 vs. 33–43); from *E. safari* sp. nov. by ratio EE/HL (0.27–0.36 vs. 0.29–0.42), HINL/AGD (0.67–0.86 vs. 0.55–0.80), and higher DTAP (52–62 vs. 38–54); from *E. boettgeri* by ratio EE/HL (0.27–0.36 vs. 0.29–0.41), and higher DTAP (52–62 vs. 33–49); from *E. robusta* sp. nov. by absence of distinct tubercles on hindlimbs (TUB = no), smaller SVL (32.9–35.2 vs. 34.4–42.6 mm), smaller ratio of BW/SVL (0.13–0.17 vs. 0.15–0.22), lower ILAB (9–10 vs. 10–11), IOS (18–21 vs. 20–23), and higher number of DTAP (52–62 vs. 46–51). Furthermore, distinguished from *E. boettgeri* and *E. safari* sp. nov. by short nasal process of premaxilla; from all other analysed *Ebenavia* except *E. robusta* sp. nov. by short premaxillary lappets of nasals; from *E. safari* sp. nov. by recognisable basal tubercle on basisphenoid; and from all other analysed *Ebenavia* by a codon insertion of AGG (Glutamine) at position 498 from the 5′ primer binding site of the PRLR fragment.

Description of the holotype: Morphometric and meristic data in Table 2. Specimen well preserved, tail partly

regenerated. Head elongate, wider than neck, but narrower than body. Snout angled downward to tip, sharply pointed. Eye round, pupil vertical, ear opening small, round to slightly oval. Body slightly depressed. Forelimbs shorter than hindlimbs, digits slightly expanded. Tail slightly shorter than SVL, nearly round in cross-section, no clear constriction at base, with sharply pointed tip. Ventral pygal section with a pair of postcloacal sacs.

Scalation: Rostral scale rectangular, much wider than high, almost twice as wide as mental. Nostril in contact with rostral, supranasal scale, 1st supralabial, and four granular postnasals on left side, with rostral, supranasal scale, 1st and 2nd supralabial, and three granular postnasals on right side. Rostral, postrostral, supralabials, and infralabials smooth. Supralabials 1, 2, and 3 larger than others, 2 widest, all wider than high, 1 highest and narrowest. Mental triangular, lateral sutures straight, bordered by infralabials and three granular scales. No distinct smooth postmental scales. Dorsolateral scales mostly granular, multicarinate. About 11 distinct rows of enlarged, trihedral tubercles beginning on the neck. Row along vertebral column with 62 tubercles. Tubercles mostly in direct contact with tubercles of the same row in the neck and anterior dorsum but separated by one or two granular scales in the posterior region. Ventral scales hexagonal, flat, and multicarinate. Pair of clearly distinct tubercles at lateral base of cloacal sacs, tubercles of left pair in direct contact, of right pair separated by a granular scale. Postpygal tail segments with dorsolateral whorls of about 6 spiny tubercles, whorls separated by 5 to 6 mostly granular scales. Additional irregular tubercles interspersed between whorls, also on ventral side. Subdigital lamellae in single rows. Pair of squarish terminal pads. Digits clawless.

Osteology: Endolymphatic sacs in the neck calcified, relatively small. Premaxilla with ca. 8 teeth, maxilla with ca. 33 teeth. Nasals fused. Nasal process of premaxilla short, parallel-sided, posterior end triangular, triangular part stretching over no more than ca. 20% of the total length of the nasal process. Premaxillary lappet of nasal short. Parietals paired, not fused. Maxilloprefrontal suture connects with nasals anterior to contact point of frontonasal suture with prefrontals. Frontonasal suture triangular, with tip in anterior direction. Basal tubercle on basisphenoid present. Presacral vertebrae 26, including 8 cervical vertebrae and 3 vertebrae connected to sternal ribs. Phalangeal formulae 2-3-4-5-3 in manus and 2-3-4-5-4 in pes.

Colouration: In life, overall brownish. Dorsal side light brownish to cream. Slightly darker brown on the head, this colouration extending as pattern of broad, irregular, and interrupted lines along the spine to the sacral region, ending in an abrupt convex (to posterior) terminal line and turning distinctly darker over the last millimetres towards this line. Two darker brown blotches on the original part of the tail, both extending over more than two whorls of tubercles, ca. twice as long as wide, darkening from anterior to posterior. Flanks of head, body, and original portion of tail dark brown, just as limbs from dorsal view, with a distinct division between dorsal and lateral colour. Bright line from rostral to eye and from eye to base of skull at this dividing line. Limbs brown as flanks, all digits with two cream annuli. Ventral side lighter brown to cream, gradually darkening from anterior to posterior, no distinct divide to lateral colour. Regenerated part of tail orange, with transversal bands of lighter and darker shadings, darker tubercles, and bright tip. Small dark spots irregularly spread over entire body. Iris golden. In preservative, colour slightly faded but largely intact except iris.

Variation: Endolymphatic sacs in the neck uncalcified in most specimens. Postcloacal tubercles more distinct in males than in female (ZSM 66/2010). ZSM 67/2010 overall darker, regenerated tail more brownish than orange, transversal bands indistinct. Colouration at night darker than during the day. Postcloacal sacs of males enlarged.

Species delimitation rationale: *Ebenavia tuelinae* sp. nov. is delimited by BPP support of ≥ 0.95, by COI divergence of at least 13.3% from all other members of the *E. inunguis* complex, by the insertion of a codon in the PRLR fragment from all other members of the *E. inunguis* complex, by diagnostic categorical morphological characters from *E. boettgeri* and *E. robusta* sp. nov., and by significant divergence in continuous morphological characters from all other members of the complex.

Etymology: A matronym dedicated to the first author's partner Tülin (alternative spelling Tuelin) for her ceaseless support of this and other works and for her excellent spotting abilities in the field.

Distribution, natural history, and conservation: See Fig. 5 for a distribution map. Observed on the Comoros Islands of Grand Comoro, Mohéli, and Anjouan, from near sea level up to 922 m a.s.l. (Fig. 5). The total terrestrial surface of the three islands on which this species occurs is 1650 km^2. Observations of any reptiles above 1000 m a.s.l. are extremely scarce in the Comoros. All specimens were observed in or near natural, mostly humid, forests (ZSM 67/2010 was found in a pineapple plantation at sea level near a stand of dry forest). The specimens of Mohéli were all collected on or around a derelict hut on the central forest ridge. A further specimen was observed, but not collected, in a fragment of degraded dry forest near Ouallah (12.3270° S, 43.6578° E, 32 m a.s.l.). While a wider acceptance of modified habitats cannot be excluded, the currently available data suggests a preference of natural habitats. Ongoing habitat destruction in the Comoros poses a severe threat to *E. tuelinae* sp. nov.

Ebenavia safari sp. nov.

LSID for this species: urn:lsid:zoobank.org:act:09C66ED5-AA63-4 BC5- 8 AC6-3D1C9BDE2244

Available names: None.

Remark: This species has been referred to as *Ebenavia* cf. *inunguis* (Hawlitschek et al. 2017a), '*Ebenavia inunguis* complex Clade B' or 'North Clade' (Hawlitschek et al. 2017b), and *Ebenavia inunguis* on Pemba (Pakenham 1983; Spawls et al. 2018), on Mayotte (Angel 1942; Blanc 1971, 1972, partim; Nussbaum and Raxworthy 1998, partim; Meirte 1999, partim; Meirte 2004; Hawlitschek et al. 2011; Hawlitschek et al. 2013), and in papers and other literature referring to *Ebenavia* in northern Madagascar.

Holotype: ZSM 2725/2010 (FGZC 3435), adult female, collected 09 January 2009, around Tungamaa, Pemba, Tanzania (5.0747° S, 39.7561° E, 9 m a.s.l.), by F. Glaw, O. Hawlitschek, and D. Rödder.

Paratypes: ZSM 2724/2010 (FGZC 3434), adult male, and ZSM 2726/2010 (FGZC 3530), adult female, collected 09 January 2009, around Tungamaa, Pemba, Tanzania (5.0747° S, 39.7561° E, 9 m a.s.l.), by F. Glaw, O. Hawlitschek, and D. Rödder.

Further material studied: ZMB 30631-01–30631-05, one adult male and three adult females, collected in Diego Suarez (Antsiranana), Diana Region, former Prov. Antsiranana, Madagascar; ZSM 533/2000 (no field number), adult female, collected 21 March 2000, at Montagne des Français, Diana Region, former Prov. Antsiranana, Madagascar (12.3167° S, 49.3333° E, ca. 250–300 m a.s.l.), by F. Glaw and K. Schmidt; ZSM 444/2005 (ZCMV 2099), collected 19 February 2005, at 'Camp Mantella', Marojejy, Sava Region, former Prov. Antsiranana, Madagascar (14.4377° S, 49.7756° E, 481 m a.sl.), by F. Glaw, M. Vences, and R.D. Randrianiaina; ZSM 1535/2008 (FGZC 1926), collected 19 April 2007, at 'Ambombofofo-Region ("trapsite 5")', Diana Region, former Prov. Antsiranana, Madagascar (12.0929° S, 49.3167° E), by M. Coates, Frontier Madagascar; ZSM 1697–1698/2008 (FGZC 1504, 3219), hatchlings, eggs collected 20 March 2008, on the slope of Mt. Benara, Mayotte, France (12.8753° S, 45.1586° E, 487 m a.s.l.), by F. Glaw and O. Hawlitschek; ZSM 209/2014 (no field number), adult female, collected on 03 May 2014, at Bungalow 10 of Nature Lodge below village, Joffreville, near Montagne d'Ambre, Diana Region, former Prov. Antsiranana, Madagascar (12.4823° S, 49.2077° E, 636 m a.sl.), by F. Glaw; ZSM 1533/2012 (FGZC 3830), adult female, collected 03 December 2012, at Ambararata, gallery forest near Andrafainkona, Sava Region, former Prov. Antsiranana, Madagascar (13.7221° S, 49.4385° E, 776 m a.s.l.), by F. Glaw, O. Hawlitschek, T. Rajoafiarison, A. Rakotoarison, F.M., Ratsoavina, and A. Razafimanantsoa; ZSM 249/2013 (FGZC 4945), adult male, and ZSM 250/2013 (FGZC 4946), adult female, collected on 27 February 2013, on Mont Mtsapéré, Mayotte, France (12.7667° S, 45.1919° E, 386 m a.s.l.), by O. Hawlitschek and F. Glaw; ZSM 251/2013 (FGZC 4947), adult male, collected on 28 February 2013, at summit of Mont Combani, Mayotte, France (12.8043° S, 45.1527° E, 481 m a.s.l.), by O. Hawlitschek and F. Glaw; ZSM 459/2016 (MSZC 0254), adult female, collected 22 November 2016 at 'Camp Mantella', Marojejy, Sava Region, former Prov. Antsiranana, Madagascar (14.4377° S, 49.7756° E, 460 m a.s.l.), by M.D. Scherz, A. Rakotoarison, M. Bletz, M. Vences, and J. Razafindraibe.

Diagnosis: Morphological data is given in Table 2 and ESM 1, a photograph of a living specimen is available in Fig. 4d, f. Distinguished from *Ebenavia maintimainty*, *E. boettgeri*, and *E. robusta* sp. nov. by rostral scale in contact with nostril (RNO = yes); from *E. maintimainty* by larger SVL (31.0–39.5 vs. ≤ 24 mm), rostral scale bordered by postrostrals distinct from posterior head scales, absence of prenasal scale between rostral and nostril, keeled abdominal scales, and lighter colour; from *E. inunguis* by higher ILAB (9–11 vs. 8–10) and DTAP (38–54 vs. 33–43); from *E. tuelinae* sp. nov. by ratio EE/HL (0.29–0.42 vs. 0.27–0.36), HINL/AGD (0.55–0.80 vs. 0.67–0.86), and lower DTAP (38–54 vs. 52–62); from *E. boettgeri* by higher DTAP (38–54 vs. 33–49); from *E. robusta* sp. nov. by absence of distinct tubercles on hindlimbs (TUB = no), smaller SVL (31.0–39.5 vs. 34.4–42.6 mm), smaller ratio of BW/SVL (0.13–0.20 vs. 0.15–0.22), and lower IOS (17–22 vs. 20–23). Furthermore, distinguished from all other analysed *Ebenavia* except *E. boettgeri* by long tapering nasal process of prevomer; from *E. tuelinae* sp. nov. and *E. robusta* sp. nov. by long premaxillary lappets of nasals; from all other analysed *Ebenavia* by absence of basal tubercle on basisphenoid; from *E. tuelinae* sp. nov. by a codon insertion of AGG (Glutamine) at position 498 from the 5′ primer binding site of the PRLR fragment; and from *E. inunguis* and *E. boettgeri* by a codon deletion of CGA (Proline) at position 438.

Description of the holotype: Morphometric and meristic data in Table 2. Specimen well preserved, original tail intact. Head elongate, wider than neck, but narrower than body. Snout angled downward to tip, sharply pointed. Eye round, pupil vertical, ear opening small, round to slightly oval. Body slightly depressed. Forelimbs shorter than hindlimbs, digits slightly expanded. Tail slightly shorter than SVL, nearly round in cross-section, no clear constriction at base, with sharply pointed tip.

Scalation: Rostral scale rectangular, much wider than high, almost twice as wide as mental. Nostril in contact with rostral, supranasal scale, 1st and 2nd supralabial, and three granular postnasals on left side, with rostral, supranasal scale, 1st and 2nd supralabial, and four granular postnasals on right side. Rostral, postrostral, supralabials, and infralabials smooth. Supralabials 1, 2, and 3 larger than others, 2 widest, 1 higher than wide, all others wider than high. Mental elongate triangular, lateral sutures slightly concave, bordered by infralabials and two granular scales. No distinct smooth postmental scales. Dorsolateral scales mostly granular, multicarinate. About 10 distinct rows of enlarged, trihedral tubercles beginning on the

neck. Row along vertebral column with 38 tubercles. Tubercles mostly in direct contact with tubercles of the same row in the neck and partly anterior dorsum but separated by one to three granular scales in the central and posterior region. Ventral scales hexagonal, flat, and multicarinate. A single, slightly enlarged tubercle at lateral base of each cloacal sac. Postpygal tail segments with dorsolateral whorls of about six (sometimes double) spiny tubercles, whorls separated by five to six granular scales, sometimes interspersed with few small tubercles. Granular scale directly anterior of spiny tubercle often replaced by smaller tubercle. Additional irregular tubercles interspersed between whorls, also on ventral side. Subdigital lamellae in single rows. Pair of squarish terminal pads. Digits clawless.

Osteology (ZSM 1533/2012): Endolymphatic sacs in the neck weakly calcified. Premaxilla with ca. 8 teeth, maxilla with ca. 34 teeth. Nasals fused. Nasal process of premaxilla extended, tapering. Premaxillary lappet of nasal long and tapering. Parietals paired, not fused. Maxilloprefrontal suture connects with nasals anterior to contact point of frontonasal suture with prefrontals. Frontonasal suture with shape similar to brace or curly bracket ('}'), with tip in anterior direction. Basal tubercle on basisphenoid almost absent. Presacral vertebrae 26, including 8 cervical vertebrae and 3 vertebrae connected to sternal ribs. Phalangeal formulae 2-3-4-5-3 in manus and 2-3-4-5-4 in pes.

Colouration: In life, overall brownish. Dorsal side light brownish. More or less distinctly visible middorsal line of darker brown spots, flanked by much less distinct lines of spots. Dorsal body colouration ending in an abrupt, roughly triangular (to posterior) terminal line with a particularly dark tip and bright margin. Another dark blotch on proximal part of tail, extending over roughly one whorl of tubercles. Flanks of head, body, and original tail darker than dorsal colour, just as limbs from dorsal view, with a separation between dorsal and lateral colour that is particularly distinct on the head and near the limbs. Bright line from rostral to eye and from eye to base of skull at this dividing line. Limbs brown as flanks, most digits with one or two brighter annuli. Irregular light brown patterns on limbs. Ventral side lighter brown to cream, gradually darkening from anterior to posterior, no distinct separation from lateral colouration. Anterior part of tail lighter than body, darkening towards the tip, with five whitish annuli. Small dark spots irregularly spread over entire body. Iris golden. In preservative, colour slightly faded but largely intact except iris.

Variation: Median postrostal scale of ZSM 459/2016 longitudinally divided, resulting in a count of four postrostrals. Maxilla of ZSM 249/2013 with ca. 46 teeth. Males with distinctly visible postcloacal tubercles, mostly in pairs, and enlarged postcloacal sacs. Hatchlings with similar colour pattern as adults, but stronger contrast. Within the available sampling, individuals from Mayotte (ZSM 249–251/2013) are distinguished from other individuals of *E. safari* sp. nov. by a higher number of dorsal tubercles in one row (50–54 vs. 38–49). Other characters vary, but no correlation of this variation to any of the sublineages could be detected.

Species delimitation rationale: *Ebenavia safari* sp. nov. is delimited by BPP support of ≥ 0.95, by COI divergence of at least 13.3% from *E. tuelinae* sp. nov. and *E. robusta* sp. nov., by the absence of the insertion of any codon in the PRLR fragment from all other members of the complex except *E. robusta* sp. nov., by diagnostic categorical morphological characters from *E. boettgeri* and *E. robusta* sp. nov., and by significant divergence in continuous morphological characters from *E. inunguis* and *E. tuelinae* sp. nov.

Etymology: 'Safari' means 'voyage' in the Kiswahili and Comoran (Shimaoré) languages spoken across the range of this species outside Madagascar. The name was chosen because this species dispersed over surprisingly long distances across the open ocean. It is treated as an unlatinised, invariable noun in apposition.

Distribution, natural history, and conservation: See Fig. 5 for a distribution map. This species has the most disjunct distribution of all recognised *Ebenavia* species so far, as it is found in the north of Madagascar and on the islands of Mayotte (Comoros Archipelago) and Pemba (off the coast of Tanzania, Fig. 5). All specimens of Pemba Island that were studied here are from the type locality Tungamaa in the north of the island. Pakenham (1983) provided a map with the localities of Chokocho (observation of 1939, georeferenced to ca. 5.4352° S, 39.6399° E, 4 m a.s.l.) in the south and Kiungajuu, Wete (observation of 1943, georeferenced to ca. 5.066° S, 39.7355° E, ca. 25 m a.s.l.) in the north. In Pemba, the three type specimens were observed in a single locality around a decayed hut made of dry leaves in a coconut plantation. This observation indicates some degree of tolerance to anthropogenic habitats, although the other localities (not verified by our work) suggest a preference for natural habitats.

Further known localities on Mayotte Island are as follows: Mont Mtsapéré (12.7684° S, 45.1891° E, 416 m a.s.l.; 12.7687° S, 45.1867° E, 501 m a.s.l.), Mont Benara (12.8766° S, 45.1572° E, 443 m a.s.l.), Hajangua (12.8615° S, 45.1948° E, 36 m a.s.l.), near Mavingoni (12.8632° S, 45.1541° E, 234 m a.s.l.), from Hawlitschek and Glaw (2014); Crête du Nord (12.7147° S, 45.0730° E, 361 m a.s.l.), Mt. Mtsapéré (12.7673° S, 45.1908° E, 422 m a.s.l.; 12.7689° S, 45.1904° E, 405 m a.s.l.), Road to Relais Forestier near Mont Combani (12.8055° S, 45.1547° E, 304 m a.s.l.), Crête de Mlima Maevodoani (12.8222° S, 45.1660° E, 340 m a.s.l.; 12.8221° S, 45.1660° E, 337 m a.s.l.), from Wang et al. (2015). In Mayotte, *E. safari* sp. nov. were almost exclusively observed in pristine forest areas, mostly in ferns or *Pandanus* plants. The eggs collected in 2008 were found as part of a common clutch of several females comprising > 10 eggs. This clutch was found in the mulch within a tree cavity. The eggs hatched within 3 days from their collection.

The following published *Ebenavia* localities most likely belong to *E. safari* sp. nov., although they are still in need of confirmation by genetic data: Nosy Lakandava (12.2327° S, 49.0621° E, ca. 0–10 m a.s.l.), an islet of 5 ha east of Nosy Hara (Metcalf et al. 2007), Diana Region, former Prov. Antsiranana, Madagascar; the five localities Ampondrabe (12.9733° S, 49.7033° E, ca. 80–580 m a.s.l.), Antsahabe (13.2000° S, 49.5500° E, ca. 350–950 m a.s.l.), Bekaraoka (13.1000° S to 13.1833° S/49.7000° E to 49.7167° E, ca. 170–360 m a.s.l.), Binara (13.2333° S to 13.2500° S/ 49.5667° E to 49.6167° E, ca. 210–1170 m a.s.l.) and Tsaramborona (12.9567° S, 49.6133° E, ca. 150–450 m a.s.l.) in the Sava Region, former Prov. Antsiranana, Madagascar (Rakotondravony 2006); Anjanaharibe, Analanjirofo Region, former Prov. Toamasina, Madagascar (Raxworthy et al. 1998); COMATSA corridor, spanning Sava, Diana, Sofia, and Analanjirofo regions, Madagascar (Rabearivony et al. 2015); and Anjanaharibe-Sud, Analanjirofo Region, former Prov. Toamasina, Madagascar (Rakotomalala and Raselimanana 2003). Typically, *E. safari* sp. nov. is observed in natural or near natural humid forests, but it has also been detected in dry forests of Ampombofofo and Montagne des Français. It is rarely seen around buildings near such areas, e.g. the bungalows at the Nature Lodge of Montagne d'Ambre.

Genetic data from Hawlitschek et al. (2017b) suggest natural colonisation of the islands of Mayotte and Pemba. Forests of Mayotte are well protected, but ongoing habitat destruction across its global range poses a severe threat to *E. safari* sp. nov. The minimum convex polygon around the localities on Madagascar is 19,150 km^2. The terrestrial areas of Pemba Island and Mayotte island are 984 km^2 and 376 km^2, respectively, resulting in a sum of 20,510 km^2. Based on these observation the species should probably be listed as Near Threatened under IUCN criteria.

Ebenavia robusta sp. nov.

LSID for this species: urn:lsid:zoobank.org:act:CD3BDBAF-FE1E-4388-9A02-A93381867957

Available names: None.

Remark: This species has been referred to as '*Ebenavia inunguis* complex Clade Ca' or 'Highland Clade' (Hawlitschek et al. 2017b) and as *E. inunguis* in all previous publications.

Holotype: ZSM 296/2010 (FGZC 4526), adult female, collected 15 April 2010, at Ambatofotsy, Alaotra-Mangoro Region, former Prov. Toamasina, Madagascar (19.5431° S, 48.3165° E, 907 m a.s.l.), by M. Pabijan, F. Randrianasolo, and S. Rasamison.

Paratypes: ZSM 332/2004 (FGZC 629), adult female, collected 2004, at 'Gilbert's Site', Alaotra-Mangoro Region, former Prov. Toamasina, Madagascar, by local collectors; ZSM 295/2010 (FGZC 4387), adult female, collected 10 April 2010, at Andasibe (Feonnyala Hotel), Alaotra-Mangoro Region, former Prov. Toamasina, Madagascar, by F. Glaw, J. Köhler, P.-S. Gehring, M. Pabijan, K. Mebert, E. Rajeriarison, F. Randrianasolo, and S. Rasamison; ZSM 297/2010 (FGZC 4635), adult female, collected 15 April 2010, at Ambatofotsy, Alaotra-Mangoro Region, former Prov. Toamasina, Madagascar (19.5431° S, 48.3165° E, 907 m a.s.l.), by M. Pabijan, F. Randrianasolo, and S. Rasamison; ZSM 156/ 2016 (FGZC 4956), adult male, collected 25 December 2012, at Vohimana (Relais de Naturaliste), Alaotra-Mangoro Region, former Prov. Toamasina, Madagascar (18.9203° S, 48.5160° E, 786 m), by F. Glaw, D. Prötzel, and L. Randriamanana.

Diagnosis: Morphological data is given in Table 2 and ESM 1, photographs of living specimens are available in Fig. 4e, g. Distinguished from *Ebenavia maintimainty* by larger SVL (34.4–42.6 vs. ≤ 24 mm), rostral scale bordered by postrostrals distinct from posterior head scales, absence of prenasal scale between rostral and nostril, keeled abdominal scales, and lighter colour; from all other *Ebenavia* by presence of distinct tubercles on hindlimbs (TUB = yes); from *E. boettgeri* by larger SVL (34.4–42.6 vs. 28.9–37.8 mm), larger ratio of BW/SVL (0.15–0.22 vs. 0.11–0.20), higher ILAB (10–11 vs. 8–11), IOS (20–23 vs. 15–21), and DTAP (46–51 vs. 33–49); from *E. inunguis*, *E. tuelinae* sp. nov., and *E. safari* sp. nov. by rostral scale mostly not in contact with nostril (RNO = yes), larger SVL (34.4–42.6 vs. 31.0–39.5 mm), larger ratio of BW/SVL (0.15–0.22 vs. 0.12–0.20), and more IOS (20–23 vs. 16–22); from *E. inunguis* by higher ILAB (10–11 vs. 8–10) and DTAP (46–51 vs. 33–43); from *E. tuelinae* sp. nov. by higher ILAB (10–11 vs. 9–10) but lower DTAP (46–51 vs. 52–62). Distinguished from *E. boettgeri* and *E. safari* sp. nov. by short nasal process of premaxilla; from all other analysed *Ebenavia* except *E. tuelinae* sp. nov. by short premaxillary lappets of nasals; from *E. safari* sp. nov. by recognisable basal tubercle on basisphenoid; from *E. tuelinae* sp. nov. by a codon insertion of AGG (Glutamine) at position 498 from the 5′ primer binding site of the PRLR fragment; and from *E. inunguis* and *E. boettgeri* by a codon deletion of CGA (Proline) at position 438.

Description of the holotype: Morphometric and meristic data in Table 2. Specimen well preserved, tail partly regenerated. Head elongate, wider than neck, but narrower than body. Snout angled downward to tip, sharply pointed. Eye round, pupil vertical, ear opening small, round to slightly oval. Body slightly depressed. Forelimbs shorter than hindlimbs, digits slightly expanded. Tail slightly shorter than SVL, nearly round in cross-section, no clear constriction at base, with sharply pointed tip. Ventral pygal section with pair of postcloacal sacs.

Scalation: Rostral scale rectangular, much wider than high, almost twice as wide as mental. Nostril in contact with postrostral scale, 1st and 2nd supralabial, and five granular postnasals on left side, with postrostral scale, 1st and 2nd supralabial, and four granular postnasals on right side.

Rostral, postrostral, supralabials, and infralabials smooth. Supralabials 1, 2, and 3 larger than others, 1 highest and narrowest, 2 widest, all wider than high. Mental bell-shaped, bordered by infralabials and two granular scales. No distinct smooth postmental scales. Dorsolateral scales mostly granular, multicarinate. About 10 distinct rows of enlarged, trihedral tubercles beginning on the neck. Row along vertebral column with 48 tubercles. Dorsal tubercles separated by one to four granular scales. Ventral scales hexagonal, flat, and multicarinate. A single enlarged tubercle at lateral base of cloacal sacs. Postpygal tail segments with dorsolateral whorls of about six spiny (mostly double) tubercles, whorls separated by five to six granular scales, interspersed with many irregular smaller tubercles. Granular scale directly anterior to spiny tubercle mostly replaced by smaller tubercle. Additional irregular tubercles interspersed between whorls, also on ventral side. Subdigital lamellae in single rows. Pair of squarish terminal pads. Digits clawless.

Osteology (Fig. 3): Endolymphatic sacs in the neck calcified, relatively small. Premaxilla with ca. 8 teeth, maxilla with ca. 35 teeth. Nasals fused. Nasal process of premaxilla short, parallel-sided, posterior end triangular with slightly concave edges, triangular part stretching over no more than ca. 20% of the total length of the nasal process. Premaxillary lappet of nasal short. Parietals paired, not fused. Maxilloprefrontal suture connects with nasals anterior to contact point of frontonasal suture with prefrontals. Frontonasal suture with shape similar to a brace or curly bracket, with tip in anterior direction. Basal tubercle on basisphenoid present. Presacral vertebrae 26, including 8 cervical vertebrae and 3 vertebrae connected to sternal ribs. Phalangeal formulae 2-3-4-5-3 in manus and 2-3-4-5-4 in pes.

Colouration: In preservative, overall dusky brownish. Dorsal side brown. Indistinct middorsal line of darker brown spots, flanked by even less distinct lines of spots. Dorsal body colouration ending in an abrupt triangular (to posterior) terminal line with a particularly dark tip. Three distinct, very dark brown blotches on original part of tail, all extending over more than two whorls of tubercles. Two anterior blotches elongate, much longer than wide, posterior blotch oblong, ca. 1.5 times as long as wide. Flanks of head, body, and original portion of tail dark brown, just as limbs from dorsal view, with a distinct separation between dorsal and lateral colour. Bright line from rostral to eye and from eye to base of skull at this dividing line, ventrally bordered by blackish line. Limbs brown as flanks, all digits with one cream annulus. Irregular cream spots on limbs. Ventral side lighter brown than flanks, but darker than dorsal side, gradually darkening from anterior to posterior, no distinct separation from the lateral colour. Original part of tail dorsally lighter than body, laterally and ventrally coloured as flanks. Small dark spots irregularly spread over entire body. Regenerated part of tail very dark brown with two white transverse bands.

Variation: Colouration of some individuals overall brighter than holotype. In life, colours more contrasting. Iris golden. In ZSM 297/2010, nostril in contact with rostral, supranasal scale, 1st and 2nd supralabial, and three granular postnasals on left side, with rostral, supranasal scale, 1st and 2nd supralabial, and four granular postnasals on right side; triplet of distinct tubercles at lateral bases of cloacal sacs. Postcloacal tubercles more visible in male (ZSM 156/2016) than in females. Male has enlarged postcloacal sacs.

Species delimitation rationale: *Ebenavia robusta* sp. nov. is delimited by BPP support of ≥ 0.95, by COI divergence of at least 13.3% from all other members of the *E. inunguis* complex, by the absence of the insertion of any codon in the PRLR fragment from all other members of the *E. inunguis* complex except *E. safari* sp. nov., by diagnostic categorical morphological characters from all other members of the complex, and by significant divergence in continuous morphological characters from all other members of the *E. inunguis* complex.

Etymology: The specific name is the feminine form of the Latin adjective 'robustus', meaning 'robust' or 'sturdy'. It was chosen because this species is the largest and most sturdily built member of this genus of small geckos.

Distribution, natural history, and conservation: See Fig. 5 for a distribution map. Current data suggests a distribution of this species in some rainforest fragments of the Central East of Madagascar, including the forests in the Andasibe area and Betampona. In Betampona this species occurs sympatrically with the much more common *E. boettgeri* and thus far it has only been recorded from Sahambendrana, an area of pristine or semi-pristine forest lying in the centre of the Reserve. Ongoing deforestation is certainly a threat to this species. As of current knowledge the minimum convex polygon around the localities of this species is ca. 367 km^2, but currently available data are insufficient for a reliable estimate of its total distribution.

Ebenavia robusta sp. nov. has been successfully kept and bred in captivity (Glaw 2016). Eggs are laid in dry rolled leaves or leaf axils of plants. Unlike many other geckos, captive specimens of *E. robusta* sp. nov. do not show any signs of territorial behaviour or any other kind of intraspecific aggression. They have been observed to emit soft purring vocalisations of unknown function (F. Glaw pers. obs. and K. Glaw, pers. comm. 2017).

Key to the species of the genus *Ebenavia*

1.a). Maximum snout-vent length ≤ 24 mm. Postrostral scales keeled, not distinct from posterior head scales. Prenasal scale between rostral and nostril present. Abdominal scales unkeeled. Almost completely blackish. ➔ *Ebenavia maintimainty.*

1.b). Snout-vent length of adults larger than 28 mm. Rostral scale bordered by postrostrals mostly smooth and distinct from keeled posterior head scales. Prenasal scale between rostral and nostril absent. Abdominal scales

keeled. Mostly brownish, flanks darker than dorsal and ventral sides. ➔ 2.

2.a). Hindlimbs with tubercles distinctly larger than surrounding scales (Fig. 2d). Tail typically with whorls of enlarged spiny tubercles, interspersed with smaller tubercles (Fig. 2f). Comparatively large and robust, snout-vent length of adults between 34.4 to 42.6 mm, ratio of body width to snout-vent length 0.15 to 0.22. Infralabials 10 to 11, interocular scales 20 to 23, dorsal tubercles in one row 46 to 51. Rostral scale normally not in contact with nostril (Fig. 2b). ➔ *Ebenavia robusta* sp. nov.

2.b). Hindlimbs without distinctly enlarged tubercles (Fig. 2c). Tail with regular whorls of spiny tubercles, not clearly enlarged (Fig. 2e). Comparatively gracile, snout-vent length of adults between 28.9 to 39.5 mm, ratio of snout-vent length to body width 0.11 to 0.20. Infralabials 8 to 11, interocular scales 15 to 21, dorsal tubercles in one row 33 to 62. Rostral scale may be in contact with nostril or not. ➔ 3.

3.a). Rostral scale not in contact with nostril (Fig. 2b). ➔ *Ebenavia boettgeri*.

3.b). Rostral scale in contact with nostril (Fig. 2a). ➔ 4.

4.a). Numerous small tubercles on dorsal side, dorsal tubercles in one row 52 to 62. Snout-vent length of adults up to 35.2 mm. Eyes relatively close to ear openings, ratio of distance eye-ear to head length 0.27 to 0.36. Hindlimbs relatively long, ratio of hindlimb length to distance between front and hindlimbs 0.67 to 0.86. ➔ *Ebenavia tuelinae* sp. nov.

4.b). Dorsal tubercles in one row 33 to 54. Snout-vent length up to 39.5 mm. Ratio of distance eye-ear to skull length 0.29 to 0.42. Ratio of hindlimb length to distance between front and hindlimbs 0.55 to 0.80. ➔ 5.

5.a). Dorsal tubercles in one row 38 to 54. Infralabial scales 9 to 11. Nasal process of prevomer long and tapering. Basal tubercle on basisphenoid mostly not detectable. ➔ *Ebenavia safari* sp. nov.

5.b). Dorsal tubercles in one row 33 to 43. Infralabial scales 8 to 10. Nasal process of prevomer short. Basal tubercle on basisphenoid visible. ➔ *Ebenavia inunguis*.

Discussion

Sampling and abundance of reptiles with cryptic habits

Though locally common, *Ebenavia* are often relatively difficult to observe and sample in large numbers. Up to five individuals of *E. boettgeri* were found at Ivoloina on the Malagasy East Coast in a single night walk; several individuals of *E. safari* sp. nov. were found on a single bungalow in Joffreville, and of *E. tuelinae* sp. nov. on a derelict building in Mohéli Island, respectively. In Betampona, ca. 40 individuals of *E. boettgeri* and one individual of *E. robusta* sp. nov. were sampled in a three-week survey of ca. 672 person-hours. On the other hand, only 12 individuals of *E. safari* sp. nov. (excluding eggs) were observed in a total of ca. 900 person-hours of surveying on Mayotte Island from 2000 to 2014 (Hawlitschek et al. 2011; Hawlitschek and Glaw 2014; Wang et al. 2015). We discovered a single *E. tuelinae* sp. nov. in 480 person-hours of dedicated search on the Anjouan Island, and only three *E. safari* sp. nov. at a single locality in 270 person-hours on Pemba Island. This often observed rarity may be due to low population density at some localities or, alternatively, to the cryptic habits of *Ebenavia*. In a study on the Mourning Gecko *Lepidodactylus lugubris*, a species similar to *Ebenavia* in habits and appearance, on the island of Guam, only 12 specimens per hectare were found by regular visual surveys. However, removing and searching all vegetation and other potential dens of the same area revealed 1777 specimens per hectare (Rodda and Campbell 2002). This relation exemplifies the skew between actual population sizes and the yield of regular surveying and may apply to *Ebenavia* as well. For taxonomic revisions of cryptic species complexes, this means that obtaining sample sizes sufficient for well-resolved molecular and morphological analyses may be difficult and time-consuming.

The performance of species delimitation algorithms in problematic datasets

Species delimitation in our *Ebenavia* dataset was complicated by several factors. First, the molecular dataset was relatively small, with only two nuclear markers available; larger datasets are recommended at least for BPP (Yang 2015; but see Yang and Rannala 2017). Second, there was a low degree of overlap between the molecular and morphological datasets, as no sequence data was available for specimens from some localities, and some molecular lineages represent individuals of which only tissue samples were collected but no voucher specimens were available. Third, only one single individual was available for some localities and molecular lineages. Fourth, and finally, clades did not form clearly delimited clusters as in many other gene trees (e.g. Hawlitschek et al. 2013), but the genetic divergences among tips were rather evenly distributed. Despite their names, species delimitation algorithms delimit phylogenetic (population) structure that is not necessarily congruent with true species (Carstens et al. 2013, Sukumaran and Knowles 2017). Delimiting the borders of structural elements in the *Ebenavia* phylogeny is certainly a difficult task also for sophisticated algorithms.

Clustering algorithms based on single mitochondrial markers are commonly employed as first step in OTU detection because of their ease of use and the readily available data

(Talavera et al. 2013). Both GMYC and BOLD have been shown to produce results highly congruent with established taxonomy, at least in arthropods (Ratnasingham and Hebert 2013). In reptiles, however, this approach appears to regularly oversplit lineages (Miralles and Vences 2013). Geckos may be particularly prone to this because of their unusually high divergences in mitochondrial markers, exemplified by COI (Nagy et al. 2012). Also in our dataset, GMYC and BOLD split up a comparably high number of OTUs. In some cases, samples from identical localities are assigned to different OTUs, e.g. in *E. boettgeri*, or in *E. safari* sp. nov. from Mayotte. However, we consider the evolutionary isolation of these sympatric and genetically close, but otherwise indistinguishable lineages unlikely, as these would be rare examples among Malagasy reptiles (Boumans et al. 2007). Additional data is clearly required to solve some of the open questions that have been raised in this taxonomic revision.

Bayesian species delimitation largely supported the results of our taxonomic revision. All newly recognised species were supported by BPP. Furthermore, BPP supported the lineage from Mananara (Analanjirofo Region, former Prov. Toamasina), now considered part of *E. boettgeri*, as a distinct OTU and detected four OTUs within *E. safari* sp. nov. We consider this result useful, as these OTUs all correspond to deeply divergent genetic lineages but are now mostly represented by only one or two samples. Future studies based on more comprehensive molecular datasets and more voucher specimens may well find more evidence for the distinction of these lineages at species or subspecies level. Overall, BPP has performed well as part of an integrative taxonomic approach despite the limited molecular data available.

The phylogenetic signals of morphological and molecular characters

Even after this revision, the species of the *Ebenavia inunguis* complex remain highly cryptic. Unambiguous identification of them in the field is difficult and will normally require the inspection of voucher specimens using a stereo microscope, and even then a reliable ID will probably not be attained in all cases. Unfortunately, also osteology did not prove helpful in this specific case and molecular tools are thus far the best methods to diagnose the different species of the *E. inunguis* complex. Future studies should explore the potential for 3D geometric morphometrics as a further aid in identifying diagnostic osteological characters of these geckos.

Other than the tubercles on the hindlimbs and partly on the tail of *E. robusta* sp. nov., the contact of nostril and rostral was the only morphological character with diagnostic value. The configuration of the scales around the nostril has been widely used to diagnose species and even delimit species groups within genera of reptiles (Mocquard 1909; Nussbaum and Raxworthy 1993). While useful at the species level, molecular phylogenies contradicted the value of this character to diagnose higher taxonomic levels, e.g. in the genus *Paroedura* (Jackman et al. 2008). The same seems to be the case in *Ebenavia*, as molecular genetics do not support a sister group relationship of *E. boettgeri* and *E. robusta* sp. nov., in neither of which the rostral contacts the nostril. This result suggests that the configuration of the scales around the nostril, which appears to change regularly during the phylogeny of geckos, may be subject to some unknown adaptive pressure.

Another potentially diagnostic character that we wanted to investigate was the presence of claws on the digits. Originally, the genus *Ebenavia* was described as clawless (Boettger 1878:8), the name 'inunguis' meaning just this (literally 'no fingernail'). Nussbaum and Raxworthy (1998:22), however, stated that "females of both species [*E. inunguis* and *E. maintimainty*] have claws on all digits of the pes". Despite careful examination we were unable to detect claws on any of the specimens studied, including specimens of SMF and BMNH studied in Nussbaum and Raxworthy (1998). The common name 'Madagascan clawless geckos' is therefore apparently not a misnomer, but we note that the German common name 'Pinselschwanzgeckos' (lit. 'brush-tailed geckos') is perhaps more apt, given the paintbrush-shaped tails that are even more colourful after regeneration than in their original state.

The absence of any haplotype sharing of nuclear genes with other species is often used as a line of evidence in species delimitation (Miralles et al. 2011). Figure S3 in Appendix 2 of Hawlitschek et al. (2017b) shows the haplotype networks of the nuclear genes analysed in this study, PRLR and RAG2. *Ebenavia maintimainty*, *E. inunguis*, and *E. tuelinae* sp. nov. do not share any haplotypes of these markers with any of the other species. This is also true, albeit only in PRLR, for *E. robusta* sp. nov. and the Mayotte and Pemba clades of *E. safari* sp. nov. There is some degree of haplotype sharing between *E. boettgeri* and *E. safari* sp. nov., which may be a result of incomplete lineage sorting or admixture and may have been detected because of the relatively higher sample sizes of these two versus all the other *Ebenavia* species.

Unlike the morphological characters studied, codon insertions in nuclear genes have proven to be of substantial diagnostic value in our study. The analysed PRLR fragment has previously been found to be highly variable in some vertebrates, including reptiles (Kato et al. 2005; Gvoždík et al. 2010, 2013; Edwards et al. 2012), and its coding regions may have an adaptive value in reproduction, at least for primates (Babb et al. 2014). We were able to delimit *E. tuelinae* sp. nov. with a codon insertion in the PRLR fragment. The clade formed by *E. inunguis* and *E. boettgeri* is delimited by another insertion, supporting the phylogeny of Hawlitschek et al. (2017b) but contrasting with the

configuration of the scales around the nostril. While many individuals of *Ebenavia* were found to be heterozygous in the PRLR fragment (Hawlitschek et al. 2017b), the codon insertions were constant throughout all individuals. This supports the high diagnostic value of these characters and also indicates some degree of adaptiveness.

Cryptic or hyper-cryptic?

Our revision increases the number of species in the genus *Ebenavia* from two to six. However, Bayesian species delimitation indicated the possible presence of many more, between 12 and 16, species in the genus. The lineage of the locality of Mananara (now part of *E. boettgeri*) and several lineages within *E. safari* sp. nov. (including those from Mayotte and Pemba) are also supported as distinct species by BPP. However, there are many voucher specimens for which no sequences are available and vice versa. As stated in the methods section, we preferred to describe only lineages for which some voucher specimens were available and postponed the description of other lineages (populations from Mananara and some lineages within *E. safari* sp. nov.). We agree with Miralles and Vences (2013) that in cases of uncertainty taxonomic changes should be applied conservatively, particularly in a morphologically conservative species whose members are difficult to diagnose and delimit. With the availability of more voucher specimens the descriptions of further taxa may be possible, at the species or—where appropriate—at the subspecies level (see Hawlitschek et al. 2012). Time will tell, not only as datasets grow but also as species delimitation methods and species concepts develop.

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