SPIXIANA	29	1	73–76	München, 01. März 2006	ISSN 0341-8391

The tadpole of the Malagasy treefrog *Boophis rufioculis*: molecular identification and description

(Amphibia, Anura, Mantellidae)

Stéphane Grosjean, Meike Thomas, Frank Glaw & Miguel Vences

Grosjean, S., M. Thomas, F. Glaw & M. Vences (2006): The tadpole of the Malagasy treefrog *Boophis rufioculis*: molecular identification and description (Amphibia, Anura, Mantellidae). – Spixiana **29/1:** 73-76

The tadpole of *Boophis rufioculis* Glaw & Vences, a species of treefrog from Madagascar, is described for the first time, based on specimens identified by DNA barcoding. The larvae were collected in a stream and were rather generalized in body shape and oral disc morphology, largely agreeing with other previously described species of the *Boophis goudoti* group. Their keratodont row formula is 1:2+2/1+1:2.

Stéphane Grosjean, Muséum National d'Histoire Naturelle, Département Systématique et Evolution, UMS 602, Taxinomie et Collections – Reptiles et Amphibiens, case 30, 25, rue Cuvier, 75005 Paris, France; e-mail: sgrosjea@mnhn.fr

Meike Thomas, Institute for Genetics, Evolutionary Genetics, University of Cologne, Weyertal 121, 50931 Köln, Germany; e-mail: meike.thomas@uni-koeln.de

Frank Glaw, Zoologische Staatssammlung, Münchhausenstr. 21, 81247 München, Germany; e-mail: frank.glaw@zsm.mwn.de

Miguel Vences, Institute for Biodiversity and Ecosystem Dynamics, Zoological Museum, University of Amsterdam, Mauritskade 61, 1092 AD Amsterdam, The Netherlands; e-mail: vences@science.uva.nl

Introduction

The larval stages of Malagasy frogs have evolved a remarkable diversity of ecological and morphological adaptations (e.g. Blommers-Schlösser 1979a,b, Glaw & Vences 1994). In many small bodies of freshwater in Madagascar, tadpoles are the predominating aquatic vertebrates, because fishes are scarce both in terms of individuals and species in small mid- and high-altitude rainforest streams and ponds. Knowledge on the ecological and morphological adaptations of anuran larvae can therefore be of relevance to understand both the ecology of aquatic ecosystems in Madagascar, and to evaluate the habitat requirements and conservation priorities of Malagasy frogs. However, tadpoles have been described for only a few species, and the descriptions are largely based on the identification of reared juveniles, an error-prone procedure considering the many morphologically similar cryptic frog species in Madagascar (Glaw & Vences 2003).

Molecular methods offer an efficient alternative to identifying larval stages of organisms (e.g. Blaxter 2004). Such DNA barcoding (Hebert et al. 2003) identifies larvae by similarities, ideally identity, in the DNA sequences of a particular gene fragment. We have recently started a research program to apply this method to the identification of Malagasy tadpoles (Thomas et al. 2005) and have obtained evidence for an acceptable reliability of the method. Here we report on one of the results, the identification of the tadpole of *Boophis rufioculis*, and provide data on its morphology.

Material and Methods

Tadpoles were collected in the field and euthanised by immersion in chlorobutanol, and subsequently divided into series based on their morphology. A small portion of the caudal fin was removed from one specimen of each series for molecular analysis. This specimen (the DNA voucher) was used for the detailed description given below. All specimens were fixed and preserved in 5 % formalin. Vouchers were deposited in the Zoologische Staatssammlung München (ZSM). Comparative specimens were examined from the Zoological Museum Amsterdam (ZMA).

Molecular identification of the tadpole followed procedures described by Thomas et al. (2005). The partial 16S rDNA sequence of an adult *Boophis rufioculis* from the type locality An'Ala (no voucher) is deposited in Genbank under the accession number AY848623. The homologous sequence of the DNA voucher tadpole (ZSM 591/2004) has the accession number DQ003334. The two sequences are identical, whereas related species (*B. boehmei, B. burgeri, B. madagascariensis, B. reticulatus;* accession numbers AY848561, AY848566, AY848576, AY848612) all had pairwise divergences of more than 5 %.

Morphological terminology follows Altig & McDiarmid (1999) and developmental stages were determined according to Gosner (1960). Keratodont row formula is given according to Dubois (1995). Measurements were taken with a graduated ocular attached to a stereomicroscope except for the total length which were measured with a hand caliper. The landmarks are those shown in Altig & McDiarmid (1999, p. 26: Fig. 3.1.), for other see Grosjean (2001). Drawings were made with the aid of a camera lucida.

The abbreviations used in the description are the following: BH maximum height of body; BL body length; BW maximum width of body; DG maximum size of dorsal papilla gap; ED maximum diameter of eye; HT maximum height of tail; LF maximum height of lower tail fin; MC maximum height of caudal muscle; NN internarial distance; NP naro-pupilar distance; ODW oral disc width; PP interpupilar distance; RN rostro-narial distance; SS distance from tip of snout to opening of spiracle; SU distance from snout to beginning of upper tail fin; TL total length; UF maximum height of upper tail fin.

Results and Discussion

A series of four tadpoles were identified as belonging to *Boophis rufioculis*. The specimens were collected in a non-protected and degraded forest named An'Ala (18°56'S, 48°28'E; altitude about 840 m above sea level), on 1 March 2003 by M. Thomas, F. Glaw and M. Puente. Tadpoles were found in a slowly running brook of about 2 m width and a variable depth of 40-70 cm. The description is based on a DNA voucher specimen at stage 25 (ZSM 591/2004, BL 10.9 mm). Because a part of the tail was taken for DNA barcoding determination and was also damaged, information upon tail fin and tip of tail was taken from the other individuals.

In dorsal view (Fig. 1a), body elliptical, widest at the level of gills, snout nearly rounded. In lateral view (Fig. 1b), body slightly depressed, BW 111 % of BH, snout rounded. Eyes of moderate size, ED 17 % of BL, bulging, not visible in ventral view, positioned and directed almost dorsally. Nares rounded, of moderate size, rimmed with a very slight mid-dorsal projection, positioned almost dorsally but directed anterolaterally and with the opening directed laterally, closer to snout than to center of eye, RN 59 % of NP; NN 66 % of PP. Spiracle sinistral, slightly conical, moderately small, attached to body wall but its tip free, laterally positioned, oriented almost posteriorly, closer to end of body than to tip of snout, SS 64 % of BL; spiracular opening oval, situated at the height of the lower part of caudal muscle. Tail musculature moderate, MC 62 % of BH and 61% of HT, gradually tapering, reaching tail tip. Tail fins of moderate size, UF 33 % of HT, LF 26 % of HT, upper fin not extending onto body, SU 120 % of BL, slightly convex, lower fin slightly convex but following the caudal muscle; point of maximum height of tail located at the first third of tail length, HT 102 % of BH, tail tip finely rounded. Anal tube moderately large, dextral, flattened tubular, directed more posteriorly than posteroventrally, proximal half linked to ventral tail fin, opening dextral. Neither lateral line nor glands visible.

Oral disc (Fig. 1c) positioned and directed anteroventrally, emarginated, of moderate size, ODW 25 % of BL and 41 % of BW. A row of marginal papillae largely interrupted medially on the upper labium, DG 65 % of ODW, lower labium with a medial notch; one submarginal papillae at a corner of upper labium, 3 on the other side at the end of A1, A2 and the third hidden by a fold, 3 to 4 on a row at each side of the lower labium leaving the median third free of submarginal papillae; papillae moderately small and conical with a rounded tip. No denticulate papillae. Keratodont row formula 1:2+2/1+1:2, rows of upper labium subequal, as are P1 and P2, P3 about two third of P2. Jaw sheaths of moderate breadth, bearing large serrations, upper jaw sheath concave with a large medial serration surrounded by a smaller on each side forming a slight convexity, brown with black serrations, lower jaw sheath V-shaped, strong, black, the part covered by the lower labium dark orange.

Tadpole transparent, all underlying organs visible. Upper side and upper flanks slightly coloured by some orange-brown diffused pigment that is present in some of the different layers of tissue. The

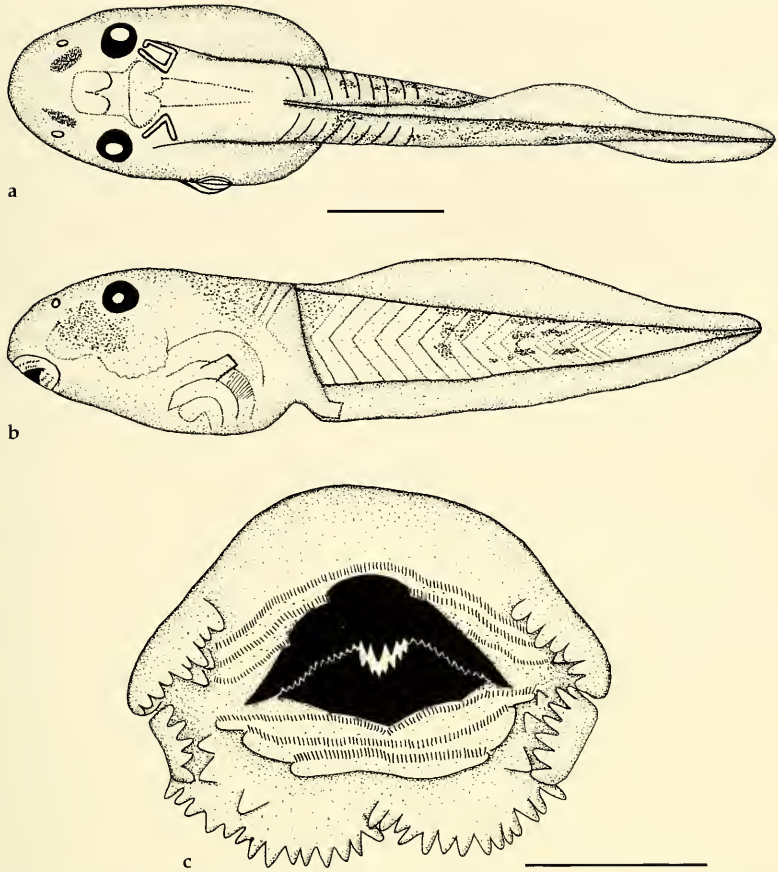

Fig. 1. Drawings of the tadpole of *Boophis rufioculis* (ZSM 591/2004). **a.** Dorsal view. **b.** Lateral view. **c.** Oral disc. Scale bars represent 5 mm in a and b, and 1 mm in c.

brain particularly well underlined by this colouration, the part anterior to eyes and the orbitohyoidien muscle coloured, a spot in the inner posterolateral side of nares. Ventral side and lower part of flanks immaculate. Upper part of caudal muscle coloured with the same tint, especially the proximal third, the lower part much less. Fins immaculate.

Variation was assessed based on three additional tadpoles at stage 25 (ZSM 592/2004-ZSM 594/2004). TL and BL of these three tadpoles are 29.9-31.1 mm (mean ± sd = 30.5 ± 0.6) and 10.9-11.6 mm (mean ± sd = 11.2 ± 0.3). The ratios vary in the following proportions: BW 116-123 % of BH; ED 16-17 % of BL; RN 53-54 % of NP; NN 57-64 % of PP; SS 55-65 % of BL; MC 49-63 % of BH; MC 53-55 % of HT; UF 32-34 % of HT; LF 24-28 % of HT; SU 81-88 % BL; HT 93-113 % of BH; ODW 24-25 % of BL; ODW 35-43 % of BW; DG 60-68 % of ODW.

Variation of the number of submarginal papillae is as follows: upper labium 2+1, 2+2, 2+2, lower labium 2+3, 4+3, 2+2. The colouration can form ill-defined bands on the proximal third of the upper part of the caudal muscle and some spots on its lower part and the rest of the upper part. Otherwise the keratodont row formula is homogenous within the sample, as well as the morphology and colouration except the small amount of variation reported above.

The genus *Boophis* has been divided into seven phenetic species groups (Blommers-Schlösser 1979b, Glaw & Vences 1994). *Boophis rufioculis* is part of the *B. goudoti* group (Glaw & Vences 1997) that contains at present eight species (Glaw & Vences 2003). This and five other species groups (all except the *B. tephraeomystax* group) belong into a clade of stream-breeding *Boophis* that is well-defined by molecular characters (Vences et al. 2002). Species of the *B. goudoti* group are usually found along streams, but tadpoles are so far only known for *B. goudoti* and *B. madagascariensis*. The finding of the tadpole of

B. rufioculis in running water confirms that this species is a rather generalized stream-breeder. Although the general morphology roughly agrees with those of *B. goudoti* and *B. madagascariensis*, the oral disc shows conspicuous differences such as a lower number of keratodont rows on the upper labium (3 in *B. rufioculis* vs. 4-7), a small papilla gap on the lower labium (also present in *B. goudoti*) and a large medial serration surrounded by a smaller one on each side forming a slight convexity on the upper jaw sheath.

Acknowledgements

We are grateful to Marta Puente, Liliane Raharivololoniaina and David R. Vieites for assistance in the field, and to the Malagasy authorities for granting research and export permits. This project was supported by the Volkswagen Foundation, and by a SYNTHESYS grant to the first author.

References

Altig, R. & R. W. McDiarmid 1999. Body plan. Development and morphology, pp. 24-51. In: McDiarmid, R. W. & R. Altig (eds.), Tadpoles. The biology of anuran larvae. – The University of Chicago Press, Chicago and London

Blaxter, M. L. 2004. The promise of a DNA taxonomy. – Phil. Trans. Roy. Soc. Lond. **B 359**: 669-679

Blommers-Schlösser, R. M. A. 1979a. Biosystematics of the Malagasy frogs. I. Mantellinae (Ranidae). – Beaufortia **352** (2): 1-77

-- 1979b. Biosystematics of the Malagasy frogs. II. The genus *Boophis* (Rhacophoridae). – Bijd. Dierk. **49**: 261-312

Dubois, A. 1995. Keratodont formulae in anuran tadpoles: proposals for a standardization. – J. Zool. Syst. Evol. Res. **33**: I- XV

Glaw F. & M. Vences 1994. A fieldguide to the amphibians and reptiles of Madagascar. 2nd edition. – Köln, Vences and Glaw Verlag

-- & -- 1997. Neue Ergebnisse zur *Boophis goudoti*-Gruppe aus Madagaskar: Bioakustik, Fortpflanzungsstrategien und Beschreibung von *Boophis rufioculis* sp. nov. – Salamandra **32** (4): 225-242

-- & -- 2003. Introduction to Amphibians. In: Goodman S. M. & J. P. Benstead (eds): The Natural History of Madagascar, pp. 883-898. – The University of Chicago Press, Chicago and London

Gosner, K. L. 1960. A simplified table for staging anura embryos and larvae with notes on identification. – Herpetologica **16**: 183-190

Grosjean, S. 2001. The tadpole of *Leptobrachium (Vibrissaphora) echinatum* (Amphibia, Anura, Megophryidae). – Zoosystema **23** (1): 143-156

Hebert, P. D. N., A. Cywinska, S. L. Ball & J. R. deWaard 2003. Biological identification through DNA barcodes. – Proc. R. Soc. Lond. B **270**:313-321

Thomas, M., L. Raharivololoniaina, F. Glaw, M. Vences & D. R. Vieites 2005. Montane tadpoles in Madagascar: molecular identification and description of the larval stages of *Mantidactylus elegans*, *M. madecassus* and *Boophis laurenti* from the Andringitra Massif. – Copeia **2005**: 174-183

Vences, M., F. Andreone, F. Glaw, J. Kosuch, A. Meyer, H.-C. Schaefer & M. Veith 2002. Exploring the potential of life-history key innovation: brook breeding in the radiation of the Malagasy treefrog genus *Boophis*. – Mol. Ecol. **11**: 1453-1463