The Stream Tadpoles of *Rhinella rumbolli* (Anura: Bufonidae)

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THE STREAM TADPOLES OF *RHINELLA RUMBOLLI* (ANURA: BUFONIDAE)

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ABSTRACT: The *Rhinella veraguensis* group is likely a paraphyletic assemblage that contains 16 species of Andean toads. To date, descriptions are available for larval stages of only three species, all of which possess a distinct sucker in the abdominal region. In this study, we describe the tadpoles of *Rhinella rumbolli*, a medium-sized Salta Toad typical of forest streams in northwestern Argentina. Thirty-three larvae (Gosner Stages 32–36) were processed for studies of the external morphology, buccal cavity, and musculoskeletal system. These larvae show a mosaic of features, some typical of the genus and some others unique to the *R. veraguensis* group. Several character states are specific to *R. rumbolli*, namely the lack of an abdominal sucker, 2–4 lingual papillae, and the absence of adrostral cartilages. Some traits are frequent in other stream tadpoles such as the muscular tail, large oral disc with complete labial rows, and the wide and robust anterior neurocranium. Bufonid tadpoles exhibit an extraordinary variation in ecology, such as preferred microhabitat, and the diversity within the family is exemplified within the genus *Rhinella*. Further comparative morphological and developmental studies, framed in the context of phylogenetic hypotheses, are needed in order to explore the pattern variation in different clades and to discuss character evolution and form–function relationships.

Key words: Buccal cavity; Lotic tadpoles; Musculoskeletal morphology; *Rhinella veraguensis* group

THE GENUS *Rhinella* is a group of Neotropical bufonids with 86 species distributed from southern Texas (USA) to southern South America. Among these, the 16 taxa tentatively assigned to the *R. veraguensis* group (sensu Duellman and Schulte, 1992) are distributed mostly along the Andes from Peru to northwestern Argentina (with the exception of *R. chrysophora*, endemic to Honduras) and occur across a gradient of 1280–3070 m in altitude (Duellman and Toft, 1979; Duellman and Schulte, 1992; Lehr et al., 2001; Pramuk and Lehr, 2005). However, only five species of this assemblage (*R. amboroensis*, *R. chavin*, *R. manu*, *R. nesiotes*, and *R. veraguensis*), have been included in phylogenetic analyses and the group remains paraphyletic in all hypotheses (Pramuk, 2006; Chaparro et al., 2007; van Bocxlaer et al., 2010; Pyron and Wiens, 2011). Adult toads of these species exhibit a diversity of life histories, with some species mostly aquatic (e.g., *R. amboroensis*), others partially (e.g., *R. chavin* and *R. veraguensis*) or mostly arboreal (e.g., *R. veraguensis* and *R. arborescandens*), and still others mainly terrestrial (e.g., *R. inca* and *R. justinianoi*; Chaparro et al., 2007). Although tadpoles of only three species have been described (*R. chrysophora*, *R. quechua*, and *R. veraguensis*), a distinctive unifying feature for them is the large sucker in the ventral region, akin to those of larval *Atelopus* (McCranie et al., 1989; Cadle and Altig, 1991; Lavilla and de Sá, 2001; Aguayo et al., 2009).

Rhinella rumbolli (Carrizo, 1992) is a medium-sized toad from subtropical forests of northwestern Argentina (Frost, 2013) and likely also Bolivia (De la Riva et al., 2000). It is a largely aquatic inhabitant of forest streams. Adults spend most of the time within the water and vocalize with half the body submerged at the stream margins where the current is slower (Carrizo, 1992). The species was previously confused with *R. quechua* and, hence, assigned to the *R. veraguensis* group, but it has never been included in phylogenetic analyses; therefore its relationships with other *Rhinella* species remain unknown. Here we describe the external, buccopharyngeal, and musculoskeletal morphology of *R. rumbolli* larvae and compare these features with those of other *Rhinella* tadpoles from different intrageneric and ecomorphological groups.

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Fig. 1.—(A) Stream habitat of *Rhinella rumbolli*. (B) Metamorphic specimen with diagnostic features evident. (C) Tadpoles in life; note the bulging regions lateral to the oral disc. Scale = 5 mm. (A color version of this figure is available online.)

Materials and Methods

Specimens

Two series of larvae were collected at the Trigo Pampa River, Ocloyas (Jujuy, Argentina; 23°55′33.2″S, 65°15′13.1″W, datum WGS84; 1500 m above sea level; Fig. 1A) during two field trips in October 2011 and September 2012. Voucher material is deposited at the herpetological collections of Fundación Miguel Lillo, San Miguel de Tucumán, Argentina (FML 25601–2: 11 tadpoles) and the Laboratorio de Genética Evolutiva, Instituto de Biología Subtropical (CONICET-UNaM), Posadas, Argentina (LGE 6253–73: tadpoles and metamorphs, 150 individuals). We assigned the larvae to *R. rumbolli* by rearing some specimens; metamorphs are easily identified because they show some diagnostic features of the species (i.e., well-developed cephalic crests, prominent and ovoid parotoid glands, and distinct coloration; Fig. 1B). Tadpoles were found at the stream margins (where water was flowing slowly) and in pools within the main course, standing close to the sandy bottom or among submerged vegetation, either with or against the current. Larvae of *Rhinella arenarum* and *Hypsiboas riojanus* (Hylidae) were found at the same localities and several *R. rumbolli* adults were also observed under rocks near to the river. Tadpoles were euthanized in the field using tricaine mesylate MS222 and then preserved in 10% formalin. Thirty-three specimens were selected, staged according to Gosner's table (1960; Stages 32–36), and used for descriptions.

External Morphology

Fifteen specimens (Stages 34–36) were used. Descriptions follow those of Altig and McDiarmid (1999), Altig (2007), and Lannoo (1987) for lateral lines. Twenty-five measurements were recorded with digital calipers (±0.1 mm) following Altig and McDiarmid (1999) and Lavilla and Scrocchi (1986): total length (TL), body length (BL), body maximum width (BMW), body width at nares (BWN), body width at eyes (BWE), body maximum height (BMH), tail length (TAL), tail muscle height (TMH), maximum tail height (MTH), nare length (N), extranarial distance (END), internarial distance (IND), eye diameter (E), extraorbital distance (EOD), interorbital distance (IOD), rostro–spiracular distance (RSD), fronto–narial distance (FND), eye–nares distance (EN), oral disc width (OD), dorsal gap length (DG), ventral gap length (VG), upper jaw sheath length (UJL), upper jaw sheath width (UJW), lower jaw sheath length (LJL), and lower jaw sheath width (LJW).
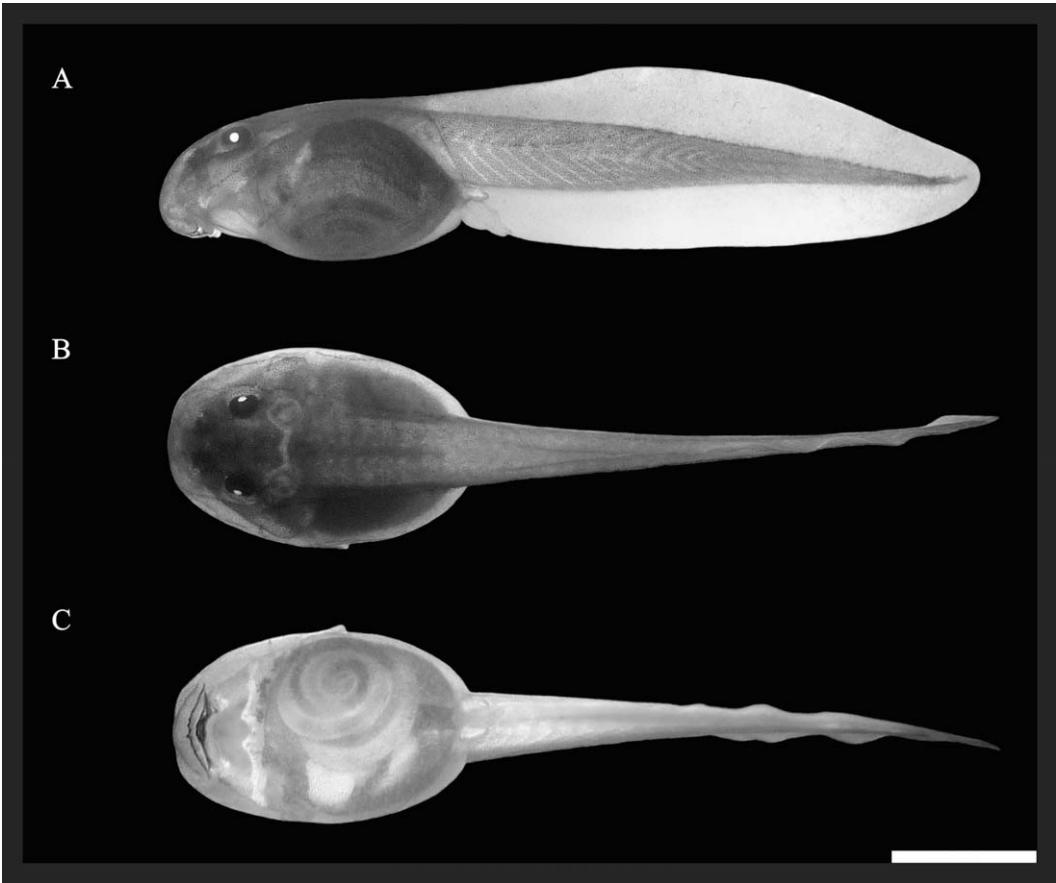
F<small>IG.</small> 2.—External morphology of *Rhinella rumbolli* (Stage 31 larva). (A) Lateral view. (B) Dorsal view. (C) Ventral view. Scale = 5 mm.

Internal Morphology

Twelve specimens (Stages 32–33) were dissected and prepared for anatomical studies. Buccopharyngeal cavity features were recorded after staining with methylene blue (Wassersug, 1976a). For description of the musculoskeletal system, samples were cleared and stained according to Wassersug's protocol (1976b). To preserve the muscles from K(OH) digestion, the procedure was interrupted at that step and Lugol's stain was used (Bock and Shear, 1972). The terminology follows that of Wassersug (1976a) for buccal morphology and that of Haas (2003) for the musculoskeletal system. In addition, six larvae (Stages 34–36) were dehydrated, critical-point dried, and metalized with gold–palladium for observation with scanning electron microscopy (SEM) to observe surface details of the ventral skin, keratinized mouthparts, and buccopharyngeal cavity.

R<small>ESULTS</small>

External Morphology (Figs. 1–4; Table 1)

The body (Fig. 2) is oval in dorsal view and slightly depressed, greater than one third of the total length, and with the maximum width at the level of the otic capsules. The snout is rounded in lateral view and truncate in dorsal view; the ventral body contour is convex at the abdominal region. A central depression delimited by bulbous lateral regions can be observed in the gular–branchial zone and is more evident when the specimen is flat against a surface (Fig. 1C). The eyes are large
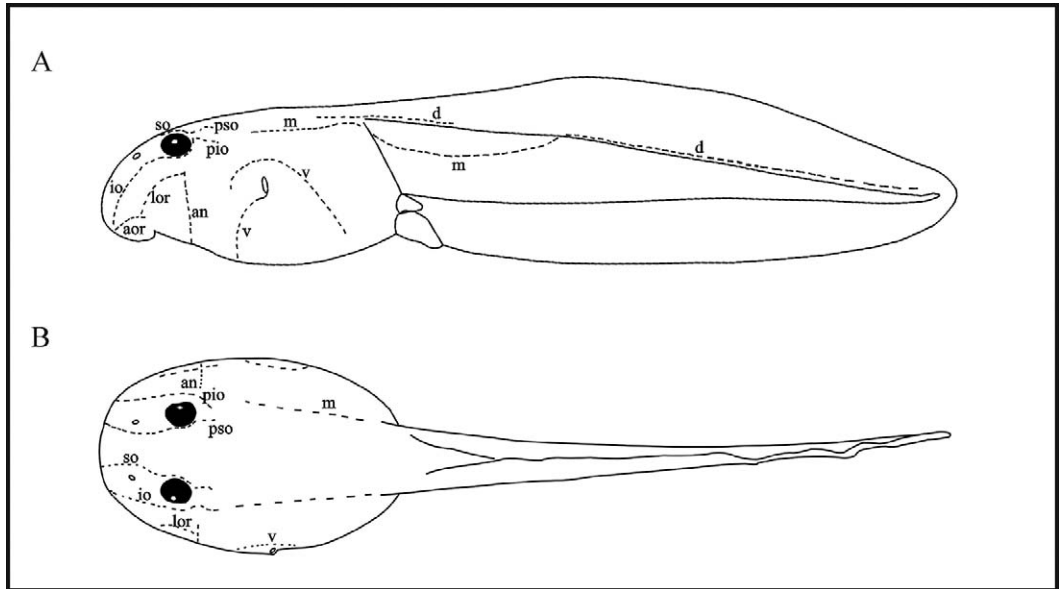
Fig. 3.—Lateral line system in *Rhinella rumbolli* (Stage 31 larva). Abbreviations: an = angular; aor = anterior oral; d = dorsal; io = infraorbital; lor = longitudinal oral; m = medial; pio = postinfraorbital; pso = post-supraorbital; so = supraorbital; v = ventral.

and located dorsolaterally. The nostrils are dorsolateral, rounded, with a slightly elevated rim, and closer to the eyes than to the tip of the snout. The pineal end organ is not evident. The spiracle is sinistral, short, visible dorsally, placed at the middle third of the body and directed posterodorsally, with the inner wall fused to the body wall. The spiracle opening is oval, smaller than that of the tube, and with its major axis oriented dorsoventrally. The vent tube is conical, reaching the margin of the ventral fin and fused to its right side; the vent opening is dextral. The tail is long and with a straight axis. Myomeres are evident through the whole caudal musculature. The dorsal fin originates before the body–tail junction, has its maximum height at the middle region, and then progressively narrows to the tip. The
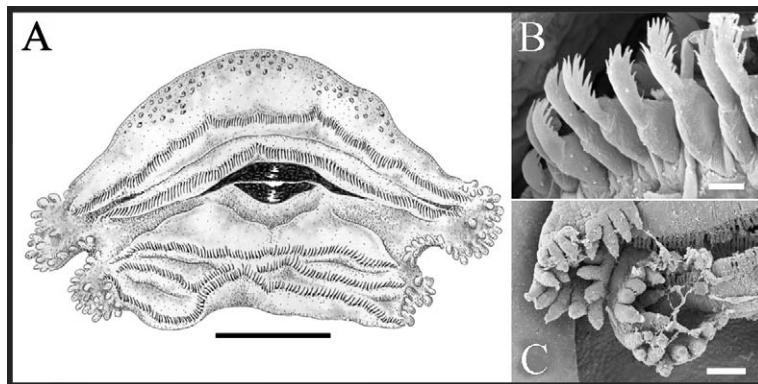

Fig. 4.—Oral disc of *Rhinella rumbolli* (Stage 33 larvae). (A) Ventral view drawing of an open oral disc. (B) Scanning electron microscopy (SEM) micrograph of labial teeth from labial row P1. (C) A SEM micrograph showing detail of marginal papillae of the right commissure. Scale: A = 1 mm; B = 10 μm; C = 100 μm.

TABLE 1.—Measurements (mm) of larval *Rhinella rumbolli*, Gosner Stages 34–36 (*n* = 15).

Morphometric variables	Mean ±1 SD	Ratio	Mean ±1 SD
Total length (TL)	28.77 ± 1.35		
Body length (BL)	10.68 ± 0.49	BL/TL	0.37 ± 0.01
Body maximum width (BMW)	7.09 ± 0.42		
Body width at nares (BWN)	4.52 ± 0.45		
Body width at eyes (BWE)	6.03 ± 0.49	BWE/BWN	1.34 ± 0.06
Body maximum height (BMH)	5.60 ± 0.33	BMH/BMW	0.79 ± 0.02
Tail length (TAL)	18.07 ± 0.99	TAL/TL	0.63 ± 0.01
Tail muscle height (TMH)	2.85 ± 0.25	TMH/BMH	0.51 ± 0.03
Maximum tail height (MTH)	6.30 ± 0.32	MTH/BMH	1.13 ± 0.04
Nare length (N)	0.35 ± 0.09	N/BWN	0.08 ± 0.01
Extranarial distance (END)	2.24 ± 0.14		
Internarial distance (IND)	1.61 ± 0.08	IND/BWN	0.36 ± 0.04
Eye diameter (E)	1.48 ± 0.11	E/BWE	0.25 ± 0.02
Extraorbital distance (EOD)	4.25 ± 0.20		
Interorbital distance (IOD)	2.78 ± 0.14	IOD/BWE	0.46 ± 0.04
Rostro–spiracular distance (RSD)	6.51 ± 0.35	RSD/BL	0.61 ± 0.02
Fronto–narial distance (FND)	0.95 ± 0.16	FND/EN	1.48 ± 0.27
Eye–nare distance (EN)	0.65 ± 0.10		
Oral disc width (OD)	4.09 ± 0.38	OD/BMW	0.58 ± 0.04
Dorsal gap length (DG)	3.24 ± 0.30	DG/OD	0.79 ± 0.04
Ventral gap length (VG)	2.54 ± 0.26	VG/OD	0.62 ± 0.04
Upper jaw sheath length (UJL)	1.52 ± 0.13		
Upper jaw sheath width (UJW)	0.15 ± 0.01		
Lower jaw sheath length (LJL)	1.05 ± 0.12		
Lower jaw sheath width (LJW)	0.08 ± 0.03		

ventral fin is uniformly tall, and its margin runs parallel to the tail axis. The tail tip is rounded. Lateral lines (Fig. 3) are clearly visible and follow the overall pattern described for anuran tadpoles, with some differences. The dorsal line is interrupted approximately at the level of the vent tube. The medial line extends only along the first portion of the tail and, instead of being parallel to the tail axis, it makes a broad ventral curve until it reaches the dorsal line. In some specimens the postinfraorbital and postsupraorbital lines appear to be continuous with the infraorbital and supraorbital lines, respectively. The ventral line is interrupted at the level of the spiracle.

The oral disc (Fig. 4; Table 1) is anteroventral, proportionately large, and emarginate. Marginal papillae are translucent, conical, arranged in a single row, and show wide dorsal and ventral gaps (80% and 60% of the oral disc length, respectively). Submarginal papillae are scarce (4–5). The jaw sheaths are thin and distally serrated; the upper jaw sheath is curved, the lower sheath is smaller and V-shaped. Labial teeth are arranged in two anterior (A1, A2) and three posterior (P1, P2, P3) tooth rows, giving a labial tooth row formula (LTRF) 2/3; tooth rows have similar length, excepting A1 which is slightly longer than the rest. Labial teeth (65–70/mm) are long and curved, with a narrow sheath and body, and a long, oblong head with numerous (10–12) marginal cusps.

Coloration.—In live specimens, the dorsum and lateral sides of the body and tail musculature are uniformly dark brown, with a few small, scattered, golden iridophores. Fins are opalescent, with brown scattered melanophores on the dorsal fin and on the posterior third of the ventral fin in some specimens. The venter is translucent, with brown melanophores and golden iridophores scattered uniformly, except for the gular region. In preserved specimens, the golden iridophores are lost and the coloration is slightly faded.

Buccopharyngeal Cavity (Fig. 5)

On the buccal roof (Fig. 5A), the prenarial arena shows 1–3 papillae. The choanae are large and arranged obliquely at an angle of 47°
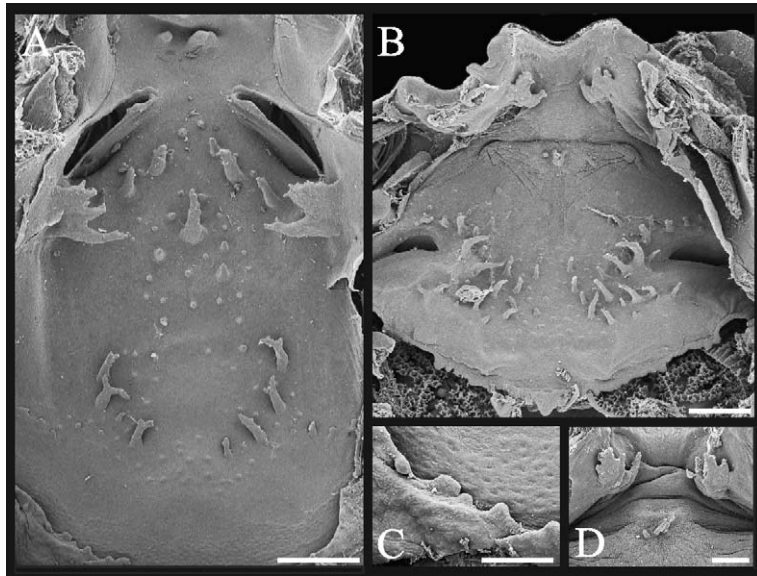
F<small>IG</small>. 5.—Buccal cavity of *Rhinella rumbolli* (Stage 35 larvae). (A) A SEM micrograph of the buccal roof. (B) A SEM micrograph of the buccal floor of the same specimen, showing 4 lingual papillae. (C) Detail of marginal projections and secretory pits of the dorsal velum. (D) Detail of the buccal floor of a second specimen, showing flap-like infralabial papillae and tongue anlage with 3 lingual papillae. Scale: A, B, D = 500 µm; C = 100 µm.

from the transverse line. The anterior margin has small prenarial papillae, and the narial valve is visible. Three paired postnarial papillae are arranged in an inverted V-shape; they are simple and conical, and the medial pair is the largest. Small pustulations are scattered among these papillae. The lateral ridge papillae are well developed, flat, and wide with 2–4 pustulate tips. The papilla-like median ridge is narrow and tall, with a pustulate tip. The buccal roof arena is defined by 3–4 pairs of papillae, often bifurcate. Small pustulations and low papillae are scattered between the median ridge and the buccal roof papillae, among the roof papillae, and posteriorly following the glandular zone. The glandular zone with large secretory pits (15–17/100 µm^2; Fig. 5C) follows a U-shaped arrangement and is posteriorly lined by a short dorsal velum. This is interrupted medially and each half bears marginal papillae that become larger towards the midline.

On the buccal floor (Fig. 5B,D) the two infralabial papillae are flap-like, flat, and wide, with 3–4 round tips oriented anteriorly. The lingual papillae are conical and vary in number from 2 to 4, most frequently 2 (70%

of the specimens examined). The buccal floor arena is delimited on both sides by 8–10 pairs of tall, conical papillae. The papillae nearest the buccal pockets are the largest and often join at their bases; the more-medial papillae follow two lines that converge anteriorly. Several pustulations and low papillae are scattered among the main papillae. Groups of 5–6 papillae diverge laterally along the anterior margin of the buccal pockets, and 5–6 conical papillae diverge laterally, delimiting the anterior edge of the ventral velum. The buccal pockets are elongated and arranged transversely. The ventral velum is semicircular and supported by spicules. Main marginal projections appear on each side, over each filter plate, and at the midline where a median notch is absent; the margin is gently undulated in the remaining regions. Secretory pits occur on the edge of the velum, at a density similar to that of the glandular zone of the buccal roof.

Chondrocranium and Hyobranchial Skeleton (Fig. 6)

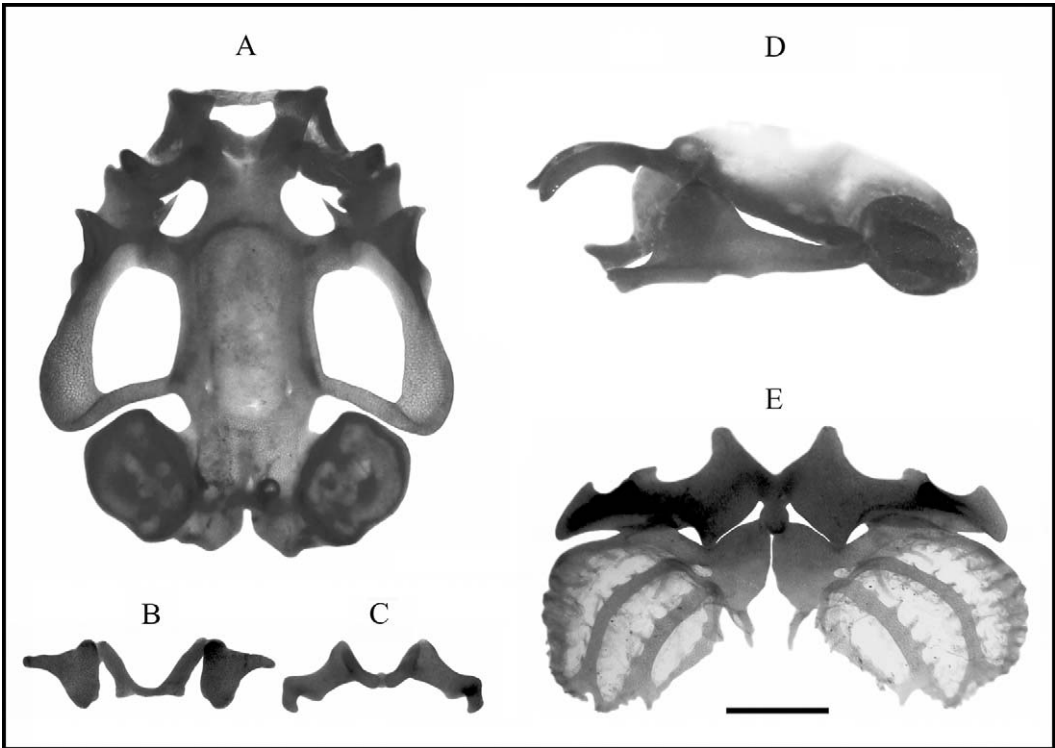The neurocranium is almost as wide as long, dorsoventrally flattened, mostly open dorsally,

Fɪɢ. 6.—Chondrocranium and hyobranchial apparatus of *Rhinella rumbolli* (Stage 35 larva). (A) Chondrocranium, dorsal view. (B) Suprarostral cartilage, frontal view. (C) Lower jaw, ventral view. (D) Chondrocranium, lateral view. (E) Hyobranchial apparatus, dorsal view. Orbital cartilages and taenia tecti are weakly developed in this specimen. Scale = 1 mm.

and heavily chondrified (Fig. 6A). No cranial ossifications are present in the stages analyzed. The suprarostral cartilage (Fig. 6B) has a single, U-shaped medial element dorsally fused to the lateral partes alares; each pars alaris is triangular and bears a well-developed dorsal posterior process. The trabecular horns are proportionately short (about 19% of the chondrocranial length); they are ventrally curved throughout their length, and the anterior margins are slightly expanded in relation to the basal width (about 1.5 times). A lateral trabecular process is present as a small projection on the ventral and outer margin of each horn. In the ethmoid region, there is a short nasal septum and a thin tectum nasi. The lamina orbitonasalis is developed, at the stages analyzed, as a pointed structure directed anterolaterally. From a lateral view, the orbital cartilages are low, with an irregular dorsal margin, and are well chondrified up to

the level of the insertion of the suspensorium (Fig. 6D). The optic and oculomotor foramina are well defined on the posterior ends of the cartilage. The prootic fissure is visible and open dorsally. The large pituitary fenestra is covered by a sheet of chondrified tissue. The foramina carotica primaria and craniopalatina are visible as small, irregular openings at the posterolateral edges of the fenestra, and they are separated from each other by a narrow band of cartilage. The otic capsules are rhomboidal structures that correspond to about 27% of chondrocranial length; the major axis of each capsule is almost parallel to the chondrocranium longitudinal axis. The fenestra ovalis occurs ventrally and the operculum is not yet developed; the jugular and inferior perilymphatic foramina are visible. The otic capsules are fused to the basicranial floor; they connect to each other dorsally by a thin but strongly chondrified

tectum synoticum. The crista parotica is absent. The taeniae tecti marginales are weakly chondrified. In older specimens, a taenia tecti medialis arises from the middle of the tectum synoticum, and a thin taenia tecti transversalis outlines at its anterior edge.

The palatoquadrate diverges anteroposteriorly from the chondrocranium longitudinal axis, describing an angle of about 18°. The anterior end of the palatoquadrate has a short, broad articular process, which articulates with the Meckel's cartilage. The muscular process is wide and rounded. The quadratocranial commissure bears a sharp and triangular quadratoethmoid process. The quadratoorbitalis commissure extends between the tip of the muscular process and the base of the quadratoethmoid process. The pseudopterygoid process is absent. The subocular bar expands posteriorly at the level of the ascending process, and it slightly overpasses the level of the anterior tip of the otic capsule; the posterior region is concave and the posterior margin forms an edge visible in dorsal view. The ascending process is thin and attaches to the chondrocranium at an angle of 70°. It fuses with the trabeculae cranii below the oculomotor foramen to form a low attachment. The larval otic process is absent. The fenestra subocularis extends for about 28% of the chondrocranial length. In the lower jaw (Fig. 6C), Meckel's cartilages are wide and curved. The lateral edge is rounded and bears a rounded retroarticular process that articulates with the articular process of the palatoquadrate. The medial edge has ventromedial and dorsomedial processes. The infrarostral cartilages are paired, curved, and connected medially through a relatively wide and chondrified symphysis. A chondrified intramandibular commissure joins the medial process of Meckel's cartilage with the respective infrarostral cartilage.

In the hyobranchial skeleton (Fig. 6E), a small, thin basihyal is present. The ceratohyals are long and narrow, strongly chondrified, and bear the typical five processes (anterior, anterolateral, lateral, and posterior processes, and the articular condyle). The anterior process is wide, and its rounded tip diverges laterally; the anterolateral process is wide, low, and quadrangular. The pars reuniens is quadrangular and very wide. The basibranchial is narrower than the former, bears a short, square urobranchial process, and fuses to the hypobranchial plates. The hypobranchial plates are well chondrified, flat, wide, and fused medially, leaving a large triangular notch at the posterior region. The four pairs of ceratobranchials are well developed and have lateral projections. Ceratobranchial I is fused to the hypobranchial plate and bears a rounded, medially oriented branchial process. Ceratobranchials II and III are joined to the hypobranchial plate through a cartilaginous bridge; ceratobranchial II has a small branchial process. The fourth ceratobranchial is not attached to the plate. The ceratobranchials are connected posteriorly by well-developed terminal commissures; proximal commissures and branchial bridges between ceratobranchials are absent. Dorsally, four long, curved spicules are present.

Musculature (Fig. 7)

Musculature is similar to that of other bufonids, with some distinctive features. Besides the typical dorsal and ventral mandibular muscles, a poorly developed m. l. m. lateralis and a short m. submentalis are present at the stages analyzed. The mandibular branch of the trigeminal nerve (V_3) runs between the mm. levatores mandibulae externus superficialis and profundus. No changes were apparent among hyoid and hyoangular muscles. In hyobranchial musculature, the m. subarcualis rectus I has three slips inserting on ceratobranchials I, II, and III, respectively. The m. subarcualis rectus II–IV shows variable configurations: in most specimens it has a lateral divergent slip (as in other bufonids); but in some specimens, it is a single muscle inserting on the distal ceratobranchial IV. In some specimens, the muscle is continuous with the middle slip of the m. subarcualis rectus I or with the m. constrictor branchialis II; also, in two specimens we observed a thin slip inserting anteriorly on ceratobranchial I. The mm. levatores arcuum branchialium I and II are wide and very close to each other. The m. rectus abdominis consists of two parallel slips: the medial slip originates almost at the level of the branchial basket, whereas the lateral slip originates at the middle region of
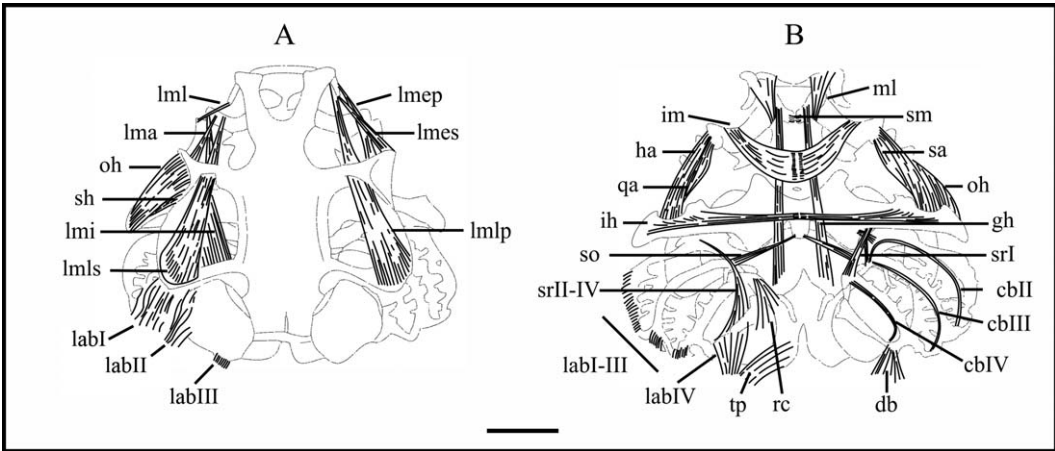
Fig. 7.—Musculature of *Rhinella rumbolli* (Stage 35 larva). (A) Dorsal view. (B) Ventral view. Abbreviations: cbII–IV = constrictor branchialis II–IV; db = diaphragmatobranchialis; gh = geniohyoideus; ha = hyoangularis; ih = interhyoideus; im = intermandibularis; labI–IV = levator arcuum branchialium I–IV; lma = levator mandibulae articularis; lmep = levator mandibulae externus profundus; lmes = levator mandibulae externus superficialis; lmi = levator mandibulae internus; lml = levator mandibulae lateralis; lmlp = levator mandibulae longus profundus; lmls = levator mandibulae longus superficialis; ml = mandibulolabialis; oh = orbitohyoideus; qa = quadratoangularis; rc = rectus cervicis; sa = suspensorioangularis; sh = suspensoriohyoideus; sm = submentalis; so = subcualis obliquus; srI = subarcualis rectus I; srII–IV = subarcualis rectus II–IV; tp = tympanopharyngeus. Scale = 1 mm.

the abdomen. Finally, mm. mandibulolabialis superior, interhyoideus posterior, diaphragmatopraecordialis, constrictor, and dilatator laryngis are absent.

DISCUSSION

Previous studies of larval morphology of species in the *Rhinella veraguensis* group (sensu Duellman and Schulte, 1992) are scarce. Of the 16 species tentatively assigned to this paraphyletic assemblage (i.e., *R. amboroensis*, *R. arborescandens*, *R. chavin*, *R. chrysophora*, *R. fissipes*, *R. gallardoi*, *R. inca*, *R. justinianoi*, *R. manu*, *R. multiverrucosa*, *R. nesiotes*, *R. quechua*, *R. rumbolli*, *R. tacana*, *R. veraguensis*, and *R. yanachaga*; Frost, 2013), only the larval external morphologies of *R. veraguensis* (Cadle and Altig, 1991), *R. chrysophora* (McCranie et al., 1989), and *R. quechua* (Aguayo et al., 2009) are known; data about internal morphology are restricted to these two latter taxa (Lavilla and de Sá, 2001; Aguayo et al., 2009). Our observations show that *R. rumbolli* larvae have features that are common among larvae of most members assigned to this genus, other traits that are shared with members of the *R.*

veraguensis group, and still others that are exclusive to this species.

Among exomorphological characters, the dark body pigmentation and the dorsal fin beginning at the body–tail junction are features typical of *Rhinella* larvae (e.g., Borteiro et al., 2006). On the other hand, the wide and truncate snout in dorsal view is distinctive of the *R. veraguensis* group (Pramuk and Lehr, 2005; Aguayo et al., 2009), although in *R. rumbolli* and *R. quechua* this trait is somewhat attenuated. Likewise, the medial vent tube is the prevalent condition in larvae from this genus (e.g., Cei, 1980; Lavilla et al., 2000; Aguilar and Gamarra, 2004; Pramuk and Lehr, 2005; Borteiro et al., 2006; Ruas et al., 2012) whereas a dextral tube is described in *R. quechua* and *R. veraguensis* (Cadle and Altig, 1991; Aguayo et al., 2009) and in *R. rumbolli* in this study. Echeverría (2004) mentioned that diversity in morphology and position of the vent tube has taxonomic and also, possibly, ecomorphological importance.

Within the genus, the abdominal sucker appears to be a distinct trait of larvae of the *R. veraguensis* group, and it is present with varied extents of development in the three

described species. Tadpoles of *R. veraguensis* have the weakest sucker, with a posterior edge less developed than in *R. chrysophora* (Pramuk and Lehr, 2005) and *R. quechua* (Aguayo et al., 2009). Tadpoles of *R. rumbolli* do not have a differentiated sucker but possess a central depression in the gular–branchial region; thus, the presence of a distinct sucker could be a synapomorphy of a less-inclusive clade within the group. Besides *Rhinella*, an abdominal sucker has been described in three other genera of Bufonidae: *Atelopus*, *Sabahphrynus*, and in *Bufo* species formerly known as *Torrentophryne* (Duellman and Lynch, 1969; Liu et al., 2000; Inger et al., 2001). Features of the *Atelopus* sucker were revisited by Kaplan (1997), who described anatomical and histological differentiation, and suggested that this could collaborate during substrate adhesion. Our SEM observations of the ventral surface of the sucker of *R. quechua* and the gular region of *R. rumbolli* reveal an undifferentiated epidermis. Regarding the ontogenetic development of the sucker, information is meager. In *Atelopus* and *Rana sauteri* (Ranidae) it begins to form between Gosner Stages 23–25 as a small shallow depression that later becomes deeper and surrounded by raised folds (Mebs, 1980; Kuramoto et al., 1984). Studies of this kind are needed to elucidate if the extents of differentiation of the abdominal region, from an undifferentiated gular depression to a proper sucker with associated muscles and ligaments, are part of the same ontogenetic trajectory and if interspecific variation in the final states are a product of evolutionary heterochronic changes.

The oral disc of *Rhinella rumbolli* is similar to those of other tadpoles of the group, a mosaic of features typical of the genus (the ventral gap and the labial tooth row formula with two anterior and three posterior labial rows) and features associated with the *R. veraguensis* group (large oral disc, 60% of the body width, and labial row A2 complete; see revision in Aguayo et al., 2009). The morphology of individual labial teeth is more similar to species of the *R. marina* group; however, with a narrow sheath and long, curved head with multiple cusps along the whole margin (e.g., Vera Candioti and Altig, 2010).

The lateral line system is rarely considered in descriptions (Echeverría and Fiorito de López, 1981; Maciel et al., 2007; Kolenc et al., 2013). In *R. rumbolli* tadpoles it is clearly visible and similar to that described by Lannoo (1987), with the exception of medial and dorsal lines that show an arrangement comparable to that of *Telmatobius atacamensis* and *Ceratophrys cranwelli* (Fabrezi and Quinzio, 2008).

Anatomical traits also show a combination of states typical of the genus, the species group, and exclusive to this species. Recently, Rodrigues de Oliveira et al. (2013) revised the buccal cavity of 12 *Rhinella* species but, unfortunately, no species of the *R. veraguensis* group were included. As compared to species examined in that paper, buccal papillae of *R. rumbolli* and *R. quechua* tadpoles (Aguayo et al., 2009) are generally scarcer, shorter, and simpler. Also, although following an overall similar pattern for the genus, two features are distinctive. The tall, papilla-like median ridges of these two species differ from the trapezoid or triangular ridges described for all other species. The lack of lingual papillae was suggested as a diagnostic trait for tadpoles of the *R. veraguensis* group (observed in *R. veraguensis* and *R. quechua*; Cadle and Altig, 1991; Aguayo et al., 2009) as opposed to the four papillae typical of *Rhinella* (e.g., Vera Candioti, 2007; Rodrigues de Oliveira et al., 2013). The presence of a variable number (2–4) of papillae on the tongue anlage of *R. rumbolli*, however, merits a more-exhaustive revision of this character within the genus, and its evaluation in a phylogenetic context, in order to define at which level the absence of lingual papillae is apomorphic.

Information on chondrocranium anatomy is scarce for species of *Rhinella*, with data available only for *R. arenarum*, *R. schneideri*, *R. marina*, *R. achavali* (*R. marina* group; Kolenc et al., 2013 and references therein), *R. spinulosa* (*R. spinulosa* group; Vera Candioti, 2007), *R. chrysophora*, and *R. quechua* (*R. veraguensis* group; Aguayo et al., 2009 and references therein). A number of features are shared among the studied species, irrespective of the species group and ecomorphological guild to which they are assigned: suprarostral cartilage with a single corpus and lateral alae

dorsally articulated, quadrato-orbital commissure, quadratoethmoid and lateral trabecular processes present, and larval otic process absent (a revision in Aguayo et al., 2009). In addition, the chondrocranium of *R. rumbolli* larvae has several traits that are attenuated compared to those of other larvae of the *R. veraguensis* group. For instance, *R. quechua* has small adrostral cartilages and *R. chrysophora* large and elliptic ones (Aguayo et al., 2009), but only a diffuse, strongly alcianophilic adrostral cartilage is observed in *R. rumbolli*; adrostral cartilages are lacking in species from other species groups. Second, trabecular horn measurements are different among *Rhinella* species and within the *R. veraguensis* group. In *R. arenarum*, *R. achavali*, and *R. spinulosa*, trabecular horns are long relative to the chondrocranium total length (22%–24%) and uniformly wide. Trabecular horns of *R. rumbolli*, like those of *R. quechua*, represent 17%–19% of the chondrocranium length and are distally wider (width at the tip/width at the base $\cong$1.5). The chondrocranium of *R. chrysophora* tadpoles, the most unusual, has horns relatively long and very wide distally (respectively, 25% and about twice as wide distally than at their base; Lavilla and de Sá, 2001). In *R. rumbolli* the articular process is shorter and wider than in species of the *R. marina* group (length/width $\cong$0.5 vs. $\cong$1), with ratios slightly higher than those measured in *R. quechua* and *R. chrysophora* ($\cong$0.4; MBH, personal observation). Finally, the angle that the ascending process describes when attaching to the neurocranium is also variable between tadpoles of the *R. veraguensis* and other groups. These variants result in a posterior curvature of the subocular bar reaching different levels regarding the otic capsules. Thus, the ascending process varies between an orientation almost perpendicular to the longitudinal axis of the chondrocranium (e.g., 80° in *R. arenarum*; MBH, personal observation) and a markedly acute angle (e.g., 45° in *R. chrysophora*; Lavilla and de Sá, 2001). In *R. rumbolli*, values of this angle are larger than those in *R. quechua* (67° vs. 56°; MBH, personal observation). In the hyobranchial skeleton, morphological variations are mainly caused by changes in proportions of the ceratohyal, hypobranchial, and cerato-

branchial areas and in the in-lever arm proportion. These areas in *R. rumbolli* have values similar to those of larvae of the *R. marina* group, but the in-lever arm proportion is comparable to that of *R. quechua* (*R. rumbolli*: in-lever arm proportion = 0.39, ceratobranchial area = 0.54, and ceratohyal area = 0.26 as compared to values of the same traits for *R. arenarum* [0.33, 0.53, 0.27] and *R. quechua* [0.38, 0.44, 0.40]; Vera Candioti, 2007; Aguayo et al., 2009).

Muscular characters are highly conserved within *Rhinella* and Bufonidae in general. Like other species in the family, tadpoles of *R. rumbolli* show an m. subarculis rectus I with three slips, an m. subarcualis rectus II–IV with a divergent slip invading the branchial septum IV, and mm. mandibulolabialis superior, interhyoideus posterior, and diaphragmatopraecordialis lacking (a revision in Haas, 2003). Compared to *R. quechua* larvae, some specimens of *R. rumbolli* are similar in that the entire m. subarcualis rectus II–IV inserts distally on the ceratobranchial IV margin, recalling the disposition of the mm. constrictores branchiales. Conspicuous differences in further aspects of muscular array of these two taxa are a consequence of the presence of the abdominal sucker in *R. quechua*. The mm. diaphragmatobranchialis, rectus cervicis, rectus abdominis, and some fibers of the mm. mandibulolabialis and intermandibularis insert on the sucker whereas in *R. rumbolli* they show their generalized trajectories. Also, thick, paired subhyoid ligaments (Noble, 1929) extend between the ceratohyals and the ventral skin of the sucker in *R. quechua*; these ligaments are distinct and well-developed in *R. rumbolli* and they probably have a similar role in helping to lift the ventral skin. Finally, the configuration of the m. rectus abdominis in *R. rumbolli* tadpoles is similar to that of other *Rhinella* larvae, although fibers are slightly more densely packed. Among other lotic environment genera–species, benthic (e.g., *Telmatobius*), suctorial (e.g., *Ascaphus*, *Boophis*, *Heleophryne*, *Hyloscirtus*), and fossorial tadpoles (e.g., *Leptobrachella mjobergi*) have well-developed mm. rectus abdominis that extend anteriorly, often reaching the mandibular region, possibly pulling the body close to the substrate and assisting in

locomotion (Noble, 1929; Gradwell, 1973; Carr and Altig, 1992; Haas and Richards, 1998; Haas et al., 2006; Vera Candioti, 2008).

Bufonid larvae are an astonishing group for studying patterns of morphological variation and their relation with ecological variables, in particular the type of habitat where the tadpoles develop (revised by van Bocxlaer et al., 2010). The diversity of genera includes terrestrial tadpoles (e.g., *Osornophryne*), micro-waterbody tadpoles (e.g., *Dendrophryniscus*), pond larvae (e.g., *Bufo*), and several guilds of lotic environments such as scarcely modified lotic–benthic (e.g., species of *Melanophryniscus*), suctorial (e.g., *Ansonia*), and gastromyzophorous tadpoles (e.g., *Atelopus*). Interestingly, this diversity is paralleled within the genus *Rhinella*, which includes terrestrial tadpoles (e.g., some *Rhinella* species previously assigned to *Rhamphophryne*), pond-type larvae (e.g., the *R. granulosa* group), micro-waterbody tadpoles (e.g., *R. castaneotica* of the *R. margaritifera* group), lotic–benthic tadpoles from zones of low and median current in streams (e.g., *R. spinulosa* and *R. ornata*), and tadpoles with an abdominal sucker (species within the *R. veraguensis* group). A set of morphological characters varies among these groups of species, from the overall body shape to particular anatomical aspects. Although assigned to an intrageneric group that comprises stream tadpoles (and all of them described so far with an abdominal sucker), *R. rumbolli* larvae are generally placed within a morphological continuum between the features typical of pond tadpoles and the modified body plan of gastromyzophorous tadpoles. As with other reophilous larvae, aspects of their body shape and musculoskeletal structure can relate to specific requirements of living in flowing water such as current avoidance (e.g., depressed body, low fins) or resistance (e.g., long and muscular tail, large oral disc with dense labial teeth in complete rows, anterior chondrocranium robust and wide, with wide trabecular horns and short articular processes, musculature and skeletal parts responsible for the buccal pumping developed; Altig and Johnston, 1989; Haas and Richards, 1998). Patterns of variation among lentic vs. lotic *Rhinella* species can be compared to those exhibited by basal bufonid tadpoles of *Melanophryniscus*. Several traits vary in the same direction (e.g., those describing body shape; Haad et al., 2011), but aspects of the skeleton show some differences. For example, trabecular horns are longer in stream *Melanophryniscus* larvae (DB, personal observation), a pattern inverse to that of *Rhinella*. Further comparative morphological and developmental studies, framed in inclusive phylogenetic hypotheses, are required in order to explore patterns of shape variation in different clades within and outside Bufonidae and to discuss character evolution and form–function relationships.

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