

A NEW FAT FOSSORIAL FROG (MICROHYLIDAE: COPHYLINAЕ: RHOMBOPHRYNE) FROM THE RAINFOREST OF THE FORÊT D'AMBRE SPECIAL RESERVE, NORTHERN MADAGASCAR

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ABSTRACT: Recent surveys of the herpetofauna in the rainforest of the Forêt d'Ambre Special Reserve, northern Madagascar, revealed a distinctive fossorial microhylid anuran species of the genus *Rhombophryne*. The new species is characterized by medium size (snout–vent length up to 49 mm in males), a stout body, short legs, and tuberculate skin on dorsal surfaces. It is most similar and closely related to *R. testudo* from the Sambirano region, but differs mainly by the absence of barbels on the lower lip, a different advertisement call, and a pairwise total sequence divergence of 8.5% the mitochondrial 16S RNA gene. We consider this species to be at particular risk from a conservation perspective because it appears to be endemic to a relatively small area of lowland transitional forest heavily altered by human activities. According to the International Union for Conservation of Nature criteria, its threat status is classified as “Endangered.”

Key words: Amphibia; Anura; Conservation; Madagascar; Microhylidae; New species; *Rhombophryne*

COPHYLINE microhylids currently comprise seven genera with more than 40 described species (Andreone et al., 2005a; Wollenberg et al., 2008). Next to the endemic Malagasy–Comoroan family Mantellidae with 12 genera and more than 170 species (Glaw and Vences, 2006; Glaw et al., 2006; Köhler et al., 2008) they represent the second largest amphibian radiation in Madagascar. Many new cophyline species have already been discovered, but still await their description (Vieites et al., 2009; Wollenberg et al., 2008).

Until recently the genus *Rhombophryne* was considered to be monotypic (*R. testudo*), but molecular genetic analyses have shown that several species formerly assigned to the genus *Plethodontohyla* actually belong to *Rhombophryne* (Andreone et al., 2005a), which currently includes eight recognized species. Recently, a considerable proportion of undescribed species diversity in the genus was identified by an integrative approach, including five so-called confirmed candidate species supported by molecular genetics and morphological differences, as well as five unconfirmed candidate species with only

molecular data available (Vieites et al., 2009). Thus far, members of the genus are considered to be largely restricted to rainforest environments or moist high-altitude habitats of northern Madagascar. However some species are found in Madagascar's east, southeast, and the central high plateau and recently a possibly undescribed species has been discovered in the dry west (Andreone and Randrianirina, 2008). The majority of species also have rather restricted distributions (Glaw and Vences, 2007), which means that they might be particularly at risk from a conservation perspective. During recent fieldwork in the extreme north of Madagascar, we discovered a distinctive fossorial species of *Rhombophryne* in the Forêt d'Ambre Special Reserve, already identified as a confirmed candidate species by Vieites et al. (2009). In this contribution we describe it as a new species, discuss its phylogenetic relationships and assess its threat status.

MATERIALS AND METHODS

We collected specimens at night, euthanized them with a chlorobutanol solution, fixed them in 90% ethanol, and preserved them in 70% ethanol. Locality information

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was recorded with global positioning system receivers. Specimens studied in this paper were deposited in the collection of the Zoologische Staatssammlung München, Germany (ZSM) and the Université d'Antananarivo, Département de Biologie Animale, Madagascar (UADBA). FGZC refers to F. Glaw field numbers.

We took the following morphological measurements (in mm) with a digital caliper (precision 0.01 mm) to the nearest 0.1 mm: snout-vent length (SVL), greatest head width (HW), head length (HL), horizontal eye diameter (ED), eye-nostril distance (E-N), nostril-nostril distance (N-N), horizontal tympanum diameter (TD), tibia length (TL), foot length (FL), and foot length including tarsus (FOOTL). Terminology and description scheme follows Glaw et al. (2007).

We recorded calls in the field with an Edirol R-09 digital recorder at a sampling rate of 44.1 kHz and 24-bit resolution and saved them as uncompressed files. Air temperature during recording was approximately 25 °C. Recordings were resampled at 22.05 kHz and 16-bit resolution and computer-analyzed using the software Adobe Audition version 1.5. We obtained frequency information through fast Fourier transformation (FFT, width 1024 points); the audiospectrogram was obtained at Hanning window function with 256 bands resolution. Temporal measurements are given in milliseconds (ms) or seconds (s), as range, with mean \pm standard deviation in parentheses. Terminology in call description follows Köhler et al. (2007).

To assess molecular differentiation we sequenced a fragment of the mitochondrial 16S rRNA gene, which is known to provide sufficient variation to distinguish among species of Malagasy frogs (Vences et al., 2005). We amplified the 550-bp fragment using primers 16Sar-L and 16Sbr-H from Palumbi et al. (1991) applying standard protocols, resolved it on automated sequencers, and compared it to a near-complete database of sequences of adult Malagasy frog species (see Vieites et al., 2009). We applied three methods of phylogenetic reconstruction to this data set: (1) Bayesian inference using the program MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) after determining the

appropriate substitution model by Akaike Information Criterion (AIC) in MrModeltest (Nylander, 2002). We performed two runs of 50 million generations (started on random trees) and four incrementally heated Markov chains (using default heating values) each, sampling the Markov chains at intervals of 1000 generations. Stabilization and convergence of likelihood values occurred after less than 1 million generations. We conservatively discarded the first 25 million generations as burn-in. (2) Heuristic searches and 2000-bootstrap replicates under the maximum likelihood optimality criterion (with 10 random addition sequence replicates and tree bisection reconnection branch swapping) were carried out in PAUP* (Swofford, 2002) after determining the appropriate substitution model with AIC (Akaike, 1974) in Modeltest (Posada and Crandall, 1998). (3) Analyses under the maximum parsimony optimality criterion were branch-and-bound searches and 2000-bootstrap replicates in PAUP* (Swofford, 2002). We deposited the newly determined DNA sequences of the new species in GenBank (accession numbers: FJ559298 for FGZC 1888 and GU195641 for FGZC 1890).

SPECIES ACCOUNT

Rhombophryne matavy sp. nov.

Holotype.—ZSM 1628/2008 (field number FGZC 1888), adult male (Fig. 1), from rainforest (12° 27' 46" S, 49° 13' 24" E, 482 m above sea level), Forêt d'Ambre Special Reserve, Antsiranana Province, northern Madagascar; collected on 27 February 2008 by N. D'Cruze, F. Glaw, J. Köhler, and local guides.

Paratypes.—ZSM 1629/2008 (FGZC 1889), UADBA (FGZC 1890), and UADBA (FGZC 1891), three adult males, same data as holotype, except FGZC 1891 collected on 28 February 2008.

Diagnosis.—A medium-sized species of the genus (SVL of adult males 39–49 mm) with a very stout body, very short legs, and tuberculate dorsum. A member of the cophyline genus *Rhombophryne*, as revealed by molecular genetic analyses of the 16S mtRNA gene. It is distinguished from the other cophyline genera *Anodonthyla*, *Cophyla*, and *Platypelis*,

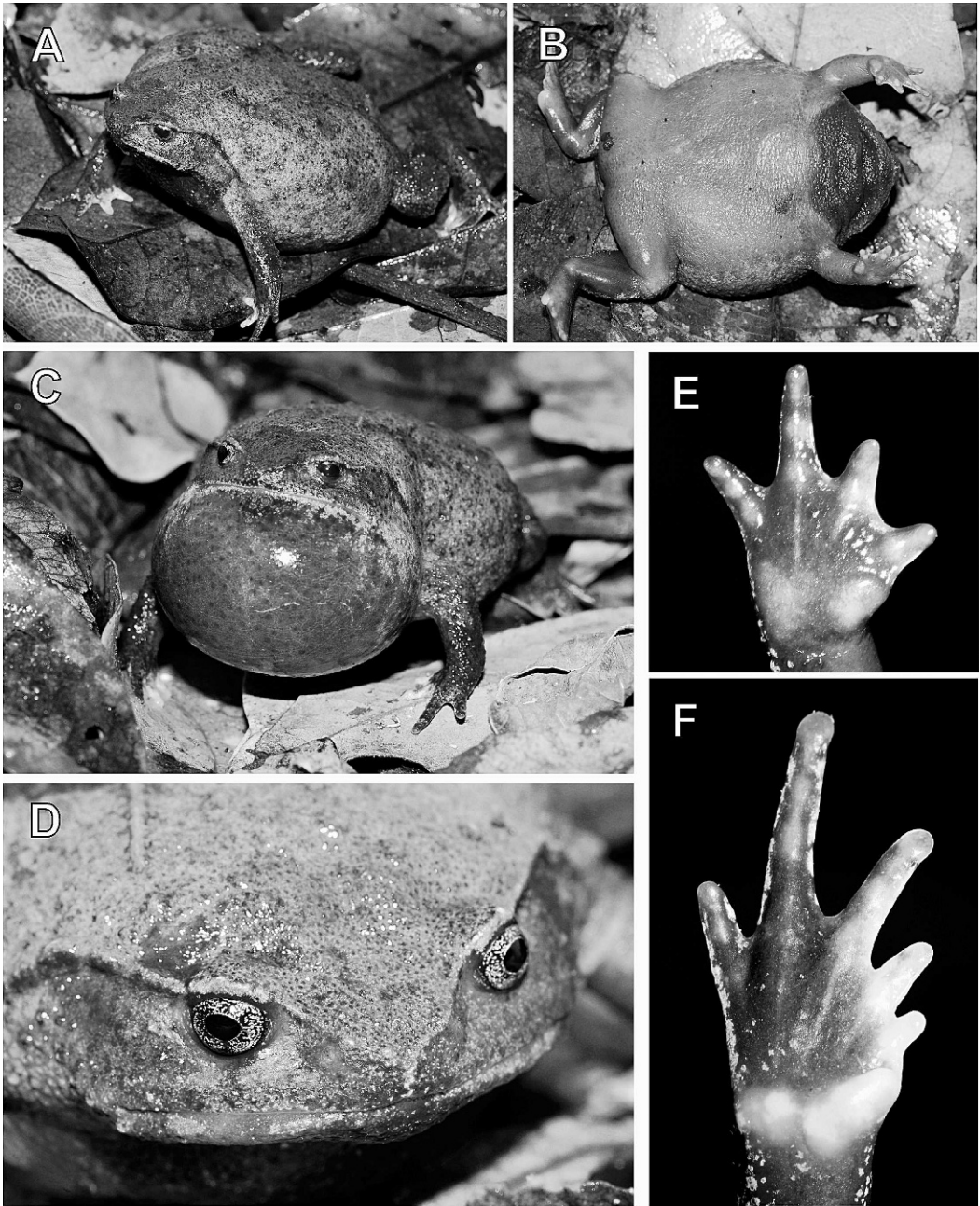


FIG. 1.—*Rhombophryne matavy* sp. nov.: (A) dorsolateral view of male holotype (ZSM 1628/2008) in life; (B) ventral view of holotype in life; (C) holotype calling (after capture, while positioned for taking photos), note nearly fully inflated dark vocal sac; (D) portrait of UADBA paratype (FGZC 1891) in life, note lack of barbels on lower lip; (E) palmar surface of right hand of paratype (ZSM 1629/2008); and (F) plantar surface of right foot of paratype (ZSM 1629/2008).

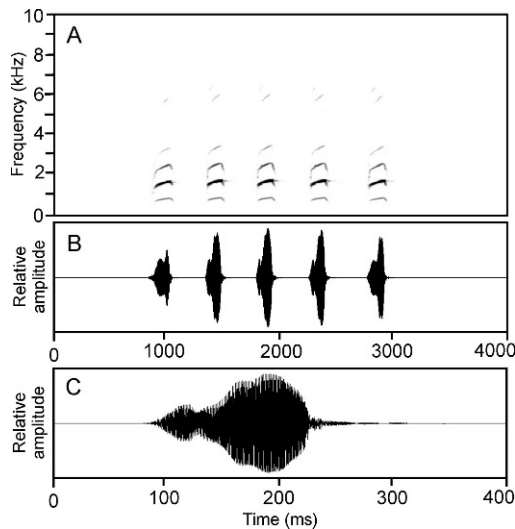


FIG. 2.—Advertisement call of *Rhombophryne matavy* sp. nov., emitted by the holotype (ZSM 1628/2008): (A) spectrogram; (B) corresponding waveform; (C) expanded waveform.

and from several *Plethodontohyla* species by the lack of expanded terminal finger disks and from *Madecassophryne* and *Stumpffia* by much larger size (SVL 39–49 mm versus 11–22 mm). It is distinguished from *Plethodontohyla* by its genetic affinities with other *Rhombophryne* species and its similarity with its type species *R. testudo*. It differs from seven of the eight other *Rhombophryne* species by strong genetic differentiation (Fig. 4, sequences of *R. guentherpetersi* not available); from five of the eight species (*R. coudreaui*, *R. coronata*, *R. guentherpetersi*, *R. minuta*, *R. serratopalpebrosa*) by larger size (39–49 mm versus 22–35 mm SVL); and probably from all eight species by shorter hind legs (tibiotarsal articulation does not reach the insertion of arms versus tibiotarsal articulation reaches between insertion of arms and nostril). *Rhombophryne matavy* differs from *R. coronata*, *R. serratopalpebrosa*, *R. guentherpetersi*, *P. alluaudi*, *P. laevipes*, and *P. minuta* by smaller eyes and the presence of a pair of short, elevated ridges, which run from behind the eye to the neck region. The new species shares the stout appearance, short legs, small eyes, tuberculate dorsum, and dorsal ridges with *R. coudreaui* and *R. testudo*. However, it differs from *R. coudreaui* by less-

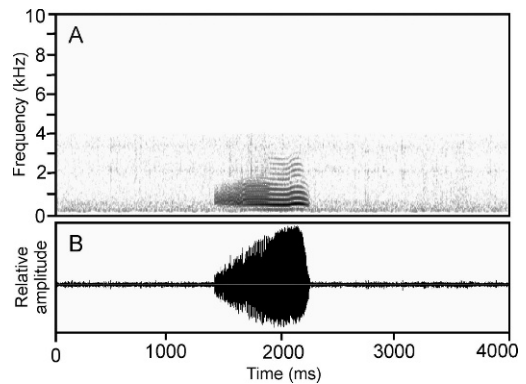


FIG. 3.—Advertisement call of *Rhombophryne testudo* from Nosy Be: (A) spectrogram and (B) corresponding waveform (same time scale as in Fig. 2A, B).

granular skin on the dorsum and larger size. *Rhombophryne matavy* most closely resembles *R. testudo* in overall appearance, but differs by the lack of barbels on the lower lip, a different advertisement call (Figs. 2 and 3), and a pairwise total sequence divergence of 8.5% in the fragment of the mitochondrial 16S RNA gene (see below).

Description of the holotype.—Adult male in good state of preservation, with a tissue sample removed from left shank. For measurements see Table 1. Body very stout, robust; head wider than long, narrower than body; snout short, rounded in dorsal and lateral views; nostrils directed laterally, slightly protuberant, nearer to tip of snout than to eye; eye small, ED 18.2% of HL; canthus rostralis relatively distinct, loreal region concave; tympanum visible but less distinct, TD 112.5% of ED; supratympanic fold distinct in life but much less distinct in preservative. Maxillary teeth absent; vomerine teeth very distinct, forming transversal rows posterior to choanae, starting close to maxillae; choanae ovoid. Arms short, robust; single subarticular tubercles; no recognizable outer metacarpal tubercles; large inner metacarpal tubercle; fingers without webbing; relative length of fingers $1 < 2 \leq 4 < 3$; finger disks not expanded, knob-like. Hind limbs very short, robust; tibiotarsal articulation not reaching insertion of arms when hind limb adpressed along body; lateral metatarsalia connected; large shovel-like inner metatarsal tubercle; indistinct outer metatarsal tubercle present;

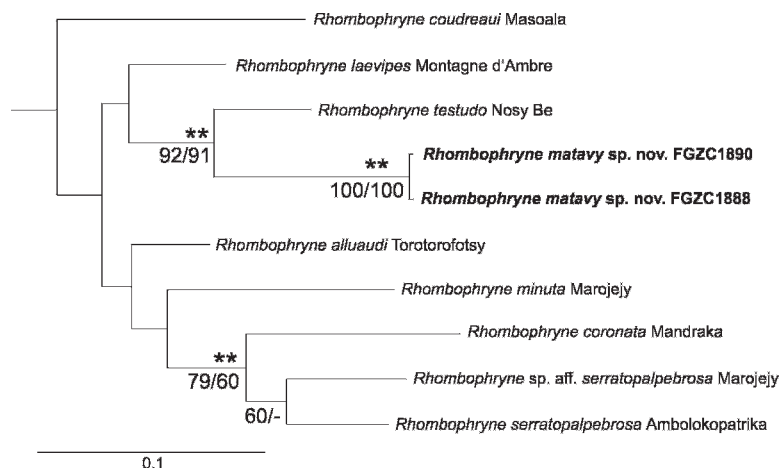


FIG. 4.—Phylogenetic tree of *Rhombophryne* species based on a 532-bp fragment of the mitochondrial 16S rRNA gene. The tree was obtained by Bayesian inference. Posterior probability values of nodes are shown if larger than 0.95; two asterisks denote a support of 0.99–1.00. Numbers at nodes are bootstrap values (%) from analyses with 2000 replicates under maximum likelihood and maximum parsimony optimality criteria, respectively (values <50% not shown). *Platyelis grandis* was used as outgroup (not shown). For sequence accession numbers except those of *R. matavy* sp. nov., see Wollenberg et al. (2008).

only traces of webbing between toes; relative length of toes $1 < 2 < 5 < 3 < 4$; third toe distinctly longer than fifth. Cloacal opening directed posteriorly. Skin on dorsal surface tuberculate, especially on the flanks; two short, slightly converging ridges behind the eyes, less distinct than in life. No dorsolateral fold or color border along the flanks. Skin on venter, chest, and throat relatively smooth.

In life, dorsum brown with irregular darker markings and flecks, partially forming two indistinct irregularly interrupted chevron-shaped markings on middorsum. Upper head, loreal region, and tympanal region slightly

darker than dorsum. Forelimbs and hind limbs dorsally dark brown with some blackish tint. No color border between dorsum and flanks. Fingers 1 and 2 and toes 1–3 dorsally yellowish cream-colored, with some brown flecking, remaining fingers and toes dorsally brown. Cloacal region whitish. Few irregular whitish spots on posterior surfaces of thighs. Venter and ventral surfaces of thighs and arms pinkish grey. Chest pinkish brown, throat blackish. Palmar and plantar surfaces pinkish brown. Palmar tubercles and subarticular tubercles on hand grey. Inner shovel-like metatarsal tubercle grayish white. Inner and

TABLE 1.—Measurements (in mm) of the type specimens of *Rhombophryne matavy* sp. nov. For abbreviations see Materials and Methods.

	Holotype		Paratypes	
	ZSM 1628/2008	ZSM 1629/2008	UADBA (FGZC 1890)	UADBA (FGZC 1891)
SVL	47.6	46.7	39.4	49.4
HW	19.0	18.9	16.1	19.5
HL	13.2	12.3	9.9	13.5
ED	2.4	2.2	1.8	2.6
TD	2.7	2.4	2.1	2.8
E–N	2.4	2.3	1.9	2.5
N–N	4.7	4.7	4.1	4.9
TL	15.1	14.1	13.3	15.5
FL	16.6	15.7	14.5	16.8
FOOTL	21.9	21.8	19.3	22.2

outer iris black with intense copper golden spotting, iris periphery black.

After 11 mo in preservative, uniform dark brown on all dorsal surfaces except the two inner fingers, the three inner toes, and the inner metatarsal tubercle, which are whitish. Throat blackish, chest, belly, and ventral parts of the shanks less dark brown. Ventral surfaces of inner fingers and toes whitish.

Variation.—Measurements of the four known adult males are provided in Table 1 (females are unknown). Apart from differences in body size, there is some slight variation in dorsal skin texture, with the smallest individual, UADBA (FGZC 1890), having a slightly more coarse appearance and apparently more spinuous tubercles. The same specimen lacked the intense copper golden spotting in the upper half of the iris and its dorsum was generally darker-colored in life. The chevron-shaped dark dorsal markings were most distinctly present in ZSM 1629/2008.

Vocalizations.—The advertisement call of the new species emitted by the holotype consists of a series of harmonic notes repeated in regular intervals (Fig. 2). Numerical call parameters are as follows: call duration, 2091–2202 ms (2130 ± 48 ; $n = 3$); number of notes per call, 5 (5 ± 0.0 ; $n = 3$); note duration, 161–209 ms (183 ± 14 ; $n = 15$); internote intervals, 267–353 ms (299 ± 22 ; $n = 12$); maximum call energy at 1588–1688 Hz (1652 ± 16 ; $n = 9$); additional harmonic frequency bands at approximately 880, 2500, 3360, 4160, 5060, and 5840 Hz. The analysis did not reveal recognizable pulses within notes. Notes exhibit distinct amplitude modulation with maximum energy present approximately in the last third of the note; frequency modulation is also recognizable, with a slight upward sweep and a fast terminal drop in each note. The call repetition rate is very variable and apparently strongly dependent on motivation. Prior to an advertisement call, males were observed emitting single soft warm-up notes. A short video sequence showing the calling behavior of the male holotype is provided on www.arkive.org.

In comparison, the advertisement call of the morphologically similar *R. testudo* consists of a single long pulsed note repeated at irregular

intervals (Fig. 3). Furthermore, the dominant frequency in calls is much lower compared to calls of the new species. Numerical parameters of a recording obtained at Nosy Be (type locality) in 1992 (Vences et al., 2006) are as follows: number of notes per call, 1 (1.0 ± 0.0 ; $n = 4$); note duration (=call duration), 823–894 ms (854 ± 30 ; $n = 4$); maximum call energy at 508–524 Hz (519 ± 7 ; $n = 4$); approximately 125 pulses per second.

Vocalizations are furthermore known and analyzed for three other species of *Rhombophryne*, namely *R. minuta*, *R. coronata*, and the undescribed *Rhombophryne* sp. aff. *minuta*, all of them differing from *R. matavy* by distinctly smaller SVL (Glaw and Vences, 2007). The call of *R. minuta* differs from that of *R. matavy* in shorter note duration (114–162 ms), distinctly longer internote intervals (4660–8050 ms), and higher dominant frequency (4030–4230 Hz). As in *R. matavy*, the call of *Rhombophryne* sp. aff. *minuta* is composed of several tonal notes, but it differs by longer internote intervals (419–822 ms) and a higher dominant frequency (3300–3550 Hz). The call of *R. coronata* is different as well, consisting of a series of 5–12 short melodious notes (note duration 65–88 ms) with complex frequency modulation pattern (Vences and Glaw, 2003).

Molecular differentiation.—In a molecular phylogenetic analysis of *Rhombophryne* based on a 532-bp fragment of the mitochondrial 16S rRNA gene, the three different analysis methods used (Bayesian inference, maximum likelihood, and maximum parsimony) yielded highly congruent results (Fig. 4), although the analyzed 16S rDNA fragment was insufficient to resolve many of the deeper relationships within *Rhombophryne*. A strict consensus of two trees selected under the maximum parsimony criterion (tree length 277 steps; consistency index 0.675, retention index 0.516) did not resolve the relationships between the clade containing *R. coronata*, *R. serratopalpebrosa*, and an undescribed species morphologically similar to *R. serratopalpebrosa*, whereas the Bayesian and maximum likelihood analyses supported a clade containing the latter two species (Fig. 4). All analysis methods supported the position of *R. matavy* sister to the morphologically similar *R. testu-*

do. However, the two (identical) included sequences of *R. matavy* had a very high uncorrected pairwise divergence of 8.5% (41 substitutions) to its closest relative, *R. testudo*.

Etymology.—The Malagasy word “matavy” translates to “fat” and refers to the body shape, especially when inflated. It is used as a noun in apposition.

Distribution and ecology.—All known specimens originate from the same lowland rainforest. We found them at night during heavy rainfall, which occurred following a period of approximately 10 days without any rain. All males were calling from the ground covered by a layer of leaf litter. The holotype was found calling from a burrow located approximately 5 cm below a thick patch of leaf litter. This burrow was located close to the base of a small tree, which provided notable canopy cover. Two of the paratypes were excavated from similar situations; the third one was found on the forest floor. Following excavation some individuals showed a defensive position with stretched hind limbs and a concave dorsum, a behavior also observed in *R. testudo* (Glaw and Vences, 2007). Despite the fact that the species was rather abundant at the type locality (a great number of males were heard calling in unison during rain at night and day), our pitfall traps failed to capture any additional specimens, arguing for a rather fossorial mode of life. Nothing is known about the species' reproduction, but a development of juveniles in burrows in the soil with some degree of parental care, as observed in *R. testudo* (Köhler et al., 1997), seems at least possible. The (remarkably large) feces of the holotype (dropped after capture in a plastic bag) contained remains of several medium-sized insects, including head capsules and elytra of Coleoptera.

The currently known range of the species is extremely small (Fig. 5). Although the actual range is very likely to comprise a larger area, we tentatively assume that the new species is endemic to the Forêt d'Ambre Special Reserve (and possibly the lower limits of the Montagne d'Ambre National Park) due to the isolation of this forest from the eastern rainforest block and the Sambirano domain. Using the same rationale and International Union for Conser-

vation of Nature/United Nations (IUCN) criteria as applied during the Global Amphibian Assessment for Malagasy amphibians (see Andreone et al., 2005b) we here classify *Rhombophryne matavy* as “Endangered” (see Discussion).

DISCUSSION

The general appearance of *R. matavy*, namely its very stout body, short limbs, and brown dorsal colors, as well as its strongly fossorial habits largely resemble the known species *R. testudo*. Apart from slight morphological differences between both taxa, pronounced differences in the advertisement call and the sequence of the mitochondrial 16S mtRNA fragment unequivocally corroborate their specific distinctness. However, molecular genetic analyses indeed revealed a close relationship of *R. matavy* and *R. testudo*, both forming a monophyletic clade (Fig. 4). The deeper relationships among *Rhombophryne* species were unresolved by this analysis, probably because the relatively short DNA fragment used and the high divergences between all species left too few informative sites for resolving the phylogeny. However, a more comprehensive analysis by Wollenberg et al. (2008), based on much larger DNA sequences in a large data set of cophyline frogs, obtained only a few supported clades of *Rhombophryne*, i.e., placing *R. alluaudi* in a clade also containing *R. laevipes*, and *R. coronata* in a clade also containing *R. serratopalpebrosa*. Clarifying the evolutionary relationships among *Rhombophryne* species thus remains as a major question that needs to be addressed to understand the biogeography and pattern of evolutionary diversification of these remarkable fossorial frogs.

Rhombophryne testudo appears to be restricted to lowland rainforest habitats and is, except for the erroneous record from La Réunion Island (Glaw and Vences, 2002), only known from the Malagasy islands Nosy Be (type locality) and Nosy Komba in the Sambirano region (Andreone et al., 2003; Fig. 5). One mainland locality, given as “massif du Marojezy” by Guibé (1978) and “Sambava-Andapa” by Blommers-Schlösser and Blanc (1991) respectively, was recently

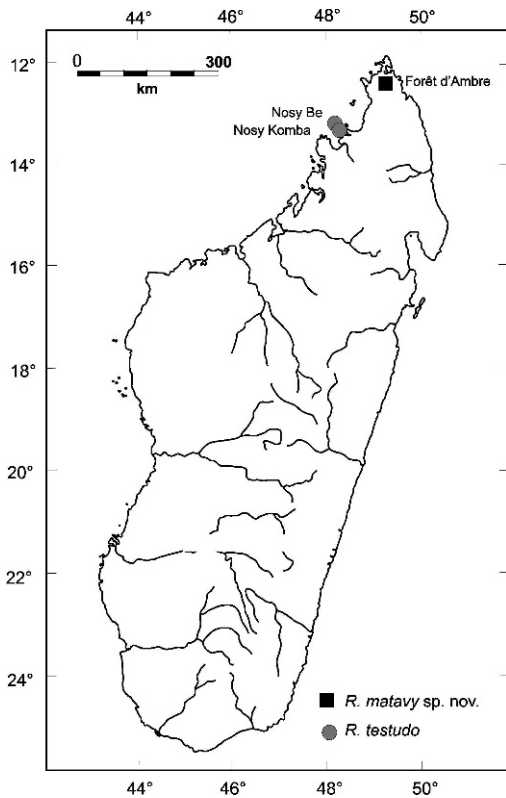


FIG. 5.—Schematic map of Madagascar showing the known distribution of *Rhombophryne matavy* sp. nov. and its closest known relative, *R. testudo*.

shown to refer to *R. coudreau* (Andreone and Randrianirina, 2008).

Rhombophryne matavy is currently known only from the Forêt d'Ambre Special Reserve. This reserve (together with the adjacent Montagne d'Ambre National Park) forms part of the Montagne d'Ambre mountain range, which is volcanic in origin and composed of basaltic rock formed about 14 million yr ago (IUCN/UNEP/WWF, 1987). Possibly, the forest within this mountain range may have been isolated for millions of years and thus may have preserved relict populations of species that disappeared from other regions of the eastern rainforest belt, or it may have facilitated speciation through geographic isolation (Raxworthy and Nussbaum, 1994). Both of these factors would have produced endemics in the Montagne d'Ambre mountain range. This is undoubtedly true for the montane rainforests of the Montagne d'Ambre National

Park, harboring a very high rate of locally endemic species. To some lesser extent, it seems to also hold true for the lowland rainforest of the Forêt d'Ambre Special Reserve, as indicated by our recent surveys and the discovery of undescribed species known from nowhere else in the country (D'Cruze et al., 2008; Köhler et al., 2008). As a result the distributional range of *R. matavy* is probably restricted to this particular area of lowland rainforest, which will have direct implications for its conservation status.

If *R. matavy* is indeed endemic to the Forêt d'Ambre Special Reserve, its area of occupancy (which is defined by the IUCN as the area within the extent of occurrence which is occupied by a taxon) would be very small (less than 50 km²). Its island-like distribution might be a further factor to consider in classifying this species as endangered. In addition, as a result of its close proximity to the administrative capital of Antsiranana province (ca. 30 km) and neighboring communes such as Sakaramy and Joffreville, the natural forest of this reserve is particularly vulnerable to numerous anthropogenic pressures. During our surveys in 2007 and 2008 we observed the following human activities within the protected area: (1) agricultural clearance for banana, coffee, khat, maize, papaya, and rice cultivation; (2) charcoal production; (3) timber production; (4) small-scale quarrying; and (5) zebu grazing. These activities have already seriously altered the forest habitat and it is clear that immediate conservation management action is required to protect the biological diversity found within the Forêt d'Ambre Special Reserve. Given the current situation and state of knowledge, we here tentatively classify *R. matavy* as being "Endangered." The same threat status was applied for the syntopic *Boophis baetkei* which is only known from Forêt d'Ambre as well (Köhler et al., 2008).

The fact that several new species were discovered in this reserve, which actually constitutes one of the oldest protected areas in Madagascar (formally created in 1958 together with the adjacent Montagne d'Ambre National Park), highlights the need for more herpetological survey work. Parallel, rapid, and increasing rates of habitat loss make

efficient protective management and reserve selection an urgent task.

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