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Two new treefrogs of the *Boophis rappiodes* group from eastern Madagascar (Amphibia Mantellidae)

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Two new sibling species of *Boophis* Tschudi 1838 are described from Andasibe in central-eastern Madagascar. Both are small greenish treefrogs with a translucent ventral skin and without lateral fringes along lower arm and tarsus, and are thereby assignable to the phenetic *B. rappiodes* group. *Boophis bottae* n. sp. is morphologically similar to *B. rappiodes* (Ahl 1928) and occurs syntopically with this species. It strongly differs from its sibling by advertisement calls (long trill notes instead of two-pulse notes), and by a reddish-brown dorsal pattern which does not fade soon in ethanol, often covering the entire back (vs a red pattern that largely fades in ethanol in *B. rappiodes*). *Boophis tasymena* n. sp. is similar to *B. erythrodactylus* (Guibé 1953) but differs in advertisement calls (notes composed of two instead of four-seven pulses) and lack of red colour on tips of fingers and toes. A lectotype is designated for *B. erythrodactylus*. The discovery of the two new species in addition to the revised distributional information for *B. rappiodes*, *B. erythrodactylus* and *B. viridis* Blommers-Schlösser 1979 confirms that mid-elevational central-eastern Madagascar is the centre of diversity for many Malagasy amphibian groups but has a relatively low degree of endemism. DNA sequence divergence was high within each pair of sibling species (6-7% in a fragment of the mitochondrial 16S rRNA gene), suggesting that their reproductive isolation was not a recent event and probably predates the Pleistocene.

KEY WORDS: Amphibia, Anura, Mantellidae, *Boophis viridis*, *Boophis rappiodes*, *Boophis erythrodactylus*, *Boophis bottae* n. sp., *Boophis tasymena* n. sp., Madagascar, advertisement calls, distribution.

INTRODUCTION

With currently more than 45 species, the treefrog genus *Boophis* is one of the most speciose groups of Malagasy anurans. It is part of an endemic frog radiation (RICHARDS et al. 2000) which has recently been defined as the family Mantellidae (VENCES & GLAW 2001). *Boophis* was first recognized as a natural unit and divided into seven species groups by BLOMMERS-SCHLÖSSER (1979). Later, BLOMMERS-SCHLÖSSER & BLANC (1991) assigned all known species in the genus to these groups, while GLAW & VENCES (1994) proposed some modifications to the grouping. According to the molecular data of RICHARDS et al. (2000), who studied representatives of five of the seven species groups (sensu GLAW & VENCES 1994), *Boophis* is a monophyletic assemblage.

One characteristic pattern in *Boophis* is the high proportion of sibling species which are well defined by advertisement calls and partly by colouration, but virtually indistinguishable by external morphology. This pattern has received particular attention by BLOMMERS-SCHLÖSSER (1979) in her treatment of the *Boophis rappiodes* group. She assigned two described species (*B. rappiodes* and *B. erythrodactylus*) to this group and described two new taxa (*B. mandraka* and *B. viridis*). She found all these species in central-eastern Madagascar, at her collecting localities Mandraka and Andasibe, and demonstrated close syntopy of two species (*B. erythrodactylus*, *B. mandraka* Blommers-Schlösser 1979) at one site (Mandraka). All species of the *B. rappiodes* group are small, basically greenish-coloured treefrogs with a translucent venter in life, reminiscent of the Neotropical Glass frogs of the family Centrolenidae. When preserved, the often diagnostic colour pattern is lost: the greenish colour changes first to pale yellow and then to whitish. If present, the red pattern often becomes brownish or fades to white. Although a few morphological characters are known to distinguish some of the species as defined by BLOMMERS-SCHLÖSSER (1979) (e.g., the position of the nostrils, which are closer to the eye in *B. mandraka* but closer to the snout tip in the other species), in general the *B. rappiodes* group exemplifies the difficulties in distinguishing sibling Malagasy frog species better than most other species assemblages (paralleled, however, by the *B. luteus* group; e.g., ANDREONE 1993, ANDREONE et al. 1995).

The recent intensive survey work carried out in several parts of eastern Madagascar revealed that the species inventory of Madagascar's herpetofauna is far from being complete (GLAW & VENCES 2000). Several of the newly discovered forms are assignable to the *B. rappiodes* group. In the present paper, we describe two of these as new species, partly revise the two species *B. rappiodes* and *B. erythrodactylus*, and provide new information about the distribution and advertisement calls of *B. viridis*. A revision of the remaining representative of the group, *B. mandraka*, and two new sibling species will be published elsewhere.

MATERIALS AND METHODS

Specimens were collected at night, mainly by locating calling males with the aid of electric torches. They were euthanised using chlorobutanol, fixed in 90% ethanol and preserved in 70% ethanol. Samples of femur muscle were preserved in pure 90% ethanol; DNA was extracted from these tissue samples, and a fragment of the mitochondrial 16S rRNA gene (560 nucleotides) was amplified and sequenced using protocols given in VENCES et al. (2000).

Morphological measurements of the preserved specimens were taken by the senior author with callipers to the nearest 0.1 mm: SVL (snout-vent length), HW (head width), HL (head length), ED (horizontal eye diameter), END (eye-nostril distance), NSD (nostril-snout tip distance), NND (nostril-nostril distance), TD (horizontal tympanum diameter), HAL (hand length), FORL (forelimb length), HIL (hindlimb length), FOL (foot length), FOTL (foot length including tarsus). The webbing formula is given according to BLOMMERS-SCHLÖSSER (1979).

Vocalizations were recorded using portable tape recorders with an external microphone (Vivanco EM 238) and were analyzed with the MEDAV sound analyzing system Spektro 3.2. Temporal measurements are given as range, with mean ± standard deviation, and number of temporal units measured, in parentheses.

Institutional abbreviations used are as follows: MNHN (Muséum National d'Histoire Naturelle, Paris); UADBA (Université d'Antananarivo, Département de Biologie Animale) (numbers given refer to the fieldnumbers of different collectors; UADBA-MICET, collection of the MICET team; UADBA-RD, collection of D. Rakotomalala; UADBA-MV, collection of M. Vences; UADBA-FG/MV, collection of F. Glaw and M. Vences); ZFMK (Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn); ZMA (Zoologisch Museum Amsterdam); ZMB (Zoologisches Museum der Universität, Berlin); ZSM (Zoologische Staatssammlung München). Coordinates and altitudes of localities are given according to GLAW et al. (2001) or given at first mention.

DNA sequences were submitted to public databases; EMBL/Genbank accession numbers are the following (voucher specimens in parentheses): *Boophis boitae* n. sp. (ZSM 344/2000), AJ314817; *Boophis erythrodactylus* (ZSM 324/2000), AJ314814; *Boophis rappiodes* (ZSM 347/2000 and UADBA-FG/MV 2000.59), AJ314815 and AJ314816; *Boophis tasymena* n. sp. (ZFMK 62888), AF215339; *Boophis viridis* (ZSM 338/2000), AJ314818.

SPECIES ACCOUNTS

Boophis viridis Blommers-Schlösser 1979

Boophis viridis BLOMMERS-SCHLÖSSER 1979. *Name-bearing type*: holotype ZMA 7100A, adult male, collected by R. Blommers-Schlösser on 14 November 1972. *Type locality*: "near Perinet (highroad R.N. 2 at km 142)" according to original description (Perinet being the old French name of Andasibe). *Other types*: paratypes ZMA 7100B, two adult males.

Identity and diagnosis. See Table 1 for morphometric measurements. A member of the *Boophis rappiodes* group as recognizable by relatively small size (SVL 29-31 mm in males, 32-35 mm in females; Table 1 and BLOMMERS-SCHLÖSSER 1979), greenish and slightly translucent dorsal colouration (during the day; at night more reddish), translucent venter (inner organs can be clearly seen through the skin in live specimens) and absence of lateral fringes along lower arm and tarsus (Fig. 1). Distinguishable from all other species of the group by the larger size of males (below 27 mm in all other species), characteristic iris colouration (inner iris area

Table 1.

Morphometric measurements (all in mm) of samples of species of the *Boophis rappiodes* group. For abbreviations of variables, see Materials and methods. Other abbreviations: HT, holotype; PT, paratype; LT, lectotype; PLT, paralectotype; M, male; F, female; SA, subadult. Relative hindlimb length (RHL) is the position reached by the tibiotarsal articulation when the hindlimb is adpressed along the body.

Catalogue number	Status	Sex	Locality	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	RHL
Boophis viridis																	
ZFMK 60012	—	M	Andasibe	29.5	11.5	10.9	1.7	4.0	2.6	1.9	2.7	18.8	8.7	50.0	20.6	12.0	beyond snout tip
ZFMK 60013	—	M	Andasibe	29.1	11.6	11.1	1.9	3.5	2.4	2.2	2.6	18.1	8.9	49.2	21.2	12.2	snout tip
ZFMK 53620	—	F	Andasibe	34.1	13.4	13.3	1.6	4.1	3.2	2.3	3.4	22.3	10.2	60.1	25.4	15.0	slightly beyond snout tip
ZFMK 60014	—	F	Andasibe	32.0	11.7	11.8	1.8	3.8	2.7	1.9	2.7	20.1	9.7	54.9	23.5	13.7	nostril
ZFMK 62211	—	F	Andasibe	35.1	12.9	12.7	2.1	3.6	2.9	2.2	3.1	22.1	10.7	62.0	25.8	15.1	beyond snout tip
Boophis mandraka																	
ZFMK 59815	—	M	Mandraka	22.1	8.8	8.0	1.3	3.1	1.8	1.9	2.8	12.7	7.7	39.9	17.0	10.3	snout tip
ZFMK 59816	—	M	Mandraka	23.2	9.3	8.3	1.4	3.3	1.5	2.1	2.9	13.8	7.7	40.0	17.4	10.7	snout tip
Boophis rappiodes																	
ZMB 30540	HT	M	Ankoraka	25.1	9.0	9.0	1.6	3.2	2.2	1.7	2.8	14.9	7.3	43.6	17.8	10.5	beyond snout tip
ZFMK 53621	—	M	Nahampoana	24.0	9.6	8.8	1.4	3.3	2.0	1.7	3.0	13.9	6.6	41.9	17.7	10.3	beyond snout tip
ZFMK 53622	—	M	Nahampoana	25.1	10.0	9.5	1.7	3.6	2.3	2.0	3.0	16.5	7.7	43.8	18.3	11.1	beyond snout tip
ZFMK 53624	—	M	Andasibe	20.4	7.7	7.4	1.4	3.1	1.5	1.3	2.3	13.9	6.9	38.3	15.8	9.2	clearly beyond snout tip
ZFMK 53625	—	M	Andasibe	21.4	8.0	8.0	1.2	3.0	1.6	1.4	2.2	13.2	6.4	36.5	14.5	8.4	snout tip
ZSM 347/2000	—	M	Andasibe	24.2	9.0	8.8	1.3	3.1	2.1	1.4	2.7	15.0	7.0	42.7	17.8	10.0	beyond snout tip
ZSM 676/2001	—	M	Andasibe	22.7	8.8	8.0	1.1	3.2	2.0	1.5	2.0	13.5	6.7	40.2	16.5	9.3	beyond snout tip
ZFMK 53623	—	F	Andasibe	33.6	11.7	12.6	1.8	4.2	2.4	2.1	3.2	21.2	10.0	60.2	26.0	15.8	beyond snout tip
ZFMK 59869	—	F	Andasibe	32.7	11.2	10.9	1.6	3.6	2.2	2.3	3.4	19.0	9.1	53.7	22.4	14.8	anterior eye corner
ZFMK 62278	—	F	Vohiparara	30.0	10.8	10.6	1.7	3.0	2.4	2.1	3.2	18.6	8.6	50.2	21.4	13.0	snout tip
Boophis bottae n. sp.																	
ZSM 678/2001	HT	M	Andasibe	24.2	9.0	8.7	1.4	3.4	2.0	1.5	2.2	15.5	6.9	43.0	17.3	10.4	beyond snout tip
ZSM 679/2001	PT	M	Andasibe	23.5	8.6	8.6	1.4	3.3	2.1	1.3	2.3	15.6	7.4	41.6	17.5	10.7	beyond snout tip
ZSM 344/2000	PT	M	Andasibe	21.9	8.1	8.2	1.4	3.3	1.6	1.4	2.4	15.0	6.9	37.7	16.3	9.6	beyond snout tip

(continued)

Table 1 *(continued)*

Catalogue number	Status	Sex	Locality	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	RHL
ZFMK 60015	PT	M	Andasibe	23.8	8.9	8.4	1.5	3.4	1.6	1.3	2.4	14.4	7.4	40.3	17.6	10.5	nostril
ZFMK 60016	PT	M	Andasibe	24.1	9.1	8.3	1.5	3.4	1.9	1.4	2.3	14.6	7.3	42.1	18.1	10.6	beyond snout tip
ZFMK 62220	PT	M	Andasibe	21.2	8.0	7.8	1.3	3.3	1.9	1.6	2.5	14.0	6.4	39.5	16.8	9.7	clearly beyond snout tip
ZFMK 62221	PT	F	Andasibe	35.1	12.7	12.5	1.8	4.0	2.4	2.2	3.4	20.7	9.8	56.7	24.2	15.0	nostril
Boophis erythrodactylus																	
MNHN 1994.1469	LT	M	Mahajeby	23.7	9.3	8.9	1.7	3.0	1.7	1.7	2.4	13.8	7.4	42.1	17.7	10.5	snout tip
MNHN 1953.171	PLT	M	Mahajeby	23.8	9.5	9.2	1.7	3.3	1.8	1.7	2.8	14.3	7.2	39.9	16.8	10.2	between eye and nostril
MNHN 1994.1470	PLT	SA	Mahajeby	21.3	7.8	7.6	1.2	2.8	1.6	1.6	2.5	12.4	6.3	37.7	15.4	9.3	snout tip
MNHN 1994.1471	PLT	SA	Mahajeby	21.2	8.2	7.6	1.4	2.8	1.7	1.2	2.2	14.8	6.1	36.9	15.6	9.4	snout tip
ZFMK 59813	—	M	Mandraka	24.6	9.6	9.1	1.7	2.7	2.0	1.5	2.6	16.4	8.0	44.0	18.8	11.4	beyond snout tip
ZSM 677/2001	—	M	Mandraka	24.2	9.2	8.5	1.7	3.3	2.0	2.0	2.8	15.9	7.4	43.2	18.0	10.5	snout tip
ZFMK 59814	—	F	Mandraka	33.0	11.9	11.1	2.2	3.1	2.5	1.7	2.9	20.4	10.0	53.7	23.8	14.2	nostril
Boophis tasymena n. sp.																	
ZSM 1085/2001	HT	M	Andasibe	22.7	8.7	8.0	1.7	3.0	1.9	1.6	2.4	13.8	7.0	39.6	17.2	10.2	snout tip
ZFMK 62225	PT	M	Andasibe	22.2	8.4	8.0	1.5	3.1	1.7	1.5	2.6	14.6	7.2	40.3	17.0	10.2	beyond snout tip
ZFMK 62243	PT	M	Mantady	21.0	8.2	7.5	1.4	2.7	1.6	1.4	2.0	13.6	6.4	38.7	16.1	9.6	clearly beyond snout tip
ZFMK 62244	PT	M	Mantady	21.9	8.3	7.5	1.5	3.0	2.0	1.5	2.3	15.2	7.0	38.9	16.3	9.8	snout tip
ZFMK 62262	PT	M	An'Ala	21.8	8.6	7.7	1.6	2.8	1.7	1.6	2.5	13.5	6.3	38.1	15.9	9.5	slightly beyond snout tip
ZFMK 62267	PT	M	An'Ala	21.8	8.5	7.8	1.5	3.0	1.7	1.2	2.3	14.9	6.6	38.0	16.2	9.6	beyond snout tip
ZFMK 62888	PT	F	Andasibe	32.3	11.1	11.0	2.2	3.0	2.4	1.7	3.1	19.0	8.7	53.3	23.1	13.5	nostril
Uncertain attribution																	
MNHN 1930.432	—	M	Vondrozo	24.5	9.3	8.7	1.5	3.1	2.3	1.8	2.6	15.6	7.7	40.8	17.5	10.3	nostril