

# Diamond in the rough: a new species of fossorial diamond frog (*Rhombophryne*) from Ranomafana National Park, southeastern Madagascar

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## Abstract

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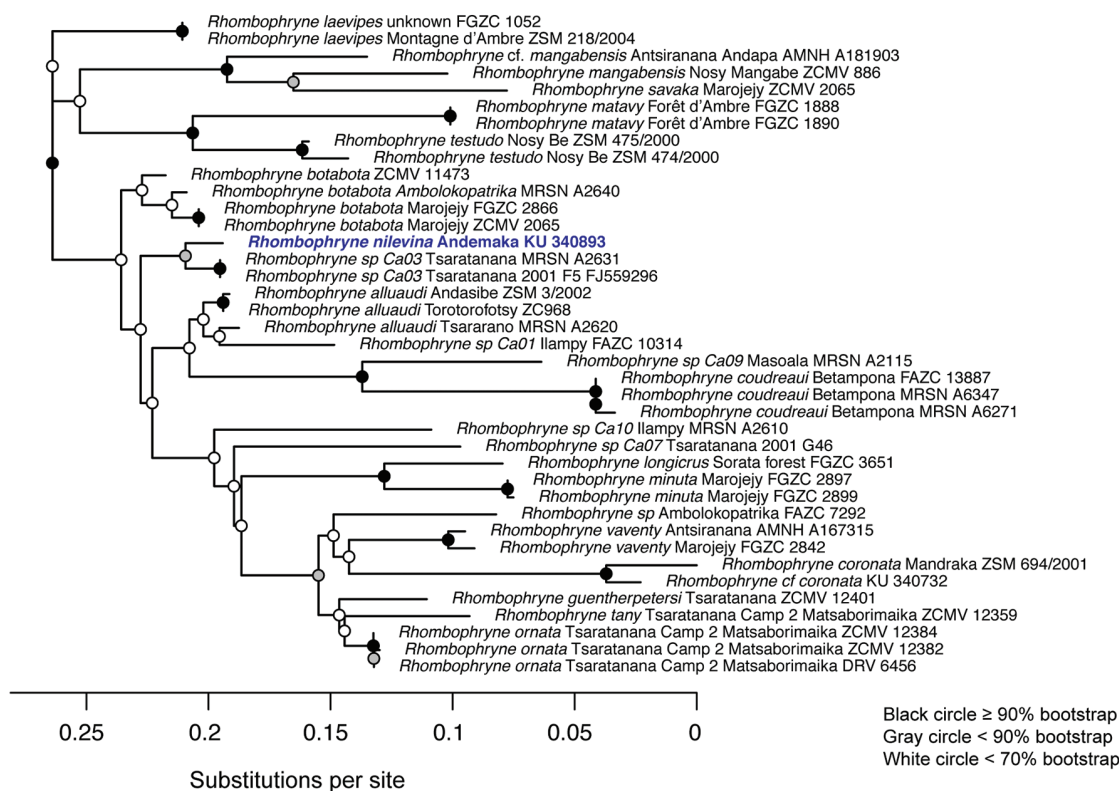
We describe a new species from the cophyline microhylid genus *Rhombophryne*, a group of fossorial and terrestrial frogs endemic to Madagascar. Found during herpetofaunal surveys of moist montane forest in the remote north of Ranomafana National Park, *Rhombophryne nilevina* **sp. n.** exemplifies two difficulties that hinder taxonomic progress in Malagasy cophyline frogs: micro-endemism and highly secretive habits. Known from only two adult male specimens, this new species is nonetheless easily distinguishable from all other known *Rhombophryne* using morphological data, and osteological data collected here via X-ray Micro-Computed Tomography, or “micro-CT”. This species is now the largest known *Rhombophryne*, and the only one known from Ranomafana National Park, which will make it the southern-most member of the genus pending a forthcoming taxonomic revision involving *Plethodontohyla* and *Rhombophryne*. Pairwise distances of the mitochondrial 16S rRNA marker show a minimum genetic distance of 4.9% from other nominal *Rhombophryne*. We also describe recordings of an advertisement call, emitted from a burrow by the holotype. *Rhombophryne nilevina* **sp. n.** is not known to be found syntopically with other *Rhombophryne*, nor to be present elsewhere in Ranomafana National Park, but it probably does co-occur with a few ecologically similar *Plethodontohyla* species. Although the type locality is within a protected area, we suggest an IUCN listing of Data Deficient for *R. nilevina* **sp. n.**, as its area of occupancy is largely undetermined within the park.

## Introduction

Over the past several decades, integrative approaches to taxonomy have shown that Madagascar’s anuran fauna is one of the most spectacular on earth, with current estimates approaching 600 species; 99.9% of which are endemic to the island (reviewed in Vieites et al. 2009, Perl et al. 2014). This estimate continues to rise as more candidate species are newly discovered, with ~465 species estimated in Vieites et al. (2009), and ~530 in Perl et al. (2014). Many recent candidate species have been found from very few localities, and are presumably restricted to small ranges (e.g., Rosa et al. 2014, Hutter et al. 2015). Among clades

of Malagasy frogs, the subfamily Cophylinae Cope, 1889 (family Microhylidae Günther, 1858) faces one of the steepest taxonomic gaps, with more candidate species existing than described species (Vieites et al. 2009, Perl et al. 2014, Scherz et al. 2016a). This phenomenon is likely explicable by the many challenges they present to systematists, including secretive habits, small range sizes, and numerous morphologically cryptic species.

*Rhombophryne* Boettger, 1880 is a particularly enigmatic cophyline genus consisting of 16 valid nominal species (Scherz et al. 2016a,b), found primarily in rainforest habitats of northern and eastern Madagascar. In addition to fossorial or otherwise secretive habits, the apparently



**Figure 1.** Phylogenetic relationships between *Rhombophryne* species estimated using maximum likelihood in RaxML using the mitochondrial 16S rRNA barcode fragment. *Rhombophryne nilevina* sp. n. is highlighted with blue bold text. Outgroups are removed from the tree figure for aesthetic purposes.

small ranges of many species have historically impeded data collection, and thus taxonomic progress, in the genus (Glaw et al. 2010). However, fueled by the application of integrative taxonomic approaches, the number of described *Rhombophryne* has swelled in recent years, with 8 species described since 2010 (D’Cruze et al. 2010, Glaw et al. 2010, Scherz et al. 2014, 2015a,b, 2016b), and only a few already-published candidate species left to be described (Scherz et al. 2016b), although several more have been identified and will be described soon (Fig. 1 and Scherz, Crottini, et al. unpubl. data).

We herein describe *Rhombophryne nilevina* sp. n., discovered during herpetofaunal surveys of moist montane forest in the remote north-western corner of Ranomafana National Park, southeastern Madagascar, in January of 2015. We diagnose *R. nilevina* sp. n. from its congeners using morphological and osteological characters collected with the help of X-ray Micro-Computed Tomography (micro-CT). We also describe the male advertisement call of the new species, which is distinct from all other known vocalizations in the genus.

## Methods

### Specimen collection

We collected specimens during the day through targeted searching, using the advertisement call to locate males. We

euthanized specimens using 20% benzocaine, fixed them in ~10% formalin solution buffered with sodium phosphate to pH 7.0, and transferred them to 70% ethanol for long-term storage after approximately two weeks. We deposited the holotype in the Biodiversity Institute of the University of Kansas (KU) and a paratype in the Mention Zoologie et Biodiversité Animal, Faculté des Sciences, Université d’Antananarivo (formerly Département de Biologie Animale of the Université d’Antananarivo; UADBA).

### DNA barcoding and phylogenetic analysis

Immediately following euthanasia, we removed the tongue and placed it in 95% EtOH. We extracted genomic DNA using standard phenol-chloroform extraction protocol and amplified a fragment of the mitochondrial rRNA marker 16S using a previously published protocol (Hutter et al. 2015). We include a total of seven newly generated 16S sequences in this study; one of *R. nilevina* sp. n., one tentatively assigned to *R. coronata*, and five outgroup sequences (Table 1). We acquired sequences of the same 16S fragment for other *Rhombophryne* from Genbank. Prior to alignment, we removed identical sequences using the “Find Duplicates” option in Geneious version 6 (Kearse et al. 2012). All retained sequences and their accession numbers are listed in Table 1. We aligned sequences with the MAFFT (Katoh and Standley 2013) plugin version 1.3 for Geneious, using the the “E-INS-i” algorithm and otherwise default settings. We inferred phylogenetic re-

**Table 1.** GenBank Accession numbers for all sequences used in phylogenetic analysis. Asterisks indicate newly generated sequences.

Species	Locality	Voucher number	Accession
<i>Platypelis barbouri</i> *	Ambatomandondona	KU 340681	KY288471
<i>Platypelis pollicaris</i> *	Torotorofotsy	KU 340614	KY288472
<i>Platypelis tuberifera</i> *	Vohidrazana	CRH 286	KY288470
<i>Plethodontohyla inguinalis</i> *	Vohidrazana	KU 340642	KY288474
<i>Rhombophryne alluaudi</i>	Andasibe	ZSM 3/2002	DQ019606
<i>Rhombophryne alluaudi</i>	Torotorofotsy	ZCMV 968	EU341105
<i>Rhombophryne alluaudi</i>	Tsararano	MRSN A 2620	AY594105
<i>Rhombophryne cf. coronata</i> *	Vohidrazana	KU 340732	KY288476
<i>Rhombophryne coronata</i>	Mandraka	ZSM 694/2001	EU341103
<i>Rhombophryne coudreaui</i>	Betampona	FAZC 13887	FJ559299
<i>Rhombophryne coudreaui</i>	Betampona	MRSN A 6271	HM364771
<i>Rhombophryne coudreaui</i>	Betampona	MRSN A 6347	HM364772
<i>Rhombophryne guentherpetersi</i>	Tsaratanana	ZCMV 12401	KU937796
<i>Rhombophryne laevipes</i>	Montagne d'Ambre	ZSM 218/2004	EU341104
<i>Rhombophryne laevipes</i>	Montagne d'Ambre	FGZC 1052	KM509189
<i>Rhombophryne longicrus</i>	Sorata forest	FGZC 3651	KR025897
<i>Rhombophryne mangabensis</i>	Nosy Mangabe	ZCMV 886	KU724181
<i>Rhombophryne matavy</i>	Foret'd Ambre	FGZC 1888	FJ559298
<i>Rhombophryne matavy</i>	Foret'd Ambre	FGZC 1890	GU195641
<i>Rhombophryne cf. mangabensis</i>	Antsiranana, Andapa	AMNH 181903	KM509192
<i>Rhombophryne minuta</i>	Marojejy	FGZC 2897	EU341100
<i>Rhombophryne minuta</i>	Marojejy	FGZC 2899	EU341106
<i>Rhombophryne ornata</i>	Tsaratanana Camp Matsaborimaika	DRV 6456	KP895582
<i>Rhombophryne ornata</i>	Tsaratanana Camp Matsaborimaika	ZCMV 12382	KP895583
<i>Rhombophryne ornata</i>	Tsaratanana Camp Matsaborimaika	ZCMV 12384	KP895584
<i>Rhombophryne savaka</i>	Marojejy	ZCMV 2065	KU724176
<i>Rhombophryne serratopalpebrosa</i>	Ambolokopatrika	FAZC 7292	EU341111
<i>Rhombophryne sp. Ca01</i>	Ilampy	FAZC 10314	FJ559295
<i>Rhombophryne sp. Ca03</i>	Tsaratanana	MRSN A 2631	AY594107
<i>Rhombophryne sp. Ca03</i>	Tsaratanana	ZSM 667/2001	FJ559296
<i>Rhombophryne botabota</i>	Ambolokopatrika	MRSN A 2640	AY594104
<i>Rhombophryne botabota</i>	Marojejy	FGZC 2866	EU341102
<i>Rhombophryne botabota</i>	Marojejy	ZCMV 2065	FJ559297
<i>Rhombophryne sp. Ca07</i>	Tsaratanana	2001 G46	EU341108
<i>Rhombophryne sp. Ca09</i>	Masoala	MRSN A 2115	AY594110
<i>Rhombophryne sp. Ca10</i>	Ilampy	MRSN A 2610	AY594111
<i>Rhombophryne nilevina sp. n. *</i>	Andemaka	KU 340893	KY288475
<i>Rhombophryne botabota</i>	Makira	ZCMV 11473	KU724173
<i>Rhombophryne tany</i>	Tsaratanana Camp Matsaborimaika	ZCMV 12359	KP895585
<i>Rhombophryne testudo</i>	Nosy Be	ZSM 474/2000	KC180070
<i>Rhombophryne testudo</i>	Nosy Be	ZSM 475/2000	EU341110
<i>Rhombophryne vaventy</i>	Antsiranana	AMNH A167315	DQ283409
<i>Rhombophryne vaventy</i>	Marojejy	FGZC 2842	EU341107
<i>Scaphiophryne marmorata</i> *	Torotorofotsy	KU 340620	KY288473

relationships with RaxML 8.2.6 (Fig. 1; Stamatakis 2014), using the -f option to search for a maximum likelihood tree and conduct 1000 rapid bootstrap replicates, under the GTR model of sequence evolution and with gamma distributed rate variation. Finally, we calculated raw pairwise genetic distances from the alignment using the dist.dna function of the ape package in R (Table 2, Paradis et al. 2004, R Development Core Team 2016).

### Morphology

We took morphological measurements using a digital caliper to 0.01 mm, rounded to 0.1 mm. We note that only the

holotype was measured, as the paratype was unavailable for study. Measurements follow the standard for this genus and are repeated here verbatim from Scherz et al. (2015b): “SVL (snout–vent length), HW (maximum head width), HL (head length, from the maxillary commissure to the anterior-most point of the mouth), ED (horizontal eye diameter), END (eye–nostril distance), NSD (nostril–snout tip distance), NND (internarial distance), TDH (horizontal tympanum diameter), TDV (vertical tympanum diameter), HAL (hand length, from the metacarpal–radioulnar articulation to the tip of the longest finger), LAL (lower arm length, from the carpal–radioulnar articulation to the

**Table 2.** Raw genetic distances at the 16s rRNA gene fragment between analysed taxa and *Rhombophryne nilevina* sp. n. (KU 340893).

Taxon	Distance
<i>Rhombophryne</i> sp. Ca03 (Tsaratanana)	3.80%
<i>Rhombophryne alluadi</i> (Andasibe, Torotorofotsy, Tsararano)	4.89–5.98%
<i>Rhombophryne botabota</i> (Ambolokopatrika, Marojejy, Makira)	5.98%
<i>Rhombophryne</i> sp. Ca01 (Ilampy)	7.61%
<i>Rhombophryne minuta</i> (Marojejy)	9.78–10.32%
<i>Rhombophryne</i> sp. Ca10 (Ilampy)	10.87%
<i>Rhombophryne tany</i> (Tsaratanana Camp 2 Matsaborimaika)	11.41%
<i>Rhombophryne laevipes</i> (Montagne d’Ambre)	11.41%
<i>Rhombophryne guentherpetersi</i> (Tsaratanana)	12.50%
<i>Rhombophryne vaventy</i> (Antsiranana, Marojejy)	11.96–12.50%
<i>Rhombophryne testudo</i> (Nosy Be)	11.96%
<i>Rhombophryne coronata</i> (Mandraka)	11.96%
<i>Rhombophryne</i> sp. Ca07 (Tsaratanana)	12.50%
<i>Rhombophryne mangabensis</i> (Nosy Mangabe)	13.04%
<i>Rhombophryne</i> sp. “Ambolokopatrika” (Ambolokopatrika)	13.04%
<i>Rhombophryne longicrus</i> (Sorata)	11.96%
<i>Rhombophryne</i> cf. <i>mangabensis</i> (Andapa)	12.50%
<i>Plethodontohyla inguinalis</i> (Vohidrazana)	13.59%
<i>Rhombophryne ornata</i> (Tsaratanana Camp 2 Matsaborimaika)	13.59%
<i>Rhombophryne coudreau</i> (Betampona)	14.13%
<i>Rhombophryne</i> sp. Ca09 (Masoala)	13.59%
<i>Platypelis pollicaris</i> (Torotorofotsy)	15.76%
<i>Rhombophryne</i> cf. <i>coronata</i> (Vohidrazana)	15.22%
<i>Platypelis barbouri</i> (Ambatomandondona)	16.30%
<i>Rhombophryne matavy</i> (Forêt d’Ambre)	19.02%
<i>Platypelis tuberifera</i> (Vohidrazana)	18.48%
<i>Scaphiophryne marmorata</i> (Torotorofotsy)	23.37%

center of the radioulna–humeral articulation), UAL (upper arm length, from the center of the radioulna–humeral articulation to the trunk, measured along the posterior aspect of the arm), FORL (forelimb length, given by the sum of HAL, LAL, and UAL), FOL (foot length, from the tarsal–metatarsal articulation to the tip of the longest toe), TARL (tarsal length, from the tarsal–metatarsal articulation to the tarsal–tibiofibular articulation), FOTL (foot length including tarsus, from the tibiotarsal articulation to the tip of the longest toe, given by the sum of FOL and TARL), TIBL (tibiofibula length), TIBW (tibiofibula width at thickest point, measured in dorsal aspect), THIL (thigh length, from the vent to the femoral–tibiofibular articulation), THIW (thigh width at thickest point, measured in supine position), HIL (hindlimb length, given by the sum FOL, TARL, TIBL, and THIL), IMCL (maximum length of inner metacarpal tubercle), IMTL (maximum length of the inner metatarsal tubercle).” A figure depicting the measurement scheme is presented in Scherz et al. (2015b).

### Osteology

We performed micro-CT scanning on a phoenix|x nanotom m cone-beam scanner (GE Measurement &

Control, Wunstorf, Germany), using a tungsten target and a 0.1 mm Cu filter. We employed settings of 140 kV and 80  $\mu$ A, with a timing of 750 ms, for 2440 projections and a total scan time of 30 minutes. We assembled the scan files in datos|x 2 reconstruct CT software (GE Measurement & Control, Wunstorf, Germany), and imported them as an unsigned 8-bit volume into VG Studio Max 2.2 (Volume Graphics GmbH, Heidelberg, Germany). We used the phong renderer with a custom color palate and rendering curve to register and visualize the scan. Using the built-in function, we took high-resolution screenshots for the production of figures. The osteological information presented is based on volume rendering. Only slightly calcified cartilage can be visualized using micro-CT, so we omit descriptions of the cartilaginous structures of the pectoral girdle (sternal features and most of the suprascapula) and those associated with the skull (the hyoid plate and nasal cartilages in particular). A Digital Imaging and Communications in Medicine (DICOM) stack of the scan files and rotational video produced in VG Studio Max 2.2 are available at the following MorphoSource [http://morpho-source.org/Detail/ProjectDetail/Show/project\\_id/263](http://morpho-source.org/Detail/ProjectDetail/Show/project_id/263)

We exported the volume as an “Analyze Volume” under standard settings in VG Studio Max 2.2, and imported the resulting .hdr file into Amira 6.1 (FEI Visualization Sciences Group, Burlington MA, USA), where a surface model was produced essentially following Ruthensteiner and Heß (2008). This model is embedded in a Suppl. material 1. The model is provided solely for reader comprehension; surface models carry inherent bias due to the manual thresholding are therefore less reliable for osteological description than volume renderings (Scherz et al. in review).

We note that skeletal comparisons to other cophylines are based on largely unpublished micro-CT data produced by MDS, which will be involved in revisions of the genera of this subfamily over the next few years. However, micro-CT-based osteological accounts for *Rhombophryne*, *Stumpffia*, *Anilany*, and *Plethodontohyla* are found in Scherz et al. (2016a) and for *Cophyla* and *Platypelis* in Rakotoarison et al. (2015).

### Bioacoustics

We recorded calls attributed to the holotype on two occasions using an Olympus LS-10 Linear PCM Field Recorder and a Sennheiser K6-ME66 super-cardioid shotgun microphone. The calls were recorded at a sampling rate of 44.1 kHz and 16 bits resolution in WAV format. Recordings were made at mid-day in overcast weather conditions. No precise temperature recordings are available, but we estimate that the ambient temperature was approximately 20° C at the time of recording. We note that the individual was not visible during the recordings, as it was calling from a burrow. We therefore cannot be completely certain that the recordings are of the same individual, however, only a single individual at a time was heard calling from this location, and the collected individual was found with distended vocal sac shortly after

**Table 3.** The advertisement call recorded for *Rhombophryne nilevina* in comparison with that of *R. testudo*. Calls were recorded from males calling during the day that were subsequently collected as vouchers. Note envelope is the ratio of the time of peak amplitude to note duration. Data are the range and then the mean  $\pm$  two standard deviations in parentheses, when appropriate. The call recording of *R. testudo* is from Vences et al. (2006).

Parameters		
Species	<i>R. nilevina</i>	<i>R. testudo</i>
Specimen number	KU 340897	NA
Locality	Ranomafana	Nosy Be
N – calls	7	4
Inter-call interval duration (s)	42.5–99.5 (68.77 $\pm$ 24.0)	5.98–10.1 (8.3 $\pm$ 2.1)
Call duration (ms)	505–544 (536 $\pm$ 1.7)	828–896 (853 $\pm$ 2.9)
Call envelope	0.601–0.787 (0.663 $\pm$ 0.073)	-
Number of amplitude peaks	3–5 (3.4 $\pm$ 0.5)	1
Fundamental frequency (Hz)	236.9–279.9 (261.5 $\pm$ 22.9)	258.4–279.9 (263.8 $\pm$ 10.8)
Dominant frequency throughout call (Hz)	528.3–538.8 (537.9 $\pm$ 9.2)	538.3–555.9 (542.8 $\pm$ 8.8)
Dominant frequency at peak amplitude (Hz)	528.3–538.8 (537.9 $\pm$ 9.2)	581.4–602.9 (586.8 $\pm$ 10.8)
First Harmonic (Hz)	775.2–818.3 (796.7 $\pm$ 17.6)	775.2–796.7 (791.3 $\pm$ 10.8)

the second recordings. Additionally, the measured call parameters from the two occasions are nearly completely overlapping (Fig. 4; Table 3).

We follow Rakotoarison et al. (2015) and define a call as individual temporally distinct segments separated by a return to the background noise between each of these segments. This definition is equivalent to single notes used in other call definitions (Duellman and Trueb 1994; mantelids: Hutter et al. 2015). We define calls as “amplitude modulated” when there are two or more clear amplitude peaks.

Following Rakotoarison et al. (2015) and Hutter and Guayasamin (2015), we report the following call variables: call duration (ms); inter-call interval (s); number of amplitude peaks; note envelope shape (time at peak amplitude / call duration); dominant frequency (Hz), measured throughout call and at peak amplitude; fundamental frequency (Hz); and first harmonic frequency (Hz). Call rate was not calculated because of insufficient sample size. We used Raven Pro 1.4 to measure temporal and spectral call characteristics. Digital recordings are deposited at the University of Kansas Biodiversity Institute digital archive and are available upon request.

### Registration of nomenclature

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that

Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>. The LSID for this publication is: urn:lsid:zoobank.org:pub:ACD2A947-B1B8-4B12-8FDF-1260C94B0AF8. The online version of this work will be archived and made available from the following digital repositories: CLOCKSS and Zenodo.

## Results

We discovered a large-bodied cophyline microhylid frog near Andemaka within Ranomafana National Park in eastern Madagascar. Several obvious differences in morphology exist between the collected specimens and all known described and undescribed cophyline microhylids. Analysis of a fragment of its mitochondrial 16S rRNA gene recovered it with a close relationship to an undescribed population of *Rhombophryne* from northern Madagascar (sp. Ca03 from Vieites et al. 2009; Fig. 1). However, this population is quite distinct from the newly collected frogs morphologically (Scherz et al., unpubl. data). We also note that the 16S tree is largely unresolved, likely due to a limited number of characters it includes. Ongoing multi-locus analyses suggest that *R. nilevina* is quite phylogenetically distinct from all known *Rhombophryne*, including sp. Ca03 (A. Crottini, pers. comm.). Our 16S analysis also shows a minimum genetic distance of 4.9% between our new taxon and all valid, nominal *Rhombophryne* species (Table 2). We therefore describe it as a new species:

### *Rhombophryne nilevina* sp. n.

<http://zoobank.org/DAD2876A-D5C4-4D7B-B712-B22013161FC4>

**Suggested common English name:** The buried diamond frog

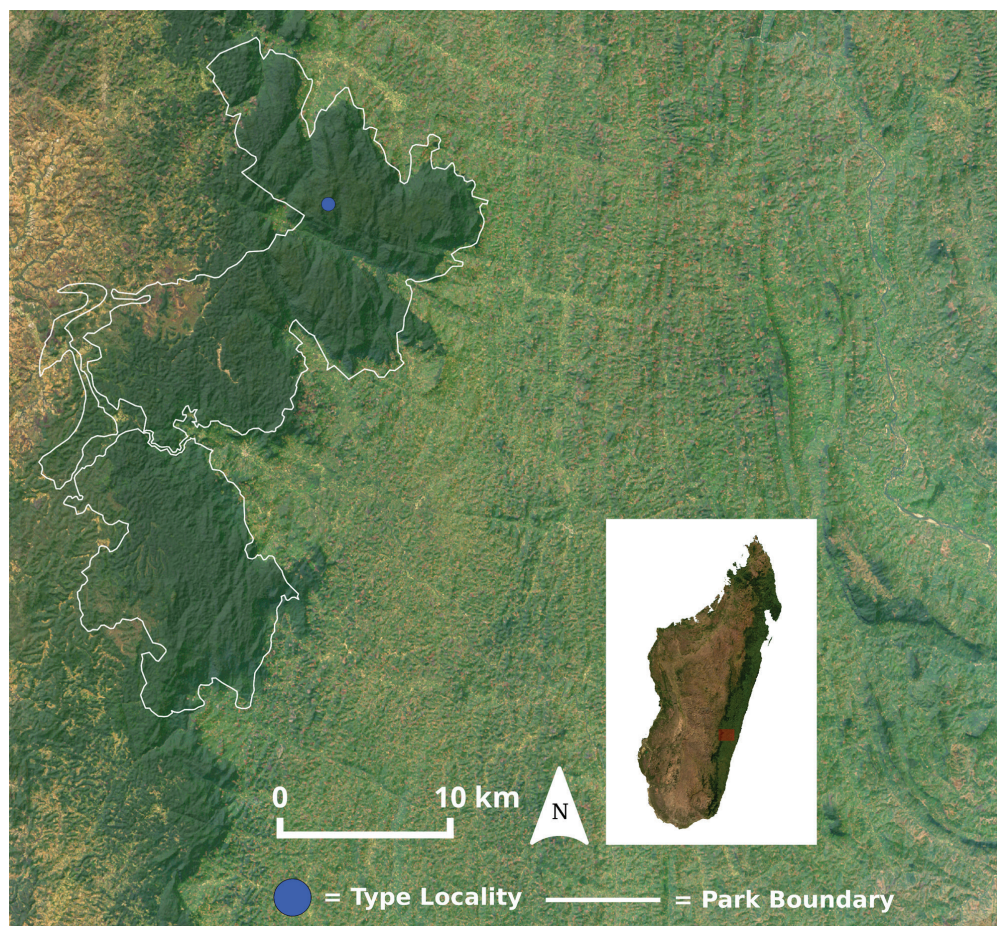
**Suggested common Malagasy name:** Sahona diamondra nilevina

**Suggested common French name:** La grenouille de diamant enterré

**Holotype.** KU 340897 (CRH 798), an adult male collected at mid-day on February 8<sup>th</sup> 2015 by Shea Maddock Lambert, Emile Rajeriarison, and Ralaivao Jean Fulgence in montane rainforest near the former village of Andemaka in Ranomafana National Park (ca. 21.1287°S, 47.5054°E, elevation ca. 1240m a.s.l.; Fig. 2).

**Paratype.** UADBA-A Uncatalogued (CRH 799), an adult male collected the morning of February 7<sup>th</sup> 2015 by Shea Maddock Lambert and Ralaivao Jean Fulgence, otherwise with the same collection information as the holotype.

**Diagnosis.** A frog assigned to the cophyline genus *Rhombophryne* on the basis of its divided vomer, the



**Figure 2.** Map of Ranomafana National Park and the type locality of *Rhombophryne nilevina* sp. n.. Map is a composite of Landsat 8 satellite imagery and a hillshade layer created from SRTM 1 Arc-Second Global digital elevation data. Data available from the U.S. Geological Survey.

possession of clavicles and knob-shaped terminal phalanges (see Scherz et al. 2016a). This species is characterized by the following suite of characters: large size (SVL at least up to 57.2 mm), wide, short head (HW 180.7% of HL), tympanum 58.6% of eye, forelimb 51.1% of SVL, tibia 42.2% of SVL, hindlimb 152.5% of SVL, large inner metacarpal and metatarsal tubercles, supratympanic fold distinct and raised, running from the posterior corner of the eye straight over the tympanum, then sharply down behind it, extending to join the front of the arm, distinct vomerine teeth forming curved rows posteromedial to the oblong choanae, separated medially by a small cleft, second finger shorter than fourth finger, fifth toe distinctly shorter than third, without finger or toe reduction, finger and toe tips not enlarged. Additionally, *R. nilevina* is separated from all nominal species of *Rhombophryne* by an uncorrected pairwise distance of at least 4.9% in the fragment of the 16S rRNA gene, and by at least 3.8% from all known candidate species in this genus.

*Rhombophryne nilevina* is the largest species in the genus *Rhombophryne*, and can be distinguished based on this character alone from all other described species (SVL 57.2 mm vs. maximums of 56.3 mm and 52.9 mm for the next two largest species, *R. laevipes* and *R. vaventy*, re-

spectively). This species differs from all of its congeners as follows: from all members of the *R. serratopalpebroso* group (*R. serratopalpebroso*, *R. coronata*, *R. vaventy*, *R. ornata*, *R. tany*, and *R. guentherpetersi*, plus two species under description by Scherz et al. in review) by the absence of superciliary spines (vs. presence); from *R. testudo*, *R. coudreaui*, and *R. matavy* by less wide head (HW 180.7% vs. 187.6–242.4% of HL in *R. testudo* and *R. matavy*), longer forelimb (FORL 51.1% vs. 35.4–49.8% of SVL), longer hindlimb (HIL 152.5% vs. 117.4–140.8% of SVL), and the possession of a clavicle (vs. lack thereof); from *R. longicrus* and *R. minima* by its wider head (HW 180.7% vs. 122.5–142.8% of HL), shorter forelimb (FORL 51.1% vs. 70.4–74.7% of SVL), and shorter hindlimb (HIL 152.5% vs. 178.5–183.8% of SVL); from *R. savaka* and *R. mangabensis* by its longer forelimb (FORL 51.1% vs. 40.9–47.9% of SVL), well ossified clavicles (vs. poorly ossified), and absence of black inguinal spots and a mid-vomerine diastema (vs. presence in *R. savaka*); and from *R. alluaudi*, *R. laevipes*, and *R. botabota* by its wider head (HW 180.7% vs. 144.2–173.8% of SVL), absence of light dorsolateral stripes (vs. presence in *R. alluaudi*), absence of a stark color border between the dorsal and lateral parts of the head (vs. presence in *R. botabota*),

absence of inguinal ocellations (vs. presence in *R. laevipes* and *R. alluaudi*).

*Rhombophryne nilevina* is morphologically similar to terrestrial members of the genus *Plethodontohyla*, but aside from being distinguishable from this genus by the combination of the possession of clavicles with knob-shaped terminal phalanges, this species can be distinguished from *P. inguinalis* by its smaller size (SVL 57.2 vs. 62.2–99.1 mm), the absence of enlarged fingertips, absence of dark inguinal spots (vs. occasional presence), and absence of a strong dorsolateral color border (vs. occasional presence); from *P. notosticta*, *P. guentheri*, *P. fonetana*, and *P. mihanika* by the absence of enlarged fingertips, absence a strong dorsolateral color border (vs. presence in all but *P. fonetana*), and shorter forelimb (FORL 51.1% vs. 57.5–71.9% of SVL); and from *P. bipunctata*, *P. tuberosa*, *P. brevipes*, and *P. ocellata* by the absence of inguinal spots (vs. presence in all but *P. tuberosa*) and larger size (SVL 57.2 vs. 24.6–44.7 mm) and from *P. tuberosa* by the presence of smooth skin (vs. granular skin).

Although the bioacoustic repertoires of cophylines is far from completely known, bioacoustically, this species' call is strongly distinct from the other known calls by being strongly amplitude modulated (Fig. 4). To the human ear, this call most closely resembles the genetically distant *R. testudo* (Table 2), but the call of *R. testudo* differs by having a much longer duration and lacking significant amplitude modulation (Fig 4). No other known calls can be confused with those of this species.

**Description of the holotype.** *Morphology of the holotype.* An adult male specimen in an excellent state of preservation. The vocal sac is still somewhat loose and malleable. The tongue was removed as a tissue sample.

Body rotund; dorsal and ventral skin smooth, with subtle bumps on the dorsal skin (more rugose in life). Head considerably wider than long (HW 180.7% of HL), snout rounded in dorsal and lateral view; nostrils protuberant, directed laterally, closer to the snout than the eye; canthus rostralis distinct and concave; loreal region concave and oblique; tympanum indistinct, oval, horizontally 58.6% of eye diameter; pupil dilated in preservative but more or less round in life (Fig. 3a, 3d); supratympanic fold distinct and raised, running from the posterior corner of the eye straight over the tympanum, then sharply down behind it, extending to join the front of the arm; tongue removed as a tissue sample, was attached anteriorly and posteriorly free; vomerine teeth distinct, forming curved rows posteromedial to the choanae; choanae relatively large, oblong.

Arms strongly built, relatively short; fingers without webbing, short, with distinct, rounded subarticular tubercles, relative lengths  $1 < 2 < 4 < 3$ , the second finger marginally shorter than the fourth (and marginally longer than the first), without enlarged terminal discs; inner metacarpal tubercle strong, oblong, 28.1% of hand length; outer metacarpal tubercle indistinct, round. Legs relatively long and thick (HIL 152.5% of SVL; TIBL 42.2% of SVL), position of the tibiotarsal articulation when adpressed along the

body not possible to assess without breaking the hindlimbs; toes long, unwebbed, with indistinct round subarticular tubercles, relative toe lengths  $1 < 2 < 5 < 3 < 4$ , third toe distinctly longer than fifth; inner metatarsal tubercle present and distinct, 12.7% of foot length; outer metatarsal tubercle absent.

*Coloration of the holotype.* In preservative, the holotype is chocolate brown dorsally with a loosely reticulated pattern of ebony to burnt umber markings, including an indistinct interocular bar. There are no inguinal spots. The loreal region has a grey marking in it. The forelimb is as the dorsum, with dark patches on the elbow and a crossband on the forearm. A distinct light annulus is present before the terminus of each finger. The hindlimb is dorsally as the back, with three dark crossbands on the thigh and shank. The posterodorsal thigh has weak cream spots, as does the anterior thigh. The dorsal foot is brown speckled with cream. The toes are even more flecked with cream, and also possess a light annulus before the terminal phalanges. The ventral abdomen is brown with numerous small cream flecks. The chin is darker and mostly solid dark brown. The ventral arms are as the trunk. The subarticular and metacarpal tubercles are lighter in color than the rest of the hand. The ventral hindlimbs are as the abdomen. The color in life was as in preservative (Fig. 3).

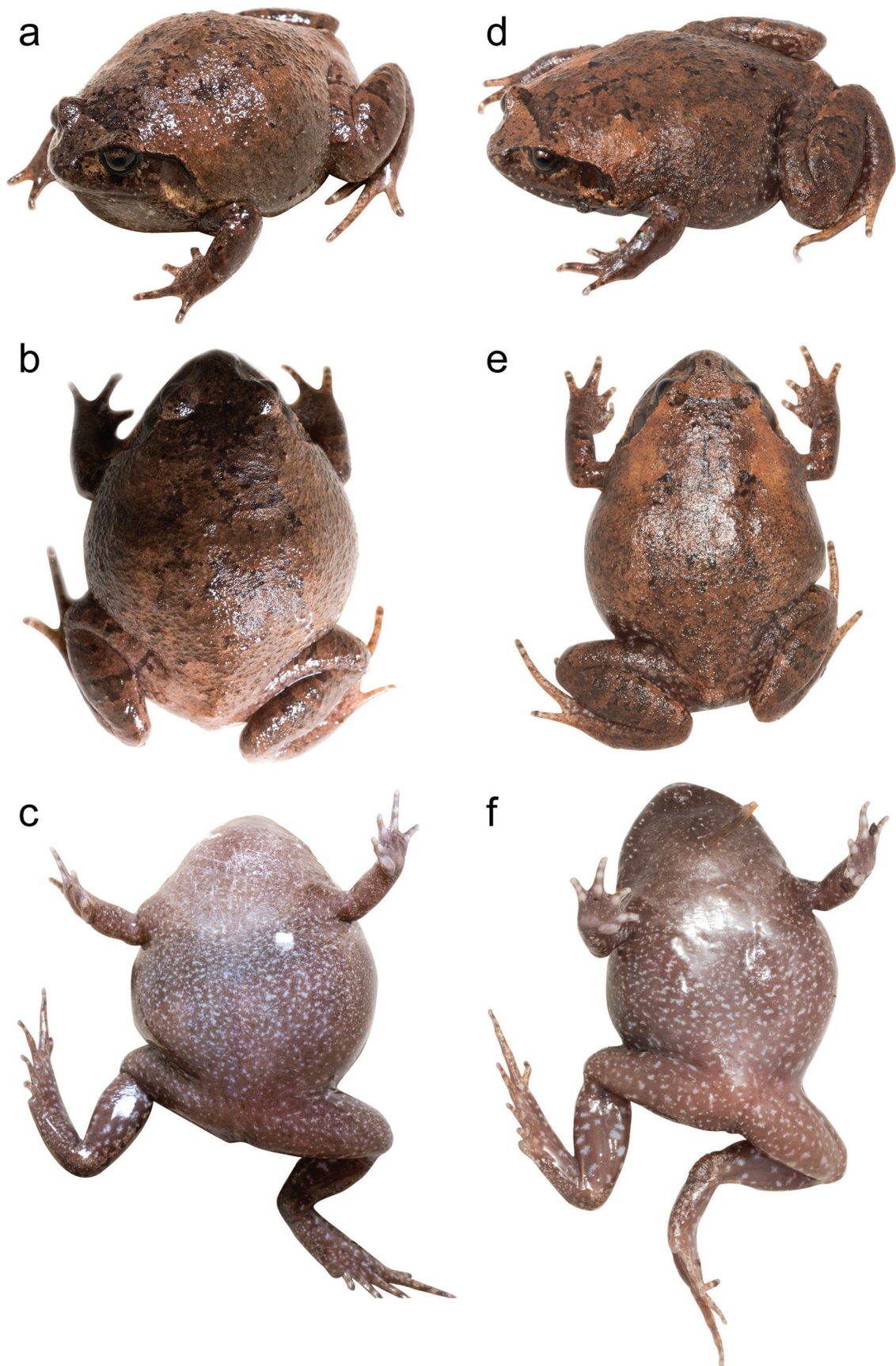
*Osteology of the holotype* (Fig. 5, Suppl. material 1). The skeleton of the holotype is typical of *Rhombophryne*. It is well ossified and robust. The right femur shows signs of an old break toward its distal end that has healed.

Anterior braincase laterally closed by the sphenethmoid. Interior braincase containing calcified material. Nasal in medial contact with contralateral and posterior contact with frontoparietal. Frontoparietal broadening anteriorly from narrow waist anterior to lateral flanges, possessing a strong, posteriorly elongated dorsal process. Prechoanal vomer simple, triradiate. Neopalatine and postchoanal vomer distinguishable. Vomerine teeth not medially fused, without diastemata, oriented oblique to antero-posterior body axis, curved. Maxillary teeth minute. Otic capsule dorsally poorly ossified.

Sternum not ossified. Clavicle robust, curved. Humerus proximally broad, distally rather narrow; possessing a well-developed crista ventralis along roughly 50% of its length; crista lateralis weak. Terminal phalanges of fingers and toes with small distal knobs. Phalangeal formula of fingers 2-2-3-3; of toes 2-2-3-4-3. Femur without cristae. Prepollex strong, blade-like, half length of first metacarpal. Prehallux strong, approximately half length of first metatarsal.

Neural spines decrease in size posteriorly, the sixth and seventh lacking spines altogether. Neural arches of atlas fused. Dorsal crest of urostyle running roughly 80% along its shaft. Iliosacral articulation type IIA sensu Emerson 1979. Iliac shafts with well developed dorsal tubercles and deep oblique grooves; dorsal crests running most of their length. Pubis partially ossified.

**Variation.** The paratype UADBA-A Uncatalogued (CRH 799) strongly resembles the holotype, but has a slightly



**Figure 3.** Photos in life of *Rhombophryne nilevina* sp. n. (a) Dorsolateral view of the holotype (KU 340893). (b) Dorsal view of the holotype. (c) Ventral view of the holotype. (d) Dorsolateral view of the paratype (CRH 799, UADBA-A Uncatalogued). (e) Dorsal view of the paratype. (f) Ventral view of the paratype.



more distinct color border between the lateral and dorsal head (see Fig. 3 for comparison).

**Bioacoustics.** We analysed a total of seven calls from *R. nilevina*, and compared these to the call of *R. testudo* (Fig. 4; Table 3). We presume that the calls we recorded come from one individual, the holotype (see **Materials and methods**). We further assume that the recorded call is an advertisement call, as no other call types (except distress calls) are known from cophylines. This call sounds like a slow groan to the human ear.

Each call is rapidly pulsed, with 3–5 ( $3.5 \pm 0.534$ ) amplitude modulated peaks occurring throughout the call, and peak amplitude occurring in the last 50% of the call. The call duration is 505–544 ( $536 \pm 1.7$ ) ms with an inter-call interval duration of 42.5–99.5 ( $68.8 \pm 24.0$ ) s. The fundamental frequency is 236.9–279.9 ( $261.5 \pm 22.9$ ) Hz. The mean dominant frequency throughout the call was 528.3–555.9 ( $537.9 \pm 9.2$ ) Hz and the first harmonic frequency is 775.2–818.3 ( $796.8 \pm 17.6$ ) Hz (Fig. 4).

**Etymology.** The specific epithet “nilevina” is a Malagasy word meaning “buried.” This name was chosen to recognize the fossorial habits of this species. It is to be treated as an invariable noun in apposition.

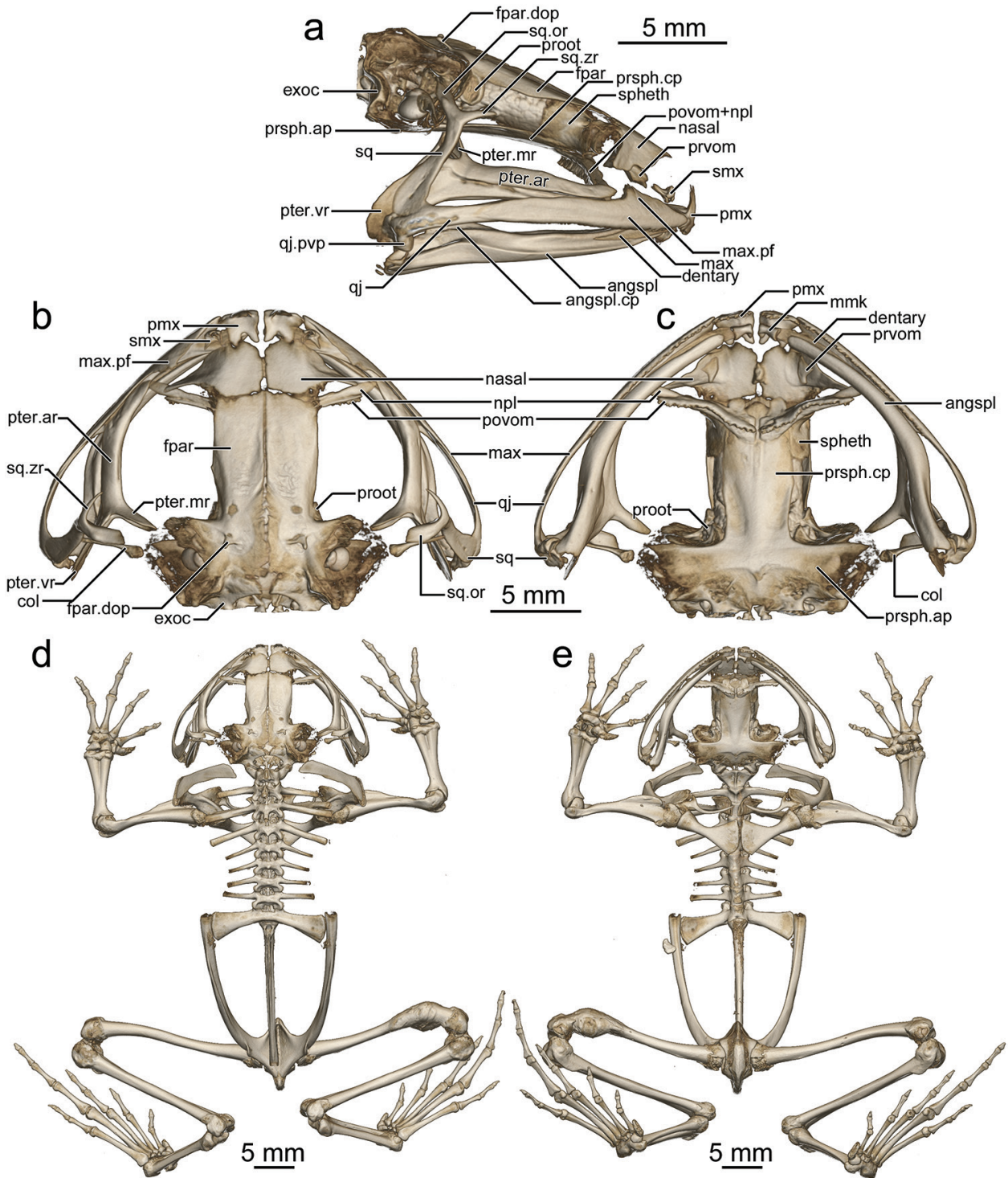
**Available names.** Due to morphological and size similarities, as well as geographic distribution, two existing names must be considered for this species: *Phrynocara laeve* Boettger, 1883, and *Plethodontohyla laevis tsianovohensis* Angel, 1936. Both of these names are currently considered to be junior synonyms of *Rhombophryne alluaudi*. We examined the morphology and osteology of the holotypes of both of these taxa (*P. laeve*: SMF 4286; *P. laevis tsianovohensis*: MNHN 1936.47), and our new species differs critically from both in the possession of a well-developed clavicle (vs. absence/strong reduction; Scherz unpubl. data). Their taxonomy, as well as that of *Rhombophryne alluaudi*, will be discussed in a future article, and we here simply rule out the possibility that they are conspecific with *R. nilevina* sp. n. based on the presence vs. absence of a clavicle. The type specimen of *P. laevis tsianovohensis* was collected from Tsianovoha, which is around 60 km south of Ranomafana, suggesting the possibility of sympatry or parapatry with *R. nilevina*.

**Natural history.** Both known specimens of *R. nilevina* were obtained from a relatively flat, poorly drained section of moist montane forest adjacent to a stream, with the holotype found along the bank of this stream. Nearby habitats include a swamp with many large *Pandanus* and steep forested slopes with relatively smaller trees. However, the calls of *R. nilevina* seemed to emanate mostly from the flatter, forested area. Males were heard calling during the day, particularly during

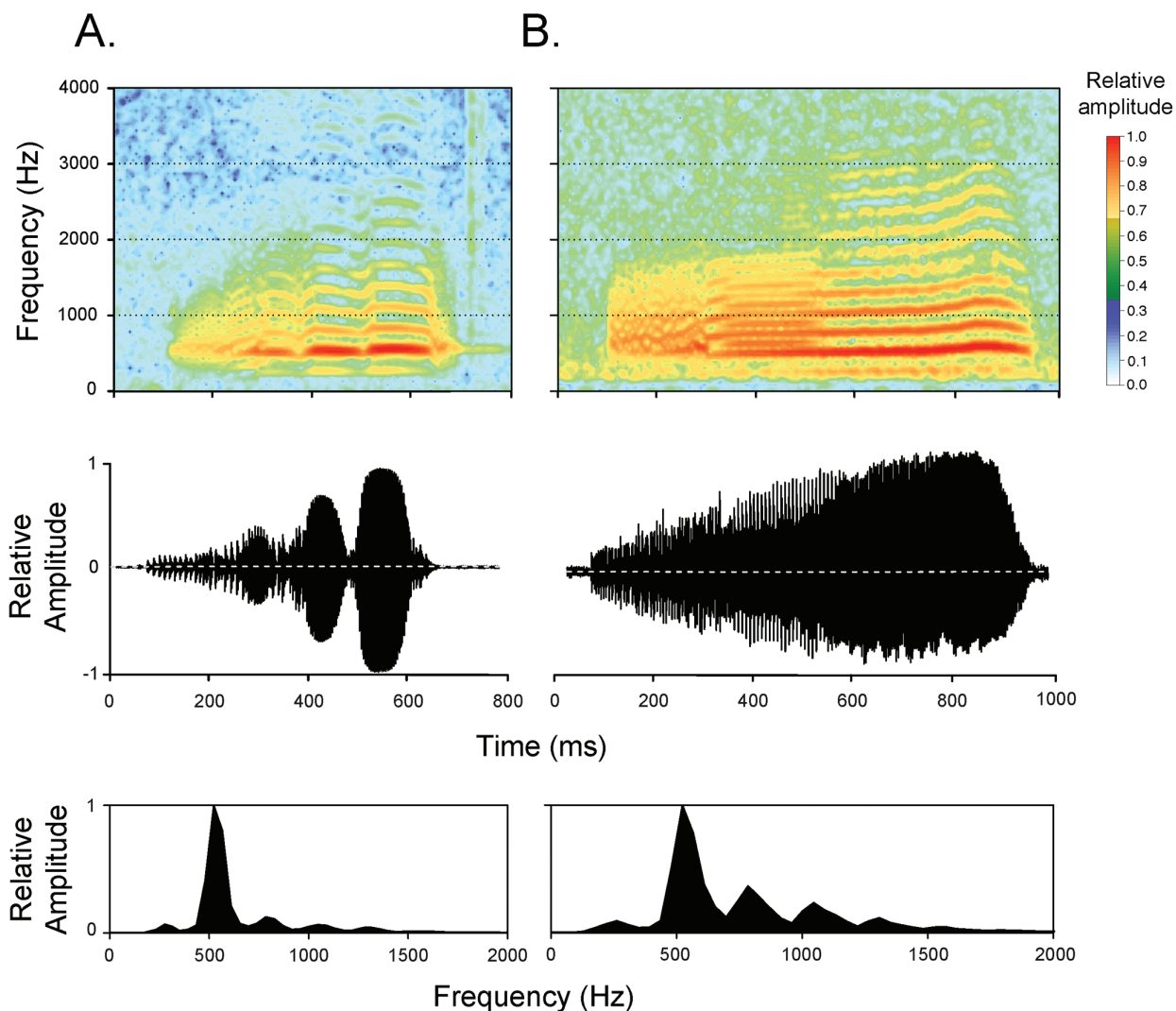
overcast conditions and after rainfall. Advertisement calls were not heard at night, however, the night-time chorus of other frogs, including *Boophis*, *Spinomantis*, *Gephyromantis*, and *Anodonthyla*, may have interfered with detection. When heard from a distance, the call is reminiscent of that of an owl. When heard from close proximity, the call sounds like a groan, and is far less melodic. Both specimens were both located by auditory tracking, and found calling from underground: one from a cavity under the roots of a large tree, and the other from a burrow in soft, moist soil alongside the stream. In order to collect the holotype from its burrow, excavation was required. Based on these observations and suggestive morphology, we presume that *R. nilevina* spend much of their lives underground, possibly coming to the surface for short periods during rainfall, similar to other fossorial *Rhombophryne* species (Glaw and Vences 2007, D’Cruze et al. 2010). We also note that *R. nilevina* was discovered in the middle of the wet season, and after a week-long period of particularly heavy, sustained rain.

**Distribution.** *Rhombophryne nilevina* has thus far been detected at a single site, near the former village of Andemaka, in the north-west of Ranomafana National Park (Fig. 2). This locality is relatively high-elevation for Ranomafana National Park (ca. 1240 m). To our knowledge, *R. nilevina* has not been detected by any previous survey, including several conducted by CRH and SML at similarly high-elevation sites in the northern (Miaramony), central (Vohiparara), and southern (Maharira) regions of Ranomafana. Nevertheless, we do not rule out here the possibility that *R. nilevina* occurs elsewhere in the park. This is in large part due to the secretive habits and potentially ephemeral activity periods of this species (see **Natural history**). In addition, much of the high-elevation forest of Ranomafana is difficult to access and thus remains sparsely or completely unsurveyed for herpetofauna. Although it is possible that *R. nilevina* has been overlooked in other eastern rainforest patches, current information suggests that this species is endemic to Ranomafana National Park, and potentially to a much smaller area within the park.

**Conservation status.** Although the type locality of *R. nilevina* is within Ranomafana National Park, its occupancy within the park is potentially highly restricted, elevationally and geographically, as it has not been detected in any other herpetological surveys of the park. However, its secretive lifestyle means that it could be easily overlooked. Given this large uncertainty in area of occupancy, we suggest an initial IUCN categorization of Data Deficient. If *R. nilevina* is for instance, restricted to the type locality, then habitat destruction, chytrid fungus (recently detected in Madagascar, Bletz et al. 2015), and/or climate change could easily place the only population of *R. nilevina* sp. n. at risk of extinction.



**Figure 4.** The osteology of *Rhombophryne nilevina* sp. n. Skull in (a) lateral, (b) dorsal, and (c) ventral view; and full skeleton in (d) dorsal and (e) ventral view. Abbreviations: angspl, angulosplenia; angspl.cp, angulosplenia coronoid process; col, columella; exoc, exoccipital; fpar, frontoparietal; fpar.dop, frontoparietal dorsal process; max, maxilla; max.pf, maxillary pars fascialis; mmk, mentomeckelian bone; npl, neopalatine; pmx, premaxilla; povom, postschoanal vomer; proot, prootic; prvom, prechoanal vomer; prsph.ap, parasphenoid alary process; prsph.cp, parasphenoid cultriform process; pter.ar, pterygoid anterior ramus; pter.vr, pterygoid ventral ramus; pter.mr, pterygoid medial ramus; qj, quadratojugal; qj.pvp, quadratojugal posteroventral process; smx, septomaxilla; spheth, sphenethmoid; sq, squamosal; sq.or, squamosal otic ramus; sq.zr, squamosal zygotic ramus.



**Figure 5.** Comparative spectrograms (top), oscillograms (center) and power spectra (bottom) between the calls of (A) *Rhombophryne nilevina* sp. n. and (B) *R. testudo* (from Vences et al. 2006). Spectrogram was created using a Hanning window size of 1024.

## Discussion

The discovery of *Rhombophryne nilevina*—never previously identified as a candidate species despite being found in one of the most well-surveyed National Parks of Madagascar—highlights the importance of continued field work for the advancement of systematics in Malagasy anurans. In particular, field surveys should help reveal diversity in clades containing species with small ranges and secretive life histories, including *Rhombophryne* and other cophyline frogs. Cophylines have already shown great promise as a model system for studying ecomorphological and reproductive mode evolution (e.g. Andreone et al. 2005, Wollenberg et al. 2008), and the continued discovery and description of novel species will only further this potential.

*Rhombophryne nilevina* is remarkable in several respects, including its morphology. Most obvious is its large size, the largest recorded for the genus, narrowly exceeding *R. laevipes* (Glaw & Vences, 2007; Scherz et al.

unpubl. data). In addition, the relatively long legs, wide head, and rotund body shape contribute to the distinctive appearance of this species. In total, the morphology of *R. nilevina* is sufficiently divergent from all other *Rhombophryne* species that it cannot be immediately assigned to a complex or species cluster.

In addition to morphological distinctiveness, *Rhombophryne nilevina* is currently the southernmost distributed species of *Rhombophryne*, excluding records of *Rhombophryne alluaudi* from the far south of Madagascar, which are due to confusion surrounding the identity of that species (Scherz, Bellati, Crottini et al. unpubl. data). It also has a strongly amplitude-modulated call unlike that of any congeners (although few call recordings are available for this genus).

Our limited genetic data suggests that *R. nilevina* may have affinities with *Rhombophryne* sp. Ca3 from Tsaratanana in northern Madagascar, but we consider this relationship tentative and ongoing multi-locus analyses suggest that *R. nilevina* represents a relatively ear-

ly-diverging, phylogenetically distinct species of *Rhombophryne* (A. Crottini, pers. comm.). Given the limited information available at this time, the phylogenetic affinities of *R. nilevina* will need to be clarified in a future revision of the genus.

## Acknowledgements

We thank the Malagasy authorities for issuing permits; field research was conducted under permit number 303/14/MEF/SG/DGF/DCB.SAP/SCB; specimens were exported under 017N-EV01/MG14. We also thank MICET and Centre ValBio for facilitating fieldwork. Finally, SML would like to thank Ralaivao Jean Fulgence and Emile Rajeriarison for their exceptional work in the field during the Andemaka expedition. If not for their dedication and ability, *R. nilevina* would surely remain undiscovered.

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## Supplementary material 1

### File S1

Authors: Shea M. Lambert, Carl R. Hutter, Mark D. Scherz

Data type: Adobe PDF file

Explanation note: This file contains a PDF-embedded interactive 3D model of the skeleton of the holotype of *Rhombophryne nilevina* sp. n., KU 340897, generated via X-ray micro-Computed Tomography. The model can be opened in Adobe® Acrobat Pro or Reader, versions IX and above. To activate it, click the image.

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