

## A new cryptic treefrog species of the *Boophis luteus* group from Madagascar: bioacoustic and genetic evidence

(Amphibia, Anura, Mantellidae)

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We describe a new sibling species of *Boophis ankaratra* from Madagascar: *Boophis schuboeae*, spec. nov. from the Ranomafana National Park in south-eastern Madagascar. The new species belongs to the *Boophis luteus* group, which is composed of green treefrogs of low morphological differentiation. Analyses of advertisement calls and of partial sequences of the mitochondrial 16S rRNA gene evidenced a high bioacoustic and genetic uniformity throughout the range of *B. ankaratra*, while *B. schuboeae* was genetically divergent (2.4-2.8 % pairwise difference) and had distinct temporal and spectral call differences. The colour patterns of *B. schuboeae* are similar to those of *B. andohahela* from far south-eastern Madagascar. Re-analyses of advertisement calls, however, indicate that this species is bioacoustically highly different and close to *B. jaegeri* from the north-west, confirming that external morphology in the *B. luteus* group is not reliable to assess relationships.

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### Introduction

Among the anurans of Madagascar, the green-coloured species of the treefrog genus *Boophis* belong to the taxonomically most complicated groups. The reasons lie in the large number of morphologically similar cryptic species, and in the fact that the often species-specific life coloration fades quickly in preservative to a largely uniform pale yellow colour. This applies to two of the seven phenetic species groups (Blommers-Schlösser & Blanc 1991, Glaw & Vences

1994): the *Boophis rappiodes* group and the *B. luteus* group. The *B. luteus* group has experienced a dramatic increase in recognized species diversity during the last decade. While only a single species, *B. luteus*, was recognized by Blommers-Schlösser & Blanc (1991), ten new species have been described since the application of bioacoustic methods (Andreone 1993, 1996, Andreone et al. 1995, Glaw & Thiesmeier 1993, Glaw & Vences 1992, 1994). Recent molecular work (Vences et al. in press) has demonstrated a rather high genetic differentiation for

a subsample of these taxa, confirming their specific validity.

One of the new species in the *Boophis luteus* group, *B. ankaratra*, was described by Andreone (1993) from Manjakatampo in the Ankaratra massif in central Madagascar. It was later recorded from the Andringitra massif about 320 km south of the type locality (Glaw & Vences 1994). Recent fieldwork has yielded records of several additional localities of this species at sites above 1000 m elevation in central eastern Madagascar, and led to the discovery of a bioacoustically divergent population from a mid-altitude site in the Ranomafana National Park. We herein provide bioacoustic and genetic evidence for the status of this population as separate species, thereby adding relevant perspective to the discussion of elevational endemism in Malagasy frogs. Considering the similarity in colour pattern of the new species to *Boophis andohahela*, we additionally provide a re-description of the divergent calls of that species and its bioacoustic sibling *B. jaegeri*.

#### Materials and methods

Frogs were collected during the night, by means of electric torches, mainly by localizing calling males. They were sacrificed using chlorobutanol, fixed in 5% formalin or 90% ethanol, and preserved in 70% ethanol. Specimens studied in this paper are deposited in the collections of the Université d'Antananarivo, Département de Biologie Animale (UADBA), Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn (ZFMK), and Zoologische Staatssammlung München (ZSM).

Morphological measurements were taken by M. V. using a caliper to the nearest 0.1 mm: SVL (snout-vent length), HW (head width), HL (head length), ED (horizontal eye diameter), END (eye-nostril distance), NSD (nostril-snout tip distance), NND (nostril-nostril distance), TD (horizontal tympanum diameter), HAL (hand length), FORL (forelimb length), HLL (hindlimb length), FOL (foot length), FOTL (foot length including tarsus).

Advertisement calls were recorded with different tape recorders using external microphones. They were analyzed either with a ME-DAV sound analyzing system with the software Spektro 3.2, or on a PC, using the software

Cooledit (Syntrillium Corp.). Temporal and metric measurements are given as range, with mean  $\pm$  standard deviation and number of measured units in parentheses.

A fragment of the mitochondrial 16S rRNA gene (up to 559 nucleotides) was amplified and sequenced using primers and protocols of Vences et al. (2000). Sequences were analyzed using PAUP\*, version 4b8 (Swofford 2001). They were deposited in public databases; EMBL/Genbank accession numbers are: *Boophis schuboeae* (ZFMK 62907; AJ315912); *Boophis ankaratra*: Andringitra (ladpole, not preserved; AF411611), Col des Tapias (ZSM 399/2000, AJ 315910), Itremo (ZSM 735/2001; AF411612), Manjakatampo (ZSM 367/2000, AJ315911), Mandraka (ZSM 400/2000, AJ315909). *Boophis* cf. *sibilans* (ZSM 327/2000, AJ315913) was used as the outgroup.

#### *Boophis schuboeae*, spec. nov.

Figs 1, 2

**Types.** Holotype: ZFMK 62299, adult male, collected by F. Glaw, D. Rakotomalala and F. Ranaivojaona on 2 March 1996 at Ranomafana National Park (ca. 21°14'S, 47°26'E), close to Ambatolahy, south-eastern Madagascar. – Paratypes: ZFMK 62906–62907, two adult males with same collecting data as holotype; ZSM 1086/2001 (originally ZFMK 62285), adult male, collected by F. Glaw, D. Rakotomalala and F. Ranaivojaona on 28 February 1996 at Vohiparara (Ranomafana National Park, at ca. 1000 m above sea level), south-eastern Madagascar.

**Diagnosis.** A member of the *Boophis luteus* group as recognizable by the green dorsal coloration with translucent shade in life (shared with the *B. rappiodes* group; opaque green in the *B. albilabris* group and in *B. microtympanum*, not green in other *Boophis* species groups), pigmented ventral side (inner organs not visible in life through a transparent skin as in species of the *B. rappiodes* group) and lateral fringes along lower arm and tarsus (absent in the *B. rappiodes* group). Within the *B. luteus* group, *B. schuboeae* is distinguished by its rather small body size (male SVL <30 mm vs. >34 mm in *B. luteus*, *B. septentrionalis*, *B. eienae*, *B. anjanaharibeensis*, *B. englaenderi*) and presence of light dorsolateral lines (known to occur only in three other species: *B. septentrionalis*, *B. andohahela*, and *B. ankaratra*). General advertisement call structure (regular series of up to 55 unharmonious



Fig. 1. *Boophis schuboeae*, spec. nov. in life.



Fig. 2. *Boophis schuboeae*, spec. nov. in life, ventral view.

notes) is fundamentally different from all species of the *B. luteus* group except for *B. ankaratra*. Differs from *B. ankaratra* by temporal and spectral call parameters (see below and Table 2), and by molecular characters.

#### Description of the holotype

SVL 26.0 mm. For measurements, see Tab. 1. Body slender; head as long as wide, much wider than body; snout rounded in dorsal and lateral views, nostrils directed dorsolaterally, slightly nearer to eye than to tip of snout; canthus rostralis indistinct; loreal region slightly concave; tympanum distinct, rounded, 47% of eye diameter; supratympanic fold not recognizable; tongue ovoid, distinctly bifid posteriorly; vomerine teeth present, in two small rounded aggregations, positioned posterolateral to choanae; choanae rounded. Arms slender, subarticular tubercles single; metacarpal tubercles not recognizable; fingers webbed; webbing formula 1 (-), 2i (-), 2e (-), 3i (-), 3e (1.75), 4 (1); relative length of fingers 1 < 2 < 4 < 3, finger 2 distinctly shorter than finger 4; finger disks enlarged; small unpigmented nuptial pads faint-

ly recognizable on inner side of first finger. Hindlimbs slender; tibiotarsal articulation reaches nostril when hindlimb is adpressed along body; lateral metatarsalia separated by webbing; inner metatarsal tubercle recognizable; no outer metatarsal tubercle; webbing formula between toes 1 (0.5), 2i (1), 2e (0), 3i (1.25), 3e (0.5), 4i (1.5), 4e (1.5), 5 (1); relative length of toes 1 > 2 > 3 > 5 > 4. Skin on the upper surface smooth; ventral skin smooth on throat, very slightly granular on belly; no distinct enlarged tubercles in the cloacal region.

After five years in preservative, uniformly yellowish-white dorsally and ventrally. Two dorsolateral rows of three dark spots each are present on the central dorsum.

Colour in life (based on slides of three males). Upper surface of head, back and flanks green. Small white or yellowish dots can be present on parts of the dorsal surface, and are most distinct on the hindlegs where they can form interrupted crossbands. The dots tend to be more yellowish above the upper lip. A yellow, sometimes incomplete or interrupted line runs from the eye to the nostril and a dorsolateral line of the same colour runs from the posterior eye corner several millimetres posteriorly (virtually absent in one specimen). The iris is golden or yellow with a reddish brown or silvery inner ring. The iris periphery is blue. An indistinct whitish line is present laterally on lower arm and tarsus. Venter white, throat pale greenish-grey to bluish-green, ventral surfaces of arms and legs bluish-green.

Variation. The morphology of the paratypes is very similar to the holotype (see Table 1). Due to tissue sampling for genetic analyses, the left forelimb is lacking in ZFMK 62906 and the right hindlimb is lacking in ZFMK 62907.

**Etymology.** *Boophis schuboeae* is dedicated to Dr. Anna Schubö, Munich.

**Natural history.** Calling males were observed at night about 2 m high in the vegetation along brooks in primary forest.

**Bioacoustic comparison of *B. schuboeae* (Fig. 3) and *B. ankaratra* (Fig. 4).** Call recordings were available of *B. schuboeae* from Ranomafana and Vohiparara, as well as of *B. ankaratra* from all six known localities (see Fig. 5). All these calls consisted of series of regularly repeated notes (but at Mandraka and the Col des Tapias we

additionally heard rapid series of 2-3 notes, possibly of territorial function, interspersed between the regular calls). Calls of *B. ankaratra* consisted of 50-105 notes, those of *B. schuboeae* of at least up to 55 notes. Notes were distinctly pulsed and unharmonious to the human ear in *B. ankaratra*, but more melodious and less distinctly pulsed on the oscillogram in *B. schuboeae*. Important differences were furthermore noted in the note repetition rate (Table 2) which was faster (3.3-3.6 notes per second) in *B. schuboeae* than in *B. ankaratra* (2.2-2.8 notes per second; 1.3 in the particularly low motivated Itremo specimens). This difference was caused by a lower duration of intervals between notes in *B. schuboeae* (mean interval duration 127-158 ms vs. 234-671 ms; Table 2). In those notes of *B. schuboeae* in which pulses were recognizable on the oscillograms, pulse number was much higher than in *B. ankaratra* (ca. 50-60 vs. 16-35). No consistent differences in note duration were

noted between both species (Table 2), despite the lower temperature at which most *B. ankaratra* recordings were made. This indicates that the differences observed between both species were not caused by influences of temperature.

**Molecular phylogeny and differentiation.** The obtained sequences presented no indels and their alignment was therefore possible without inclusion of gaps. 23 nucleotides (nt) in the first and last sections could not be read in one or more sequences and were therefore excluded from the analysis. Of 536 included characters, 479 were constant, 49 were variable but parsimony-uninformative, and eight were variable and parsimony-informative. A Maximum Parsimony analysis (exhaustive search) yielded five equally most parsimonious trees; a strict consensus of those is shown in Fig. 6. The included sequences of *Boophis ankaratra* were grouped as monophylum, strongly supported by bootstrap

**Tab. 2.** Temporal and spectral characteristics of available calls of *Boophis ankaratra* and *B. schuboeae*. The divergent calls of the Itremo population of *B. ankaratra* were recorded from lowly motivated males late at night when calling activity had almost stopped. Calls heard (but not recorded) earlier the same night had clearly a faster repetition rate by subjective impression.

Species	Locality	Recording date	Recording temperature	Note duration [ms]	
<i>Boophis schuboeae</i>	Vohiparara	28 February 1996	ca. 22-23°C	96-182 (146±21, n=55)	
	Ranomafana, specimen 1	2 March 1996	23°C	141-168 (153±9, n=10)	
	Ranomafana, specimen 2	2 March 1996	23°C	154-170 (163±5, n=9)	
	Ranomafana, specimen 3	2 March 1996	23°C	57-170 (117±27, n=52)	
<i>Boophis ankaratra</i>	Manjakatempo	8 January 1994 (21:30 h)	ca. 17°C	116-129 (125±4, n=10)	
	Ambohitately	7 February 1996(20:05 h)	-	122-198 (151±26, n=12)	
	Mandraka	6 March 2000	18°C	151-204 (170±16, n=10)	
	Col des Tapias	26 March 2000	19.5°C	125-165 (146±13, n=10)	
	Itremo (low motivated call)	10 March 2001	21.1°C	94-191 (144±44, n=11)	
	Andringitra	15 January 1994	ca. 18°C	125-142 (133±4, n=21)	
Species	Inter-note interval duration [ms]	Note repetition rate	pulses per note	Frequency [Hz]	Dominant frequency [Hz]
<i>Boophis schuboeae</i>	111-221 (140±21, n=54)	3.4/s	ca. 50-60	2300-4000	3350-3500
	131-154 (141±7, n=9)	3.3/s	-	-	-
	112-139 (127±10, n=8)	3.5/s	-	2900-3900	3300-3550
	121-283 (158±38, n=51)	3.6/s	-	2850-3800	3200-3400
<i>Boophis ankaratra</i>	251-263 (257±4, n=10)	2.5/s	21-23 (22.4±0.7, n=10)	2350-3350	2750-3000
	166-343 (234±45, n=12)	2.8/s	-	1700-3500	1900-3000
	220-293 (246±23, n=10)	2.2/s	24-30 (28±2, n=10)	2000-2900	2350-2700
	227-269 (249±16, n=10)	2.4/s	ca. 30	2100-3400	2500-3100
	533-954 (671±149, n=9)	1.3/s	16-35 (28±8, n=5)	2650-3600	2800-3150
	229-309 (262±19, n=20)	2.5/s	24-26 (24.9±0.7, n=10)	1750-3400	2500-2700

**Tab. 1.** Morphometric measurements (all in mm) of holotype and paratypes of *Boophis schuboeae*. For abbreviations of measured variables, see Materials and methods; RHL (relative hindlimb length) gives the point reached by tibiotarsal articulation when the hindlimb is adpressed along the body.

Specimen	ZFMK 62299	ZSM 1086/2001	ZFMK 62906	ZFMK 62907
Sex	male	male	male	male
Status	holotype	paratype	paratype	paratype
SVL	26.0	24.0	25.3	24.0
HW	9.0	9.5	9.3	8.9
HL	9.0	8.9	9.2	8.6
ID	1.4	1.8	1.5	1.3
FD	3.0	3.3	2.9	2.8
END	1.8	1.8	1.9	1.7
NSD	1.9	2.2	2.3	2.0
NND	3.1	3.0	2.5	2.5
HAL	7.5	8.0	7.3	7.4
FORL	15.9	15.6	15.7	15.6
HIL	42.7	40.9	42.2	40.9
FOTL	18.6	17.8	17.6	17.8
FOL	11.0	10.9	10.7	10.6
RHL	nostril	nostril	between eye and nostril	snout tip

