

EXTERNAL MORPHOLOGY, CHONDROCRANIUM, CRANIAL MUSCLES, AND BUCCOPHARYNGEAL FEATURES OF TADPOLES OF *PLEURODEMA THAUL* (ANURA: LEIUPERIDAE): A COMPARISON WITH *P. BUFONINUM*

DIEGO A. BARRASSO^{1,4}, LEANDRO ALCALDE², LIZA B. MARTINAZZO³, AND NÉSTOR G. BASSO³

¹Laboratorio de Ecología Molecular, Centro Regional de Estudios Genómicos, Universidad Nacional de La Plata, Avenida Calchaquí km 23.5, Piso 4 (CP1888) Florencio Varela, Buenos Aires, Argentina and CONICET

²Área Sistemática, Sección Herpetología, Instituto de Limnología "Dr. Raúl A. Ringuelet" (ILPLA-CONICET), Avenida Calchaquí km 23.5 (CP1888), Florencio Varela, Buenos Aires, Argentina

³Centro Nacional Patagónico (CENPAT-CONICET), Bulevar Brown 2915 (CP9120), Puerto Madryn, Chubut, Argentina

ABSTRACT: This article describes the external and buccopharyngeal morphology, chondrocranium, and cranial muscles in tadpoles of *Pleurodema thaul* from Argentina. Further comparison was made with larvae of other *Pleurodema* species, particularly *P. bufoninum*. The larvae of *P. thaul* possess: (1) tooth row formula 2(2)/3(1), (2) single to double row of marginal papillae with wide rostral gap, (3) presence of long processus pseudopterygoideus, (4) pars alaris and pars corpora of the cartilago suprarostralis joined by both proximal and distal connections, and (5) larval processus oticus present. In the light of the available evidence, the larvae of *Pleurodema* are characterized by (1) medium size (total length = 35.4–48.7 mm); (2) medium-sized tail (0.63 times of total length); (3) rounded snout; (4) nostrils closer to the eyes than to the snout; (5) vent tube opening medial; (6) tail tip rounded; and (7) oral disc subterminal with angular constrictions and rostral gap present. In Leiuperidae, the cartilaginous distal connection between pars alaris and pars corpora of the cartilago suprarostralis is present only in *Pleurodema*. In addition, all *Pleurodema* studied to date have processus pseudopterygoideus (shared with *Eupemphix* and *Physalaemus marmoratus*). Finally, within *Pleurodema*, the larval processus oticus is present only in *P. thaul* and *P. bufoninum*.

Key words: Buccal papillation; Chondrocranium; Cranial muscles; External morphology; Larvae; Leiuperidae

THE GENUS *Pleurodema* currently contains 15 species; 10 of them occur in Argentina, 3 of which are distributed in the Patagonian region (Cei, 1980; Weigandt et al., 2004; Ferraro and Casagrande, 2009; Frost, 2011). Although *Pleurodema thaul* (Lesson, 1826) has a wide altitudinal and latitudinal range in Chile (Cei, 1962; Correa et al., 2007; Correa Quezada et al., 2010), in Argentina it is restricted mainly to the areas of *Nothofagus* forest in the southern provinces of Chubut, Neuquén, and Rio Negro (Úbeda, 1998, 2001). There are only a few records in the ecotone of this biome with the Patagonian steppe, at localities where it can be found in sympatry with *Pleurodema bufoninum* (Weigandt et al., 2004; Ferraro and Casagrande, 2009).

The phylogenetic position of *Pleurodema* was addressed by many precladistic works (i.e., Parker, 1927; Dunn, 1949; Heyer, 1975). *Pleurodema* was then considered to be related

either to *Edalorhina* and *Physalaemus*, or to *Pseudopaludicola* and *Physalaemus* (Dunn, 1949). The third of the trees constructed by Heyer (1975) shows a group containing *Physalaemus* and *Pleurodema*. More recently, Frost et al. (2006) found *Pleurodema* within the monophyletic Leptodactylidae, which also included *Adenomera*, *Edalorhina*, *Engystomops*, *Eupemphix*, *Hydrolaetare*, *Leptodactylus*, *Lithodytes*, *Paratelmatobius*, *Physalaemus*, *Pseudopaludicola*, *Scythrophrys*, *Somuncuria*, and *Vanzolinius*. Almost simultaneously, Grant et al. (2006) reassigned *Pleurodema* to Leiuperidae, together with *Edalorhina*, *Engystomops*, *Eupemphix*, *Physalaemus*, *Pseudopaludicola*, and *Somuncuria*. Interestingly, both studies found a sister relationship between *Pleurodema* and a clade formed by *Physalaemus* and *Edalorhina*. Correa et al. (2006) analyzed a set of the leiuperids studied by Grant et al. (2006) and recovered *Pleurodema* as the basal taxon of a clade formed by *Edalorhina*, *Pseudopaludicola*, and *Physalaemus*.

⁴ CORRESPONDENCE: e-mail, diegobarrasso@gmail.com

In contrast to the recent advances produced by the DNA-based phylogenetic analyses, the gross morphology of several anuran groups remains poorly studied. As demonstrated by many works (Larson and de Sá, 1998; Faivovich, 2002; Haas, 2003; Pugener et al., 2003; Alcalde, 2007), larval characters are particularly important for providing additional morphological support for most of the recently described clades based on DNA data.

External morphological features were described for the larvae of most species of *Pleurodema* (Fernández and Fernández, 1921; Fernández, 1927; Cei, 1962, 1980; León-Ochoa and Donoso-Barros, 1969–1970; Rada, 1981; Peixoto, 1982; Fabrezi and Vera, 1997; Úbeda, 1998; Nomura et al., 2003; Weigandt et al., 2004; Lynch, 2006; Kolenc et al., 2009). However, the study of internal features remains incomplete: The buccal cavity was described for *P. borellii*, *P. bufoninum*, *P. brachyops*, *P. cinereum*, and *P. nebulosum* (Wassersug and Heyer, 1988), and few aspects of the chondrocranium and related muscles were reported for *P. borellii*, *P. cinereum*, *P. kriegi*, and *P. tucumanum* (Fabrezi and Vera, 1997; Palavecino, 2000; Haas, 2003).

The external larval morphology of *P. thaul* was never described in detail. Using the name *Paludicola bibronii*, Fernández (1927) reported some measurements and spiracle, vent-tube, and oral-disc characteristics of *P. thaul*. More recently, Cei (1962, 1980) and Úbeda (1998) provided a little additional information about the external features of *P. thaul* (see discussion).

The key goals of this project were (1) to provide a detailed description of the external, buccal, chondrocranial, and cranial musculature of the larvae of *P. thaul*; (2) to identify key characters that would be useful for distinguishing between larval stages of *P. thaul* and *P. bufoninum*, the unique other species of *Pleurodema* that, in some regions, may share the same ponds with the former; and (3) to give an account of larval characters that would be useful for distinguishing *Pleurodema* from other leiuperids, as the diagnosis of this genus has been so far based on the adult stage.

MATERIALS AND METHODS

We collected tadpoles of *P. thaul* on 13 January 2008 in a temporary pond at Ñorquinco,

on Neuquén province, Argentina (39°49'51.94"S, 71°16'17.70"W, 1080 meters above sea level [masl], datum = WGS84). We anesthetized the larvae after capture and immediately fixed them in 10% buffered formalin. The tadpoles of *P. bufoninum* were collected near Road No.12, at 75 km of Gobernador Gregores (Santa Cruz province, Argentina, 48°30'30.0"S, 69°40'44.6"W (616 masl), and at Lago Fontana (Chubut, Argentina). To identify the species, we reared some tadpoles of both species until metamorphosis was complete. We identified recently metamorphosed froglets with the use of the external descriptions provided by Cei (1980); the specimens are now in the amphibian collection of the Museo de La Plata (MLP, Buenos Aires, Argentina): MLP.A-5376-77 for *P. thaul* and MLP.A-5378-79 for *P. bufoninum*. Larvae were staged according to Gosner (1960) and are also housed at MLP. We used 10 tadpoles of *P. thaul* (MLP.A-5262) and five of *P. bufoninum* (MLP.A-5261) for the external morphology descriptions (stages 35–40). Following the technique of Taylor and Van Dyke (1985), we double-stained three additional tadpoles of *P. thaul* (MLP.A-5263) and another three of *P. bufoninum* (MLP.A-5112) at Stage 37 in order to study the chondrocranium and associated muscles. We observed the muscles before clearing the specimens, and studied the chondrocranium after clearing them. Additionally, we prepared one Stage 37 specimen of each species for scanning electronic microscope (SEM) examination of the buccal cavity and oral disc features, following Alcalde and Blotto (2006). We took measurements (to the nearest 0.1 mm) under a stereomicroscope. Terminology follows d'Heursel and de Sá (1999) and Haas (1995) for the chondrocranium, Alcalde and Rosset (2003) for the chondrocranial measurements, Haas (2001) for the mandibular muscles, Haas (2003) and Haas and Richards (1998) for the hyobranchial muscles, Schlosser and Roth (1995) for the innervation, Wassersug (1980) for the buccal cavity features, Lavilla (1983) and Van Dijk (1966) for the external morphology, Johnston and Altig (1986) for the oral-disc morphology, and Altig and Johnston (1989) for the tadpole ecomorphological types.

In addition, we choose 25 morphological measurements to be taken externally: total

TABLE 1.—Morphometric measurements (mm) for 10 tadpoles of *Pleurodema thaul* from Ñorquinco, Neuquén, Argentina (MLP.A-5262), and five tadpoles of *Pleurodema bufoninum* from Gobernador Gregores, Santa Cruz, Argentina (MLP.A-5261). Mean, standard deviation, and range given in parentheses. Asterisks* mark statistically significant differences based on Student's *t*-tests ($P < 0.01$).

Character	<i>P. thaul</i>	<i>P. bufoninum</i>
Total length (TL)	44.93 ± 4.0 (35.4–48.7)	42.04 ± 2.32 (40.4–46.1)
Body length (BL)	16.39 ± 1.0 (13.8–17.2)	18.42 ± 1.54 (17.4–21.1)
Tail length (TAL)*	28.52 ± 3.06 (21.6–29.8)	23.58 ± 0.95 (25.0–27.7)
Maximum tail height (MTH)*	11.51 ± 0.80 (9.5–12.3)	9.72 ± 0.91 (8.5–10.8)
Tail-muscle height (TMH)	5.61 ± 0.75 (4.3–6.9)	4.5 ± 1.05 (3.6–6.3)
Tail-muscle width (TMW)	4.10 ± 0.48 (3.0–4.8)	3.38 ± 0.48 (3.0–4.2)
Internarial distance (IND)*	1.35 ± 0.11 (1.2–1.5)	1.62 ± 0.13 (1.5–1.8)
Interorbital distance (IOD)*	5.55 ± 0.38 (4.8–5.9)	4.82 ± 0.43 (4.5–5.5)
Body maximum width (BMW)	11.32 ± 1.06 (8.7–12.5)	11.96 ± 1.0 (13.5–10.8)
Body width at nostrils (BWN)	6.32 ± 0.64 (5.2–7.2)	7.08 ± 1.08 (6.4–8.8)
Body width at eye level (BWE)	9.39 ± 0.90 (7.5–10.5)	9.68 ± 1.04 (8.2–11.1)
Body maximum height (BMH)	10.21 ± 0.78 (8.6–11.2)	9.68 ± 0.89 (8.5–10.9)
Rostrospiracular distance (RSD)	10.33 ± 0.98 (8.3–11.4)	10.38 ± 0.96 (8.5–10.9)
Frontonasal distance (FN)	2.03 ± 0.25 (1.5–2.4)	2.04 ± 0.23 (1.8–2.4)
Eye–nostril distance (END)*	1.27 ± 0.07 (1.2–1.4)	1.5 ± 0.07 (1.4–1.6)
Nostril major axis (N)	0.48 ± 0.06 (0.5–0.6)	0.48 ± 0.08 (0.4–0.6)
Eye diameter (E)	2.05 ± 0.21 (1.6–2.3)	1.48 ± 0.05 (1.8–1.9)
Extranasal distance (EN)	2.31 ± 0.14 (2.0–2.5)	2.5 ± 0.17 (2.2–2.6)
Intraocular distance (IO)	2.90 ± 0.13 (2.7–3.0)	2.74 ± 0.60 (2.3–3.8)
Oral-disc width (OD)*	3.89 ± 0.37 (3.1–4.4)	3.06 ± 0.32 (2.8–3.6)
Dorsal-gap length (DG)	2.12 ± 0.25 (1.7–2.6)	1.88 ± 0.33 (1.7–2.4)
Dorsal-fin height (DFH)	3.55 ± 0.31 (3.0–4.1)	3.22 ± 0.57 (2.8–4.1)
Ventral-fin height (VFH)	3.48 ± 0.47 (2.6–4.1)	3.24 ± 0.71 (4.3–2.4)
Snout–dorsal-fin-origin distance (SDF)*	10.07 ± 0.84 (8.6–11.3)	13.42 ± 1.0 (12.4–15.0)

length (TL), body length (BL), tail length (TAL), maximum tail height (MTH), tail-muscle height (TMH), tail-muscle width (TMW), internarial distance (IND, with modifications: measured between the internal edges of narial apertures), interorbital distance (IOD, with modifications: measured between the external edges of pupils; all them from Altig and McDiarmid, 1999); body maximum width (BMW), body width at nostrils (BWN), body width at eye level (BWE), body maximum height (BMH), rostrorspiracular distance (RSD, measured horizontally from the tip of the snout to the posterior edge of the spiracular tube), frontonasal distance (FN, from the tip of the snout to the anterior edge of nostrils), eye–nostril distance (END, from the posterior edge of nares to the anterior edge of eyes), nostril major axis (N), eye diameter (E), extranasal distance (EN, distance between external edges of nares), intraocular distance (IO, distance between interior edges of eyes), oral-disc width (OD), and dorsal-gap length (DG; taken from Lavilla and Scrocchi, 1986); dorsal-fin height (DFH) and ventral-fin height (VFH), both measured at a position coincident with MTH

(after Kolenc et al., 2009), and the new variable snout to dorsal-fin-origin distance (SDF, measured in lateral view from the tip of the snout to the anterior edge of dorsal fin). All measurements are in mm and are presented as mean value ± 95% confidence limits. We compared each variable between species with the use of a Student's test (see details in Table 1). In the Results section, we describe all features for the larvae of *P. thaul*; the traits that present different states in *P. bufoninum* follow the description of the corresponding states of *P. thaul*.

RESULTS

External Morphology

Pleurodema thaul (MLP.A-5262).—Tadpoles Type IV, exotrophic, lentic, and benthic larvae (Fig. 1); total length 44.93 ± 4.0 mm; body ovoid, slightly depressed (BMH/BMW = 0.90 ± 0.05); body length about 36% of total length (44% in *P. bufoninum*); body shape oval in dorsal view with maximum width at the posterior portion of the abdominal region. Snout rounded in dorsal and lateral

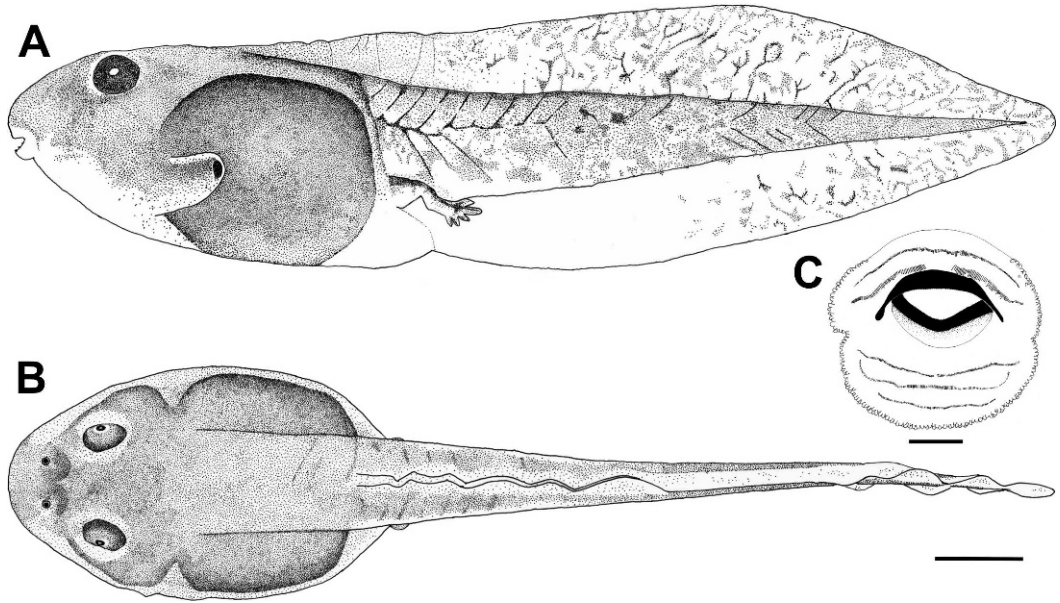


FIG. 1.—External morphology of the tadpole of *Pleurodema thaul* at Stage 37. (A) Lateral, (B) dorsal views (scale bar = 5 mm), and (C) oral disc (scale bar = 1 mm).

profile. Eyes relatively small ($E = 2.05 \pm 0.21$), dorsally located and larger than interorbital distance ($IOD/E = 2.73 \pm 0.28$), eye diameter represents 21–22% of body width at eye level ($BWE = 9.39 \pm 0.9$). Interorbital distance represents 59% of body width at eye level. Nostrils circular, dorsal, closer to eye than tip of snout. Nostril diameter ($N = 0.48 \pm 0.06$) 17–18% of body width at nostril level ($BWN = 6.32 \pm 0.64$). Frontonasal distance ($FN = 2.03 \pm 0.25$) larger than eye–nostril distance ($END = 1.29 \pm 0.07$). Internarial distance ($IND = 1.35 \pm 0.12$) smaller than interorbital distance ($IOD = 5.55 \pm 0.38$). Extranasal distance ($EN = 2.31 \pm 0.14$) smaller than interocular distance ($IO = 2.90 \pm 0.13$). Spiracle sinistral, slightly separated from the lateral body wall at its distal end (Fig. 1A). Spiracular tube caudally projected, slightly curved toward the dorsum, and laterally opened. Spiracular opening rounded, its diameter smaller than the tube diameter. Rostrospiracular distance ($RSD = 10.33 \pm 0.98$) 63% of body length. Vent tube medial, attached to the ventral fin; tip of tube does not overpass the free margin of the ventral fin. The tail is medium-sized ($TAL = 28.52 \pm 3.06$), represents 63–64% of total length (56%

in *P. bufoninum*). The maximum tail height ($MTH = 11.51 \pm 0.80$) occurs in its middle third, and both fins are slightly higher than the body height ($MTH/BMH = 1.13 \pm 0.08$). Both fins have regularly convex margins, and their maximum height is almost equal ($DFH/VFH = 1.03 \pm 0.10$). The dorsal fin extends slightly onto the body ($SDF/BL = 0.61 \pm 0.04$; in *P. bufoninum*: $SDF/BL = 0.73 \pm 0.04$). The tail axis is straight, and the tail smoothly stretches towards the tip in its last half, ending rounded. Caudal-musculature width ($TMW = 4.10 \pm 0.48$) is narrower than the caudal-musculature height at the beginning of the tail ($TMH = 5.61 \pm 0.75$), the latter representing about 54% of body height. Myomeres noticeable but slightly obscured by pigmentation, with the most posterior ones more diffuse.

Oral disc subterminal (Fig. 1A), ventrally positioned, not visible dorsally, and emarginated. Width of disc ($OD = 3.89 \pm 0.37$) about 0.34 times of maximum body width ($OD/BMW = 0.34 \pm 0.03$). The marginal papillae are single, in alternate disposition laterally, and double in mental region (Fig. 2A), without ventral gap but with medium to large rostral gap ($DG/OD = 0.55 \pm 0.05$; in *P. bufoninum*:

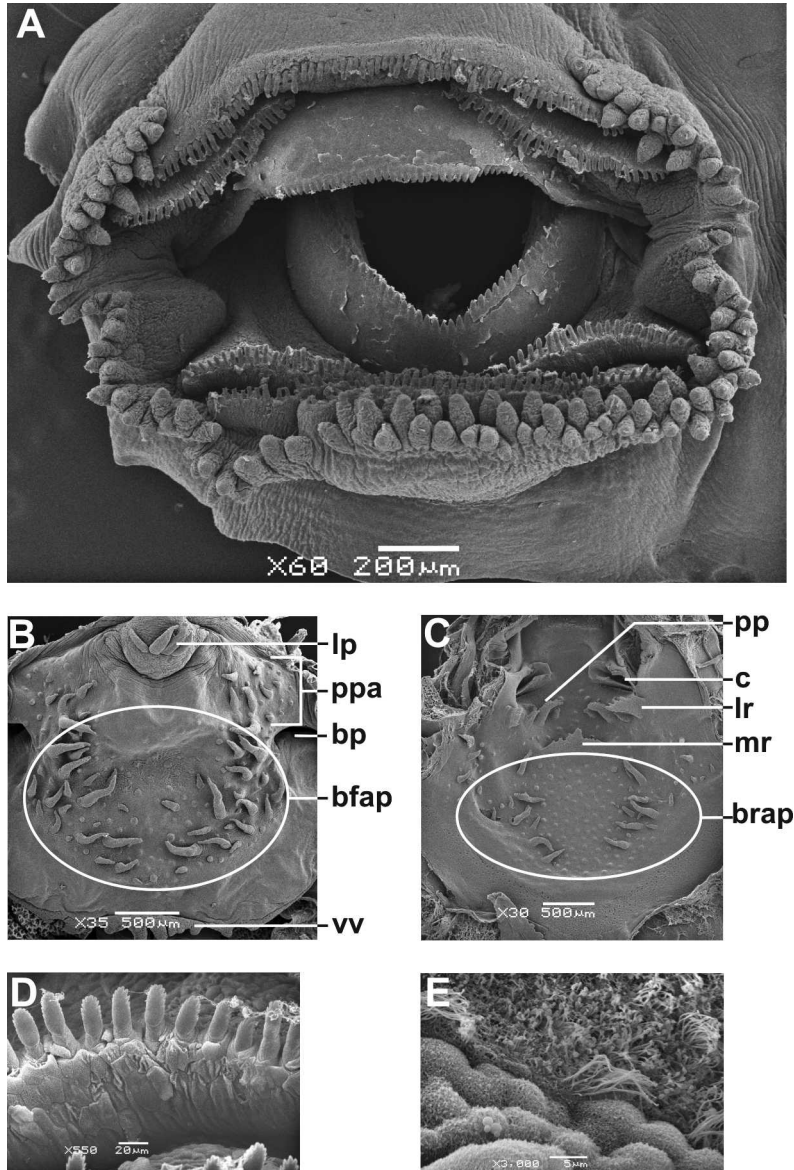


FIG. 2.—Scanning-electron-microscope photographs of the oral disc (A), buccal floor (B), and roof (C) papillation of *Pleurodema thaul* at Stage 37. (D) Shows keratodonts of the mental rows and (E) shows the ciliated epithelium of the left choana. Abbreviations: bfap = buccal floor papillae; bp = buccal pocket; brap = buccal roof papillae; c = choana; lp = lingual papillae; lr = lateral ridge; mr = median ridge; pp = postnarial papillae; ppa = prepocket papillae; vv = ventral velum.

DG/OD = 0.61 ± 0.06). Intramarginal papillae scarce or absent in supra-angular regions. The keratodonts are spatulated and serrated, arranged in a tooth-row formula 2(2)/3(1) (Fig. 1C). The relative lengths of the rows are $A_2 > A_1$ and $P_1 > P_2 > P_3$. Both arch-shaped upper jaw and V-shaped

lower jaw sheaths are robust and bear marginal serrations (Fig. 2A).

In preservative, the body exhibits a brown dorsal region with irregularly scattered dark-brown blotches; the flanks are of the same color as the dorsal region. Intestinal mass appears as a dark area visible through the

unpigmented skin of the ventral region. The dorsal and ventral fins are mostly transparent and deeply scattered with brown blotches. The muscular portion of the tail is brown and pigmented with some darker blotches, except in ventral anterior region near the body-tail junction, in which pigment is absent. Hyobranchial apparatus is visible through the transparent skin of the gular region.

Buccal Cavity Morphology

Buccal floor (Fig. 2B) with two pairs of long, simple infralabial papillae (not visible in Fig. 2B) and four long, simple lingual papillae. Buccal floor bears 16–18 pairs of large papillae that form two arches, which are almost parallel to the midline. There are 30–40 pustulations in the central area of the buccal floor. The well-marked and arched buccal pockets are anteriorly limited by 14 pairs of pustulations and 6–8 pairs of long prepocket arena papillae. Ventral velum with serrated margins and secretory pits.

Buccal roof with a transverse crest of small pustulations in the prenarial area (Fig. 2C). The transverse and slender choana are limited by both anterior and posterior elevated and serrated margins. The exposed epithelium of the medial region of the choana is completely covered by cilia and mucus (Fig. 2E). The postnarial area has 8–10 randomly distributed pustulations and three large and serrated papillae obliquely arranged from midanterior to lateroposterior directions, with the medial one the largest. The well-developed, almost triangular and serrated medial ridge is smaller than the rectangular-shaped and distally serrated lateral ridges. The buccal roof has nine pairs of long conical papillae aligned in parallel along each side of the midline. The central area of the buccal roof is completely covered by small pustulations. The dorsal velum bears secretory pits.

Chondrocranium and Cranial Muscles

Neurocranium oval (width/length = 0.91) and depressed (height/width = 0.30), with greatest width at level of the arcus subocularis. The tetrapartite cartilage suprarostralis has the pars corpora medially joined by a cartilaginous distal bridge (Fig. 3D). Similar proximal and distal bridges connect the pars

corpora with the respective pars alaris at each side. The cornu trabeculae diverges anterolaterally from the planum ethmoidale and comprises 25% of chondrocranial length; they are uniformly wide and bear a well-developed processus lateralis. Both the lamina orbitonasalis and the septum nasi are present at the studied stages. The fenestra frontoparietalis is limited posteriorly by the tectum synoticum, laterally by the taenia tecti marginalis, and anteriorly by the taenia tecti ethmoidalis. The taenia tecti transversalis divides the fenestra into two parts, the frontal and parietal ones, with the latter subdivided into left and lateral fenestrae by the taenia tecti medialis. The cartilaginous lateral walls formed by the cartilagine orbitalis are open at levels of the optic, metoptic, trochlear foramina, and fisura prootica. At the studied stages, the basi cranii is not completely ossified by the presence of a wide pituitary fenestra and a pair of foramina carotica primaria. The capsula auditiva comprises 28% of the neurocranial length and its anterior copula is slightly overlapped with the dorsum of the processus ascendens. The larval crista parotica is well developed. The medial wall of the capsula auditiva is alcian-blue negative and its openings could not be observed. The superior perilymphatic foramen opens in the posterior wall of the capsula. The operculum is present.

The palatoquadrate bears processus articularis quadrati, processus muscularis quadrati, commissura quadrato-cranialis anterior, processus quadrato-ethmoidalis, long processus pseudopterygoideus, processus ascendens, and larval processus oticus. The triangular-shaped processus quadrato-ethmoidalis is slender and narrow (wider in *P. bufoninum*; Fig. 3A,B). The processus ascendens meets the pila antotica just posterior to the oculomotor foramen (intermediate union), forming a straight angle with the cranial floor. The larval processus oticus is uniformly wide between the capsula and the posterior part of the arcus subocularis (in *P. bufoninum* the larval processus oticus is wide near the capsula and slender near the arcus subocularis; Fig. 3A,B).

The lower jaw is composed by cartilagine infraorbitales and cartilago meckeli, the latter with processus retroarticularis, dorsomedialis, and ventromedialis well developed. The

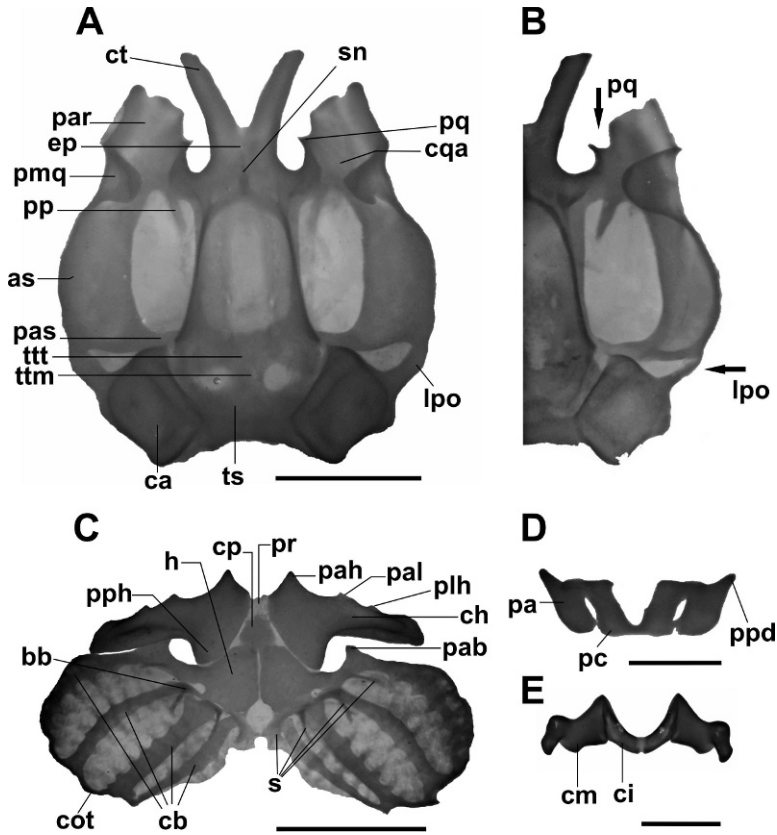


FIG. 3.—Chondrocranium of *Pleurodema thaul* at Stage 37. (A) Dorsal and (B) right half of the chondrocranium of *P. bufoninum* at Stage 37 (note the differences), scale bar = 3 mm; (C) ventral view of hyobranchial apparatus, scale bar = 3 mm; (D) frontal view of cartilago suprarostralis, scale bar = 1 mm; (E) frontal view of cartilago infraostrale and cartilage meckeli, scale bar = 1 mm. Abbreviations: as = arcus subocularis; bb = branchial bridge; ca = capsula auditiva; cb = ceratobranchiales; ch = ceratohyale; ci = cartilago infraostrale; cm = cartilago meckeli; cot = commissura terminalis; cp = copula posterior and processus urobranchialis; cqa = commissura quadrato-cranialis anterior; cs = cartilago suprarostralis; ct = cornua trabeculae; ep = ethmoid plate; h = hypobranchiale; lpo = larval processus oticus; pa = pars alaris; pb = processus anterior branchialis; pah = processus anterolateralis hyalis; par = processus articularis; pas = processus ascendens; pc = pars corporis; plh = processus lateralis hyalis; pmq = processus muscularis quadrati; pp = processus pseudopterygoideus; ppd = processus posterior dorsalis; pph = processus posterior hyalis; pq = processus quadrato-ethmoidalis; pr = pars reuniens; s = spicula I–IV; sn = septum nasi; ttm = taenia tecti medialis; ts = tectum synoticum; ttt = taenia tecti transversalis.

commissura intramandibularis is alcian-blue negative (Fig. 3D,E).

The hyobranchial apparatus lacks copula I. All ceratohyale processes (anterohyal lateral, anterohyal, posterohyal, and articular) are well developed. The ceratohyalia is joined medially by an alcian-blue negative pars reuniens. The copula II bears a short processus urobranchialis. All ceratobranchiales are continuous to the planum hypobranchiale (1 and 2 clearly joined, 3 and 4 by a smooth connection; Fig. 3C). Although the commissura proximalis is absent

in all ceratobranchials, all of ceratobranchials are distally joined by a double distal commissure. The processus branchialis is closed and the four spiculae are well developed. The exoccipital, frontoparietal, parasphenoid, and prootic are the only ossification centers present at the studied stages.

With respect to the cranial muscles, the ramus mandibularis of the nervus trigeminus runs laterally to all mm. (musculi) levatorae mandibulae (see Table 2 for details about origin and insertion of each muscle).

DISCUSSION

The external morphology of *P. thaul* larvae is similar to the descriptions of Fernández (1927), Úbeda (1998), and Cei (1980), with the following exceptions: (1) vent tube opening is medial instead of being projected to the left (Fernández, 1927; Cei, 1962, 1980); (2) tail length represents 63–64% of total length, which differs from the description of Cei (1980); and (3) the LTRF 2(2)/3(1) that we observed agrees with previous descriptions of Fernández and Fernández (1921) and Úbeda (1998). Cei (1980) also mentioned a LTRF 2(2)/3(1), but illustrated it as being 2(1–2)/3(1). The reported maximum total lengths were 60 mm at Stage 37 (Úbeda, 1998), 37 mm (inferred from the figure of Cei, 1980), 32 mm (Fernández, 1927), and 48.7 mm at Stage 40 in this study; these lengths are likely influenced by sample size and environmental circumstances.

The comparison of the external larval traits of *P. thaul* with those previously published for this species and for the larvae of *P. borellii*, *P. bibroni*, *P. brachyops*, *P. bufoninum*, *P. cinereum*, *P. dipolister*, *P. guayape*, *P. kriegi*, *P. marmoratum*, *P. nebulosum*, *P. thaul*, and *P. tucumanum* (Fernández and Fernández, 1921; Fernández, 1927; Cei, 1962, 1980; León-Ochoa and Donoso-Barros, 1969–1970; Rada, 1981; Peixoto, 1982; Fabrezi and Vera, 1997; Úbeda, 1998; Weigandt et al., 2004; Lynch, 2006; Kolenc et al., 2009) produced the following larvae characterization of the genus *Pleurodema*: tadpoles Type IV, exotrophic, lentic and benthic larvae, medium-sized tadpoles (TL = 20.5–63.7mm), with medium-sized tail (50–65% of TL) with rounded tip, rounded snout, dorsal nostrils that are closer to the eye than to the snout, medial vent tube opening (in *P. bibroni* may also open to the right), and subterminal oral disc with LTRF 2(2)/3(1) or 2(2)/2, laterally emarginated and with a wide rostral gap in the marginal papillae. Some features may vary within species (e.g., the narrow gap in P-1 may be present or absent in *P. bufoninum*). We found that two external characters distinguish *P. thaul* from *P. bufoninum*: (1) the vent tube in *P. bufoninum* extended beyond the margin of the ventral fin, but not in *P. thaul*, (2) in both species the dorsal fin originates near of

body–tail junction, but in *P. thaul* is slightly more anterior than in *P. bufoninum*.

The buccal-cavity papillation has been described for *P. bufoninum*, *P. borellii*, *P. brachyops*, *P. cinereum*, and *P. nebulosum* (Wassersug and Heyer, 1988). There are no particular character states in the buccal-cavity features that support the clades proposed by the phylogenetic analysis for *Pleurodema* by Duellman and Veloso (1977). Based on the study of buccal features, Wassersug and Heyer (1988) found three species groups of *Pleurodema*: one formed by *P. borellii*, *P. brachyops*, and *P. bufoninum*; the *P. cinereum* group; and the *P. nebulosum* group. In a more-inclusive context, there are also no buccal cavity features supporting *Pleurodema* with respect to other leiuperid genera (*Euglyptomops*, *Eupemphix*, *Physalaemus*, *Pseudopaludicola*; Wassersug and Heyer, 1988; Nomura et al., 2003; Alcalde et al., 2006; Miranda and Ferreira, 2009; *Edalorhina* and *Somuncuria* remain unstudied).

Comparing the chondrocranium of *P. thaul* to the descriptions by Fabrezi and Vera (1997) and Haas (2003) allowed us to recognize some interesting features that vary within *Pleurodema*: (1) processus anterolateralis of crista parotica short, not forming a larval processus oticus (*P. kriegi*, *P. tucumanum*, *P. borellii*), or larval processus oticus present (*P. bufoninum*, *P. thaul*); (2) the connection between the processus muscularis quadrati and the lateral wall of the ethmoidal region of the neurocranium may be by ligaments (*P. bufoninum*, *P. thaul*), sometimes with a conspicuous larval processus antorbitalis (*P. kriegi*, *P. tucumanum*), or is completely cartilaginous with a low commissura quadrato-orbitalis (*P. borellii*); and (3) the processus pseudoterygoideus is long in some species (*P. bufoninum*, *P. thaul*) but short in others (*P. borellii*, *P. kriegi*, *P. tucumanum*). We found two chondrocranial features that vary between *P. bufoninum* and *P. thaul*: the relative width of the larval processus oticus and the relative size of the processus quadrato-ethmoidalis (see in results and Fig. 3A,B).

Within the Leiuperidae, chondrocranial descriptions are available for the genera *Eupemphix*, *Physalaemus*, and *Pleurodema* (Fabrezi and Vera, 1997; Haas, 2003; Alcalde

TABLE 2.—Origin and insertion of the cranial muscles in tadpoles of *Pleurodema thaul* and *Pleurodema bufoninum* at Stage 37.

Muscle	Origin	Insertion
Nervus trigeminus (cranial nerve V), mandibular musculature		
Levator mandibulae internus	Processus ascendens	Cartilago meckeli
Levator mandibulae longus superficialis	Arcus subocularis	Cartilago meckeli
Levator mandibulae longus profundus	Arcus subocularis	Both muscles insert together in the
Levator mandibulae externus profundus	Processus muscularis quadrati	pars alaris by a common tendon
Levator mandibulae externus superficialis	Processus muscularis quadrati	Cartilago meckeli
Levator mandibulae articularis	Processus muscularis quadrati	Cartilago meckeli
Levator mandibulae lateralis	Processus articularis	Pars alaris
Submentalis	Absent at the studied stages	
Intermandibularis	Cartilago meckeli	Median raphe
Mandibulolabialis inferior	Cartilago meckeli	Oral disc
Mandibulolabialis superior	Absent	
Nervus facialis, (cranial nerve VII), hyoid musculature		
Suspensoriohyoideus	Processus muscularis quadrati and arcus subocularis	Ceratohyale
Suspensorioangularis	Processus muscularis quadrati	Cartilago meckeli
Orbitohyoideus	Processus muscularis quadrati	Ceratohyale
Quadratoangularis	Anteroventral on palatoquadrate	Cartilago meckeli
Hyoangularis lateralis	Ceratohyale	Cartilago meckeli
Hyoangularis medialis	Absent	
Interhyoideus	Ceratohyale	Median raphe
Interhyoideus posterior	Tiny sheet of randomly arranged fibers below the branchial chamber, posterior to m. interhyoideus	
Diaphragmatopraecordialis	On m. interhyoideus posterior	Pericardium
Nervus glossopharyngeus (cranial nerve IX), branchial musculature		
Levator arcuum branchialium I	Arcus subocularis	Ceratobranchial I
Subarcualis rectus I	Dorsal head on ceratobranchial I ventral heads on ceratobranchial II and branchial bridge	Ceratohyale
Constrictor branchialis I	Absent	
Nervus vagus (cranial nerve X), branchial musculature		
Constrictor branchialis II	Proximal on ceratobranchial II	Commissura terminalis I–II
Constrictor branchialis III	Branchial bridge	Commissura terminalis II–III
Constrictor branchialis IV	Proximal on ceratobranchial III	Distal end ceratobranchial III
Diaphragmatobranchialis	Peritoneal wall	Commissura terminalis III–IV
Levator arcuum branchialium II	Arcus subocularis	Commissura terminalis II–III
Levator arcuum branchialium III	Posterolateral on capsula auditiva	Commissura terminalis II–III
Levator arcuum branchialium IV	Posteroventral on capsula auditiva	Ceratobranchial IV
Subarcualis obliquus II	Branchial bridge	Processus urobranchialis
Subarcualis rectus II–IV	Ceratobranchial IV	Branchial bridge
Tympanopharyngeus	m. levator arcuum branchialium IV	Pericardium
Dilatator laryngis	Posteroventral on capsula auditiva	Constrictor laryngis
Constrictor laryngis	Forms an annulus rounding the larynx	
Transversus ventralis IV	Absent	
Nervus hypoglossus (spinal nerve II), hypobranchial musculature		
Geniohyoideus	Hypobranchial at level of ceratobranchial III	Cartilago infarostrale
Rectus cervicis	Peritoneal wall	Branchial bridge

et al., 2006; Prado, 2006; Vera Candioti, 2007). In contrast to most species of *Physalaemus* studied to date, *Pleurodema* and *Eupemphix* share a processus pseudopterygoideus (also present in *Physalaemus*

marmoratus). In addition, the partes alares and corpora of the cartilago supraparostalis of *Eupemphix* and *Physalaemus* lack the distal connection that is present in most *Pleurodema*.

The hyomandibular muscles were described for seven species of *Pleurodema*: *P. bufoninum* and *P. thaul* (this study); *P. diplolister*, only mandibular muscles (Starrett, 1968); *P. borellii*, *P. cinereum*, and *P. tucumanum* (Palavecino, 2000); *P. kriegi* (Haas, 2003). The hyomandibular muscles were also described for six other leiuperids: *Physalaemus fernandezae* (Alcalde et al., 2006); *P. biligonigerus* and *P. cuqui* (Palavecino, 2000); *P. santafecinus* (Vera Candioti, 2007); and *Edalorhina perezii* and *Engystomops pustulosus* (Starrett, 1968). *Engystomops pustulosus* shows the same condition of hyomandibular muscles as the species of *Pleurodema* that we studied. *Pleurodema diplolister* and *Edalorhina perezii* differ from other known leiuperids by the lack of the m. (musculus) levator mandibulae externus superficialis (see Starrett, 1968). Branchial and hypobranchial muscles were described for a few leiuperids, including *P. bufoninum* and *P. thaul* (this study), *P. kriegi* and *P. biligonigerus* (Haas, 2003), *P. fernandezae* (Alcalde et al., 2006), and *P. santafecinus* (Vera Candioti, 2007). These muscles show more variability than the hyomandibular muscles: (1) the second ventral head of the m. subarcualis rectus I and the insertion of the m. subarcualis rectus II–IV originate on ceratobranchiale II (most species), but in *P. thaul* and *P. bufoninum* its origin is displaced towards the branchial bridge formed between ceratobranchiales II and III; (2) the m. subarcualis obliquus II of *Physalaemus fernandezae*, *Pleurodema thaul*, and *P. bufoninum* inserts on the branchial bridge, but in *P. kriegi*, *P. santafecinus*, and *P. biligonigerus* it inserts on the ceratobranchiale II; and (3) the m. rectus cervicis may insert either on the branchial bridge (*P. thaul*, *P. bufoninum*), on branchial process III and medial region of ceratobranchiale IV (*Physalaemus santafecinus*), or at ceratobranchiales II and III (*Physalaemus fernandezae*, *P. biligonigerus*, *Pleurodema kriegi*).

Finally, we wish to remark that the monophyly of the Leiuperidae indicated in the most recent phylogenetic analyses (see Grant et al., 2006) needs to be complemented with the study of additional morphological characters. Knowledge of larval morphology in leiuperids, although described in detail for a

few species (e.g., *P. santafecinus*: Vera Candioti, 2007), is still fragmentary. For instance, there are no published data on the chondrocranium and cranial muscles in *Pseudopaludicola* and *Somuncuria*. In addition, some larval features in the Leiuperidae are highly variable, such as the presence of gaps in the marginal papillation of the oral disc (Nomura et al., 2003; Alcalde et al., 2006; Laufer and Barreneche, 2008; Kolenc et al., 2009). We think that studies to obtain the lacking information should be a priority, and will contribute to a better understanding of the relationships and character evolution of the highly variable traits present in this group of frogs.

Acknowledgments.—We thank three anonymous reviewers and C. Borteiro for valuable comments made on the early version of the manuscript, J. Viale for assistance in the fieldwork, and J.D. Williams (MLP) for allowing the study of comparative material under his care. The authors are grateful to Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) for the continuous support of our research. This work was partially supported by grant PIP 6476 from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and grant BID 1728/OC-AR PICT 506 from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) of Argentina.

LITERATURE CITED

- Alcalde, L. 2007. Desarrollo del Esqueleto Larvario y Musculatura Asociada en el Género *Batrachyla* Bell 1843 (Anura: Neobatrachia) y Sus Relaciones Filogenéticas Interespecíficas. Ph.D. Dissertation, Universidad Nacional de La Plata, Argentina.
- Alcalde, L., and B.L. Blotto. 2006. Chondrocranium, cranial muscles and buccopharyngeal morphology on tadpoles of the controversial leptodactylid frog *Limnomedusa macroglossa* (Anura: Leptodactylidae). *Amphibia-Reptilia* 27:241–253.
- Alcalde, L., and S.D. Rosset. 2003. Descripción y comparación del condrocáneo en larvas de *Hyla raniceps* (Cope, 1862), *Scinax granulatus* (Peters, 1871) y *Scinax squalirostris* (A. Lutz, 1925) (Anura: Hylidae). *Cuadernos de Herpetología* 17:35–51.
- Alcalde, L., G.S. Natale, and R. Cajade. 2006. The tadpole of *Physalaemus fernandezae* (Anura: Leptodactylidae). *Herpetological Journal* 16:203–211.
- Altig, R., and G.F. Johnston. 1989. Guilds of anuran larvae: Relationships among developmental modes, morphologies, and habitats. *Herpetological Monographs* 3:81–109.
- Altig, R., and R.W. McDiarmid. 1999. Body Plan: Development and Morphology. Pp. 24–51 in R.W. McDiarmid and R. Altig (Eds.), *Tadpoles: The Biology of Anuran Larvae*. University of Chicago Press, Illinois, USA.

- Cei, J.M. 1962. Batracios de Chile. Universidad de Chile, Chile.
- Cei, J.M. 1980. Amphibians of Argentina. *Monitore Zoologico Italiano, Italian Journal of Zoology, Monografia* 2.
- Correa, C., A. Veloso, P. Iturra, and M.A. Mendez. 2006. Phylogenetic relationships of Chilean leptodactylids: A molecular approach based on mitochondrial genes 12S and 16S. *Revista Chilena de Historia Natural* 79:435–450.
- Correa Quezada, C., E. Riveros, G. Lobos, and N. Velásquez. 2010. Amphibia, Anura, Leiuperidae, *Pleurodema thaul* (Lesson, 1827): Altitudinal distribution extension and new records from its northern range. *CheckList* 6:10–12.
- Correa, C.L., M. Sallaberry, B.A. González, E.R. Soto, and M.A. Méndez T. 2007. Amphibia, Anura, Leiuperidae, *Pleurodema thaul*: Latitudinal and altitudinal distribution extension in Chile. *CheckList* 3:267–270.
- d'Heursel, A., and R.O. de Sá. 1999. Comparing the tadpoles of *Hyla geographica* and *Hyla semilineata*. *Journal of Herpetology* 33:353–361.
- Duellman, W.E., and A.M. Veloso. 1977. Phylogeny of *Pleurodema* (Anura: Leptodactylidae): A biogeographic model. *Occasional Papers of the Museum of Natural History, The University of Kansas* 64:1–46.
- Dunn, E.R. 1949. Notes on the South American frog genus *Edalorhina*. *American Museum Novitates*, 1–10.
- Fabrezi, M., and R. Vera. 1997. Caracterización morfológica de larvas de anuros del Noreste Argentino. *Cuadernos de Herpetología* 11:37–49.
- Faivovich, J. 2002. A cladistic analysis of *Scinax* (Anura: Hylidae). *Cladistics* 18:367–393.
- Fernández, K., and M. Fernández. 1921. Sobre la biología y reproducción de algunos batracios argentinos I. *Cystignathidae*. *Anales de la Sociedad Científica Argentina* 41:97–139.
- Fernández, K. 1927. Sobre la biología y reproducción de batracios argentinos (segunda parte). *Del Boletín de la Academia Nacional de Ciencias en Córdoba* 29:271–328.
- Ferraro, D.P., and D.M. Casagrande. 2009. Geographic distribution of the genus *Pleurodema* in Argentina (Anura: Leiuperidae). *Zootaxa* 2024:33–55.
- Frost, D.R. 2011. Amphibians species of the world: An online reference Version 5.5. Available at <http://research.amnh.org/herpetology/amphibia/index.html>. American Museum of Natural History, New York, USA.
- Frost, D.R., T. Grant, J. Faivovich, R.H. Bain, A. Haas, C.F.B. Haddad, R.O. de Sá, A. Channing, M. Wilkinson, S.C. Donnellan, C.J. Raxworthy, J.A. Campbell, B.L. Blotto, P. Moler, R.C. Drewes, R.C. Nussbaum, J.D. Lynch, D.M. Green, and W.C. Wheeler. 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History* 297:1–370.
- Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190.
- Grant, T., D.R. Frost, J.P. Caldwell, R. Gagliardo, C.F.B. Haddad, P.J.R. Kok, D.B. Means, B.P. Noonan, W.E. Schargel, and W.C. Wheeler. 2006. Phylogenetic systematics of Dart-Poison Frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). *Bulletin of the American Museum of Natural History* 299:1–262.
- Haas, A. 1995. Cranial features of dendrobatid larvae (Amphibia: Anura: Dendrobatidae). *Journal of Morphology* 224:241–264.
- Haas, A. 2001. Mandibular arch musculature of anuran tadpoles, with comments on homologies of amphibian jaw muscles. *Journal of Morphology* 247:1–33.
- Haas, A. 2003. Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics* 19:23–89.
- Haas, A., and S.J. Richards. 1998. Correlations of cranial morphology, ecology, and evolution in Australian suctorial tadpoles of the genera *Litoria* and *Nyctimystes* (Amphibia: Anura: Hylidae: Pelodyadinae). *Journal of Morphology* 238:109–141.
- Heyer, W.R. 1975. A preliminary analysis of the intergeneric relationships of the frog family Leptodactylidae. *Smithsonian Contributions to Zoology* 199:1–55.
- Johnston, G.F., and R. Altig. 1986. Identification characteristics of anuran tadpoles. *Herpetological Review* 17:36–37.
- Kolenc, F., Borteiro, C., Baldo, D., Ferraro, D.P., and C.M. Prigioni. 2009. The tadpoles and advertisement calls of *Pleurodema bibroni* Tschudi and *Pleurodema kriegi* (Müller), with notes on their geographic distribution and conservation status (Amphibia, Anura, Leiuperidae). *Zootaxa* 1969:1–35.
- Larson, P.M., and R.O. de Sá. 1998. Chondrocranial morphology of *Leptodactylus* larvae (Leptodactylidae: Leptodactylinae): Its utility in phylogenetic reconstruction. *Journal of Morphology* 238:287–305.
- Laufer, G., and J.M. Barreneche. 2008. Re-description of the tadpole of *Pseudopaludicola falcipes* (Anura: Leiuperidae), with comments on larval diversity of the genus. *Zootaxa* 1760:50–58.
- Lavilla, E.O. 1983. Sistemática de larvas de Telmatobiinae (Anura: Leptodactylidae). Ph.D. Dissertation, Universidad Nacional de Tucumán, Argentina.
- Lavilla, E.O., and G.J. Scrocchi. 1986. Morfometría larval de los generos de Telmatobiinae (Anura: Leptodactylidae) de Argentina y Chile. *Physis* 44:39–43.
- León-Ochoa, J., and R. Donoso-Barros. 1969–1970. Desarrollo embrionario y metamorfosis de *Pleurodema brachyops* (Cope) (Salientia-Leptodactylidae). *Boletín de la Sociedad Biológica de Concepción* 42:355–379.
- Lesson, R.P. 1826. Voyage autour du monde pendant les années 1822, 1823, 1824 et 1825. *Zoologie*. Pp. 1–65 in R.P. Lesson and R.P. Garnot (Eds.), *Voyage autour du monde, exécuté par ordre du Roi, sur la Corvette de La Majesté La Coquille, pendant les années 1822, 1823, 1824 et 1825*. Arthus Bertrand, France.
- Lynch, J.D. 2006. The tadpoles of frogs and toads found in the lowlands of northern Colombia. *Academia Colombiana de Ciencias* 30:443–457.
- Miranda, N.E.O., and A. Ferreira. 2009. Morfología oral interna de larvas dos gêneros *Eupemphix*, *Physalaemus* e *Leptodactylus* (Amphibia: Anura). *Biota Neotropical* 9:165–176.
- Nomura, F., D.D. Rossa-Feres, and V.H. Mendonça Do Prado. 2003. The tadpole of *Physalaemus fuscumaculatus* (Anura: Leptodactylidae), with a description of internal oral morphology. *Zootaxa* 370:1–8.
- Palavecino, P. 2000. Desarrollo de la musculatura mandibular e hioidea en Leptodactylinae del noroeste

- argentino. Ph.D. Dissertation, Universidad Nacional de Tucumán, Argentina.
- Parker, W.H. 1927. A revision of the frogs of the genera *Pseudopaludicola*, *Physalaemus*, and *Pleurodema*. *Annals and Magazine of Natural History* 9:450–478.
- Peixoto, O.L. 1982. Observações sobre la larva de *Pleurodema diplolistris* (Peters, 1870) (Amphibia, Anura, Leptodactylidae). *Revista Brasileira de Biologia* 42:631–633.
- Pugener, L.A., A.M. Maglia, and L. Trueb. 2003. Revisiting the contribution of larval characters to an analysis of phylogenetic relationships of basal anurans. *Zoological Journal of the Linnean Society* 139:129–155.
- Prado, V.H.M. 2006. Similaridade ecológica em comunidades de girinos (Amphibia, Anura): o papel de componentes históricos (filogenéticos) e contemporâneos (ecológicos). Master thesis, Universidade Estadual Paulista, Brazil.
- Rada, D. 1981. Renacuajos de algunos anfibios de Clarines (Edo. Anzoátegui, Venezuela). *Memorias de la Sociedad de Ciencias Naturales La Salle* 41:57–76.
- Schlosser, G., and G. Roth. 1995. Distribution of cranial and rostral nerves in tadpoles of the frog *Discoglossus pictus* (Discoglossidae). *Journal of Morphology* 226:189–212.
- Starrett, P.H. 1968. The phylogenetic significance of the jaw musculature in anurans amphibians. Ph.D. Dissertation, University of Michigan, USA.
- Taylor, W.R., and G.C. Van Dyke. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium* 9:107–119.
- Úbeda, C.A. 1998. Batracofauna de los bisques templados patagónicos: Un enfoque ecobiogeográfico. Ph.D Dissertation, Universidad Nacional de Buenos Aires, Argentina.
- Úbeda, C.A. 2001. Geographic distribution. *Pleurodema thaul*. *Herpetological Review* 32:272.
- Van Dijk, D.E. 1966. Systematic and field keys to the families, genera and described species of Southern African Anuran tadpoles, with preliminary inclusion of certain undescribed and inadequately described tadpoles. *Annals of Natal Museum* 18:231–286.
- Vera Candiotti, M.F. 2007. Anatomy of anuran tadpoles from lentic water bodies: Systematic relevance and correlation with feeding habits. *Zootaxa* 1600:1–175.
- Wassersug, R.J. 1980. Internal oral features of larvae from eight families: Functional, systematic, evolutionary and ecological considerations. *Miscellaneous Publications, Museum of Natural History, University of Kansas* 68:1–146.
- Wassersug, R.J., and W.R. Heyer. 1988. A survey of internal oral features of leptodactyloid larvae (Amphibia: Anura). *Smithsonian Contributions to Zoology* 457:1–99.
- Weigandt, M., C.A. Úbeda, and M. Diaz. 2004. The larva of *Pleurodema bufoninum* Bell, 1843, with comments on its biology and on the egg strings (Anura, Leptodactylidae). *Amphibia-Reptilia* 2:429–437.

Accepted: 17 September 2011

Associate Editor: Richard Lehtinen