

New treefrog of the genus *Boophis* Tschudi 1838 from the northwestern rainforests of Madagascar

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A new species of the *Boophis goudoti* group is described from elevations of 680-1100 m in the Tsaratanana and Manongarivo Massifs in north-western Madagascar. *Boophis axelmeyeri* n. sp. is morphologically similar to *B. burgeri* Glaw & Vences 1994, but differs by its eye coloration which has a red outer iris area. It is strongly differentiated in a fragment of the mitochondrial 16S rRNA gene (6.6% pairwise sequence divergence) and has advertisement calls with longer note durations as compared to *B. burgeri*. Specimens from the Marojejy Massif in north-eastern Madagascar, previously attributed to *B. burgeri*, are placed sister to *B. axelmeyeri* by molecular phylogenetic analysis. They differ by 1.9% DNA sequence divergence, and by a brown versus a red upper iris area, but are here tentatively assigned to *B. axelmeyeri*. This differentiation among mid-altitude amphibian species of Marojejy and Tsaratanana/Manongarivo may be indicative of a process of allopatric isolation among populations from these massifs.

KEY WORDS: Amphibia, Anura, Mantellidae, *Boophis axelmeyeri* n. sp., systematics, phylogeny.

Introduction	238
Materials and methods	238
<i>Boophis axelmeyeri</i> n. sp.	239
Discussion	247
Acknowledgements	248
References	248

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INTRODUCTION

Among the endemic radiation of mantellid frogs from Madagascar, most arboreal species are attributed to the genus *Boophis* Tschudi 1838. With currently almost 50 species (GLAW & VENCES 2003), *Boophis* is the second largest anuran genus from Madagascar. Although *Boophis* have evolved an important diversity in morphology and ecological adaptations (BLOMMERS-SCHLÖSSER & BLANC 1991, CADLE 2003), they still are all characterized by a typical treefrog morphology with enlarged terminal disks of fingers and toes, broad head, and relatively large eyes. They have a generalized reproductive behavior with aquatic egg deposition and tadpoles developing in free water. Based on their reproduction, *Boophis* can be divided into one clade specialized to breed in running water, and an assemblage of lentic breeders (VENCES et al. 2002, 2003b).

The recent steep increase in recognized species numbers of Malagasy frogs was especially distinct in *Boophis*. The *B. luteus* group and the *B. majori* group, thought to contain one and three species less than 15 years ago (BLOMMERS-SCHLÖSSER & BLANC 1991) are now known to contain 12 and eight species, respectively (GLAW & VENCES 2003). The new discoveries are due to a combination of increased survey work and the application of bioacoustic techniques which allowed distinguishing many species that are morphologically similar but that subsequently turned out to be genetically highly divergent (VENCES et al. 2002).

In this paper we describe a new species assigned to the *B. goudoti* group, one out of the seven groups of stream-breeding *Boophis*. We diagnose the new taxon, discovered during surveys in the Sambirano region in north-western Madagascar, by morphological, chromatic and bioacoustic characters. We provide a hypothesis of its phylogenetic relationships based on DNA sequences from the mitochondrial 16S rRNA gene.

MATERIALS AND METHODS

Frogs were captured by locating calling males during the night. They were euthanised using a 1% chlorobutanol solution, fixed in either 5% formalin or 95% ethanol, and preserved in 70% ethanol. Institutional abbreviations are as follows: MRSN, Museo Regionale di Scienze Naturali, Torino; ZFMK, Zoologisches Forschungsmuseum A. Koenig, Bonn; ZMA, Zoological Museum Amsterdam; ZSM, Zoologische Staatssammlung München.

Measurements were taken to the nearest 0.1 mm using a caliper: snout-vent length (SVL); maximum head width (HW); head length from tip of snout to posterior edge of snout opening (HL); horizontal tympanum diameter (TD); horizontal eye diameter (ED); distance between anterior edge of eye and nostril (END); distance between nostril and tip of snout (NSD); distance between both nostrils (NND); forelimb length, from limb insertion to tip of longest finger (FORL); hand length, to the tip of the longest finger (HAL); hindlimb length, from the cloaca to the tip of the longest toe (HIL); tibia length (TIL); foot length including tarsus (FOTL); foot length (FOL). Webbing formula follows MYERS & DUELLMAN (1982) and SAVAGE & HEYER (1997). To facilitate comparisons, in the holotype description we also give the formula used by BLOMMERS-SCHLÖSSER (1979) and most subsequent authors who published accounts on Malagasy anurans.

Muscle tissue samples were taken from freshly killed adult and larval specimens in the field and preserved in pure ethanol. DNA was extracted and a fragment of the mitochondrial 16S rRNA gene amplified and sequenced using the primers 16SA-L and 16SB-H (PALUMBI et al.

1991). Sequences were aligned to those of other *Boophis* species, as obtained by VENCES et al. (2002) and available from Genbank, using the software Sequence Navigator (Applied Biosystems). Because several regions of the alignment required the inclusion of gaps to account for multiple indels and could therefore not reliably aligned, we performed analyses after excluding these hypervariable sections. Phylogenetic analysis was carried out using PAUP, version 4b10 (SWOFFORD 2003). We performed a maximum likelihood analysis after determining the substitution model that best fits our data through hierarchical likelihood ratio tests as implemented in Modeltest (POSADA & CRANDALL 1998). Furthermore, we carried out unweighted maximum parsimony heuristic searches, with tree-bisection reconnection branch swapping, and random sequence addition with 100 replicates. Robustness of nodes was tested by full heuristic bootstrapping, with 500 pseudoreplicates under maximum likelihood and 2000 pseudoreplicates (and 10 random addition sequence replicates) under maximum parsimony. DNA sequences were deposited in Genbank (accession numbers of *B. axelmeyeri* from Tsaratanana and Manongarivo: DQ118669 and DQ118668; accession numbers of the other species used for comparison included in the series AJ315909-315920 and AY847959-AY848683).

Advertisement calls were recorded with different tape recorders and microphones, and were analysed with the program CoolEdit (Syntrillium Corp.) on a PC. Temporal measurements in the call description are given in milliseconds, as range with mean \pm standard deviation and number of measured temporal units in parentheses.

Boophis axelmeyeri n. sp.

Holotype. Adult male, ZSM 627/2001 (Figs 1-2) from Manarikoba forest, Camp I (Antsahamanara), Tsaratanana Massif, Marovato Fivondronana, Antsiranana Faritany (Diégo Suarez Province), northwestern Madagascar, 14°02'42"S, 48°47'04"E, ca 1000 m above sea level, collected by F. Andreone, F. Mattioli, J. Randrianirina and M. Vences on 3.II.2001.

Paratypes. 15 adult males. ZSM 626/2001, same collection data as holotype; MRSN A2509-2515, same collecting data as holotype except earlier collecting date (1.II.2004); ZSM 628-631/2001, same collecting data as holotype except later collecting date (4-9.II.2001); MRSN A2516, same collecting data as holotype except later collecting date (5.II.2001); MRSN A2508, from Tsaratanana, Antsamahanintsy (no coordinates recorded), collected by J.E. Randrianirina on 21.XII.2000. ZSM 837/2003, collected at Manongarivo Special Reserve, northwestern Madagascar, 13°58'32"S, 48°25'36"E, 688 m, by F. Glaw, R.-D. Randrianiana and M. Vences on 2.II.2003.

Other specimens. Three males from the Marojejy Massif, collected on 26 February 1995 at an elevation of ca 700 m by F. Glaw and O. Ramilison, are here attributed to *B. axelmeyeri* in a preliminary way, based on molecular and bioacoustic similarities (see below): ZFMK 59904, 59905 and 59923.

Diagnosis. A species of *Boophis* based on the presence of intercalary element between ultimate and penultimate phalanges of fingers and toes (as verified by external examination), presence of nuptial pads in males, and phenetic similarities to species in the *Boophis goudoti* group. Assigned to the *Boophis goudoti* group by webbing between fingers and evidence of heel spine (a combination not found in any other *Boophis* group), and network of dermal ridges on back (unique to a subset of species in the *B. madagascariensis* group). Within the group, distinguished from *B. goudoti* Tschudi 1838 and *B. periegetes* Cadle 1995 by much smaller size (SVL of males 36-43 vs 50-75 mm), presence of heel spine (vs absence) and of dorsal ridge network (vs absence); from *B. madagascariensis* (Peters 1874) and *B.*



Fig. 1. — Holotype of *Boophis axelmeyeri* (ZSM 627/2001) from Antsahamanara, Manarikoba forest, Tsaratanana Massif, in life, dorsal view.



Fig. 2. — Holotype of *Boophis axelmeyeri* (ZSM 627/2001) from Antsahamanara, Manarikoba forest, Tsaratanana Massif, in life, ventral view.

brachychir (Boettger 1882) by smaller size (36-43 vs > 60 and 46-49 mm) and presence of dorsal ridge network (vs absence); from *B. boehmei* Glaw & Vences 1992 by much larger size (male SVL 36-43 vs 25-30 mm); from *B. reticulatus* Blommers-Schlösser 1979 and *B. rufiocularis* Glaw & Vences 1997 by larger size (36-43 vs 29-37 mm) and different iris color. The new species is morphologically similar to *Boophis burgeri*, but differs from this species by a distinct red area in the upper iris (vs uniformly light beige iris), shorter hindlimb length (ratio TIBL/SVL 0.49-0.56 vs 0.56-0.58), and by advertisement calls (see below; longer duration of notes both of type 1 and type 2). Furthermore, the strong molecular differentiation of the new species relative to all other species of the *B. goudoti* group corroborates its specific status.

Description of the holotype. Adult male (collected while calling) in good state of preservation. Muscle tissue from left femur removed for molecular analysis. For measurements see Table 1. Body moderately slender; head longer than wide, slightly wider than body; snout rather sharply pointed in dorsal and lateral view; nostrils directed laterally, protuberant, closer to tip of snout than to eye; canthus rostralis very distinct, slightly concave; loreal region concave; tympanum distinct, small, its diameter 45% of eye diameter; supratympanic fold distinct and straight; tongue ovoid, distinctly bifid posteriorly; vomerine teeth as one distinct oblong group posterolateral of each choana, the two groups not in median contact; choanae large, elliptical. Forelimbs slender; subarticular tubercles single; inner and outer metacarpal tubercles not recognizable. Webbing among fingers: $\text{II}^2 - ^3\text{III}^{2+} - ^2\text{IV}$. Webbing according to the notation of BLOMMERS-SCHLÖSSER (1979): 1(traces), 2i(traces), 2e(1), 3i(2), 3e(1.25), 4i(1); relative finger length $1 < 2 < 4 < 3$; finger disks enlarged; unpigmented nuptial pads on inner side of first finger. Legs slender, when legs are adpressed along body, the tibiotarsal articulation reaches between eye and nostril; lateral metatarsalia separated; inner metatarsal tubercle small, outer metatarsal tubercle absent; webbing of the foot: $\text{I}^1 - ^2\text{II}^1 - ^2\text{III}^1 - ^2+\text{IV}^{2+} - ^1\text{V}$; webbing according to the notation of BLOMMERS-SCHLÖSSER (1979) 1(0), 2i(1); 2e(0); 3i(1); 3e(0); 4i(1.25), 4e(1.5), 5(0); relative toe length $1 < 2 < 3 < 5 < 4$. Skin on the dorsum quite smooth, with a little prominent but distinct irregular network of dermal ridges. Ventral skin slightly granular on throat and chest, granular on belly. Distinct whitish tubercles in the cloacal region. Distinct short dermal spine on heel, small tubercle also on elbow.

Color of the holotype in life (Figs 1-2) dorsally dark brown; legs and arms grayish brown with dark brown crossbands. Iris beige with brown speckles next to pupilla, and a bright red upper outer iris area (Fig. 1). Iris periphery turquoise. Ventrally white with dark brown speckles on throat, chest and belly, translucent blueish with brown speckles on limbs.

After 3 years in preservative, dorsum uniformly grayish brown. Hindlimbs and forelimbs light gray brown with dark brown crossbands (8-9 on femur, 5-6 on tibia, 10-15 on tarsus and foot, 4-5 on forelimb). Tympanic region light brown, sharply separated from darker dorsal color by supratympanic fold. Dorsally whitish with fine brown speckles externally on throat, on belly and limbs.

Variation. All paratypes agree morphologically with holotype. In some specimens, the dark crossbands on limbs are less distinct. The dorsal color is more reddish brown in some individuals and more grayish in others. Isolated small dark patches are present on the dorsum of a few individuals. The specimens from

Table 1.

Morphological measurements of type specimens of *Boophis axelmeyeri* and of the similar species *B. boehmei*, *B. burgeri*, *B. reticulatus* and *B. rufioculis*.

Species and specimens	Status	Sex	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	TIL	RHL
<i>Boophis axelmeyeri</i>																	
ZSM 627/2001	HT	M	40.0	14.7	15.6	2.5	5.6	3.1	3.6	4.1	23.9	12.7	64.3	28.1	16.1	20.2	2
ZSM 626/2001	PT	M	35.5	13.6	13.7	2.4	5.1	2.6	3.0	3.8	23.4	10.7	61.5	19.7	14.9	19.8	5
ZSM 628/2001	PT	M	36.2	14.3	14.3	2.3	5.0	2.6	3.2	4.4	24.2	11.5	65.1	27.5	15.8	20.3	4
ZSM 629/2001	PT	M	37.2	14.0	15.0	2.2	5.2	2.7	3.4	4.5	24.2	11.5	62.0	27.0	15.8	19.5	2
ZSM 630/2001	PT	M	40.3	15.3	15.8	2.4	5.0	3.2	3.3	4.6	24.6	12.7	65.4	28.2	16.9	20.7	2
ZSM 631/2001	PT	M	41.8	15.0	15.8	2.6	5.0	3.0	3.0	4.8	26.2	13.3	65.7	28.5	17.1	20.4	2
MRSN A2511	PT	M	38.8	14.1	15.0	2.5	4.8	2.8	3.4	4.3	22.3	11.7	62.3	26.6	16.0	20.0	3
MRSN A2513	PT	M	40.3	14.3	15.0	2.6	4.2	2.8	3.4	4.7	22.6	12.5	65.0	27.2	16.0	20.6	3
MRSN A2514	PT	M	38.2	13.9	14.2	2.3	4.6	2.6	3.1	4.0	21.7	11.2	62.7	26.6	15.8	19.8	3
MRSN A2512	PT	M	43.1	15.1	15.8	2.1	4.8	3.0	3.2	3.9	25.5	13.2	66.3	28.8	17.3	21.3	2
MRSN A2509	PT	M	40.7	14.8	14.9	2.1	4.9	2.9	3.6	4.3	23.4	12.8	63.4	27.7	16.2	20.2	3
MRSN A2515	PT	M	41.2	15.2	15.5	2.2	5.7	3.0	3.3	4.4	25.4	12.1	66.7	28.4	17.0	20.9	2
MRSN A2510	PT	M	41.2	14.5	15.5	2.4	5.0	2.6	3.0	4.3	25.6	12.6	66.1	28.8	17.2	21.2	1
MRSN A2516	PT	M	36.2	13.0	13.6	1.8	4.4	2.3	3.0	3.8	23.0	12.0	63.0	27.8	16.3	20.0	3
MRSN A2508	PT	M	36.1	14.7	14.3	2.4	4.4	2.7	3.1	4.5	24.7	12.1	65.3	28.3	16.8	20.2	3
ZSM 837/2003	PT	M	41.2	14.7	16.5	2.6	5.6	3.0	3.3	4.9	26.0	12.6	67.3	28.0	16.9	21.7	3
<i>Boophis boehmei</i>																	
ZFMK 53642	HT	M	29.4	11.3	11.9	2.0	3.9	2.4	2.3	3.2	17.9	9.0	48.4	21.2	12.4	16.4	3
ZFMK 52639	PT	M	28.5	10.7	11.6	2.0	3.6	2.2	2.2	3.0	18.4	9.3	49.3	21.3	12.5	15.6	3

ZFMK 52637	PT	M	27.8	10.8	11.5	2.0	4.0	2.2	2.3	3.0	17.1	8.5	46.6	19.9	11.2	15.3	3
ZFMK 52638	PT	M	28.7	11.4	11.7	2.1	4.2	2.3	2.4	3.6	18.2	8.9	49.2	20.6	12.3	14.9	3
<i>Boophis burgeri</i>																	
ZFMK 53641	HT	M	37.9	13.7	14.5	2.6	5.0	3.3	3.0	4.2	23.3	11.9	68.3	29.1	16.9	22.0	5
ZFMK 57405	PT	M	38.1	13.5	14.0	2.3	5.0	3.0	3.0	4.5	22.0	11.2	66.3	27.3	15.3	21.3	5
<i>Boophis reticulatus</i>																	
ZMA 7101A(945)	HT	M	33.0	12.4	12.4	2.4	4.1	2.9	2.3	3.6	22.7	11.4	59.8	26.3	16.0	18.2	
ZMA 7101B (947)	PT	M	31.0	11.6	12.0	2.0	4.4	2.5	2.4	3.5	21.0	10.8	56.4	24.1	13.6	17.4	
ZMA 7101B (948)	PT	M	31.0	11.1	11.9	2.3	4.6	2.5	2.4	3.3	18.8	10.2	55.2	24.0	14.0	17.3	3
ZMA 7101B (975)	PT	M	29.3	10.7	11.8	2.4	4.3	2.5	2.3	3.5	21.2	10.0	54.8	23.4	14.4	17.4	3
ZMA 7101B (974)	PT	M	29.7	10.9	11.7	2.2	4.4	2.1	2.3	3.4	18.6	10.1	54.4	23.8	14.2	16.9	4
ZMA 7101B (946)	PT	M	35.0	12.4	12.4	2.2	4.3	2.8	2.3	4.0	21.7	11.4	57.6	25.4	15.1	17.7	3
ZMA 7101B (973)	PT	M	33.0	11.3	12.4	2.2	4.4	2.2	2.5	3.9	22.0	11.1	58.6	25.8	15.5	18.4	4
ZMA 7101B (1024)	PT	M	30.1	10.7	12.0	2.0	4.5	2.5	2.3	3.5	19.9	9.3	53.3	23.0	13.7	17.0	4
ZMA 7101B (944)	PT	M	33.2	12.1	12.5	2.3	4.8	2.3	2.4	3.5	19.0	10.5	57.4	25.4	15.3	17.7	3
<i>Boophis rufioculis</i>																	
ZFMK 60079	HT	M	32.9	12.5	13.4	2.2	4.2	3.4	2.2	3.6	20.7	10.3	58.6	25.0	14.0	19.1	5
ZFMK 60080	PT	M	34.9	13.7	13.7	2.7	5.0	3.1	2.9	4.1	22.0	11.1	61.7	26.9	15.7	19.6	5
ZFMK 62250	PT	M	32.6	12.5	13.3	2.6	4.8	2.9	2.5	3.6	21.3	10.8	59.2	25.4	15.1	19.0	5
ZFMK 62249	PT	M	47.3	15.7	17.0	3.1	5.5	3.6	3.7	5.0	28.2	14.9	77.5	34.0	20.3	25.4	2

For abbreviations of measurements see Materials and methods. Additional abbreviations used: HT, holotype; PT, paratype; M, male; F, female; RHL, relative hindlimb length, given as point reached by the tibiotarsal articulation when hindlimb is addressed along body. RHL is coded as follows: tibiotarsal articulation reaches (1) the anterior eye corner, (2) between eye and nostril, (3) nostril, (4) tip of snout, (5) beyond tip of snout.

Marojejy agree in general morphology but in life have a browner inner iris and a dark brown (not red) color on the upper part of the iris.

Etymology. We are pleased to dedicate this species to Axel Meyer, Konstanz, in recognition of his important contributions to the understanding of the origins and diversity of the Malagasy fauna, especially cichlid fishes and amphibians. The red eye color of the new frog appropriately representing desperate days of discussion and exhaustive nights of work that we shared with our workaholic friend and colleague.

Natural history. Calling males were observed at night, at perch heights of 2-3 m on branches of bushes and trees along small rainforest streams (1-2 m width).

Advertisement calls. Vocalizations were recorded at the type locality on 5.II.2001 at an air temperature of 20.5 °C. As in other species of the *Boophis goudoti* group (e.g., NARINS et al. 2000) their structure was complex and they were composed of different unharmonious note types. Single notes of type 1 (Fig. 3a) were usually emitted after irregular intervals of several seconds or minutes. Note duration was 132-161 msec (150 ± 10 msec; $n = 7$). In one case two such notes were emitted after a short interval of 830 msec. Frequency was between 1500-3000 Hz. Notes of type 2 were emitted in short series (Fig. 3b). Two such series contained 10 notes each and had durations of 1802-2004 msec. Note duration was 38-80 msec (61 ± 9 msec; $n = 14$), duration of intervals between notes was 104-157 msec (135 ± 15 msec; $n = 12$). Note duration tended to increase towards the end of the series. These notes had an indistinctly pulsed structure; 10-14 (12 ± 2 ; $n = 8$) pulses were recognizable on the oscillograms. Frequency of these notes was 1800-2500 Hz.

Advertisement calls of related species. Calls of *Boophis burgeri*, the species that is morphologically more similar to *B. axelmeyeri*, were undescribed so far. Recordings were made on 7.XII.2001, 18:30-19:00 hr, at Andasibe, central eastern Madagascar, 24.8 °C air temperature. The calls consisted of two unharmonious note types. Notes of type 1 were emitted singly (Fig. 4) or in short series of 6-8 notes. Note duration was 42-78 msec (56 ± 10 msec; $n = 10$), duration of intervals between notes in a series was 602-871 msec (733 ± 79 msec; $n = 10$). Note intensity in a series first increased and then decreased towards the end of a series. Note structure was indistinctly pulsed, with 12-17 (14 ± 2 ; $n = 7$) pulses recognizable per note. Frequency was 1750-3000 Hz, with some weak frequency bands up to 6000 Hz. Notes of type 2 were typically arranged in a short series of 2-3 notes preceded by one note of type 1 (Fig. 4). Note duration was 21-37 msec (27 ± 5 msec; $n = 8$), duration of intervals between notes of type 2 was 65-95 msec (76 ± 12 msec; $n = 5$), duration of intervals between first note of type 2 and preceding note of type 1 was 76-92 msec (82 ± 9 msec; $n = 3$). Notes of type 2 were composed of 47 (5 ± 1 ; $n = 6$) indistinct pulses and had frequencies of 1750-4000 Hz.

Molecular relationships. The alignment of the 16S rDNA sequences had a length of 539 characters. After exclusion of short segments that could not be reliably aligned due to multiple insertions and deletions, 523 characters were included in the analysis. Of these, 371 were constant and 103 were parsimony-informative. Maximum parsimony recovered two equally parsimonious trees (not shown) with a length of 347 steps and a consistency index of 0.591. A strict consensus of these

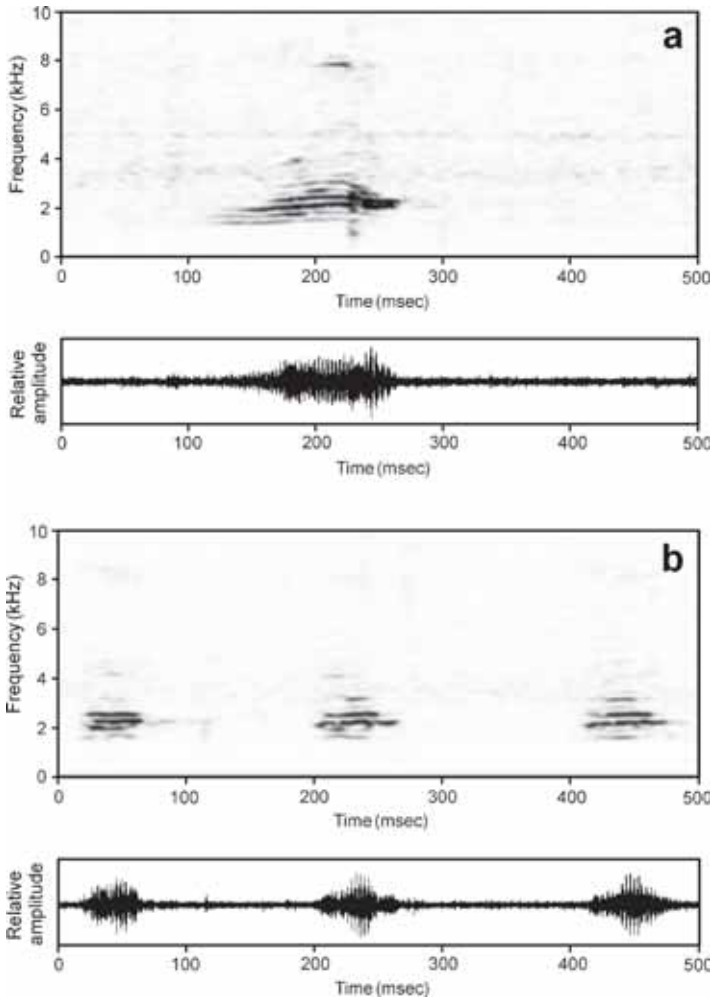


Fig. 3. — Sonograms and oscillograms of the advertisement call of *Boophis axelmeyeri*: (a) single note of type 1, (b) part of a series of notes of type 2, recorded at Antsahamanara, Tsaratanana Massif.

grouped the species of the *B. goudoti* group except for *B. rhodoscelis* as monophyletic group. *B. rhodoscelis* was placed with two species belonging to the *B. microtyimpanum* group (*B. microtyimpanum* and *B. williamsi*). Within the *B. goudoti* group, the following monophyletic clades were recovered: (1) a clade with the two specimens of *B. axelmeyeri* and a third specimen from Marojejy possibly belonging to this species; (2) a clade with *B. brachychir* as sister group of *B. goudoti* and *B. cf. periegetes*; (3) a clade with *B. reticulatus* and *B. rufioculis*. A maximum likelihood tree, calculated using a general time-reversible (GTR + G) substitution model selected by Modeltest (POSADA & CRANDALL 1998), with empirical base frequencies and substitution rates and a gamma distribution shape parameter of 0.1704, recovered

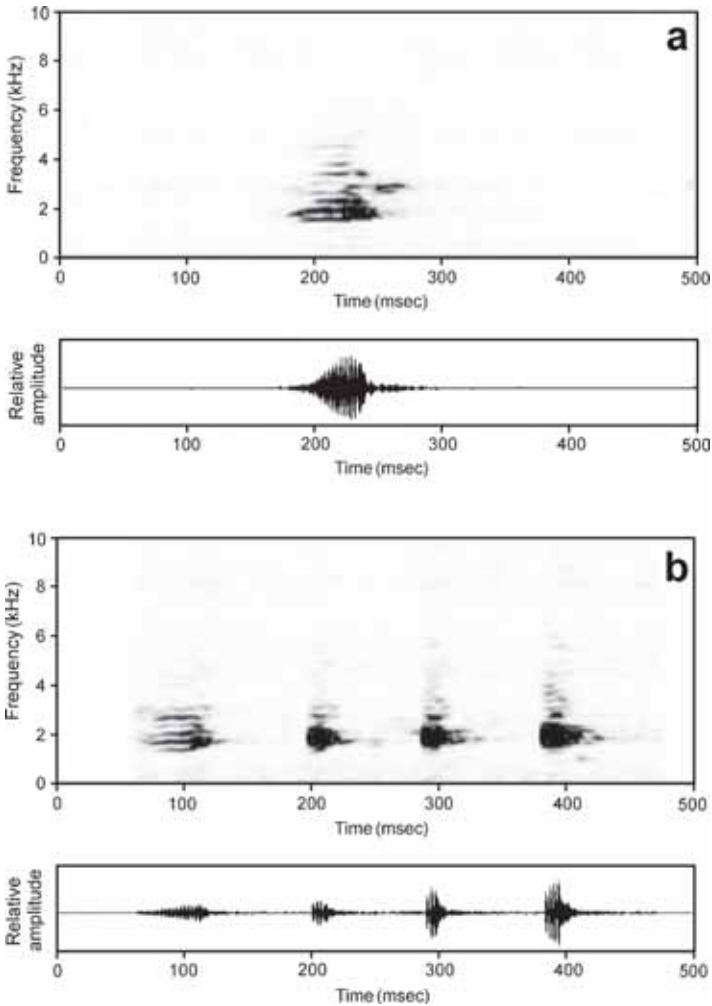


Fig. 4. — Sonograms and oscillograms of the advertisement call of *Boophis burgeri*, recorded at Andasibe: (a) single note of type 1, (b) one note of type 1, followed by three notes of type 2.

a topology (Fig. 5) largely compatible with that of the maximum parsimony tree. Maximum likelihood and maximum parsimony bootstrap analysis indicated that most groupings in the tree were not sufficiently supported (Fig. 5). The uncorrected genetic pairwise divergences of *B. axelmeyeri* (all characters included) to the other species included in the *B. goudoti* group were 6.4–9.3% (6.6% to the morphologically closest species *B. burgeri*), whereas the divergences among these species ranged from 2.7–11.4%. The divergence of the *B. axelmeyeri* specimens from Manongarivo and Tsaratanana to that from Marojejy was 1.9%. The two sequences from Manongarivo and Tsaratanana were fully identical.

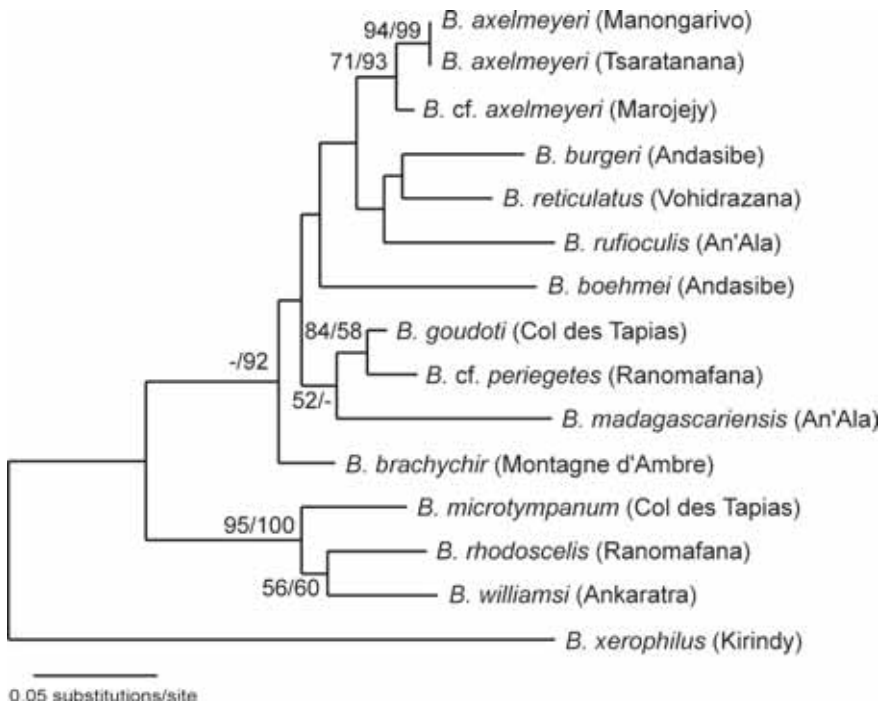


Fig. 5. — Phylogenetic relationships of species in the *Boophis goudoti* group and the *B. microtympanum* group. The phylogram was calculated under the maximum likelihood optimality criterion based on 523 base pairs of the mitochondrial 16S rRNA gene, after exclusion of hypervariable and gapped sites. Numbers are support values in percent from a maximum likelihood analysis (500 replicates) and from a maximum parsimony analysis (2000 replicates); values below 50% not shown. Localities of specimens sequenced are given in parentheses after the species name.

DISCUSSION

In a previous study, GLAW & VENCES (1997) analyzed specimens from Marojejy that they attributed to *Boophis burgeri* in a preliminary way, already stressing their different iris coloration. However, at that state no call recordings of *Boophis burgeri* from its type locality were available, and a direct bioacoustic comparison of the Marojejy population was therefore not possible. The molecular data presented here (Fig. 5) grouped the Marojejy species clearly with *B. axelmeyeri* but also indicated a relevant differentiation (1.9% pairwise 16S divergence) between the two forms. However the few available bioacoustic data on the Marojejy population as described in GLAW & VENCES (1997) are not conclusive; note duration was 48–65 msec, but it is not sufficiently clarified whether these notes, emitted by specimens of probably low motivation, are homologous to notes of type 1 or 2 as defined herein. More data are necessary to ascertain whether the Marojejy specimens represent an allopatric population of *B. axelmeyeri* of substantial genetic differentiation, or are yet another species of this treefrog group.

A previous study of frogs in the genus *Mantidactylus* Boulenger 1895 indicated that populations of the low-altitude species *M. granulatus* (Boettger 1881) from Tsaratanana and Andapa (near Marojejy) show a lower genetic differentiation than we here report for *B. axelmeyeri* and *B. cf. axelmeyeri* from the two regions (VENCES et al. 2003a). Although we found *B. axelmeyeri* at Manongarivo below 700 m elevation, the species seems to be absent from low-altitude localities in north-western Madagascar, such as Nosy Be (ANDREONE et al. 2003) and Benavony (GLAW & VENCES 1994). Unlike *Mantidactylus granulatus*, *B. axelmeyeri* also appears to have been unable to disperse to the isolated Montagne d'Ambre rainforest in extreme northern Madagascar (RAXWORTHY & NUSSBAUM 1994). This might be seen as an indication that the species is specialized to mid-altitude rainforest. The examples of *M. granulatus* and *B. axelmeyeri* could indicate a trend of increasing endemism with increasing elevation among frogs from north-eastern and north-western Madagascar. According to WELLS (2003) the north-western Monsoon rainforest of Madagascar may have originated only in the Late Miocene or Pliocene, and the initial colonization of the area by rainforest-specialized groups of frogs may have occurred in this period. Ancestral populations of *B. axelmeyeri* may have subsequently remained isolated on the Marojejy and Tsaratanana/Manongarivo Massifs and evolved their distinctive chromatic and molecular features in allopatry.

Populations of *B. axelmeyeri* were found within the boundaries of two protected areas, namely the Tsaratanana Strict Nature reserve and the Manongarivo Special Reserve. The third known population, attributed to *B. axelmeyeri* in a preliminary way, also is located within a reserve (Marojejy National Park). The mid-altitude forests in these reserves are relatively extensive and no immediate threats for *B. axelmeyeri* are currently obvious despite the apparent specialization of the species to undisturbed forest. However, the presence of these and other local endemics in Manongarivo and Tsaratanana highlights the need for effective protection of the north-western rainforests of Madagascar.

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REFERENCES

- ANDREONE F., GLAW F., NUSSBAUM R.A., RAXWORTHY C.J., VENCES M. & RANDRIANIRINA J.E. 2003. The amphibians and reptiles of Nosy Be (NW Madagascar) and nearby islands: a case study of diversity and conservation of an insular fauna. *Journal of Natural History* 37: 2119-2149.

- BLOMMERS-SCHLÖSSER R.M.A. 1979. Biosystematics of the Malagasy frogs. I. Mantellinae (Ranidae). *Beaufortia* 352: 1-77.
- BLOMMERS-SCHLÖSSER R.M.A. & BLANC C.P. 1991. Amphibiens (première partie). *Faune de Madagascar* 75: 1-379.
- CADLE J.E. 2003. Genus *Boophis*, pp. 916-919. In: Goodman S.M. & Benstead J.P., Edits. The Natural History of Madagascar. *Chicago and London: The University of Chicago Press*, 1709 pp.
- GLAW F. & VENCES M. 1994. A fieldguide to the amphibians and reptiles of Madagascar, 2nd Ed. *Köln, Germany: Vences and Glaw Verlag*, 480 pp.
- GLAW F. & VENCES M. 1997. Neue Ergebnisse zur *Boophis goudoti*-Gruppe aus Madagaskar: Bioakustik, Fortpflanzungsstrategien und Beschreibung von *Boophis rufioculis* sp. nov. *Salamandra* 32: 225-242.
- GLAW F. & VENCES M. 2003. Introduction to Amphibians, pp. 883-898. In: Goodman S.M. & Benstead J.P., Edits. The Natural History of Madagascar. *Chicago and London: The University of Chicago Press*, 1709 pp.
- MYERS C.W. & DUELLMAN W.E. 1982. A new species of *Hyla* from Cerro Colorado, and other tree frog records and geographical notes from western Panama. *American Museum Novitates* 2752: 1-32.
- NARINS P.M., LEWIS E.R. & MCCLELLAND B.E. 2000. Hyperextended call note repertoire of the endemic Madagascar treefrog *Boophis madagascariensis* (Rhacophoridae). *Journal of Zoology, London* 250: 283-298.
- PALUMBI S.R., MARTIN A., ROMANO S., MCMILLAN W.O., STICE L. & GRABOWSKI G. 1991. The Simple Fool's Guide to PCR, Version 2.0. *Privately published document compiled by S. Palumbi, Department of Zoology, University of Hawaii, Honolulu*.
- POSADA D. & CRANDALL K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- RAXWORTHY C.J. & NUSSBAUM R.A. 1994. A rainforest survey of amphibians, reptiles and small mammals at Montagne d'Ambre, Madagascar. *Biological Conservation* 69: 65-73.
- SAVAGE J.M. & HEYER W.R. 1997. Digital webbing formulae for anurans: a refinement. *Herpetological Review* 28: 131.
- SWOFFORD D.L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods), Version 4b10. *Sunderland, Massachusetts: Sinauer Associates*.
- VENCES M., ANDREONE F., GLAW F., KOSUCH J., MEYER A., SCHAEFER H.-C. & VEITH M. 2002. Exploring the potential of life-history key innovation: brook breeding in the radiation of the Malagasy treefrog genus *Boophis*. *Molecular Ecology* 11: 1453-1463.
- VENCES M., ANDREONE F., GLAW F. & RANDRIANIRINA J.E. 2003a. Molecular and bioacoustic divergence in *Mantidactylus granulatus* and *M. zavona* sp. n. (Anura: Mantellidae): bearings for the biogeography of northern Madagascar. *African Zoology* 38: 67-78.
- VENCES M., VIEITES D.R., GLAW F., BRINKMANN H., KOSUCH J., VEITH M. & MEYER A. 2003b. Multiple overseas dispersal in amphibians. *Proceedings of the Royal Society of London (B)* 270: 2435-2442.
- WELLS N.A. 2003. Some hypotheses on the Mesozoic and Cenozoic paleoenvironmental history of Madagascar, pp. 16-34. In: Goodman S.M. & Benstead J.P., Edits. The Natural History of Madagascar. *Chicago and London: The University of Chicago Press*, 1709 pp.