Development of the nidicolous tadpoles of *Eupsophus emiliopugini* (Anura: Cycloramphidae) until metamorphosis, with comments on systematic relationships of the species and its endotrophic developmental mode

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Abstract

Vera Candioti, M.F., Nuñez, J.J. and Úbeda, C. 2011. Development of the nidicolous tadpoles of *Eupsophus emiliopugini* (Anura: Cycloramphidae) until metamorphosis, with comments on systematic relationships of the species and its endotrophic developmental mode. — *Acta Zoologica* (Stockholm) **92**: 27–45.

Species of *Eupsophus* are unique within Alsodinae in having nidicolous tadpoles. They are characterized by traits typical of generalized exotrophic (e.g., oral disc and spiracular tube) and endotrophic larvae (e.g., scant pigmentation and large hind limbs). The larval morphology and development of *E. emiliopugini*, including external, buccal, and musculoskeletal features, is described herein. Like the larvae of other alsodines, these larvae have four lingual and four infralabial papillae, quadratoethmoid process, and an m. rectus cervicis with a double insertion. Among the traits exclusive to the genus are: the absence of the pseudopterygoid process and quadrato-orbital commissure; presence of the m. subarcualis rectus I with two slips; and presence of the m. subarcualis rectus II–IV inserting on Ceratobranchial II. The development and metamorphosis of *Eupsophus* include some characters that develop later (e.g., degeneration of mouthparts and chondrocranium with minimum calcification), characters that develop earlier (e.g., hind-limb appearance and jaw and suspensorium ossification), and characters that develop at the same time (e.g., most external features and cranial muscles) than in most exotrophic species. Some distinctive characters (third lower labial ridge absent, general configuration of the hyobranchial skeleton, skeletal development with retention of larval traits) resemble those of other endotrophic species, and the precocious ossification of jaws and suspensorium is shared with several direct-developing species among recent amphibians.

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Introduction

Ground frogs of the genus *Eupsophus* Fitzinger (1843) are widely distributed in *Nothofagus* forests and constitute one of the most diverse genera within the austral batrachofauna. On the basis of advertisement call, karyotypical, allozymic, morphometric, and molecular data, two intrageneric groups have been defined—the *E. vertebralis* Group, including *E. vertebralis* and *E. emiliopugini*, and the *E. roseus* Group, including the eight remaining species (Formas *et al.* 1983; Fernández de la Reguera 1987; Formas 1992a; Formas and Brieva 1994; Nuñez 2004; Veloso *et al.* 2005). Notwithstanding the lack of information on intrageneric relationships, the monophyly of this genus is supported by molecular data (Correa *et al.* 2006; Frost *et al.* 2006 updated in Frost 2009), and it is hypothesized that *Eupsophus* is the sister group of *Alsodes*.

Research on anatomic and developmental complexity of the larvae can provide important insights into systematics and functional diversification in closely related taxa of anurans (e.g., Larson and de Sá 1998; Haas 2003; Púgener *et al.* 2003; Larson 2005, 2008; Vera Candioti 2007). The tadpoles of *Eupsophus* species are noticeable and unusual because after hatching, they remain in dark conditions in small, water-filled holes or chambers near streams or flooded ground (Úbeda

and Nuñez 2006). They are assigned to the endotrophic nidicolous guild as defined by Altig and Johnston (1989; i.e., eggs oviposited terrestrially, and embryos developed from oogenic energy sources, producing various sorts of free-living, nonfeeding larvae), and have been characterized by few keratinized buccal structures and scarce pigmentation (Formas 1989a,b; Formas 1992b; Nuñez and Úbeda 2009; Vera Candioti *et al.* 2005). The traits mentioned facilitate to distinguish *Eupsophus* tadpoles from other anuran tadpoles that inhabit the Patagonian forests, and in a general context, they agree with the hypothesis of *Eupsophus* as a monophyletic assemblage. Nevertheless, information about larval morphology is derived mainly from descriptions of tadpoles of the *E. roseus* Group (Formas and Pugín 1978; Formas 1989a; Vera Candioti *et al.* 2005; Cárdenas-Rojas *et al.* 2007a; Nuñez and Úbeda 2009). The only available data for larvae of the *E. vertebralis* Group are descriptions of external morphology of *E. emiliopugini* (Formas 1989a) and *E. vertebralis* (Formas 1992b).

Herein, the larval external morphology, anatomy and development of *Eupsophus emiliopugini* are described to: (1) provide morphological data of a member of the *E. vertebralis* Group; (2) discuss morphological features in the context of the systematics of *Eupsophus* and Alsodinae; (3) compare the anatomy and developmental patterns of *Eupsophus* larvae with other endotrophic anurans.

Materials and Methods

A sample of 88 eggs of *Eupsophus emiliopugini* was collected in a forest in Guabún (41°50′ S, 74°01′ W; Chiloé Province, Chile). The clutch was found under moss in a hollow full of water on the banks of a stream, and it was transferred to the laboratory. There, the embryos were reared to different stages of development during 64 days at constant temperature (16°C). They were periodically euthanized using an overdose of benzocaine in aqueous solution and then fixed in 10% neutral-buffered formalin. Specimens were deposited in the Herpetological Collection of Instituto de Zoología, Universidad Austral de Chile, but after the 2007 fire, only 30 specimens remain (*E. emiliopugini* developmental series IZUA 3542). External morphology was studied on the complete series, although many intermediate stages were not observed. We took measurements with the aid of a stereoscopic microscope (Wild type 308700 Heerbrugg, Switzerland) with a micrometric eyepiece. Measurements and terminology are based on those of Altig and McDiarmid (1999a) and Vera Candioti *et al.* (2005).

Five specimens at Stages 31, 34, 37, 42/43, and 46 (as defined by limb development and mouth angle; Gosner 1960) were dissected for anatomic studies. Specimens were prepared according to a clearing-staining protocol (Wassersug 1976a), but interrupting the procedure before the immersion in glycerol. Muscles were colored with Lugol's solution, to obtain a better contrast with the skeleton (Böck and Shear

1972). Buccopharyngeal cavity features were recorded after coloration with methylene blue (Wassersug 1976b). Drawings were made with a stereomicroscope equipped with a camera lucida. The terminology employed to describe oral, buccal, and musculoskeletal structures is that suggested by Altig (2007), Wassersug (1976b), and Haas (2003), respectively.

Results

Early development (n = 8, Stages ≅ 19–24; Fig. 1)

The eggs are 6.7–7.2 mm in diameter. The non-pigmented embryos have a large amount of yolk that gives the abdomen a spherical shape. External gills are present and hind-limb

Fig. 1—Development of the external morphology of *Eupsophus emiliopugini*. Days after hatching and approximate Gosner stages are indicated.

development corresponds to Stage 27/28; forelimbs are not developed. Hatchlings are about 12 mm in total length. External gills and abdominal yolk are present. The stomodeum is still closed, and elements of the oral disc (oral papillae and keratinized mouthparts) are not yet developed. The vent tube is medial and oblique. The tail is proportionally long (61.5% of the total length), and fins are formed; the tip of the tail is rounded. Three days after hatching, two hatchlings have their gills covered by the operculum. The total length increases to 13.9–14 mm. The skin is mostly non-pigmented, but several isolated chromatophores appear on dorsal surfaces of the head, body, and anterior third of the tail. The eyes are scarcely pigmented and can be seen through the translucent skin. The oral disc begins to form lateral emarginations but no papillae; tiny labial teeth appear arranged in one upper and one lower row. The nares open in a small, depressed zone. The tail achieves about 63% of the total length. Both fins are developed; the dorsal fin starts as a narrow crest at the posterior half of the body, and the ventral fin originates anterior to the anal tube. The vent tube lengthens and opens in some specimens.

Tadpole description

External morphology (n = 12, Stages 31–36; Fig. 1 and Table S1, Supporting information). The short, elliptical body is nearly twice as long as high and slightly depressed. In lateral view, the ventral profile is convex. In ventral view, vitellum can be seen in the coiled gut through the translucent body wall. Some chromatophores are present around Stage 33. The forelimb buds are observed through the translucent skin at Stage 34. The snout is rounded in dorsal view and slightly truncated in lateral view. The nostrils are small and circular in

shape, and have a low rim; they are located in a small depression, dorsolateral and slightly closer to the snout than to the eyes. The internarial distance is less than the interocular distance. The large, dorsolateral eyes have circular pupils. The spiracular tube is single, sinistral, and ventrolateral, and lacks an inner wall. The vent tube is medial and large and slightly wider at the middle region; its opening is medial. The tail is large, high, and the tail axis is straight. The tail fins are equally developed with slightly sinuated margins. The maximum height of each tail fin is at the mid-length of the tail; the tail tip is rounded. The dorsal fin starts on the posterior third of the trunk; the ventral fin starts posteriorly to the vent tube. The caudal musculature is moderately robust, decreasing slightly in size toward the posterior half; the musculature does not reach the tip of tail. Myotomes of caudal musculature are visible but not strongly marked.

Coloration. In life, dorsal and ventral fins are transparent. The dorsum of the body is creamy white with scattered chromatophores. The venter is transparent and the internal organs are visible. The oral disc and papillae are unpigmented, and keratinized structures are black. The iris is black, and the spiracle is transparent. At Stage 35, the coloration intensifies, and the dorsal and lateral regions are light brown. A whitish orbitonasal line is scarcely evident.

Oral disc and buccopharyngeal cavity (n = 12 and n = 2, respectively, Stages 31–36; Fig. 2). The oral disc (Fig. 2A) is subterminal, small, and slightly emarginate. It is lined by a single row of rounded, marginal papillae; a wide dorsal gap occupies the entire upper lip. The labial teeth are arranged on two anterior (A-1 and A-2) and two posterior (P-1 and P-2) labial
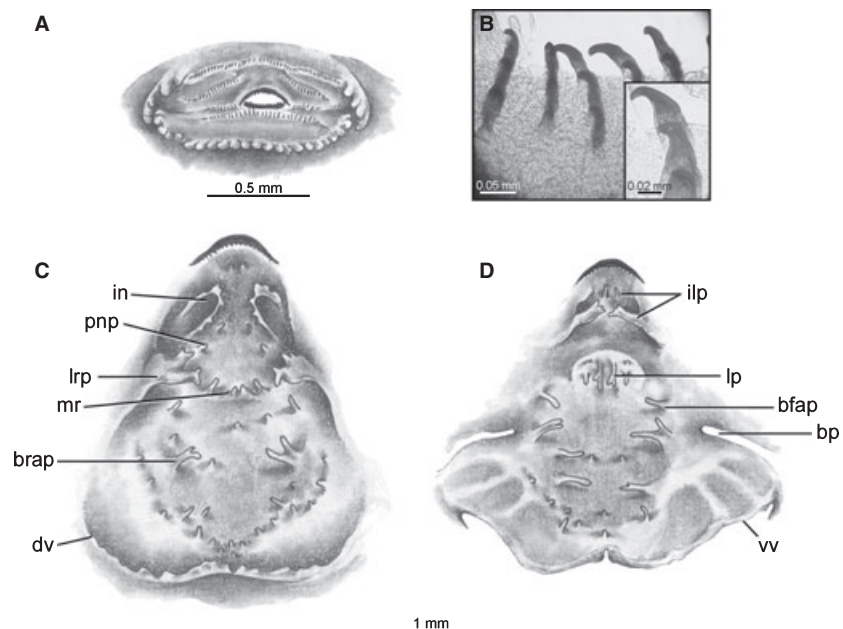

Fig. 2—Oral apparatus and buccal cavity of *Eupsophus emiliopugini*. Stage 31: — **A.** Oral disc, frontal view. — **B.** Detail of labial teeth; three series of labial teeth are observed: those erupted from the labial ridge, and two replacement series of teeth, with the heads interdigitated into the sheath of the tooth above. Stage 34: — **C.** Buccal roof. — **D.** Buccal floor. bfap, buccal floor arena papilla; bp, buccal pocket; brap, buccal roof arena papilla; dv, dorsal velum; ilp, infralabial papilla; in, internal nare; lp, lingual papilla; lrp, lateral ridge papilla; mr, median ridge; pnp, postnarial papilla; vv, ventral velum.

ridges. Row A-1 is the longest with its length equaling that of the upper lip. Rows A-2 and P-1 bear medial gaps equivalent in width to the jaw sheath; the gap in P-1 is narrower than that of A-2. Corresponding labial tooth row formula (LTRF) is 2(2)/2(1), a fractional designation that indicates the total number of anterior (numerator) and posterior (denominator) labial tooth rows; rows with medial gaps are indicated with parentheses. The individual labial teeth (Fig. 2B) are small, sparsely placed (25–35 teeth/mm), and replacement elements are observed below those emerging. The head of each labial tooth is short, curved and narrow, scarcely differentiated from the tooth body. Fine features of the head shape could not be resolved with a light microscope, but cusps seem to be poorly developed or absent. Jaw sheaths are very thin and serrated.

The buccal roof (Fig. 2C) is triangular and unpigmented. The prenarial arena has one pair of small pustules. The internal nares are arranged at an angle of about 54° to the transverse axis, and the anterior margins have small prenarial papillae; the narial valve is not developed. The postnarial arena has one pair of pustules that are anteriorly adjacent to one or two pairs of short papillae. The lateral ridge papillae are wide, flat, and bifurcate, with the caudal point longer and with pustulate tip. The low, semicircular median ridge has a crenulated margin and is flanked on each side by conical papillae. The buccal roof arena is ovoid and defined by four or five pairs of simple papillae (those of the second pair bifurcate), scarce pustules, and small papillae. A glandular zone is not evident. The short dorsal velum has a slightly uneven margin and a broad median notch.

The buccal floor (Fig. 2D) is short and unpigmented. Anteriorly, there are four infralabial papillae. The anterior two are conical and located near one another; the posterior two are medially oriented, but not overlapping, and located on the medial edge of the Meckel's cartilage. The posterior papillae are longer, flatter, and wider than the anterior pair, and their margins are pustulate. There are four lingual papillae on the tongue anlage; the medial papillae are long, cylindrical, and bifid, whereas the papillae of the lateral pair are slightly more caudal, simple, conical, and smaller. Additionally, one pair of pustules appears laterally, aligned with the medial pair of lingual papillae. The buccal pockets are transverse and shallow, and lack any pustulation or papillae. The buccal floor arena is poorly defined, surrounded by five or six pairs of conical papillae (the second pair bifid) and a few pustules. The short, semicircular ventral velum has a slightly uneven margin and a deep median notch; secretory pits are not evident.

Skeleton (n = 2, Stages 31 and 34; Fig. 3). The oval chondrocranium (Fig. 3A) is narrow anteriorly. The suprarostral (Fig. 3A–C) is composed of two rectangular cartilages in a V-shaped configuration and triangular alae with rounded dorsal processes. The trabecular horns articulate at the level of the body-ala junction. They are divergent, short (barely 13% of the chondrocranium length), thin, and uniform in width. The thin orbital cartilages (Fig. 3B) have large optic and oculomotor foramina. The trochlear foramen is present, but not well-defined. The taeniae tecti marginales are thin, and their caudal edge does not reach the otic capsule; the pro-otic foramen is open dorsally. The cranial floor is completely chondrified, with thin cartilage in the central region. The small, round carotid foramina are located near the point of attachment of the ascending process. The craniopalatine foramina are not evident. On the posterior margin of the braincase floor, the notochordal canal reaches about 30% of the chondrocranium length. The otic capsules are oval and
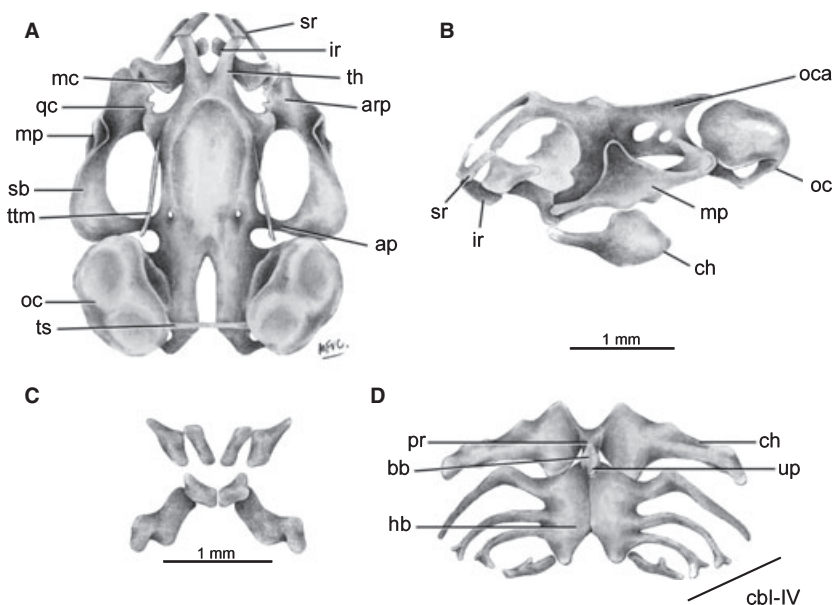

Fig. 3—Chondrocranium and hyobranchial skeleton of *Eupsophus emiliopugini*, Stage 34. — **A.** Chondrocranium dorsal view. — **B.** Chondrocranium lateral view. — **C.** Detail of the suprarostrals (top) and infrarostrals plus Meckel's cartilage (bottom). — **D.** Hyobranchial skeleton ventral view (dorsal spicules poorly developed not illustrated). ap, ascending process; arp, articular process; bb, basibranchial; cbI–IV, Ceratobranchials I–IV; ch, ceratohyal; hb, hypobranchial; ir, infrarostral; mc, Meckel's cartilage; mp, muscular process; oc, otic capsule; oca, orbital cartilage; pr, pars reuniens; qc, quadratocranial commissure; sb, subocular bar; sr, suprarostral; th, trabecular horn; ts, tectum synoticum; ttm, taenia tecti marginalis; up, urobranchial process.

large (36% of the chondrocranium length). The fenestra ovalis is located ventrolaterally, and the operculum, crista parotica, and larval otic process are not defined. The capsules are joined through the thin, scarcely alcianophilic tectum synoticum.

The palatoquadrate has three definite regions. The articular process is short and wide, and on its anterior margin there is a small sinus where the Meckel's cartilage articulates. The muscular process is triangular, low, with a wide base and a rounded tip. Ventrally, the region of articulation with the ceratohyal is evident, as a short, rounded protuberance. The subocular bar widens caudally, and its lateral margin is smooth and rounded. There are two points of articulation between the palatoquadrate and the neurocranium— the quadratocranial commissure, with a small quadratoethmoid process, and the ascending process, attached at the level of the oculomotor foramen. Meckel's cartilage (Fig. 3A,C) is sigmoid, with a well-developed retroarticular process. The infrarostrals (Fig. 3A–C) are oblong, dorsally curved, and joined by a non-chondrified commissure.

In the hyobranchial skeleton (Fig. 3D), the ceratohyals have a triangular, wide anterior process, a low, wide anterolateral process, and a subtriangular, wide posterior process. The articular condyle is stout and rounded. The pars reuniens and the basibranchial are continuous, and on the caudal edge, the quadrangular urobranchial process is present. The basihyal is absent. The hypobranchial cartilages are continuous with the basibranchial, and diverge posteriorly, leaving a caudal

triangular notch. Ceratobranchials I–III are fused to the hypobranchial plate; Ceratobranchial IV is wider and articulates to the hypobranchial plate through a non-chondrified commissure. Ceratobranchials are curved caudally, and lack lateral projections. Distally, the terminal commissures are poorly developed, making the ceratobranchials appear bifurcate. The examination with light microscope reveals small, poorly developed cartilaginous spicules.

Musculature (n = 2, Stages 31 and 34; Fig. 4). The larval muscular configuration in this species is summarized in Table 1. Besides the muscles listed, the tissues that will differentiate as the mm. geniohyoideus medialis, genioglossus ventralis, and hyoglossus are distinguishable.

Development to metamorphosis

External morphology (n = 5, Stages 37–46; Fig. 1 and Table S1, Supporting information). At Stage 37, the tadpole total length and the hind-limb length increase noticeably. The dorsal fin starts at the body–tail junction; both fins are well developed. The coloration is similar to that of the Stage 35 larva, but slightly more intense; a pale orbitonasal line is evident.

At Stage 42/43, the forelimbs have emerged from the branchial chamber, and a thin operculum still covers the proximal region. The snout is truncated in lateral and dorsal views. Most of the keratinized structures on the oral disc have disappeared, but some labial teeth and a well-defined papillar
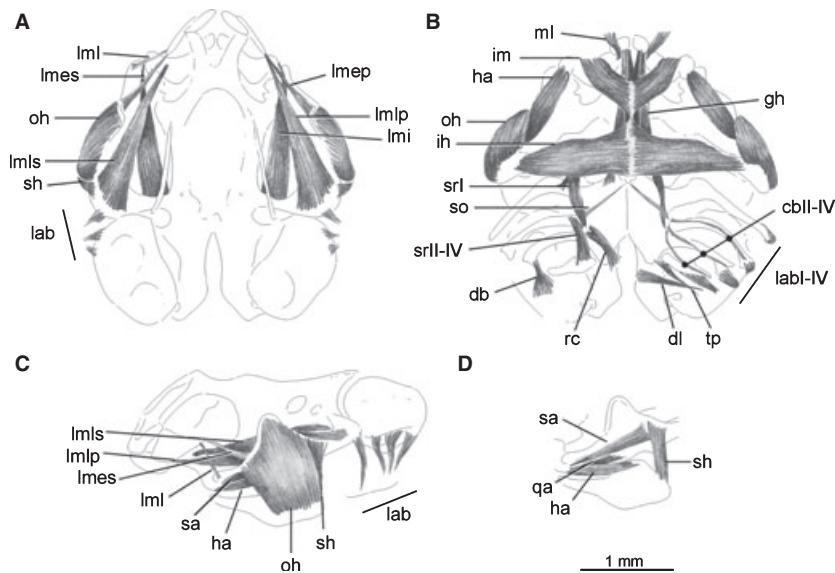

Fig. 4—Larval musculature of *Eupsophus emiliopugini*, Stage 34. — **A.** Dorsal view. — **B.** Ventral view. — **C.** Lateral view. — **D.** Detail of the angularis muscles, lateral view. cbII–IV, constrictor branchialis II–IV; db, diaphragmatobranchialis; dl, dilatator laryngis; gh, geniohyoideus; ha, hyoangularis; ih, interhyoideus; im, intermandibularis; labI–IV, levator arcuum branchialium I–IV; 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.

Table 1 Larval musculature of *Eupsophus emiliopugini*, Stage 34

Muscles	Insertion sites
Mandibular muscles	
Intermandibularis	Anterior margin of Meckel's cartilage—median aponeurosis
Levator mandibulae articularis	Medial surface of the muscular process—lateral edge of Meckel's cartilage
Levator mandibulae externus profundus	Medial surface of the muscular process, medial to m. levator mandibulae externus superficialis—ventral margin of the suprarostral ala. The anterior insertion is through a tendon common to the m. levator mandibulae longus profundus
Levator mandibulae externus superficialis	Medial surface of the muscular process—lateral edge of the suprarostral ala. It is very thin, constituted by scarce fibers
Levator mandibulae internus	Ventral surface of the ascending process—lateral edge of Meckel's cartilage. The insertion on Meckel's cartilage is through a well-developed tendon
Levator mandibulae lateralis	Dorsolateral edge of the articular process—lateral edge of the suprarostral ala. It is very thin, formed of 3 or 4 fibers
Levator mandibulae longus profundus	Posterolateral and posterior margin of the subocular bar, ventral to the m. levator mandibulae longus superficialis—ventral margin of the suprarostral ala
Levator mandibulae longus superficialis	Posterolateral and posterior margin of the subocular—dorsomedial point of Meckel's cartilage
Mandibulolabialis	Ventromedial edge of Meckel's cartilage—lower lip of the oral disc. It is represented by a single slip, corresponding to the m. mandibulolabialis inferior
Submentalis	Ventral surface of the infrarostrals, without median aponeurosis
Hyoid muscles	
Hyoangularis	Dorsal point on the lateral edge of the ceratohyal—retroarticular process
Interhyoideus	Lateral edge of the ceratohyal –median aponeurosis. This muscle aponeurosis joins that of the m. intermandibularis, forming a X-shaped structure
Interhyoideus posterior + diaphragmatopraecordialis	The presence of these muscles could not be verified after observation with stereomicroscope; a histologic study would be needed
Orbitohyoideus	Anterior, dorsal, and posterior margin of the muscular process—dorsolateral edge of the ceratohyal
Quadratoangularis	Medial point of the ventral region of the muscular process—retroarticular process
Suspensorioangularis	Posterior point of the lateral surface of the muscular process—retroarticular process
Suspensoriohyoideus	Posterior margin of the muscular process and part of the subocular bar—dorsolateral edge of the ceratohyal
Branchial muscles	
Constrictor branchialis II	Proximal edge of Ceratobranchial II—distal edge of Ceratobranchial II. It is disposed on Ceratobranchial II; some fibers are continuous with those of the mm. levator arcuum branchialium II and subarcualis rectus II–IV
Constrictor branchialis III	Proximal edge of Ceratobranchial II—distal edge of Ceratobranchial III. It is disposed on the Ceratobranchial III; some fibers are continuous with those of the m. levator arcuum branchialium III
Constrictor branchialis IV	Proximal edge of Ceratobranchial II—distal edge of Ceratobranchial IV. It is disposed on Ceratobranquial IV; some fibers are continuous with those of the m. levator arcuum branchialium IV
Constrictor laryngis	Sphincter–like disposition, surrounding the glottis
Diaphragmatobranchialis	Peritoneum—distal edge of Ceratobranchial III
Dilatator laryngis	Posterolateral surface of the otic capsule—lateral margin of the glottis
Levator arcuum branchialium I	Posterolateral point of the subocular bar—distal edge of Ceratobranchial I
Levator arcuum branchialium II	Lateral surface of the otic capsule—distal edge of Ceratobranchial II
Levator arcuum branchialium III	Lateral surface of the otic capsule—distal edge of Ceratobranchial III
Levator arcuum branchialium IV	Posterolateral surface of the otic capsule—distal and medial surface of Ceratobranchial IV
Subarcualis obliquus	Urobranchial process—proximal point of Ceratobranchial II. It is very thin, almost unnoticeable
Subarcualis rectus I	Point lateral to the base of the posterior hyal process –proximal point of Ceratobranchial I (dorsal slip) and proximal point of Ceratobranchial III (ventral slip). The dorsal slip is short and very thin
Subarcualis rectus II–IV	Ventral, proximal surface of Ceratobranchial IV—proximal point of Ceratobranchial II. Some fibers are continuous with those of the m. constrictor branchialis II
Tympanopharyngeus	Posterolateral surface of the otic capsule—proximal edge of Ceratobranchial IV and posterolateral margin of the hypobranchial plate. The origin of this slip is fully independent from that of the m. levator arcuum branchialium IV
Hypobranchial muscles	
Geniohyoideus	Ventral surface of the infrarostral—hypobranchial plate, next to the junction point with Ceratobranchial II. Some fibers insert on Ceratobranchial II, and are continuous with those of the m. rectus cervicis
Rectus abdominis	Peritoneum, at the level of the posterior region of the branchial basket—pelvic girdle
Rectus cervicis	Peritoneum—proximal point of Ceratobranchial II and connective tissue between Ceratobranchials II and III. Two very close slips are distinguished; some fibers are continuous with those of a medial slip of the m. rectus abdominis

Fig. 5—Skeletal development of *Eupsophus emiliopugini*, chondrocranium dorsal view. — **A.** Stage 37. — **B.** Stage 42/43. — **C.** Stage 46. Arrows point those places with morphological changes most relevant regarding to the previous developmental stage, mainly concerning the taeniae transversalis and medialis, the ethmoid region, the lengthening of the lower jaw elements, and the restructuring and reorientation of the palatoquadrate.
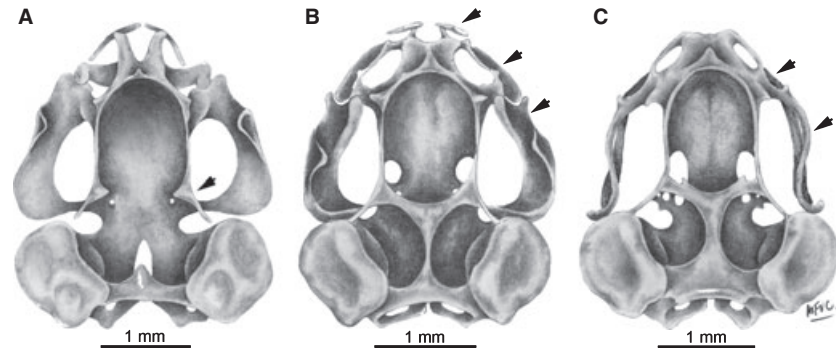
margin remain at the commissural region and on the lower lip. The eyes protrude slightly and are oriented anterolaterally; eyelids are developed. The limbs are completely developed, and the manus and pes bear metacarpal, metatarsal, and subarticular tubercles, respectively. The vent tube is absent. The tail is still very long but distal myotomes have begun to disappear. The caudal fins are regressing; the origins are coincident with the third or fourth tail myotome, and the height of each fin is diminished. The patterns of dorsal and limb coloration are evident. The abdominal region is smooth and the tail is scarcely pigmented; the fins remain unpigmented.

At Stage 44, the abdomen is still rounded and filled with vitellum. The mouth is slitlike, and some marginal papillae still remain on the commissural regions; also, remnants of the first labial ridge with labial teeth can be seen in lateral regions. Dorsally, the skin becomes more verrucose, and the definitive adult coloration is achieved.

A juvenile at Stage 46 resembles an adult in external morphology. The acuminate snout protrudes over the lower jaw; the mouth is defined and the tongue is present. The canthus rostralis is defined. The eyes protrude anterolaterally. A

tympanic membrane is absent. Large amounts of vitellum remain in the gut. The tail bud is completely reabsorbed. The dorsal skin and the seat patch are verrucose, and the entire adult coloration is evident.

Skeleton (n = 3, Stages 37, 42/43 and 46; Figs 5–7). At Stage 37 (Fig. 5A), the septum nasi and the antorbital processes are present in the ethmoid region. Additionally, the taeniae tecti transversalis and medialis are present.

At Stage 42/43 (Figs 5B and 6A), the anterior region of the chondrocranium, the lower jaw, and the palatoquadrate change profoundly. The suprarostral cartilages are almost reabsorbed, leaving two small cartilaginous remnants anteriorly. The infrarostrals and Meckel's cartilages extend transversally and curve caudally, and the ventromedial and retroarticular processes have disappeared from Meckel's cartilages. In the ethmoid region, the antorbital processes lengthen laterally. The trabecular horns shorten and reorganize, and prenasal cartilages and the crista subnasalis are present. The tectum, and anteriorly, the inferior prenasal cartilages are present. In lateral and ventral views of the chondrocranium,
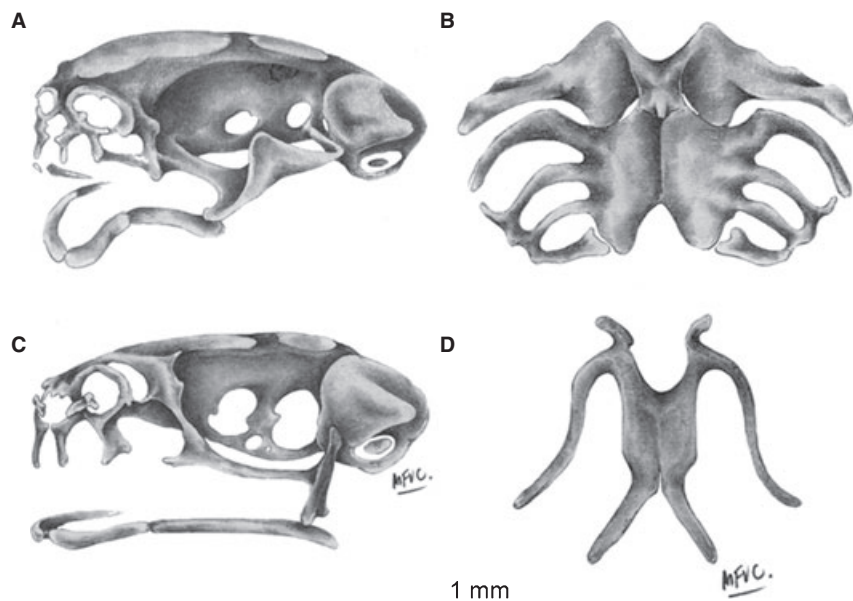

Fig. 6—Skeletal development of *Eupsophus emiliopugini*. Stages 42/43. — **A.** Chondrocranium, lateral view. — **B.** Hyobranchial skeleton, ventral view. Stage 46. — **C.** Chondrocranium, lateral view. — **D.** Hyobranchial skeleton, ventral view.
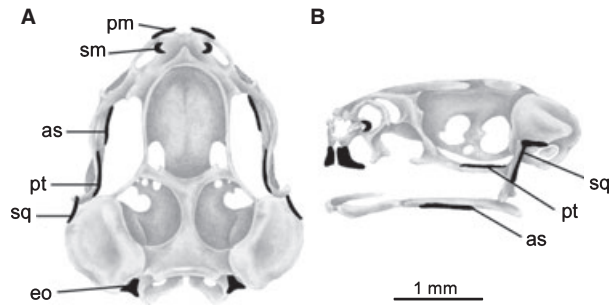
Fig. 7—Cranial ossifications present at metamorphed specimen (Stage 46). — **A.** Dorsal view. — **B.** Lateral view. as, angulosplenial; eo, exoccipitals; pm, premaxilla; pt, pterygoid; sm, septomaxilla; sq, squamosal.

three foramina are visible —the optic foramen, confluent with the craniopalatine foramen; the oculomotor foramen, which includes the carotid foramen; and the prootic foramen. The palatoquadrate is obliquely oriented, and all its regions are still distinguishable. The muscular process is reduced in its height, and the ascending process is oriented more vertically. In the otic capsules, the operculum is evident in the fenestra ovalis. Dorsally, the taeniae tecti transversalis and medialis are completely formed, although scarcely alcianophilic. The hyobranchial skeleton (Fig. 6B) lengthens anteroposteriorly, but the profound changes that will result in the adult configuration are not yet evident. Terminal commissures II, III, and IV are formed.

Finally, in the juvenile at Stage 46 (Figs 5C and 6C), the suprarostral cartilage is absent. In the nasal region, the oblique cartilages are joined with the lamina inferior of the antorbital process. The alary and inferior prenasal cartilages are present. The short, acute anterior maxillary process and long, slender posterior maxillary process projected from the antorbital plate. The posterior process fuses to the quadratoethmoid process, forming the pterygoid process. The supraorbital process is evident on the posteromedial margin of the antorbital plate. The elements of the lower jaw are longer and thinner, and the region of articulation between the infrarostral and the Meckel's cartilage is still noticeable. The symphysis between infrarostrals remains conjunctive. The palatoquadrate is vertically oriented and looses connection with the neurocranium. The posterior region of the subocular bar has disappeared, and the articular and muscular processes and the quadratocranial commissure are absent. Finally, the hyobranchial skeleton (Fig. 6D) is completely restructured. The ceratohyals are very thin and sigmoid, and constitute the hyal cartilages; the anterior, posterior, and lateral processes have disappeared. The anterior processes of the hyale appear from new cartilaginous centers. The basibranchial is fused completely to the hyobranchial plate, resulting the hyoid plate; the urobranchial process is absent. Posterolateral and posteromedial processes are observed. Ceratobranchials are absent.

Regarding cranial ossifications (Fig. 7), at Stage 46, premaxillae and septomaxillae are ossified. The angulosplenials have begun to ossify in the lower jaw. The ventral and otic rami of the squamosal are present, and the pterygoid is a slender sliver dorsal to the pterygoid process. In the posterior region of the otic capsules, the exoccipitals begin to mineralize.

Musculature (n = 3, Stages 37, 42/43, and 46; Figs 8 and 9). At Stage 37 (not shown), the mm. geniohyoideus medialis and lateralis are evident. The m. geniohyoideus medialis is scarcely developed, and extends between a point lateral to the basibranchial and the ventromedial edge of the infrarostral cartilage and intramandibular commissure. Additionally, the mm. orbitohyoideus and suspensoriohyoideus are indistinguishable.

At Stage 42/43 (Fig. 8), the mm. submentalis and intermandibularis grow longer, accompanying the elongation of the infrarostral and Meckel's cartilages (Fig. 8A). The m. geniohyoideus medialis (Fig. 8B) is completely developed, and reaches the hypobranchial plate with its caudal insertion. The m. hyoglossus originates on the tongue anlage and inserts on the anterior region of the hypobranchial plate. The m. genioglossus ventralis originates on the tongue anlage and inserts on the infrarostral ventral surface. The jaw levator muscles (Fig. 8C) have a more dorsoventral orientation. The mm. levator mandibulae externus and longus profundus have not yet changed their insertions to the Meckel's cartilage; they are still anteriorly inserted on connective tissue next to the suprarostral cartilage remnants. The m. levator mandibulae externus superficialis is absent. Among angularis muscles, mm. suspensorioangularis, quadratoangularis and hyoangularis are still independent (Fig. 8D).

Finally, in the juvenile at Stage 46 (Fig. 9), the m. intermandibularis forms a band constituted by transverse fibers that extend between the ventromedial surface of Meckel's cartilages; an anterior supplementary element characterized by oblique fibers is evident. The m. interhyoideus parallels the development of the hyoid (Fig. 9A). The mm. geniohyoideus are well developed and positioned longitudinally between the mandibular symphysis and the medial portion of each half of the lower jaw, and the base of the posterolateral process of the hyoid (Fig. 9B). The m. hyoglossus is very long, inserting on the posteromedial process of the hyoid. The jaw levator muscles are dorsoventrally oriented, a typical configuration of adult anurans (Fig. 9C). The mm. levator mandibulae longus superficialis and profundus constitute a single muscle, the m. levator mandibulae longus, which originates on the anterodorsal surface of the otic capsule, runs ventrally, and inserts on a posterior point of the inner surface of the lower jaw. The m. levator mandibulae internus changes its origin to the anteromedial surface of the otic capsule, and inserts on the inner surface of the lower jaw, medial to the m. levator mandibulae longus. The m. levator mandibulae articularis is a short muscle placed caudal to the m. levator mandibulae longus, and
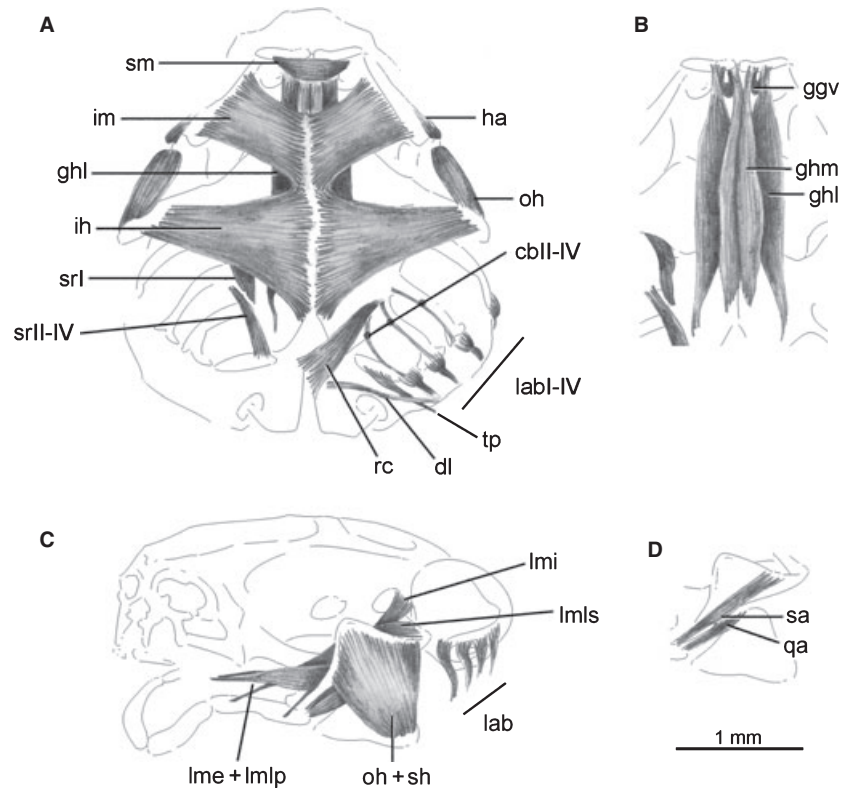
Fig. 8—Musculature of *Eupsophus emiliopugini*, Stage 42/43. — **A.** Ventral view. — **B.** Detail of mm. geniohyoideus and genioglossus. — **C.** Lateral view. — **D.** Detail of angularis muscles. cbII–IV, constrictor branchialis II–IV; dl, dilatator laryngis; ggv, genioglossus ventralis; ghl, geniohyoideus lateralis; ghm, geniohyoideus medialis; ha, hyoangularis; ih, interhyoideus; im, intermandibularis; labI–IV, levator arcuum branchialium I–IV; lme, levator mandibulae externus; lmi, levator mandibulae internus; lmlp, levator mandibulae longus profundus; lmls, levator mandibulae longus superficialis; oh, orbitohyoideus; qa, quadratoangularis; rc, rectus cervicis; sa, suspensorioangularis; sm, submentalis; srI–IV, subcualis rectus I–IV; tp, tympanopharyngeus.

extends between the anterolateral surface of the otic capsule and the dorsomedial, posterior margin of the lower jaw (Fig. 9D). The m. levator mandibulae externus is the most superficial muscle, and it includes possibly the fibers of the larval mm. levator mandibulae externus superficialis and profundus. The muscle originates on the ventral ramus of the squamosal bone, descends parallel to it, and inserts on the dorsolateral, posterior region of the lower jaw, concealing the m. articularis and the inferior portion of the mm. levator mandibulae longus and internus. At this stage, the m. levator mandibulae lateralis is a fan-shaped muscle originating from a small external surface of the quadrate, and inserting on the posterolateral of the lower jaw. The jaw depressor muscles are oriented dorsoventrally (Fig. 9C). The mm. orbitohyoideus and suspensoriohyoideus are joined forming a single muscle that extends between the otic ramus of the squamosal and the lateral surface of the otic capsule, and the posterior edge of the lower jaw. The mm. quadratoangularis, suspensorioangularis, and hyoangularis join to form a cylindrical slip located between the ventral margin of the otic ramus of the squamosal and the posterior edge of the lower jaw. Among the branchial muscles (Fig. 9A), the mm. subcualis obliquus, subcualis recti, and diaphragmatobranchialis are absent. Five slips are still distinguishable running from the edge of the hyale, the edge of the posteromedial process, and the connective tissue between them, to the ventral surface of the otic capsule; they correspond to the mm. levator arcuum branchialium I–IV and

tympanopharyngeus, and probably incorporate fibers of the mm. constrictores branchiales. The mm. dilatator and constrictor laryngis maintain the typical disposition of larval stages. Finally, the m. rectus cervicis constitutes the adult m. sternohyoideus, extending between the hyoid and the developing sternum.

Discussion

Within Alsodinae (Cycloramphidae), species of *Eupsophus* are distinguished by having nidicolous tadpoles, characterized by a combination of traits that typify generalized exotrophic (e.g., oral disc with keratinized mouthparts, spiracular tube and opening) and endotrophic larvae (e.g., scant pigmentation, large hind limbs, small size at metamorphosis) (Thibaudeau and Altig 1999; Vera Candioti *et al.* 2005). Larval stage descriptions are available for six of the ten *Eupsophus* species: *E. roseus* (Formas and Pugín 1978), *E. calcaratus* (Formas 1989a; Vera Candioti *et al.* 2005), *E. emiliopugini* (Formas 1989b), *E. vertebralis* (Formas 1992b), *E. queulensis* (Cárdenas-Rojas *et al.* 2007a), and *E. nahuelbutensis* (Nuñez and Úbeda 2009). Although these larvae are qualitatively similar, some characters discriminate among them. With respect to tadpoles of *E. vertebralis* (*E. vertebralis* Group), Formas (1992b) mentioned differences in the snout and tail shape, dorsal pigmentation, and several variables related to tadpole size. Nuñez and Úbeda (2009) show morphometric
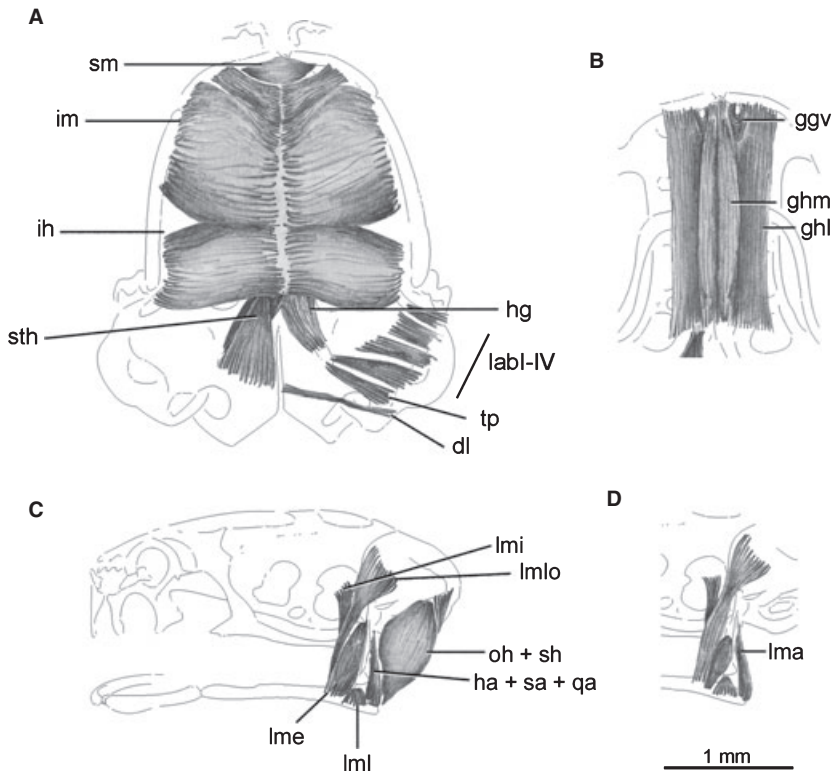
Fig. 9—Musculature of *Eupsophus emiliopugini*, Stage 46. — **A.** Ventral view. — **B.** Detail of mm. geniohyoideus and genioglossus. — **C.** Lateral view. — **D.** Detail of the m. levator mandibulae articularis. dl, dilatator laryngis; ggv, genioglossus ventralis; ghl, geniohyoideus lateralis; ghm, geniohyoideus medialis; ha, hyoangularis; hg, hyoglosuss: ih, interhyoideus; im, intermandibularis; labI–IV, levator arcuum branchialium I–IV; lma, levator mandibulae articularis; lme, levator mandibulae externus; lmi, levator mandibulae internus; lml, levator mandibulae lateralis; lmlo, levator mandibulae longus; oh, orbitohyoideus; qa, quadratoangularis; sa, suspensorioangularis; sh, suspensoriohyoideus; sm, submentalis; sth, sternohyoideus; tp, tympanopharyngeus.

differences between larvae of *E. emiliopugini*, *E. nahuelbutensis*, *E. vertebralis*, and *E. roseus*, mainly in the tail height/total length proportion.

Table 2 lists morphological features of *Eupsophus emiliopugini* in comparison with other *Eupsophus* species, exotrophic and endotrophic forms within Cycloramphidae, and nidicolous tadpoles from unrelated families. The oral disc of *E. emiliopugini* tadpoles closely resembles those described for congeneric larvae. The labial tooth row formula 2/2 is a deviation from the subfamily general pattern; a third labial ridge appears in other alsodines, and a vestigial third lower labial tooth row was observed in one Stage-36 tadpole of *E. calcaratus* (Vera Candioti et al. 2005). Likewise, exotrophic larvae in Cycloramphinae have a third lower labial ridge, whereas endotrophic species show some reduction in the oral apparatus. In this context, the oral apparatus apparently generalized of *Eupsophus* tadpoles, like that of the endotrophic cycloramphines, would represent a reduction with respect to the usual condition in the family (i.e., a complete provision of three lower labial ridges; Fig. 10), probably associated to the lack of active feeding. The third lower labial ridge is the last to form embryologically, and Altig (2006) interpreted its lost in several species or species groups as a product of a truncated development. Reduced or absent oral discs also characterizes other nidicolous tadpoles within Aromobatidae, Bufonidae, Hemiphractidae, and Leptodactylidae (Table 2). Wassersug and Duellman (1984) found a progressive loss of oral papillation, keratinized mouthparts, and several buccopharyngeal

features in *Gastrotheca* species, from those producing free-living tadpoles to those direct-developers. By comparing oral and buccal ontogenies, these authors also found that advanced stages in direct-developing species resemble early embryos of tadpole-producing species, and concluded that morphological patterns in direct-developing forms can be accounted for by truncation of tadpole development. This could be also the case with *Eupsophus* characteristics, although detailed studies on oral ontogeny of related genera (e.g., sister taxa *Alsodes*) would be needed to confirm it. Finally, in a recent paper by Correa et al. (2006), *Insuetophrynus acarpicus* has been related to *Rhinoderma*, and thus included in Cycloramphidae. This exotrophic species is characterized by a reduced oral apparatus (see Table 2), and should its inclusion in Cycloramphidae be confirmed, it would disprove the relationship between endotrophy and oral reduction, at least in this family.

The buccopharyngeal cavity of *Eupsophus emiliopugini* is typical of most generalized larvae, although an overall scarcity of papillation is evident. Most Alsodinae tadpoles share the presence of four infralabial papillae and four lingual papillae (Fig. 10), whereas the configuration of the remaining buccal structures is highly variable. As compared with exotrophic species, endotrophic tadpoles show a marked reduction or absence of filtering and entrapping structures (see Table 2).

With respect to the cartilaginous skeleton, *Eupsophus emiliopugini* tadpoles resemble those of other *Eupsophus* species in several features; some minor differences appear in supra- and

Table 2 Morphological features of *Eupsophus emiliopugini*, as compared with (1) other *Eupsophus* species— major differences in previously described *Eupsophus* tadpoles are noted; (2) alsodine genera— shared (=) and different (≠) characters are noted; variable features are summarized; variable features are detailed for each genera; (3) cycloramphine genera— distinctive characters of exotrophic and endotrophic forms are noted; and (4) unrelated endotrophic nidicolous tadpoles— similar characters are noted

	DM	Oral disc	Buccal cavity	Larval skeleton	Larval musculature
Eupsophus emiliopugini (Formas 1989b; this work)	NI	Small, subterminal, slightly emarginate; marginal papillae with dorsal gap; LTRF 2/2; sparse labial teeth with reduced or absent cusps	All typical structures present, although an overall scarce papillation is evident; four infralabial papillae; four lingual papillae, the medial pair often larger	Supra- and infrarostral cartilages with independent elements, short trabecular horns (10–13% of the chondrocranium length); dorsally open prootic foramen; quadrato-orbital commissure absent; larval otic process absent; large otic capsules (33–37% of the chondrocranium length); thin tectum synoticum; quadratoethmoid process present; hyobranchial skeleton present; hyobranchial skeleton without basihyal; bar-like ceratobranchials without lateral projections; spicules reduced or absent	All mandibular, hyoid, branchial, and hypobranchial muscles typical of generalized larvae; m. subarcualis obliquus inserted on Ceratobranchial II; m. rectus cervicis with a double proximal insertion; m. tympanopharyngeus fully independent from m. levator arcuum branchialium IV; mm. constrictores branchiales and levatores arcuum branchiales aligned on the same ceratobranchial, often with continuous fibers; ventral slip of the m. subarcualis rectus I inserted on the proximal edge of Ceratobranchial III; m. subarcualis rectus II–IV inserted on the proximal edge of Ceratobranchial II
(1) Other *Eupsophus* species E. *calcaratus* (Formas 1989a; Vera Candioti et al. 2005) E. *nahuelbutensis* (Nuñez and Úbeda 2009) E. *queulensis* (Cárdenas-Rojas et al. 2007a) E. *roseus* (Formas and Pugín 1978; Wassersug and Heyer 1988) E. *vertebralis* (Formas 1992b)	NI	Not emarginated in E. *queulensis*	*Eupsophus roseus* with glandular zone, more buccal papillation, and ventral velum with well-developed spicular support, secretory pits, and marginal projections	Suprarostral with fused elements in E. *queulensis*, infrarostral with fused elements in E. *nahuelbutensis*, both cartilages with fused elements in E. *calcaratus*; prootic foramen closed in E. *nahuelbutensis*	*Eupsophus calcaratus* with constrictores branchiales and levatores arcuum branchiales not aligned on the same ceratobranchial, and m. subarcualis rectus II–IV converging on the proximal edge of Ceratobranchial II[1]
(2) Other alsodines		≠ LTRF 2/3; labial teeth labial teeth with numerous cusps	= Four infralabial papillae, four lingual papillae, the medial pair often larger	= Quadratoethmoid process present; ≠ quadrato-orbital commissure present; long pseudopterygoid process; ceratobranchials with lateral projections	= M. mandibulolabialis superior absent; mm. levator mandibulae externus superficialis and profundus present; m. tympanopharyngeus present; m. subarcualis obliquus inserted on Ceratobranchial II; m. rectus cervicis with a double proximal insertion; ≠ m. subarcualis rectus I with three portions; m. subarcualis rectus II–IV inserted on Ceratobranchial I

Table 2 (*Continued*)

	DM	Oral disc	Buccal cavity	Larval skeleton	Larval musculature
Alsodes (Díaz and Valencia 1985; Echeverría *et al.* 2001; Formas and Brieva 2004; Lavilla 1992; Wassersug and Heyer 1988)	EX			Suprarostral with independent elements; adrostral cartilages; trabecular horns >20% of the chondrocranium length; larval otic process present in *A. barrioi*	ND
Hylorina (Wassersug and Heyer 1988; Cárdenas-Rojas *et al.* 2007b)	EX			Suprarostral with independent elements; trabecular horns >20% of the chondrocranium length	ND
Limnomedusa (Alcalde and Blotto 2006)	EX		Infralabial papillae absent	Suprarostral tripartite; trabecular horns ≤20% of the chondrocranium	ND
Macrogenioglottus (Abravaya and Jackson1978; cited in Altig and McDiarmid1999b; Wassersug and Heyer 1988)	EX			ND	ND
Odontophrynus (Wassersug and Heyer 1988; Lavilla and Scrocchi 1991; Echeverría and Filipello1994; Echeverría and Montanelli 1995; Rossa-Feres and Jim 1996; Fabrezi and Vera 1997; Palavecino 1999; Haas 2003)	EX			Suprarostral with independent elements; adrostral cartilages in *O. achalensis* and *O. americanus*; trabecular horns >20% of the chondrocranium length	Mm. interhyoideus posterior and diaphragmatopraecordialis arranged in an angled pattern
Proceratophrys (Wassersug and Heyer 1988; Eterovick and Sazima 1998; Giaretta *et al.* 2000; de Sá and Langone 2002)	EX			ND	ND
Thoropa (Wassersug and Heyer 1983, 1988; Caramaschi and Sazima 1984) (3) Cycloramphines	EX		Two infralabial papillae	ND	ND
Crossodactylodes (Peixoto 1981; Wassersug and Heyer 1988)	EX	LTRF 2/3	8–10 infralabial papillae; four lingual papillae; postnarial, lateral ridge, and buccal roof and floor papillae present	ND	ND
Cycloramphus (Heyer 1983a; Wassersug and Heyer 1988; Lavilla 1991)	EX	LTRF 2/3	Four lingual papillae; postnarial papillae present; numerous buccal roof and floor papillae	ND	ND
	NI	LTRF 2/2	Reduction or absence of lingual, infralabial, lateral ridge, and buccal roof and floor papillae; glandular zone and secretory pits absent	Short trabecular horns; quadrato-orbital commissure absent; basihyal absent; bar-like ceratobranchials without lateral projections; spicules absent	ND

Table 2 (*Continued*)

	DM	Oral disc	Buccal cavity	Larval skeleton	Larval musculature
Insuetophrynus (Rabanal and Formas 2009)	EX	LTRF 2/2; weak, sparse labial teeth with reduced or absent cusps	Five lingual papillae; dense buccal roof and floor papillation	Trabecular horns >20% of the chondrocranium; adrostral cartilages; quadrato-orbital commissure present; ceratobranchials without lateral projections; spicules present	ND
Rhinoderma (Lavilla 1987; Wassersug and Heyer 1988)	EV	LTRF 0/3; third labial ridge very short; overall lack of keratinization	Infralabial and lateral ridge papillae absent; 3/4 lingual pustules; numerous buccal roof and floor papillae; glandular zone and secretory pits absent	Short trabecular horns; quadrato-orbital commissure absent; basihyal absent; ceratobranchials without lateral projections; spicules reduced	ND
(4) Other endotrophic nidicolous anurans					
Zachaenus (Lutz 1944; Heyer 1969; Heyer and Crombie 1979)	NI	LTRF 1/1; reduced total number of labial teeth	ND	ND	ND
Allobates (Aromobatidae; Caldwell and Lima 2003; Kaiser and Altig 1994)	NI	Absent or with reduced keratinization	ND	ND	ND
Anomaloglossus (Aromobatidae; Caldwell and Lima 2003; Juncá et al. 1994)	NI	Absent or with reduced keratinization	ND	ND	ND
Flectonotus (Hemiphractidae; Duellman and Gray 1983; Haas 1996a; Wassersug and Duellman 1984)	NI	Reduced or absent keratinized mouthparts and/or marginal papillae	Ventral velum and branchial food traps reduced	Short trabecular horns; basihyal absent; bar-like ceratobranchials without lateral projections; spicules reduced	Mm. levator mandibulae externus superficialis, subarcualis rectus I (dorsal head), and interhyoideus posterior absent; mm. constrictors branchiales poorly developed
Syncope antenori (Microhylidae; Krügel and Richter 1995)	NI	LTRF 0/0[2]	Food traps absent	ND	ND
Pelophryne (Bufonidae; Inger 1960; Leong and Teo 2009)	NI	LTRF 1/0	ND	ND	ND
Leptodactylus (Leptodactylidae; De la Riva 1995; Heyer 1975; Heyer and Silverstone 1969; Kokubum and Giaretta 2005; Wassersug and Heyer 1988)	NI	Some species with poorly developed or absent jaw sheaths, and labial teeth absent	Lingual papillae reduced; prepocket, and buccal roof and floor papillae absent; glandular zone, dorsal velum, and secretory pits absent	ND	ND

Endotrophic tadpole categorization follows the scheme by Thibaudeau and Altig (1999).
DM, developmental mode; EX, exotrophic; EV, endotrophic exoviviparous; NI, endotrophic nidicolous; LTRF, labial tooth row formula; ND, no data.
[1]Examination of Stage-31 and 36 *E. calcaratus* specimens with a light microscope revealed that muscles reported as absent (mm. levator mandibulae externus superficialis, levator mandibulae lateralis and subarcualis obliquus) are in fact present, and because of their small size and poor development have been overlooked during previous observations with stereomicroscope.
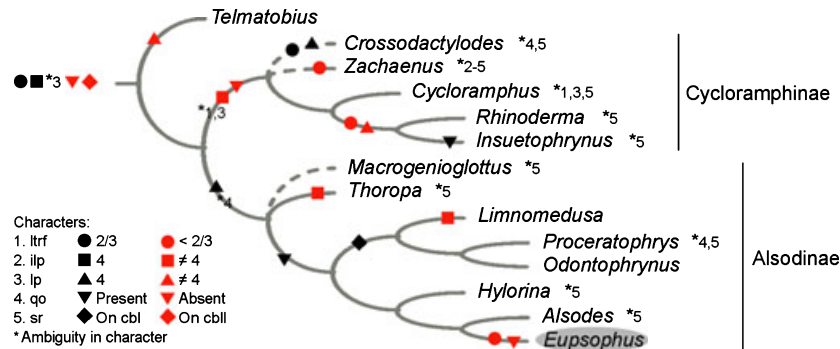[2]Like most microhylids.

Fig. 10—Distribution of some morphological features within Cycloramphidae. The tree is based on Frost *et al.* (2006), updated in Frost 2009) and Correa *et al.* (2006) for *Insuetophrynus* placement. *Rupirana* was not included because its location within Cycloramphidae remains provisional. Taxa with dashed lines have unresolved phylogenetic relationships within subfamilies. The tree is rooted in *Telmatobius* (Ceratophryidae), and character coding followed references in Table 2 for cycloramphids and Vera Candioti (2008) for the outgroup. Optimization was made using Winclada (Nixon 1999). cbI, Ceratobranchial I; cbII, Ceratobranchial II; ilp, infralabial papillae; lp, lingual papillae; ltrf, labial tooth row formula; qo, quadrato-orbital commissure; sr, m. subcualis rectus II–IV. Ambiguity is because of lack of data in all cases excepting characters 1 and 3 of *Cycloramphus*, in which exotrophic and endotrophic species have different states.

infrarostral cartilages (Table 2). The only morphological feature that appears to be constant within Alsodinae is the presence of the quadratoethmoid process; unlike *Eupsophus*, the remaining alsodines described have a quadrato-orbital commissure (Fig. 10) and a long pseudopterygoid process, which may be independent of the neurocranium or fused to its floor. Other characters vary widely among genera, e.g., the configuration of the suprarostral cartilage, the presence of adrostral cartilages, the extension of the trabecular horns, and the presence of larval otic process. On the other hand, *Eupsophus* and other nidicolous tadpoles share the short trabecular horns and the general configuration of the hyobranchial skeleton. The hyobranchial skeleton resembles also those of embryos of direct-developing *Philautus silus* and *Eleutherodactylus coqui* (Hanken *et al.* 1992; Kerney *et al.* 2007), and those of exotrophic macrophagous tadpoles (e.g., *Dendropsophus microcephalus*, *D. nanus*, *Lepidobatrachus llanensis*; Vera Candioti 2007). The reduction of the skeletal structures that support the filtering and entrapment structures is consistent with the fact that these species obtain nutrients through mechanisms other than by filter-feeding.

The larval myological configuration of *Eupsophus emiliopugini* includes all mandibular, hyoid, branchial, and hypobranchial muscles typical of generalized larvae, with only minor differences having been noted in the branchial muscles of *E. calcaratus* (Table 2). Unlike *Eupsophus*, other Alsodinae tadpoles described have a m. subcualis rectus I with three heads, and a m. subcualis rectus II–IV inserted on Ceratobranchial I. This latter trait was proposed as a synapomorphy of Cycloramphidae (including hylodines) by Frost *et al.* (2006); the alternate condition in *Eupsophus* resembles that of the species in the sister clade Ceratophryidae (Fig. 10; Haas 2003; Vera Candioti 2007, 2008). Finally, the only other nidicolous larva for which there are myological data is *Flectonotus goeldii*, and unlike *Eupsophus*, this species has a poorly developed branchial musculature that lacks several muscles (see Table 2).

Eupsophus species share a reproductive mode characterized by small clutches of large, yolk-rich, nonpigmented eggs deposited in varied humid sites, a relatively short larval development (<90 days), and a small size at metamorphosis (<10 mm) (Formas and Vera 1980; Díaz 1986; Vera Candioti *et al.* 2005). In a group of recent papers, Fabrezi and collaborators (Fabrezi and Quinzio 2008; Fabrezi and Goldberg 2009; Fabrezi *et al.* 2009a, 2009b) discussed patterns of development and metamorphosis in several exotrophic species, within the frame of heterochronic changes and uncoupling of developmental events. The development of nidicolous larvae differs from that of most exotrophic larvae, by including features characteristic of these larvae that either fail to develop or that do not develop fully (e.g., adhesive glands, external gills, oral apparatus, coiled intestine, lateral line system, and spiracle; Caldwell and Lima 2003; Leong and Teo 2009; Thibaudeau and Altig 1999 and cites therein; Vera Candioti *et al.* 2005), as well as characters that differ in time and sequence of appearance and disappearance.

Figure 11 is a schematic representation of some landmark events taking place during the development of *Eupsophus emiliopugini*, as compared with other species. Bininda-Emonds *et al.* (2007) revised the relative timing of fore- vs. hind-limb development in vertebrates, and suggested that plesiomorphically, forelimbs would develop earlier than hind limbs. Among lissamphibians studied, the hind limbs of *Xenopus laevis* and *Eleutherodactylus coqui* develop earlier than forelimbs, whereas *Pseudis platensis*, *Discoglossus pictus*, and *Rana temporaria* have an isochronic onset of limb buds (Bininda-Emonds *et al.* 2007; Fabrezi *et al.* 2009). In *E. emiliopugini*, hind limbs begin developing early relative to forelimbs and to general development (prior to the loss of gills), and almost reach juvenile proportions at Stage 42/43. Likewise, in *Allobates chalcopis*,
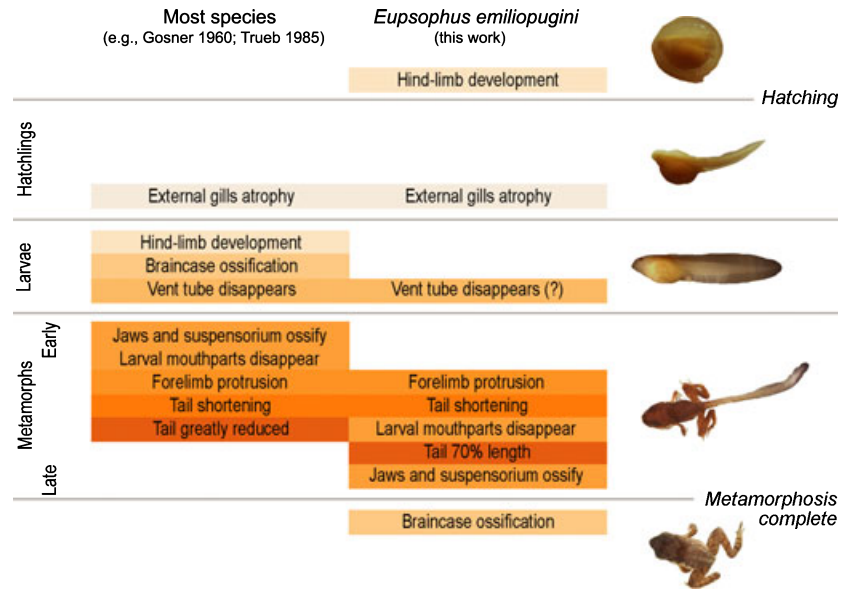
Fig. 11—Comparison of sequence heterochronies between most exotrophic species (e.g., Gosner 1960; Trueb 1985) and *Eupsophus emiliopugini*. Events appear with different shades to highlight different pattern in *Eupsophus*.

hind limbs of embryos before hatching are at Stage 26/27 (Kaiser and Altig 1994), and in *Syncope antenori*, limb bud development occurs earlier in time than in pond-dwelling microhylids (Krügel and Richter 1995). Also, hind limbs are comparatively much larger than in exotrophic forms (Thibaudeau and Altig 1999; Caldwell and Lima 2003; Vera Candioti *et al.* 2005; Leong and Teo 2009). Species of *Batrachyla* (Ceratophryidae) have an intracapsular developmental period in humid ground, and hind limbs appear and gills become internal while the embryo is still within the egg (Cei and Capurro 1958; Formas and Pugín 1971). Also, hind limbs of some semiterrestrial tadpoles develop earlier relative to general development (e.g., Clarke 1983; Drewes *et al.* 1989; Altig and McDiarmid 1999a). The precocious development of hind limbs in these exotrophic tadpoles points out a relationship with terrestrial breeding rather than with endotrophic developmental mode. In most species, even the congeneric *E. calcaratus*, the disappearance of larval mouthparts occurs before forelimb protrusion at Stage 40/41 (Gosner 1960; Hall *et al.* 1997; Grosjean 2005; Vera Candioti *et al.* 2005), but in *E. emiliopugini*, this takes place much later, and metamorph at Stage 44 still has remnants of marginal papillae and labial ridges with teeth. A delayed degeneration of larval oral structures also characterizes the metamorphosis of other endotrophic and exotrophic larvae—e.g., nidicolous *Pelophryne signata* (Bufonidae; Leong and Teo 2009), and torrent-dwelling *Ansonia* and *Meristogenys* (Bufonidae and Ranidae, respectively; Nodzenski and Inger 1990). Finally, the tail in *E. emiliopugini* attains its maximum length at Stage 37, and then undergoes resorbtion; at Stage 44, it is still about 70% of the body length. This is a fairly slow tail regression in contrast to that of other species (e.g., Gosner 1960; Grosjean 2001; Buchholz and Hayes 2002; Fabrezi and Quinzio 2008), although not as striking as recorded in *P. platensis* tadpoles

that early in Stage 45 show no reduction in the length of the tail (Fabrezi *et al.* 2009).

The timing and sequence of chondrocranial and muscular development in *Eupsophus emiliopugini* and *E. calcaratus* (Vera Candioti *et al.* 2005) are similar; unlike *E. emiliopugini*, the m. depressor mandibulae of *E. calcaratus* is a single, compact muscle at Stage 46. Regarding the osteocranial development, in the postmetamorphic specimen of *E. emiliopugini*, the braincase is almost completely cartilaginous, and ossifications present are the premaxillae, septomaxillae, angulosplenials, squamosal, pterygoid, and exoccipitals. Unlike *E. calcaratus*, maxillae and dentaries are not yet ossified. Compared with other exotrophic forms, the cephalic skeleton of *Eupsophus* (and also that of the nidicolous *Flectonotus goeldii*, Haas 1996a) retains some larval traits, such as the overall shape, the persistent parietal fenestrae, absence of an adult otic process, and minimal calcification. Paedomorphosis has been reported in direct-developing species (e.g., loss of cranial bones that develop late in ontogeny in *Pipa pipa*— Trueb *et al.* 2000; frontoparietals fused only posteriorly in *Oreophrynella nigra*— McDiarmid 1971). Additionally, cranial metamorphosis in *Eupsophus* follows a different pattern in the ossification sequence, wherein the first elements to appear are those of the upper and lower jaws, and suspensorium, while there is no ossification on the ventral and dorsal regions corresponding to the elements that are the first to ossify in most species (frontoparietals and parasphenoid, e.g., de Sá 1988; Fabrezi 1988; Fabrezi and Goldberg 2009; Haas 1996b; Perotti 2001; Púgener and Maglia 1997; Sheil 1999; Sheil and Alamillo 2005; Trueb 1985; Wiens 1989; Wild 1997, 1999). Likewise, direct-developing species studied (e.g., *Eleutherodactylus coqui*, *P. pipa*, *Philautus silus*; Hanken *et al.* 1992, 1997; Kerney *et al.* 2007; Trueb *et al.* 2000) show an accelerated formation of upper and lower jaw bones and the jaw suspension with

respect to the remaining skull bones. Based on observations on *Eleutherodactylus* and pipids, Yeh (2002) suggested that early jaw ossification occurs only in anurans with ontogenetically advanced larval jaw morphology if adult-like feeding also commences early. This does not seem to be the case with *Eupsophus*, in which the persistence of vitellum remnants after metamorphosis would delay the beginning of active feeding. Finally, the general myological developmental pattern in *Eupsohus* agrees with that described for exotrophic larvae from several neobatrachian families (e.g., Starrett 1968; Fabrezi 1988; Haas 2001), although an overall slower development is evident in some muscles (e.g., two slips constituting the m. depressor mandibulae are still present at Stage 46). Conversely, jaw muscle development in direct-developing *E. coqui* differs considerably from that observed in metamorphosing anurans (Hanken *et al.* 1997).

To conclude, some final considerations about the genus *Eupsophus* and the endotrophic developmental mode are summarized as follows: (1) intrageneric division of *Eupsophus* is based on features of the advertisement call, and karyotypical, allozymic, morphometric, and molecular data. Comparative analysis of larval internal morphology of *Eupsophus* species does not support this arrangement conclusively, and until data on the remaining species from both groups are available, differences reported might be interpreted as interspecific variation unrelated to monophyletic species groups. (2) *Eupsophus* tadpoles are the only endotrophic forms within Alsodinae. These larvae share several morphological features with other alsodine larvae, such as the number of lingual papillae, the presence of a quadratoethmoid process, and the configuration of the mm. mandibulolabialis, levator mandibulae externus, tympanopharyngeus, subarcualis obliquus, and rectus cervicis. Conversely, other features are unique to the genus *Eupsophus*, such as the absence of pseudopterygoid process and quadrato-orbital commissure, and the configuration of the mm. subarcuales recti. Compared with most exotrophic anurans, the development and metamorphosis of *Eupsophus* include some characters that develop late (e.g., degeneration of larval mouthparts, chondrocranium shape and minimum calcification, and depressor mandibulae configuration), characters that develop earlier (e.g., appearance of hind limbs, ossification of jaws, and suspensorium), and characters that develop at the same time (e.g., most external features and most cranial muscles). The divergence of *Eupsophus* might have included also an ecomorphological component (*sensu* Giannini 2003) linked to the developmental mode, because some of the distinctive characteristics (e.g., the reduction of labial ridges, the general structure of the hyobranchial apparatus, and the development with retention of larval traits) resemble those of other endotrophic species. (3) The clade Cycloramphidae includes species with both exotrophic and endotrophic developmental mode. Most exotrophic species have pond-type larvae (e.g., *Odontophrynus*), but semiterrestrial (subaerial) tadpoles characterize the genus *Thoropa* and *Cycloramphus* spp. (Heyer 1983a,b; Wassersug and Heyer

1983, 1988). Endotrophic development, in two nidiculous and exoviviparous variants (Thibaudeau and Altig 1999), has evolved in entire genera (*Eupsophus*, *Zachaenus*, *Rhinoderma*; Formas and Vera 1980; Lavilla 1987; Lutz 1944) and also within one genus (*Cycloramphus*; Heyer and Crombie 1979). Direct development (i.e., complete absence of larval stages) has not been reported in this family thus far. Based on some shared features, such as the early development of limb buds and the usual lack of several larval traits (e.g., Callery *et al.* 2001; Elinson 2001; Desnitskiy 2004), nidicolous-like patterns have been suggested to occur during the evolution of direct development (Thibaudeau and Altig 1999). More research on other developmental patterns (e.g., the altered cranial ossification sequence typical of both nidicolous and direct-developing species), comparative data of embryos of other endotrophic guilds, and the integration of these results within a phylogenetic, ecologic and historic framework will be useful to understand the diversity and evolution of reproductive and developmental modes in anurans.

Supporting Information

Additional supporting information may be found in the online version of this article.
Table S1 Measurements of external morphology in *Eupsophus emiliopugini*, stages 31–46. Number of specimens (N), average (α), and standard deviation (SD). Tadpoles at stage 34 did not have upper jaw sheath, so the cell appears with no data (N/D).
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