SALAMANDRA **61**(1): 70–94
15 February 2025 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

A preliminary assessment of the diversity in the frog genus *Anilany* (Microhylidae: Cophylinae) with description of a new species from western Madagascar

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Manuscript received: 9 September 2024
Accepted: 6 November 2024 by Stefan Lötters

Abstract. The cophyline microhylid frog genus *Anilany* was established as a monotypic genus in 2016 for the miniaturised species *Anilany helenae* (Vallan, 2000), from the type locality Ambohitantely, a patch of rainforest surrounded by savannah in central Madagascar. Fieldwork conducted over the past two decades identified three unexpected populations from Bemaraha, Mahajanga, and Beanka from limestone caves near sea level in arid western and northwestern Madagascar, which were assigned to the genus *Anilany* based on diagnostic morphological features. We generated new data for specimens of the Ambohitantely, Bemaraha, and Mahajanga populations to test if all three populations belong to one or multiple species, studying their genetic variability based on mitochondrial (16S rRNA, COI) and nuclear (BDNF, RAG-1) genes, as well as morphological and osteological data. We identify several previously unknown diagnostic characters of the skeleton of *Anilany* compared to its closest relatives, *Rhombophryne* and *Stumpffia*, but no skeletal features that can be used to reliably distinguish among *Anilany* populations. Based on concordant genetic and morphological differences, we formally describe the population from the limestone karsts of Tsingy de Bemaraha National Park, formerly considered candidate species *Anilany* sp. Ca14, as a new species, *Anilany karsticola* sp. n. It can be distinguished from other *Anilany* lineages by larger size and shorter relative tibia length, uncorrected p-distances in DNA sequences of the 16S gene amounting to 2.6% and a lack of allele sharing in the analysed fragments of the nuclear genes BDNF and RAG-1. More data, especially advertisement call recordings and additional specimens, are required from other locations for a more thorough assessment of the genus and the distribution of its species.

Key words. Amphibia, Anura, *Anilany karsticola* sp. n., citizen science, iNaturalist, molecular genetics, morphology, osteology, systematics.

Introduction

Madagascar's miniaturized cophyline microhylids are amongst the most poorly known frogs on the island. Earlier DNA barcoding work revealed over 30 undescribed species from the Madagascar-endemic genus *Stumpffia* Boettger, 1881 alone (Vieites et al. 2009, Perl et al. 2014), which led to a flurry of taxonomic work (Rakotoarison et al. 2017, 2019, 2022, Crottini et al. 2020, Mullin et al. 2022a). More significantly, recent attempts to resolve the unstable superspecific taxonomy of the Cophylinae Cope, 1889 (Peloso et al. 2016, 2017, Scherz et al. 2016, 2017b, 2019) revealed the existence of several genera that have been mistaken for *Stumpffia* in the past based on their small size, but probably converged on this miniaturized morphology (Scherz et al. 2016, 2019).

One of these cryptic genera was subsequently described as *Anilany* Scherz, Vences, Rakotoarison, Andreone, Köhler, Glaw & Crottini, 2016. The exact phylogenetic relationships of this genus are still in question, but certainly it belongs to a clade within the Cophylinae that also includes *Rhombophryne* Boettger, 1880 and *Stumpffia* (Scherz et al. 2016). In some analyses, *Stumpffia tridactyla* Guibé, 1975 has also been found to cluster with *Anilany* (Scherz et al. 2016, Peloso et al. 2017), but this relationship is probably artefactual (Scherz et al. 2017b). Peloso et al. (2017) proposed to synonymise *Anilany* with *Rhombophryne*, but it was reinstated by Scherz et al. (2017b) and its position as distinct genus was found to be highly supported in subsequent work (Tu et al. 2018).

In the genus description (Scherz et al. 2016), *Anilany* was defined by a curved clavicle (straight or reduced in all examined *Stumpffia* species), broad, flat, curving neopalatine/vomer complexes (straight or curving but narrow in both other genera), and small size coupled with expanded terminal digit discs and T-shaped terminal phalanges (most *Rhombophryne* are larger and *Stumpffia* typically do not have widely expanded terminal discs or T-shaped terminal phalanges).

A single *Anilany* species has hitherto been known, *A. helenae* (Vallan, 2000). It was originally thought that this species was microendemic to Ambohitantely Special Reserve on the central plateau of Madagascar, but recent surveys revealed that it also occurs in the nearby Ankafobe forest fragments (Mullin et al. 2021). A complete mitochondrial genome of this species was recently published (Mullin et al. 2022b). *Anilany helenae* is listed as Critically Endangered by the International Union for the Conservation of Nature's (IUCN) Red List, under IUCN Criteria (IUCN 2012) based on the species' range size (estimated at 29 km² in 2016; IUCN SSC Amphibian Specialist Group 2016a, before it was known from Ankafobe), known number of localities (one, prior to knowledge from Ankafobe), and ongoing threats and habitat declines (habitat destruction is rampant in this area). Due to its genetic distinction and threat level, it is also the 43rd species on the top 100 Evolutionarily Distinct, Globally Endangered (EDGE) amphibian species (EDGE 2023).

Specimens assignable to *Anilany* by their external morphology (hand with expanded terminal phalanges on digits 3 and 4, lateral head with a distinct colour border to dorsum) were collected in the Tsingy de Bemaraha National Park (Glaw et al. 2007, Raselimanana 2008, Bora et al. 2010). DNA sequencing revealed that the specimens from Bemaraha were indeed closely related to *A. helenae*, and this lineage was called *Stumpffia* sp. 8 by Wollenberg et al. (2008), and subsequently *Stumpffia* sp. 14 by Vieites et al. (2009) and *Stumpffia* sp. Ca14 in Perl et al. (2014). Since the erection of the genus *Anilany*, it has been referred to as *Anilany* sp. Ca14 (Scherz et al. 2016), though it is the only candidate species awaiting taxonomic attention within the genus *Anilany* at present. Its taxonomic status has not yet been examined in detail, but based on biogeographic separation and genetic divergence, it has been assumed that this lineage may constitute a distinct species. Hence, it was not included in the aforementioned IUCN and EDGE assessments. Raselimanana (2013) also recorded '*Stumpffia* aff. *helenae*' from Beanka forest, ca 70 km north of Bemaraha, but this population has not been studied, and it has likewise been omitted from subsequent work.

In this study, we provide a preliminary revision of the genus *Anilany* based on an integrative taxonomic analysis including morphometry, external morphology, osteology, mitochondrial DNA, and nuclear DNA data for three geographically isolated populations from Ambohitantely/Ankafobe, Bemaraha, and Mahajanga, as well as records found in the literature and on iNaturalist. The results substantially expand upon the original species description (Vallan 2000a) and the brief first original description of the genus (Scherz et al. 2016), but further reveal a new species and suggest a broader range for the whole genus than previously known.

Methods

iNaturalist record collection

We searched iNaturalist records (excluding our own) for microhylid frogs observed in a rectangle between 19.6889° S, 43.7591° E (southwest corner) and 13.8243° S, 48.2916° E (northeast corner). Photos associated with records were surveyed superficially for specimens assignable to *Stumpffia* or *Anilany*, and then examined in close detail for diagnostic features of *Anilany*: broadened fingertips of fingers 3 and 4, distinct colour border on lateral head, and fourth finger not strongly reduced. A map of all known locations (Fig. 1) was compiled in QGis v3.26 (QGIS Development Team 2022).

Sample collection

Specimens studied genetically in this paper were collected during multiple expeditions to Madagascar to Ambohitantely (2005, 2019), Tsingy de Bemaraha (2006), Mahajanga (2018), and Ankafobe (2020). Toe clips and swabs were

taken from live individuals in Ambohitantely in 2019 and Ankafobe in 2020. Following anaesthesia and subsequent euthanasia using chlorobutanol or MS222, specimens were fixed in 90% ethanol, and thereafter transferred to 70% ethanol for long-term storage. Tissue samples were taken from the euthanised animals by extracting pieces of the thigh muscle, tongue or whole parts of the extremities (for small sized individuals) and preserved in pure ethanol. FGZC refers to the field numbers of Frank Glaw, and KAMU (S/T) to the field numbers of Katherine Mullin (KAMU refers to specimen vouchers, KAMUS to swabs, and KAMUT to tissue samples). Specimens were deposited in the collections of the Université d'Antananarivo, Département de Biologie Animale (now Mention Zoologie et Biodiversité Animale) (UADBA-A) in Antananarivo, Madagascar, and the Zoologische Staatssammlung München (ZSM) in Munich, Germany.

Morphological examination

External morphological measurements were taken to 0.01 mm using a digital calliper, and rounded to 0.1 mm. Ratios were calculated before rounding. Measurements taken generally follow those given by Scherz et al. (2015): snout–vent length, from the tip of the snout to the cloaca, measured in dorsal aspect (SVL); head width, measured at the widest point (HW); head length, measured along the mouth from the anterior-most point to the rictus (HL), eye diameter, measured horizontally (ED); eye–nostril distance, from the anterior-most corner of the eye to the centre of the nostril (END); nostril–snout tip distance, from the centre of the nostril to the anterior-most tip of the snout (NSD); nostril–nostril distance, from the centres of the nostrils (NND); horizontal tympanum diameter (TDH); vertical tympanum diameter (TDV); hand

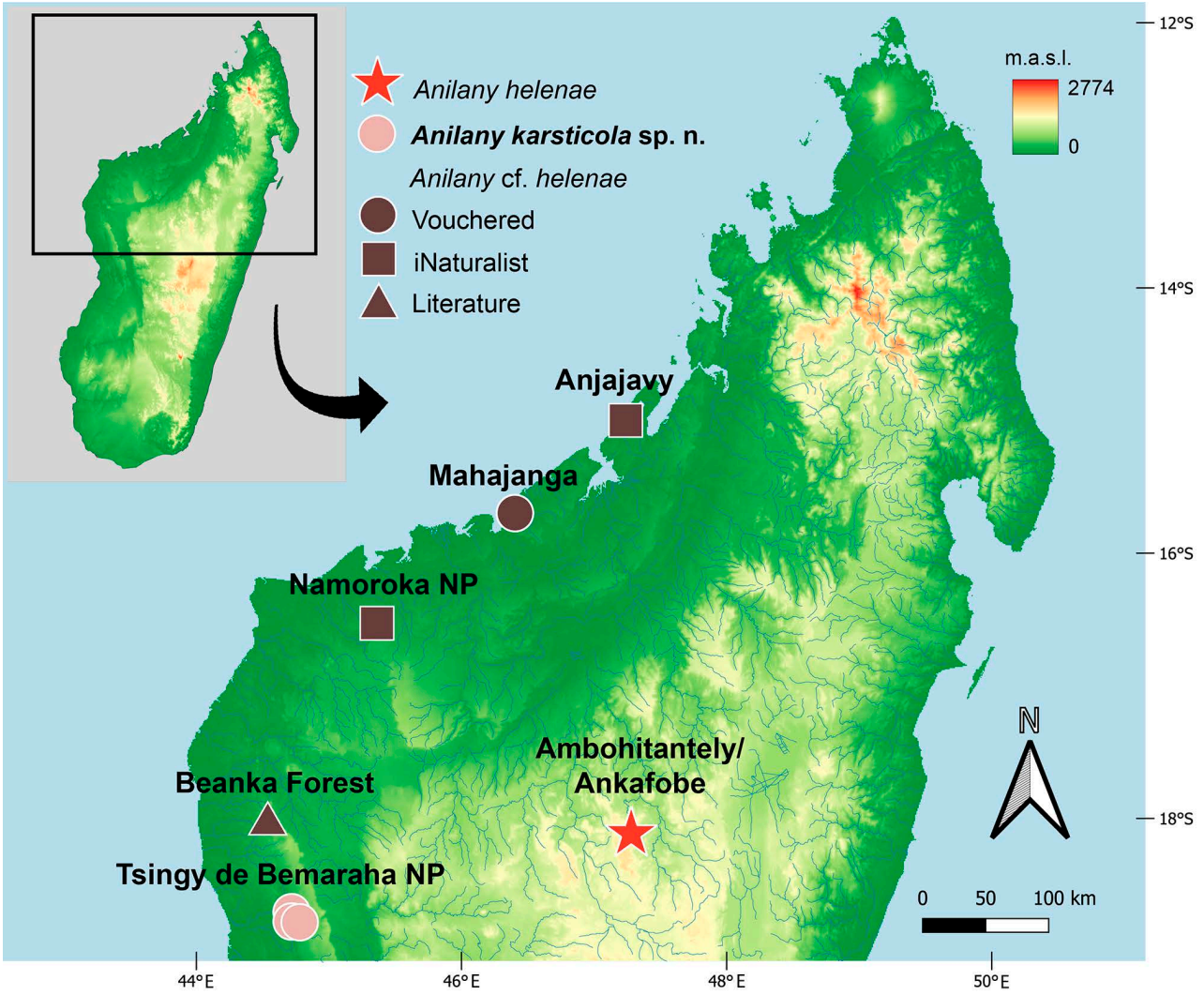

Figure 1. Map of Madagascar indicating locality records of *Anilany helenae* (Ambohitantely and Ankafobe), *A.* cf. *helenae* (Mahajanga), *A. karsticola* sp. n. (Bemaraha) and unsampled records based on literature (Beanka forest) and on iNaturalist observations (Namoroka and Anjajavy). The base map is the USGS SRTM 1-Arc second digital elevation model. Coordinates are given in datum WGS84 format.

length, from the base of the inner metacarpal tubercle to the tip of the third finger (HAL); upper arm length, from the insertion of the arm to the articulation of the elbow, measured in ventral aspect (UAL); lower arm length, from the articulation of the elbow to the base of the inner metacarpal tubercle (LAL); thigh length, from the cloaca to the knee (THIL); thigh width at the widest point lateral to the hip (THIW); tibia length, from the knee to the articulation with the tarsus (TIBL); tibia width at the widest point in dorsal view (TIBW); tarsus length, from the tibiotarsal articulation to the tarsal-metatarsal articulation (TARL); foot length, from the tarsal-metatarsal articulation to the tip of the fourth toe (FOL); inner metacarpal tubercle length (IMCL); inner metatarsal tubercle length (IMTL). Note that this scheme differs in several aspects from that of Rakotoarison et al. (2017) and other works on *Stumpffia* (e.g. by measuring the limbs piece-by-piece, rather than stretching them and measuring the whole limb at once), and therefore not all measurements are fully comparable among these works. External morphological characters were inspected by naked eye and at 6.5–40× magnification through a stereo dissecting microscope. Specimens were sexed based on field records, presence of an enlarged inner metacarpal tubercle and flared humeral crests (in males), or gonad investigation. Specimens that were substantially smaller than all definitively sexed adults were deemed to be subadults.

Osteological description: A specimen of topotypical *Anilany helenae* (ZSM 370/2005), three specimens of *Anilany* sp. Ca14 from Tsingy de Bemaraha (ZSM 137/2006, ZSM 21/2006, and UADBA-A 17849) and one specimen of *Anilany* sp. from Mahajanga (ZSM 240/2018) were scanned using a phoenix|x nanotom m micro-Computed X-ray Tomography (micro-CT) machine (GE Measurement & Control, Wunstorf, Germany), using methods described in Scherz et al. (2017a). 3D volumes of the scans were refined in VG Studio Max 2.2 (Volume Graphics GMBH, Heidelberg, Germany), removing artefacts with clipping planes and/or segmentation. Subsequently, the volumes were rendered using the Phong renderer with a custom pre-set and screenshots of different perspectives were taken in orthographic view. The scans of all five specimens were exported as DICOM image stacks and are available on MorphoSource (https://www.morphosource.org/projects/000661751). Osteological terminology follows that used by Scherz et al. (2017a), which in turn is based largely on Trueb (1968, 1973) and Fabrezi & Alberch (1996).

Molecular phylogenetics

DNA sequencing: Available data for *Anilany* specimens from Ambohitantely and Bemaraha (Scherz et al. 2016, Rakotoarison et al. 2017) was supplemented by unpublished sequences and newly generated data by extracting genomic DNA from twelve tissue samples (see Table 1 for details) following standard protocols in the DNeasy Blood and Tissue Kit (Qiagen Inc., Venlo, Netherlands) for *A. helenae* or by using an SPRI Bead Protocol (Phyletica Lab 2024) for specimens from Bemaraha and Mahajanga. The yield in DNA was quantified using the Agilent Genomic DNA Screen Tape assay on an Agilent 2200 TapeStation (Agilent Technologies, Santa Clara, California USA) and DNA subsequently amplified for two adjacent fragments of the 16S rRNA gene that cover the 3' and 5' termini of the 16S rRNA gene, respectively (here called 16S3' and 16S5'), cytochrome oxidase subunit 1 (COI), a fragment of the nuclear recombination-activating gene 1 (RAG-1), and a fragment of the nuclear brain-derived neurotrophic factor (BDNF). PCR amplifications were carried out in a total volume of 25 µl consisting of 12.5 µl nuclease-free water, 5 µl 5x Green GoTaq Flexi Buffer (Promega Madison, Wisconsin USA), 4 µl of 25 mM MgCl2 (Promega), 0.4 µl dNTPs (10 mM) (Invitrogen/Thermo Fisher Scientific, Waltham, Massachusetts USA), 1 µl each of forward and reverse primers (10 pM) (Thermo Fisher Scientific, Waltham, Massachusetts USA), 0.1 µl 5 U/µl GoTaq Flexi DNA Polymerase (Promega) and 1 µl of extracted DNA. Details of individual primer sequences and PCR conditions can be found in Table 2.

PCR products were purified using the Min Elute PCR Purification Kit (Qiagen) and the respective amplicon size for each marker was verified through running a gel-electrophoresis on a 2% agarose gel. Sequencing PCRs were run under the thermal profile 96 °C (90s), [96 °C (20s), Tm (15s), 60 °C (240s)] × 35 on a Biometra T3000 or a T1 Cycler (Analytik Jena, Jena, Germany) in a total volume of 10 µl, comprising 2 µl 5x Sequencing Buffer, 0.5 µl BigDye v.3.1, 0.5 µl forward or reverse primer, 6 µl HPLC-water and 1 µl purified PCR product. The respective annealing temperature (T_m) was chosen according to the specifics of each marker (Table 2). The PCR products were purified using gel filtration (Sephadex G-50 Superfine; Sigma-Aldrich via Merck KGaA, Darmstadt, Germany) on a MultiScreen-HTS-HV plate and subsequently sequenced in forward and reverse direction on a 3500 Genetic Analyzer (Applied Biosystems, Waltham, Massachusetts USA). Chromatograms were verified by eye and sequences corrected manually, if necessary, in Geneious Prime® 2022.0.1 (Biomatters Ltd., Auckland, New Zealand). All newly generated sequences were submitted to NCBI GenBank (accession numbers provided in Table 1).

Phylogenetic analyses: A species-identification dataset was compiled including available and newly generated sequences of 16S3', 16S5', and COI gene fragments for all *Anilany* specimens. The 16S3' fragment was mainly used for species identification, being the most widely used marker for molecular taxonomic identification (DNA barcoding) in amphibians from Madagascar (Vences et al. 2005) and having consequently the largest reference database for Malagasy frogs. The 16S5' and COI datasets were used to verify taxonomic identifications and as alternative information when 16S3' data were not available. All alignments were generated using the MAFFT local pair algorithm implemented in AliView v1.26 (Larsson 2014).

Phylogenetic inferences were based on the concatenated alignment of all three mitochondrial markers, 16S3', 16S5' and COI (1694 bp), which was compiled using Concatenator v0.2.1 from the iTaxoTools toolkit 0.1 (Vences et al. 2021, 2022). PartitionFinder v2.1.1 (Lanfear et al. 2017) was used to infer the best suited models of molecular evolution on the basis of the AIC, using the input configuration file generated in Concatenator v0.2.1 including five partitions, corresponding to one partition each for the two rRNA gene fragments (16S3' and 16S5'), and individual codon positions for the protein-coding gene (COI). The partitions and models identified as optimal are as follow: (1) 16S5': GTR+I; (2) 16S3': GTR+I; (3) COI_1st: GTR+I; (4) COI_2nd: HKY; and (5) COI 3rd: GTR. The multi-gene phylogeny was inferred using a Maximum Likelihood (ML) approach under 1000 replicates for the ultrafast bootstrap analysis in IQ-TREE v1.6.12 (Nguyen et al. 2015, Chernomor et al. 2016, Hoang et al. 2018). To control for over-parametrisation, we ran the same analysis based on a simple JC69 model, which retrieved a nearly identical topology (not shown).

Despite the attempt to generate sequences of all five markers for all 19 individuals collected for the genus *Anilany* to date (see Table 1), gene sequences could not be obtained for all of them due to exhausted tissue samples. Sequences for those individuals were coded as missing (?) for the respective marker in the concatenated dataset. The tree was rooted using available sequences from *Scaphiophryne marmorata* (ZCMV 2212) and *S. menabensis* (ZSM 89/2006) as outgroups. The assignment of specimens to mitochondrial lineages was further based on individual uncorrected pairwise distances (p-distances) calculated in TaxI2, part of the iTaxoTools toolkit (Vences et al. 2021), based on the individual alignments of the 16S3', 16S5', and COI markers to avoid ambiguities due to missing data. Potential species status was assessed based on inter-specific thresholds of more than 2.5% genetic distance for both fragments of 16S and 6% for COI as criteria, following Fouquet et al. (2007), Vieites et al. (2009), and Perl et al. (2014), which are consistent, for example, with distances between some closely related *Stumpffia* species (Rakotoarison et al. 2017).

Diversity in nuclear-encoded DNA: The protein-coding nuclear genes BDNF (376 bp) and RAG-1 (730 bp) were analysed separately using a haplotype network approach in the program Hapsolutely from the iTaxoTools toolkit (Vences et al. 2021) to gather evidence for lineage distinction from unlinked loci. One specimen (ZSM 21/2006 from Bemaraha) had to be omitted from the RAG-1 dataset due to several undetermined sites (Ns), which cannot be phased. Alleles (haplotypes) were initially inferred using the PHASE algorithm (Stephens et al. 2001) implemented in Hapsolutely, using a phase (-p) and allele (-q) threshold of 0.5 with 100 MCMC iterations. Subsequently, networks for both markers were built from the phased alignments based on the TCS algorithm (Clement et al. 2000).

Molecular diagnosis: We determined molecular diagnostic sites differentiating all three populations for the mitochondrial markers 16S3', 16S5' and COI using MolD (Fedosov et al. 2022), as implemented in iTaxoTools (Vences

Table 1. Samples of *Anilany* specimens used for phylogenetic analyses, including locality information and GenBank accession numbers of the respective sequence for each marker. Newly generated sequences are highlighted in boldface. We use the name *A. karsticola* sp. n. (formerly *A.* sp. Ca14) in anticipation of our chief results.

Lineage	Collection number	Field number	Locality	Latitude	Longitude	16S3'	16S5'	COI	BDNF	RAG–1
A. helenae	ZSM 370/2005	FGZC 2101	Ambohitantely	-18.19	47.28	EU341070	EU341070	MF768136	**PQ787188**	EU341125
A. helenae	not collected	KAMUT2	Ambohitantely	-18.18522	47.2875	**PQ773877**	–	**PQ773471**	–	–
A. helenae	not collected	KAMUT6	Ambohitantely	-18.20958	47.28031	**PQ773878**	–	**PQ773472**	–	–
A. helenae	not collected	KAMUT9	Ambohitantely	-18.17019	47.28011	**PQ773879**	–	**PQ773473**	–	–
A. helenae	not collected	KAMUT10	Ambohitantely	-18.1753	47.30911	**PQ773880**	–	**PQ773474**	–	–
A. helenae	Mol. Ecol. Lab, Cardiff University, uncatalogued	KAMU21	Ambohitantely	-18.19694	47.28413	MZ751042	MZ751042	MZ751042	–	–
A. helenae	not collected	KAMUT22	Ambohitantely	-18.2095	47.28038	**PQ773881**	–	**PQ773475**	–	–
A. helenae	not collected	KAMUS441	Ankafobe	-18.11095	47.19429	MW561474	–	–	–	–
A. helenae	not collected	KAMUS444	Ankafobe	-18.11095	47.19429	MW561476	–	–	–	–
A. karsticola sp. n.	ZSM 21/2006	FGZC 711	Bemaraha	-18.70861	44.71889	**PQ773882**	MF768224	**PQ773476**	**PQ787189**	–
A. karsticola sp. n.	ZSM 22/2006	FGZC 712	Bemaraha	-18.70861	44.71889	–	MF768225	–	–	**PQ787194**
A. karsticola sp. n.	UADBA-A 25660	FGZC 713	Bemaraha	-18.77889	44.71889	EU341069	EU341069	**PQ773477**	**PQ787190**	**PQ787195**
A. karsticola sp. n.	UADBA-A 25663	FGZC 714	Bemaraha	-18.78417	44.71889	**PQ773883**	**PQ773872**	**PQ773478**	**PQ787191**	**PQ787196**
A. karsticola sp. n.	ZSM 114/2006	FGZC 899	Bemaraha	-18.77889	44.71889	–	MF768226	–	–	**PQ787197**
A. karsticola sp. n.	ZSM 137/2006	FGZC 941	Bemaraha	-18.78417	44.77944	–	MF768227	–	–	–
A. cf. *helenae*	UADBA uncatalogued	FGZC 5751	Mahajanga	-15.69869	46.40386	**PQ773884**	**PQ773873**	**PQ773479**	**PQ787192**	**PQ787198**
A. cf. *helenae*	UADBA uncatalogued	FGZC 5752	Mahajanga	-15.69869	46.40386	–	**PQ773874**	–	–	–
A. cf. *helenae*	ZSM 239/2018	FGZC 5753	Mahajanga	-15.69869	46.40386	–	**PQ773875**	–	–	–
A. cf. *helenae*	ZSM 240/2018	FGZC 5754	Mahajanga	-15.69869	46.40386	**PQ773885**	**PQ773876**	**PQ773480**	**PQ787193**	**PQ787199**

et al. 2021). For this purpose, sequences of *Kaloula borealis* served as indexing reference, which were extracted from the complete mitochondrial genome available on Genbank (JQ692869.1). Alignment positions only covered by the reference sequence were removed and sequences were trimmed to equal length. Gaps were not considered as diagnostic sites (Gaps_as_chars=no) and a percent difference of 2% was used for the inference of rDNC's (Pdiff=2). The reference alignments and output files from MolD are available from the Zenodo repository (DOI: 10.5281/zenodo.14502386).

Nomenclatural act

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new name contained herein is available under that Code from the electronic edition of this article. This published work and the nomenclatural act it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: urn:lsid:zoobank.org:pub:E6F87FC9-1508-494A-B6E2-F9C941A0F06A. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: salamandra-journal.com, zenodo.org.

Results

iNaturalist and other new records

Our survey of iNaturalist observations (not including observations made by us) resulted in additional records of *Anilany* specimens from four localities. Two observations were from Ambohitantely Special Reserve, by Martin Mandak on 19 November 2012 (https://www.inaturalist.org/observations/247269) and Len de Beer in September 2010 (https://www.inaturalist.org/observations/105928493). One observation from the Tsingy de Bemaraha by Len de Beer from February 2012 is also assignable to this genus (https://www.inaturalist.org/observations/108939466). Populations of *Anilany* from Ambohitantely and Tsingy de Bemaraha are vouchered based on our own collections as well, and are analysed in detail below.

Our iNaturalist search yielded further observations from two new localities, from which we had no voucher specimens or tissues: On 20 November 2016, Jonhson William Clovis Ratsimanadino observed a specimen in Anjajavy, north-western Madagascar (14.9906° S, 047.2335° E) that, based on the lateral head colour border and hand shape, is clearly an *Anilany* (https://www.inaturalist.org/observations/4627638). Two further specimens probably assignable to *Anilany* were also observed in Anjajavy (14.9983° S, 047.2317° E, ± 1.57 km) by Justin Gerlach on 10 and 13 April 2019 (https://www.inaturalist.org/observations/93144780 and https://www.inaturalist.org/observations/93144861, respectively; Fig. 2).

Table 2. DNA fragments analysed, with an overview of their approximate fragment sizes (bp), primer names and sequences, and thermal cycling profiles used. Thermal cycling schemes start with temperature (in °C) of each step, followed by the time in seconds between parentheses.

Marker	Direction	Primer	Primer Sequence (5'-3')	PCR conditions	Reference
16S3' (530 bp)	F	16SAR-L	CGCCTGTTTATCAAAAACAT	94 (90), [94 (45), 55 (45), 72 (90)] x33, 72 (600)	Palumbi et al. (1991)
	R	16SBR-H	CCG GTCTGAACTCAGATCACGT		Palumbi et al. (1991)
16S5' (470 bp)	F	16SL3	AGCAAAGAHYWWACCTCGTACCTTTTGCAT	94 (90), [94 (45), 55 (45), 72 (90)] x33, 72 (600)	Hedges (1994): modified
	R	16SA-H	ATGTTTTTGATAAACAGGCG		Palumbi et al. (1991)
COI (650 bp)	F	LCO1490	GGTCAACAAATCATAAAGATATTGG	94 (90), [94 (30), 49 (45), 72 (90)] x35, 72 (600)	Folmer et al. (1994)
	R	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA		Folmer et al. (1994)
BDNF (700 bp)	F	BDNF.Amp.F1	ACCATCCTTTTCCTTACTATGG	96 (120), [95 (25), 52 (25), 72 (120)] x45, 72 (600)	van der Meijden et al. (2007)
	R	BDNF.Amp.R1	CTATCTTCCCCTTTTAATGGTC		van der Meijden et al. (2007)
RAG-1 (500 bp)	F	Rag1_Coph_F1	CGTGATCGGGTAAAAGGTGT	94 (120), [94 (20), 53 (50), 72 (180)] x 35, 72 (600)	Rakotoarison et al. (2015)
	R	Rag1_Coph_R1	TCGATGATCTCTGGAACGTG		Rakotoarison et al. (2015)
(700 bp)	F	RagF1	AGCTGCAGYCARTACCAYAARATGTA	94 (120), [94 (20), 54 (50), 72 (180)] x 35, 72 (600)	San Mauro et al. (2004)
	R	RagR1	AACTCAGCTGCATTKCCAATRTCACA		San Mauro et al. (2004)

In August 2018, Olivier Testa (and colleagues) observed two specimens in Tsingy de Namoroka National Park, western Madagascar (16.53° S, 045.36° E; https://www.inaturalist.org/observations/37504632; Fig. 3) that, based on their lateral head colour border, hand shape, and close resemblance to specimens from Bemaraha, are as-

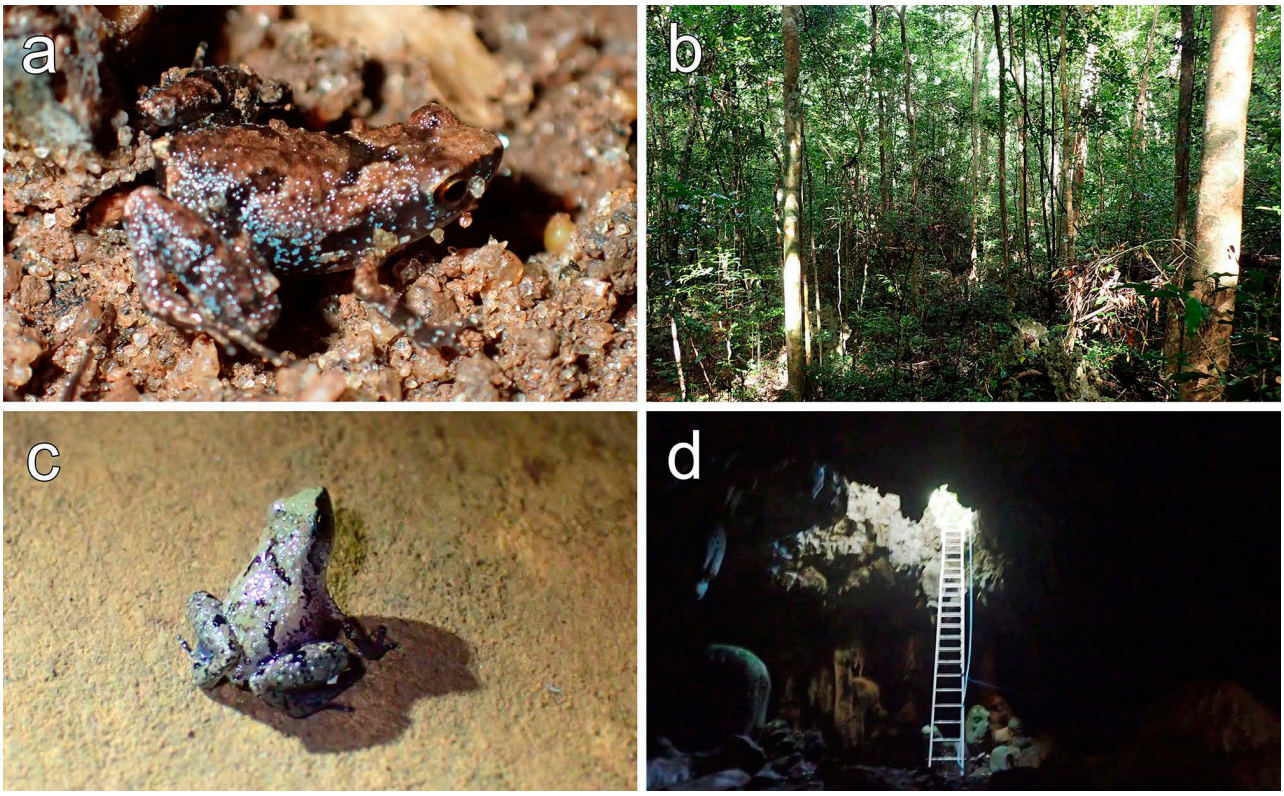

Figure 2. *Anilany* cf. *helenae* specimens from Anjajavy: (a) a putatively adult specimen in dorsolateral view showing contrasting chevron patterning dorsally, and visible expansion of terminal finger discs, typical of this genus. This specimen was observed in dry coastal forest (b); (c) an adult specimen in posterodorsolateral view, showing the dark dorsal chevron and expanded finger discs. This specimen was observed in a cave (d), above which was forest like that shown in (b). Photographs by Justin Gerlach (used with permission).

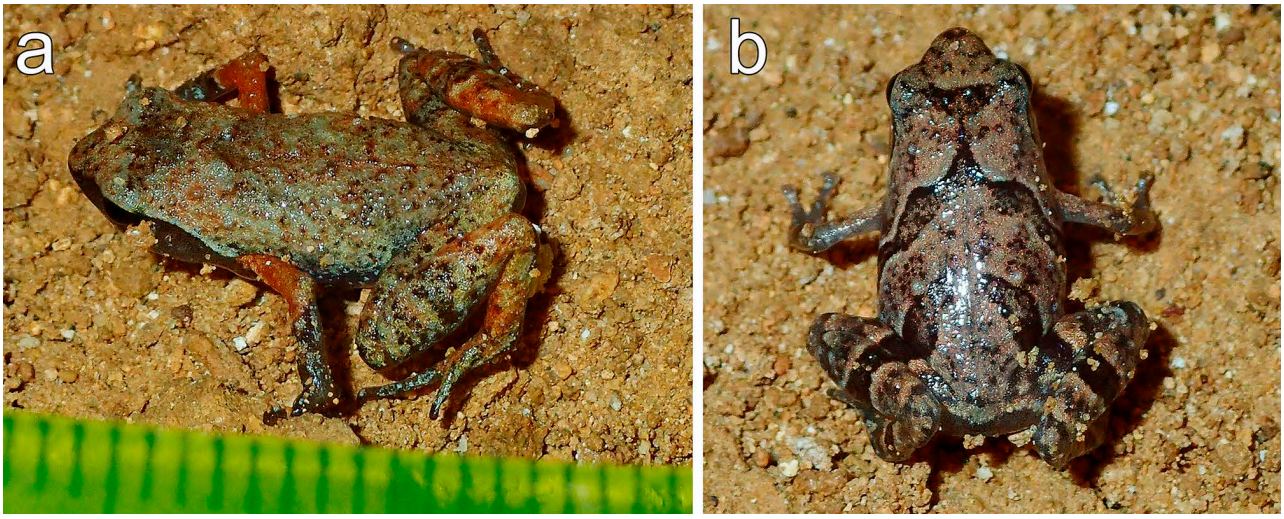

Figure 3. *Anilany* cf. *helenae* specimens from Namoroka National Park: (a) an adult specimen in dorsolateral view showing weak patterning dorsally, exhibiting the distinct lateral head surface typical of this genus; (b) an adult specimen in dorsal view with strong dorsal patterning, including leg crossbands. Photographs by Josiane Lips (used with permission).

signable to this genus. These frogs were observed during exploration of the tsingy systems on an expedition of the French Federation of Speleology (FFS N°16/2018) (Sibert et al. 2019). They were recorded in close proximity to one another, and both measured ca. 12 mm in SVL (Josiane Lips pers. comm.).

The Anjajavy and Namoroka specimens substantially expand our knowledge of the distribution of *Anilany* (Fig. 1). In late 2023, voucher material was collected from Anjajavy, but it could not be integrated herein this study due to time constraints and delays in obtaining export permits.

External morphology, morphometrics, and colouration

The specimens from iNaturalist, as far as is visible from the photographs, are all morphologically similar, though they vary quite substantially in colour pattern.

Morphometric data based on our own measurements of *Anilany* specimens from Ambohitantely (type locality of *A. helenae*; n = 3), Tsingy de Bemaraha (locality of *A.* sp. Ca14; n = 10), and Mahajanga (locality of *A.* cf. *helenae*; n = 2) are provided in Table 3. Subtle differences among these three populations are present in relative lengths of the hindlimbs (TIBL/SVL = 0.48 in *A. helenae* sensu stricto vs 0.42–0.46 in *A.* sp. Ca14 vs 0.47–0.50 in *A.* cf. *helenae*) and SVL (13.8–15.0 mm vs 15.7–17.4 mm vs 12.7–12.7 mm).

Colouration in *Anilany* is remarkably variable (Figs 2–7). A dorsal stripe has been observed in some specimens from Ambohitantely (Fig. 4a) and Ankafobe (Fig. 5). Large dark chevrons are sometimes present (Figs 2a, c, 3b, 4c, i, 6c, h). The upper arms are sometimes reddish (Figs 3a, 5b, d, 6c, e). Dorsolateral brown stripes can be present (Figs 6e, 7a). Crossbands on hindlimbs range from indistinct (Figs 3a, 4a, h, 5d, 6a, 7a) to highly distinct (Figs 2c, 3b, 4i, 5c, 6c). Ventral colour ranges from uniformly beige (Fig. 4b) to largely white (Fig. 6b) through a range of speckling (Figs 4d, 6d, f, i), but has not been documented for most specimens. At present, insufficient photographs from each population are available to assess the extent of variability of each, and whether or not any colour patterns might be consistently different.

Osteology

In the following, we provide a comparative osteological account of the sampled *Anilany* populations (Figs 8, 9), and also elaborate on some of the important differences between *Anilany* and the closely related *Rhombophryne* and *Stumpffia*.

Neurocranium: The neurocranium is typical of a small to miniaturised cophyline: the skull is generally narrow and triangular, and the lateral elements exhibit some verticalization, probably associated with miniaturisation (Yeh 2002). The sphenethmoid is weakly to moderately well ossified. It is rarely in contact with the vomer, and is too far anterior to the ossified portion of the parasphenoid to touch it. The exoccipitals and prootics are widely separated from each other; they are rather robust, and do not seem to differ substantially from *Stumpffia*, though a thorough investigation of these bones across Cophylinae may reveal interesting patterns, as examination of them so far has always been superficial. The septomaxilla is small and spiralled, but its small size and low scan resolution prevents detailed comparative analysis of its structure (Scherz et al. 2017a). The columella runs obliquely anteroventrolaterally from pars interna plectra to the pars media plectra. It exhibits surprisingly great variability in specimens from Bemaraha.

Dorsal investing bones: The nasals are widely separated at the midline. Laterally, they are strongly curved, with a broad maxillary process. This process is much narrower relative to the rest of the bone in all *Rhombophryne*, and in all *Stumpffia* examined except the extremely miniaturised *S. tridactyla*, *S. yanniki*, *S. obscoena*, and *S. contumelia* (unpublished data); its shape is therefore also significant for distinction of these genera. The frontoparietal is broad, covering most of the dorsal skull, and long, extending over most of the length of the braincase. The inter-frontoparietal cleft is broad. In several specimens, a diamond-shaped gap is present where the developmental fusion of the frontal and parietal elements has occurred; this is particularly evident in ZSM 240/2018 from Mahajanga. The frontoparietal is more completely ossified in specimens from Bemaraha than the other populations examined, especially posterolaterally where it overlays the prootic.

Ventral investing and palatal bones: The parasphenoid cultriform process is remarkably short, much shorter and less ossified than in all *Rhombophryne* and *Stumpffia* species, giving it also diagnostic power among these genera. The lateral parasphenoid alary processes are weakly flared, and the posterior process approaches but does not participate in the fenestra ovalis.

In *Stumpffia* and *Rhombophryne*, the anterolateral palate consists of the neopalatine and the posterior (postchoanal) portion of the vomer, with the vomer lying ventral to the neopalatine, but invariably contacting it laterally. Medially, the vomer is in contact with the cultriform process of the parasphenoid. In *Anilany*, a single laminar bone is present across this area. The bone runs horizontally initially before deflecting at ca 30° around its midpoint. It is broadest at its medial end, and narrows laterally. There is no evidence of a joint or fusion of two bones. Thus, it seems that either the neopalatine or the posterior vomer has been lost in this genus, leaving a single element. Based on the proximity of the bone to the midline, and the spatulate medial end, we hypothesise that it is the vomer that has been retained, and the neopalatine has been lost or, more likely, is completely unossified. To confirm this hypothesis, more specimens are needed in order to investigate the cartilages of the skull more closely by clearing and staining. The anterior portion of the vomer is present as a small, triradiate element, distantly separated from all other bones.

Table 3. Morphological measurements (in mm) of *Anilany* specimens from Ambohitantely, Tsingy de Bemaraha, and Mahajanga. * = measurements are sourced from Vallan (2000a). Sex: A = unsexed adult, M = male, F = female, F? = probable female, sa = subadult; NM = not measured; HT = holotype; PT = paratype. For other abbreviations see Methods.

Lineage	Collection number	Field number	Locality	Sex	SVL	HW	HL	ED	END	NSD	NND	TDH	TDV	HAL	UAL	LAL	THIL	THIW	TIBL	TIBW	TARL	FOL	IMCL	IMTL
A. helenae	NMBE 1044802 (HT)*	–	Ambohitantely	M	13.8	4.6	NM	1.3	0.9	0.6	NM	0.7	NM	2.1	NM	NM	NM	NM	6.6	NM	NM	5.4	NM	NM
A. helenae	NMBE 1044801 (PT)*	–	Ambohitantely	F	15.0	5.1	NM	1.6	0.9	0.7	NM	1.0	NM	3.1	NM	NM	NM	NM	7.2	NM	NM	6.2	NM	NM
A. helenae	ZSM 370/2005	FGZC 2101	Ambohitantely	M	14.5	5.0	3.7	1.4	0.8	NM	1.7	0.8	0.6	3.1	2.1	2.7	5.9	2.4	7.0	2.3	3.9	6.7	1.0	0.5
A. karsticola sp. n.	ZSM 21/2006 (HT)	FGZC 711	Tsingy de Bemaraha	F	16.3	5.4	3.6	1.9	0.9	NM	1.7	0.9	0.9	3.0	2.6	3.4	7.6	3.1	7.0	2.2	3.4	6.0	0.4	0.0
A. karsticola sp. n.	ZSM 22/2006 (PT)	FGZC 712	Tsingy de Bemaraha	F?	15.9	5.2	3.9	1.8	1.1	0.9	1.7	0.9	1.0	3.0	2.9	3.0	6.7	2.9	6.7	2.4	3.6	6.2	0.4	0.4
A. karsticola sp. n.	UADBA-A 25660 (PT)	FGZC 713	Tsingy de Bemaraha	F?	16.1	5.0	3.7	1.6	1.1	0.9	1.8	0.9	1.1	3.1	2.8	3.6	6.5	2.7	7.3	2.3	3.8	7.0	0.5	0.5
A. karsticola sp. n.	UADBA-A 25663 (PT)	FGZC 714	Tsingy de Bemaraha	F?	17.4	5.2	3.9	1.7	1.1	1.0	1.8	1.0	1.0	3.4	2.8	3.5	7.9	2.7	7.2	2.4	4.0	6.5	0.5	0.3
A. karsticola sp. n.	ZSM 137/2006 (PT)	FGZC 941	Tsingy de Bemaraha	sa	13.0	4.8	3.5	1.6	0.8	0.8	1.4	0.7	0.7	2.6	2.3	3.3	6.3	2.3	6.3	1.8	3.5	5.9	0.0	0.2
A. karsticola sp. n.	UADBA-A 17850 (PT)	APR 01226	Tsingy de Bemaraha	F	16.4	5.5	3.9	1.7	1.1	0.9	1.7	1.1	1.1	3.4	3.4	3.7	7.4	2.4	7.3	2.4	4.1	7.0	0.4	0.5
A. karsticola sp. n.	UADBA-A 17847 (PT)	APR 01040	Tsingy de Bemaraha	M	16.1	5.3	4.0	1.7	1.1	0.9	1.7	1.1	1.1	3.7	2.8	3.6	7.3	3.1	7.1	2.5	4.0	7.0	0.9	0.5
A. karsticola sp. n.	UADBA-A 18751 (PT)	APR 00219	Tsingy de Bemaraha	F?	16.7	5.9	4.0	1.9	1.2	1.0	1.8	1.0	1.0	3.6	3.1	3.6	7.7	2.5	7.7	2.2	4.1	7.7	0.5	0.5
A. karsticola sp. n.	UADBA-A 17846 (PT)	APR 01030	Tsingy de Bemaraha	M	15.7	5.1	3.6	1.6	1.1	0.9	1.5	0.8	0.9	3.7	2.8	3.4	6.6	3.2	7.0	1.9	3.6	6.8	1.0	0.4
A. karsticola sp. n.	UADBA-A 17849 (PT)	APR 01099	Tsingy de Bemaraha	M	16.7	5.5	3.9	1.7	1.1	1.1	1.7	1.0	1.0	3.9	3.2	3.6	7.1	2.7	7.6	2.3	4.2	8.0	1.1	0.4
A. cf. *helenae*	ZSM 240/2018	FGZC 5754	Cave near Mahajanga	F?	12.7	4.4	3.7	1.7	1.0	1.0	1.6	0.6	0.8	2.9	2.8	3.1	6.1	2.5	6.4	2.1	3.6	5.8	0.4	0.0
A. cf. *helenae*	ZSM 239/2018	FGZC 5753	Cave near Mahajanga	A	12.7	4.6	3.5	1.6	0.6	0.8	NM	0.7	0.7	3.0	3.0	2.8	5.5	2.1	6.0	1.6	3.0	5.8	0.4	0.5

Figure 4. *Anilany helenae* from the type locality (Ambohitantely) in life: (a, b) ZSM 370/2005 (FGZC 2101), in dorsolateral (a) and ventral (b) views; (c, d) KAMUT2 in dorsal (c) and ventral (d) views; (e) juvenile in dorsal view (not sampled); (f) KAMUS59 in lateral view (molecular data not available), (g) the manus of KAMUT23 in dorsal view (molecular data not available), with marked differences in digit expansion between digits 1+2 and 3+4; (h) KAMUT37 in dorsal view (molecular data not available); (i) KAMUT55 in lateral and dorsolateral views.

Overall, the palate of *Anilany* is reminiscent of *Anodonthyla* (Noble & Parker 1926, Parker 1934). Both have short parasphenoid processes, and both lack vomerine teeth and have lost an element of the vomer–neopalatine complex. However, unlike *Anilany*, it seems that the post-choanal vomer of *Anodonthyla* has been lost, and the neopalatine retained (Noble & Parker 1926, Parker 1934). Thus, the supposed state in *Anilany* is unique within Cophylinae.

Maxillary arcade: Teeth are totally absent from the maxillary arcade. The palatine and lateral processes of the premaxilla's pars palatina are short and relatively broad, the angle of the palatine process being much more acute than that of the lateral process. The ascending alary process is vertical or slightly concave posteriorly in lateral view, and ascends dorsolaterally, away from the midline. The maxilla is long and thin, with a broad facial process that does not closely approach the maxillary process of the nasal. Its pars palatina is narrow. It is in only brief contact posteriorly with the quadratojugal posteriorly (contact is longer in ZSM 370/2005 than other individuals; specimens from Bemaraha exhibit substantial variation), and medially is separated from the pterygoid by the pterygoid fossa.

Suspensory apparatus: The suspensory apparatus appears delicate, with only brief contact among its bony components. The pterygoid has the typical three rami. The anterior ramus is rather thin, curved in both the horizontal and vertical planes, and ventrolaterally sculpted to run along the pterygoid fossa of the maxilla. The medial ramus is short and posteriorly concave, as is typical. The posterior ramus is broad and flat, and very upright. The quadratojugal is club-like. Its lateral surface is sculpted. Its ventral edge is concave, and its dorsal edge convex, giving it a slight curve. The ventral extent is also longer than the dorsal edge, giving it an acuminate tip, where it contacts the maxilla at the very point. It has a slightly bulbous posteroventral process, with a mineralised connection with the posterior ramus of the pterygoid. Dorsally, near its posterior end, it has a receiving facet for the ventral ramus of the squamosal, and is in brief connection with it (more substantial in ZSM 21/2006). The squamosal is slender. Its shape is surprisingly variable among individuals (see Fig. 9). The ventral ramus is straight to slightly curved, with a posterior crest on its lower half in some individuals. As described above, there is some variation in the extent of its contact with the quadratojugal. In general, the otic and zygomatic rami are at a right-angle to one another in lateral view, the zygomatic ramus substantially shorter than the otic ramus. In individuals from Bemaraha, the otic ramus curves towards the ear capsule; in ZSM 240/2018 from Mahajanga the head of the squamosal is twisted so that both rami are on a single line oriented towards the ear capsule; and in ZSM 370/2005 from Ambohitantely, they are oriented more parallel to the sagittal axis. More specimens are

Figure 5. *Anilany helenae* from Ankafobe in life: (a, b) KAMUT97 in anterior (a) and dorsolateral (b) views (molecular data not available); (c) KAMUS441 in dorsolateral view; (d) KAMUT95 in dorsolateral view (molecular data not available).

Figure 6. *Anilany karsticola* sp. n. from Tsingy de Bemaraha National Park in life: (a, b) adult female holotype ZSM 21/2006 (FGZC 711) in dorsolateral (a) and ventral (b) views; (c, d) paratype ZSM 22/2006 (FGZC 712) in dorsolateral (c) and ventral (d) views; (e–g) paratype UADBA-A 25660 (FGZC 713) in dorsolateral (e) and ventral (f) views, with a closeup of the hand (g) illustrating expanded digits only present on fingers 3 and 4; (h, i) paratype UADBA-A 25663 (FGZC 714) in dorsolateral (h) and ventral (i) views.

needed to ascertain if these differences in angles are consistent in the different populations.

Mandible: The angulosplenial is long and narrow, with a strongly concave posterolateral shelf and a pronounced coronoid process. Most of the anterior portion of the bone is straight, but it makes a weak sigmoid curve posteriorly. Anterolaterally it is traced by the laminar and narrow dentary, which approaches, and in some cases contacts, the mentomeckelian. The mentomeckelians are small, bow-shaped elements, hanging slightly below the level of the dentary and angulosplenial.

Hyoid: The posteromedial hyoid processes are well ossified. They bear a flared base and a distinct medial crest. Analyses of the cartilaginous components of the hyoid apparatus are needed, but too little material of *Anilany* is currently available to allow such study. No ossified parahyoid is present.

Vertebral column: The vertebral column typically contains 8 presacral vertebrae, but UADBA-A 17849 exhibits fusion of presacrals I–III, with a single pair of transverse processes. This is presumed to be a developmental anomaly, as is quite common in frogs (Haas et al. 2021). The

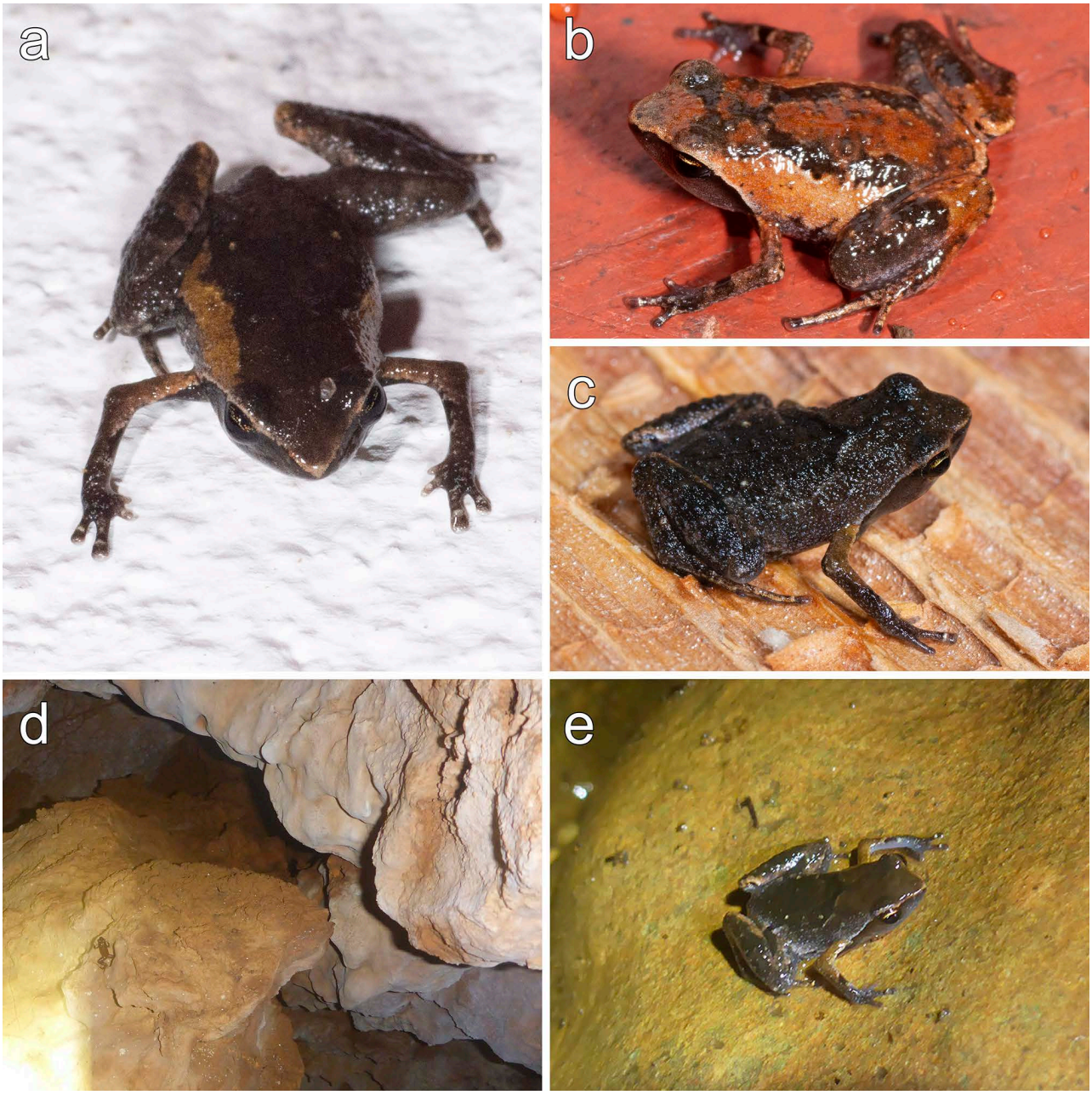

Figure 7. *Anilany* cf. *helenae* (a–c, e) from a cave (d) near Mahajanga along the road to Betsako. Assignment of figured specimens to voucher specimens is uncertain.

transverse processes of all vertebrae are rather short. Those of presacrals II and VIII are oriented anteriorly (as seen in dorsal view), those of VII laterally, and all others posteriorly. Transverse processes of presacrals II–IV are substantially longer than those of V–VIII. The dimensions of the centrae are remarkably variable; those of most speci-

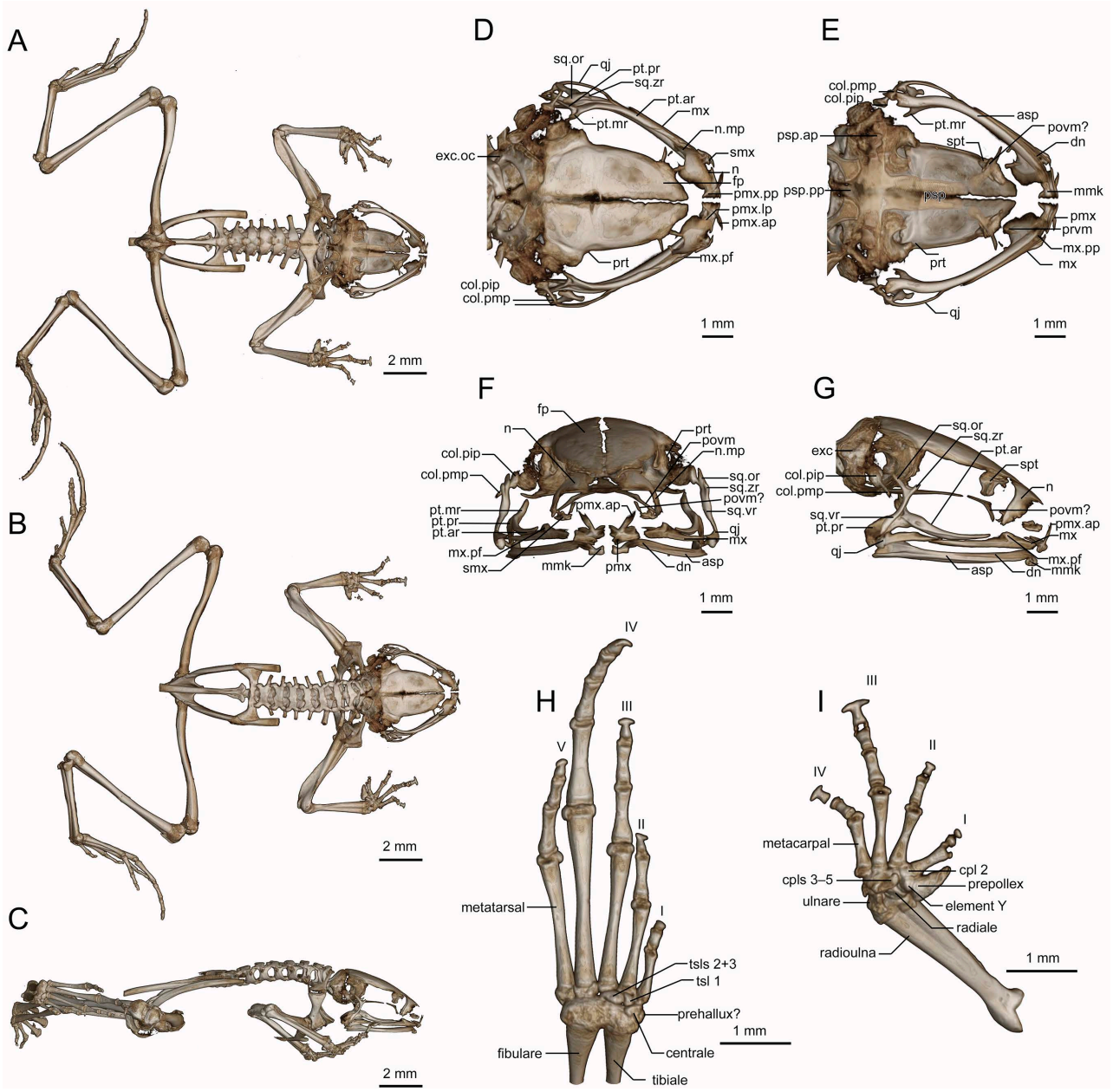

Figure 8. Micro-CT scan images showing the osteology of *Anilany helenae* (ZSM 370/2005). (a–c) Full skeleton in ventral (a), dorsal (b), and lateral (c) view; (d–g) skull in dorsal (d), ventral (e), anterior (f), and lateral (g) view; (h) foot in ventral view; and (i) hand in ventral view. Abbreviations: asp = angulosplenial; col.pip = pars interna plectri of columella; col.pmp = pars media plectri of columella; cpl(s) = carpal(s); dn = dentary; exc = exoccipital; exoc.oc = occipital condyle of exoccipital; fp = frontoparietal; mmk = mentomeckelian; mx = maxilla; mx.pf = pars facilialis of maxilla; mx.pp = pars palatina of maxilla; n = nasal; n.mp = maxillary process of nasal; pmx = premaxilla; pmx.ap = ascending process of premaxilla; pmx.lp = lingual process of premaxilla; pmx.pp = palatine process of premaxilla; povm = postchoanal portion of vomer; prt = prootic; prvm = prechoanal portion of vomer; psp = parasphenoid; psp.ap = alary process of parasphenoid; psp.pp = posterior process of parasphenoid; pt.ar = anterior ramus of pterygoid; pt.mr = medial ramus of pterygoid; pt.pr = posterior ramus of pterygoid; qj = quadratojugal; smx = septomaxilla; spt = sphenethmoid; sq.or = otic ramus of squamosal; sq.vr = ventral ramus of squamosal; sq.zr = zygomatic ramus of squamosal; tsl(s) = tarsals.

mens are substantially broader than they are long, whereas those of ZSM 21/2006 are roughly equally broad and long. Neural arch of presacral I can be complete or incomplete. No neural spines are present. The sacral diapophyses are broadly flared, with concave anterior and posterior edges, and convex lateral surfaces. The articulation between the sacrum and the urostyle is bicondylar. The urostyle has a broad head with distinct crests, behind which it becomes very narrow, gently flaring towards the posterior tip.

Pectoral girdle: The zonal portion of the pectoral girdle consists of the coracoids, clavicles, scapulae, and cleithra. The clavicle is Y-shaped, with a curved thin medial portion, and two short arms laterally. The lateral arms reach the pars acromialis of the scapula. The coracoid is bow-shaped, with a flattened medial end and a rounded glenoid end, the medial end substantially broader than the glenoid. The anterior edge is much more concave than the posterior edge, and more strongly curved than the clavicles. The scapula is also bow-shaped. It has a broad, medially angled pars acromialis, articulating with the clavicle, and the pars glenoidalis forming the glenoid socket via its contact with the coracoid. The contact surface for the cleithrum is broad. The cleithrum is hatchet-shaped, with a broad ventral edge and a narrow anterior ossified element. The suprascapula is not ossified.

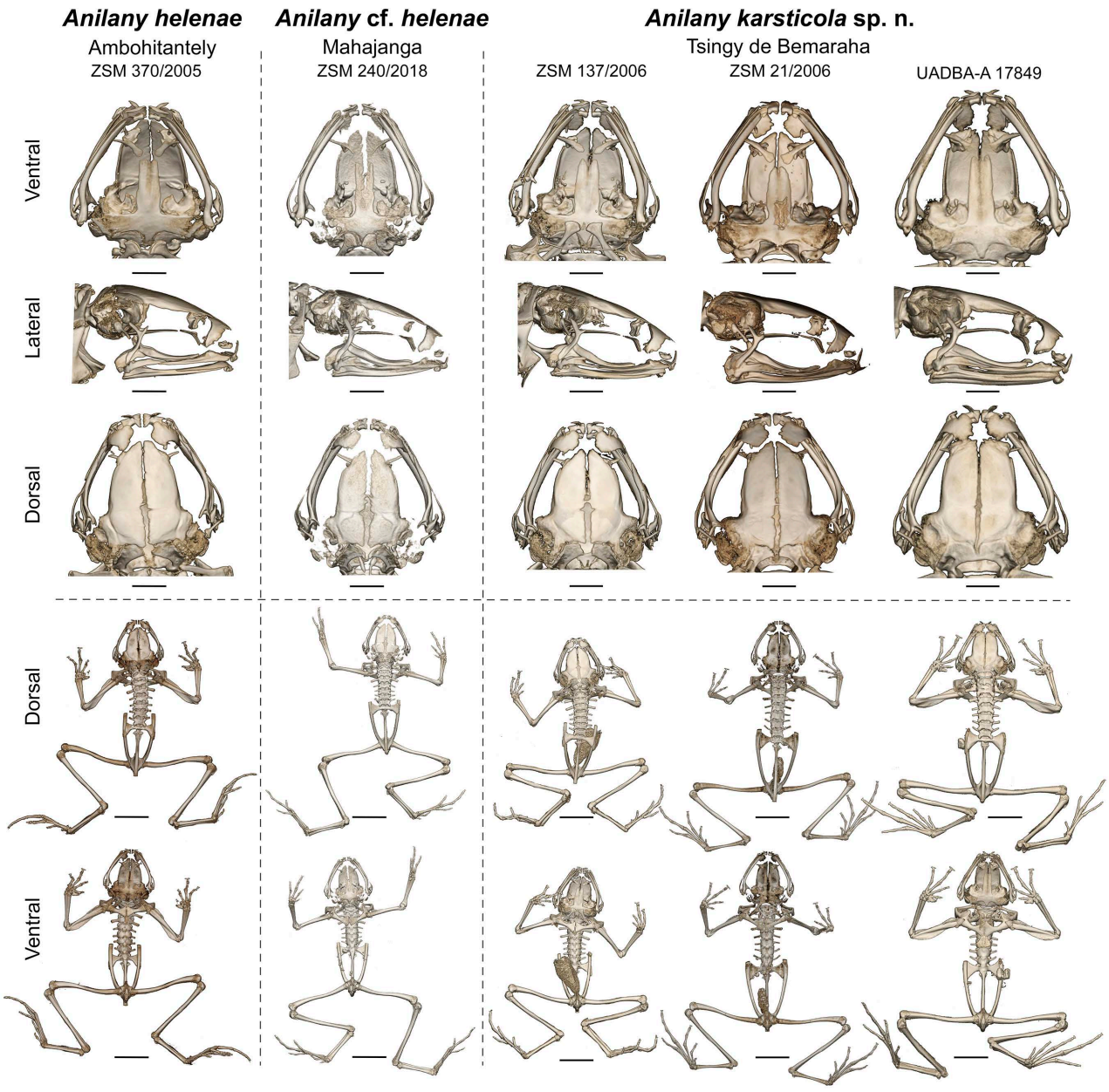

Figure 9. Micro-CT scan images showing comparative osteology of *Anilany* specimens. Scale bars indicate 1 mm (skulls) or 3 mm (full skeletons).

Forelimb and manus: The humerus is one of the most remarkable features of *Anilany*. In contrast to *Stumpffia* and *Rhombophryne*, where there are at most moderate crests, the humerus of *Anilany* males is highly flared, bearing enormous cristae ventralis, lateralis, and medialis. Presumed females (ZSM 21/2006, ZSM 137/2006, ZSM 240/2018) have a distinct crista ventralis and a visible crista lateralis, but lack a crista medialis, and are thus more similar to *Stumpffia* and *Rhombophryne*. The extreme, sexually dimorphic cristae, within the subfamily Cophylinae, are otherwise present only in the distantly related *Anodonthyla*; a second feature convergently similar with that genus – the first being the overall appearance of the palate. The radio-ulna is simple, rather slender, with a distinct medial furrow. In males, it bears a low dorsal crest. These are probably the only ornamented radio-ulnae in this subfamily; *Anodonthyla* species lack crests on the radio-ulna, even in species with extremely hypertrophied humeral crests, such as *A. jeanbai* and *A. rouxae* (based on our own observations; unpublished data). The carpus consists of the ulnare, radiale, element Y, carpal 2, and a large, probably fused element consisting of carpals 3–5. Males exhibit a very large prepollex, as long or longer than the first metacarpal, and substantially broader at the base. Pairs of small, round, ossified elements (presumably sesamoids) are present at most of the joints of the fingers, especially visible between the metacarpals and proximal phalangeal bones. These structures may be homologous with the epiphyses of other cophylines (Abdala et al. 2019); more detailed studies are needed on these. Finger phalangeal composition is standard (2-2-3-3), although the distal phalange of the first finger is highly reduced and almost lost in ZSM 370/2005 and ZSM 240/2018. The distal phalanges of fingers I and II are roughly square, weakly flared distally, whereas those of fingers III and IV are T-shaped. These clearly underly the terminal discs present on fingers III and IV and absent from the other fingers, which characterises this genus externally.

Pelvic girdle: The pelvic girdle is robust. The ischium, pubis, and ilium are well fused. The pubis is generally less ossified than the other elements. The iliac shafts pass ventrolateral to the sacral diapophyses. There is no distinct dorsal crest. The dorsal prominence is only weakly raised, and the oblique groove distinct.

Hindlimb and pes: The femur is gently sigmoid, lacking crests. The tibiofibula is likewise unadorned, with a distinct medial furrow proximally and distally. The tibiale-fibulare is proximally and distally fused. The tarsus consists of tarsals 1 and a broad element presumably comprising 2+3 tarsals. A centrale is present, and a short, pyramidal prehallux is also present (indistinct in scans of ZSM 137/2006 and ZSM 240/2018, probably due to low absorbance). Phalangeal formula is standard (2-2-3-4-3). Both phalanges of digit I are highly reduced, but there is little other evidence of digital reduction in the foot. The terminal phalange of toe I is simple, but all other toes have T-shaped phalanges.

Summary: No consistent differences in osteology were found among populations that exceeded the degree of variation that was observed among specimens from Bemaraha, which was substantial, especially in the suspensory apparatus and maxillary arcade. The only tentatively consistent difference appears to be the angles of the dorsal rami of the squamosal, but more scans are required to confirm this as being diagnostically reliable.

The following features are revealed here to be diagnostic characters of *Anilany*, which reinforce and expand upon those given in the original description of the genus by Scherz et al. (2016): (1) broad maxillary process of nasals, (2) very short parasphenoid cultriform process, (3) a single element in the vomerine region, probably representing the postchoanal portion of the vomer, (4) strongly flared crests on the humeri of males, (5) crests on the radio-ulnae in males, and (6) strongly developed prepollex in males.

Molecular phylogenetics

The ML phylogenetic tree inferred from concatenated mitochondrial dataset (16S3'+16S5'+COI) shown in Figure 10a visualizes the genetic divergence among the 19 sampled individuals of the genus *Anilany*, belonging to three different populations from Ambohitantely/Ankafobe, Bemaraha, and Mahajanga (see details in Table 1). Members of all three populations cluster in agreement with their respective location, forming monophyletic groups with strong support (Ambohitantely/Ankafobe BP = 89; Bemaraha BP = 97; Mahajanga BP = 99). The newly sampled individuals from Mahajanga cluster as sister to the Ambohitantely/Ankafobe lineage but with weak support (BP = 69).

The three sampled populations differ significantly in their intrapopulational variation. Whereas all nine specimens from Ambohitantely/Ankafobe are almost genetically identical (uncorrected p-distance in 3' 16S rRNA fragment 0.00–0.21%), more genetic divergence was found amongst the specimens from Bemaraha (0.00–0.88%) and especially amongst individuals from Mahajanga (0.17–1.05%) (Fig. 10; Tables 4, 5, 6).

Uncorrected pairwise distances (p-distances) between the three populations are comparatively low, generally below the 3% divergence threshold for 16S typically used to identify candidate species of amphibians in Madagascar (Vieites et al. 2009). This applies especially for 16S3' (488 bp; Table 4). Genetic distances between members of all three populations ranged between 0.82% (Ambohitantely KAMU21 vs. Mahajanga FGZC 5751) and 2.63% (Ambohitantely ZSM 370/2005 vs. Bemaraha FGZC 713), the latter value approaching the 3% threshold for defining candidate species (Fouquet et al. 2007, Vieites et al. 2009). The 16S5' alignment (592 bp) shows greater genetic distances, ranging from 1.81–2.44% between Ambohitantely/Ankafobe and Mahajanga, 4.11%–4.56% between Ambohitantely/Ankafobe and Bemaraha, and 4.96%–5.60% between Mahajanga and Bemaraha. The reason for the difference in genetic distance between both fragments of the 16S marker might be determined by the number of parsimony informative (PI) sites per alignment; although of similar length, the 16S5' terminus has 34 PI sites, three times as many as

the 16S3' alignment. There is slightly more missing data in the 16S5' (38.25%) than in the 16S3' alignment. P-distances were also computed for the COI alignment (582 bp) to account for the differences in distance values found for both 16S fragments and gather additional evidence for lineage distinction. Distance values among all three populations, however, are rather low, and fall far below the 6% divergence threshold generally applied for this marker in Malagasy amphibians (Perl et al. 2014), ranging from 1.72% between Ambohitantely KAMUT6 vs. Mahajanga FGZC 5751, to 4.12% between Ambohitantely KAMUT9 and Bemaraha FGZC 713 (Table 6). Thus, altogether, mitochondrial differentiation among populations is comparatively low.

Diversity in DNA sequences of nuclear-encoded markers was assessed based on haplotype networks inferred from alleles of the nuclear protein-coding markers BDNF (376 bp alignment length; 6 individuals) and RAG-1 (730 bp alignment length; 7 individuals). Both networks (Fig. 10b) show no haplotype sharing among individuals of the three populations. However, the two nuclear markers differ substantially in haplotype diversity found within each population. Comparable to mitochondrial data, the highest diversity for RAG-1 was found for individuals from Bemaraha, with each of the four individuals holding two unique alleles differing from each other by one (ZSM 144/2006) to seven (ZSM 22/2006) mutational steps. By contrast, all three individuals share a single nuclear haplotype for BDNF. The single individual from Ambohitantely for which nuclear data could be obtained is characterized by a single haplotype for BDNF and two distinct alleles for RAG-1, which differ by a single mutation. Although this is from a single specimen, it nonetheless aligns with the low intra-populational mitochondrial diversity among individuals from Ambohitantely and Ankafobe. The individuals of the Mahajanga population sampled for RAG-1 or BDNF can be characterized for both markers by two different alleles, differing from each other at one or two polymorphic sites, being separated from the Ambohitantely/Ankafobe specimens by 9–12 mutational steps in RAG-1 and two to three mutational steps in BDNF.

In summary, there is complete concordance between mitochondrial and nuclear data in the genetic differentiation of

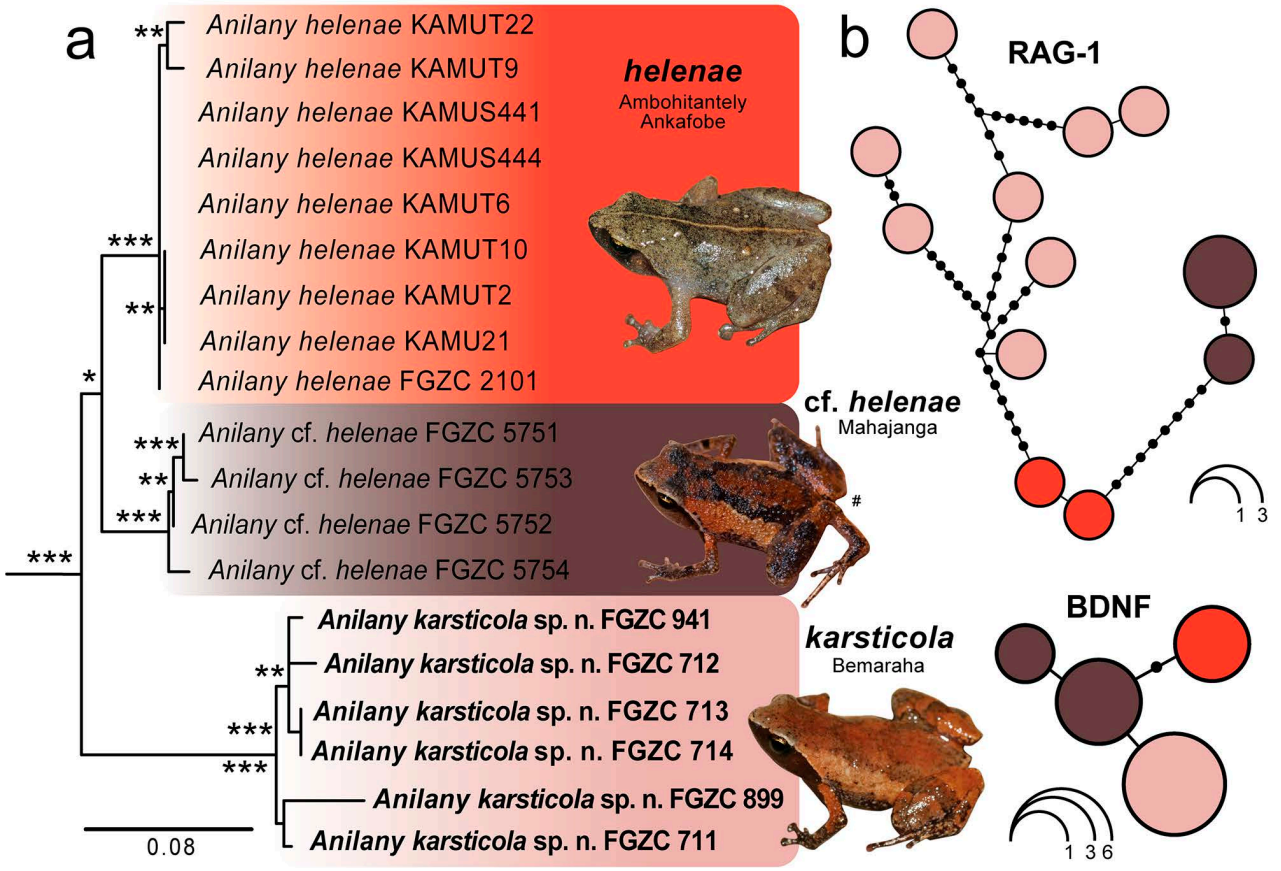

Figure 10. Molecular differentiation of lineages included in the genus *Anilany*. (a) Maximum Likelihood tree calculated from the concatenated alignment (1694 bp) of three mitochondrial markers (16S3', 16S5', COI). Asterisks on the nodes mark bootstrap support: *55–69, ** 70–84, *** 85–100. Photographs not to scale. # = original photograph mirrored. (b) Haplotype networks inferred from the phased DNA sequences of the nuclear genes BDNF (376 bp) and RAG-1 (730 bp). Circles represent haplotypes, with size proportional to their frequency in the individuals sequenced. Dots on branches indicate inferred haplotypes between sampled haplotypes; connections between dots indicate mutational steps.

Table 4. Uncorrected genetic distances calculated for the alignment of the 16S rRNA 3' terminus (16S3') fragment including 14 individuals of the genus *Anilany*. All values are given in percentage. See Table 1 for more information on the analysed individuals.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Anilany helenae* (Ambohitantely) ZSM 370/2005, FGZC 2101	NA													
2	*Anilany helenae* (Ambohitantely) KAMU21	0.00	NA												
3	*Anilany helenae* (Ambohitantely) KAMUT10	0.00	0.00	NA											
4	*Anilany helenae* (Ambohitantely) KAMUT22	0.24	0.21	0.21	NA										
5	*Anilany helenae* (Ambohitantely) KAMUT2	0.00	0.00	0.00	0.21	NA									
6	*Anilany helenae* (Ambohitantely) KAMUT6	0.00	0.00	0.00	0.21	0.00	NA								
7	*Anilany helenae* (Ambohitantely) KAMUT9	0.00	0.00	0.00	0.21	0.00	0.00	NA							
8	*Anilany helenae* (Ankafobe) KAMUS441	0.00	0.00	0.00	0.21	0.00	0.00	0.00	NA						
9	*Anilany helenae* (Ankafobe) KAMUS444	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.00	NA					
10	*Anilany karsticola* sp. n. (Bemaraha) ZSM 21/2006, FGZC 711	2.40	2.07	2.07	2.27	2.07	2.07	2.07	2.07	2.07	NA				
11	*Anilany karsticola* sp. n. (Bemaraha) UADBA-A 25660, FGZC 713	2.63	2.3	2.3	2.51	2.3	2.3	2.3	2.3	2.3	0.21	NA			
12	*Anilany karsticola* sp. n. (Bemaraha) UADBA-A 25663, FGZC 714	2.62	2.26	2.26	2.46	2.26	2.26	2.26	2.26	2.26	0.21	0.00	NA		
13	*Anilany* cf. *helenae* (Mahajanga) UADBA-A-FGZC 5751	0.95	0.82	0.82	1.03	0.82	0.82	0.82	0.82	0.82	1.65	1.88	1.85	NA	
14	*Anilany* cf. *helenae* (Mahajanga) ZSM 240/2018, FGZC 5754	1.26	1.08	1.08	1.08	1.08	1.08	1.08	1.08	1.08	1.94	2.2	2.16	0.22	NA

the sampled *Anilany* populations. Although the mitochondrial divergence is comparatively low, the variation in nuclear-encoded markers is in line with what has been observed in sister species in other cophyline frogs (e.g. *Stumpffia*; Rakotoarison et al. 2017). The population from Bemaraha is phylogenetically sister to the other two sampled populations and is quite distinct from them genetically, without haplotype sharing in the nuclear encoded markers.

Taxonomic conclusion

In light of (a) concordant mitochondrial and nuclear divergence of *Anilany* individuals from Bemaraha compared to other populations, and (b) morphological differences given above with non-overlapping values in body size and relative hindlimb length, and despite the comparatively low mitochondrial divergence, we conclude that the observed pattern is best reflected by recognizing specimens of the Bemaraha population as an undescribed species, for which we here provide a formal taxonomic description. The population from Mahajanga we tentatively consider to represent *A. helenae*. Populations from Anjajavy, Namoroka, and Beanka require detailed future study, but the proximity between the Beanka population and the Bemaraha population make us think these two are likely to be conspecific.

***Anilany karsticola* sp. n.**

(Figs 6, 9)

ZooBank LSID: urn:lsid:zoobank.org:act:E8391FE1-CoE6-421F-84AE-D1A7BCB3DC4B

Remark: This species was previously treated as *Stumpffia* sp. (Glaw et al. 2007), *Stumpffia* sp. aff. *helenae* "Bemaraha" (Glaw & Vences 2007), *Stumpffia* aff. *helenae* (Raselimanana 2008, 2013), *Stumpffia* sp. (aff. *helenae*) (Bora et al. 2010), *Stumpffia* sp. 8 (Wollenberg et al. 2008, Tu et al. 2018), *Stumpffia* sp. Ca14 (Vieites et al. 2009, Perl et al. 2014, Scherz et al. 2016), and *Anilany* sp. Ca14 (Scherz et al. 2016, Belluardo et al. 2022).

Holotype: ZSM 21/2006 (FGZC 711), an adult female, collected in the Grotte Crystal near Andranopasazy (18.7086° S, 44.7189° E), 146 m a.s.l., Tsingy de Bemaraha

Table 5. Uncorrected genetic distances calculated for the alignment of the 16S rRNA 5' terminus (16S5') fragment including 12 individuals of the genus *Anilany*. All values are given in percentage. See Table 1 for more information on the analysed individuals.

		1	2	3	4	5	6	7	8	9	10	11	12
1	*Anilany helenae* (Ambohitantely) KAMU21	NA											
2	*Anilany helenae* (Ambohitantely) ZSM 370/2005, FGZC 2101	0.00	NA										
3	*Anilany karsticola* sp. n. (Bemaraha) ZSM 114/2006, FGZC 899	4.41	4.29	NA									
4	*Anilany karsticola* sp. n. (Bemaraha) ZSM 137/2006, FGZC 941	4.24	4.11	1.53	NA								
5	*Anilany karsticola* sp. n. (Bemaraha) ZSM 21/2006, FGZC 711	4.24	4.11	1.19	0.68	NA							
6	*Anilany karsticola* sp. n. (Bemaraha) ZSM 22/2006, FGZC 712	4.56	4.29	1.75	0.53	0.88	NA						
7	*Anilany karsticola* sp. n. (Bemaraha) UADBA-A 25660, FGZC 713	4.11	4.11	1.61	0.36	0.72	0.54	NA					
8	*Anilany karsticola* sp. n. (Bemaraha) UADBA-A 25663, FGZC 714	4.24	4.11	1.53	0.34	0.68	0.53	0.00	NA				
9	*Anilany* cf. *helenae* (Mahajanga) UADBA-A-FGZC 5751	2.25	2.19	5.36	5.36	5.36	5.37	5.47	5.35	NA			
10	*Anilany* cf. *helenae* (Mahajanga) UADBA-A-FGZC 5752	1.88	1.81	5.15	4.97	4.97	4.96	5.06	4.97	0.34	NA		
11	*Anilany* cf. *helenae* (Mahajanga) ZSM 239/2018, FGZC 5753	2.44	2.21	5.58	5.58	5.58	5.6	5.52	5.57	0.17	0.52	NA	
12	*Anilany* cf. *helenae* (Mahajanga) ZSM 240/2018, FGZC 5754	2.08	2.01	5.2	5.2	5.2	5.56	5.3	5.2	0.87	0.52	1.05	NA

National Park, western Madagascar, on 19 March 2006 by F. Glaw, J. Köhler, P. Bora, and H. Enting.

Paratypes: ZSM 22/2006 (FGZC 712), UADBA-A 25660 (FGZC 713), and UADBA-A 25663 (FGZC 714), three adult females, with the same collection data as the holotype; ZSM 137/2006 (FGZC 941), a presumed subadult, collected at Andafiabe on the Beboka river (18.7842° S, 44.7794° E), 177 m a.s.l., within Tsingy de Bemaraha National Park on 31 March 2006 by F. Glaw, J. Köhler, P. Bora, and H. Enting; ZSM 114/2006 (FGZC 899), a presumed subadult, collected in a small cave near Bendrao (18.7789° S, 44.8783° E), 444 m a.s.l., in the Tsingy de Bemaraha National Park on 29 March 2006 by F. Glaw, J. Köhler, P. Bora, and H. Enting; UADBA-A 17850 (APR 1226), an adult female, collected in a narrow canyon in Andolombazimba south side of Manambolo river (19.1483° S, 44.8283° E), 120 m a.s.l, within Tsingy de Bemaraha National Park on 05 December 2001 by A. P. Raselimanana and H. Rakotondravony; UADBA-A 18751 (APR 00219), a presumed adult female, collected on the ground in a cave at about 80 m from the entrance of the cave in Anjohimbazimba forest (18.6947° S, 44.7152° E), ca 100 m a.s.l., within Tsingy de Bemaraha National Park on 19 February 2001 by A. P. Raselimanana and D. Rakotomalala; UADBA-A 17847 (APR 01040), an adult male, collected whilst calling on a log in Kidroadroa forest on the north side of Manambolo river (19.1332° S, 44.8098° E), ca 130 m a.s.l., within Tsingy de Bemaraha National Park on 23 November 2001 by A. P. Raselimanana and H. Rakotondravony; UADBA-A 17846 (APR 01030), an adult male, collected during a rainy evening, calling on a log lying on tsingy at 50 cm above the ground in Kidroadroa forest on the north side of Manambolo river (19.1332° S, 44.8098° E), ca 120 m a.s.l., within Tsingy de Bemaraha National Park on 22 November 2021; UADBA-A 17849 (APR 01099), an adult male, collected whilst calling on tsingy in a narrow canyon in Kidroadroa forest on the north side of Manambolo river (19.1332° S, 44.8098° E), ca 130 m a.s.l., within Tsingy de Bemaraha National Park on 25 November 2001 by A. P. Raselimanana and H. Rakotondravony.

Diagnosis: The new species is assigned to the genus *Anilany* on the basis of its small size combined with expanded terminal discs and T-shaped terminal phalanges, short parasphenoid, broad, angled postchoanal vomers, absence of a neopalatine, and close genetic affinities with *A. helenae*. It can be distinguished from populations of its only described congener, *A. helenae*, by larger adult body size (adult SVL 15.7–17.4 mm vs. 13.8–15.0 mm Ambohitantely and 12.7 mm Mahajanga) and relative shorter tibia length (TIBL/SVL 0.42–0.46 vs. 0.48 Ambohitantely and 0.47–0.50 Mahajanga). Furthermore, *A. karsticola* sp. n. is distinguished from all other genetic lineages of *Anilany* by the

Table 6. Uncorrected genetic distances calculated for the alignment of the cytochrome oxidase subunit I (COI) gene fragment including 12 individuals of the genus *Anilany*. All values are given in percentage. See Table 1 for more information on the analysed individuals.

		1	2	3	4	5	6	7	8	9	10	11	12
1	*Anilany helenae* (Ambohitantely) KAMUT10	NA											
2	*Anilany helenae* (Ambohitantely) KAMUT22	0.52	NA										
3	*Anilany helenae* (Ambohitantely) KAMUT2	0.00	0.18	NA									
4	*Anilany helenae* (Ambohitantely) KAMUT6	0.17	0.34	0.18	NA								
5	*Anilany helenae* (Ambohitantely) KAMUT9	0.69	0.52	0.35	0.52	NA							
6	*Anilany helenae* (Ambohitantely) KAMU21	0.00	0.52	0.00	0.17	0.69	NA						
7	*Anilany helenae* (Ambohitantely) ZSM 370/2005, FGZC 2101	0.19	0.39	0.20	0.00	0.58	0.19	NA					
8	*Anilany karsticola* sp. n. (Bemaraha) ZSM 21/2006, FGZC 711	3.63	3.81	3.56	3.46	3.98	3.63	3.48	NA				
9	*Anilany karsticola* sp. n. (Bemaraha) UADBA-A 25660, FGZC 713	3.78	3.95	3.71	3.61	4.12	3.78	3.48	0.52	NA			
10	*Anilany karsticola* sp. n. (Bemaraha) UADBA-A 25663, FGZC 714	3.78	3.95	3.71	3.61	4.12	3.78	3.48	0.52	0.00	NA		
11	*Anilany* cf. *helenae* (Mahajanga) UADBA-A-FGZC 5751	1.89	2.06	1.77	1.72	2.23	1.89	1.74	3.11	3.26	3.26	NA	
12	*Anilany* cf. *helenae* (Mahajanga) ZSM 240/2018, FGZC 5754	2.07	2.24	1.95	1.89	2.41	2.07	1.74	3.29	3.44	3.44	0.17	NA

following diagnostic sites in mitochondrial genes used for molecular taxonomic identification (positions relative to the whole respective markers from the mitochondrial genome of *Kaloula borealis*, GenBank reference JQ692869): in COI, 'T' at site 249, 'T' at site 261, and 'C' at site 285; in the 5' fragment of 16S, 'C' at site 352, 'C' at site 576, and 'T' at site 632; and in the 3' fragment of 16S (without statistical support), 'C' at site 1009, 'C' at site 1093, 'T' at site 1177, 'T' at site 1220, 'C' at site 1248.

Although superficially similar, *Anilany karsticola* sp. n. can be distinguished from all nominal *Stumpffia* species, except *S. be*, *S. hara*, *S. staffordi*, and *S. megsoni*, by the possession of expanded terminal discs and T-shaped terminal phalanges. From these species, it can be distinguished with ease by its smaller adult size (maximum SVL 17.4 mm vs. 21.0–27.9 mm), and expanded terminal finger discs being present only on the third and fourth fingers.

Description of the holotype: Specimen in an excellent state of preservation, tongue removed as a DNA sample. Body elongate; head wider than long (HW/HL = 1.50), 33% of body length; snout rounded in dorsal view, slightly pointed in lateral view; nostrils directed laterally, slightly protuberant, further from tip of snout than from eye; canthus rostralis distinct, concave; loreal region slightly concave, vertical; tympanum distinct, 50% of eye diameter; supratympanic fold distinct, running straight over the tympanum, and then diagonally to the anterior of the insertion of the arm; maxillary teeth absent; vomerine teeth absent; choanae oval. Forelimbs slender; subarticular tubercles single, indistinct; outer metacarpal tubercle small and round; inner metacarpal tubercle small and round; hand without webbing; first finger strongly reduced, second finger slightly reduced; relative length of fingers 1<2<4<3, fourth finger distinctly longer than second; tips of third and fourth fingers expanded into discs. Hind limbs robust; tibiotarsal articulation reaching the eye when hind limb adpressed along body; TIBL 42.8% of SVL; lateral metatarsalia strongly connected; inner metatarsal tubercle small, indistinct, oblong; outer metatarsal tubercle absent; no webbing between toes; first toe reduced; toe tips slightly expanded; relative length of toes 1<2<5<3<4; fifth toe distinctly shorter than third; subarticular tubercles indistinct, single. Skin on dorsum smooth, without distinct dorsolateral folds. Ventral skin smooth.

After 10 years in 70% ethanol, the dorsum was light pink. A faint dark chevron was present in the suprascapular region. Large dark spots were present in the inguinal region. The dorsal surface of the head was as the back, with a faint dark interocular bar. The nostrils were brown. The lateral surface of the head was brown. The flanks faded from the dorsal colouration to the cream of the venter. A few additional dark markings were present on the flanks as well. The ventral trunk was posteriorly cream, anteriorly mottled with light brown, fading to brown on the chin. The ventral legs were as the belly. The sole of the foot was light brown inter-

nally, darker externally, with the dark brown forming a heel spot on the shank. Dorsally, the legs were as the dorsum, with two dark crossbands on the dorsal thigh (one much more distinct), three on the shank, and one at the tarsal–metatarsal joint. The foot was dorsally light brown. The toes were mottled light and dark brown, with a fine light stripe before their tips. The cloacal region was dark brown. The arms were as the dorsum. A dark spot was present on the forearm, not forming a crossband. The internal dorsal surface of the hand was light brown, becoming darker laterally. The fingers had small light and dark flecks. The underside of the arm was anteriorly dark brown, continuous with the lateral face, and posteriorly cream. Colour in life as in preservative, but the dorsal base colour was a reddish-brown, and faint small bluish spots were present on the flanks.

Variation: For variation in measurements among specimens, see Table 3. In general, all examined specimens agree strongly with the holotype in morphology. The toe pads of ZSM 22/2006 are slightly more expanded than those of the holotype, while those of the subadult ZSM 137/2006 are less expanded. ZSM 22/2006 is plumper than the other specimens. There is strong sexual dimorphism in crests of the humerus and radio-ulna, as detailed in the Osteology section above. The nostril is approximately equidistant between the nostril and the eye in the paratypes. A high degree of variability was noted in the colouration of individuals (see Fig. 6).

Etymology: The species epithet *karsticola* is a first-declension noun derived from the German word 'Karst' (a craggy limestone formation), and the Latin lexical suffix '-cola' meaning 'inhabitor', in reflection of ecology of this species, which has only been found in and near caves in limestone karst within the Tsingy de Bemaraha National Park. The species name is considered as a noun in apposition.

Natural history: The species inhabits a karstic limestone environment, with all eleven specimens collected near or within caves at Tsingy de Bemaraha National Park. It is quite common in Bemaraha on both sides of Manambolo river. This species was very active after rain in late afternoon and in the evening. Males called sitting on logs or on tsingy rocks at 0.5–1 m above forest floor. Individuals were never seen calling from the leaf litter on the forest floor. The advertisement call of the species remains unrecorded.

Discussion

Most Malagasy microhylids are micro-endemic, as many species are characterized by rather small distribution ranges (Wollenberg et al. 2008, Rakotoarison et al. 2012). Furthermore, it has been shown that range sizes of Malagasy frogs strongly correlate with body size (Brown et al. 2016), that small frog species usually have limited dispersal capacities (Wollenberg et al. 2011), and that smaller frog species tend to have larger genetic divergence between populations (Pabijan et al. 2012). *Anilany* are small ground-dwelling and climbing frogs, with an SVL ranging between 12.7 and 17.4 mm, and it has therefore been expected that they, too, were microendemic to the known area around Ambohitantely in the central highlands. The recent range extension to the Ankafobe Special Reserve 10 km north of Ambohitantely (Mullin et al. 2021) was no major surprise, given the proximity of these sites, though it was welcome news given the dire conservation outlook that the species was thought to be facing.

Here, we have found evidence for a much greater distribution of the genus, spanning multiple habitat types. Ambohitantely Special Reserve, located on the central plateau of Antananarivo (Fig. 1), and the nearby Ankafobe Private Reserve, are mostly covered by grasslands with fragmentary patches of humid montane forest (Goodman et al. 2018, Mullin et al. 2021, Barata et al. 2022). In contrast, the areas of the Tsingy de Bemaraha National Park and Beanka in central-western Madagascar (Fig. 1), is dominated by a 'stone forest' consisting of karstic landscapes, caves, narrow canyons, and limestone cliffs, partly covered by dry and deciduous forest (Goodman et al. 2018); humid habitats within arid environments, which may be key for amphibians. The limestone caves near Mahajanga on the road to the village Betsako were also in a small karst formation and ecologically similar to those in Namoroka and Tsingy de Bemaraha (Fig. 7d). Anjajavy, like Mahajanga, also includes some small karst formations and caves (Fig. 2d). The substantial geographic distance between the known sites hosting such microhabitat, and the large extent of non-suitable intervening matrix in the rather arid western biomes of Madagascar, argue against the occurrence of a single widespread species of miniaturised microhylid frog with genetically cohesive populations in this part of the island.

As expected, given the substantial geographical distances of > 200 km between several populations we analysed, we encountered some degree of differentiation in molecular and morphological characters. But translating the encountered pattern into taxonomy proved to be a complex endeavour. Firstly, despite an intensive examination of osteological and external characters, the only inter-populational morphological differences were the body size and relative tibia length difference of the Bemaraha specimens in comparison to topotypical *A. helenae* from Ambohitantely and Ankafobe. Secondly, the mitochondrial genetic differentiation between populations was surprisingly small, with uncorrected pairwise distances in the 3' fragment of the mitochondrial 16S rRNA not reaching 3% and in COI not reaching 6%, thus remaining below the thresholds usually used for preliminary identification of candidate species in Malagasy frogs (Vieites et al. 2009, Perl et al. 2014). Thirdly, however, the three major mitochondrial clades were congruently diverged also in the two nuclear-encoded markers analysed (BDNF, RAG-1), without any haplotype sharing between lineages. And fourthly, advertisement call recordings are only available from Ambohitantely (Vences et al. 2006) but not from any of the western populations.

Deciding which taxonomic status to assign the various *Anilany* populations ultimately depends on assessing their identity as independent evolutionary lineages. For this, bioacoustic data would be very helpful, as calls typically differ substantially among closely related cophyline species (D'Cruze et al. 2010, Vences et al. 2010, Rakotoarison et al. 2017). Also, restricted hybridization in zones of contact or syntopy could provide conclusive evidence about reproductive isolation (Dufresnes et al. 2021, Vences et al. 2024) but current data are too sparse for such analyses. In a clade of mantellid frogs (*Gephyromantis* subgenus *Phylacomantis*), we have recently argued for a subspecies status of an isolated population related to *G. corvus* in the North West of Madagascar despite high mitochondrial divergence, given mixed evidence from haplotype sharing of nuclear genes and absence of any detectable morphological differentiation (Scherz et al. 2024). In the case of *Anilany*, the concordant mitochondrial and nuclear signals, along with subtle but consistent morphological differentiation, led us to favour the taxonomic scenario hypothesizing that the Bemaraha population is not conspecific with *A. helenae* from its type locality and should thus be considered as the separate species, *A. karsticola*. We will in the following discuss aspects of conservation and biogeography based on this hypothesis. If future studies suggest admixture over a geographically wide hybrid zone, an alternative scenario considering *A. karsticola* as a subspecies may be warranted, but in each of these scenarios, scientifically naming the Bemaraha population highlights its genetic differentiation and makes it accessible as a separate unit for conservation management.

The description of a new *Anilany* species from the Tsingy de Bemaraha National Park enriches the sparse cophyline diversity of western Madagascar by another described species (though previously acknowledged by Bora et al. 2010). Together with *Plethodontohyla fonetana,* they represent the only two cophyline taxa known from Bemaraha (Glaw et al. 2007), but they are not the sole cophylines to inhabit a karstic limestone environment. For instance, the species *Stumpffia be*, *S. hara*, *S. megsoni*, and *S. staffordi* are all affiliated with karst or exposed volcanic rock (Köhler et al. 2010, Rakotoarison et al. 2022). In contrast to their congeners, all four of these *Stumpffia* species exhibit enlarged terminal finger discs and are relatively large-bodied, which led to the conclusion that these morphological features may be related to the occupation of this novel ecological niche (Köhler et al. 2010). This assumption is corroborated by the presence of enlarged finger discs and body-sizes in other cave-dwelling frog species, including *Anilany karsticola*.

The taxonomic identity of the disparate populations of *Anilany* has major implications for the conservation status of the species involved. *Anilany helenae* is considered a Critically Endangered micro-endemic of the highlands of Madagascar. It is undisputable that the highland populations from Ambohitantely and Ankafobe are highly threatened (Vallan 2000b, Goodman et al. 2018, Mullin et al. 2021, 2022b, Barata et al. 2022), but these populations represent only a small part of the genetic diversity of *Anilany,* according to our results. The taxonomic status of this diversity needs careful re-assessment.

The remarkable distribution of these populations over very different habitats spanning an enormous area, compared to relatively low genetic diversity and only little morphological differentiation, complicates taxonomic treatment of the clade. The great geographic distance and the apparent rarity of suitable habitat between the locations where *Anilany* have so far been found may favour/have favoured isolated lineages to become evolutionarily independent units. This, however, could only be confirmed for the Bemaraha population, which can be distinguished by morphological and genetic traits from individuals of the Ambohitantely/Ankafobe and Mahajanga population. Given that *Anilany karsticola* is only known from a few caves located in the Tsingy de Bemaraha National Park, the species might be endemic to this region, which would be in line with the high level of microendemism found in cophyline frogs (Wollenberg et al. 2008). Following the assessment for the Bemaraha-endemic *Plethodontohyla fonetana* (IUCN SSC Amphibian Specialist Group 2016b), the species may qualify as Endangered on the IUCN Red List of Endangered Species.

Assessing the conservation status of *Anilany helenae*, on the other hand, is more difficult, and may have to wait until more data are available from the populations that we have identified here in western and northwestern Madagascar. Based on the low differentiation we recovered between the populations of Ambohitantely and Mahajanga, and the lack of evidence of morphological distinction, we consider these populations likely conspecific. In consequence, the distribution of *A. helenae* sensu lato is already considerably expanded. If this is also true of populations from Namoroka, and Anjajavy, *A. helenae* would occupy the broadest range of any miniaturised frog in Madagascar, increasing the Extent of Occurrence (EOO) from 29.4 km² (IUCN SSC Amphibian Specialist Group 2016a) to > 80,000 km². Individuals of the Beanka Forest population, on the other hand, are probably conspecific with *Anilany karsticola* given their proximity to Tsingy the Bemaraha National Park (Fig. 1).

Although the EOO will capture that there is a strong spread of risk to *A. helenae*, it is mostly comprised of unsuitable habitat, and thus fails to capture the diminutive and declining area of suitable habitat for the species. Calculating the Area of Occupancy (AOO) should therefore be a priority for a revised conservation assessment of the species, but it will be challenging to assess this parameter due to uncertainty about the extent of suitable habitat in Mahajanga and other areas. Several of the known populations are in imminent danger of extirpation; Ambohitantely and Ankafobe have decreased substantially in size in recent years due to fires (KEM pers. obs.). This must be taken into account for a revised assessment, as should the uncertainty of the overall taxonomic conclusions due to pending samples from additional population records.

It is further important to note that many areas in central western Madagascar remain poorly explored, with the inventory of Madagascar's amphibian fauna being far from

complete. It is possible that even more populations of *Anilany* exist in other small, yet-unsurveyed forest fragments across the highlands and western lowlands of Madagascar. Considering the ongoing deforestation of Madagascar's last refugia for biodiversity (Vieilledent et al. 2018), improving the data on spatial occurrences is not only interesting from an evolutionary point of view, but also of paramount importance for appropriate conservation measures to mitigate biodiversity declines, as both Red List assessments and on-the-ground conservation efforts strongly rely on data of geographic occurrences for each species.

Acknowledgements

Fieldwork was carried out in the framework of cooperation accords among the Departément de Biologie Animale, Université d'Antananarivo, the Association Nationale pour la Gestion des Aires Protegées, the Technische Universität Braunschweig, Durrell Wildlife Conservation Trust, Missouri Botanical Gardens, and the Zoologische Staatssammlung München and supported by grants from Global Wildlife Conservation (now Re:wild), chameleon assets, Volkswagenstiftung, Freunde der Zoologischen Staatssammlung München, the Hessisches Landesmuseum Darmstadt, and UKRI NERC-funded GW4+ Doctoral Training Partnership NE/L002434/1 (K. Mullin). We are grateful to J. Gerlach and J. Lips who provided photos of *Anilany* from Anjajavy and Namoroka, and to the following persons for their support and assistance in the field: P. Bora, H. Enting, M. G. Rakotomanga, T. Raditra, D. Rakotomalala, and H. Rakotondravony. Permits for collection and export of specimens were kindly issued by the Ministère des Eaux et Forêts of Madagascar. We further want to thank M. Preick and S. Abelt for the assistance with laboratory work. We thank V. Gorin for reviewing an earlier version of this manuscript. This study was supported by a grant (SCHE 2181/1-1) from the German Research Foundation (DFG) in the framework of the priority program SPP 1991: TAXON-OMICS (447176041). Portuguese National Funds through FCT (Fundação para a Ciência e a Tecnologia) support the research contract to AC [https://doi.org/10.54499/2020.00823.CEECIND/CP1601/CP1649/CT0002].

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