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# Larval morphology and development of the Malagasy frog *Mantidactylus betsileanus*

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**Abstract.** The Mantellidae is a species-rich family of neobatrachian frogs endemic to Madagascar and Mayotte. Although tadpoles have been described from many mantellids, detailed studies of their early embryonic development are rare. We provide a documentation of the developmental stages of *Mantidactylus betsileanus*, a common mantellid frog of Madagascar's eastern rainforests, based on clutches deposited and raised in captivity. Metamorphosis was completed after 89 days on average. External gills were not recognizable in the embryos, similar to three other, previously studied mantellids, which apparently constitutes a difference to the mantellid sister group, the Rhacophoridae. We also provide updated descriptions of the species' larval morphology at stage 25 and stage 36, respectively, from captive bred and wild-caught individuals, and report variations in the keratodont row formula from 0/2, 1/1, 1/3 to 1:1+1/3.

Key words. Amphibia, Anura, Mantellidae, Madagascar, tadpole staging, developmental biology.

## Introduction

Tadpoles, the larval stages of anuran amphibians, often constitute a crucial component of numerous freshwater ecosystems (e.g., Connelly et al. 2008, Flecker et al. 1999, Kupferberg 1997, Ranvestel et al. 2004, Whiles et al. 2006), but compared to adult frogs, very limited information is available on the natural history of tadpoles (Wells 2007). In Madagascar, tadpole communities in rainforest streams can be particularly rich in species and individuals (Strauß et al. 2010, 2013), possibly due to the absence of fishes from many of these streams.

Tadpole communities offer a great potential to study general questions of ecology and evolution and to efficiently monitor Madagascar's frog diversity also outside the peak breeding activity (Vences et al. 2008), and the routine use of molecular identification tools has made these life history stages available to such research projects (Strauß et al. 2010).

Madagascar's native amphibian fauna consists of 292 nominal species plus more than 230 undescribed candidate species (Perl et al. submitted). All of these are frogs, as caecilians and salamanders are absent, and they belong to five independent endemic clades that colonized Madagascar during the Caenozoic (Crottini et al. 2012). As far as is known, the vast majority of these, if not all, have exotrophic (feeding) or endotrophic (non-feeding) larval stages, while true direct development most likely does not occur on the island (Randrianiaina et al. 2011a, b, 2012). The last years have seen large progress being made in the descriptive morphology of tadpoles, especially of the largest clade endemic to the Malagasy biogeographical region, the family Mantellidae (e.g., Altig & McDiarmid 2006, Grosjean et al. 2011a, b, Knoll et al. 2007, Randrianiaina et al. 2011a, b, Schmidt et al. 2009, Thomas et al. 2005), but these studies only referred to the external morphology of the free-swimming larval stages. In fact, knowledge on earlier embryonic and larval development in these frogs is scarce in general. The first descriptions and drawings of various embryonic stages were published by Arnoult (1966) for *Mantella aurantiaca* and by Blommers-Schlösser (1975) for *Guibemantis liber*. Later, these types of data were expanded by studies on a few additional species of *Blommersia, Guibemantis, Mantidactylus* and *Boophis* (Blommers-Schlösser 1979a, b), and by an account on the endotrophic (non-feeding) larvae of *Gephyromantis* (Randrianiaina et al. 2011b). Altig & McDiarmid (2007) published data on the clutches of several mantel-

lids and emphasised the importance of this information for comprehensively understanding the natural history of these frogs.

The embryonic and larval development of anurans is typically summarised according to generalised staging tables, in which the ontogenetic appearance of certain traits is used as an indicator for a larva having attained a certain stage. The staging table for the Neotropical toad *Incilius valliceps* by Gosner (1960) serves as a standard (e.g., Duellman & Trueb 1986, McDiarmid & Altig 1999), although for some specialized taxa, alternative schemes have since been proposed, such as the one by Del Pino & Escobar (1981) for endotrophic larva.

The subgenus *Brygoomantis* in the genus *Mantidactylus* contains a number of riparian and semi-aquatic mantellid frogs that are widespread in Madagascar's humid forests. While the taxonomy of some species in this clade is well understood, many cryptic taxa and undescribed lineages are known and require in-depth taxonomic revision (Vieites et al. 2009, Perl et al. submitted). The calls of most *Brygoomantis* are composed of pulsed notes of species-specific duration, arrangement and relatively low intensity, emitted during day and night from a usually concealed position next to water with a slow to moderately strong current (Glaw & Vences 2007). *Brygoomantis* are members of the subfamily Mantellinae, which is characterised by the presence of so-called femoral glands in males that produce volatile compounds, probably constituting sexual pheromones (Poth et al. 2012). Tadpoles of numerous species and candidate species in the subgenus have been described (Blommers-Schlösser 1979a, Thomas et al. 2005, Knoll et al. 2007, Schmidt et al. 2009), but so far, the embryonic development remains unexplored, except for a brief mention of the lack of external gills in embryos of one species, identified as *Mantidactylus ulcerosus,* by Blommers-Schlösser (1979a).

In this paper, we report on the embryonic and larval development of *Mantidactylus* (*Brygoomantis*) *betsileanus,* based on specimens bred and reared in captivity. We illustrate the developmental stages in this species by microscope photographs and compare their ontogeny with Gosner's (1960) standard account. In addition, we provide an extensive and updated tadpole description for this species on the basis of specimens collected in the wild and identified by means of DNA barcoding. To investigate the possible variation in tadpole morphology, we compare our results with the descriptions provided by Blommers-Schlösser (1979a) and Knoll et al. (2007).

## Materials and methods

### Captive breeding and rearing

Individuals of *Mantidactylus betsileanus* had been obtained from the pet trade in 2009, were since kept and bred at the Technische Universität (TU) Braunschweig (Brunswick), Germany, and from 2011, offspring from the Brunswick stock was kept and bred in the Amphibian Breeding Unit at Cologne Zoo, Germany. DNA sequences from the initial breeding stock confirmed that the frogs belonged to the lineage of *M. betsileanus* known from the Northern Central East region of Madagascar (roughly in the Moramanga region), where amphibians are regularly collected for the pet trade. The breeding groups also included a few additional specimens belonging to the North East mitochondrial lineage of *M. betsileanus,* but the majority of offspring almost certainly descended from the Northern Central East breeding stock.

At Cologne Zoo, the individuals were accommodated in the Amphibian Breeding Unit, in which room and terrarium temperatures were kept between 22–25°C. Water temperature ranged from 22–26°C, and water parameters were pH 8.3, carbonate hardness 2–4, total hardness 6–8, and conductivity 320 µS. About 20–25 adult frogs with an unknown sex ratio were each kept in three terraria of 60 × 45 × 40 cm (length × width × height). In order to maintain a constant humidity of 80–100%, the terraria were sprayed daily with rainwater for about 30 seconds with a manual pump sprayer. Illumination was provided by twin T5-fluorescent lamps (Osram FQ, 865 Lumilux, daylight: 24 Watt) for twelve hours in summer (April to September) and ten hours during winter (October to March). The terraria were each subdivided into a terrestrial and an aquatic part. The terrestrial part, 60 × 32 cm (L × W), consisted of a foam mat, 60 × 32 × 1 cm, on top of a light grid, 60 × 32 × 1 cm, and contained various live plants (*Ctenidium molluscum*, *Polystichum polyblepharum, Ficus pumila*). Rear and side walls of the terraria were covered with a dark synthetic material (Juwel® structured background) to emulate a more naturalistic environment. The bottom of the aquatic parts, 60 × 13 × 2–4 cm each, was covered with river sand and the water was exchanged partially as necessary, but at least once a week. To facilitate individual observations, each tadpole was housed in a small, perforated box, 10 × 8 × 4 cm (L × W × H), integrated in the aquatic part of the terraria, water depth inside the boxes was 2 cm. When the tadpoles had grown to a total length of about 20 mm, they were transferred to a Makrolon® box, 46 × 26 × 15 cm, with a water level of 10 cm. Just like the terraria, it was filled with well water and a constant influx and drain of water provided a continuous water exchange. Illumination was provided by a single T5-fluorescent lamp (Osram FQ, 865 Lumilux daylight: 54 Watt) and the photoperiod was equivalent to that of the terraria. After forelimbs emerged, the metamorphosing tadpoles were moved to portable terraria, 18 × 11 × 11 cm and 35 × 22 × 21 cm, both outfitted similar to the adult terraria.

At the TU Brunswick, individuals were kept in four terraria with sizes ranging from 60 × 30 × 30 cm to 80 × 40 × 40 cm at temperatures between 20 and 27°C. Each terrarium was sprayed daily with tap water. Adults, juveniles and tadpoles were kept together at densities of between 6 and 35 specimens and randomly assigned to the different terraria. The terraria were filled with tap water at levels ranging from 3–5.5 cm. The whole ground area was covered with cork sheets, H 2–5 cm, floating on the water. To enable ac-

cess to the water beneath, holes with an approximate diameter of 5 cm had been punched into the cork sheets. Shelter and hiding places were provided in the shape of pieces of bark or cork, stones, halved coconut shells and various plants such as silver vine (*Scindapsus aureus*), bromeliads of the genera *Vriesea* and *Neoregelia*. Water was exchanged when necessary, but at least once a month. To improve the water quality, dried leaves of sea almond (*Terminalia catappa*) were placed into the water. Illumination was provided by T5-fluorescent lights, and the photoperiod was twelve hours all year round.

Adults and juveniles at Cologne Zoo were fed three times a week with fruitflies (*Drosophila*) and small crickets (*Acheta domesticus*), fortified with mineral powder (Calca-Mineral® Pego). The tadpoles were fed three times a week with fish flakes (TetraMin®); they also grazed on the algae growing on the walls of the tank.

Feeding of adults and subadults at TU Brunswick took place every two to four days with fruitflies (*Drosophila*), buffalo worms (*Alphitobius diaperinus*), pea aphids (*Acyrthosiphon pisum*), bean weevils (*Bruchus quadrimaculatus*), and small crickets (*Acheta domesticus*), dusted with vitamin powder (Herpetal Amphib®). Tadpoles were fed with "Wels-Chips" (Sera®) and fish flakes (TetraMin®) as per the same feeding schedule.

### Data acquisition from captive-bred specimens

At Cologne Zoo, egg deposition and larval development were monitored from May 2012 until the end of October 2012. Documentation of tadpole development was conducted once a week during the first weeks upon discovery of a clutch and three times a week after hind limb buds were visible. Tadpoles were photographed from a ventral perspective on millimetre-scaled paper and measured to the nearest millimeter from snout to tail tip. Data for identification of morphological characteristics, as well as classification of developmental stages, was acquired from photographs of tadpoles, taken in glass vessels from ventral, lateral and dorsal views. Digital photographs were taken with an OLYMPUS E-600 (DG MACRO 105 mm 1:2.8 lens, SIGMA) and a Pentax K-30 camera (DG MACRO 100 mm 1:2.8 lens, SIGMA), respectively.

At TU Brunswick, data concerning egg deposition and larval development was recorded for seven weeks, from September 2012 until the end of October 2012. During specimen collection, the terraria were checked daily for new clutches. In order to achieve a complete documentation of developmental stages, one egg, or later hatchling, was euthanised with an overdose of MS222 every day, transferred into an Eppendorf tube and preserved in Roti®-Histofix 4% (acid free pH 7 – phosphate buffered 4% formaldehyde solution). Preserved eggs and hatchlings were photographed from a dorsal, ventral and lateral perspective for measuring of size and identification of developmental stage and characteristic morphological traits. All photographs were taken with a digital camera connected to a stereomicroscope (EMS-Zeiss, Discovery. V12 SteREO Zeiss). Pictures were taken and edited using Zeiss AxioVision Rel 4.8 (06-2009) software. The measuring of specimens from head to tail tip was conducted with the program's integrated measuring tool. If the mouthparts or spiracle were poorly visible, the specimens were stained with methylene blue.

All measurements of the captive-bred specimens were taken by a single person (S. Scheld). Mean and standard deviation were calculated for each measurement.

### Data acquisition from wild-caught specimens

Tadpoles were collected in the field and euthanised by immersion in chlorobutanol solution. A tissue sample from the tail musculature or fin of each tadpole was taken and preserved in 99% ethanol. All detailed morphological tadpole characterisations and drawings are based on one DNA voucher specimen (field number ZCMV 4664 – ZSM 1416/2007) and were done by the same person (R.D. Randrianiaina), whereas variation is described based on additional DNA voucher specimens. After tissue collection, all specimens were preserved in 5% formalin. Specimens were deposited in the Zoologische Staatssammlung München, Germany (ZSM). The tadpole used for the description was identified in the study of Strauß et al. (2010), using a DNA barcoding approach based on a fragment of the mitochondrial 16S rRNA gene, which is known to be sufficiently species-indicative among the species of Malagasy frogs (Thomas et al. 2005).

For detailed morphological examination, especially to identify developmental stages and assess characters of the oral disk, the preserved tadpole was stained slightly with methylene blue. Morphological description, measurements and drawings were executed on the basis of digital pictures of the preserved tadpoles taken with the stereomicroscope mentioned above, following the markers, terminology and definitions provided by McDiarmid & Altig (1999) and Randrianiaina et al. (2011a, b, 2012), except that we use the term keratodonts instead of labial teeth.

### Staging methodology and terminology

We follow the terminology of larval morphology suggested by McDiarmid & Altig (1999), Altig (2007) and Altig & McDiarmid (2007). Identification of morphological characteristics, as well as appraisal of developmental stages were conducted according to Gosner (1960), as reproduced in Duellman & Trueb (1986) and McDiarmid & Altig (1999). After hatching, the embryos are referred to as 'tadpoles'. Our specification of keratodont row formulae follows the scheme proposed by Altig & McDiarmid (1999). Tadpoles from Cologne Zoo could not be referred to specific clutches, as they were only discovered upon hatching, for which reason the ages of tadpoles from stage 25 onwards were calculated as follows: the mean age of the

individuals at stage 25 observed at the TU Brunswick is added to the number of days since the discovery of tadpoles from Cologne Zoo.

The following abbreviations are used: $A_1$ (first upper keratodont row), $A_2$ (second upper keratodont row), $A_{2gap}$ (medial gap in $A_2$), BH (maximum body height), BL (body length), BW (maximum body width), DF (dorsal fin height at mid-tail), DG (size of the dorsal gap devoid of marginal papillae), DMTH (distance of the point of maximum tail height from the tail-body junction), ED (eye diameter), EH (eye height – measured from the lower curve of the belly to the centre of the eye), HAB (height of the point where the axis of the tail myotomes contacts the body – measured from the lower curve of the belly), IND (internarial distance – measured from the centre of the nares), IOD (interorbital distance – measured between the centres of the eyes), JW (maximum jaw sheath width), LTRF (keratodont row formula), MCL (length of the medial convexity of the upper sheath), MP (marginal papillae), MTH (maximum tail height), ND (naris diameter), NH (naris height – measured from the lower curve of the belly to the centre of the naris), NP (naris–pupil distance), ODW (maximum oral disk width), $P_1$ (first lower keratodont row), $P_2$ (second lower keratodont row), $P_3$ (third lower keratodont row), RN (rostro–narial distance), SBH (distance between snout and the point of maximum body height), SBW (distance between snout and the point of maximum body width), SE (snout–eye distance), SH (spiracle height – measured from the lower curve of the belly to the centre of the spiracle), SL (spiracle length – measured between the visible edges), SMP (submarginal papillae), SS (snout–spiracle distance), TAL (tail length), TH (tail height at the beginning of the tail), THM (tail height at mid-tail), TL (total length), TMH (tail muscle height at the beginning of the tail), TMHM (tail muscle height at mid-tail), TMW (tail muscle width at the beginning of the tail), VF (ventral fin height at mid-tail), VL (vent–tube length).

## Results

### Pre-mating, egg deposition and larval development

Adult males appear to be highly territorial. They seem to preferably sit at least partially concealed, e.g., under a rock or piece of bark. Calling takes place from open spots and mainly during the night, but was also observed at other times of the day. No seasonality was noted in captivity. Females usually deposit their clutches under some or other structure, such as moss, leaves or a piece of bark (Figs 1A+B). The site of egg deposition is not necessarily close to water and it seems that females use different deposition sites. The males guard the clutches until the eggs start to lose their compact structure at stage 22 or later.

All clutches were exposed clumps of 3–26 eggs (averaging 11 eggs per clutch), with each (freshly laid) egg having an approximate diameter of 2.0 mm excluding the gelatinous capsule surrounding it; these gelatinous capsules fuse during development to form a single jelly mass in which the embryos develop (Figs 1A+B). Embryonic development was observed in the majority of the discovered eggs; only occasionally would some eggs grow mould and not develop any further. From time to time, single eggs or egg clutches were found in the aquatic parts of the terraria, but none of those eggs developed. During the observation period, twelve clutches in total were discovered, of which nine were found as eggs and three as hatched tadpoles.

Stages 1 to 8, as well as stage 12 were not observed in any of the collected specimens as a result of the clutches being discovered only several hours or even days after deposition. The earliest stage observed was stage 9, at which embryos were on average less than one day old. The animal and vegetal poles were clearly distinguishable by colour: the animal pole was grey-brownish and the vegetal pole light cream (Fig. 2A). Within the first day, the surface began to smoothen and only few cells were distinguishable (Fig. 2C). Stages 13, 15, 16 and 17 were not identified and are therefore not described herein. At stage 14, the embryos were on average one day old, and the neural folds started to develop and became distinguishable from the yolk sack, while the whole embryo was of a grey-brownish colour. In the region of the neural fold, where the head develops, the distance between the two folds increased while they formed a rounded tip (Fig. 2D).

After two days, embryos reached stage 18, at which somites became visible and the yolk sack began to become distinct from the embryo by turning lighter in colour (Fig. 2E). The embryos of *M. betsileanus* showed no gill buds at stage 19, which was also reached after approximately two days. However, somites became very prominent and the colouration of the yolk sack became increasingly lighter (Fig. 2F). As tail elongation began at stage 20, the embryos started to grow around the yolk sack, which was now assuming a whitish colour (Fig. 2G). The number of somites increased and two elevated streaks appeared along the dorsal side of the head, reaching from the future location of the eyes to the caudal part of the head. Dark pigmentation started to appear on the head, yolk sack and dorsal tail parts proximate to the head. In addition, first movements were detected when the gelatinous mass was touched. At stage 21, pigmentation on the head and yolk sack increased and also expanded farther onto the tail (Fig. 2H). The tail fins started to become distinguishable. Embryos were still coiled around the yolk sack, and the dorsally elevated ridges were still prominent (Fig. 2H). At stage 22, approximately seven days after oviposition, the embryos were no longer coiled around the yolk sack. The gelatinous layers surrounding the individual eggs melted into a mass in which the embryos moved about freely. The iris and pupils became apparent and the iris darkened. Dark brown pigmentation increased on the entire embryo; some (four out of sixteen) individuals now also showed early stages of iridescent pigmentation. The tail fins turned transparent and fin circulation began. On the cranial side of the yolk sack, tissue of the embryo began to overgrow the front of the yolk sack. The shape of the yolk sack altered from globular to oval (Fig. 2I). Entering stage 23, labia developed in

all embryos; in some individuals, jaw sheaths, and lower tooth rows were distinguishable, and papillae began to develop. The tail was still growing in length and increasing in height; fin circulation was visible in all embryos. The dark brown pigmentation was spreading further and iridescent pigmentation increased, now resembling the pigmentation typical for adult *M. betsileanus*. Even though pigmentation was increasing, blood vessels underneath transparent parts of the head and yolk sack became visible (Fig. 2J). About one-third of the embryos observed now left the jelly mass and moved into the water. Because no development of external gills, an important trait to distinguish stages 23 and 24, was observed in any of the tadpoles, stage 24 could not be documented. By the time the embryos reached stage 25, they had all moved into the water. Mouthpart structures with papillae, tooth rows and jaw sheaths, which darkened with increasing age, developed into the typical shape of a benthic tadpole (Fig. 2K). The yolk sack was completely atrophied by now and intestines became visible through the skin of the ventral body side. The dark body pigmentation increased and light, iridescent pigmentation on the iris began to develop (Figs 1C and 4A+C).

At stage 26, at a minimum age of 24 days, hind limb buds were visible (Fig. 3A) and kept increasing in length and volume until developing the foot paddle at stage 31 after approximately 34 days. The age of tadpoles at stages 26–31 ranged between 24 and 54 days. During stage 30, the appearance of dark pigmentation on the hind limbs was first documented in one individual. At stage 31, blood vessels on the outlines of the foot paddle were discernible in some (four out of eleven) of the tadpoles (Fig. 3F). At stage 32, the number of blood vessels visible on the hind limbs increased in all individuals (Fig. 3G). During stage 33, dark hind limb pigmentation began to show in all individu-

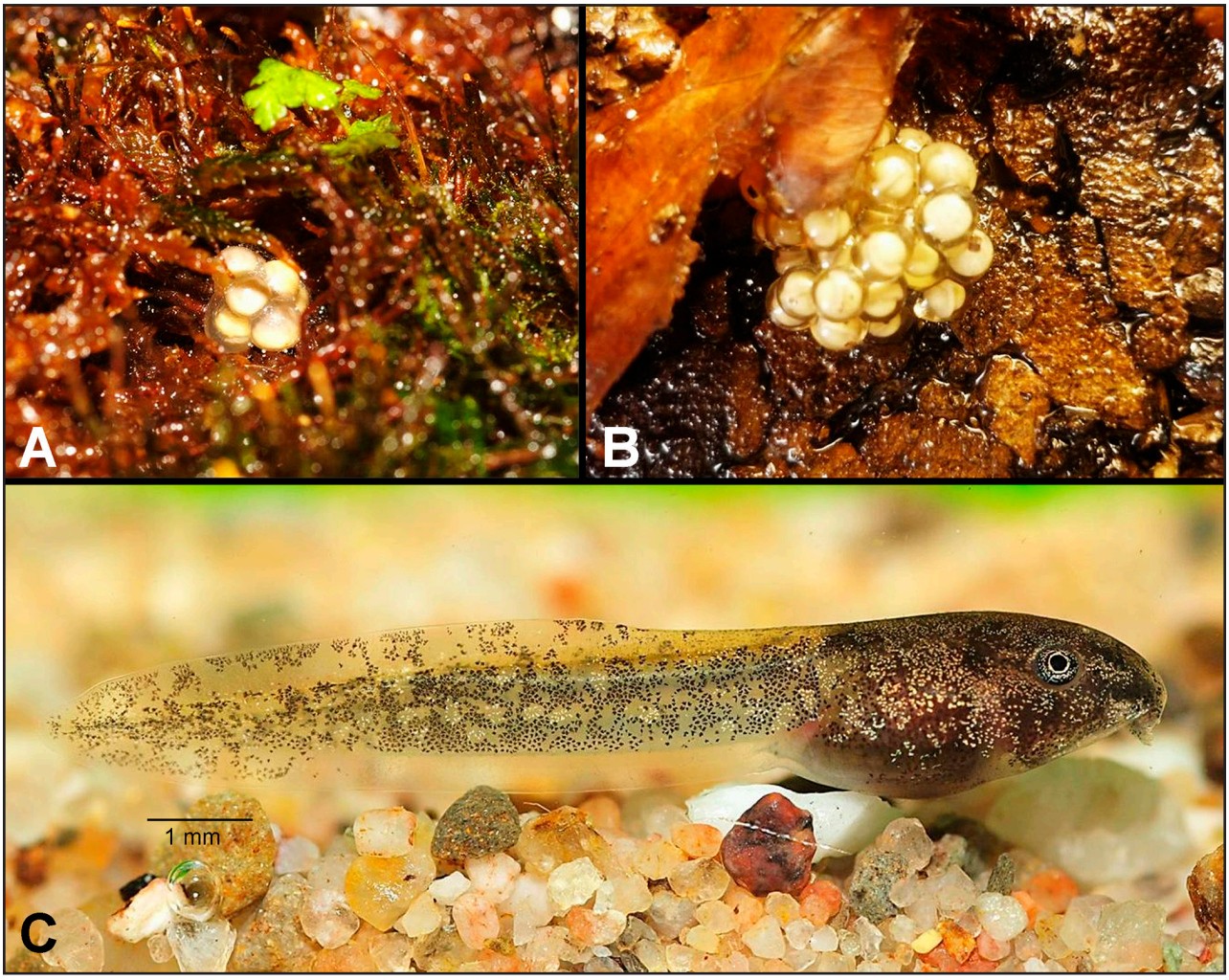


Figure 1. Pictures of a live tadpole of *Mantidactylus betsileanus* at stage 25 and two clutches at different oviposition sites. A) eggs of clutch number four, found inside a moss pit; B) eggs of clutch number three discovered beneath a leaf, embryos are already distinguishable from their yolk sacks; C) lateral view, picture taken on 03 June 2012.

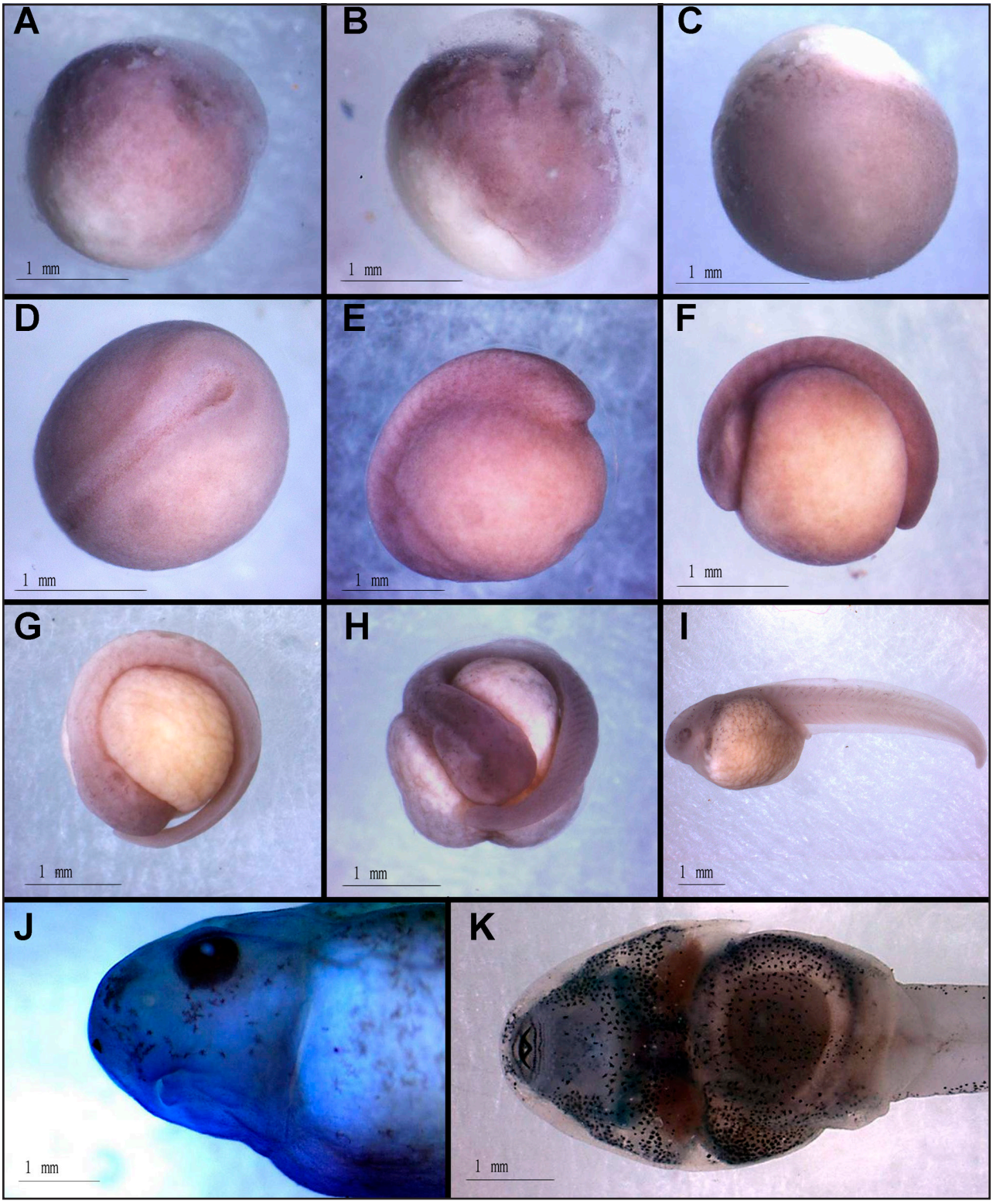


Figure 2. Embryos of *Mantidactylus betsileanus* at stages 9 to 25. A: stage 9, preserved on 18 October 2012; B: stage 10, preserved on 18 October 2012; C: stage 11, preserved on 19 October 2012; D: stage 14, preserved on 19 October 2012; E: lateral view of an embryo at stage 18, preserved on 24 October 2012; F: lateral view of an embryo at stage 19, preserved on 20 October 2012; G: lateral view of an embryo at stage 20, preserved on 27 September 2012; H: frontal view of an embryo at stage 21, preserved on 22 October 2012; I: lateral view of an embryo at stage 22, preserved on 23 October 2012; J: close up lateral view of an embryo at stage 23, preserved on 26 October 2012; K: close up ventral view of a tadpole at stage 25, preserved on 28 October 2012.

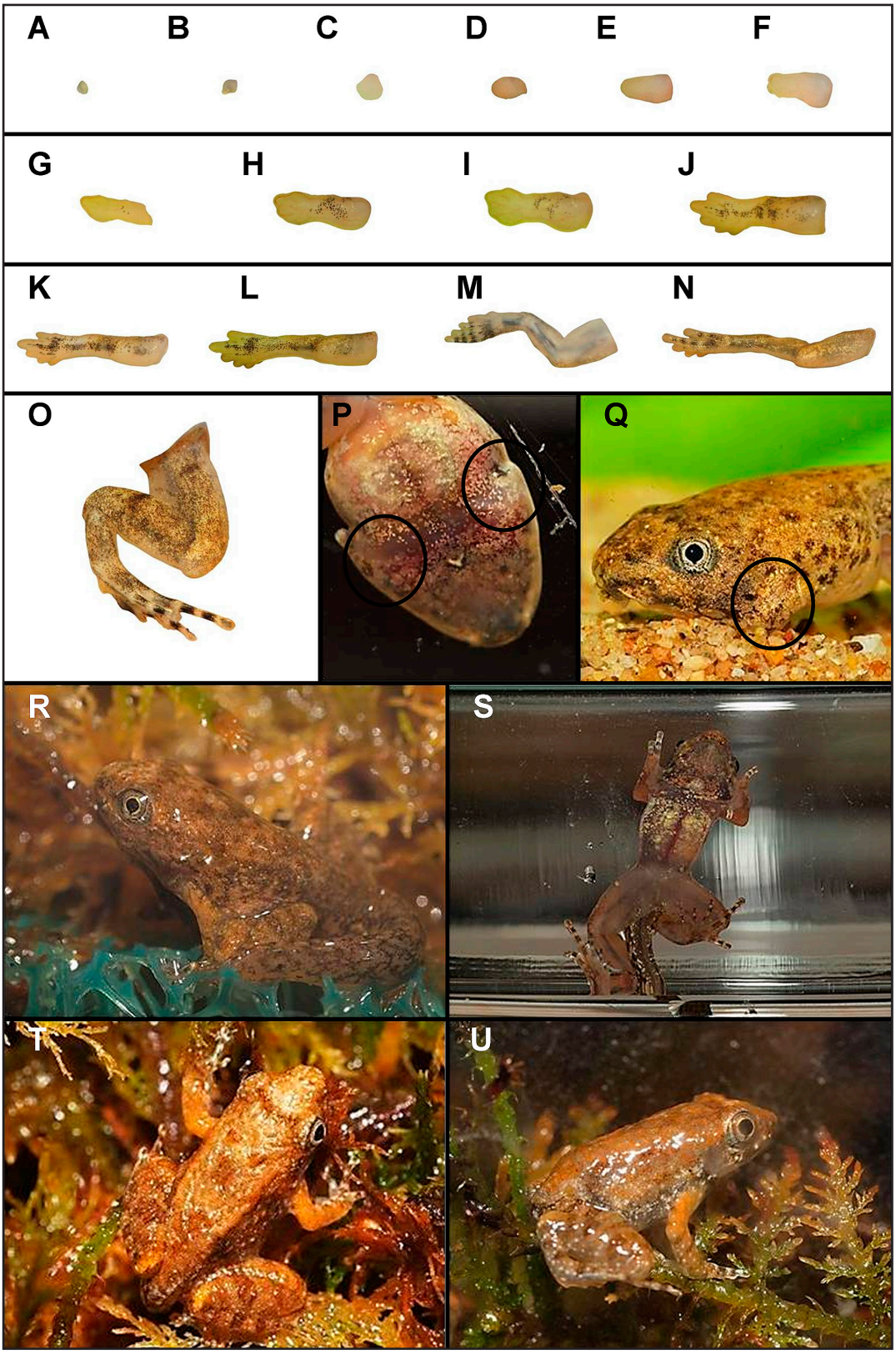


Figure 3. Overview of hind limb development in tadpoles of *Mantidactylus betsileanus*. A: stage 26; B: stage 27; C: stage 28; D: stage 29; E: stage 30; F: stage 31; G: stage 32; H: stage 33; I: stage 34; J: stage 35; K: stage 36; L: stage 37; M: stage 38; N: stage 39; O: stage 40; P: stage 41; Q: stage 42; R: stage 43; S: stage 44; T: stage 45; U: stage 46; sizes of hind limbs are not to scale.

als, with two out of four individuals also developing light brown pigments (Fig. 3H). At stage 34, iridescent pigmentation on the hind limbs appeared in five out of seven individuals (Fig. 3I). During stage 35, blood vessels were now also visible in the toes, and the knee joint became discernible (Fig. 3J). Reaching stage 36, the pigmentation expanded onto the toes (Fig. 3K). All toes were separated after an average of 66 days, during which the individual lengths of the toes increased, the femur became more elongated, and the hind limbs grew thinner (Fig. 3L). During stage 38, the tadpoles began to hold their hind limbs in a bent configuration. Furthermore, light brown pigmentation on the hind limbs was increasing and dark cross bands appeared on the hind limbs (Fig. 3M). By the time the development of the hind limbs was completed at stage 40, this banded pattern had extended over the whole hind limbs in all individuals (Fig. 3O), and the iris was fully pigmented. At stage 41, forelimbs started to develop, now visible ventrally through the skin and laterally as bumps (Fig. 3P). Mouthparts were still prominent, but the vent tube was already reduced in most of the tadpoles. On reaching stage 42, the forelimbs had fully emerged and the tadpoles began to respond with their typical behaviour to being touched, reminiscent of feigning death. Atrophy of the mouthparts began to show, and the number of marginal papillae decreased (Fig. 3Q). Mouthparts were completely atrophied after 85–93 days (stage 43), and the corners of the mouth were now situated below the nostrils and eyes (Fig. 3R). The pigmentation of the skin changed to a more reddish colour, and the inner organs were no longer visible through the skin (Fig. 3R). The tail began to atrophy and became equal in length to the body of the tadpole. In addition, the tadpoles had begun to explore the terrestrial parts of the terraria and showed the typical posture of an adult frog. During stage 44, tail length decreased further (Fig. 3S). At stage 45, only a tail stub was left and the tadpoles had nearly the same physical appearance as an adult frog, including the typical granular surface of the skin (Fig. 3T). Furthermore, they now lived mainly on the terrestrial parts of their terraria and started to prey upon *Drosophila*. On average, our tadpoles had passed through metamorphosis and reached stage 46 eighty-nine days after oviposition. The tail was completely resorbed and the froglets had adapted to the terrestrial life of the adults (Fig. 3U).

## Larval description based on captive-bred specimens

The following larval description is based on one tadpole at stage 25 preserved on 27 October 2012; variation is described based on eight tadpoles of the same clutch and stage, preserved successively on 20–28 October 2012.

In dorsal view, the body of the tadpole is of elliptical shape, with a pointed snout, and the total length is 17.1 mm with the maximum body width at the level of the base of the spiracle (SBW 50% of BL) (Fig. 4A). Nares small, positioned dorsally, proximate to the snout rather than the eyes (RN 50% of NP) and a moderately wide distance between the nares (IND 60% of IOD) (Figs 4A+C). Eyes are of medium size (ED 16% of BL), situated dorsally in the first third of the body (SE 28% of BL) and directed dorsolaterally; a moderately wide distance between the eyes (IOD 50% of BW) (Figs 4A+C). Two elevated ridges along the body from the tail-body junction to the eyes (Fig. 4D).

In lateral view, body of tadpole depressed (BW 142% of BH), maximum body height after second third of body length (SBH 60% of BL) (Fig. 4C). Spiracle located sinistrally, visible in both dorsal and ventral view, its base situated ventrolaterally in the second third of the body, its oval opening ending dorsolaterally rather than ventrolaterally (SH 67% of BH) proximate to the last third of the body (SS 57% of BL), oriented posteriorly (Figs 4A–C). Vent tube positioned at the ventral tail-body junction, adherent to lower tail fin. Tail of medium length (TAL 249% of BL), maximum tail height is higher than maximum body height (MTH 110% of BH), tail musculature fairly moderately developed (TMW 43% of BW, TMH 71% of BH and 65% of MTH), gradually tapering towards the end of the tail, not reaching tail tip; tail tip rounded, myotomes of the tail musculature V-shaped (Figs 1C & 4D+C). Tail fins approximately one fifth of MTH; ventral tail fin beginning at base of the tail, dorsal fin at second fifth of tail, increasing in height towards tail tip (Fig. 1C).

Oral disk anteroventrally positioned, of moderate size (ODW 30% of BW), generalised and emarginated; marginal papillae on upper labium with a large medial gap (DG 60% of ODW), five marginal papillae and two submarginal papillae on each side, lower labium with 23 marginal papillae and 14 submarginal papillae, all papillae less than 0.2 mm in length each, with rounded tips; jaw sheaths black and serrated, upper jaw sheath M-shaped, lower jaw sheath V-shaped with elongated rounded ends; LTRF 2(2)/3, keratodont density about 42 per mm, keratodont row length reduced drastically from a very long $A_1$ row to a shorter $A_2$ with a large $A_{2gap}$ ($A_{2gap}$ 30% of $A_2$) and from $P_1$ to $P_3$ only slightly (Figs 4E+F).

Colour in preservative: tan, whole body slightly transparent (Figs 4A–C). Dorsal parts of body covered with dense, irregular, dark brown dots and small, more regularly scattered iridescent pigment, creating a patchy pattern of different shades of brown (Fig. 4A). Ventral side fairly densely pigmented with irregular dark brown dots and iridescent pigments (Fig. 4B). Skin near mouthparts and vent tube nearly free of pigmentation (Figs 4B–F). The whole ventral side slightly transparent and intestines visible (Fig. 4B). Lateral parts with less dense pigmentation than dorsal side but denser than on the ventral side (Fig. 4C). Pigments as described above, but with the density of the iridescent pigmentation decreasing towards the ventral side (Fig. 4C). Few iridescent pigments visible on eyes (Fig. 4C). Posterior part of the tail, tail muscle and fins irregularly patterned with dark brown dots and iridescent pigments, concentrated in larger patches (Fig. 4D). No pigmentation on the cranial ventral and lower lateral sides, where the skin is transparent with main blood vessels being visible (Fig. 4D).

Colour in life of captive-bred specimens: The following description is based on one live tadpole (picture taken on 03 June 2012, age: 11 days) (Fig. 1C): whole body slightly transparent, predominant colour of dorsal portion is tan, created by many small dark brown pigments that are densely overlain with irregular iridescent pigments. Iridescent pigmentation decreasing from dorsal towards ventral side of the body. Dark brown pigmentation reduced on ventral side with only very few iridescent pigments; skin more clearly transparent than on the rest of the body and intestines visible. Vent tube apparently free of pigmentation. Dense iridescent pigmentation of the eyes. Dorsal fins and tail muscle densely dotted with dark brown, interrupted by several unpigmented patches, especially on the fins. Iridescent pigmentation concentrated in a few larger patches scattered between the dark brown areas. Last third of ventral fins covered with a few dark brown pigment dots, while the rest of the ventral fins is transparent with neither pigmentation nor blood vessels visible.

Variation (9 tadpoles; measurements in mm): All specimens of the same colouration; in some, the pigmentation on the ventral side of the body is more dense than in others, as is the iridescent pigmentation in the eyes; BL 4.5 ± 0.4; TAL 11.4 ± 0.4; TL 16.0 ± 0.7; BW 2.6 ± 0.3; ED 0.5 ± 0.1; IOD 1.4 ± 0.2; IND 0.9 ± 0.2; TMW 1.1 ± 0.2; BH 1.9 ± 0.1; TMH 1.4 ± 0.1; MTH 2.1 ± 0.2; ODW 0.8 ± 0.1; MP and SMP length 0.1 ± 0.0; LTRF: 0/2, 1/1, 1/2, 1/3, 2(2)/3; density of keratodonts: 0 to 42 per mm; position, general morphological traits, and oral disk only vary in size.

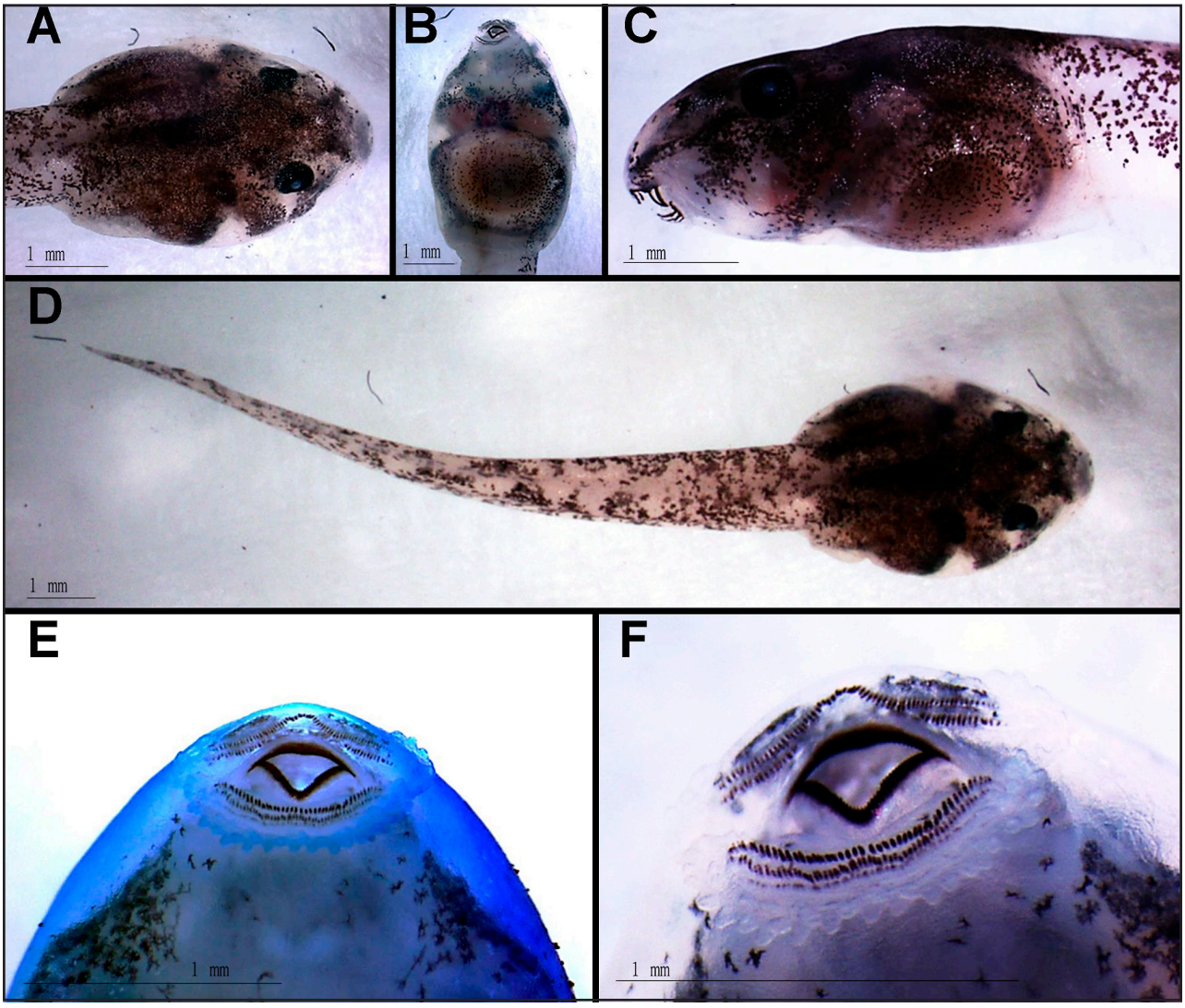


Figure 4. Preserved captive-bred tadpole of *Mantidactylus betsileanus* at stage 25. Tadpole preserved on 27 October 2012; A: close-up dorsal view; B: close-up lateral view; C: close-up ventral view; D: overall dorsal view, with the following measurements in mm: BL 4.9; TAL 12.2; TL 17.1; BW 3.0; IOD 1.5; IND 0.9; TMW 1.3; E + F: close-up ventral view on the oral apparatus (E: dyed with methylene blue; F: natural colour), with the following measurements in mm: ODW 0.9; MP & SM length 0.2.

Larval description based on wild-caught specimens

The following description refers to one tadpole at developmental stage 36 (field number ZCMV 4664 – ZSM 1416/2007; BL 10.6 mm, TL 35.8 mm) from Ambatovaky in the Ranomafana National Park. The 16S rDNA sequence of this specimen (GU975167) was 100% identical to reference sequences of adult specimens of *Mantidactylus betsileanus* from the Ranomafana area (see Strauß et al. 2010).

In dorsal view, body elliptical, maximum body width at between 2/5 and 3/5 of body length (SBW 55% of BL), snout narrow and rounded (Fig. 5A). In lateral view, body depressed (BW 156% of BH), maximum body height at between 3/5 and 4/5 of body length (SBH 76% of BL), snout narrow and rounded (Fig. 5C). Eyes moderately large (ED 12% of BL), not visible in ventral view, positioned high dorsally (EH 76% of BH) and directed dorsolaterally, situated between 3/10 and 4/10 of the body length (SE 33% of BL), distance between eyes moderately wide (IOD 59% of BW) (Figs 5A–C). Nares moderately large and rounded (ND 2% of BL), with a marginal rim, positioned high dorsally (NH 70% of BH) and oriented anterolaterally, situated closer to snout than to eyes (RN 88% of NP) and lower than eyes (NH 92% of EH), distance between nares moderately wide (IND 59% of IOD), dark spot posterior to the nares absent, ornamentation absent (Figs 5A+C). Spiracle short, sinistral (SL 14% of BL), directed posterodorsally, visible in dorsal and ventral views, and obvious in lateral view; inner wall detached from body and formed so that its aperture opens laterally instead of posteriorly, opening rounded, situated between 3/5 and 4/5 of the tail length (SS 65% of BL), located high on the body (SH 61% of BH) at the height of the point where the axis of the tail myotomes contacts the body (SH 97% of HAB) (Figs 5A–C). Vent tube moderately long, dextral, inner wall absent (VL 13% of BL), attached to ventral fin. No glands. Tail moderately long (TAL 239% of BL), maximum tail height higher than body height (MTH 120% of BH), tail height at mid-tail higher than body height and nearly as high as maximum tail height (THM 116% of BH and THM 97% of MTH), tail height at the beginning of the tail higher than body height (TH 105% of BH) (Figs 5D+E). Caudal musculature moderately developed (TMW 49% of

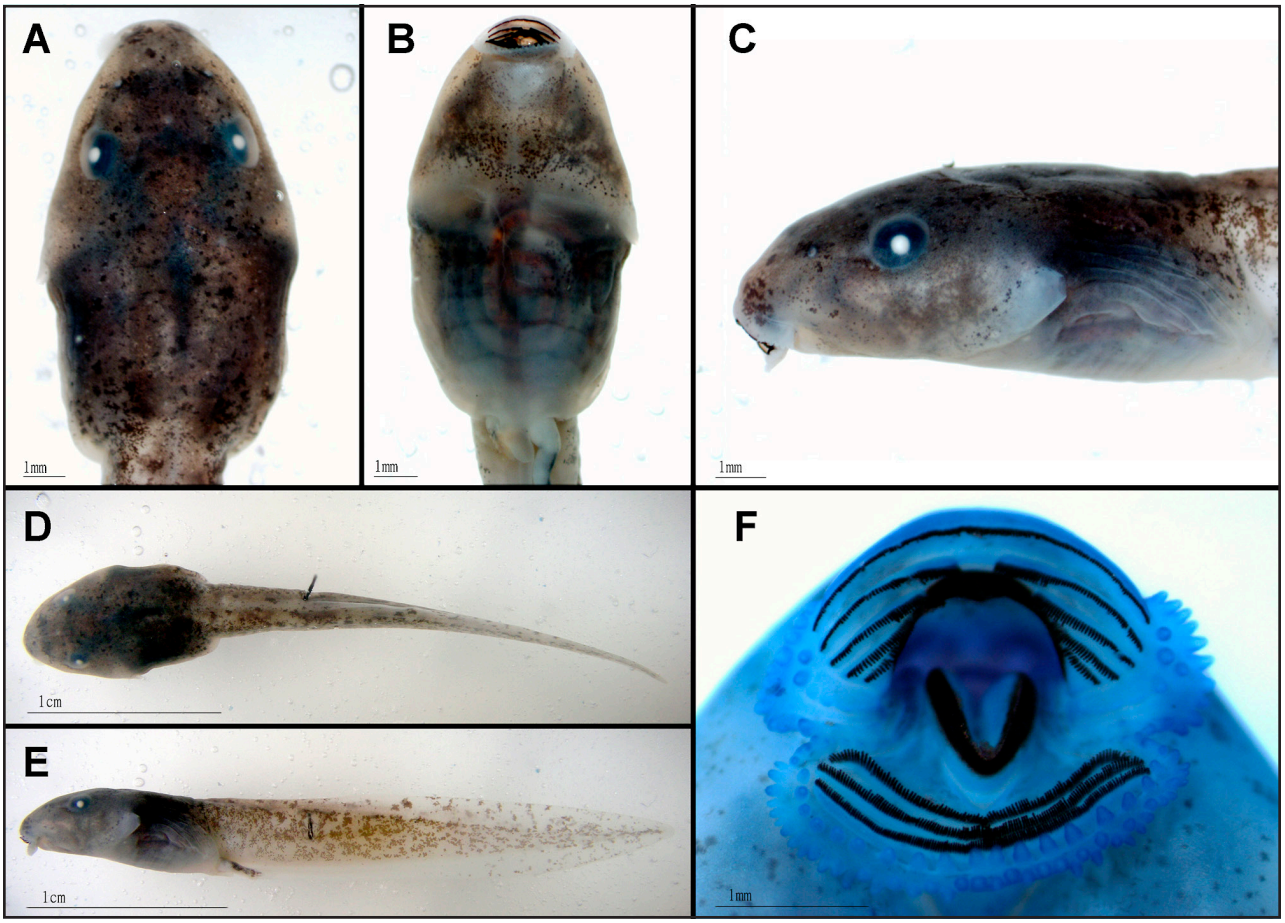


Figure 5. Close-ups and an overall lateral view of a preserved wild-caught tadpole of *Mantidactylus betsileanus* at stage 36 (Field number ZCMV 4664 – ZSM 1416/2007). A: close-up dorsal view; B: close-up ventral view; C: close-up lateral view; D: overall dorsal view; E: overall lateral view; F: close-up ventral view of the oral apparatus, stained with methylene blue.

Table 1. Description of developmental stages 1 to 17 of captive-bred *Mantidactylus betsileanus*. Stage = stages according to Gosner (1960); n = number of individuals in the corresponding Gosner (1960) stage; Age [d] = age in days; Diameter embryo [mm] = diameter of embryo (M ± SD) in mm; Notes = observed characteristics per stage, diagnostic traits for each stage according to Gosner (1960) are italicised; – = no data available; * = characteristic trait according to Gosner (1960), not observed in any individuals. Descriptions are based on specimens from different clutches.

| Stage | n | Age [d] | Diameter of embryo [mm] | Notes |
|---|---|---|---|---|
| 1–8 | – | – | – | *Fertilisation** (stage 1)*; grey crescent** (stage 2)*; 2-cell** (stage 3)*; 4-cell** (stage 4)*; 8-cell** (stage 5)*; 16-cell** (stage 6)*; 32 cell** (stage 7)*; mid-cleavage** (stage 8)*;* no individuals found at these stages |
| 9 | 2 | 0 | 2.0 < 0.0 | *Late cleavage;* animal pole grey-brownish, vegetal pole light cream coloured; surface rough, with multiple defined cells visible; egg diameter including gelatinous mass: 6.5 ± 2.2 mm |
| 10 | 2 | 1 | 2.1 < 0.0 | *Dorsal lip*; surface rough, with multiple defined cells visible; egg diameter including gelatinous mass: 6.5 ± 2.2 mm |
| 11 | 2 | 0 | 2.1 < 0.0 | *Yolk plug*; surface smoothening, only a few defined cells distinguishable; animal pole extending to vegetal pole; egg diameter including gelatinous mass: 5.0 ± 0.9 mm |
| 12 | – | – | – | *Late gastrula*;* no individuals found at this stage |
| 13 | – | – | – | *Neural plate**; no individuals found at this stage |
| 14 | 4 | 1 | 2.1 < 0.0 | *Neural fold;* neural folds distinguishable from yolk sack, forming a rounded tip where the head will develop; embryo uniformly grey-brownish; egg diameter including gelatinous mass: 4.4 ± 0.6 mm |
| 15–17 | – | – | – | *Rotation*; elongation** (stage 15); *neural tube*; gill plates** (stage 16); *tail bud*; adhesive gland** (stage 17); no individuals found at these stages |

BW, TMH 74% of BH, TMH 70% of TH and 62% of MTH, TMHM 47% of THM and 45% of MTH). Tail muscle reaches tail tip. Very low fins (DF 62% of TMHM, VF 55% of TMHM), dorsal fin higher than ventral fin (DF 113% of VF) at mid-tail (Fig. 5E). Dorsal fin begins at the dorsal body–tail junction, increases regularly to maximum tail height, and then continues at a constant height to mid-tail where it starts to decline towards the tail tip (Fig. 5E). Ventral fin begins at the ventral terminus of the body, extends slowly to the 3/4 of the tail length, and then gradually decreases in height towards the tail tip (Fig. 5E). Maximum tail height at between 1/5 and 2/5 of tail length (DMTH 35% of TAL), lateral tail vein subtle and myosepta recognizable in the anterior half of the tail musculature, at the point where the axis of the tail myotomes contacts the body in the upper half of the body (HAB 63% of BH), axis of the tail myotomes parallel to the long axis of the body. Tail tip narrow, rounded (Fig. 5E). Moderately wide, generalised oral disk (ODW 44% of BW), positioned ventrally and directed anteroventrally, emarginated, maximum width across the upper labia (Figs 5C+F). Oral disk visible in dorsal view; the upper labium is a continuation of the snout (Fig. 5A). Single row of marginal papillae interrupted by a very wide gap in the upper labium (DG 66% of ODW), gap absent in the lower labium, total number of marginal papillae 72 (Fig. 5F). Twenty-three submarginal papillae, positioned ventrally and laterally on the lower, and laterally on the upper labium (Fig. 5F). Papillae short, large, conical, with rounded tipa; the longest marginal and submarginal papillae both measure 0.14 mm (Fig. 5F). LTRF 5(2–5)/2(1) (formula after Altig & McDiarmid 1999). A single row of keratodonts per ridge (Fig. 5F). $A_1$ row very long (81% of ODW). Density of keratodonts varies from 45 to 68/mm, density on $A_1$ 65/mm (total 149). Gap in the first anterior interrupted row very narrow ($A_{2gap}$ 10% of $A_2$) (Fig. 5F). Row alignment regular. Keratodonts short (0.09 mm) but discernible, with distal keratodonts being shorter than those in the middle; distinct space between marginal papillae and keratodont rows (Fig. 5F). Partially keratinised jaw sheath; only the half section close to the edge is black in colour and the remainder whitish; finely pointed serrations; narrow jaw sheath (JW 28% of ODW) with a very short, narrow, rounded (MCL 4% of JW), medial convexity on the upper sheath (Fig. 5F). Lower jaw sheath V-shaped, partially keratinised and partially hidden by the upper jaw sheath (Fig. 5F).

## Discussion

Overall, the larval development of *M. betsileanus* corresponded at stages 9, 10, 11 and 14 observed in this study with the characteristic developmental traits proposed by Gosner (1960) based on *Incilius valliceps*.

Muscular response, observed during specimen collection, was only detected as early as at stage 20 instead of stage 18 (Gosner 1960). Heartbeats were not observed in any embryos because we did not examine live specimens. A

Table 2. Description of developmental stages 18 to 46 of captive-bred *Mantidactylus betsileanus*. Stage = stages according to Gosner (1960); n = number of individuals at the corresponding stage; Age [d] = age in days; TL [mm] = total length (M ± SD) in mm; Notes = observed characteristics per stage, diagnostic characteristics according to Gosner (1960) are italicised. Descriptions are based on specimens from different clutches.

| Stage | n | Age [d] | TL [mm] | Notes |
|---|---|---|---|---|
| 18 | 1 | 2 | 3.9 ± 0.0 | *Muscular response*; olfactory pits visible**; somites (13 ± 1) visible; colour of yolk sack lighter; egg diameter including gelatinous mass: 5.4 ± 0 mm |
| 19 | 2 | 2 | 4.1 ± 0.0 | *Heart beat*; gill buds*;* somites very distinctive (12); colour of yolk sack becoming increasingly whiter; egg diameter including gelatinous mass: 4.6 ± 0 mm |
| 20 | 11 | 2–6 | 5.4 ± 0.9 | *Gill circulation**; *tail elongation*; embryo growing around the yolk sack; number of visible somites increasing (12–18); two elevated ridges along dorsal side of head; dark pigmentation appearing (n = 2); first movements; egg diameter including gelatinous mass: 5.9 ± 1.4 mm; diameter of yolk sack: 1.9 ± 0.3 mm |
| 21 | 7 | 3–13 | 6.6 ± 1.8 | *Cornea transparent; mouth opens*; increase in pigmentation (n = 5); tail fins distinguishable (n = 2); embryos still coiled around yolk sack (n = 10); egg diameter including gelatinous mass: 4.8 ± 0.4 mm; diameter of yolk sack: 1.9 ± 0.1 mm |
| 22 | 16 | 5–11 | 8.3 ± 1.6 | *Tail fins transparent, fin circulation* (n = 12); no longer coiled around yolk sack (n = 15); gelatinous layer melted into mass with gummy surface, in which embryos move freely; iris and pupils distinguishable, iris darkening; early signs of iridescent pigmentation (n = 4); tissue of embryo overgrowing cranial side of the yolk sack, yolk sack oval; width of yolk sack: 1.9 ± 0.4 mm |
| 23 | 14 | 6–12 | 10.3 ± 2.7 | *Operculum covers gill bases*; labia and teeth differentiate;* jaw sheaths distinguishable (n = 3), development of lower tooth rows and papillae (n = 2); fin circulation completed, tails increasing in height; pigmentation resembling typical larval pigmentation of *M. betsileanus*; blood vessels on head and yolk sack visible; embryos hatch into water (n = 5); width of yolk sack: 1.6 ± 0.4 mm |
| 24 | 10 | – | – | *External gills atrophy*; operculum closes on the right*;* no individuals found at this stage |
| 25 | 27 | 13–45 | 15.5 ± 2.2 | *Mouthparts obvious; spiracle forms on left;* all embryos in the water; yolk sack completely atrophied; intestines visible; appearance of coloured iris pigmentation |
| 26 | 2 | 2–54 | 22.0 ± 3.5 | *Hind limb buds discernible (L < ½ D);* dorsal elevations gone |
| 27 | 4 | 24–44 | 20.9 ± 1.0 | *Hind limb buds increasing in size (L > ½ D)* |
| 28 | 3 | 24–44 | 23.5 ± 1.5 | *Hind limb buds increasing in size (L > D)* |
| 29 | 13 | 31–36 | 25.0 ± 1.4 | *Hind limb buds increasing in size (L > 1½ D)* |
| 30 | 7 | 34–46 | 26.4 ± 1.7 | *Hind limb buds increasing in size (L > 2 D)*; appearance of dark pigmentation on hind limbs (n = 1) |
| 31 | 11 | 34–54 | 29.6 ± 1.8 | *Foot paddle develops;* appearance of pigmentation on foot paddle; blood vessels visible on foot paddle |
| 32 | 3 | 40–52 | 29.7 ± 0.8 | *Indentation between toes 4 and 5;* visible blood vessels on hind limbs increasing in number |
| 33 | 4 | 43–57 | 31.3 ± 1.5 | *Indentation between toes 3 and 4;* beginning and expansion of pigmentation, dark and light brown, on hind limbs |
| 34 | 7 | 46–60 | 30.8 ± 3.6 | *Indentation between toes 2 and 3;* development of iridescent pigments on hind limbs |
| 35 | 14 | 46–66 | 34.1 ± 2.8 | *Indentation between toes 1 and 2;* blood vessels in toes also visible; knee joint discernible |
| 36 | 12 | 55–89 | 34.4 ± 2.8 | *Toes 3–5 separated*; pigmentation expanding onto toes |
| 37 | 17 | 52–88 | 37.1 ± 1.8 | *All toes separated*; femur elongating; individual lengths of toes increasing; hind limbs thinning and elongating |
| 38 | 4 | 64–70 | 38.5 ± 1.7 | *Metatarsal tubercle*; hind limbs bent; light brown pigmentation on hind limbs increasing; striped pattern distinguishable |
| 39 | 19 | 61–88 | 39.1 ± 1.7 | Subarticular patches |
| 40 | 16 | 64–87 | 39.1 ± 1.9 | *Foot tubercle; vent tube present;* striped pattern on whole hind limb; iris fully pigmented |
| 41 | 19 | 68–89 | 38.7 ± 1.3 | *Mouthparts atrophy*; forelimbs visible; vent tube gone* (n = 18); mouthparts still prominent |
| 42 | 12 | 71–95 | 38.6 ± 3.0 | *Mouth corners anterior to the nostrils*; forelimbs emerge;* right forelimb emerging first (n = 12); typical stress posture, feign death when touched; marginal papillae decreasing in number |
| 43 | 3 | 85–93 | 34.3 ± 2.5 | *Mouth corners beneath nostril and eye; tail atrophies;* mouthparts completely resorbed; pigmentation changing slightly towards red/orange in colour, skin seems thicker; typical posture of adults; exploring terrestrial parts |
| 44 | 2 | 78–80 | 28.0 ± 7.8 | *Mouth corners beneath eye; tail greatly reduced* |
| 45 | 4 | 91–93 | – | *Mouth corners posterior to eye; tail stub;* physical appearance resembles adults; terrestrial lifestyle; preying on *Drosophila* flies |
| 46 | 5 | 89 | – | *Tail resorbed; metamorphosis complete* |

major deviation from the classical Gosner (1960) classification was observed during stages 18 to 25. Embryos at those stages showed no development of external gills, a phenomenon previously described and known from other mantellid tadpoles, too (Blommers-Schlösser 1975, 1979a).

While the early tadpole stages of frogs of most of the major clades (families) have external gills, a strong reduction or absence of external gills is found in at least one very basal taxon, *Ascaphus truei* (Ascaphidae), as well as in some derived neobatrachians, such as the direct-developing *Oreobates barituensis* (Strabomantidae) (Goldberg et al. 2012, Noble & Putnam 1931). Warkentin (2000) experimentally observed the regression of external gills during the development of *Agalychnis callidryas* and suggested that the ontogenetic reduction of external gills in this species may be due to an increased exposure to oxygen (Warkentin 2000). However, in contrast to the natural oviposition sites of, e.g., *Ascaphus truei*, namely cold fast-flowing water, those of *M. betsileanus* are not characterised by particularly high oxygen concentrations, suggesting that, in this species, the absence (or extreme reduction) of external gills might be a phylogenetic constraint rather than an adaptation. The apparent absence of external gills in embryos of *M. betsileanus* and other mantellids points to a general lack of studies on the physiological relevance of these structures in early anuran stages. Blommers-Schlösser (1975, 1979a) recorded a lack of external gills in larvae of *Boophis madagascariensis* and *B. microtympanum*. Arnoult (1966) also reported a development without external gills in the larvae of *Mantella aurantiaca*. Hence, external gills might be absent or extremely reduced in all mantellids, and given the presence of external gills in larval salamanders, caecilians, lungfish and basal actinopterygians (Clemens 1894), their reduction must be considered a derived state.

In the development of *M. betsileanus*, correlation with Gosner stages 18, 19, 20 and 23 was only possible due to somitogenesis (stages 18 and 19), differentiation and elongation of the tail (stage 20), and differentiation of labia (stage 23), while the other traits would have pertained to the gills and muscular responses (Tab. 2). Due to the absence of external gills, stage 24 could not be assigned to any embryo. On the other hand, stages 21, 22 and 25 agreed well with stages 21, 22 and 25 of *I. valliceps*. Pigmentation began to show at stage 20 and therefore earlier than recorded by Gosner (1960) (stages 23–25) and pigment patterns of advanced tadpoles had formed by reaching stage 23 (compared to stage 32). Differentiation of the oral disk during stages 23 to 25 corresponded with the development in *I. valliceps*, and tooth rows also developed gradually (Gosner 1960). In *M. betsileanus*, the tooth ridges of the lower labium became discernible only at stage 23, and tooth rows only during stage 25, whereas in *I. valliceps*, tooth rows starting differentiating at stage 23 (Gosner 1960). Nevertheless, the tooth rows differentiated gradually in both species. Hatching in *M. betsileanus* occurred during stages 23–25, i.e., later compared to most other species, which hatch between stages 17–20 (Gosner 1960). On reaching stage 25, the yolk sack had also disappeared in all larvae, marking the transition from embryo to feeding tadpole, just as in *I. valliceps* (Gosner 1960). Identification of the earliest appearance of hind limb buds was difficult, due to their being of small size and undifferentiated colour at stages 26–27. Nevertheless, as far as was discernible, hind limb development corresponded well with the proposed stages by Gosner (1960). Total length increased gradually until tadpoles reached stage 41, and from this stage onwards, TL decreased as tadpoles began their metamorphosis and the tail was being reduced. Metamorphosis was similar to *I. valliceps* only to some extent. Forelimbs only became visible at stage 41, so that their early development could not be properly monitored, and they emerged at stage 42. Unlike tadpoles of *I. valliceps,* the mouthparts of *M. betsileanus* were still prominent up to stage 42 and only resorbed during stage 43.

The larval development of *M. betsileanus* took much longer than in *I. valliceps*. Tadpoles of *I. valliceps* had passed metamorphosis after about 27.7 days (Gosner 1960), whereas *M. betsileanus* tadpoles took more than three times as long and passed metamorphosis only after about 89 days. Furthermore, the embryonic and larval developmental periods, as well as the time required for metamorphosis, differed from Gosner (1960).

Duration of embryonic development from stages 1 to 19 was similar in the two species, with an average duration of two days in *M. betsileanus* and 1.7 days in *I. valliceps* (Gosner 1960). However, compared to the entire developmental period, the embryonic development in *M. betsileanus* was more rapid, taking only 2% of the time as opposed to 6% in *I. valliceps* (Gosner 1960). Likewise, tadpoles of *M. betsileanus* metamorphosed faster, spending only 7% of their whole developmental time on metamorphosis as compared to 25% of the time needed by *I. valliceps* (Gosner 1960). While larvae of *M. betsileanus* spent on average about 20% of their whole developmental time at stages 20–25 and about 70% at stages 26–40, the larvae of *I. valliceps* spent only 12% of development time at stages 20–25 and 58% at stages 26–40 (Gosner 1960). This longer developmental period almost certainly correlates with the fact that *M. betsileanus* larvae develop in quite stable streams rather than in shallow lentic waters as *I. valliceps* does. Thus, there is no pressure on tadpoles to complete metamorphosis before ponds or streams dry out, which has been reported to decrease larval developmental times up to metamorphosis for other anuran tadpoles (Denver et al. 1998, Loman 1999, Newman 1989). Low food and spatial resources have also been reported to decrease this period (Gromko et al. 1973, Reques & Tejedo 1995). However, this does not seem to be of relevance for the *M. betsileanus* tadpoles in our study, as they were kept at low densities and had unlimited access to food in the Makrolon-box. A distinct variation in development period was detected among the individuals of this study. Tadpoles from the first clutch discovered at Cologne Zoo in particular showed a prolonged development in comparison to the tadpoles of the two following clutches, which both originated from the same terrarium.

Captive-raised larvae of *M. betsileanus* at stage 25, despite similar body sizes, showed variation of the keratodont row formula, as was already reported by Knoll et al. (2007) for wild-caught specimens. Knoll et al. (2007) observed a keratodont row formula of 1:3+3/1+1:2 for tadpoles at stage 25, whereas we documented fewer keratodont rows for this stage, ranging from 0/2, 1/1, 1/3 to 1:1+1/3. As ascertained by Vences et al. (2012) for another mantellid tadpole (*Boophis luteus*) and also known from other species of frogs (e.g., "*Bufo*" *americanus*, Tubbs et al. 1993), keratodont numbers per row and number of anterior keratodont rows are more strongly correlated with body size than with developmental stage, i.e., new keratodonts and keratodont rows are added as the tadpole grows, independently of its developmental stage. Additional variation of similarly-sized specimens as observed herein might be also due to environmental effects (e.g., nutrition, temperature) which might affect the duration of the larval development and thus delay the development of larval traits such as differentiation of tooth rows and keratodonts (Denver et al. 1998, Gromko et al. 1973, Loman 1999, Newman 1989, Vences et al. 2002).

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**References**

Altig, R. (2007): A primer for the morphology of anuran tadpoles. – Herpetological Conservation and Biology, **2**: 71–74.

Altig, R. & R. W. McDiarmid (2006): Descriptions and biological notes on three unusual mantellid tadpoles (Amphibia: Anura: Mantellidae) from south-eastern Madagascar. – Proceedings of the Biological Society of Washington, **119**: 418–425.

Altig, R. & R. W. McDiarmid (2007): Morphological diversity and evolution of egg and clutch structure in amphibians. – Herpetological Monographs, **21**: 1–32.

Arnoult, J. (1966): Contribution a l'étude des batraciens de Madagascar. Écologie et développement des *Mantella aurantiaca* Mocquard 1900. – Bulletin du Muséum national d'Histoire Naturelle Paris, **37**: 931–940.

Blommers-Schlösser, R. M. A. (1975): A unique case of mating behaviour in a Malagasy tree frog, *Gephyromantis liber* (Peracca 1893), with observations on the larval development (Amphibia, Ranidae). – Beaufortia, **23**: 15–23.

Blommers-Schlösser, R. M. A. (1979a): Biosystematics of the Malagasy frogs, I. Mantellinae (Ranidae). – Beaufortia, **29**: 1–77.

Blommers-Schlösser, R. M. A. (1979b): Biosystematics of the Malagasy frogs. II. The genus *Boophis* (Rhacophoridae). – Bijdragen tot de Dierkunde, **49**: 261–312.

Clemens, P. (1894): Die äusseren Kiemen der Wirbeltiere. – Beiträge und Referate zur Anatomie und Entwickelungsgeschichte, **5**: 51–155.

Connelly, S., C. M. Pringle, R. J. Bixby, R. Brenes, M. R. Whiles, K. R. Lips, S. Kilham & A. D. Huryn (2008): Changes in stream primary producer communities resulting from large-scale catastrophic amphibian declines: Can small-scale experiments predict effects of tadpole loss? – Ecosystems, **11**: 1262–1276.

Crottini, A., O. Madsen, C. Poux, A. Strauß, D. R. Vieites & M. Vences (2012): Vertebrate time-tree elucidates the biogeographic pattern of a major biotic change around the K–T boundary in Madagascar. – Proceedings of the National Academy of Sciences of the USA, **109**: 5358–5363.

Del Pino, E. M. & B. Escobar (1981): Embryonic stages of *Gastrotheca riobambae* (Fowler) during maternal incubation and comparison with development of other marsupial frogs. – Journal of Morphology, **167**: 277–295.

Denver, R. J., N. Mirhadi & M. Phillips (1998): Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondi* tadpoles to habitat dessication. – Ecology, **79**: 1859–1872.

Duellman, W. E. & L. Trueb (1986): Biology of amphibians. – New York: McGraw-Hill.

Flecker A. S., B. P. Feifarek & B. W. Taylor (1999): Ecosystem engineering by a tropical tadpole: density-dependent effects on habitat structure and larval growth rates. – Copeia, **1999**: 495–500.

Glaw, F. & M. Vences (2007): A field guide to the amphibians and reptiles of Madagascar. Third Edit. – Vences & Glaw Verlags GbR.

Goldberg J., F. V. Candioti & M. S. Akmentins (2012): Direct-developing frogs: Ontogeny of *Oreobates barituensis* (Anura: Terrarana) and the development of a novel trait. – Amphibia-Reptilia, **33**: 239–250.

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

Gromko, M. H., F. S. Mason & S. J. Smith-Gill (1973): Analysis of the crowding effect in *Rana pipiens* tadpoles. – Journal of Experimental Zoology, **186**: 63–72.

Grosjean, S., R. D. Randrianiaina, A. Strauß & M. Vences (2011a): Sand-eating tadpoles in Madagascar: morphology and ecology of the unique larvae of the treefrog *Boophis picturatus*. – Salamandra, **47**: 63–76.

Grosjean, S., A. Strauß, J. Glos, R. D. Randrianiaina, A. Ohler & M. Vences (2011b): Morphological and ecological uniformity in the funnel-mouthed tadpoles of Malagasy litter frogs, subgenus *Chonomantis*. – Zoological Journal of the Linnean Society, **162**: 149–183.

Knoll, A., J. Köhler, F. Glaw, M. Teschke & M. Vences (2007): Larval morphology in four species of Madagascan frogs of the subgenus *Brygoomantis* (Mantellidae: *Mantidactylus*). – Zootaxa, **59**: 49–59.

Kupferberg, S. (1997): Facilitation of periphyton production by tadpole grazing: functional differences between species. – Freshwater Biology, **37**: 427–439.

Loman, J. (1999): Early metamorphosis in common frog *Rana temporaria* tadpoles at risk of drying: an experimental demonstration. – Amphibia-Reptilia, **20**: 421–430.

McDiarmid, R. W. & R. Altig (1999): Tadpoles. The biology of anuran larvae. – Chicago: University of Chicago Press.

Newman, R. A. (1989): Developmental plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. – Ecology, **70**: 1775–1787.

Noble, G. K., G. Phillips & P. G. Putnam (1931): Oberservation on the life history of *Ascaphus truei* Stejneger. – Copeia, **1931**: 97–101.

Perl, R. G. B., Z. T. Nagy, G. Sonet, F. Glaw, K. C. Wollenberg & M. Vences (submitted): DNA barcoding Madagascar's amphibian fauna. – Amphibia-Reptilia.

Poth, D., K. C. Wollenberg, M. Vences & S. Schulz (2012): Volatile amphibian pheromones: macrolides of mantellid frogs from Madagascar. – Angewandte Chemie International Edition, **51**: 2187–2190.

Randrianiaina, R. D., A. Strauß, J. Glos, F. Glaw & M. Vences (2011a): Diversity, external morphology and 'reverse taxonomy' in the specialized tadpoles of Malagasy river bank frogs of the subgenus *Ochthomantis* (genus *Mantidactylus*). – Contributions to Zoology, **80**: 17–65.

Randrianiaina, R. D., K. C. Wollenberg, T. Rasolonjatovo Hiobiarilanto, A. Strauß, J. Glos & M. Vences (2011b): Nidicolous tadpoles rather than direct development in Malagasy frogs of the genus *Gephyromantis*. – Journal of Natural History, **45**: 2871–2900.

Randrianiaina, R. D., A. Strauß, J. Glos & M. Vences (2012): Diversity of strongly rheophilous tadpoles of Malagasy tree frogs, genus *Boophis* (Anura, Mantellidae), and identification of new candidate species via larval DNA sequence and morphology. – ZooKeys, **178**: 59–124.

Ranvestel A. W., K R. Lips, C. M Pringle, M R. Whiles & R. J. Bixby (2004): Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. – Freshwater Biology, **49**: 274–285.

Reques, R. & M. Tejedo (1995): Negative correlation between length of larval period and metamorphic size in natural populations of natterjack toads (*Bufo calamita*). – Journal of Herpetology, **29**: 311–314.

Schmidt, H., A. Strauß, F. Glaw, M. Teschke & M. Vences (2009): Description of tadpoles of five frog species in the subgenus *Brygoomantis* from Madagascar (Mantellidae: *Mantidactylus*). – Zootaxa, **1988**: 48–60.

Strauß, A., E. Reeve, R. D. Randrianiaina, M. Vences & J. Glos (2010): The world's richest tadpole communities show functional redundancy and low functional diversity: ecological data on Madagascar's stream-dwelling amphibian larvae. – BMC Ecology, **10**: article 12.

Strauß, A., R. D. Randrianiaina, M. Vences & J. Glos (2013): Species distribution and assembly patterns of frog larvae in rainforest streams of Madagascar. – Hydrobiologia, **702**: 27–43.

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*, *Mantidactylus madecassus*, and *Boophis laurenti* from the Andringitra Massif. – Copeia, **2005**: 174–183.

Tubbs, L. O. E., R. Stevens, M. Wells & R. Altig (1993): Ontogeny of the oral apparatus of the tadpole of *Bufo americanus*. – Amphibia-Reptilia, **14**: 333–340.

Vences, M., M. Puente, S. Nieto & D. R. Vieites (2002): Phenotypic plasticity of anuran larvae: environmental variables influence body shape and oral morphology in *Rana temporaria* tadpoles. – Journal of Zoology, **257**: 155–162.

Vences, M., Y. Chiari, M. Teschke, R. D. Randrianiaina, L. Raharivololoniaina, P. Bora, D. R. Vieites & F. Glaw (2008): Which frogs are out there? A preliminary evaluation of survey techniques and identification reliability of Malagasy amphibians. – pp. 233–253 in: Andreone, F. (Ed.): A Conservation Strategy for the Amphibians of Madagascar. – Monografie del Museo Regionale di Scienze Naturali di Torino, **45**.

Vences, M., R. D. Randrianiaina, A. Strauß, D. C. Yoheswara, H. Beims, D. Donath, M. Ebert, S. Gebauer, J. Herrmann, J. Klages, F. Kollmeier, M. Messal, C. Momberg, S. Parlow & C. Zumpe (2012): External morphology of the tadpoles of the Malagasy treefrog *Boophis luteus*: ontogenetic variation of keratodont numbers and uniformity between genealogical lineages. – Herpetology Notes, **5**: 325–334.

Vieites, D. R., K. C. Wollenberg, F. Andreone, J. Köhler, F. Glaw & M. Vences (2009): Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. – Proceedings of the National Academy of Sciences of the U.S.A., **106**: 8267–8272.

Warkentin, K. M. (2000): Environmental and developmental effects on external gill loss in the Red-Eyed Tree Frog, *Agalychnis callidryas*. – Physiological and Biochemical Zoology, **73**: 557–565.

Wells, K. D. (2007): The ecology and behavior of amphibians. – The University of Chicago Press, Chicago.

Whiles, M. R., K. R. Lips, C. M. Pringle, S. S. Kilham, R. J. Bixby, R. Brenes, S. Connelly, J. C. Colon-Gaud, M. Hunte-Brown, A. D. Huryn, C. Montgomery & S. Peterson (2006): The effects of amphibian population declines on the structure and function of neotropical stream ecosystems. – Frontiers in Ecology and the Environment, **4**: 27–34.