Papéis Avulsos de Zoologia

Museu de Zoologia da Universidade de São Paulo

Volume 53(15):211-224, 2013 **www.mz.usp.br/publicacoes** **ISSN impresso: 0031-1049**
http://portal.revistasusp.sibi.usp.br **ISSN *on-line:* 1807-0205**
www.scielo.br/paz

Tadpoles of the High-Andean *Hyloxalus subpunctatus* (Anura: Dendrobatidae) with description of larval variation and species distinction by larval morphology

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ABSTRACT

This study redescribes the tadpoles of Hyloxalus subpunctatus *(Anura: Dendrobatidae) based on larvae from the type locality and over most of its geographic range, including all stages of ontogenetic development. I describe tadpoles in the three developmental phases: (1) back-riding tadpoles, (2) free-swimming tadpoles, (3) froglets or individuals in metamorphosis. The larval morphology showed at least two types of variation: ontogenetic variation and variation within each developmental phase. In back-riding tadpoles, the variation in labial tooth rows suggests a pattern of the labial tooth row formation. In free-swimming tadpoles there is variation in the disposition of marginal papillae,* i.e., *in the number of rows on the margin of the lips, but this variation has no effect on the general aspect of the tadpoles. Moreover, I compared the tadpoles of* H. subpunctatus *with tadpoles of potential sympatric species and populations previously identified as* H. subpunctatus. *Interspecific differences and larval characters were found that were useful in delimiting those species.*

Key-Words: Tadpoles; Larval differentiation; Ontogenetic variation; Sequence of labial tooth row formation.

INTRODUCTION

The diversity of larval morphology in the family Dendrobatidae (Dendrobatoidea of Grant *et al.,* 2006) is unknown (Caldwell *et al.,* 2002, Castillo-Trenn, 2004). For example, for the largest genus of dendrobatids, *Hyloxalus* with 58 species (Frost, 2011), there are only 25 species with described tadpoles (43.1% of the genus); and of the 25 tadpoles described in *Hyloxalus,* 64% of the descriptions are based on a single individual and 40% are based only on back-riding stages (Table 1). Although other descriptions included free-swimming tadpoles, there has been little emphasis on variation and on details of morphology. Only eight *Hyloxalus* tadpoles (out of 21 reported for Colombia) are known and the available descriptions are very short, general, and unspecific. The situation reflects the scarce interest in tadpole biology and their morphology (external and internal) as a taxonomic tool or as source of potential phylogenetic characters.

Hyloxalus subpunctatus (Cope, 1899) is a dendrobatid frog of cryptic coloration, which lives in the Altiplano Cundiboyasense of the Cordillera Oriental of Colombia, between 1750 to 4020 meters in altitude (Bernal & Lynch, 2008). The species has received considerable attention: adults were twice redescribed

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TABLE 1: List of the descriptions of the tadpoles of species of *Hyloxalus* showing type of tadpole employed for the description, stage(s) and number of tadpoles (*n*). Legends: **(?)** signifies undetermined or not specified in literature; **(*)** description based on back-riding tadpoles and **(**)** free-swimming tadpoles.

Species	Tadpoles description	Type tadpoles	Stage(s)	*n*
Hyloxalus anthracinus	Coloma, 1995	Free-swimming	26	1
Hyloxalus awa	Coloma, 1995	Free-swimming	35	1
Hyloxalus azureoventris	Lotters *et al.,* 2000	Free-swimming	37, 39, 40	3
Hyloxalus bocagei	Páez-Vacas *et al.,* 2010	Back-riding	28	1
Hyloxalus chlorocraspedus	Caldwell, 2005	Free-swimming	25	1
Hyloxalus delatorreae	Coloma, 1995	Back-riding	25	1
Hyloxalus edwardsi	Lynch, 1982	Free-swimming	?	?
Hyloxalus elachyhistus	Edwards, 1971	Free-swimming	31-41	?
Hyloxalus eleutherodactylus	Duellman, 2004	Back-riding	25	5
Hyloxalus exasperatus	Duellman & Lynch, 1988	Back-riding	23-25	?
Hyloxalus fascianigrus	Grant & Castro-H., 1998	Back-riding	25-26	7
Hyloxalus idiomelus	Duellman, 2004	Free-swimming	34	1
Hyloxalus infraguttatus	Coloma, 1995	Back-riding	24	1
Hyloxalus insulatus	Duellman, 2004	Free-swimming	34	1
Hyloxalus italoi	Páez-Vacas *et al.,* 2010	Free-swimming	25	1
Hyloxalus leucophaeus	Duellman, 2004	Free-swimming	28	1
Hyloxalus maculosus	Páez-Vacas *et al.,* 2010	Back-riding	28	1
Hyloxalus pulchellus	Edwards, 1974	Back-riding and free-swimming	25-26 * and 28-37**	?
	Coloma, 1995	Back-riding	25	1
Hyloxalus sauli	Edwards, 1974	Free-swimming	25-26	?
	Duellman, 1978	Free-swimming	36	1
Hyloxalus sordidatus	Duellman, 2004	Free-swimming	25	1
Hyloxalus subpunctatus	Stebbins & Hendrickson, 1959	Back-riding and free-swimming	?	19
Hyloxalus sylvaticus	Duellman & Wild, 1993	Free-swimming	33	1
Hyloxalus toachi	Coloma, 1995	Back-riding	25	1
Hyloxalus vertebralis	Coloma, 1995	Back-riding	25	1
Hyloxalus yasuni	Páez-Vacas *et al.* 2010	Free-swimming	27	1

(Stebbins & Hendrickson, 1959; Cochran & Goin, 1970), tadpoles were described by Stebbins & Hendrickson (1959), and other contributions about its natural history have been published (*e.g.,* Navas & Bevier, 2001; Fandiño *et al.,* 1997).

The description of 19 (between back-riding and free-swimming tadpoles) larvae of *Hyloxalus subpunctatus* from the municipality of Bogotá, Colombia, by Stebbins & Hendrickson (1959) reports very general larval features that are common to all tadpoles of the genus *Hyloxalus* and do not allow the distinction of the tadpoles of *H. subpunctatus* from other species of the genus. Nevertheless, I reviewed a large series of tadpoles of *H. subpunctatus* that included all the developmental stages. Here, I present larval features in the free-living tadpoles, to permit the distinction of the tadpoles from those of other species (either species sympatric with *H. subpunctatus* or from populations previously identified as *H. subpunctatus*). Moreover, the larval morphology of *H. subpunctatus* shows different types of variation (*e.g.,* ontogenetic variation), which are described herein. A re-description of the tadpoles of *H. subpunctatus* with variation and ontogenetic change is provided, highlighting characters useful for distinction and diagnosis of free-living larvae.

MATERIAL AND METHODS

Tadpoles

I reviewed a total of 347 tadpoles of *H. subpunctatus:* 143 back-riding tadpoles, *i.e.,* tadpoles captured with the carrying adults (stages 25 to 27, Gosner, 1960), 196 free-swimming tadpoles (stages 26 to 41) and eight individuals in metamorphosis. Staging of tadpoles follows Gosner (1960). For identification of the tadpoles, I used tadpoles from localities where *H. subpunctatus* is the only dendrobatid found (*e.g.,* the campus of the Universidad Nacional de Colombia, Bogotá), and I identified larval characters of this species; this allowed me to identify tadpoles of *H. subpunctatus* from other places. This characterization was then applied to samples for three other situations:

(1) larvae from other places where adults of *H. subpunctatus* have been collected, (2) larvae from highland localities in the Cordillera Oriental de Colombia where *H. subpunctatus* might potentially occur, and (3) tadpoles from other highland populations of the country where *H. subpunctatus* is not expected but other species are known (for example, *H. pulchellus*). These three sets of tadpoles were compared or contrasted with my reference samples from the campus of the Universidad Nacional de Colombia in Bogotá.

Description and variation

A composite description is provided, mainly based on free-swimming tadpoles (stages 25-41), since all morphological features are fully developed in those stages and with slight variation. Larval terminology follows Altig & McDiarmid (1999) and the format of the description and measurements follows Caldwell *et al.* (2002) and Castillo-Trenn (2004), except for the following measurements: nostril length (measured between the lateral edges of the nostril), nostril-snout distance (from the anterior edge of narial opening to the tip of snout), nostril-eye distance (from the posterior edge of the narial opening to the anterior edge of eye), internarial distance (between internal edges of the nostrils), eye diameter (only the eye, not the ocular orbit), spiracle height (height taken at base of the spiracle), upper jaw sheath length (measured from the tips of the lateral process), and lower jaw sheath length (measured from the tips of the edges). The taxonomy for the generic level follows Grant *et al.* (2006) and only one family, Dendrobatidae, is recognized, following Santos *et al.* (2009) and Pyron & Wiens (2011).

Measurements were taken from eleven tadpoles in stage 26, ten tadpoles in stage 36 and five in stage 40 to evaluate variation in proportions during ontogeny. Nevertheless, only the measurements and proportions in stage 36 are used in the description, because the range of the proportions is overlapped between stages and remains relatively stable throughout the larvae phase, *i.e.,* between stages 25-41 (Table 2). Papillae were counted on the right side of the anterior labium and in a space of 1 mm on the posterior labium. Furthermore, for 194 larvae with stage of development between free-swimming tadpoles and metamorphic tadpoles the total length, body length and maximum tail height were taken. Measurements were taken with a caliper (0.01 mm) and with the ocular micrometer of a Wild M8 stereomicroscope (0.001 mm). All measurements are reported in millimeters (mm.).

TABLE 2: Range in variation of proportions (in percentage) for 11 tadpoles in stage 26, 10 tadpoles in stage 36 and 5 tadpoles in stages 40 of the tadpoles of *Hyloxalus subpunctatus.* Abbreviations: **(BH)** body height, **(BL)** body length, **(BW)** body width, **(IND)** internostril distance, **(IOD)** interorbital distance, **(ODW)** oral disc width, **(TAL)** tail length, **(TL)** total length, **(VT)** vent tube.

Proportions	Stage 26 (11)	Stage 36 (10)	Stage 40 (5)
BL/TL	39-47	34-46	36-38
BH/BW	71-92	70-99	68-75
BW/BL	63-74	58-78	64-73
Snout-Spiracle/BL	53-73	52-66	60-68
TAL/LT	53-61	54-66	62-64
VT/BL	8-17	9-13	11-15
Nostril/Eye	36-51	21-34	19-28
Eye/IOD	24-29	26-41	36-45
IOD/BW	43-56	34-43	40-45
IND/IOD	59-67	56-85	54-58
Height/length Spiracle	49-90	64-98	52-87
Width/height Tail	53-86	72-97	72-99
ODW/BW	37-43	25-39	33-37
Anterior Lip gap/ODW	54-71	50-71	67-79
A-2 gap/ODW	3-8	4-15	3-10

Three phases of development are defined: (1) back-riding tadpoles, (2) free-swimming tadpoles, and (3) metamorphic tadpoles; ontogenetic variation and variation within each of the phases is described. Each phase is identifiable and defined by abrupt changes in larval morphology and changes in life habit (Anganoy-Criollo, 2010). The number of rows of marginal papillae varies among lips, among tadpoles of the same stage (random individual variation), and between stages of free-swimming tadpoles. This variation was evaluated as follows: first, the oral disc was divided for each lip (anterior and posterior) and by the position of the papillae on each lip; and second, the number of papillae rows was quantified as one row, one biseriated row, two rows and three or more rows of papillae (see Figure 3 in Sánchez, 2010).

For external neuroreceptive cells, I followed Lannoo (1999) and Altig & McDiarmid (1999, Figure 3.5) for the attached type of vent tube and spiracle.

Comparisons

Free-swimming tadpoles of *H. subpunctatus* were compared with tadpoles of other potentially sympatric species (*Rheobates palmatus* and *H. edwardsi*) and other similar species that were previously confused within it (*H. pulchellus;* Cochran & Goin, [1970: 64] suggested that *H. s. subpunctatus* is in southern Colombia, but those populations are now considered

H. pulchellus). Comparisons between species were made between tadpoles of the same phase (= *semaphoront*), *i.e.,* stages among free-swimming tadpoles, but tadpoles in different semaphoronts should not be compared/confused (*e.g.,* back-riding tadpoles at stage 25 with free-swimming tadpoles at the same stage).

All tadpoles are preserved and stored in 10% formalin (formaldehyde) and housed in the Colección de Anfibios, Instituto de Ciencias Naturales, Universidad Nacional de Colombia (ICN). Museum number and localities of tadpoles examined are given in Appendix A.

RESULTS

Description of the tadpoles

Tadpoles between stages 28-39 of moderate size, total length from 7.5-26.1 mm (12.5 ± 5.5, *n* = 51) at stage 26 to 30.1-32.8 mm (31.9 ± 1.5, *n* = 3) at stage 41 (Fig. 1). In stage 36, total length 29.5-42.2 mm (35.9 ± 3.8), body length 10.9-17.6 mm (14.1 ± 2.1), body width 7.5-12.3 mm (9.6 ± 1,4), and body height 5.6-9.8 mm (8 ± 1.3); body length 34-46% total length, body width 58-78% total length, body width 58-78% body length, and body height 70-99% body width.

Body ovoid in dorsal view and depressed in lateral view (Fig. 2). Snout rounded in dorsal and lateral view. Lateral-line system symmetric on both sides of body, with infraorbital, supraorbital, postsupraorbital, longitudinal oral, and angular lines. Stitches on lateral lines rounded, separated by equal distances. Two pairs of rounded pale whitish spots on ventral region of body (= *cumuli of neuromasts*) of equal to variable size, the first pair of spots on angular line in ventral region and second pair on ventral body line near body-tail junction. Nostril large, dorsal on body with dorso-anterolateral direction, from rounded to slightly oval; nostril length 0.3-0.4 (0.3 ± 0.1), 21-34% of eye diameter. Inner margin of the nostril with a thick fleshy ring and with low fleshy projection dorsally. Nostril located about midway between eyes and snout tip. Internarial distance 1.9-3.2 (2.6 ± 0.3), 56-85% of interorbital distance and interorbital distance 2.6-5.3 (3.6 ± 0.8), 34-43% of body width. Eyes dorsal, directed dorsolaterally, eye diameter sub-equal to nostril-eye distance, eye diameter 1-1.6 (1.2 ± 0.2), 26-42% of interorbital distance. Spiracle sinistral, conical, directed posterodorsally, located below longitudinal midline and slightly posterior to vertical midline of body; snout-spiracular opening distance 6.9-11.4 (8.7 ± 1.6) *i.e.,* opening ending 52-66% of body length (from tip of snout). Spiracle length longer than (in early stages, < stage

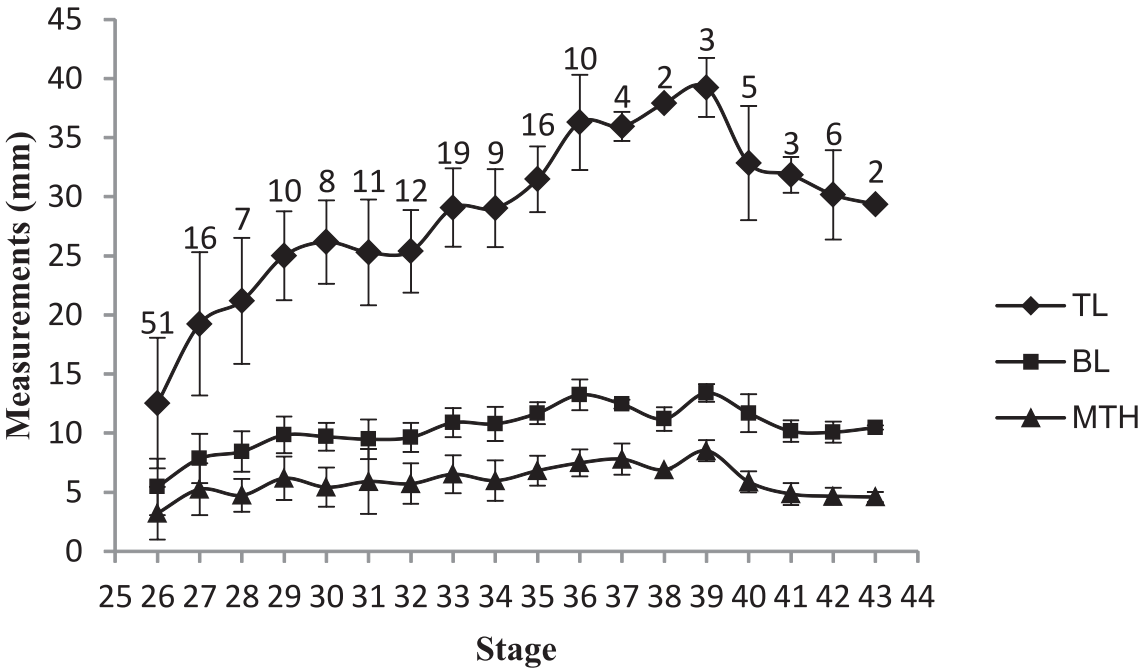

FIGURE 1: Variation in total length **(TL)**, body length **(BL)** and maximum tail height **(MTH)** in the free-swimming tadpoles of *Hyloxalus subpunctatus.* Bars represent standard deviation, and the numbers on TL are the number of individuals employed for the three measurements. Stages refer to Gosner (1960).

37) or equal to (in late stages, > stage 38) spiracle height, spiracle length 1.4-2.2 (1.8 ± 0.2) and spiracle height 1.2-2 (1.4 ± 0.3). Opening of spiracle small, 22-49% of spiracle height. Inner edge of spiracular opening separate from body. Vent tube short, 9-13% of body length; vent tube attached to right side of the ventral fin in A-type (see Altig & McDiarmid 1999, Figure 3.5) and opening of the vent tube with dextral direction and without indentations. Intestines visible through translucent skin, disposed from central, center-sinistral to transversal to body in ventral view (Fig. 3). Tail moderate, 54-66% total length. Caudal musculature thin and myotomes higher than (commonly) or as high as wide at body-tail junction, narrowing gradually until tip of tail; tail muscle height 38-53% body height. Dorsal fin height equal to ventral fin height and myotomes equal to or less than fin height at mid-tail. Dorsal fin originates at tail-body junction of equal height through tail or higher at mid-tail. Tip of tail rounded. Maximum tail height equal to the body height, from 5.6 to 10.4 (8.4 ± 1.4).

Oral disc positioned and directed anteroventrally, emarginate and surrounded by marginal papillae, except on anterior lip; without sub-marginal papillae (Fig. 4A). Oral disc width 2.4-4.0 mm (3.2 ± 0.4), 25-39% of body width. Anterior lip gap 1.4-2.4 mm (2.1 ± 0.3), 45-71% of oral disc width. Marginal papillae disposed in rows on lips that vary by lip and stage from one row to three or more rows (see Table 3 and Figure 5 and see variation section). Papillae abundant on anterior and posterior lip, from 12 to 23 papillae on right side of anterior lip and from 16 to 21 papillae

FIGURE 2: Tadpole of *Hyloxalus subpunctatus* in lateral **(A),** dorsal **(B)** and ventral **(C)** view at Gosner (1960) stage 31. Total length 31.3 mm and body length 11.5 mm (ICN 55281).

TABLE 3: Variation in the number of marginal papilla rows in the free-swimming tadpoles of *Hyloxalus subpunctatus.* Variation is reported as the range, average ± standard error, and the mode. Conventions: **(A)** papillae on anterior lip, **(B)** papillae on postero-lateral side of posterior lip and **(C)** papillae on middle of posterior lip; **(1)** one row, **(2)** one biseriated row, **(3)** two rows and **(4)** three or more rows of papillae. Stages refer to Gosner (1960).

Stages	*n*	Parameters	A	B	C
25-26	15	*Range*	1-3 (1.44 ± 0.1)	1-2 (1.46 ± 0.1)	1-3 (1.78 ± 0.2)
		Mode	1	1	2
27-32	21	*Range*	1-2 (1.46 ± 0.1)	1-3 (1.76 ± 0.1)	1-4 (2.14 ± 0.2)
		Mode	1	1	3
33-39	24	*Range*	1-3 (2 ± 0.1)	1-4 (2.4 ± 0.14)	1-4 (2 ± 0.2)
		Mode	2	2	1

(in 1 mm) on posterior lip in stage 36. Papillae thin, elongate, and low on anterior and posterior lip; papilla width (at base) about half of papilla height. Tips of papillae rounded to sub-acuminate. Labial tooth row formulae 2(2)/3[1]; A-2 gap short, from 4 to 15% of oral disc width; first lower row sometimes narrowly interrupted medially, space equal to one to two denticles. Length of first upper row equal second upper row commonly; first lower row equal to second lower row and third lower row slightly shorter than first and second lower rows; upper rows sub-equal to first and second lower rows. Jaw sheath keratinized and fully pigmented black or dark brown. Upper jaw sheath moderately robust with short, thin lateral processes. Upper jaw sheath width 44-57% of oral disc width. Upper jaw sheath slightly concave, anterior edge strongly curved and free posterior edge weakly curved with low serrations; lateral processes without serrations. Tips of serrations rounded on middle of upper jaw sheath and acuminate towards ends. No shelf on concealed upper jaw sheath. Lower jaw sheath (LJS) thin, in V-shape with its ends weakly open; LJS is not entirely pigmented, pigmentation area is at least half of the height of what is pigmented in the upper jaw sheath. Serrations on lower jaw sheath low and with rounded tips.

Color in life

Field notes of M. Anganoy-C, August 23, 2009, deposited at ICN. Free-swimming tadpoles with dorsum and flanks dark brown with cream reticulations. Venter translucent to dark brown; when are visible, intestines dark brown and region between oral disc and intestines reddish. Myotomes of the tail cream with light brown spots and dorsal and ventral fins are translucent. Dorsal fin with light brown spots. Eyes black. Back-riding tadpoles with dorsum and flanks

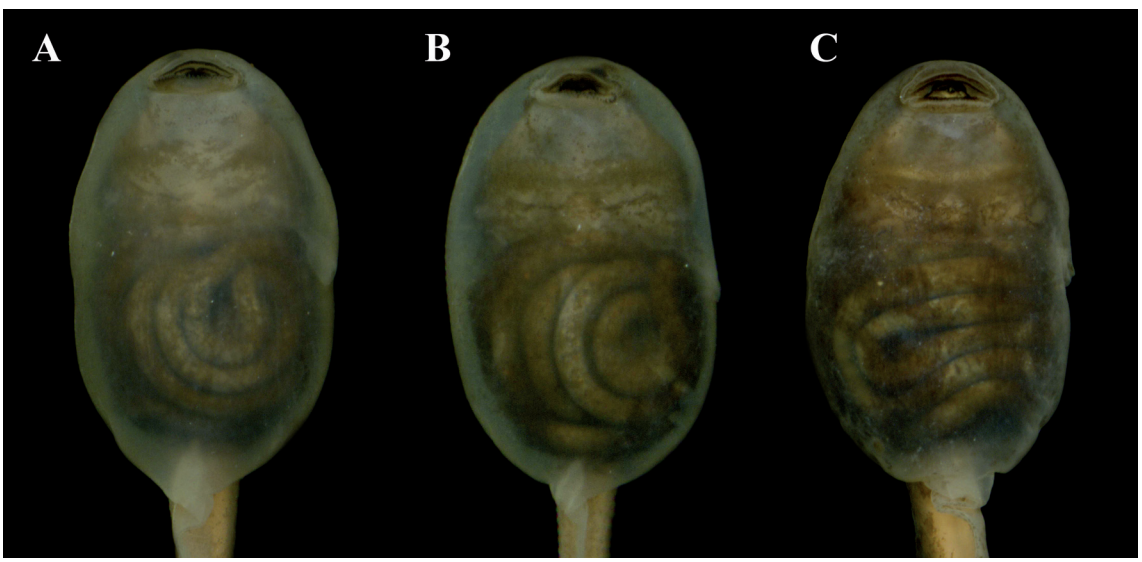

FIGURE 3: Variation in the disposition of intestines in the free-swimming tadpoles of *Hyloxalus subpunctatus.* Central **(A),** centro-sinistral **(B)** and longitudinal **(C)** intestines. **(A)** ICN 45566, stage 27, total length 27 mm (approx.) and body length 11 mm. **(B)** ICN 45566, stage 26, total length 19.5 mm and body length 8.4 mm. **(C)** ICN 32500, stage 31, total length 27 mm (approx.) and body length 12.5 mm.

dark brown; venter translucent and intestines yellowish cream; myotomes cream with dull black spots and fins translucent.

Color in preservative (Formaldehyde 10%)

Dorsum and flanks of free-swimming tadpoles from light brown to dark brown or pale cream. Spiracle and vent tube translucent. Translucent skin of the venter from pale white to dark brown with light to dark brown dots. When visible, intestines dark brown to black. Caudal coloration from translucent cream to dark brown, anterior more intense than posterior; dorsal and ventral fins translucent, dorsal fin with brown spots and ventral fin with few light brown spots. Eyes black and region surrounding the eyes cream. Papillae of the oral disc cream with light brown stippling.

In back-riding tadpoles, body light brown to pale dark brown. Tail cream with light brown spots. In metamorphic tadpoles, dorsum dark brown with cream vertebral line; dorsolateral flanks light brown and flanks brown with slightly oblique lateral stripe from pale brown to pale cream, extended from groin to the eyelid and sometimes to the nostril. Anterior myotomes brown and posterior myotomes light brown with brown spots. Dorsal and ventral fins white with brown spots. Limbs pale brown, forelimbs with dark brown dots and hind limbs with diffuse to intense dark brown bands. Hands and feet pale cream with dark brown dots.

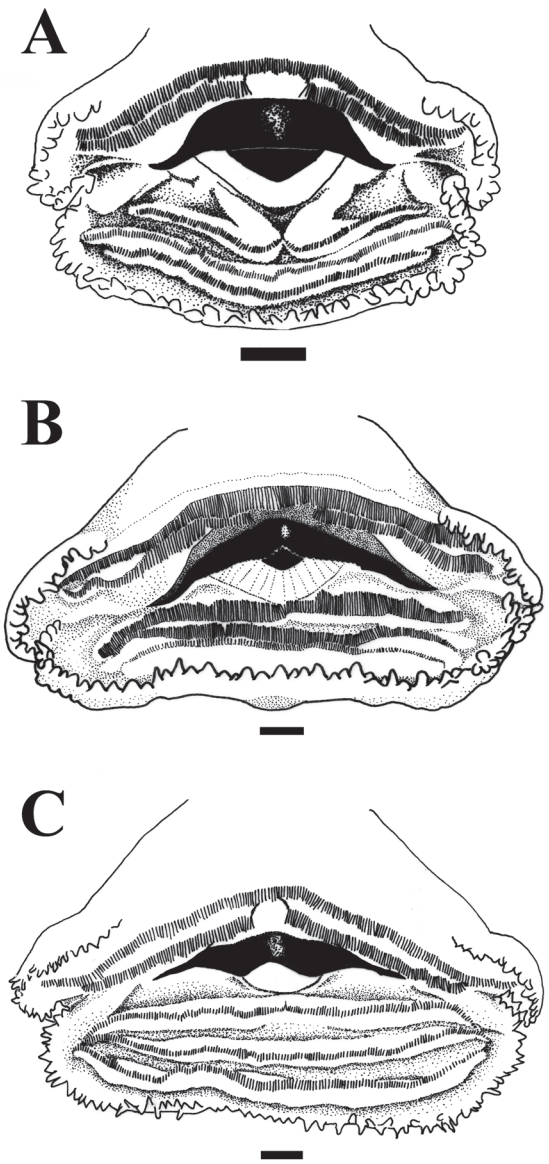

FIGURE 4: Oral discs of free-swimming tadpole of *Hyloxalus subpunctatus* (Stage 27, ICN 45567) **(A)** *Hyloxalus pulchellus* (Stage 30, ICN 9682) **(B)** and of *Rheobates palmatus* (Stage 35, ICN 23311) **(C)**. Scale bar equal to 0.5 mm.

Variation

The variation is divided and described as ontogenetic changes and for each of the three different phases already defined (back-riding, free-swimming and metamorphic tadpoles; Fig. 6):

Ontogenetic variation

Stitches and lateral lines are not visible in back-riding tadpoles, but in free-swimming tadpoles, the stitches and lateral lines are visible and fully developed. In metamorphic tadpoles, the stitches are on the posteroventral region of the body and lateral lines on the posterior body and between the nostril and eyes there is a naso-ocular line. The narial opening is dorsolateral in back-riding tadpoles and dorsoanterolateral to anterolateral in free-swimming and in metamorphic tadpoles. There is a low fleshy rim around of the nostril in back-riding tadpoles, it is fully developed in free-swimming tadpoles and low or atrophied in metamorphic tadpoles. The spiracle opening is attached to skin of body and is higher than long in back-riding tadpoles and it is separated from the skin during the later stages of back-riding tadpoles to the beginning of metamorphosis. The spiracle is atrophied at metamorphosis. The dorsal fin height is the same throughout the tail in back-riding tadpoles, higher at mid-tail in free-swimming tadpoles, and after the beginning of metamorphosis is atrophied.

Oral disc is ventral in back-riding tadpoles, anteroventral in free-swimming tadpoles and anterior on body after initiating metamorphosis (stages > 42). The oral disc is weakly emarginated in back-riding tadpoles, with evident emargination in free-swimming tadpoles, and without emargination in metamorphic tadpoles. The A-2 gap moderately long, 30-37% of oral disc width in back-riding tadpoles, 4-15% of oral disc width in free-swimming tadpoles, and in metamorphic tadpoles the labial tooth rows are atrophied (including A-2). There are 3 to 10 marginal papillae on anterior lip in back-riding tadpoles, from 10 to 24 in free-swimming tadpoles, and after the beginning of metamorphosis the papillae are atrophied. Jaw sheaths are not fully pigmented in back-riding tadpoles, fully pigmented in free-swimming tadpoles, and atrophied in metamorphosis. Denticles do not cover all ridges of the labial tooth rows in back-riding tadpoles, are on the entire length of ridges in free-swimming tadpoles, and in metamorphic tadpoles the labial tooth rows are

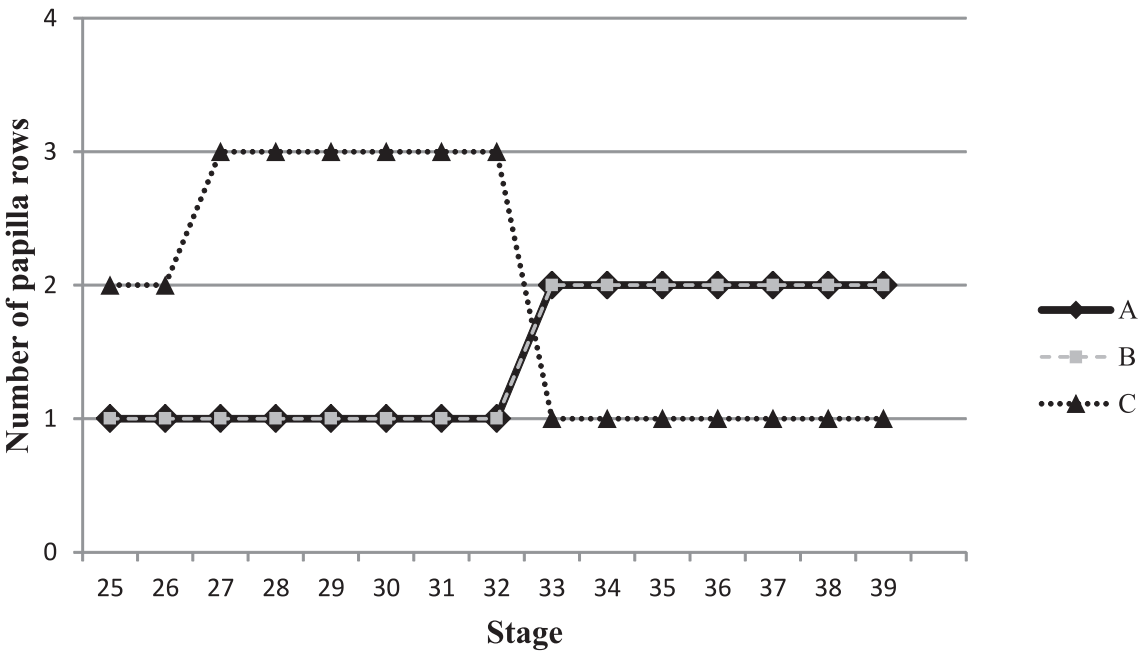

FIGURE 5: Pattern of variation in the number of marginal papillae rows against Gosner's (1960) stages in free-swimming tadpoles of *Hyloxalus subpunctatus*. Values represent the mode in each stage; see Table 3 for the size sample. Conventions: **(A)** papillae on anterior lip, **(B)** papillae on postero-lateral side of posterior lip and **(C)** papillae on medial region of the posterior lip; **(1)** one row, **(2)** one biseriated row, **(3)** two rows and **(4)** three or more rows of papillae.

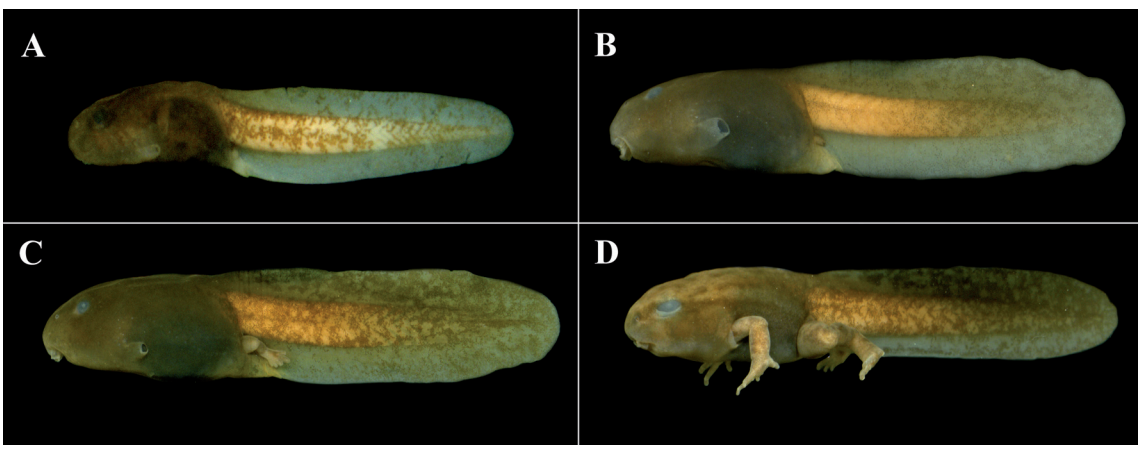

FIGURE 6: Ontogenetic changes in free-living tadpoles of *Hyloxalus subpunctatus.* **(A)** Stage 26, TL = 11.5 mm, ICN 34083; **(B)** Stage 28, TL = 25.4 mm, ICN 45567; **(C)** Stage 35, TL = 33.4, ICN 45567; **(D)** Stage 43, TL = 29.8, ICN 45567. Not to scale.

atrophied. A weak notch at middle of the free-edge of the upper jaw sheath, sometimes not visible in back-riding tadpoles.

Variation within each phase of development

Variation among back-riding tadpoles: The tadpoles carried on the back of adults are in stage 25 (*n* = 23, 16.8%), stage 26 (*n* = 116, 81.1%) and in stage 27 (*n* = 3, 2.1%). The total length of all the back-riding tadpoles is 6.1-12.6 mm (9.1 ± 0.8) and the body length is 2.8-5.5 mm (3.5 ± 0.4). Coiling of intestines from central to sinistral; opening of the vent tube commonly dextral and rarely central. Oral disc with one row to one biseriated row of marginal papillae; labial tooth row formula from 0/0 to 2/2 in stage 25, 0/0 to 2/3 in stage 26 and 2/3 in stage 27 (Table 4). Back-riding tadpoles that are in stage 25 and that have labial tooth row formulae 0/0 and 1/2 with body wider at posterior body level, snout sub-acuminate in dorsal view and truncate in lateral view. Addition of the labial teeth on posterior tooth ridges (P-1 and P-2) from middle to lateral ends of the ridge.

Variation among free-swimming tadpoles: External morphology changes slightly in free-swimming tadpoles, but does not affect general morphology. The opening of the vent tube is central in one tadpole in stage 28, one in 30 and one in 36, and two tadpoles in stage 33. Tooth row length varies from having the same length in all posterior rows (P-1 = P-2 = P-3) to different lengths, P-2 being larger than P-1 and P-3, or P-1 equal to P-2. At stage 40, denticles on P-3 ridge do not cover the entire ridge. The papillae on the lips are disposed from a single row to three rows, but variation depends on the lip and on the stages as follow (Table 3, mode is reported): papillae on anterior lip and papillae on postero-lateral side of posterior lip in one row from stage 25 to 32, and in one biseriated row from stage 33 to 39; papillae on medial region of the posterior lip in one biseriated row from stage 25 to 26, two rows from stage 27 to 32, and in one row from stage 33 to 39 (Fig. 5).

Variation in metamorphic tadpoles: Froglets in stage 42 (*n* = 6) and 43 (*n* = 2). Total length 25.8-36 (29.8 ± 2) and body length 8.7-11.3 (10.3 ± 0.5). The body is wider at cephalic region and the snout is slightly truncate in dorsal view. The vent tube, spiracle, oral disc, and keratinized structures such as the upper and lower jaw sheaths are atrophied; moreover, labial tooth row formula is 0/0 from stages 41 and the papillae are only on lateral sides of disc oral from stages 42. Froglets have some characters typical of the adults, such as basal toe webbing between toes II-III-IV, banded pattern on the legs, and a cream pale oblique lateral stripe (Fig. 6D).

TABLE 4: Variation in labial tooth row formulae in back-riding tadpoles of *Hyloxalus subpunctatus* (*n* = 99). Data are number of tadpoles for each formula.

Stage	Labial Tooth Row Formula						
	0/0	1/0	0/1	1/1	1/2	2/2	2/3
25	4				1	3	
26	5	1	1	2	1	34	44
27							3

Comparisons with tadpoles of other dendrobatids

Tadpoles of four species of dendrobatids, formerly *Colostethus sensu lato,* inhabitant the Altiplano Cundiboyacense on Cordillera Oriental de Colombia, *R. palmatus, H. vergeli, H. edwardsi* and *H. ruizi.* Tadpoles of *H. vergeli* and *H. ruizi* are unknown and tadpoles *R. palmatus* (Dunn, 1944: under the name *H. granuliventris,* described tadpoles from base of the eastern flank of the Cordillera Oriental, Buenavista, Villavicencio, 1230 m) and of *H. edwardsi* (Lynch, 1982) were described briefly. Nevertheless, tadpoles of *R. palmatus* and *H. edwardsi* are available in Laboratorio de Anfibios, Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá.

The tadpoles of *H. subpunctatus* differ from tadpoles of *R. palmatus* in the following ways: notch on upper jaw sheath absent (present in *R. palmatus;* Fig. 4C), lateral processes short (long in *R. palmatus;* Fig. 4C), shelf absent (present in *R. palmatus*), oral disc anteroventral on body (ventral in *R. palmatus*), caudal musculature slender at body-tail junction (robust in *R. palmatus*), stitches rounded (ovoid in *R. palmatus*), and nostril rounded (elliptic in *R. palmatus*) without fleshy projection (present in *R. palmatus*); from *H. edwardsi* in: notch on upper jaw sheath absent (present in *H. edwardsi*), lateral processes thin and short (thin and long in *H. edwardsi*), serrations on jaw sheath low (higher and evident in *H. edwardsi*), and spiracle located slightly posterior to middle body (located in the anterior first third of the body in *H. edwardsi;* data based on direct observation of ICN 6391 and original descriptions, see Lynch, 1982, figure 5).

The highland populations of southern Colombia (Puracé, Cauca) have been confused with *H. subpunctatus* (Cochran & Goin, 1970: 64) because adults shared some features such as complete oblique lateral

stripe, basal or absent toe webbing and Finger I longer than Finger II. The tadpoles from highlands of southern Colombia and northern Ecuador (for localities see Appendix A) match with the description of the tadpoles of *H. pulchellus sensu* Coloma (1995), but differ from tadpoles *sensu* Edwards (1974) in the coloration pattern on the tail (spotted in tadpoles from southern Colombia and reticulate in tadpoles of *H. pulchellus sensu* Edwards, 1974). To date, the tadpoles of the populations from southern Colombia can be assigned to *H. pulchellus,* but the taxonomic status of these populations is doubtful (see comment in Coloma, 1995: 47, 49). Tadpoles of *H. subpunctatus* differ from tadpoles of *H. pulchellus* from southern Colombia in the following ways: notch on upper jaw sheath absent (present in *H. pulchellus sensu* Coloma, 1995), lateral processes thin and short (thin and large in *H. pulchellus sensu* Coloma, 1995. Fig. 4B), nostril round (ovoid in *H. pulchellus sensu* Coloma, 1995), and dorsal fin curved and higher at mid-tail (not curved and of equal height throughout entire tail in *H. pulchellus sensu* Coloma, 1995).

Rivero & Serna (1989 "1988") grouped the species with complete oblique lateral stripe, basal or absent toe webbing and Finger I longer than Finger II in their group IX. However, their groups are defined by combinations of characters present and variables between groups, and not by synapomorphies. In Colombia, group IX is represented by *H. borjai, H. breviquartus, H. lehmanni, H. pinguis, H. ramosi, H. ranoides, H. sauli* and *C. yaguara* and *"Colostethus" ramirezi.* Tadpoles of *H. sauli* have been described by Edwards (1974) and Duellman (1978), but the tadpoles of the other species are not known. Tadpoles of *H. subpunctatus* differ from tadpoles of *H. sauli* in the following ways: notch on upper jaw sheath absent (present), papillae abundant on anterior lip (reduced in *H. sauli,* see Edwards, 1974: figure 3C), and dorsal fin arises from body-tail junction (absent on the anterior first third of the tail in *H. sauli*).

DISCUSION

Knowledge of the tadpoles of dendrobatid frogs is limited due to the scarce number of tadpoles in amphibian collections (excepting back-riding tadpoles) and the apparent conservative morphology, reducing interest in larval morphology as a taxonomic tool. Moreover, most of the available descriptions are based on back-riding tadpoles or on one single free-living tadpole, which does not permit assessment of variation. However, in free-swimming tadpoles of *H. subpunctatus,* the morphology is relatively stable, *i.e.,* variation occurs but with slight modifications in the general pattern and their larval morphology differs from other tadpoles when the same semaphoront is compared.

Cochran & Goin (1970) described two subspecies for *H. subpunctatus* (*H. subpunctatus subpunctatus* and *H. subpunctatus walesi*) and reported the subspecies *H. s. subpunctatus* from Villavicencio-Meta. In my revision of tadpoles from the Cordillera Oriental de Colombia, I found tadpoles of *H. subpunctatus* in the Departments of Boyacá, Cundinamarca, and southern Santander, between 2015 to 3850 meters (see Appendix A), and did not find geographic variation or inter-populational differences in the larval morphology. Therefore, based on larval morphology, there is no reason to justify two (sub)species in *H. subpunctatus;* moreover, tadpoles from lowlands of the eastern flank of the Cordillera Oriental de Colombia, deposited in the ICN amphibian collection from Villavicencio and neighboring localities, do not show characters similar to the larvae of *H. subpunctatus* (*e.g.,* no notch on upper jaw sheath), and it is likely that these or at least some populations (previously reported as *H. subpunctatus*) correspond to *H. ranoides.*

Stebbins & Hendrickson (1959) noted a peculiar small gap in the middle of the posterior lip in tadpoles of *H. subpunctatus;* however, all tadpoles observed of *H. subpunctatus* have papillae on posterior lip without a gap and it is likely that at least some larvae observed by them had an atrophied posterior lip. Tadpoles of the other species present near Bogotá, Colombia (*Centrolene buckleyi, Dendropsophus labialis, Hyloscirtus bogotensis*) that might be confused with tadpoles of *H. subpunctatus* do not have a gap on the posterior labium.

Donnelly *et al.* (1990) proposed a labial tooth row formation sequence for *Phyllobates lugubris,* P-1, A-1, P-2, A-2 and P3. Interestingly, the variation present in labial tooth rows of back-riding tadpoles of *H. subpunctatus* (0/1 or 1/0, 1/1, 1/2, 2/2, 2/3; Table 4) would support the pattern detected in *Phyllobates.* However, the data are inadequate to determine the formation and appearance of P-1 before A-1 or vice-versa, and therefore the order is ambiguous. Nevertheless, the sequence of formation P-1 (first?), A-1 (second?), P-2, A-2 and P-3 is different from the typical and commonly accepted A-1, P-2, P-1, A-2 and P-3 for tadpoles with labial tooth row formulae 2/3 (Thibaudeau & Altig, 1988; Altig & McDiarmid, 1999), suggesting that the family Dendrobatidae has a sequence of labial tooth row formation distinct from other families.

Ontogenetic variation in number of marginal papillae was described for *Allobates kingsburyi* (Castillo-Trenn, 2004), but variation in the number of rows of papillae on the edge of lips through the stages has not been described. This feature (number of rows of papillae) was employed to differentiate species (*e.g.,* Savage, 1968). The variation described in the number of rows of papillae in tadpoles of *H. subpunctatus* suggests that the disposition is not homogeneous and is independent of stages and on each lip, but the pattern in the number of papillae rows can be detected when large samples are available; otherwise caution must be taken. I suggest that variation in the number of papillae rows should be included in the description of tadpoles.

The tadpoles of *H. subpunctatus* present typical morphology of larvae of other species, formerly *Colostethus sensu lato:* body moderately depressed; snout rounded in lateral and dorsal view; nostril about midway between eyes and tip of snout; eyes and nostril on dorsum of the body and directed dorsolaterally; nostril with fleshy rim and fleshy projection; spiracle sinistral; vent tube short, conical and dextral to the ventral fin; caudal musculature moderately robust; fins low and commonly of equal height; coiling of intestines not central in ventral view; oral disc emarginated, not umbelliform and without papillae on anterior lip; labial tooth row formula 2(2)/3[1] with A-2 gap short or narrow and upper jaw sheath with lateral processes defined. These species are now included in the genera *Aromobates, Mannophryne, Rheobates* (Aromobatidae of Grant *et al.,* 2006), *Colostethus* and *Hyloxalus* (Dendrobatidae of Grant *et al.,* 2006) and there are no differences at the familial or generic levels, but at species level there are differences. Grant *et al.* (2006: 158) mentioned, "that all species of Aromobatidae lack the ability to sequester alkaloids", which was a reason to justify the division of the dendrobatids in two families (Aromobatidae and Dendrobatidae); however, this datum is unknown for most of the species formerly included in *Colostethus* (*sensu lato*) (except *C. panamensis* and *C. ucumari*). Furthermore morphological synapomorphies or characters in adults or larvae that support both families of dendrobatids are unknown.

In addition to the characters mentioned above, a notch in the middle upper jaw sheath is commonly present in species of *Hyloxalus* (see Fig. 4B and 4C) and in the tadpoles of other dendrobatids of the genera *Anomaloglossus, Ameerega, Aromobates, Colostethus, Epipedobates, Mannophryne, Rheobates, Silverstoneia,* and some *Allobates* and *Phyllobates* (Sánchez, *in prep.,* and personal observation of author). However, the notch is absent in free-swimming tadpoles of *H. subpunctatus.* The absence also has been described in other tadpoles of *Hyloxalus* such as *H. azureiventris* (Lötters *et al.,* 2000), *H. chlorocraspedus* (Caldwell, 2005), and *H. sylvaticus* (Duellman & Wild, 1993). Moreover, tadpoles of *Adelphobates, Dendrobates, Oophaga, Ranitomeya,* and some *Allobates* and *Phyllobates* lack the notch (Sánchez, *in prep.,* and personal observation of the author). Considered that the absence or presence of the notch are states of a same characters, until otherwise is proved (Hennig, 1968), then the absence or present of the notch in middle upper jaw sheath is phylogenetically polymorphic within *Hyloxalus* and is interpreted as convergent when the relationships for dendrobatids proposed by Grant *et al.* (2006) are considered.

Grant *et al.* (2006) proposed three character-states in larval caudal coloration: vertical stripes (state 0), scattered melanophores clumped to form irregular blotches (1) and evenly pigmented (2). The variation detected in the coloration of the tail in tadpoles of *H. subpunctatus* shows that states 1 and 2 are not independent, since both states are present in stages of the same semaphoront (free-swimming tadpoles) of *H. subpunctatus* and herein is interpreted as inter-population variation. Furthermore, presence or absence of the lateral line system and the associated stitches (character 98 of Grant *et al.,* 2006) in one semaphoront must be taken with caution; apparently, variation (*i.e.,* presence or absence) is due to the effects of fixation, preservation, and observation methods.

As a result of my early studies on dendrobatid larvae, at the inter-specific level, the major larval differences are found in the upper jaw sheath, pattern and shape of stitches, nostrils and their ornamentation, caudal musculature, and fins. However, the available descriptions are so generalized that details of these anatomical points cannot be detected in those descriptions. I suggest that within the descriptions of dendrobatid tadpoles, these characters and the conditions present in the tadpoles of each species be included as additional data of larval morphology to distinguish between species.

RESUMEN

En este estudio se redescriben los renacuajos de la rana nodriza venenosa Hyloxalus subpunctatus *(Anura: Dendrobatidae) con base en material larvario proveniente de la localidad tipo y de la mayoría de su rango de distribución, y se incluye toda la secuencia de estadios de desarrollo ontogenético. Describo los renacuajos en las*

tres fases de desarrollo: (1) renacuajos de la espalda del adulto, (2) renacuajos nadadores libres y (3) renacuajos o individuos metamórficos. Dos tipos de variación hay en la morfología larval de esta rana: variación ontogenética y variación dentro de cada fase de desarrollo. En renacuajos de espalda, la variación en hileras dentales labiales sugiere un patrón de variación de formación de hileras dentales labiales. En los renacuajos de vida libre hay variación en la disposición de las papilas marginales, es decir en el número de hileras sobre los márgenes de los labios, pero la variación no tiene efectos sobre el aspecto general de los renacuajos. Además, comparé los renacuajos de H. subpunctatus *con los renacuajos de especies potencialmente simpátricas y con renacuajos de poblaciones previamente identificadas como* H. subpunctatus *y encontré diferencias inter-específicas y caracteres larvarios útiles para delimitar estas especies.*

Palabras-Clave: Renacuajos; Diferenciación larvaria; Cambios ontogenéticos; Secuencia de formación de hileras de dientes labiales.

ACKNOWLEDGMENTS

To John D. Lynch for his help, support and valuable discussion on neotropical tadpoles, and for allowing access to the tadpole collection of the Laboratorio de Anfibios, Instituto de Ciencias Naturales (ICN), Universidad Nacional de Colombia. To David Sánchez for sharing his knowledge of dendrobatid tadpoles. To the herpetology group (Belisario Cepeda, Diana Mora, Ernesto Pérez and Mileidy Betancourth) of the Universidad de Nariño, Pasto, for supporting my studies in frogs and tadpoles. To Lina María Escobar for her help in catching additional tadpoles. To Albertina Lima, John D. Lynch, Enrique La Marca, Jhon Jairo Ospina-Sarria, and David Sánchez for valuable comments on this manuscript and to Marco Rada for his comments on the initial version. Partial economic support for the year 2009 was provided by the Vicerrectoria de Investigaciones, Posgrados y Relaciones Internacionales of the Universidad de Nariño, Pasto, Colombia.

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Aceito em: 09/01/2013
Publicado em: 30/06/2013

APPENDIX A

Tadpoles Examined

The following reports the locality, the collection number, the number of tadpoles (*n*) and the range of the stages Gosner (1960), for each lot of tadpoles. (*) Designed back-riding tadpoles.

Hyloxalus subpunctatus

Colombia: **Cundinamarca,** Albán, Tres Marías, ICN 14348, *n* = 9, stages 26-27; Bogotá, Universidad Nacional de Colombia, ICN 55305*, *n* = 16, stages 26-27, ICN 18202*, *n* = 20, stage 26; Bogotá, Universidad de los Andes, ICN 45778, *n* = 13, stages 25-27; Bogotá, barrio el Salitre, ICN 55281, *n* = 18, stages 25-43, ICN 55282, *n* = 1, stage 44; Bogotá, road to Pacho, ICN 35567, *n* = 24, stages 25-27, ICN 35567a*, *n* = 12, stage 25; Bogotá, antiguo cementerio del sur de Bogotá, ICN 55280*, *n* = 2, stage 26; Fomeque, road La Calera-Fómeque, km 44.5, ICN 32500, *n* = 32, stages 30-42, ICN 32500a, *n* = 2, stages 36-37; Fusagasugá, Agua Bonita, ICN 55307, *n* = 2, stages 25-26; Guasca, ICN 45780*, *n* = 4, stage 26; Junín, Terama, ICN 37077*, *n* = 11, stages 25-26, ICN 37082, *n* = 6, stage 26; La Calera, Páramo de Palacio, ICN 33753, *n* = 25, stages 28-34, ICN 34083, *n* = 3, stages 26-27; Páramo Cruz Verde, ICN 35569, *n* = 33, stages 25-26, ICN 45894, *n* = 4, stages 35-42; Usme, Laguna Chisacá, ICN 45779*, *n* = 7, stage 25; Usme-Bogotá, Páramo de Chisacá, ICN 35598*, *n* = 11, stage 26; **Boyacá,** Aquitania, Páramo de Toquilla, ICN 32497, *n* = 11, stages 26-34; Duitama, Páramo de la Rusia, ICN 55306*, *n* = 11, stage 26, ICN 55308*, *n* = 7, stage 26, ICN 19773, *n* = 9, stages 27-32, ICN 35568, *n* = 7, stage 26; Duitama, road to Peñas Negras, ICN 42727*, *n* = 9, stage 25; Duitama, road to Charalá, ICN 42829, *n* = 4, stages 31-42; Pajarito, farm Comijoque, ICN 20751, *n* = 8, stage 25, ICN 55279, *n* = 28, stage 25-38; Sotaquirá, Callizal, ICN 45565*, *n* = 4, stages 25-26, ICN 45564*, *n* = 14, stages 25-26, ICN 45566, *n* = 17, stages 25-38, ICN 45567, *n* = 12, stages 26-33; **Santander,** Albania, Guacos, ICN 45517, *n* = 10, stage 26-42; Berbeo, ICN 45782, *n* = 2, stages 28-39.

Hyloxalus edwardsi

Colombia: **Cundinamarca,** La Calera, Los Patios, Cueva de las Moyas, ICN 6391, *n* = 2, stage 25.

Hyloxalus pulchellus

Colombia: **Cauca,** Páez, km 35.5 Belarcázar-Tacueyó Road, 2400 m, ICN 6980, *n* = 10, stages 26-31; Puracé, Paletará, 2960 m, ICN 9682, *n* = 6, stages 27-42; Totoró, Totoró-Inzá Road km 7, La Pedrera, 2900 m, ICN 55309*, *n* = 9, stages 25-26. *Ecuador:* **Carchi,** Tulcan, km 16 Tulcan-Tufiño Road, 3130-3160 m, ICN 12137, *n* = 52, stages 25-40.

Rheobates palmatus

Colombia: **Boyacá,** Garagoa, ICN 45801, *n* = 1, stage 43; Miraflores, EL Tunjito, ICN 14359, *n* = 2, stage 28; Moniquirá, ICN 32499, *n* = 2, stage 26-30; Paipa y Duitama, Laguna el Palmar, ICN 9654, *n* = 1, stage 33; Tununga, Santa Rosa, ICN 45516, *n* = 6, stage 26-36; **Cundinamarca,** Albán, Tres Marías, ICN 14368*, *n* = 9, stages 25-26; ICN 14357, *n* = 1, stage 30; ICN 23311, *n* = 101, stages 25-45; ICN 45582, *n* = 1, stage 29; road Bogotá-Fusagasugá, ICN 19770, *n* = 1, stage 26; Sibaté, ICN 35490, *n* = 1, stage 28; **Santander,** Berbeo, ICN 45783, *n* = 30, stages 25-35, ICN 45781, *n* = 4, stages 29-34; Charalá, Virolín, ICN 9721, *n* = 7, stage 25-40; ICN 14366, *n* = 1, stage 27; ICN 14379, *n* = 1, stage 35, ICN 33193, *n* = 5, stage 25-26; ICN 45580, *n* = 1, stage 40; Confines, Oiba-Socorro Road km 11.2, ICN 45562, *n* = 2, stages 26-35; Gámbita, Río Guillermo, ICN 19739, *n* = 1, stage 36; ICN 45707, *n* = 1, stage 26; San Vicente del Chucurí, La Primavera, ICN 55302, *n* = 113, stage 25-46, ICN 55303, *n* = 9, stages 27-36, ICN 55304, *n* = 2, stages 26-27; Toná, Bucaramanga-Pamplona Road km 22, ICN 33447b, *n* = 1, stage 26.