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# Cranial anatomy of tadpoles of five species of *Scinax* (Hylidae, Hylinae)

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## Abstract

We studied the oral apparatus, buccal cavity and musculoskeletal features in tadpoles of five species of the genus *Scinax* (*S. acuminatus*, *S. uruguayus*, *S.* aff. *pinima*, *S. aromothyella*, and *S. berthae*). Observed variation is mainly related to intrageneric grouping. *Scinax acuminatus* (*S. ruber* clade, sister taxon of *S. rostratus* group) has a distinctive combination of a mental gap in the margin of oral papillae, straight labial teeth with few or absent cusps, processus muscularis acute and posteriorly directed, and m. subarcualis rectus I with two slips. *Scinax uruguayus* and *S.* aff. *pinima* (*S. uruguayus* group) have keratinized sheets ventrolateral to the lower jaw sheath, well-developed infralabial and lateral ridge papillae, robust jaw cartilages, cornua trabeculae with short and widely divergent free portions, processus articularis short and wide, processus muscularis thin and directed anteriorly. *Scinax aromothyella* and *S. berthae* (*S. catharinae* group) have poorly developed, non-colored spurs behind the lower jaw sheath, long and thin processus articularis, wide and rounded processus muscularis, and tripartite cartilago suprarostralis. Anatomical features described are congruent with current phylogenetic arrangements based on molecular, chromosomal, and morphological data, and provide a source of information that can be useful to solve interspecific relationships within *Scinax*.

**Key words:** anuran larvae, buccal cavity, chondrocranium, cranial muscles, hyobranchial apparatus

## Introduction

Tadpole morphology is a valuable source of information to infer anuran evolution. External morphological characters have been traditionally considered informative for the higher classification of anurans (e.g., Orton 1953). More recently, the search for additional larval characters, mainly from oral cavity and musculoskeletal morphology, resulted in new hypotheses about anuran relationships at different taxonomic levels (Sokol 1975; Wassersug 1980; Wassersug & Heyer, 1988). Recent cladistic analyses have been the source of remarkable changes in anuran systematics, but most of them have been mainly based on molecular data (e.g., Frost *et al*. 2006; Grant *et al*. 2006; Roelants *et al*. 2007), and the lack of suitable morphological data sets is a recognized weak point of some of these studies (Faivovich *et al*. 2005; Frost *et al*. 2006).

Larval characters have rarely been used for extensive phylogenetic analysis, except for a few works (Haas 2003 and references therein). The phylogenetic analysis by Haas (2003) based on larval characters suggested the non monophyly of the widespread family Hylidae, as was also the case of some of its genera as then recognized. In a recent taxonomic review of this family, many larval characters were proposed as synapomorphic for different level taxonomic categories, highlighting the relevance of the knowledge of larval morphology (Faivovich *et al*. 2005).

Within Hylinae, the Neotropical genus *Scinax* is one of the largest, comprising almost 100 species (Frost 2009). Two major clades are recognized within the genus, the *S. catharinae* and the *S. ruber* clades (Faivovich

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2002; Faivovich *et al*. 2005). The first clade is composed of the *S. catharinae* and the *S. perpusillus* groups, while the second includes the *S. rostratus* and the *S. uruguayus* groups and more than 40 species without group assignment. Synapomorphies supporting these clades include several characters from larval external morphology. The internal morphology of *Scinax* tadpoles is poorly known and mostly limited to some species of the *S. ruber* clade (Appendix I). For instance, there are no published data on larval internal morphology for the species in the *S. uruguayus* group, and the same applies to musculoskeletal features of species in the *S. catharinae* clade.

The goal of the present study is to provide original information on the larval morphology of five species of *Scinax*, enlarging the data set of informative characters for the systematics of this genus. The buccal cavity and musculoskeletal system of two species in the *S. catharinae* group and two species in the *S. uruguayus* group are described for the first time, together with a redescription of these features in *S. acuminatus*, the sister taxon of the *S. rostratus* group. We then review our findings and the available information in the context of current phylogenetic knowledge of the genus.

## Material and methods

Tadpoles used in morphological descriptions (stages 28–39; Gosner 1960) were collected at different localities in Argentina and Uruguay, euthanized in the field using MS222 anesthetic and then fixed in 10% neutral-buffered formalin. Species assignation was corroborated by rearing some larvae until metamorphosis was complete. The specimens examined are deposited at the amphibian collections of the Museo de La Plata (MLP). *Scinax acuminatus*.— MLP DB-5309, *n* = 1 stage 39 (oral disc, OD, and buccal morphology, BM); *n* = 4 stages 36–39 (cranial morphology, CM). *Scinax aromothyella*.— MLP DB-8789, *n* = 2 stages 29 and 31 (OD, BM); *n* = 4 stages 28–30 (CM). *Scinax berthae*.— MLP A-4993, *n* = 1 stage 38 (OD, BM); n = 2 stages 38–39 (CM), MLP DB-8790, *n* = 1 stage 36 (OD, BM); *n* = 4 stages 30–35 (CM). *Scinax* aff. *pinima*.— MLP DB-4294, *n* = 1 stage 37 (OD, BM), *n* = 2 stage 35 and 37 (CM); MLP DB-5595, *n* = 1 stage 37 (OD, BM), and *n* = 2 stage 36 (CM). *Scinax uruguayus*.— MLP DB-8792, *n* = 1 stage 37 (OD, BM), *n* = 4 stages 30–36 (CM); MLP DB-8791, *n* = 1 stage 37 (OD, BM), *n* = 3 stage 34–35 (CM). See Appendix II for collection sites.

**Oral disc and buccal cavity morphology.** Nine larvae were prepared for scanning electron microscope examination of the oral disc and buccal cavity after Wassersug (1976) and Alcalde and Blotto (2006). Buccal terminology follows Wassersug (1976; 1980).

**Musculoskeletal morphology.** Twenty five larvae were stained for bone and cartilage examination, after Taylor and Van Dyke (1985). The process was interrupted before clearing and tadpoles were dissected for observation of muscles, then clearing was achieved for chondrocranium description. Observations and measurements were made using a Reichert Wien stereomicroscope, with measurement accuracy up to the nearest 0.1 mm. Terminology follows those of Schlosser and Roth (1995), d'Heursel and de Sá (1999), and Haas (2003). Measurements were taken according to Alcalde and Rosset (2003) and Haas (2003), and we included additional measurements defined as follows: 1) length of the free portion of the cornu trabeculae: on the chondrocranial midline, between the anteriormost point of the chondrocranium and the point of divergence of the cornua trabeculae; 2) width of the free portion of the cornu trabeculae: taken perpendicularly to the axis of the cornu, on its midlength; 3) width of the processus articularis: taken at the base of the processus; 4) length of the processus articularis: taken between the anteriormost point and the base of the processus, on its mid width; 5) length, 6) width, and 7) angle regarding the midline of the cartilagines infrarostrales; 8) length, 9) width, and 10) angle regarding the midline of cartilagines meckeli. Linear measurements are shown in Fig. 1. Representative morphological data from the aforementioned character systems are summarized in a character list and a matrix in the Appendix III.

## Results

Oral disc. The oral discs of four of the species studied herein were previously described: *Scinax acuminatus* (Mercolli *et al*. 1994), *S. berthae* (de Sá *et al*. 1997), *S. uruguayus* (Kolenc *et al*. 2003), and *S. aromothyella* (Kolenc *et al*. 2007). General features of the disc of *S.* aff. *pinima* are the same as in *S. uruguayus*. These five species share non-emarginate oral discs, almost terminal in *S. acuminatus* and anteroventral in the remaining species; labial tooth row formula is 2(2)/3(1) in *S. acuminatus*, *S. uruguayus*, and *S.* aff. *pinima*, and 2(2)/3 in *S. aromothyella* and *S.*

*berthae*. Marginal papillae are arranged in single (*S. berthae*, *S. uruguayus*) or multiple rows (*S. acuminatus*, *S. aromothyella*), and those of the mental region of *S. uruguayus* and *S.* aff. *pinima* are particularly large and widely spaced (Fig. 2). Mental gap is present only in *S. acuminatus*. Submarginal papillae are generally numerous (*S. aromothyella* and *S. berthae*) or few (*S. acuminatus*, *S. uruguayus* and *S.* aff. *pinima*).

Regarding keratinized structures, labial teeth are curved, with a long, convex, spatulate head with numerous triangular cusps, except for *Scinax acuminatus* in which labial teeth are either devoid of cusps or have 2–4 very smooth cusps (Fig. 2). Jaw sheaths are very well developed in *S. uruguayus* and *S.* aff. *pinima*, intermediate in *S. acuminatus*, and comparatively tiny in *S. aromothyella* and *S. berthae*. *Scinax uruguayus* and *S.* aff. *pinima* larvae have a pair of keratinized sheets ventrolateral to the lower jaw sheath (Fig. 3).

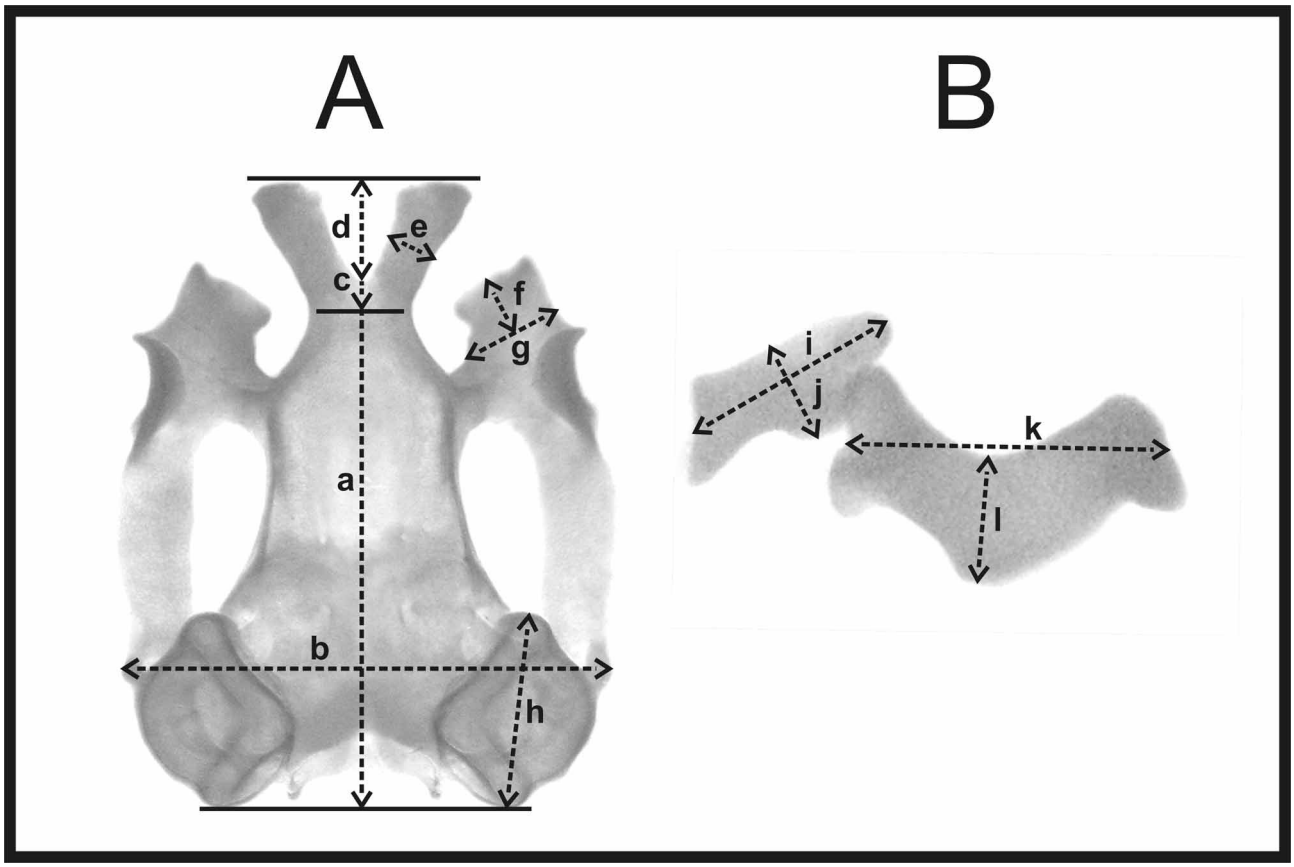


**FIGURE 1.** Measurements taken on (A) dorsal view of the chondrocranium and (B) ventral view of the lower jaw cartilages (only right half is shown). References: a, total length of the chondrocranium; b, maximum width of the chondrocranium; c, length of the cornu trabeculae; d, length of the free portion of the cornu trabeculae; e, width of the free portion of the cornu trabeculae; f, length of the processus articularis; g, width of the processus articularis; h, length of the capsula auditiva; i, length of the cartilago infrarostralis; j, width of the cartilago infrarostralis; k, length of the cartilago meckeli; l, width of the cartilago meckeli.

Buccal cavity. In the buccal floor, keratinized spurs (Fig. 3) are present in *Scinax acuminatus*, *S. uruguayus*, and *S.* aff. *pinima*; they project medially behind the posterior edges of the lower jaw sheath and can be uni or multicuspidate, more pointed and larger in *S. acuminatus*. In *S. aromothyella* and *S. berthae*, small, hard, pointed, non-colored spurs were present. *Scinax acuminatus*, *S. aromothyella*, and *S. berthae* bear one pair of infralabial papillae projecting medially from the posteromedial region of the cartilago meckeli, accompanied by one pair of pustules or low papillae on the cartilago infrarostralis (Fig. 4). In *S. uruguayus* and *S.* aff. *pinima*, large, wide, multifid papillae project from the whole medial region of the cartilago meckeli, overlapping each other at the midline (Fig. 5). The lingual anlage lacks lingual papillae in all species examined (Fig. 4). The prepocket papillation varies among species (Table 1), *S. acuminatus* bearing more pustules and papillae than the rest. The buccal pockets of most species are narrow, transverse, medially curved slits; in *S. acuminatus* they are comparatively wider. The buccal floor arena is delimited by 8–14 papillae in *S. uruguayus* and *S.* aff. *pinima*, and appears poorly defined in the other species

(Table 1). The ventral velum is sinuous, with three conspicuous undulations on each side in all species (Fig. 4), except for *S. berthae*. The median notch is absent in *S. berthae*. A glandular epithelium with large secretory pits is present at the posterior, dorsal margin of the velum. The glottis is exposed in *S. acuminatus*, *S. berthae* and *S. uruguayus*, whereas it is concealed by the velum in *S. aromothyella* and *S.* aff. *pinima* (Fig. 4).

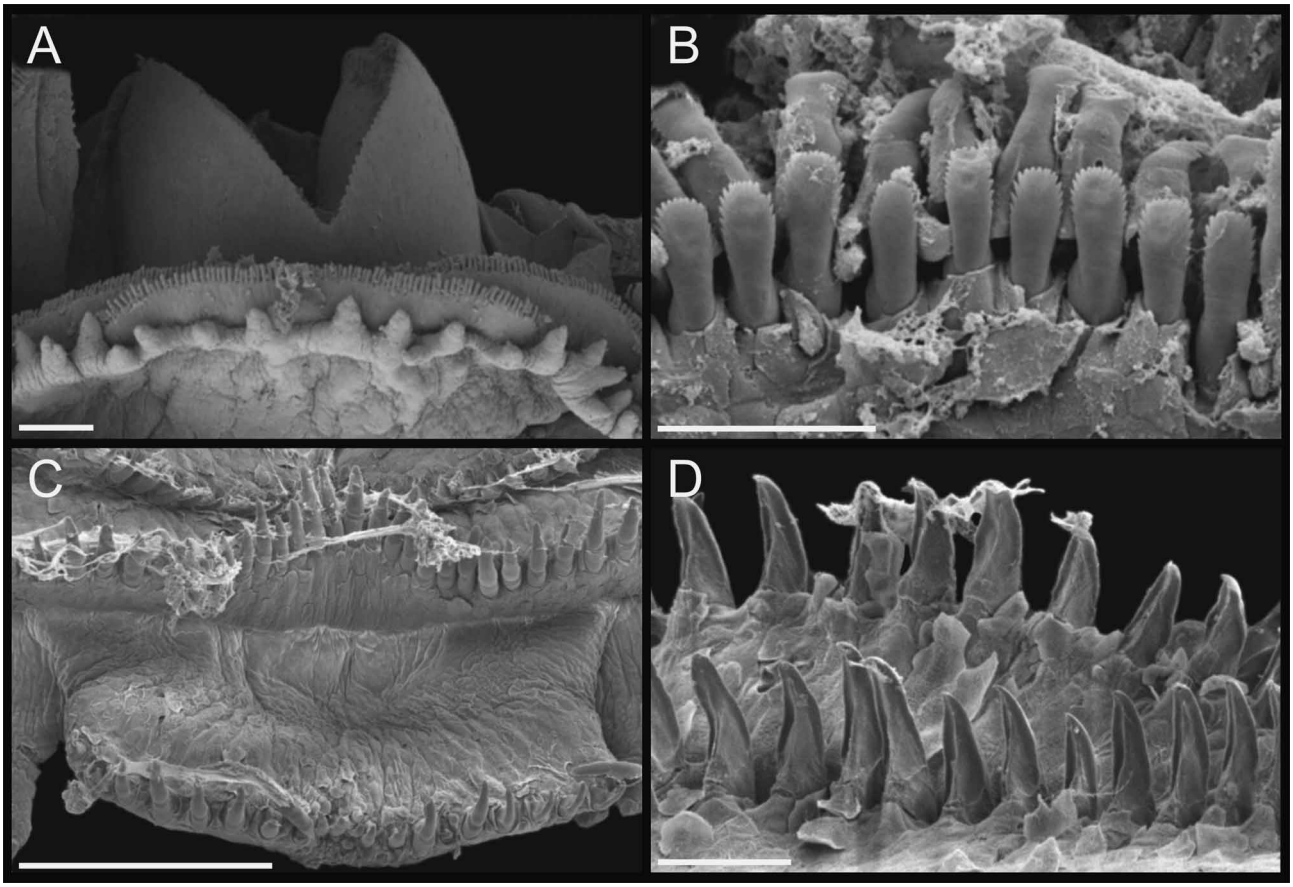


**FIGURE 2.** Scanning electron photomicrographs (SEM) of the oral discs of *Scinax* species. (A) *S. uruguayus* stage 37, frontal view showing lower jaw sheath, lower labial tooth rows, and widely-spaced mental marginal papillae; (B) *S. aromothyella* stage 31, detail of multicusped labial teeth; (C) *S. acuminatus* stage 39, detail of the lower labial tooth rows and mental gap; (D) *S. acuminatus* stage 39, detail of upper labial tooth with cusps reduced or absent. Bars (A, C) = 0.25 mm and (B, D) = 0.05 mm.

**TABLE 1.** Summary of most varying features of the buccal cavity of five *Scinax* species. Abbreviations: BFA: buccal floor arena, BRA: buccal roof arena, PA: papillae, PPA: prepocket arena, PTNA: postnarial arena, PU: pustules.

| | *S. uruguayus* | *S.* aff. *pinima* | *S. acuminatus* | *S. aromothyella* | *S. berthae* |
|---|---|---|---|---|---|
| PPA | ~28 PU; 6 short PA; 4 long conical PA | 10 PU; 4 short PA; 4 long conical PA | ~50-60 PU; 8 short PA | 8 PU; 2 short PA | 10 PU; 2 short PA |
| BFA | defined; ~34 PU; no short PA; 14 long PA (2 digitiform) | defined; ~40 PU; no short PA; 8 long PA | poorly defined; ~50 PU; 6 short PA | poorly defined; ~20 PU; 14 short PA; 2 digitiform PA | poorly defined; ~14 PU; no short PA; 2 digitiform PA |
| PTNA | 1 PU; no short PA; 6 long ramified PA | 7 PU; no short PA; 6 long ramified PA | ~20 PU; no short PA; 2 narial ridges | No PU; 10 short PA | 7–10 PU |
| BRA | not defined; ~130 PU | not defined; ~50 PU | not defined; ~120 PU | not defined; ~70 PU | not defined; ~60–70 PU |

In the buccal roof (Fig. 6) the prenarial arena bears a single, rounded medial pustule only in *Scinax aromothyella* and in Uruguayan specimens of *S. berthae*. The choanae are similar in all species; they are oblique slits (transversal in *S. acuminatus*), with the anterior margin bearing small prenarial papillae and the posterior margin forming narial valves. The papillation of the postnarial arena also varies between species (Table 1). The most divergent is *S. acuminatus* that bears two pustulate narial ridges presumably formed by the fusion of the postnarial papil-

lae. A typical median ridge is present in almost all species, but in *S. aromothyella* it is reduced to some long conical papillae. The median ridges have a pustulate margin and are high and triangular (*S. uruguayus*, *S.* aff. *pinima*, and *S. berthae* from Uruguay) or low, wide and rounded (*S. acuminatus* and *S. berthae* from Argentina). The lateral ridge papillae are wide, flat and pustulate in *S. uruguayus* and *S.* aff. *pinima*, and smaller, conical and narrow in the remaining species. The buccal roof arena is not defined in any species, the whole zone is scattered with numerous, small pustules; groups of 4–6 small lateral roof papillae can be seen in *S. acuminatus* and *S. uruguayus*. The dorsal velum is short, smooth and exhibits large secretory pits in a band-like array in the anterior zone.

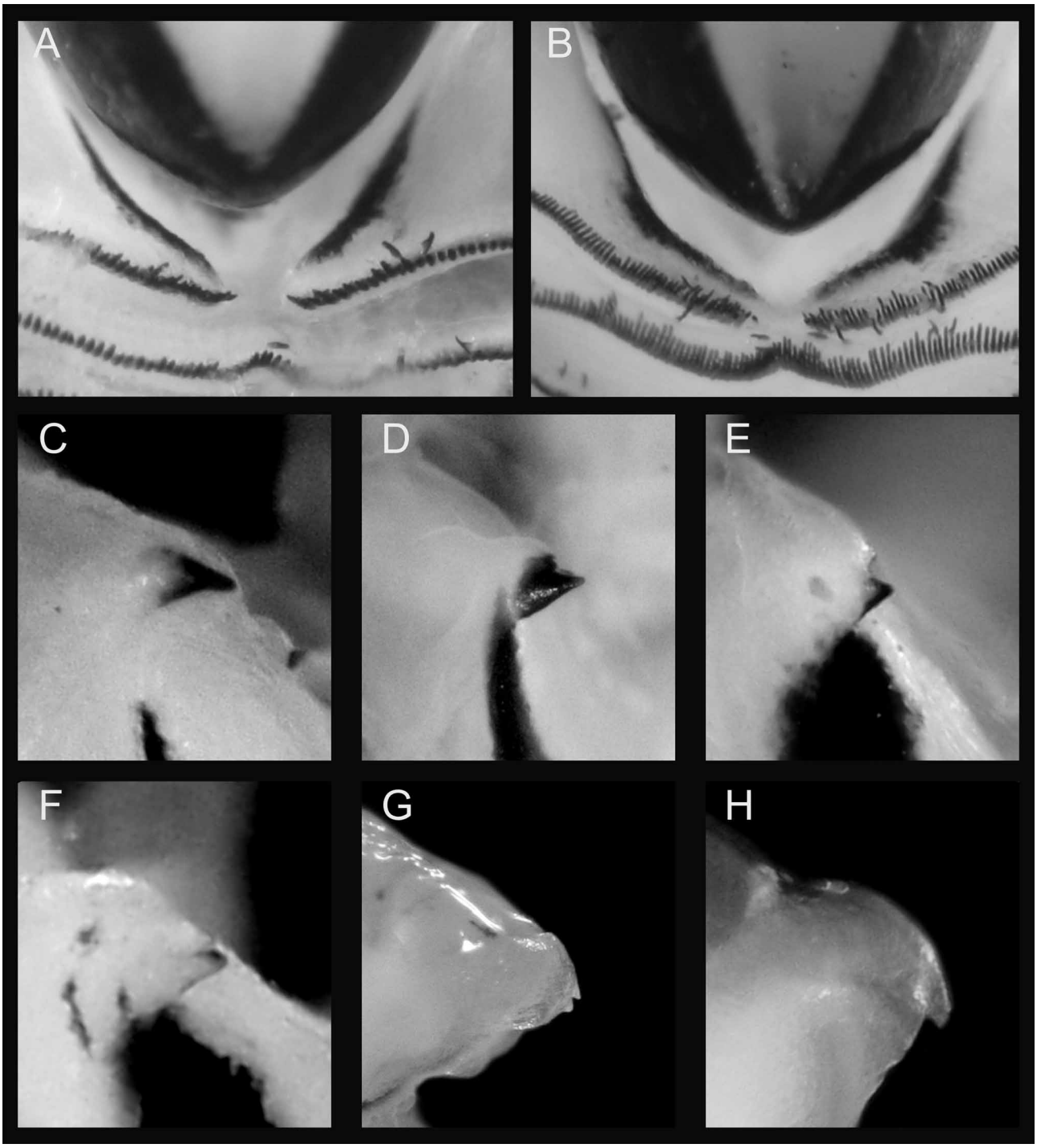


**FIGURE 3.** Keratinized sheets associated to the lower jaw sheath of: (A) *Scinax uruguayus* and (B) *S.* aff. *pinima*. Spurs associated to the lower jaw sheath of: (C) *S. acuminatus*, (D) *S. nasicus*, (E) *S. uruguayus*, (F) *S.* aff. *pinima*, (G) *S. aromothyella*, and (H) *S. berthae*. Pictures are not to scale.

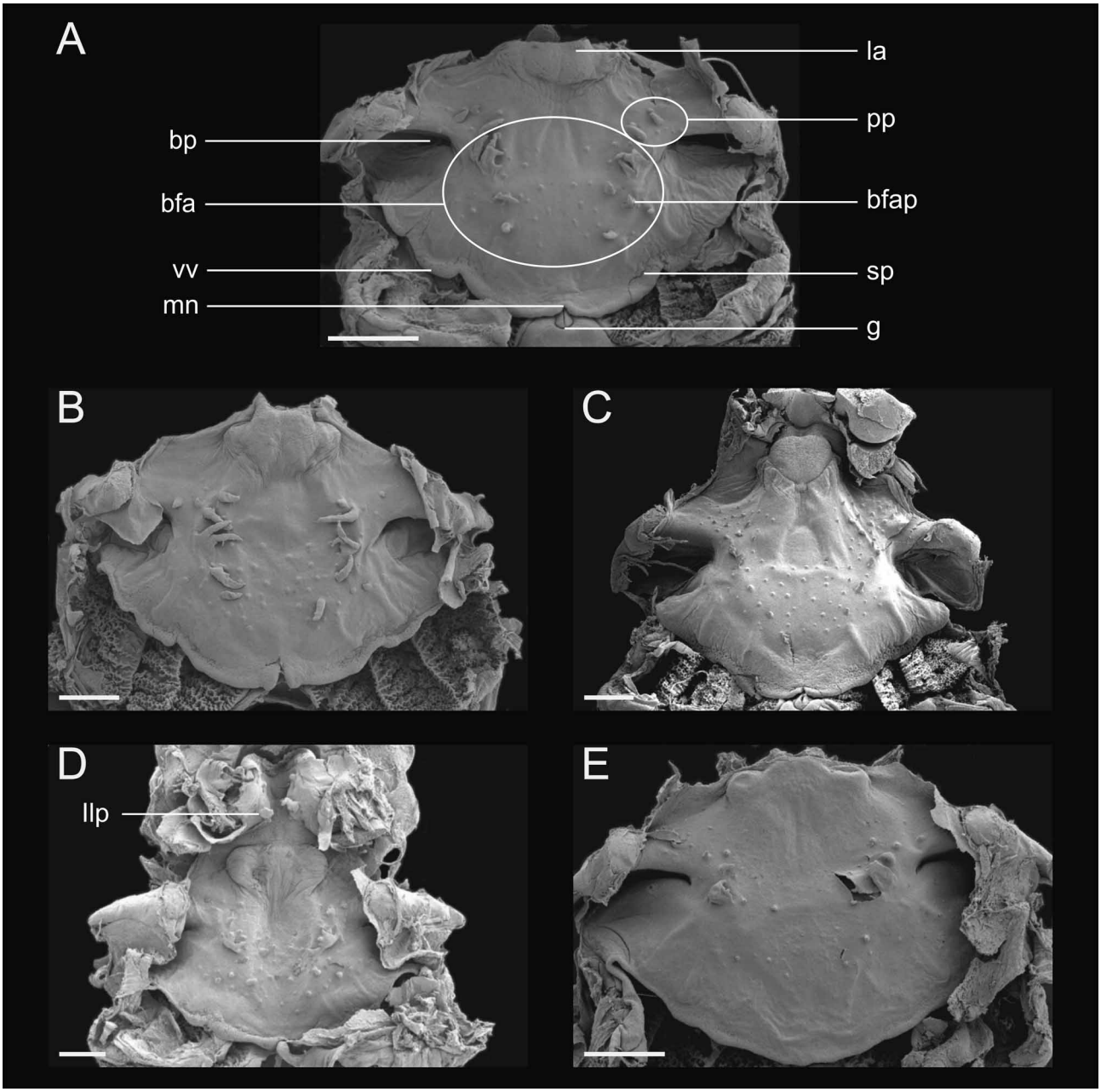


**FIGURE 4.** SEM photomicrographs of the buccal floors of *Scinax* species. (A) *Scinax uruguayus* stage 37; (B) *S.* aff. *pinima* stage 37; (C) *S. acuminatus* stage 39; (D) *S. aromothyella* stage 31; (E) *Scinax berthae* stage 38. References: bfa, buccal floor arena; bfap, buccal floor arena papillae; bp, buccal pocket; g, glottis; ilp, infralabial papilla; la, lingual anlage; mn, median notch; pp, prepocket papillae; sp, secretory pits; vv, ventral velum. Bar = 1 mm.

**Skeleton.** The neurocranium (Fig. 7) is rectangular (width / length = 0.72–0.87) in *Scinax uruguayus*, *S.* aff. *pinima*, *S. aromothyella*, and *S. berthae*, whereas it is square-shaped (width / length = 1.02) in *S. acuminatus*. The neurocranium is depressed (height / width = 0.34–0.5) in all species, being widest at the level of the arcus subocularis. In *S. acuminatus*, *S. uruguayus* and *S.* aff. *pinima* the cartilago suprarostralis is massive and the pars corporis and pars alaris are completely fused. *Scinax aromothyella* and *S. berthae* have the partes corpores and the partes alares joined by a syndesmosis, and both partes corpores are connected to each other by a cartilaginous distal bridge. These two latter species also bear a small medial projection on the medial margin of each pars alaris, oriented towards the pars corporis. The processus posterior dorsalis of the partes alares is present in all studied species and is particularly developed in *S. uruguayus* and *S.* aff. *pinima*.

The cornua trabeculae (Fig. 7) represent about 23% of the chondrocranium length and the general aspect differs between species. The free portions are short (< 60% of the cornu length) and wide (> 70% of the free portion length) in *Scinax uruguayus* and *S.* aff. *pinima*, long and intermediately wide in *S. aromothyella* and *S. berthae* (> 70%, 50–60%), and long and narrow in *S. acuminatus* (> 70%, ~ 40%). Additionally, cornua are almost uniformly wide in *S. acuminatus*, *S. uruguayus*, and *S.* aff. *pinima*, and distally widened in *S. aromothyella* and *S. berthae*. A ventral processus lateralis is seen in the five species.

In *Scinax acuminatus* (stage 36) and *S. aromothyella* (stage 29), the cranial floor is widely open by the presence of the pituitary fenestra and the foramina craneopalatina and carotica primaria are not yet developed. In *S. uruguayus* (stage 32), *S.* aff. *pinima* (stage 36), and *S. berthae* (stage 38) the floor is almost completely chondrified and the foramina craneopalatina and carotica primaria are well defined.

The cranium is roofed only by the tectum synoticum between the capsulae auditivae at early stages of most species. Conversely, in *Scinax aromothyella* the taeniae tecti medialis, transversalis, and marginales begin to form at stage 29, delimiting the fenestra frontoparietalis anteriorly and two fenestrae parietales posteriorly. The tectum parietale is well developed in *S. acuminatus* and outlined in *S.* aff. *pinima* and *S. berthae* (Fig. 7).

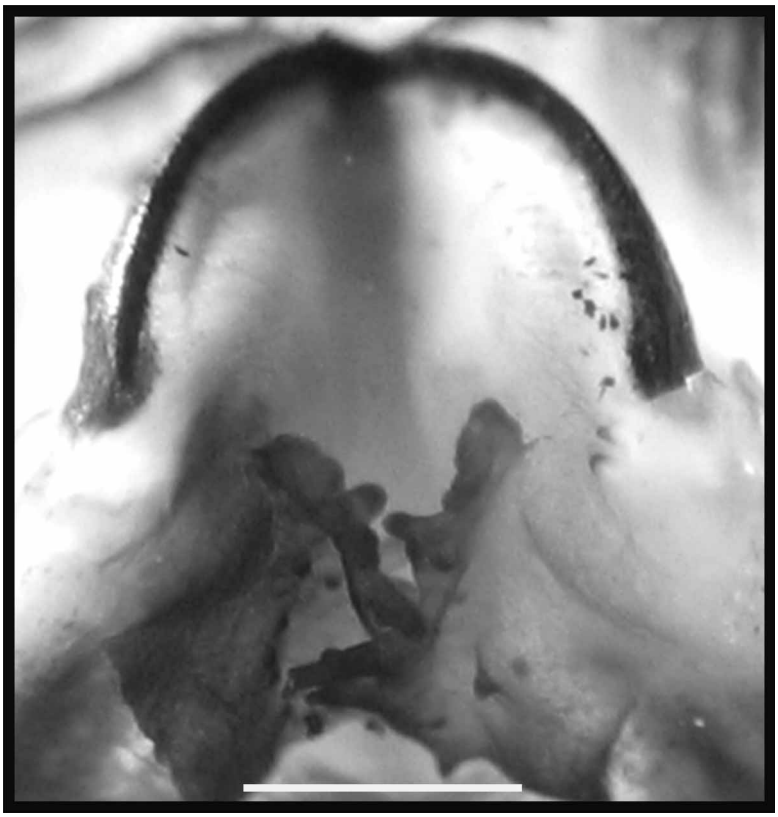

**FIGURE 5.** Detail of the infralabial papillae of *Scinax uruguayus*. Bar = 0.5 mm.

Cartilagines orbitales are formed by the pila preoptica, pila metoptica and pila antotica in *Scinax acuminatus* (stage 36), *S.* aff. *pinima*, *S. aromothyella* (stage 29) and *S. berthae* (stage 38). In *S. uruguayus* (stage 32) and *S. berthae* (stage 30) the lateral walls are formed only by the pila antotica and the pila metoptica. The processus antorbitalis is present only in *S. aromothyella* and *S. berthae*.

The capsulae auditivae (Fig. 7) are spherical and account for about 27–30% of the chondrocranial length. The larval crista parotica shows a vertical processus anterolateralis that reaches the palatoquadrate forming the larval processus oticus. A small, triangular, ventrally projected processus posterolateralis of the larval crista parotica is present in *Scinax acuminatus*, *S. uruguayus*, *S.* aff. *pinima*, and *S. berthae*; the absence in *S. aromothyella* may be result of having analyzed earlier developmental stages. The medial walls of the capsulae auditivae are pierced by the foramen acusticum, foramen endolymphaticum and foramen perilymphaticum inferius. The foramen perilymphaticum superius opens in the posterior wall of each capsula auditiva. The operculum is chondrified in *S. acuminatus*, *S.* aff. *pinima*, and *S. berthae*.

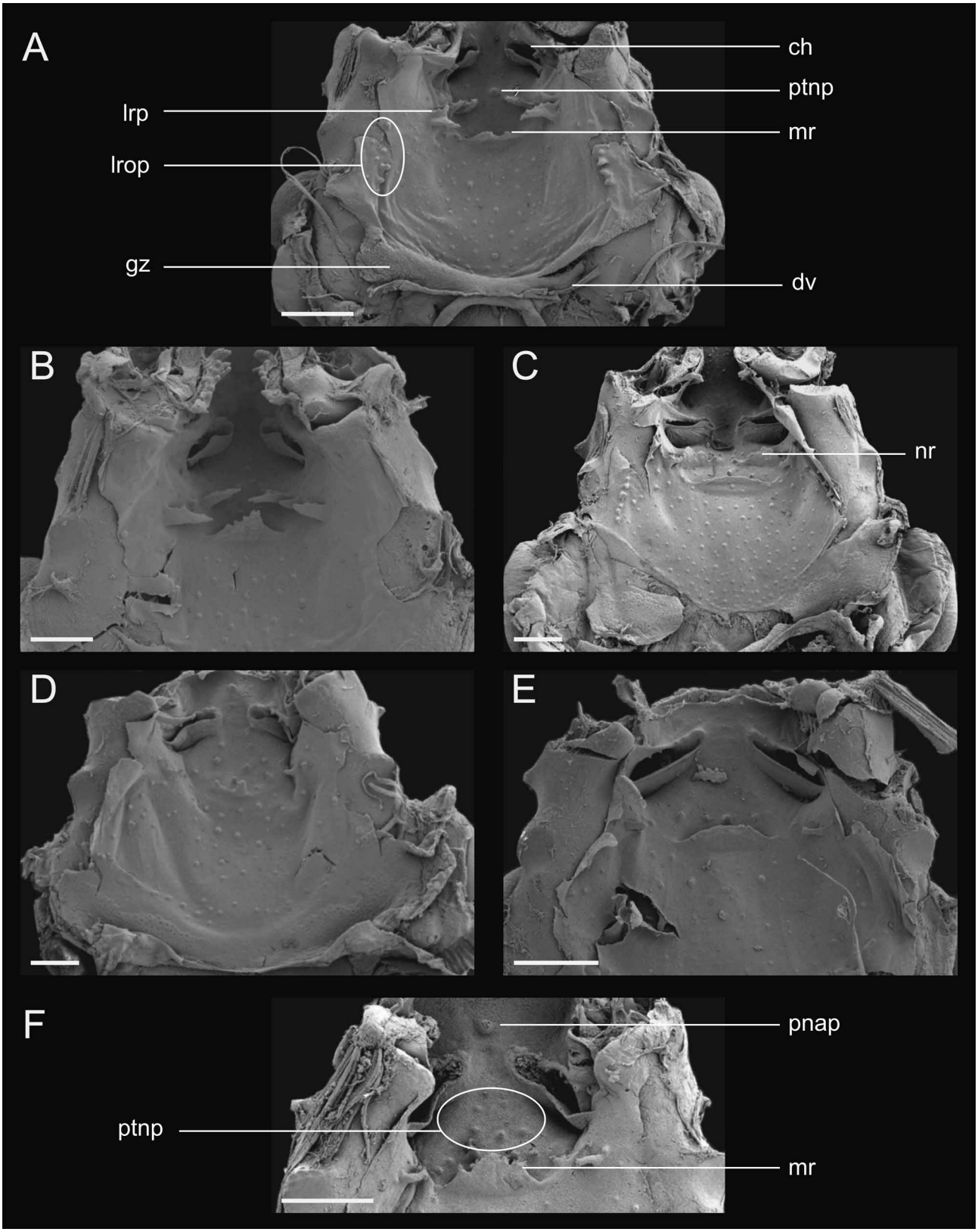


**FIGURE 6.** SEM photomicrographs of the buccal roofs of *Scinax* species. (A) *S. uruguayus* stage 37; (B) *S.* aff. *pinima* stage 37; (C) *S. acuminatus* stage 39; (D) *S. aromothyella* stage 31; (E) *S. berthae* stage 38; (F) *S. berthae* (from Uruguay) stage 36, detail of the anterior region of the buccal roof. References: ch, choana; dv, dorsal velum; gz, glandular zone; lrop, lateral roof papillae; lrp, lateral ridge papillae; mr, median ridge; nr, narial ridge; pnap, prenarial arena papilla; ptnp, postnarial papillae. Bar = 1 mm.

The palatoquadrate (Fig. 7) bears a processus articularis, processus muscularis, commissura quadrato-cranialis anterior, processus ascendens, and larval processus oticus; it lacks the commissura quadrato-orbitalis and processus pseudopterygoideus. The palatoquadrate is located farther laterally in *Scinax aromothyella* and *S. berthae* and the fenestra subocularis appears wider. The processus articularis varies among the species. It is very short and wide (5% and > 17% of the chondrocranium length), and its anterior margin bears three processes in *S. uruguayus* and *S.* aff. *pinima*; it is long and narrow (11% and 14%, respectively), and has two processes in *S. acuminatus*; in *S. aromothyella* and *S. berthae* values are intermediate (6–7% and ~15%, respectively) and two processes are visible. The processus quadrato-ethmoidalis is outlined in *S. aromothyella* and *S. berthae*, and absent in the remaining species. The processus muscularis is narrow, rounded, and directed anteromedially in *S. uruguayus* and *S.* aff. *pinima*, wide, rounded, and directed medially in *S. aromothyella* and *S. berthae*, and narrow, triangular, and directed posteromedially in *S. acuminatus*. In *S. acuminatus*, the lateral margin of the arcus subocularis is irregular. The processus ascendens joins the pila antotica near to the trabecula cranii (low attachment).

The cartilago meckeli bears well-developed processus ventromedialis and dorsomedialis and a short processus retroarticularis (Fig. 7). The cartilago meckeli articulates with the cartilago infrarostralis by the alcian blue negative commissura intramandibularis. In *Scinax uruguayus* and *S.* aff. *pinima*, the lower jaw cartilages are massive (width / length > 0.60 in both cartilagines infrarostrales and meckeli) and arrange describing an acute angle (~ 50º) regarding the midline. In the remaining species, lower jaw cartilages are slender (0.30–0.40) and more divergent (> 60º).

In the hyobranchial apparatus (Fig. 8), copula I is very reduced or absent in all species examined. All ceratohyale processes typically present in tadpoles are well developed. The processus anteriores are rounded in most species, but triangular in *Scinax aromothyella* and *S. berthae*, species which also have medially curved processus anterolaterales. Ceratohyalia are medially joined by a rectangular alcian blue negative pars reuniens. The copula II is oval and bears a short processus urobranchialis. The connection between ceratobranchiale I and planum hypobranchiale is always synchondrotic but the connection of the ceratobranchialia II-IV may be synchondrotic (*S. uruguayus* and *S.* aff. *pinima*) or syndesmotic (remaining species). The ceratobranchialia II-III (also III and IV in *S. aromothyella* and *S. berthae*) are joined to each other by commissurae proximales. The processus branchialis is open and four spicula are well developed. Spicula I–III are bifid in most specimens of the five species; the fourth spiculum is wider than the others and fused to the planum hypobranchiale; the tip of spiculum IV is rounded in *S. uruguayus*, *S.* aff. *pinima* and some *S. berthae*, and bifid in *S. acuminatus*, *S. aromothyella* and some *S. berthae*.

**Cranial muscles.** The cranial muscles of *Scinax uruguayus* larvae are shown in Fig. 9 and Table 2. The ramus mandibularis of the nervus trigeminus runs dorsally to all mm. levatores mandibulae in this species. The following deviations from the pattern in *S. uruguayus* were detected: *S. acuminatus* larvae differ by the following features: ramus mandibularis of the trigeminus nerve running ventral to m. levator mandibulae externus superficialis, and m. submentalis absent at the studied stages. *Scinax berthae* larvae differ by: m. submentalis absent at the stages examined and m. levator mandibulae internus originating at the processus ascendens and capsula auditiva. *Scinax aromothyella* is the most divergent species by having: the m. levator mandibulae internus originated at the processus ascendens and capsula auditiva, m. geniohyoideus originated posterior to the ceratobranchiale IV-planum hypobranchiale junction, m. subarcualis rectus I with three slips on ceratobranchialia I, II and III, and mm. submentalis and levator mandibulae lateralis absent at studied stages.

A summary of states for 20 characters from oral disc, buccal cavity and musculoskeletal system is shown in Appendix III, along with a matrix coding the five *Scinax* species studied.

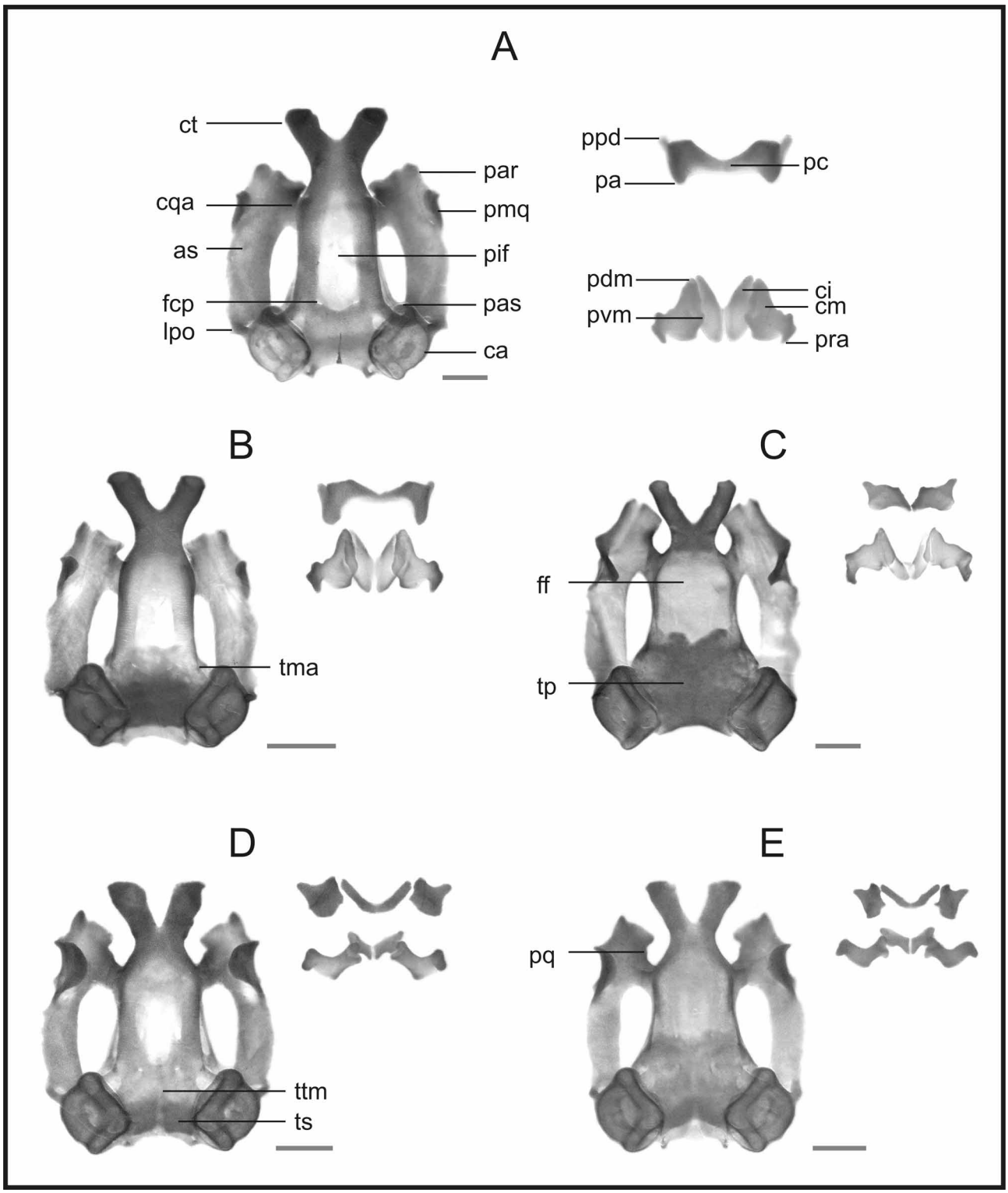


**FIGURE 7.** Larval neurocrania and palatoquadrates (dorsal view, left), cartilago suprarostralis (frontal view, right top), and lower jaw cartilages (dorsal view, right bottom) of *Scinax* species. (A) *S. uruguayus* stage 36; (B) *S.* aff. *pinima* stage 35; (C) *S. acuminatus* stage 36; (D) *S. aromothyella* stage 29; and (E) *S. berthae* stage 38. References: as, arcus subocularis; ca, capsula auditiva; ci, cartilago infrarostralis; cm, cartilago meckeli; cqa, commissura quadratocranialis anterior; ct, cornu trabeculae; fcp, foramen caroticum primarium; ff, fenestra frontoparietalis; lpo, larval processus oticus; pa, pars alaris; par, processus articularis; pas, processus ascendens; pc, pars corporis; pdm, processus dorsomedialis; pif, pituitary fenestra; pmq, processus muscularis; ppd, processus posterior dorsalis; pq, processus quadratoethmoidalis; pra, processus retroarticularis; pvm, processus ventromedialis; tma, taenia tecti marginalis; tp, tectum parietale; ts, tectum synoticum; ttm, taenia tecti medialis. Bar = 1 mm.

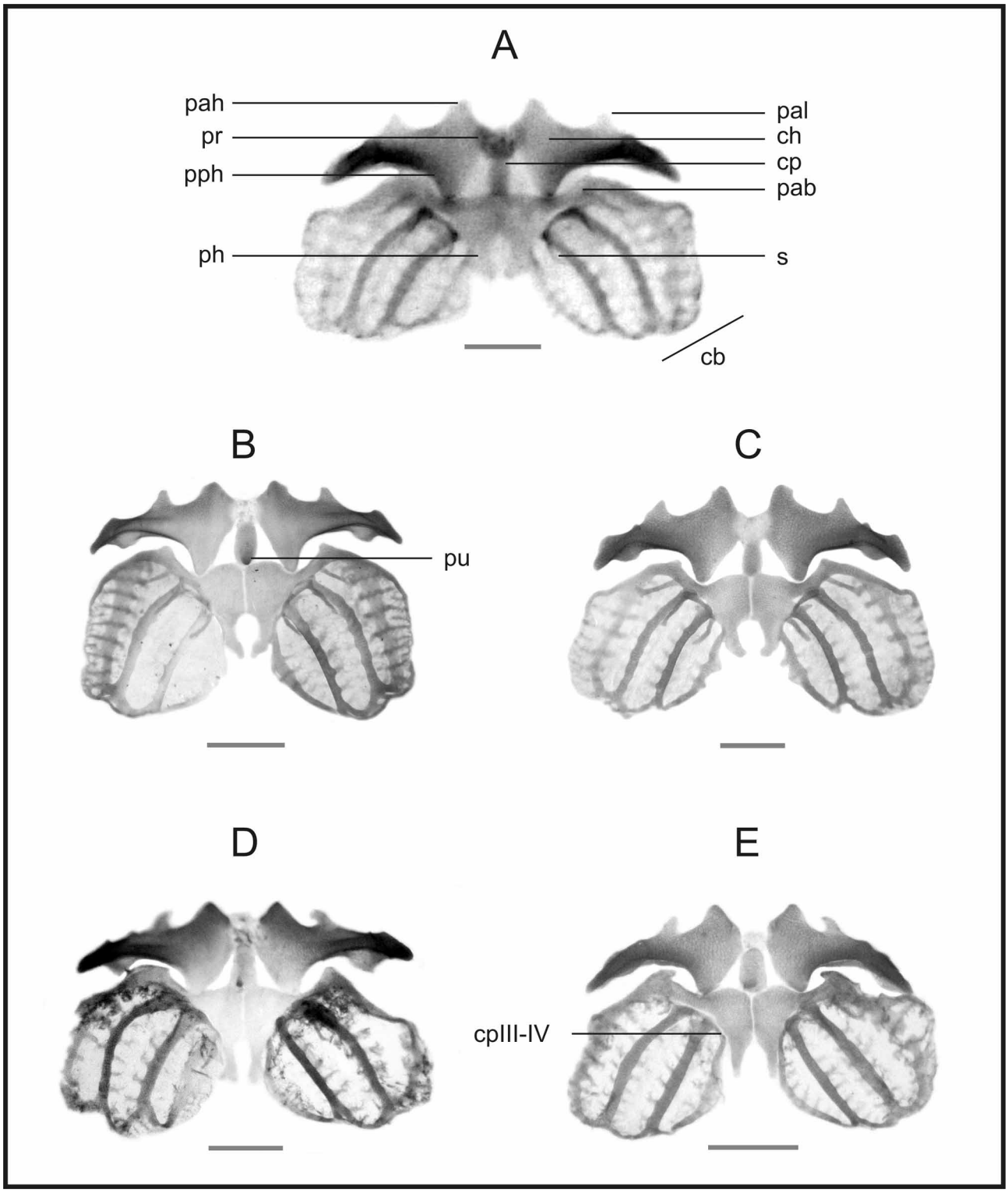


**FIGURE 8.** Hyobranchial apparatus of *Scinax* species, ventral view. (A) *S. uruguayus* stage 36, (B) *S.* aff. *pinima* stage 35, (C) *S. acuminatus* stage 36, (D) *S. aromothyella* stage 29, and (E) *S. berthae* stage 38. References: cb, ceratobranchialia; ch, ceratohyale; cp, copula II; cpIII–IV, commissura proximalis ceratobranchialia III–IV; pab, processus anterior branchialis; pah, processus anterior hyalis; pal, processus anterolateralis hyalis; ph, planum hypobranchiale; pph, processus posterior hyalis; pr, pars reuniens; pu, processus urobranchialis; s, spicula I–IV. Bar = 1 mm.

**TABLE 2.** Cranial muscles of *Scinax uruguayus* larvae at Gosner stage 36.

| Muscle | Origin | Insertion |
|---|---|---|
| Nervus trigeminus (cranial nerve V), mandibular musculature | | |
| Levator mandibulae internus | Processus ascendens, trabecula cranii and capsula auditiva | Cartilago meckeli |
| Levator mandibulae longus superficialis | Arcus subocularis | Cartilago meckeli |
| Levator mandibulae longus profundus | Arcus subocularis | Both muscles insert together in the pars alaris through a common tendon |
| Levator mandibulae externus profundus | Processus muscularis | |
| Levator mandibulae externus superficialis | Processus muscularis | Ligamentum mandibulo-suprarostralis |
| Levator mandibulae articularis | Reduced. On processus muscularis | Cartilago meckeli |
| Levator mandibulae lateralis | Processus articularis | Pars alaris |
| Submentalis | Cartilago infrarostralis | Median raphe |
| Intermandibularis | Well-developed. On cartilago meckeli | Median raphe |
| Mandibulolabialis inferior | Well-developed. On cartilago meckeli | Ventral and lateral on the oral disc |
| Mandibulolabialis superior | Cartilago meckeli | Dorsolateral on the oral disc |
| Nervus facialis (cranial nerve VII), hyoid musculature | | |
| Suspensoriohyoideus | Processus muscularis and arcus subocularis | Ceratohyale |
| Orbitohyoideus | Processus muscularis | Ceratohyale |
| Suspensorioangularis | Processus muscularis | Cartilago meckeli |
| Quadratoangularis | Anteroventral on the palatoquadrate | Cartilago meckeli |
| Hyoangularis | Ceratohyale | Cartilago meckeli |
| Interhyoideus | Ceratohyale | Median raphe |
| Interhyoideus posterior | Diffuse. Ventral wall of the branchial chamber. Some fibers lay anterior to the m. interhyoideus | |
| Diaphragmatopraecordialis | Medial to the m. interhyoideus posterior | Pericardium |
| Nervus glossopharyngeus (cranial nerve IX), branchial musculature | | |
| Levator arcuum branchialium I | Arcus subocularis | Ceratobranchiale I and commissura terminalis I |
| Subarcualis rectus I | Dorsal head on ceratobranchiale I. Ventral head on ceratobranchiale II | Ceratohyale |
| Nervus vagus (cranial nerve X), branchial musculature | | |
| Constrictor branchialis II | Ceratobranchiale I | Commissura terminalis I |
| Constrictor branchialis III | Ceratobranchiale II | Commissura terminalis II |
| Constrictor branchialis IV | Ceratobranchiale III | Commissura terminalis III |
| Diaphragmatobranchialis | Peritoneal wall | Ceratobranchiale III |
| Levator arcuum branchialium II | Arcus subocularis | Commissura terminalis I |
| Levator arcuum branchialium III | Lateral on capsula auditiva and larval processus oticus | Commissura terminalis III |
| Levator arcuum branchialium IV | Ventral on capsula auditiva | Ceratobranchiale IV |
| Subarcualis obliquus II | Double on ceratobranchialia II and III | Processus urobranchialis |
| Subarcualis rectus II–IV | Ceratobranchiale IV | Ceratobranchiale II |
| Tympanopharyngeus | M. levator arcuum branchialium IV | Pericardium posterior to planum hypobranchiale |
| Dilatator laryngis | Capsula auditiva | Larynx |
| Constrictor laryngis | Sphincter-like around the larynx | |

continued next page

**TABLE 2.** (continued)

| Muscle | Origin | Insertion |
|---|---|---|
| Nervus hypoglossus (spinal nerve II), hypobranchial musculature | | |
| Geniohyoideus | Planum hypobranchiale between ceratobranchialia III and IV | Cartilago infrarostralis |
| Rectus cervicis | Peritoneal wall | Ceratobranchiale III |

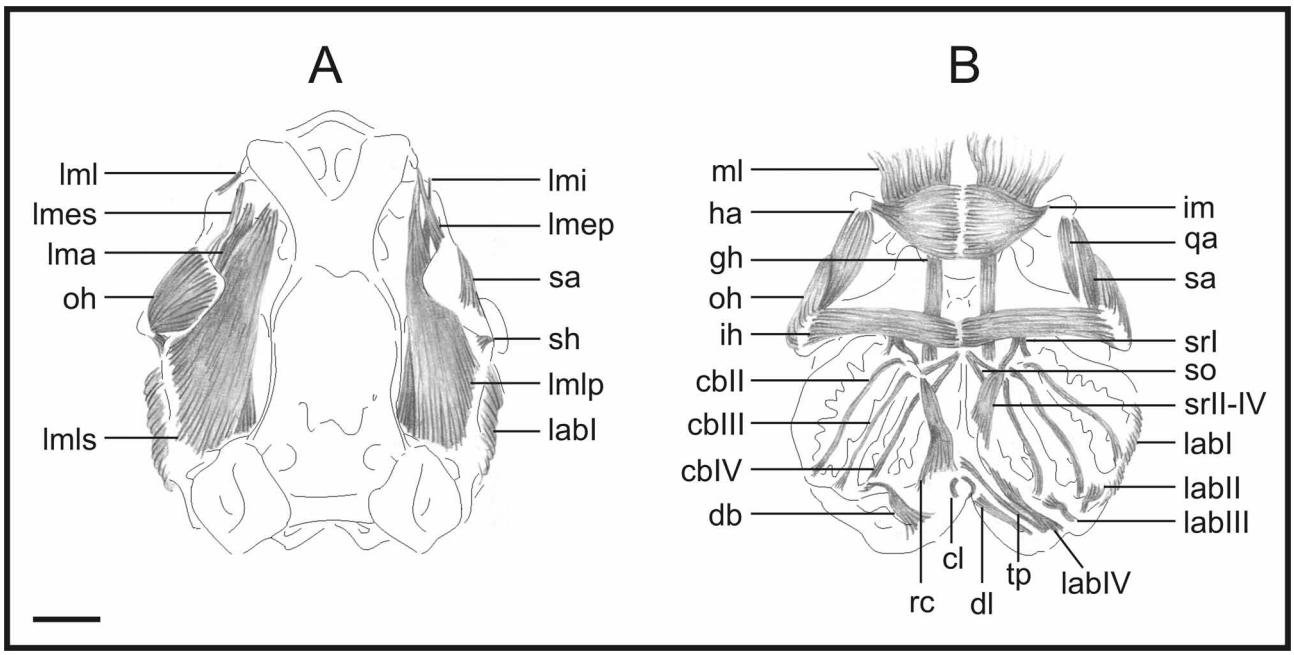


**FIGURE 9.** Cranial muscles of *Scinax uruguayus* stage 35. (A) Dorsal and (B) ventral views. References: cbII–IV, constrictor branchialis II–IV; cl, constrictor laryngis; db, diaphragmatobranchialis; dl, dilatator laryngis; gh, geniohyoideus; ha, hyoangularis; ih, interhyoideus; im, intermandibularis; 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; so, subarcualis obliquus; srI–IV, subarcualis rectus I–IV; tp, tympanopharyngeus. Bar = 1 mm.

## Discussion

The variation in larval characters of *Scinax* species studied herein is congruent with current phylogenetic arrangements based on molecular, chromosomal, and morphological data of adult and larvae (Faivovich 2002; Faivovich *et al*. 2005; Wiens *et al*. 2010). Available data about *Scinax* tadpoles are summarized in Appendix I. The keratinized spurs behind the lower jaw sheaths were considered absent in the *S. catharinae* clade (Faivovich 2002; Conte *et al*. 2007), although reported for *S. berthae* (Spirandeli-Cruz 1991) and *S. luizotavioi* (Bertoluci *et al*. 2007). This character should be carefully revised, as weakly-developed spurs like we observed in *S. aromothyella* and *S. berthae* can be easily overlooked. The buccopharyngeal cavity of the *S. catharinae* group shares numerous characters with other *Scinax*, such as the absence of lingual papillae, scarce or absent buccal roof papillation, presence of postnarial papillae, one pair of lateral ridge papillae and of major infralabial papillae with pustulations or low papillae (Spirandeli-Cruz 1991; Faivovich *et al*. 2005; Conte *et al*. 2007; Vera Candioti 2007 and references therein). Additionally, *S. aromothyella* and *S. berthae* have a poorly-defined buccal floor arena with a conspicuous pair of wide, multifid papillae at the level of the buccal pockets, prenarial arena with pustulations, and small lateral ridge papillae (see also Spirandeli-Cruz 1991). Larvae of *S. catharinae* possess some similar traits, but prepocket and buccal floor arena papillae are numerous (Spirandeli-Cruz 1991; Conte *et al*. 2007).

Previous descriptions of the larval skeleton are not available for the *Scinax catharinae* group. Those of *S. aromothyella* and *S. berthae* are very alike and share several traits, such as the long and slightly divergent free portion of the cornua trabeculae, the long and thin processus articularis, the wide and rounded processus muscularis, the tripartite cartilago suprarostralis, slender lower jaw cartilages, a medially directed processus anterolateralis and ceratobranchialia III and IV joined by commissurae proximales. Regarding cranial muscles, in *S. aromothyella* the m. subarcualis rectus I is composed by three slips, as in some species of the *S. ruber* clade not assigned to groups and also in several other hylid genera (Haas 2003; Vera Candioti 2007 and references therein). The m. tympanopharyngeus is clearly distinguishable from the m. levator arcuum branchialium IV in all studied species, a frequent trait within Hylidae (Haas 2003; Alcalde 2005; Vera Candioti 2007).

In the *Scinax ruber* clade, the group of *S. uruguayus* presents as a distinctive feature keratinized sheets on both sides of the lower jaw sheath (Faivovich *et al*. 2005; present work, Fig. 4). These sheets were observed in larvae of *Kassina senegalensis* (Hyperoliidae), *Trichobatrachus robustus* (Arthroleptidae), and species of the *Lithobates pipiens* group (Ranidae), and are apparently related with the presence of robust jaw sheaths (for review see Altig 2006). Keratinized spurs behind the lower jaw sheath are present in both species of the *S. uruguayus* group examined herein, and also in *S. pinima* (see Kolenc *et al*. 2003). This character is shared with some species of the *S. catharinae* group and the *S. ruber* clade except for the *S. rostratus* group (Faivovich 2002). The characteristic large and widely-spaced ventral marginal papillae in the oral disc of the *S. uruguayus* group (Faivovich *et al*., 2005), are present also in the third and undescribed species of it, *S.* aff. *pinima*. In this species and *S. uruguayus*, distinctive traits in the buccal cavity are the multifid and overlapping infralabial papillae, large lateral ridge papillae, and well-defined buccal floor arena and ventral velum. Several musculoskeletal features seem to be also characteristic of the *S. uruguayus* group, e.g., cornua trabeculae with short and wide free portions, processus articularis short and wide, processus muscularis narrow and directed anteriorly, cartilago suprarostralis forming a single structure, lower jaw cartilages massive, ceratobranchialia II–IV continuous with the planum hypobranchiale, and m. subarcualis rectus I formed by two slips.

A monophyletic clade joins *Scinax acuminatus* with the *S. rostratus* group, being shared larval characters the terminal oral disc with mental gap, tooth row P3 placed on the margin of the disc and about 1/2 the length of P2, and intestinal coiling axis subparallel to the main body axis (Faivovich 2002). However, there are conspicuous differences between these species, i.e., lateral gaps in the oral disc, labial arm present, length of P3 1/4 the length of P2, and spurs absent in the *S. rostratus* group. Some other traits of *S. acuminatus* are shared with *S. boulengeri*, like the straight labial teeth with cusps weakly developed or absent, the cornua trabeculae with long and narrow free portions, the processus muscularis acute and posteriorly directed, and mm. subarcualis rectus I with two slips (Fabrezi & Lavilla 1992; Vera Candioti 2007; Vera Candioti & Altig 2010).

Some characters in the *Scinax uruguayus* group resemble those seen in the clade formed by *S. acuminatus* and the *S. rostratus* group, yet different from other *Scinax*, e.g., short P3, the coiling of the gut, and the configuration of the cartilago suprarostralis. In addition, the lower jaw cartilages are massive in the *S. uruguayus* group and in *S. boulengeri* (Vera Candioti 2007). The varied oral disc configurations in these species (e.g., typical papillar margin / mental papillae large and widely spaced / mental gap; P3 within the oral disc margin / aligned with the oral disc margin / or on a labial arm —sensu McDiarmid & Altig 1989–1990) raise several questions on the different developmental processes involved. Their study would help to establish relationships between these species and also with related taxa presenting similar features (e.g., *S. boesemani* with labial arm plus mental papillae; de Sá *et al*. 1997).

Among those species of the *Scinax ruber* clade yet not assigned to any group an important variation is observed in the length of tooth rows and oral disc papillation (e.g., Bokermann 1967; Cardoso & Sazima 1980; Wogel *et al*. 2000; Pugliese *et al.* 2004; Vera Candioti 2007). Labial teeth have spatulate, convex heads with numerous marginal cusps (Vera Candioti 2007, and references therein), similar to those seen in the *S. catharinae* clade and the *S. uruguayus* group. Buccal cavity features resemble species of the *S. catharinae* clade; *S. granulatus* is distinctive within *Scinax* by having a well-defined buccal roof arena (see Vera Candioti 2007). Variable features of larval skeletons include the shape of the cartilagines suprarostrales, size and shape of the processus articularis, presence of operculum and processus posterolateralis in the capsulae auditivae, presence of copula I, and the type of junction between ceratobranchialia and planum hypobranchiale, among others (Fabrezi & Vera 1997; Haas 1996; 2003; Alcalde & Rosset 2003; Vera Candioti 2007). Finally, muscular configuration is almost identical between species, excepting the m. subarcualis rectus I that has two or alternatively three slips (Alcalde 2005; Vera Candioti 2007).

Our knowledge about evolutionary relationships between *Scinax* species is still preliminary. The *S. uruguayus* group emerge as peculiar, with many characteristic features which may be not shared with other *Scinax*, and others relating it to *S. acuminatus* and the *S. rostratus* group. Future work should address the description of the internal larval morphology in additional species of the scarcely studied *S. catharinae* clade, especially the phytotelm-dwelling tadpoles of the *S. perpusillus* group, from which virtually nothing is known. The broad variation in the larval morphology and ecology of *Scinax* provides an exceptional tool for study of the evolution of this species-rich genus of frogs.

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**APPENDIX I.** Summary of research on internal morphology of the genus *Scinax*.

| Species | Oral cavity | Chondrocranium | Cranial muscles |
|---|---|---|---|
| *Scinax catharinae* clade, *S. catharinae* group | | | |
| *S. aromothyella* | This work | This work | This work |
| *S. berthae* | Spirandeli-Cruz 1991; This work | This work | This work |
| *S. catharinae* | Spirandeli-Cruz 1991; Conte *et al*. 2007 | Unknown | Unknown |
| *Scinax ruber* clade, *S. rostratus* group | | | |
| *S. boulengeri* | Vera Candioti 2007 | Vera Candioti 2007 | Vera Candioti 2007 |
| *Scinax ruber* clade, *S. uruguayus* group | | | |
| *S.* aff. *pinima* | This work | This work | This work |
| *S. uruguayus* | This work | This work | This work |
| *Scinax ruber* clade, unassigned to species groups | | | |
| *S. acuminatus* | Sandoval 2000; This work | Fabrezi & Lavilla 1992; This work | This work |
| *S. fuscovarius* | Spirandeli-Cruz 1991; Echeverría & Montanelli 1992; Fabrezi & Vera 1997 | Fabrezi & Vera 1997 | Unknown |
| *S. granulatus* | Echeverría 1997 | Alcalde & Rosset 2003 | Alcalde 2005 |
| *S. nasicus* | Fabrezi & Vera 1997; Vera Candioti *et al*. 2004 | Vera Candioti 2007 | Vera Candioti *et al*. 2004; Alcalde 2005; Vera Candioti 2007 |
| *S. ruber* | Unknown | Haas 1996, 2003 | Haas 1996, 2003 |
| *S. similis* | Spirandeli-Cruz 1991 | Unknown | Unknown |
| *S. squalirostris* | Unknown | Alcalde & Rosset 2003 | Alcalde 2005 |

**APPENDIX II.** Geographic coordinates and altitudes of the collection sites of the specimens examined.

| Species | Collection number | Collection site | Coordinates | Altitude (m asl) |
|---|---|---|---|---|
| *S. acuminatus* | MLP DB-5309 | Argentina**,** Corrientes, Estancia El Oscuro, | 29º10’S, 58º33’W | 48 |
| *S. aromothyella* | MLP DB-8789 | Uruguay, Treinta y Tres, Quebrada de los Cuervos | 32º52’S, 54º28’W | 252 |
| *S. berthae* | MLP-4993 | Argentina, Buenos Aires, Camping Municipal de Atalaya | 35º01’S, 57º29W | 10 |
| | MLP DB-8790 | Uruguay, San José, Delta del Tigre | 34º46’S, 56º21’W | 3 |
| *S.* aff. *pinima* | MLP DB-4294, 5595 | Argentina, Misiones, National Route Nº 12, Km 1329 | 27º 26’S, 56º01’W | 146 |
| *S. uruguayus* | MLP DB-8792 | Uruguay, Treinta y Tres, Quebrada de los Cuervos | 32º58’S, 54º27’W | 147 |
| | MLP DB-8791 | Uruguay, Maldonado, Sierra de Carapé, Route 109 | 34º20’S, 54º38’W | 330 |

**Appendix III.** (A) Summary of qualitative characters scored on five species of *Scinax* included in this study; (B) Matrix.

(A)

*Oral disc*

(1) Mental region. 0: papillae like in other regions of the lower lip; 1: papillae large and widely spaced, differentiated from those of the rest of the lower lip; 2: without papillae.
(2) LTRF. 0: 2(2)/3(1); 1: 2(2)/3.
(3) Labial teeth. 0: multicusped; 1: cusps reduced or absent
(4) Keratinized sheets ventrolateral to the lower jaw sheath. 0: absent; 1: present.

*Buccal cavity*

(5) Spurs. 0: well developed and colored; 1: weak and not colored.
(6) Main infralabial papillae. 0: short and simple; 1: large, wide, multifid and overlapping each other at the midline.
(7) Buccal floor arena. 0: poorly defined; 1: defined by numerous (> 8) buccal floor arena papillae.
(8) Median notch. 0: present; 1: absent.
(9) Postnarial papillae. 0: reduced or absent; 1: long and pustulate; 2: narial ridges.
(10) Lateral ridge papillae. 0: short and simple; 1: wide, flat and pustulate.

*Skeleton*

(11) Cartilago suprarostralis. 0: tripartite; 1: partes corpores and alares fused.
(12) Processus articularis. 0: with three processes; 1: with two processes.
(13) Processus quadrato-ethmoidalis. 0: present; 1: absent.
(14) Processus muscularis. 0: narrow, rounded, and directed anteromedially; 1: narrow, triangular, and directed posteromedially; 2: wide, rounded, and directed medially.
(15) Processus anterolateralis of the ceratohyale. 0: directed anteriorly; 1: medially curved.
(16) Connection between planum hypobranchiale and ceratobranchialia II–IV. 0: synchondrotic; 1: syndesmotic.
(17) Commissura proximalis between ceratobranchialia III and IV. 0: absent; 1: present.

*Musculature*

(18) Ramus mandibularis of the trigeminus nerve. 0: dorsal to all levator muscles; 1: ventral to m. levator mandibulae externus superficialis.
(19) Origin of the m. geniohyoideus. 0: planum hypobranchiale between ceratobranchialia III and IV; 1: posterior to the ceratobranchiale IV-planum hypobranchiale junction.
(20) M. subarcualis rectus I. 0: with two slips on ceratobranchialia I and II; 1: with three slips on ceratobranchialia I, II and III.

(B)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. uruguayus* | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *S.* aff. *pinima* | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *S. acuminatus* | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 |
| *S. aromothyella* | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 1 | 1 | 0 | 1 | 1 |
| *S. berthae* | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 1 | 1 | 0 | 0 | 0 |