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Molecular and bioacoustic differentiation of deep conspecific lineages of the Malagasy treefrogs *Boophis tampoka* and *B. luteus*

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Abstract. During herpetological surveys in various largely degraded areas of northern Madagascar, in particular on the western slopes of the Makira plateau and the highlands bordering the southern slopes of the Tsaratanana massif, we collected at four localities specimens of green treefrogs that we assigned to *Boophis tampoka*, a species previously only known from its type locality, the Tsingy de Bemaraha in western Madagascar. We here present details of this finding, including call descriptions of the new populations, morphometric measurements of voucher specimens, and a molecular genetic analysis. The new northern populations of *B. tampoka* were morphologically, chromatically and bioacoustically similar to the Bemaraha population but had a strong molecular differentiation, with a divergence of 3.3% (uncorrected p-distance) in a fragment of the mitochondrial 16S rRNA gene. We conclude that despite this substantial genetic differentiation, the populations are most appropriately seen as deep conspecific lineage rather than as cryptic species. A similar situation is found in *Boophis luteus*, the sister species of *B. tampoka*, for which we also review the molecular and bioacoustic evidence. Also in this species, two genealogical lineages exist which show no consistent bioacoustic differentiation. We conclude that these lineages, similar to those in *B. tampoka*, should be considered as deep conspecific lineages (DCL). The new localities of *Boophis tampoka* constitute range extensions of almost 500 km to the north and we therefore suggest that the IUCN threat status of this species should be changed to "Near Threatened".

Keywords. Amphibia, Anura, Mantellidae, Madagascar, *Boophis tampoka*, *Boophis luteus*, Deep Conspecific Lineages, range extension.

Introduction

Green-colored treefrogs of the genus *Boophis* (family Mantellidae) are a group of convoluted taxonomic history, mainly because of their low differentiation in external morphological characters and because the color and pattern of most species completely fade to uniform yellowish and eventually whitish after preservation, making it impossible to recognize the (anyway limited) interspecific color differences in preserved individuals (Köhler et al. 2007). However, given that these treefrogs have typically intense and species-specific advertisement calls, a bioacoustic species delimitation has proven to be very efficient to distinguish distinct evolutionary entities that in most cases were also characterized by high molecular divergences (e.g., Glaw & Vences 2002; Glaw et al. 2010). In the *Boophis luteus* group which includes all of the larger-sized *Boophis* species with translucent green dorsal color, Vieites et al. (2009) distinguished nine species and a further seven yet undescribed candidate species, one of which was subsequently described by Glaw et al. (2010).

Similar to the situation in the genus *Boophis*, almost all nominal species of Malagasy amphibians are

Figure 1. Western slope of the Makira plateau with largely deforested areas except some gallery forest along streams. This site is located about 20 km from Antsatramidola and close to Sahaovy, two of the new collection localities of *Boophis tampoka.*

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Table 1. Temporal and spectral parameters of advertisement calls of *Boophis tampoka* from different localities.

	note duration [ms]	internote interval duration [ms]	notes / second	dominant frequency [Hz]
Antambato (n = 14)	168-200 (178 ± 10)	180-225 (205 ± 14)	2.58	3280-3330
Befandriana (n = 10)	162-197 (178 ± 15)	195-218 (206 ± 8)	2.59	3220-3290
Antsatramidola (n = 23)	190-267 (224 ± 30)	194-310 (256 ± 36)	2.12	3240-3323
Andafiabe (n = 50)	159-180 (168 ± 7)	ca. 140-150	3.26	3131-3623

distinguished from their closest relatives by deep genetic divergences. However, Vieites et al. (2009) also identified numerous populations which from at least a mitochondrial perspective represent deeply differentiated genealogical lineages, but on the basis of other evidence (lack of morphological or bioacoustic divergence, signatures of genetic admixture in some populations) they probably do not represent fully isolated evolutionary entities and thus should not be considered as distinct species but as deep conspecific lineages (DCL) *sensu* Vieites et al. (2009). So far, few studies have addressed the degree of bioacoustic and molecular differentiation among such DCLs in detail.

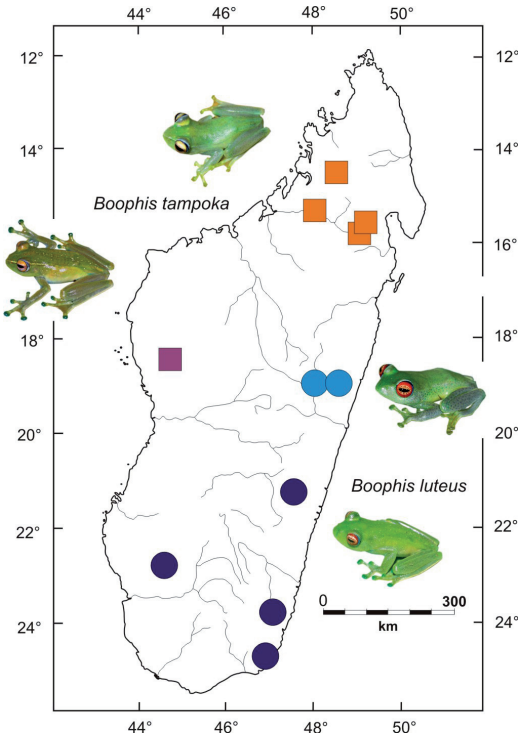

Figure 2. Map showing the distribution of *Boophis luteus* (circles) and *B. tampoka* (squares), based on specimens reliably identified using bioacoustics and molecular data. The colors represent deep conspecific lineages of the two species as shown in Fig. 4. The four new locality records (in orange) of *B. tampoka* are (from north to south) Antambato, Antsohihy-Befandriana, Sahaovy, and Antsatramidola.

Boophis tampoka is a representative of the *Boophis luteus* group which was described from the karstic limestone massif of the Tsingy de Bemaraha, in western Madagascar (Köhler et al. 2007). Interestingly, molecular phylogenetic data (Köhler et al. 2007; Vieites et al. 2009) indicated that this species is sister to *Boophis luteus*, a widespread species in the rainforests of northern central east, southern central east, and south east of Madagascar. In addition, Vieites et al. (2009) provided a phylogenetic tree based on DNA sequences of the mitochondrial 16S rRNA gene according to which two DCLs exist within *B. luteus*.

During recent surveys in several areas of northern Madagascar, new material became available and suggested that *B. tampoka* is probably not endemic to the Tsingy de Bemaraha area but considerably more widespread. The goal of this paper is to report on these new populations, present bioacoustic and molecular data for them, and provide additional data suggesting that the variation in *B. tampoka* as well as in its sister species *B. luteus* is at present adequately addressed by the DCL concept rather than reflecting the existence of cryptic species.

Material and Methods

Frog specimens were collected at night by opportunistic searching and by locating calling males, using torches and head lamps. Specimens were euthanized in a chlorobutanol solution, fixed in 5% formalin or 95% ethanol, and preserved in 70% ethanol. Specimens were deposited in the collections of the Université d'Antananarivo, Département de Biologie Animale, Antananarivo, Madagascar (UADBA) and the Zoologische Staatssammlung München, Germany (ZSM). DRV and ZCMV refer to D. R. Vieites field numbers and M. Vences field numbers.

Calls were recorded using an Edirol R09 digital recorder. Call

Table 2. Morphological measurements of specimens of *Boophis tampoka* from the new localities in northern Madagascar. Measurements (in millimetres) were all done by M. Vences with a digital caliper to the nearest 0.1 mm. Abbreviations are: SVL (snout-vent length), HW (maximum 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, actually referring not to the tibia bone but to the shank), HAL (hand length), HIL (hindlimb length), FOL (foot length), FOTL (foot length including tarsus), FORL (forelimb length), and RHL (relative hindlimb length). RHL is given as the point reached by the tibiotarsal articulation when the hindlimb is adpressed along body.

Field number	Locality	Sex	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	TIL	RHL
ZCMV 12617	betw. Antsohihy / Befandriana	M	36.4	12.8	13.1	1.7	4.9	2.1	3.1	4.4	24.5	10.9	60.5	27.3	16.5	19.1	nostril
ZCMV 12618	betw. Antsohihy / Befandriana	M	32.5	11.6	11.8	1.8	4.9	2.3	3.1	3.6	19.3	10.0	54.9	24.0	14.7	16.9	nostril
ZCMV 12221	Antambato	M	29.0	9.9	10.4	1.6	4.1	1.9	2.5	3.4	17.8	8.1	45.6	20.4	11.9	14.3	between eye and nostril
ZCMV 12222	Antambato	M	29.9	10.0	10.2	1.8	4.3	2.2	2.7	3.2	17.6	8.2	46.4	21.0	12.8	14.3	anterior eye corner
ZCMV 11450 (ZSM 483/2009)	Sahaovy	M	30.3	9.9	10.8	1.6	4.3	2.1	2.7	3.3	18.0	8.8	47.8	21.2	13.2	15.5	between eye and nostril
ZCMV 11439 (ZSM 481/2009)	Antsatramidola	M	31.3	10.4	11.8	2.0	4.7	2.2	2.8	3.3	19.5	8.8	52.9	23.0	14.2	16.1	nostril
ZCMV 11441 (ZSM 479/2009)	Antsatramidola	M	26.8	8.9	9.8	1.5	3.5	2.2	2.2	3.0	16.3	8.2	43.2	18.3	10.8	13.5	between nostril and snout tip
DRV 5786 (ZSM 484/2009)	Antsatramidola	M	33.8	11.7	12.2	2.0	4.3	2.4	3.0	3.8	22.4	10.4	58.1	25.1	15.3	17.9	snout tip
ZCMV 11440 (ZSM 482/2009)	Antsatramidola	F	41.6	14.5	15.3	2.9	4.8	3.0	3.4	4.8	25.6	11.0	67.9	30.7	18.5	21.1	nostril

recordings were analyzed on a personal computer with Windows XP Professional operating system using the software Adobe Audition version 1.5. Frequency information was obtained through Fast Fourier Transformation (FFT; width 1024 points). Spectrograms were obtained at Hanning window function with 256 bands resolution. Temporal measurements are given as range, with mean ± standard deviation in parentheses. Classification of call types was done according to the social context observed during calling and the researchers' experience.

We retrieved previously analyzed DNA sequences of the mitochondrial 16S rRNA gene as used for *B. luteus* and all other species of the *B. luteus* group by Vieites et al. (2009), and for *B. tampoka* by Köhler et al. (2007), and complemented these with newly determined sequences for several of the novel northern localities of *B. tampoka*. Tissue samples were taken in the field and preserved in 95-99% ethanol. DNA was extracted and a fragment of the mitochondrial 16S rRNA gene amplified using standard protocols (see Vences et al. 2005), and subsequently resolved on automated sequencers. Newly determined sequences were deposited in GenBank (accession numbers JF793628-JF793632). Sequences were aligned using the Clustal algorithm implemented in MEGA 4.0 (Tamura et al. 2007) and all positions with gapped or missing data in one or several sequences were excluded from further analysis. We ran a Minimum Evolution analysis under the Kimura-2-Parameter model of sequence evolution to visualize the clustering of specimens and populations, and we emphasize that our goal here is not to provide a thorough analysis of phylogenetic relationships (for which more comprehensive data sets with longer sequences from more than one gene would be required) but just to indicate (by graphical comparison of branch lengths) the degree of genetic differentiation among populations.

Uncorrected pairwise distances between specimens and populations were calculated in MEGA, with pairwise exclusion of gapped and missing stretches of the sequences.

Results and Discussion

New localities for B. tampoka

For the purpose of surveying the herpetofauna of the Makira reserve in north-eastern Madagascar in June of 2009 an expedition led by MV accessed the reserve from the west, and on the way also surveyed some sites in the dry biome of lowland north-western Madagascar, including sites where this type of habitat borders the slope of the Makira plateau (Fig. 1). Similar types of habitat were opportunistically surveyed in June 2010 on the way to the Tsaratanana National Park in northern Madagascar. During these surveys, numerous new amphibian and reptile records were obtained which will be reported elsewhere. At four different sites (Fig. 2), green *Boophis* specimens of the *B. luteus* group were collected (Fig. 3) which by a combination of molecular and bioacoustic data (Figs 4-5) resulted to represent range extensions of *Boophis tampoka* of almost 500 km, and a separate DCL of this species. These four localities, in a north-south direction, were as follows:

(1) Antambato village, between Bealanana and Ambatoria (14°29'34.9'' S, 48°52'7.1'' E, 1188 m above sea level). Observations were made on 6 June 2010 by M. Vences, D. R. Vieites, R. D. Randrianiaina, F. Ratsoavina, E. Rajeriarison and T. Rajofiarison. Specimens were collected and calls recorded in a shallow, sandy stream immediately bordering the village, in a largely degraded landscape with some remaining primary vegetation as gallery forest along the stream. Voucher specimens: ZCMV 12221-12222, to be catalogued in ZSM.

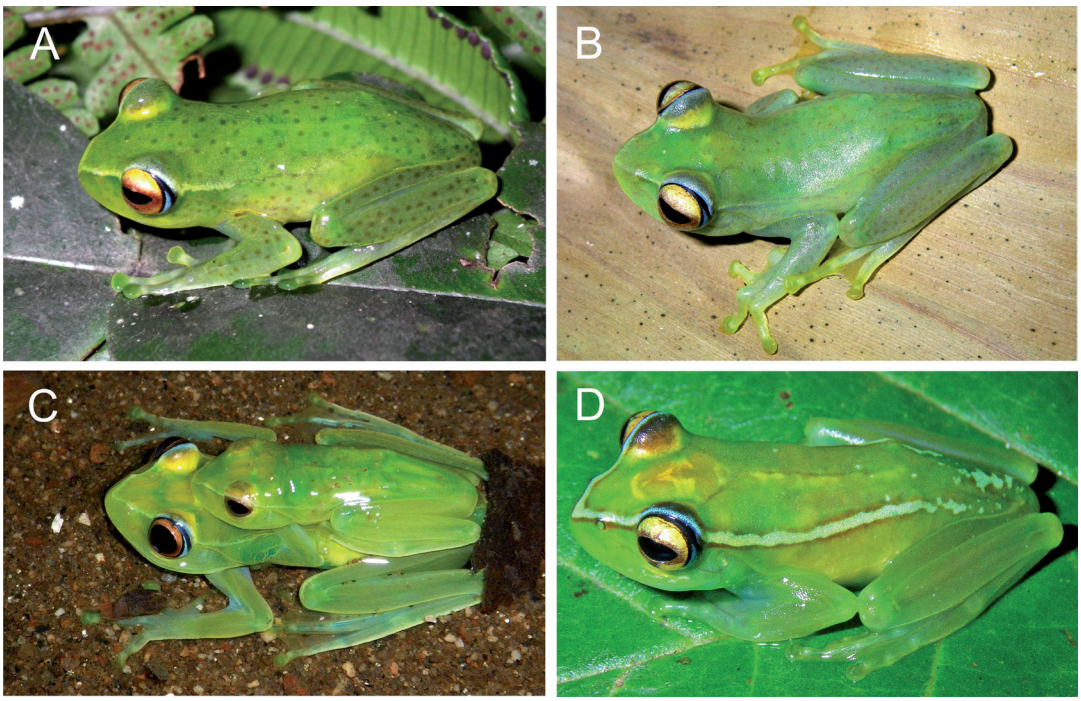

Figure 3. Specimens of *Boophis tampoka* from new northern localities in life. (A) Specimen from Antambato, (B) male from along the road Antsohihy-Befandriana, (C) amplecting couple from Antsatramidola, and (D) ZSM 479/2009 (ZCMV 11441), male from Antsatramidola that was observed emitting the click call type documented in Fig. 5.

(2) Sahaovy Campsite, on the western slope of the Makira plateau, outside of Makira reserve (15°29'19.9" S, 49°04' 42.6" E, 607 m a.s.l.). Observations were made on 20 June 2009 by M. Vences, D. R. Vieites, R. D. Randrianiaina, F. Ratsoavina, J. Patton, C. Patton, E. Rajeriarison and T. Rajofiarison. Calls were heard and one specimen was collected along a large, relatively fast flowing stream with sandy bottom and large boulders, in a degraded area but with numerous large trees along the stream. Voucher specimen: ZSM 483/2009 (ZCMV 11450).

(3) Antsatramidola village (15°38'02.5"S, 48°58'03.1" E, 404 m a.s.l.). Observations were made on 19 June 2009 by the same team as in Sahaovy. Calls were recorded and specimens collected from the vegetation along a shallow, relatively broad stream with sandy bottom which was directly bordering Antsatramidola village. At the time of our survey, during the dry season, the stream had numerous very shallow areas and was comparatively slow flowing. The water was clean and transparent, despite the fact that the whole area was largely deforested. Along the stream was a narrow fringe of 10-30 meters of vegetation, comprising some large trees and areas of dense bushes. Calls were heard from elevated positions at least 2-3 m above the ground. One amplecting couple (Fig. 3) was collected in the stream. Voucher specimens: ZSM 479/2009 (ZCMV 11441) and ZSM 481-482/2009 and 484/2009 (ZCMV 11439-11440 and DRV 5786).

(4) Bridge at km 27 of road between Antsohihy and Befandriana (15°03'11.6" S, 48°12'23.l" E, 140 m a.s.l.). Observations were made on 29 June 2009 by the same team as in Sahaovy. The habitat is a small stream with slow current, bordered by degraded dry forest. Compared to the remaining habitats, this site is in an area of much drier and hotter climate. Specimens were seen and recorded calling at night. Voucher specimens: ZCMV 12617 and 12618, to be catalogued in ZSM.

These new localities for *B. tampoka* in northern Madagascar, which constitute a significant range extension, and its tolerance of a significant degree of habitat destruction suggest that the species is less threatened than believed so far. However, we have observed very severe deforestation going on in the northwestern range of the species. It is clear that in areas where also the remaining gallery forest around streams is destroyed and where streams are polluted due to erosion, this species will not survive. We therefore propose to change its IUCN threat status to "Near Threatend" (currently "Endangered"), because it is

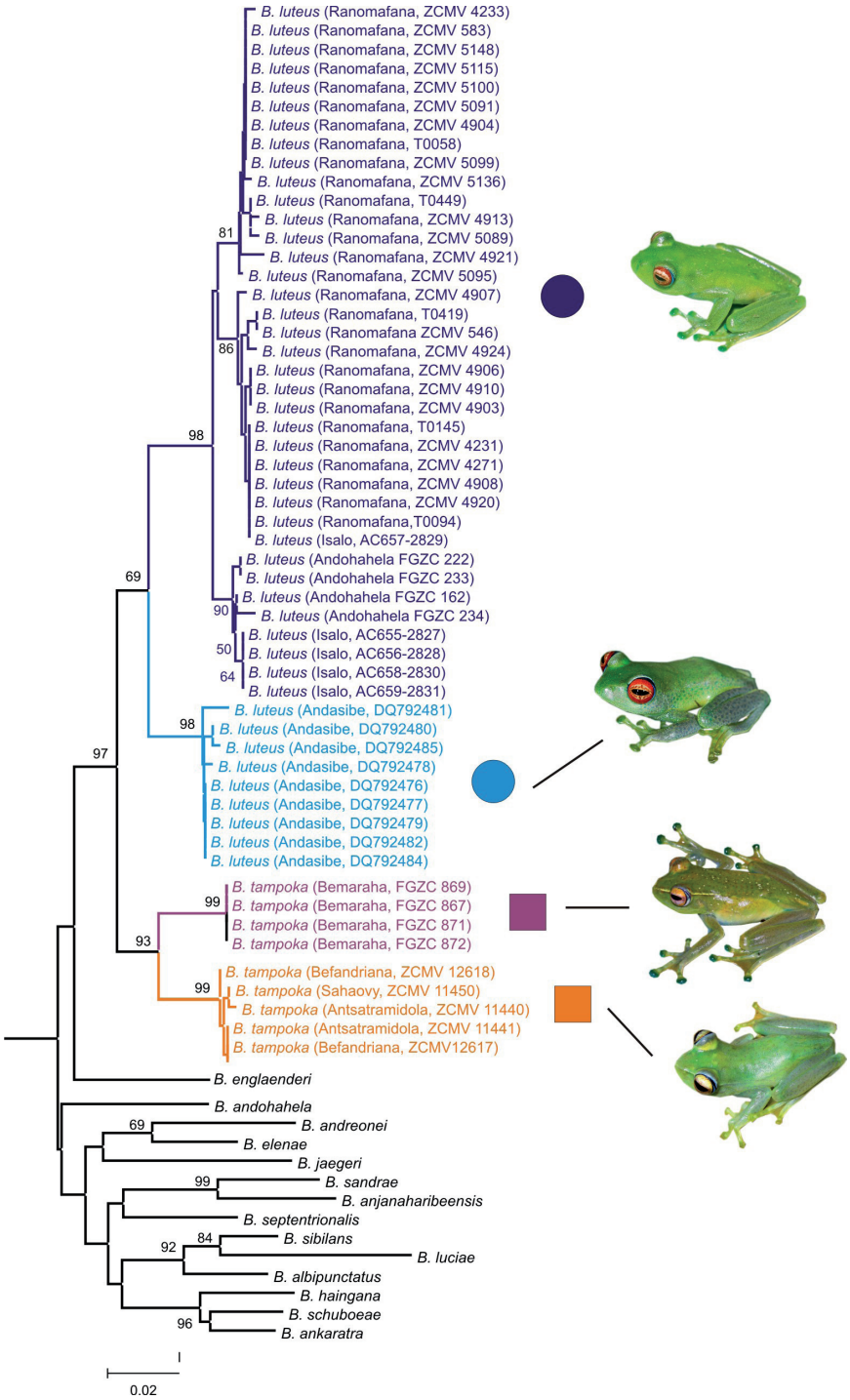

Figure 4. Phylogram of species in the *Boophis luteus* group, with an emphasis on *B. luteus* and *B. tampoka*. The tree is based on a 536 bp alignment of DNA sequences of the 16S rRNA gene, and was reconstructed under the Minimum Evolution optimality criterion using a Kimura-2-Parameter substitution model. Numbers at branches are support values in percent from a bootstrap analysis (2000 replicates; shown only if >60%). The tree was rooted using *B. doulioti* of the *B. tephraeomystax* group as outgroup (not shown). Symbols and inset photos of DCLs of focal species as in Fig. 3. Note that this tree is presented as a means to visualize the clustering of specimens and their genetic differentiation as proportional to branch lengths, and not as an estimate of phylogenetic relationships.

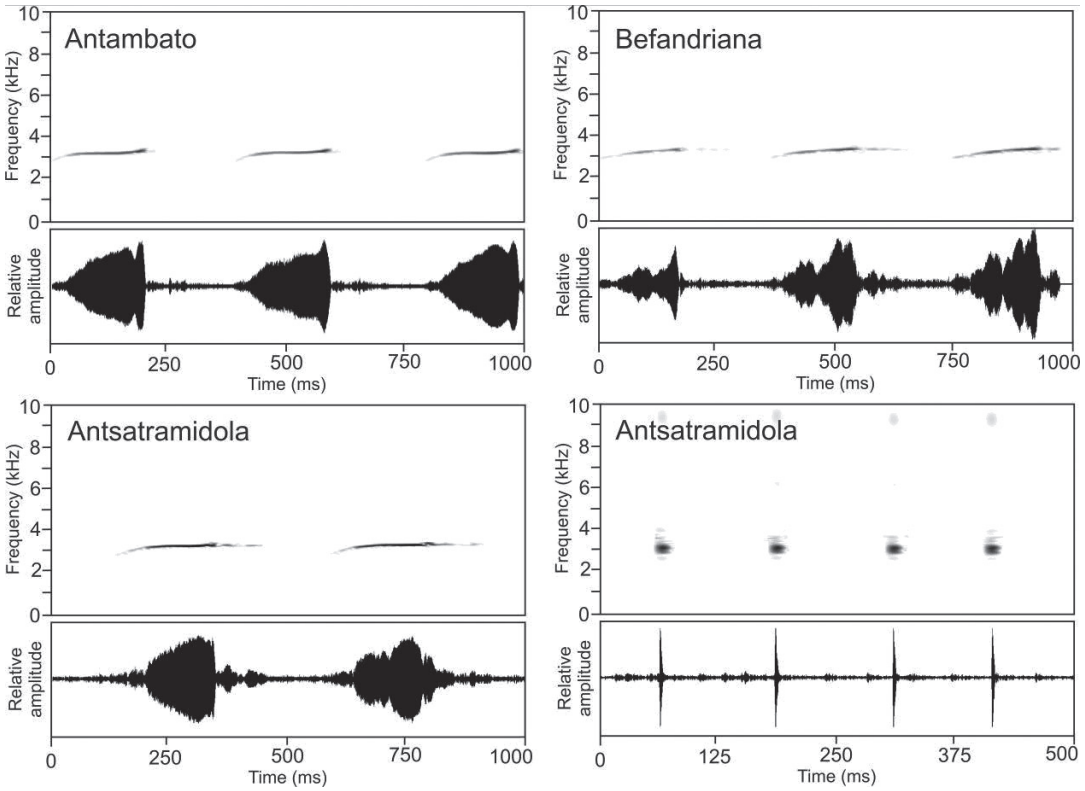

Figure 5. Spectrograms and corresponding oscillograms of advertisement calls (upper row and lower left) of *Boophis tampoka* recorded at different localities in northern Madagascar, and "click" call type recorded at Antsatramidola (from specimen ZSM 479/2009; lower right).

known from less than 10 locations and because there is continuing decline in the extent and quality of its habitat.

Bioacoustic data of B. tampoka

The *Boophis* specimens at these four sites were associated to calls that sounded similar to those previously recorded at the Tsingy de Bemaraha for *B. tampoka.* At three of the sites, calls were recorded and their spectral and temporal parameters measured (Fig. 5). Two different call types were recorded. At most localities, specimens emitted a series of melodious notes. This call type, classified by us as the advertisement call of *B. tampoka,* is a regular series of whistling notes, as described in detail by Köhler et al. (2007). The advertisement calls from new localities studied here generally agree in temporal and spectral characters with the call from the type locality (Andafiabe, Bemaraha). Note repetition rate in calls from new localities is 2.1-2.6/s and thus is slightly lower compared to the Andafiabe calls. Among all populations studied, note duration varies from 159-267 ms, with notes being shortest at the type locality in Bemaraha and being longest in Antsatramidola. Inter-note interval duration varies from ca. 140-150 (Andafiabe) to 310 ms (Antsatramidola). However, differences in individual male motivation and air temperature are likely to explain these temporal differences (see Tab. 1). Apart from these differences, dominant frequency varies in the similar narrow range among all known populations, as all calls show a characteristic upward frequency modulation within notes.

The second call type was recorded from one specimen (ZSM 479/2009 [ZCMV 11441]) from Antsatramidola and consists of a single, very short click note repeated at regular intervals and emitted in long series. Note repetition rate within series is approximately 8.1/s. Frequency is distributed within a broad band from approximately 1500–11000 Hz, with harmonics recognizable at around 6200 Hz and above 9000 Hz. Numerical parameters are as follows (range followed by mean ± SD in parentheses): note duration 3–8 ms (3.6 ± 1.2; n = 65), inter-note interval 91–137 ms

(111.0 ± 11.3; n = 65); duration of call series (= note series) 1750–6890 ms (4237 ± 1967; n = 7); dominant frequency range 2500–4000 Hz; maximum call energy at 2940–3154 Hz (3049 ± 99; n = 57). Call series may start with soft notes having less energy. Call series were emitted at irregular intervals.

Such a "click call-type" has also been described for *B. tampoka* from Bemaraha, its type locality (Köhler et al. 2007). In comparison to the call from Antsatramidola, the click call from Bemaraha is very similar in note duration, frequency range and general character. Note repetition rate was slightly faster at Bemaraha and note series were significantly shorter, with series consisting of 3-7 notes only, a fact possibly explainable with differences in individual male motivation.

Morphological and chromatic characters

The collected specimens of *B. tampoka* associated with the described calls presented some chromatic differences. All had a dorsal ground color that was green with translucent shade (Fig. 3), and a whitish to yellowish, non-transparent venter. In most specimens, a thin white line runs from the snout tip over the nostrils to the eye, and is then continued as a dorsolateral line in the anterior 1/4 to 1/3 of the body. This line is more yellowish and distinct in the photographed specimen from Antambato while it is very faint in specimens from Antsohihy-Befandriana. The Antambato specimen furthermore has distinct dark green dorsal spots, wheras in other populations these spots are either reddish or absent at all. Most deviant is a single specimen from Antsatramidola that, unlike other specimens from the same locality, had much broader dorsolateral stripes continued until the inguinal region, and was also smaller and had a less broad head than other specimens (Fig. 3D). Interestingly, this specimen (ZSM 479/2009) was the only one observed emitting the second call type, but molecular DNA sequences (obtained several times from independent tissue samples of this specimen) indicated it does not represent an independent lineage or species (Fig. 4).

In external morphology and body proportions, no obvious differences of the specimens from the new populations to *B. tampoka* from the type locality, Tsingy de Bemaraha, were noted. Adult snout-vent lengths of males ranged from 26.8-36.4 mm (vs. 31.6-34.8 in the type series), and snout-vent length of the single female was 41.6 mm (40.8-45.8 in the type series). For additional morphometric measurements of the newly collected specimens, see Table 2.

Molecular differentiation

The molecular data (Fig. 4) indicate that the northern populations of *B. tampoka* clearly represent a separate mitochondrial lineage which was separated by 3.3% uncorrected pairwise genetic distance (p-distance) from the specimens collected at the type locality, Tsingy de Bemaraha. This value is above the threshold of 3% used by Vieites et al. (2009) to distinguish candidate species of Malagasy frogs. However, due to the absence of any consistent morphological or bioacoustic difference accompanying the molecular differences, we conclude that the two genealogical lineages within *B. tampoka* are best regarded as deep conspecific lineages (DCL). The 16S divergence of the two DCL of *B. tampoka* from its sister species *B. luteus* was 3.7-6.2%.

Deep conspecific lineages in Boophis luteus

Vieites et al. (2009) had also defined two DCL for *B. luteus,* one from the area around Andasibe in the northern central east of Madagascar (and probably comprising the populations from Andasibe, Ankeniheny, and Mandraka, for which bioacoustic data are available), and a second DCL for populations from the southern central east and south-east. The 16S divergence among these DCL amounts up to 4%. The spectrograms presented by Vieites et al. (2009) suggest a possible quantitative difference in the calls of these two DCL (with southern populations having a faster note repetition rate), and we therefore undertook a review of available data on the calls of *B. luteus* (e.g., Vences et al. 2006).

According to the available bioacoustic data from populations of the Andasibe region, the note-repetition rates are 5.5/s (recording temperature 18°C) at Andasibe (Glaw & Vences 1992), 5.5/s (temperature unknown) at Mandraka (Glaw & Vences 1994), 7/s (temperature unknown) at Mandraka (Blommers-Schlösser 1979), and 5.5/s (22°C) at Ankeniheny (pers. obs.).

Note-repetition rate from the southern populations are 4.8-5.8/s (21°C) at Ranomafana (Andreone 1993), 9.3/s (25°C) near Tolagnaro (Glaw & Vences 1992), and 6.1/s (recording temperature unknown) at Isalo (Glaw & Vences 1994, described under *B. albilabris occidentalis*). Considering the high temperature dependency of note repetiton rate in frog calls, these data suggest that there is no distinct difference in note repetition rate between the northern and southern clades of *Boophis luteus*. We therefore conclude that also in this case, for the time being the genetically divergent mitochondrial lineages should be considered as DCL.

Conclusion

The two cases reported here are exemplary for high molecular divergences in amphibians which in our opinion do not warrant taxonomic consequences. Because we are aware of cases of broad geographic admixture of deep haplotype lineages in tropical frogs (e.g., Robertson et al. 2009; Hauswaldt et al. 2011), we suggest that seeking for concordance between mitochondrial and nuclear markers, or of molecular with morphological or bioacoustic characters, is preferable before new species of frogs are erected in groups of poorly or moderately differentiated species complexes (see also Padial et al. 2010; Vences et al. 2010). Careful evaluation of the evidence in such cases is crucial to understand whether the observed entities really represent independent evolutionary entities that warrant recognition as separate species.

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References

Andreone, F. (1993): Two new treefrogs of the genus *Boophis* (Anura: Rhacophoridae) from central-eastern Madagascar. Boo. Mus. Reg. Sci. nat. Torino **11**: 289–313.

Blommers-Schlösser, R.M.A. (1979): Biosystematics of the Malagasy frogs. II. The genus *Boophis* (Rhacophoridae). Bijdr. Dierk. **49**: 261–312.

Glaw, F., Köhler, J., De la Riva, I., Vieites, D.R., Vences, M. (2010): Integrative taxonomy of Malagasy treefrogs: combination of molecular genetics, bioacoustics and comparative morphology reveals twelve additional species of *Boophis*. Zootaxa **2383**: 1-82.

Glaw, F., Vences, M. (1992): A Fieldguide to the Amphibians and Reptiles of Madagascar. Cologne, Vences & Glaw Verlag, 331 pp.

Glaw, F., Vences, M. (1994): A Fieldguide to the Amphibians and Reptiles of Madagascar. 2nd edition. Cologne, Vences & Glaw Verlag, 480 pp.

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.

Hauswaldt, J. S., Ludewig, A.-K., Vences, M., Pröhl, H. (2011): Widespread co-occurrence of divergent mitochondrial haplotype lineages in a Central American species of poison frog (*Oophaga pumilio*). J. Biogeogr. **38**: 711-736.

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. Trop. Zool. **20**: 215-227.

Padial, J. M., Miralles, A., De la Riva, I., Vences, M. (2010): The integrative future of taxonomy. Frontiers Zool. **7**: article 16.

Robertson, J.M., Duryea, M.C., Zamudio, K.R. (2009): Discordant patterns of evolutionary differentiation in two Neotropical treefrogs. Mol. Ecol. **18**: 1375–1395.

Tamura, K., Dudley, J., Nei, M., Kumar, S. (2007): MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24**: 1596-1599.

Vences, M., Glaw, F., Márquez, R. (2006): *The Calls of the Frogs of Madagascar.* 3 Audio CDs and booklet, 44 pp. Alosa-Fonozoo, Barcelona.

Vences, M., Köhler, J., Crottini, A., Glaw, F. (2010): High mitochondrial sequence divergence meets morphological and bioacoustic conservatism: *Boophis quasiboehmei* sp. n., a new cryptic treefrog species from south-eastern Madagascar. Bonn zool. Bull. **57**: 241-255.

Vences, M., Thomas, M., Bonett, R.M., Vieites, D.R. (2005): Deciphering amphibian diversity through DNA barcoding: chances and challenges. Phil. Trans. Roy. Soc. B **360**: 1859–1868.

Vieites, D.R., Wollenberg, K.C., Andreone, F., Köhler, J., Glaw, F., Vences, M. (2009): Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. Proc. Natl. Acad. Sci. USA **106:** 8267-8272.

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