

# Two new species of the *Boophis mandraka* complex (Anura, Mantellidae) from the Andasibe region in eastern Madagascar

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**Abstract.** The treefrog genus *Boophis* is represented in Madagascar by 50 described species. In this paper we describe two new cryptic species: *B. liami* sp. nov. and *B. solomaso* sp. nov. are similar to *B. mandraka* in morphology and colouration, but are clearly different in bioacoustic and genetic characters. The existence of many sibling species within *Boophis* shows the importance of detailed studies to clarify the distribution and the ecological requirements of the species in this genus.

**Résumé.** Les anoures arboricoles du genre *Boophis* sont représentés à Madagascar par 50 espèces. Dans cet article nous décrivons deux nouvelles espèces cryptiques : *B. liami* sp. nov. et *B. solomaso* sp. nov. ressemblent à *B. mandraka* par la coloration et la morphologie, mais ce distinguent clairement par la bioacoustique et le caractères génétiques. L'existence de beaucoup de "sibling species" dans le genre *Boophis* montre l'importance des études détaillées pour qu'on puisse clarifier les questions concernant la répartition et les demandes écologiques des espèces de ce genre.

## Introduction

Considering all recent descriptions (Andreone et al., 1995; Cadle, 1995; Andreone, 1996; Glaw and Vences, 1997a, b; Vallan et al., 1998; Glaw et al., 2001; Glaw and Vences, 2002; Vences and Glaw, 2002), the genus *Boophis* Tschudi, 1838 is currently represented in Madagascar by 50 described species. Most *Boophis* are nocturnal treefrogs of rather secretive habits. Seven phenetic species groups are distinguished in the genus, based on their external morphology.

One of these, the *Boophis rappiodes* group, contains six nominal species characterized by a translucent ventral skin which, as in species of the Neotropical family Centrolenidae, allows the direct observation of internal organs of living individuals (Blommers-Schlösser, 1979b; Blommers-Schlösser and Blanc, 1991): *Boophis rappiodes* (Ahlf, 1928), *B. erythro-dactylus* (Guibé, 1953), *B. mandraka* Blommers-Schlösser, 1979, *B. viridis* Blommers-Schlösser, 1979, *Boophis bottae* Vences and Glaw, 2002, and *Boophis tasymena* Vences and Glaw, 2002. Representatives of the group are largely distributed in the rainforests of central eastern Madagascar (Blommers-Schlösser and Blanc, 1991) but have also been found in the south-east (Glaw and Vences, 1994) and north-east (Raxworthy et al., 1998; Andreone et al., 2000; Raselimanana et al., 2000). They are rather small frogs (snout-vent length 20-35 mm) that breed in brooks and streams and have so far mostly been diagnosed by their colouration in life. However, their green colour rapidly fades to yellow and finally to white in alcohol. Consequently much of the characteristic pattern is not visible in preserved specimens, enhancing the difficulties for taxonomic revisions without new field observations.

Recent studies have revealed the existence of undescribed cryptic taxa close to *B. rappiodes* and *B. erythro-dactylus* (Vences and Glaw, 2002) and have casted doubt on the monophyly of the group as a whole (Vences et al., 2002). During ongoing surveys we collected specimens with external characters of *Boophis mandraka* which, by bioacoustic and molecular analyses, actually resulted to be separate species. In the present paper we describe these two new taxa and discuss their relationships.

## Material and methods

Animals were searched for by means of opportunistic surveys during the night with the help of hand torches and headlamps. After anaesthesia the specimens were fixed in 90% ethanol and preserved in 70% ethanol. Measurements were taken by the senior author following Duellman (1970), with exceptions as noted below. All measurements were taken with dial callipers to a precision of 0.1 mm. The following parameters were measured: SVL, snout-vent length; HW, head width; FORL, forelimb length; HAL, hand length (from the tubercle at the base of finger 1 to the tip of the longest digit); TIL, tibia length; FOL, foot length (from the base of the metatarsal tubercle to the tip of toe 4); TARL, tarsus length; TD, horizontal tympanum diameter; ED, horizontal eye diameter; END, distance from eye to nostril; NSD, distance from nostril to snout tip. Webbing formulae are given according to Blommers-Schlösser (1979a). Advertisement calls were recorded with different tape recorders and microphones, and were analysed with the MEDAV sound analyzing system Spektr 3.2 or with the program CoolEdit (Syntrillium Corp.) on a PC. Temporal and metric measurements in the call descriptions are given as range, with mean  $\pm$  standard deviation and number of measured units in parentheses. Colour photographs were taken to document colouration in life. A section of the mitochondrial 16S rRNA gene (555 nucleotides) was sequenced using primers and protocols of Vences et al. (2000). Sequences have been submitted to public databases; EMBL/Genbank accession numbers and voucher specimens used for molecular analyses are: *Boophis mandraka*, ZSM 346/2000 (museum catalogue number), AJ315921 (EMBL/Genbank accession number); *B. liami*, ZSM 310/2000, AJ315919; *B. solomaso*, NMBE 1046008, AJ315920.

The following institutional abbreviations are used: NMBE, Naturhistorisches Museum Bern; UADBA, Université d'Antananarivo, Département de Biologie Animale; ZFMK, Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn; ZSM, Zoologische Staatssammlung München. As no definitive UADBA catalogue numbers are available yet, the corresponding specimens are listed by their field numbers of M. Vences (MV).



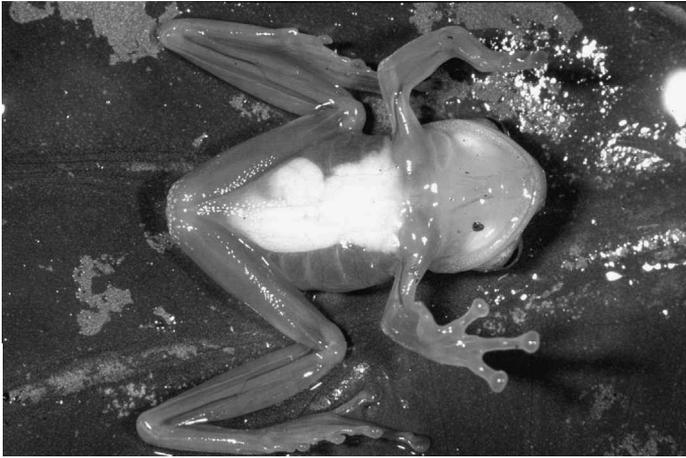
**Figure 1.** Dorsal view of *Boophis liami* holotype (ZFMK 60123).

***Boophis liami* sp. n.** (figs 1 and 2)

**Diagnosis.** A tree frog assigned to the *Boophis rappiodes* group based on (a) presence of nuptial pads and absence of femoral glands in breeding males, (b) light green dorsal colour and translucent ventral skin, (c) small size (male SVL < 22 mm), and (d) absence of lateral fringes along lower arm and tarsus. Assigned to the *Boophis mandraka* complex based on molecular data (Vences et al., 2002). It shares with *B. mandraka* the position of nostrils (nearer to eye than to snout tip in most specimens vs. always nearer to snout tip in other species of the *B. rappiodes* group) and the general absence of red pigment on the dorsal surface (mostly present in all other species of the *B. rappiodes* group). Distinguished from *B. mandraka* by (1) slightly smaller size (SVL of males 19–21 mm in *B. liami*, 21–26 mm in *B. mandraka*), (2) the lack of a distinct net of brown “vessels” in the iris, (3) brownish or silvery lines from the eye to the snout tip (yellow in *B. mandraka*), (4) advertisement calls (regular series of long, whistling notes vs. irregular short chirps), and (5) by a high genetic differentiation (see below). For distinction from *B. solomaso*, see diagnosis of that species.

**Holotype.** ZFMK 60123, adult male, collected at the edge of the river Ranomena near Ambavaniasy (a site called Befody), 18°57,3'S, 48°30,7'E, 760 m elevation, Moramanga Fivondronana, Toamasina Province, central eastern Madagascar, on 2 April 1995 by F. Glaw and D. Vallan.

**Paratypes.** ZFMK 60124 and ZFMK 60125, adult males, same locality and collection data as holotype; NMBE 1034197 and NMBE 1034198, adult males, same locality as holotype, collected 24 March 1995 by D. Vallan; NMBE 1035448, adult male, from the edge of the river Ranomena in the forest of Vohidrazana near Ambavaniasy (18°57'54"S, 48°30'36"E, 800 m elevation, collected 16 March 1996 by D. Vallan; NMBE 1046016



**Figure 2.** Ventral view of *Boophis liami* holotype (ZFMK 60123).

same locality as holotype, collected 8 February 1997 by D. Vallan; NMBE 1046017 same locality as holotype, collected 6 April 1997 by D. Vallan; ZSM 310/2000 and ZSM 311/2000, adult males, from Vohidrazana ( $18^{\circ}57'57''\text{S}$ ,  $48^{\circ}30'37''\text{E}$ , 731 m elevation), collected 10 April 2000 by F. Glaw; ZSM 673-674/2001 and UADBA-MV 2001.173-174, four adult males, collected by M. Vences, D.R. Vieites and F. Mattioli on 17 February 2001 at Vohidrazana ( $18^{\circ}57'58''\text{S}$ ,  $48^{\circ}30'35''\text{E}$ , 810 m elevation).

*Morphology of holotype.* A small, slender frog of 20.2 mm SVL (for other measurements see table 1). Head broader than long. Skin on the back smooth, on ventral side slightly granular. Finger tips and toe tips enlarged. Relative finger length:  $1 < 2 < 4 < 3$ . Relative length of toes:  $1 < 2 < 3 = 5 < 4$ . Weakly developed webbing between the fingers: 1(1), 2i(2), 2e(1), 3i(2.5), 3e(1.5), 4(1). Feet with well developed webbing: 1(0), 2i(0.5), 2e(0), 3i(1), 3e(0), 4i(1), 4e(0.5), 5(0). A small inner metatarsal tubercle is present, an outer is absent. Lateral metatarsalia are separated. Small nuptial pads are present. The nostrils are nearer to the eyes than to the tip of the snout. The tibiotarsal articulation reaches the tip of the snout. Tympanum/eye ratio is 41%.

*Colour of holotype in life.* The dorsal side and the flanks were of a light pastel green and yellowish-green colour. The eyelids were light brown. A light brown to silvery line ran from the tip of the snout above the eye and tympanum ending behind the tympanum. The dorsum and the dorsal sides of the limbs were lightly mottled with small light blue to turquoise patches and dark dots. Flanks, hands and feet were only mottled with small white dots. The flanks, the middle of the back and the middle of the head were of a yellowish-green. Blood vessels were visible through the skin of the dorsal as well as the ventral side of body and limbs. The ventral side of the body, the arms and the legs were greenish-blue and transparent, the inner organs partly shining through. The ventral side of hands and

**Table 1.** Characteristics and morphometric measurements (in mm) of specimens of *Boophis liami* and *B. solomaso* (all males). For abbreviations, see Materials and methods. Further abbreviations: HT, holotype; PT, paratype; RHL, relative hindlimb length. RHL is given as the point reached by the tibiotarsal articulation when the limb is adpressed along the body and coded as follows: a, snout tip; b, slightly beyond snout tip; c, beyond snout tip.

Specimen	Status	SVL	HW	FORL	HAL	TIL	TARL	FOL	TD	ED	END	NSD	RHL
<i>B. liami</i> (ZSM 311/2000)	PT	21.3	9.1	4.3	7.2	12.2	6.8	8.8	0.9	2.9	1.7	1.9	b
<i>B. liami</i> (ZSM 310/2000)	PT	19.8	8.1	4.4	6.3	11.5	6.0	8.8	1.2	2.9	1.4	1.7	a
<i>B. liami</i> (ZFMK 60123)	HT	20.2	8.2	4.0	6.5	11.5	6.1	9.4	1.1	2.7	1.5	1.9	a
<i>B. liami</i> (ZFMK 60124)	PT	19.0	8.0	4.0	6.4	11.9	6.3	9.4	1.0	3.0	1.5	1.8	c
<i>B. liami</i> (ZFMK 60125)	PT	19.9	8.3	4.2	6.7	11.8	6.3	9.5	1.1	2.8	1.4	1.8	b
<i>B. liami</i> (NMBE 1034198)	PT	20.6	8.5	4.2	7.0	12.4	6.7	9.8	0.9	3.1	1.5	2.0	b
<i>B. liami</i> (NMBE 1046016)	PT	20.8	8.7	3.9	5.7	12.1	6.7	9.6	1.3	3.7	1.4	2.0	a
<i>B. liami</i> (NMBE 1046017)	PT	20.6	8.0	4.3	6.6	12.0	6.5	9.7	1.0	3.3	1.3	1.9	b
<i>B. liami</i> (NMBE 1034197)	PT	19.3	8.0	4.1	6.3	11.3	6.2	9.0	1.0	3.1	1.4	1.7	a
<i>B. liami</i> (NMBE 1035448)	PT	20.1	8.5	4.0	6.5	11.6	6.3	9.2	1.2	3.3	1.3	1.5	a
<i>B. solomaso</i> (NMBE 1046010)	HT	20.4	8.4	4.2	6.1	11.6	6.2	8.8	1.1	3.0	1.5	1.9	a
<i>B. solomaso</i> (NMBE 1046008)	PT	21.6	9.1	4.0	6.7	12.2	6.9	9.3	1.1	3.3	1.3	1.7	a

feet was yellowish-green. The blue colour of the thigh, the breast and the throat was a bit more intensive. The horizontal pupil was surrounded by a dirty white iris that was slightly mottled with small brown dots in the inner area. The colour of the iris periphery was blue.

*Colour of preserved holotype.* The green colour of the back and the flanks became yellow as well as the light brown eyelids. The small white and light bluish spots disappeared or became also yellow. The small dark dots remained unchanged. The greenish transparent skin on the ventral surface became light yellow and opaque. The blue colour on the thigh, the breast and the throat disappeared.

*Morphology of paratypes.* SVL of the paratypes (all adult males) varies between 19.0 and 21.3 mm (see table 1). The tibiotarsal articulation reaches the tip of the snout or slightly beyond. Only in ZFMK 60124 the tibiotarsal articulation reaches clearly beyond the tip of the snout. Concerning the webbings of hands and feet there are just little differences. Webbing formula of hands: 1(1-2), 2i(1.5-2), 2e(1), 3i(2-2.5), 3e(1-1.5), 4(1). Webbing formula of feet: 1(0-0.5), 2i(0.5-1), 2e(0-0.5), 3i(1), 3e(0-0.5), 4i(1), 4e(0.5-1.5), 5(0-0.5).

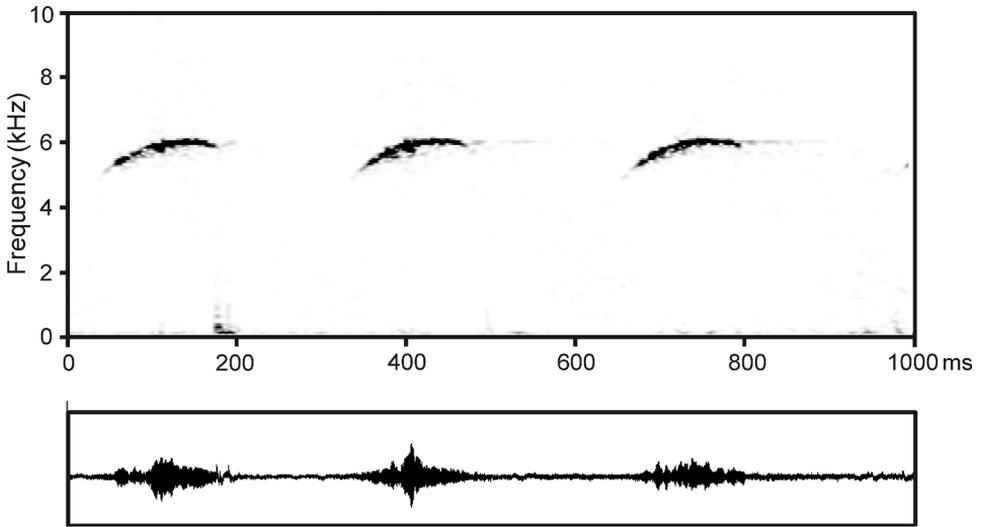
*Colour of paratypes in life.* The colour of *Boophis liami* is very variable. The ground colour of the back in the paratypes was always green ranging from a pastel green (NMBE 1034198) to a little bit more vivid but always light green (NMBE 1035448). The flanks were a little lighter than the back. The back and dorsal side of the limbs were mottled with brown to black spots and/or patches. It ranged from just a few patches and spots evenly distributed over the body (NMBE 1035448) to more patches and spots concentrated mainly on the anterior part of the dorsum (NMBE 1034197, NMBE 1034198 and NMBE 1046016). The brown patches were sometimes so frequent that they fused (ZFMK 60124 and ZFMK 60125), the predominant colour of the back becoming brown. Only very few spots and patches were present on the flanks. In ZFMK 60124 and ZFMK 60125 a fine yellow line ran from the posterior end of the eye over the tympanum along the border between back and flanks ending at the middle between insertion of the arms and legs. In NMBE 10341997 this line was less developed and ended above the insertion of the arms. In all specimens small, pale whitish-green and inconspicuous dots covered the green colour of the back. The fingertips were greenish-blue (in NMBE 1046016). The eyelids were brown (NMBE 1034198 and NMBE 1035448) to reddish brown (ZFMK 60124, ZFMK 60125, NMBE 1034197 and NMBE 1046016). In NMBE 1034198 the brown eyelid was bordered by a broad white stripe at the edge to the eyeball. This stripe extended to the tip of the snout. Also in ZFMK 60124, ZFMK 60125, NMBE 1034197 and NMBE 1046016 the colour of the eyelid continued as a stripe ending on the tip of the snout. In all the other paratypes there was also present a line or a stripe from the eye to the tip of the snout that was lighter than the eyelid. No brown patches were present on the forearm of all paratypes. In NMBE 1046016 the underside of the fingertips was greenish-blue. The horizontal pupil was surrounded by a dirty white iris. Brown pigment was sometimes present on the inner area of the iris (ZFMK 60125 and NMBE 1034197) or on both, inner and outer iris area (ZFMK 60124, NMBE 1035448 and NMBE 1046016). This brown pigment was sometimes nearly absent (NMBE 1034198). The colour of the iris periphery was turquoise in all paratypes.

*Colour of preserved paratypes.* The changes were exactly as described for the holotype.

*Etymology.* This species is dedicated to Liam Nicolas Vlasimsky, on behalf of his father Stan M. Vlasimsky, and in recognition of financial support to the study of Madagascar's biodiversity through the BIOPAT programme.

*Genetic differentiation.* In the 555 nucleotides of the partial sequences of the mitochondrial 16S rRNA gene, we observed 38 pairwise substitutions (+ 3 indels) between *B. mandraka* and *B. liami*. Total pairwise sequence divergence was thus 7.4%.

*Habitat and habits.* *Boophis liami* seems to be a rather adaptable species. It has been found in disturbed rainforest (Vohidrazana) and in secondary, herbaceous vegetation (Vohidrazana and Befody). Calling males were observed during the night 1-2 m above the ground in bushes along broad brooks. The specimens were partly found at considerable



**Figure 3.** Sonogram and oscillogram of a call (three notes of a typical series of regularly repeated notes) of *Boophis liami* from Vohidrazana.

distances (up to 10 m) from the water. One individual was found during the day resting on a branch.

**Call.** Advertisement calls were recorded on 2 April 1995 almost certainly from the holotype at the type locality. They consisted of a series of 8-10 ( $9 \pm 1$ ,  $n = 8$ ) melodious whistling notes (fig. 3). Total call duration extended up to 3670 ms. Note duration was 139-232 ms ( $187 \pm 14$  ms,  $n = 72$ ), duration of inter-note intervals was 168-342 ms ( $208 \pm 33$  ms,  $n = 63$ ), with the longest interval preceding the last note of a series. Note repetition rate was 2.5-3/s. The notes were frequency-modulated in a characteristic way: they started at a frequency of 4700 Hz, followed by a steep increase up to 6400 Hz, followed by a slight decrease in the second half of the note. The first note of a series often had a lower frequency (4300-5800 Hz). Calls heard and recorded on 17 February 2001 at Vohidrazana had very similar temporal and spectral characteristics (fig. 3).

**Distribution.** *Boophis liami* is known from the region around Ambavaniasy about 10 km east of Andasibe. It was found at two sites: Befody and Vohidrazana.

### ***Boophis solomaso* sp. n.** (figs 4 and 5)

**Diagnosis.** A tree frog assigned to the *Boophis rappiodes* group based on (a) presence of nuptial pads and absence of femoral glands in breeding males, (b) yellowish-green dorsal colour and translucent ventral skin, (c) small size (male SVL < 22 mm), and (d) absence of lateral fringes along lower arm and tarsus. Assigned to the *Boophis mandraka* complex based on molecular data (Vences et al., 2002). It shares with *B. mandraka* and *B. liami* the



**Figure 4.** Dorsal view of *Boophis solomaso* holotype (NMBE 1046010).



**Figure 5.** Ventral view of *Boophis solomaso* holotype (NMBE 1046010).

position of nostrils (nearer to eye than to snout tip vs. nearer to snout tip in other species of the *B. rappiodes* group) and the general absence of distinct red pigment on the dorsal surface (mostly present in all other species of the *B. rappiodes* group). Distinguished from *B. mandraka* by (1) the presence of a brown bar between the eyes, (2) the lack of a distinct net of brown “vessels” in the iris, (3) slightly smaller size (SVL of males 20–22 mm vs. 21–26 mm), (4) advertisement calls (see below), and (5) by a high genetic differentiation (see below). Distinguished from the sympatric *B. liami* by (1) the presence of a brown bar between the eyes (vs. absence), (2) advertisement calls (see below), and (3) a high genetic differentiation (see below).

*Holotype.* NMBE 1046010, adult male, collected at a site called Analambalotra near Ambavaniasy, 18°57'36"S, 48°30'00"E, about 880 m elevation, Moramanga Fivondronana, Toamasina Province, central eastern Madagascar, on 10 February 1997 by D. Vallan.

*Paratype.* NMBE 1046008, adult male, same locality and collection data as holotype.

*Morphology of holotype.* A small, slender frog of 20.4 mm SVL (for other measurements see table 1). Head broader than long. Skin on the back smooth, on the ventral side slightly granular. Finger tips and toe tips enlarged. Relative finger length: 1 < 2 < 4 < 3. Relative length of the toes: 1 < 2 < 3 = 5 < 4. Weakly developed webbing between the fingers: 1(2), 2i(2), 2e(1), 3i(2.5), 3e(1.5), 4(1). Feet with well developed webbing: 1(0.5), 2i(1), 2e(0), 3i(1), 3e(0.5), 4i(1), 4e(1), 5(0). A small inner metatarsal tubercle is present, an outer is absent. The lateral metatarsalia are separated. Small nuptial pads are present. The nostrils are nearer to the eyes than to the tip of the snout. The tibiotarsal articulation reaches the tip of the snout. Tympanum/eye ratio is about 37%.

*Colour of holotype in life.* The back was light green with several distinct brown spots. A brown bar was present between the eyes. Eyelids were also brown, bordered by a white line along the dorsal part of the eyeball. A brown line connected the snout tip with the eye. The green colour of the back faded to a lighter green on the flanks and became white near the belly. On the flanks between the back and the belly there was no sharp colour border. No brown spots were present on the flanks. The dorsal side of the arms and legs were of the same colour as the back, and were also spotted. Only the upper arm had a lighter colour and no spots. The fingers and toes were of a lighter green than the rest of the limbs, with the exception of the tips that were of the same colour. On the ventral side of the arms, the legs and the body were white and transparent, the inner organs partly shining through. The ventral sides of hands and feet were light yellowish-green. Thigh, breast and throat had a light touch of blue. The horizontal pupil was surrounded by a light brown iris. The colour of the iris periphery was light blue, that of the eye periphery dark blue.

*Colour of preserved holotype.* The green colour became yellow. The brown spots on the back and on the limbs remained unchanged. The transparent skin on the ventral side became opaque and light yellow. The blue colour on thigh, breast and throat disappeared.

*Morphology of paratype.* The morphology of the paratype is similar to the holotype (for measurements see table 1). Small differences were found concerning the webbing; hand: 1(2), 2i(2), 2e(1), 3i(2.5), 3e(1), 4(1); foot: 1(0.5), 2i(1), 2e(0.25), 3i(1), 3e(0.5), 4i(1.5), 4e(1), 5(0). A small piece of the muscle of the right thigh was removed for DNA analyses.

*Colour of paratype in life.* The colouration of the paratype corresponded more or less to that of the holotype. There were fewer brown spots on the back and on the dorsal side of the limbs, and the brown bar between the eyes was less pronounced and shorter, not connecting the brown patches on the eyelids. The brown colour of the eyelids was less developed and the white lines along the dorsal part of the eyeballs were shorter. The brown line between

the tip of the snout and the eye was also shorter than in the holotype, reaching neither the eye nor the tip of the snout.

*Colour of preserved paratype.* The changes were exactly as described for the holotype.

*Variation.* A further specimen probably belonging to this species has been found on the 19 March 1996 by the senior author also in the forest of Analambalotra resting on the ground about 8 meter away from a brook. The colouration was similar to the one of the paratype. Only small differences existed: the shape of the dots on the dorsum was longish (versus round) and dots on the thigh were absent.

*Etymology.* The specific name is derived from *solomaso* (Malagasy: eyeglasses) and refers to the dark band between the eyes, which apparently is rather typical for this species. The name is used as invariable noun in apposition to the generic name.

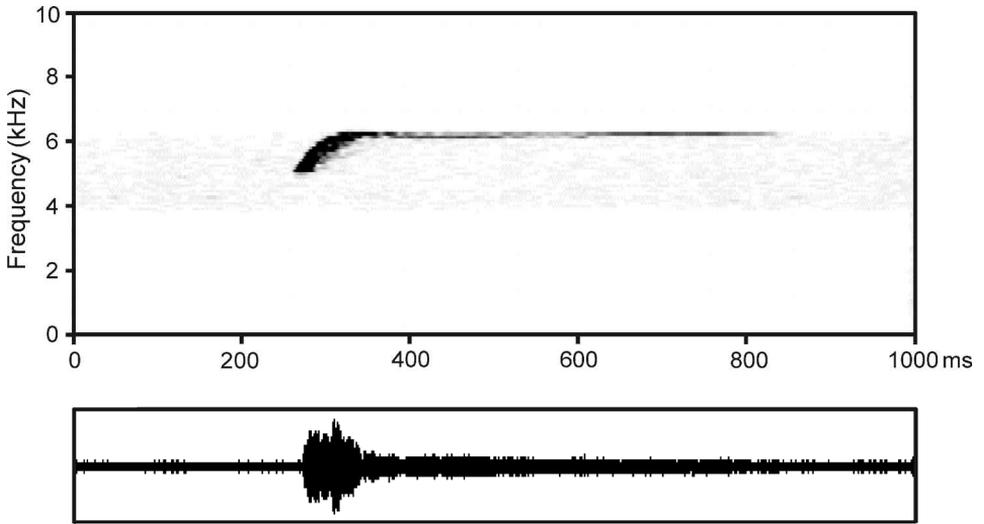
*Genetic differentiation.* In the 555 nucleotides of the partial sequences of the mitochondrial 16S rRNA gene, we found 39 pairwise substitutions (+ 3 indels) between *B. mandraka* and *B. solomaso*, and 45 between *B. liami* and *B. solomaso*. Total pairwise sequence divergence was thus 7.6% and 8.1%.

*Habitat and habits.* The two known specimens were found during the night in a 25 years old secondary forest bordering an intact rain forest. Both frogs called sitting on a leaf at 20.55 h. One frog called sitting on a leaf four meters above a brook. The other one was two meters distant from a brook in two meters height. A third animal was heard at 19.00 h. The animals were calling while it was foggy or during drizzle. It seems that *Boophis solomaso* is a strictly nocturnal frog, since it was never heard or seen active during the censuses conducted during the day at the same locality. Only one specimen attributed to this species was found resting on the ground on the leaf litter during the day.

*Boophis solomaso* may prefer a habitat with a more or less closed canopy and the presence of brooks or streams (at least during the reproductive season). Since it has not been found elsewhere in the surrounding places it is also possible that it depends on the presence of pristine forest. Its occurrence in the secondary forest of Analambalotra could be due to the immediate vicinity of a pristine rainforest, the old age (25 years) of the secondary vegetation with a more or less closed canopy and the presence of a brook.

*Call.* Calls that were emitted by *B. solomaso* were recorded from one of the type specimens on 10 February 1997, 20.55 h at the type locality (at an air temperature of 19°C). Two note types can be distinguished on the available recordings. Since both note types were occasionally emitted at the same time and have a similar intensity on the sonagrams, the recordings include obviously vocalizations of at least two specimens, which were closely sitting to each other. Although very likely, it is not totally clear if both note types belong to the same species.

Note type 1 (fig. 6) is a long whistle that is arranged in series of 4-5 notes ( $n = 3$ ). Note duration is 422-571 ms ( $477 \pm 34$  ms,  $n = 17$ ). Interval duration between two notes of a



**Figure 6.** Sonogram and oscillogram of one note of a call of *Boophis solomaso*.

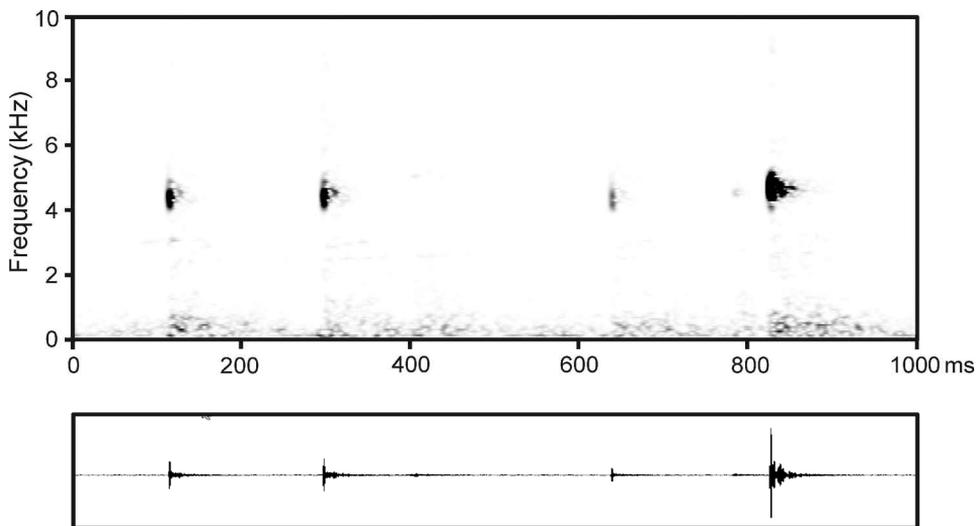
series is 229-274 ms ( $247 \pm 15$  ms,  $n = 11$ ) and note repetition rate is 1.4/s. Each note can be classified in two parts. The first part has a duration of 81-128 ms ( $105 \pm 15$  ms,  $n = 20$ ) and is characterized by a distinct frequency modulation, starting at 5800 Hz and reaching 6600 Hz. The second part is much longer and the frequency is nearly constant at 6250-6350 Hz. In one case two closely neighboured specimens emitted note type 1 at the same time.

Note type 2 is a short click that is arranged in series of 1-4 notes ( $n = 7$ ). Note duration is 18-57 ms ( $39 \pm 14$  ms,  $n = 19$ ), interval duration between two notes of a series 84-128 ms ( $106 \pm 16$  ms,  $n = 12$ ), note repetition rate is 6.6-7.7/s. The frequency of the first note of a series is lower (4400-5400 Hz) than that of the following notes (about 5700 Hz). It is remarkable that the frequency of note type 2 is distinctly lower than that of type 1.

*Distribution.* This species is only known from the type locality.

### **Calls of *Boophis mandraka* Blommers-Schlösser, 1979**

In order to allow a better comparison, we here provide an updated description of the advertisement calls of *B. mandraka*. They were recorded from the specimen ZSM 346/2000 on 8 February 2000 at 18.4°C air temperature. The call is composed of short melodious chirping notes of rather variable duration (fig. 7). These can be emitted singly and repeated after relatively irregular intervals, or in short series of three to five notes. In single notes, note duration was 54-98 ms ( $70 \pm 14$  ms,  $n = 8$ ), and duration of inter-note intervals was 2060-3865 ms ( $2881 \pm 626$  ms,  $n = 6$ ). In notes arranged in series, note duration was 25-



**Figure 7.** Sonogram and oscillogram of a call (four irregularly repeated notes) of *Boophis mandraka*.

141 ms ( $63 \pm 44$  ms,  $n = 7$ ), and duration of inter-note intervals 351-652 ms ( $522 \pm 103$  ms,  $n = 5$ ). Frequency was 4000-5300 Hz.

Blommers-Schlösser (1979b) originally described the call of *B. mandraka* as consisting of three loud melodious clicks followed by a very soft click (call duration about 750 ms, dominant frequency 3500 Hz). Her recordings, which she kindly made available to us for re-analysis, differed from our own recordings, and were possibly made from specimens in captivity. In these recordings, calls consisted of notes which were composed of three click pulses, respectively. Each pulse had a duration of 14-27 ms ( $18 \pm 4$  ms,  $n = 12$ ), duration of intervals between pulses was 102-134 ms ( $118 \pm 12$  ms,  $n = 8$ ). Calls were emitted after intervals of 658-1042 ms ( $844 \pm 192$  ms,  $n = 3$ ). Frequency was between 2900-4300 Hz, dominant frequency 3300-3800 Hz.

### Key to living males of the species of the *Boophis rappiodes* group

- 1a Snout-vent length 29-30 mm; inner iris area dark brown, outer iris area blue; no line from eye to nostril — *Boophis viridis*
- 1b Snout-vent length 19-26 mm; iris colouration different; a yellow, red or light brown line from eye to nostril present or absent — **2**
- 2a Dorsal surface with distinct red dots or spots; nostril nearer to snout tip than to eye — **3**
- 2b Dorsal surface without distinct red dots or spots (the dorsal surface of the eyeball may be reddish-brown and reddish-brown lichen-like spots rarely may occur); nostril nearer to eye than to snout tip — **6**
- 3a Entire dorsal surface with many small, red, and evenly distributed dots — **4**

- 3b Back with larger and irregularly distributed markings or with only few red spots — **5**
- 4a At least some finger and toe tips red; calls consist of 4-7 short pulses with a relatively low frequency (1850-3500 Hz) — *Boophis erythroductylus*
- 4b Finger and toe tips not red; calls consist of 2-3 short pulses with a relatively high frequency (3700-6100 Hz) — *Boophis tasymena*
- 5a Back often with yellow, red and black spots; a red line or no line from eye to nostril; calls include long trills with a duration of up to 2.8 s — *Boophis bottae*
- 5b Back often with only little red pigment and without black pigment; generally a yellow line from eye to nostril; calls consist of short double clicks which are repeated after 2.8-5.4 s — *Boophis rappiodes*
- 6a A brown bar between the eyes; calls consist of series of 4-5 long (duration 422-571 ms) whistling notes with a repetition rate of 1.4/s — *Boophis solomaso*
- 6b Generally no brown bar between the eyes; calls consists of much shorter notes — **7**
- 7a Snout-vent length 21-26 mm; iris with a net of brown “vessels”; a yellow line, often bordered by brown colour from the eye to the snout tip; calls consist of isolated and irregularly repeated notes or of short series of 3-5 short (duration 25-141 ms) chirping notes — *Boophis mandraka*
- 7b Snout-vent length 19-21 mm; iris without a net of brown “vessels”; a light brownish or silvery line from the eye to the snout tip; calls consist of 8-10 moderately long (duration 139-232 ms) whistling notes with a repetition rate of 2.5-3/s — *Boophis liami*

## Discussion

Our data on morphological variability emphasize the difficulty of species diagnoses based on morphology alone. For example, specimens of the *Boophis mandraka* complex are generally recognizable by the position of their nostrils (closer to the eye than to the snout tip). However, the differences are small, often less than 0.5 mm and a reliable measurement is therefore difficult without direct comparative examination of other species. Also the relative length of the hindlimbs is rather variable in our sample. This character is in some cases useful for identification but in other cases, such as the *B. rappiodes* group, too variable to discriminate between sibling species. Additional care should therefore be applied when a morphological diagnosis is based on single specimens and not on larger series that represent the variability of a species. To account for these difficulties, the key to the *B. rappiodes* group presented here includes morphological characters as well as colouration in life and advertisement calls.

The discovery of new sibling species of *Boophis mandraka* as reported in the present paper confirms a general trend in Malagasy frogs. The phenomenon of cryptic sibling species is widespread in this group and implies serious problems for field studies or

taxonomic revisions if no bioacoustic or genetic data are available. The high genetic distances found between these (Vences et al., 2002) demonstrate that they constitute well defined and reproductively isolated entities which certainly will in part differ in their ecological requirements. Past biogeographic or ecological analysis which lumped sibling species into single species (e.g., Blommers-Schlösser and Blanc, 1993) must therefore be carefully re-evaluated after clarification of the identity of the respective vouchers. In the case of the *B. mandraka* complex, it is unknown to which of the three known species (if any) the populations from northern Madagascar do belong: Marojejy, Anjanaharibe-Sud, Tsararano and Ambolokopatrika (Raxworthy et al., 1998; Andreone et al., 2000; Raselimanana et al., 2000). Their correct identification will be crucial to attain a better understanding of the reasons for the strikingly different species composition of northern rainforests as compared to those of the central east.

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