

Descriptions of the tadpoles of seven species of Malagasy treefrogs, genus *Boophis*

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Abstract

The genus *Boophis* is a species-rich group of treefrogs within the family Mantellidae Laurent, endemic to Madagascar. The larval morphology of these frogs is an important trait to understand the evolution of reproductive modes and larval morphologies in the mantellid radiation and can provide important information to compare adaptations of tadpoles and adults, and elucidate possible covariation, and convergent evolution of these traits. We here assign seven previously unknown or insufficiently described *Boophis* tadpoles to species via DNA barcoding, and provide detailed morphological descriptions based mainly on the unambiguously identified DNA voucher specimens. All described tadpoles are stream-adapted, exotrophic tadpoles of a relatively generalized morphology. Applying our previous classification for stream-breeding *Boophis* based on relative oral disk width and the number of papillae and keratodonts we attempt an assignment of all species into ecomorphological guilds. Our results show that this previous definition of guilds (in *Boophis*) based on only three characters was an oversimplification, and that the variation in these tadpoles is more complex. In a phylogenetic context we found that species within at least two species groups of *Boophis* are heterogeneous in their assignment to the ecomorphological guilds confirming the probable non-monophyly of these groups.

Key words: Amphibia, Anura, Mantellidae, *Boophis*, *B. albilabris*, *B. andreonei*, *B. bottae*, *B. brachychir*, *B. guibei*, *S. septentrionalis*, *B. ulftunni*, Madagascar, tadpoles, ecomorphological guild

Introduction

Frogs of the family of Mantellidae Laurent found on Madagascar are a prime example for a species-rich radiation on an island (Bossuyt & Milinkovich 2001, Vences & Glaw 2003). The up-to-date knowledge of the phylogeny based on four mitochondrial and one nuclear gene reveals 11 genera within this family (Glaw & Vences 2006), in which the genus *Boophis* Tschudi represents a group of treefrogs (Cadle 2003). Species of *Boophis* are typically characterised by large eyes and enlarged disks on fingers and toes. They separate into two subgenera, *Boophis* and *Sahona* (Glaw & Vences 2006). While members of the latter breed in stagnant water bodies, most of the species belonging into the subgenus *Boophis* breed in running water. Within the subgenus *Boophis*, species aggregate into nine phenetic species groups, based on morphological and ecological traits of the adult frogs (Glaw & Vences 1994, 2007). While some of these groups are probably monophyletic lineages, the phylogenetic status of others is uncertain, and some are almost certainly

heterophyletic (Vences *et al.* 2002; Glaw & Vences 2006).

So far, out of the over 50 described species of *Boophis*, the tadpoles of about 30 species have been described. Compared to other groups of Malagasy amphibians, this is a rather high percentage, and completing the larval morphological database of these treefrogs would be an important contribution to understand the evolution of reproductive modes and larval morphologies in the mantellid radiation.

Morphological data on frogs is in general unbalanced: while detailed descriptions are usually available for adults, tadpoles have often been neglected. However, tadpoles must not be reduced to frog add-ons as they represent an essential phase in anuran life history. In fact, the larval stage of frogs can show more distinct morphological characters than the adults, often clear synapomorphies (Haas 2003).

In *Boophis*, all tadpoles known so far are exotrophic and most of them are relatively generalized in their morphology. Besides a single species (*B. picturatus*) with completely reduced labial teeth (see Altig & McDiarmid 2006) and two related species with some degree of labial tooth reduction (*B. majori* and *B. sp. aff. majori*; Schmidt *et al.* 2008), some species show obvious adaptations to running water bodies, mainly in size and structure of the oral disk. Raharivololoniaina *et al.* (2006) undertook a first attempt to divide these stream tadpoles into three ecomorphological guilds that they named A, B and C. The key characteristics used for classification were relative oral disk width, the number of oral papillae, and the number of keratodonts in the first anterior keratodont row. Such ecomorphological guilds on a more inclusive level have been defined before (Altig and Johnston 1989) and provide important information to compare adaptations of tadpoles and adults, possible covariation, and convergent evolution of these traits. Raharivololoniaina *et al.* (2006) concluded that *Boophis* tadpole guilds appear to be generally conserved within those phenetic species groups that are known to be monophyletic, while very variable larval morphology occurs in the paraphyletic *B. majori* group. At least one guild morphology (that of guild C) probably evolved convergently in several species groups.

In the present study we assign seven previously unknown or insufficiently described *Boophis* tadpoles to species via DNA barcoding, and provide detailed morphological descriptions of these larvae. By extending the number of described tadpoles in this genus, we discuss the applicability of key traits and their present definitions for assigning stream-breeding *Boophis* tadpoles to guilds.

Materials and methods

Tadpoles were collected in the field, euthanised by immersion in chlorobutanol solution, and immediately sorted into homogeneous series based on morphological characters. From each series one specimen was selected and a tissue sample from its tail musculature or fin taken and preserved in 99% ethanol. This specimen is here named “DNA voucher”. All detailed tadpole descriptions and drawings are based on this DNA voucher, whereas variation is described based on further specimens of the series. After tissue collection, all specimens were preserved in 5% formalin. Specimens were deposited in the Zoologische Staatssammlung München, Germany (ZSM).

Tadpoles were identified using a DNA barcoding approach based on a fragment of the mitochondrial 16S rRNA gene, which is known to be sufficiently variable among species of Malagasy frogs (Thomas *et al.* 2005). The ca. 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. Identification was considered to be unequivocal when the tadpole sequence was 99–100% identical to an adult specimen from the same geographical region, and clearly less similar to all sequences from other species. DNA sequences were deposited in Genbank (accession numbers EU717859-EU717876; accession numbers of comparative adult specimens are included in the sequence sets AY847959-AY848683 and AJ315909-AJ315913).

The molecular results identified the tadpoles examined here as belonging to *B. albilabris* (Boulenger), *B. andreonei* Glaw & Vences, *B. septentrionalis* Glaw & Vences, *B. brachychir* Vences & Glaw, *B. bottae*

Vences & Glaw, *B. ulftunni* Wollenberg, Andreone, Glaw & Vences, and *B. guibei* (McCarthy). Of these taxa, the tadpoles of *B. albilabris* and *B. guibei* have been described before (Blommers-Schlösser 1979; Raharivololoniaaina *et al.* 2006). However, the existing description of the *B. albilabris* tadpole referred to specimens from central eastern Madagascar and we here add a second description referring to specimens from a north-western population. The former *B. guibei* description is relatively brief and does not include many characters (such as number of papillae and of keratodonts) which are important for comparative purposes. This tadpole is therefore redescribed based on new material.

Tadpoles of *B. albilabris* and *B. andreonei* were collected in the Special Reserve of Manongarivo. All *B. andreonei* tadpoles and some *B. albilabris* were collected on 31 January 2003 at the bank of the Maromalo River ($13^{\circ}56'53''$ S, $48^{\circ}27'28''$ E, and 288 m above sea level). There, the water was calm and had a maximum depth of approx. 50 cm. Other *B. albilabris* tadpoles were collected on 01 February 2003 in the Special Reserve of Manongarivo at a new campsite named by us "campsite 0" ($13^{\circ}58'32''$ S, $48^{\circ}23'36''$ E; 688 m a.s.l.) in a little stream downstream of a waterfall. The brook had a depth of approx. 30 cm. *B. septentrionalis* and *Boophis brachychir* were collected on 17 February 2003 in the river upstream of the Antakarana waterfall in Montagne d'Ambre National Park (coordinates at river not taken, but not far from $12^{\circ}31'37''$ S, $49^{\circ}10'19''$ E, 1050 m a.s.l.). The current was strong and the river had a maximum depth of approximately 100 cm. Tadpoles of *B. bottae* were collected on 04 December 2001 at the border of Analamazaotra Special Reserve that contains a mixture of degraded vegetation, secondary forest and primary rainforest ($18^{\circ}56'50''$ S, $48^{\circ}25'07''$ E, 900–1000 m a.s.l.). They were collected in a relatively slow-moving stretch of the Analamazaotra River around the bridge on the road leading to the village of Andasibe. Tadpoles of *B. ulftunni* were collected on 16 February 2005 at Camp Simpona of Marojejy National Park ($18^{\circ}49'48''$ S, $48^{\circ}25'56''$ E, 966 m a.s.l.). They were found in a relatively small brook inside a primary forest in 100% open canopy. The brook had a maximum depth of approx. 40 cm, and its floor was rocky and contained some gravel. Tadpoles of *B. guibei* were collected at An'Ala forest (18.91926° S, 48.48796° E, 889 m) in a small, probably temporary pond of maximal 30 cm depth and a muddy ground.

Developmental stages (DS) are described following Gosner (1960). Morphological measurements were taken by using a graduated ocular attached to a stereomicroscope, following landmarks, terminology and definitions of Altig & McDiarmid (1999). The formula of labial tooth rows (LTRF) is given according to Altig & McDiarmid (1999) and Dubois (1995). Drawings and photographs of the preserved tadpoles are represented in Figures 1–8.

The following abbreviations are used: BH (maximum height of body), BL (body length), BW (maximum width of body), DG (maximum size of dorsal papillae gap), ED (maximum diameter of eye), IOD (interocular distance), MTH (maximum tail height), NN (internarial distance), NP (distance from nare to eye), ODW (oral disk width), PP (interpupillary distance), RN (rostro-narial distance), SS (distance from tip of snout to opening of spiracle), TAL (tail length), TMH (tail muscle height), TMW (tail muscle width), TL (total length).

Results

Boophis albilabris

(Figures 1 and 8)

The following description refers to one tadpole in developmental stage 26 (field number FG/MV 2002.1916, ZSM 699/2004, TL 23.3 mm, BL 8.6 mm), from site "campsite 0" in the Special Reserve of Manongarivo. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of an adult specimen (Genbank accession AY848556) from the same locality.

In dorsal view, body ovoid, snout rounded. In lateral view, body depressed, BW 117% of BH. Eyes of moderate size, ED 11.6% of BL, not visible in ventral view, positioned dorsally and directed dorsolaterally, situated at about 1/3 of body length. Nares elliptical, moderately sized, prominent with marginal rim and dark

black spots, positioned dorsally, oriented anterolaterally and nearer to snout than to eye. RN 74% of NP, NN 58% of PP. Spiracle sinistral, inner wall free and formed such that the aperture opens laterally instead of posteriorly, visible in dorsal view, spiracular opening elliptical, positioned laterally, directed dorsally, situated at the height of the longitudinal axis of caudal musculature and the aperture is much closer to the end of the body than to the snout, SS 61% of BL. Medial vent tube with lateral displacement, tubular, short. Caudal musculature moderately developed, TMH 52% of BH and 59% of MTH, TMW 36% of BW; its height 2/3 of total height at midlength of tail. Dorsal fin origins at the tail body junction, increases gradually to attain its maximum height, then declines abruptly towards the tail tip. Ventral fin origins at the ventral terminus of the body, decreases meticulously to the 1/3 of tail, then increases to attain the maximum height of the tail, and decreases gradually towards the tail tip. Tail tip pointed, MTH 89% of BH, lateral line visible only on proximal 1/6 of the tail. Oral disk of moderate size, ODW 25% of BL and 38% of BW, positioned ventrally, directed anteroventrally, emarginated. A single row of marginal papillae interrupted by a large gap on the upper labium (DG 72% of ODW) and a small medial gap on the lower labium; total number of marginal papillae 56 (28 on each side). 15 submarginal papillae positioned in the lateral parts of the anterior and posterior labium. Papillae of moderate size, round or conical with rounded tips. No denticulate papillae. LTRF 1:3+3/1+1:2 after Dubois (1995) and 4(2–4)/3(1) after Altig & McDiarmid (1999). The density of keratodonts on A_1 is about 57 per millimeter (a total of ca. 100). The length of interrupted anterior keratodont rows (A_2 , A_3 and A_4) decreases gradually towards the center of the OD, P_3 about 5/6 of P_2 . Both jaw sheaths coarsely serrated and completely black; upper jaw sheath with a large medial serration surrounded by a smaller one on each side, reversed U-shaped; lower jaw sheath V-shaped. Coloration in preservative. Dorsally: body and tail musculature whitish, covered with two types of spots. First, condensed spots, darkened in some parts. Second, large, generally round, irregularly distributed spots. Some concentrated pigmentation forming dark patches between eyes. Laterally: intestinal coils and spiracle well visible; caudal musculature whitish with large, round, black spots, irregularly distributed along the tail muscle. Spot density and size diminish towards tail tip. Fins translucent with a few black and round spots. Ventrally: the whole surface of ventral body translucent, including the branchial and cardial region that is well visible through the ventral integument which is regularly spiral-shaped.

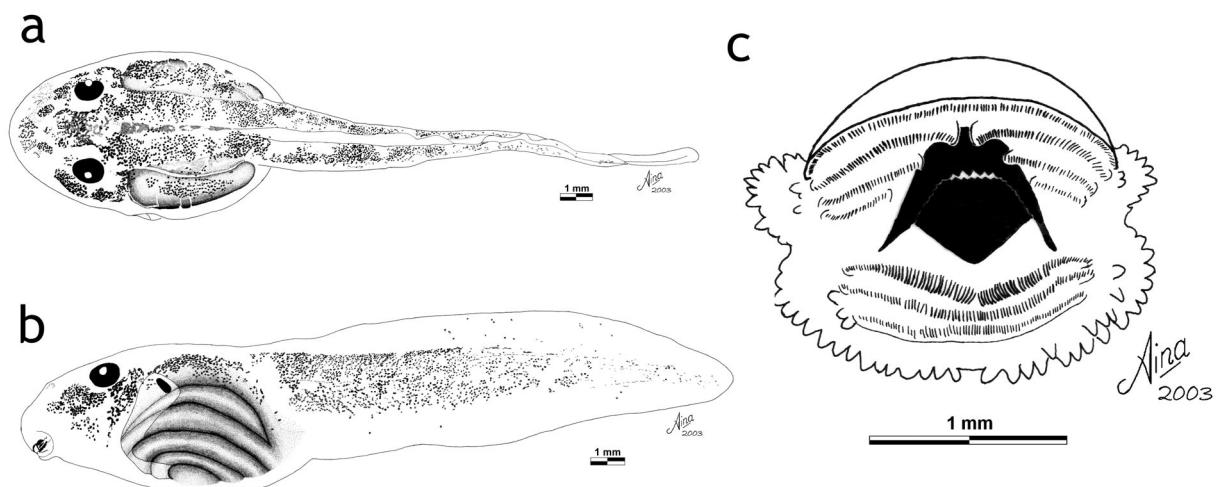


FIGURE 1. Drawings of preserved tadpole specimen of *Boophis albilabris* (ZSM 699/2004) from Manongarivo Special Reserve (developmental stage 26). (a) dorsal view; (b) lateral view; (c) oral disk.

Variation: TL and BL of 83 tadpoles at stages 25–39, from the Site Maromalo (FG/MV 2002.1905 – ZSM 680, 681, 682, 1021/2004; FG/MV 2002.1910 – ZSM 685, 686/2004; FG/MV 2002.1911 – ZSM 687,

688/2004; FG/MV 2002.1912 – ZSM 689, 690, 691, 1022/2004; FG/MV 1913 – ZSM 692, 693, 694, 1023/2004) and the campsite 0 (FG/MV 2002.1916 – ZSM 700, 701, 702, 1024/2004; FG/MV 2002.1922- ZSM 712, 713/2004; FG/MV 2002.1923- ZSM 714/2004; FG/MV 2002.1928 – ZSM 719, 720/2004) are 18–46 mm and 5–17 mm, respectively. BW 95–136% of BH; ED 9–18% of BL; RN 44–109% of NP; NN 48–143% of PP; SS 49–73% of BL; TMH 23–65% MTH; TMW 33–37% of BW; MTH 68–113% of BH; ODW 15–35% of BL; ODW 26–59% BW. LTRF: 1:3+3/1+1:2, 1:4+4/1+1:2, 1:5+5/1+1:2 after Dubois (1995) and 4(2–4)/3(1), 5(2–5)/3(1), 6(2–6)/3(1) after Altig & McDiarmid (1999).

Boophis andreonei

(Figures 2 and 8)

The following description refers to one tadpole in developmental stage 25 (field number FG/MV 2002.1903 – ZSM 675/2004, TL 22 mm, BL 8 mm), from the site Maromalo in the Special Reserve of Manongarivo. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of an adult specimen (accession AY848449) from the same locality.

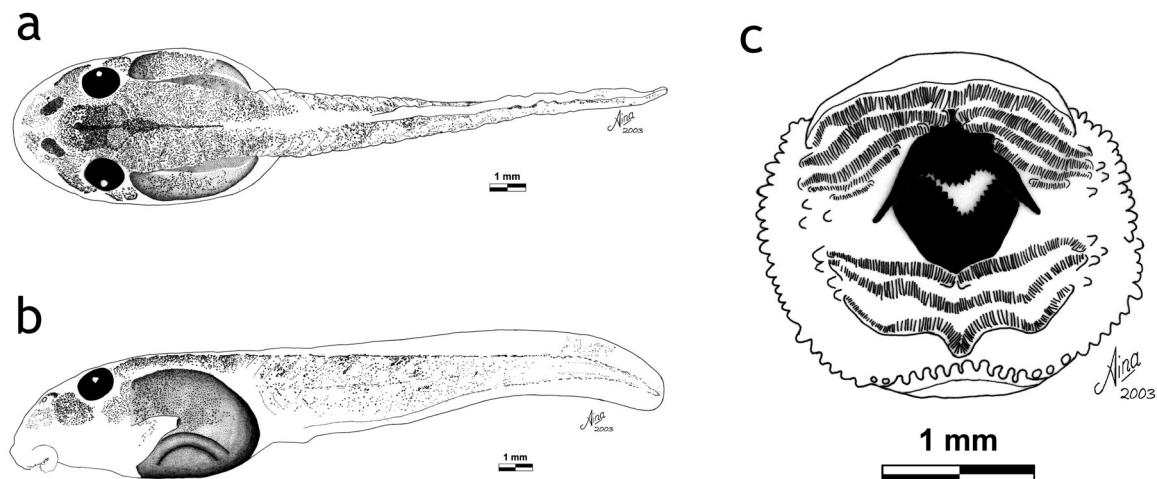


FIGURE 2. Drawings of preserved tadpole specimen of *Boophis andreonei* (ZSM 675/2004) from Manongarivo Special Reserve (developmental stage 25). (a) dorsal view; (b) lateral view; (c) oral disk.

In dorsal view, body elliptical, snout large. In lateral view, body depressed, BW 114% of BH, snout pointed. Eyes of moderate size, ED 16% of BL, not visible in ventral view, positioned dorsally and directed dorsolaterally, situated at about 2/5 of the body length. Nares elliptical, moderately sized, prominent with a marginal rim and dark black spots, positioned dorsally, oriented anterodorsally and nearer to snout than to eye. RN 72% of NP, NN 55% of PP. Spiracle sinistral, inner wall free and formed such that the aperture opens posteriorly, not visible in dorsal view, spiracular opening oval, positioned laterally, directed dorsally, situated under the height of the longitudinal axis of caudal musculature and the aperture is much closer to the end of the body than to the snout, SS 61% of BL. Medial vent tube with lateral displacement, tubular, short. Caudal musculature well developed, TMH 56% of BH and 80% of MTH, TMW 39% of BW; its height 1/2 of total height at midlength of tail. Height of dorsal fin two times that of ventral fin at maximum tail height. Dorsal fin originates at the tail body junction, increases gradually to attain its maximum height, then declines slightly towards the tail tip. Ventral fin originates at the ventral terminus of the body, remains more or less constant towards the tail tip. Tail tip rounded, MTH 71% of BH, lateral line visible on the proximal half of the tail. Oral

disk relatively large, ODW 34% of BL and 56% of BW, positioned ventrally, directed ventrally, not emarginated, round. A single row of marginal papillae interrupted by a large gap on the upper labium (DG 67% of ODW), no medial gap on the lower labium; total number of marginal papillae 65. 14 submarginal papillae positioned in the lateral parts of the anterior and posterior labium. Papillae small, round or conical with rounded tip. No denticulate papillae. LTRF 1:4+4/1+1:2 after Dubois (1995) and 5(2–5)/3(1) after Altig & McDiarmid (1999). The density of keratodonts on A_1 is about 58 per millimeter (a total of ca. 108). The length of interrupted anterior keratodont rows (A_2 , A_3 , A_4 and A_5) decreases gradually towards the center of the OD, P_2 and P_3 with same size. Keratodonts have the same size except on A_4 that has smaller keratodonts. Keratodonts are more or less connected. Both jaw sheaths coarsely serrated and completely black; upper jaw sheath with a large medial serration surrounded by a smaller serration on each side, more opened reversed U-shaped, medial convex; lower jaw sheath broadly U-shaped. Coloration in preservative. Dorsally: body whitish, with black round spots which are irregularly distributed, spots condensed between eyes to form a dark patch, tail musculature whitish speckled, density and size of the spots diminish towards the tail tip. Laterally: body blackish, intestinal coils and spiracle visible; transparent, dorsal fin with speckled pattern that is condensed around midtail. Ventrally: transparent, intestine visible and regularly spiral-shaped.

Variation: TL and BL of 10 tadpoles at stages 25–27, all from the site Maromalo (FG/MV 2002.1903 – ZSM 676/2004, 677/2004, 1020/2004 and FG/MV 2002.1914 – ZSM 696/2004, 697/2004) are 17–26 mm and 5–10 mm, respectively. BW 102–117% of BH; ED 11–18% of BL; RN 58–97% of NP; NN 54–71% of PP; SS 47–67% of BL; TMH 47–83% of MTH; TMW 33–47% of BW; MTH 67–109% of BH; ODW 21–38% of BL; ODW 42–68% of BW. LTRF: 1:3+3/1+1:2, 1:4+4/1+1:2 after Dubois (1995) and 4(2–4)/3(1), 5(2–5)/3(1) after Altig & McDiarmid (1999).

Boophis septentrionalis

(Figures 3 and 8)

The following description refers to one tadpole in developmental stage 26 (field number FG/MV 2002.1961 – ZSM 786/2004), TL 37 mm, BL 13.9 mm), from Montagne d'Ambre National Park. The 16S rDNA sequence of this specimen was 100% identical to a reference sequence of an adult specimen (accession AY848507) from the same locality.

In dorsal view, body ovoid, snout rounded. In lateral view, body depressed, BW 121% of BH, snout pointed. Eyes of moderate size, ED 14% of BL, not visible in ventral view, positioned dorsally and directed laterally, situated at about 1/3 of body length. Nares elliptical, moderately sized, marked with the black spots, positioned dorsally, oriented anterodorsally and nearer to snout than to eye. RN 67% of NP, NN 49% of PP. Spiracle sinistral, inner wall free from body and formed such that aperture opens posteriorly, visible in dorsal view, spiracular opening oval, positioned ventrolaterally, directed posteriorly, situated under the height of the longitudinal axis of caudal musculature and the aperture is much closer to the end of the body than to the snout, SS 56% of BL. Medial vent tube with web between tube and fin, tubular, short. Caudal musculature well developed, TMH 74% of BH and 81% of MTH, TMW 58% of BW; its height 2/5 of total height at midlength of tail. Upper fin higher than lower fin. Dorsal fin originates at the proximal 1/5 of the tail muscle, increases gradually to attain its maximum height, and then diminishes gradually towards the tail tip. Ventral fin origins at the ventral terminus of the body, diminishes gradually towards the tail tip. Tail tip slightly rounded, MTH 92% of BH, lateral line visible on the proximal 3/4 of the tail, myosepta visible mainly in the proximal half of the tail. Oral disk relatively large, ODW 31% of BL and 53% of BW, positioned ventrally, directed ventrally, not emarginated, elliptical. Two rows of marginal papillae ventrally and dorsolaterally, interrupted by a large gap on the upper labium (DG 77% of ODW), small medial gap on the lower labium; total number of marginal papillae 88 (45 on the left side, 43 on the right side). 25 submarginal papillae positioned in the lateral parts of the anterior and posterior labium. Papillae of moderate size, ventral papillae

elongated, lateral papillae elongated or rounded with rounded tip. No denticulate papillae. LTRF 1:4+4/1+1:2 after Dubois (1995) and 5(2–5)/3(1) after Altig & McDiarmid (1999). The density of keratodonts on A₁ is about 39 per millimeter (a total of ca. 152). The length of interrupted anterior keratodont rows (A₂, A₃, A₄ and A₅) decreases gradually towards the center of the disk, P₂ and P₃ have the same length. Same sized keratodonts except in A₃. Keratodonts are more or less connected, difficult to distinguish. Both jaw sheaths coarsely serrated and completely black; upper jaw sheath with a large medial and rounded serration, surrounded by a smaller serration on each side, more open reversed U-shaped with medial convexity; lower jaw sheath U-shaped. Coloration in preservative. Dorsally: body whitish, with irregular and different sized spots that are condensed forming dark patches between eyes and on the dorsum. Tail musculature whitish, with many irregular and different sized spots that are irregularly scattered, their size and density diminishing towards the tail tip. Laterally: body blackish, intestinal coils not visible, spiracle visible; upper fin transparent with some spots. Ventrally: transparent, intestine visible. Variation: TL and BL of 39 tadpoles at stage 25–28, all from the National Park of Montagne d'Ambre FG/MV 2002.1961- ZSM 787, 788, 1036/2004) are and 36–48.25 mm 13.15–14.60 mm, respectively. BW 117–126% of BH; ED 12–22% of BL; RN 50–65% of NP; NN 48–54% of PP; SS 46–57% of BL; TMH 67–86% of MTH; TMW 56–58% of BW; MTH 83–99% of BH; ODW 20–28% of BL; ODW 38–47% of BW.

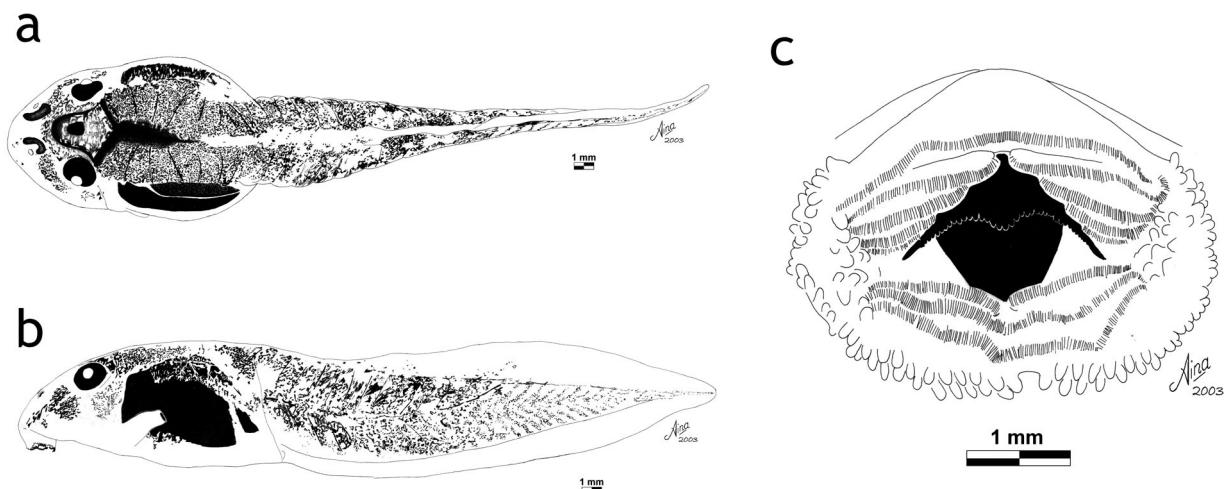


FIGURE 3. Drawings of preserved tadpole specimen of *Boophis septentrionalis* (ZSM 786/2004) from Montagne d'Ambre National Park (developmental stage 26). (a) dorsal view; (b) lateral view; (c) oral disk.

Boophis brachychir

(Figures 4 and 8)

The following description refers to one tadpole in developmental stage 26 (field number FG/MV 2002.1953 – ZSM 765/2004), TL 54 mm, BL 20 mm), from Montagne d'Ambre National Park. The 16S rDNA sequence of this specimen was 100% identical to a reference sequence of an adult specimen (accession AY848563) from the same locality.

In dorsal view, body elliptical, snout large. In lateral view, body depressed, BW 112% of BH, snout pointed. Eyes of moderate size, ED 11% of BL, not visible in ventral view, positioned dorsally and directed laterally, situated at about 1/3 of the body length. Nares elliptical, moderately sized, marked with black spots, positioned dorsally, oriented dorsolaterally and nearer to snout than to eye. RN 85% of NP, NN 50% of PP. Spiracle sinistral, inner wall free from body and formed such that aperture opens posteriorly, visible in dorsal view, spiracular opening rounded, positioned ventrolaterally, directed posteriorly, situated under the extension of the longitudinal axis of caudal musculature and the aperture is much closer to the end of the body than to the snout, SS 49% of BL. Dextral vent tube, tubular, short. Caudal musculature well developed, TMH 78% of

BH and 85% of MTH, TMW 60% of BW; its height 1/2 of total height at midlength of tail. Upper fin higher than the lower fin. Dorsal fin originates at the proximal 1/4 of the tail muscle, increases quickly to attain the maximum height, then diminishes abruptly towards the tail tip. Ventral fin originates at the ventral terminus of the body, increases slightly, and then diminishes gradually towards the tail tip. Tail tip slightly pointed, MTH 91% of BH, lateral line visible on the proximal 3/5 of the tail. Oral disk of moderate size, ODW 19% of BL and 35% of BW, positioned ventrally, directed ventrally, not emarginated. One row of marginal papillae interrupted by a large gap on the upper labium (DG 69% of ODW), small medial gap on the lower labium; total number of marginal papillae 67 (33 on the left side, 34 on the right side). 8 submarginal papillae positioned in the lateral parts of the anterior and posterior labium. Papillae of moderate size, rounded with rounded tip. No denticulate papillae. LTRF 1:5+5/1+1:2 after Dubois (1995) and 6(2–6)/3(1) after Altig & McDiarmid (1999). The density of keratodonts on A₁ is about 70 per millimeter (a total of ca. 206). The length of interrupted anterior keratodont rows (A₂, A₃, A₄, A₅ and A₆) decreases gradually towards the center of the disk, P₂ and P₃ have the same size. A₁ and P₃ with small keratodonts. In all other rows, keratodont size declines from the center to the periphery. Both jaw sheaths coarsely serrated and completely black; upper jaw sheath wide with a large medial and pointed serration surrounded by a smaller serration on each side; lower jaw sheath U-shaped. Coloration in preservative. Dorsally: body with two different pigmentation patterns. First, fine spots forming a network of patches. Second, darker, irregular and differently sized patches, condensed to form a network of patches on some parts of the dorsum. Laterally: body dorsolaterally with same colour as dorsally, intestinal coils visible, spiracle visible. Tail musculature: whitish with many differently formed and irregularly sized patches, condensed to form a network of patches. Density and size of patches diminish towards the tail tip. Ventrally: translucent, intestine visible and regularly spiral-shaped. Variation: TL and BL of 6 tadpoles at stage 26, all from the National Park of Montagne d'Ambre (FG/MV 2002.1953; ZSM 766, 767, 768, 1033/2004) are 43–54 mm and 17–20 mm, respectively. BW 107–131% of BH; ED 11–12% of BL; RN 84–88% of NP; NN 49–52% of PP; SS 49–56% of BL; TMH 73–89% of MTH; TMW 50–73 of BW; MTH 87–99% of BH; ODW 19–25% of BL; ODW 35–43% of BW. LTRF: 1:5+5/1+1:2, 1:4+5/1+1:2 and 1:5+4/1+1:2 after Dubois (1995) and 6(2–6)/3(1) after Altig & McDiarmid (1999).

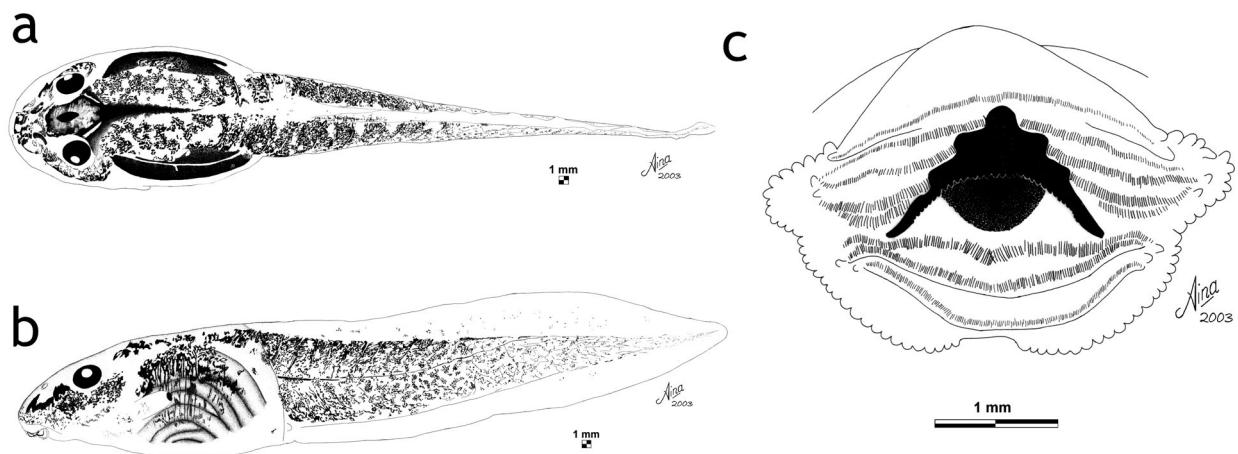


FIGURE 4. Drawings of preserved tadpole specimen of *Boophis brachychir* (ZSM 765/2004) from Montagne d'Ambre National Park (developmental stage 26). (a) dorsal view; (b) lateral view; (c) oral disk.

Boophis bottae (Figures 5 and 8)

The following description refers to one tadpole in developmental stage 36, ZSM 488/2008 (field number LR 247) (TL 30 mm, BL 11 mm) from Analamazaotra Special Reserve. The 16S rDNA sequence of this

specimen differs in 2 substitutions over an alignment of 525 bp from a reference sequence of an adult specimen (accession AJ314817) from the same locality.

In dorsal view, body elongated ovoid, snout rounded. In lateral view, body depressed, BW 143% of BH, snout as an almost uniform curve. Eyes moderately large, ED 13% of BL, not visible in ventral view, not bulging, positioned more laterally than dorsolaterally but directed dorsolaterally. Pineal ocellus absent. Nares nearly oval, moderately-sized, rimmed, positioned dorsolaterally, directed dorsally, much closer to the eyes than to the snout. RN 136% of NP, NN 57% of PP. Spiracle sinistral, moderately small, slightly conical, inner wall free from body, spiracular opening oval and directed more posteriorly than posterodorsally, the aperture is situated much closer to vent tube than to tip of snout, SS 70% of BL, situated below the longitudinal axis of tail musculature. Vent tube short, dextral, opening at ventral edge of fin, medial vent tube with lateral displacement. Caudal musculature moderately strong, TMH 55% of BH and 56% of MTH, TMW 45% of BW, height of caudal musculature almost the half of total tail height at mid-length of tail, reaching almost tail tip. Caudal fins regular with straight edges, very shallow, MTH 98% of BH; dorsal fin originating at the dorsal tail body junction and slightly taller than ventral fin at mid-length of tail; ventral fin originating at the level of the ventral terminus of the body, following the caudal muscle; tail tip nearly pointed. Oral disk moderately large, ODW 25% of BL and 45% of BW, positioned anteroventrally and directed ventrally, emarginated. Oral disk surrounded by a single row of 66 (32 on the left side and 34 on the right side) marginal papillae interrupted by a large gap on the upper labium (DG 61% of ODW), and by a small medial gap on the lower labium. Five submarginal papillae positioned in the lateral and posterolateral parts of the lower labium. Papillae of moderate size, round or conical with rounded tips. LTRF 1:4+4/1+1:2 after Dubois (1995) and 5(2–5)/3(1) after Altig & McDiarmid (1999). The number of keratodonts on A₁ is about 70/mm (a total of 123). The length of interrupted anterior keratodont rows (A₂, A₃, A₄ and A₅) decreases gradually towards the center of the disk, P₁ and P₂ of about similar size, slightly longer than P₃. Both jaw sheaths coarsely serrated; upper jaw completely black with a large medial serration surrounded by a smaller on each side forming a slight convexity; lower jaw partially pigmented, V-shaped.

Coloration in preservative. Dorsally: body and tail musculature brownish, covered by scattered dark patches, almost uniform. Intestinal coils not visible dorsally.

Laterally: intestinal coils obscure but partially visible on the lower part of the body; dorsal fin sparsely pigmented; ventral fin clear. Ventrally: bronchial and cardial region not visible through ventral body wall. Intestinal coils dextral, well visible ventrally with regular spiral shape

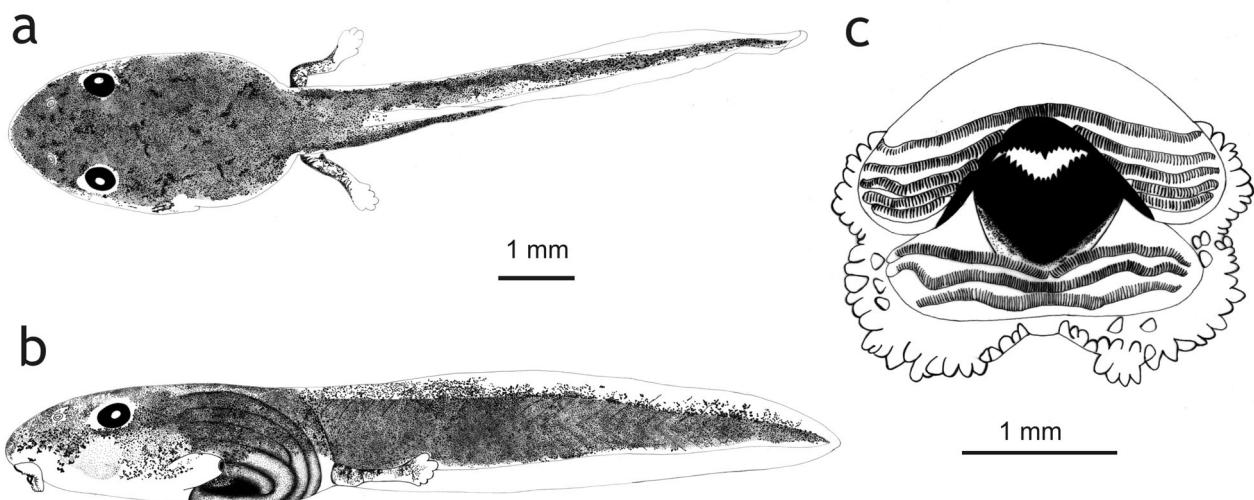


FIGURE 5. Drawings of preserved tadpole specimen of *Boophis bottae* from Analamazaotra Special Reserve (developmental stage 36). (a) dorsal view; (b) lateral view; (c) oral disk. Not drawn to scale.

Boophis ulftunni
(Figures 6 and 8)

The following description refers to one tadpole in developmental stage 25, ZSM 535/2008 (field number FGZC 2279) (TL 35 mm, BL 13.5 mm) from Camp Simpona in the Marojejy National Park. The 16S rDNA sequence of this specimen differed in 2 substitutions over an alignment of 497 bp from a reference sequence of an adult specimen from the same locality (accession EU252144).

In dorsal view, body ovoid, snout uniformly rounded. In lateral view, body depressed, BW 115% of BH, snout as a slope decreasing gradually up to the level of oral disk. Eyes of moderate size, ED 11% of BL, slightly bulging, not visible in ventral view, positioned dorsolaterally but directed almost laterally. Pineal ocellus present between eyes. Nares nearly oval, moderately-sized, rimmed with a flat mediadorsal projection positioned dorsolaterally, directed anterolaterally, closer to anterior edge of eyes than to tip of snout. RN 115% of NP, NN 52% of PP. Spiracle sinistral, inner wall free from body, well visible from dorsal view, spiracular opening oval, directed posterodorsally, the aperture is closer to vent than to snout, SS 57% of BL, situated below the longitudinal axis of tail musculature. Vent tube short, opening dextral at ventral edge of fin, medial vent tube with lateral displacement. Caudal musculature moderately strong, TMH 58% of BH and 64% of MTH, TMW 38% of BW, at mid-length of tail its height about 9/5 of the total tail height, reaching almost tail tip. Caudal fins shallow anteriorly, deepest at about midlength of tail, their height decreasing progressively up to tail tip, MTH 90% of BH; dorsal fin originating at the dorsal tail body junction and taller than ventral fin at mid-length of tail; tail tip bluntly pointed.

Oral disk of moderate size, ODW 26% of BL and 44% of BW, positioned and directed anteroventrally, emarginated. A single row of marginal papillae interrupted by a large gap on the upper labium (DG 55% of ODW) and a small medial gap on the lower labium; total number of marginal papillae 81 (39 on the left side, 42 on the right side). No submarginal papillae present. LTRF 1:2+2/1+1:2 after Dubois (1995) and 3(2–3)/3(1) after Altig & McDiarmid (1999). The density of keratodonts on A_1 is about 60 per millimetre (total ca. 130). The length of interrupted anterior keratodont rows (A_2 and A_3) decreases gradually towards the center of the disk, P_1 and P_2 of about similar size, slightly longer than P_3 . Both jaw sheaths serrated; upper jaw with a large arch, convex medially and fully black pigmented; lower jaw sheath V-shaped and partially pigmented.

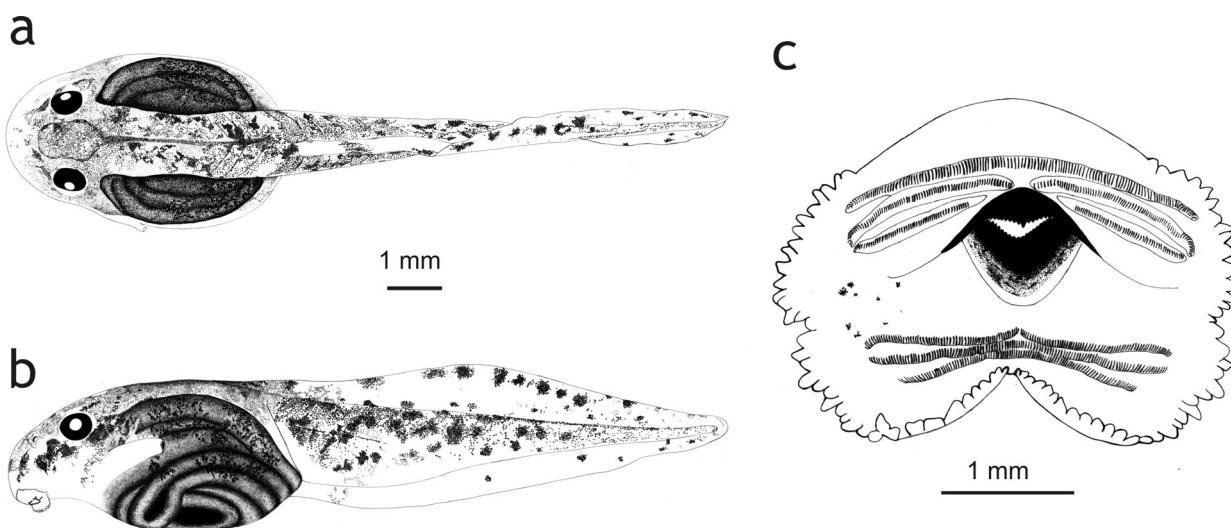


FIGURE 6. Drawings of preserved tadpole specimen of *Boophis ulftunni* from Marojejy National Park (developmental stage 25). (a) dorsal view; (b) lateral view; (c) oral disk. Not drawn to scale.

Coloration in preservative. Dorsally: body and tail musculature brownish, covered by scattered black spots. Some pigmentation concentrated forming one dark patch between eyes and one on mid-dorsal area of

body. A dark line slightly visible mid-dorsally of body.

Laterally: intestinal coils well visible. Spiracle not pigmented. Body and caudal musculature covered by scattered dark patches. Musculature junctions slightly conspicuous anteriorly. Dorsal fin with sparse black dots. Ventral fin almost translucent. Ventrally: branchial and cardial region opaque through ventral body wall; intestinal coils well visible.

Variation: TL and BL of one further tadpole at stage 25 are 21 mm and 13 mm. The ratios are: BW 135% of BH, ED 8% of BL, RN 116% of NP, NN 48% of PP, SS 62% of BL, TMH 48% of BH, TMH 53% of MTH, TMW 36% of BW, MTH 91% of BH, ODW 27% of BL, ODW 42% of BW.

Boophis guibei

(Figure 7)

The following description refers to one tadpole in developmental stage 36 (field number FG/MV 2003-2018, ZSM 1609/2004, TL 38 mm, BL 15 mm) from Ranomafana National Park. The 16S rDNA sequence of one specimen from the same batch differed in 18 substitutions over an alignment of 512 bp from a reference sequence of an adult specimen that originated from another locality near Ranomafana (accession AY848480).

In dorsal view body ovoid. In lateral view body depressed, snout almost uniformly curved, BW 115% of BH. Moderately large eyes, ED 12% of BL, positioned dorsally, directed laterally, situated at the anterior 1/3 of the body. Nares elliptical, prominent with marginal rim, positioned dorsolaterally, oriented anterolaterally, much closer to snout than to eyes, RN 58% of NP and NN 42% of PP. Wide and short sinistral spiracle, inner wall free and formed such that the aperture opens laterally instead of posteriorly not visible from dorsal view. The elliptical opening is situated close to the origin of the lower part of the tail musculature and oriented posterodorsally, the aperture is closer to end of body than to snout, SS 57% of BL. Dextral vent tube, the aperture is under a flap. Strong caudal musculature in the anterior half, TMH 54% of BH and TMW 45% of BW. Dorsal fin higher than ventral fin. At midlength of tail, caudal musculature represents about 1/4 of total tail height. Dorsal fin origins before the tail body junction and ventral fin originates at the ventral terminus of the body.

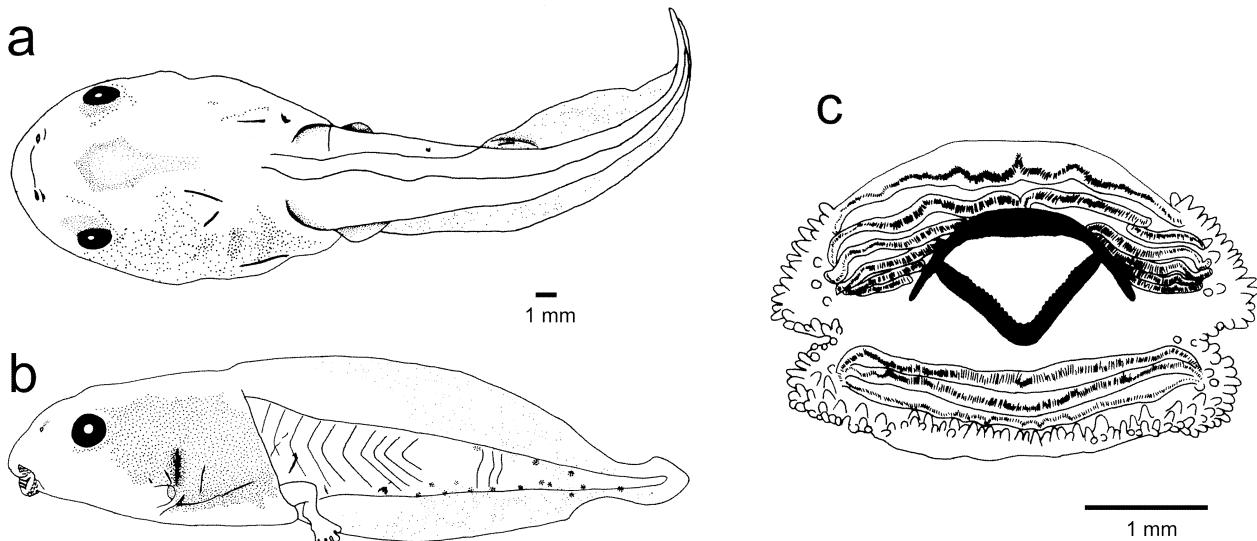


FIGURE 7. Drawings of preserved tadpole specimen of *Boophis guibei* (ZSM 1609/2004) from Ranomafana National Park (developmental stage 36). (a) dorsal view; (b) lateral view; (c) oral disk.

Oral disk moderately large, ODW 24% of BL and 36% of BW, positioned ventrally and directed anteroventrally, emarginated. One row of marginal papillae that is interrupted along by a large gap on the upper labium (DG 69% of ODW); a total of 102 marginal papillae, density 18 papillae/mm, 34 submarginal

papillae positioned in the lateral parts of the anterior and posterior labium (19 on the left side and 17 on the right side. Papillae of moderate size, round or conical with rounded tips. LTRF 1:5+5/1+1:3 after Dubois (1995) and 6(2–6)/3(1) after Altig & McDiarmid (1999). A₂ has 58 keratodonts/mm (a total of 217 keratodonts), A₁ has a total of 238 keratodonts (88 keratodonts/mm). Jaw sheaths partially pigmented and coarsely serrated, serrations rounded. Upper jaw sheath M-shaped, lower jaw sheath V-shaped.

Coloration in preservation. Dorsally: small brown patches between the nares and at the beginning of the caudal musculature. The patches are condensed around and between the eyes. In lateral view, half-moon shaped brown patches behind the eye. Intestinal coils visible in ventral and lateral view, regularly spiral-shaped. Some brown dots on dorsal and ventral fin.

Variation: TL and BL of five tadpoles at stages 25–42 are 19–38 mm and 10–15 mm, respectively. BW 113–122% of BH; ED 9–22% of BL; RN 83–181% of NP; NN 39–50% of PP; TMH 48–79% of BH; TMW 35–83% of BW; ODW 23–28% of BL; ODW 33–41% of BW; SS 40–61% of BL; TMH 45–62% of MTH; MTH 104–120% of BH.

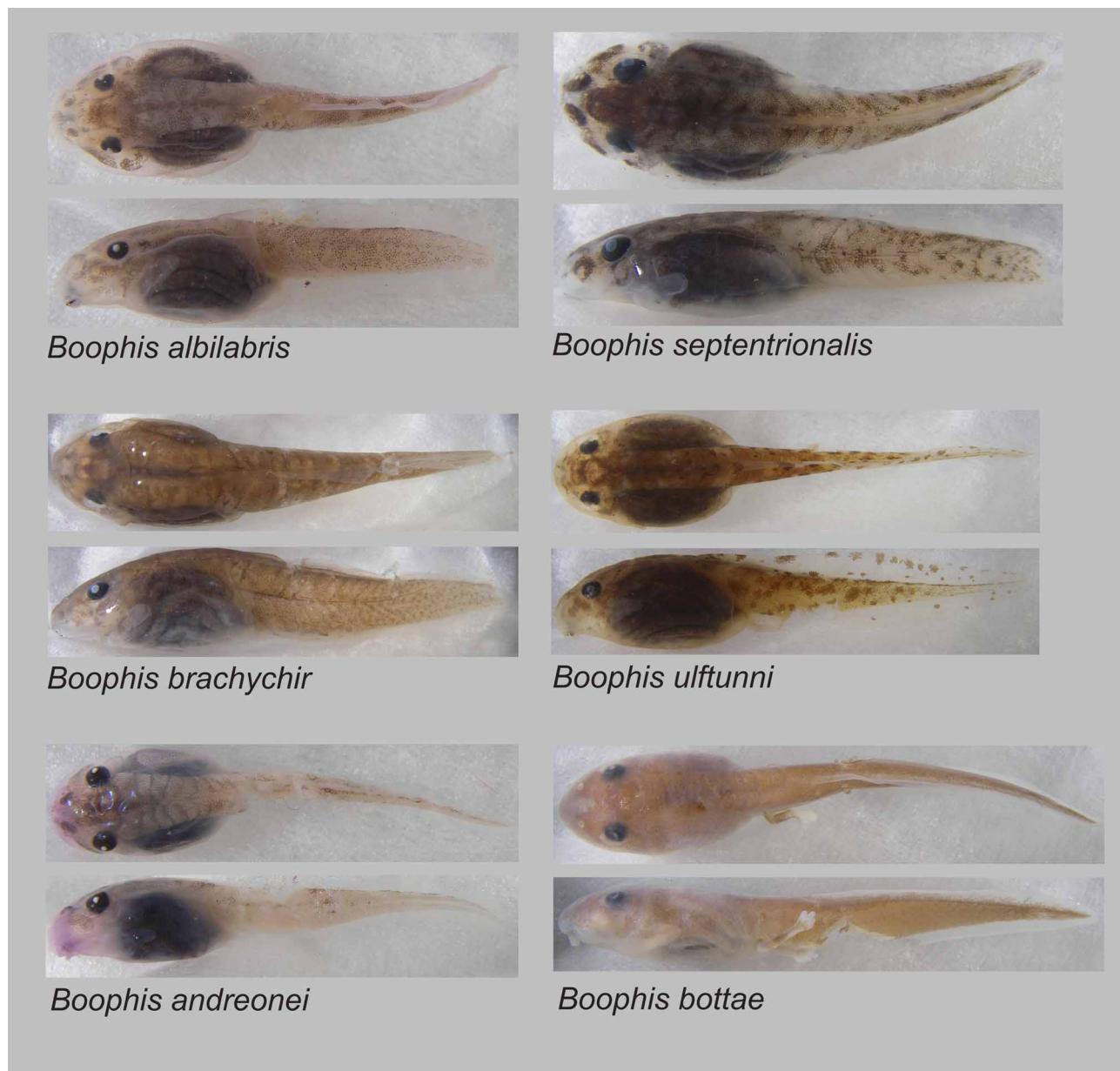


FIGURE 8. Photographs of the preserved DNA voucher specimens of six *Boophis* tadpoles described herein, in dorsal and lateral views.

Discussion

Comparisons with previous descriptions

This paper describes the tadpoles of seven species of *Boophis*, of which two are redescriptions. The larvae of *B. albilabris* have previously been described by Raharivololoniaina *et al.* (2006) based on specimens collected near Andasibe in central-eastern Madagascar. The description provided here refers to specimens collected at Manongarivo in the Sambirano region in northern Madagascar. The 16S rDNA sequences of the specimens from these two populations (as deposited in Genbank) show only a very limited differentiation, indicating that *B. albilabris* qualifies as a widespread species in Madagascar with limited phylogeographic variation. The *B. albilabris* tadpoles from Andasibe have some similarities to those described here: the size, ventral position and emarginated shape of the oral disk, the presence of a single row and the dorsal gap of marginal papillae, the low number of lateral sub-marginal papillae, the black color of the jaw sheath, the depressed body shape, and the sinistral spiracle which is closer to end of body than to snout. However, there are relevant differences in some other characters in specimens from Manongarivo vs. Andasibe: the oral disk is oriented anteroventrally (vs. ventrally), the keratodont formula is 4(2–4)/3(1) (vs. 5(2–5)/3(1)), the spiracle is positioned laterally and oriented dorsally (vs. positioned below the longitudinal axis and oriented posterodorsally), the medial vent tube has a lateral displacement and is compressed ventrolaterally (vs. dextral). While some of these differences may be due to different interpretation of certain structures by different observers, others clearly are indicative of an important larval variability in *B. albilabris*.

The tadpoles of *B. guibei* have previously been described by Blommers-Schlösser (1979) under the name *B. granulosus*. The published description is thorough but misses some important characters that have been reported in subsequent tadpole studies on other Malagasy species. Our description largely agrees with Blommers-Schlösser (1979) who examined 65 tadpoles in Gosner stages 25–40. The specimens described by this author were slightly smaller with TL and BL values of 8–36 mm and 3–12 mm respectively. The LTRF was 5(2–5)/3(1) or 6(2–6)/3(1). Coloration in life was yellowish with brown spots and a silvery belly.

The tadpoles here assigned to *B. brachychir* were collected from Montagne d'Ambre. The status of *B. brachychir* is currently uncertain because the holotype (from Nosy Be) is lost, and at least two species exist in northern Madagascar that roughly fit the very short original description (Boettger 1882; see Glaw & Vences 2007), and a further similar, undescribed species of large body size occurs in this area as well (named *B. sp. aff. madagascariensis* "North" by Glaw & Vences 2007). The tadpoles described here agree genetically with the species named *B. sp. aff. brachychir* in Glaw & Vences (2007) but which in fact may represent the real *B. brachychir* (own, unpublished data). Independent from the nomenclatural and taxonomic fate of the various species in the *B. brachychir* / *B. madagascariensis* complex, the DNA sequences of our tadpole specimens will act as a molecular tag that will allow tracing their species appartenance unambiguously.

Published information (Blommers-Schlösser 1979; Glaw & Vences 1994; Glos and Linsenmaier 2005; Thomas *et al.* 2005, 2006; Raharivololoniaina *et al.* 2006; Grosjean *et al.* 2006) and the new data provided herein agree that *Boophis* in general have rather generalized tadpoles with well developed jaw sheaths and keratodonts. Two exceptions are known to date: the larva of *B. picturatus* is unique in lacking jaw sheaths and keratodonts but having long papillae, and the species appears to ingest sand and other sediment (Altig & McDiarmid 2006); the tadpole of *B. majori* has strongly modified jaw sheaths and a tendency to reduce the first anterior row of labial teeth which furthermore is unusual in often being divided (Schmidt *et al.* 2008). Except for *B. picturatus*, all *Boophis* tadpoles have 3 posterior rows of labial teeth, with the first row being usually divided (rarely uninterrupted). Known numbers of anterior rows are 3–8.

Ecomorphological guilds in *Boophis* tadpoles

A major step towards understanding the ecological and evolutionary implications of morphological variation among anuran larvae is their classification into ecomorphological guilds (Altig and Johnston 1989). In a previous paper summarizing the state of knowledge on stream-breeding tadpoles in the genus *Boophis*, Raharivololoniaina *et al.* (2006) attempted to define three ecomorphological guilds (named Guild A, B and C)

based on mainly three characters: relative width of oral disk, number of inframarginal papillae, and number of keratodonts on the first anterior row. Guild C was characterized by high relative oral disk width (63–89% of BW), and high numbers of papillae and keratodonts, the latter being beyond 200. Guild A had low values for all three characters (31–43% ODW/BW; 48–81 papillae; 58–144 keratodonts) and Guild B was intermediate (43–63% ODW/BW; 69–164 papillae; 164–238 keratodonts). It needs to be highlighted that these guilds partly but not completely correspond to the guilds “clasping”, “adherent”, and “suctorial” as defined by Altig and Johnston (1989) (see below).

Of the tadpoles described herein, one species smoothly fit into these guilds: *B. albilabris* in Guild A. A second species, *B. septentrionalis*, would fit into Guild B if the definition would be extended to include a number of keratodonts in A₁ down to 150 keratodonts, a range between Guild A and Guild B that previously was left unassigned. *Boophis albilabris* was previously been assigned to Guild B but a possible appartenance to Guild A was already discussed (Raharivololoniaina *et al.* 2006). Further problems with assignations to guilds A and B were noted in four other species, *B. andreonei*, *Boophis brachychir*, *B. bottae*, and *B. ulftunni* which show inconsistencies in the defining characteristics (see Table 1).

The very deviant tadpole of *B. picturatus* as described by Altig & McDiarmid (2006) certainly does not belong into any of the three *Boophis* guilds defined before and would need to be included into a further guild if all *Boophis* were to be classified in this manner. The same is true for *B. majori*, whereas the tadpole of the closely related *B. sp. aff. majori* lacks many of the specializations of *B. majori* and may again be a case of a transitional morphology between guilds. Furthermore, our data on *B. guibei*, the only pond-breeding species examined herein, also indicate that more characters need to be included to define pond-breeding *Boophis* tadpoles. In fact, the number of keratodonts on A₁ (238) and of papillae (167) in the pond-dwelling *B. guibei* tadpole is quite high and thereby almost approaches values in the species of Guild C that are clearly adapted to fast-flowing streams, while the relative oral disk width in this species is smaller.

Several of the classification problems certainly are caused by the different developmental stages of the tadpoles examined. The number of keratodonts is known to increase with development and the same is probable for papillae (Vater and Kovac 1996). In *B. albilabris*, the specimen examined here was in stage 26 (BL 8.6 mm) whereas the specimen examined by Raharivololoniaina *et al.* (2006) was in stage 33 (BL 16.1 mm).

Independent from these ontogenetic considerations, it seems clear that our previous definitions of ecological guilds in *Boophis* based on only three characters (Raharivololoniaina *et al.* 2006) were an oversimplification, and that variation in these tadpoles is more complex. Especially the differences between guilds A and B, meant as distinguishing tadpoles adapted to slow-moving parts of streams from those adapted to moderate current, needs to be improved by including further characters and will likely not be possible in an unambiguous way.

From a phylogenetic perspective, our data add to some previous general considerations (Raharivololoniaina *et al.* 2006). Species of the *B. goudotii* group described so far have been assigned to Guild A, including *Boophis brachychir* described herein, and with the sole exception of *B. madagascariensis* which was tentatively included in Guild B, with a mention of their possible appartenance to Guild A (Raharivololoniaina *et al.* 2006). The tadpole of *B. bottae* is the third tadpole of the *B. rappioides* group showing a Guild A–B morphology, far from the Guild C morphology described for *B. erythrodactylus* (Blommers-Schlösser 1979) which may have been a confusion with the tadpole of another species. In the *B. majori* group, the morphology of *B. marojezensis* stands in stark contrast to at least four other species, i.e., *B. pyrrhus* that has Guild A tadpoles, *B. sp. aff. majori* and *B. majori* that show an increasing number of oral disk specializations that makes their attribution to any guild difficult, and *B. picturatus* has a completely deviant tadpole (Raharivololoniaina *et al.* 2006; Altig & McDiarmid 2006; Schmidt *et al.* 2008). This heterogeneity of larvae is in agreement with the probable non-monophyly of this group.

TABLE 1. Comparisons of morphological and ecological key characters of seven species of *Boophis* tadpoles described herein. For abbreviations, see Materials and Methods. The last four rows show characters used for guild definition by Raharivololoniaina *et al.* (2006) and indicate the guild (A, B or C) into which each of the variables would assign the respective tadpole. LTRF is given after (1) Dubois (1995) and (2) Altig & McDiarmid (1999).

Species	<i>B. albilabris</i>	<i>B. andreoni</i>	<i>B. septentrionalis</i>	<i>B. brachychir</i>
Group	<i>B. albilabris</i>	<i>B. luteus</i>	<i>B. luteus</i>	<i>B. goudotii</i>
Field number	FG/MV 2002.1916	FG/MV 2002.1903	FG/MV 2002.1961	FG/MV 2002.1953
ZSM number	ZSM 699/2004	ZSM 675/2004	ZSM 786/2004	ZSM 765/2004
Stage	26	25	26	26
Breeding habitat	River, stream	River	River	River
BL	8.6	8	13.9	20
BL/TL	36%	36%	37%	37%
TL/BL	1.69	5.01	1.66	1.73
TMH/BH	52%	56%	74%	78%
TMH/MTH	59%	80%	81%	85%
TMW/BW	36%	39%	58%	60%
MTH/BH	89%	71%	92%	91%
BW/BH	117%	114%	121%	112%
ODW/BW	38%	56%	53%	35%
OD orientation	Anteroventrally	Ventrally	Ventrally	Ventrally
LTRF(1)	1:3+3/1+1:2	1:4+4/1+1:2	1:4+4/1+1:2	1:5+5/1+1:2
LTRF(2)	4(2–4)/3(1)	5(2–5)/3(1)	5(2–5)/3(1)	6(2–6)/3(1)
LTRF variations	1:5+5/1+1:2 1:4+4/1+1:2	1:3+3/1+1:2		1:4+5/1+1:2 1:5+4/1+1:2
Number of keratodonts in row A ₁	100	108	152	206
Marginal papillae rows	single	single	double	single
Marginal papillae	56	65	88	67
Submarginal papillae	15	14	25	8
ODW	A	B	B	A
keratodonts in row A ₁	A	A	between A and B	B
Papilla rows	A	A /B	B	A
Number of papillae	A	A	B	A

continued.

Species	<i>B. bottae</i>	<i>B. ulftunni</i>	<i>B. guibei</i>
Group	<i>B. rappiodes</i>	<i>B. ulftunni</i>	<i>B. tephraeomystax</i>
Field number	LR 247	FGZC 2279	FG/MV 2003-2018
ZSM number	ZSM 488/2008	ZSM 535/2008	ZSM 1609/2004
Stage	40	25	36
Breeding habitat	River	Stream	Pond
BL	11	13.5	15
BL/TL	36%	38%	39%
TL/BL	1.73	1.63	1.53
TMH/BH	55%	58%	54%

TMH/MTH	56%	64%	
TMW/BW	45%	38%	45%
MTH/BH	98%	90%	
BW/BH	143%	115%	115%
ODW/BW	45%	44%	36%
OD orientation	Ventrally	Anteroventrally	Anteroventrally
LTRF(1)	1:4+4/1+1:2	1:2+2/1+1:2	1:5+5/1+1:3
LTRF(2)	5(2–5)/3(1)	3(2–3)/3(1)	6(2–6)/3(1)
LTRF variations			
Number of keratodonts in row A ₁	123	130	238
Marginal papillae rows	single	single	single
Marginal papillae	66	81	102
Submarginal papillae	6	no	34
ODW	B	A	A
keratodonts in row A ₁	B	A	B
Papilla rows	A/B	A/B	A/B
Number of papillae	A	B	> B

TABLE 2. Morphometric measurement (all in mm) of the DNA vouchers or reference specimens of seven species of *Boophis*. For abbreviations, see Materials and Methods (n.m., not measured).

	<i>B. albilabris</i> ZSM 699/2004	<i>B. andreonei</i> ZSM 675/2004	<i>B. septentrionalis</i> ZSM 786/2004	<i>B. brachychir</i> ZSM 765/2004	<i>B. bottae</i> ZSM 488/2008	<i>B. ulftunni</i> ZSM 535/2008	<i>B. guibei</i> ZSM 1609/2004
Stage	26	25	26	26	40	25	36
BL	8.65	8.00	13.90	19.75	11.00	13.15	14.7
ODW	2.20	2.70	4.30	3.85	2.70	3.50	8.0
IOD	3.20	2.20	5.75	6.50	3.70	4.30	5.2
ED	1.00	1.25	2.00	2.20	1.40	1.50	1.9
TMW	2.15	1.90	4.90	6.35	2.70	3.00	3.3
MTH	4.00	2.75	5.65	8.55	4.10	5.00	8.2
TMH	1.50	1.40	3.35	3.25	2.30	4.00	5.5
TAL	14.65	14.00	23.10	34.10	19.00	21.50	23.4
length of keratodont row A ₁	1.75	1.85	3.25	2.85	1.75	2.18	n.m.
length of keratodont row A ₂	0.80	1.00	1.75	1.60	0.87	1.13	n.m.

General ecomorphological guilds of tadpoles were first defined by Altig and Johnston (1989). The new definitions by Raharivololoniaina *et al.* (2006) were not meant to replace these original guilds nor to refine them, but rather to achieve a complementary, more quantitative classification that would fit the variation in *Boophis* tadpoles better. Linking the two sets of guild definitions is not easy, because the criteria used to elaborate them were partly different. Furthermore, some criteria of Altig and Johnston (1989) are not found in *Boophis* tadpoles.

According to Altig and Johnston (1989), clasping tadpoles have a dorsal gap of marginal papillae, labial tooth rows commonly 5 but as numerous as 8/8, usually with anterior rows more numerous than posterior rows, and a globular to slightly depressed body. They inhabit medium to slow currents and the position

maintenance via the oral disc is minor. All the tadpoles in guilds A and B of Raharivololoniaina *et al.* (2006) fit in this clasping guild because they have a depressed body, anterior rows more numerous than posterior rows, at least a dorsal gap of marginal papillae (some have also ventral gap). As far as known they live in a medium or slow current and the position maintenance via oral disc is minor.

The adherent and suctorial guilds are defined by some similar criteria, such as the complete rows of small marginal papillae, the continuous position maintenance via oral disc, and the depressed body shape. The difference concerns mainly the labial tooth rows, with a LTRF of commonly 2/3 for adherent tadpoles, and from 2/3 to a maximum of 17/21 in the suctorial guild. These tadpoles are supposed to inhabit faster water currents in the succession clasping - adherent - suctorial. Whether tadpoles of *Boophis* Guild C belong into the adherent or suctorial guild sensu Altig & McDiarmid (1989) is not easy to decide. All *Boophis* tadpoles have more anterior than posterior keratodont rows, but the high numbers of rows in Guild C agree more closely with the suctorial guild.

Clearly, a full understanding of ecomorphological adaptations in *Boophis* tadpoles will require detailed descriptions of a yet more complete array of species, and multivariate comparisons of both morphological and ecological characters. In concert with DNA barcoding which allows reliable identification of these larvae to species, the analytical tools for such analyses are now available (Candioti 2007) and will certainly lead to new insights into the evolutionary and ecological implications of tadpole variation.

Reliability of tadpole identification in field series

In our study we followed a protocol in which tadpoles were, in the field, first assigned to series of morphospecies. Subsequently, one specimen out of each morphospecies was identified by DNA barcoding (the DNA voucher). This protocol has proven its efficiency and has been of great aid to identify reliably numerous Malagasy tadpoles to species (Thomas *et al.* 2005, 2006; Raharivololoniaina *et al.* 2006; Vejarano *et al.* 2006a,b,c; Grosjean *et al.* 2006, 2007; Glos *et al.* 2007; Knoll *et al.* 2007; Randrianiaina *et al.* 2007; Schmidt *et al.* 2008, 2009a, b; Grosjean & Vences 2009), but it has an obvious shortcoming: attribution of the non-sequenced specimens to a series is based solely on field identification. While this is certainly a very reliable method in areas of low species diversity such as montane habitats (Thomas *et al.* 2005) where the few tadpoles furthermore are easily recognizable at first glance, its reliability is questionable at rainforest sites of high species diversity where over 25 species of tadpoles can occur simultaneously in one stretch of a stream, partly in high densities (Vences *et al.* 2008). In order to obtain a full picture of the tadpole diversity in the streams, we usually partition morphologically similar tadpoles into several series, which often allows us to have several DNA voucher specimens for description and assessment of variability. Nevertheless, when interpreting our descriptions, it is crucial to understand which parts are based always on the unambiguously identified DNA voucher specimen (all textual descriptions, and usually the measurements in the tables, and drawings) and which on other specimens of the series which may or may not be correctly identified (all or most reports of morphological variation). In fact, once that morphological data of more tadpoles become available and a clearer picture emerges of which species have very similar or very distinctive larvae, it will be fruitful to test the efficiency of morphospecies sorting in the field and adjust the sampling protocol as necessary.

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