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Correspondence

Race against desiccation: rapid larval development in *Melanophryniscus klappenbachi* (Anura: Bufonidae)

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Klappenbach's Red-bellied Frog (*Melanophryniscus klappenbachi*) was first described by Prigioni & Langone in 2000 and is one of the currently 26 recognized species (Frost 2013) in the genus *Melanophryniscus* Gallardo, 1961. The genus is arranged in three (Cruz & Caramaschi 2003) to four species groups (Cespedez & Motte 2001). Among these, the *Melanophryniscus stelzneri* species group currently contains nine species, i.e., *M. atroluteus* (Miranda-Ribeiro, 1920), *M. cupreuscapularis* Céspedez & Alvarez, 2000, *M. dorsalis* (Mertens, 1933*), M. fulvoguttatus* (Mertens, 1937), *M. klappenbachi* Prigioni & Langone, 2000, *M. krauczuki* Baldo & Basso, 2004, *M. montevidensis* (Philippi, 1902), *M. rubriventris* (Vellard, 1947), and *M. stelzneri* (Weyenbergh, 1875).

Melanophryniscus klappenbachi is distributed in northern Argentina in the area of Chaqueña, to the province of Santiago del Estero and northward to the province of Santa Fe and in the Chaco of Paraguay (Parque Defensores del Chaco) (Céspedez & Motte 2001). It has recently been reported from Porto Murtinho, Mato Groso do Sul, Brazil (Amaral et al. 2012). Its habitats are highly seasonal in terms of humidity, providing suitable conditions for reproduction only during a few weeks per year for most species strongly depending on rainfall (Bustos Singer & Gutierrez 1997, Pereyra et al. 2011). As a special adaptation to the high seasonality, larval development is very rapid in many species of the genus that prefer ephemeral water bodies (Pereyra et al. 2011), with metamorphosis commonly being completed within 30 days, as has been reported for *M. stelzneri* (Bustos Singer & Gutierrez 1997). It is listed as 'least concern' (Aquino et al. 2004, IUCNredlist.org).

Basic information about the larval development and breeding behaviour of *M. klappenbachi* is currently not available. Herein we present our experience with breeding this species and present for the first time a detailed, illustrated description of the early embryonic stages and tadpole development of this species.

In 2011, we received a group of 12 *M. klappenbachi* from a pet shop, which had been imported from Paraguay according to the trader. The specimens were housed in an outdoor terrarium measuring 80 × 40 × 40 cm (l × w × h), outfitted with a water container of about 30 × 30 × 10 cm and wild grass, herbs and mosses taken from the garden of the ZFMK as described in Kurth et al. (2013). The specimens were transferred to a refrigerator (8°C) in October 2012 for hibernation. After 3 weeks, the specimens were transferred to an indoor terrarium (40 × 50 × 40, ¼ water of around 5 cm in depth, ¾ land outfitted with leaf litter, branches and moss cushions) in a new amphibian breeding facility at ZFMK, where it was possible to automatically emulate mist and heavy rains. After a few hours of artificial heavy rains every day and increasing humidity, calling activity started in mid-November, peaking in the morning hours, as described by Kurth et al. (2013) and two or three pairs in amplexus were observed. In the evening of 21 November 2012, we discovered spawn in the aquatic section of the terrarium (Fig. 1). The spawn was transferred to a small water container (10 × 10 × 10 cm) and a few eggs were isolated and transferred to a petri dish for detailed observation. Pictures of the eggs were taken with a Canon EOS 600D at regular intervals to monitor their growth, first manually at intervals of 30 minutes, later automatically, using the Canon EOS Utility for PC. Temperatures ranged between 23 and 25°C. One voucher egg is deposited in the herpetological collection of the Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK 94213).

To document and plot the growth of the tadpoles, we used a novel tool programmed on the open source statistics platform R (R Developmental Core Team 2013), which facilitates the semiautomatic processing of standardized im-

age files: SAISAQ (Kurth et al. 2014). Based on standardized high-resolution digital images, this technique allows to compute the surface area of a tadpole, which is highly correlated with the tadpole's body mass (Kurth et al. 2014). The technique is non-invasive and therefore most suited for repeated measuring. Eggs and tadpoles were staged according to Gosner (1960, as cited in McDiarmid & Altig 1999) from each interval photograph taken. Ten specimens at stages from 34–41 were preserved in 4% formalin for the herpetological collection of the ZFMK (ZFMK 94971).

The first eight external features of our tadpole descriptions follow Altig & McDiarmid (1999): total length (TL), body length (BL), tail length (TAL), tail musculature height (TMH), maximum tail height (MTH), internarial distance (IND, measured between the centres of narial apertures), interorbital distance (IOD, distance between the centres of pupils), and tail musculature width (TMW). The remaining characters were recorded as per Lavilla & Scrocchi (1986, as cited in Mijares-Urrutia 1998): body maximum width (BMW), body width at nostrils level (BWN), body width at eye level (BWE), body maximum height (BMH), rostro–spiracular distance (RSD, measured horizontally from the tip of the snout to the posterior edge of the tube), fronto–nasal distance (FN, from the tip of the snout to the anterior edge of the nostrils), eye–nostril distance (END, from the posterior edge of the nostril to the anterior edge of the eye), nostril major axis (N), extra nasal distance (EN, distance between the external edges of the nares), eye diameter (E), extraocular distance (EO, measured between the external edges of the pupils), oral disc width (OD, disc measured in a folded state), dorsal gap length (DG), and ventral gap length (VG). These characters are those that have been used most frequently to describe tadpoles of *Melanophryniscus* species (Ahl 1938, Fernández 1927, Garrido-Yrigaray 1989, Echeverría 1992, Bustos Singer & Gutiérrez 1997, Lavilla & Vaira 1997, Baldo & Basso 2004, Bokermann 1967, Starrett 1967, Prigioni & Arrieta 1992, Prigioni & Langone 1990, Bernardo-Silva et al. 2010; as summarized in Baldo et al. 2010). The larval description is based on 10 speci-

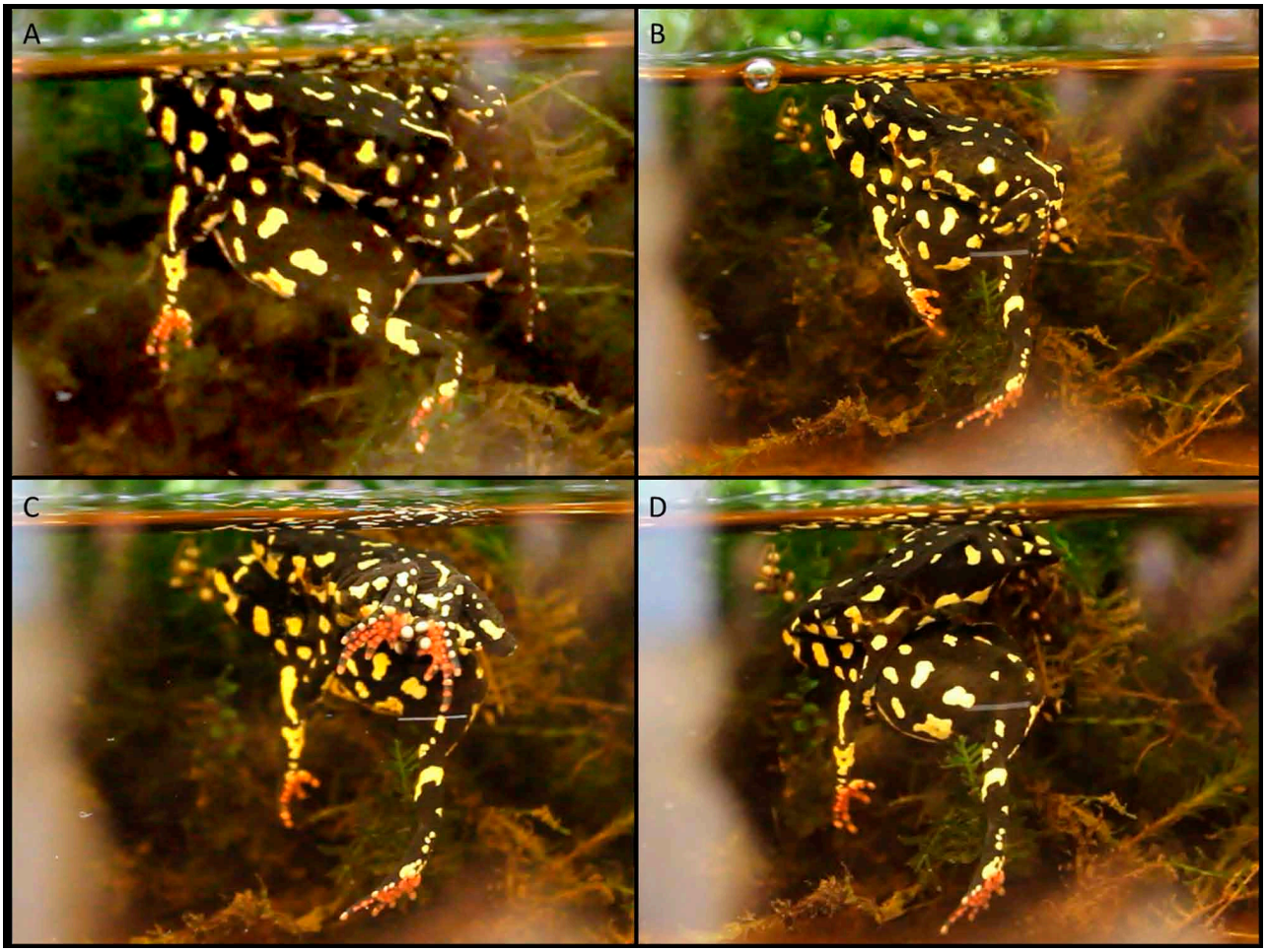

Figure 1. Detailed sequence of spawn deposition. The female crooks its back (A) and the male rubs the cloaca of the female (B). The clutch leaves the cloaca of the female and is fertilized by the male attaching the clutch to its feet (C). The male searches for submerged vegetation to attach the fertilized clutch by moving its body in half circles (C, D).

mens at stages 34–41, with the diagnosis following Baldo et al. (2010). The following measurements were taken using a binocular ZEISS Stemi 2000-C and a micrometric ocular (10 mm) and given in millimetres as (min–max) average ± standard deviation.

Summaries of the development of the spawn and the tadpoles are provided in Figure 2. Clutches comprised several smaller groups of about 10 eggs each, which were very sticky and attached to vegetation and substrate. Embryonic development was generally very fast, with Gosner stage 1–2 being reached after about 10 min. after spawning (Fig. 2A), stage 3 after 2 min., stage 4 after 43 min., stage 5 after 45 min. (Fig. 2B), stage 7 after 1 h 12 min., stage 8 after 2 h 13 min., stage 9 after 4 h 16 min., stage 10 after 5 h 18 min. (Fig. 2C), stage 11 after 14 h 12 min., stage 12 after 15 h 04 min., stage 13 after 18 h 45 min., stage 14 after 23 h 13 min., stage 15 after 1 d 0 h 58 min., stage 16 after 1 d 1 h 58 min. (Fig. 2D), stage 17 after 1 d 3 h, stage 18 after 1 d 5 h 40 min., stage 19 after 1 d 19 h 34 min. (Fig. 2E), and stage 20 after 1 d 22 h (Fig. 2F). The coloration of the eggs

Figure 2. Clutch morphology and embryonic development of *Melanophryniscus klappenbachi*. Numbers refer to the total time passed since fertilization. Gosner stage 1–2 (A), stage 5 (B), stage 10 (C), stage 16 (D), stage 19 (E), stage 20 (F).

Table 1. Morphometric data of *Melanophryniscus klappenbachi* tadpoles; measurements in mm; N = sample size; n/a = not available.

Abbreviation	Definition	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8	No.9	No.10
Gosner stage		37-38	39	34-35	39-40	36	40-41	37-38	35	36-37	38-39
TL	total length	15.26	18.00	14.22	19.20	14.90	18.30	13.05	15.15	15.75	15.15
BL	body length	5.88	7.08	6.00	7.30	6.00	7.35	6.75	5.10	6.30	5.55
TAL	tail length	9.38	10.92	8.22	11.9	8.90	10.95	9.30	10.05	9.45	9.95
TMH	tail musculature height	1.54	1.68	1.48	1.80	1.32	1.62	1.32	1.22	1.38	1.32
MTH	maximum tail height	3.92	3.94	3.06	n/a	n/a	3.50	3.24	3.01	3.12	3.45
IND	internarial distance	1.20	1.20	1.17	1.35	0.96	1.32	1.20	1.02	1.44	0.72
IOD	interorbital distance	2.60	2.64	2.16	2.79	2.40	2.85	2.76	2.25	2.64	2.22
TMW	tail musculature width	1.68	1.68	1.44	1.74	1.14	1.68	1.56	1.50	1.44	1.40
BMW	body maximum width	4.80	5.28	4.56	5.58	4.74	5.40	5.58	4.32	4.86	4.80
BWN	body width at nostrils level	2.80	2.52	2.10	2.70	1.98	2.94	2.40	2.46	2.52	2.10
BWE	body width at eye level	4.20	4.56	3.60	4.62	3.48	4.50	4.26	3.60	4.08	3.70
BMH	body maximum height	3.92	3.60	3.12	4.30	3.54	3.66	3.60	3.05	3.12	3.75
RSD	rostro-spiracular distance	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	3.40
FN	fronto-nasal distance	0.42	0.36	0.66	0.90	0.48	0.48	0.76	0.79	0.66	0.66
END	eye-nostril distance	0.28	0.32	0.12	0.40	0.15	0.36	0.30	0.36	0.24	0.30
N	nostril major axis	0.30	0.36	0.29	0.36	0.24	0.36	0.36	0.24	0.30	0.27
EN	extra nasal distance	1.90	1.44	1.38	1.62	1.32	1.62	1.50	1.26	1.38	1.29
E	eye diameter	0.96	1.02	0.72	1.10	0.54	1.08	1.02	0.91	0.72	0.93
EO	extraocular distance	2.80	3.06	2.46	3.06	2.58	3.12	2.94	2.46	2.82	2.46
OD	oral disc width	2.30	2.34	1.86	2.16	2.10	2.52	2.04	2.04	2.16	1.86
VG	ventral gap length	1.50	1.56	1.08	0.78	1.14	1.62	1.14	0.96	n/a	0.70
BL/TL		0.39	0.39	0.42	0.38	0.40	0.40	0.52	0.34	0.40	0.37
BMH/BMW		0.82	0.68	0.68	0.77	0.75	0.68	0.65	0.71	0.64	0.78
E/BWE		0.23	0.22	0.20	0.24	0.16	0.24	0.24	0.25	0.18	0.25
IOD/BWE		0.62	0.58	0.60	0.60	0.69	0.63	0.65	0.63	0.65	0.60
EO/BWE		0.67	0.67	0.68	0.66	0.74	0.69	0.69	0.68	0.69	0.66
EN/BWN		0.68	0.57	0.66	0.60	0.67	0.55	0.63	0.51	0.55	0.61
FN/END		1.50	1.13	5.50	2.25	3.20	1.33	2.53	2.19	2.75	2.20
TAL/TL		0.61	0.61	0.58	0.62	0.60	0.60	0.71	0.66	0.60	0.66
OD/BMW		0.48	0.44	0.41	0.39	0.44	0.47	0.37	0.47	0.44	0.39
VG/OD		0.65	0.67	0.58	0.36	0.54	0.64	0.56	0.47	n/a	0.38
OD/BL		0.39	0.33	0.31	0.30	0.35	0.34	0.30	0.40	0.34	0.34
VG/BL		0.26	0.22	0.18	0.11	0.19	0.22	0.17	0.19	n/a	0.13
MTH/BMH		1.00	1.09	0.98	n/a	n/a	0.96	0.90	0.99	1.00	0.92
OD/IND		1.92	1.95	1.59	1.60	2.19	1.91	1.70	2.00	1.50	2.58
OD/IOD		0.88	0.89	0.86	0.77	0.88	0.88	0.74	0.91	0.82	0.84
IND/N		4.00	3.33	4.03	3.75	4.00	3.67	3.33	4.25	4.80	2.67
EN/IOD		0.73	0.55	0.64	0.58	0.55	0.57	0.54	0.56	0.52	0.58
EN/EO		0.68	0.47	0.56	0.53	0.51	0.52	0.51	0.51	0.49	0.52
LTRF		2/3(1)	2/3	2/3	2/3(1)	2/3(1)	2/3(1)	2/3(1)	2/3	2/3(1)	2/3

changed rapidly from greyish in the upper and whitish in the lower parts to uniform dark grey. The diameter of the nuclei was about 1 mm. During the observation period, it became apparent that the relative developmental rates were highly variable even within the same clutch. The reported developmental rates therefore refer to the first egg reaching the respective stage.

After hatching, the developmental rates of the tadpoles were monitored using image-based surface analysis by photographing five randomly selected specimens at 2–3 d intervals. The total development period amounted to about 23 days with a burst within the first two weeks. External gills were resorbed within the first one or two days, limb buds became visible between days 2 and 22, forelimbs

emerged around day 19, and the tail resorption was completed about 23 days after hatching (Fig. 3). Differences in densities in the upper panel of Figure 3 can be attributed to variations in growth rates. Three photos were excluded from the final analysis due to their insufficient quality.

Tadpoles of *Melanophryniscus klappenbachi* at stages 34–41 have a total length of about 15.90 mm (13.05–19.20 ± 1.86). Their body length is slightly larger than a third of the total length (BL/TL = 0.40 ± 0.03); body slightly depressed (BMH/BMW = 0.72 ± 0.06), ventral contour of body concave in the branchial regions and convex in the abdominal region; shape ovoid in dorsal view, with the maximum body width in the posterior portion of the head or in the abdominal region; snout rounded in dorsal and lateral views, not pointed; eyes large in relation to body width (E/BWE = 0.22 ± 0.03) and placed dorsally (IOD/BWE = 0.62 ± 0.03); nostrils placed dorsolaterally (EN/BWN = 0.60 ± 0.05), closer to eyes than to snout (FN/END = 2.46 ± 1.18), slightly oval, internarial distance approximately 40% of interorbital distance; spiracle single, lateral, sinistral, short, gut loop on left side of abdominal region; vent tube opening medially; tail medium-sized (TAL/TL = 0.62 ± 0.04), slightly lower than body (MTH/BMH = 0.98 ± 0.06); tail

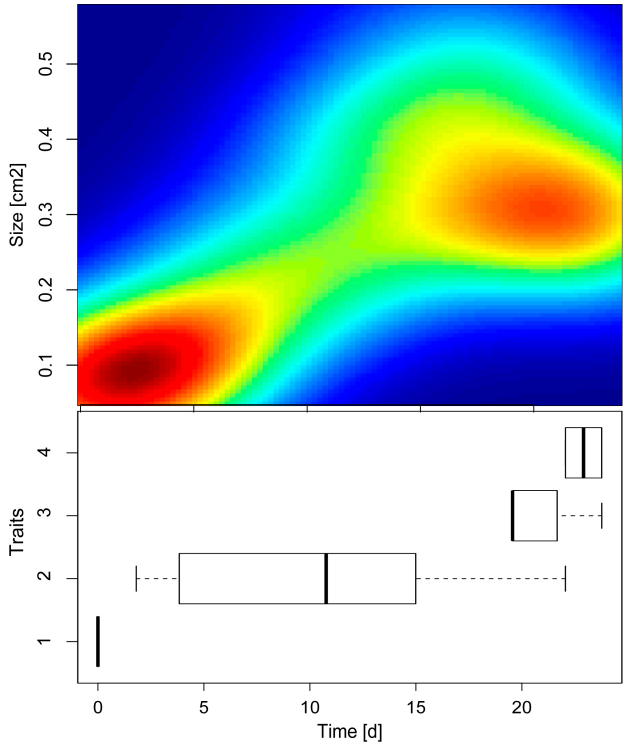

Figure 3. Temporal development of *Melanophryniscus klappenbachi* tadpoles between hatching and metamorphosis. The upper panel shows the gain in surface area as a proxy of body mass. Warmer colours indicate higher densities of samples. Note the decrease in surface gains after 15 days, which is related to tail resorption. The lower panel illustrates the temporal occurrence of the following traits: 1 = external gills present; 2 = hind limb bud development; 3 = fore limb development; 4 = tail resorption completed.

axis straight, caudal musculature clearly defined with clearly visible myotomes, becoming narrower towards tip of tail, not reaching the end of tail; fins slightly convex, dorsal fin originating on body, and ventral fin originating at the middle of the vent tube; tail tip rounded; oral disc emarginate (Figs 4–5), anteroventrally positioned, small (OD/BMW = 0.43 ± 0.04), not visible dorsally; anterior labium slightly larger than lower; ventral gap medium-sized (VG/OD = 0.54 ± 0.11); labial tooth row formula (LTRF) 2/3 (see Figs 4–5) in four specimens and 2/3(1) in six specimens.

In preserved specimens, the skin is translucent, the dorsum brown, and the venter translucent with scattered brownish spots and patches, tail muscle light brown, fins transparent with some brown vertical lines or spots, more concentrated in the ventral fin. Coloration in life: Dorsum dark grey to blackish with golden spots, venter translucent with few greyish spots, fin transparent with some greyish vertical lines, tail muscle dark grey (Fig. 5).

Measurements (in mm). TL = 13.05–19.20, 15.9 ± 1.86; BL = 5.10–7.35, 6.33 ± 0.73; TAL = 8.22–11.9, 9.90 ± 1.04; TMH = 1.22–1.80, 1.47 ± 0.18; MTH = 3.01–3.94, 3.41 ± 0.34; IND = 0.72–1.44, 1.16 ± 0.20; IOD = 2.16–2.85, 2.53 ± 0.24; TMW = 1.14–1.74, 1.53 ± 0.17; BMW = 4.32–5.58, 4.99 ± 0.42; BWN = 1.98–2.94, 2.45 ± 0.30; BWE = 3.48–4.62, 4.06 ± 0.41; BMH = 3.05–4.30, 3.57 ± 0.37; RSD (N = 1) = 3.4; FN = 0.36–0.90, 0.62 ± 0.17; END = 0.12–0.40, 0.28 ± 0.09; N = 0.24–0.36, 0.31 ± 0.05; EN = 1.26–1.90, 1.47 ± 0.19; E = 0.54–1.10, 0.90 ± 0.17; EO = 2.46–3.12, 2.78 ± 0.25; OD = 1.86–2.52, 2.14 ± 0.20; VG = 0.70–1.62, 1.16 ± 0.31. A LTRF of 2/3 was observed in four specimens, and a LTRF of 2/3(1) in six specimens.

Being well adapted to a highly seasonal environment, the tadpoles hatch after less than 48 hours (Fig. 2) and metamorphosis is completed after 10–28 days (Figs 3, 6) depending on temperature, water-level (Bustos Singer & Gutiérrez 1997) and availability of food.

The tadpoles of nine species of *Melanophryniscus* have been described by now (Baldo et al. 2010). All of these belong to the benthic ecomorphological guild (Section II: A: 1 of McDiarmid & Altig 1999). Morphologically, this is reflected in the general body shape of the tadpoles, whereas *Melanophryniscus* species with lotic breeding habitats have tadpoles that are more elongated with a smaller body width than those breeding in lentic habitats (Haad et al. 2011). Within this categorisation, tadpoles of *M. klappenbachi* can be characterized as belonging to the lentic type.

Field identification of *Melanophryniscus* tadpoles remains difficult given the currently available data. Reviewing all published tadpole descriptions, it became apparent that sample sizes were generally low with widely varying Gosner stages being analysed (Supplemental Material S1). Furthermore, morphometrics were performed based on different sampling regimes and following different authors (e.g., Altig & McDiarmid 1999, Lavilla & Scrocchi 1986) where a most comprehensive morphometric analysis should be preferred. Baldo et al. (2010) suggested differences in TL to be the best separator between *Melano-*

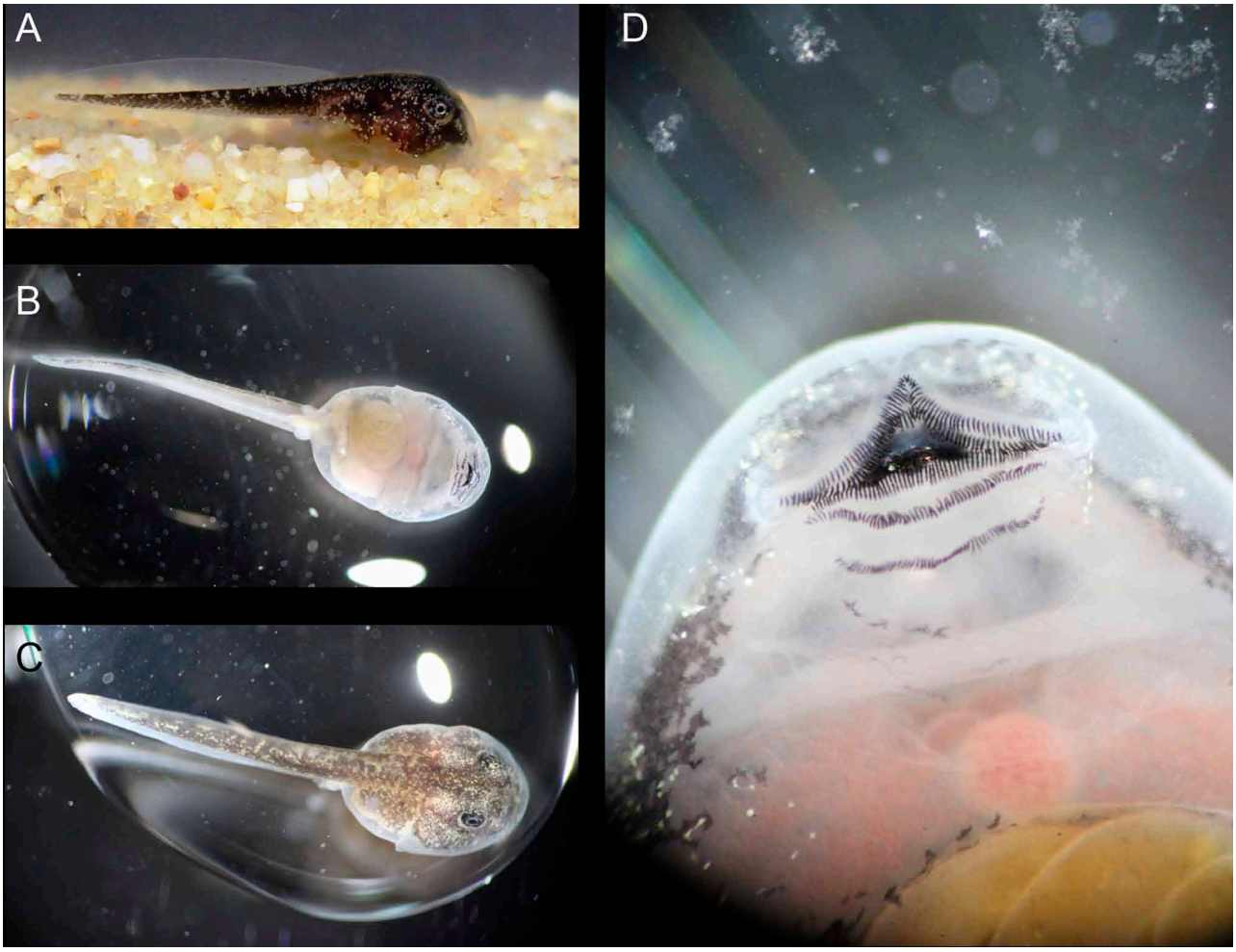

Figure 4. Lateral (A), ventral (B) and dorsal (C) view of a live tadpole of *Melanophryniscus klappenbachi*, and detailed view of the oral disc (D).

phryniscus taxa, however, this necessitates having tadpoles of comparable Gosner stages at hand. Identification is further complicated by the available published data being inconsistent. Labial tooth row formulae are also only of limited use for identification, because they may be as variable as in our study species. The only *Melanophryniscus* species that is clearly separated from *M. klappenbachi* is *M. sanmartini* (1(1)/3(1); Prigioni & Arrieta 1992 cited in Baldo et al. 2010. As phenotypic plasticity may influence tadpole morphology, habitat conditions and/or captive breeding conditions such as stagnant or flowing water bodies and water levels need to be taken into consideration for an assessment of the environmental factors that might affect the specimens studied.

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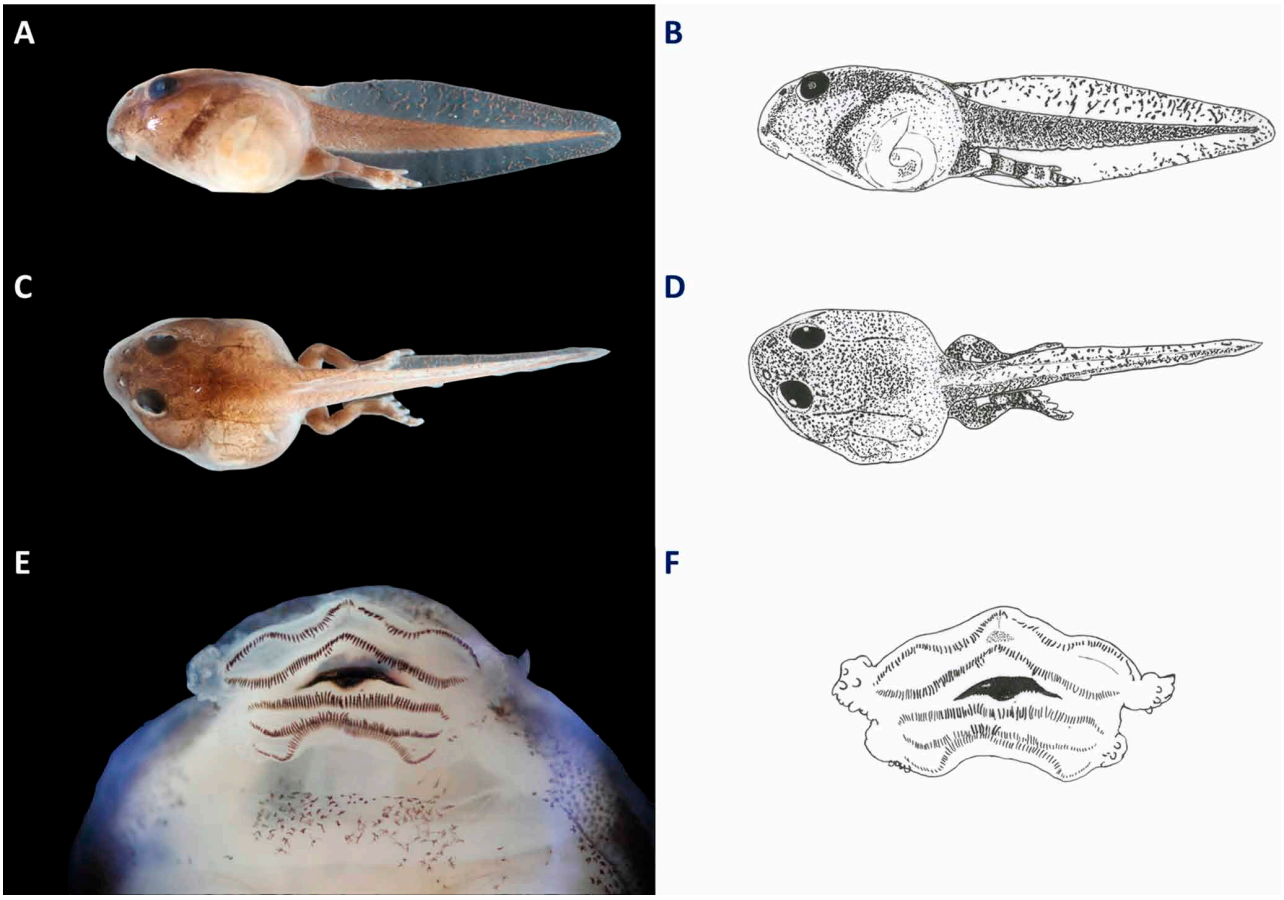

Figure 5. Pictures and drawings of preserved specimens of *Melanophryniscus klappenbachi* tadpoles. Lateral (A, B) and dorsal (C, D) views as well as a detailed view of the oral disc (E, F).

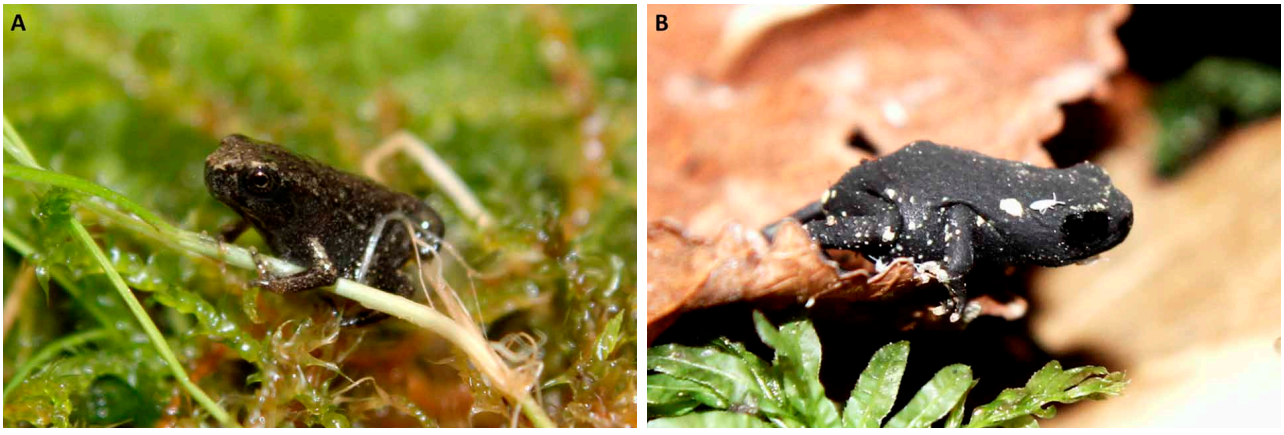

Figure 6. Froglets of *Melanophryniscus klappenbachi* approximately two months after fertilization of their eggs.

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Supplementary material

Table S1. Summary of morphometric data on *Melanophryniscus* tadpoles.

Online Supplementary data

Kurth, M., D. Hörnes & D. Rödder (2014): Race against desiccation: rapid larval development in *Melanophryniscus klappenbachi* (Anura: Bufonidae). – Salamandra, **50**(2): 117–124.

Supplementary Material S1. Summary of morphometric data on *Melanophryniscus* tadpoles. Note that the definitions of morphometric characters are not necessarily comparable among studies. References: 1 – this study; 2 – Baldo et al. (2010); 3 – Haad et al. (2011), 4 – Bernardo-Silva et al. (2010); 5 – Prigioni & Langone (1990); 6 – Garrido-Yrigaray (1989); 7 – Lavilla & Vaira (1997); 8 – Bokermann (1967); 9 – Bustos Singer & Gutiérrez (1997).

Abbreviation	*M. klappenbachi*	*M. atroluteus*		*M. cambaraensis*	*M. orejasmirandai*		*M. montevidensis*		*M. rubriventris*		*M. moreirae*	*M. s. stelzneri*	*M. sanmartini*
Reference	1	2	3	4	3	5	6	3	7	3	8	9	3
Gosner stage	34–41	31–36	31–37	30–37	31–37	36	34	31–37	31–34	31–37	39	37	31–37
N	10	15	7	16	10	1	1	6	18	4	1	1	3
TL	15.90 ± 1.86 (13.05–19.20)	12.00 (9.39–13.79)				21.30	13.80		16.70±1.16 (14.50–18.20)		22	22.4	
BL	6.33 ± 0.73 (5.10–7.35)	4.89 (3.96–5.80)	5.99±1.73	6.70±0.50	7.72±0.83	7.60	8.20	5.62±0.57	6.30±0.33 (5.70–6.80)	5.50±0.41			5.90 ±0.56
TAL	9.90 ± 1.04 (8.22–11.9)	7.20 (5.19–7.99)		11.80±1.00		13.7			10.30±0.94 (8.10–11.4)				
TMH	1.47 ± 0.18 (1.22–1.80)	0.89 (0.85–1.20)				2.50			1.35±0.12 (1.20–1.50)				
MTH	3.41 ± 0.34 (3.01–3.94)	2.69 (2.13–3.13)		3.20±0.30		4.30			3.70±0.20 (3.20–4.00)				
IND	1.16 ± 0.20 (0.72–1.44)	0.71 (0.60–0.80)		1.10±0.10		1.16			0.81±0.07 (0.70–0.90)				
IOD	2.53 ± 0.24 (2.16–2.85)	1.05 (0.75–1.30)		1.80±0.30		1.16			1.13±0.08 (1.00–1.3)				
TMW	1.53 ± 0.17 (1.14–1.74)	0.79 (0.60–0.95)											
BMW	4.99 ± 0.42 (4.32–5.58)	3.39 (2.80–3.87)		4.30±0.30		5.50	3.80		4.50±0.24 (4.10–4.9)				
BWN	2.45 ± 0.30 (1.98–2.94)	2.25 (1.93–2.53)				2.50			3.00±0.25 (2.40–3.30)				
BWE	4.06 ± 0.41 (3.48–4.62)	3.12 (2.73–3.53)				4.90			3.90±0.27 (3.40–4.30)				
BMH	3.57 ± 0.37 (3.05–4.30)	2.46 (2.00–3.20)		3.40±0.30		3.70	3.20		3.70±0.30 (3.30–4.30)				
RSD	3.40	3.45 (2.80–4.13)				5.20			4.40±0.28 (3.90–5.00)				
FN	0.62 ± 0.17 (0.36–0.90)	0.60 (0.50–0.85)				1.58			1.16±0.11 (1.10–1.30)				
END	0.28 ± 0.09 (0.12–0.40)	0.50 (0.35–0.55)				0.50			0.38±0.04 (0.30–0.40)				
N	0.31 ± 0.05 (0.24–0.36)	0.13 (0.10–0.17)				0.17			0.29±0.05 (0.20–0.40)				
EN	1.47 ± 0.19 (1.26–1.90)	1.00 (0.80–1.15)				1.50			1.37±0.09 (1.20–1.50)				
E	0.90 ± 0.17 (0.54–1.1)	0.64 (0.50–0.80)		0.70±0.10		1.00			0.69±0.07 (0.60–0.80)				
EO	2.78 ± 0.25 (2.46–3.12)	2.00 (1.60–2.30)				2.80			2.36±0.12 (2.20–2.60)				
OD	2.14 ± 0.20 (1.86–2.52)	1.75 (1.50–2.05)	1.71±0.15	2.00±0.20	2.30±0.38	2.32		1.75±0.15	1.99±0.12 (1.80–2.10)	1.79±0.11			1.72±0.35
DG		1.50 (1.25–1.85)	1.46±0.19		2.16±0.37	2.04		1.40±0.13	1.46±0.13 (1.20–1.70)	1.43±0.09			1.42±024
VG	1.16 ± 0.31 (0.7–1.62)	0.86 (0.60–1.15)	0.91±0.13		1.38±0.30	1.36		0.80±0.15	1.22±0.09 (1.10–1.40)	1.03±0.06			0.68±0.03
LTRF		2/3		2/3	2/3(1)	2/3(1)	2/3		2/3		2/3	2/3	