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Larval development and morphology of six Neotropical poison-dart frogs of the genus *Ranitomeya* (Anura: Dendrobatidae) based on captive-raised specimens

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Abstract. Larval development is a crucial step during the ontogeny of amphibians, concomitantly it is the most sensitive life phase in this group. Due to the complex morphological, physiological and anatomical changes, in addition to their susceptibility to the environment changes, this phase is known as one of the most critical period of development as well as an obstacle in ex-situ breeding programs. Tadpole growth rates can be used to predict the effects of biotic interactions, as well as to predict the survival rate on environmental changes. The assessment of the mortality rate during this phase can be performed using a non-invasive image-based tool, programmed on the open source statistical platform R, SAISAQ (semi-automatic quantification of image-based surface area). It allows analyzing semi-automatically a sequence of standardized image files in order to quantify growth rates. However, the current literature lacks estimates of the larval growth rates for the most species of amphibians, which is also true for species of the genus *Ranitomeya* Bauer, 1986. Herein, we present the data of the complete larval development of *Ranitomeya amazonica* (Schulte, 1999)*, R. benedicta* Brown, Twomey, Pepper & Sanchez-Rodriguez, 2008*, R. imitator* (Schulte, 1986)*, R. reticulata* (Boulenger, 1884)*, R. sirensis* (Aichinger, 1991) and *R. vanzolinii* (Myers, 1982), assisted by photographs, drawings and tables with detailed information about the metamorphosis. In addition, we provide a new larval description for *R. benedicta*. The results presented here also provide new data of the larval development and morphology for the target species, based on a sample series for each species. With this information, we want to contribute to a better understanding of the group and provide important data to help solve the systematic relationships puzzle. Providing also a baseline to improve further research on captive breeding, our results may have important implications for conservation breeding programs.

Keywords. Amazon, conservation, ex-situ, *Ranitomeya benedicta,* SAISAQ, tadpole.

Resumen. El desarrollo larvario es un periodo crucial en la ontogenia de los anfibios y al mismo tiempo la fase más sensible de la vida de este grupo. Debido a los complejos cambios morfológicos, fisiológicos y anatómicos, además de la susceptibilidad a los cambios ambientales, esta fase es conocida como uno de los periodos más críticos de desarrollo así como un obstáculo en los programas de reproducción ex-situ. Las tasas de crecimiento de los renacuajos pueden ser empleadas para predecir los efectos de las interacciones bióticas, así como predecir la tasa de supervivencia de los anfibios a los cambios ambientales. Se puede realizar la evaluación de la tasa de mortalidad de los renacuajos mediante una herramienta no invasiva basada en imágenes, programada en la plataforma estadística de código abierto R, SAISAQ (Semi-Automatic Surface Image-Based Quantification). Por medio de este, es posible obtener una secuencia semi automática de archivos de imagen estandarizados, con el fin de evaluar el crecimiento en relación al tiempo. Sin embargo, la literatura actual presenta una carencia de estimaciones de la tasa de crecimiento basadas en imágenes del desarrollo larvario para la mayoría de las especies de anfibios, algo igualmente observado para las especies del género *Ranitomeya* Bauer, 1986. Así, presentamos aquí los datos del desarrollo larvario completo de *Ranitomeya amazonica* (Schulte, 1999)*, R. benedicta* Brown, Twomey, Pepper & Sanchez-Rodriguez, 2008*, R. imitator* (Schulte, 1986)*, R. reticulata* (Boulenger, 1884)*, R. sirensis* (Aichinger, 1991) y *R. vanzolinii* (Myers, 1982), asistidos por fotografías, ilustraciones y tablas con información detallada acerca de la metamorfosis. Además proporcionamos una descripción larvaria inédita para *R. benedicta*. Los resultados aquí presentados también proporcionan nuevos datos acerca de la morfología y el desarrollo larvario de las especies aquí estudiadas, basados en una serie de muestras para cada especie. Estas informaciones contribuyen para una mejor comprensión del grupo y proporcionan datos importantes para ayudar a resolver el tan complejo rompecabezas de las relaciones sistemáticas. Además promueven una base de conocimiento para futuras investigaciones sobre la cría en cautividad de anfibios, una importante herramienta en los programas de cría en cautividad para la conservación de especies de anfibios amenazadas.

Palabras clave. Amazónia, cría en cautividad, ex situ, renacuajo, *Ranitomeya benedicta*, SAISAQ.

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INTRODUCTION

Anurans currently include more than 7,000 described species (Frost 2020) and are by far the most species-rich and evolutionary successful group among the extant amphibians. This evolutionary success is promoted by their wide diversity of survival and reproductive strategies, as well as their life histories (Duellman & Trueb 1986; Wells 2007). The huge variety of functional types, including terrestrial, aquatic, fossorial and arboreal forms and their morphological adaptations allowed anurans to occupy a wide array of terrestrial and fresh-water niches (Duellman & Trueb 1986; Wells 2007). However, due to their thin and permeable skin as well as their ectothermy, they are highly susceptible to humidity and temperature variations, the most important abiotic factors within their habitats (Wells 2007).

Poison-dart frogs belong to the superfamily Dendrobatoidea Cope, 1865 and include about 328 known species divided into the families Aromobatidae and Dendrobatidae (Grant et al. 2006; Brown et al. 2011; Frost 2020). All members have a small to medium size of 2–6 cm snout-vent length (SVL) in combination with a highly complex social and reproductive behavior (Lötters et al. 2007; Brown et al. 2011). The genus *Ranitomeya* Bauer, 1986, which is placed within the family Dendrobatidae, consists of 16 species arranged into four species groups, namely the *R. defleri* Twomey & Brown, 2009, *R. reticulata* (Boulenger, 1884), *R. vanzolinii* (Myers, 1982) and *R. variabilis* (Zimmermann & Zimmermann, 1988) groups (Brown et al. 2011). Species of that genus are characterized by their diminutive size with SVL less than 21 mm, their bright aposematic coloration and an almost smooth to slightly granular dorsal surface (Daly et al. 1987; Vences et al. 2003; Brown et al. 2011). Furthermore, the first finger is reduced and shorter than the second, which is the largest of all four fingers (Brown et al. 2011).

In 2011, Brown et al. published a revision of the genus *Ranitomeya*, which represents the actual and widely accepted classification for this group (e.g., Sánchez 2013; Vargas-Salinas et al. 2014; Krings et al. 2017). Within their study, systematic arrangements and the history of that genus are explained, based on molecular phylogenetics in combination with adult and larval morphology. For understanding the systematic and phylogenetic relationships, it is a general consensus that morphological data is an indispensable tool. Regarding taxonomic and phylogenetical purposes, we cannot neglect the knowledge about the morphology of the amphibian larval phase (i.e., tadpoles), where many of the descriptions of the larval stage are quite incomplete, based on a single sample or data are totally absent.

For the genus *Ranitomeya*, many of the tadpole descriptions are based on back riding tadpoles during the transport by the adults to water bodies (Sánchez 2013). At this stage of development, the tadpoles often lack fully developed tooth rows or other specific characteristic traits of the species (Brown et al. 2011). Furthermore, clutches of *Ranitomeya* species are very small, which makes it difficult to obtain a larger number of specimens at a time, and tadpoles that have already been carried by adults to a water site where they will develop are hard to find. One way to locate tadpoles at this stage is observing the adults, which are shy and therefore hard to discover (Sánchez 2013). In a captive breeding framework, higher numbers of tadpoles can be analyzed across a long time span making it possible to document the complete development. Furthermore, as conservation breeding becomes more and more important detailed information on developmental rates can be very helpful for ex-situ breeding programs. By optimizing husbandry conditions, potential effects of artificial nutrition and artificial environmental conditions can be minimized, although they may occur. Thus, results obtained from captive bred specimens should be ideally complemented and confirmed by data collected in the field.

The aim of this study is to provide, as accurately as possible, data concerning the complete larval morphology and development of the tadpoles of *Ranitomeya amazonica* (Schulte, 1999), *R. benedicta* Brown, Twomey, Pepper & Rodriguez, 2008, *R. imitator* (Schulte, 1986), *R. reticulata*, *R. sirensis* (Aichinger, 1991) and *R. vanzolinii* based on specimens obtained from captive breeding. In the study by Brown et al. (2011), descriptions of *R. amazonica* tadpoles are presented based on a tadpole in Gosner's stage 29, as well as those of *R. imitator* based on a tadpole in stage 26, of *R. reticulata* based on a tadpole in stage 30 and of *R. vanzolinii* based on a tadpole in stage 38. The tadpole description of *R. benedicta* includes only a mouthpart description which was presented by Brown et al. (2008). As stated by the authors the sample ended up being ruined by the fixation process, making it impossible to describe the tadpole (Brown et al. 2008). The tadpole of *R. sirensis* from the stages 25–36 was described in detail by May et al. (2008a) under the name *Ranitomeya biolat* (Morales, 1992) (c.f. Brown et al. 2011) which is considered to be a synonym of *R. sirensis* (Frost 2020).

We present in this study the first larval description of *R. benedicta* and provide image-based growth rate estimates of larval development for all target species, based on the SAISAQ (Semi Automatic Image based Surface Area Quantification, Kurth et al. 2014) tool. In addition, all data presented herein will contribute to fill knowledge gaps of the amphibian larval development, which is also useful for ex-situ breeding programs, in response to the biodiversity crisis, which requires a moral and ethical obligation for proactive interventionist conservation actions to assist species recovery and reduce the population decline.

MATERIALS AND METHODS

Captive Management and Breeding

Adult specimens of the species *R. amazonica*, *R. benedicta*, *R. imitator*, *R. reticulata*, *R. sirensis* and *R. vanzolinii* were acquired from pet trade and kept in customized terraria at the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK) in Bonn, Germany. According to the venders the specimens were captive bread as F_2 from specimens exported from the countries of origin. Correct taxonomy was confirmed by comparing the external morphology with the respective original descriptions.

For the course of the study, groups of five specimens of *Ranitomeya amazonica*, two specimens of *R. imitator*, two specimens of *R. reticulata*, four specimens of *R. sirensis* and four specimens of *R. vanzolinii* were kept in terraria of 40x50x40 cm, while three specimens of *R. benedicta*, four specimens of *R. imitator* and four specimens of *R. vanzolinii* were kept in terraria of 60x50x50 cm size. *Ranitomeya* individuals did not have contact with other individuals of the other species of the genus, also kept in terrariums, thus avoiding hybridization between species.

Each terrarium was equipped on the base with a filter mat which was covered with local leaf litter. The rear and one of the sides were covered with cork tile (Lucky Reptile©, Schwarzkorkrückwände). Artificial lighting was promoted with a LED light (Solar Stinger, 1100 Sunstrip Dimmable Driver, 25W) and daily, artificial daylight was provided from 8 a.m. to 8 p.m. In addition, the terrarium had a bottom irrigation system, a small body of standing water with a drain in a sieve form, located in one of the front corners of the terrarium and a misting system. The misting system was activating three times a day for 120 seconds, divided into twelve alternating intervals of ten seconds spraying, followed by ten seconds pause. Average air and water temperature fluctuated between 22 and 26 °C.

In order to ensure a finely storied vertical structure and provide opportunities to refuge and breeding sites, the terraria were heavily planted with *Ficus pumila* L., *Scindapsus* sp. as also *Neoregelia* sp. "fireball bromeliad" which provides a natural phytotelm for the anurans, important resource for laying the eggs. Additionally, the micro ambient was equipped with stones, roots and film containers (35mm) thus providing additional artificial phytotelms. The amphibians were fed with a diet of *Drosophila melanogaster* Meigen, 1830 or collembolans, every two to three days, and the food was enriched with vitamins and minerals (Herpetal© Mineral + Vitamin D3, Korvimin ZVT + Reptil).

Detected clutches were removed with aid of water and pipettes and placed into petri dishes. To ensure stable conditions, the clutches were transferred into an environmental test chamber (MLR-352H-PE, Panasonic Biomedical Sales Europe BV, Netherlands), which was set to a humidity of 80%, a temperature of 24 °C and a twelve hour photoperiod from 8 a.m. – 8 p.m. Every second day all eggs were wetted, except the clutches of *R. amazonica* which were completely covered with enriched pure water, attending the reproductive behavior of the species, as e.g. mentioned in Poelman & Dicke (2007) and Poelman et al. (2013).

After hatching, the larvae were kept separated within small translucent plastic containers (10 x 10x 10 cm), which were filled with enriched pure water and several oak leaves as well as a stem of *Ceratophyllum demersum*. Each container was placed into the environmental test chamber and got a specific identification number. Every two to three days, two thirds of the water were exchanged, in order to preserve the favorable environment for the tadpole. The larvae were fed with a finely ground ration of several types of algae and fish food *ad libitum,* which is different from the natural food. These species are known for their oophageal and predatory behavior in the natural environment (Lötters 2007; Poelman & Dicke 2007). In addition, specimens could graze on biofilm and algae that naturally grew within their container.

When the forelimbs emerged, a small piece of cork tile was placed on the water surface in order to provide a small "land area". During the latest steps of metamorphosis, when the tail was resorbed, the froglets were transferred to a new container with a huge "land area", covered with oak leafs, as well as a small water body (18 x 13 x 6 cm). The container was sealed with a perforated cover to ensure air exchange. From this moment, the froglets were fed with a diet of *Drosophila melanogaster* and collembolans.

The climate at each known location of the six different species, as defined by Brown et al. (2011), was obtained using ArcGIS (Environmental Systems Resource Institute, ArcGIS 10.2.2, Redlands, California). In order to do so, the longitudinal and latitudinal values of the locations were received through georeferencing of available distribution maps. On the one hand, these coordinates were used to generate a new map which contains the distribution areas of the study organisms (Fig. 1). Expert range maps provided by IUCN were randomly sampled every 10 km^2 to estimate annual mean, and monthly minimum and maximum temperatures within the geographic range of each species. Modern climate data with a spatial resolution of 30 arc sec was obtained from the free global online database CHELSA (Karger et al. 2017a, b).

Measurements

During their development, the growth rate of the active and mobile tadpoles was documented using a photo camera (EOS 600D, Canon© Deutschland GMBH, Krefeld, Germany), which was mounted on a table-top tripod in a fixed distance to the object. Photographs were taken

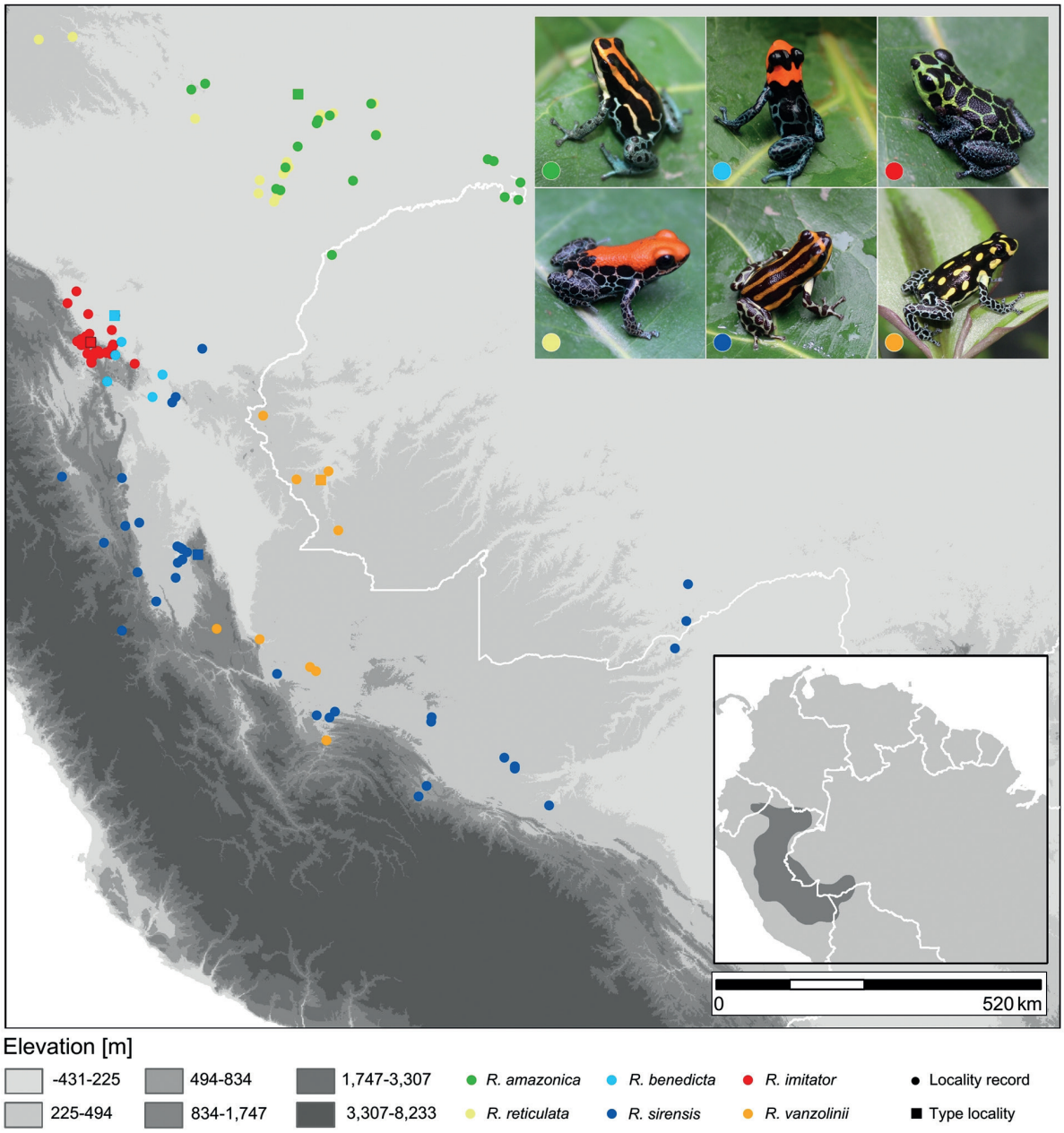

Fig. 1. Known distribution of *Ranitomeya amazonica* (green), *R. benedicta* (light blue), *R. imitator* (red), *R. reticulata* (yellow), *R. sirensis* (blue) and *R. vanzolinii* (orange). Darker shades of gray indicate higher elevations. The inset map displays the distribution of all six compared species, which are shown in the upper right corner of that figure with the corresponding color code.

three times a week, on alternate days. For this purpose, the larvae were placed into a translucent petri dish on top of a light source (CL 6000 LED, Carl Zeiss© Microscopy GMBH, Jena, Germany) which was modified by an alabaster glass. Thus, the light was homogenously distributed and therefore ensured a high contrast between the object and the background.

In all photography sessions, first a standard picture was taken to calibrate an image analysis software programmed in the open source statistics platform R (R Development Core Team, 2014), which allows a semiautomatic procession of standardized image files (SAISAQ, Kurth et al. 2014). Subsequently the settings and distances were kept constant and all tadpoles were photographed. The software measured the surface area of each tadpole, which is strongly correlated to the body mass. Therefore, a picture series of the same individual documenting its development leads to a graph which represents the growth rate. Every tadpole was photographed with four different settings, ranging in light intensity and different sensitivities

of the camera (Appendix I) which allows observing the tadpole in more details and assisting the software to set the threshold between the object and the background more efficiently. Dorsal and ventral high-resolution pictures, of each tadpole, were created with a special camera setup (Canon EOS 7D mounted on a P-51 Cam-Lift, Dun Inc., Virginia, USA), which perform automatically multiple pictures in different depths and stacks the photos in order to create a final clear image.

Length based measurements were taken to the nearest of 0.1 mm with a stereomicroscope and its integrated eyepiece (Stemi 2000 C, Carl Zeiss© Microscopy GmbH, Jena, Germany) or ImageJ (National Institutes of Health, ImageJ 1.42q, Bethesda, Maryland). The morphological terminology, characters and measurements are determined following McDiarmid & Altig (1999) (Fig. 2) extended by larval measurements from Lavilla & Scrocchi (1986) as cited in Mijares-Urrutia (1998).

Characters and measurements following McDiarmid & Altig (1999; Fig. 2) are: first anterior tooth row (A1); second anterior tooth row (A2); medial gap in first anterior tooth row (A1-GAP); anterior (upper) labium (AL); body length, measured from the tip of the snout to the junction of the posterior body wall with the axis of the tail myotomes (BL); internarial distance, measured between centers of narial apertures (IND); interorbital distance, measured between centers of pupils (IOD); lower jaw sheath (LJ); lateral process of upper jaw sheath (LP); labial tooth row formula (LTRF); mouth (M); marginal papillae (MP); maximum tail height (MTH); oral disc (OD); posterior (lower) labium (PL); first posterior tooth row (P1); second posterior tooth row (P2); third posterior tooth row (P3); medial gap in first posterior tooth row (P1-GAP); tail length (TAL); tail muscle height at base (TMH); tail muscle width at base (TMW); total length (TL); submarginal papillae (SM); upper jaw sheath (UJ).

Characters and measurements following Lavilla & Scrocchi (1986) as cited in Mijares-Urrutia (1998) are: body width at eye level (BWE); body width at nostril level (BWN); horizontal eye diameter (ED); eye nostril distance (END); maximum body height (MBH); maximum body width (MBW); oral disc width (ODW); rostro-eye distance, from tip of snout to the center of the eye in lateral view (RED); rostro-nasal distance, from tip of snout to the center of the nostril in lateral view (RND); rostro-spiracle distance, from tip of snout to center of the spiracle in lateral view (RSD).

Besides that, staging of the development process took place according to Gosner (1960), voucher specimens were euthanized using a saturated solution of Chlorobutanol, subsequently preserved in 6% formalin, and after an ascending alcohol series, preserved in 70% ethanol at the herpetological section of ZFMK. The voucher numbers of each tadpole are provided in the results section. A brief description of the natural history of each species covered in this study can be found in Appendix II.

RESULTS

Species Accounts

Ranitomeya amazonica (Schulte, 1999)

Breeding behavior in captivity. The breeding pairs, among the five specimens of *R. amazonica,* placed the clutches of four to six anthracite eggs in the bromeliad phytotelm. While those egg depositions had no clear frequency, later depositions in a water filled film container occurred every two to five days. Thereby, the clutches were placed directly underneath the water surface of the vertically orientated container, which was placed on the ground next to a large stone. Moreover, at one day a single tadpole at stage 25 was found at the ground of the container, beneath a newly produced clutch.

Larval morphology. The description of the tadpole is based on one specimen at stage 41 (ZFMK 97374). Further voucher specimens are ZFMK 97357, 97362, 97366, 97370–97373. According to McDiarmid & Altig (1999), *R. amazonica* tadpoles belong to the exotrophic, lentic, benthic, arboreal larval type. All measurements that were used to calculate the following proportions and its comparison with the other species of this study, can be found in Appendix III.

Dorsal view: Body shape is oval and moderately elongated (MBW/BL=0.75). The snout is short and moderately pointed (RED/BL=0.23, BWN/BWE=0.56), nares are small and elliptical, positioned dorsally and orientated laterally. Nares are situated closer to snout than to eyes (RND/RED=0.43). Eyes are large (ED/BL=0.12), positioned dorsally and orientated laterally. Internarial distance is smaller than interorbital distance (IND/IOD=0.52). Single, sinistral spiracle is not visible in dorsal view.

Lateral view: Body is depressed (MBH/MBW=0.59), snout is rounded. The spiracle is positioned below the longitudinal axis, at the second half of the body (RSD/BL=0.64), the inner wall is free from the body, opening is round and the spiracle tube is short. The maximum body height is situated between the eyes and the tail. The tail is long and narrowly rounded (TAL/BL=1.95, TAL/TL=0.66). The musculature is well developed (TMH/MTH=0.58, TMW/MBW=0.33). The "V"-shaped myosepta are visible along the whole length of the tail, particularly at the first half. Upper fin originates posterior to the tail-body junction and the margin of the lower fin. Upper fin is slightly higher than the lower fin. Ventral tube is dextral, emergence from abdomen sagittal, opening is rounded. Hindlimbs are fully developed. Oral apparatus is visible in lateral view.

Oral apparatus: Oral disc is elliptical, positioned ventrally and covers nearly one third of the body width (ODW/MBW=0.27), emarginated. Marginal, ensiform,

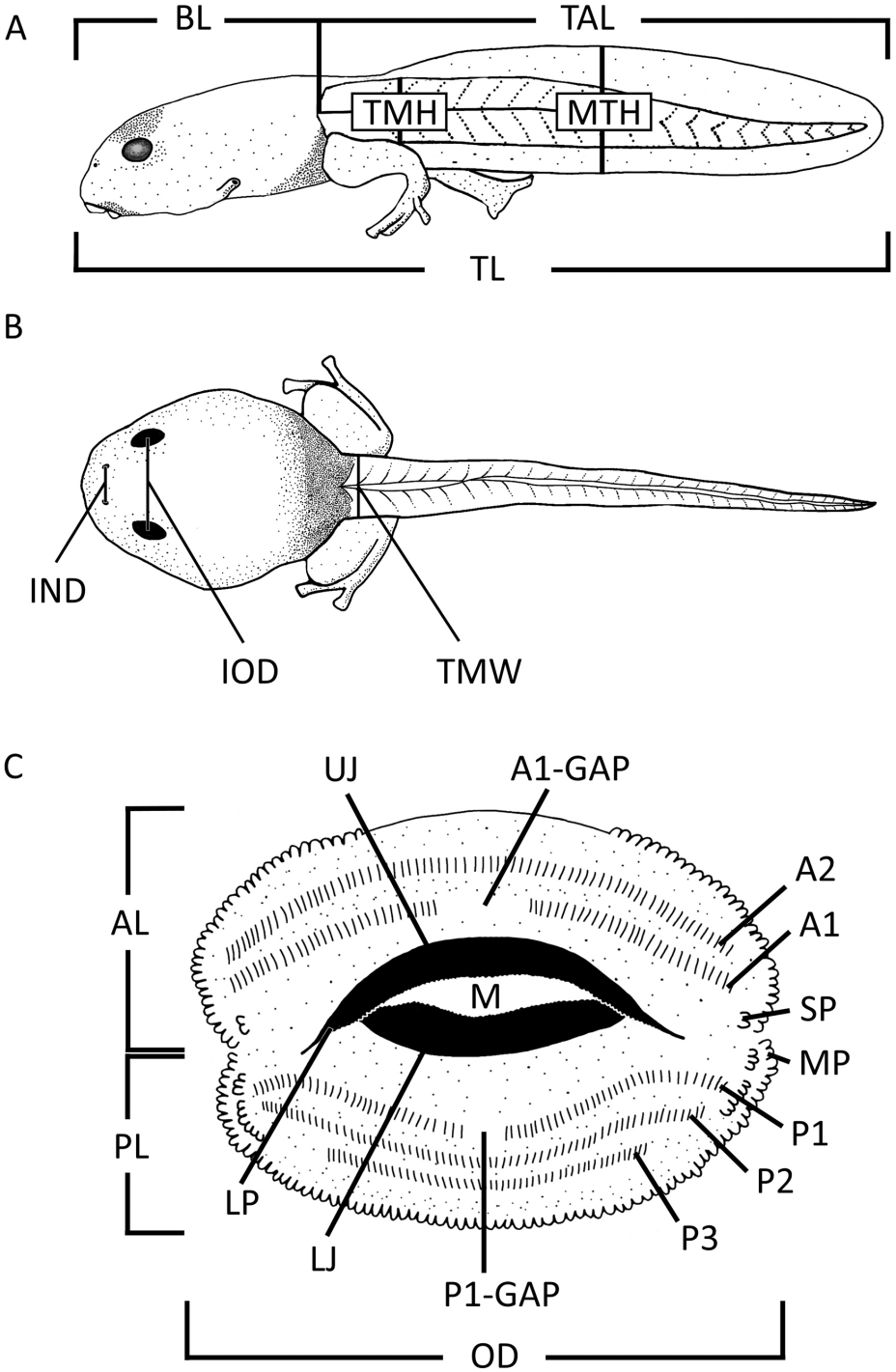

Fig. 2. Landmarks and measurements of a tadpole body and definitions of the oral apparatus. **A**. Lateral view. **B**. Dorsal view. **C**. Oral apparatus. Abbreviations A–B: BL = body length; TAL = tail length; TL = total length; TMH = tail muscle height; MTH = maximum tail height; IND = internarial distance; IOD = interorbital distance; TMW = tail muscle width. The dotted line indicates the accurate progression of the measurement which represents the body length**.** Abbreviations C: A1 = first anterior tooth row; A2 = second anterior tooth row; A2-GAP = medial gap in second anterior tooth row; AL = anterior (upper) labium; LJ = lower jaw sheath; LP = lateral process of upper jaw sheath; M = mouth; MP = marginal papillae; OD = oral disc; PL = posterior (lower) labium; P1 = first posterior tooth row; P2 = second posterior tooth row; P3 = third posterior tooth row; SM = submarginal papillae; UJ = upper jaw sheath.

Table 1. *Ranitomeya amazonica* (n=5) larvae morphometric measurements at the stages 26–41. Not all stages are represented; all measurements are given in [mm]. Abbreviations: BL=body length; BWE=body width at eye level; BWN=body with at nostril level; ED=horizontal eye diameter; END=eye nostril distance; INFD=internarial distance; IOD=interorbital distance; MBH=maximum body height; MBW=maximum body width; MTH=maximum tail height; ODW=oral disc width; TAL=tail length; TMH=tail muscle height at base; TMW=tail muscle width at base; TL=total length; RED=rostro-eye distance, from tip of snout to the center of the eye in lateral view; RND=rostro-nasal distance, from tip of snout to the center of the nostril in lateral view; RSD=rostro-spiracle distance.

Stage	26	31	34	38	41
BL	5.15	7.12	8.33	8.33	9.38
BWE	3.79	4.24	4.70	5.00	5.57
BWN	2.73	2.73	3.03	3.33	3.14
ED	0.45	0.61	0.76	0.83	1.13
END	0.91	1.21	1.52	1.52	1.23
IND	1.14	1.36	1.52	1.59	1.57
IOD	1.97	2.42	2.73	3.08	3.00
MBH	2.05	3.03	3.18	3.18	4.14
MBW	3.94	5.15	5.76	5.91	7.00
MTH	1.97	3.00	3.33	3.03	3.71
ODW	1.14	1.79	1.97	1.97	1.86
TAL	10.30	13.79	15.15	15.15	18.29
TMH	0.91	1.67	1.82	1.74	2.14
TMW	1.06	1.67	1.97	2.12	2.29
TL	15.45	20.91	23.48	23.48	27.67
RED	1.52	1.97	2.42	2.42	2.15
RND	0.61	0.76	0.98	0.98	0.92
RSD	3.26	4.85	5.68	5.76	6.00
MBW/BL	0.76	0.72	0.69	0.71	0.75
RED/BL	0.29	0.28	0.29	0.29	0.23
ED/BL	0.09	0.09	0.09	0.10	0.12
RND/RED	0.40	0.38	0.41	0.41	0.43
IND/IOD	0.58	0.56	0.56	0.52	0.52
TMW/MBW	0.27	0.32	0.34	0.36	0.33
MBH/MBW	0.52	0.59	0.55	0.54	0.59
TAL/BL	2.00	1.94	1.82	1.82	1.95
TAL/TL	0.67	0.66	0.65	0.65	0.66
TMH/MTH	0.46	0.56	0.55	0.58	0.58
TMW/MBW	0.27	0.32	0.34	0.36	0.33
ODW/MBW	0.29	0.35	0.34	0.33	0.27
RSD/BL	0.63	0.68	0.68	0.69	0.64
BWN/BWE	0.72	0.64	0.65	0.67	0.56

rounded and transparent papillae are present at the posterior side, with a moderate medial gap, and absent at the anterior side, except the most lateral part (seven papillae). Submarginal papillae are absent. Anterior labium contains two tooth rows of the same width (A1, A2), large medial gap in second anterior tooth row (A2-GAP). Posterior labium contains three tooth rows (P1, P2, P3), moderate medial gap in first tooth row (P1-GAP). Black jaw sheaths, both with serrations. The upper jaw sheath is wider than the lower jaw sheath. The labial tooth row formula is 2(2)/3(1) (Fig. 3D). Characteristic traits and the correlated proportions do not change during the development stages 26 to 41 (Table 1).

Table 2. *Ranitomeya amazonica* (n = 4): development stages of embryos and hatchlings.

n = 4	Day	Stage	Traits
Embryos	1	8	egg diameter 1.5 mm; eggs anthracite to dark gray; swam beneath the water surface; transparent egg integument; highly glutinous; no pigmentation
	2	10	eggs with brown pigmentation; dorsal lip visible
	3	13	neural plate visible
	4	–	
	5	19	large yolk sack present; embryonic body assumes larval shape; head and tail region visible; larva dun, spotted beige; gill buds present; mouth slightly perceptible
Hatchlings	6	20	elongation of the tail; gills present, circulation recognizable; tail fins slightly visible; myosepta visible; vent tube bud visible
	7	21–22	elongation of the tail and the gills; tail pointed; overall body size increased; upper and lower tail fins more transparent; denser pigmentation of body and tail region
	8	22	eyes visible; nares discernible; atrophy of the yolk sack initiated
	9	23–24	reduction of the right gill; oral apparatus discernible; yolk sack almost fully atrophied
	10	24–25	gills absent in 75% of the clutch; yolk sack completely atrophied
	11–12	25	gills absent; spiracle forming on the left

Coloration of a living tadpole of *R. amazonica* (ZFMK 97374). The dorsum is black to grey, with a yellowish green median stripe and two dorsolateral stripes of the same color, which run parallel to the longitudinal axis, and two lateral stripes (Fig. 3A1, A2). The two dorsolateral stripes originate at one point posterior to the nares, become separated and run next to the eyes to the base of the tail, with a moderate gap on eye level. The median one lies in between the two others, starts at eye level and ends prior to the tail-body junction. The lateral stripes are situated differently. One of the lateral stripes is situated at the first half of the body below the longitudinal axis, while the other one is located above the longitudinal axis at the second half of the body. The hindlimbs are dark bluish with large black spots. The tail shows a brownish coloration and is covered with dark and bright spots, the second half is brighter than the first half. Fins are transparent and spotted with beige dots. The density of dots wanes till the tip.

During metamorphosis, the dorsal coloration of tadpoles changed in regard to the different development stages (Fig. 4). Reaching stage 25 some specimens displayed a few isolated yellowish green spots while the majority showed no coloration. At stage 28 some parts of the medial and dorsolateral stripes were present at the first half of the dorsum. In comparison to the final coloration, those areas were yellowish green instead of yellow and lacked a continuous connection. At stage 36 the color pattern was yellow, the dorsolateral stripes reached the second half of the body and the medial stripe ended close to the posterior margin of the eyes. While the dorsolateral stripes were continuous, the medial stripe was spotted. At stage 41, the dorsolateral stripes reached the tail-body junction and the medial line ended at the second half of the body. Moreover, each flank displayed the initiation of the ventrolateral stripes posterior to the forelimb pouches, which were visible in dorsal view, as well as the typical color pattern of the hindlimbs (Fig. 3C1, C2).

Coloration of a preserved tadpole of *R. amazonica* (ZFMK 97374). The dorsum is dark gray, with a brownish area at the forelimb pouches. Dorsolateral and median stripes are whitish and run on top or parallel to the longitudinal axis, clearly discernible on the head and the first half of the body. Dorsolateral stripes originate and bifurcate at one point posterior to the nares and run next to the eyes, with a moderate gap on eye level. The median stripe runs in between the eyes, not fusing with the origin of the dorsolateral stripes. The hindlimbs are bluish gray, spotted with dark dots. The tail is brownish; the first half is darker than the second one, which is almost transparent. Fins are transparent and spotted with beige dots. Ventral side has a dark grey to brown coloration, except one bright spot at the chin, posterior to the oral disc.

Larval staging. During their embryonic development, all four to six eggs of the same clutch develop at the same pace, except the reduction of the gills. While the majority of the eggs swam separately beneath the water surface, two in each clutch stayed as a pair (Fig. 5B). Eggs up to stage 10 were not pigmented (Fig. 5A). At stage 10, when the dorsal lip was visible, the pigmentation started and the eggs became brownish. After three days, the neural plate was discernible (Fig. 5C). Reaching stage 18, a whitish yolk sack was present at the ventral side of the embryo and body parts were slightly differentiat-

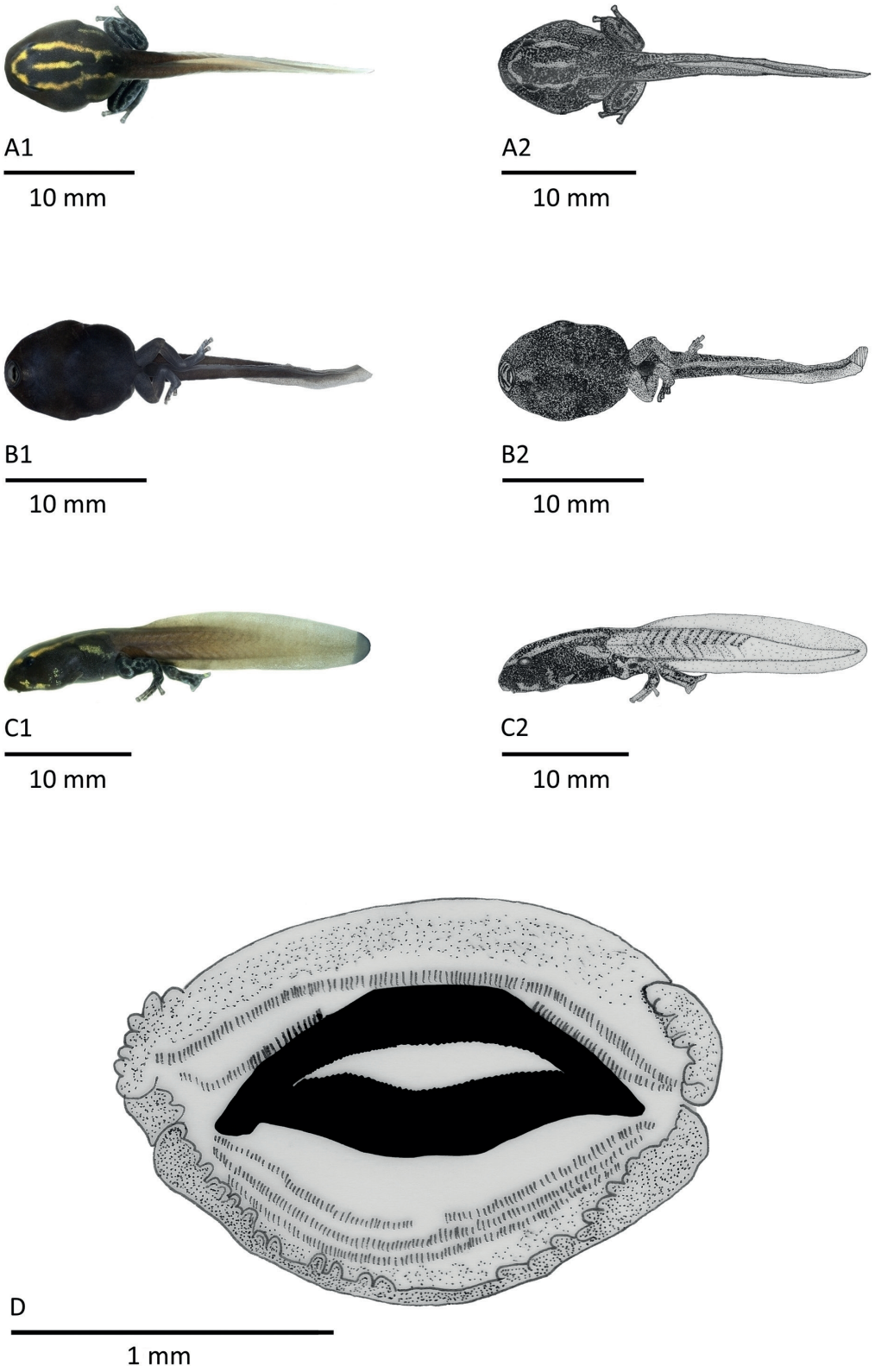

Fig. 3. Illustrations of the tadpole of *Ranitomeya amazonica,* stage 41 of Gosner (1960). **A1**. Dorsal view, photograph. **A2**. Dorsal view drawing. **B1**. Ventral view, photograph. **B2**. Ventral view, drawing. **C1**. Lateral view, photograph. **C2**. Lateral view, drawing. **D**. Drawing of the oral disc. LTRF = 2(2)/3(1).

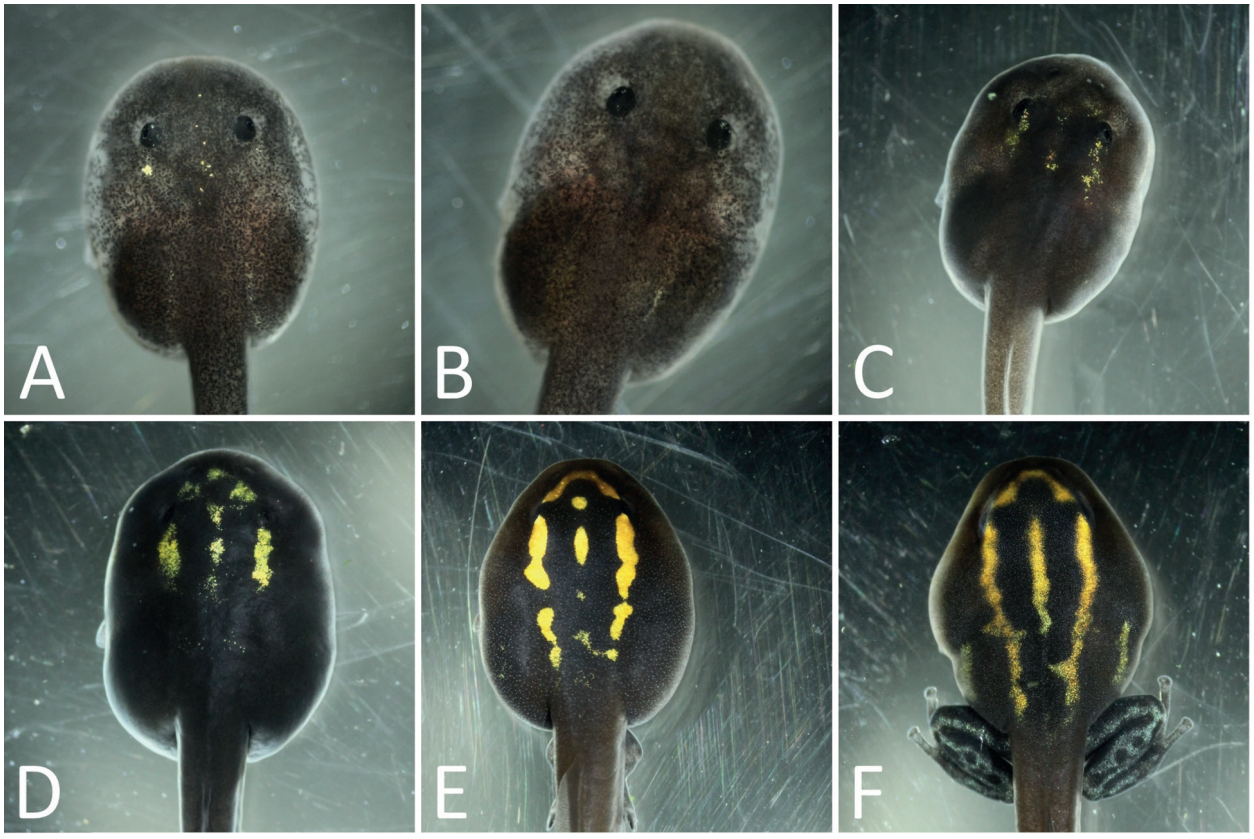

Fig. 4. Development of the color pattern of *Ranitomeya amazonica*. **A**. Tadpole at stage 25 with isolated colored spots. **B**. Tadpole at stage 25 without isolated colored spots. **C–D**. Different densities at Gosner stage 30. **E**. Tadpole at Gosner stage 36. **F**. Tadpole at Gosner stage 41, typical color pattern on hindlimbs.

ed (Fig. 5D). At stage 19, the embryo slowly assumed a larval shape. The head and tail region were visible and the larva had a dun coloration with beige spots. While the gill buds, the opening of the mouth and the ventral tube emerged, the eyes were absent. At stage 20, the gills and the correlated circulation were present while the whole body was elongated (Fig. 5E, Table 2). Upper and lower tail fins were slightly visible and the myosepta were present. Between stages 21 and 22, the tail and the gills were even more elongated, the overall body size increased and the pigmentation of all structures was denser. Tail fins were transparent, the tail was pointed. At stage 22, eyes were visible, nares were discernible and the decrease of the yolk sack was initiated (Fig. 5F). During the transition from stage 23 to 24, the sinistral gills were present while the dextral gills were completely reduced (Fig. 5G). The yolk sack was almost fully atrophied and the oral apparatus was formed. The transition to a free living and mobile tadpole started at stage 25, while the majority of the clutch was no longer enclosed by the jelly layer and the yolk sack was fully reduced. The spiracle was formed on the left, and after eleven to twelve days of development, the hatchlings swam freely within the water column (Fig. 5H–I).

Right after hatching, the free-swimming larvae had a surface area of 0.10 ± 0.02 cm² (Table 3). Between the stages 25 to 27, while the hindlimb bud was slightly developed, the surface increased by 330%, resulting in an area of 0.33 ± 0.20 cm². At stage 28, which half of the tadpoles had reached after 49 to 67 days (median = 56 days), they had a mean surface area of 0.74 ± 0.12 cm² (Table 3). The hindlimb bud was as long as wide and the dorsal color pattern was slightly visible at the first half of the body. Between stages 29 to 40, where the completion of the hindlimb development took place, the larvae had a mean surface area of 0.98 ± 0.19 cm². Thus, all toes, the metatarsal tubercles and the subarticular patches were discernible. With an area of 1.17 ± 0.12 cm², half of all tadpoles reached stage 41 after 69 to 88 days (median = 84 days). Forelimb buds were visible and the hindlimbs showed the typical color pattern of the adult frog (Fig. 3A1, B1). Furthermore, colored dorsolateral stripes were discernible and the ventral tube as well as the oral apparatus was still present. While the forelimbs grew inside the body, during the transition from stage 41 to 42, the larvae reached their maximum size with a surface area of 1.19 ± 0.12 cm². After 82 to 94 days (median = 89 days), 50% of all metamorphs had emerged

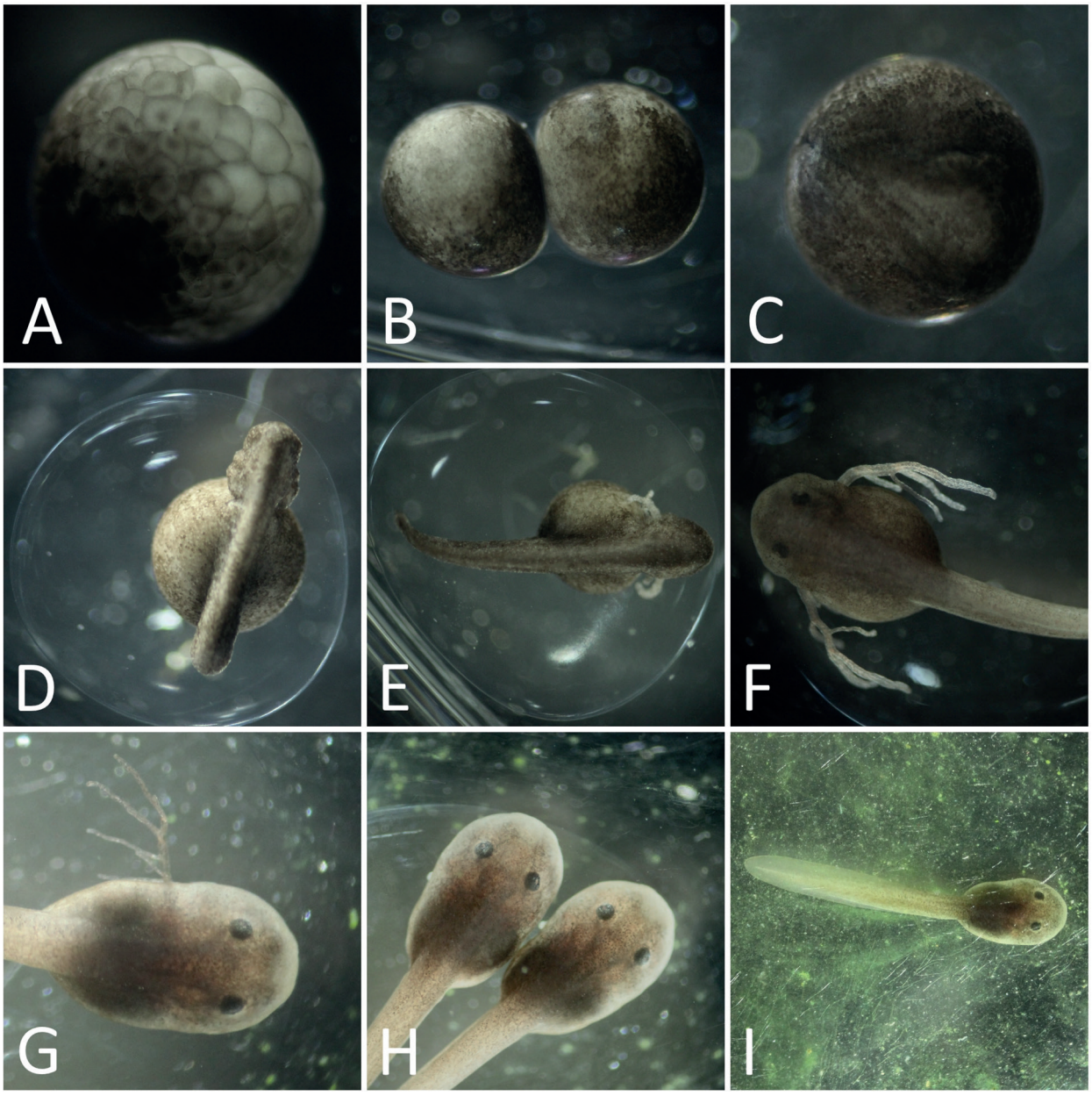

Fig. 5. Embryos and hatchlings of *Ranitomeya amazonica*. **A**. Isolated egg at stage 8. **B**. Egg pair at stage 9–12. **C**. Embryo at stage 13–14. **D**. Embryo at stage 18–19. **E**. Hatchling at stage 20. **F**. Hatchlings at stage 22. **G**. Hatchling at stage 23–24. **H**. Hatchlings at stage 25. **I**. Free swimming larva at stage 25–26.

forelimbs, while the surface area decreased to 1.08 ± 0.17 cm². The resorption of the tail started after 91 to 99 days (median = 96 days), while the tadpoles had a mean surface area of 0.86 ± 0.15 cm². During the next days, the tail atrophied until the larva completed the metamorphosis, whereby the area of the larva was reduced to 0.82 ± 0.15 cm². Thus, the transition from a free-swimming larva to a froglet with a remnant of the tail lasted 91 to 99 days (median = 96 days), while some individuals needed less (84 days) and others more time (105 days, Fig. 6).

An additional and more detailed staging table, based on stereomicroscopic determinations of 17 specimens between stages 25 to 41, can be found in the Table 4.

The complete development, from the embryogenesis through hatching and larval period to metamorphosis, was observed under constant conditions with a temperature of 24 °C while the annual mean temperature within the natural distribution area of *R. amazonica* is slightly higher (T_{Mean} = 25.2 °C, T_{Max} = 28.5 °C, T_{Min} = 21.8 °C: Karger et al. 2017a,b; Fig.7)

Table 3. *Ranitomeya amazonica* (n = 16) larvae and metamorphs development stages based on image analyses. Area [cm²] is highly correlated with body mass.

n = 16	Stage (n)	Traits	Area [cm²]
Larvae	25 (16)	spiracle present; oral apparatus clearly visible; typical dorsal color pattern absent	0.10 ± 0.02
	25–27 (16)	hindlimb bud slightly developed, diameter < length; typical dorsal color pattern slightly visible at the first half of the body	0.33 ± 0.20
	28 (14)	length of the hindlimb bud equal to the diameter, no pigmentation	0.74 ± 0.12
	28–40 (14)	hindlimb bud length > diameter; foot paddle slightly visible; indentation between toes 4–5 and 3–4; indentation between toes 4–5, 3–4 and 2–3; Indentation between toes 4–5, 3–4, 2–3 and 1–2; toes 3–5 separated; all toes separated; metatarsal tubercle present; subarticular patches present; hindlimbs with pigmentation; typical dorsal color pattern present	0.98 ± 0.19
	41 (14)	forelimb buds present; typical color pattern on hindlimbs present; lateral stripes present	1.17 ± 0.12
Metamorphs	41–42 (14)	enlargement of the forelimb buds	1.19 ± 012
	42 (9)	forelimbs emerged	1.08 ± 0.17
	43 (10)	initiation of tail resorption	0.86 ± 0.15
	43–46 (10)	reduction of the tail until metamorphosis was completed	0.82 ± 0.15

Table 4. *Ranitomeya amazonica* (n = 17) larval development stages based on stereomicroscopic determinations. Area [cm²] is highly correlated with body mass.

n = 17	Stage (n)	Traits	Area [cm²]
Larvae	25 (3)	spiracle present; oral apparatus clearly visible; typical dorsal color pattern is absent	0.25 ± 0.01
	28 (5)	hind limb bud slightly developed, diameter ≤ length; typical dorsal color pattern slightly visible at the first half of the body	0.49 ± 0.09
	29 (9)	length of the hind limb bud 1.5 times of the diameter	0.59 ± 0.08
	30 (4)	length of the hind limb bud two times of the diameter	0.66 ± 0.07
	31 (4)	foot paddle is slightly visible, slight pigmentation at the base of the hind limb	0.75 ± 0.11
	33 (7)	indentation between toes 4–5 and 3–4	0.80 ± 0.14
	35 (9)	indentation between toes 4–5, 3–4, 2–3 and 1–2	0.87 ± 0.14
	36 (3)	toes 3–5 are separated; dorsal color pattern is denser and exceeds the first half of the body	1.28 ± 0.11
	39 (4)	all toes are separated; metatarsal tubercle is present; subarticular patches are present	1.30 ± 0.08
	41 (2)	forelimb buds are present; typical color pattern on hind limbs present; lateral stripes are present	1.36 ± 0.12

Ranitomeya benedicta Brown, Twomey, Pepper & Sanchez-Rodriguez, 2008

Breeding behavior in captivity. The breeding pair among the three available specimens mainly deposited their clutches of two to three eggs inside a dry and horizontally orientated film container which was attached to the cork tile. In rare cases, they placed the clutches within the bromeliad phytotelm. Reproduction did not obey a standardized way.

Larval morphology. Tadpole description is based on one individual at stage 41 (ZFMK 97363). Further voucher specimens are ZFMK 97367 and 97376. According to McDiarmid & Altig (1999), the larva belongs to the exotrophic, lentic, benthic and arboreal larval type. All measurements that were used to calculate the following

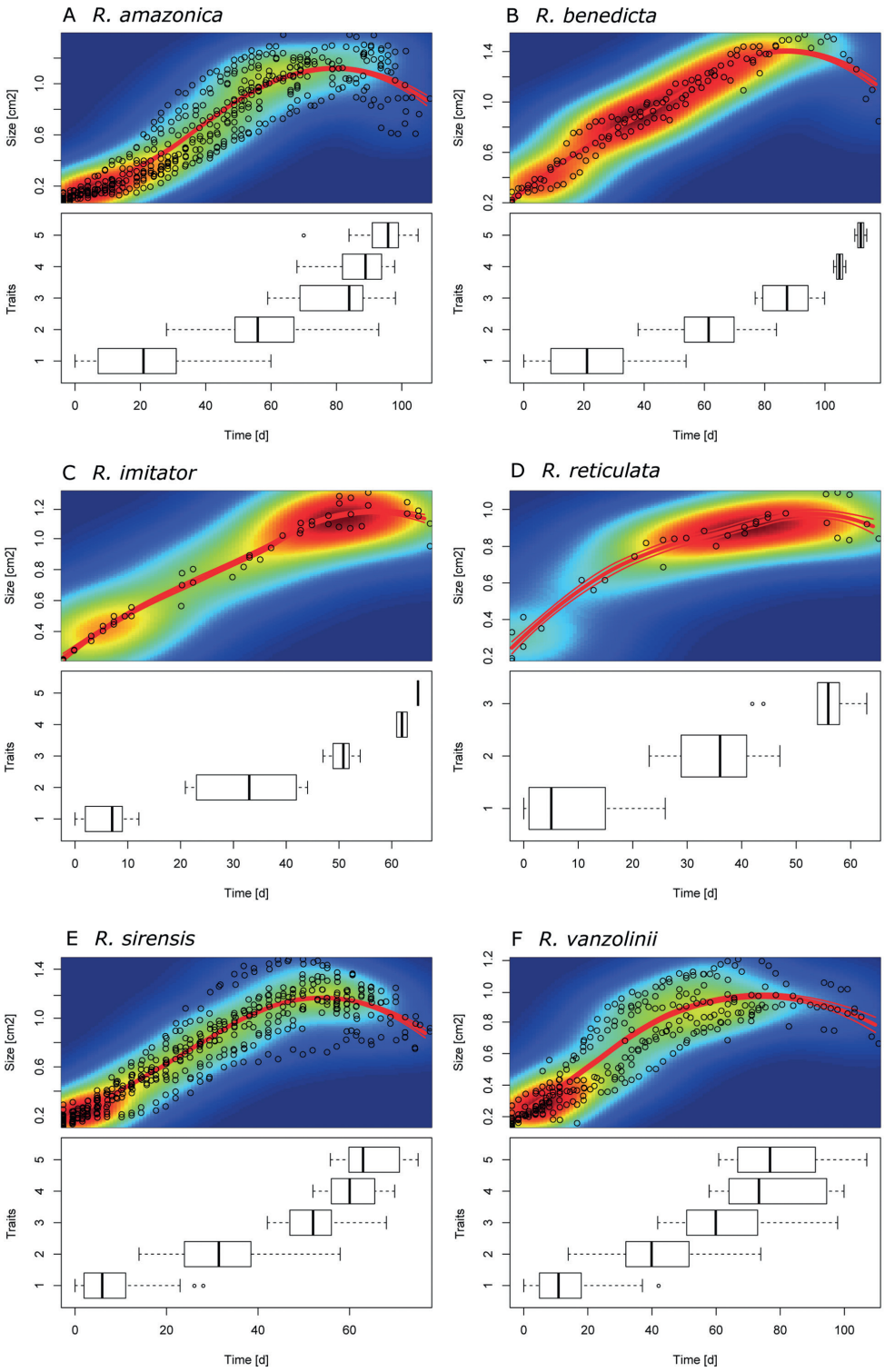

Fig. 6. Developmental series of six *Ranitomeya* species during the transition from a hatchling to a froglet. The upper panel illustrates the increase of surface area over time, whereby the surface area [cm²] is highly correlated with the body mass. Warmer colors indicate a higher sample density; each circle represents one data point. Loess function represented by the red lines, whereby the outer lines display the 95% confidence interval. The lower panel illustrates the temporal occurrence of the following traits: 1 = no limb bud discernible; 2 = hindlimb bud discernible; 3 = forelimb pouches discernible; 4 = forelimbs emerged; 5 = initiation of tail resorption.

proportions and its comparison with the other species of this study, are to be found in Appendix III.

Dorsal view: The body is oval shaped and moderately elongated (MBW/BL=0.78). The snout is short and rounded (RED/BL=0.18, BWN/BWE=0.57). The nares are positioned and orientated laterally; their shape is not visible in dorsal view. A skin fold connects the nares with the anterior edge of the eyes. Nares are closer to the snout than to the eyes (RND/RED=0.41). The eyes are large (ED/BL=0.11), positioned dorsally and orientated laterally. Internarial distance is smaller than the interorbital distance (IND/IOD=0.40). The single and sinistral spiracle is not visible in dorsal view.

Lateral view: The body is depressed (MBH/MBW=0.59), the snout pointed. Nares are small and elliptical. The spiracle is situated below the longitudinal axis, at the second half of the body (RSD/BL=0.56). The inner wall of the spiracle is free from the body, the opening is round, and the spiracle tube is short. Maximum body height is situated posterior to the eye. The tail is long and rounded (TAL/BL=2.06, TAL/TL=0.67), the musculature well developed (TMH/MTH=0.62, TMW/MBW=0.33). The "V"-shaped myosepta are visible along the whole length of the tail. Both tail fins are of the same height and originate posterior to the tail-body junction, the lower fin slightly anterior to the upper fin. The ventral tube is small, dextral; emergence from abdomen is sagittal, opening is elliptical. Hindlimbs are fully developed. Oral apparatus is visible in lateral view.

Oral apparatus: The oral disc is elliptical, positioned ventrally and covers nearly one third of the body width (ODW/MBW=0.29), emarginated. Marginal, ensiform, rounded and transparent papillae are present at the posterior labium and absent at the anterior labium, except the most lateral part (five papillae). Submarginal papillae are absent. The anterior labium contains two tooth rows (A1, A2) of the same width, whereas the second row is divided by a large medial gap (A2-GAP). The posterior labium contains three rows of teeth (P1, P2, P3) with a moderate medial gap in the first tooth row (P1-GAP). P1 P2 and P3 have the same width. Both jaw sheaths are black and serrated. The upper jaw sheath is wider than the lower jaw sheath. Lateral processes are present, extending barely past the lower jaw. The tooth row formula is 2(2)/3(1) (Fig. 8D).

Coloration of a living tadpole of *R. benedicta* (ZFMK 97363). The basic color of the dorsum is black to dark gray, with a reddish area anterior and posterior to the eyes, which starts posterior to the nares and ends at the first half of the body in dorsal view (Fig. 8A1, A2). In between both eyes light coloration is lacking, except one narrow stripe which connects the posterior and anterior part of the color pattern. The hindlimbs are as black as the dorsum. The tail is brownish beige and covered with darker dots. Fins are transparent and spotted with beige dots.

Coloration of a preserved tadpole of *R. benedicta* (ZFMK 97363). The basic color of the dorsum is dark gray, except some brighter areas at the forelimb pouches and at the muscle attachment of the tail. Additionally, there is a bright area anterior and posterior to the eyes. While the anterior part is bright orange, the posterior part is auburn. Both parts are fused medially, creating a face mask. The hindlimbs are as gray as the dorsum, with some slightly bright areas at the tip of the toes. The tail is beige, covered with grayish dots; the first half is brighter

Table 5. *Ranitomeya benedicta* (n=4) larvae and metamorphs development stages based on image analyses. Area [cm²] is highly correlated with body mass.

n=4	Stage (n)	Traits	Area [cm²]
Larvae	25(4)	sinistral spiracle present; oral apparatus clearly visible	0.23 ± 0.04
	25–27(4)	hindlimb bud slightly visible, length < diameter; body depressed	0.60 ± 0.24
	28 (3)	length of the hindlimb bud equal to the diameter, no pigmentation	1.01 ± 0.11
	28–40 (3)	hindlimb bud length > diameter; foot paddle slightly visible; indentation between toes 4–5 and 3–4; indentation between toes 4–5, 3–4 and 2–3; Indentation between toes 4–5, 3–4, 2–3 and 1–2; toes 3–5 separated; all toes separated; metatarsal tubercle present; subarticular patches present; hindlimbs with pigmentation; typical dorsal color pattern present	1.20 ± 0.16
	41 (3)	forelimb buds present; typical color pattern on hindlimbs present	1.38 ± 0.13
Metamorphs	41–42 (3)	enlargement of the forelimb buds	1.45 ± 0.09
	42 (1)	forelimbs emerged	1.39
	43 (1)	initiation of tail resorption	1.03 ± 0.00
	43–46 (1)	reduction of the tail until metamorphosis was completed	1.00 ± 0.13

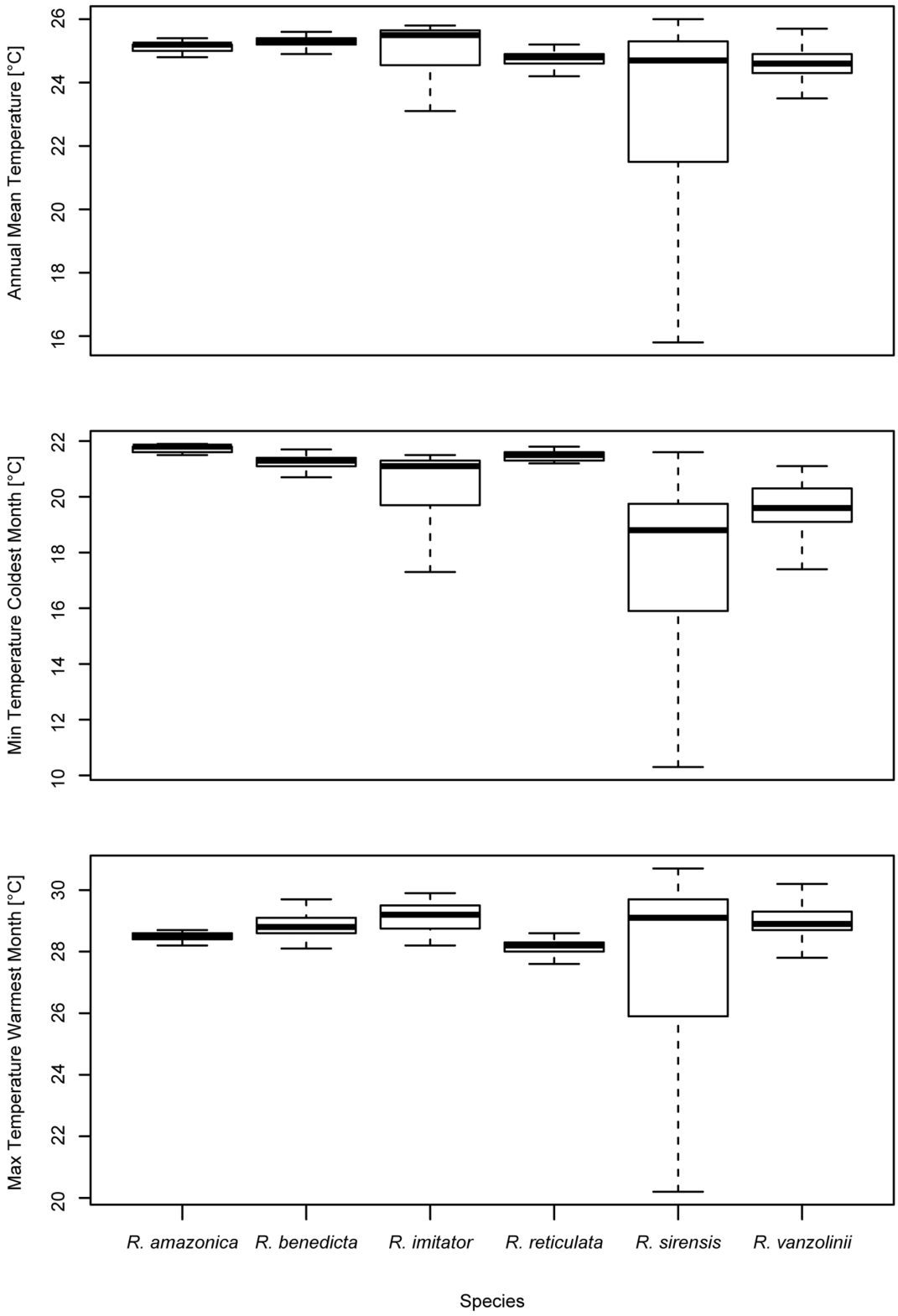

Fig. 7. Climatic characteristics within the geographic ranges of six *Ranitomeya* species in terms of annual mean temperature, minimum and maximum temperature of the coldest / warmest month. Boxplots show the 95% range, lower and upper hinge enclosing 50% of the samples and the median based on a random sample per 10 km^2.

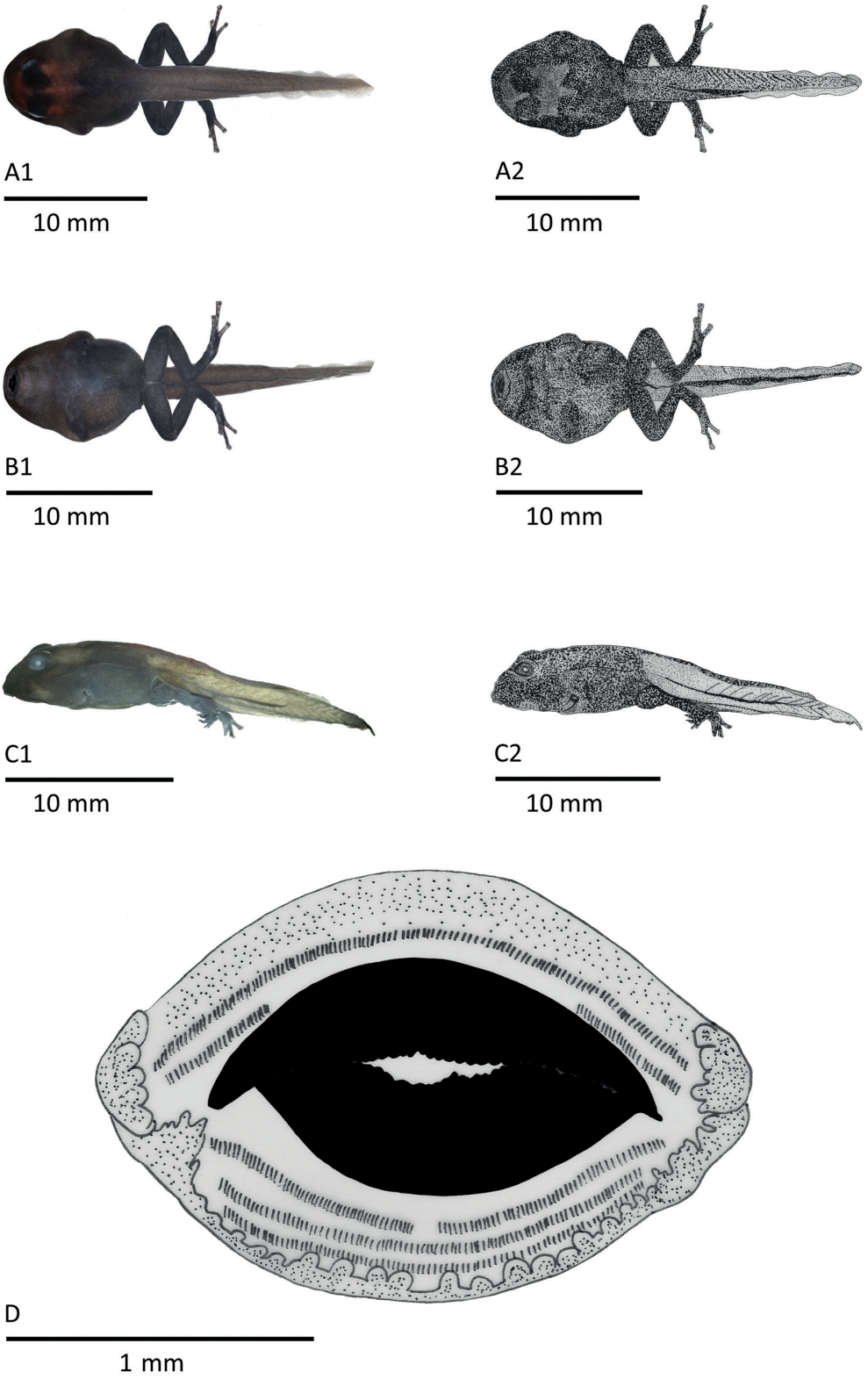

Fig. 8. Illustrations of the tadpole of *Ranitomeya benedicta*, stage 41 of Gosner (1960). **A1**. Dorsal view, photograph. **A2**. Dorsal view, drawing. **B1**. Ventral view, photograph. **B1**. Ventral view, drawing. **C1**. Lateral view, photograph. **C2**. Lateral view, drawing. **D**. Drawing of the oral disc. LTRF = 2(2)/3(1).

than the second half. The ventral side is as gray as the dorsum, with an auburn area around the oral disc which fades till the tail-body junction.

Larval staging. Right after hatching, the free-swimming larvae had a surface area of 0.23 ± 0.04 cm² (Table 5). During the transition from stage 25 to 27, while the hindlimb buds were slightly visible, the surface area increased to 0.60 ± 0.24 cm² (Table 5). This development period lasted for at least 54 to 70 days (median = 61 days), when half of all individuals reached stage 28. At this point the hindlimb buds were equal in length and diameter and therefore clearly discernible, while the tadpoles had reached a surface area of 1.01 ± 0.11 cm². Between stages 29 to 40, where the completion of the hindlimb development took place, the tadpoles had a mean surface area of 1.20 ± 0.16 cm². Thus, all toes, the metatarsal tubercles as well as the subarticular patches were discernible. After 80 to 94 days (median = 88 days), 50% of the individuals reached stage 41. The forelimb pouches were visible and the larvae had a surface area of 1.38 ± 0.13 cm². While the forelimbs evolved within the body, during the transition from stage 41 to 42, the tadpoles reached the peak of their growth with an area of 1.45 ± 0.09 cm². After 105 days, the forelimbs emerged and the remaining larva reached stage 42. Seven days later, the resorption of the tail was initiated, ensuring the transition from a hatchling to a young froglet. Therefore, the area of the metamorph was reduced to a size of 1.00 ± 0.13 cm². Altogether, the development from a free-swimming larva to a young froglet lasted around 114 days (Fig. 6).

The development was observed under constant conditions with a temperature of 24 °C, while the annual mean temperature within the natural distribution area of *R. benedicta* is slightly higher (T_{Mean} = 25.3 °C, T_{Max} = 28.8 °C, T_{Min} = 21.3 °C: Karger et al. 2017a, b; Fig. 7).

Ranitomeya imitator (Schulte, 1986)

Breeding behavior in captivity. The single breeding pair deposited the clutches of one to two whitish to beige eggs directly in the bromeliad phytotelm or in a horizontally orientated film container, which was attached to the side wall of the terrarium, which was kept moist by the misting system. Reproduction did not obey a standardized way**.**

Larval morphology. Description of the tadpole is based on two specimens at stage 41 (ZFMK 97358). Further voucher specimens are ZFMK 97364, 97368 and 97377. According to McDiarmid & Altig (1999), the larvae belong to the exotrophic, lentic, benthic and arboreal larval type. All measurements that were used to calculate the following proportions and its comparison with the other species of this study, can be found in Appendix III.

Dorsal view: The body is shaped elliptically and slightly elongated (MBW/BL = 0.75). The snout is short, rounded and moderately pointed (RED/BL = 0.26, BWN/BWE = 0.65). The shape of the nares is not visible in dorsal view. A skin fold, which originates at the nares, ends close to the anterior margin of the eyes; the two landmarks are not connected. Nares are located closer to the snout than to the eyes (RND/RED = 0.39). Eyes are large (ED/BL = 0.09), situated dorsally and orientated laterally. Internarial distance is smaller than the interorbital distance (IND/IOD = 0.46). The single sinistral spiracle is not visible in dorsal view.

Lateral view: Body is depressed (MBH/MBW = 0.73), snout is pointed. Nares are round, positioned and orientated dorsally. The spiracle is positioned below the longitudinal axis, at the posterior part of the body (RSD/BL = 0.56), the inner wall is free from the body and the opening is round, spiracle tube is short. The maximum body height is situated between the eyes and the tail-body junction. The tail is long and the tip is broadly rounded (TAL/BL = 1.83, TAL/TL = 0.65). The musculature is well developed (TMH/MTH = 0.49; TMW/MBW = 0.34). The "V"- shaped myosepta are visible along the whole length of the tail, particularly in the first half. The upper fin originates anterior, the lower posterior to the tail-body junction. Upper fin is slightly higher than lower fin. Ventral tube partially absorbed, dextral; emergence from the abdomen sagittal, the opening is triangular and has smooth edges. Hindlimb development is completed. Parts of the oral apparatus are visible in lateral view, particularly the margins.

Oral apparatus: The oral disc is shaped elliptically, positioned ventrally, emarginated and covers almost one third of the body width (ODW/MBW = 0.31). Two rows of marginal, ensiform, rounded and transparent papillae are present at the posterior labium (around 20 papillae) and except one short row at the most lateral part, absent at the anterior labium (three to five papillae). Submarginal papillae are absent. The anterior labium contains two tooth rows of equal width (A1, A2) with a large medial gap in the second row (A2-GAP). The posterior labium contains three tooth rows (P1, P2, P3) with a moderate medial gap in the first tooth row (P1-GAP). Black jaw sheaths, both serrated. Upper jaw sheath is wider than the lower jaw sheath. Lateral processes are present, extending slightly past the lower jaw. Tooth row formula is 2(2)/3(1) (Fig. 9D).

Coloration of a living tadpole of *R. imitator* (ZFMK 97358). The basic color of the dorsum is beige, heavily covered with puce to black dots. Additionally, the first half of the body is strongly dotted with yellowish green spots, which are able to reflect the light and become golden yellow, while the second half is almost completely covered with black dots, except some single yellowish green spots. The hindlimbs are beige with dark spots. The

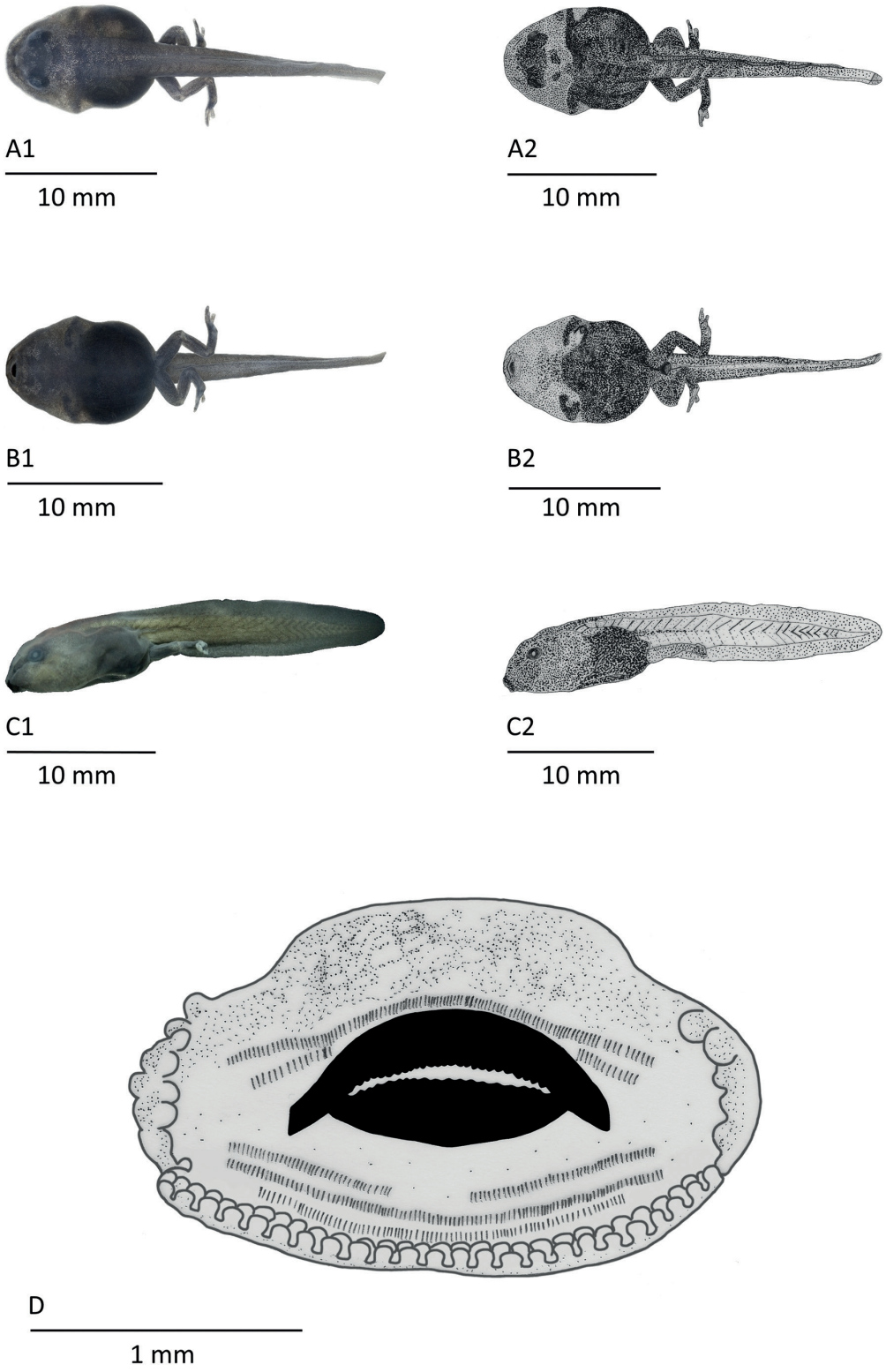

Fig. 9. Illustrations of the tadpole of *Ranitomeya imitator*, stage 41 of Gosner (1960). **A1**. Dorsal view, photograph. **A2**. Dorsal view, drawing. **B1**. Ventral view, photograph. **B2**. Ventral view, drawing. **C1**. Lateral view, photograph. **C1**. Lateral view, drawing. **D**. Drawing of the oral disc. LTRF = 2(2)/3(1).

tail is dun and spotted with puce dots; the second half is brighter than the first one. Fins are transparent and spotted with beige dots.

Coloration of a preserved tadpole of *R. imitator* (ZFMK 97358). Dorsum is beige, densely spotted with gray dots, with some brighter areas at the forelimb pouches and the muscle structures at the tail-body junction. The hindlimbs and the tail are of the same color as the dorsum, spotted with gray dots. While the dots on the hindlimbs are evenly distributed along their length, the pigmentation of the tail decreases towards the tip. The fins are transparent and spotted with gray dots. The ventral side is beige and spotted with gray dots, while the concentration of that pigmentation increases to the tail-body junction.

Larval staging. One egg with a diameter of around 1.2 mm was found within a bromeliad phytotelm, where it swam beneath the water surface. At this time, it was not pigmented, had a transparent egg integument and was encompassed by a highly glutinous layer (Fig. 10A).

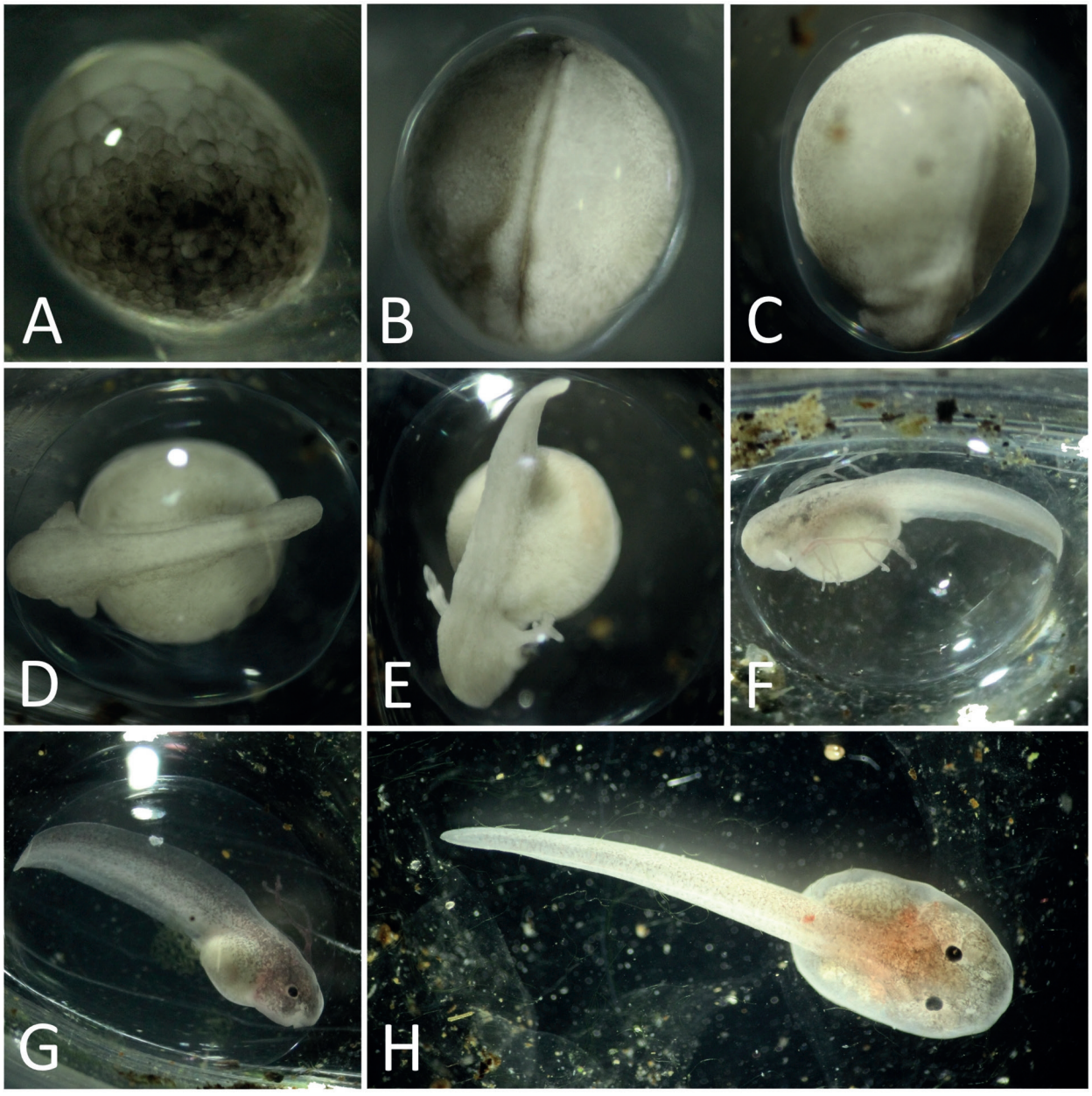

Fig. 10. Embryos and hatchlings of *Ranitomeya imitator*. **A**. Isolated egg at stage 8. **B**. Embryo at stage 14–15. **C**. Embryo at stage 16–17. **D**. Embryo at stage 19. **E**. Embryo at stage 20. **F**. Hatchling at stage 21. **G**. Hatchling at stage 24. **H**. Free swimming larva at stage 25–26. Picture C is from a different clutch.

Table 6. *Ranitomeya imitator* (n = 1) embryo and hatchling development stages.

n = 1	Day	Stage	Traits
Embryos	1	8	egg diameter 1.2 mm; egg whitish to beige; cells moderately large; swam beneath the water surface; transparent egg integument; highly glutinous; no pigmentation
	2	9	egg coloration paler than before; higher number of smaller cells
	3	11	yolk plug visible
	4	14	neural fold present
	5	19	large yolk sack present; embryonic body assumes larval shape; head and tail region visible; larva pale; gill buds present
Hatchlings	6	20	elongation of the tail; gills present; circulation recognizable; tail fins slightly visible; myosepta visible; vent tube bud visible
	7	21–22	elongation of the tail and the gills; tail pointed; overall body size increased; upper and lower tail fins more transparent; denser pigmentation of body and tail region
	8	22	elongation of the gills; eyes visible; atrophy of the yolk sack initiated
	9	22	tail fins are higher; pigmentation of the body denser; nares are discernible; yolk sack covered with blood vessels
	10	23	tail fins transparent and spotted with dots; yolk sack almost completely atrophied; oral apparatus discernible
	11–12	24	dextral gills absent, sinistral gills present; pigmentation of body and tail denser, spotted with beige dots; upper tail fin spotted with dark dots, lower tail fin with bright dots; anterior and posterior labia discernible; papillae present
	13	24	sinistral gill partially reduced; yolk sack completely atrophied; maximum body width in the second half of the body
	14–15	25	gills absent; spiracle forming on the left
	16	25	upper and lower jaw sheath visible (black); larva hatched

Table 7. *Ranitomeya imitator* (n = 3) larvae and metamorphs development stages based on image analyses. Area [cm²] is highly correlated with body mass.

n = 3	Stage (n)	Traits	Area [cm²]
Larvae	25 (3)	spiracle present; oral apparatus clearly visible; typical dorsal color pattern absent	0.22 ± 0.00
	25–27 (3)	hindlimb bud slightly developed, diameter < length; typical dorsal color pattern slightly visible at the first half of the body	0.39 ± 0.12
	28 (3)	length of the hindlimb bud equal to the diameter, no pigmentation	0.69 ± 0.11
	28–40 (3)	hindlimb bud length > diameter; foot paddle slightly visible; indentation between toes 4–5 and 3–4; indentation between toes 4–5, 3–4 and 2–3; Indentation between toes 4–5, 3–4, 2–3 and 1–2; toes 3–5 separated; all toes separated; metatarsal tubercle present; subarticular patches present; hindlimbs with pigmentation; typical dorsal color pattern present	0.89 ± 0.16
	41 (3)	forelimb buds present; typical color pattern on hindlimbs present	1.11 ± 0.04
Metamorphs	41–42 (2)	enlargement of the forelimb buds	1.18 ± 0.09
	42 (2)	forelimbs emerged	1.20 ± 0.17
	43(2)	initiation of tail resorption	1.03 ± 0.10
	43–46 (2)	reduction of the tail until metamorphosis was completed	1.03 ± 0.10

Table 8. *Ranitomeya imitator* (n = 4) larval development stages based on stereomicroscopic determinations. Area [cm²] is highly correlated with body mass.

n = 4	Stage (n)	Traits	Area [cm²]
Larvae	25(1)	sinistral spiracle present; oral apparatus clearly visible; first half of the body brighter than the second half; first half of the tail darker than second half	0.20
	26 (1)	hindlimb bud slightly developed, length < ½ diameter	0.25
	28 (1)	length of the hindlimb bud equal to the diameter	0.40
	29 (1)	length of the hindlimb bud 1.5 times of the diameter	0.59
	30 (1)	length of the hindlimb bud two times of the diameter	0.69
	31 (1)	foot paddle slightly visible	0.75
	33 (1)	indentation between toes 4–5 and 3–4	0.82
	34(1)	indentation between toes 4–5, 3–4 and 2–3; dorsal color pattern slightly visible	1.02± 0.03
	35 (1)	indentation between toes 4–5, 3–4, 2–3 and 1–2; hindlimbs with pigmentation	1.09
	36 (2)	toes 3–5 separated	1.15 ± 0.09
	37 (2)	all toes separated; metatarsal tubercle present; subarticular patches present	1.16 ± 0.05
	41	forelimb buds present; typical color pattern on hind limbs present	1.18 ± 0.09

After one day it reached stage 9, the coloration became paler and the number of discernible cells increased (Table 6). At day three the egg reached stage 11 and the yolk plug was visible, followed by the neural fold at day four (Fig. 10B). A large yolk sack was discernible and the embryonic body assumed a larval shape at stage 19 (Fig. 10D). Thus, the head and tail region became visible and the gill buds were present. After six days of development the gills were discernible and the tail underwent several changes. The upper and lower tail fins together with the myosepta were slightly visible, while the whole tail was elongated (Fig. 10E). That elongation went on until day eight, as the hatchling reached stage 22.

The tail was pointed, the overall body size and the area of the gills increased, whereby the yolk sack atrophied. The pigmentation of the body and tail region became denser; the tail fins more transparent (Fig. 10F). At day nine, the hatchling was still at stage 22. The tail fins were higher, the nares discernible and the yolk sack was covered with blood vessels. When the larva reached stage 23, the transparent tail fins were spotted with beige dots, the oral apparatus was clearly visible and the yolk sack was almost completely atrophied. During day eleven and twelve, at stage 24, the dextral gill was reduced while the sinistral gill was still present (Fig. 10G). Additionally, the pigmentation of the body and tail region became denser and the anterior and posterior labia together with the papillae were discernible. At stage 25, both gills were absent while the sinistral spiracle was present. After 16 days of development the tadpole hatched from the jelly layer and swam free in the water body (Fig. 10H). At this time it had a surface area of 0.22 cm² (Table 7).

Between stages 25 to 27, where the hindlimb bud was slightly discernible, the larvae had a surface area of 0.39 ± 0.12 cm² (Table 7). After 24 to 43 days (median = 34 days), half of all individuals had a hindlimb bud that was equally in width and length and a surface area of 0.69 ± 0.11 cm² (Table 7). Between stages 28 to 40, the tadpoles had a surface area of 0.89 ± 0.16 cm². During this development period, the hindlimbs grew, all toes became separated and the typical dorsal color pattern was discernible. After 48 to 53 days (median = 51 days), 50% of the tadpoles reached stage 41, with a surface area of 1.11 ± 0.04 cm². The forelimb buds were clearly perceptible and the hindlimbs displayed their typical reticulated color pattern. The forelimbs emerged after around 63 days, while the larvae reached their peak of growth with a surface area of 1.20 ± 0.17 cm², followed by the resorption of the tail after 67 days. During the transition to a young froglet, the surface area decreased to a mean value of 1.03 ± 0.10 cm² (Fig. 6, Table 7). A more detailed staging table based on stereomicroscopic determinations of four specimens between stages 25 to 37 can be found within Table 8.

The development was observed under constant conditions with a temperature of 24 °C, while the annual mean temperature within the natural distribution area of *R. imitator* is slightly higher (T_{Mean} = 25.5 °C, T_{Max} = 29.2 °C, T_{Min} = 21.1 °C: Karger et al. 2017a,b; Fig. 7).

Ranitomeya reticulata (Boulenger, 1884)

Breeding behavior in captivity. The breeding pair deposited the clutches, consisting of an egg, within the bromeliad phytotelm. Reproduction was occasionally.

Larval morphology. Description is based on three tadpoles at developmental stage 41 (ZFMK 97359). Further voucher specimens are ZFMK 97360, 97365 and 97378. According to McDiarmid & Altig (1999), the larvae belong to the exotrophic, lentic, benthic and arboreal larval type. All measurements that were used to calculate the

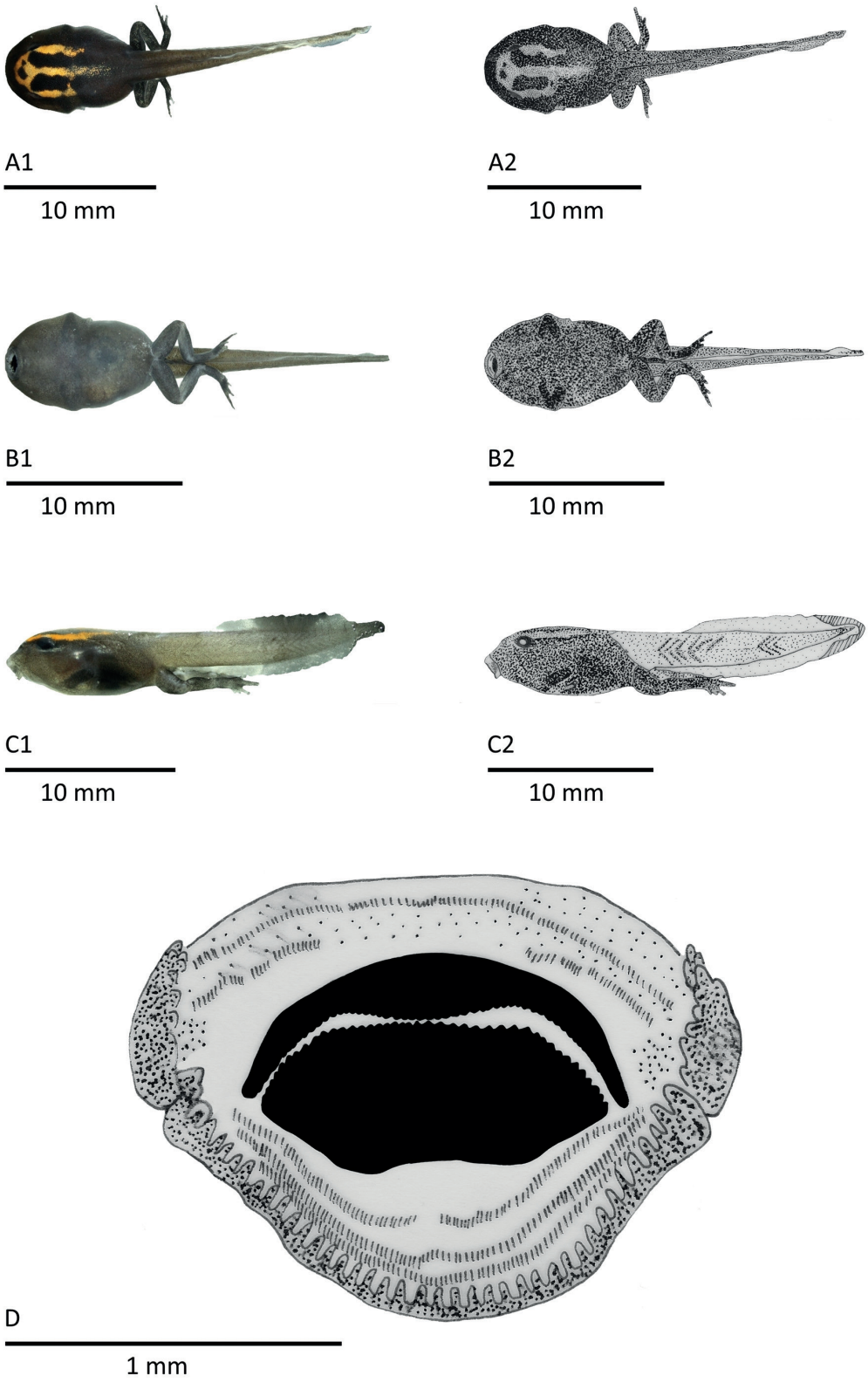

Fig. 11. Illustrations of the tadpole of *Ranitomeya reticulata*, satage 41 of Gosner (1960). **A1**. Dorsal view, photograph. **A2**. Dorsal view, drawing. **B1**. Ventral view, photograph. **B2**. Ventral view, drawing. **C1**. Lateral view, photograph. **C2**. Lateral view, drawing. **D**. Drawing of the oral disc. LTRF = 2(2)/3(1).

Table 9. *Ranitomeya reticulata* (n = 3) larvae development stages based on image analyses. Area [cm²] is highly correlated with body mass. Note that data concerning metamorphs is missing as no specimen reached this stage.

n = 3	Stage (n)	Traits	Area [cm²]
Larvae	25 (3)	spiracle present; oral apparatus clearly visible; typical dorsal color pattern absent	0.23 ± 0.09
	25–27 (3)	hindlimb bud slightly developed, diameter < length	0.32 ± 0.11
	28 (2)	length of the hindlimb bud equal to the diameter, no pigmentation	0.81 ± 0.01
	28–40 (2)	hindlimb bud length > diameter; foot paddle slightly visible; indentation between toes 4–5 and 3–4; indentation between toes 4–5, 3–4 and 2–3; Indentation between toes 4–5, 3–4, 2–3 and 1–2; toes 3–5 separated; all toes separated; metatarsal tubercle present; subarticular patches present; hindlimbs with pigmentation; typical dorsal color pattern discernible	0.88 ± 0.06
	41 (2)	forelimb buds present; typical color pattern on hindlimbs present	1.02 ± 0.11

Table 10. *Ranitomeya reticulata* (n = 5) larval development stages based on stereomicroscopic determinations. Area [cm²] is highly correlated with body mass.

n = 5	Stage (n)	Traits	Area [cm²]
Larvae	31 (3)	foot paddle is present; hindlimb bud length is two times of the diameter	0.53 ± 0.01
	34 (2)	indentation between toes 4–5, 3–4 and 2–3	0.64 ± 0.13
	36 (5)	indentation between toes 4–5, 3–4, 2–3 and 1–2; toes 3–5 are separated; dorsal color pattern extends from the posterior edge of the nares to the second half of the body	0.72 ± 0.08
	37 (4)	all toes are separated	0.77 ± 0.06
	39 (5)	subarticular patches present; dorsal color pattern is denser, has cross connections and ends at tail-body junction	0.80 ± 0.05
	41 (5)	forelimb buds are visible; vent tube is still present	0.78 ± 0.02

following proportions and its comparison with the other species of this study, are be found in the Table 13.

Dorsal view: The body is oval and elongated (MBW/BL = 0.62). The snout is short and moderately pointed (RED/BL = 0.28, BWN/BWE = 0.65). The shape of the nares is not visible in dorsal view, nares are closer to the snout than to the eyes (RND/RED = 0.40). A skin fold, which originates at the nares, ends close to the anterior margin of the eyes; the two landmarks are not connected. The eyes are large (ED/BL = 0.09), positioned dorsally and orientated laterally. The internarial distance is smaller than the interorbital distance (IND/IOD = 0.53). The single, sinistral spiracle as well as parts of the oral apparatus are visible in dorsal view.

Lateral view: Body is slightly depressed (MBH/MBW = 0.68), the snout is pointed. Nares are round, located and orientated dorsally. The spiracle is situated below the longitudinal axis, at the second half of the body (RSD/BL = 0.64), the inner wall is free from the body and the opening is round. The maximum body height is located posterior to the eye. The tail is long and moderately pointed (TAL/BL= 1.64, TAL/TL = 0.62). The "V"-shaped myosepta are visible along the whole length of the tail. The upper fin originates posterior to the lower fin and the tail-body junction, the margin of the lower fin is nearly parallel to the margin of the tail muscle. Ventral tube is strongly atrophied, emergence from abdomen sagittal. Hindlimbs are completely developed. Oral apparatus is visible in lateral view.

Oral apparatus: The oral disc is elliptical, emarginated, located anteroventrally and covers more than one third of the maximum body width (ODW/MBW = 0.40). Marginal, pointed and pigmented papillae are present at the posterior labium and except the most lateral part, absent at the anterior labium. Submarginal papillae are absent. The anterior labium contains two tooth rows of an equal width (A1, A2), the second tooth row has a large medial gap (A2-GAP). The posterior labium contains three tooth rows (P1, P2, P3), with a moderate gap in the first row (P1-GAP). Tooth row P1 and P2 are of the same width, the width of the P3 was not discernible. Both jaw sheaths are black and serrated. The tooth row formula is 2(2)/3(1) (Fig. 11D).

Coloration of a living tadpole of *R. reticulata* (ZFMK 97359). The dorsum has an anthracite basic color, with three golden to orange stripes running on top or parallel to the longitudinal axis (Fig. 11A1, A2). The two dorso-

lateral stripes originate at one point posterior to the nares, bifurcate and run close to the eyes, with a moderate gap on eye level. The medial stripe runs in between the two others, situated on the symmetry line of the body. Depending on the specimen, the medial and the dorsolateral stripes are fused, originating from one point posterior to the nares and anterior to the eyes. The distance between the stripes decreased at the second half of the body. The hindlimbs and the tail are as anthracite as the dorsum, spotted with darker dots. Fins are transparent and spotted with grayish dots.

Coloration of a preserved tadpole of *R. reticulata* (ZFMK 97359). The dorsum has a beige basic color, with some darker areas at the outermost part of the forelimb pouches and one small line at the anterior margin of the dorsolateral stripes. The area in between the dorsolateral stripes, which extends to the tail-body junction, is of the same color as the dark areas mentioned beforehand. The dorsolateral and median stripes are clearly discernible on the head and the first half of the body, running on top or parallel to the longitudinal axis. The dorsolateral stripes originate and bifurcate at one point posterior to the nares and run next to the eyes, with a moderate gap on eye level. The whitish median stripe originates in between the eyes, not fusing with the origin of the dorsolateral stripes. Anterior to the eye, the dorsolateral stripes are beige, posterior they are whitish. The hindlimbs and the tail are as beige as the dorsum, spotted with some dark dots. Fins are transparent and spotted with dark dots. The ventral side is beige, spotted with gray dots. The hindlimbs' ventral side is brighter than the dorsal side.

Larval staging. At stage 25, right after hatching, the tadpoles had a surface area of 0.23 ± 0.09 cm². During the transition from stage 25 to 27, where the hindlimb buds were slightly visible, the surface area increased to 0.32 ± 0.11 cm². After 29 to 41 days (median = 36 days), half of all larvae had developed a hindlimb bud that was equally in diameter and length and reached a surface area of 0.81 ± 0.01 cm². Between the stages 28 to 40, the larvae had a surface area of 0.88 ± 0.06 cm². During this development period, the hindlimbs grew, all toes became separated and the typical dorsal color pattern was present. The forelimb pouches were discernible after a minimum of 42 and a maximum of 63 days, while half of all individuals reached that development stage after 54 to 58 days (median = 56 days). At this point, the tadpoles had a surface area of 1.02 ± 0.11 cm². Not a single larva completed the full metamorphosis to a young froglet (Fig. 6, Table 9). A more detailed staging table based on stereomicroscopic determinations of five specimens from an external source between stages 25 to 37 can be found within the Table 10.

The development was observed under constant conditions with a temperature of 24 °C, while the annual mean temperature within the natural distribution area of *R. reticulata* is slightly higher (T_{Mean} = 24.8 °C, T_{Max} = 28.2 °C, T_{Min} = 21.5 °C: Karger et al. 2017a,b; Fig. 7).

Ranitomeya sirensis (Aichinger, 1991)

Breeding behavior in captivity. The breeding pairs among the four specimens deposited clutches of up to two eggs in the bromeliad phytotelm. Reproduction occurred occasionally.

Larval morphology. The description is based on lateral and dorsal pictures of one specimen at stage 29. Thus, no voucher specimen is available. According to McDiarmid & Altig (1999), the tadpole belongs to the exotrophic, lentic, benthic and arboreal larval type. All measurements that were used to calculate the following proportions and its comparison with the other species of this study, can be found in Appendix III.

Dorsal view: Body shape is oval and slightly elongated (MBW/BL = 0.86). The snout is short and round (RED/BL = 0.24, BWN/BWE = 0.88). Nares are oval in dorsal view. The eyes are large (ED/BL = 0.09), located dorsally

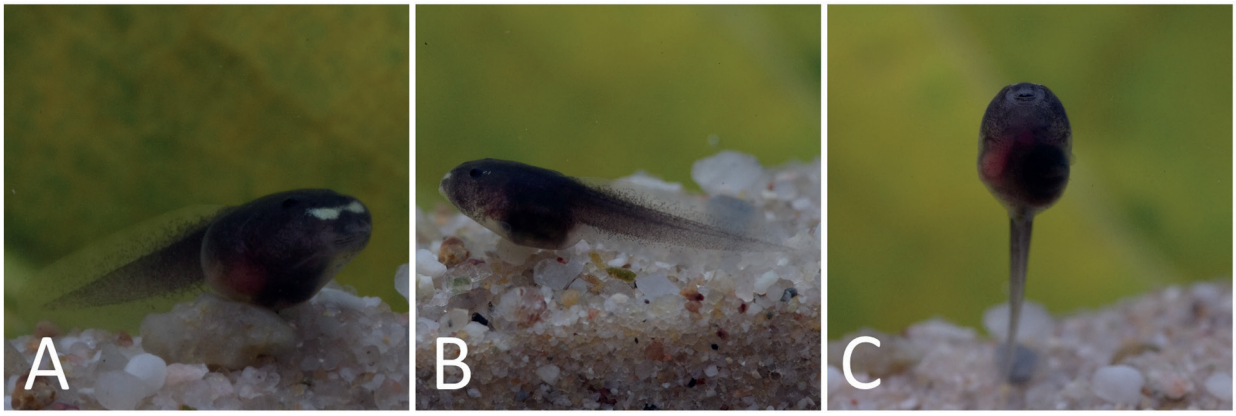

Fig. 12. Illustrations of the tadpole of *Ranitomeya sirensis*, stage 29 of Gosner (1960). **A**. Frontolateral view. **B**. Lateral view. **C**. Ventral view. Photo credit: Morris Flecks.

Table 11. *Ranitomeya sirensis* (n = 31) embryos and hatchlings development stages based on image analyses. Area [cm²] is highly correlated with body mass.

n = 31	Stage (n)	Traits	Area [cm²]
Larvae	25 (31)	sinistral spiracle present; oral apparatus clearly visible	0.16 ± 0.04
	25–27 (31)	hindlimb bud slightly visible, length < diameter	0.29 ± 0.13
	28 (15)	hindlimb bud length = diameter	0.58 ± 0.11
	28–40 (15)	hindlimb bud length > diameter; foot paddle slightly visible; indentation between toes 4–5 and 3–4; indentation between toes 4–5, 3–4 and 2–3; Indentation between toes 4–5, 3–4, 2–3 and 1–2; toes 3–5 separated; all toes separated; metatarsal tubercle present; subarticular patches present; hindlimbs with pigmentation; typical dorsal color pattern present	0.86 ± 0.23
	41 (15)	forelimb buds present; typical color pattern on hindlimbs present; lateral stripes present	1.15 ± 0.17
Metamorphs	41–42 (15)	enlargement of the forelimb buds	1.18 ± 0.20
	42 (15)	forelimbs emerged	1.20 ± 0.16
	43 (15)	initiation of tail resorption	1.04 ± 0.16
	43–46 (15)	reduction of the tail until metamorphosis was completed	1.01 ± 0.16

and oriented dorsolaterally. Internarial distance in smaller than interorbital distance (IND/IOD = 0.48). Sinistral spiracle is clearly visible in dorsal view.

Lateral view: The body is depressed (MBH/MBW = 0.71), the snout is moderately pointed. Nares are almost round. Sinistral spiracle is situated below the longitudinal axis, at the second half of the body (RSD/BL = 0.53), oriented laterally with an elliptical opening, whereas the inner wall of the spiracle is free from the body wall. The maximum body height is situated posterior to the spiracle. The tail is long and broadly rounded (TAL/BL = 1.90, TAL/TL = 0.66). The tail musculature is well developed (TMH/MTH = 0.51), "V"-shaped myosepta are visible at the first two thirds of the tail. Both fins are equal in height and originate at the tail body junction. The ventral tube is situated dextrally, the emergence from the abdomen is sagittal and the opening is oval. Hindlimb development is not completed (length ≥ 150% of the diameter). Upper and lower labia are clearly visible in lateral view.

Oral Apparatus: The oral disc is emarginated, elliptical, positioned ventrally and covers more than one third of the maximum body width (ODW/MBW = 0.39). Marginal papillae are present at the posterior labium and at the outermost parts of the anterior labium. The anterior labium contains two tooth rows of the same width (A1, A2), with a large gap in the second row (A2-GAP). The posterior labium contains three tooth rows (P1, P2, P3), of which the first has a moderate medial gap (P1-GAP). The first two rows (P1, P2) have the same width, while the third one (P3) is slightly shorter. The tooth row formula is 2(2)/3(1).

Coloration of living tadpole of *R. sirensis.* The basic color is beige, densely coverered with dark dots. Two light blue spots anterior to the nares, fused medially (Fig. 12A). The first half of the dorsum is brighter than the second half, additionally slightly transparent below the longitudinal axis (Fig. 12B). Inner organs are visible in ventral and lateral view (Fig. 12C). The hindlimb buds are white, slightly pigmented at the base. The tail has the same coloration as the dorsum, the color density of the dark pigmentation wanes to the posterior end. The tip of the tail lacks any pigmentation. Fins are transparent and spotted with brown dots. The density of those dots decreases to the tip.

Larval staging. During the stages 25 to 27, before the hindlimb buds were clearly discernible, the larvae had a mean surface area of 0.29 ± 0.13 cm² (Table 11). After 24 to 39 days, half of the tadpoles reached stage 28 (median = 31 days). At this time, the hindlimb buds were almost equal in width and length and the surface area increased to 0.58 ± 0.11 cm². In between the stages 29 to 40, the hindlimb development was completed and the larvae had a mean surface area of 0.86 ± 0.23 cm². After 47 to 56 days, 50% of the individuals reached stage 41 (median = 52 days). The forelimb buds were perceptible and the tadpoles had a surface area of 1.15 ± 0.17 cm². While the forelimbs grew inside the dorsum, the larval growth rate decreased. After 56 to 65 days, half of the tadpoles reached stage 42 and the forelimbs emerged through the body wall (median = 60 days). At this time, the tadpoles reached their peak of growth with a surface area of 1.20 ± 0.16 cm². Afterwards, between day 60 and 71 (median = 63 days), the resorption of the tail was initiated. Close to the end of the metamorphosis, when the froglets

had just a short remnant of the tail, the metamorphs had a surface area of 1.01 ± 0.16 cm² (Fig. 6, Table 11).

The development was observed under constant conditions with a temperature of 24 °C, while the annual mean temperature within the natural distribution area of *R. sirensis* is slightly higher (T_{Mean}=24.7 °C, T_{Max}=29.1 °C, T_{Min}=18.8 °C: Karger et al. 2017a,b; Fig. 7).

Ranitomeya vanzolinii (Myers, 1982)

Breeding behavior in captivity. Successful reproductions were observed in two different terraria, each inhabited by four specimens. While the breeding pairs of the first tank deposited the clutches of two to three whitish to beige eggs in a horizontally orientated and dry film container, the breeding pairs of the second tank placed their clutches of similar size in the bromeliad phytotelm. In rare cases, tadpoles at different development stages were found within the bromeliad phytotelm. Reproduction occurred occasionally.

Larval morphology. The description is based on a single specimen at stage 41 (ZFMK 97361). Further voucher specimens are ZFMK 97369 and 97379. According to McDiarmid & Altig (1999), the tadpole belongs to the exotrophic, lentic, benthic and arboreal larval type. All measurements that were used to calculate the following proportions and its comparison with the other species of this study, can be found in Appendix III.

Dorsal view: Body shape is oval and elongated (MBW/BL=0.76). The snout is short and moderately pointed (RED/BL=0.23, BWN/BWE=0.65). The shape of the nares is not visible in dorsal view. A skin fold connects the nares with the anterior margin of the eyes. Eyes are large (ED/BL=0.10), located dorsally and orientated laterally. Internarial distance is smaller than interorbital distance (IND/IOD=0.48). The single sinistral spiracle is not visible in dorsal view.

Lateral view: Body is depressed (MBH/MBW=0.71), snout is round. Nares are shaped elliptically, located laterally and orientated ventrolaterally. The single, sinistral spiracle is situated below the longitudinal axis, at the second half of the body (RSD/BL =0.61), and is oriented laterally. The inner wall is free from the body and the opening is round. The maximum body height is situated posterior to the eye. The tail is long and broadly rounded (TAL/BL=1.87, TAL/TL=0.65). The musculature is well developed (TMH/MTH=0.54, TMW/MBW=0.33). "V"-shaped myosepta are visible along the whole length of the tail, particularly in the second half. At the maximum tail height, the upper fin is nearly double as high as the lower fin. Both fins originate at the tail-body junction. Ventral tube is slightly reduced, dextral, emergence sagittal from abdomen. Hindlimb development is completed. Upper and lower labia are visible in lateral view.

Oral apparatus: The oral disc is elliptical, emarginated, positioned ventrally and covers nearly one third of the maximum body width (ODW/MBW=0.27). Marginal, transparent and rounded papillae are present at the posterior labium and except of six to seven papillae at the most lateral part, absent at the anterior labium. Submarginal papillae are absent. The anterior labium contains two tooth rows of the same width (A1, A2) with a large medial gap in the second row (A2-GAP). The posterior labium contains three tooth rows (P1, P2, P3) of which the first row has a moderate medial gap (P1-GAP). P2 is slightly shorter than P1, P3 is slightly shorter than P2. Both jaw sheaths are black and serrated, lateral processes of the upper jaw sheath are present and extend barely past

Table 12. *Ranitomeya vanzolinii* (n=13) larvae and metamorphs development stages based on image analyses. Area [cm²] is highly correlated with body mass.

n=13	Stage (n)	Traits	Area [cm²]
Larvae	25 (13)	sinistral spiracle present; oral apparatus clearly visible	0.19 ± 0.05
	25–27 (13)	hindlimb bud slightly visible, length < diameter	0.32 ± 0.11
	28 (11)	length of the hindlimb bud equal to the diameter	0.53 ± 0.15
	29–40 (11)	hindlimb bud length > diameter; foot paddle slightly visible; indentation between toes 4–5 and 3–4; indentation between toes 4–5, 3–4 and 2–3; Indentation between toes 4–5, 3–4, 2–3 and 1–2; toes 3–5 separated; all toes separated; metatarsal tubercle present; subarticular patches present; hindlimbs with pigmentation	0.76 ± 0.13
	41 (9)	forelimb buds present	0.98 ± 0.09
Metamorphs	41–42 (9)	enlargement of the forelimb buds	1.00 ± 0.10
	42 (9)	forelimbs emerged	1.03 ± 0.11
	43 (9)	initiation of tail resorption	0.92 ± 0.11
	43–46 (9)	reduction of the tail until the completion of the metamorphosis	0.86 ± 0.13

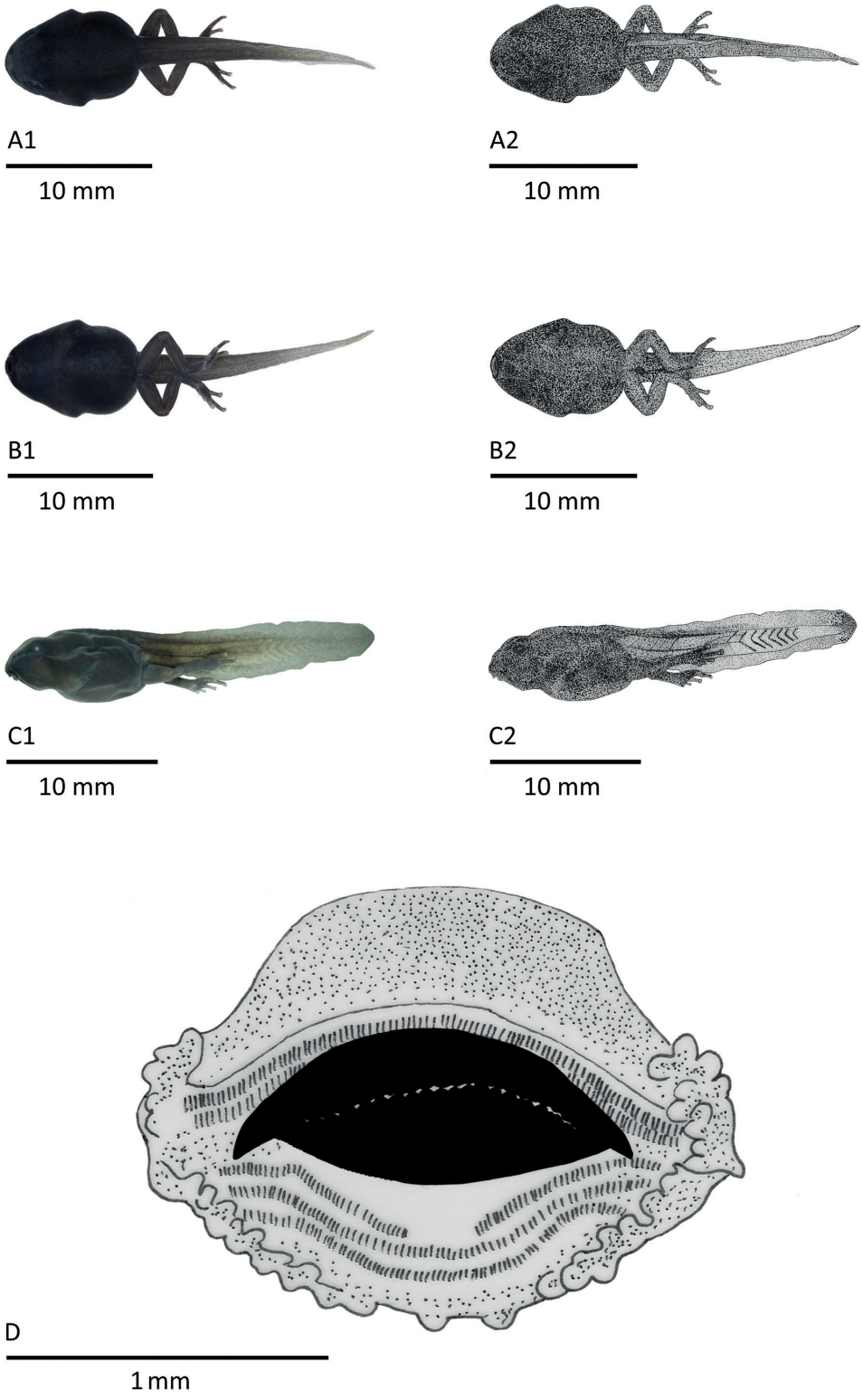

Fig. 13. Illustrations of the tadpole of *Ranitomeya vanzolinii*, stage 41 of Gosner (1960). **A1**. Dorsal view, photograph. **A2**. Dorsal view, drawing. **B1**. Ventral view, photograph. **B2**. Ventral view, drawing. **C1**. Lateral view, photograph. **C1**. Lateral view, drawing. **D**. Drawing of the oral disc. LTRF = 2(2)/3(1).

the lower jaw sheath. The tooth row formula is 2(2)/3(1) (Fig. 13D).

Coloration of a living tadpole of *R. vanzolinii* (ZFMK 97361). The basic color of the dorsum is dark gray to black and lacks any pattern of another color (Fig. 13A1, A2). Hindlimbs are equally colored. The tail is brighter than the dorsum, with a color gradient between the first and the second half of the tail, whereas the color becomes brighter till the tip. The transparent fins are spotted with dark dots.

Coloration of a preserved tadpole of *R. vanzolinii* (ZFMK 97361). The basic color of the dorsum is anthracite, with some beige spotted areas at the forelimb pouches and the muscle attachment of the tail as well as a light gray area which originates at the tip of the snout and extends to the posterior margin of the eyes. The hindlimbs are of the same color as the dorsum, slightly spotted with beige dots. The tail is beige; the anterior half is darker than the posterior one. Fins are transparent and spotted with gray dots. The ventral side is as anthracite as the dorsal side, slightly spotted with beige dots.

Larval staging. At stage 25, right after hatching, the tadpoles had a surface area of 0.19 ± 0.05 cm². During the transition from stage 25 to 27, when the hindlimb bud was just slightly visible in some rare cases, the tadpoles had a surface area of 0.32 ± 0.11 cm² (Table 12). After 32 to 52 days, 50% of the larvae had reached stage 28. At this time, while the hindlimb bud was as long as wide and therefore clearly discernible, the tadpoles had a mean surface area of 0.53 ± 0.15 cm². In between the stages 29 to 40, the development of the hindlimbs was completed and the larvae had a mean surface area of 0.76 ± 0.13 cm². All toes became separated and the hindlimbs pigmented. After 51 to 73 days (mean = 60 days), half of all tadpoles had reached stage 41 (Fig. 6). The forelimb buds were clearly discernible and the larvae had a mean surface area of 0.98 ± 0.09 cm². While the forelimbs grew inside the body, the larval growth rate decreased. After 64 to 94 days (median = 73 days), half of the tadpoles reached stage 42 and the forelimbs emerged. At this time the tadpoles reached their peak of growth with a surface area of 1.03 ± 0.11 cm². Afterwards, as a part of the ongoing metamorphosis during the stages 43 to 46, the tail was reduced and the mean surface area decreased to a value of 0.86 ± 0.13 cm² (Fig. 6, Table 12). Altogether, the transition from a free living larva to a metamorph which initiated the resorption of the tail lasted 61 to 107 days, while half of all tadpoles reached that development period after 66 to 91 days (median = 77 days).

The development was observed under constant conditions with a temperature of 24 °C, while the annual mean temperature within the natural distribution area of *R. vanzolinii* is slightly higher (T_{Mean} = 24.6 °C, T_{Max} = 28.9 °C, T_{Min} = 19.6 °C: Karger et al. 2017; Fig. 7).

DISCUSSION

We presented new data on the tadpole morphology and development of six *Ranitomeya* species allowing for the first time the identification of specimens in different developmental stages in a captive breeding setup. The development, as studied herein, strongly coincides with the tadpole staging system provided by Gosner (1960). However, few morphological variations between the herein studied tadpoles and a generalized tadpole at Gosner stage 41 were not compatible with those reported in the literature. We observed a delay among the atrophy of the ventral tube on *R. amazonica* tadpoles as well as a delay among the atrophy of the oral apparatus on *R. vanzolinii* tadpoles. The ventral tube in *R. amazonica* was still fully developed, different from that observed for the tadpoles of the other species studied herein, where the ventral tube was partially absorbed. The tadpoles of *R. vanzolinii* in this study displayed a complete oral apparatus, including all anterior and posterior rows of teeth, different from what is reported in Brown et al. (2011) where the tooth rows of *R. vanzolinii* are irregular at stage 40.

The complete metamorphosis was described for five of the six species studied here, as unfortunately none of the tadpoles of *R. reticulata* completed the full metamorphosis. In *R. amazonica*, a species of the *variabilis* group, the tadpoles needed 91–99 days for the complete metamorphosis. The tree species of the *vanzolinii* group studied herein, namely *R. sirensis*, took 60–71 days, *R. imitator* grew up within 67 days and *R. vanzolinii* needed 66–91 days for the complete metamorphosis. *R. benedicta* of the *variabilis* group needed 114 days for the complete metamorphosis.

Waldram (2008) stated that tadpoles of *R. sirensis* (as *R. biolat*) needed 58 days until they completed the metamorphosis in a natural environment. Herein we observed a difference in relation of these results, where the tadpoles of *R. sirensis*, which were bred at a constant temperature of 24 °C with an artificial food resource needed 60–71 days before the absorption of the tail was initiated. In the natural environment, anuran larvae respond to temperature variation by an alteration of their growth and developmental rates (Alvarez & Nicieza 2002; Smith-Gill & Berven 1979). In our study, all clutches were bred under constant conditions (24 °C) and in equivalent water chemistry and nutrition. However, Kam et al. (2001) noticed that the fluctuations in the temperature of phytotelmata mirrored fluctuations in air temperature and hence the water temperatures in the phytotelms are not likely to be constant. Findings of Poelman et al. (2013) support this assumption, as the water temperatures reported in the studied phytotelms present similar averages as the data

of air temperatures obtained from the CHELSA data set (Karger et al. 2017a,b). It needs to be noted that the nutritional conditions in a natural environment are different from that provided during our study.

However, even if the development of the tadpoles studied herein took place in an environment which is different from natural conditions, our results suggest only small morphological differences compared to other descriptions based on tadpoles collected in the field. Therefore, we suggest that if the temperature in the climatic test chamber mimics as closely as possible the known temperatures within the natural habitats of the species, the pace of larval development is presumably more accurate under artificial conditions as they can be easily standardized.

While the coloration of the eggs can be used to distinguish the *vanzolinii* clade from all remaining groups, the coloration of tadpoles does not allow this. Nevertheless, the typical color pattern and the reticulation of the hindlimbs verify the assignment of the specimens to the genus *Ranitomeya*. The provided pictures, drawings and descriptions of the tadpoles should allow to at least the identification of specimens on genus level, which could be useful to help customs officials to recognize CITES protected animals in earlier development stages and therefore reduce the illegal trade.

Methods criticism: Advantages, limitations and efficiency

Recent studies provide growth rates in order to classify the fitness of a species and therefore predict the effects of changing environmental conditions or biotic factors (e.g., as reviewed by Dmitriew 2011). They are either obtained by length-based measurements, quantified by weight or image-based approaches (Relyea 2004; Davis et al. 2008; Pham et al. 2015). As the body length of the tadpole at Gosner stage 35 or greater is highly correlated with the SVL of the young froglet, growth rates based on the former approach usually end at this point of development, as seen in *Amolops cremnobatus* (Inger & Kottelat 1998) (McDiarmid & Altig 1999; Pham et al. 2015). Thus, it implies that the change in body size over time stops as well, although shape changes alter the tadpoles' body mass as well as the surface area during this period. Moreover, repeated measurements of living specimens, either with calipers or integrated eye pieces, are stressful for fragile individuals like tadpoles. Studies based on the latter approach use the surface area of a tadpole as a proxy for its body mass. SAISAQ, the method used in this study to generate growth rates of the tadpoles, was introduced by Kurth et al. in 2014, extending the image-based concept of Davis et al. (2008). Instead of manually analyzing images with software packages like Fovea Pro or ImageJ, the implementation into the open source statistic platform R allows a semiautomatic procession based on standardized image files. Nonetheless, the capabilities of this method are limited. The emerging forelimbs at Gosner stage 41 may affect the dorsal surface to mass relationship, which could falsify the results of the actual and subsequent stages. Moreover, the calibration of the camera in a fixed angle and distance to the object influences the picture quality. While the surface area is sharp and high in contrast, the depth of field decreases. Thus, staging tables based on those pictures need conspicuous traits, reducing their resolution. Nevertheless, SAISAQ allows to document, quantify and monitor the tadpole development in a time-efficient way, obtaining huge amounts of data which could be used to extend our current knowledge of several anuran species.

In order to maximize the sample size while reducing the mortality rate, documentations of the embryogenesis as well as microscopic determinations of the developmental stages were neglected until the growth rate related data acquisition was done. Species which stopped their reproduction at this time of the study lack these measurements (*Ranitomeya benedicta*, *R. sirensis* and *R. vanzolinii*). Thus, staging tables that allow a comparison of all six species are based on image analyses of the growth rate related photographs, which are less detailed than microscopic examinations. Therefore, further studies should either start with the documentation of detailed staging tables and the embryonic development, or conduct both methods simultaneously to add to the completeness of current descriptions. Additionally, an adjustment of the climatic test chamber in regard to the temperatures of the macro-habitats of the species could prove the statement that specimens develop faster if the artificial environment mirrors their natural conditions.

In times of a global biodiversity crisis and wide spread population declines in amphibians, conservation breeding programs become increasingly important. Our data may provide a baseline for further research how to optimize captive breeding in *Ranitomeya* species. The developmental staging tables and growth rates can be used to compare different husbandry and breeding setups. Furthermore, we hope that the detailed larval descriptions are also useful for the identification of specimens by customs.

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APPENDIX I.

Different camera settings (1–4) with regard to light intensities and ISO-Settings.

Setting	**1**	**2**	**3**	**4**
Light intensity [%]	50	66	80	100
ISO	1600	1600	1600	100

APPENDIX II.

A brief description of the natural history of the target species of this study

Ranitomeya amazonica Schulte (1999) is a poison dart frog placed in the *variabilis* group of the genus *Ranitomeya* (Brown et al. 2011). The basic color is black, with a yellowish orange pattern of medial, dorsolateral and lateral stripes. Frequently, the black component forms a "Y" on the back, which begins at the anterior margins of the eyes and ends at the cloaca (Fig. 1, green color code). The limbs and the ventral side are teal and dotted with black spots. Known are two widely separate populations of *R. amazonica*: east population, distributed in extreme southern Guyana; eastern French Guiana; region of the mouth of the Amazon in Brazil and west population, distributed in northwestern Amazonian Peru (Loreto), extreme southeastern Colombia (Amazonas) and expected in the adjacent borderlands of Brazil (Frost 2006). In the present study we used data from individuals of the western population (Fig. 1). They inhabit primary and secondary rainforests, limited to sparse stands of stunted trees (Lötters et al. 2007). Clutches of two to six eggs can be found in water filled leaf axils of those bromeliads (Lötters et al. 2007). Due to continuous doubts concerning the taxonomic validity of *R. amazonica*, the extent of its occurrence as well as the ecological requirements are unknown. Therefore, the IUCN lists this species as data deficient (IUCN 2015).]

Ranitomeya benedicta (Brown et al. 2008), also known as blessed poison frog, is placed within the *reticulata* group of the genus *Ranitomeya* (Brown et al. 2011). The predominant color is black, covered by a blue reticulation. Except for the black spots surrounding the eyes, the head region is red and extends posterior to the shoulders (Fig. 1, light blue color code). In some individuals the black areas around the eyes are medially fused and extend to the tympanum, forming a "W"-shaped face mask. Limbs and the ventral side show the same coloration as the dorsum, whereas the throat region is covered by a black marbling. They are distributed throughout the lowland forests of southern Loreto and eastern San Martín, Peru (Brown et al. 2011). While they primarily occur in

forests which are located 150 m above sea level, some individuals have been sighted in areas over 315–405 m elevation (Brown et al. 2008; von May et al. 2008b). Clutches consist of two to six eggs and can be found within the leaf litter covering the forest floor (Brown et al. 2008). Due to an estimated extent of occurrence of about 19.000 km², declining habitats as well as negative effects of the international pet trade, the IUCN list this species as vulnerable (IUCN 2015).

Ranitomeya imitator (Schulte 1986), also known as mimic poison frog, is placed in the *vanzolinii* group of the genus *Ranitomeya* (Brown et al. 2011). There are three different forms, whereby the study organism was a highland form, which is called the *variabilis* or "two dots" type. The ground color of the dorsal side is teal, covered with large black dots. The nostrils are surrounded by two black dots, which extends to the snout and therefore are responsible for the name "two dots". The ventral side is grayish blue, while the throat is usually yellowish. The limbs are teal and covered with irregular small black dots. The "two dots" or highland form is distributed in Cordillera Oriental, the east of the Departamento San Martín, Peru, 250–1000 m a.s.l. with temperatures fluctuating between 22–26 °C (Lötters et al. 2007) (Fig. 1). The frogs usually inhabit moist premontane primary and secondary forests, but are also able to live along roads or at the margins of plantations, usually found in vegetation heights between 0.5 and 1.5 m above the ground (Lötters et al. 2007). Clutches consist of one to two white eggs, which are placed in a rolled up leaf (Schulte 1986; Brown et al. 2011). Due to its wide distribution range with many suitable habitats and large populations, the IUCN list them as least concern (IUCN 2015).

Ranitomeya reticulata (Boulenger, 1884) is placed within the *reticulata* group of the genus *Ranitomeya* (Brown et al. 2011). The head and the back are usually copper red to reddish brown, while the limbs, the lower sides and the flanks up to the dorsal and sacral region are covered with irregular sized black spots on a bluish background (Fig. 1, yellow color code). The species is distributed throughout the lowland forests of the Departamento Loreto, Peru, 150–200 m a.s.l to the province of Pastaza, Ecuador, 200–340 m a.s.l. (Brown et al. 2011). In the vicinity of Iquitos, Departamento Loreto, the frogs occur in syntropy to *R. amazonica* in the "varillales". While *R. amazonica* is more common in moist environments, individuals of *R. reticulata* can be found more frequently in dryer ones, where they perched in vegetation up to 2 m height. In captivity, clutches of one to five eggs are deposited in dark and horizontal places (Lötters et al. 2007). Due to its wide distribution with presumable large populations, the IUCN list them as least concern (IUCN 2015).

Ranitomeya sirensis (Aichinger, 1991), also known as the Sira-poison frog, nowadays comprises the two former species *R. biolat* and *R. lamasi* and is placed in the *vanzolinii* group of the genus *Ranitomeya* (Brown et al. 2011). The ground color of our breeding group was black, with an orange pattern that consists of five thin stripes. The median and the dorsolateral stripes originate anterior to the margin of the eyes and end at the cloaca and at the margin of the thighs respectively, while the two lateral stripes originate at the tip of the snout in between the nostrils and end next to the dorsolateral stripes (Fig. 1, blue color code). Limbs are sage and covered with black spots. The species is distributed from the Amazonian Basin in central eastern and south eastern Peru (Departamentos Loreto, San Martín, Ucayali, Pasco, Junín, Huánuco, Cusco, Madre de Dios) to the southern part of Brazil (State of Acre) and the northern part of Bolivia (Departamento of Pando) (Brown et al. 2011). They usually inhabit premontane and montane primary and secondary forests at elevations between 250–1560 m a.s.l. with an annual precipitation between 1000–7000 mm (Schulte 1999; von May et al. 2008b; Brown et al. 2011). Depending on the elevation, the species inhabits bromeliads or bamboo forests, but also tolerates modulated habitats such as coffee plantations (Lötters et al. 2007). Due to its wide distribution with presumable large populations in combination with the fact that many habitats are protected, the IUCN list this species as least concern (IUCN 2015).

Ranitomeya vanzolinii (Myers, 1982), also known as the Brazilian poison frog, is a member of the *vanzolinii* group of the genus *Ranitomeya* (Brown et al. 2011). The basic color is black, covered with irregular yellow spots which are sometimes fused to lines, especially close to the eyes and at the flanks, or create a marbled pattern at the ventral side. Limbs are teal and covered with black spots. The throat is yellow, whereas a wide dark line crosses the entire width of the posterior part. The species distribution ranges from central eastern parts of Peru (Departamentos Loreto, Huánuco and Pasco) to western Amazonian parts (Estado Acre) of Brazil (Brown et al. 2011) (Fig. 1). They usually inhabit primary lowland forests at an elevation between 200–400 m, except one locality which is situated in the premontane and moist cloud forests between Río Pachitea and Río Ucayali at an altitude of 1300 m (von May et al. 2008b, Brown et al. 2011). As a tree-dwelling species, specimens can be found on trunks, branches and leaves in heights of up to 4 m, or on the ground (Lötters et al. 2007). Clutches consist of one to two light colored eggs which are produced by the same pair that regularly spawns together (Caldwell 1997; Brown et al. 2011). Although the population trend is decreasing, the IUCN still list them as least concern, because they are widely distributed and the populations are presumed to be large enough (IUCN 2015).

APPENDIX III.

Measurements of six different species of the genus *Ranitomeya*. **1**. *R. amazonica.* 2. *R. benedicta.* 3. *R. imitator.* 4. *R. reticulata.* 5. *R. sirensis.* 6. *R. vanzolinii*. Measurements were taken from voucher specimens at stage 41, except *R. sirensis* which was at stage 29. All measurements are given in millimeters [mm]. Abbreviations: BL=body length; BWE=body width at eye level; BWN=body with at nostril level; ED=horizontal eye diameter; END=eye nostril distance, IND=internarial distance; IOD=interorbital distance; MBH=maximum body height; MBW=maximum body width; MTH=maximum tail height; ODW=oral disc width; TAL=tail length; TMH=tail muscle height at base; TMW=tail muscle width at base; TL=total length; RED=rostro-eye distance, from tip of snout to the center of the eye in lateral view; RND=rostro-nasal distance, from tip of snout to the center of the nostril in lateral view; RSD=rostro-spiracle distance.

Species	1	2	3	4	5	6
BL	9.38	9.38	9.69	8.38	6.37	9.00
BWE	5.57	5.00	5.29	4.58	4.33	4.86
BWN	3.14	2.86	3.43	2.96	3.83	3.14
ED	1.13	1.00	0.74	0.80	0.55	0.86
END	1.23	1.00	1.08	1.20	1.06	1.31
IND	1.57	1.43	1.43	1.57	1.05	1.57
IOD	3.00	3.57	3.29	2.93	1.74	3.29
MBH	4.14	4.29	5.15	3.48	3.87	4.86
MBW	7.00	7.29	7.14	5.16	5.47	6.86
MTH	3.71	4.14	4.43	3.07	3.30	4.00
ODW	1.86	2.14	2.14	2.04	2.12	1.86
TAL	18.29	19.29	17.14	13.79	12.10	16.86
TMH	2.14	2.57	2.43	1.64	1.69	2.14
TMW	2.29	2.43	2.29	1.87	1.60	2.29
TL	27.67	28.67	26.84	22.17	18.47	25.86
RED	2.15	1.69	2.08	1.79	1.55	2.08
RND	0.92	0.69	1.00	2.37	0.49	0.77
RSD	6.00	5.23	5.92	5.38	3.40	5.46
MBW/BL	0.75	0.78	0.75	0.62	0.86	0.76
RED/BL	0.23	0.18	0.26	0.28	0.24	0.23
ED/BL	0.12	0.11	0.09	0.09	0.09	0.10
RND/RED	0.43	0.41	0.39	0.40	0.32	0.37
IND/IOD	0.52	0.40	0.46	0.53	0.60	0.48
TMW/MBW	0.33	0.33	0.34	0.36	0.29	0.33
MBH/MBW	0.59	0.59	0.73	0.68	0.71	0.71
TAL/BL	1.95	2.06	1.83	1.64	1.90	1.87
TAL/TL	0.66	0.67	0.65	0.62	0.66	0.65
TMH/MTH	0.58	0.62	0.49	0.53	0.51	0.54
TMW/MBW	0.33	0.33	0.34	0.36	0.29	0.33
ODW/MBW	0.27	0.29	0.31	0.40	0.39	0.27
RSD/BL	0.64	0.56	0.56	0.64	0.53	0.61
BWN/BWE	0.56	0.57	0.65	0.65	0.88	0.65