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Montane Tadpoles in Madagascar: Molecular Identification and Description of the Larval Stages of *Mantidactylus elegans*, *Mantidactylus madecassus*, and *Boophis laurenti* from the Andringitra Massif

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The larval stages of three species of frogs from montane habitats in the Andringitra Massif, southern central Madagascar at 2100–2500 m above sea level, were identified through mitochondrial DNA sequences and are described herein. The tadpoles of *Boophis laurenti* agree with the previously known tadpoles of the closely related *Boophis microtympanum*, whereas the tadpoles of *Mantidactylus madecassus* are similar to those of other species of the subgenus *Brygoomantis* that occur at lower altitudes. The tadpoles of *Mantidactylus elegans* are very large (up to 106 mm total length) and show mouthparts largely agreeing with those of species of the subgenus *Guibemantis*, with a relatively high number of upper labial tooth rows (one continuous and six interrupted). These tadpoles are uniformly blackish on the dorsum, indicating a possible general trend of high frequency of dark color and melanism in montane amphibians. Molecular identification provides a fast and very efficient tool to identify larval stages of amphibians, especially in cases of specialized tadpoles from remote areas in which rearing is difficult.

THE central high plateau of Madagascar encompasses several mountain massifs which locally reach altitudes higher than 2800 m above sea level. The three highest massifs (Tsaratanana, Ankaratra, and Andringitra) harbor a highly specialized and endemic montane fauna, which is poorly known in terms of ecology and biology (Raxworthy and Nussbaum, 1996b). Virtually nothing is known about the amphibians of Tsaratanana in northern Madagascar (Blommers-Schlösser and Blanc, 1991; Raxworthy and Nussbaum, 1996b), whereas recent inventories of the Ankaratra mountains in central Madagascar have resulted in a reasonable state of knowledge (Glaw and Vences, 1994; Vences and Glaw, 1999; Vences et al., 2002a). The third high-altitude massif, Andringitra, has been intensively surveyed (Raxworthy and Nussbaum, 1996a; Raselimanana, 1999; Rasolonandrasana and Goodman, 2000), but detailed ecological data of the montane herpetofauna specialized to altitudes above 2000 m is so far lacking.

According to present knowledge, three amphibian species (*Anodonthyla montana*, *Boophis laurenti*, and *Mantidactylus madecassus*) and one reptile (*Lygodactylus intermedius*) are endemic to high-elevations of Andringitra massif (Vences and Glaw, 1999; Vences et al., 2002a). The local populations of several further amphibians (e.g., *Scaphiophryne madagascariensis*, *Mantidactylus brevipalmatus*, and *Mantidactylus curtus*) appear to be either genetically or morphologically distinct and may also represent separate entities at the specific or subspecific level. The status of *Boophis laurenti*, which is morphologically close to *Boophis microtympanum*, remains to be clarified.

We recently started a project to describe the larval stages of Malagasy frogs, based on their identification through mitochondrial DNA sequences (e.g., Hebert et al., 2003; Blaxter and Floyd, 2003; Tautz et al., 2003). This method is considerably faster and potentially more reliable than identification through rearing of tadpoles and determination of juveniles.

Knowledge on the morphology and habitat of tadpoles is highly relevant for the understanding of the ecological requirements and natural history of frog species. Recent global trends of amphibian declines (Kiesecker et al., 2001) seem to especially affect montane species (e.g., Young et al., 2001). Hence, the understanding of the natural history of montane Malagasy amphibians bears relevance for conservation biology, because these species have a very limited distribution, and several may qualify for a threatened category in terms of IUCN categories (IUCN, 2001).

In this paper, we provide the first descriptions of the tadpoles of three frogs from the Andringitra Massif belonging to the family Mantellidae: *B. laurenti*, *Mantidactylus elegans*, and *Mantidactylus madecassus*. We discuss our findings in the context of the evolution of larval morphology among mantellids and review the utility of genetic identification of larval stages.

Materials and Methods

Tadpoles were collected with dip nets, euthanized using chlorobutanol, and assigned to morphotype categories in the field. Subsequently a piece of tail or fin was taken as a tissue sample for DNA extraction from one specimen of each tadpole series, and all tadpoles were preserved in 4% buffered formalin. Institutional abbreviations follow Leviton et al. (1985).

Fieldwork was carried out at the beginning of February 2003 in the Andringitra National Park. The first locality, the sampling site of the tadpole series ZSM 608/2003, ZSM 609/2003, and ZSM 611/2003, was at a slowly running stream at an altitude of 2488 m above sea level located in a depression named ''Cuvette Boby'' (22°11′41″ S/46°53′23″E). The second site was at a stream on the Andohariana plateau (22°10′49″S/46°54′01″E; 2114 m above sea level), sampling site of the tadpole series ZSM 610/2003. The water of both sites was cold and clear; the streams widths were 1.5–2.0 m; and average stream depth was 0.5 m. At some places, the depth dropped down to 2 m. The bottoms of the brooks were covered by gravel; aquatic plants and algae were rare.

For tadpole identification, we amplified a fragment of up to 550 bp of the mitochondrial 16S rRNA gene of each sample. We compared these sequences of tadpoles with homologous sequences of morphologically and bioacustically well-identified adult specimens from the same population. According to a large unpublished dataset of this gene fragment available to us, including sequences retrievable from Genbank as of May 2004, pairwise sequence divergences are high among species of mantellid frogs (2–15%), especially if these belong to distinct genera and species group as in the case study reported here. However, there are very low sequence divergences among conspecific individuals belonging to the same population; usually these sequences are 100% identical. Hence, identification of larvae is unequivocal if full sequence identity between larvae and adults is encountered.

DNA was amplified by PCR in 50 μl volumes, applying 34 cycles of 30 sec at 95 C, 60 sec at 55 C, 60 min at 72 C with 20–100 ng DNA-template, 1,5 μl of 50mM $MgCl_2$, 5 μl dNTPs, and 1 U of Taq-Polymerase, using 5 pmol of primers 16SA-L and 16SB-H (Vences et al., 2000) as originally described by S. R. Palumbi, A. Martin, S. Romano, W. O. McMillan, L. Stice, and G. Grabowski (The Simple Fool's Guide to PCR, Vers. 2.0, 1991, unpubl.). Annealing temperature was at 55 C. Double-stranded sequences were resolved on an ABI 3730 capillary sequencer. DNA sequences are deposited in GenBank (accession numbers: *M. elegans* AY659959; tadpole of *M. elegans* AY659960; *M. madecassus* AY659961; tadpole of *M. madecassus* AY659962; *B. laurenti* AY659963; tadpole of *B. laurenti* AY659964).

To detect possible mistakes in assigning tadpoles to series in the field, we compared the DNA voucher once again with all other specimens of the series using a stereo microscope. Drawings and descriptions in this paper are based on the DNA vouchers, whereas other representative specimens of the same series were examined to supplement structures missing because of tissue sampling. To assess morphological variability, measurements were taken from all specimens of each series. All tadpoles were staged according to Gosner (1960). Terminology and measurements follow Altig and McDiarmid (1999) with some modifications. The following measurements were taken to the nearest 0.1 mm with dial calipers: body length (distance from the tip of the snout to the body terminus, which is the junction of the posterior body wall with the tail axis); tail length (the distance from the body terminus to the absolute tip of the tail); total length (the sum of body length and tail length); body width (measured at the widest point of the ''head'' right behind the eyes, not in the intestinal part); maximum eye diameter; interorbital distance (measured between the centers of the pupils); internarial distance (measured between the centers of the nares); distance between tip of snout and naris (up to the centre of the naris); distance between naris and eye (from the center of naris to the anterior edge of the eye); distance between tip of snout and spiraculum (up to the center of the spiracular aperture); tail muscle height (first, measured vertically from the junction of the body wall with the ventral margin of the tail muscle; second, measured at midtail); tail height (including fins and caudal musculature, taken at its maximal vertical extent); and dorsal-fin origin (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). Values given throughout are number of individuals (*N*) and means of the morphometric measurements ± standard deviations (SD) as well as maximal and minimal values.

Results

In our comparisons of sequences of tadpoles with those of adult specimens, we found three series of larvae showing identical sequences to

TABLE 1. MORPHOMETRIC MEASUREMENTS OF THREE TADPOLES OF *Mantidactylus elegans* FROM CUVETTE BOBY, ANDRINGITRA MASSIF (ZSM 608/2003). Data refer to one specimen of stage 35 and two specimens of stage 25. All values in millimeters.

Character	Specimen 1 (stage 35)	Specimen 2 (stage 25)	Specimen 3 (stage 25)
Body length	40.4	38.4	30.5
Tail length	65.6	67	47.7
Total length	106	105.4	78.2
Body width	20.3	19	15
Eye diameter	3.4	3.4	2.5
Interorbital distance	13.6	13.5	13.5
Internarial distance	6	5	5
Distance snout-naris	3.3	4	2.4
Distance naris-eye	5.6	5.4	4.1
Distance snout-spiraculum	15.3	15.2	13.5
Tail muscle height 1	13.2	12.4	10
Tail muscle height 2	9	8	6
Tail height	21	19	14

well-identified adult frogs, which were collected at the same locality. A tadpole belonging to the series ZSM 609/2003 showed a 100% identity over a fragment of 528 bp as compared to a sequence of an adult specimen of *M. madecassus* (ZSM 755/2001). For the tadpole ZSM 608/2003, the best hit of sequences was with a subadult *M. elegans* (ZSM 971/2003), with 498 of 498 bases being identical. The sequence of an adult specimen of *B. laurenti* (ZSM 727/2001) was 100% identical over 528 bp with that of a tadpole from the series ZSM 610/2003 and 611/2003 (specimens from both series showing similar morphologies).

Description of the tadpole of Mantidactylus elegans.— The following description is based on three tadpoles of *M. elegans* (ZSM 608/2003), which are in an excellent state of preservation (a small piece of the lower fin is removed for DNA extraction), two specimens in stage 25 and one in stage 35. The tadpoles of *M. elegans* are very large exotrophic, benthic tadpoles of Orton (1953) Type IV. They are consistently dark pigmented. The coloration varies from dark brown to black. The intestinal spiral is just slightly visible through the also pigmented abdominal wall. This wall shows a blue glimmer. Most of the observed specimens show golden spots on the dorsum as well as a few on the caudal musculature. The fins are also dark pigmented, but sometimes this becomes less evident on the posterior part of the tail. The only body part totally lacking pigmentation is the lower labium.

Detailed morphometric data of the specimens is given in Table 1. Total length 106 mm (stage 35), 105.4 mm (stage 25), 78.2 mm (stage 25); body shape oval in dorsal view (Fig. 1A), body width about 0.5 times body length; snout nearly rounded; rimmed, bulging upper lip visible from dorsally; eyes relatively small (diameter about 0.08 time body length), internarial distance between 0.38–0.52 times interorbital distance, directed laterally, positioned dorsolaterally, in ventral view not visible; nares small, rounded, directed anterolaterally, positioned much closer to snout than to eyes. Body shape in lateral view (Fig. 1B) moderately low; snout rounded; spiracle sinistral, completely attached to body wall, positioned laterally (closer to venter than to dorsum), oriented posterodorsally; spiracular opening oval, situated slightly below level of apex of myotomes of tail musculature; tail musculature strong, gradually tapering, almost reaching tail tip; fins are moderate; dorsal fin originates near dorsal tail body junction, anteriorly concave shaped, posteriorly convex, ventral-fin shape convex; point of maximum height located at midtail; anal tube short, dextral, tubular, opening directed posteriolaterally, left wall dorsally displaced.

Oral apparatus (Fig. 1C) generalized; positioned almost completely ventrally, laterally emarginated; upper labium with large medial papillae gap (covers almost whole upper lip, as wide as length of first upper teeth row); rest of oral disc bordered by dense row of short marginal papillae; submarginal papillae positioned in lateral parts of upper and lower labia; tooth row formula 1:6 + 6/1 + 1:2; about 60 labial teeth per mm, tooth rows in upper labium become continuously shorter from UTR_2 to UTR_7; UTR_3 first row that touches beak; LTR_1 with short medial gap; LTR_2 longest tooth row; jaw

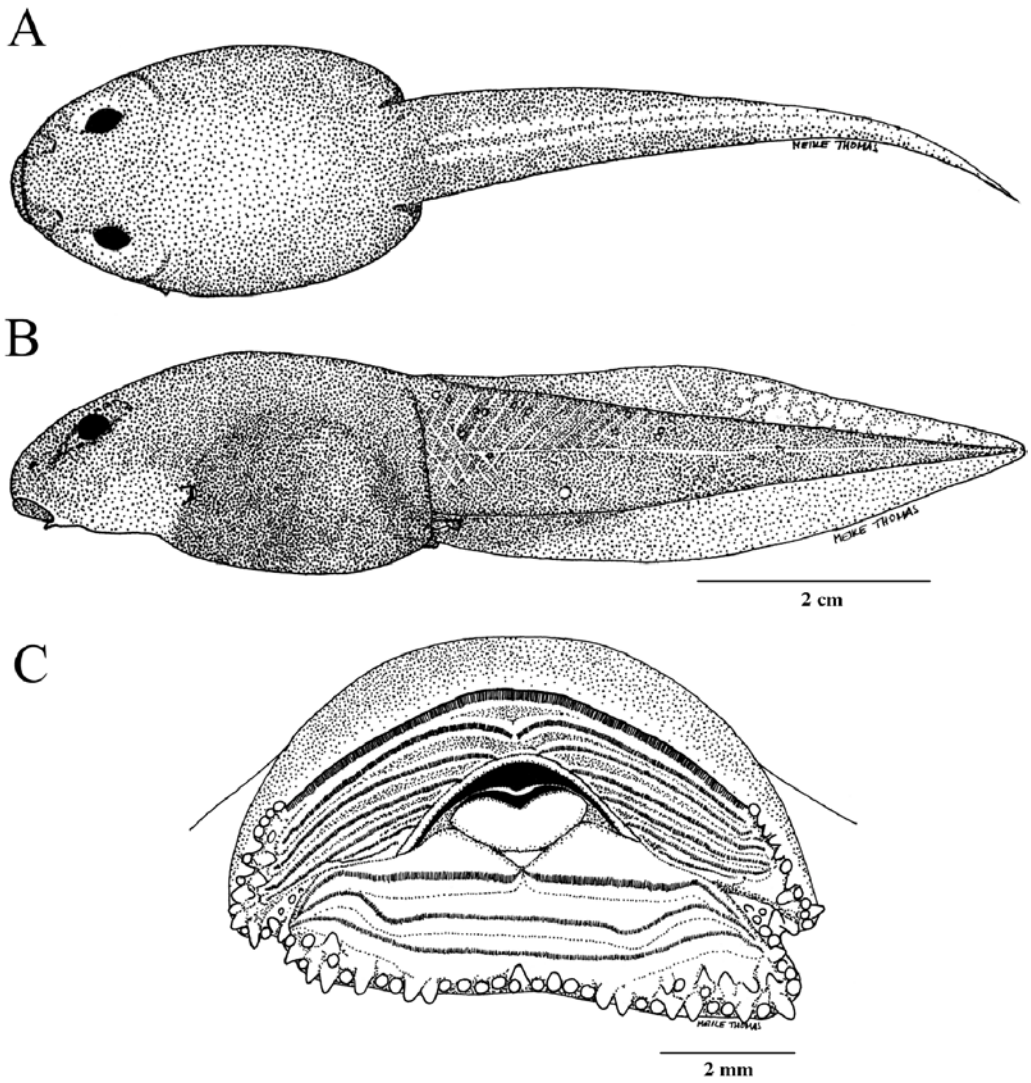

Fig. 1. Drawings of a tadpole of *Mantidactylus elegans* from the series ZSM 608/2003. (A) The specimen is shown in dorsal view with its enormous total length of more than 10 cm; in lateral view (B), the strong tail musculature is visible; the oral apparatus (C) shows that even these huge larvae from montane streams are generalized tadpoles.

sheaths slightly serrated, coloration white with black pigmentation along the sheaths, upper beak with a larger black colored part; upper beak wide opened reversed U-shape, lower beak compact element with slight V-shaped central groove.

Description of the tadpole of Mantidactylus madecassus.—Based on seven tadpoles of *M. madecassus* (ZSM 609/2003). All tadpoles are in stage 25 and in an excellent state of preservation (in one specimen the tip of the tail was removed as a DNA-tissue). *Mantidactylus madecassus* has typical exotroph, benthic tapoles of Orton (1953) Type IV. A dark pigmentation colors the dorsum and the tail almost completely black. Just the anterior part of the ventral fin and the outer skin of the venter are not pigmented. The intestinal spiral is slightly visible though the pigmented inner abdominal wall.

Detailed morphometric data is given in Table 2. Tadpoles of moderate size (body length 12.0 ± 1.3 mm [SD]); body shape long oval, almost straight laterally, in dorsal view (Fig. 2A), body width about 0.5 times body length; upper ''lip'' overhangs the snout; eyes moderately sized (di-

TABLE 2. MORPHOMETRIC MEASUREMENTS OF SEVEN TADPOLES OF *Mantidactylus madecassus* IN STAGE 25 FROM CUVETTE BOBY, ANDRINGITRA MASSIF (ZSM 609/2003). All measurements in millimeters.

Character	Mean	SD	Minimum	Maximum
Body length	11.96	1.13	9.6	13
Tail length	17.43	2.56	12.9	20.5
Total length	29.45	3.75	22.5	33.5
Body width	5.94	0.79	4.6	6.9
Eye diameter	1.37	0.19	1	1.6
Interorbital distance	3.99	0.39	3.4	4.6
Internarial distance	2.16	0.26	1.6	2.4
Distance snout-naris	2.04	0.28	1.5	2.3
Distance naris-eye	1.86	0.3	1.2	2.1
Distance snout-spiraculum	6.26	0.88	5.1	7.5
Tail muscle height 1	3.14	0.47	2.3	3.8
Tail muscle height 2	2.15	0.23	1.8	2.5
Tail height	5.35	0.72	4.3	6.5

ameter about 0.11 times body length), internarial distance 0.54 times interorbital distance, directed laterally, positioned dorsolaterally, in ventral view not visible; nares rounded, rimmed, directed dorsally, positioned closer to eyes than to snout. Body shape in lateral view (Fig. 2B) relatively low, slightly flattening toward snout; skin fold separates venter from oral disc; spiracle sinistral, positioned median laterally, oriented posterodorsally, completely attached to body wall; spiracular opening oval, situated a bit below level of apex of myotomes of tail musculature; tail musculature moderate, gradually tapering, almost reaching obtuse tail tip; dorsal fin originates at body-tail junction; dorsal and ventral fin convex, maximum height in second half of tail length; anal tube dextral, very short, opens posterolaterally, left wall dorsally displaced.

Oral apparatus (Fig. 2C) generalized, positioned anteroventrally, laterally emarginated; marginal papillae with large medial anterior and small (sometimes very small) medial posterior gap; rest of oral disc bordered by a row of marginal papillae (serrated row in lower labium); some submarginal papillae in lateral parts of both labia; tooth row formula 1:4 + 4/1 + 1:2, about 26 labial teeth per mm, tooth rows in upper labium become continuously shorter from UTR_2 to UTR_5, UTR_2 first row that touches beak, LTR_1 with short medial gap; beaks relative massive, almost completely covered by dark pigmentation, slightly serrated; sheath of upper beak M-shaped, lower beak very compact, only slightly grooved.

Description of the tadpole of Boophis laurenti.—Based on a series of seven tadpoles of *B. laurenti* (ZSM 610/2003 and ZSM 611/2003), all in stage 27. The tadpoles are exotroph, benthic tadpoles of Orton (1953) Type IV. They show a dark pigmentation on the dorsum, with diverse clustered darker spots to the lateral and anterior side. The tail and the fins are not consistently pigmented. There is an irregular pattern of dark and light areas.

Detailed morphometric data of the specimens is given in Table 3. Body length varies between 21.8 and 26.1 mm; oval body shape in dorsal view (Fig. 3A), about 0.53 times as wide as long; snout nearly rounded; eyes relatively small, eye diameter about 0.12 times body length, internarial distance 0.57 times interorbital distance, eyes directed laterally, positioned dorsolaterally, not visible in ventral view; nares rounded, rimmed, directed dorsally, positioned closer to eyes than to snout. In lateral view (Fig. 3B), body shape also oval, snout rounded; spiracle sinistral, completely attached to body wall, positioned laterally (closer to venter than to dorsum), oriented posterodorsally; spiracular opening oval, situated almost in level of apex of myotomes of tail musculature; tail musculature moderate, gradually tapering, almost reaching obtuse tail tip; dorsal fin originates at dorsal tail body junction, anterior in the first third of the tail concave, behind this area convex; point of maximal tail extension before mid tail; anal tube short, dextral, tubular, opening directed posterolaterally, left wall displaced.

Oral apparatus (Fig. 3C) directed anterioventrally; oral disc not laterally emarginated; upper and lower labium with medial papillae gap, in upper part gap covers almost whole shape of labium, most papillae in lateral parts, one marginal row of papillae, diverse rows of submarginal papillae; tooth row formula not consistent, two variants 1:5 + 5/1 + 1:2 and 1:6 + 6/1 +

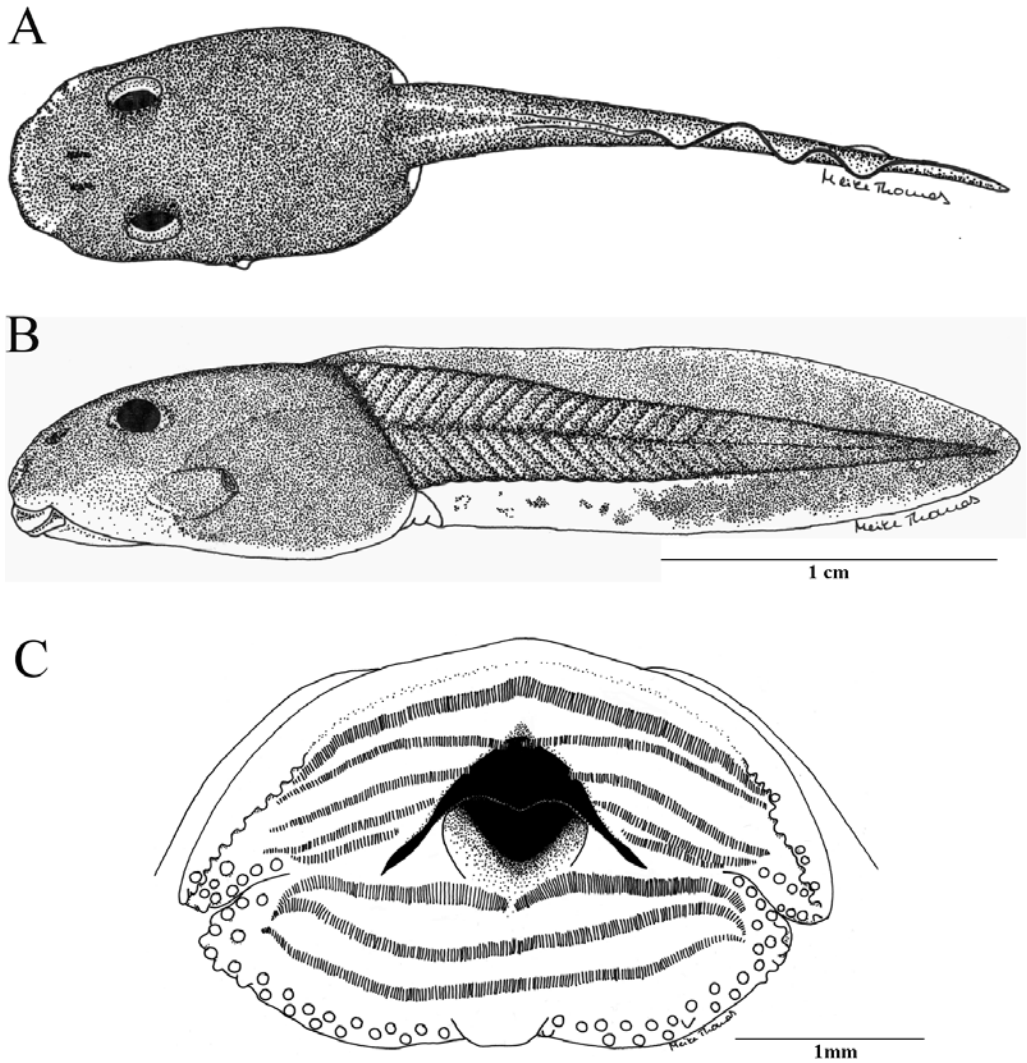

Fig. 2. Drawings of a tadpole of *Mantidactylus madecassus* from the series ZSM 609/2003. Dorsally (A) the larva has a uniformly dark coloration, fading into lighter ventral color on the flanks as visible in lateral view (B). The oral apparatus (C) is generalized and the lower marginal papillae show a large medial gap.

1:2, about 45 labial teeth per mm; tooth rows in upper labium become continuously shorter from UTR_2 to UTR_6, UTR_2 first row that touches beak, LTR_1 shows short medial gap; jaw almost completely colored black, slightly serration at its sheaths; upper beak M-shaped, lower beak with a central V-shaped groove.

Discussion

Montane adaptations of tadpoles.—The three tadpoles described herein were collected in montane habitat, characterized by ericoid vegetation as typical for Malagasy mountains above 2000 m (Raxworthy and Nussbaum, 1996b) and by granitic rocks. Suitable breeding habitats for frogs available at these altitudes are mostly streams, which, in the Cuvette Boby, are in general slow-running water bodies. However, because of the large granite rocky surface areas, they show extreme fluctuations in water level and during heavy rainfalls can quickly become very large and fast-running. The tadpoles of *B. laurenti*, *M. madecassus*, and *M. elegans* were collected in such a high-altitude stream habitat, and they show adaptations to lotic conditions to a variable degree.

Boophis laurenti is almost certainly a close rel-

TABLE 3. MORPHOMETRIC MEASUREMENTS OF SEVEN TADPOLES OF *Boophis laurenti* IN STAGE 27 FROM THE ANDRINGITRA MASSIF (ZSM 610/2003 AND ZSM 611/2003). Tail measurements refer to 4–5 individuals only because of damaged tails in some specimens. All measurements in millimeters.

Character	Mean	SD	Minimum	Maximum
Body length	23.61	1.67	21.8	26.1
Tail length	37.45	3.34	33	40.0
Total length	61.4	5.13	55	65.7
Body width	12.51	1.04	11	14.2
Eye diameter	2.76	0.29	2.4	3.2
Interorbital distance	6.97	1.44	4	8.3
Internarial distance	3.81	0.29	3.4	4.2
Distance snout-naris	3.66	0.24	3.4	4
Distance naris-eye	3.2	0.27	2.9	3.7
Distance snout-spiraculum	13.16	0.57	12.4	13.9
Tail muscle height 1	6.6	0.93	5.6	7.9
Tail muscle height 2	4.38	0.73	3.5	5.5
Tail height	10.58	1.11	9.8	12.5

ative of *B. microtympanum*. In the adult stage, it has beige to light green dots on a dark brown dorsum, whereas *B. microtympanum* usually is olive green with brown vermiculations. Different calls have been described from the two species (Glaw and Vences, 1994), but the call of *B. microtympanum* is known to be highly variable. In January 2001, at the Andohariana plateau (approximately 2000 m a.s.l.), we observed several mixed couples in amplexus. However, at the Cuvette Boby, the tadpole collecting site, only *B. laurenti* has been found thus far, and the DNA sequences of the tadpoles agreed with *B. laurenti*. A more detailed genetic study analyzing various adult specimens with characteristics of *B. laurenti* and *B. microtympanum* is necessary to clarify whether these two taxa are merely color morphs of a single species or two closely related species that occur in syntopy. Independent from these considerations, the tadpole of *B. laurenti* as described herein agrees perfectly with the one of *B. microtympanum* from the Ankaratra Massif (Blommers-Schlösser, 1979b), corroborating their close relationships between these two forms. The relatively broad oral disc, anterior gap in marginal papillae, and relatively high number of labial tooth rows are characteristic for the ''clasping'' ecomorphological guild of McDiarmid and Altig (1999) and, thereby, typical for lotic waters. The relatively large size of *B. laurenti* tadpoles indicates that they might require at least one year to complete metamorphosis, as it is known for *Boophis microtympanum* and *Boophis williamsi* from the Ankaratra massif (Blommers-Schlösser, 1979b).

Mantidactylus elegans is a very poorly known species that apparently is restricted to montane habitat with granitic rocks and to rocky outcrops in high-altitude rain forest (Glaw and Vences, 1994). So far, no adult males of this species are known, whereas only a few adult females (part of the type series) have been found. Hence, nothing is known about the reproductive biology of this species, although Glaw and Vences (1994) report the finding of a large juvenile with remains of the tail and a snout–vent length of 34 mm. The enormous size of the tadpoles of *M. elegans* agrees with this record and indicates that they spend at least one year, possibly longer, in the water before metamorphosis.

Mantidactylus elegans has been included in the subgenus *Guibemantis* (Glaw and Vences, 1994), but its similarities to species of *Spinomantis* have been stressed (Andreone et al., 1998). The mouthparts of the *M. elegans* tadpole agree very well with those of the tadpoles of *Mantidactylus depressiceps* and *Mantidactylus tornieri* in the subgenus *Guibemantis*: seven upper and three lower labial tooth rows and lateral (rather than central) rows of submarginal papillae. In contrast, the tadpoles attributed to *Mantidactylus aglavei*, the only ones of the subgenus *Spinomantis* that so far have been described (Blommers-Schlösser, 1979a), are characterized by a tooth row formula of 2:1 + ½, or 2/2 which is unique in *Mantidactylus*.

Mantidactylus madecassus is a representative of the subgenus *Brygoomantis*. These semiaquatic mainly stream-breeding frogs are widespread over Madagascar (Blommers-Schlösser and Blanc, 1991). They are generally very abundant, and the various species are morphologically rather similar. Taxonomy within the subgenus is not well assessed, but the two montane species (*M. madecassus* and *Mantidactylus pauliani*) are easily recognized by their relatively small size

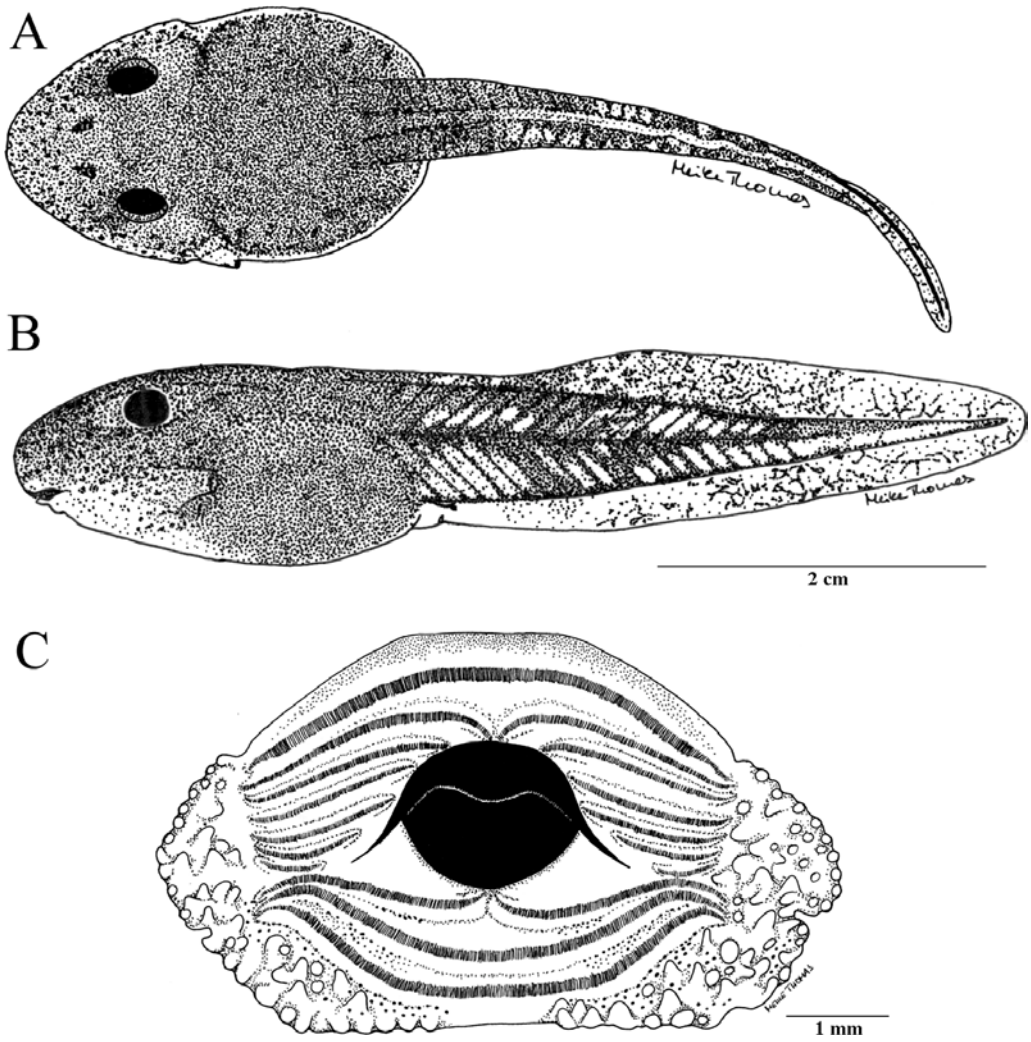

Fig. 3. Drawings of a tadpole of *Boophis laurenti* from the series ZSM 610/2003. The dorsum (A) shows a dark pigmentation, with diverse clustered darker spots to the lateral and anterior side; tail and the fins have an irregular pattern as visible in lateral view (B); the oral disc (C) is not laterally emarginated and is characterized by a large number of lateral papillae, the lower labium showing a medial gap of papillae.

and their extremely short snout (Vences and Glaw, 1999). Similar to other *Brygoomantis*, the tadpoles of *M. madecassus* have a weak expression of papillae and in the tooth row formula (Blommers-Schlösser, 1979a). This lack of apparent adaptations to running waters may indicate that they are restricted to still water in sidearms of the streams and pools at the stream margins.

One obvious character of all three tadpoles described herein is their generally dark coloration. This is especially remarkable for the larvae of *M. elegans*, which had an almost uniformly blackish tone. This further confirms a trend of prevalence of blackish color in montane tadpoles (Vences et al., 1998), which agrees with the frequent occurrence of melanism in adult amphibians and reptiles at high elevations. Whether the major function of this dark color is protection against ultraviolet radiation or improved thermoregulation (Vences et al., 2002b), or possibly even has a function in predator deterrence, needs further exploration.

Molecular identification of tadpoles.—The identification of tadpoles is a major step in the characterization of the biology of frogs. Except for cases in which eggs can be obtained from a mat-

ing couple of well-determined adults, or in which the coloration of the juveniles agrees with an unique adult pattern (Raharivololoniaina et al., 2003), tadpoles are usually identified by time-consuming captive rearing and tentative determination of juveniles. Especially in hyperdiverse tropical anuran communities, this bears the danger of misidentification. The many tadpoles that Blommers-Schlösser (1979a,b) described after painstaking captive rearing may to a large part have been misidentified because additional sibling species have been subsequently discovered at the same localities (e.g., Glaw and Vences, 1994). These cryptic taxa are very difficult to distinguish in the adult stage by morphology alone, and a reliable attribution of juveniles to any of these taxa is virtually impossible.

Herein, we used mitochondrial DNA sequences to identify tadpoles. For practical reasons we chose a fragment of the 16S rRNA gene, which already had been sequenced from many Malagasy frogs and can be very reliably amplified and sequenced. Mitochondrial sequences as ''DNA barcodes'' have also been proposed by Hebert et al. (2003), whereas Tautz et al. (2003) advocated the use of nuclear rDNA sequences for DNA taxonomy.

Tadpole identification through mitochondrial sequences has already been carried out several times (Malkmus and Kosuch, 2000; Ziegler, 2002). In the present work, we used this tool to identify tadpoles in a depauperate montane species assemblage from which only four species (*Anodonthyla montana*, *B. laurenti*, *M. madecassus*, and *M. elegans*) were known (Glaw and Vences, 1994). These species all belong to very distinct lineages of frogs, and hybridization or haplotype sharing among them is extremely unlikely. However, before mitochondrial identification of tadpoles can be applied at a large scale, and especially to anuran communities with a large number of closely related species, these factors need to be analyzed more in detail. The frequency of hybridization and haplotype sharing strongly differs among organisms (Avise, 2000) and, therefore, must be assessed for each group to which DNA taxonomy with mitochondrial sequences is to be applied. In cases in which these phenomena are exceptional, the identification via mitochondrial markers is usable. The identification of larval stages through fast comparison of mitochondrial sequences bears the potential of becoming one of the most important and most efficient applications of DNA barcoding.

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