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Two new species of terrestrial microhylid frogs (Microhylidae: Cophylinae: *Rhombophryne*) from northeastern Madagascar

Mark D. Scherz[1], Frank Glaw[1], Miguel Vences[2], Franco Andreone[3] & Angelica Crottini[4]

[1)] Zoologische Staatssammlung München (ZSM-SNSB), Münchhausenstr. 21, 81247 München, Germany
[2)] Zoologisches Institut, Technische Universität Braunschweig, Mendelssohnstr. 4, 38106 Braunschweig, Germany
[3)] Museo Regionale di Scienze Naturali, Via G. Giolitti, 36, 10123 Torino, Italy
[4)] CIBIO, Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, Nº 7, 4485-661 Vairão, Portugal

Corresponding author: Mark D. Scherz, e-mail: mark.scherz@gmail.com

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Abstract. We describe two new microhylid frog species of the genus *Rhombophryne* from the humid forests of northeastern Madagascar: *Rhombophryne botabota* sp. n. and *R. savaka* sp. n. The former is a medium-sized species, characterised by darkened lateral sides of the head (present in only one other congener, *R. laevipes*) and a unique combination of morphological, osteological, and molecular characters. The latter is a rather small species, characterised by medially undivided vomerine teeth with two large lateral diastemata, and presence of inguinal spots. *Rhombophryne savaka* sp. n. is the first species of the genus known from Makira Natural Park, and is reported also from Marojejy National Park and Ambolokopatrika (Betaolana Forest). Although its distribution range is relatively large compared to those of congeners, its known extent of occurrence is less than 2,000 km². As deforestation and habitat degradation persist as threats despite formal legal protection, we suggest an IUCN Red List status of Vulnerable for this species. *Rhombophryne savaka* sp. n. is possibly endemic to the Marojejy National Park, like several other *Rhombophryne* species, but is unusual in being found at a relatively low altitude. As such, it is likely to be at high risk of habitat loss and decreasing range, and we propose a status of Endangered for it. We discuss the affinities of these new species and the variability of calls in this genus.

Key words. Amphibia, Anura, bioacoustics, Makira Natural Park, Marojejy National Park, Ambolokopatrika, *Rhombophryne mangabensis*, *R. alluaudi*, new species, COMATSA.

Introduction

Madagascar's diamond frogs, genus *Rhombophryne* Boettger, 1880 (Microhylidae: Cophylinae), consist of 14 currently valid nominal species (AmphibiaWeb 2016, Scherz et al. 2015a). They were recently recognized as having a high proportion of undescribed diversity, with more candidate species than named species (Wollenberg et al. 2008, Vieites et al. 2009, Perl et al. 2014). We have worked on reducing this taxonomic gap, both to improve our understanding of this genus and to improve the conservation prospects of its species. Seven new species of *Rhombophryne* have been described since the year 2000, but several new ones are still awaiting formal description.

We here describe two new species from northeastern Madagascar. The phylogenetic position of one of these has already been resolved in a previous study based on a multigene dataset of DNA sequences (Scherz et al. in press); we here provide an assessment of the genetic variation of these two new species based on the mitochondrial 16S rRNA gene fragment, which is traditionally used for the taxonomic identity assessment of Malagasy amphibians. X-ray Micro-Computed Tomography (micro-CT) was used to aid in identifying differences between the new species and their congeners.

Materials and methods

Individuals were collected by targeting calling specimens and by pitfall trapping. They were euthanised by immersion in 0.5% MS 222, fixed in 90% ethanol or 10% buffered formalin, and subsequently transferred to 70% ethanol for permanent storage. ZCMV and MVTIS refer to the zoo-

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logical collection of Miguel Vences and FGZC to that of Frank Glaw, while FAZC and FN refer to the zoological collection of Franco Andreone. Specimens were deposited in the herpetological collections of the Université d'Antananarivo Département de Biologie Animale (UADBA), the Zoologische Staatssammlung München (ZSM), and the Museo Regionale di Scienze Naturali, Torino (MRSN).

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Sound recordings were made on a Tascam DR07 digital field recorder with its built-in microphone, sampled at 44.1 kHz, and saved at 24-bit uncompressed resolution. Audio files were processed and analysed in Audacity® 2.1.0 (Audacity Team 2014), including noise reduction and manual silencing of noisy segments. Frequency information was obtained through Fast Fourier Transformation (FFT; width 512 points). We define 'core call duration' as the duration of the main peak in amplitude of a call to the exclusion of a trailing tail. Figures were produced in R (R Core Team 2014) using the spectro() function in the Seewave package (Sueur et al. 2008). We follow Glaw & Vences (1994) and Rakotoarison et al. (2015) in considering a 'call' of these frogs to consist of a single note, and will use the terms 'inter-call interval' and 'call duration' accordingly. All times were rounded to the nearest ms, and frequencies to the nearest Hz.

Morphometric data was measured using a digital caliper accurate to 0.01 mm, rounded to the nearest 0.1 mm. Morphometric ratios were calculated prior to rounding. We measured the following characters: SVL (snout–vent length), HW (maximum head width), HL (head length, from the maxillary commissure to the snout tip along the jaw), 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 metacarpal–radioulnar articulation to the radioulnar–humeral articulation), UAL (upper arm length, from the radioulnar–humeral articulation to the trunk, measured along the posterior aspect of the arm), FORL (forelimb length as the sum of HAL + LAL + 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 as the sum of FOL + TARL), TIBL (tibiofibula length), TIBW (tibiofibula width), THIL (thigh length, from the vent to the femoral-tibiofibular articulation), THIW (thigh width at thickest point, measured in supine position), HIL (hind limb length as the sum of FOL + TARL + TIBL + THIL), IMCL (maximum length of inner metacarpal tubercle), IMTL (maximum length of the inner metatarsal tubercle). See Scherz et al. (2015b) for a figure showing this measurement scheme.

Micro-CT scanning was conducted using a phoenix nanotom m (GE Measurement & Control, Wunstorf, Germany). Specimens were mounted on a polystyrene baseplate using wooden struts, and placed inside a polyethylene vessel. Scans were conducted at a voltage of 140 kV and current of 80 mA, for 2,440 projections over 20 minutes. Volumes initially rendered in phoenix|x reconstruction software (GE Measurement & Control) were examined in VG Studio Max 2.2 (Volume Graphics GMBH, Heidelberg, Germany). Surface models were produced in Amira 6.0. Our osteological terminology follows Trueb (1968, 1973). Supplementary PDF-embedded 3D models were constructed using the Adobe® 3D Toolkit and Adobe® Acrobat X Pro. Micro-CT does not render cartilage, and cartilage structures are therefore omitted from our osteological descriptions.

Four samples of *R. botabota* sp. n., one sample of *R. alluaudi* (Mocquard, 1901), two samples of *R. savaka* sp. n., one sample of *Rhombophryne* sp. from Sorata, four samples of *R. mangabensis* Glaw, Köhler & Vences, 2010, and one of *R. guentherpetersi* (Guibé, 1974) were newly analysed genetically for this study. Toe clippings or thigh muscle were collected as tissue samples. Total genomic DNA was extracted from the tissue samples by applying proteinase K digestion (10 mg/ml concentration) followed by a standard salt extraction protocol (Bruford et al. 1992). We sequenced a fragment of ca 550 bp of the 3' terminus of the mitochondrial rrnL (large ribosomal RNA, or 16S rRNA gene). For primers used and cycling protocols applied, see Crottini et al. (2011). Standard polymerase chain reactions were performed in a final volume of 11 µl and using 0.3 µl each of 10 pmol primer, 0.25 µl of total dNTP 10 mM (Promega, Fitchburg, WI, USA), 0.08 µl of 5 U/ml GoTaq, and 2.5 µl 5X Green GoTaq Reaction Buffer (Promega). Successfully amplified PCR products, treated with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) to inactivate remaining primers and dNTPs, were directly used for the cycle sequencing reaction, using dye-labelled terminators (Applied Biosystems, Foster City, CA, USA) with the amplification primers. Labelled fragments were analysed on an ABI 3130 automated DNA sequencer (Applied Biosystems). Sequences were compared with GenBank sequences and chromatographs were visually checked and edited, when necessary, using CodonCode Aligner 3.7.1 (CodonCode Corporation, Centerville, MA, USA). This alignment required the inclusion of gaps to account for indels in only a few cases. For GenBank accession numbers of the sequences used, see Table 1.

Molecular analyses included one sequence of each described *Rhombophryne* species, plus all sequences available to us of *R. botabota* sp. n., *R. savaka* sp. n., and their closest relatives, *R. alluaudi* and *R. mangabensis* (see Table 1). We also included one sequence each of two unde-

Table 1. List of samples included in the present study for molecular analyses (ID), species identification, localities, GenBank accession numbers.

ID	Species	Locality	16S
MRSN A2620	*R. alluaudi*	Tsararano	AY594105
ZSM 3/2002	*R. alluaudi*	Andasibe	DQ019606
ZCMV 2209	*R. alluaudi*	Andasibe	KU724170
ZCMV 968	*R. alluaudi*	Torotorofotsy	EU341105
FGZC 3631	*R.* sp.	Sorata	KU724171
FGZC 3651	*R. longicrus*	Sorata	KR025897
DRV 5836	*R. botabota*	Makira	KU724172
ZCMV 11473	*R. botabota*	Makira	KU724173
ZCMV 11474	*R. botabota*	Makira	KU724174
FGZC 2896	*R. botabota*	Marojejy	FJ559297
MRSN A2640	*R. botabota*	Ambolokopatrika	AY594104
MRSN A2956	*R. botabota*	Ambolokopatrika	KU724175
ZCMV 2065	*R. savaka*	Marojejy	KU724176
ZCMV 2079	*R. savaka*	Marojejy	KU724177
ZSM 694/2001	*R. coronata*	Mandraka	EU341103
FAZC 13887	*R. coudreaui*	Betampona	FJ559299
DRV 6220	*R. guenther-petersi*	Tsaratanana	KU724178
FGZC 423	*R. laevipes*	Montagne d'Ambre	EU341104
ZCMV 886	*R. mangabensis*	Nosy Mangabe	EU341109
FGZC 1888	*R. matavy*	Forêt d'Ambre	J559298
FGZC 2899	*R. minuta*	Marojejy	EU341106
ZCMV 12384	*R. ornata*	Tsaratanana	KP895584
FAZC 7292	*R.* sp.	Ambolokopatrika	EU341111
ZCMV 12359	*R. tany*	Tsaratanana	KP895585
ZSM 475/2000	*R. testudo*	Nosy Be	AY594125
FGZC 2842	*R. vaventy*	Marojejy	EU341107
MVTIS 2001/G54	*S. psologlossa*	Benavony	EU341066
ZCMV 2118	*R. mangabensis*	Nosy Mangabe	KU724179
ZCMV 2119	*R. mangabensis*	Nosy Mangabe	KU724180
ZCMV 886	*R. mangabensis*	Nosy Mangabe	KU724181
ZCMV 891	*R. mangabensis*	Nosy Mangabe	KU724182

scribed species from Ambolokopatrika and Sorata, respectively, in order to confirm whether these new species are different from the ones we describe here. A homologous 16S rRNA gene sequence of *Stumpffia psologlossa* Boettger, 1881 from Benavony was added to the 16S gene fragment alignment for outgroup rooting in the phylogenetic analyses. The purpose of the presented phylogenetic analyses is to show the closest relationships of the two new species to *R. mangabensis* and *R. alluaudi*, rather than provide a strongly supported phylogenetic hypothesis of the relationships between all *Rhombophryne* species.

Uncorrected pairwise distances (p-distance transformed into percentage) between individuals of the same species and between analysed *Rhombophryne* species and *S. psologlossa* were computed using MEGA 6.06 (Tamura et al. 2013) (see Table 2).

Bayesian inference searches of the mitochondrial 16S rRNA gene fragment (Fig. 1) were conducted in MrBayes 3.2.1 (Ronquist et al. 2012). The simple JC model of substitution was used because this non-overparametrised model produced a more realistic topology for this short gene segment. We performed two runs of 10 million generations (started on random trees) and four incrementally heated Markov chains (using default heating values), sampling the Markov chains at intervals of 1000 generations. Stabilization and convergence of likelihood values were checked by visualizing the log likelihoods associated with the posterior distribution of trees in the software Tracer (Rambaut & Drummond 2007), and occurred after about 3.5–4 million generations. The first four million generations were therefore discarded, and six million trees were retained post burn-in and summarized to generate a majority-rule consensus tree.

Results

Two candidate species of *Rhombophryne* have been recognized from Marojejy, Ambolokopatrika, and Makira based on DNA sequence data (Vieites et al. 2009, Perl et al. 2014): *R.* sp. Ca2 and *R.* sp. Ca4. These taxa are superficially similar in external appearance, and thus far they have had an unstable phylogenetic position, mostly due to the lack of a robust phylogeny of the genus. However, based on our newly obtained DNA sequence data, we were able to correct previous sequences and provide a new hypothesis on the position of these candidate taxa (see Fig. 1). The two Bayesian analyses resulted in identical trees. *Rhombophryne* sp. Ca2, hereinafter referred to as *R. botabota* sp. n., was recovered as a member of the *R. alluaudi* complex (posterior probability 0.99), and *R.* sp. Ca4, hereinafter called *R. savaka* sp. n., was recovered as the sister species of *R. mangabensis* (posterior probability 1.00). Our data also reveal genetic differentiation between the populations of *R. botabota* from Makira and Marojejy + Ambolokopatrika (see Fig. 1), with an intraspecific mean uncorrected p-distance of 1.2% (Table 2). At interspecific level, the lowest mean uncorrected p-distances were observed between *R. botabota* sp. n. and *R. alluaudi* (3.6%) and between *R. savaka* sp. n. and *R. mangabensis* (8.4%); the highest mean uncorrected p-distances were observed between *R. botabota* sp. n. and *R. savaka* sp. n. versus *R. matavy* D'Cruze, Köhler, Vences & Glaw, 2010 (10.7%, 13.9%, respectively) (for intraspecific comparisons and comparisons with other *Rhombophryne* species, see Table 2).

In conclusion, the two new species, *R. botabota* sp. n. and *R. savaka* sp. n., are genetically distinct from all congeners (mitochondrial genetic differentiation ≥ 3.6%; see Fig. 1 and Table 2). This genetic distinction is corroborated by osteological and morphological differences, and we here provide their formal taxonomic description.

Table 2. Within- (bold) and among-species genetic divergence values of the analysed 16S rRNA mitochondrial gene fragment, based on uncorrected pairwise-distance for the analysed species of the genus *Rhombophryne* and *Stumpffia psologlossa*.

	R. alluaudi	*R.* sp. Sorata	*R. longicrus*	*R. botabota* sp. n.	*R. savaka* sp. n.	*R. coronata*	*R. coudreaui*	*R. guentherpetersi*	*R. laevipes*	*R. mangabensis*	*R. matavy*	*R. minuta*	*R. ornata*	*R.* sp. Ambolokopatrika	*R. tany*	*R. testudo*	*R. vaventy*	*S. psologlossa*
R. alluaudi	1.2%																	
R. sp. *Sorata*	9.2%	–																
R. longicrus	8.2%	10.8%	–															
R. botabota sp. n.	3.6%	9.6%	8.6%	**1.2%**														
R. savaka sp. n.	10.0%	12.9%	13.5%	9.0%	**0.0%**													
R. coronata	9.2%	9.2%	11.5%	10.3%	12.0%	–												
R. coudreaui	9.0%	10.9%	11.6%	8.9%	12.8%	11.9%	–											
R. guentherpetersi	7.3%	5.6%	8.2%	7.1%	11.3%	8.2%	10.6%	–										
R. laevipes	6.8%	9.4%	9.3%	5.8%	10.9%	10.8%	11.7%	8.9%	–									
R. mangabensis	10.5%	13.6%	13.1%	10.1%	8.4%	13.8%	13.6%	12.9%	11.1%	**0.0%**								
R. matavy	10.2%	13.1%	10.5%	10.7%	13.9%	12.2%	13.9%	12.2%	9.5%	14.1%	–							
R. minuta	9.5%	9.1%	7.1%	8.7%	11.8%	10.6%	12.2%	9.4%	10.3%	11.9%	12.2%	–						
R. ornata	7.1%	5.8%	8.8%	6.3%	10.5%	9.1%	10.6%	2.2%	8.4%	12.3%	10.6%	9.6%	–					
R. sp. *Ambolokopatrika*	8.3%	6.1%	9.7%	7.8%	13.1%	8.3%	11.4%	6.1%	9.3%	13.2%	12.0%	10.3%	6.1%	–				
R. tany	7.8%	5.8%	10.0%	7.2%	11.3%	8.6%	10.7%	4.2%	9.9%	12.4%	12.1%	9.5%	3.8%	6.7%	–			
R. testudo	8.2%	11.4%	11.2%	8.5%	10.8%	11.7%	13.3%	10.8%	7.3%	11.2%	8.5%	10.9%	9.8%	11.3%	10.3%	–		
R. vaventy	8.1%	6.6%	10.3%	7.8%	12.6%	9.1%	11.0%	4.7%	8.4%	13.4%	12.0%	11.2%	4.2%	6.2%	6.6%	10.9%	–	
S. psologlossa	11.7%	12.3%	12.4%	10.8%	14.0%	11.8%	12.3%	10.5%	12.2%	13.8%	12.7%	12.6%	12.0%	11.0%	10.9%	13.9%	12.5%	–

***Rhombophryne botabota* sp. n.**
(Figs 1–5)

ZooBank LSID: urn:lsid:zoobank.org:act:B95E889C-C2E2-4362-B20C-37E8C7999A81
Suggested common name: Chubby diamond frog

Holotype: ZSM 358/2005 (FGZC 2896), an adult male collected in Marojejy National Park ('Camp Simpona'), 14.4364° S, 49.7433° E, 1,326 m above sea level (a.s.l.), Sava Region, northeastern Madagascar, on 18 February 2005 by F. Glaw, M. Vences, and R. D. Randrianiaina.

Paratypes: ZSM 538/2009 (ZCMV 11473), ZSM 539/2009 (ZCMV 11474), two presumably immature males collected at Angozongahy, on the western side of the Makira plateau (Camp 1), 15.4370° S, 49.1186° E, 1,009 m a.s.l., Analanjirofo Region, northeastern Madagascar, on 21 June 2009 by M. Vences, D. R. Vieites, F. Ratsoavina, R. Randrianiaina, E. Rajeriarison, T. Rajofiarison, and J. Patton; MRSN A2956 (FN 7164), A2640 (FN 7238), A2954 (FN 7281), A2955 (FN 7300), two males and two females collected at a site known as 'Andranomadio' on the Ambolokopatrika/Betaolana ridge which connects the massifs of Marojejy and Anjanaharibe-Sud (Andreone et al. 2000, Rakotomalala & Raselimanana 2003), 14.5304° S, 49.4383° E, 860 m a.s.l., Sava Region, northeastern Madagascar, on 9–15 December 1997 by F. Andreone, G. Aprea, and J. E. Randrianirina.

Remark: This species was included in the phylogenies produced by Vieites et al. (2009) and Perl et al. (2014) as *R.* sp. 2 (EU341102) and *R.* sp. Ca2 (KF611585), respectively. The GenBank accession number erroneously given in the Supplementary Information of Vieites et al. (2009) for *R.* sp. 4 (FJ559297) also referred to *R. botabota* (FGZC 2896) and the sequence of *R.* sp. Ca4 in Scherz et al. (2015b) therefore represents *R. botabota* as well.

Diagnosis: A Malagasy microhylid frog assigned to the genus *Rhombophryne* on the basis of its possessing clavicles coupled with knobbed rather than Y-shaped terminal phalanges (Scherz et al. in press) and phylogenetic position based on our analysis of the 16S rRNA gene. *Rhombophryne botabota* sp. n. is characterised by the following combination of features: medium size (SVL 24.2–

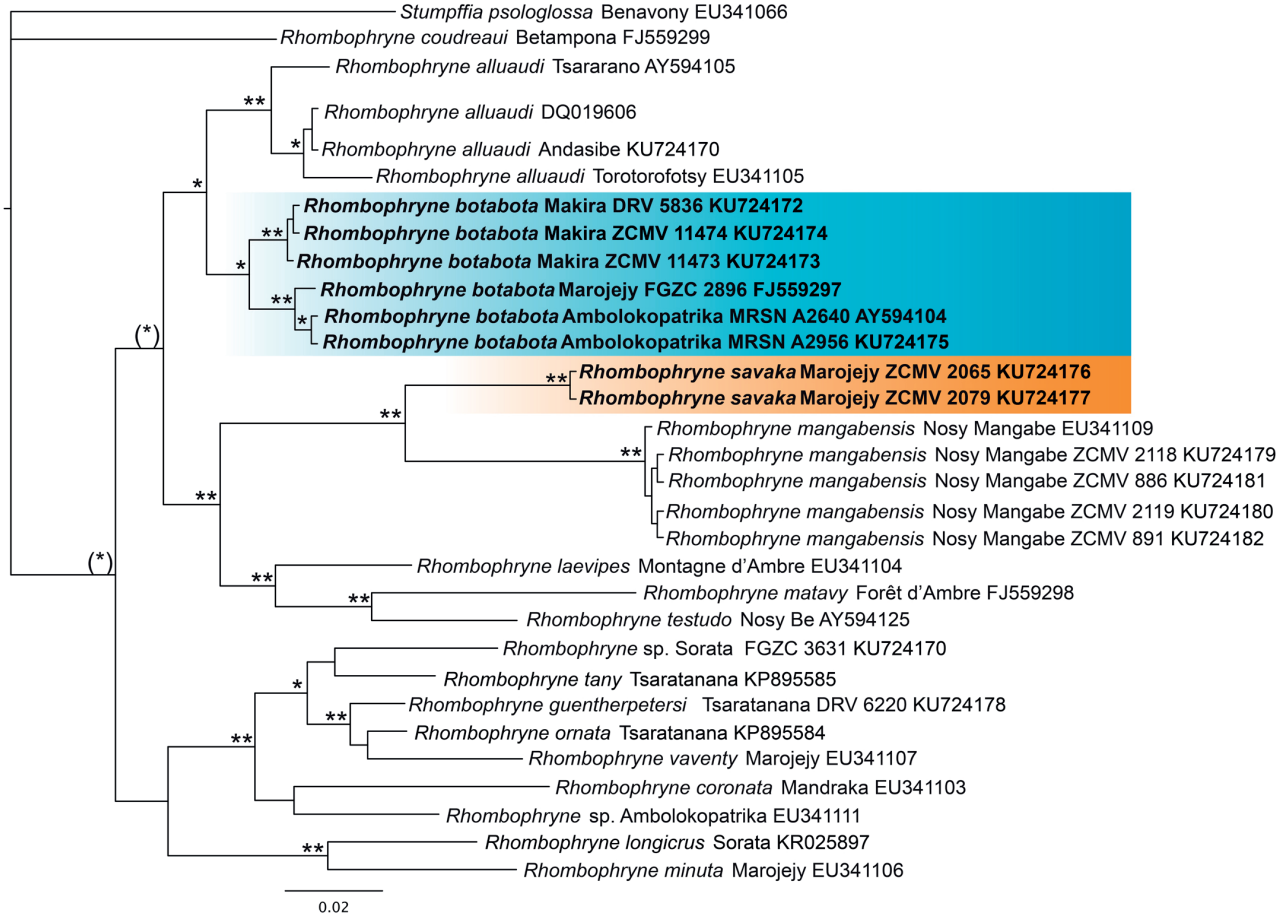

Figure 1. Bayesian inference tree of *Rhombophryne* species based on 545 bp of the mitochondrial 16S rRNA gene fragment. Asterisks denote Bayesian posterior probabilities values: (*): 97–98%; *: 99%; **: 100%. The two new species described herein are highlighted.

32.2 mm); TDH 51.0–70.5% of ED; FORL 43.5–55.2% of SVL; TIBL 38.4–45.7% of SVL; TIBW 26.5–36.0% of TIBL; HIL 141–164% of SVL; HW 144.2–169.5% of HL; tibiotarsal articulation reaching the tympanum or the eye; distinct colour border between lateral head and dorsum in most specimens; possession of curved clavicles, and maxillary and vomerine teeth; vomerine teeth sigmoidal, medially separated by a small cleft; an uncorrected p-distance of at least 3.6% in the analysed 16S rRNA gene fragment, and a unique call (see below).

Within the genus *Rhombophryne*, *R. botabota* sp. n. may be distinguished from the *R. serratopalpebrosa* group (*R. serratopalpebrosa*, *R. vaventy*, *R. coronata*, *R. ornata*, *R. tany*, and *R. guentherpetersi*) by the absence of superciliary spines (but see below for additional comments on its differentiation from *R. guentherpetersi* in which the superciliary spines are small). It is distinguished from all other described *Rhombophryne* species except *R. laevipes* by typically having a distinct colour border between its dorsum and lateral head. Additionally, *R. botabota* sp. n. may be distinguished from *R. minuta* and *R. mangabensis* by its larger SVL (24.2–32.2 mm vs 15.4–23.2 mm); from *R. laevipes* and *R. alluaudi* by its smaller SVL (24.2–32.2 mm vs 36.4–56.3) and the absence of inguinal ocelli (vs presence); from *R. minuta* and *R. longicrus* by its shorter forelimbs (FORL 43.5–55.2% vs 70.4–74.7% of SVL), robust legs (vs slim), larger tympanum (TDH 51.0–70.5% vs 39.5–48.3% of ED), and wider head (HW 144.2–169.5% vs 122.5–142.8% of HL); from *R. minuta*, *R. longicrus*, and *R. laevipes* by its shorter TIBL (TIBL 38.4–45.7% vs 48.3–52.3% of SVL) and shorter HIL (HIL 141–164% vs 175–184% of SVL); from *R. mangabensis*, *R. testudo*, *R. matavy*, and *R. coudreaui* by its smooth skin (vs tubercular), possession of ossified clavicles (vs not ossified, reduced, or absent), and narrower vertebral transverse processes; from *R. mangabensis* by its higher call repetition rate (inter-call interval 2,359 vs 6,420 ms), lower dominant call frequency (1,272 Hz vs 2,800–7,800 Hz), and a call without frequency modulation (vs with); and from *R. testudo*, *R. matavy*, and *R. coudreaui* by its longer TIBL (TIBL 38.4–45.7% vs 30.3–37.2% of SVL), and tibiotarsal articulation reaching the tympanum or eye (vs not exceeding the axilla). Some specimens of *R. botabota* sp. n. superficially resemble *R. guentherpetersi*, the superciliary spines of which are sometimes difficult to observe, but can easily be distinguished from this species by the absence of tibial glands. For a distinction from *R. savaka* sp. n., see the description of that species, below.

Rhombophryne botabota sp. n. is most similar to *R. alluaudi* (which is also its sister species). It may be distinguished from that species (as it is currently understood; see Discussion) by the absence of light dorsolateral markings (vs presence), absence of inguinal ocelli (vs presence), smaller SVL (24.2–32.2 mm vs 36.4–42.6 mm), higher dominant call frequency (1,272 ± 13 Hz vs 798 ± 23 Hz), and higher fundamental call frequency (621 ± 11 Hz vs 379 ± 34 Hz). One micro-CT scanned specimen of *R. alluaudi* (ZSM 3/2002) differed from *R. botabota* sp. n. in having a less ossified skeleton, fusion of presacral VIII and sacrum (vs not fused), and the dorsal crest of urostyle running almost its full length (vs ~75% of its length). More micro-CT scans of *R. alluaudi* are needed to identify non-variable skeletal characters useful for distinguishing it from *R. botabota* sp. n.

Rhombophryne botabota sp. n. differs from all species of *Plethodontohyla* Boulenger, 1882 except *P. mihanika*, *P. inguinalis*, and *P. fonetana* by possessing a clavicle; from *P. mihanika* and *P. inguinalis* by the absence of expanded digital discs and a dorsolateral colour border, and from these two species and *P. fonetana* by possessing knob-like terminal phalanges (vs Y-shaped). In its external morphology, this species could be confused with *P. brevipes*, but its supratympanic fold is dark (vs whitish), and it lacks inguinal spots (vs usually present). As far as is known, the two species do not overlap in distribution, so confusion in the field should not occur.

Description of holotype: The specimen is in a good state of preservation. A tissue sample was taken from the right thigh for molecular analyses. An incision running from side to side across the posterior abdomen was made to check sex and access gut contents, leaving the testes clearly visible.

Body robust. Head wider than long. Pupils small, round. Snout rounded in dorsal and lateral views. Canthus rostralis distinct, concave. Loreal region concave. Nostril closer to eye than to tip of snout, slightly protuberant. Tympanum indistinct, rounded, TDH 51.0% of ED. Supratympanic fold distinct, weakly raised, curving slightly from posterior corner of eye over tympanum toward axilla. Superciliary spines absent. Tongue very broad, attached anteriorly, unlobed. Maxillary and vomerine teeth present, vomerine teeth distinct, forming two curved rows posteromedial to choanae, separated by a small medial gap. Choanae oblong.

Forelimbs relatively thick. Fingers without webbing, relative lengths 1 < 2 < 4 < 3, fourth finger slightly longer than second; finger tips not enlarged; subarticular tubercles faint, single; nuptial pads absent; inner metacarpal tubercle present, outer metatarsal tubercle absent. Hind limb thick; tibiotarsal articulation reaching tympanum; tibia length 38.4% of SVL. Inner metatarsal tubercle present, slightly enlarged; outer metatarsal tubercle absent. Toes not webbed; relative lengths 1 < 2 < 5 < 3 < 4, fifth toe distinctly shorter than third; without subarticular tubercles; toe tips not enlarged.

Osteology of holotype: The skeleton of the holotype is fairly typical of *Rhombophryne* (see Scherz et al. 2015a, b for detailed accounts of other species in this genus). It is well ossified, without any broken bones. Unusually, the inter-vertebral spaces are highly X-ray absorbent, giving the impression of an almost ankylosed spine.

Anterior braincase laterally and anteriorly closed by sphenethmoid (or an intersphenethmoid calcium deposit). Vomerine teeth consisting of a sigmoidal row on either side of the parasphenoid, separated at the midline by a small gap. Maxillary teeth small, poorly defined.

Sternum not ossified. Clavicle well developed, curved. Humerus with crista ventralis roughly 50% of its length;

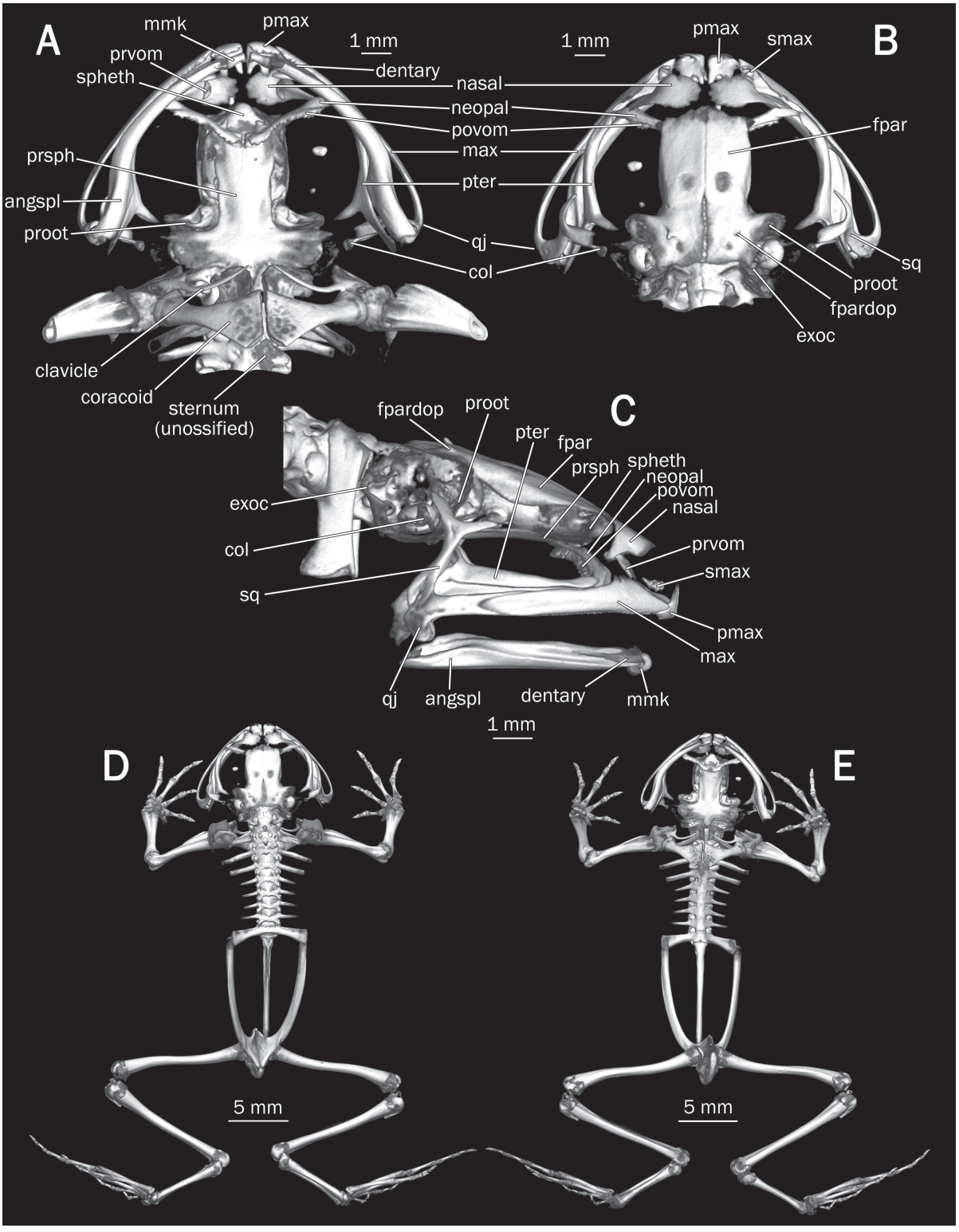

Figure 2. Osteology of *Rhombophryne botabota* sp. n. (holotype, ZSM 358/2005), showing the skull in A) ventral, B) dorsal, and C) lateral views, and the full skeleton in D) dorsal and E) ventral views. Abbreviations as follows: angspl – angulosplenial; col – columella; dentary – dentary bone; exoc – exoccipital; fpar – frontoparietal; fpardop – dorsal process of frontoparietal; max – maxilla; mmk – mentomeckelian bone; nasal – nasal bone; neopal – neopalatine; povom – postchoanal vomer; pmax – premaxilla; proot – prootic; prsph – parasphenoid; prvom – prechoanal vomer; pter – pterygoid; qj – quadratojugal; smax – septomaxilla; spheth – sphenethmoid; sq – squamosal. A PDF-embedded 3D model is provided in the supplementary material online.

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 ossified, relatively small. Prehallux not ossified.

Neural spines decreasing in size posteriorly. Dorsal crest of urostyle running roughly 75% along its shaft. Iliosacral articulation type IIA sensu Emerson (1979). Iliac shafts bearing weak dorsal tubercles, with dorsal crests running roughly 90% of their length. Pubis ossified.

Colour pattern of holotype: After ten years in preservative, specimen dorsally light brown (Fig. 3). Symmetric markings present behind scapular region. Thin cream stripes run obliquely over each scapula. Dorsum faintly striped with darker and lighter brown, in lines, running roughly from inguinal region obliquely toward midline. Inguinal spots absent. Lateral side of head dark brown, strongly distinct from dorsal coloration, limited by canthus rostralis, eyelid, and supratympanic fold. On flanks, the dorsal brown merges with the translucent cream ventral coloration. Ventral belly immaculate cream. Chin cream, heavily flecked with brown. Cloacal region dark brown. Legs dorsally light brown with two dark cross-bands on the thigh (oblique), three on the shank, two on the tarsus. Thighs ventrally cream, flecked with brown anteriorly and posteriorly, anterodorsally brown with cream flecks, posteriorly proximally cream, distally becoming dark brown and ocellated with cream. Shank ventrally distally and externally cream, flecked lightly with brown, but brown ocellated with cream toward the inside of the knee. Tarsus ventrally cream with brown flecks. External foot as the tarsus, with one dark brown area. Sole of foot brown with small cream flecks. Toes speckled cream and light brown. Toe tips dark brown. Arms ventrally cream with brown flecks, dorsally light brown with a dark brown cross band on the lower arm. Interior hand finely speckled with cream and brown. Fingers light brown with small dark brown and cream regions. Colour in life as in preservative (see Figs 4a, b).

Variation: In general, all paratypes agree strongly with the holotype in morphology, but not in coloration. For full details of variation in measurements, see Table 3. SVL ranges from 24.2 to 32.2 mm, FORL from 43.5 to 55.2% of SVL, TIBL from 38.4 to 45.7% of SVL, TIBW from 26.5 to 36.0% of TIBL, HIL from 141 to 164% of SVL, TDH from 65.0 to 80.8% of ED, and HW from 144.2 to 169.5% of HL. Finger ratios vary slightly, but in general the second and fourth finger are subequal in length. The two specimens from Makira (ZSM 538/2009 and 539/2009) are considerably smaller than the other specimens. The paratypes vary quite strongly in coloration. In all specimens, light, oblique, thin lines are present across the scapular region. The dark spots posterior to these are present in specimens MRSN A2955 and A2956, but absent from all others. In all specimens, the side of the head is darker than the dorsum. The posterior dorsal/inguinal stripes are present in MRSN A2954–2956, but absent from all other paratypes. The ocelli on

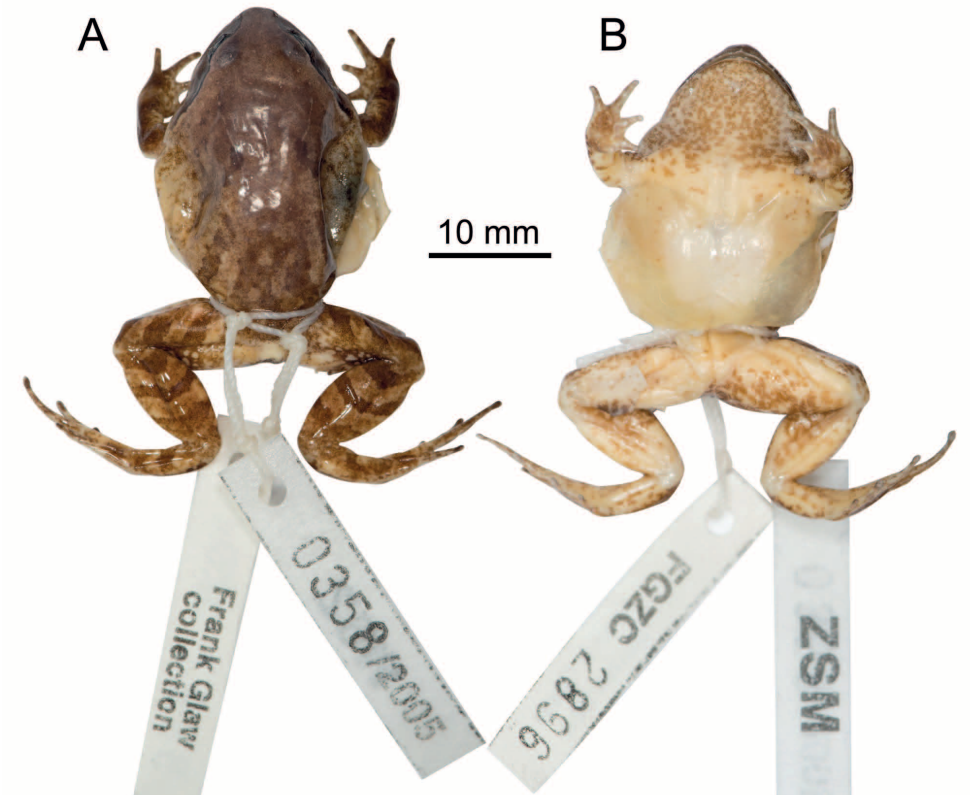

Figure 3. The holotype of *Rhombophryne botabota* sp. n. (ZSM 358/2005) in preservative, in A) dorsal and B) ventral views.

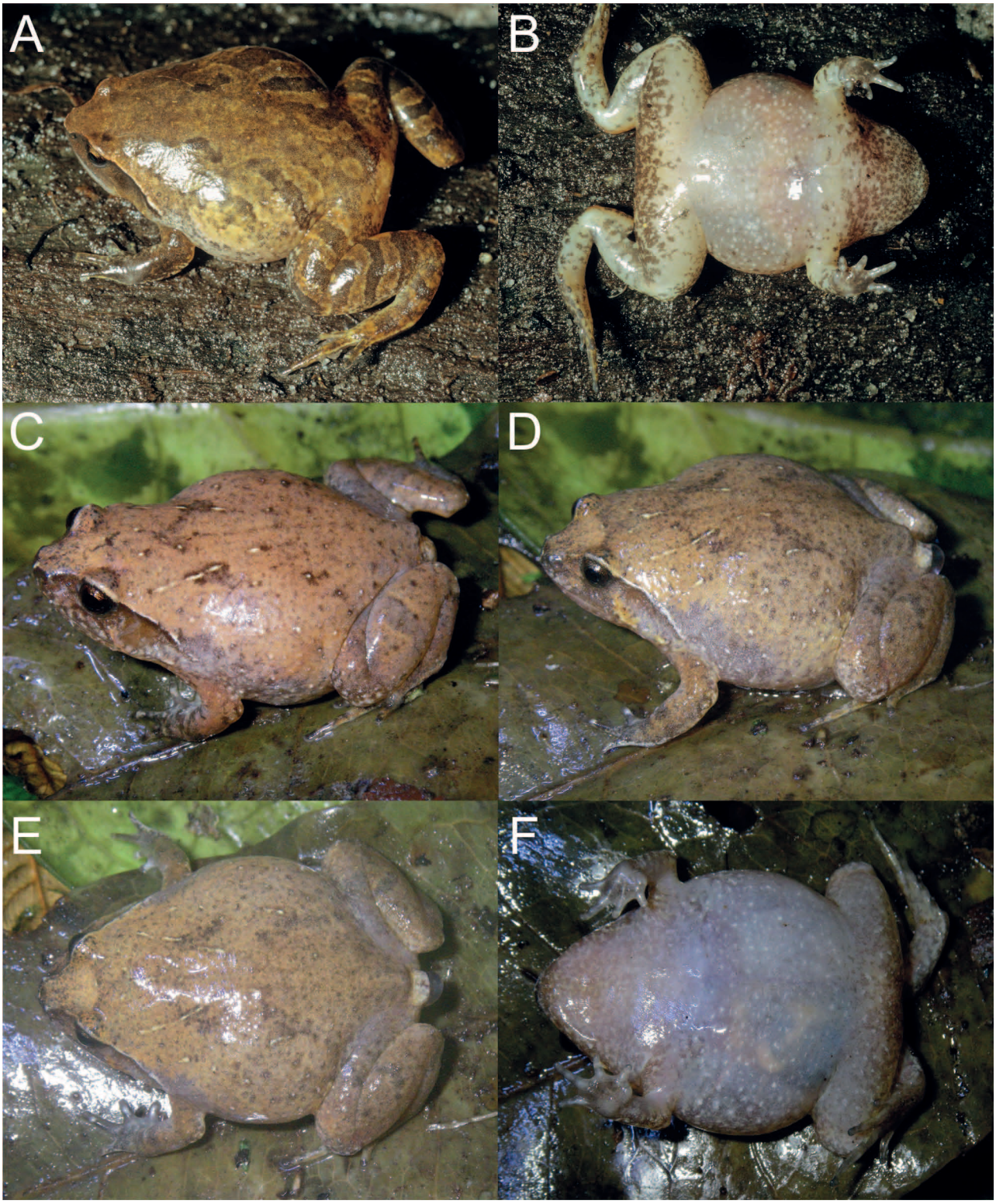

Figure 4. *Rhombophryne botabota* sp. n. in life. A–B) ZSM 358/2005 (holotype) from Marojejy National Park in dorsolateral and ventral views; C) ZSM 539/2009 from Angozongahy (Makira) in dorsolateral view; D–F) ZSM 538/2009 from Angozongahy (Makira) in dorsolateral, dorsal, and ventral views.

Table 3. Morphological measurements of *Rhombophryne botabota* sp. n. and *R. savaka* sp. n. Measurements were rounded to the nearest 0.1 mm. Abbreviations are defined in the chapter Materials and methods except HT (holotype), PT (paratype), M (male), and F (female).

Collection No.	ZSM 358/2005	MRSN A2640	MRSN A2954	MRSN A2955	MRSN A2956	ZSM 538/2009	ZSM 539/2009	ZSM 468/2005
Field No.	FGZC 2896	FN 7238	FN 7281	FN 7300	FN 7164	ZCMV 11473	ZCMV 11474	ZCMV 2065
Species	*R. botabota*	*R. botabota*	*R. botabota*	*R. botabota*	*R. botabota*	*R. botabota*	*R. botabota*	*R. savaka*
Locality	Marojejy	Ambolokopatrika	Ambolokopatrika	Ambolokopatrika	Ambolokopatrika	Makira	Makira	Marojejy
Status	HT	PT	PT	PT	PT	PT	PT	HT
Sex	M	M	F	M	F	M	M	M
SVL	30.2	30.4	32.2	30.8	28.6	25.4	24.2	20.4
HW	11.5	12.3	13.3	12.7	11.8	10.9	9.6	9.2
HL	6.8	7.7	7.9	7.8	7.5	6.8	6.6	5.1
ED	2.6	2.5	2.6	2.7	2.6	2.4	2.4	2.0
END	1.6	1.9	2.3	2.1	1.7	1.9	1.6	1.2
NSD	1.6	1.4	1.6	1.5	1.5	1.1	1.1	1.1
NND	2.9	2.9	3.0	2.8	2.7	2.4	2.2	2.5
TDH	1.3	1.5	1.8	1.6	1.7	1.4	1.4	1.2
TDV	1.7	1.8	1.9	1.7	1.6	1.7	1.5	1.4
HAL	6.5	7.0	6.8	7.1	6.0	5.9	5.1	3.7
UAL	2.4	3.9	3.5	2.7	3.4	1.6	3.2	1.7
LAL	4.3	5.9	5.0	5.0	3.6	4.8	4.4	3.5
FORL	13.1	16.8	15.3	14.8	13.1	12.3	12.7	8.8
THIL	11.4	13.1	13.4	13.6	13.4	11.2	10.7	8.1
THIW	5.7	5.4	6.1	5.5	5.1	4.6	4.8	5.3
TIBL	11.6	13.5	13.9	12.8	13.1	10.2	10.2	7.6
TIBW	4.2	4.2	3.7	4.2	4.0	3.2	3.3	3.6
TARL	7.1	6.5	7.4	6.7	7.0	6.3	6.4	4.4
FOL	12.5	14.4	15.0	13.4	13.4	11.0	10.9	7.8
FOTL	19.5	20.9	22.4	20.0	20.5	17.3	17.3	12.2
HIL	42.5	47.5	49.7	46.4	46.9	38.7	38.2	27.9
IMCL	1.4	1.4	1.5	1.2	1.3	1.1	1.0	0.8
IMTL	1.5	1.5	1.4	1.5	1.1	1.0	1.1	0.9

the posterior thigh are present in MRSN A2954 and ZSM 538/2009 and 539/2009, weakly expressed in MRSN A2955 and A2956, and absent in MRSN A2955 and A2640. Thigh cross-bands are present only in MRSN A2955 and the holotype. Shank cross-bands are present in most specimens, but vary in number (one in MRSN A2955, three in MRSN A2954), and intensity (almost invisible in ZSM 538/2009, clearly visible in MRSN A2954). Ventral coloration is more or less consistent across all specimens.

In the paratype ZSM 538/2009, the spine is malformed: first and second presacrals fused but atypically so, sacral articulation with urostyle differentiated to form a regular vertebral articulation instead of the typical bicondylar articulation found in this genus (Scherz et al. 2015a,b). Urostyle possessing a lateral parapophysis anteriorly on its right side.

Advertisement call: A single individual was recorded by MV during the day on 24 June 2009 in Makira (Angozongahy site), calling from the leaf litter in dense primary rainforest at an estimated ambient temperature of 22–24°C. The individual in question was not seen during the recording, but we are confident that the call belongs to this species because of its resemblance to the call of *R. alluaudi* and because this was the only *Rhombophryne* found near the location of the call. In fact, more calling individuals were heard and the two paratypes were collected, all from the same small area of an estimated 50 m². The advertisement call of this species consists of a series of harmonious honking notes repeated at regular intervals (Fig. 5). The following analysis is based on a recording of just four calls and is therefore tentative. Call duration was 505 ± 76 ms (range: 405–582 ms, n = 4), but this includes a long tapering tail; the core-call duration was 151 ± 18 ms (134–175 ms, n = 4). Inter-call intervals were 2359 ± 44 ms (range: 2313–2363 ms, n = 3). Fundamental frequency was 621 ± 11 Hz (610–634, n = 4). Dominant frequency peaked at 1272 ± 13 Hz (1259–1289, n = 4), i.e., the first harmonic. A harmonic band appeared at 1,893 ± 24 Hz (1,869–1,923) in all calls; one call had further harmonics at 2,537 and 3,185 Hz. No frequency modulation or pulsing was recognizable.

Ecology: The stomach of one of the paratypes (ZSM 538/2009) contained a small snail (tentatively identified as a member of Subulinidae, possibly the non-native *Subulina octona*) measuring 8.6 mm (measured in VG Studio Max 2.2). This is the first record of *Rhombophryne* predating on gastropods. The paratypes currently housed in MRSN were collected using pitfall traps, as described by Andreone et al. (2000). This provides further support for a fossorial lifestyle and rather secretive behaviour of this species.

IUCN Red List status: This species has been found at three localities: Marojejy, Ambolokopatrika, and Makira. These areas span a distance of 128.5 km. A simple minimum convex polygon (triangle) of the three collection sites covers an area of 1,457 km². Current records include two large protected areas: Makira Natural Park, consisting of 3,850 km² of protected forest surrounded by community-managed protected zones; and Marojejy National Park, consisting of 597.5 km² of protected forest. Ambolokopatrika is at present unprotected, but forms part of a proposed protected area encompassing the forest corridor connecting Marojejy with Tsaratanana (COMATSA; see Rabearivony et al. 2015). Based on these records, the altitudinal range of this species extends from 860 to 1,326 m a.s.l.

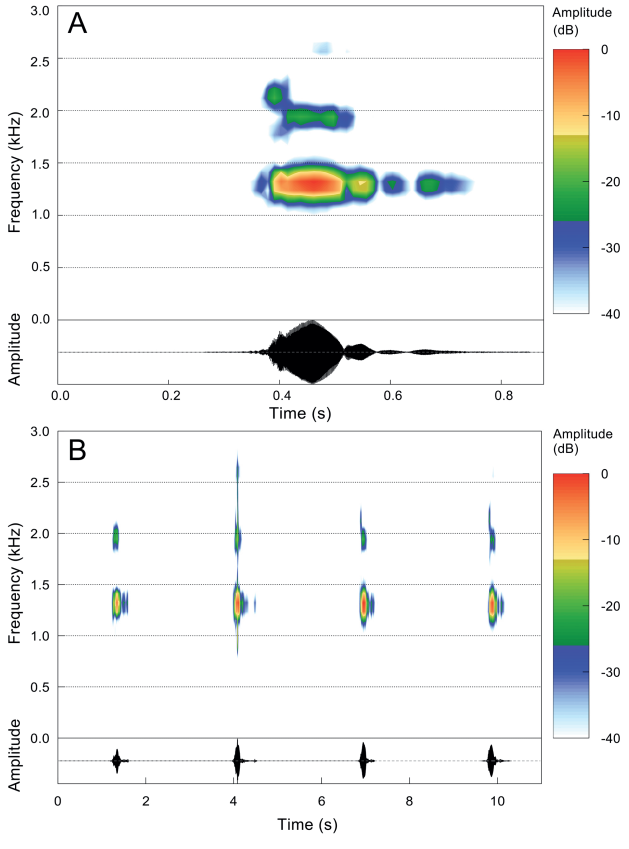

Figure 5. Oscillograms and spectrograms of *Rhombophryne botabota* sp. n., showing A) the structure of a single call and B) the structure of a call series. The second call is probably overmodulated.

Within the protected areas, anthropogenic activities continue to compromise the quality and coverage of forest (Patel & Welch 2013). This is even more true for the expanses of forest between them, including the Anjanaharibe-Sud Special Reserve and COMATSA. Mining and harvesting of hardwood trees (Randriamalala & Liu 2010, Patel & Welch 2013) are the two most important factors diminishing the quality of these forests.

Current knowledge provides an extent of occurrence (EOO) of 1,457 km², but this is probably an underestimate. Two of the three known localities are relatively well protected, but threats, including declining forest expanse and quality, persist. We propose a status of Vulnerable for this species, under IUCN Red List Criteria B1ab(iii) (IUCN 2012).

Etymology: The specific epithet *botabota* (pronounced 'buddha-buddha') is a Malagasy word meaning 'chubby' in allusion to the plump appearance of this frog. It is to be considered a noun in the nominative singular in apposition to the generic name.

Rhombophryne savaka sp. n.

(Figs 1, 6, 7)

ZooBank LSID: urn:lsid:zoobank.org:act:D75D0A22-35EE-41C4-A385-1D02F896E6DB

Suggested common name: Savaka diamond frog

Holotype: ZSM 468/2005 (ZCMV 2065), a male collected in Marojejy National Park ('Camp Marojejia'), 14.4350° S, 49.7605° E, 746 m a.s.l., Sava Region, northeastern Madagascar, on 18 February 2005 by F. Glaw, M. Vences, and R. D. Randrianiaina.

Paratype: UADBA-A uncatalogued (ZCMV 2079), a specimen of unknown age and sex with the same collection data as the holotype. This specimen could not be examined for this study, but its 16S sequence was 100% identical with the holotype, and we are therefore sure of its assignment to this taxon.

Remark: This species was included as *R.* sp. Ca4 in the phylogenies produced by Vieites et al. (2009) and Perl et al. (2014). However, the sequence accession number FJ559297 given in the Supplementary Information of Vieites et al. (2009) for this species had incorrect voucher information and in reality referred to a sequence of *R. botabota* (specimen FGZC 2896).

Diagnosis: A Malagasy microhylid frog assigned to the genus *Rhombophryne* on the basis of possessing clavicles coupled with knobbed, rather than Y-shaped terminal phalanges (Scherz et al. in press) and phylogenetic position based on our analysis of the 16S rRNA gene. *Rhombophryne savaka* sp. n. is characterized by the following combination of features: small size (SVL 20.4 mm), TDH

60.0% of ED; FORL 43.3% of SVL; TIBL 37.4% of SVL; TIBW 47% of TIBL; HIL 137% of SVL; HW 179.5% of HL; tibiotarsal articulation reaching the tympanum; possession of inguinal spots; possession of thin, curved clavicles, and maxillary and vomerine teeth; vomerine teeth with large lateral diastemata, medially fused; well-ossified braincase; well-developed prehallux; and an uncorrected p-distance of at least 8.4% in the analysed 16S rRNA gene fragment.

Within the genus *Rhombophryne*, *R. savaka* sp. n. is unique in having inguinal spots and medially fused vomers, and has the largest lateral vomerine diastemata yet observed. This species may be distinguished from the *R. serratopalpebrosa* group (*R. serratopalpebrosa*, *R. vaventy*, *R. coronata*, *R. ornata*, *R. tany*, and *R. guentherpetersi*) by the absence of superciliary spines; from *R. longicrus*, *R. laevipes*, *R. alluaudi*, *R. testudo*, *R. matavy*, *R. coudreaui*, and *R. botabota* possibly by its smaller size (SVL 20.4 vs 23.8–56.3 mm); from *R. longicrus*, *R. minuta*, and *R. botabota* by its broader head (HW 179.5% vs 122.5–169.4% of HL); from *R. testudo* by its narrower head (HW 179.5% vs 200.4–259.9% of HL); from *R. minuta* and *R. longicrus* by its larger tympanum (TDH 60.0% vs 39.5–48.3% of ED) and shorter forelimbs (FORL 43.3% vs 70.4–74.7% of SVL); from *R. minuta*, *R. longicrus*, and *R. laevipes* by its shorter tibia length (TIBL 37.4% vs 47.2–52.3% of SVL); from *R. minuta*, *R. longicrus*, *R. laevipes*, and possibly *R. alluaudi* by its shorter hind limbs (HIL 137% vs 146–184% of SVL); and from *R. testudo*, *R. matavy*, *R. mangabensis,* and *R. coudreaui* by its possessing clavicles (vs absence) and smooth skin (vs tubercular). As for *R. botabota*, this species can be easily distinguished from *R. guentherpetersi* by the absence of tibial glands.

Rhombophryne savaka sp. n. is most similar to *R. mangabensis* (which is also its sister species) and some individuals of *R. botabota* sp. n., described above. It may be distinguished from either species by the presence of inguinal spots (vs absence), possibly slightly shorter hind limbs (HIL 137% vs 141–164% of SVL), and possessing medially fused vomerine teeth with a large mid-row diastema on either side (vs medially separated with either no or just a small diastema), and from *R. mangabensis* by the presence of clavicles (vs absence), shorter forelimbs (FORL 37.4% vs 43.9–45.9% of SVL), and smooth skin (vs tubercular).

Rhombophryne savaka sp. n. differs from all *Plethodontohyla* species except *P. mihanika*, *P. inguinalis*, and *P. fonetana* by possessing a clavicle; from *P. mihanika* and *P. inguinalis* by the absence of expanded digital discs, and from these two species and *P. fonetana* by possessing knob-like terminal phalanges (vs Y-shaped). Externally, it resembles *P. bipunctata* and *P. brevipes*, but can be distinguished from these species by its shorter relative forelimb length (FORL 43.3% vs 48.1–54.3% of SVL), and broader shanks (TIBW 47.2% vs 29.4–40.8% of TIBL). It is not known to co-occur with either of these two taxa.

Description of holotype: Specimen in an excellent state of preservation. A small tissue sample for sequencing was taken from the right thigh. A small incision for sexing was made on the left flank, revealing that the testes are large and distinct.

Body robust. Head wider than long. Pupils small, round. Snout rounded in dorsal and lateral views. Canthus rostralis distinct, concave. Loreal region concave. Nostril closer to eye than to tip of snout, directed laterally, slightly protuberant. Tympanum indistinct, rounded, TDH 60.0% of ED. Supratympanic fold distinct, not raised, indicated by a dark marking, running from the posterior corner of the eye and over the tympanum, curving toward but not extending to the axilla. Superciliary spines absent. Maxillary teeth present. Vomerine teeth distinct, forming a broad, U-shaped central patch and two additional patches laterally that are clearly separated by a small diastema. Choanae oblong.

Forelimbs stubby. Fingers without webbing, relative lengths $1 < 2 = 4 < 3$; finger tips not expanded; fingers not reduced; nuptial pads absent; inner metacarpal tubercle distinct, outer metacarpal indistinct; subarticular tubercles faint. Hind limbs short and strongly built; tibiotarsal articulation reaching the tympanum; tibia length 37.4% of SVL. Inner metatarsal tubercle present, outer metatarsal tubercle present, light in colour but not raised. Toes not webbed; relative lengths $1 < 2 < 5 < 3 < 4$, fifth toe distinctly shorter than third. Toe tips not expanded.

Osteology of holotype: The osteology of the holotype is typical of *Rhombophryne*. It is well ossified, without any broken bones.

Anterior braincase laterally closed by sphenethmoid, anteriorly with a small fenestra. Vomerine teeth medially fused in a central patch, laterally bearing large (0.5 mm) diastemata in the middle of each lateral extension, so that three patches of vomerine teeth are present (one central and two lateral ones). Maxillary teeth small. Otic capsule dorsally partly ossified.

Sternum not ossified. Clavicle thin, not well ossified, curved. Humerus with crista ventralis roughly 60% 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 ossified and relatively small. Prehallux well developed.

Neural spines decreasing in size posteriorly. Dorsal crest of urostyle running roughly 60% along its shaft. Iliosacral articulation type IIA sensu Emerson (1979). Iliac shafts with almost no dorsal tubercles or oblique grooves; dorsal crests running roughly 90% of their length. Pubis well ossified.

Colour of holotype: After ten years in preservative, light brown dorsally, cream ventrally (Fig. 7). A lighter scapular region bordered anteriorly and posteriorly by darker areas. Dark supratympanic fold running from posterior corner of eye and curving over the tympanum but not reaching the axilla. Large, comma-shaped, dark inguinal spots. Dorsal coloration dissolving increasingly into speckles before fading to cream ventrally. Ventrally immaculate cream posterior to the chin. Chin cream with brown speckling. Cloacal region dark brown. Legs light brown dorsally, cream ventrally. Two dark cross bands on the thigh, one on the shank, one on the tarsus. Posterior side of thighs translu-

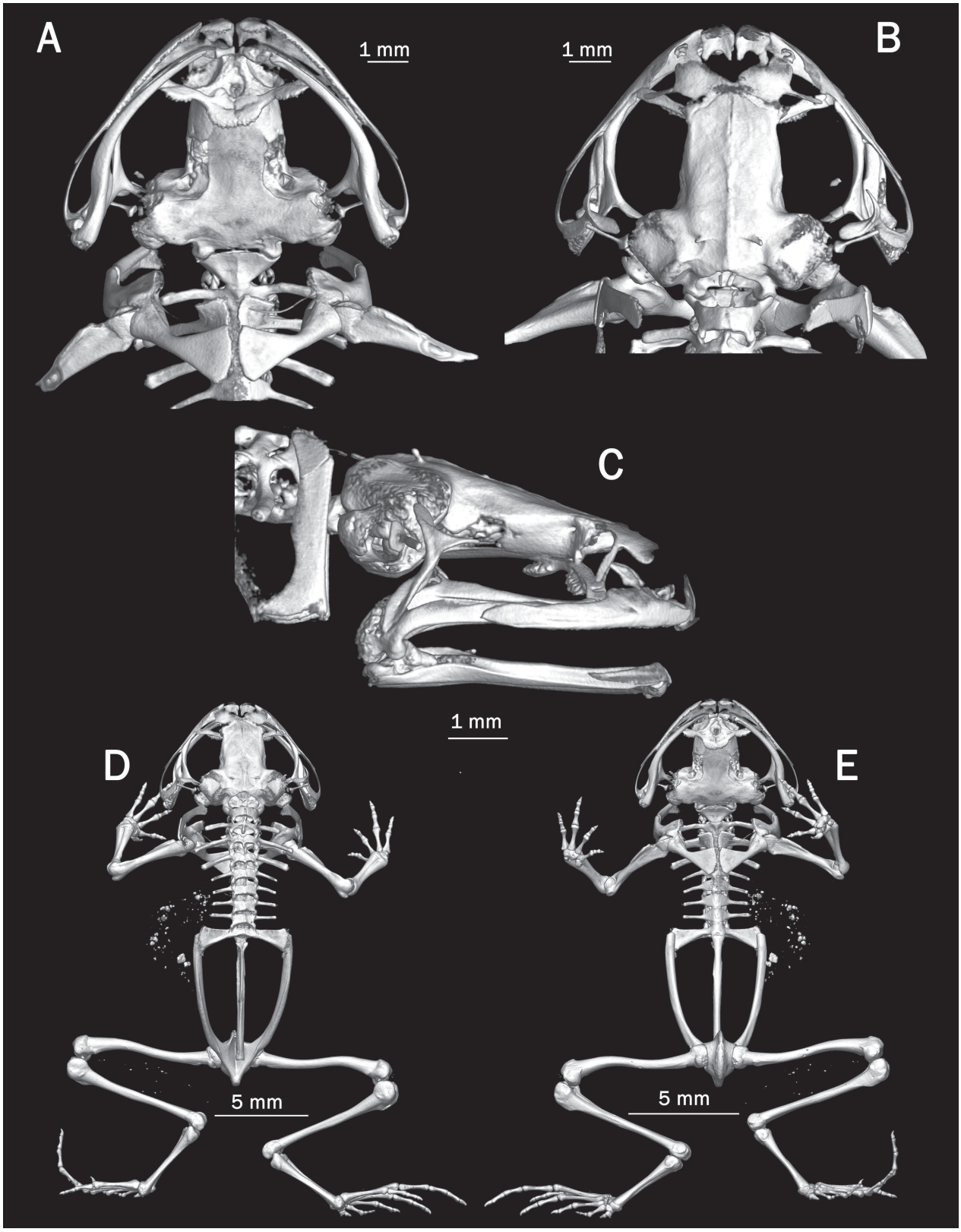

Figure 6. Osteology of *Rhombophryne savaka* sp. n. (holotype, ZSM 468/2005) from Marojejy National Park, showing the skull in A) ventral, B) dorsal, and C) lateral views, and the full skeleton in D) dorsal and E) ventral views. See Fig. 2 for bone names. A PDF-embedded 3D model is provided in the supplementary material online.

cent cream. Tarsus with a dark brown posterior face. Sole of foot light brown. Toes light brown with cream stripes anterior to the tips. Arms dorsally light brown with a single cross band on the lower arm, ventrally cream. Fingers lightly striped with cream.

Ecology: The sole two known specimens were captured in pitfall traps in primary rainforest suggesting terrestrial or fossorial habits. No further field observations are available. The gut of the holotype specimen contained the head of a large ant of the genus *Mystrium* (Yoshimura & Fisher 2014).

IUCN Red List status: This species is known only from two individuals captured at 746 m a.s.l. in the Marojejy National Park. The location of capture is roughly 600 m from degraded forest to the east, and 4.9 km in a straight line from the edge of the protected area and the forest. Most *Rhombophryne* species are microendemic to narrow altitudinal ranges and areas (Wollenberg et al. 2008). As this species occurs in a forest around 746 m a.s.l., it might be less strictly restricted to the Marojejy Massif than higher altitude species (e.g., *R. vaventy* and *R. serratopalpebrosa*), and could possibly be found in other parts of the northern rainforest chain, too. However, as the majority of species of *Rhombophryne* are known from fewer than five localities, we think it unlikely that it occurs in an area much larger than the size of the Marojejy National Park, which is 597.5 km². Therefore, due to a projected small extent of occurrence (< 5,000 km²), its being known from just one threatened location and the higher rate of forest alteration at lower altitudes in this area (Patel & Welch 2013, Rabearivony et al. 2015), this species qualifies as Endangered B1ab(iii) under the IUCN Red List criteria (IUCN 2012).

Etymology: The specific epithet *savaka* is a Malagasy word meaning 'diastema' in reference to the diastemata in the vomerine teeth of this species. It is to be considered a noun in the nominative singular in apposition to the genus name.

Discussion

Vieites et al. (2009) identified ten candidate species in the genus *Rhombophryne* that were possibly in need of description. This number was increased to twelve by Perl et al. (2014) after the discovery of two new candidate species of this genus from Tsaratanana. Here, we have described candidates *R.* sp. Ca2 and *R.* sp. Ca4 as *R. botabota* and *R. savaka*, respectively. Ca5 was described by Glaw et al. (2010) as *R. mangabensis*, Ca6 by Scherz et al. (2014) as *R. vaventy*, Ca8 by D'Cruze et al. (2010) as *R. matavy*, and Ca11 and Ca12 were described by Scherz et al. (2015a) as *R. ornata* and *R. tany*, respectively. We have recently demonstrated that *R.* sp. Ca7 probably represents a member of a new genus of miniaturized frogs (Scherz et al. in press). Thus, only candidates 1, 3, 9, and 10 still remain to be described. These potential species – and others discovered since the major barcode studies on Madagascar's amphibians (Vieites et al. 2009, Perl et al. 2014) – will be the subject of a revision of the genus *Rhombophryne* (Crottini et al. in prep.). We also anticipate that the status and definition of *R. alluaudi* will likely need to be adjusted in the course of forthcoming revisions. Nevertheless, we here chose to keep the definition of *R. alluaudi* in line with current taxonomy as it was established by Blommers-Schlösser (1975), who first defined stout microhylids from the Northern Central East of Madagascar (i.e., the region around Andasibe) as being referrable to this species.

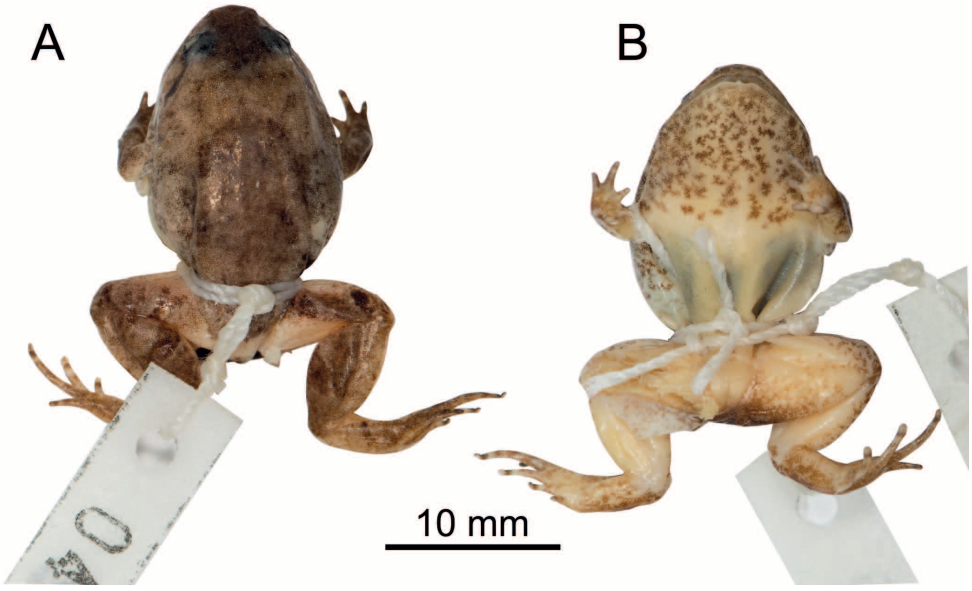

Figure 7. The holotype of *Rhombophryne savaka* sp. n. (ZSM 468/2005) in preservative, in A) dorsal and B) ventral views.

The skeletons of microhylids are notoriously variable (Noble & Parker 1926, Parker 1934, Duellman & Trueb 1986). *Rhombophryne* has proven to be no exception to this pattern (Scherz et al. 2014, 2015a, b, in press). The new species *R. savaka* is sister to *R. mangabensis*, but unlike that species, it possesses fully developed, albeit poorly ossified, clavicles. Three other species, *R. testudo*, *R. matavy*, and *R. coudreaui* also lack clavicles, while all other known members of this genus possess them. Although our 16S rRNA gene fragment phylogeny could not resolve the position of *R. coudreaui*, the monophyly of these three clavicle-lacking species is suggested by their morphology and ecology (Glaw & Vences 2007, M. D. Scherz unpubl. data). *Rhombophryne mangabensis* is related to this group, but clearly more closely to *R. savaka*. Because *R. savaka* possesses clavicles, we may infer that *R. mangabensis* has lost its clavicles independently. It might be tempting to conclude that the most fossorial species of *Rhombophryne* tend to have lost their clavicles, but clavicles are otherwise absent in arboreal taxa of other genera within the Cophylinae (Blommers-Schlösser & Blanc 1991, Rakotoarison et al. 2015, Scherz et al. in press), and there is little or no correlation between ecology and pectoral girdle morphology in frogs in general (Emerson 1984).

The new species *R. botabota* has most often been recovered as being closely related to the *R. alluaudi* complex (Vieites et al. 2009, Perl et al. 2014), although in Scherz et al. (in press) it is recovered as the sister of *R. mangabensis* and another undescribed miniaturized *Rhombophryne* from Andapa, without support. *Rhombophryne botabota* resembles *R. alluaudi* and *R. laevipes* in external morphology, but it is smaller and lacks inguinal ocelli. Like other members of this species complex, *R. botabota* occurs at moderate altitudes. It is remarkable that this species apparently also occupies a moderately large distributional range compared to other members of the genus *Rhombophryne*, although this range is still much smaller than that of *R. laevipes*.

In addition to its morphology, the call of *R. botabota* also closely resembles *R. alluaudi* (Vences et al. 2006) in that it has long inter-call intervals, a low frequency, an unmodulated note structure, and is emitted during daylight hours from concealed positions in the leaf litter. In these aspects it is also quite similar to those of *R. matavy* and *R. testudo* (Vences et al. 2006, D'Cruze et al. 2010), and rather dissimilar to those of *R. mangabensis* and *R. minuta* (Vences et al. 2006, Glaw et al. 2010). Together, osteology and bioacoustics seem to have a strong potential for taxonomic differentiation within this genus.

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Supplementary material

Additional information is available in the online version of this article at http://www.salamandra-journal.com

PDF-embedded 3D models of the skeletons of the holotypes of *Rhombophryne botabota* sp. n. and *Rhombophryne savaka* sp. n.