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1329

Comparative larval morphology in Madagascan frogs of the genus *Guibemantis* (Amphibia: Mantellidae)

SILVIA VEJARANO[1], MEIKE THOMAS[2] & MIGUEL VENCES[1,3]*

[1]*Institute for Biodiversity and Ecosystem Dynamics, Zoological Museum, University of Amsterdam, Mauritskade 61, 1092 AD, Amsterdam, The Netherlands*
[2]*University of Cologne, Department of Genetics, Zülpicher Str. 47, 50674 Cologne, Germany*
[3]*Current address: Zoological Institute, Technical University of Braunschweig, Spielmannstr. 8, 38106 Braunschweig, Germany*
**Corresponding author. E-mail m.vences@tu-bs.de*

Abstract

We describe the tadpole morphology for four species of frogs classified in the endemic Madagascan genus *Guibemantis*, based on larval specimens identified by DNA barcoding. The tadpoles of *Guibemantis kathrinae* and *G. tornieri* are reported for the first time. The tadpole of *G. kathrinae* has a heterogeneous coloration, emarginated oral disc bordered with papillae and one row of submarginal papillae. Labial tooth row formula is 6(2–6)/3(1). Number of labial teeth per millimetre is variable in each row, ranging from 36 to 64. The tadpole of *G. tornieri* is very similar to that of *G. timidus* (previously considered conspecific with *G. tornieri*) except for the patched coloration of *G. tornieri* (vs. rather uniform in *G. timidus*). The tadpole of *G. depressiceps* is characterized by having a higher number of teeth per millimetre in all tooth rows than the other species of the group. The tadpole of *Guibemantis liber* differs from the other species by having a lower number of upper labial tooth rows (two, three or four vs. five or more). No morphological differences were found between larvae of *G. liber* from two separate localities, Ranomafana and Andasibe. In general, the *Guibemantis* larvae examined (except *G. liber*) are morphologically similar to each other but several of the characters examined were highly variable within populations and species, highlighting the usefulness of molecular tools for their identification.

Key words: Amphibia, Mantellidae, *Guibemantis*, tadpole description, systematics

Introduction

Within the family Mantellidae, endemic to Madagascar and the Comoros, *Mantidactylus* Boulenger, 1895 was long seen as the most species-rich and morphologically heterogeneous genus (Glaw & Vences 2003). The large number of species with a high

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diversity in morphology and natural history made it a complex group that had been divided into 12 subgenera (Glaw & Vences 1994). In a recent paper, Glaw and Vences (2006) partitioned this heterogeneous (and paraphyletic) genus into seven monophyletic genera, based on the results of a molecular phylogenetic analysis. The classification proposed by these authors has also been applied in a recent bioacoustic soundguide to Malagasy frogs (Vences *et al.* 2006), and will be followed herein as well. The genus *Guibemantis* Dubois, 1992, according to Glaw & Vences (2006), is subdivided in two subgenera, *Guibemantis* and *Pandanusicola* Glaw & Vences, 1994. The subgenus *Guibemantis* (*Guibemantis*) has four species: *G. depressiceps* (Boulenger, 1882), *G. tornieri* (Ahl, 1928), *G. kathrinae* (Glaw, Vences and Gossmann, 2000), and the recently described *G. timidus* (Vences & Glaw, 2005). These species are largely arboreal frogs that breed in stagnant water in forest or at forest edges. Eggs are deposited on leaves hanging above the water. After embryonic development, the tadpoles drop into the water, and complete metamorphosis in the pond (Blommers-Schlösser 1975, 1979). Two species, *Guibemantis depressiceps* and *G. kathrinae*, are characterized by conspicuously white eggs and jelly masses (Blommers-Schlösser 1979; Glaw *et al.* 2000).

Phylogenetic relationships within the subgenus *Guibemantis*, based on femoral gland morphology, size, connection of lateral metatarsalia, egg color and call structure, suggested a close relationship between *G. depressiceps* and *G. kathrinae*, these two species being sister to *G. tornieri* (Glaw *et al.* 2000). Molecular data have instead placed *G. kathrinae* close to *G. tornieri* (Vences *et al.* 2005).

A further species to be considered in this context is *Guibemantis liber* (Peracca, 1893). This species has a similar reproductive mode and was considered to be closely related to the species of the subgenus *Guibemantis* (Glaw & Vences 1994), but was found to belong into the group of phytotelm-breeding Pandanus frogs, subgenus *Pandanusicola* (Lehtinen & Nussbaum 2003; Vences *et al.* 2003; Lehtinen *et al.* 2004).

Considering the strong differentiation of various mantellid subgroups in terms of reproductive biology and larval morphology (Blommers-Schlösser 1979; Glaw & Vences 1994), knowledge on their tadpole is relevant to better understand their evolutionary relationships and systematics. In the subgenus *Guibemantis*, larval morphology is known for *G. depressiceps* and *G. timidus* (the latter under the name *Mantidactylus tornieri*; Blommers-Schlösser 1979), and the tadpole of *Guibemantis liber* has been described as well (Blommers-Schlösser 1975). Here we provide a more extensive comparison of larval stages of species in the subgenus and of *G. liber*, also investigating whether there are morphological differences between the tadpoles of *G. liber* from central eastern and south-eastern Madagascar (Ranomafana and Andasibe regions), and describing for the first time the tadpoles of *G. kathrinae* and *G. tornieri*.

Materials and methods

Tadpoles were collected in the field, euthanised by immersion in chlorobutanol solution, and immediately sorted into homogeneous series based on morphological characters. From each series one specimen was selected and a tissue sample from its tail musculature or fin taken and preserved in 99% ethanol. This specimen is here named "DNA voucher". After tissue collection, all specimens were preserved in 4% formalin. Specimens were deposited in the Zoologische Staatssammlung München, Germany (ZSM); comparative specimens were examined from the herpetological collection of the Zoological Museum Amsterdam, Netherlands (ZMA).

Tadpoles were identified using a DNA barcoding approach based on a fragment of the mitochondrial 16S rRNA gene, which is known to be sufficiently variable among species of Malagasy frogs (Thomas *et al.* 2005). The ca. 550 bp fragment was amplified using primers 16Sa-L and 16Sb-H from Palumbi *et al.* (1991) applying standard protocols, resolved on automated sequencers, and compared to a near-complete database of sequences of adult Malagasy frog species. Identification was considered to be unequivocal when the tadpole sequence was 99–100% identical to an adult specimen from the same geographical region, and not more similar to any sequence from another species. DNA sequences were deposited in Genbank (accession numbers DQ054492–DQ054503; accession numbers of comparative adult specimens included in the sequence sets AY847959–AY848683 and AY684185–AY684191).

Drawings and descriptions of the tadpoles are based only on the DNA vouchers. All specimens of each series were examined to complete information on the structures missing for the tissue sampling and to assess morphological variability. Developmental stages are based on Gosner (1960). Morphological terminology follows Altig and McDiarmid (1999). Labial tooth row formula is in addition also given according to the notation of Dubois (1995). The measurements taken using a stereo microscope with measuring device and converted later into millimetres are: BL = body length (from the tip of the snout to the junction of the posterior body wall with the axis of the tail myotomes); BW = body width (the widest point of the ‘head’ right behind the eyes); ED = eye diameter; IOD = interorbital distance (from the centre of each pupil); TH = tail height (at the beginning of the tail); TMW = caudal muscle width (at the beginning of the tail); ODW = maximum oral disc width; DGMP = dorsal gap of the marginal papillae. The mouth parts include: MP = number of marginal and SMP = submarginal papillae; LMP = length of the marginal papillae; UPT = upper tooth row (1 is the most external); LTR = lower tooth row (1 is the most internal); NT = number of teeth; LLTR = length of the labial tooth row.

Descriptions

Guibemantis liber (Peracca), central-eastern Madagascar

Series examined: ZSM 466–467/2004 (field number 2002.2014; Genbank accession number DQ054499), series of 14 specimens, ZSM 468/2004 (fieldnumber 2002.2016; DQ054500) one specimen, ZSM 469/2004 (fieldnumber 2002.2017; DQ054501), one specimen, ZSM 470/2004 (fieldnumber 2002.2019; DQ054502), series of two specimens and ZSM 659/2004 (fieldnumber 2002.1610; DQ054503) series of 34 specimens, all from An'Ala forest (18°56'S, 48°28'E, 840 m above sea level), collected in different shallow ponds not deeper than 50 cm with swampy and leaf-covered grounds on 1 March 2003.

Description (based on the DNA voucher of series ZSM 466-467/2004). Tadpole in stage 34. Coloration in preservative light brown without any distinct pattern. The intestinal wall also pigmented and intestinal spiral visible. Ventrolateral and ventral parts of the body lack pigmentation. Anterior part of the tail with the same coloration of the body. Most of the tail and caudal fin missing because of tissue sampling. Tadpole type IV (Orton 1953). Body shape oval. Snout in dorsal view almost rounded. Eyes small (about 0.06 of body length) dorsally positioned (fig. 1a and b). Spiracle sinistral. Oral disc laterally emarginated, and ventrally positioned (fig. 1c). Dorsal gap of the marginal papillae 1.03 mm. Oral disc bordered by 46 short marginal papillae more or less equal in length (˜0.05 mm). No submarginal papillae. LTRF 1: 3+3/3 (Dubois 1995) or 4(2–4)/3(–) (Altig & McDiarmid 1999). The lower lip and tooth rows possibly damaged by fixation or preservation; LTR_1 continues on the left side until it meets LTR_3 and appears as one single continuous row; LTR_2 incomplete on the left side. UTR ca. 52–80 teeth per mm and LTR ca. 13–16 teeth per mm. From UTR_2 on, they are separated by the upper jaw sheath. Jaw sheaths distinct and black, with serrated edges (ca. 55 on each jaw). Sheath of upper jaw not curved in the middle, lower jaw sheath V-shaped.

Variation within the series. Series ZSM 466–467/2004 with 13 additional tadpoles in stages 27–40. Number of marginal papillae variable (31–64); submarginal papillae 0–3 on each side of the upper lip and 1–4 on the lower lip, except one specimen with seven on the right side of the lower lip. LTRF 3(2–3)/3(1) or 4(2–3)/3(1), independent from the stage. In the remaining specimens, general and oral morphology very similar but certainly different from the DNA voucher specimen in the oral disc (differences might be artefacts).

Series ZSM 468/2004 and 469/2004 only consisting of their respective DNA voucher specimen. Series ZSM 470/2004 with two specimens, the DNA voucher in stage 27 and a second specimen in stage 31 that has LTRF 5(2–5)/3(1), 53 marginal and three submarginal papillae on the left side of the upper and lower lips and one on the right side. Series ZSM 659/2004 with 34 tadpoles in stages 28–41. LTRF 4(2–4)/3(1) in most specimens except five with LTRF 3(2–3)/3(1) and one with 5(2–5)/3(1) in which UTR_5 has only four teeth on each side. In two specimens the oral disc is not in a good state of

preservation, and misses parts of the lips or tooth rows. Coloration varies between individuals from light to dark brown, but the pattern is the same as in the DNA voucher of series ZSM 466–467/2004. Marginal papillae 32–65, but in most specimens 51–59. Number of submarginal papillae on each side of the upper lip usually 2–3, lacking in some specimens, and four in other individuals; number in the lower lip varies more, 1–8 on each side. This series is very homogeneous, in spite of the large number of specimens and their different developmental stages. Morphometric data of all DNA vouchers of *G. liber* are shown in tables 1 and 2.

TABLE 1. Morphometric data (all in mm) of DNA voucher specimens of *Guibemantis liber*. * DNA vouchers used for descriptions and drawings. ** No oral disc morphology data because of advanced developmental stage.

	Central-Eastern Madagascar				
	* ZSM 466-467/2004	ZSM 659/2004	ZSM 470/2004	ZSM 468/2004	** ZSM 469/2004
Stage	34	36	27	34	43
BL	8.1	10.00	6.50	9.00	10.00
BW	5.0	5.50	4.00	5.00	4.00
ODW	1.9	2.58	1.43	1.98	2.65
DGMP	1.1	1.35	1.08	1.33	1.38
IOD	2.9	3.69	2.44	2.50	4.00
ED	0.6	1.38	0.75	1.06	1.75
TH	2.7	1.56	2.25	2.56	3.00
TMW	2.1	2.63	1.75	2.03	2.00
MP	0.05	0.13	0.10	0.13	0.05
LTRF	4(2–4)/3(?)	3(2–3)/3(1)	3(2–3)/3(1)	4(2–4)/3(1)	
UTR_1	1.3	1.69	0.90	1.78	
UTR_2	0.4	0.81	0.30	0.78	
UTR_3	0.2	0.25	0.18	0.38	
UTR_4	0.08	-	-	0.18	
LTR_1	2.5	0.69	0.40	0.68	
LTR_2	2.6	1.63	1.05	1.18	
LTR_3	4.0	1.38	0.75	0.80	
$LLTR_3$		0.125	0.050	0.050	
$LUTR_1$		0.075	0.063	0.075	

to be continued.

TABLE 1 (continued).

	South-Eastern Madagascar			
	* ZSM 433/2004	ZSM 452/2004	ZSM 451/2004	ZSM 449/2004
Stage	33	27	27	27
BL	8.3	5.50	5.50	6.00
BW	6.1	3.50	3.50	3.00
ODW	1.9	1.45	1.50	1.58
DGMP	1.2	0.88	0.75	1.00
IOD	1.7	1.69	1.93	1.90
ED	1.0	0.69	0.73	0.68
TH	2.2	0.90	0.73	0.73
TMW	2.2	0.65	0.48	0.48
MP	0.13	0.10	0.08	0.10
LTRF	4(2–4)/3(1)	2(2)/3(1)	2(2)/3(1)	2(2)/3(1)
UTR_1	1.6	1.05	0.95	0.93
UTR_2	0.6	0.39	0.30	0.30
UTR_3	0.3	-	-	-
UTR_4	0.2	-	-	-
LTR_1	0.7	0.41	0.40	0.40
LTR_2	1.3	0.85	0.88	0.98
LTR_3	1.2	0.70	0.65	0.80
$LLTR_3$		0.025	0.025	0.075
$LUTR_1$		0.050	0.050	0.075

Variation among the series. Complete morphometric data of all DNA voucher specimens are shown in table 1. DNA voucher of series ZSM 468/2004 in stage 34; LTRF 4 (2–4)/3(1); 45 marginal papillae; 1–2 submarginal papillae on each side of the upper lip and one on each side of the lower lip; in coloration and general morphology agreeing with the DNA voucher specimen of series ZSM 466–467/2004. DNA voucher of series ZSM 469/2004 in stage 43. Oral disc and mouth parts modified. Dorsal coloration of the body agreeing with the DNA voucher specimen of series ZSM 466–467/2004; tail and posterior legs with dark brown spots. DNA voucher of series ZSM 470/2004 in stage 27; LTRF 3(2–3)/3(1); 44 marginal papillae; two submarginal papillae on each side of the lower lip and one on the right side of the upper lip. Coloration of the body brown, but softer and lighter than in the DNA vouchers of all other series. DNA voucher of series ZSM 659/2004 in stage 36. LTRF 3(2–3)/3(1); 51 marginal papillae; two on each side of the upper lip and four on each side of the lower lip. Coloration of the body dark brown. In other

characteristics, as far as recognizable, there are no relevant differences among the DNA voucher specimens of all series.

TABLE 2. Number of marginal and submarginal papillae (MP, SMP) and number of teeth per row of all DNA voucher specimens of *Guibemantis liber*. For rows divided by a gap, the number of teeth presented on the table is the average of the numbers on each side of the tooth row. * DNA vouchers used for descriptions and drawings. ** No oral disc morphology data because of advanced developmental stage.

	Central-Eastern Madagascar				
	* ZSM 466-467/2004	ZSM 659/2004	ZSM 470/2004	ZSM 468/2004	** ZSM 469/2004
MP	46	51	44	45	34
SMP	0	11	5	5	5
UTR_1	68	99	62	104	
UTR_2	26	29	25	42	
UTR_3	16	18	11	21	
UTR_4	6	-	-	9	
LTR_1	33	39	28	28	
LTR_2	41	89	66	66	
LTR_3	54	68	48	48	

continued.

	South-Eastern Madagascar			
	* ZSM 433/2004	ZSM 452/2004	ZSM 451/2004	ZSM 449/2004
MP	52	36	35	40
SMP	39	7	6	8
UTR_1	68	47	40	45
UTR_2	25	16	13	15
UTR_3	12	-	-	-
UTR_4	6	-	-	-
LTR_1	29	22	18	19
LTR_2	90	43	38	39
LTR_3	88	34	29	37

Previous descriptions. The tadpole of *G. liber* has been described by Blommers-Schlösser (1975) under the name *Gephyromantis liber*: 68 tadpoles collected in Andasibe, the Mandraka valley, and Tampoketsa d'Ankazobe (all localities in central eastern Madagascar) were examined at that time, and the only noticeable difference between the

stages was the size (body length 3.3–13 mm); general body shape was as a typical type IV tadpole (Orton 1953), the oral disc emarginated, directed downwards and surrounded by small papillae except the dorsal margin. LTRF was variable between 4(2–4)/3(1), 3(2–3)/3(1) and 5(2–5)/3(1).

Guibemantis liber (Peracca), south-eastern Madagascar

Material examined: ZSM 433/2004 (fieldnumber 2002.1825; Genbank accession DQ054495), series of 19 specimens from Samalaotra (21°14.282'S/47°23.753'E), collected in a pond on 20 January 2003; ZSM 452/2004 (fieldnumber 2002.1893; DQ054498), series of 22 specimens, ZSM 451/2004 (fieldnumber 2002.1892; DQ054497), one specimen, and ZSM 449/2004 (fieldnumber 2002.1888; DQ054496), series of eight specimens from Ranomafana National Park (Park entrance, 21°15'38"S/47°25'13"E, 846 m above sea level), collected in a temporary pond with maximum depth of 30 cm and many leaves in the bottom, on 17 January 2003.

Description (based on the DNA voucher of series ZSM 433/2004). Tadpole in stage 33. Coloration in preservative light brown without any distinct pattern. The intestinal wall also pigmented and intestinal spiral visible. Ventral part of the body lacks pigmentation. Anterior part of the tail with the same coloration of the body. The end of the tail and caudal fin missing because of tissue sampling. Tadpole type IV (Orton 1953). Body shape oval. Snout in dorsal view almost rounded. Eyes medium size (about 0.1 of body length) dorsally positioned (fig. 1d and e). Spiracle difficult to recognize. Oral disc laterally emarginated, and ventrally positioned (fig. 1 f). Dorsal gap of the marginal papillae 1.23 mm. Oral disc bordered by 52 marginal papillae more or less equal in length (˜0.13 mm). 4-5 submarginal papillae on each side of the lower lip, and 2–3 on the upper lip. LTRF 1:3+3/1+1:2 (Dubois 1995) or 4(2–4)/3(1) (Altig & McDiarmid 1999); LTR_1 and LTR_2 with thinner, longer and more teeth per millimetre (ca. 70/mm) than other tooth rows (ca. 30–42/mm) and becoming shorter at the borders. From UTR_2 on, they are separated by the upper jaw sheath. LTR_1 with a short gap. Jaw sheaths distinct and black, with serrated edges (ca. 55 on each jaw). Sheath of upper jaw softly curved in the middle, lower jaw sheath V-shaped.

Variation within series. The series ZSM 433/2004 contains 18 additional tadpoles in stages 26-35. One specimen was discarded because it shows no resemblance with the DNA voucher or others. Number of marginal papillae highly variable (33–71) but mainly around 55. Number of submarginal papillae also variable (0-4 on each side of the upper lip, and 0-7 on the lower). In almost all specimens in stages under 30 LTRF is 3(2–3)/3(1), different from the DNA voucher, but agreeing in every other respect with it. Other tadpoles in stages above 30 have the same LTRF as the DNA voucher and agree in morphology and oral parts. A further specimen in stage 28 has LTRF 3(2–3)/3(2–3).

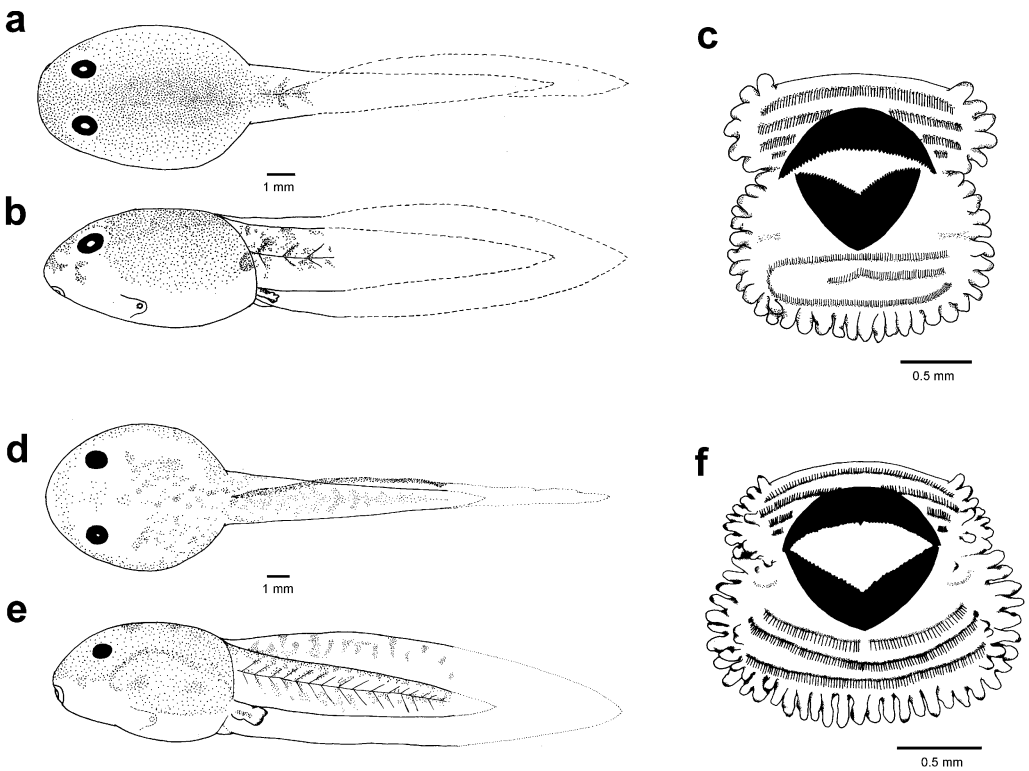

FIGURE 1. Drawings of the preserved DNA voucher tadpoles in (dorsal and lateral view, and oral disk) of *Guibemantis liber* from central-eastern Madagascar (a,b,c; series ZSM 466–467/2004) and from south-eastern Madagascar (d, e, f; series ZSM 433/2004).

The series ZSM 452/2004 contains 22 tadpoles in stages 26–28. Four specimens were discarded because of sustained differences with the DNA voucher. In all other specimens LTRF varies among individuals 3(2–3)/3(1) or 2(2)/3(1). 31–44 marginal papillae, except two specimens with 48. All specimens with 0-4 submarginal papillae on each side of upper and lower lip. The series ZSM 449/2004 contains eight tadpoles in stages 27 except one in stage 28. One specimen was discarded because of sustained differences with the rest of the series. LTRF is 3(2–3)/3(1) in 3 specimens (including the one in stage 28) and 2(2)/3(1) in the rest. 27–44 marginal papillae; 0–3 submarginal papillae on each side of the upper lip and 2–4 on each side of the lower lip. The series FG/MV ZSM 451/2004 contains only the DNA voucher specimen.

Variation among series. DNA voucher of series ZSM 449/2004, 451/2004, and 452/2004, are in stage 27 and have LTRF 2(2)/3(1). Coloration, general and oral morphology is similar among them and to the DNA voucher description of series ZSM 433/2004, except for the numbers of marginal and submarginal papillae which are highly variable (table 2). DNA voucher of series ZSM 452/2004, 36 marginal papillae; one submarginal papilla on each side of the upper lip and 2-3 on each side of the lower lip. Coloration of the DNA voucher of series ZSM 449/2004 is lighter than that of other series, but the pattern is the

same; 40 marginal papillae; one submarginal papilla on the left side of the upper lip and three on each side of the lower. DNA voucher of series ZSM 451/2004, 35 marginal papillae; one small submarginal papilla on the right side of the upper lip and 2-3 on each side of the lower lip.

As shown in table 1, the measurements and number of labial teeth between the series from the same geographic region show no strong variation. Variation among individuals of different series is mainly related to the developmental stage.

***Guibemantis depressiceps* (Boulenger)**

Series examined: ZSM 425–429/2004 (fieldnumber 2002.1811; Genbank accession DQ054492) series of 6 specimens from Vohiparara, Kidanavo bridge (21°13'50"S/ 47°22'08"E, 1115 m above sea level) collected in a relatively slow-moving brook on 21 January 2003.

Description based on the DNA voucher specimen. Tadpole in stage 26. For morphometric data see tables 3 and 4. Colour in preservative homogeneous brown. The intestinal wall also pigmented and the intestinal spiral visible. Ventral part of the body lacks pigmentation. Anterior part of the tail with faint transverse stripes, but the rest of the tail and caudal fin are missing because of tissue sampling. Tadpole type IV (Orton 1953). Body shape oval. Snout in dorsal view almost rounded. Eyes medium-sized (about 0.1 of body length) dorsally positioned (fig. 2a and b). Spiracle difficult to recognize. Oral disc laterally emarginated, and positioned ventrally (fig. 2c). Dorsal gap of the marginal papillae long (2.13 mm). Oral disc bordered by 51 marginal papillae (˜0.1 mm) that decrease in size towards the emargination. One row of 14 submarginal papillae on the lower lip, three on the right side of the upper lip and only a small one on the left side. LTRF 1:6+6/1+1:2 (Dubois 1995) or 7(2–7)/3(1) (Altig & McDiarmid 1999); UTR_1 with thinner, longer and more teeth per millimetre (ca. 75/mm) than the rest (ca. 45–65/mm) and becoming shorter at the borders. From UTR_2 on, they are separated by the upper jaw sheath. LTR_1 with a short gap. Jaw sheaths distinct and black, with serrated edges (ca. 60 on each jaw). Sheath of upper jaw strongly curved in the middle, and lower jaw sheath resembling a wide V.

Variation within the series. 5 specimens in stages 25–28. LTRF 5(2–5)/3(1) in 2 specimens in stage 25 and 6(2–6)/3(1) in the rest. 45–61 marginal papillae 2–3 on each side of the upper lip and 4–8 on each side of the lower. Coloration, general and oral disc morphology agree in all specimens with the DNA voucher.

Previous descriptions. The tadpole of *G. depressiceps* has been described by Blommers-Schlösser (1979) from specimens collected in central eastern Madagascar (Andasibe, Mandraka and Moramanga-Anosibe) between 900–1200 m above sea level; tadpoles in stages 25–30 measured up to 10 mm body length and up to 15 mm in stage 38;

they were typical type IV tadpoles (Orton 1953); mouth small, directed downwards; oral disc indented laterally, and papillary border with a wide dorsal gap; labial tooth row formula (LTRF) was 6(2–6)/3(1) or 7(2–7)/3(1).

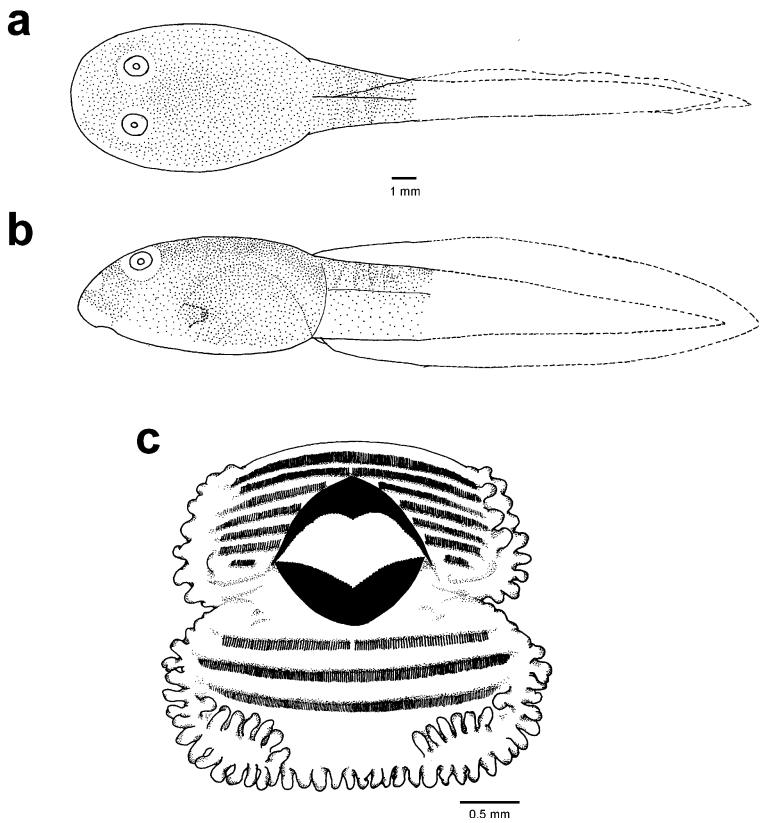

FIGURE 2. Drawings of the preserved DNA voucher tadpole of *Guibemantis depressiceps*, series ZSM 425–429/2004; (a) dorsal view, (b) lateral view, (c) oral disc.

Guibemantis kathrinae Glaw, Vences & Gossmann, 2000

Series examined: ZSM 460/2004 (fieldnumber 2002.1984; DQ054493), series of 3 specimens from An'Ala forest (18°56'S/48°28'E) at an altitude of about 840 m, all collected in one shallow pond not deeper than 30 cm with a swampy and leaf-covered ground on 1 March 2003.

Description based on the DNA voucher specimen. Tadpole in stage 25. For morphometric data see tables 3 and 4. Colour in preservative brown, not homogeneous but with a patch on top of the head and transverse stripes behind. The intestinal wall is also pigmented and the intestinal spiral visible. Ventral part of the body lacks pigmentation. Anterior part of the tail with transverse stripes, but the rest of the tail and caudal fin are missing because of tissue sampling. Tadpole type IV (Orton 1953). Body shape oval.

Snout in dorsal view almost rounded. Eyes medium-sized (about 0.1 of body length) dorsally positioned (fig. 3a and b). Spiracle sinistral, positioned ventrolaterally at mid body. Oral disc completely ventral, laterally emarginated (Fig. 3c). Dorsal gap of the marginal papillae large (2.2 mm). The rest of the oral disc bordered completely by 52 marginal papillae; ventral (posterior) ones about 0.1 mm long, diminishing in length as they reach the middle. One row of 28 submarginal papillae on the lower lip; three lateral on each side of the upper lip. LTRF 1:5+5/1+1:3 (Dubois 1995) or 6(2–6)/3(1) (Altig & McDiarmid 1999); Number of teeth per millimetre varies in each row from 36 to 64. From UTR_2 on, the rows are separated by the upper jaw sheath. LTR_1 with a short gap. Jaw sheaths distinct and black, with serrated edges (ca. 80 on each jaw). Sheath of upper jaw gently curved in the middle, and lower jaw sheath resembling a wide V.

TABLE 3. Morphometric measurements (all in mm) of DNA vouchers of *Guibemantis tornieri*, *G. depressiceps* and *G. kathrinae*. For rows divided by a gap, the number of teeth presented on the table is the average of the numbers on each side of the tooth row.

	G. tornieri	*G. depressiceps*	*G. kathrinae*
Stage	26	26	25
bl	7.3	10	10.8
bw	4.7	5.0	7.0
odw	2.3	3.3	3.5
dgmp	1.5	2.1	2.2
iod	2.9	4.5	4.8
ed	1.1	1.3	1.3
th	2.9	3.8	4.6
tmw	2.1	2.6	3.3
mp	0.1	0.1	0.1
UTR_1	1.6	2.0	2.7
UTR_2	0.8	1.2	1.3
UTR_3	0.5	1.0	1.0
UTR_4	0.3	0.9	0.8
UTR_5	1.2+	0.8	0.6
UTR_6	-	0.6	0.4
UTR_7	-	0.2	-
LTR_1	0.8	1.2	1.2
LTR_2	1.6	2.8	2.5
LTR_3	1.7	2.9	2.7

+ UTR_5 only on the right side of the oral disc.

TABLE 4. Number of marginal and submarginal papillae (MP, SMP) and number of labial teeth per row in the DNA vouchers of *Guibemantis tornieri*, *G. depressiceps* and *G. kathrinae*. For rows divided by a gap, the number of teeth presented on the table is the average of the numbers on each side of the tooth row.

	G. tornieri	*G. depressiceps*	*G. kathrinae*
MP	41	56	52
SMP	12	17	28
LTRF	4–5(2–5)/3(1)	7(2–7)/3(1)	6(2–6)/3(1)
UTR_1	128	208	99
UTR_2	41	70	71
UTR_3	32	53	64
UTR_4	19	48	45
UTR_5	9+	36	38
UTR_6	-	29	24
UTR_7	-	13	-
LTR_1	40	55	69
LTR_2	97	166	116
LTR_3	119	190	154

+ UTR_5 only on the right side of the oral disc.

Two other specimens of the series in stages 25 and 27. The latter with UTR_7 on the right side, 72 marginal papillae, two submarginal papillae on each side of the upper lip and 9–10 on the lower lip. The other specimen with 55 marginal papillae, three on each side of the upper lip and 8–9 on the lower lip. Coloration, general and oral disc morphology agree with the DNA voucher specimen.

Guibemantis tornieri (Ahl, 1928)

Note. Previous tadpole descriptions for this species were from specimens collected at the East Coast of Madagascar (Foulpointe) where *G. tornieri* does not occur. These descriptions almost certainly refer to *G. timidus* (see below).

Series examined: ZSM 424/2004 (fieldnumber 2002.1740; DQ054494) series of two specimens from Ranomafana village collected in an artificial pond near the "Hotel Thermal" (21°15.602S/47°27.031E, 293 m above sea level) on 25 January 2003.

Description is based on the DNA voucher from this series. For morphometric data see

tables 3 and 4. Tadpole in stage 26. Colour in preservative brown, with small dorsal patches. The intestinal wall is also slightly pigmented and the intestinal spiral visible. Ventral part of the body lacks pigmentation. Anterior part of the tail with transverse stripes, but the rest of the tail and caudal fin are missing because of tissue sampling. Tadpole type IV (Orton 1953). Body shape oval (fig. 4a and b). Snout in dorsal view almost rounded. Eyes medium-sized (about 0.1 of body length) dorsally positioned. Spiracle difficult to recognize. Oral disc laterally emarginated, completely ventral, but

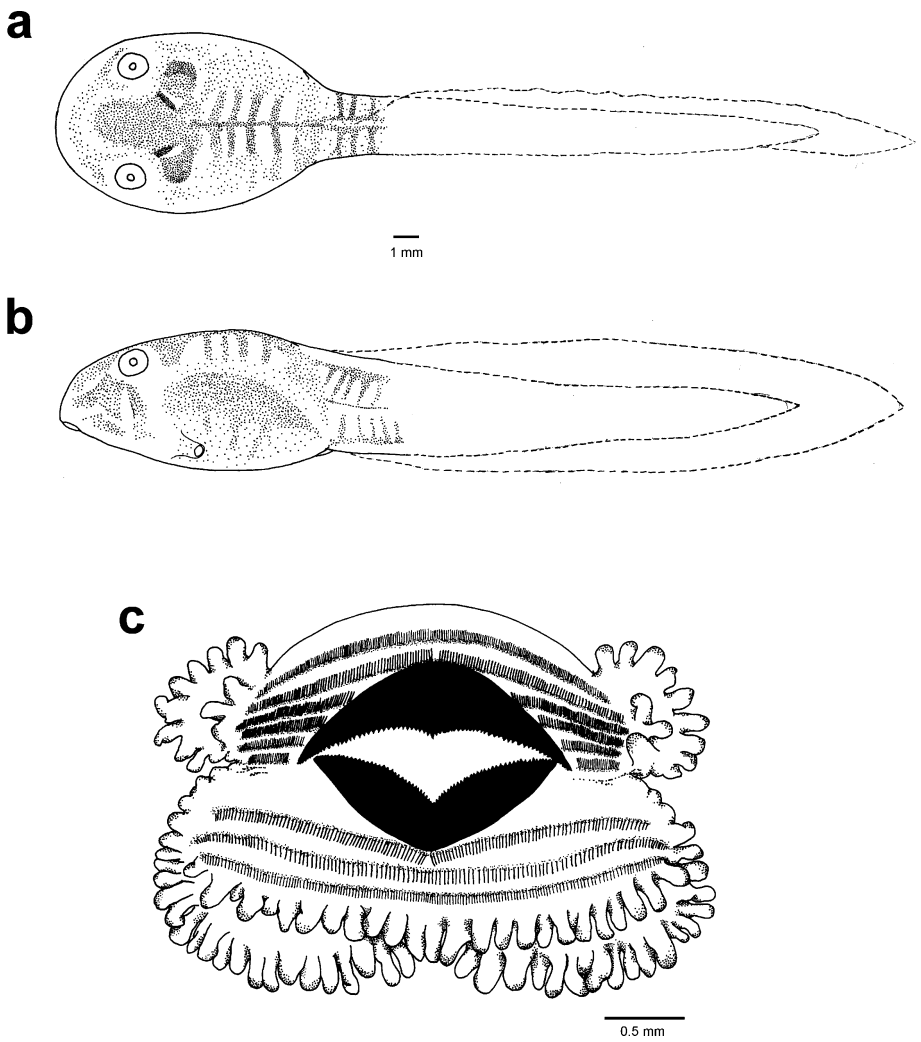

FIGURE 3. Drawings of the preserved DNA voucher tadpole of *Guibemantis kathrinae*, series ZSM 460/2004; (a) dorsal view, (b) lateral view, (c) oral disc.

localized in the exact end of the snout. (fig. 4c). Dorsal gap of the marginal papillae 1.5 mm. Oral disc bordered by 41 (˜0.1 mm) marginal papillae except a short part near the

emargination. One row of 10 submarginal papillae on the lower lip and one on each side of the upper lip. LTRF 1:3+4/1+1:2 (Dubois 1995) or [4–5](2–5)/3(1) (Altig & McDiarmid 1999); UTR_1 and LTR_3 with thinner and more teeth per millimetre (ca. 70–80/mm) than the rest (ca. 55–64/mm) and becoming shorter at the borders. From UTR_2 on, they are separated by the upper jaw sheath. UTR_5 only present on the right side but with big teeth (only 7/mm) LTR_1 with a short gap. Jaw sheaths distinct and black, with serrated edges (ca. 60 on each jaw). Sheath of upper jaw gently curved in the middle, and lower jaw sheath resembling a wide V.

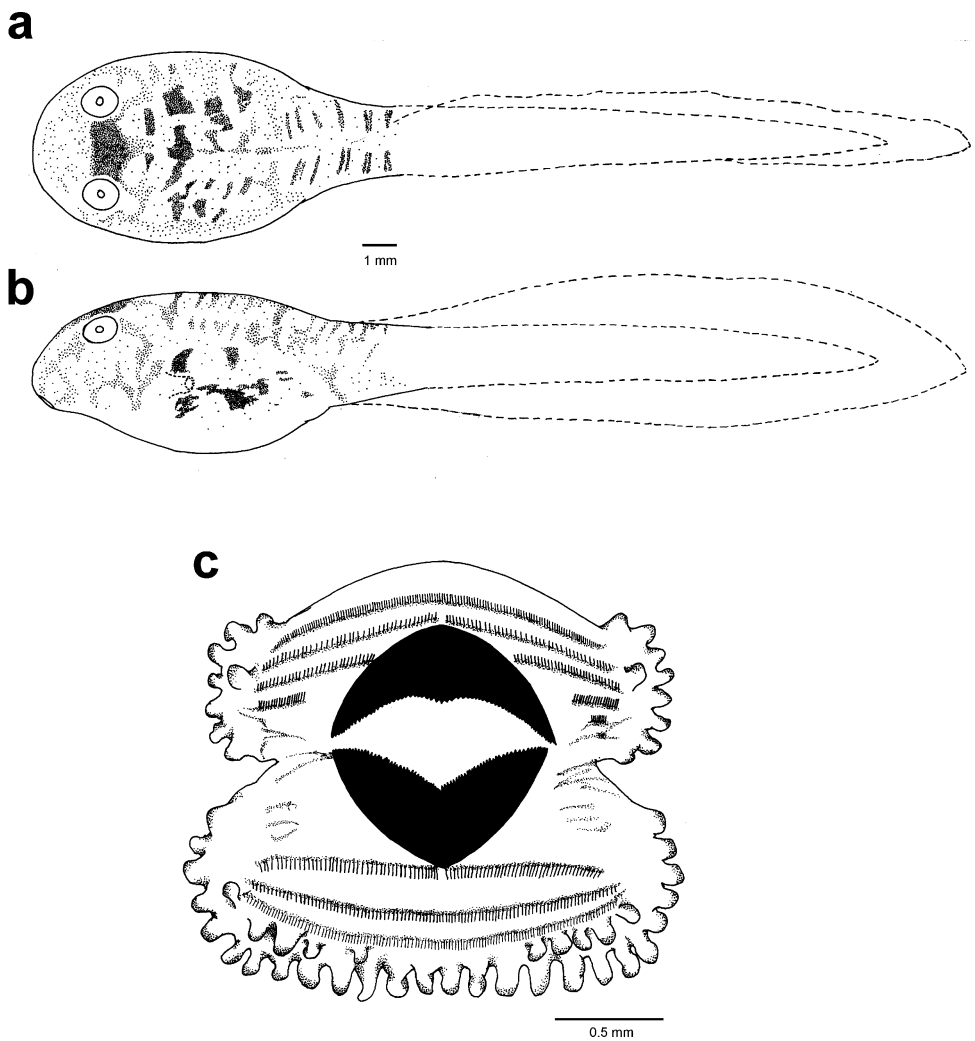

FIGURE 4. Drawings of the preserved voucher tadpole of *Guibemantis tornieri*, series ZSM 424/2004; (a) dorsal view, (b) lateral view, (c) oral disc.

Second specimen of the series in stage 25; LTRF 5(2–5)/3(1); 64 marginal papillae; 2–4 submarginal papillae on each side of the upper lip and 12–13 on the lower lip. Coloration, general and oral disc morphology similar to the DNA voucher specimen.

***Guibemantis timidus* Vences & Glaw, 2005**

Tadpoles of this species have been described by Blommers-Schlösser (1979) under the name *G. tornieri*. The specimens had been collected in Foulpointe (sea level) at the East Coast of Madagascar. They had body lengths of 3.5–4 mm in stage 25, 8 mm in stage 27 and measured up to 15 mm in stages 31–40; general body shape as a typical type IV tadpole (Orton 1953), oral disc laterally emarginated and bordered with papillae except for a wide dorsal gap; LTRF 1:4+4/1+1:2 to 1:5+5/1+1:2 (Dubois 1995) or 5(2–5)/3(1) to 6(2–6)/3(1) (Altig & McDiarmid 1999). For a more complete description see Blommers-Schlösser (1979).

Discussion

With the data provided herein, the tadpoles of all species currently in the subgenus *Guibemantis* have been described, providing a basis for morphological comparisons and evaluation of character variability. They also allow for a detailed comparison with the tadpole of *Guibemantis (Pandanusicola) liber* which had previously been placed into the subgenus *Guibemantis* (Glaw & Vences 1994).

The examined tadpoles of *G. tornieri*, *G. depressiceps* and *G. kathrinae* show clear differences to those of *G. liber* in having a higher number (5 or more) of upper tooth rows, although according to Blommers-Schlösser (1975), the tadpoles of *G. liber* could also have five UTRs. The tadpole of *G. liber* also differs from those of the other species in the shape of the sheath of the upper jaw, which is more rounded versus pointed. *Guibemantis liber* had formerly been assigned to the *Mantidactylus pulcher*-group (Blommers-Schlösser 1979), now subgenus *Pandanusicola* (Glaw & Vences 1994), but it was transferred to the *M. depressiceps* group, now genus *Guibemantis*, by Glaw and Vences (1992) based on its breeding in ponds (not in phytotelmes) and its large relative hand length (derived character of what is now the subgenus *Guibemantis*). Glaw *et al.* 2000 suggested that, based on femoral gland morphology, *G. liber* differs from *Pandanusicola*. Molecular evidence has recently shown that *G. liber* is more closely related to *Pandanusicola*, in agreement with the original assumption of Blommers-Schlösser (1979) and Blommers-Schlösser & Blanc 1991). Hence, the separate status of *G. liber* from other species in the subgenus *Guibemantis* is also supported by tadpole morphology in agreement with its placement into the subgenus *Pandanusicola* (Glaw & Vences 2006), although, on the other hand, its tadpoles also lack resemblance to the elongated, specialized phytotelmic tadpoles of other

Pandanusicola as described by Blommers-Schlösser (1979). Nevertheless, from molecular data it seems clear that *G. liber* belongs into the subgenus *Pandanusicola*, and may even be nested within that lineage (Lehtinen & Nussbaum 2003; Vences *et al.* 2003; Lehtinen *et al.* 2004; Glaw & Vences 2006).

The tadpole of *Spinomantis elegans* Guibé, 1974, a species previously placed within the subgenus *Guibemantis* of the genus *Mantidactylus* (Blommers-Schlösser & Blanc 1991; Glaw & Vences 1994; Andreone *et al.* 1998) has a morphology similar to the other species of the group, except for the dark pigmentation which is an adaptation to montane habitats (Thomas *et al.* 2005); despite of this, molecular data confirm that *S. elegans* does not belong to *Guibemantis* but to the genus *Spinomantis* (Vences & Glaw 2005).

The tadpole of *G. kathrinae* appears to have a higher number of submarginal papillae on the lower lip as compared to those of other *Guibemantis*, but since the number of these papillae is highly variable within species we do not consider this as reliable diagnostic character. The coloration pattern of the body is very similar in all examined specimens of the species; it is slightly similar to the coloration of *G. tornieri* and different from that of *G. depressiceps*; another difference to the latter species is the number of tooth per millimetre (between 40 and 100 fewer teeth) in *G. kathrinae* in UTR_1, LTR_2 and LTR_3.

The most distinctive characteristic of *G. depressiceps* is the elevated number of teeth per millimetre in all tooth rows. A further character, a higher number of upper tooth rows (seven), is present only in the DNA voucher specimen. All other specimens in the series as well as those examined by Blommers-Schlösser (1979) have five or six upper tooth rows. In every other respect, general and oral disc morphology, the DNA voucher of *G. depressiceps* agrees with the description of the specimens examined by Blommers-Schlösser (1979). It has a very uniform coloration, in this being different from *G. tornieri* and *G. kathrinae*, but similar to *G. liber*.

The tadpole of *G. tornieri* largely agrees with the descriptions published by Blommers-Schlösser (1979) which however referred to the recently described *G. timidus* (Vences & Glaw 2005). The only recognizable difference is the coloration that appears to be more uniform in the drawings of *G. timidus*. The presence of a distinct color pattern in both *G. tornieri* and *G. kathrinae* tadpoles is in agreement with the close molecular relationships of these species (Vences & Glaw 2005).

Despite the presence of a relatively high genetic differentiation among *G. liber* specimens from central eastern and south-eastern Madagascar (3.4% pairwise sequence divergence in the 16S rRNA gene; sequence accession numbers AY848080–AY848088), the tadpoles examined of this species show no consistent differences. There is high variability in the number of marginal and submarginal papillae and in the LTRF. Coloration and general shape of the body and oral disc is very similar in all tadpoles of the different series, except series ZSM 466–467/2004 from An'Ala forest in central eastern Madagascar, where pigmentation is a little lighter the rest. In all species of the subgenus *Guibemantis*, LTRFs vary among individuals, and might not be a distinctive character to

separate species. Only in *G. kathrinae* all three specimens examined have the same LTRF 6(2–6)/3(1). As a conclusion, many of the characters examined here appear to be highly variable even among individuals of the same series, not to mention among series (in the case of *G. liber*). This is certainly in part due to differences in developmental stages, but can also be a consequence of environmental plasticity (e.g., Vences *et al.* 2002 and references therein). A reliable and fast morphological identification of tadpoles in *Guibemantis* to species appears difficult, and the use of DNA barcoding methods at a larger scale will therefore be necessary to understand more in detail whether these largely sympatric larvae show differences in ecology.

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