

Diamond frogs forever: a new species of *Rhombophryne* Boettger, 1880 (Microhylidae, Cophylinae) from Montagne d'Ambre National Park, northern Madagascar

Mark D. Scherz^{1,2,3}

¹ Zoologische Staatssammlung München (ZSM-SNSB), Münchhausenstr. 21, 81247, München, Germany

² Zoologisches Institut, Technische Universität Braunschweig, Mendelssohnstr. 4, 38106, Braunschweig, Germany

³ Department of Biology, Universität Konstanz, Universitätsstr. 10, 78464, Konstanz, Germany

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Corresponding author: Mark D. Scherz (mark.scherz@gmail.com)

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Abstract

Although taxonomic progress on the frogs of Madagascar is currently proceeding at an unprecedented pace, the goal of completing the amphibian inventory of this hyper-diverse island is still far off. In part this is because more new species continue to be discovered at a high rate, in some cases within well-studied areas. Here, I describe *Rhombophryne ellae* **sp. nov.**, a new species of diamond frog discovered in Montagne d'Ambre National Park in northern Madagascar in 2017. This new species is highly distinctive in having orange flash-markings on its hindlimbs (not known from any described species of *Rhombophryne*), and large, black inguinal spots (larger than in all other described *Rhombophryne* species). It is separated from all named species of *Rhombophryne* by a substantial uncorrected pairwise distance in the 16S rRNA mitochondrial barcode marker (> 7%) and is most closely related to an undescribed candidate species from Tsaratanana in northern Madagascar. *Rhombophryne ellae* **sp. nov.** adds another taxon to the growing list of cophyline microhylids that have red to orange flash-markings, the function of which remains unknown and which has clearly evolved repeatedly in this radiation. The discovery of such a distinctive species within a comparatively well-studied park points toward the low detectability of semi-fossorial frogs and the role of inclement weather in increasing that detectability.

Key Words

Amphibia, Anura, micro-CT, molecular genetics, osteology *Rhombophryne ellae* sp. nov., systematics, taxonomy

Introduction

The diamond frogs, genus *Rhombophryne* Boettger, 1880, are a group of fossorial and terrestrial microhylid frogs in the subfamily Cophylinae. These frogs show substantial ecological variation, with most species being terrestrial or semi-fossorial, but several species being fully fossorial (*R. testudo* Boettger, 1880, *R. matavy* D'Cruze, Köhler, Vences & Glaw, 2010, and *R. coudreaui* (Angel, 1938)), and one being highly miniaturised (*R. proportionalis* Scherz, Hutter, Rakotoarison, Riemann, Rödel, Ndriantsoa, Glos, Roberts, Crottini, Vences & Glaw, 2019). In this respect they capture a significant portion of the

variation within the Cophylinae (Andreone et al. 2005; Wollenberg et al. 2008). DNA barcoding studies revealed considerable undescribed diversity within *Rhombophryne* (Vieites et al. 2009; Perl et al. 2014), triggering increased taxonomic attention. The known species diversity of the genus has more than doubled in the last 10 years, as numerous new species have been described from the rainforests of northern and northeastern Madagascar (Vences and Glaw 2003; D'Cruze et al. 2010; Scherz et al. 2014, 2015a, 2015b, 2016a, 2016b, 2017a, 2019). Despite the increased rate of taxonomic description of *Rhombophryne* species, several undescribed candidate species of *Rhombophryne* are still awaiting description (Vieites et

al. 2009; Scherz et al. 2016b), and recent fieldwork efforts in well-studied and poorly studied areas (both in terms of herpetofaunal survey work and biological survey work in general) have continued to yield new discoveries.

Montagne d'Ambre National Park in northern Madagascar is the island's oldest protected area (Goodman et al. 2019). In general it is considered well studied, but only two published herpetofaunal studies have been conducted in the park, one focussing on the rainforest at elevations above 700 m a.s.l. (Raxworthy and Nussbaum 1994), and the other on drier, more deciduous forest at elevations below 700 m a.s.l. in the former Forêt d'Ambre Special Reserve (now subsumed within the national park) (D'Cruze et al. 2008). Until now, two *Rhombophryne* species, *R. laevipes* (Mocquard, 1895) and *R. matavy*, have been known from the park, although reference to a *Plethodontohyla* from the park in the past (Raxworthy and Nussbaum 1994) may have referred to other *Rhombophryne* species, given that these two genera were taxonomically re-arranged only afterwards (Andreone et al. 2005; Wollenberg et al. 2008) and there are no known *Plethodontohyla* species present in the park (Glaw and Vences 2007; my own unpublished data). During fieldwork in 2017, a relatively small *Rhombophryne* was collected that was immediately recognisable as an undescribed species. Not only was it considerably smaller than an adult *R. laevipes*, but it also differed considerably in colouration and morphology from that species. I show here that this animal is genetically highly distinct from all other *Rhombophryne* species and describe it as a new species.

Methods

The new specimen was collected during fieldwork in Montagne d'Ambre in December 2017. It was photographed in life before being anaesthetised and subsequently euthanised with an aqueous solution of MS-222. A tissue sample was taken from the right thigh and deposited in 99% ethanol. The specimen was fixed with 90% ethanol and transferred to 70% ethanol for long-term storage. The specimen has been deposited in the Zoologische Staatssammlung München (ZSM) in Munich, Germany (ZSM 76/2018). Other institutional and field numbers are used in Table 1, and these are: AMNH-A are the amphibian series numbers of the American Museum of Natural History, New York NY, USA; FAZC and FN are field numbers of Franco Andreone; FG/MV, FGZC, ZCMV, and MV are field numbers of Frank Glaw and Miguel Vences; KU is the zoological collection of Kansas University, Lawrence KS, USA; MSZC is my own field number series; and RAX are the field numbers of Christopher J. Raxworthy.

Morphological examination of the specimen followed that used in my previous work on this genus; an illustrated measurement scheme is presented in Scherz et al. (2015a). For practicality, I reiterate this measurement scheme here: SVL, snout-vent length; HW, head width at the widest point; HL, head length, measured diagonally from the jaw commissure to the anterior-most point of the

jaw; ED, eye diameter; END, eye-nostril distance; NSD, nostril-snout-tip distance; NND, nostril-nostril distance; TDH, horizontal tympanum diameter; TDV, vertical tympanum diameter; HAL, hand length, from the base of the hand to the tip of the longest (third) finger; UAL, upper arm length, from the insertion of the arm to the elbow; LAL, lower arm length, from the elbow to the base of the hand; FORL, forelimb length, given by the sum of HAL, UAL, and LAL; FARL, forearm length, given by the sum of HAL and LAL; THIL, thigh length, from the cloaca to the knee; THIW, width of the thigh at its widest point; TIBL, tibia length, from the knee to the tibiotarsal articulation; TIBW, width of the tibia at its widest point; TARL, tarsus length, from the tibiotarsal articulation to the base of the foot (end of the inner metatarsal tubercle); FOL, foot length, from the base of the foot to the end of the longest (fourth) toe; FOTL, given by the sum of FOL and TARL; HIL, given by the sum of THIL, TIBL, TARL, and FOL; IMCL, inner metacarpal tubercle length; OMCL, outer metacarpal tubercle length; IMTL, inner metatarsal tubercle length; OMTL, outer metatarsal tubercle length.

A micro-computed tomography (micro-CT) scan of the specimen was produced using a phoenix|x nanotom m cone beam scanner (GE Measurement & Control, Wunstorf, Germany). The scan was made at a voltage of 140 kV and current of 80 μ A, using a tungsten target with a 0.1 mm copper filter. 2440 projections each were captured at 750 ms for a total scan duration of 30 minutes. The scan was reconstructed in `datos|x reconstruct` (GE Measurement & Control) and examined in VG Studio Max 2.2 (Volume Graphics GmbH, Heidelberg, Germany). Screenshots used here are from volumetric renderings of the skeleton, following recommendations laid out in Scherz et al. (2017a). A DICOM stack of the scan has been deposited on MorphoSource.org at http://www.morpho-source.org/Detail/ProjectDetail/Show/project_id/1005.

Total genomic DNA was extracted from the tissue sample using a standard salt extraction (Bruford et al. 1992). A segment of the 3' end of the 16S rRNA mitochondrial barcoding marker was amplified using the primers 16Sar-L and 16Sbr-H (Palumbi et al. 1991) following protocols used by Vences et al. (2003), and a further segment from the cytochrome oxidase I (COI) marker with the primers dgLCO1490 and dgHCO2198 (Meyer et al. 2005) following protocols used by Perl et al. (2014). Sequences were resolved on an ABI 3130xl automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). The newly determined sequences are deposited in GenBank under accession numbers **MT371794** (16S) and **MT372330** (COI).

A phylogeny of the genus *Rhombophryne* was constructed using the newly determined sequences and a matrix of all other species and several candidate species for the same genes from GenBank (Table 1). This resulted in a matrix of 27 ingroup terminals plus *Anodonthyla montana* Angel, 1925 used as an outgroup. 16S sequences were available from all included terminals, and COI sequences from 18 terminals. Sequences of the two loci were aligned in AliView 1.21 (Larsson 2014) using MUSCLE (Edgar 2004), and the alignment visually proofed. 16S rRNA and

Table 1. Uncorrected pairwise distance (p-distance) in the 3' fragment of the 16S rRNA mitochondrial marker, and GenBank accession numbers of sequences used to reconstruct phylogenetic relationships within *Rhombophryne* in Figure 1. For the full p-distance table (all comparisons) see Appendix II, which is based on the more comprehensive sampling of specimens for 16S given in Appendix I.

Genus	Species	Specimen number	16S p-distance	3' 16S	COI
<i>Anodonthyla</i>	<i>montana</i>	FG/MV 2001-530	–	AY594090	GU177056
<i>Rhombophryne</i>	<i>botabota</i>	FGZC 2896	7.1–7.9	EU341102	KF611585
<i>Rhombophryne</i>	<i>coronata</i>	MV2001-199	11.5–11.6	EU341103	KM509856
<i>Rhombophryne</i>	<i>coudreaui</i>	FAZC 13887	11.8	FJ559299	–
<i>Rhombophryne</i>	<i>diadema</i>	FGZC 3631	10.4	KU724171	–
<i>Rhombophryne</i>	<i>ellae</i> sp. nov.	MSZC 0534	–	MT371794	MT372330
<i>Rhombophryne</i>	<i>guentherpetersi</i>	ZCMV 12401	9.0–9.4	KU937796	–
<i>Rhombophryne</i>	<i>laevipes</i>	FGZC 1052	8.5–9.6	KM509189	KM509857
<i>Rhombophryne</i>	<i>longicrus</i>	FGZC 3654	9.5	KR025898	–
<i>Rhombophryne</i>	<i>mangabensis</i>	ZCMV 886	12.7–13.0	EU341109	KF611588
<i>Rhombophryne</i>	<i>matavy</i>	FGZC 1888	11.0	FJ559298	KF611589
<i>Rhombophryne</i>	<i>minuta</i>	FGZC 2897	10.9–11.3	EU341100	KF611590
<i>Rhombophryne</i>	<i>nilevina</i>	KU 340893	7.7	KY288475	–
<i>Rhombophryne</i>	<i>ornata</i>	ZCMV 12384	7.4–9.5	KP895584	KF611583
<i>Rhombophryne</i>	<i>proportionalis</i>	ZCMV 12404	8.8	KU937808	KF611640
<i>Rhombophryne</i>	<i>regalis</i>	FN 7292	10.3	EU341111	–
<i>Rhombophryne</i>	<i>savaka</i>	ZCMV 2065	11.4	KU724176	KF611594
<i>Rhombophryne</i>	<i>tany</i>	ZCMV 12359	9.1	KP895585	KF611582
<i>Rhombophryne</i>	<i>testudo</i>	FG/MV 2000-277	9.1–9.9	AY594125	EF396066
<i>Rhombophryne</i>	<i>vaventy</i>	FGZC 2842	9.8	EU341107	KF611595
<i>Rhombophryne</i>	sp. aff. <i>coronata</i>	KU 340732	10.6	KY288476	–
<i>Rhombophryne</i>	sp. aff. <i>vaventy</i>	AMNH-A167315	8.8	DQ283409	KM509853
<i>Rhombophryne</i>	sp. Ca01	FAZC 10314	8.7	FJ559295	–
<i>Rhombophryne</i>	sp. Ca03	MV2001-131	7.5–8.0	FJ559296	KF611592
<i>Rhombophryne</i>	sp. Ca07	MV2001-G46	6.0	EU341108	–
<i>Rhombophryne</i>	sp. Ca10	FAZC 10312	7.4	AY594111	–
<i>Rhombophryne</i>	sp. ex-alluaudi	ZCMV 968	7.6–8.1	EU341105	KF611584
<i>Rhombophryne</i>	sp. RAX 10368	RAX 10368	9.6	KM509192	KM509860

COI alignments were concatenated to increase phylogenetic signal, yielding an alignment of 1134 positions, of which 327 were parsimony informative. GTR + I + G was determined to be the best model using model testing in MEGA X (Kumar et al. 2018). Phylogenetic analysis was performed under Maximum Likelihood (ML) in MEGA X and Bayesian inference (BI) in MrBayes 3.2 (Ronquist et al. 2012). ML analysis was conducted with 500 nonparametric bootstrap replicates, using SPR level 5 branch swapping. For BI analysis, two parallel runs were carried out, each with four heated chains, for a total of 10 million generations. Trees were sampled every 10,000 generations and 10% were discarded as burn-in after checking for convergence in Tracer 1.5 (Rambaut and Drummond 2007). A second matrix of 51 ingroup terminals plus *A. montana* was constructed for the 3' 16S marker alone, including a larger number of specimens for each species (full list in Appendix I). This too was aligned in AliView 1.21 using MUSCLE, yielding 530 positions, of which 159 were parsimony informative, and from it uncorrected pairwise distances (p-distances) were calculated in MEGA X (Appendix II).

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Results

The BI and ML reconstructions of *Rhombophryne* largely agreed with one another (Fig. 1) and with most previous work on this genus (Scherz et al. 2015a, 2016a, 2017a; Lambert et al. 2017), although support was generally low, and it is evident that the phylogeny of the genus cannot be resolved based on these two short mitochondrial markers alone (*R. coudreaui* for example is very unstable in its placement, but on morphological grounds is thought to belong to the *R. testudo*+*R. matavy* clade). The BI tree was better resolved and better matched previous hypotheses, so it is shown in Figure 1. It is intended here to serve as a guide only to illustrate the degree of divergence of the new taxon from other *Rhombophryne* species, and not as an evolutionary hypothesis.

The new specimen collected in Montagne d'Ambre (ZSM 76/2018) is recovered in a clade together with *R. sp. Ca07* from Tsaratanana with high support (bootstrap support (BS) = 94%, posterior probability (PP) = 1). The position of this clade within the genus is variable; in BI

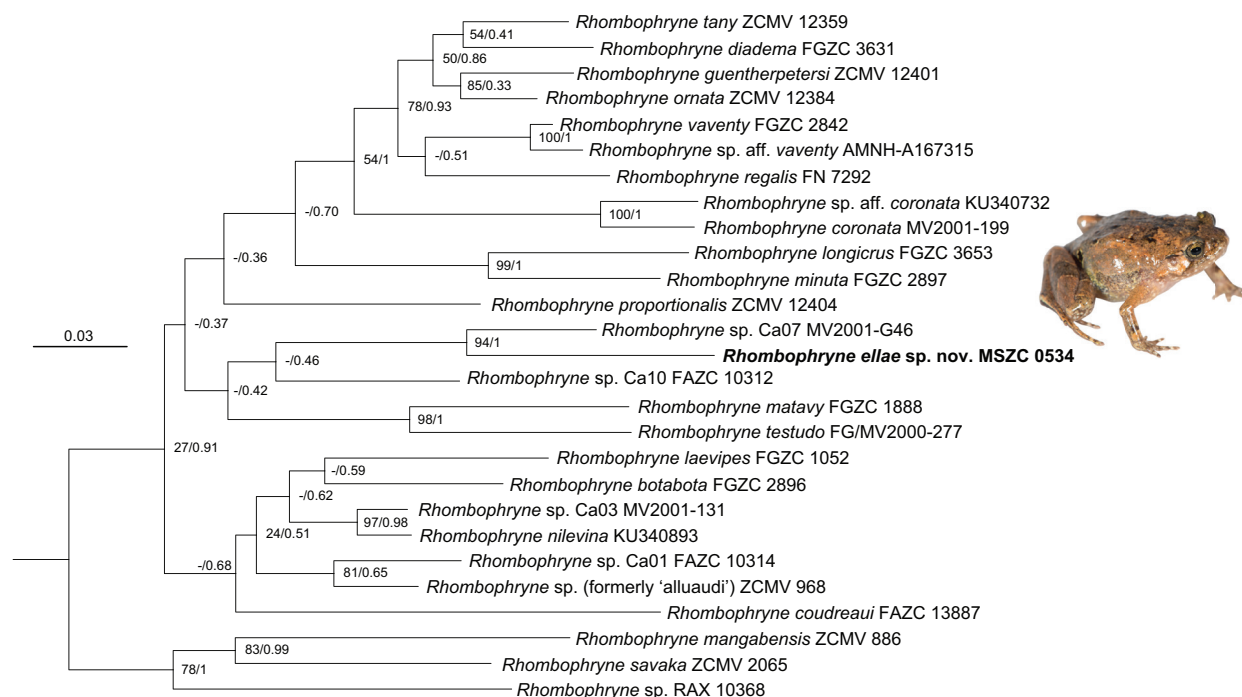


Figure 1. Bayesian inference phylogeny of *Rhombophryne* based on concatenated sequences of the mitochondrial markers 16S rRNA and COI (1134 bp). Values at nodes indicate percent bootstrap support (BS)/Bayesian posterior probability (PP); ‘-’ indicates clades not recovered in the ML tree. *Anodonthyla montana* was used as outgroup (not shown for graphical reasons). *Rhombophryne serratopalpebrosa* is the only described species missing from this phylogeny, as no sequences of that species are available.

analysis it was found to be sister to *R. sp. Ca10*, whereas in ML analysis it was found to be sister to the *R. laevipes* species group. The placement of *Rhombophryne* sp. Ca07 has been problematic before (see Scherz et al. 2016b, 2017b), so its affinities will need further work to clarify. However, it is unambiguously clear that the specimen from Montagne d’Ambre is highly distinct from all described species in the genus, and indeed from all known candidate species.

The new frog from Montagne d’Ambre is separated from *R. sp. Ca07* by an uncorrected pairwise distance (p-distance) of 6% in the 3’ fragment of the mitochondrial 16S rRNA gene analysed here, and from all other species of *Rhombophryne* by at least 7.1% (Table 1; Appendix II). This distance is much higher than the 3% 16S p-distance threshold usually used for candidate species recognition in Madagascar’s frogs (Vieites et al. 2009), and within the genus *Rhombophryne* is comparable with several species pairs (e.g. *R. minuta* (Guibé, 1975) and *R. longicrus* Scherz, Rakotoarison, Hawlitschek, Vences, & Glaw, 2017 at 6.2–6.3%) and much greater than some others (e.g. *R. ornata* Scherz, Ruthensteiner, Vieites, Vences & Glaw, 2015 and *R. guentherpetersi* (Guibé, 1974) at 2.2–3.8%; see Scherz et al. 2017a).

The specimen of the new frog is an adult or subadult female, based on the presence of small eggs in its ovaries and thickened, white oviducts. Morphologically, the individual from Montagne d’Ambre most closely resembles *R. savaka* Scherz, Glaw, Vences, Andreone & Crottini, 2016 and *R. mangabensis* Glaw, Köhler & Vences, 2010 among described species but differs from them in a number of aspects that I detail below. Most characteristically, its thighs have

bright flash-markings, which are not present in any described *Rhombophryne* so far (but see the Discussion for more on this topic), and it has distinct black inguinal spots, which are rare among *Rhombophryne* species. Both flash-markings and inguinal spots are notably present in *R. sp. Ca07*, further supporting the close affinities of these two species (*R. sp. Ca07* will be described elsewhere in the context of a larger revision). *Rhombophryne* species show little sexual dimorphism, and as bioacoustic recordings of males are rare, species are described based on holotypes of either sex.

Based on the congruence of strong genetic divergence and morphological differences from all nominal species, the new specimen from Montagne d’Ambre unambiguously represents an undescribed species, and I here provide its diagnosis and description.

Rhombophryne ellae sp. nov.

<http://zoobank.org/A656F828-D640-4C7D-B3D3-BCCE6D0E11B8>

Figures 1–3

Holotype. ZSM 76/2018 (MSZC 0534), adult or subadult female, collected on 28 December 2017 in Montagne d’Ambre National Park (12.5066°S, 49.1746°E, 892 m a.s.l.), Antsiranana Region, northern Madagascar by M. D. Scherz, J. H. Razafindraibe, A. Razafimanantsoa, O. Randriamalala, S. M. Rasolonjavato, R. Tiavina, E. Z. Lattenkamp, and A. Rakotoarison.

Diagnosis. *Rhombophryne ellae* sp. nov. is assigned to the genus *Rhombophryne* based on its plump body shape,

presence of vomerine and maxillary teeth, curved clavicle, knob-shaped terminal phalanges, and phylogenetic relationships. It is distinguished by the following unique combination of characters: (1) adult or subadult female SVL 24.9 mm, (2) distinctly enlarged inner metatarsal tubercle, (3) absence of superciliary spines, (4) orange flash-markings on its posterior thighs in life, and (5) presence of large and distinct black inguinal spots.

The new species can be distinguished from all described members of the genus *Rhombophryne* by the orange colouration on its posterior thighs. Additionally, it may be distinguished from all members of the *Rhombophryne serratopalpebrosa* species group (*R. serratopalpebrosa* (Guibé, 1975), *R. guentherpetersi*, *R. coronata* (Vences & Glaw, 2003), *R. vaventy* Scherz, Ruthensteiner, Vences & Glaw, 2014, *R. ornata*, *R. tany* Scherz, Ruthensteiner, Vieites, Vences & Glaw, 2015, *R. diadema* Scherz, Hawlitschek, Andreone, Rakotoarison, Vences & Glaw, 2017, and *R. regalis* Scherz, Hawlitschek, Andreone, Rakotoarison, Vences & Glaw, 2017) with ease by absence of superciliary spines; from *R. testudo*, *R. matavy*, and *R. coudreaui* by smoother dorsal skin, a longer and less broad head (HW/HL 1.48 vs 1.88–2.42), relatively longer legs (HIL/SVL 1.77 vs 1.17–1.41), and less-developed inner metatarsal tubercle; from *R. savaka* and *R. mangabensis* by its longer forelimb (FORL/SVL 0.55 vs 0.41–0.48), longer hindlimb (HIL/SVL 1.77 vs 1.49–1.60), and less broad head (HW/HL 1.48 vs 1.54–1.86); and from *R. minuta* and *R. longicrus* by its shorter hindlimb (HIL/SVL 1.77 vs 1.79–1.84), shorter forelimb (FORL/SVL 0.55 vs 0.70–0.75), and wider head (HW/HL 1.48 vs 1.22–1.43). Morphologically, *Rhombophryne ellae* sp. nov. is similar to *R. laevipes*, *R. nilevina* Lambert, Hutter & Scherz, 2017, and *R. botabota* Scherz, Glaw, Vences, Andreone & Crottini, 2016, especially young individuals, but in addition to large genetic distances, it differs by absence of ocelli on the hidden portions of the legs (vs presence), presence of large black inguinal spots (vs absence), and absence of distinct colouration of the lateral surface of the head (vs presence in *R. botabota* and some *R. laevipes*), and also in its smaller body size from *R. laevipes* and *R. nilevina* (presumed adult female SVL 24.9 mm vs at least 28.6 mm and generally > 30 mm).

Rhombophryne ellae sp. nov. is also distinguished from all described *Rhombophryne* species by an uncorrected p-distance of > 7.1% in a fragment of the 16S rRNA gene (Table 1; Appendix II).

The new species can also be distinguished from all known members of the morphologically similar but not closely related genus *Plethodontohyla* on the basis of the orange colouration on its posterior thighs.

Holotype description. Morphology. An adult or subadult female specimen in a good state of preservation, its hindlimbs and toe tips slightly dehydrated. Tissue samples taken from left thigh for sequencing. A small incision is present on the right side and in the wall of the gut. Developing eggs are visible in the ovaries, and the oviduct is thick and white.

Body robust. Head wider than long (HW/HL = 1.48). Pupils more or less round. Snout rounded in dorsal and lateral view. Canthus rostralis distinct, concave. Loreal region concave, without dermal folds. Nostrils nearer to snout tip than to eye (END/NSD = 0.87), directed laterally, slightly protuberant. Tympanum distinct, TDH/ED = 0.6. Supratympanic fold distinct, rounded over the tympanum from the posterior corner of the eye, ending anterior to the insertion of the forelimb. Superciliary spines absent. Vomerine teeth distinct, in a straight row on either side of the palate, varying in height, approaching each other medially but separated by a small gap. Choanae diminutive, unusually close to the neopalatine.

Arms rather slender. Fingers without webbing, relative lengths $1 < 4 < 2 < 3$; fourth finger slightly shorter than second; finger tips not expanded; fingers not reduced (Fig. 2c); nuptial pads absent; inner metacarpal tubercle present, outer metacarpal tubercle absent; subarticular tubercles round and flat, undivided. Hindlimbs fairly slender (also in life, not an artefact of preservation); tibiotarsal articulation reaches the eye when the hindlimb is adpressed forward along the body; TIBL/SVL = 0.46. Inner metatarsal tubercle present, round, slightly enlarged, outer metatarsal tubercle present, rather weak, distinct, round. Toes unwebbed; relative lengths $1 < 2 < 5 < 3 < 4$, fifth toe distinctly shorter than third. Toe tips not expanded, second, third, and fourth toe tips slightly pointed. Dorsal and ventral skin smooth in preservative, but with a few dispersed pustules in life (Fig. 2). Dorsolateral folds absent.

Colouration. After just over a year in preservative, specimen dorsally brown, with two darker spots above the suprascapulae. A faint dark brown chevron is present on the posterior portion of the dorsum. Distinct black inguinal spots present. A russet brown spot is present on the posterodorsal portion of the tympanum, which is otherwise dirty cream. On the left side of the snout there is a further lighter area that is not present on the right side. A light interocular bar is present. The dorsal hindlimbs are as the dorsum in colour, with faint, dark-grey crossbands on the thigh and shank. The feet are highly mottled with cream and grey-brown, with a whitish annulus before each toe tip. The hidden surfaces of the thigh are cream, and there is a distinct black trapezoid in the cloacal region. The forelimbs are similar to the dorsum in dorsal colourations, with a dark-grey crossband on the antebrachium, followed by a white spot distally. The hands are mottled like the feet, also with light annuli before each fingertip. Ventrally, the abdomen is translucent cream, the scapular region and chin are mottled cream and olive-brown, and the hindlimbs and forelimbs are brown, flecked with cream, forming larger blotches more distally. The soles of the hands and feet are dark-grey, mottled with cream. The subarticular and carpal and tarsal tubercles are likewise cream. Colouration in life is shown in Figure 2.

Osteology. Skeleton resembling other *Rhombophryne* species (Scherz et al. 2017a); what follows is thus a brief summary of remarkable features of the skeleton (Fig. 3). Frontoparietal robust, bearing dorsal processes nearly forming a ridge. Otic capsule not dorsally ossified. Nasal

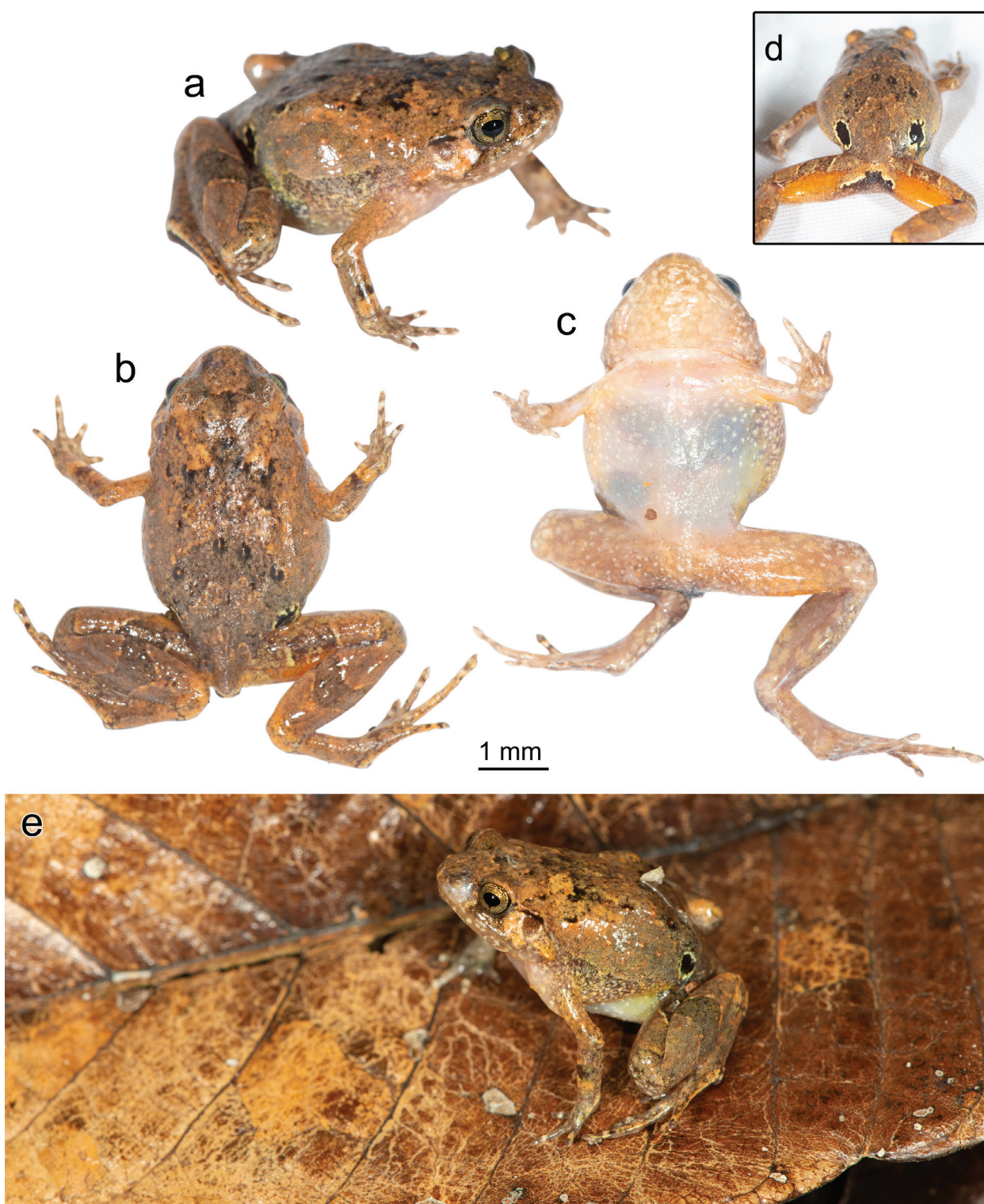


Figure 2. The holotype of *Rhombophryne ellae* sp. nov. in life. **a.** In dorsolateral view; **b.** In dorsal view; **c.** In ventral view; **d.** In posterior view showing the orange flash-markings on the thighs and the distinctive inguinal spots; **e.** On leaf litter.

broad with a long and cuneate maxillary process, widely separated from the contralateral. Post-choanal vomer bearing teeth with a distinct diastema either side of the cultriform process of the parasphenoid. Parasphenoid alae nearly the breadth of its cultriform process. Neopalatine broad. Sphenethmoid strongly ossified, bounding around half the length of the braincase. Exoccipitals widely separated dor-

sally. Premaxilla and maxilla bearing teeth. Maxilla with a broad connection to the quadratojugal. Quadratojugal anteriorly broad, its bulbous posteroventral process thick, dorsally with broad contact to the squamosal. Squamosal with a distinctly flared ventral ramus, a nearly vertical otic ramus, and a shorter, anteromedially curving zygomatic ramus. Pterygoid anterior ramus distinctly bowed, ventral

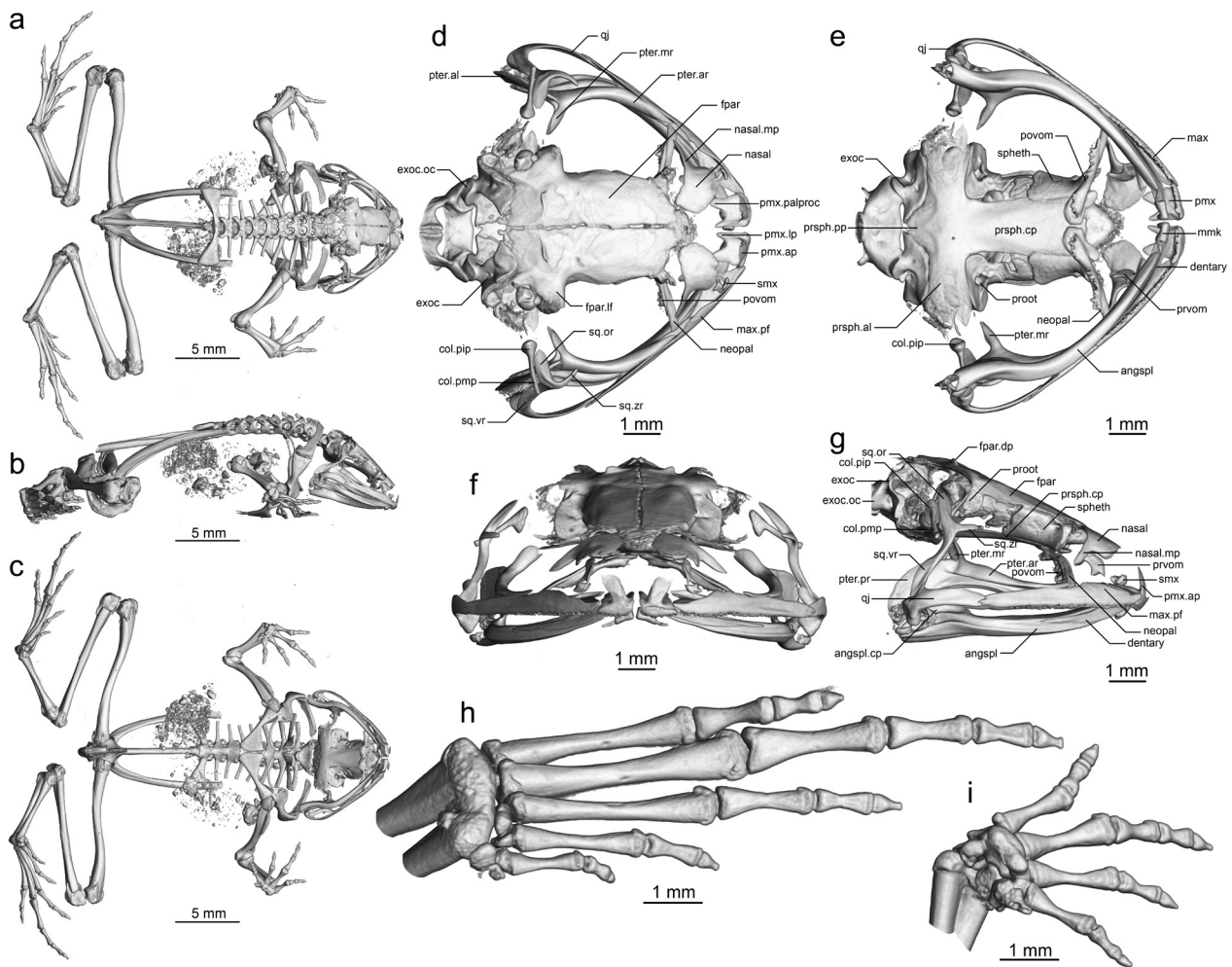


Figure 3. Volumetric renderings of micro-CT scans of the skeleton of the holotype of *Rhombophryne ellae* sp. nov. (ZSM 76/2018). **a–c.** Full skeleton in (a) dorsal, (b) lateral, and (c) ventral view; **d–g.** Skull in (d) dorsal, (e) ventral, (f) anterior, and (g) lateral view; **h.** Foot in ventral view; **i.** Hand in ventral view. Abbreviations: **angspl** – angulosplenial; **angspl.cp** – coronoid process of the angulosplenial; **col.pip** – pars interna plectri of columella; **col.pmp** – pars media plectra of columella; **exoc** – exoccipital; **exoc.oc** – occipital condyle of exoccipital; **fpar** – frontoparietal; **fpar.dp** – dorsal process of frontoparietal; **fpar.lf** – lateral flange of frontoparietal; **max** – maxilla; **max.pf** – pars facialis of maxilla; **mmk** – mentomeckelian; **nasal.mp** – maxillary process of nasal; **neopal** – neopalatine; **pmx** – premaxilla; **pmx.ap** – ascending process of premaxilla; **pmx.lp** – lingual process of premaxilla; **pmx.palproc** – palatine process of premaxilla; **povom** – postchoanal portion of vomer; **proot** – prootic; **prsph.al** – parasphenoid alae; **prsph.cp** – cultriform process of parasphenoid; **prsph.pp** – posterior process of parasphenoid; **prvom** – prechoanal portion of vomer; **pter.ar** – anterior ramus of pterygoid; **pter.mr** – medial ramus of pterygoid; **pter.pr** – posterior ramus of pterygoid; **qj** – quadratojugal; **smx** – septomaxilla; **spheth** – sphenethmoid; **sq.or** – otic ramus of squamosal; **sq.vr** – ventral ramus of squamosal; **sq.zr** – zygomatic ramus of squamosal.

ramus very deep. Mandible robust, the receiving surface of the angulosplenial (coronoid process) somewhat laterally flared to receive the quadratojugal. Clavicle robust and curved. Coracoid slender at its midpoint. Humerus with a strong crista ventralis and a broad epicondylus ulnaris. Urostyle with a dorsal crest running circa two-thirds of its length. Ilium with a low dorsal crest and a rather shallow oblique groove. Pubis semi-ossified. Centrale of the foot large, but the prehallux is not enlarged. Terminal phalanges of fingers and toes knobbed. Prepollex short and triangular. Hand bone configuration as in the *R. serratopalpebrosa* species group (Scherz et al. 2017a).

Measurements (all in mm). SVL 24.9, HW 9.6, HL 6.5, ED 2.5, END 1.3, NSD 1.5, NND 3.1, TDH 1.5, TDV 1.6, HAL 5.3, UAL 3.6, LAL 4.7, FORL 13.7, FARL 10.0,

THIL 12.5, THIW 3.5, TIBL 11.5, TIBW 3.3, TARL 6.96, FOL 11.6, FOTL 18.6, HIL 42.5, IMCL 1.0, OMCL 1.1, IMTL 1.0, OMTL 0.6.

Natural history, distribution, and conservation status.

The holotype was collected at 892 m a.s.l. in rainforest on Montagne d'Ambre during the day actively jumping away from trampling feet during moderate to heavy rain brought about by Cyclone Ava. Its gut contents included three whole ants and one ant head, seemingly belonging to two different species (one of the whole ants is diminutive), the head of a jumping spider (Salticidae), and the elytra and other body parts of a beetle. Nothing more is known of the ecology of this species, though it is probable that its reproductive mode and ecology is similar to other lit-

ter-dwelling *Rhombophryne* species (Scherz et al. 2016a). As the species is known from a single individual, its Red List status cannot be confidently estimated. However, the syntopically occurring *Stumpffia* species have been suggested to be Near Threatened due to their small range and presumed micro-endemicity within a well-protected forest, and this likely applies to *R. ellae* sp. nov. as well.

Etymology. It is with great pleasure that I dedicate this charming little frog to my partner, Dr Ella Z. Lattenkamp, in appreciation of her love, support, and infinite patience, and in celebration of the completion of her PhD.

Available names. No names are currently available for the family Cophylinae that could refer to this species.

Discussion

The bright orange flash-markings on the thighs of *R. ellae* are a particularly notable character. It is the first described *Rhombophryne* species to possess this trait, though it should be noted that it is also present in *R. sp. Ca07* (depicted by Glaw and Vences 2007: 119, photograph 5, as *R. guentherpetersi*, though not showing the flash markings), as well as another undescribed species from Marojejy that seems more closely allied with *R. mangabensis* and *R. savaka* (unpublished data). *Rhombophryne ornata* also has dark reddish colouration on its legs, although that colouration is less clearly demarcated and may serve some other purpose. These three lineages are not closely related, and the species between them do not have flash-markings, indicating repeated evolution of this trait within *Rhombophryne*.

Beyond *Rhombophryne*, red to orange thighs are also present in various other Malagasy microhylids (e.g. *Stumpffia* be Köhler, Vences, D'Cruze & Glaw, 2010 and several *Platypelis* species; Glaw et al. 2020), not to mention mantellids, and indeed numerous other groups of frogs worldwide. The fact that this trait is so widespread, and has clearly been evolved numerous times, implies some functional significance. Yet, at present I am not aware of any tests of its function, which must be imagined to be either intraspecific communication (sexual selection) or, more likely, in predator deterrence. The pairing of the bright flash-markings with inguinal 'eye' spots, as is the case for *R. ellae*, would tend to support the latter hypothesis. Yet, in most other Malagasy frogs with orange thighs, such inguinal markings are missing. Behavioural observations and detailed studies will be needed on individual groups of frogs to establish the function of the flash-markings and whether that function is common to all taxa that have developed it, even when the shade of red can vary substantially (e.g. the recently described *Platypelis ranjomena* Glaw, Scherz, Rakotoarison, Crottini, Raselimanana, Andreone, Köhler & Vences, 2020 has deep blood-red flash-markings, as opposed to the bright orange of *R. ellae*; see Glaw et al. 2020).

Rhombophryne ellae is another highly distinct member of the genus *Rhombophryne*, discovered in 2017. It is of comparable divergence to *R. longicrus*, which was discov-

ered in 2012 and described in 2015 (Scherz et al. 2015a). Unlike the latter, which was found in an area that has seldom been surveyed before, the new species described here was found in Montagne d'Ambre National Park, an area that is generally considered well surveyed. Fossorial and semi-fossorial frogs are particularly prone to being missed by short-term and localised survey efforts, and are still more so when they are limited to extremely small ranges, as is often the case among Madagascar's microhylids, including several *Rhombophryne* species; I presume that this will also be the case of *R. ellae*. This often means that specimen numbers are few, and in the present case, only a single specimen of the new species has so far been collected (examples of other recently described frog species based on singletons include *R. tany*, *Anodonthyla eximia* Scherz, Hutter, Rakotoarison, Riemann, Rödel, Ndriantsoa, Glos, Roberts, Crottini, Vences & Glaw, 2019, *Oreobates yanucu* Köhler & Padial, 2016, and all three species of *Vietnamophryne* Poyarkov, Suwannapoom, Pawangkhanant, Aksornneam, Duong, Korost & Che, 2018).

Although the usage of singletons for species descriptions is not ideal, it can be necessary, given that rarity is natural and common (Lim et al. 2012). The combined substantial improvement in our knowledge of the genus *Rhombophryne* over the last decade (D'Cruze et al. 2010; Scherz et al. 2014, 2015a, 2015b, 2016a, 2016b, 2017a, 2019) and the establishment of DNA barcoding in these frogs (Vieites et al. 2009), allows us to be highly confident in the recognition of species-level lineages in this genus. Micro-CT even allows us to access data on the internal anatomy of such singletons without harming the specimens, yielding a wealth of data that would formerly have required at least two specimens, with one intact and one dissected or cleared and stained. Köhler and Padial (2016) discussed this point in some detail, highlighting the comparative robustness of species described using multiple lines of evidence compared to those based solely on morphology, even when specimens are plentiful (although, as they say, 'large series of specimens are always desirable'). In the case of *R. ellae*, the diagnosis and description was facilitated by the highly unique appearance of the frog and its very large genetic distances from congeners.

Of course, much remains unknown about singleton species, such as variability, sexual dimorphism, ecology, and adult body size distributions (for further discussion see Lim et al. 2012 and Köhler and Padial 2016); future work must continue to build on the taxonomic foundation, though it may be difficult when they are so rarely encountered. Fortunately, the detection probability of (semi-) fossorial frogs seems to increase in inclement weather. In Madagascar, cyclones appear to act as a particular stimulus for the activity of such frogs: three species that my colleagues and I have recently described, *R. nilevina*, *R. ellae*, and *Anodonthyla eximia*, were all collected during cyclonic weather (Lambert et al. 2017; Scherz et al. 2019). This emphasises the importance of rainy-season studies in even well-surveyed areas like Montagne d'Ambre in order to fully capture the diversity of these areas. Even so, many taxa are liable to be overlooked; continuous monitoring is

probably the most effective way to ensure near-total sampling of such areas, but even with substantial manpower and resources, some rare and ephemeral species may go unnoticed. Conservation strategies must account for this detectability problem among potentially undescribed species by protecting habitat at the landscape level.

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Appendix I

GenBank numbers for all 16S sequences included in Appendix II.

Species	Genbank Number
<i>Anodonthyla montana</i>	AY594090
<i>Rhombophryne botabota</i>	AY594104
	EU341102
	KU724172
	KU724173
	KU724174
	KU724175
<i>Rhombophryne</i> sp. Ca01	AY594106
	FJ559295
<i>Rhombophryne</i> sp. Ca03	AY594107
	FJ559296
<i>Rhombophryne</i> sp. Ca07	EU341108
<i>Rhombophryne</i> sp. Ca10	AY594111
<i>Rhombophryne coronata</i>	EU341103
	KM509188
<i>Rhombophryne coudreai</i>	FJ559299
	HM364771
<i>Rhombophryne diadema</i>	KU724171
<i>Rhombophryne ellae</i>	MT371794
<i>Rhombophryne guentherpetersi</i>	KU724178
	KU937796
<i>Rhombophryne laevipes</i>	EU341104
	KM509189
<i>Rhombophryne longicrus</i>	KR025897
	KR025898
<i>Rhombophryne mangabensis</i>	EU341109
	KU724179
	KU724180
	KU724182
<i>Rhombophryne matavy</i>	FJ559298
	GU195641
<i>Rhombophryne minuta</i>	EU341100
	EU341106
<i>Rhombophryne nilevina</i>	KY288475
<i>Rhombophryne ornata</i>	KP895582
	KP895583
	KP895584
<i>Rhombophryne proportionalis</i>	KU937808
<i>Rhombophryne regalis</i>	EU341111
<i>Rhombophryne savaka</i>	KU724176
	KU724177
<i>Rhombophryne</i> sp. aff. <i>coronata</i>	KU340732
<i>Rhombophryne</i> sp. aff. <i>vaventy</i>	DQ283409
<i>Rhombophryne</i> sp. ex-alluaudi	AY594105
	AY594112
	EU341105
	KU724170
<i>Rhombophryne</i> sp. RAX 10368	KM509192
<i>Rhombophryne tany</i>	KP895585
<i>Rhombophryne testudo</i>	AY594125
	KC180070
<i>Rhombophryne vaventy</i>	EU341107

Appendix II

Uncorrected pair-wise distances (in percent) of the mitochondrial 16S rRNA gene among the genus *Rhombophryne*. Numbers along the diagonal refer to intraspecific distances. The new species is bolded. GenBank numbers for the respective species are listed in Appendix I.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1 <i>R. botabota</i>	0.0–1.8																										
2 <i>R. laevipes</i>	5.1–5.9	0.0																									
3 <i>R. nilevina</i>	3.3–4.4	6.6–6.7	na																								
4 <i>R. sp. Ca03</i>	3.1–4.8	5.8–6.6	2.0–2.2	0.0																							
5 <i>R. testudo</i>	7.7–9.3	7.7–8.6	9.1–9.9	8.5–9.7	1.1																						
6 <i>R. coudeaudi</i>	8.7–9.5	11.1–11.2	8.7	8.9–9.1	13.0–13.1	0.0																					
7 <i>R. malavy</i>	10.3–10.7	10.0–10.1	10.9	10.7–12.5	7.9–8.9	13.0	0.0																				
8 <i>R. longicrus</i>	7.9–8.8	8.5–9.6	8.9	8.5–9.2	10.7–11.7	10.7	10.7	0.0																			
9 <i>R. minuta</i>	7.3–8.6	9.1–9.4	9.4–9.6	8.5–9.5	10.4–11.8	11.7–11.8	11.9–12.4	6.2–6.3	0.6																		
10 <i>R. diadema</i>	8.7–9.7	9.4–9.7	9.1	9.1–9.7	11.0–11.7	10.6–10.7	13.1	9.6	8.9–9.4	na																	
11 <i>R. guentherpetersi</i>	7.0–8.5	9.2–9.3	7.2–7.8	7.4–8.0	10.8–11.7	11.3	12.3–12.5	8.0–8.2	9.2–9.3	5.0–5.3	0.8																
12 <i>R. ornata</i>	5.8–10.9	8.1–11.9	7.0–11.3	7.0–13.0	9.6–11.7	10.5–14.1	10.6–14.0	8.2–9.8	8.9–10.2	5.2–6.3	2.2–3.8	0.0–0.2															
13 <i>R. regalis</i>	7.1–8.2	8.8–8.9	7.5	7.7–8.2	11.2–12.3	11.1	11.9	9.2	9.9–10.1	5.8	6.1–6.6	5.9–8.0	na														
14 <i>R. vaventy</i>	7.6–8.1	8.7	9.1	7.8–8.7	10.9–11.8	11.5	12.8	9.8	10.6–10.7	6.6	5.1–6.0	4.7–6.0	6.4	na													
15 <i>R. sp. aff. vaventy</i>	6.8–7.5	8.1–8.2	7.2	7.4–7.8	10.3–11.0	10.0	12.0	8.4	9.8–9.9	6.9	4.3–5.1	4.0–4.6	5.9	1.5	na												
16 <i>R. coronata</i>	9.7–10.0	10.2–10.3	8.5–8.6	9.3–9.6	11.8–13.0	11.9	12.5	11.7–11.9	10.5–10.6	8.8–9.4	7.6–8.3	8.6–13.3	7.8	9.2–9.3	8.6–8.7	0.0											
17 <i>R. sp. aff. coronata</i>	9.3–9.8	8.9–9.1	8.7	9.1–9.6	10.8–11.8	11.5–11.6	11.5	10.8	11.5–11.6	9.4	7.5–7.8	8.1–12.9	7.8	8.1	7.7	3.2–3.3	na										
18 <i>R. sp. ex. alluaudi</i>	3.1–4.1	6.6–6.7	3.7–4.1	4.3–5.2	7.9–9.1	8.9–9.4	9.9–10.2	7.5–8.5	8.1–8.5	8.7–9.3	7.0–8.2	7.0–11.5	7.5–8.3	7.6–8.5	6.6–7.6	8.8–9.1	8.1–9.1	0.0–1.6									
19 <i>R. tany</i>	7.1–7.9	9.7–9.8	6.9	6.9–7.4	10.1–10.9	10.7	11.7	8.7	9.1	5.2	4.3–4.4	4.0–6.0	6.5	7.0	6.2	7.9–8.1	8.0	7.5–7.9	na								
20 <i>R. mangabensis</i>	9.3–10.4	11.4–11.5	10.9–11.1	10.3–11.2	11.0–12.0	13.5–13.7	13.6–14.1	12.5–13.0	11.5–11.6	13.0–13.3	13.3–13.6	12.1–15.2	12.9–13.1	13.6–13.3	13.0–13.3	13.8–13.9	12.6–13.1	10.1–10.5	12.3–12.6	0.0							
21 <i>R. sp. RAX 10368</i>	7.2–8.0	9.1–9.3	8.4	8.2–9.0	9.0–9.7	11.8	11.4	12.0	10.7–11.2	11.7	10.3	9.9–13.3	10.7	12.2	11.0	11.3–11.4	10.8	7.4–7.9	9.4	7.4	na						
22 <i>R. savaka</i>	8.7–9.9	10.6–10.7	9.5	9.0–9.5	11.0–11.3	12.7–12.8	13.6	12.9	11.1–11.6	12.5	11.7	10.7–14.0	12.9	12.8	11.8	11.9	12.2	9.3–9.8	11.3	8.3–8.4	8.0	0.0					
23 <i>R. sp. Ca01</i>	4.5–5.2	7.0–7.1	4.9	4.7–5.2	8.7–9.5	8.9	10.5	8.3	8.5	9.7	8.0–8.2	8.3–13.1	7.9	9.3	8.2	9.2–9.4	8.9	3.3–3.7	8.5	10.9–11.1	8.6	10.1	0.0				
24 <i>R. sp. Ca10</i>	6.2–6.6	7.1	6.3	6.0–6.2	9.1–9.5	10.8	10.0	10.1	10.9–11.1	9.6	7.2–7.7	6.8–9.9	9.0	8.5	8.4	10.4–10.5	9.8	6.4–6.7	8.5	12.2–12.4	10.8	11.2	6.7	na			
25 <i>R. proportionalis</i>	6.2–7.1	7.7–7.8	6.8	6.5–6.8	10.2–11.0	9.9–10.0	10.2	9.6	9.5	9.0	6.6–6.7	5.7–9.6	7.6	9.0	7.8	8.8–8.9	8.3	6.8–7.1	7.5	11.4–11.6	9.0	10.4	7.0	7.0	na		
26 <i>R. sp. Ca07</i>	7.6–8.1	7.2	7.8	8.5–9.5	9.6–10.4	11.5–11.6	11.1	9.1	10.7	9.6	7.9–8.9	7.5–9.4	8.8	8.7	7.9	9.8–9.9	10.0	7.4–7.8	8.9	12.6	10.1	12.6	8.9	7.9	8.2	na	
27 <i>R. ellae sp. nov.</i>	7.1–7.9	8.5–9.6	7.7	7.5–8.0	9.1–9.9	11.8	11.0	9.5	10.9–11.3	10.4	9.0–9.4	7.4–9.5	10.3	9.8	8.8	11.5–11.6	10.6	7.6–8.1	9.1	12.7–13.0	9.6	11.4	8.7	7.4	8.8	6.0	na

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