

Larval Anatomy of Andean Toads of the Rhinella spinulosa Group (Anura: Bufonidae)

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Herpetological Monographs

Larval Anatomy of Andean Toads of the Rhinella spinulosa Group (Anura: Bufonidae) --Manuscript Draft--

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Full Title:	Larval Anatomy of Andean Toads of the Rhinella spinulosa Group (Anura: Bufonidae)
Article Type:	Research Article
Keywords:	Buccal cavity; Chondrocranium; Musculature; Tadpoles
Abstract:	The Rhinella spinulosa group is a clade of toads that inhabit the Andes mountains from northern Ecuador to Patagonia. Its taxonomy was recently revised, and in its new arrangement comprises nine species, including Rhinella gallardoi traditionally placed in a different intrageneric group. In this work we studied the larval external and internal morphology in this group, by describing for the first time tadpoles of R. achalensis , R. gallardoi , and R. vellardi , and then summarizing morphological data for R. altiperuviana , R. limensis , R. papillosa , R. spinulosa , and R. trifolium . Although we found no diagnostic larval features for the whole clade, two distinct morphs were identified. Most tadpoles were highly pigmented and slender, and their oral discs showed a long gap in the second labial tooth row; conversely, tadpoles of R. limensis and R. vellardi shared a globose body and a very short gap. Buccal and musculoskeletal features were highly conserved within the group and regarding other Rhinella , and included four lingual papillae, non-keratinized spurs, tripartite suprarostral cartilages, quadratoorbital commissure, and in musculature, m. subarcualis rectus II–IV invading the branchial septum III and laryngeal muscles reduced or absent.
Response to Reviewers:	

1	Larval Anatomy of Andean Toads of the Rhinella spinulosa Group (Anura: Bufonidae)
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21	RRH: VERA CANDIOTI ET ALLARVAL ANATOMY OF RHINELLA SPINULOSA GROUP

22	ABSTRACT: The Rhinella spinulosa group is a clade of toads that inhabit the Andes
23	mountains from northern Ecuador to Patagonia. Its taxonomy was recently revised, and in its new
24	arrangement comprises nine species, including Rhinella gallardoi traditionally placed in a
25	different intrageneric group. In this work we studied the larval external and internal morphology
26	in this group, by describing for the first time tadpoles of <i>R</i> . <i>achalensis</i> , <i>R</i> . <i>gallardoi</i> , and <i>R</i> .
27	vellardi, and then summarizing morphological data for R. altiperuviana, R. limensis, R. papillosa,
28	R. spinulosa, and R. trifolium. Although we found no diagnostic larval features for the whole
29	clade, two distinct morphs were identified. Most tadpoles were highly pigmented and slender,
30	and their oral discs showed a long gap in the second labial tooth row; conversely, tadpoles of R .
31	limensis and R. vellardi shared a globose body and a very short gap. Buccal and musculoskeletal
32	features were highly conserved within the group and regarding other Rhinella, and included four
33	lingual papillae, non-keratinized spurs, tripartite suprarostral cartilages, quadratoorbital
34	commissure, and in musculature, m. subarcualis rectus II-IV invading the branchial septum III
35	and laryngeal muscles reduced or absent.
36	Key words: Buccal cavity; Chondrocranium; Musculature; Tadpoles
37	
38	THE NEOTROPICAL genus of toads Rhinella currently comprises 92 species assigned to
39	seven phenetic groups, the R. acrolopha /R. festae, R. crucifer, R. granulosa, R. margaritifera, R.
40	marina, R. spinulosa, and R. veraguensis groups (Duellman and Schulte 1992; Pramuk 2006;
41	Grant and Bolivar-G 2014; Moravec et al. 2014), some of which are still poorly diagnosed
42	(Pereyra et al. 2015). The Rhinella spinulosa species group includes medium to large-sized toads,
43	very resistant to dryness and altitude (Cei 1960), distributed on both sides of the Andes mountain
44	range and in sub-Andean mountain systems, from northern Ecuador to the Patagonian forests of
45	southern Argentina and Chile, and from sea level to 5000 m asl. These toads inhabit and

reproduce mainly in rivers and streams, oviposit strings of eggs (uniserial, biserial, or multiserial)
in a gelatinous tube (see Pereyra et al. 2015), and have exotrophic larvae, which usually develop
in lotic environments.

49 The taxonomy of the species of the *Rhinella spinulosa* group was highly controversial and 50 different authors disagreed on the specific or subspecific status of some taxa (e.g., Vellard 1959; 51 Cei 1971; Duellman and Schulte 1992; Córdova 1999; Haas 2002). Traditionally, this group was 52 composed of nine species: R. achalensis, R. amabilis, R. arequipensis, R. arunco, R. atacamensis, 53 R. limensis, R. rubropunctata, R. spinulosa (including several subspecies), and R. vellardi (Frost 54 2019). However, the validity of the group was argued following recent molecular phylogenies 55 that place *R. arunco* and *R. atacamensis* outside of the monophyletic group joining the remaining 56 species (e.g., van Bocxlaer et al. 2010; Pyron 2014; Jetz and Pyron 2018). In addition, R. 57 gallardoi is morphologically similar to R. spinulosa but it was not assigned to the R. spinulosa 58 group due the presence of wide frontoparietals typical of species of the R. veraguensis and R. 59 margaritifera groups (Carrizo 1992). Recent molecular evidence (M.O. Pereyra, personal 60 observation) supports a restricted monophyletic R. spinulosa group composed of nine species: 1) 61 four original members, i.e., R. achalensis, R. amabilis, R. limensis, and R. vellardi; 2) R. 62 altiperuviana, R. papillosa, and R. trifolium erected at species level; 3) R. spinulosa sensu stricto, 63 including *R. arequipensis*; and 4) *R. gallardoi* now recovered within this group. Features of larval 64 external morphology are known for most of these species (Fernández 1927; Cei 1980; Sinsch 65 1986; Haas 2002; Angulo and Aguilar 2003; Aguilar and Gamarra 2004; Aguilar et al. 2007a), 66 whereas data on internal morphology were described for only two of them (Aguilar and Pacheco 67 2005; Vera Candioti 2007).

In this work we revised larval external and internal morphology of the *Rhinella spinulosa*group. We first included formal description of the unknown tadpoles of three species *R*.

achalensis, *R. gallardoi*, and *R. vellardi*, and then we compared and summarized morphological
descriptions of five more, clarifying taxonomic and nomenclatorial issues in previous literature.
We finally explored buccal and musculoskeletal features to discuss the scarce information about
larval internal morphology of the *R. spinulosa* group, and compared against what is known in
other species groups in *Rhinella*.

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- 76

MATERIALS AND METHODS

77 We studied 14 lots of tadpoles belonging to eight nominal species (*Rhinella achalensis*, *R*. 78 altiperuviana, R. gallardoi, R. limensis, R. papillosa, R. spinulosa, R. trifolium, and R. vellardi), 79 and two populations from Argentina (Catamarca and Mendoza Provinces, now on Rhinella aff. 80 spinulosa CAT and MZA respectively). We collected tadpoles in several localities of Argentina 81 and Perú; their taxonomic identity was confirmed by the combination of specimens reared to 82 metamorphosis, DNA barcoding (16S gen), and unequivocal geographic distributions. We 83 euthanized larvae with the anaesthetic MS222, and preserved them in 4% formalin. Voucher 84 material is housed at herpetological collections of Laboratorio de Genética Evolutiva (IBS, 85 CONICET - UNAM; LGE), Museo Argentino de Ciencias Naturales (CONICET; MACN), and 86 Museo de Historia Natural (UNMSM; MUSM). Details of each lot are consigned in the 87 Appendix.

We used two to twenty specimens per species (stages 29–38; Gosner 1960) for description and measurements of external morphology with a Leica M205 stereomicroscope. We colored tadpoles with a 1% solution of methylene blue to enhance visualization of some soft tissues (e.g., oral structures, spiracle). Measurements follow those proposed by Lavilla (1983) and Altig and McDiarmid (1999): total length, body length, maximum body width, body width at nares, body width at eyes, maximum body height, tail length, tail muscle height, maximum tail height, naris

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94	length, extranarial distance, internarial distance, eye diameter, extraorbital distance, interorbital
95	distance, rostro-spiracular distance, fronto-narial distance, eye-nares distance, oral disc width,
96	dorsal gap width, and ventral gap width.
97	We dissected one to six specimens per species (stages 31–38) for internal anatomy
98	description. We exposed buccopharyngeal cavity through a longitudinal cut below the tadpole
99	eye, and used methylene blue to stain buccal papillae (Wassersug 1976a). For description of the
100	musculoskeletal system, we used specimens cleared and stained according to Wassersug's
101	protocol (1976b). To preserve the muscles from KOH digestion, we interrupted the procedure
102	before that step and used Lugol's stain (Bock and Shear 1972) to visualize orange muscles
103	against blue cartilages. External morphology terminology follows that of Altig and McDiarmid
104	(1999), Altig (2007), and Lannoo (1987) for lateral lines. Terminology for buccal cavity and
105	musculoskeletal features follow those of Wassersug (1976a) and Haas (2003), respectively.
106	Unless they are not available, we used English names for skeletal structures instead of Latin
107	terms.
108	
109	RESULTS
110	External Morphology
111	Tadpoles of Rhinella achalensis, R. gallardoi, and R. vellardi were fully described in the
112	paragraphs below. Morphological variations in the already described tadpoles of R. limensis
113	(Angulo and Aguilar 2003; Aguilar et al. 2007a; as Bufo limensis), R. papillosa (Cei 1980; as B.
114	spinulosus papillosus), R. spinulosa (Aguilar and Gamarra 2004; as B. arequipensis), R. trifolium
115	(Sinsch 1986; Haas 2002; Aguilar and Gamarra 2004; Aguilar et al. 2007a; as R. spinulosus or R.
116	s. flavolineatus, R. s. trifolium, and R. s. spinulosus), R. altiperuviana and R. aff. spinulosa CAT

117 (Fernández 1927; as *B. spinulosus*), and *R. aff. spinulosa* MZA (Cei 1980; as *B. s. spinulosus*) are
118 summarized next.

119

120 **Rhinella achalensis**.—(Figs. 1A, 2A; n = 8, stages 32–35) Body depressed and oval from 121 dorsal view; maximum width at the otic region. Snout slightly truncate in dorsal view and sloped 122 in lateral view. Ventral body contour with a shallow depression in the gular region and convex in 123 the abdomen. Nostrils large (6.5% of maximum width of body), dorsal, rounded, with an elevated 124 marginal rim and a triangular medial projection; nostrils closer to the tip of the snout than to the 125 eyes. Eyes large (20% of maximum width of body) and dorsolateral. Pineal end organ not visible. 126 Spiracle sinistral and placed at the posterior half of the body, short, conical, posterodorsally 127 oriented; external wall slightly folded dorsal and ventrally, giving an appearance of spiracle free 128 from the body wall, but inner wall absent; small oval opening with its major axis oriented 129 dorsoventrally. Gut switchback point medial or slightly displaced to the left of the abdominal 130 cavity. Vent tube short and cylindrical, directed medially and opened dextral, margin fused to the 131 ventral fin. Tail 1.6 longer than the body, with maximum height near to the distal third; dorsal fin 132 originating at the body-tail junction, with the first portion low and almost parallel to the 133 longitudinal body axis and then curved and taller; ventral fin as tall as the dorsal fin and 134 uniformly high; tail tip widely rounded. Tail as high as the body (ratio between body maximum 135 height and maximum tail height is about 1). Caudal musculature thick, tapering distally 136 without reaching the tail tip. Lateral lines not visible. Oral disc (Fig. 2A) large (43% of maximum 137 width of body), ventral, and emarginated; marginal papillae in a single row, with wide dorsal and 138 ventral gaps (84% and 58% of the oral disc width, respectively); submarginal papillae scarce (1– 139 3) and mainly on the upper commissure; jaw sheaths darkly colored, serrated, and with similar 140 width, the upper sheath gently curved with distal ends oriented posterolaterally, and the lower

141 sheath smaller and V-shaped. Labial tooth row formula 2(2)/3(1); gap in A2 row about 20 teeth 142 wide, and gap in P1 very short, about 4–5 teeth wide; labial teeth are curved, with a wide sheath, 143 scarcely marked neck, and head with 12–14 marginal cusps (Fig. 4A). Coloration: in preserved 144 specimens, dorsum and lateral sides of the body uniformly brown, venter opaque grey and brown 145 with melanophores concentrated on the gular region where the adhesive gland regressed. A dark, 146 straight dorsal line (which in early embryos points out the distribution of cells of the hatching 147 gland) still visible at the analyzed stages, from the nares level to the base of the tail muscles. Tail 148 musculature lighter, lacking melanophores at the muscle-fin junctions; dorsal fin and the 149 posterior third of the ventral with melanophores uniformly distributed, and remaining portions of 150 the ventral fin with disperse melanophores.

151

152 *Rhinella gallardoi*.—(Figs. 1B, 2B; *n* = 10, stages 29–31) Body slightly depressed and 153 oval from dorsal view; maximum width at the otic region. Snout widely truncate in dorsal view 154 and rounded in lateral view. Ventral body contour slightly convex in the abdomen. Nostrils large 155 (7% of maximum width of body), dorsal, rounded, with a slightly elevated rim and a barely 156 outlined medial projection; nostrils closer to the tip of the snout than to the eyes. Eyes large (17% 157 of maximum width of body) and dorsolateral. Pineal end organ not visible. Spiracle sinistral and 158 placed at the middle third of the body, short, conical, posterodorsally oriented; inner wall absent 159 and small oval opening with its major axis oriented dorsoventrally. Gut switchback point medial 160 or slightly displaced to the left of the abdominal cavity. Vent tube short and cylindrical, directed 161 medially and opened dextral, margin fused to the ventral fin. Tail is 1.4 larger than the body, with 162 maximum height near to the distal third; dorsal fin originating at the body-tail junction, with the 163 first portion low and almost parallel to the longitudinal body axis and then curved and taller; 164 ventral fin lower than the dorsal fin and uniformly high; tail tip narrowly rounded. Tail slightly

165 lower than the body (ratio between body maximum height and maximum tail height is about 1.1). 166 Caudal musculature thick, tapering distally without reaching the tail tip. Lateral lines not visible. 167 Oral disc (Fig. 2B) mid-sized (41% of maximum width of body), ventral, scarcely pigmented, 168 and emarginated; marginal papillae in a single row, with wide dorsal and ventral gaps (83% and 169 51% of oral disc width, respectively); submarginal papillae scarce (1-3), on the upper and also 170 frequently on the lower commissure; jaw sheaths darkly colored, serrated, and with similar width, 171 the upper sheath gently curved with distal ends oriented posterolaterally, and the lower sheath 172 smaller and V-shaped. Labial tooth row formula 2(2)/3; gap in A2 row 5-10 teeth wide, P1 and 173 P3 slightly shorter than P2; labial teeth are curved, with a wide sheath, scarcely marked neck, and 174 head with 10-12 marginal cusps (Fig. 4B). Coloration: in preserved specimens, dorsum and 175 lateral sides of the body light brown, venter translucent with a few melanophores scattered on the 176 gular region. A dark, straight dorsal line (which in early embryos points out the distribution of 177 cells of the hatching gland) still visible at the analyzed stages, from the nares level to the otic 178 region. Tail musculature lighter; fins mostly unpigmented, excepting a narrow band at the fin-179 muscle junction.

180

181 **Rhinella vellardi**.—(Figs. 1C, 2C; n = 2, stage 37) Body slightly depressed and oval from 182 dorsal view; maximum width at the otic region. Snout widely truncate in dorsal view and rounded 183 in lateral view. Ventral body contour slightly convex in the abdomen. Nostrils large (5% of 184 maximum width of body), dorsal, rounded, with a slightly elevated rim and a barely outlined 185 medial projection; nostrils closer to the tip of the snout than to the eyes. Eyes large (19% of 186 maximum width of body) and dorsolateral. Pineal end organ not visible. Spiracle sinistral and 187 placed at the middle third of the body, short, conical, posterodorsally oriented; external wall 188 slightly folded dorsal and ventrally, giving an appearance of spiracle free from the body wall, but

189 inner wall absent; small oval opening with its major axis oriented dorsoventrally. Gut switchback 190 point medial or slightly displaced to the left of the abdominal cavity. Vent tube short and 191 cylindrical, directed medially and opened dextral, margin fused to the ventral fin. Tail 1.5 larger 192 than the body, with maximum height at half its length; dorsal fin originating at the body-tail 193 junction, with the first portion low and almost parallel to the longitudinal body axis and then 194 abruptly curved and taller; ventral fin lower than the dorsal fin and uniformly high; tail tip 195 narrowly rounded. Tail taller than the body (ratio between body maximum height and maximum 196 tail height is about 0.8). Caudal musculature thick, tapering distally without reaching the tail tip. 197 Lateral lines not visible. Oral disc (Fig. 2C) mid-sized (37% of the body width), ventral, scarcely 198 pigmented, and emarginated; marginal papillae in a single row, with wide dorsal and ventral gaps 199 (81% and 57% of oral disc width, respectively); submarginal papillae scarce (1–3) on both 200 commissures; jaw sheaths darkly colored, serrated, and with similar width, the upper sheath 201 gently curved with distal ends oriented posterolaterally, and the lower sheath smaller and V-202 shaped. Labial tooth row formula 2(2)/3; gap in A2 row about 10 teeth wide, P3 slightly shorter 203 than P2; labial teeth are curved, with a wide sheath, scarcely marked neck, and head with 12–14 204 marginal cusps (Fig. 4G). Coloration: in preserved specimens, dorsum and lateral sides of the 205 body light brown, venter translucent with a few melanophores scattered on the gular and 206 branchial regions. Dark dorsal line outlined between the nares and the otic region. Tail 207 musculature lighter with several unpigmented spots; fins mostly unpigmented, excepting a 208 narrow band at the fin-muscle junction.

209

Tadpoles of *Rhinella altiperuviana*, *R. limensis*, *R. papillosa*, *R. spinulosa*, *R. trifolium*,
and the two populations of *R.* aff. *spinulosa* were pictured in Figure 3. All tadpoles had labial
tooth row formula (LTRF) 2(2)/3, excepting some specimens of *R. altiperuviana* (3 of 16)

213 tadpoles examined) and R. trifolium (2 of 29) that showed LTRF 2(2)/3(1). Submarginal papillae 214 were absent or scarce (1-3) and frequently arranged only in the upper commissures, excepting R. 215 *limensis* where all specimens analyzed had papillae on both commissures. Main variations 216 included body shape and the length of the gap in row A2. Tadpoles of *R. limensis* had a globose 217 body shape, with high tail fins taller than the body (ratio between body maximum height and 218 maximum tail height about 0.9); the row A2 in these tadpoles showed a very short gap, about 4–5 219 teeth wide. Conversely, tadpoles of R. altiperuviana, R. papillosa, R. spinulosa, R. trifolium, and 220 R. aff. spinulosa were more streamlined with lower fins (ratio between body maximum height 221 and maximum tail height 1–1.26), body highly pigmented, and the gap in A2 was much longer, 222 about 15-30 teeth wide. Labial teeth were similar in all these taxa (Fig. 4). Lateral lines were 223 usually not evident. The exceptions were R. aff. spinulosa MZA, where all lines were visible 224 without staining, R. altiperuviana and R. papillosa with dorsal lines evident but ventral lines 225 diffuse, and some specimens of *R. trifolium* where dorsal and some ventral lines were visible. 226 Lateral lines of R. aff. spinulosa MZA were pictured in Figure 5 and arranged as follows. The 227 dorsal line extended between the midbody, at the level of the origin of the epaxial musculature, 228 and the first third of the dorsal fin. The medial line originated slightly anterior to the dorsal line 229 and ran ventral and parallel through the caudal muscles; it described a wide curve and returned to 230 the dorsal margin of the musculature to almost reach the tip of the tail. The ventral line originated 231 in a medial point in the abdominal region, ran transversely to the side of the body and then directed caudally to end in a region anterior to the limb base; on the left side it was interrupted at 232 233 the spiracle level. The angular line ran transversely between a ventral point next to the 234 peribranchial wall level and a region below the eye. Four lines originated around the oral disc. 235 The longitudinal oral line originated dorsolateral to the disc and ran caudally for a short tract 236 reaching the eye level; in some specimens it joined the angular line. The oral line began lateral to

237	the disc and ran ventral and caudally through the gular region, up to the level of the peribranchial
238	wall; in some specimens this line diverged from the longitudinal oral line. The supraorbital line
239	originated dorsal to the oral disc and ran medial to the nares reaching the posterior margin of the
240	eye. The infraorbital line in turn began dorsolateral to the disc and directed longitudinally below
241	the eye up to its posterior margin. A short postsupraorbital line occurred caudal to the
242	supraorbital line, in some specimens diverging from its posterior edge. Likewise, a very short
243	postinfraorbital line appeared below and posterior to the infraorbital line. Finally, in some
244	specimens an extra line ran longitudinally between the infraorbital and longitudinal oral lines.
245	
246	Buccopharyngeal Cavity
247	The buccal cavity of tadpoles of Rhinella altiperuviana was described by Vera Candioti
248	(2007; as Chaunus spinulosus) and some features of the buccal cavity of R. limensis tadpoles
249	were described by Aguilar and Pacheco (2005; as Bufo limensis). In the paragraphs below we
250	described comparatively features of all tadpoles examined in this work. Figures 6 and 7 showed
251	the general aspects and some morphological variations.
252	On the buccal roof (Figs. 6A, 7A–C), the prenarial arena showed 1–5 pustulations
253	arranged transversely (Rhinella achalensis, R. altiperuviana, R. gallardoi, R. spinulosa, R.
254	trifolium, R. aff. spinulosa, and some specimens of R. limensis) or a transverse ridge (in R.
255	papillosa, R. vellardi, and some specimens of R. limensis). The choanae were large and arranged
256	obliquely at an angle of about 45° from the longitudinal axis; the anterior margin had small
257	prenarial papillae, and the narial valve was smooth and thick. Three to five paired postnarial
258	papillae were arranged in an inverted V-shape; they were simple and conical, and the second pair
259	was usually the largest; small pustulations were scattered among these papillae. The lateral ridge
260	papillae were well developed, flat, wide, and branched with 2-4 pustulate tips. The median ridge

261 was marginally ornamented and varied in shape, including high triangular (in *R. achalensis*, *R.* 262 limensis, R. papillosa, R. trifolium, R. vellardi, and R. aff. spinulosa CAT) and rectangular (in R. 263 altiperuviana, R. gallardoi, R. spinulosa, and R. aff. spinulosa MZA). The buccal roof arena was 264 defined by 3–5 pairs of papillae, conical or bifurcate; a group of 2–3 papillae or small 265 pustulations diverged laterally from the caudal edge of the arena, parallel to the glandular zone in 266 all species but R. gallardoi, R. trifolium, and R. vellardi. Small pustulations and low papillae 267 were scattered on the buccal roof arena and between roof papillae. The glandular zone was 268 evident in all species, with large secretory pits disposed in a wide U-shaped arrangement. The 269 dorsal velum was short, medially interrupted, smooth (in R. achalensis, R. papillosa, R. vellardi, 270 and R. aff. spinulosa MZA) or with small marginal papillae (in R. atiperuviana, R. gallardoi, R. 271 limensis, R. spinulosa, R. trifolium, and R. aff. spinulosa CAT).

272 On the buccal floor (Figs. 6B, 7D–F), non-keratinized spurs occurred at the edges of the 273 lower jaw sheath, with curved, medially oriented tips, sometimes bifurcate (Rhinella achalensis 274 and R. spinulosa). Paired infralabial papillae were placed on the internal surface of Meckel's 275 cartilage and were wide, flap-like, flat, with 3-4 rounded tips oriented anteriorly; in most 276 specimens a few very small pustulations arranged transversely on the internal aspect of the 277 infrarostral cartilages. On the tongue anlage, four lingual papillae were conical (excepting in the 278 examined specimens of R. papillosa, R. trifolium, and R. vellardi where some were bifid) and 279 tall; laterally, tadpoles of R. gallardoi, R. limensis, R. vellardi, and R. aff. spinulosa CAT showed 280 two rounded lingual pads. The buccal floor arena was delimited on both sides by 8–10 pairs of 281 tall, mostly conical papillae; the papillae nearest to the buccal pockets were the largest and often 282 flat and deeply branched. Several pustulations and low papillae were scattered on the arena and 283 among main papillae. Groups of 3-6 prepocket papillae diverged laterally along the anterior 284 margin of the buccal pockets, and caudally, 3–6 small papillae diverged laterally delimiting the

285	anterior edge of the ventral velum. The buccal pockets were elongated and arranged oblique. The
286	ventral velum was semicircular and supported by spicules. Main marginal projections appeared
287	on each side over filter plates, and at the midline where a median notch was absent; the margin
288	was gently undulated in the remaining regions. Secretory pits develop on the ventral margin of
289	the velum, and are mainly noticeable in specimens of R. altiperuviana and the two populations of
290	R. aff. spinulosa.
291	
292	Cranial Skeleton
293	Aspects of larval cranial skeleton were described only in Rhinella altiperuviana (Vera
294	Candioti 2007; as Chaunus spinulosus). In the paragraphs below we summarized morphological
295	variations in all species examined (Figs. 8, 9).
296	The neurocranium was almost as wide as long, dorsoventrally flattened, and mostly open
297	dorsally (Figs. 8A,E, 9A–D). The suprarostral cartilage (Figs. 8B,F, 9E,F) had a single, U-shaped
298	medial element dorsally fused to the lateral alae; corpora cartilages appeared comparatively
299	thinner in Rhinella limensis and R. vellardi. Each ala was triangular and had well-developed
300	dorsal anterior and posterior processes. In some specimens of R. achalensis, R. altiperuviana, R.
301	gallardoi, and R. spinulosa the suprarostrals showed a narrow proximal connection between ala
302	and corpus. The trabecular horns represented about 23% of the chondrocranial length, and they
303	diverged describing an angle of 60° in average; the anterior margins were slightly expanded, and
304	a small lateral trabecular process projected on the ventrolateral margin in all specimens except for
305	R. vellardi. In the ethmoid region, the nasal septum and lamina orbitonasales were outlined in all
306	species excepting R. limensis. The orbital cartilages were weak and low with an irregular dorsal
307	margin. The taeniae tecti marginales were slightly chondrified and did not reach the anterior
308	margin of the otic capsules. The optic and oculomotor foramina were defined on the posterior

309 ends of the cartilage, and the prootic fissure was visible and open dorsally. The large pituitary 310 fenestra was covered by a sheet of chondrified tissue but the carotid foramen was not always 311 completely encircled by cartilage (e.g., in specimens of *R. gallardoi*). The otic capsules were 312 rhomboidal structures that corresponded to about to 27% the of chondrocranial length; the major 313 axis of each capsule was almost parallel to the chondrocranium longitudinal axis. The fenestra 314 ovalis occurred ventrally and the operculum was not yet developed; the jugular and inferior 315 perilymphatic foramina were visible. The otic capsules were fused to the basicranial floor; they 316 connected to each other dorsally by a thin chondrified tectum synoticum; they showed a thin 317 crista parotica in all species, but a small and pointed anterolateral process was evident only in R. 318 achalensis, R. altiperuviana, R. spinulosa, and R. trifolium.

319 The palatoquadrate arranged slightly divergent anteroposteriorly from the 320 chondrocranium longitudinal axis. The articular process was long and wider than the rest of the 321 cartilage; the anterior margin, which articulated with the Meckel's cartilage, was smooth, and a 322 long, thin quadratoethmoid process projected from the medial margin. The muscular process was 323 wide and rounded, and a thick quadratoorbitalis commissure extended between its tip and the 324 base of the quadratoethmoid process. The pseudopterygoid process was absent. The subocular bar 325 expanded posteriorly at the level of the ascending process; the posterior region was concave, with 326 a posterolateral margin angular (more evident in *Rhinella achalensis*, *R. altiperuviana*, *R.* 327 spinulosa, and R. trifolium) or rounded (as in R. gallardoi and R. limensis), and it showed a thick, 328 dorsal edge that was continuous with the ascending process; a pointed otic process was evident at 329 the posterolateral corner in R. achalensis, R. spinulosa, and R. trifolium. The ascending process 330 was thin and attached almost perpendicularly to the chondrocranium in an intermediate 331 attachment. The larval otic process was absent (excepting a unilateral process in the analyzed 332 specimen of *R. vellardi*). The fenestra subocularis extended for about 27% of the chondrocranial

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333	length. In the lower jaw (Fig. 8C,G), Meckel's cartilages were slightly curved, with a dorsolateral
334	edge rounded and a robust retroarticular process; the medial edge had ventromedial and
335	dorsomedial processes. The infrarostral cartilages were paired, thin, and connected medially
336	through an alcianophilic symphysis.
337	In the hyobranchial skeleton (Fig. 8D,H), the ceratohyals were strongly chondrified and
338	had five tall, robust processes. The anterior processes were triangular and oriented slightly lateral;
339	the anterolateral processes were wider, lower, and oriented medially. The posterior processes had
340	a wide base, and their tips diverge slightly lateral contacting the hypobranchial plate. On the
341	lateral edge of the ceratohyals, small lateral processes projected from the anterior margin, and
342	stout articular condyles were seen on the dorsal surface. The pars reuniens joining the ceratohyals
343	was quadrangular, wide, and formed of a different type of cartilage (Fig. 9G); the basibranchial
344	was narrower and shorter, fused to the hypobranchial plate, and had a long, square urobranchial
345	process. The basihyal was absent. The hypobranchial plates were flat and articulate medially
346	leaving a large triangular notch at the posterior region. The four pairs of ceratobranchials were
347	long, thin, and curved, with numerous marginal projections. Ceratobranchials I and II were fused
348	to the hypobranchial plate and had branchial processes; in some specimens of Rhinella achalensis
349	a branchial process III was outlined. In most specimens (excepting R. trifolium) ceratobranchial
350	III articulated with the hypobranchial plate through a syndesmotic union. Ceratobranchial IV was
351	not attached to the plate. The ceratobranchials were connected posteriorly by thin terminal
352	commissures; proximal commissures and branchial bridges between ceratobranchials were
353	absent. Dorsally, four long, curved spicules were present, but the fourth was usually very weak
354	and not connected to the ceratobranchial IV; in R. altiperuviana and R. trifolium there was a
355	cartilaginous bridge joining spicules III and IV (Fig. 9H).

No cranial ossifications were present at the stages analyzed in most species, excepting
frontoparietals, parasphenoid, and exoccipitals that occur in individuals of *Rhinella spinulosa*(Fig. 9A). Subhyoid ligament was evident in all specimens, on the ventral surface of the
ceratohyals (Fig. 10B).

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Musculature

362 Larval musculature was previously described for Rhinella altiperuviana (Vera Candioti 363 2007; as Chaunus spinulosus). Muscle configuration was almost identical in all species analyzed, 364 and followed the same pattern already known in other bufonids. Typical features were the m. 365 subarcualis rectus I with three slips, the m. subarcualis rectus II-IV with fibers invading the third 366 branchial septum, the presence of all seven mm. levatores mandibulae, and the absence of mm. 367 mandibulolabialis superior, interhyoideus posterior, diaphragmatopraecordialis, and constrictor 368 and dilatator larvngis. Table 2 summarized descriptions of the 31 muscles as seen in R. 369 achalensis, and Figure 10 illustrated some distinct features in the genus. Minor variations in other 370 species were commented in the lines following. Medial and ventral slips of the m. srI were not 371 well defined in *R. limensis* and *R. trifolium*. The lateral fibers of the m. srII-IV ran for half the 372 ceratobranchial IV length in R. limensis. In R. papillosa specimens, some caudal fibers of the m. 373 subarcualis obliquus inserted on ceratobranchial III. Subhyoid ligament was not well-developed 374 in *R. limensis*, and fibers of the m. rectus abdominis did not reach it. Finally, a very poorly 375 developed, almost indistinguishable m. dilatator laryngis appeared in one specimen of R. 376 trifolium.

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DISCUSSION

379 Comparative studies have coincided when highlighting the conservative larval 380 morphology in bufonids (e.g., Haas 2003). Within *Rhinella*, excepting some clades with distinct 381 features (such as the *R. veraguensis* and *R. granulosa* groups; Blotto et al. 2014; Haad et al. 2014; 382 Grosso et al., in press), the intrageneric groups apparently lack diagnostic larval traits. In our 383 study considering almost all tadpoles now assigned to the *Rhinella spinulosa* group, we found no 384 diagnostic features for the clade, but two distinct morphs among species. Most tadpoles are 385 highly pigmented and slender, with low tail fins lower or as high as the body, and their oral 386 morphology shows a long gap in the second anterior labial tooth row. This morphology is typical 387 of tadpoles of R. achalensis, R. altiperuviana, R. papillosa, R. spinulosa, R. trifolium, and the two 388 populations of R. aff. spinulosa. Conversely, tadpoles of R. limensis and R. vellardi share a 389 globose body morphology, high tail fins and a very short gap in tooth row A2. In turn, tadpoles of 390 *R. gallardoi* have a combination of streamlined body and A2 gap with intermediate length. The 391 original assigning of this species to the R. veraguensis group (Carrizo 1992) was not supported 392 by larval morphology, since known tadpoles of this group show a set of distinct external and 393 internal features (e.g., undivided row A2, gular depression or abdominal sucker, adrostral 394 cartilages; Aguayo et al. 2009; Haad et al. 2014), none of which are present in R. gallardoi. 395 While the distinction of two morphs in body shape within the *Rhinella spinulosa* gr. was 396 not discussed in previous literature, variations in the length of the gap in A2 row were already 397 noticed by Aguilar and collaborators when comparing tadpoles of *R. limensis* with other Peruvian 398 larvae (Angulo and Aguilar 2003; Aguilar and Gamarra 2004; Aguilar et al. 2007a). Interpreting 399 these two characters and their relationship along the phylogenetic hypothesis for the *R. spinulosa* 400 gr. (M. O. Pereyra, personal observation) shows that the globose morph could define the clade 401 composed of *R. limensis* and *R. vellardi*, and that the slender morph characterizes the large clade

402 including all the remaining species. A long gap in row A2 would have evolved within this latter,403 in the clade joining all species but *R. gallardoi*.

404 The oral discs vary slightly in other features as well. These variations are not 405 unambiguously related to the said morphs, and should be checked in wider samples to interpret 406 their distribution. For instance, a small gap in row P1 appeared in 20% of the analyzed specimens 407 of Rhinella altiperuviana, and was reported in tadpoles of R. trifolium (Sinsch 1986), 20% of R. 408 limensis studied by Angulo and Aguilar (2003), and all R. limensis studied by Aguilar and 409 Gamarra (2004). In addition, submarginal papillae were scarce and mostly on the upper 410 commissure in tadpoles with slender morphology, whereas they occurred also in the lower 411 commissure in all the examined globose tadpoles (see also Angulo and Aguilar 2003; Aguilar et 412 al. 2007a) and in some specimens of R. aff. spinulosa (Fernández 1927; Cei 1980). Larval 413 measurements were very similar interspecifically, excepting the aforementioned differences in 414 tail and body heights, and an outlined discrimination in naris relative size and position (slightly 415 larger, caudal, and more distant to each other in slender tadpoles). 416 The ecological significance of variations in traits discussed, if any, should be further 417 explored. On one hand, a streamlined body shape is frequent in anuran larvae from lotic 418 environments (e.g., Altig and McDiarmid 1999), including related (e.g., species of the R. 419 veraguensis group, Nannophryne cophotis; Aguilar and Gamarra 2004; Aguayo et al. 2009) and 420 unrelated Andean tadpoles (e.g., Telmatobius atahualpai; Aguilar et al. 2007b). However, all 421 tadpoles of R. spinulosa group, irrespective of their morphotype, develop in similar water bodies, 422 in general shallow pools and slow-moving rivulets at margins of streams. Additionally, tadpoles

423 with the globose morph inhabit different environments, with *R. limensis* restricted to Peruvian

424 coastal deserts and *R. vellardi* distributed in dry forests at higher elevations (Cei 1972). In this

425 scenario, it seems likely that if an ecomorphological correlation between body shape and type of

426 environment occurred, it was early during the evolution of the *R. spinulosa* group, and later 427 diversification allowed for different phenotypes to appear and persist in those same 428 environments. On the other hand, the loss of labial teeth (naturally occurring, in tadpoles affected 429 by pathogens, or after surgery removal) has functional consequences in oral disc kinematics 430 compromising feeding efficiency (Venesky et al. 2010a,b,c). While the differences in length of 431 the gaps in the A2 tooth row of slender vs. globose tadpoles would appear to be too subtle, slight 432 variations in the anchoring of tooth ridges to substrates during foraging cannot be ruled out. 433 Available information on internal morphology of tadpoles of the Rhinella spinulosa group 434 is scarce. Some buccopharyngeal traits were mentioned in *R. limensis* (Aguilar and Pacheco 435 2005), and morphology of the buccal cavity and musculoskeletal system were described in larvae 436 of R. altiperuviana (Vera Candioti 2007). Rodrigues de Oliveira et al. (2013) studied 437 comparatively buccal features of 12 Rhinella species of several intrageneric groups, and their 438 data allowed for identifying general aspects and some interspecific comparisons. Tadpoles of the 439 Rhinella spinulosa group share with most congeneric species the ornamentation of the prenarial 440 arena, a single pair of infralabial papillae, and four lingual papillae. As noted by Rodrigues de 441 Oliveira et al. (2013), in general infralabial papillae are branched in a distinct way, so that a deep, 442 U-shaped notch bifurcates each papilla leaving uneven halves with secondary projections. In 443 addition, in all tadpoles studied here the skin that covers the distal edges of the infrarostral 444 cartilages folds in a particular manner defining a pair of pointed, curved projections medially 445 oriented. These projections were referred as non-keratinized spurs by Vera Candioti (2007) and 446 Kolenc et al. (2013) in some *Rhinella* tadpoles, but were not mentioned in descriptions of larvae 447 of the *R. veraguensis* group (Aguayo et al. 2009; Haad et al. 2014). A re-examination of *R.* 448 quechua and R. rumbolli tadpoles revealed very small projections, and scanning electronic 449 micrographs of *R. abei*, *R. icterica*, and *R. ornata* in Rodrigues de Oliveira et al. (2013) also

450	showed them, suggesting that this is a feature universally present in the genus. Unlike in other
451	clades (hylid Scinax, ranid Hoplobatrachus; Grosjean et al. 2004; Alcalde et al. 2011), the spurs
452	are never keratinized in Rhinella tadpoles.
453	Variable buccal features in the genus include the presence and number of infrarostral
454	ornamentation, shape of lingual papillae, extent of papillation of buccal arenas, shape of the
455	median ridge, and configuration of the dorsal and ventral vela (see Tables in Rodrigues de
456	Oliveira et al. 2013). Lingual papillae are in general simple and conical, but Y-shaped papillae
457	appear in tadpoles of Rhinella icterica, R. ornata, R. pombali, R. diptycha, two species of the R.
458	granulosa group (Rodrigues de Oliveira et al. 2013), and specimens of R. papillosa and R.
459	vellardi here studied. Finally, most tadpoles of the R. spinulosa group (excepting the specimens
460	of R. gallardoi, R. trifolium, and R. vellardi) have a line of buccal roof arena papillae or
461	pustulations that diverge laterally parallel to the anterior margin of the glandular zone. These are
462	evident in other Rhinella tadpoles (e.g., R. abei, R. crucifer, R. icterica), but further observations
463	with wider samples would confirm whether they are not intraspecifically variable.
464	With the exception of larvae of the Rhinella veraguensis group that show several distinct
465	features (e.g., adrostral cartilages, oblique ascending processes, short and wide articular
466	processes; revised in Haad et al. 2014), the general aspect of the chondrocrania and hyobranchial
467	skeleton is similar among Rhinella tadpoles (e.g., Vera Candioti 2007; Kolenc et al. 2013;
468	Rodrigues de Oliveira et al. 2014). Shared features are the tripartite suprarostral cartilages,
469	quadratoethmoid and lateral trabecular processes present, perpendicular ascending processes,
470	quadratoorbital commissures present, and larval otic process absent. Slight variations, apparently
471	not related to intrageneric groups, are the presence of anterolateral processes of the crista parotica
472	(in R. diptycha, R. granulosa, and within the R. spinulosa group, in R. achalensis, R.
473	altiperuviana, R. spinulosa, and R. trifolium), cartilaginous bridges between suprarostral corpus

474 and alae (in *R. achalensis*, *R. altiperuviana*, *R. gallardoi*, and *R. spinulosa*), and small 475 palatoquadrate otic processes (in *R. achalensis*, *R. spinulosa*, and *R. trifolium*). Likewise, 476 muscular characters are highly conserved in *Rhinella* and bufonids in general, and the two 477 synapomorphies recovered by Haas (2003) for the family (i.e., m. diaphragmatopraecordialis 478 absent and m. subarcualis rectus II-IV with a lateral, diverging slip) occur in all species of the R. 479 spinulosa group. Laryngeal muscles are absent in almost all tadpoles examined here. Although 480 the distribution of these muscles was not analyzed in a phylogenetic context, data on other genera 481 (e.g., Melanophryniscus; Baldo et al. 2014) suggested a widespread absence or deep reduction of 482 mm. constrictor et dilatator larynges that could be related to the general reduction and late 483 development of lungs in bufonid tadpoles (Haas 2003). Finally, the configuration of the m. rectus 484 abdominis, with medial slips reaching or overpassing the branchial region, seems to be similar in 485 Rhinella tadpoles described; some differences in myotome thickness and packing of fibers could 486 be revealed after detailed quantification, at least regarding tadpoles of the R. granulosa group (F. 487 Vera Candioti, personal observation).

Finally, three species formerly assigned to the *Rhinella spinulosa* group, *R. arunco*, *R. atacamensis*, and *R. rubropunctata*, were excluded from the group (M. O. Pereyra, personal observation). Larval external morphology in all of them shows features that are similar to those of the *R. gallardoi* + *R. spinulosa* clade (i.e., a rather slender body and A2 gap mid-sized to long; Cei 1962; Formas and Pugín 1978), but a thorough examination and coding in these and other related *Rhinella* would be required to assess phylogenetic status for these features.

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624	Appendix
625	Specimens Examined
626	Rhinella achalensis.—ARGENTINA: CÓRDOBA: Departamento San Alberto: Pampa de
627	Achala, Puesto Cuello near to El Volcán, LGE 04235, $n = 14$, stages 32–36. Skeleton (6, 35–36);
628	muscles (1, 34); buccal cavity (1, 34).
629	Rhinella altiperuviana.—ARGENTINA: JUJUY: Departamento Humahuaca: Chucalezna,
630	Río Grande, Quebrada de Humahuaca, LGE 03871 and Departamento Susques: Susques, Salar de
631	Olaroz, LGE 22111, $n = 16$, stages 35–38. Skeleton (4, 35); muscles (2, 35 and 36); buccal cavity
632	(1, 37).
633	Rhinella gallardoi.—ARGENTINA: JUJUY: Departamento Doctor Manuel Belgrano:
634	Tilquiza, LGE 09887, $n = 11$, stages 29–31. Skeleton (2, 31); muscles (1, 31); buccal cavity (1,
635	31).
636	Rhinella limensis.—PERÚ: LA LIBERTAD: Cajabamba, Sanagorán, LGE 22101 and LIMA:
637	Provincia Huarochirí: Barba Blanca, LGE 22102, $n = 5$, stages 30–38. Skeleton (1, 33); muscles
638	(2, 33 and 35); buccal cavity (1, 33).
639	Rhinella papillosa.—ARGENTINA: NEUQUÉN : Departamento Minas: Camalón, MACN
640	50401, <i>n</i> = 17, stages 30–31. Skeleton (2, 31); muscles (1, 31); buccal cavity (1, 31).
641	Rhinella spinulosa.—PERÚ: AREQUIPA: Provincia Arequipa: Sabandía, LGE 22103, n =
642	2, stages 37. Skeleton (1); muscles (2); buccal cavity (1).
643	<i>Rhinella trifolium.</i> —PERÚ: PASCO: Paucartambo, MUSM 17815–6, LGE 22104, $n = 29$,
644	stages 30–34. Skeleton (2, 33); muscles (2, 34); buccal cavity (2, 34).
645	Rhinella vellardi.—PERÚ: AMAZONAS: Provincia Chachapoyas: Balsas, Quebrada
646	Honda, LGE 22105, $n = 2$, stages 37. Skeleton (1); muscles (1); buccal cavity (1).

- 647 *Rhinella* aff. *spinulosa*.—ARGENTINA: CATAMARCA: Departamento Andalgalá:
- 648 Capillitas, LGE 22112, n = 7, stages 31–38. Skeleton (1, 37); muscles (1, 37–38); buccal cavity
- 649 (1, 37); MENDOZA: Departamento Malargüe: Ruta Provincial N° 226, on the way to Paso
- 650 Vergara, margins of the Río Valenzuela, LGE 17106, n = 6, stages 35–38. Skeleton (2, 35);
- 651 muscles (1, 37); buccal cavity (1, 37).

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- 652 TABLE 1.—External morphology measurements, presented as average (standard deviation), in species of the *Rhinella spinulosa* group.
- 653 The two last columns correspond to populations of *R*. aff. *spinulosa* from Argentinean Provinces Catamarca and Mendoza (CAT and
- 654 MZA respectively).

	R. achalensis	R. altiperuviar	ıaR. gallardoi	R. limensis	R. papillosa	R. spinulosa	R. trifolium	R. vellardi	R. CAT	R. MZA
	<i>n</i> = 8	<i>n</i> = 10	<i>n</i> = 10	<i>n</i> = 3	<i>n</i> = 10	n = 1	<i>n</i> = 10	<i>n</i> = 1	n = 7	<i>n</i> = 7
	stages 32-33	stages 36-37	stages 30-37	stages 30-37	stages 30-31	stage 36	stages 32-34	stage 36	stages 30-32	stages 35-37
Snout vent length	8.80 (0.39)	13.86 (0.78)	9.92 (1.03)	12.86 (0.80)	8.94 (0.29)	11.89	9.14 (0.71)	11.49	8.65 (0.47)	12.27 (1.70)
Maximum body height	3.98 (0.37)	6.60 (0.42)	4.66 (0.79)	6.55 (0.77)	4.69 (0.20)	5.82	4.36 (0.33)	5.23	4.41 (0.22)	5.84 (0.84)
Maximum body width	5.07 (0.41)	8.73 (0.74)	5.70 (0.92)	8.32 (0.54)	5.53 (0.22)	7.67	5.67 (0.35)	6.94	4.95 (0.22)	7.37 (1.05)
Body width at eyes	4.37 (0.24)	6.52 (0.47)	4.67 (0.60)	6.69 (0.29)	4.50 (0.17)	6.06	4.63 (0.30)	5.89	4.13 (0.30)	5.67 (0.77)
Body width at nares	3.01 (0.17)	4.66 (0.36)	3.25 (0.39)	4.60 (0.20)	3.18 (0.15)	4.29	2.91 (0.23)	3.89	2.80 (0.22)	4.08 (0.57)
Dorsal gap width	1.83 (0.12)	2.42 (0.16)	1.92 (0.13)	2.15 (0.10)	1.74 (0.07)	2.09	1.6 (0,11)	2.08	1.73 (0.16)	2.30 (0.26)
Eye diameter	1.01 (0.06)	1.28 (0.17)	0.95 (0.22)	1.33 (0.13)	0.91 (0.04)	1.20	1.06 (0.11)	1.35	0.92 (0.08)	1.21 (0.17)
Eye-nares distance	1.69 (0.08)	2.54 (0.14)	1.88 (0.12)	2.54 (0.21)	1.66 (0.09)	2.39	2.12 (0.10)	1.79	1.84 (0.13)	2.45 (1.00)
Extranarial distance	0.87 (0.05)	1.55 (0.14)	1.16 (0.32)	1.37 (0.06)	0.93 (0.06)	1.15	1.00 (0.10)	0.96	0.95 (0.06)	1.34 (0.23)
Extraorbital distance	3.09 (0.10)	4.46 (0.26)	2.88 (0.57)	4.52 (0.48)	2.90 (0.09)	3.90	3.33 (0,25)	3.80	2.92 (0.35)	4.09 (0.59)
Fronto-narial distance	0.57 (0.10)	1.01 (0.19)	0.70 (0.17)	0.84 (0.04)	0.83 (0.12)	0.86	0.72 (0,10)	0.65	0.58 (0.09)	1.19 (0.26)
Internarial distance	1.20 (0.09)	1.66 (0.13)	1.36 (0.07)	1.97 (0.17)	1.14 (0.04)	1.52	1.48 (0.08)	1.16	1.21 (0.13)	1.83 (0.74)
Interorbital distance	1.89 (0.26)	3.39 (0.21)	1.72 (0.55)	3.43 (0.28)	1.92 (0.13)	2.99	2.08 (0.14)	1.90	1.94 (0.18)	3.00 (0.40)

Maximum tail height	4.04 (0.30)	5.32 (0.35)	4.27 (0.70)	6.74 (0.70)	4.00 (0.16)	5.74	4.33 (0.25)	6.19	3.49 (0.28)	5.47 (0.79)
Naris length	0.33 (0.10)	0.50 (0.09)	0.38 (0.09)	0.50 (0.09)	0.37 (0.03)	0.59	0.5 (0.06)	0.35	0.44 (0.06)	0.55 (0.40)
Oral disc width	2.17 (0.12)	2.89 (0.20)	2.32 (0.17)	2.74 (0.27)	1.91 (0.08)	2.54	2.04 (0.09)	2.56	2.09 (0.16)	2.71 (0.19)
Rostro-spiracular distance	5.40 (0.19)	7.14 (0.62)	5.22 (0.65)	7.18 (0.64)	4.81 (0.31)	6.51	5.31 (0.48)	6.31	5.17 (0.27)	6.74 (0.93)
Tail length	14.14 (0.42)	21.88 (1.24)	15.14 (1.12)	20.10 (5.15)	12.38 (0.51)	18.37	14.84 (1.36)	16.98	13.74 (1.33)	19.00 (2.72)
Total length	22.94 (0.53)	35.85 (1.83)	24.02 (3.62)	26.26 (11.94)	20.44 (2.69)	30.25	23.98 (1.98)	30.25	20.13 (3.72)	28.91 (4.69)
Tail muscle height	1.65 (0.10)	2.62 (0.18)	2.07 (0.14)	3.07 (0.41)	1.57 (0.11)	2.86	1.84 (0.13)	2.11	1.91 (0.11)	2.47 (0.47)
Ventral gap width	1.27 (0.12)	1.62 (0.25)	1.19 (0.16)	1.60 (0.14)	1.01 (0.06)	1.12	0.95 (0.09)	1.45	1.22 (0.16)	1.75 (0.26)

655

Muscle	Attachments
Levator mandibulae longus superficialis	posterolateral margin of subocular bar – dorsomedial
	edge of Meckel's cartilage
Levator mandibulae longus profundus	posterolateral margin of subocular bar, ventral to
	levator mandibulae longus superficialis – frontal
	aspect of suprarostral ala, next to lateral margin,
	through a long, thin tendon
Levator mandibulae externus superficialis	medial point at the ascending margin of muscular
	process, at about half its length – posterior surface of
	the dorsolateral edge of suprarostral ala; ramus V3 is
	ventral to this muscle
Levator mandibulae externus profundus	inner surface of the ascending margin of muscular
	process, at about half its length – frontal surface of
	suprarostral ala, next to lateral margin, through a
	tendon common with that of levator mandibulae
	longus profundus
Levator mandibulae internus	ventral surface of the ascending process and
	anteroventral surface of the otic capsule –
	dorsolateral edge of Meckel's cartilage
Levator mandibulae articularis	inner surface of muscular process, ventral to levator
	mandibulae externus profundus –dorsolateral edge of
	Meckel's cartilage, medial to levator mandibulae

656 TABLE 2.—Larval musculature in *Rhinella achalensis*.

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	internus
Levator mandibulae lateralis	lateral edge of articular process -dorsolateral edge of
	suprarostral ala
Mandibulolabialis	ventromedial edge of Meckel's cartilage - lower lip,
	in a small, mental area
Intermandibularis	ventrolateral edge of Meckel's cartilage – median
	aponeurosis attached to the ventral skin
Submentalis	ventral surface of infrarostral cartilages
Orbitohyoideus	dorsal edge of muscular process -lateral edge of
	ceratohyal
Suspensoriohyoideus	inferior region of the ascending margin of muscular
	process -posterolateral surface of the lateral edge of
	ceratohyal
Hyoangularis	dorsal surface of lateral process of ceratohyal -
	retroarticular process
Suspensorioangularis	ventral half of the descending margin of muscular
	process -posterolateral surface of the lateral edge of
	ceratohyal; it occupies the lower half of muscular
	process
Quadratoangularis	ventral surface of muscular process - retroarticular
	process
Interhyoideus	lateral edge of ceratohyal – median aponeurosis
Geniohyoideus	ventral surface of infrarostral – hypobranchial plate

	at the level of ceratobranchial III
Constrictor branchialis II	branchial process II – terminal commissure I;
	disposed on ceratobranchial I
Constrictor branchialis III	branchial process II – terminal commissure II;
	disposed on ceratobranchial II
Constrictor branchialis IV	branchial process II – distal edge of ceratobranchial
	III; disposed on ceratobranchial III
Levator arcuum branchialium I	lateral margin of subocular bar – lateral margin of
	ceratobranchial I
Levator arcuum branchialium II	posterolateral margin of subocular bar -terminal
	commissure I
Levator arcuum branchialium III	lateral and posterolateral margins of otic capsule -
	terminal commissure II; a posterior slip inserts more
	dorsally on the capsule
Levator arcuum branchialium IV	ventral surface of otic capsule – medial distal margin
	of ceratobranchial IV
Tympanopharyngeus	a few fibers that diverge medially from levator
	arcuum branchialium IV, to insert on the connective
	tissue anterior to glottis
Subarcualis rectus I	three slips originated at the posterior surface of the
	posterior process of the ceratohyal; ventral slip
	inserts on branchial process III, middle slip on
	branchial process II, and dorsal slip on proximal area

of ceratobranchial I. The distinction between middle and ventral slips is not well defined at the insertion on ceratohyal Subarcualis rectus II-IV ceratobranchial II –lateral margin of proximal area of ceratobranchial IV; very thin. At the anterior insertion, some fibers are continuous with those of ceratobranchial II; at the posterior insertion, a thin slip diverges laterally following the ceratobranchial reaching its distal edge Subarcualis obliquus urobranchial process – branchial process II **Rectus cervicis** branchial process III – peribranchial peritoneum; short and thin Diaphragmatobranchialis distal edge of ceratobranchial III – peribranchial peritoneum; short and very thin **Rectus** abdominalis pelvic girdle – peritoneum. Lateral slips originate at the level of spiracle, and middle slips reach the pericardic region; more medial slips form a compact slip that continues as a fibrous connective tissue that reaches the subhyoid ligament, whereas the remaining medial fibers are loose and insert caudal and dorsally on the diaphragm

658	FIGURE CAPTIONS
659	FIG. 1.—The tadpoles of (A) Rhinella achalensis (stage 33, LGE 04235), (B) R. gallardoi
660	(stage 31, LGE 09887), and (C) R. vellardi (stage 37, LGE 22105), showing lateral, dorsal, and
661	ventral views. Scale bars $= 1 \text{ mm}$.
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664	FIG. 2.—Details of the oral discs of same specimens of (A) Rhinella achalensis, (B) R.
665	gallardoi, and (C) R. vellardi. Scale bars = 0.5 mm .
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668	FIG. 3.—Comparative external morphology in tadpoles of the Rhinella spinulosa group.
669	Lateral views and detail of the oral disc are shown. (A) R. altiperuviana (stage 37, LGE 22111),
670	(B) R. limensis (stage 34, LGE 22101), (C) R. papillosa (stage 31, MACN 50401), (D) R.
671	spinulosa (stage 37, LGE 22103), (E) R. trifolium (stage 32, MUSM 17815-6), (F) R. aff.
672	spinulosa CAT (stage 31, LGE 22112), (G) R. aff. spinulosa MZA (stage 38, LGE 17106). Scale
673	bars = 1 mm. Note the two morphotypes differentiating the tadpoles of <i>R. limensis</i> (globose body
674	and small gap in A2) vs. all the remaining tadpoles (slender bodies and larger gaps in A2).
675	
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677	FIG. 4.—Labial tooth morphology in tadpoles of the Rhinella spinulosa group, lateral
678	views of individual teeth and details of head cusps. (A) R. achalensis (stage 33, LGE 04235), (B)
679	R. altiperuviana (stage 37, LGE 22111), (C) R. gallardoi (stage 31, LGE 09887), (D) R. limensis
680	(stage 34, LGE 22101), (E) R. papillosa (stage 31, MACN 50401), (F) R. spinulosa (stage 37,
681	LGE 22103), (G) R. trifolium (stage 32, MUSM 17815-6), (H) R. vellardi (stage 37, LGE

682	22105), (I) R. aff. spinulosa CAT (stage 31, LGE 22112), (J) R. aff. spinulosa MZA (stage 38,
683	LGE 17106). Scale bars, main divisions = 0.05 mm. Note the similar curved teeth with numerous
684	marginal cusps.
685	
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687	FIG. 5.—Lateral lines, as seen in a tadpole of Rhinella aff. spinulosa MZA (stage 37, LGE
688	17106). AN = angular, D = dorsal, IO = infraorbital, LOR = longitudinal oral, M = median, OR =
689	oral, PIO = postinfraorbital, PSO = postsupraorbital, SO = supraorbital, V = ventral.
690	
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692	FIG. 6.—Buccal roof (A) and floor (B) of <i>Rhinella achalensis</i> (stage 34, LGE 04235).
693	BFAP = buccal floor arena papillae, BP = buccal pocket, BRAP = buccal roof arena papillae, DV
694	= dorsal velum, GZ = glandular zone, ILP = papillae, IN = internal nare, LP = lingual papillae,
695	LRP = lateral ridge papilla, MR = median ridge, PNA = prenarial arena, PPP = prepocket
696	papillae, $PTNP = postnarial papillae$, $S = spur$, $VV = ventral velum$. Scale bars = 1 mm.
697	
698	
699	FIG. 7.—Details of buccal cavities of other species of the Rhinella spinulosa group,
700	showing some distinctive features. Anterior region of the buccal roof of (A) R. papillosa (stage
701	31, MACN 50401) and (B) R. spinulosa (stage 37, LGE 22103); note the different shapes of the
702	median ridges. (C) Posterior region of the buccal roof of R. limensis (stage 33, LGE 22102),
703	showing the dorsal velum and glandular zone. (D) Anterior region of the buccal floor of R .
704	altiperuviana (stage 37, LGE 22111), showing the spurs, infralabial and lingual papillae. (E)
705	Detail of the tongue anlage of <i>R. gallardoi</i> (stage 31, LGE 09887), showing the lingual pads on

706	both sides of the tongue. (F) Detail of the buccal pockets of R. aff. spinulosa CAT (stage 37, LGE
707	22112), showing the prepocket papillae and general papillae of the floor arena. BFAP = buccal
708	floor arena papillae, BP = buccal pocket, DV = dorsal velum, ILP = infralabial papillae, LP =
709	lingual papillae, LRP = lateral ridge papilla, MR = median ridge, P = lingual pad, PPP =
710	prepocket papillae, $S = spur$, $SP = secretory$ pits. Scale bars = 1 mm.
711	
712	
713	FIG. 8.—Larval skeleton in Rhinella achalensis (stage 35, LGE 04235) and R. gallardoi
714	(stage 31, LGE 09887). (A,E) Chondrocranium; (B,F) Suprarostral cartilage; (C,G). Lower jaw
715	cartilages; (D,H) Hyobranchial skeleton. A = ala, AP = articular process, ASP = ascending
716	process, $C = corpus$, $CB = ceratobranchials$, $CH = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $IR = ceratohyals$, $HP = hypobranchial plate$, $HP = hyp$
717	infrarostral cartilage, LT = lateral trabecular process, MC = Meckel's cartilage, MP = muscular
718	process, OC = otic capsule, PAL = anterolateral process of the otic capsule, PQ = palatoquadrate,
719	PR = pars reuniens, QEP = quadratoethmoid process, QOC = quadratoorbital commissure, SB =
720	subocular bar, $TH =$ trabecular horns, $TS =$ tectum synoticum. Scale bars = 0.5 mm.
721	Chondrocrania are very similar, excepting the angular margin of subocular bar and the small
722	anterolateral process of the otic capsules in R. achalensis.
723	
724	
725	FIG. 9.—Details of larval skeletons of other species of the Rhinella spinulosa group,
726	showing some distinctive features (asterisks). Chondrocranium in (A) R. spinulosa (stage 37,
727	LGE 22103), ventral view; note the small processes in the subocular bars and the otic capsules.
728	(B) R. papillosa (stage 31, MACN 50401), ventral view; (C) R. aff. spinulosa MZA (stage 35,
729	LGE 17106), dorsal view; note the pointed lateral trabecular processes in the two species. (D) R.

730	vellardi (stage 37, LGE 22105), ventral view; note the thin, unilateral larval otic process.
731	Suprarostral cartilages in frontal view of (E) <i>R. limensis</i> (stage 33, LGE 22102) and (F) <i>R</i> .
732	spinulosa (stage 37, LGE 22103); note the cartilaginous bridges between corpus and ala in the
733	latter. (G) Detail of pars reuniens of the specimen of R. spinulosa, showing the different type of
734	cartilaginous tissue. (H) Detail of four spicules in R. altiperuviana (stage 35, LGE 22111),
735	showing the cartilage bridge between spicules III and IV. Scale bars $= 1$ mm.
736	
737	
738	FIG. 10.—Larval cranial musculature in tadpoles of species of the Rhinella spinulosa
739	group. (A) Lateral view of the muscular process of R. gallardoi (stage 31, LGE 09887), showing
740	details of mm. levatores mandibulae; thin levator mandibulae lateralis and externus superficialis
741	are present in all species. (B) Ventral view of the mandibular region of R. aff. spinulosa CAT
742	(stage 37, LGE 22112), showing ventral muscles and the subhyoid ligament; when the ventral
743	skin is lifted, the attachment of the anterior portion of the m. intermandibularis to the lower lip
744	skin is evident. (C) Ventral view of the left branchial basket of R. altiperuviana (stage 35, LGE
745	22111), showing branchial muscles; note the lateral slip of the m. subarcualis rectus II-IV
746	invading the third branchial septum in a constrictor-like arrangement. (D) Detail of the left
747	branchial basket of <i>R</i> . aff. spinulosa MZA (stage 37, LGE 17106), showing the three slips of the
748	m. subarcualis rectus I. (E) Ventral view of the abdominal region of the specimen of R. gallardoi,
749	showing the general configuration of the m. rectus abdominis with three types of fibers. CBII-IV
750	= constrictor branchialis II-IV, GH = geniohyoideus, HA = hyoangularis, IM =
751	intermandibularis, LIG SH = subhyoid ligament, LMA = levator mandibulae articularis, LMEP =
752	levator mandibulae externus profundus, LMES = levator mandibulae externus superficialis, LML
753	= levator mandibulae lateralis, LMLS = levator mandibulae longus superficialis, MLI =

- 754 mandibulolabialis inferior, OH = orbitohyoideus, QA = quadratoangularis, RAL = rectus
- abdominis lateral fibers, RAM1 = rectus abdominis medial, compact fibers, RAM2 = rectus
- abdominis medial, loose fibers, SO = subarcualis obliquus, SRI = subarcualis rectus I, SRID =
- subarcualis rectus I dorsal slip, SRII-IV = subarcualis rectus II-IV, SRIM = subarcualis rectus I
- 758 median slip, SRIV = subarcualis rectus I ventral slip, V3 = mandibular branch of the trigeminal
- nerve. Scale bars = 0.5 mm excepting (E), 1 mm.

























