



Two additional treefrogs of the *Boophis ulftunni* species group (Anura: Mantellidae) discovered in rainforests of northern and south-eastern Madagascar

JÖRN KÖHLER¹, FRANK GLAW² & MIGUEL VENCES³

¹Hessisches Landesmuseum Darmstadt, Department of Natural History – Zoology, Friedensplatz 1, 64283 Darmstadt, Germany. E-mail: j.koehler@hlmd.de

²Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 München, Germany. E-mail: Frank.Glaw@zsm.mwn.de

³Division of Evolutionary Biology, Zoological Institute, Technical University of Braunschweig, Spielmannstr. 8, 38106 Braunschweig, Germany. E-mail: m.vences@tu-bs.de

Abstract

We describe two new frog species of the endemic Malagasy-Comoroan genus *Boophis*. One species, described as *Boophis baetkei* sp. n., originates from Forêt d'Ambre Special Reserve in northernmost Madagascar, whereas *Boophis liliana* sp. n. was discovered near Ifanadiana and Ranomafana in the Southern Central East of the island. Both new species have very deep genetic divergences in the 16S rRNA gene that complicate the assessment of their phylogenetic affinities but are here tentatively assigned to the recently defined *Boophis ulftunni* species group based on phenetic similarity and preliminary results of analyses of other genes. All three species known in this group share a green dorsum with translucent shade in life, a pigmented venter and, most characteristic, pink markings in life and in preservative. *Boophis liliana* sp. n. is the smallest species of *Boophis* known so far (SVL of adult male 18.3 mm, ovigerous female 20.0 mm). Phylogenetic relationships, distribution and threat status of the new species are discussed.

Key words: Amphibia, Anura, Mantellidae, *Boophis ulftunni* group, new species, phylogeny, Madagascar

Introduction

Malagasy frogs of the genus *Boophis* Tschudi constitute a species-rich endemic radiation, occurring in almost all types of habitats known on the island. The genus has recently been partitioned in the two subgenera *Boophis* and *Sahona* (Glaw & Vences 2006). Within the subgenus *Boophis*, these authors defined eight species groups, among them six groups of predominantly green-coloured species: the *Boophis albipunctatus* group, *B. luteus* group, *B. mandraka* group and *B. rappiodes* group, which all have a green dorsal ground colour with a translucent shade; and the *B. albilabris* group and *B. microtypanum* group, both with an opaque green dorsal colour and including individuals of brownish colour. All of these groups contain morphologically similar cryptic species, which mostly differ by bioacoustic and genetic characters (e.g. Glaw & Vences 2002, Vences & Glaw 2005, Köhler *et al.* 2007). Recently, Wollenberg *et al.* (2008) described a green species of *Boophis* from the Masoala peninsula, with translucent shade and with a pinkish colour pattern, which turned out to represent an independent phylogenetic lineage: it did not belong to any of the above mentioned species groups, but instead appeared to be related to the *B. microtypanum* group. Consequently, Wollenberg *et al.* (2008) erected a new species group for this single species, the *B. ulftunni* group.

During fieldwork in 2006 and 2007, we discovered two additional undescribed species of green *Boophis* in northern and south-eastern Madagascar which were not immediately assignable to any of the species

groups. Molecular phylogenetic analyses tentatively revealed that both are probably related to *B. ulftunni* and thus are best considered as additional members of the *B. ulftunni* species group. Herein, we describe these two species and briefly discuss biogeographic and phylogenetic aspects of this discovery.

Material and methods

Frog specimens were collected at night, euthanized in a chlorobutanol solution, fixed in 90% ethanol, and preserved in 70% ethanol. Locality information was recorded with GPS receivers. Specimens studied in this paper are deposited in the collection of the Zoologische Staatssammlung München, Germany (ZSM). FGZC and ZCMV refer to F. Glaw and M. Vences field numbers.

Morphological measurements (in millimetres) were taken with a digital caliper (precision 0.01 mm) to the nearest 0.1 mm. Used abbreviations are: SVL (snout-vent length), HW (greatest 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), TL (tibia length), HAL (hand length), FOL (foot length), FOTL (foot length including tarsus). Terminology and description scheme follows Glaw & Vences (2002) and Glaw & Vences (1997) for eye colouration. Webbing formulae follow Blommers-Schlösser (1979).

Calls were recorded in the field with an Edirol R-09 digital recorder at a sampling rate of 44.1 kHz and 24-bit resolution and saved as uncompressed files. Recordings were resampled at 22.05 kHz and 16-bit resolution and computer-analysed using the software Adobe Audition version 1.5. Frequency information was obtained 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 (msec) or seconds (sec), as range, with mean \pm standard deviation in parentheses. Terminology in call description follows Köhler (2000). Seven calls of two individuals were analysed.

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). The 550 bp fragment was amplified using primers 16Sar-L and 16Sbr-H from Palumbi *et al.* (1991) applying standard protocols, resolved on automated sequencers, and compared to a near-complete database of sequences of adult Malagasy frog species. Newly determined DNA sequences were deposited in Genbank (see below for accession numbers).

Taxonomy

Boophis baetkei sp. n.

Holotype. ZSM 2051/2007 (field number FGZC 1391), adult male, from Forêt d'Ambre Special Reserve, 12°28'00" S, 49°13'37" E, 470 m above sea level, Antsiranana Province, northern Madagascar, collected on 12 March 2007 by P. Bora, F. Glaw and J. Köhler.

Paratype. ZSM 1638/2008 (field number FGZC 1874), adult male, same locality as holotype, collected on 27 February 2008 by N. D'Cruze, F. Glaw and J. Köhler.

Remark. The holotype was figured as *Boophis* sp. aff. *rappiodes* by Glaw & Vences (2007: page 446).

Diagnosis. A member of the *Boophis ulftunni* species group (sensu Wollenberg *et al.* 2008). *Boophis baetkei* is distinguished from other species groups containing green species as follows. From members of the *B. albilabris* and *B. microtympanum* species groups, the new species differs by green dorsal colouration with

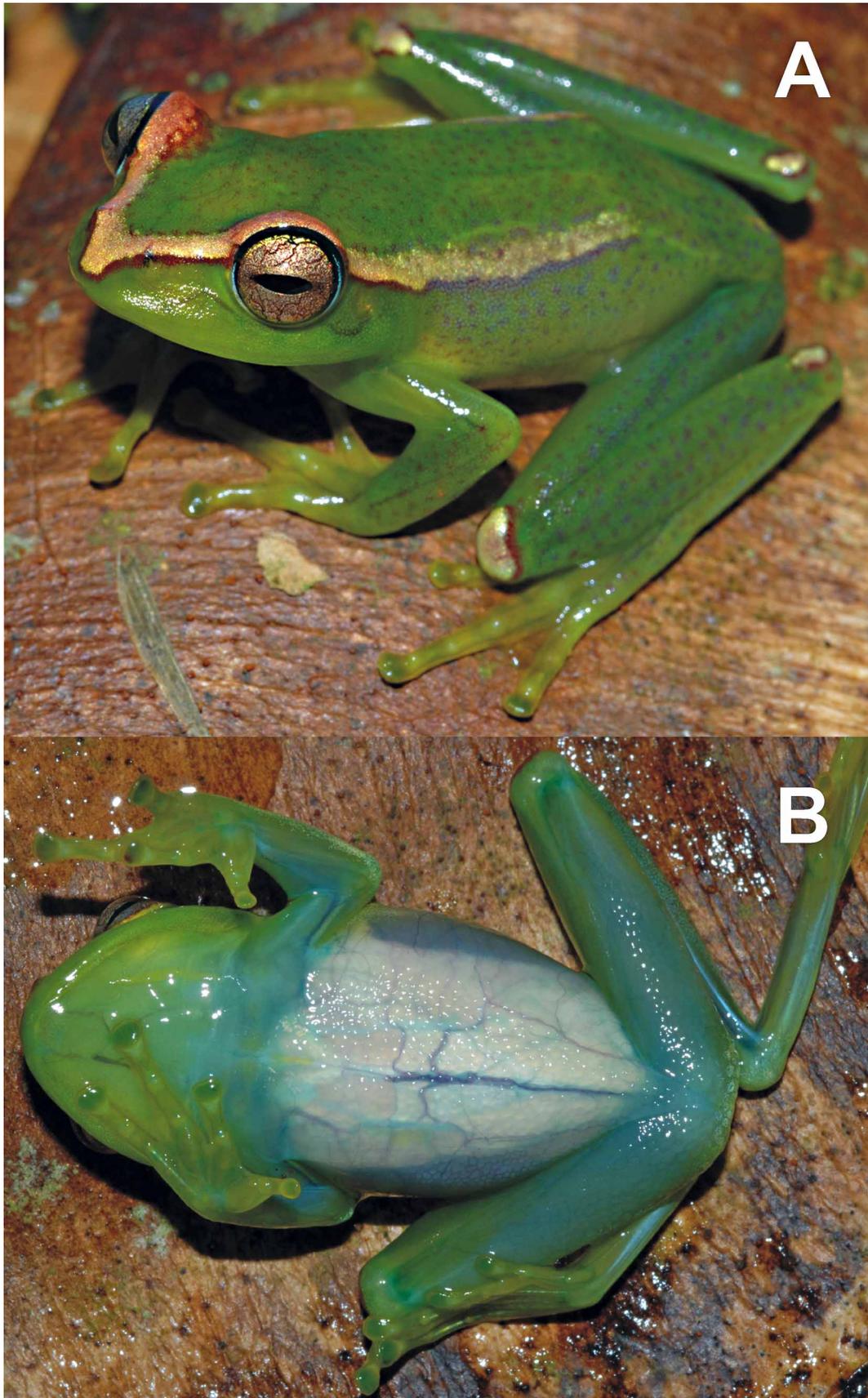


FIGURE 1. Male holotype of *Boophis baetkei* sp. n. (ZSM 2051/2007) in life. **A.** Dorsolateral view, **B.** Ventral view. SVL 30.8 mm.

translucent shade in life (versus opaque green) and smaller size. It mainly differs from all species in the *B. rappiodes* and *B. mandraka* groups by a pigmented ventral side (versus inner organs visible through transparent ventral skin). *Boophis baetkei* differs from all species of the *B. albipunctatus* and *B. luteus* groups by advertisement call (see below), colouration in life and lack of lateral dermal fringes along lower arm and tarsus. From the other two species in the *B. ulftunni* group, *B. baetkei* differs as follows: From *B. ulftunni* by the lack of reddish brown markings and flecks on mid-dorsum and between eyes, silvery grey iris with brown spots and reticulation (versus bicoloured iris with outer golden-yellow ring and inner purple ring), more extensive webbing between toes, larger adult male size (30.8 versus 21–24 mm SVL), and differences in the advertisement call (see below). From *B. lilianae* (described below) by presence of distinct patches of vomerine odontophores, relatively larger tympanum, rounded snout in dorsal view (versus mucronate) and larger adult male size (30.8 versus 18.3 mm SVL). Furthermore, *B. baetkei* differs strongly from all the species contained in the mentioned groups by molecular characters.

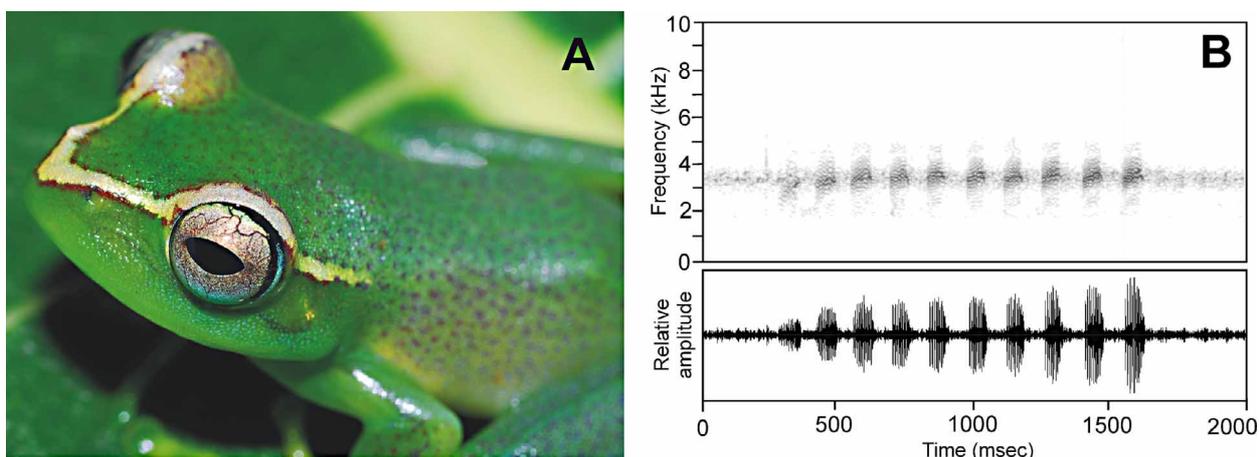


FIGURE 2. A. Male paratype of *Boophis baetkei* sp. n. (ZSM 1638/2008) in life, lateral aspect of head. Note lack of dorsolateral stripe on posterior body. B. Audiospectrogram and corresponding waveform of the advertisement call of *Boophis baetkei* sp. n., emitted by the holotype.

Description of the holotype. Adult male, SVL 30.8 mm. Body slender; head slightly wider than long, wider than body; snout rounded in dorsal view, obtuse in lateral view, nostrils directed laterally, nearer to tip of snout than to eye; canthus rostralis rounded, loreal region slightly concave; tympanum distinct, rounded, TD 43% of ED; supratympanic fold distinct; tongue removed for tissue sample; vomerine odontophores distinct, well separated in two round patches, positioned posteromedian to choanae; choanae medium-sized, rounded. Arms slender, subarticular tubercles single, round; metacarpal tubercles not recognizable; fingers with basal webbing and lateral dermal fringes; webbing formula 1(1.5), 2i(1.5), 2e(1), 3i(2), 3e(1.5), 4(0.5); relative length of fingers $1 < 2 < 4 < 3$; finger discs moderately enlarged; distinct, medium-sized nuptial pad on inner side of first finger, unpigmented. Hindlimbs slender; tibiotarsal articulation reaching nostril when hindlimb is adpressed along body; lateral metatarsalia separated by webbing; inner metatarsal tubercle distinct, elongated; no outer metatarsal tubercle; webbing between toes well developed, lateral dermal fringes present; webbing formula 1(0.5), 2i(0.75), 2e(0.25), 3i(1), 3e(0), 4i(1), 4e(1), 5(0); relative length of toes $1 < 2 < 5 = 3 < 4$; toe discs small, only slightly enlarged. Skin smooth on dorsal surfaces, very finely granular on throat, smooth on chest, coarsely granular on belly, glandular around cloacal opening; no distinct enlarged tubercles in the cloacal region.

Measurements (in mm): SVL 30.8, HW 11.1, HL 10.6, ED 3.7, END 2.7, NSD 1.9, NND 2.8, TD 1.6, TL 17.1, HAL 9.3, FOL 13.5, FOTL 23.1.

After six months in preservative, ground colour of flanks, dorsal and ventral surfaces creamy yellow. Upper surface of head, dorsum, flanks and upper surfaces of limbs covered by regularly scattered small pink spots, most distinct on posterior dorsum. A continuous pink stripe running from the tip of snout along the canthus rostralis (above nostrils) to the upper eyelid and continuing as a supratympanal and dorsolateral stripe which fades at the urostyle. Inner margin of upper eyelid with irregular brown fleck. Nostril bordered with black dorsally. Irregular pink flecks on heel and knee, the first having half the size of the latter.

In life, ground colour of upper surface of head, dorsum and flanks translucent green. Small regularly scattered reddish to purplish dots on dorsum, flanks and upper surfaces of limbs. A continuous pinkish golden stripe running from the tip of snout along the canthus rostralis (above nostrils) to the upper eyelid and continues as a supratympanal and dorsolateral stripe which fades at midbody. The pinkish golden stripe on canthus, upper eyelid and above the tympanum is bordered by a dark red line anteriorly and by red spots posteriorly. This red line and the spots become purplish dorsolaterally posterior to tympanum. Upper edge of nostril bordered with fine black line. Pinkish golden flecks encircled by reddish brown line present on heel and knee, the first having half the size of the latter. Dorsal surfaces of fingers and toes yellowish green, terminal discs green. Ventral surfaces of limbs translucent bluish green, chest translucent turquoise green, throat translucent yellowish green. Belly white. Webbing yellowish green. Bones bluish green. Iris silvery grey with fine brown spotting and reticulation. Posterior iris periphery black, followed by light blue. Eye periphery black dorsally (Fig. 1).

Variation. Measurements (in mm) of the male paratype are as follows: SVL 30.0, HW 10.7, HL 11.5, ED 3.9, END 2.6, NSD 1.8, NND 3.1, TD 2.0, TL 16.6, HAL 9.1, FOL 13.4, FOTL 21.9. After ten days in preservative, very fine red spotting evenly distributed on dorsal surfaces, more distinct when compared to holotype. In life, pinkish golden stripe running from the tip of snout along the canthus rostralis to the upper eyelid and continues as a supratympanal stripe, fading posterior to tympanum, not continuing as dorsolateral stripe as in the holotype (Fig. 2A). Pinkish golden fleck on heel very small, that on knee distinctly smaller and less distinct compared to the holotype. Females are unknown.

Molecular differentiation. A 16S rRNA sequence (517 bp) of the holotype of *B. baetkei* has been deposited in Genbank (accession number EU314954). This sequence had an uncorrected sequence divergence of 5.9% (30 substitutions) as compared to *B. ulftunni*.

Vocalization. The advertisement call of *Boophis baetkei* (recorded from the holotype at an air temperature of ca. 24°C) consists of a series of strongly pulsed notes repeated in regular intervals (Fig. 2B). Temporal and spectral call parameters are as follows: call duration, 1026–1963 msec (1565 ± 341); number of notes per call, 8–13 (10.83 ± 1.72); note duration, 58–88 msec (70.13 ± 7.97); note repetition rate within calls, 6.41–7.11 notes/sec (6.92 ± 0.34); number of pulses per note, 9–15 (10.75 ± 1.71); pulse repetition rate within notes, 133–241 pulses/sec (189.17 ± 40.91); maximum call energy at 3108–3910 Hz (3573 ± 248); dominant frequency range approximately 2800–4600 Hz. Calls are repeated at a rate of approximately 11 calls/minute. Amplitude of notes is increasing towards the end of the call. Terminal pulses within notes are sometimes clustered and partly fused. When less motivated, males may emit single notes only in irregular intervals.

In comparison, calls of *B. ulftunni* are composed of a single pulsed note only, exhibiting much longer intervals between pulses. Consequently, note duration is much longer in *B. ulftunni* when compared to *B. baetkei*, but overall call duration is significantly shorter. Furthermore, with 5247–6413 Hz, maximum call energy in *B. ulftunni* calls is higher pitched (see Wollenberg *et al.* 2008).

Distribution and natural history. Only known from the type locality. Both type specimens were collected at night in heavily disturbed transitional forest (that could also be characterized as relatively dry rainforest) at the edge of the Forêt d'Ambre Special Reserve. The small patch of forest was encircled by an artificial irrigation channel, not containing any water at the time of collection in 2007, and by a stream (width ca. 3–4 m, maximum depth ca. 0.5 m) at one side which might be used for breeding (tadpoles are unknown). Several males of *B. baetkei* were heard calling at night from trees and bushes along the edge of this stream in

2007 and 2008. Lowest calling position of males was at approximately two metres height (i.e. of the type specimens), but most males were calling from far higher positions in trees which were not accessible and prevented further collection. *Boophis baetkei* occurs in syntopy with *B. septentrionalis* and *B. brachyichir*. The stream contained large numbers of individuals of the threatened endemic fish *Pachypanchax sakaramyi* in 2007, but only few in 2008 (at considerably lower water level).

Etymology. We are pleased to dedicate this species to Claus Bätke (Deutsche Gesellschaft für Technische Zusammenarbeit – GTZ). Acting as leader of the "Tropenökologisches Begleitprogramm" (TÖB) his personal efforts were crucial for the establishment of BIOPAT in 1999. Therewith, he decisively contributed to the support of biodiversity research and nature conservation in tropical countries, including Madagascar.

***Boophis lilianae* sp. n.**

Holotype. ZSM 201/2006 (field number ZCMV 2864), adult male, from km 6 on road Ifanadiana–Tolongoina, 21°21'21" S, 47°36'47" E, 468 m above sea level, Fianarantsoa Province, Southern Central East of Madagascar, collected on 22 February 2006 by L. Raharivololoniaina.

Paratype. ZSM 202/2006 (field number ZCMV 2865), adult female, same data as holotype.

Remark. This species was referred to as *Boophis* sp. aff. *rappiodes* "South" by Glaw & Vences (2007: pages 174-175).

Diagnosis. A species of the *B. ulftunni* species group (sensu Wollenberg *et al.* 2008). *Boophis lilianae* is so far the smallest known species in the genus. It is distinguished from other species groups of *Boophis* containing green species as follows. From members of the *B. albilabris* and *B. microtypanum* species groups, the new species differs by green dorsal colouration with translucent shade in life (versus opaque green) and much smaller size. It mainly differs from all species in the *B. rappiodes* and *B. mandraka* groups by a pigmented ventral side (versus inner organs visible through transparent ventral skin) and smaller size. *Boophis lilianae* differs from all species of the *B. albipunctatus* and *B. luteus* groups by smaller size and lack of lateral dermal fringes along lower arm and tarsus. Furthermore, *B. lilianae* strongly differs from all the species contained in the mentioned groups by molecular characters. From the other two species in the *B. ulftunni* group, *B. lilianae* mainly differs as follows: From *B. ulftunni* by smaller size (SVL 18.3 versus 21-24 mm in males, 20 versus 32–38 mm in females), iris colouration (inner iris ring silvery grey versus purple) and mucronate snout shape in males. From *B. baetkei* by smaller adult male size (30.8 versus 18.3 mm SVL), mucronate male snout shape in dorsal view (versus rounded) and a relatively smaller tympanum. *Boophis lilianae* furthermore differs from *B. baetkei* and *B. ulftunni* by the lack of prominent vomerine odontophores.

Description of the holotype. Adult male, SVL 18.3 mm. Body slender; head as wide as long, wider than body; snout mucronate in dorsal view, obtuse in lateral view; nostrils directed laterally, distinctly protruding, as close to tip of snout as to eye; canthus rostralis rounded, loreal region slightly concave; tympanum distinct, rounded, TD 30% of ED; supratympanic fold weak; tongue narrowly cordiform, posteriorly distinctly bifurcated and half free; vomerine odontophores not evident; choanae small, rounded. Arms slender, subarticular tubercles small, single, round, weakly developed; metacarpal tubercles not recognizable; fingers with basal webbing and lateral dermal fringes; webbing formula 1(1), 2i(2), 2e(1), 3i(2), 3e(1.5), 4(1); relative length of fingers 1<2<4<3; finger discs moderately enlarged; distinct, medium-sized nuptial pad on inner side of first finger, unpigmented. Hindlimbs slender; tibiotarsal articulation reaching snout tip when hindlimb is adpressed along body; lateral metatarsalia separated by webbing; inner metatarsal tubercle distinct, elongated; no outer metatarsal tubercle; webbing between toes well-developed, lateral dermal fringes present; webbing formula 1(1), 2i(1), 2e(0.25), 3i(2), 3e(0), 4i(0.75), 4e(0.75), 5(0.5); relative length of toes 1<2<5=3<4; toe discs moderately enlarged. Skin smooth on dorsal surface with widely scattered minute tubercles, smooth on throat and chest, coarsely granular on belly, glandular around cloacal opening; no distinct enlarged tubercles in the cloacal region.

Measurements (in mm): SVL 18.3, HW 6.5, HL 6.5, ED 2.7, END 1.0, NSD 1.0, NND 1.9, TD 0.8, TL 9.4, HAL 5.4, FOL 7.0, FOTL 12.2.

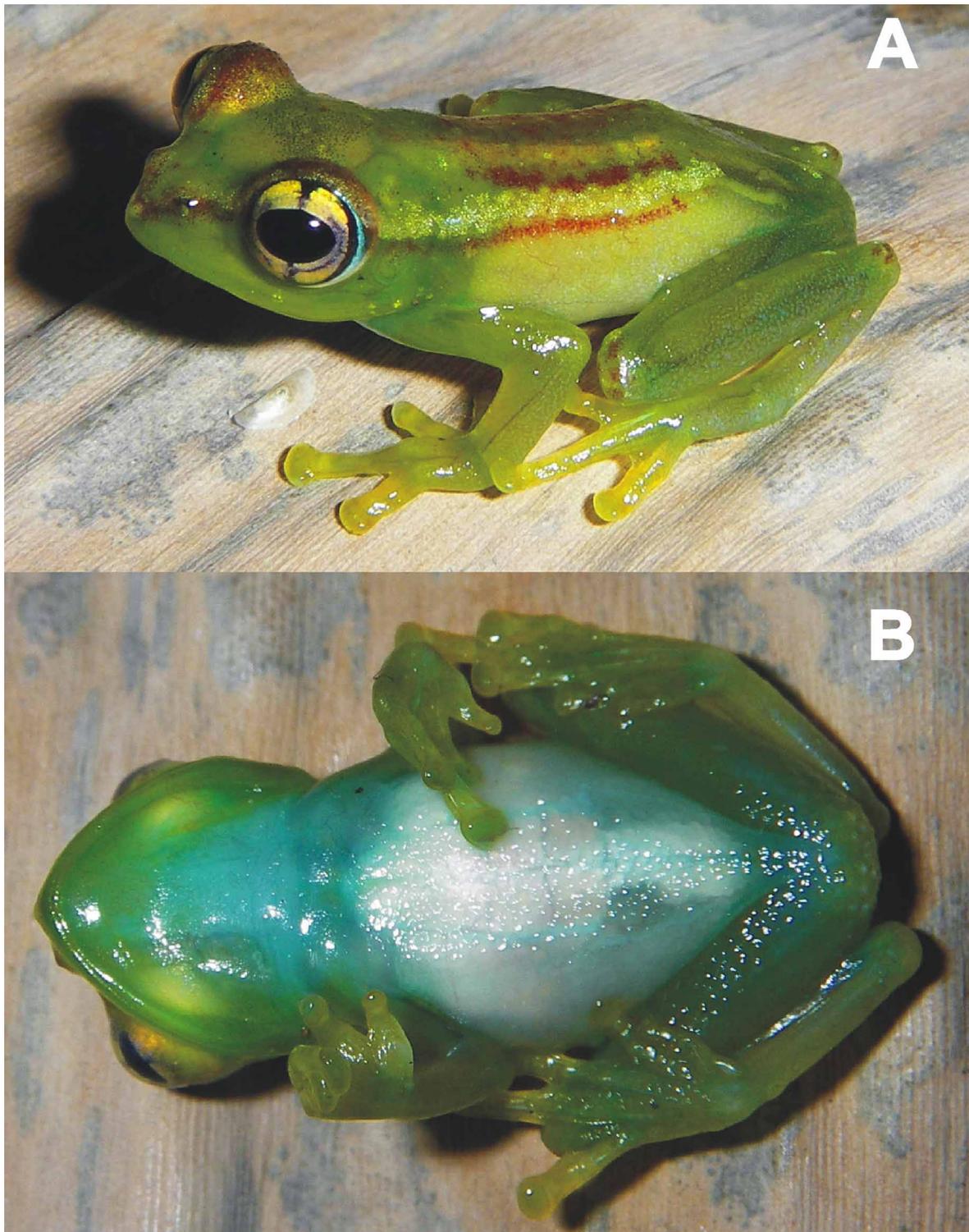


FIGURE 3. Male holotype of *Boophis lilianae* sp. n. (ZSM 201/2006) in life. **A.** Dorsolateral view, **B.** Ventral view. SVL 18.3 mm.

After nineteen months in preservative, ground colour of flanks, dorsal and ventral surfaces creamy yellow. A pink canthal stripe running from tip of snout to upper eyelid. Nostril encircled by thin brown line. Upper

eyelid covered by brown blotch, bordered by pink spot posteriorly. Triangular brown fleck between eyes, its tip directed posteriorly. Thin pink supratympanal line continuing as thin dorsolateral line, fading at level of urostyle. Two fine longitudinal parallel stripes on mid-dorsum formed by rows of pink spots, extending from behind scapular region to level of sacral vertebra. Few minute pink spots irregularly scattered on dorsum. Small pink fleck on knee.

In life, ground colour of upper surface of head, dorsum and flanks translucent yellowish green, slightly transparent in groin. A reddish canthal stripe running from tip of snout to upper eyelid. Thin reddish supratympanic line continuing as fine red dorsolateral line and fading at level of urostyle. Upper eyelid covered by reddish brown blotch. Weak brownish triangular fleck between eyes, its tip directed posteriorly. Two fine longitudinal parallel stripes on mid-dorsum formed by rows of reddish to pink spots, extending from behind scapular region to level of urostyle. Between these stripes, some irregular pink marbling on mid-dorsum. Few minute irregularly scattered reddish brown spots on dorsum. Irregular reddish brown spots on heel and knee. Dorsal surfaces of fingers, toes and terminal discs yellowish green. Ventral surfaces of limbs, chest and posterior half of throat translucent turquoise green, anterior half of throat translucent yellowish green. Belly white with a turquoise shade. Bones green. Inner iris silvery grey, with a fine brownish circular line. Outer iris golden yellowish, with a black triangular fleck median above and below pupil, respectively. Posterior iris periphery black, followed by light blue (Fig. 3).

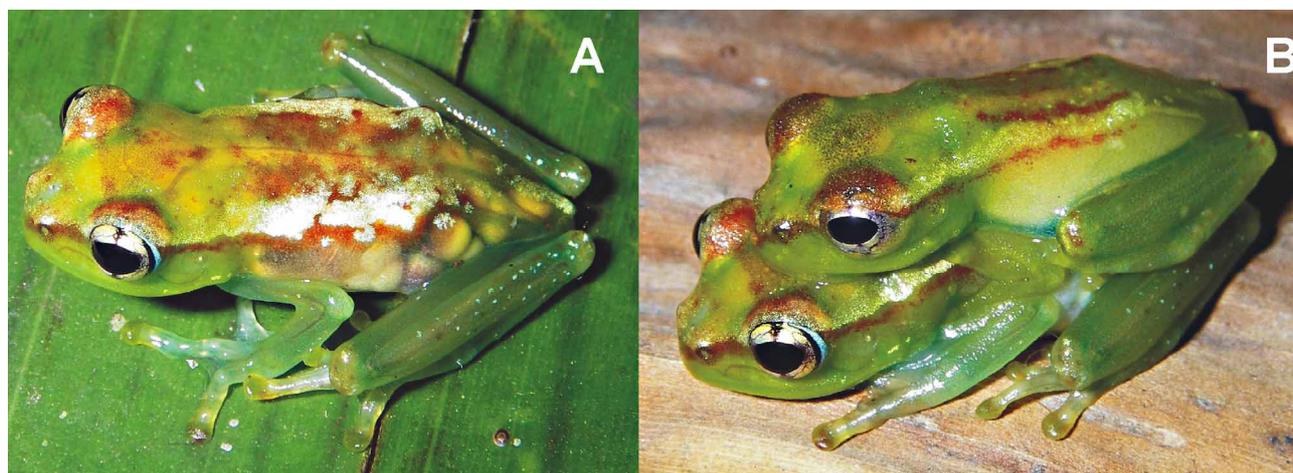


FIGURE 4. A. Female paratype of *Boophis lilianae* sp. n. (ZSM 202/2006) in life, dorsolateral view. Note eggs visible through transparent skin. SVL 20.0 mm. B. Male and female of *Boophis lilianae* sp. n. in amplexus, showing the minimal sexual dimorphism in body size.

Variation. Measurements (in mm) of the female paratype are as follows: SVL 20.0, HW 7.2, HL 7.1, ED 2.7, END 1.5, NSD 1.3, NND 1.9, TD 1.0, TL 11.0, HAL 5.8, FOL 8.3, FOTL 13.6. The snout is rather truncate in dorsal view in the paratype, compared to the distinctly mucronate shape in the holotype. In preservative, the paratype exhibits more extensive pink colour on dorsum, formed by several irregular blotches and flecks on mid-dorsum. The pink dorsolateral lines are partly interrupted, generally wider and more irregular in outline. The brown flecks on upper eyelids contain a layer of pink colour. Two distinctly separated pink mid-dorsal spots are present posteriorly to the triangular brown fleck between eyes. In life, the red dorsolateral stripes are bordered by bold silvery white blotches. Minute white spots are scattered on dorsal surfaces of limbs. The skin in groin and posterior dorsum is almost completely transparent (Fig. 4A). Tentatively judging from the two available specimens, a difference of only 8.5% in the SVL between the adult male and adult female, sexual dimorphism in body size is less pronounced than usually observed in species of green *Boophis* (see Fig. 4B).

Molecular differentiation. A 16S rRNA sequence (462 bp) of the holotype of *B. liliana*e has been deposited in Genbank (accession number EU314953). This sequence had an uncorrected sequence divergence of 8.7% (40 substitutions) as compared to the holotype of *B. baetke*i, and of 10.0% (46 substitutions) to *B. ulftun*ni.

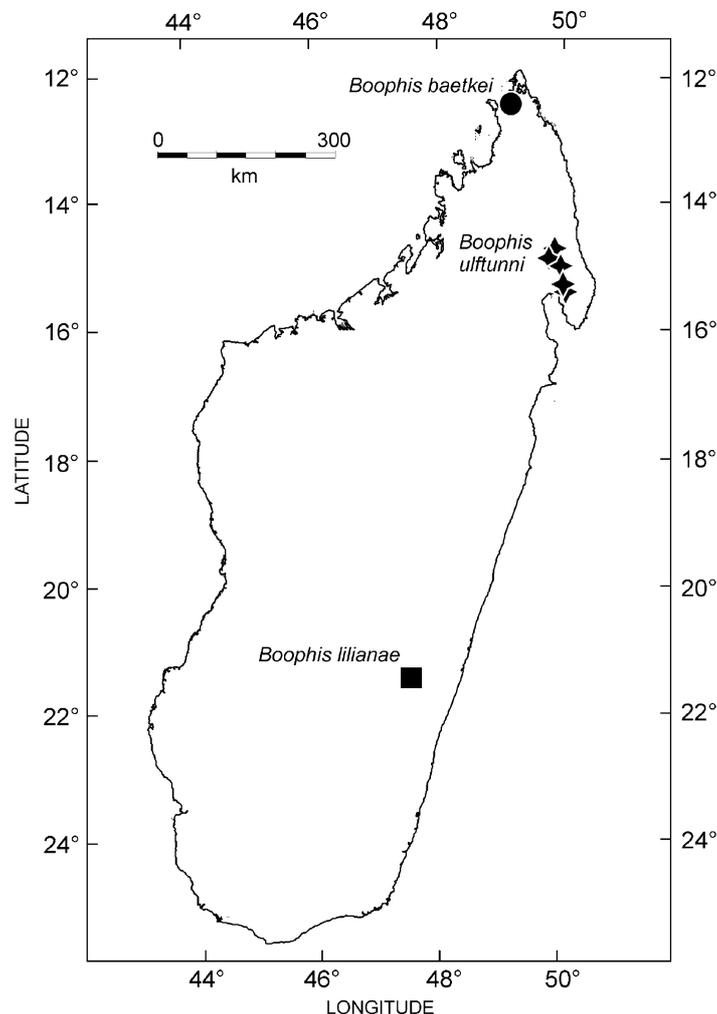


FIGURE 5. Schematic map of Madagascar showing the known distribution of species in the *Boophis ulftunni* group.

Distribution and natural history. So far only collected from the type locality. The two type specimens were collected at night in amplexus. They were sitting on low vegetation in a swampy area formed by a slowly moving stream that flooded large parts of a small patch of dense palm forest next to highly degraded rainforest. The female contains yellowish eggs (each approximately 1.6 mm in diameter) in its oviducts as visible through the transparent skin on flanks, indicating that reproduction was taking place at the time of collecting (during the rainy season). *Boophis liliana*e occurs in sympatry with *B. madagascariensis*, *B. opisthodon*, and *B. pyrhus*. Despite intensive searches at the type locality, on the same day the amplexing specimens were collected, and on at least 5 other occasions, no further specimens could be found and no calls potentially assignable to this species were heard. In 2007, we collected, at the type locality, tadpoles assignable to this species which will be described elsewhere. One further pair in amplexus, tentatively assignable to this species, has been observed and photographed by A. Strauß and J. Glos on 22 January 2008 at a site locally known as Imaloka in the Ranomafana National Park (21°14'32" S, 47°27'55" E, approximately 900-1000 m a.s.l.). The specimens were sitting about 1.5 m high on leaves of a shrub above a small, rather slow moving stream with

sandy substrate. According to our observations, *B. lilianae* may either be very secretive or seasonal in its calling behaviour.

Etymology. This species is dedicated to Liliane Raharivololoniaina who collected the two type specimens, in recognition of her contribution to the study of Madagascan amphibians and her continuous help during our field expeditions. The terminal "e" in "Liliane" has intentionally been omitted in the species name for easier pronunciation.

Discussion

The new species described are two further examples of genetically, morphologically, and as far as known bio-acoustically highly divergent yet previously undiscovered lineages of Madagascan frogs (compare Glaw *et al.* 2006). In previous revisions of the *Boophis rappiodes* and *B. mandraka* groups (Vences & Glaw 2002, Vallan *et al.* 2003) we had examined most of the available material of small-sized and green-coloured *Boophis* from major historical collections (i.e., those housed in Paris, Amsterdam and Bonn) without finding any instance of specimens resembling *Boophis baetkei* or *B. lilianae*. These two species may be geographically restricted to regions that have been poorly surveyed, and may have partly seasonal or secretive habits, thus evading discovery so far.

Nevertheless, it is remarkable that a whole cluster of morphologically similar treefrogs (the *Boophis ulftunni* group) has remained unperceived so far in Madagascar. These frogs have unique and partly conspicuous calls, are morphologically easily recognizable (being the only small green species without transparent ventral skin), and *B. lilianae* is even the smallest of all *Boophis* species known so far. Furthermore, the genetic differentiation of these species is remarkable: not only are they highly divergent from all other *Boophis*, also the genetic divergences within the group are very high (compare Köhler *et al.* 2005), indicating that these species are evolutionary ancient. The differentiation among *B. baetkei*, *B. lilianae* and *B. ulftunni* is so massive that phylogenetic analyses based on the 16S rRNA gene alone fail to group them as monophyletic (results not shown), although all methods used placed them relatively close to each other and indicated possible relationships with the *B. microtympanum* group (see also Wollenberg *et al.* 2008). These results indicate that the division of stream-breeding *Boophis* into phenetic species groups, and the inclusion of the two new species in the *B. ulftunni* group, is to be seen as a tentative and preliminary classification. However, further analyses of other genes within this taxonomic group (K.C. Wollenberg *et al.* in prep.) seem to be supportive of the classification proposed herein.

The distribution of the three species of the *Boophis ulftunni* group spans over most of Madagascar's rainforest areas, and a particular biogeographic pattern is not apparent from the few collecting localities known so far. From a conservation perspective, *Boophis lilianae* clearly qualifies for the status "Data Deficient" because its extent of occurrence and area of occupancy are totally unknown. So far, this species is known from the unprotected type locality and from one site within Ranomafana National Park. Records of adults refer to single mating pairs in both cases, and the calls have not been heard so far. This indicates that the species is secretive and may have passed unperceived at other previously surveyed rainforest sites in eastern Madagascar. For *Boophis baetkei*, we consider it more likely that it actually is a regional endemic, as are many species occurring in the far north of Madagascar. Although this species can be easily distinguished from *B. ulftunni* by its larger size, it has not been detected elsewhere during intensive surveys of northern and north-eastern Madagascar (e.g. Andreone *et al.* 2000), despite its rather characteristic advertisement call. We therefore consider this species to be restricted to lower altitudes of the the Ambre massif. Its only known locality, the Forêt d'Ambre Special Reserve, is legally protected but under heavy pressure, resulting in logging and agriculture within the reserve area (D'Cruze *et al.* unpubl.). For these reasons (extent of occurrence is less than 5,000 km², all individuals are in only one location, and there is heavy decline in the extent and quality of its forest habi-

tat), *B. baetkei* must be considered as "Endangered" according to IUCN criteria (see also Andreone *et al.* 2005, Andreone *et al.* in press).

Acknowledgements

We are grateful to Liliane Raharivololoniaina, Roger-Daniel Randrianiaina, Parfait Bora, Ylenia Chiari, Angeluc Razafimanantsoa, Hildegard Enting, Angelika Knoll, Neil D'Cruze, and Axel Meyer for their help in the field, and to Katharina Wollenberg for molecular analyses. Axel Strauß and Julian Glos provided unpublished information on the second locality of *B. liliana*. The work was carried out in the framework of collaboration accords of the author's institutions with the Département de Biologie Animale, Université d'Antananarivo, and the Association Nationale pour la Gestion des Aires Protégées (ANGAP). We are grateful to the Malagasy authorities, in particular the Ministère de l'Environnement et des Eaux et Forêts, for research and export permits. Funding was provided by the Volkswagen Foundation, the German Science Foundation (DFG), the European Association of Zoos and Aquaria (EAZA), Act for Nature, and the Hessisches Landesmuseum Darmstadt.

References

- Andreone, F., Randrianirina, J.E., Jenkins, P.D. & Aprea, G. (2000) Species diversity of Amphibia, Reptilia and Lipotyphla (Mammalia) at Ambolokopatrika, a rainforest between the Anjanaharibe-Sud and Marojejy massifs, NE Madagascar. *Biodiversity and Conservation*, 9, 1587–1622.
- Andreone, F., Cox, N., Glaw, F., Köhler, J., Rabibisoa, N.H.C., Randriamahazo, H., Randrianasolo, H., Raxworthy, C. J., Stuart, S., Vallan, D. & Vences M. (in press) Update of the Global Amphibian Assessment for Malagasy amphibians in light of new species discoveries, taxonomic changes, and new field information. *A conservation strategy for the amphibians of Madagascar* (Andreone, F., editor). Monografia XLV, Museo Regionale di Scienze Naturali, Torino.
- Andreone, F., Cadle, J.E., Cox, N., Glaw, F., Nussbaum, R.A., Raxworthy, C.J., Stuart, S.N., Vallan, D. & Vences, M. (2005) Species review of amphibian extinction risks in Madagascar: conclusions from the Global Amphibian Assessment. *Conservation Biology*, 19, 1790–1802.
- Blommers-Schlösser, R.M.A. (1979) Biosystematics of the Malagasy frogs. II. The genus *Boophis* (Rhacophoridae). *Bijdragen tot de Dierkunde*, 49, 261–312.
- Glaw, F., Hoegg, S. & Vences, M. (2006) Discovery of a new basal relict lineage of Madagascan frogs and its implications for mantellid evolution. *Zootaxa*, 1334, 27–34.
- Glaw F. & Vences, M. (1997) Anuran eye colouration: definitions, variation, taxonomic implications and possible functions, pp. 125–138. In: Böhme, W., Bischoff, W. and Ziegler T., Eds. *Herpetologia Bonnensis*. SEH Proceedings, Bonn.
- Glaw, F. & Vences, M. (2002) A new cryptic treefrog species of the *Boophis luteus* group from Madagascar: bioacoustic and genetic evidence (Amphibia, Anura, Mantellidae). *Spixiana*, 25, 173–181.
- Glaw, F. & Vences, M. (2006) Phylogeny and genus-level classification of mantellid frogs. *Organisms Diversity & Evolution*, 6, 236–253.
- Glaw, F. & Vences, M. (2007) *A Field Guide to the Amphibians and Reptiles of Madagascar. Third edition*. Vences & Glaw Verlag, Cologne, 496 pp.
- Köhler, J. (2000) Amphibian diversity in Bolivia: a study with special reference to montane forest regions. *Bonner zoologische Monographien*, 48, 1–243.
- Köhler, J., Glaw, F. & Vences, M. (2007) A new green treefrog, genus *Boophis* Tschudi 1838 (Anura Mantellidae), from arid western Madagascar: phylogenetic relationships and biogeographic implications. *Tropical Zoology*, 20, 215–227.
- Köhler, J., Vieites, D.R., Bonett, R.M., Hita Garcia, F., Glaw, F., Steinke, D. & Vences, M. (2005) New amphibians and global conservation: a boost in species discoveries in a highly endangered vertebrate group. *BioScience*, 55, 693–696.
- 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. Dept. Zoology, Univ. Hawaii, Honolulu.
- Vallan, D., M. Vences & Glaw, F. (2003) Two new species of the *Boophis mandraka* complex (Anura, Mantellidae) from

the Andasibe region in eastern Madagascar. *Amphibia-Reptilia*, 24, 305–319.

- Vences, M. & Glaw, F. (2002) Two new treefrogs of the *Boophis rappiodes* group from eastern Madagascar. *Tropical Zoology*, 15, 141–163.
- Vences, M. & Glaw, F. (2005) A new cryptic frog of the genus *Boophis* from the northwestern rainforests of Madagascar. *African Journal of Herpetology*, 54, 77–84.
- Vences, M., Thomas, M., Van der Meijden, A., Chiari, Y. & Vieites, D.R. (2005) Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology*, 2, article 5.
- Wollenberg, K.C., Andreone, F., Glaw, F. & Vences, M. (2008) Pretty in pink: a new treefrog species of the genus *Boophis* from north-eastern Madagascar. *Zootaxa*, 1684, 58–68.