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Description of the tadpole of the Malagasy treefrog *Boophis andohahela*

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We describe the larval stages of the Malagasy treefrog *Boophis andohahela*, based on specimens identified by their DNA sequences. The tadpoles were collected in a stream pool under a waterfall and were dwelling on submerged rocks. They show a rather distinctly flattened and convex body shape. Their oral disk structure and labial tooth row formula (2:4+4/1+1:2) is similar to those of other representatives of the *Boophis luteus* species group.

INTRODUCTION

The genus *Boophis* Tschudi, 1838 contains a radiation of treefrogs which belongs to the endemic family Mantellidae from Madagascar and the Comoro island of Mayotte (VENCES et al., 2003). The genus currently contains about 48 species (GLAW & VENCES, 2003), but new taxa are continuously being discovered, and many species have been already identified and await formal description (VALLAN et al., 2003). Frogs of this genus are arboreal, with typical treefrog habitus: enlarged finger discs, broad and anteriorly rounded head, large eyes and no dorsolateral ridge (GLAW et al., 2001). According to BLOMMERS-SCHLÖSSER & BLANC (1991) and GLAW & VENCES (1994), seven phenetic species groups are distinguished in the genus.

Within *Boophis*, two major clusters can be distinguished depending on the site of reproduction: the pond breeders of the *Boophis tephraeomystax* group appear to be characterized by ancestral states of several characters (VENCES et al., 2002) but they were grouped as a homophyletic group in a more recent analysis (VENCES et al., 2003). It is clear, however, that

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the species-rich assemblage of brook-breeders is a homophyletic, probably monophyletic group (RICHARDS et al., 2000; VENCES et al., 2002, 2003).

One of the species assemblages in this lotic lineage is the *Boophis luteus* group that contains a number of morphologically extremely similar, medium-sized green-coloured treefrogs. The number of species in this group has climbed up from one (BLOMMERS-SCHLÖSSER & BLANC, 1991) to 12 (GLAW & VENCES, 2002). Larval stages are known for only three of these, *Boophis luteus* (Boulenger, 1882), *Boophis ankaratra* Andreone, 1993 and *Boophis jaegeri* Glaw & Vences, 1992 (BLOMMERS-SCHLÖSSER, 1979; GLAW & VENCES, 1994). We here describe the tadpole of one further species of the *B. luteus* group, *Boophis andohahela* Andreone, Nincheri & Piazza, 1995.

MATERIAL AND METHODS

Specimens were collected in January 2003 in Ranomafana National Park, Fianarantsoa Province, southeastern Madagascar, from a brook in the rainforest. The habitat was a pool underneath a waterfall (ca. 847 m above sea level; 21°15.77'S, 47°24.78'E), which dropped down about five meters along rocks. The pool was very deep (more than 2 metres) and had a diameter of at least seven metres. Specimens were attached to the submerged rocks in the pool and were found on rocks in quiet water areas as well as on rocks positioned in strong current. Collected specimens were anesthetized and killed in a solution of highly concentrated chlorobutanol. The dead tadpoles were assigned to morphotype categories using a stereomicroscope. From one specimen of each of these categories a piece of tail was taken as a DNA tissue sample. Subsequently all tadpoles were preserved in 4 % buffered formalin. Adult and larval voucher specimens were deposited in the herpetological collections of the Université d'Antananarivo, Département de Biologie Animale (UADBA), Zoologische Staatssammlung München (ZSM) and the Zoological Museum Amsterdam (ZMA).

Species identification was based on DNA sequences. We amplified a fragment of about 500 bp of the mitochondrial 16S rRNA gene of each tadpole sample, using primers and protocols described in THOMAS et al. (2005), and compared it with homologous sequences of adult specimens. DNA sequences were deposited in Genbank (accession numbers AY863216-AY863217 for the two tadpole DNA vouchers, and AY848447-AY848448 and AY848456 for three comparative adult specimens).

Drawings and descriptions are based on the DNA voucher, and other representative specimens of the same series were used to supplement structures missing because of tissue sampling. In order to assess morphological variability, measurements were taken from six specimens of the series using dial calipers; values were taken to the nearest 0.1 mm. All tadpoles were staged according to GOSNER (1960). Terminology is based on ALTIG & MCDIARMID (1999) with some modifications. Body length is estimated by measuring the distance from the tip of the snout to the body terminus, which is the junction of the posterior body wall with the tail axis (ALTIG & MCDIARMID, 1999). Tail length is defined as the distance from the body terminus to the absolute tip of the tail (ALTIG & MCDIARMID, 1999). Total length is the sum of body length and tail length. Body width is measured at the widest point

of the "head" right behind the eyes, not in the intestinal part. Eye diameter is the maximum width of the orbit. Interorbital distance is measured between the centres of the pupils; internarial distance is measured between the centres of the nares. The distance between tip of snout and naris is taken to the centre of the naris. Distance between naris and eye is measured from the centre of naris to the anterior edge of the eye. Distance between tip of snout and spiraculum is also taken up to the centre of the spiracular aperture. Tail muscle height is first measured vertically from the junction of the body wall with the ventral margin of the tail muscle and secondly measured at midtail. Tail height including fins and caudal musculature is taken at its maximal vertical extent. Dorsal fin origin is defined relatively to the tail body junction. The formula of labial tooth rows follows Dubois (1995). The mouthparts include upper tooth rows (UTR) and lower tooth rows (LTR).

Results

Boophis andohahela was described from Andohahela National Park in south-eastern Madagascar (Andreone et al., 1995). Our surveys of south-eastern rainforests yielded, in 2003 and 2004, several specimens that agreed with this species in general morphology and coloration: (1) at Ambatolahy forest next to Ranomafana National Park, 21°14.632'S, 47°25.573'E, 915 m a.s.l. (specimens ZMA 20017-20018 and 20304, collected in February 2004); (2) close to the first locality, between Vohiparara and the entrance of Ranomafana National Park, no coordinates taken (specimens ZSM 665.2003, collected on 17 January 2003); (3) at Vevembe forest, close to Vondrozo, 22°47.686'S, 47°11.228'E, 581 m a.s.l. (specimens ZMA 20019 and 20125-20126, and UADBA 24292, collected on 10 February 2004). Specimens from Vevembe were observed calling, their advertisement calls fully corresponding to those of topotypical specimens as described by Andreone et al. (1995). DNA from three of these adult specimens was sequenced, the two sequences from the Ranomafana region (specimens ZMA 20018 and ZSM 665.2003) resulting fully identical, the one from Vevembe (ZMA 20125) having 6 substitutions compared to those from Ranomafana (1.2 % pairwise sequence divergence).

Two tadpole series from Ranomafana with the field numbers FG/MV 2002.1802 (catalogued as ZSM 667.2004) and FG/MV 2002.1803 (catalogued ZSM 668.2004) had sequences fully identical with the adult sequences from Ranomafana, and their sequences strongly differed from all other frog species studied in this region. In terms of DNA barcoding we therefore consider these tadpoles to be reliably identified. We based the following description on a subset of the specimens from one of these series (ZSM 667.2004). Specimens from the second series agreed in general morphological features.

Larvae of *B. andohahela* are exotrophic and benthic tadpoles of Orton's (1953) type IV. The coloration shows irregular pattern of dark areas on a light ground. The intestinal spiral is clearly visible through the abdominal wall. In life, most of the observed specimens showed a yellow coloration on the tail: the fins were almost without pigmentation, just a yellow glimmer was visible.

We selected the DNA voucher of the series ZSM 668.2004 and five additional tadpoles of this same series, of representative size and stage, and in good state of preservation, for the

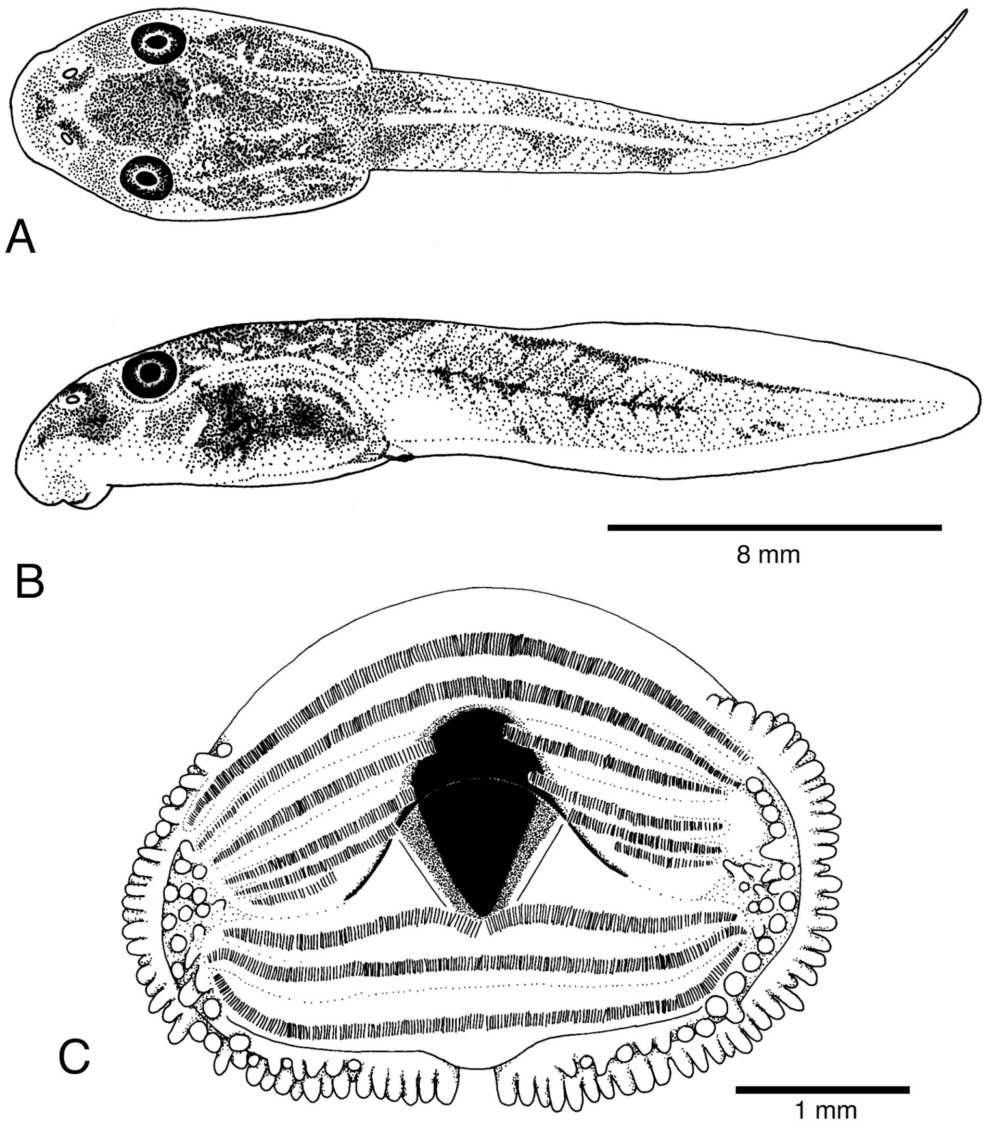

Fig. 1. – Drawings of a tadpole of *Boophis andohahela* from the series ZSM 667.2004 . On top (A) the specimen is shown in dorsal view with its relatively large eyes, in lateral view (B) the very low body shape is visible; the oral apparatus (C) shows the dense row of marginal papillae with its large medial gap in the upper labium and its small gap in the lower labium.

description. The DNA voucher specimen had a part of the tail removed for DNA extraction. All specimens were in stage 25. Detailed morphometric data of the specimens are given in tab. 1. The larvae of *B. andohahela* have a total length of 21.84 ± 0.76 mm (mean ± standard deviation). They show an oval to more of less rhombic body shape in dorsal view (fig. 1A) and the body width is about 58 % of body length. The snout is flatly rounded, and the upper

Table 1. – Morphometric measurements (mm) of six tadpole specimens of *Boophis andohahela* (series ZSM 667.2004, all in stage 25) collected in Ranomafana National Park.

Character	*n*	Mean	Standard deviation	Minimum	Maximum
Body length	6	8.03	0.66	7.3	8.9
Tail length	5	13.98	0.58	13.5	14.9
Total length	5	21.84	0.76	20.8	22.6
Body width	6	4.66	0.52	4.3	5.7
Eye diameter	6	1.20	0.11	1.1	1.4
Interorbital distance	6	3.02	0.19	2.9	3.4
Internarial distance	6	1.37	0.05	1.3	1.4
Distance snout-naris	6	1.25	0.12	1.0	1.3
Distance naris-eye	6	1.78	0.12	1.6	1.9
Distance snout-spiraculum	6	4.82	0.38	4.3	5.4
Tail muscle height 1	6	2.60	0.09	2.5	2.7
Tail muscle height 2	6	2.05	0.08	2.0	2.2
Fin height	6	3.30	0.28	2.8	3.6

mouthpart is anterior. The eyes are relatively large (diameter about 15 % of body length). They are positioned dorsally and directed dorsolaterally. In ventral view the eyes are not visible. The internarial distance is about 45 % of the interorbital distance. The rounded naris is moderate in size, directed dorsally and positioned closer to the snout than to the eyes. In lateral view (fig. 1B), the body shape is very depressed and in some specimens shows an extreme concave shape ventrally. The snout is rounded. The spiracle is sinistral and ¾th of the tube are attached to the body wall; it is positioned laterally (closer to venter than to dorsum) and oriented posterodorsally. The spiracular opening is oval and situated slightly below the level of the apex of myotomes of tail musculature. The tail musculature is strong, of almost uniform height until the midtail; in the distal half of the tail the musculature is gradually tapering and almost reaches the tail tip. The fins are moderate. The dorsal fin originates near the dorsal tail body junction, but really expands just after one fourth of the tail length. Like the ventral fin, the dorsal fin has a concave shape. The point of maximum fin height is located in the third fourth of the tail. The anal tube is short, tubular and medial with a lateral displacement to the right, the opening is directed posterolaterally.

The oral apparatus (fig. 1C) is generalized. It is positioned ventrally and there is no lateral emargination present. The upper labium shows a large medial papillae gap. The rest of the oral disc is bordered by a dense row of marginal papillae, except a small part in the middle of the lower labium. Submarginal papillae are present in the lateral parts and cover almost the whole lower labium, just a small area in the middle being free of submarginal papillae. The labial tooth row formula is 2:4+4/1+1:2. In the upper labium, the tooth rows become continuously shorter from UTR_2 to UTR_6. UTR_3 is the first row that touches the beak. LTR_1 has a short medial gap. The jaw sheaths are slightly serrated; the coloration is white with black pigmentation. On the upper labium the beak has a wide opened reversed U-shape, whereas the lower beak is a compact element with a slight V-shaped grooving.

DISCUSSION

DNA barcoding has proven to be a valuable tool to assign larval stages to adult species, especially in cases were rearing would be very time-consuming (HEBERT et al., 2004; THOMAS et al., 2005). In the case study reported here, we have even used this method first to assess the conspecificity of adult specimens from several localities, and in a second step to verify tadpole identification. In *Boophis andohahela*, as in other species of the *B. luteus* group, the original green colour quickly fades to yellow and later to white, with the slight species-specific chromatic characters totally vanishing. Even living frogs have few diagnostic characters, and the most distinct one (light dorsolateral lines on the anterior part of the body) can also be found in other species. Hence, the only adult specimens in our collection that could be reliably identified using traditional methods were those from Vevembe, because here we could collect them while emitting their diagnostic advertisement calls (described by ANDREONE et al., 1995). These differ clearly from those of all other representatives of the *B. luteus* group, except *B. jaegeri* (see GLAW & VENCES, 2002) which strongly differs genetically. Adult specimens collected at Ranomafana were assigned to *B. andohahela* because of agreement in live coloration and low genetic differences to a specimen from Vevembe. In turn, tadpoles from Ranomafana were identical in their DNA sequence to adults from this region. Altogether five DNA sequences of *B. andohahela* (two tadpoles and three adults) were available, and the differences among these were much lower than to all other species of *Boophis*, confirming the validity of molecular taxonomy to identify larval stages of tropical anurans.

According to BLOMMERS-SCHLÖSSER (1979) and GLAW & VENCES (1994), the tadpoles assigned to *B. luteus* and *B. jaegeri* are characterized by the following morphologies: labial tooth formula 1:5+5/3 or 1:4+4/3 with a large number of papillae, gap in papillae on the upper labium and median gap on the lower labium; body not conspicuously flattened in *B. luteus*, slightly flattened in *B. jaegeri.* Hence, the general oral morphology of *B. andohahela* agrees relatively well with its close relatives. Its rather flattened, almost concave ventral body shape might be an adaptation to adhesion to submerged rocks in strong currents and reminds tadpoles of *B. ankaratra* (as briefly described in GLAW & VENCES, 1994) and of representatives of other species groups: *Boophis majori* (Boulenger, 1896) (*Boophis majori* group), *Boophis erythrodactylus* (Guibé, 1953) and *Boophis mandraka* Blommers-Schlösser, 1979 (*Boophis rappiodes* group). This indicates that several characters of the tadpole morphology in *Boophis* have undergone extensive parallel evolution in similar habitats. Deciphering the pathways and ecological correlates of the recurrent adaptations to more or less extreme lotic conditions must await a better knowledge on the phylogeny of these frogs, and the descriptions of the larval stages of more species.

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