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Description of the tadpole of *Agalychnis hulli* (Anura: Hylidae)

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Abstract

Description of the tadpole of *Agalychnis hulli* (Anura: Hylidae). The collection of larval specimens from Amazonian Ecuador allows for the scientific description of the tadpole of *Agalychnis hulli*. The tadpole is unusual among Phyllomedusinae and its external morphology appears more similar to species of *Hylomantis* than other species of *Agalychnis,* suggesting the species may well represent an important evolutionary link between these genera. The tadpole, which to date has remained undescribed, can be distinguished from all other *Agalychnis* tadpoles in having an oral disc (i) that is directed anteroventrally and extends to more than half the width of the body, (ii) which is modified into a short funnel-shaped structure, (iii) and surrounded by a single row of marginal papillae.

Keywords: Amphibia, Larvae, Morphology, Phyllomedusinae.

Resumen

Descripción del renacuajo de *Agalychnis hulli* (Anura: Hylidae). Se recolectaron larvas en la Amazonia Ecuatoriana, las cuales permiten la descripción científica del renacuajo de *Agalychnis hulli.* El renacuajo es inusual entre los Phyllomedusinae y su morfología externa es más similar a las especies de *Hylomantis* que a otras especies de *Agalychnis,* lo que sugiere que la especie bien puede representar un vínculo evolutivo importante entre estos géneros. El renacuajo, que hasta la fecha no ha sido descrito, se puede distinguir de todos los demás renacuajos de *Agalychnis* por tener un disco oral (i) que se dirige anteroventralmente y se extiende a más de la mitad del ancho del cuerpo, (ii) que se modifica en una estructura corta en forma de embudo, (iii) y que está rodeado por una sola fila de papilas marginales.

Palabras clave: Amphibia, Larva, Morfología, Phyllomedusinae.

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Resumo

Descrição do girino de *Agalychnis hulli* (Anura: Hylidae). A coleta de espécimes larvais da Amazônia equatoriana permitem a descrição científica do girino de *Agalychnis hulli*. O girino é incomum entre os Phyllomedusinae e sua morfologia externa parece mais similar à das espécies de *Hylomantis* do que a de outras espécies de *Agalychnis*, sugerindo que essa espécie podem representar um importante elo evolutivo entre estes gêneros. O girino, que até hoje não havia sido descrito, pode ser distinguido de todos os outros girinos de *Agalychnis* por apresentar um disco oral (i) dirigido anteroventralmente e estendendos-e para mais da metade da largura do corpo, (ii) modificado em uma estrutura curta em forma de funil, e (iii) rodeado por uma única fileira de papilas marginais.

Palavras-chave: Anfíbios, Larvas, Morfologia, Phyllomedusinae.

Introduction

Agalychnis hulli (Duellman and Mendelson, 1995) is a small to medium sized hylid, originally described from a specimen collected at a small forest pond in the Province of Loreto, Amazonian Peru (Frost 2022). The species was originally placed in the *Phyllomedusa buckleyi* group (Cannatella 1980), subsequently in the *Hylomantis buckleyi* group (Faivovich *et al.* 2005), and later moved to *Agalychnis* Cope, 1864 without further assignment to a particular species group (Faivovich *et al.* 2010). Although it is not a common frog, unlike other *Agalychnis* species it is known to occur in the rainforest lowlands (200–997 m a.s.l.) of the Amazonian region of northeastern Peru and Ecuador; in the latter in Sucumbíos, Orellana, Napo, Pastaza, Morona Santiago, and Zamora Chinchipe provinces (Vigle 2008, Beirne and Whitworth 2011, Ron and Read 2019). The species is easily distinguished from other phyllomedusines from these areas in having coarsely granular skin on both its dorsal and ventral surfaces. The species' reproduction is characteristic of phyllomedusines, where eggs are laid on leaves overhanging temporary ponds into which the developing tadpoles drop upon hatching. All tadpoles currently described (10 species, including this description) that belong to species of the genus *Agalychnis* (14 species) are characterized in having mouthparts of typical suspension feeding phyllomedusines, whereas the mouthparts of *Hylomantis* tadpoles (two species) are modified into short funnel-shaped structures considered to resemble an intermediate morphological condition to those of *Phasmahyla* Cruz, 1991, which have mouthparts modified into an anterodorsal funnel-shaped structure (Pimenta *et al*. 2007). However, to date nothing has been published on *A. hulli* tadpole morphology. Herein we describe the external morphology and mouthparts of the tadpole of *Agalychnis hulli*, which distinctly differ from the tadpoles of other species of *Agalychnis*.

Materials and Methods

Measurements and remarks of ontogenetic changes are based on six tadpoles (MM.D.1299.1, MM.D.1299.2, MM.D.1299.3, MM.D.1299.4, CJ 11821, CJ 4741a) ranging from stages 26 to 44 (Gosner 1960). One of them (CJ 4741a) was field-collected and five were first generation descendent tadpoles born under laboratory conditions at the Centro Jambatu de Investigación y Conservación de Anfibios, Ecuador (CJ).

The description is based on a tadpole specimen (CJ 4741a) at Stage 36, which was collected with others from a pond close to Río Pucayacu, Reserva Otoyacu, (01°22'16.4" S, 77°51'23.4" W; 922 m a.s.l.), Provincia Pastaza, Ecuador, on 12 June 2016 by Elicio E. Tapia, Nadine Dupérré and Anabelle Tapia-Dupérré. The pond was approximately 40 m long × 20 m wide × 80 cm deep and located close to the

river. The pond was artificially modified by the obstruction of a creek due to a road construction, and the marginal vegetation was secondary, with 15–20 m high trees and many bushes surrounding it. The tadpoles were collected at the margins of the pond and transported to CJ to be raised to adulthood.

A resulting female specimen (CJ4741) was successfully raised to adulthood at CJ and maintained in laboratory with adult males from the same series, and also with a wild male collected at Comunidad Yarentaro (01°03'01.3" S, 76°11'37.5" W; 244 m a.s.l.), Parque Nacional Yasuní, Provincia Orellana, Ecuador, by Morley Read and Yerka Sagredo-Nuñez, which had been found perched on a branch 30 cm above ground near a small pond around a petroleum platform on 09 August 2021. The reproducing pair at CJ were not observed while in amplexus or when egg laying so we could not identify if the male in question was from Pucayacu or Yasuní. Therefore, the laboratory-bred tadpoles produced, including five specimens the details of which are incorporated in the measurements and remarks on ontogeny section, were first generation descendants of the Pucayacu female (CJ 4741), and either a male from the same series or the male from Yasuní.

The group of 25 descendant tadpoles was reared within a plastic container measuring 35.5 cm long × 27.5 cm wide, containing water 13.5 cm deep (12 L). They were kept in the lab at temperatures varying from 19°C (at night) to 24°C (during the day). Tadpoles were fed with a mix of *Taraxacum officinale* and Zoo Med's Aquatic Frog Food. A minimum number of specimens (4: MM.D.1299.1, MM.D.1299.2, MM.D.1299.3, MM.D.1299.4) were preserved in formalin (10%).

Two live specimens (the original CJ 4741a) and a descendant (CJ 11821) were reared to adulthood in the laboratory affording the documentation of ontogenetic variation. Adult specimens were identified as *Agalychnis hulli* and distinguished from *Agalychnis buckleyi* (Boulenger, 1882) by having coarsely granular skin on the dorsum (minutely granular skin on the dorsum) and by lacking a small calcar (present). Thus, our specific allocation of the tadpoles described herein is based on the adult similarity of the Reserva Otoyacu population to *A. hulli* from the type locality at Teniente López, Provincia Loreto, Peru. Both localities are at about 240 km airline distance.

Museum acronyms used in the text are MM for Manchester Museum, University of Manchester, and CJ for Centro Jambatu de Investigación y Conservación de Anfibios, Ecuador. Terminology and measurements follow Altig and McDiarmid (1999) and Pimenta *et al.* (2007). Abbreviations used are as follows: TL (total length); BL (body length); TAL (tail length); MTH (maximum tail height); TMH (tail muscle height); TMW (tail muscle width); IND (internarial distance: straight line distance between the inner margins of the nostrils); IOD (interorbital distance: straight line distance between the inner margins of the eyes); BH (greatest body height); BW (greatest body width); ED (eye diameter: horizontal diameter of the eye in lateral view); ODW (oral disc width: greatest horizontal width of the oral disc in front view); LTRF (Labial Tooth Row Formula): A1; A2 (anterior tooth rows); P1, P2 and P3 (posterior tooth rows): DFH (dorsal fin height: greatest height of dorsal fin); VFH (ventral fin height: greatest height of ventral fin); END (eye–nostril distance: straight line distance between the anterior margin of nostril and the tip of snout). The measurements (in mm) were taken using a calipers (to the nearest 0.01 mm) and through the use of a Zeiss stereomicroscope.

Results

Tadpole Description (CJ 4741a), Stage 36 (Figures 1 and 2)

Body shape: Ovoid in dorsal view and slightly triangular in lateral view, about equal in height and width, highest and widest at the midpoint of the body (Figure 1). Body length: 30.4% of the total length (BL = 10.5

mm; TL = 34.5 mm). Snout shape: Rounded in dorsal view and sub-elliptical in lateral view. Eyes: large, located immediately above the midline of the body, directed laterally (ED = 1. 9 mm) representing 25.7% of the interorbital distance and 14.7% of the body length, visible from below. Nostrils: small, ovoid, dorsolaterally positioned, with external openings directed anterolaterally, situated closer to snout than eyes (IND = 2.1 mm representing approximately 28.4% of the interorbital distance). Spiracle: ventral, opening scallop shaped, flap-like, transparent, directed backwards, located mid-body on the left side near the transversal midline, 1.4 mm long, widest towards the opening (1.5 mm wide). Ventral surface with silver covering: internal organs including intestinal tube not visible through the ventral and lateral surfaces of the body: Anal tube: short, dextral, right wall attached to the ventral fin, opening posteroventrally. Tail: tail and body of about equal height. Caudal musculature well developed, slender, becoming progressively thinner caudally. Myosepta and caudal vein defined until about the first half of the tail. Dorsal fin considerably lower than ventral fin (DFH = 1.5 mm, VFH = 2.5 mm), weakly arched, does not extend onto the body, origins anterior third of the tail. Ventral fin approximately 66.7% deeper than the dorsal, weakly arched distally, origins posterior quarter of the body, widest point at approximately midpoint, corresponding with dorsal; both tail fins graduate to a distal point. Caudal tip ends in long flagellum. For measurements see Table 1.

Table 1. Morphometric measurements (in mm) of the tadpole of *Agalychnis hulli* during ontogeny. Developmental stages are according to Gosner (1960).

Specimen number	**MM.D1299.1**	**CJ4741a**	**MM.D.1299.2 CJ11821**	**MM.D.1299.3 MM.D.1299.4 CJ11821**	**CJ4741a**
Stage	26 (*N* = 1)	36 (*N* = 1)	41(*N* = 2)	43 (*N* = 3)	44 (*N* = 1)
Total length	34.5	43.9	52.0–55.0	42.7–56.0	43.4
Body length	10.5	12.9	16.0–16.2	16.3–17.6	22.0
Body width	5.1	6.8	9.0–10.5	8.8–9.7	-
Body height	5.3	6.4	8.0–11.5	4.8–7.2	8.0
Tail length	24.0	31.0	35.8–39.0	26.4–39.0	21.4
Tail muscle height	3.6	4.3	6.0–7.1	3.9–5.2	5.6
Dorsal fin height	-	1.5	1.6	1.6	-
Ventral fin height	2.0	2.5	3.2	2.6	-
Snout–nostril distance	1.3	1.7	2.2–2.4	1.8–2.0	-
Internarial distance	2.0	2.1	2.4–2.6	3.0–3.1	-
Eye–nostril distance	2.2	3.5	3.9–4.0	3.4–3.5	3.8
Eye diameter	1.5	1.9	2.5–2.6	2.6–3.0	3.6
Interorbital distance	4.8	7.4	7.0–8.2	7.9–8.2	-
Oral disc width	2.1	3.7	4.1	3.4–4.2	-
Anal tube length	1.4	2.4	3.0	-	-

Mouthparts

The oral disc is positioned and directed anteroventrally, the outer edge of the labium forming a short funnel-shaped structure, excepting a median dorsal gap in the upper labium being a continuation of the snout (Figure 2A). Except for the median dorsal gap, the labium is bordered by one continuous uniserial row of about 80–100 small conical-shaped marginal papillae along the outer edge, and the inner surfaces have a uniform scattering of papillae which are indiscreet, barely breaking the otherwise smooth surface (being clearly visible within the translucent labium when lit from beneath) (Figure 2B).

The oral disc is large (ODW = 3.7 mm) representing 53.5% of the body width, having a small median emargination in the lower labium. There are two anterior and three posterior rows of teeth; LTRF is 2(2)/3(1). Teeth on the first anterior row (A1) form a broad arch and are smaller in size than in the second anterior row (A2), which is interrupted medially; the anterior rows are longer than the first (P1), second (P2), and third (P3) posterior rows, which reduce in length sequentially; the first posterior row (P1) has a small medial gap, the second (P2) and third (P3) rows are complete. Upper and lower jaw sheaths are robust, heavily pigmented, and both bear fine serrations; upper jaw sheath medially convex; moderately long lateral processes; lower jaw sheath V-shaped (Figure 2A).

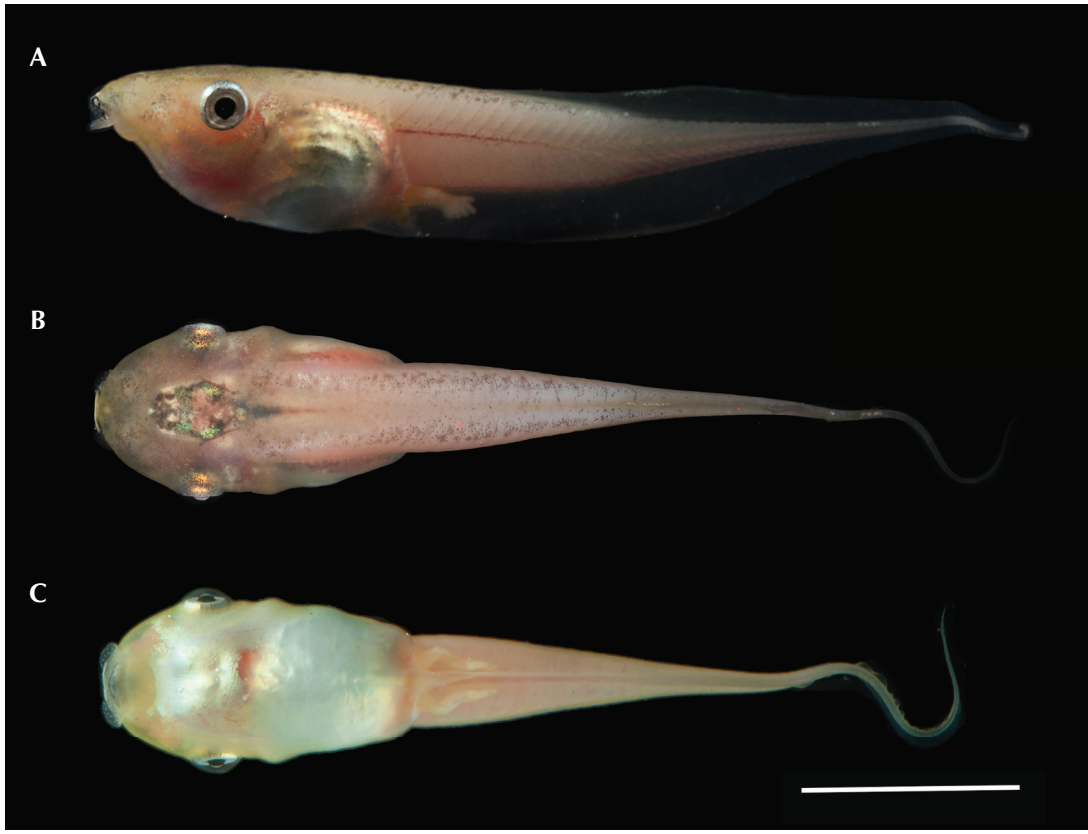

Figure 1. Tadpole of *A. hulli* (CJ 4741a) Stage 36 in lateral (**A**), dorsal (**B**), and ventral (**C**) views. Scale bar 1 cm.

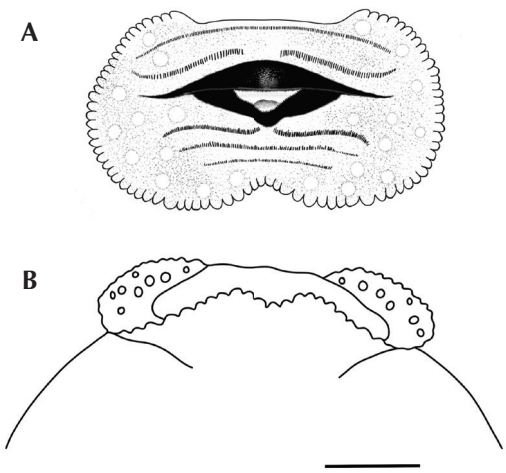

Figure 2. Mouthparts of the tadpole of *A. hulli* (CJ 4741a) Stage 36: (**A**) Oral disc; (**B**) detail of funnel-shaped labia. Scale bar 1 mm.

Coloration

In life.—Background color of body at Stage 36 flesh-pink, having fine dark brown speckling on the dorsal surfaces of the body, extending along caudal musculature at the anterior of the tail. Tail musculature flesh pink, caudal vein dark red until about the first half of the tail. Dorsal and ventral fins transparent. Ventral surface of body silver. Silver covering to internal organs; thus, organs are not visible through the ventral and lateral surfaces of the body. Chondrocranial elements visible dorsally, dark metallic-brown polygon-shaped. Iris silver-grey, periphery with fine silver reticulations. Limb buds and anal tube follow coloration of the body.

In preservative.—Background coloration of body and tail musculature becomes whitish-cream; dorsal surfaces of body and anterior of tail with greyish speckling. Intestinal mass dark grey, clearly visible through lateral and left ventral body walls; caudal vein black until about the first half of the tail. Spiracle, dorsal and ventral fins remain translucent. Iris black. Oral disc and papillae translucent-white, oral surfaces of upper and lower jaw sheaths black.

Ontogenetic Changes

At stages 25–26 the distance between the nostrils and the eye are of about equal width, whereas from Stage 26 the distance between the eye and nostril increases considerably compared to that between each nostril. Throughout development the bilateral myotonic muscle masses and the caudal vein in the tail musculature remain clearly defined. At Stage 36 the pupil has developed an elliptical shape, the spiracle opening is evident although not prominent, and a shallow groove from the posterior edge of the nostril to the eye is observed. Between stages 36–41 a notable increase in the growth of the ventral fin height is seen compared with that of the dorsal fin. This, and growth in the musculature, result in a notable overall change in tail height compared to tail length from about 26.8% at Stage 36 to about 30.6% at Stage 41. From Stage 41, with the onset of metamorphosis, the spiracle and the anal tube disappear, the tail begins to shrink and the nostrils become defined. The overall tail muscle height reduces but the caudal vein remains prominent, the eye to nostril distance starts to decrease, the anterior limbs appear, and fine dorsal granulations seen in the adult also begin to appear. The nostrils become more pronounced, although the shallow groove between the nostril and the eye remains minimal, and a small notch appears at the bottom of the pupil in respect of initiating the pupils' vertical development. One specimen in Stage 44 (CJ 4741a) which emerged during the final stages of metamorphosis showed an TL (total length) of 43.4 mm, including its partially absorbed tail (Figure 3).

Discussion

Faivovich *et al.* (2010) considered it reasonable to assume that a close relationship existed between *Agalychnis hulli* and *A. buckleyi*. This

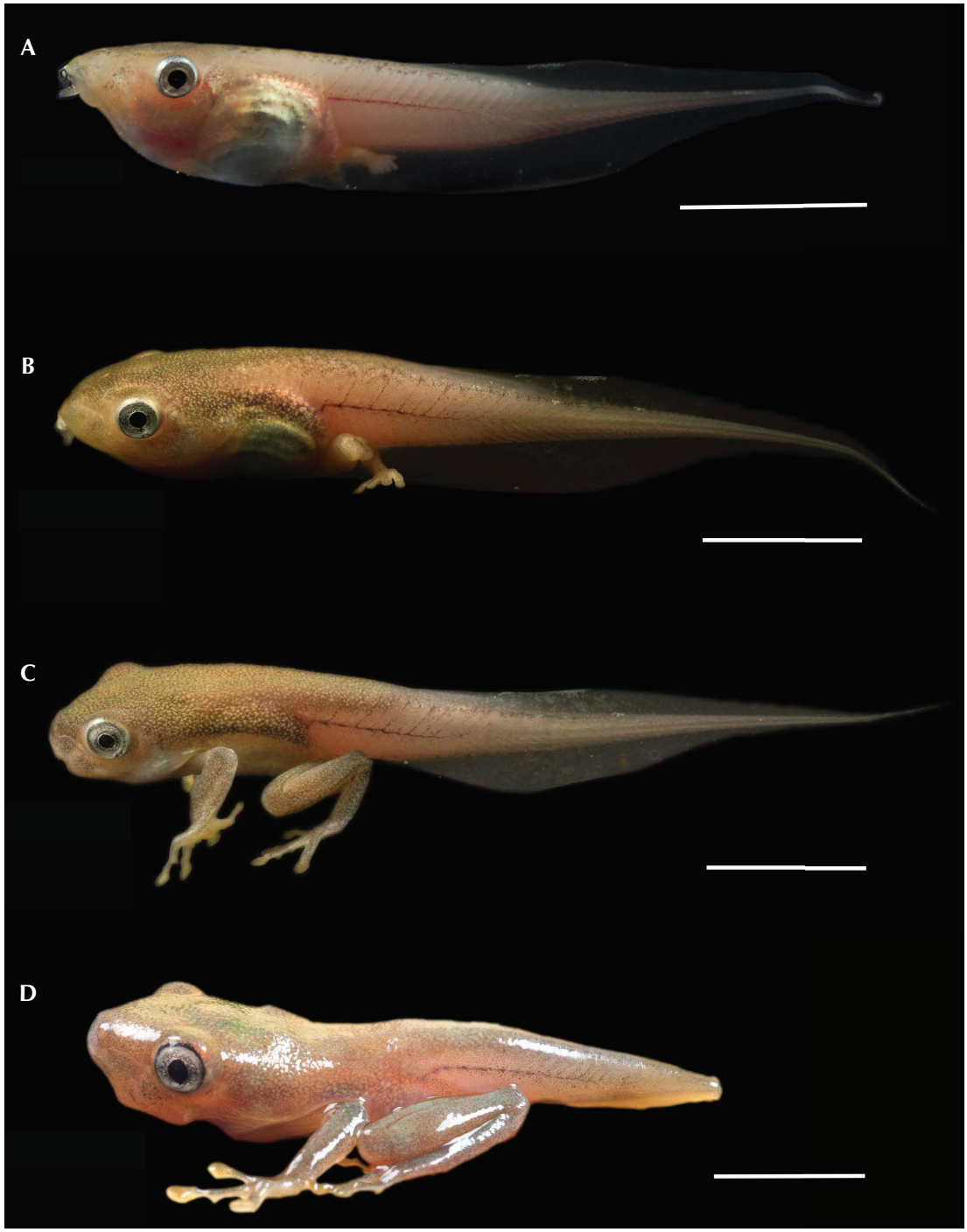

Figure 3. Development in the tadpole of *A. hulli* (CJ 11821) to metamorphosis. Scale bar 1 cm. Stages: (**A**) 36, (**B**) 40, (**C**) 42, (**D**) 43. Photos: Steven Guevara Salvador.

assumption was based on anatomical similarities in adult specimens. Both do look similar and both have an amount of tuberculated skin: *A. hulli* has extensive granulated skin, coarsely granular skin on the dorsum (Duellman and Mendelson 1995); *A. buckleyi* has minutely granular skin on the dorsum (Cannatella 1980). As such, *A. hulli* was placed with *A. buckleyi* within the genus *Agalychnis,* along with all other members that had previously been hypothesized as belonging in *Hylomantis* (Faivovich *et al.* 2010). However, the tadpole described herein differs from the tadpole of *A. buckleyi* (characters in parentheses) described and illustrated by Cannatella (1980) from Río Azuela and Río Salado, Provincia Napo, Ecuador, by having an oral disc being modified into an anteroventrally directed short funnel-shaped structure (not funnel-shaped) and a single row of marginal labial papillae (2–3 rows). These two features of *A. hulli* are unusual within Phyllomedusinae. The funnel-shaped oral disc is however more similar to that seen in both members of the genus *Hylomantis* from Brazil: being most similar to *Hylomantis aspera* Peters, 1873, and to a lesser extent *Hylomantis granulosa* (Cruz 1989). At Stage 36 the mouth of the tadpole of *A. hulli* is large, representing more than half the width of the tadpole, whereas in all other *Agalychnis* tadpoles the mouth lacks the funnel-shape and is thus considerably smaller. The single row of marginal labial papillae possessed by the tadpole of *A. hulli* is a further characteristic seen in *Hylomantis* and not found in any other *Agalychnis* species.

Interestingly, when Pimenta *et al.* (2007) evaluated the oral disc characteristic in the closely related species to *Hylomantis aspera* and *H. granulosa* they considered the distinct anteroventrally directed oral disc (funnel shaped and 50%+ the width of the tadpole) reflected such a distinct morphological character that it could well present a synapomorphy for *Hylomantis*. Although the tadpoles of *H. aspera, H. granulosa,* and *A. hulli* are morphologically similar in this way, we acknowledge that the two species in *Hylomantis* have a wide geographical separation from *A. hulli* (Duellman *et al.* 2016) and there is also the possibility that a resemblance to each other could be due to homoplasy, possibly through these species occurring and reproducing in temporary ponds inside or at forest edges which have a substrate of dead leaves. Nonetheless, according to the molecular phylogenetic analysis of Faivovich *et al*. (2010), *Agalychnis hulli* (from Ahuano, Provincia Napo, Ecuador) is the sister taxon of *Hylomantis aspera* + *H. granulosa* from northeastern Brazil. So, the two tadpole features previously mentioned supports the molecular topology hypothesized by Faivovich *et al.* (2010), and the inclusion of *A. hulli* within *Hylomantis.* However, we refrain of doing the latter generic change given that genetic data are needed from the hypothetically closely related taxa (*A. buckleyi*, *Agalychnis danieli* Ruiz-Carranza, Hernández-Camacho, and Rueda-Almonacid, 1988*, Agalychnis medinae* Funkhouser, 1962*,* and *Agalychnis psilopygion* Cannatella, 1980) before the phylogenetic relationships can be clarified for a suitable classification. It is clear that further molecular work is required in order to clarify some of the hypothesized phylogenetic relationships within the *Agalychnini* (sensu Dubois *et al*. 2021), particularly between *Hylomantis* and those species previously referred to as belonging to the '*buckleyi* group' that are currently placed within *Agalychnis.*

Additionally, the phylogenetic hypothesis of Faivovich *et al*. (2010) indicate that *Hylomantis* (sensu Duellman *et al.* 2016) probably represents an early divergence event in the ancestral lineage that also gave rise to *Agalychnis.* Thus, the mouth feature in *A. hulli* could well be a retained ancestral characteristic, which contrasts with those of other *Agalychnis* species, including *A. buckleyi, Agalychnis lemur* Boulenger, 1882, and *A. psilopygion,* which all have several rows of marginal papillae (Cannatella 1980) and typical oral discs associated with the suspension-feeding tadpoles of most other phyllomedusines (Pimenta *et al.* 2007).

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