SALAMANDRA **48**(3) | 147–156 | 30 October 2012 | ISSN 0036–3375

Tadpole descriptions of three *Cardioglossa* species from southwestern Cameroon (Amphibia: Anura: Arthroleptidae)

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Manuscript received: 9 March 2012

Abstract. Tadpoles of *Cardioglossa* were collected in streams of the Mount Manengouba region, southwestern Cameroon during the rainy seasons of 2010 and 2011. We examined our samples together with material collected by J.-L. Perret from the same region. Within *Cardioglossa* tadpoles, we distinguished three different morphotypes. We assigned these morphotypes to known *Cardioglossa* species in that region by applying DNA-barcoding (16S rRNA): *C. melanogaster*, *C. pulchra*, and *C. manengouba*. The tadpoles of *C. pulchra* and *C. melanogaster* are described for the first time. Additionally, the existing description of *C. manengouba* tadpoles is supplemented with our new data. We argue that published morphological data and drawings of *C. gracilis* tadpoles are inconclusive and might represent *C. melanogaster*. The *Cardioglossa* tadpoles described herein are exotrophic and share several morphological characters, like a long muscular tail with narrow fins, a long spiracle, and mouthparts lacking any labial tooth rows. Nevertheless, the body shape, length and position of the spiracle, and the number and shape of the conspicuous papillae at the posterior lip differ among the examined species. These traits enable the identification of *Cardioglossa* species already at larval stage.

Key words. Cameroon highlands, *Cardioglossa manengouba, Cardioglossa melanogaster, Cardioglossa pulchra*, DNA-barcoding, Mount Manengouba, larval morphology.

Introduction

The genus *Cardioglossa* Boulenger, 1900 currently comprises 16 species, which occur in tropical sub-Saharan Africa (Amiet 1972a, Blackburn 2008a, Blackburn et al. 2008). All species live close to rainforest streams (Amiet 1972a, b; Rödel et al. 2001), and it is presumed that tadpoles are exotrophic and adapted to a free-living lifestyle, although the tadpoles of most species of the genus *Cardioglossa* are still unknown. Descriptions and/or illustrations are so far only available for *C. occidentalis* Blackburn, Kosuch, Schmitz, Burger, Wagner, Gonwouo, Hillers & Rödel, 2008 (Lamotte 1961 as *C. leucomystax*), *C. gracilis* Boulenger, 1900 (Perret 1966), and *C. manengouba* Blackburn, 2008 (Blackburn 2008b).

The Cameroon volcanic line is the area with the highest *Cardioglossa* species diversity. This applies in particular to the Nkongsamba-Mount Manengouba region in southwestern Cameroon from where 10 species have been recorded (Amiet 1972a & b, 1975, Blackburn 2008a, own unpubl. data), i.e., *C. elegans* Boulenger, 1906, *C. gracilis*, *C. gratiosa* Amiet, 1972, *C. leucomystax* Boulenger, 1903, *C. manengouba*, *C. melanogaster* Amiet, 1972, *C. nigromaculata* Nieden, 1908, *C. pulchra* Schiøtz, 1963, *C. trifasciata* Amiet, 1972, and *C. venusta* Amiet, 1972.

Species of the genus *Cardioglossa* occur in forested areas and appear to cope with threats like forest loss and agricultural expansion to different extents (own unpubl. data). Some species like, e.g., *C. pulchra* and *C. melanogaster* are already listed in the IUCN category Endangered (Schiøtz & Amiet 2004), additionally, the species *C. trifasciata* and *C. manengouba* are endemic to the Mount Manengouba (Amiet 1972a, Blackburn 2008b). Only the presence of tadpoles can actually confirm if a stream is appropriate for the reproduction of a species. It follows that the larval stage of a species needs to be known by its morphological characters.

We collected tadpoles in various rivers at Mount Manengouba (Fig. 1) in 2010 and 2011, including those of different *Cardioglossa* species. Herein we describe for the first time the tadpoles of *C. pulchra* and *C. melanogaster*, provide additional morphological details of *C. manengouba* tadpoles, and compare the known tadpoles within this genus.

Material and methods

The tadpoles were collected with dip nets by Mareike Hirschfeld on Mount Manengouba in 2010 (November and December) and 2011 (August to October). Immediately after collection, the tadpoles were anaesthetised with chlorobutanol. Tail tissue samples were taken and stored in ethanol (96%) for genetic analysis. The tadpoles were preserved in 8% formalin and subsequently stored in 75% ethanol. All collected individuals are stored at the Museum für Naturkunde Berlin (ZMB). Additionally, we examined tadpoles from the Museum of Natural History in Geneva (MHNG), collected by J.-L. Perret in 1960. According to Perret (1966), the respective specimens were the larvae of *C. gracilis*.

Tadpoles were identified using a DNA-barcoding approach, i.e., by analysing sequences of 422-550 *bp* of mitochondrial 16S ribosomal RNA. DNA was extracted using High Pure PCR Template Preparation kits (Roche). We used the primers 16sar-L and 16sbr-H to amplify the 16S rRNA gene (Palumbi 1996). Standard PCR protocols were used and PCR products were purified using the High Pure PCR Product Purification kit (Roche). Purified templates were directly sequenced using an automated sequencer (ABI 3100). Raw chromatograms were displayed and sequences validated using Chromas 2.31 (Technelysium Pty Ltd, Helensvale, Australia). Sequences were aligned with the Clustal option of the software BioEdit (Hall 1999) and subsequently adjusted manually. Uncorrected pairwise sequence divergences were calculated using PAUP* 4.0b10 (Swofford 2002). We assigned the tadpoles to species known from adults in the region, stored at the Museum für Naturkunde Berlin (ZMB) and Museum of Comparative Zoology (Harvard University, MCZ). The respective DNA sequences of these adults have already been published (Blackburn 2008a, Blackburn et al. 2008). Individuals included in the DNA-barcoding approach are listed in Tab. 1.

The tadpole descriptions are based on the genotyped specimens and additional individuals of the respective developmental stage and morphotype (see e.g. Strauss et al. 2010). The following measurements were taken from each voucher: TL: total length (comprising body and tail length), BL: body length, TAL: total tail length, BH: maximum body height, BW: maximum body width, ED: eye diameter, IOD: interorbital distance, IND: internarial distance, RN: nostril–snout distance, NP: eye–nostril distance, SE: eye–snout distance, ODW: oral disc width (including papillae), ESPD:

Figure 1. Habitats of *Cardioglossa* tadpoles on Mount Manengouba, Cameroon; a) stream where we recorded larval *C. pulchra* (05°03'27.9" N, 009°49'39.0" E, 1,719 m a.s.l.); b) larval habitat of *C. melanogaster* (05°00'46.5" N, 009°46'05.8" E, 1,372 m a.s.l.); c) one of three rivers where *C. manengouba* tadpoles were collected (05°01'04.6" N, 009°51'54.9" E, 2,100 m a.s.l.).

Table 1. *Cardioglossa* specimens and GenBank data analysed in our 16S DNA-barcoding analysis.

Species	Collection Number	GenBank Number	Stage	Origin	References
C. manengouba	ZMB 77587	JQ711153	tadpole	Cameroon	this study
C. manengouba	ZMB 77588	JQ711154	tadpole	Cameroon	this study
C. manengouba	ZMB 77592	JQ711155	tadpole	Cameroon	this study
C. manengouba	ZMB 77593	JQ711156	tadpole	Cameroon	this study
C. manengouba	ZMB 77594	JQ711157	tadpole	Cameroon	this study
C. manengouba	ZMB 77596	JQ711158	tadpole	Cameroon	this study
C. manengouba	MCZ A 137909	FJ151121	adult	Cameroon	Blackburn 2008a
C. manengouba	MCZ A 137910	FJ151149	adult	Cameroon	Blackburn 2008a
C. melanogaster	ZMB 77621	JQ711160	tadpole	Cameroon	this study
C. melanogaster	ZMB 77607	JQ711161	tadpole	Cameroon	this study
C. melanogaster	ZMB 77609	JQ711162	tadpole	Cameroon	this study
C. melanogaster	ZMB 77608	JQ711163	tadpole	Cameroon	this study
C. melanogaster	ZMB 77612	JQ711164	tadpole	Cameroon	this study
C. melanogaster	ZMB 77623	JQ711165	tadpole	Cameroon	this study
C. melanogaster	ZMB 77610	JQ711166	tadpole	Cameroon	this study
C. melanogaster	MCZ A 137907	FJ151120	adult	Cameroon	Blackburn 2008a
C. melanogaster	MCZ A137905	FJ151145	adult	Cameroon	Blackburn 2008a
C. melanogaster	MCZ A 137906	FJ151146	adult	Cameroon	Blackburn 2008a
C. pulchra	ZMB 70426	EF640995	adult	Cameroon	Blackburn et al. 2008
C. pulchra	ZMB 70427	EF640996	adult	Cameroon	Blackburn et al. 2008
C. pulchra	ZMB 70429	EF640997	adult	Cameroon	Blackburn et al. 2008
C. pulchra	ZMB 70423	EF640999	adult	Cameroon	Blackburn et al. 2008
C. pulchra	ZMB 70424	EF641000	adult	Cameroon	Blackburn et al. 2008
C. pulchra	ZMB 77633	JQ711159	tadpole	Cameroon	this study
C. pulchra	MCZ A 137924	FJ151134	adult	Cameroon	Blackburn 2008a
C. pulchra	MCZ A137916	FJ151126	adult	Cameroon	Blackburn 2008a

eye-end of spiracle distance, SP: spiracular tube length (distance from skin fold to posterior end of tube), ESP: eye–spiracle skin fold distance, TMH: tail muscle height (measured at body/tail junction, termed also "tail axis" in the text), TMW: tail muscle width (measured at body/tail junction), MTH: maximum tail height (tail muscle height including dorsal and ventral fins). Measurements including eyes and nostrils were taken from their respective centres. Tadpole stages were determined following Gosner (1960). Morphological terminology of tadpoles is based on Altig & McDiarmid (1999) and Dubois (1994). Photographs of tadpoles are multifocus-photos of preserved specimens (see e.g. Lohrmann & Ohl 2010). They were taken with a Leica® DFC420 digital camera on a Leica® MZ 95 dissecting microscope. Single exposures were combined using the Automontage® software version 5.03.0061 (Syncroscopy).

Results

All collected *Cardioglossa* tadpoles could be assigned to one of three distinctly different morphological types. According to the 16S sequences of adult *Cardioglossa* collected in the study area (see Tab. 1), the tadpoles could be identified as *C. melanogaster* (sequence divergence between tadpole and adult: 0.00–0.69%, $N_{adult} = 3$, $N_{tadpole} = 10$), *C. pulchra* (sequence divergence 0.00–0.45%, $N_{adult} = 1$, $N_{tadpole} = 7$), and *C. manengouba* (sequence divergence 0.00–0.18%, $N_{adult} = 2$, $N_{tadpole} = 6$), respectively (Tab. 1).

Cardioglossa melanogaster

Material examined (tadpole series including genotyped specimens marked with an *) – We examined 26 tadpoles from the Mount Manengouba area: ZMB 77607 (tadpole* Gosner stage 25, Ebonemin, 5°01'33.4" N, 9°45'46.7" E, 1,356 m a.s.l., 13 December 2010); ZMB 77608 (tadpole* Gosner stage 27, Ebonemin, 5°00'33.0" N, 9°46'30.0" E, 1,365 m a.s.l., 14 December 2010); ZMB 77609 (10 tadpoles*, Gosner stages 25–27, Ebonemin, 05°00'46.5" N, 009°46'05.8" E, 1,372 m a.s.l., 16 December 2010); ZMB 77610 (tadpole* Gosner stage 34, Ebonemin, 5°01'16.8" N, 9°52'53.2" E, 1,459 m a.s.l., 8 October 2011); ZMB 77612 (tadpole* Gosner stage 25, M'Bouroukou, 05°04'03.5" N, 009°51'56.2" E, 1,492 m, 22 September 2011); ZMB 77621 (tadpole* Gosner stage 31, Pola, 5°03'27.8" N, 9°50'08.9" E, 1,742 m a.s.l., 3 December 2010); ZMB 77622 (Figs. 2a, 3a

Figure 2. Lateral views of *Cardioglossa* tadpoles; a) *C. melanogaster* (ZMB 77622, Gosner stage 35); b) *C. pulchra* (ZMB 77634, Gosner stage 29); c) *C. manengouba* (ZMB 77589, Gosner stage 27); scale bars = 2 mm.

Figure 3. Dorsal views of *Cardioglossa* tadpoles; a) *C. melanogaster* (ZMB 77622, Gosner stage 35); b) *C. pulchra* (ZMB 77634, Gosner stage 29); c) *C. manengouba* (ZMB 77589, Gosner stage 27), scale bars = 2 mm.

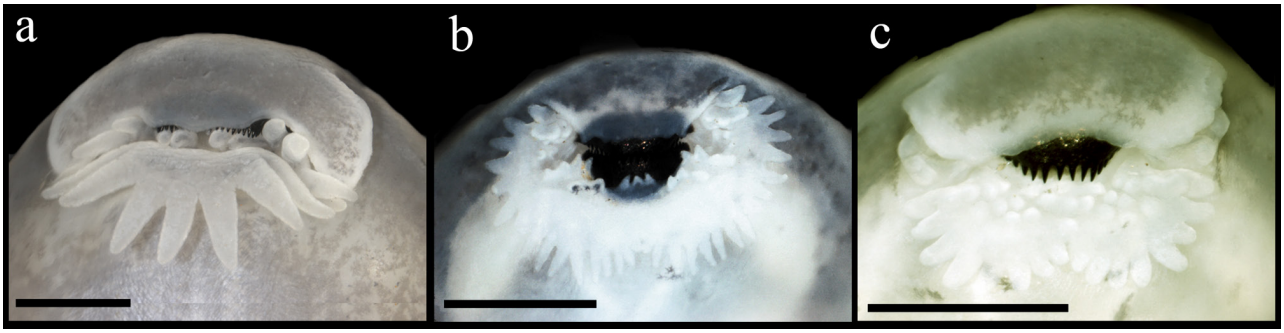

Figure 4. Mouth parts of *Cardioglossa* tadpoles in ventral views; a) *C. melanogaster* (ZMB 77622, Gosner stage 35); b) *C. pulchra* (ZMB 77634, Gosner stage 29); c) *C. manengouba* (ZMB 77589, Gosner stage 27); scale bars = 1 mm; the colour balance of b and c was modified to enhance the contrast of the papillary structures.

& 4a, Gosner stage 35, same site and date as ZMB 77621); ZMB 77623 (tadpole* Gosner stage 36, Pola, 05°03'27.9" N, 009°49'39.0" E, 1,719 m a.s.l., 20 October 2011); MHNG 1039.094 (9 tadpoles, Gosner stages 24–35, Nsoung, J.-L. Perret 1960).

The tadpoles were collected in low- to mid-altitude streams within forest fragments and strongly degraded areas with farms nearby. We found all *C. melanogaster* tadpoles in those parts of the streams where the current was very slow (Fig. 1b). We collected the specimens during the day when most individuals were hiding under stones or dead leaves in the riverbed. They frequently co-occurred with larvae of *Leptodactylodon mertensi* Perret, 1959.

The description of *C. melanogaster* tadpoles is based on two individuals at Gosner stage 27 (from tadpole series ZMB 77608 and 77609; see Tab. 1). The first values refer to a genotyped individual (tadpole from series ZMB 77608); proportions including total or tail lengths are only available for non-genotyped individuals. Body robust and elongated in dorsal and lateral views (Figs. 2a, 3a), body length -/26.8% of total length; body height 42.2/44.4% of body length; body width 51.1/55.6% of body length; maximum body width on level of eyes; snout semicircular in dorsal view; nostrils hardly visible at earlier Gosner stages, small and oval and situated dorsally; nostrils closer to snout tip than to eyes (RN/NP = 0.63/0.45), distance snout-nostrils 13.3/10.0% of body length; eyes positioned dorsolaterally; eye diameter 3.3/5.5% of body length; interorbital distance exceeds the internostril distance by a factor of 1.5/1.7; tail long (-/74% of total length) with narrow fins; dorsal fin originating just posterior to half of tail length; ventral fin originates at tail base; fin height along first two thirds of tail higher in ventral part, equally high in last third of tail; maximum tail height including fin equal to body height; muscular part of the tail axis broad and muscular, narrowing towards tail tip after about two thirds of tail length; maximum height of tail axis 69/72% of total tail height; fin tip rounded; median vent tube; spiracle sinistral, visible in dorsal view, originating at the posterior third of the body; spiracle tube length 31.1/36.7% of body length; mouth opens anteroventrally; oral disc width (Fig. 4a) half of body width (54.3/52.0%); labial tooth row formula 0/0; upper jaw distinctly and strongly serrated, heavily keratinised part forms a semi-circle; lower jaw also distinctly serrated, but less pronounced in comparison to upper jaw; heavily keratinised part forms a semicircle as well; anterior lip bulging, without papillae, overlaps upper jaw almost completely; posterior lip dominated by a single row of long papillae; body and tail colour almost uniform light brownish with white dots all over (visible in live and preserved individuals); fin faintly dotted, like posterior part of tail; spiracle translucent; shiny in life. Measurements of all examined specimens are provided in Tab. 2.

Cardioglossa pulchra

We examined six tadpoles from one stream in the Mount Manengouba area (tadpole series including genotyped specimens marked with an *): ZMB 77633 (5 tadpoles*, Gosner stages 25–28, 05°03'27.9" N, 009°49'39.0" E, 1,719 m a.s.l., 3 December 2010), ZMB 77634 (Figs. 2b, 3b & 4b, Gosner stage 29, same location and date as ZMB 77633).

The mid-altitude stream, from which our tadpoles were collected, was dominated by several small cascades. The banks of the small river were heavily overgrown by weeds that completely covered the water surface at the beginning of the dry season (Fig. 1a). The surroundings consisted of typical farmbush vegetation and there were no trees growing on the riverbanks. All *Cardioglossa pulchra* tadpoles were collected during the day. They were located near the riverbank, hiding in the sandy mud or between stones. They shared this habitat with tadpoles of the genus *Astylosternus*.

The description is based on the genotyped individual at Gosner stage 25 (first value) and two individuals at stage 28 (latter values; all individuals ZMB 77633); proportions including total or tail length are only available for the non-genotyped individuals. Body depressed and ellipti-

Table 2. Morphometrics of *Cardioglossa melanogaster* tadpoles; G = Gosner stages; measurements in mm. The respective range is given if three or more tadpoles of the same stage were collected; n = sample size; n/a = not available; abbreviations are explained in Material and Methods; *Cardioglossa melanogaster* tadpoles formerly described as *C. gracilis* and collected by J.-L. Perret (1966; MHNG 1039.094) are added to the table.

Measurements/Gosner stage	*Cardioglossa melanogaster* (ZMB series)							*Cardioglossa melanogaster* (formerly *Cardioglossa gracilis,* MHNG 1039.094)			
G	25 min–max (n)	27	27	31	34	35	36	24	25 min–max (n)	26	35
BL	4.8–8.4 (11)	9.0	9.0	10.9	10.7	10.6	11.6	6.4	5.0–7.5 (5)	7.2	11.9
BH	2.0–3.4 (11)	4.0	3.8	4.7	4.2	4.4	5.5	2.9	2.5–3.1 (5)	3.1	5.8
BW	2.9–4.5 (11)	5.0	4.6	5.5	4.5	5.9	7.1	3.9	3.6–4.7 (5)	4.5	7.4
TL	19.2–27.3 (5)	33.6	n/a	n/a	n/a	40.6	n/a	21.8	20.0–23.6 (4)	n/a	43.6
TAL	14.4–19.8 (5)	24.7	n/a	n/a	n/a	30.2	n/a	15.3	14.5–16.2 (4)	n/a	32.1
ED	0.2–0.4 (11)	0.5	0.3	0.7	0.5	1	0.9	0.4	0.4–0.5 (6)	0.5	0.7
IOD	1.8–2.7 (11)	3.5	3.0	4.3	3.4	4.1	4.3	2.2	2.0–2.9 (6)	2.5	4.5
IND	1.0–1.8 (4)	2.1	2.0	2.4	2.2	2.5	2.2	1.4	1.2–1.8 (5)	1.5	2.4
TMH	1.5–2.5 (11)	2.7	2.8	3.8	2.7	3.4	3.6	2.1	1.8–2.4 (6)	2.3	4.5
TMW	1.1–1.9 (11)	2.3	2.3	3.7	2.5	3.2	3.3	1.4	1.2–1.8 (6)	1.5	3.6
MTH	2.0–3.4 (11)	3.9	3.9	5.7	4.0	4.9	4.3	3.0	2.5–3.1 (6)	3.0	4.7
RN	0.4–1.0 (4)	0.9	1.2	1.4	1.2	1.1	1.3	0.8	0.8–1.1 (4)	1.0	1.4
NP	1.1–1.7 (4)	2	1.9	2.4	2.4	2.4	2.7	0.6	0.6–1.8 (5)	1.8	2.4
SE	1.6–2.7 (11)	3	3.1	3.7	3.5	3.7	4.2	2.4	2.1–3.0 (5)	2.9	3.9
ODW	1.5–2.5 (11)	2.6	2.5	3.1	2.8	2.8	2.9	1.8	1.7–2.3 (5)	2.5	3.1
ESPD	3.6–6.0 (11)	7.1	6.7	9.3	4	8.6	4.6	n/a	2.5–3.5 (5)	3.0	4.9
SP	1.8–3.4 (11)	3.3	2.8	3.9	4.2	4	4.4	n/a	1.8–3.6 (3)	3.1	4.8
ESP	1.5–7.1 (11)	3.7	3.9	4.7	8.2	4.9	8.8	n/a	4.5–6.0 (3)	5.8	9.2

cal in dorsal and lateral views (Figs. 2b, 3b); body length -/28.2/29.2% of total length; body height 32.7/35.1/34.4% of body length; body width 57.1/59.6/62.5% of body length; maximum body width on the level of the spiracle insertion; snout rounded in dorsal view; nostrils round and situated dorsolaterally, closer to snout tip than eyes (RN/NP = 0.36/0.38/0.50), distance snout-nostrils 8.2/8.8/10.9% of body length; eyes positioned dorsolaterally; eye diameter 6.1/7.0/6.2% of body length; interorbital distance exceeds inter-nostril distance by a factor of 2.1/2.2/1.8; long tail (-/69/66% of total length) with moderately pronounced fins; dorsal fin originating just posterior of tail base; ventral fin originates at tail base; fin height along first half higher in ventral part, equal in second half of tail; body height 66.7/71.4/81.5% of total tail height; tail axis moderate and muscular, narrowing towards tail tip after about first half of tail length; maximum height of tail axis 54.2/53.6/55.6% of total tail height; fin tip broadly rounded; median vent tube; spiracle sinistral, barely visible in dorsal view, originating at second third of the body; spiracle tube length 20.4/21.1/30.3% of body length; mouth opens anteroventrally; oral disc width (Fig. 4b) less than half of body width (42.9/41.2/37.5%); labial tooth row formula 0/0; upper jaw distinctly and strongly serrated, heavily keratinised part forms a semi-circle; lower jaw also distinctly serrated, but less pronounced in comparison to upper jaw; heavily

Table 3. Morphometrics of *Cardioglossa pulchra* tadpoles; G = Gosner stages; measurements in mm; n = sample size; n/a = not available; abbreviations are explained in Material and Methods.

Measurements/Gosner stage						
G	25	26	27	28	28	29
BL	4.9	5.6	6.7	5.7	6.4	7.8
BH	1.6	2.1	2.4	2	2.2	3.5
BW	2.8	2.8	4.1	3.4	4	5.1
TL	n/a	19	21.8	20.2	21.9	25.2
TAL	n/a	14.3	16	14	14.4	17
ED	0.3	0.4	0.4	0.4	0.4	0.5
IOD	1.9	2.2	2.6	2.4	2.4	3.3
IND	0.9	1.2	1.4	1.1	1.3	1.7
TMH	1.3	1.6	1.8	1.5	1.5	2.1
TMW	1.2	1.3	1.6	1.3	1.7	1.8
MTH	2.4	2.6	2.8	2.8	2.7	3.3
RN	0.4	0.7	0.7	0.5	0.7	1
NP	1.1	1.3	1.4	1.3	1.4	1.8
SE	1.5	1.9	2.2	1.8	2.2	2.6
ODW	1.2	1.4	1.6	1.4	1.5	2
ESPD	2.7	2.9	3.4	3.1	3.4	4.1
SP	1	1	1.7	1.2	1.3	1.6
ESP	1.7	1.9	1.9	1.9	2.1	2.6

keratinised part also forms a semi-circle; anterior lip without papillae, overlaps upper jaw marginally; posterior lip covered densely with small papillae; body coloured greyish dorsally in preserved specimen, tail axis brownish, lighter than body, fin greyish without any pattern; spiracle translucent. Measurements of all examined specimens are provided in Tab. 3.

Cardioglossa manengouba

We examined 35 tadpoles from three different rivers in the Mount Manengouba area (tadpole series including genotyped specimens marked with an *): ZMB 77587 (1 tadpole*, Gosner 34, near summit, 5°00'35.3" N, 9°51'24.8" E, 2,135 m a.s.l., 7 November 2010); ZMB 77588 (4 tadpoles*, Gosner stages 27–28, same site and day as ZMB 77587); ZMB 77589 (Figs. 2c, 3c & 4c, Gosner stage 27, same site and day as ZMB 77587); ZMB 77592 (2 tadpoles*, Gosner stage 25–26, near summit, 05°01'04.6" N, 009°51'54.9" E, 2,100 m a.s.l., 6 August 2011); ZMB 77593 (17 tadpoles*, Gosner stages 25–29, near summit, 05°01'04.6" N, 009°51'54.9" E, 2,100 m a.s.l., 7 August 2011); ZMB 77594 (3 tadpoles*, Gosner stages 30–31, near summit, 05°02'07.6" N, 009°50'46.8" E, 2,088 m a.s.l., 25 September 2011); ZMB 77595 (2 tadpoles, both Gosner stage 29, near summit, 5°00'35.3" N, 9°51'24.8" E, 2,135 m a.s.l., 28 September 2011); ZMB 77596 (5 tadpoles*, near summit, Gosner stages 25–34, 05°01'04.6" N, 009°51'54.9" E, 2,100 m a.s.l., 29 September 2011).

The tadpoles were collected at high altitudes from rivers of small and medium sizes. The rivers were located in small secondary forest fragments (Fig. 1c). At some streams, the surrounding vegetation was strongly degraded by grazing cattle. *Cardioglossa manengouba* tadpoles were caught during daytime in parts with a less strong water current. Most tadpoles were hiding between small stones, in the sand, or under the leaf litter in the river bed. At the type locality of this species, some of the tadpoles were collected in open water. In this stream, they shared the microhabitat with *Phrynobatrachus* (undescribed species) and *Werneria bambutensis* tadpoles (Hirschfeld et al. 2012).

The description is based on two individuals at Gosner stage 28 (both from tadpole series ZMB 77587); first values refer to the genotyped individual; proportions including total or tail lengths are only available for the non-genotyped individual. Body robust and elongated in dorsal and lateral views (Figs. 2c, 3c); body length -/29.8% of total length; body height 41.0/42.6% of body length; body width 54.1/54.4% of body length; maximum body width at half of body length; snout nearly rounded in dorsal view; nostrils small, round and situated dorsally, closer to snout tip than to eyes (RN/NP = 0.38/0.33), distance snout-nostrils 8.2/8.8% of body length; eyes positioned dorsolaterally; eye diameter 6.6/5.9% of body length; interorbital distance exceeds internostril distance by a factor of 1.7/1.6; long tail (-/72% of total length) with moderately pronounced fins; dorsal and ventral fins originate at level of tail base; fin height higher in ventral fin along anterior half of tail, higher in dorsal fin in posterior half of tail; body height 78.1/87.9% of total tail height; tail axis moderate and muscular, narrowing towards tail tip after about ca. 66% of tail length; maximum height of tail axis 79.7/65.6% of total tail height; fin tip narrowly rounded; median vent tube; spiracle sinistral, not visible in dorsal view, originating at second third of the body; spiracle tube length 24.6/32.4% of body; mouth opens ventrally; oral disc width (Figs. 4c) nearly half of body width (45.9/45.5%); labial tooth row formula 0/0; upper jaw distinctly and strongly serrated; strongly keratinised (black) part forms a semicircle; lower jaw also distinctly and serrated, but less pronounced in comparison to the upper jaw; heavily keratinised part forms a semicircle as well; anterior lip overlaps upper jaw marginally; anterior lip crescentic in shape, lacking papillae; posterior lip densely covered with short and round papillae; colour of body and tail almost uniform light brownish; tail axis lighter than body; fin transparent without pattern; spiracle translucent. Measurements of all examined specimens are provided in Tab. 4.

Discussion

Tadpoles of three *Cardioglossa* species have been described: *C. occidentalis* (Lamotte 1961, tadpole described as *C. leucomystax*), known from forests in Sierra Leone to Ghana (Rödel et al. 2001, Blackburn et al. 2008), *C. gracilis* (Perret 1966), occurring in lowland forests from eastern Nigeria to Gabon, including the area studied herein (Amiet 1972a), and *C. manengouba,* which is endemic to high-altitude sites on Mount Manengouba (Blackburn 2008b). According to the description, colouration and drawing of the oral disc in Perret (1966), tadpoles of *C. gracilis* are most similar to those of *C. melanogaster* described in this paper. We therefore re-examined the tadpoles collected by J.-L. Perret near Nsoung (Mount Manengouba, MHNG 1039.094, nine tadpoles, Gosner stages 24–35, Tab. 2) in 1960 to use them as reference for a detailed species comparison. The anterior lip bulges, the posterior lip of *C. gracilis* tadpoles is dominated by few but thick and long papillae (Fig. 5a). These morphological features are also found in the larvae of *C. melanogaster* (Fig. 4a). Additionally, the body shape, fin height, as well as the position and length of the spiracle are shared features and very similar in both series (MHNG and ZMB). As our three *Cardioglossa* tadpoles distinctly differ from each other and various *Cardioglossa* species occur on Mount Manengouba in syntopy, we doubt the correct species allocation of these *C. gracilis* tadpoles, which was solely based on evidence from an ontogenetic series (Perret 1966). Instead we suggest that the MHNG tadpoles in fact belong to *C. melanogaster.* Amiet (1972a) provides a lateral-view drawing of *C. gracilis* (Fig. 5b). However, it is uncertain if he assigned this tadpole to *C. gracilis* by himself or relied on the description by to Perret (1966). Neither author applied genetic means of identification (not available at that time), and did not state

Table 4. Morphometrics of *Cardioglossa manengouba* tadpoles; G = Gosner stages; measurements in mm. The respective range is given if three or more tadpoles of the same stage were collected; n = sample size; n/a = not available; abbreviations are explained in Material and methods.

Measurements/Gosner stage									
G	25 min–max (n)	26 min–max (n)	27 min–max (n)	28	28	29 min–max (n)	30	31	34 min–max (n)
BL	5.2–7.6 (6)	8.1–9.5 (4)	6.0–9.3 (12)	6.8	6.1	7.9–9.4 (5)	8.5	8.4	8.7–9.6 (3)
BH	2.1–2.4 (6)	3.4–4.0 (4)	2.5–3.8 (12)	2.9	2.5	3.4–3.9 (5)	3.7	3.8	3.9–4.2 (3)
BW	2.7–4 (6)	4.6–5.1 (4)	3.4–5.0 (12)	3.7	3.3	4.2–5.5 (5)	4.2	5.0	5.0–5.6 (3)
TL	19.9–25.3 (5)	27.8–29.9 (3)	16.6–28.9 (11)	22.8	n/a	26.7–30.2 (4)	28.0	28.4	30.4–30.9 (2)
TAL	13.2–16.8 (5)	19.7–21.1 (3)	14.1–20.3 (11)	16.4	n/a	18.5–19.8 (4)	18.5	19.4	21.6–23.5 (2)
ED	0.3–0.5 (6)	0.4–0.6 (4)	0.3–0.5 (12)	0.4	0.4	0.3–0.6 (5)	0.5	0.6	0.6–0.8 (3)
IOD	2.0–2.8 (6)	3.2–3.5 (4)	2.2–3.5 (12)	2.4	2.2	2.7–3.7 (5)	3.0	3.2	3.5–3.9 (3)
IND	1.0–1.5 (6)	1.6–1.8 (4)	1.3–2.0 (12)	1.5	1.3	1.3–1.9 (5)	1.6	1.4	1.5–2.0 (3)
TMH	1.5–2.1 (6)	2.2–2.4 (4)	1.7–2.5 (12)	2.3	2.1	2.4–2.7 (5)	2.2	2.5	2.4–3.0 (3)
TMW	1.2–1.4 (6)	1.7–2.1 (4)	1.2–2.2 (12)	1.6	1.5	1.7–1.8 (5)	2.0	1.9	1.9–2.4 (3)
MTH	2.3–3.5 (6)	3.3–4.0 (4)	2.9–4.4 (12)	3.3	3.2	3.3–4.4 (5)	4.0	4.5	4.1–4.6 (3)
RN	0.5–0.9 (6)	0.7–1.2 (4)	0.4–1.0 (12)	0.6	0.5	0.8–1.2 (5)	1.0	0.9	1.1–1.3 (3)
NP	1.2–1.6 (6)	1.8–2.1 (4)	1.4–2.4 (12)	1.8	1.3	1.7–2.1 (5)	1.9	2.0	1.6–2.2 (3)
SE	1.7–2.5 (6)	2.8–3.0 (4)	2.0–3.0 (12)	2.4	1.8	2.8–3.0 (5)	3.1	2.9	2.9–3.1 (3)
ODW	1.3–2.1 (6)	2.1–2.3 (4)	1.3–2.4 (12)	1.7	1.5	2.0–2.5 (5)	2.1	2.2	2.1–2.6 (3)
ESPD	1.8–2.5 (6)	2.4–3.9 (4)	2.5–4.5 (12)	4.6	4.0	2.4–3.7 (5)	2.7	3.2	3.1–6.5 (3)
SP	1.5–2.6 (6)	2.3–4.3 (4)	1.5–3.1 (12)	2.2	1.5	2.4–3.5 (5)	3.3	2.9	3.0–3.6 (3)
ESP	3.2–5.5 (6)	5.7–7.9 (4)	2.2–6.6 (12)	2.4	2.5	5.7–7.1 (5)	6.0	5.8	3.6–6.9 (3)

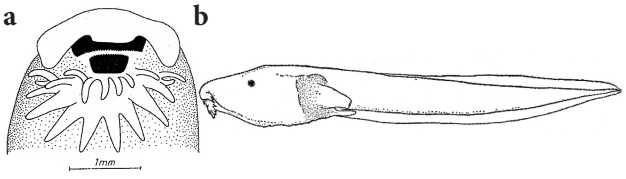

Figure 5. Drawings of *Cardioglossa* tadpoles originally described by Perret (1966) and illustrated by Perret (1966a) and Amiet (1972a, b) as *C. gracilis*; a) oral disc, individual collected by J.-L. Perret near Nsoung, Mount Manengouba in 1960; b) lateral view possibly illustrating the same individual as shown in a.

whether their tadpoles were reared from known parents, or were reared until unambiguous species identification was possible (all adult characters visible). Of course the possibility cannot be precluded that tadpoles of *C. melanogaster* and *C. gracilis* are morphologically indistinguishable. However, based on obvious differences among other tadpoles within this genus, this is very unlikely. Therefore we consider the *C. gracilis* tadpoles described by Perret (1966) and Amiet (1972a) as representing *C. melanogaster.*

Cardioglossa pulchra occurs in scattered mid-altitude patches in western Cameroon and adjacent Nigeria (Schiøtz 1963, Amiet 1972a). The tadpole of this species has hitherto been unknown. While tadpoles from Mount Manengouba show little genetic variation to each other and to adults from the same area (max. p-distance 0.45%), a sequence based on material from Mount Oku (GenBank: FJ151134) differed by a genetic divergence of 0.78-1.21% from the Manengouba vouchers. This difference might result from the geographical distance of both mountains to each other (approximately 150 km). *Cardioglossa pulchra* tadpoles differ in some characters from the other species described herein. The posterior lip of *C. pulchra* tadpoles is covered with small thin papillae that encircle two thirds of the oral disc. In *C. melanogaster,* on the other hand, the anterior lip lacks any papillae and makes up two thirds of the oral disc. The anterior lip of *C. pulchra* is less pronounced and in particular not bulging, as is the case in *C. melanogaster*. The spiracle of *C. pulchra* is shorter and originates from a more ventral position compared to *C. melanogaster*.

Blackburn (2008b) described *Cardioglossa manengouba* including morphological data of its tadpole. We collected additional individuals of this species at the type locality and further high-altitude streams on Mount Manengouba. They all agree with the description and drawings in Blackburn (2008b). The posterior lip is dominated by a high number of short, rounded and closely arranged papillae (Fig. 4c). This was less clear in the drawing provided by Blackburn (2008b, Fig. 5d in this paper). The oral disc of *C. melanogaster* possesses fewer, but longer and thicker papillae compared to *C. manengouba*. The posterior lip of *C. pulchra,* on the other hand, is equally covered with twice as many and more filamentous papillae, like in *C. manengouba* tadpoles. The anterior lip of all *C. manengouba* tadpoles is bulged in shape, in ours as well as in the individuals examined by Blackburn (2008b). The lip of *C.*

manengouba tadpoles is less bent and of an even more robust shape compared to *C. melanogaster*, i.e., bordering the mouth opening only anteriorly and not along the lateral parts of the mouth as is the case in *C. melanogaster*. Among the species examined, *C. manengouba* has the strongest, most heavily keratinised jaws with the largest serrations. In *C. manengouba* tadpoles, the spiracle originates in the last third of the body, at about mid height. The length and position of the spiracle thus further facilitates the differentiation between tadpoles of this genus, since it is much longer in *C. melanogaster* and originates more ventrally in *C. pulchra*.

The differences in body shape of the tadpoles of the three species examined are illustrated in Figs. 2 and 3. The body of *C. melanogaster* and *C. manengouba* is elongated whereas the body of *C. pulchra* is ellipsoid. In *C. melanogaster* and *C. manengouba* tadpoles, the body transforms smoothly into the muscular tail, whereas in *C. pulchra*, there are clear body and tail sections, which are distinct from each other.

Based on our results and former descriptions of *Cardioglossa* tadpoles (Lamotte 1961, Blackburn 2008b), tadpoles of this genus share several character states. These similarities have partially been mentioned by Amiet (1971) before: All tadpoles have a stream-adapted eel-like shape with long, muscular tails, narrow fins, and a long spiracle. These morphological characters may facilitate a life between stones and in the substrate of a riverbed. Most *Cardioglossa* tadpoles from Manengouba support this hypothesis, as they were collected while hiding in this type of habitat. The posterior lip is dominated by conspicuous papillae. The tadpoles lack labial teeth (0/0) and the upper and lower jaws are distinctly and strongly serrated. Unfortunately, there is currently no information on their feeding mode and food resources available.

Various species belonging to the family Arthroleptidae have tadpoles that show a lateral skin expansion in the posterior part of the body (Amiet 1970, 1971). This structure can be inflated (mechanism so far unknown) and might facilitate the movement and lifestyle of these tadpoles in habitats such as sand- and gravel-dominated stream channels. *Cardioglossa melanogaster* and *C. manengouba* likewise exhibit a laterally expanded skin fold, whereas this structure is not recognisable in *C. pulchra*. This morphological dissimilarity and the obvious differences in oral disc morphology might be indicative of the use of different microhabitats. This cannot so far be validated, though, as detailed knowledge of the microhabitats utilised and the biology of these species is lacking. Future studies on main food resources and particular characteristics of the microhabitat are needed to clarify whether the noted morphological variation is related to differences in species-specific biology.

Acknowledgements

We thank the Cameroonian Ministry of Forestry and Wildlife (MINFOF) for issuing research, collection and export permits, and Andreas Schmitz (MHNG) for sending specimens on loan. We are indebted to our local field assistants for their help during data collection. Mareike Hirschfeld was supported by scholarships from the Federal State of Berlin (Elsa-Neumann-Stipend) and the German Academic Exchange Service (DAAD). Eureka! provided the camping equipment. Further financial support was provided by the Leipzig branch of the German Society for Herpetology and Terrariology (DGHT) and the newspaper "Seesener Beobachter".

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