The Tadpole of the Glassfrog *Chimerella mariaelenae* (Anura: Centrolenidae)

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ABSTRACT

We describe the tadpole of the glassfrog *Chimerella mariaelenae*, including information about its ontogenetic variation. The tadpole is characterized mainly by having a brown coloration that, in later stages turns yellowish-green, and nearly straight jaw sheaths with serrations of uniform length. Ontogenetic changes of *C. mariaelenae* tadpoles are conspicuous in terms of coloration and oral apparatus morphology (i.e., number of papillae and degree of keratinization). Mouth morphology and coloration patterns are likely to provide enough characters to identify glassfrog tadpoles at the species level, but more descriptions are necessary to assess their intra and interspecific variation.

KEYWORDS

Centrolenidae, Ecuador, lateral line system, ontogeny, tadpole.

RESUMEN

Se describe el renacuajo de la rana de cristal *Chimerella mariaelenae*, incluyendo información de su variación ontogenética. El renacuajo se caracteriza principalmente por presentar una coloración café que en estadíos de desarrollo posteriores se vuelve verde amarillenta, y por queratostomas casi rectos con serraciones de tamaño uniforme. Los cambios ontogenéticos del renacuajo de *C. mariaelenae* son conspicuos sobretodo en la coloración y morfología del aparato oral (i.e., número de papilas y grado de queratinización). Es posible que la morfología oral y los patrones de coloración permitan identificar a los renacuajos de las ranas de cristal al nivel de especie; sin embargo, se requieren muchas más descripciones para entender la variación intra e interespecífica de estos caracteres.

PALABRAS CLAVE

Centrolenidae, Ecuador, ontogenia, renacuajo, sistema de líneas laterales.



Introduction

Glassfrogs are endemic to the Neotropical region and a particularly diverse group in the Tropical Andes [1, 2], with 51 reported species in Ecuador [3]. Knowledge on tadpoles of glassfrogs and most other Neotropical anurans is scarce (but see [4]), albeit their important ecological roles in streams [5].

Chimerella mariaelenae is a species restricted to the Amazonian slopes of the Ecuadorian and Peruvian Andes [6, 7, 8]. The species was described by [6], but the tadpole remains unknown. Herein, we describe the tadpole of *C. mariaelenae* and include information on its ontogenetic variation.

Methods

Egg clutch collection

An egg clutch, found attached to the upper side of a leaf, was collected at Río Hollín (0.694633 S, 77.730083 W; 1061 masl), Napo province, slopes of the Cordillera Oriental of Ecuador, on 24 November 2008 by Elicio E. Tapia. Mean annual temperature of this locality is 20.7° C and annual precipitation is 4009 mm³ [9].

Laboratory rearing of eggs and tadpoles

Rearing took place at the facilities of Balsa de los Sapos, Pontificia Universidad Católica del Ecuador, under the supervision of Luis Coloma and Diego Almeida. The egg clutch, including the leaf to which it was attached, was transferred to a small plastic container with 3 cm of filtered water. Once the larvae hatched, they were transferred into a plastic aguarium of 13.6 (height) \times 22.5 $(length) \times 14.5$ (width) cm. The aquarium was filled with filtered water, 50% of which was changed daily. Tadpoles were fed daily with Superalimento de Renacuajos (SAR) with 24.5% of protein content [10]. Tadpoles in Stages 39, 40, 41 and 42 sensu [11] were preserved in formalin for the description. All specimens are housed at the Museo de Zoología of the Pontificia Universidad Católica del Ecuador (QCAZ).

Description of the tadpoles

Tadpoles were staged following the methodology

of [11]. The general description is based on an individual at Stage 39. The ontogenetic variation was documented using a total of three tadpoles: 1 in Stage 40, 1 in Stage 41, and 1 in Stage 42. The terminology for traits (body shape, tail shape, oral disc size, etc.) used in the description was taken from [12] and [5]. We measured the morphological characters suggested by [13] and [14], using a Tresna digital caliper (resolution \pm 0.01). Meristic characters were: (1) total length (TL) = distance from tip of snout to tip of tail; (2) body length (BL) = distance from tip of shout to beginning of caudal musculature; (3) tail length (TAL) =distance from beginning of caudal musculature to tip of tail; (4) body width (BW) = width of body at the level of spiracle; (5) body height (BH) = height of the body behind the eyes; (6) internarial distance (IND) = distance between centers of narial apertures; (7) nostril-snout distance (NSD) = distance between center of nostril to tip of snout; (8) nostril-eye distance (NED) = distance between center of nostril aperture to anterior edge of eye; (9) interorbital distance (IOD) = distance between the internal border of the eyes; (10) spiracle aperture diameter (SAD); (11) spiracle-snout distance (SSD) = distance between the tip of snout and posterior border of spiracle; (12) eye-snout distance (ESD) = distance between tip of snout andanteriormost border of eye; (13) maximum tail height (MTH) = height of tail, including the fins, where the tail reaches its maximum height; (14) tail muscle height (TMH) = height of caudal musculature at the beginning of the tail; (15) tail muscle width (TMW) = width of muscle at the beginning of the tail; (16) dorsal fin height (DFH) = maximum height of dorsal fin; (17) ventral fin height (VFH) = maximum ventral fin height; (18)Oral disc width (ODW) = transverse diameter of oral disc; (19) upper jaw sheath width (UJW) = upper jaw sheath transverse width, including lateral processes; (20) lower jaw sheath width (LJW); (21) vent tube length (VTL) = distance between beginning of vent tube and its aperture; (22) vent tube width (VTW) = transverse width of vent tube.The oral apparatus of some specimens was stained with methylene blue to facilitate visualization of the structures. The taxonomy for Centrolenidae follows the proposal by [1]. The nomenclature of the lateral line system follows the description of [15]; individuals were dyed with methylene blue to highlight the lines, otherwise lines were not evident.



Results

Tadpole description of *Chimerella mariaelenae* (QCAZ 43622b, Stage 39, Fig. 1)

TL = 35.5 mm; BL = 9.9 mm (27.9% of TL). Body elongated and oval-depressed (sensu [12]), wider (body width = 5.1 mm) than higher (body height)= 3.8 mm). Chondrictial elements not visible. Snout rounded in dorsal and lateral views. Lateral-line system visible; lateral lines formed by several stitches parallel or perpendicular to the longitudinal axis of the body. The stitches are parallel to each other or placed in order to form a discontinuous line (Fig. 2). Eyes dorsal, ESD = 2.9 mm; small narial apertures, NSD = 1.9 mm. IND = 2.2 mm. NED = 0.9 mm. Interorbital distance shorter than internarial distance. IOD = 1.0 mm. Short spiracle, single, sinistral, located at posterolateral region of body; spiracular aperture with dorsoposterior orientation, with inner wall present as a low ridge (sensu [5]); SAD = 0.3 mm. SSD = 7.9 mm, spiracle located at 80.4% of body length from tip of snout. Vent tube short and abdominal, free posteriorly, opening directed posteriorly; VTL = 1.1 mm, VTW = 0.3 mm. Tail long, TAL = 26.1 mm (2.6 times the BL) with subacute tip. Myotomes visible throughout entire length of tail; straight medial line visible, separating dorsal and ventral myotomes. TMW = 2.6 mm; TMH = 2.5 mm; MTH = 4.6 mm; DFH = 1.4, VFH = 1.6 mm. Dorsal fin originating at about mid-length of tail; height (except for beginning) more or less uniform until distal end, where it decreases abruptly. Proximally, ventral fin originating at base of tail muscle, reaching its maximum height after midlength of tail.

Medium-sized oral disc, ODW = 2.6 mm (51.2% of BW), not-emarginate and anteroventral, surrounded by 49 marginal uniserial papillae. Only ventral and lateral papillae present, lacking dorsal

papillae (Fig. 3A). Lengths of lateral papillae (N = 15) 0.04-0.16 mm (mean = 0.10 ± 0.03), width $0.07-0.16 \text{ mm} (\text{mean} = 0.09 \pm 0.02); \text{ lengths of}$ ventral papillae (N = 23) 0.02-0.16 mm (mean = 0.11 ± 0.03), width 0.07–0.18 mm (mean = 0.09 \pm 0.03). Upper and lower jaw sheaths nearly straight and completely keratinized, with serrated edge. Lower jaw sheath placed behind upper jaw. UJW = 1.3 mm (50.9% of ODW), LJW = 1.0 mm (38.2% of ODW). Labial tooth row formula 2(1)(2)/3; gap in tooth row A-1 could be artificial (because of teeth loss). Tooth rows about equal in length. Tooth row A-1 = 2.3 mm long, number of teeth = 208; tooth row A-2 interrupted medially by upper jaw sheath, each side with length = 0.6mm, number of teeth = 86; tooth row P-1 = 2.2mm, number of teeth = 154; tooth row P-2 = 2.1mm, number of teeth = 192; tooth row P-3 2.2 mm, number of teeth = 207.

Color in life (Fig. 1, Stage 39)

Based on a digital color photo by Luis A. Coloma. Dorsally, the tadpole is brownish with two areas without pigmentation at the anterolateral border of the eye. Some reddish pigmentation is seen in the anterior half of the dors um because of its transparency. The most anterior part of the head is gray. Iridophore aggregations are present on the dorsum, along the vertebral column. The iris is bronze.

Color in preservative (formalin, Stage 39)

Based on QCAZ 43622b. The tadpole is cream with several brown dots on the dors um. The venter is translucent with few white dots that are more abundant posteriorly. Dorsally, the tail is brown; laterally, myotomes are brown, with its coloration fading as it approaches the ventral surface of tail. The dorsal fin has brown flecks; the ventral fin has also brown flecks, but only in the portion next to the caudal musculature.

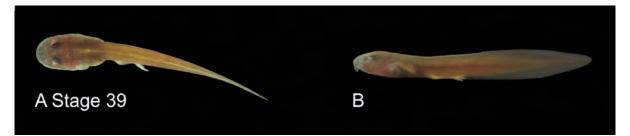


Figure 1. Tadpoles of Chimerella mariaelenae (QCAZ 43622b) in life, Stage 39. (A) Dorsal view. (B) Lateral view. Photo by L.A. Coloma



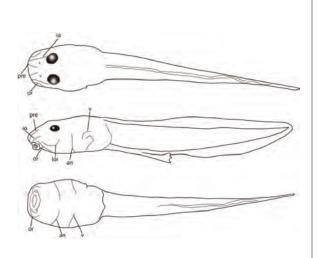


Figure 2. Lateral line system pattern of Chimerella mariaelenae (QCAZ 43622c) at larval Stage 40 in dorsal, lateral and ventra 1 views. Discontinuous lines represent stitches (orientation and arrangements of stitches are not represented in the figure). Abbreviations: an, angular line; d, dorsal line; io, infraorbita l ihe; lor, oral longitudinal line; m, medial line; or, oral line; pre, preorbital line; so, supraorbital; v, ventral line.

Ontogenetic variation

Variation of 18 meristic characters from three tadpoles in Stages 41 and 42 is shown in Table 1. The tadpole on Stage 40 lacks keratin on the jaw sheaths; also, most of the tooth rows have lost their keratin (few teeth are visible with coloration; Fig. 3B). Coloration is very similar to that on Stage 39 (Figs. 4A, 4B). The vent tube is no longer visible.

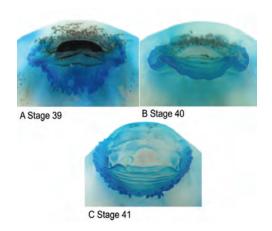


Figure 3. Oral apparatus of the tadpole of Chimerella mariaelenae. (A) Stage 39, QCAZ 43622b. (B) Stage 40, QCAZ 43622c. (C) Stage 41, QCAZ 43622d. Blue coloration is caused by the use of alcian blue to dye the papillae.

	Stage 40 (<i>n</i> = 1)	Stage 41 $(n = 1)$	Stage 42 ($n = 1$)
TL	33.8	36.3	35.3
BL	9.6	10.3	9.6
TAL	24.4	27.7	25.9
BW	5.1	5.8	5.1
BH	3.6	4.2	4.0
IND	1.5	1.1	1.1
NSD	1.8	1.9	1.4
IOD	1.0	1.4	1.3
NED	2.8	1.2	1.3
SAD	0.6	-	-
SSD	7.7	-	-
ESD	2.8	3.0	2.7
MTH	4.7	4.8	4.1
TMH	2.3	2.5	2.2
TMW	2.2	2.4	2.0
DFH	1.5	1.5	1.1
VFH	1.2	1.4	1.3
ODW	2.5	2.5	2.2
# of papillae	36	46	51

Table 1. Dimensions in mm of the larvae of Chimerella mariaelenae, QCAZ 43622c-e. Abbreviations: total length (TL), body length (BL), tail length (TAL), body width (BW), body height (BH), internarial distance (IND), nostril-snout distance (NSD), interorbital distance (IOD), nostril-eye distance (NED), spiracle aperture diameter (SAD), snout-spiracle distance (SSD), eye-snout distance (ESD), maximum tail height (MTH), tail muscle height (TMH), tail muscle width (TMW), dorsal fin height (DFH), ventral fin height (VFH), oral disc width (ODW).

The oral apparatus in tadpoles at Stages 41 and 42 have ridges instead of tooth rows; tadpoles at Stage 41 have few teeth on tooth rows A1 and P1 (Fig. 3C). Individual QCAZ 43622d already has one free arm and the other is still inside the body, with a bud seen externally; individual QCAZ 43622e has the four extremities already free. In preservative, coloration at Stages 41 and 42 is slightly different from that at Stages 39 and 40, with brown coloration of the dorsum restricted along the middorsal area of the body. There are five iridophore aggregations forming marks on the beginning of the caudal musculature on the dorsum. Shanks have some brown dots on its dorsal surface. In life, the tadpole at Stage 40 is brown, but slightly greenish (Figs. 4A, 4B). Individuals at Stages 41 and 42 have lost part of the brown pigments and have a yellowish-green (Figs. 4A, 4B)



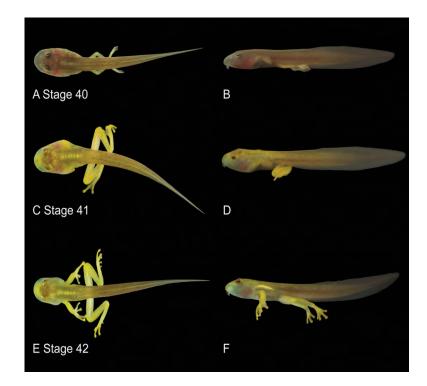


Figure 4. Ontogenetic variation of the tadpole of *Chimerella mariaelenae.* (A, B) QCAZ 43622c; (C, D) QCAZ 43622d; (E, F) QCAZ 43622e. Photo by L.A. Coloma.

and bluish-green (Figs. 4E, 4F) coloration, respectively. The snout in both individuals is bluish-green and the tail remains brown. Finally, the eyes are no longer inside the skin; they resemble more to the eyes of an adult. The iris is reddish. Number of papillae in the oral disc varies in the three individuals: Stage 41 = 36 papillae, Stage 41 = 46 papillae, and Stage 42 = 51 papillae.

Discussion

Comparisons among tadpoles of centrolenid species are difficult because there are few published descriptions and also because available descriptions are based mostly on few individuals at early Gosner stages; thus, lacking trait information related to the morphology at more developed stages. Description of ontogeny is very important because some features, like coloration and oral apparatus, change with the development of the tadpole [13, 14, 16]. Therefore, it is important to make comparisons between individuals at similar stage. Some centrolenid species, because of their fossorial habits, posses a high vascularized skin to obtain oxygen. This vascularization gives them a reddish coloration [13]. However, coloration varies with the development of the tadpole; in general, individuals at early stages present more brown flecks or dots on the dorsum and/or tail. As the individuals grow, they lose gradually the brown coloration as they acquire the adult coloration.

Similarly, some authors report that the number of papillae varies ontogenically with a tendency of an increasing number of papillae as the tadpole develops [14, 16]. Although, there is also variation between individuals in the same stage, *C. mariaelenae* presents an increment in the number of papillae.

Lateral line systems have not been described for any other centrolenid species, thus comparisons within the family are not possible. The presence of stitches forming the lateral lines are typical in species with midwater suspension feeding forms, such as *Xenopus*, *Rhinophrynus*, *Phrynomantis* [17]. Nevertheless, centrolenid larvae are not sus-



pension feeders, they are lotic: burrower [5]. Besides, some lines are absent or incomplete. In C. mariaelenae dorsal and medial lines are absent, and the preorbital line does not reach the eye (it ends at the nostrils). The reduction and loss of lateral lines are a derived condition and they may be correlated with larval ecology or with historical and developmental constraints [18]. The ventral line does not have connection with that above the spiracle, thus a more accurate name for it could be supraspiracular, as suggested by [18] for Spea intermontana. Finally, we would like to stress the importance of describing tadpoles since they show a different set of traits that those found in adults, and are constrained by very dissimilar ecological and environmental conditions.

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References

[1] Guayasamin, J. M., Castroviejo-Fisher, S., Trueb, L., Ayarzagüena, J., Rada, M., and Vilà, C. 2009. Phylogenetic systematics of glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni.* Zootaxa 2100: 1–97.

[2] Castroviejo-Fisher, S., Guayasamin, J. M., Gonzalez-Voyer, A., and Vilá, C. 2014. Neotropical diversification seen through glassfrogs. Journal of Biogeography 41: 66–80.

[3] Centro Jambatu. 2011–2014. Anfibios de Ecuador. Fundación Otonga. Quito, Ecuador. Available at: http://www.anfibios webecuador.ec/anfibios ecuador.aspx [4] Hoffmann, H. 2010. The glass frog tadpoles of Costa Rica (Anura: Centrolenidae): A study of morphology. Abhandlungen der Senckenberg Gesellschaft für Naturforschung 567: 1–78.

[5] McDiarmid, R. W., and Altig, R. 1999. Tadpoles. The Biology of Anuran Larvae. The University of Chicago Press, Chicago.

[6] Cisneros-Heredia, D. E., and McDiarmid, R. W. 2006. A new species of the genus *Centrolene* (Amphibia: Anura: Centrolenidae) from Ecuador with comments on the taxonomy and biogeography of Glassfrogs. Zootaxa 1244: 1–32.

[7] Cisneros-Heredia, D. F., and Guayasamin, J. M. 2006. Amphibia, Anura, Centrolenidae, *Centrolene mariaelenae:* Distribution extension, Ecuador. Check List 2:93–95.

[8] Catenazzi, A., and Venegas, P. 2013. Anfibios y reptiles/ Amphibians and reptiles. In: Peru:
Cerros de Kampankis (N. Pitman, E. Ruelas Inzunza, D. Alvira et al., eds.). Rapid Biological Inventories. The Field Museum, Chicago.

[9] Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., and Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965–1978.

[10] Coloma, L. A., and Almeida-Reinoso, D. 2012. Manejo ex situ de cinco especies remanentes de *Atelopus* en Ecuador-Reporte de progresos. AArk Boletin Informativo 20: 9–12.

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

[12] Mijares-Urrutia, A. 1998. Los renacuajos de los anuros (Amphibia) altoandinos de Venezuela: morfología externa y claves. Revista de Biología Tropical 46: 119-143.



[13] Rada, M., Rueda-Almonacid, J. V., Velásquez-Alvarez, A. A., and Sánchez-Pacheco, S. J. 2007. Descripción de los renacuajos de dos centrolénidos (Anura: Centrolenidae) del noroccidente de la Cordillera Oriental, Colombia. Papéis Avulsos de Zoología 47: 259–272.

[14] Castillo-Trenn, P. 2004. Description of the tadpole of *Colostethus kingsburyi* (Anura: Dendrobatidae) from Ecuador. Journal of Herpetology 38: 600 - 606.

[15] Fabrezi, M., and Quinzio, S. I. 2008. Morphological evolution in Ceratophryinae frogs (Anura, Neobatrachia): the effects of heterochronic changes during larval development and metamorphosis. Zoological Journal of the Linnean Society 154: 752–780.

[16] Terán-Valdez, A., Guayasamin, J. M., and Coloma L. A. 2009. Description of the tadpole of Cochranella resplendens and redescription of the tadpole of *Hyalinobatrachium aureoguttatum* (Anura: Centrolenidae). Phyllomedusa 8: 105–124.

[17] Lannoo, M. J. 1987. Neuromast topography in anuran amphibians. Journal of Morphology 191: 115–129.

[18] Hall, J. A., Larsen, J. H., and Fitzner, R. E. 2002. Morphology of the prometamorphic larva of the spadefoot toad, *Scaphiopus intermontanus* (Anura: Pelobatidae), with an emphasis on the lateral line system and mouthparts. Journal of Morphology 252: 114–130.

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