

THE TADPOLE OF *PHYSALAEMUS FERNANDEZAE* (ANURA: LEPTODACTYLIDAE)

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This paper describes the external and buccopharyngeal morphology, chondrocranium and cranial muscles in tadpoles of *Physalaemus fernandezae*. The data are compared with those for other species of *Physalaemus* to improve the diagnosis of the "species group" within the genus. Species of the "*P. biligonigerus*" group have four infralabial papillae, two semicircular arches of pustulations in a V-shaped pattern on the prenarial arena, 6–8 conical papillae and 40–60 pustulations on the buccal roof arena, four postnarial papillae, a semicircular median ridge, claw-shaped lateral ridges and larval crista parotica with a poorly-developed anterior process. Species of the "*P. pustulosus*" group possess four infralabial papillae (shared with the *P. biligonigerus* group), tooth row formula 2(2)/3, four lingual papillae, two postnarial papillae, twelve conical papillae and 16–20 pustulations on the buccal roof arena, short lateral ridges with rough concave margins and larval crista parotica with a well-developed anterior process and reduced posterior process. Species of the "*P. cuvieri*" group present two infralabial papillae, three pustulations and two serrated papillae on the prenarial arena, five pustulations and two serrated papillae on the postnarial arena, four long and bifid papillae and more than 60 pustulations on the buccal roof arena, and lack larval crista parotica. In species of the "*P. signiferus*" group both medial and lateral mental gaps are absent, and the tooth row formula is 2(2)/3(1).

Key words: amphibian, larvae, frog, morphology, musculature

INTRODUCTION

The neotropical genus *Physalaemus* comprises a group of small toad-like leptodactylid frogs distributed from Mexico to northern Argentina (Frost, 2004). Following Lynch (1970), four species groups of *Physalaemus* are currently recognized: the *P. cuvieri*, *P. biligonigerus*, *P. pustulosus* and *P. signiferus* groups.

At present, anuran tadpole morphology is receiving increasing attention in phylogenetic analyses (Larson & de Sá, 1998; Faivovich, 2002; Haas, 2003). Of the 48 species of *Physalaemus* (Caramaschi *et al.*, 2003; Cruz & Pimenta, 2004; Frost, 2004; Haddad & Sazima, 2004; Ron *et al.*, 2004, 2005), the tadpoles of only 20 have been described (Nomura *et al.*, 2003; Pimenta *et al.*, 2005). The buccopharyngeal morphology, chondrocranium and cranial muscles of *Physalaemus* larvae remain poorly known (Larson & de Sá, 1998; Palavecino, 2000; Nomura *et al.*, 2003).

Physalaemus fernandezae belongs to the "*P. cuvieri*" group and inhabits flooded grasslands in northeastern Argentina and southwestern Uruguay (Langone, 1994). Several studies have been carried out concerning the mating call, natural history and adult morphology of this species (Gallardo, 1963; Barrio, 1964, 1965; Lobo, 1992) but a detailed description of its tadpole is not available. Gallardo (1963), Barrio (1964), Cei (1980) and Langone (1994) give some information about total length and general aspects of the oral disc.

The aim of this paper is to describe the external and buccopharyngeal morphology, chondrocranium and cranial muscles of *Physalaemus fernandezae* tadpoles in the context of the other *Physalaemus* species. This information will be used to improve the diagnosis of the *Physalaemus* species group, which so far has been based only on adult characters.

MATERIALS AND METHODS

Between May and July 2001, we collected tadpoles of *Physalaemus fernandezae* at Punta Lara (Buenos Aires province, Argentina). Some of them ($n=13$) were fixed after capture in 10% buffered formalin and then staged using Gosner's (1960) table. The material examined is deposited in the amphibian collection of the Museo de La Plata (MLP). The remaining tadpoles were reared until metamorphosis to corroborate the species identification. Seven tadpoles were employed for oral disc and external morphology descriptions (stages 32, 35, 36, 37 and 38, MLP 3333). Two stage 35 (MLP 3334) and three stage 40 (MLP 3335) specimens were stained following the technique of Taylor & Van Dyke (1985). The process was interrupted before clearing; tadpoles were dissected for observation of muscles and then cleared for chondrocranium description. One tadpole (stage 39) was dehydrated in a graded ethanol series (30%: three 15-minute baths; 50%: a week; 70%: three 15-minute baths; 100%: 15 minutes prior to the critical point) for scanning electronic microscope examination of the buccopharyngeal morphology and keratinized structures of the oral disc. The tadpole was sectioned according to Wassersug (1980) and critical point dried

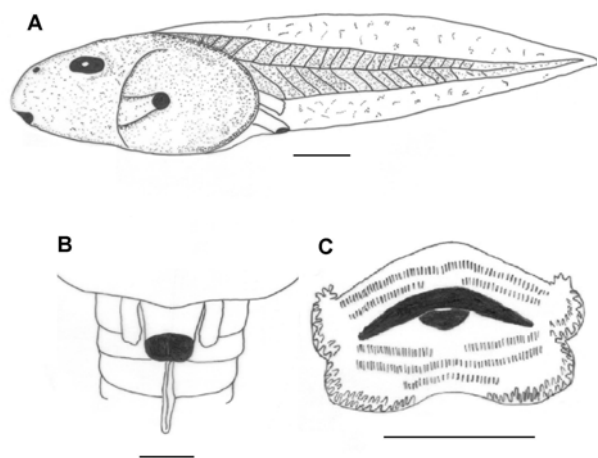


FIG. 1. External morphology of the tadpole of *Physalaemus fernandezae* at stage 38 (MLPA 3333). A) Lateral view; B) ventral view of the vent tube; C) oral disc. Scale bars=1 mm.

in carbon dioxide using amyl acetate as intermediate liquid, mounted on a double-face Carbon tape and sputter-coated with 400 Å thick gold-palladium using a Model Ion Sputter Fine Coat JFC-1100 (Jeol System). Photographs were taken using a Jsm-T100 scanning electron microscope at 5–15 kV equipped with an Ilford camera. The buccopharyngeal morphology of a stage 35 tadpole was also examined under a stereomicroscope. Observations, measurements and drawings referring to external morphology, chondrocranium and cranial muscles were made under a Reichert Wien stereomicroscope with measuring equipment (accurate to the nearest 0.1 mm) and camera lucida.

Terminology follows D'Heursel & de Sá (1999) and Haas (1995) for chondrocranium structures, Alcalde & Rosset (2003) for chondrocranial measurements, Haas (2001) for mandibular musculature, Haas & Richards (1998) and Haas (2003) for branchial and hyoid musculature, Schlosser & Roth (1995) for muscular innervation, Wassersug (1980) for buccopharyngeal morphology, Van Dijk (1966) and Lavilla (1983) for external morphology, Johnston & Altig (1986) for oral disc morphology and Altig & Johnston (1989) for tadpole ecomorphological types.

RESULTS

EXTERNAL MORPHOLOGY

The following description is based on seven specimens at developmental stages 32–38. External morphology is illustrated in Fig. 1. Measurements are in mm (arithmetic mean \pm 95% confidence limits). Percentages were calculated based on the maximum and minimum values of each variable.

Type IV, extrophic, lentic and benthic tadpoles. Size small, total length 26.84 mm (\pm 1.92), body length (8.73 \pm 0.84) one-third of total length; body shape oval, body length 50–60% of body height (4.96 \pm 0.60), and body width (5.23 \pm 0.32) 80–100% of body height, without constrictions between head and trunk; snout rounded in dorsal and lateral profile; eyes relatively

large, dorsolaterally placed; eye diameter (1.21 \pm 0.11) 27–28% of body width at eye level (4.34 \pm 0.42), and 87–93% of interorbital distance (1.39 \pm 0.10); interorbital distance 29–32% of body width at eye level; rostro-orbital distance 5.86 \pm 0.26. Nostrils subcircular, dorsal, elevated, closer to tip of snout than to eye, nostril diameter (2.76 \pm 0.4) 13–14% of body width at nostril level, rostronasal distance (0.71 \pm 0.11) 55–61% of orbitonasal distance (1.06 \pm 0.14); nostril diameter (0.37 \pm 0.06) 37–45% of internarial distance (0.94 \pm 0.10), and internarial distance 66–73% of interorbital distance; extranarial distance (1.55 \pm 0.10) 45–47% of extraorbital distance (3.37 \pm 0.23). Spiracle sinistral, spiracular tube and opening lateral, spiracular opening rounded, rostro-spiracular distance

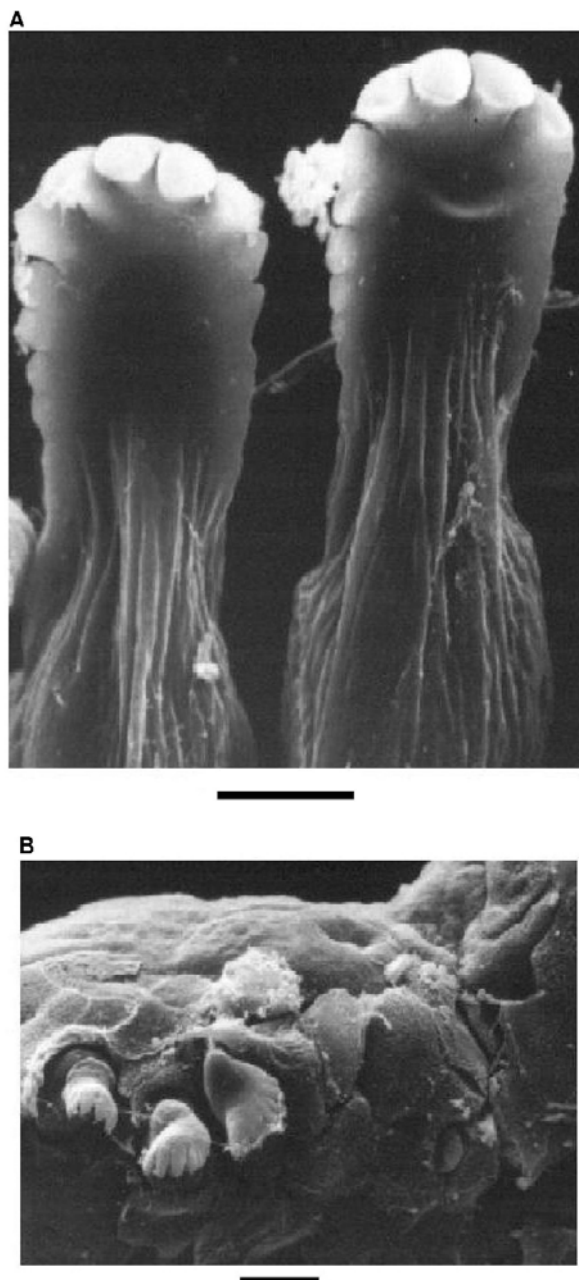


FIG. 2. Scanning electron microscope photographs of the keratodonts of the first mental row (A) and of the third left marginal papilla bearing small keratodonts (B) of *Physalaemus fernandezae* at stage 39. Scale bars 10 μ m.

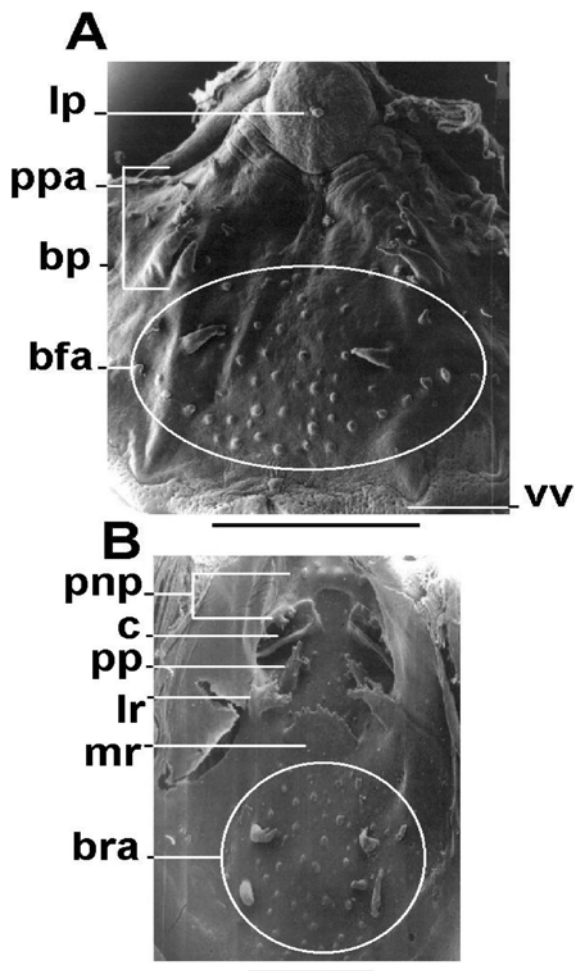


FIG. 3. Scanning electron microscope photographs of the buccal floor (A) and buccal roof (B) papillation of *Physalaeemus fernandezae* at stage 39. In A, infralabial papillae are not visible. In B, infrarostral papillae are not visible. Scale bars 1 mm. References: bfa, buccal floor arena; bp, buccal pocket; bra, buccal roof arena; c, choana; lp, lingual papilla; lr, lateral ridge; mr, median ridge; pnp, prenarial papillae; pp, postnarial papillae; ppa, prepocket papillae; vv, ventral velum.

(5.86 ± 0.26) 63–71% of body length. Vent tube length (2.08 ± 0.75) 14–29% of body length; vent opening medial. Tail length (15.79 ± 1.57) 59–63% of total length, tail height at the base of the tail 5.39 ± 0.55 , tail height at the tip of the caudal musculature 0.70 ± 0.26 ; dorsal and ventral fins well developed, with slightly curved margins; maximum tail height approximately at middle length and lower than body height; tail axis straight and tip of tail rounded. Caudal musculature height at the base of the tail (2.74 ± 0.36) 55–56% of body height, caudal musculature width at the base of the tail 2.51 ± 0.27 ; myotomes clearly visible, the posteriormost ones not reaching the end of the tail.

Oral disc sub-terminal, not visible dorsally; oral disc width 1.91 ± 0.14 , disc small, about 36–38% of maximum body width; disc with angular constrictions; an irregular double row of triangular and rounded marginal papillae in lateral regions; small mental gap present (0.43 ± 0.11); with medium-sized rostral gap (1.16 ± 0.08), about 61% of oral disc width;

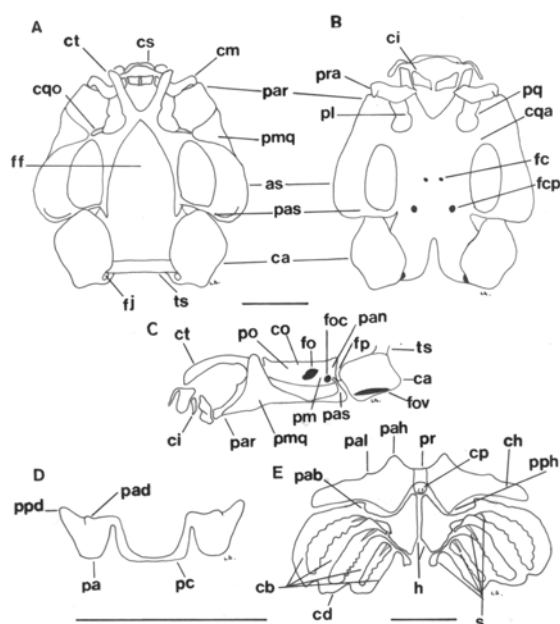


FIG. 4. Chondrocranium of *Physalaeemus fernandezae* at stage 35 (MLPA 3334). A) Dorsal, B) ventral and C) lateral views of the neurocranium and mandibular arch. D) Frontal view of cartilago suprarostralis. E) Ventral view of hyobranchial apparatus. Dark areas represent cranial fenestrations. Scale bars 1 mm. References: as, arcus subocularis; ca, capsula auditiva; cb, ceratobranchiales; cd, commissura terminalis; ch, ceratohyalis; ci, cartilago infrarostrale; cm, cartilago meckeli; co, cartilago orbitale; cp, copula posterior and processus urobranchialis; cqa, commissura quadrato-cranialis anterior; cqo, commissura quadrato-orbitalis; cs, cartilago suprarostralis; ct, cornu trabeculae; fc, foramen cranoopalatinum; fcp, foramen trabeculae primarium; ff, fenestra frontoparietalis; fj, foramen jugulare; fo, foramen opticum; foc, foramen oculomotorium; fov, fenestra ovalis; fp, fissura prootica; h, hypobranchiales; pa, pars alaris; pab, processus anterior branchialis; pad, processus anterior dorsalis; pah, processus anterior hyalis; pal, processus anterolateralis hyalis; pan, pila antotica; par, processus articularis; pas, processus ascendens; pc, pars corporis; pl, processus lateralis; pm, pila metoptica; pmq, processus muscularis quadrati; po, pila preoptica; ppd, processus posterior dorsalis; pph, processus posterior hyalis; pq, processus quadrato-ethmoidalis; pr, pars reuniens; pra, processus retroarticularis; s, spicula I, II III and IV; ts, tectum synoticum.

intramarginal papillae absent; tooth row formula 2(2)/3(1), rostradonts well developed and keratinized, margins serrated (Fig. 1C); keratodonts spatulated and serrated (Fig. 2A). One specimen bears small keratodonts on the marginal papillae (Fig. 2B).

In life, dorsum and lateral body sides uniformly greyish, darker dorsally than laterally; ventral region grey, peribranchial zone paler than abdominal region, abdomen rich in guanophores producing silvery and golden sheens; fins scantily pigmented, transparent, and dotted with few irregular rows of melanophores; caudal musculature darker with melanophores arranged more densely than on fins. In preservative, creamy white tail with few isolated brown spots more abundant in the hypaxial musculature. Body darker than tail, dorsally dark-brown, ventrally pale brown. Intestinal mass visible through transparency.

Buccopharyngeal morphology. The buccal floor (Fig. 3A) possesses two short and rounded infralabial papillae (not visible in Fig. 3A), and one triangular lingual papilla. The buccal floor arena has more than 60 pustulations and six large and serrated papillae (Fig. 3A). There are 12 short and conical papillae (sometimes serrated) and few pustulations on the prepocket arena. The ventral velum presents secretory pits.

The buccal roof (Fig. 3B) possesses two trifold infrastral papillae (not visible in Fig. 3B). Prenarial arena with three pustulations and two long and trifold papillae. The postnarial arena presents three central pustulations and two lateral and serrated papillae placed anteriorly to the median ridge, and one small pustulation anterior to each lateral ridge. The well-developed median ridge is subcircular and serrated. The lateral ridges are rectangular and serrated. There are more than 60 central pustulations, and four long and bifid papillae on the buccal roof arena.

Chondrocranium. Neurocranium almost rectangular (width/length=0.86) and depressed (height/width=0.4), with greatest width at level of the arcus subocularis. Medial corpora of the cartilago suprarostralis connected by a distal bridge (Fig. 4D). Lateral partes alares and partes corpora joined by a proximal connection. Well-developed processus posterior dorsalis. Cornua trabeculae forming about 19% of chondrocranial length, uniformly wide, with well-developed processus lateralis. The cranium is roofed only between the capsula auditivae by the tectum synoticum. Lateral walls (cartilagine orbitales) formed by the pila ethmoidea (*sensu de Beer, 1985*), pila preoptica, pila metoptica and pila antotica (Fig. 4C). Basi cranii closed and pierced by the foramina carotica primaria and craneopalatina (Fig. 4B). Capsulae auditivae subspherical representing about 38% of the chondrocranial length; dorsally coupled with the processus ascendens and lacking larval crista parotica. Medial walls of the capsula auditivae pierced by the acoustic and the endolymphatic foramina. No inferior perilymphatic foramen at the studied stages. Superior perilymphatic foramen opening in the posterior wall of the capsula auditiva, just in front of the jugular foramen. Operculum not chondrified.

Palatoquadrate with processus articularis quadrati, processus muscularis quadrati, commissura quadrato-cranialis anterior, processus quadrato-ethmoidalis and processus ascendens, but lacking commissura quadrato-orbitalis, processus pseudopterygoideus and larval processus oticus. No lateral projections on the margins of the arcus subocularis, and processus ascendens joined to pila antotica by an intermediate union (Fig. 4C).

Lower jaw consisting of cartilago meckeli and cartilagine infrastrales. Processus retroarticularis of cartilago meckeli short and articulating with processus articularis quadrati. Both processus ventromedialis and dorsomedialis of the cartilago meckeli articulating with

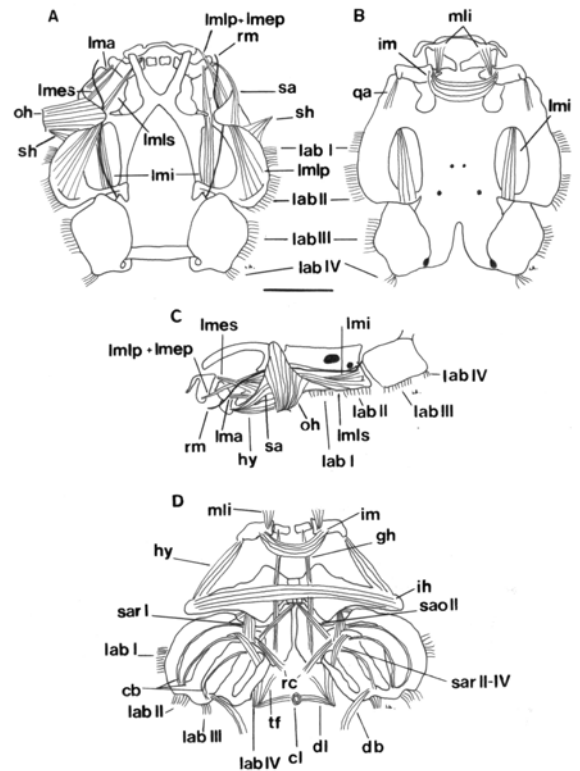


FIG. 5. Cranial muscles of *Physalaemus fernandezae* at stage 35 (MLPA 3834). A) Dorsal, B) ventral and C) lateral views of muscles related to neurocranium and mandibular arch. d) Ventral view of muscles related to the hyobranchial apparatus. In A, mm. levator mandibulae externus profundus, levator mandibulae longus profundus, suspensorioangularis (left side), levator mandibulae longus superficialis, levator mandibulae externus superficialis and orbitohyoideus (right side) were removed. Dark areas represent cranial fenestrations. Scale bar 1 mm. References: cb, constrictor branchiales II, III and IV; cl, constrictor laryngis; db, diaphragmatobranchialis; dl, dilatator laryngis; gh, geniohyoideus; hy, hyoangularis lateralis; ih, interhyoideus; im, intermandibularis; lab I, levator arcuum branchialium I; lab II, levator arcuum branchialium II; lab III, levator arcuum branchialium III; lab IV, levator arcuum branchialium IV; lma, levator mandibulae articularis; lmp, levator mandibulae externus profundus; lms, levator mandibulae externus superficialis; lmi, levator mandibulae internus; lmp, levator mandibulae longus profundus; lms, levator mandibulae longus superficialis; mli, mandibulolabialis inferior; oh, orbitohyoideus; qa, quadratoangularis; rc, rectus cervicis; rm, ramus mandibularis of trigeminus nerve; sa, suspensorioangularis; sao II, subarcualis obliquus II; sar I, subarcualis rectus I; sar II-IV, subarcualis rectus II-IV; sh, suspensoriohyoideus; tf, tympanopharyngeus.

the cartilagine infrastrales by the sindesmotomic commissura intramandibularis (Fig. 4B).

Copula I absent. All ceratohyale processes are well developed, except the very short processus anterolateralis hyalis. Ceratohyalia medially joined by a rectangle-shaped pars reuniens. Copula II bearing a short processus urobranchialis. Ceratobranchiale I continuous with the hypobranchiale; the remaining ceratobranchiales sindesmotomically joined to the hypobranchiale (Fig. 4E). Ceratobranchiales III and IV

TABLE 1. Origin and insertion of each mandibular and hyobranchial muscle on tadpoles of *Physalaemus fernandezae*.

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 pars alaris by a common tendon.
Levator mandibulae externus profundus	Processus muscularis quadrati	Pars alaris
Levator mandibulae externus superficialis	Processus muscularis quadrati	Cartilago meckeli
Levator mandibulae articularis	Processus muscularis quadrati	Cartilago meckeli
Levator mandibulae lateralis	Absent at the studied stages	
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
Quadratoangularis	Anterior and ventral on the palatoquadrate	Cartilago meckeli
Hyoangularis lateralis	Ceratohyale	Cartilago meckeli
Hyoangularis medialis	Absent	
Interhyoideus	Ceratohyale	Median raphe
Interhyoideus posterior	These muscles were not found under dissections, but they may be observable in histological sections	
Diaphragmatopraecordialis		
NERVUS GLOSSOPHARYNGEUS (CRANIAL NERVE IX), BRANCHIAL MUSCULATURE		
Levator arcuum branchialium I	Arcus subocularis	Commissura terminalis I
Subarcualis rectus I	The dorsal head on ceratobranchiale I The ventral heads on ceratobranchiales II and III	Ceratohyale
Constrictor branchialis I	Absent	
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 II
Levator arcuum branchialium III	Capsula auditiva	Commissura terminalis III
Levator arcuum branchialium IV	Capsula auditiva	Ceratobranchiale IV
Subarcualis obliquus II	Between ceratobranchiales II and III	Processus urobranchialis
Subarcualis rectus II-IV	Ceratobranchiale IV	Ceratobranchiale II
Tympanopharyngeus	M. levator arcuum branchialium IV	Pericardium
Dilatator laryngis	Capsula auditiva	Larynx
Constrictor laryngis	Forms an annulus rounding the larynx	
Transversus ventralis IV	Absent	
NERVUS HYPOGLOSSUS (SPINAL NERVE II), HIPOBRANCHIAL MUSCULATURE		
Geniohyoideus	Hypobranchiale	Cartilago infarostrale
Rectus cervicis	Peritoneal wall	Ceratobranchiales II and III

joined by a commissura proximalis. Processus branchiales not closed. All spiculae well developed.

Ossifications. The parasphenoid is the only bone present at the studied stages.

Cranial muscles. The cranial muscle pattern of *Physalaemus fernandae*'s tadpoles is shown in Figure 5. Table 1 provides details about the origin and insertion of each muscle. The ramus mandibularis of the nervus trigeminus runs laterally to all muscles levatorae mandibulae.

DISCUSSION

External tadpole morphology has been described for four species of the "*Physalaemus biligonigerus*" group: *Physalaemus biligonigerus* (Férrandez & Férrandez, 1921; Cei, 1980), *P. fuscomaculatus* (Nomura *et al.*, 2003), *P. nattereri* (Vizzoto, 1967; Cei, 1980) and *P. santafecinus* (Perotti & Céspedes, 1999); nine of the "*P. cuvieri*" group: *P. aguirrei* (Pimenta & Cruz, 2004), *P. albonotatus* (Kehr *et al.*, 2004), *P. centralis* (Rossa-Feres & Jim, 1993), *P. cuqui* (Perotti, 1997), *P. cuvieri* (Bokermann, 1962; Cei, 1980; Heyer *et al.*, 1990), *P. enesefae* (Duellman, 1997), *P. gracilis* (Langone, 1989), *P. henselii* (Barrio, 1964; Cei, 1980) and *P. riograndensis* (Prigioni & Garcia, 2001); three of the "*P. pustulosus*" group: *P. coloradorum* (Cannatella & Duellman, 1984), *P. petersi* (Duellman, 1978) and *P. pustulosus* (Breder, 1946); and five of the "*P. signiferus*" group: *P. atlanticus* (Haddad & Sazima, 2004), *P. bokermanni* (Cardoso & Haddad, 1985), *P. camacan* (Pimenta *et al.*, 2005), *P. maculiventris* (Bokermann, 1963) and *P. spiniger* (Haddad & Pombal, 1998). The tadpole of *Physalaemus rupestris* is also known but does not belong to any of the four species groups (Nascimento *et al.*, 2001).

We compared these tadpoles' descriptions with the tadpole of *Physalaemus fernandae* in order to obtain a characterization of the known larvae of *Physalaemus*. In light of the present knowledge, *Physalaemus* larvae are small (total length=14.8–31.5 mm), possess medium-sized tail (43–68% of total length), ovoid body, rounded snout, dorsolateral eyes, dorsal fin higher than ventral fin and sub-terminal emarginated oral disc with rostral gap.

Some features of the larvae of *Physalaemus*, such as the vent tube opening, the mental gap, the marginal papillae row and the tooth row formula, are highly variable and do not exhibit unique states for each of the species groups proposed by Lynch (1970). The vent tube of most larvae is positioned medially, but its opening may be medial (*P. atlanticus*, *P. bokermanni*, *P. camacan*, *P. fernandae*, *P. rupestris*, *P. spiniger*) or dextral (*P. albonotatus*, *P. cuqui*, *P. fuscomaculatus*, *P. maculiventris*, *P. nattereri*). The vent opening of *P. centralis* may be medial, dextral or sinistral within the same population. Previous authors have not made a clear difference between the position of the vent tube and the vent opening for other species. In them, the vent tube position (or the vent opening?) should be dextral

(*P. biligonigerus*, *P. cuvieri*, *P. gracilis*, *P. riograndensis*, *P. santafecinus*) or sinistral (*P. enesefae*).

The marginal papillae is present as a single row in tadpoles of most species of *Physalaemus* (*P. albonotatus*, *P. biligonigerus*, *P. bokermanni*, *P. centralis*, *P. cuqui*, *P. cuvieri*, *P. fuscomaculatus*, *P. maculiventris*, *P. nattereri*, *P. petersi*, *P. pustulosus*, *P. riograndensis*, *P. rupestris*). In other species, the marginal papillae row may be ventrally double and laterally single (*P. atlanticus*, *P. spiniger*), completely double (*P. gracilis*), ventrally single and double at some areas of the lateral region (*P. fernandae*), ventrally single and double at the infra-angular areas (the internal rows of *P. fuscomaculatus* and *P. santafecinus* were described as intramarginal papillae by Perotti & Céspedes, 1999, and Nomura *et al.*, 2003), ventrally and laterally single but double or triple at the lateral folds (*P. coloradorum*), or laterally single but double or triple at mental region (*P. camacan*).

In anuran tadpoles, the marginal papillae row may be incomplete for lacking either the anterior (rostral gap) or posterior papillae (mental gap – here identified as medial mental gap). Some species of *Physalaemus* have two ventrolateral gaps on each side of the oral disc, here identified as lateral mental gaps. According to these types of mental gaps, larvae of *Physalaemus* may possess four oral disc configurations: (1) medial mental gap present and lateral mental gaps absent (*P. fernandae*, *P. henselii*); (2) both medial and lateral mental gaps present (*P. albonotatus*, *P. cuqui*); (3) both mental gaps absent (*P. atlanticus*, *P. biligonigerus*, *P. bokermanni*, *P. camacan*, *P. coloradorum*, *P. enesefae*, *P. gracilis*, *P. maculiventris*, *P. nattereri*, *P. petersi*, *P. pustulosus*, *P. riograndensis*, *P. rupestris*, *P. santafecinus*, *P. spiniger*); and (4) only lateral mental gaps present (*P. centralis*). *Physalaemus fuscomaculatus* is unique in having configurations 3 and 4 within a single population. Contradictory information has been published about this character state for *P. cuvieri*. This species was described as possessing configurations 1 (Bokermann, 1962) and 4 (Heyer *et al.*, 1990).

There are seven tooth row formulae in *Physalaemus*: 2(2)/3(1) (*P. albonotatus*, *P. atlanticus*, *P. bokermanni*, *P. camacan*, *P. cuqui*, *P. fernandae*, *P. gracilis*, *P. maculiventris*, *P. nattereri*, *P. spiniger*); 2(2)/2 (*P. biligonigerus*, *P. centralis*); 2(2)/2(1) (*P. fuscomaculatus*, *P. riograndensis*, *P. santafecinus*); 2/3(1) (*P. cuvieri*, *P. henselii*); 2(1)/3 (*P. enesefae*); 2(2)/3 (*P. coloradorum*, *P. petersi*, *P. pustulosus*), or 2(2)/3(1-2) (*P. rupestris*). The tooth row formula 2(2)/3 is unique to the species assembled in the "*P. pustulosus*" species group.

The buccopharyngeal papillation has been described for *Physalaemus biligonigerus*, *P. fuscomaculatus*, *P. nattereri*, and *P. santafecinus* ("*P. biligonigerus*" group), *P. petersi* and *P. pustulosus* ("*P. pustulosus*" group) (Wassersug & Heyer, 1988; Spirandeli-Cruz,

TABLE 2. Summary of buccal floor variation for the species of *Physalaemus* in which buccopharyngeal morphology has been described. Abbreviations: PA, papillae; PU, pustulations.

"Species group"	Species	Lingual papillae				Prepocket papillae				Buccal floor arena
		1	2	3	0	1	1-2	2-3	6	
"Physalaemus biligonigerus" group	<i>P. biligonigerus</i> (Perotti & Céspedes, 1999)	X			X					8-10 PA, 30-35 PU
	<i>P. santafecinus</i> (Perotti & Céspedes, 1999)	X			X					
	<i>P. fuscomaculatus</i> (Nomura <i>et al.</i> , 2003)		X					X		8-12 PA, 30-40 PU
	<i>P. nattereri</i> (Spirandeli-Cruz, 1991)				X		X			>24 PA, several PU
"P. pustulosus" group	<i>P. petersi</i> (Wassersug & Heyer, 1988)		data unknown					X		data unknown
	<i>P. pustulosus</i> (Wassersug & Heyer, 1988)				X					12 PA, 20 PU
"P. cuvieri" group	<i>P. fernandezae</i>	X							X	6 PA, >60 PU

1991; Fabrezi & Vera, 1997; Perotti & Céspedes, 1999; Nomura *et al.*, 2003). No information on the buccopharyngeal morphology of species of the "*P. signiferus*" group is available. These features, in particular those from the buccal floor, are highly variable within some species groups (see Table 2). The buccal floor arena papillae and pustulations are putative characters for delimiting species groups within *Physalaemus*, but it would be interesting to know the range of variation for these characters.

On the other hand, other buccopharyngeal structures seem to be useful for the characterization of the "*P. biligonigerus*", "*P. cuvieri*" and "*P. pustulosus*" species groups: (1) species of the "*P. biligonigerus*" group possess four infralabial papillae; two semicircular arches of pustulations separated by a moderate notch and arranged in a V-shaped pattern on the prenarial arena; 6-8 conical papillae and 40-60 pustulations on the buccal roof arena; four postnarial papillae; a semicircular median ridge; and claw-shaped lateral ridges; (2) species of the "*P. pustulosus*" group are characterized by the possession of four infralabial papillae (shared with the "*P. biligonigerus*" group); four lingual papillae; two postnarial papillae; 12 conical papillae and 16-20 pustulations on the buccal roof arena; and short lateral ridges with rough and concave margins; (3) species of the "*P. cuvieri*" group differ from other species of the genus in having two infralabial papillae; three pustulations and two serrated papillae on the prenarial arena; three central and two lateral pustulations and two serrated papillae on the postnarial arena; four long and bifid papillae and up to 60 pustulations on the buccal roof arena.

The chondrocranium is known for *Physalaemus cuqui* ("*P. cuvieri*" group), *P. biligonigerus* ("*P. biligonigerus*" group), and *P. pustulosus* ("*P. pustulosus*" group) (Fabrezi & Vera, 1997; Larson & de Sá, 1998; Haas, 2003). Comparisons among these species and *P. fernandezae* allow a preliminary recognition of the following chondrocranial patterns within the genus: (1) the larval crista parotica is absent ("*P. cuvieri*" group), represented by a poorly developed anterior process ("*P. biligonigerus*" group), or possesses a well developed anterior process and a very reduced posterior process ("*P. pustulosus*" group); (2) closed commissura quadrato-orbitalis (*P. biligonigerus*, *P. cuqui* and *P. pustulosus*), or open (*P. fernandezae*); and (3) the processus anterolateralis hyalis of the ceratohyale may be reduced (*P. fernandezae*) or well developed (*P. cuqui*, *P. biligonigerus*). Larson & de Sá (1998) did not report this character for *Physalaemus pustulosus*.

The larval cranial musculature has been described for *Physalaemus cuqui* ("*P. cuvieri*" group), *P. biligonigerus* ("*P. biligonigerus*" group) and *P. pustulosus* ("*P. pustulosus*" group) (Starrett, 1968; Palavecino, 2000; Haas, 2003). The only difference among these species is that the muscle levator mandibulae lateralis is absent at advanced developmental stages in *P. fernandezae*, but it is present from stage 31 in the remaining species (Palavecino, 2000).

ACKNOWLEDGEMENTS

We wish to thank D. Baldo, F. Nomura, B. Pimenta and C. Haddad for the loan of bibliographical material and C. Morgan for her help in English translation. This

paper is scientific contribution N° 766 of the Instituto de "Limnología Dr. R. A. Ringuelet". L. Alcalde acknowledges UNLP for financial support (Doctoral Scholarship 2004). G. S. Natale acknowledges CONICET for financial support (Doctoral Scholarship 2002).

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