Giant Tadpole and Delayed Metamorphosis of *Pseudis platensis* Gallardo, 1961 (Anura, Hylidae)

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Abstract.—*Pseudis platensis*, like *Pseudis paradoxa*, is a Neotropical aquatic frog with a giant tadpole. Despite being one of the few anuran species whose larvae grow larger than adults, knowledge of its larval development is limited to prometamorphic stages. This study describes qualitative and quantitative variation through *P. platensis* larval development, including descriptions of various traits (lateral line, oral disc, vent tube, limbs) and their transformations during metamorphosis. We found similarities with the development of other anurans at larval stages up to the end of prometamorphosis but not with their metamorphic stages. For this species, we described key features to characterize 13 ontogenetic stages as follows: four for prometamorphic larval morphologies, eight stages covering metamorphic changes, and one defining the posmetamorphic adult stage. At the end of prometamorphosis, tadpole body size in *P. platensis* is similar to adult size, but the tail shows extra development. Unlike most anurans, the disappearance of two larval traits (i.e., vent tube and tail) is delayed until later in metamorphosis. Larval development of *P. platensis* exhibits unique features not reported for other anurans and offers a model to study developmental and evolutionary aspects of anuran metamorphosis.

Pseudis paradoxa is well known because its immature stage is larger than the adult stage. However, the genus *Pseudis* is now comprised of 10 other species of aquatic frogs from the lowlands of Guianas, northeastern Venezuela, Trinidad, southern Brazil, Paraguay, southeastern Peru, eastern Bolivia, northeastern Argentina, and Uruguay (Aguiar et al., 2007; Frost, 2007). *Pseudis paradoxa* was the first species of the genus to be described (Linnaeus, 1758) and was distributed across almost the entire range reported for the genus. Different populations of *P. paradoxa* had been nominated as subspecies, with *Pseudis paradoxus platensis* from Argentina being the smallest (Cei, 1981). Recently, Aguiar et al. (2007) proposed that the *P. paradoxa* subspecies actually represent full species (*P. paradoxa* and *Pseudis platensis*).

Despite the surprising size of the *P. paradoxa* tadpole, which may reach 220 mm, only Emerson (1988) studied the correlation of size and shape between larval and adult stages and interpreted possible proximal mechanisms (hormonal) producing changes in the larval morphology of this species. Data on tadpole ecology, larval development, and morphology in *Pseudis* are limited to prometamorphic stages (Emerson, 1988; Dixon et al., 1995; Fabrezi and Barg, 2001; Alcalde and Barg, 2006). Metamorphic changes in size and shape from tadpoles to adult stages compared to other genera is largely uninvestigated. Miranda-Ribeiro (1926) described and illustrated a metamorphic specimen of *P. paradoxa* having bizarre features (e.g., larval mouthparts, forelimbs protruding, a deep tail with reduction of caudal fins, and disproportionately large hind limbs), while Fabrezi and Quinzio (2008) described metamorphic stages in *P. platensis*. They found that the adult mouth is well differentiated even when the vent tube and tail persist, thus suggesting a delayed metamorphosis.

Here, we present a table of stages of larval development of *P. platensis* based on external features. We use quantitative data from the different development stages to explore previous hypotheses and generalizations about the relative size of larvae and adults of this species. Comparison of larval development of *P. platensis* with that of other anurans demonstrates that dissociation of metamorphic events that led to the unique morphologies observed during metamorphosis in this species. We hope this study sets the basis for further analysis of larval development in other species of *Pseudis*, which can then help to elucidate the evolution of larval development in the genus.

Materials and Methods

We studied a sample of 430 tadpoles of *P. platensis* collected from November to April 1997–07 in ephemeral ponds along National Route 81 (23°10′–14′S, 63°21′–39′W) in San Martin Department, Salta (Argentina). Speci-

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mens were fixed in 10% formalin in the field and are deposited in the Herpetological Collection of the Museo de Ciencias Naturales (MCN), Universidad Nacional de Salta (Argentina) with the following collecting dates (collection numbers in parentheses); February 1997 (597), February 1998 (682), January 2000 (683), November 2000 (684), December 2001 (1,128), December 2003 (1,056), March 2004 (1,015, 1,130, 1,137), December 2004 (964, 968, 972, 973, 1,181), February 2005 (988, 1,060, 1,114, 1,182, 1,183), March 2005 (1,055, 1,176), April 2005 (1,139, 1,142, 1,143), November 2005 (1,038), December 2005 (1,012), March 2006 (1,138, 1,171), November 2006 (1,110), March 2007 (1,196), and April 2007 (1,197).

Measurements were made with dial calipers (0.02 mm) and are given in millimeters. Terminology for external morphology is that of Altig et al. (1998). Topography of neuromasts follows descriptions in Lannoo (1987) with some modifications. Most morphological features were observed with a stereomicroscope, line drawings were made with a camera lucida, and pictures were obtained with a digital camera. Descriptions of neuromasts and photographs were made with a Scanning Electron Microscopy (SEM) at the LASEM (Laboratorio de Microscopía Electrónica de Barrido, ANPCyT/UNSa/CONICET), Salta, Argentina.

We divided larval development into 12 stages. Stage XIII represents sexually mature adult specimens. Stages I–IV correspond to Stages 26–41 of Gosner (1960), and Stages V–XII (Fig. 1) include metamorphosis that differs from the metamorphic stages in Gosner's table (1960) and those described for selected taxa (Taylor and Kollros, 1946; Nieuwkoop and Faber, 1956; Hall et al., 1997). Table 1 compares key features for staging anuran larval development (Gosner, 1960) with those identified herein to describe *P. platensis* larval development.

We also present observations on the developing forelimbs. Forelimb development characterizes larval morphologies, despite the forelimbs being beneath the body skin, and observations on their development allows us to make comparisons with development of the hind limbs. For this, manual dissection was required.

Results

Stage I.—At this stage tadpoles are in Gosner Stages 26–30 according to features of the hind limb buds (Table 1). Body size (mean = 13.91 mm SVL) and total length (mean = 41.73 mm TL) varied from 6.6–22.84 mm and 17.58–66.12 mm, respectively ($N = 134$, Fig. 2). Tadpoles at this stage are present from November to March.

The shape of *P. platensis* tadpole is rhomboid in dorsal view, with a compressed body and lateral eyes. The transparent tail is twice the body length with tall and thick dorsal and ventral fins which end in an acuminate tip. The dorsal fin originates on the head. Spiracle is sinistral, oriented and curved posterodorsally, with an opening smaller than the tube size beyond the posterior margin of eye. The long, funnel-shaped vent tube (Fig. 3A) covers hind limb buds, is fused to ventral fin, and has a medial opening.

Oral disc is emarginated, small, and subterminal. Its features can be observed during Stages I–IV (Fig. 4A). The well-separated marginal papillae occur in a single row with a dorsal gap, have wide bases, and rounded tips. Few submarginal papillae are placed laterally. Jaw sheaths are well keratinized, strongly serrated, and wider than high. The upper jaw sheath is narrowly U-shaped with prominent serrations, and the lower sheath is V-shaped. The long, curved labial teeth have acute tips, and the labial tooth row formula is 1(1+1)/(1+1)2 (Fig. 4A, B).

The anterior lateral line system presents four pairs of neuromast lines on the larval head: infraorbital, from the lateral margin of oral disc to behind and below the eyes; preorbital, from the upper labium between nostrils to behind the eyes; oral, from the lateral margin of oral disc descending to ventral region; and angular, from below the eye extending ventrally and joining in ventral region (Fig. 5A–C). The posterior lateral line system is formed by dorsal line, arising lateral to the keel of dorsal fin and extended along the base of dorsal fin; medial line, from behind and below the eye along the caudal musculature; and ventral lines, bordering the abdominal cavity and converging near the vent tube. The left ventral line is interrupted by the spiracle (Fig. 5A–C). Neuromasts are aggregated in longitudinal stitches (neuromasts aligned along their long axis) with a variable number of neuromasts per stitch (between 12 and 20; Fig. 6A, B). These features are observed in subsequent stages (Fig. 6C).

Both hind-limb and forelimb buds appear simultaneously. The hind limb is first noticeable as a hemispheric and symmetrical bud (Fig. 7A), which then elongates and orientates ventrally and perpendicular to caudal musculature. The forelimb bud also has a distal hemispheric end, although the primary axis is a little more noticeable.

Stage II.—Tadpoles at this stage are in Gosner Stages 31–34 (Fig. 1B, Table 1). Body size (mean = 22.69 mm SVL) and total length (mean = 70.39 mm TL) varied from 13.24–44.50 mm and 31.52–101.00 mm, respectively ($N = 106$, Fig. 2).
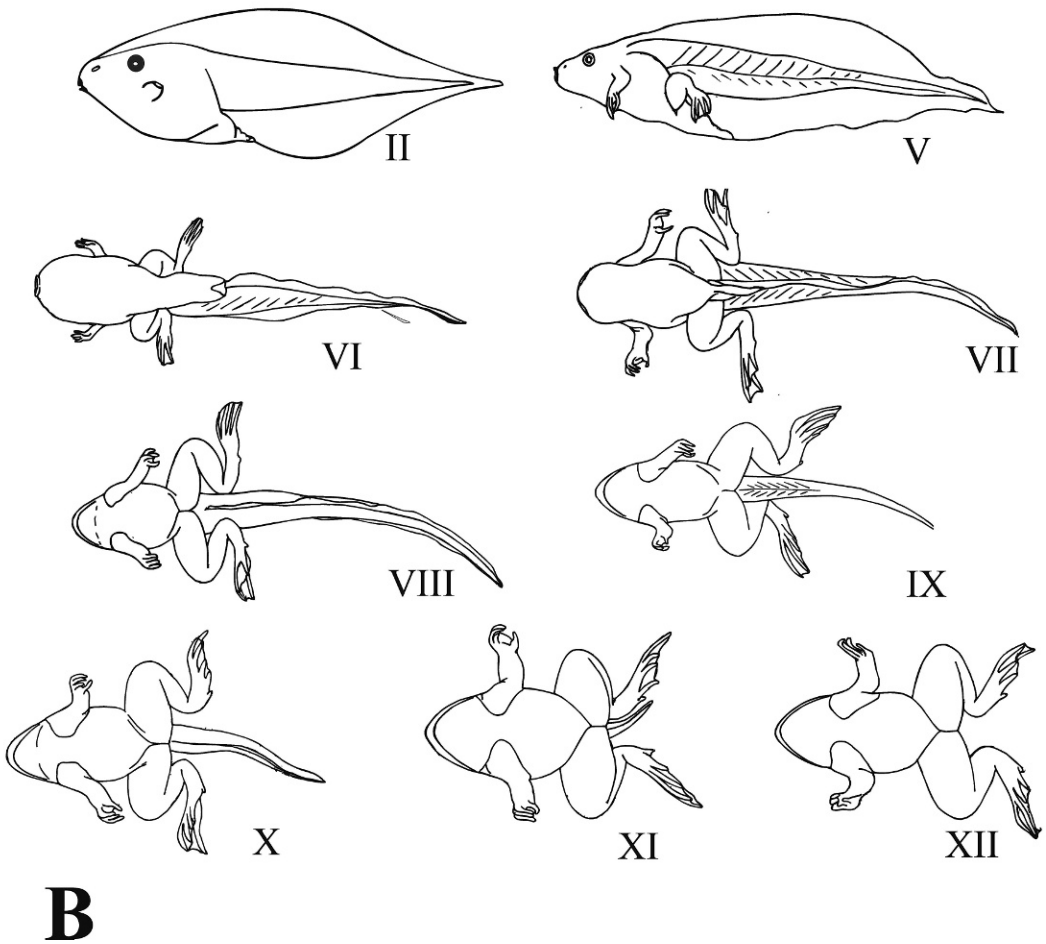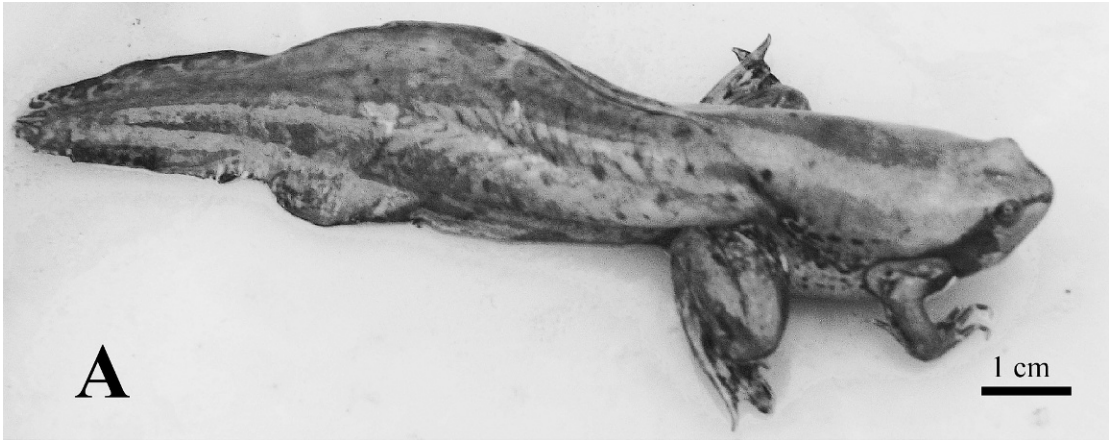
Fig. 1. (A) Specimen of *Pseudis platensis* at Stage VIII. Note that the tail is still high, with dorsal and ventral fins well developed; yet the angle of mouth is placed at level of the posterior margin of the eye. The metamorphosis of the tail lags well behind the metamorphosis of the head. (B) Larval morphologies of *P. platensis* through metamorphic Stages V–XII.

TABLE 1. Comparisons of key features between anuran larval stages by Gosner (1960) and larval stages of *Pseudis platensis* (this paper). Those single Stages 42, 44, and 45 of Gosner (1960) that comprise two larval stages of *P. platensis* are indicated between parentheses.

Gosner larval stages	*Pseudis platensis* stages
26–30: Limb bud length from one-half diameter to twice diameter.	I: Limb bud length from one-half diameter to twice diameter.
31–34: Complete mouth parts. Paddle-like foot. Interdigital indentations.	II: Complete mouth parts. Paddle-like foot. Interdigital indentations.
35–37: Stabilized pigmentary patterns. Toe development.	III: Pigmentary patterns become stabilized. Appearance of individual toes
38–41: Metatarsal and subarticular tubercles. Maximum larval size. Breakdown of larval mouth parts. Loss of vent tube.	IV: Metatarsal and subarticular tubercles. Maximum larval size.
42: Forelimbs emergence. Total absence of larval mouth parts. Angle of mouth anterior to the nostril.	V (42): Forelimbs emergence. Breakdown of larval mouth parts. Maximum size of vent tube. Nictitating membrane.
—	VI (42): Total absence of larval keratinized mouth parts. Angle of mouth anterior to the nostril.
43: Angle of mouth between nostril and the midpoint of eye. Presence of tongue. Regression of dorsal and ventral fins.	VII: Absence of oral papillae. Angle of mouth between nostril and the midpoint of eye. Presence of tongue. Lower eyelid. Loss of neuromasts of anterior lateral lines. Vent tube collapses.
44: Angle of mouth between the midpoint and the posterior margin of eye Caudal musculature greatly reduced.	VIII (44): Angle of mouth between the midpoint and the posterior margin of eye. Reduction of vent tube.
—	IX (44): Upper eyelid. Neuromasts of dorsal line are still present.
45: Angle of mouth at the posterior margin of eye. Tail reduced to stub.	X (45): Angle of mouth at the posterior margin of eye. Regression of dorsal and ventral fins. Loss of the vent tube.
—	XI (45): Angle of mouth caudal to the posterior margin of eye. Tympanic membrane. Caudal musculature greatly reduced.
46: Tail completely resorbed.	XII: Tail completely resorbed.

Tadpoles at this stage are present from November to April. Tail is higher and longer than body. Both dorsal and ventral fins are taller, and dorsal fin bears a hard keel on the head. Limb bud elongation is evident and a new proximal segment is defined by appearance of both anterior and posterior constrictions, which delimit stylopodium and zeugopodium from autopodium (Fig. 7B, C, F, G). The latter preserves its distal hemispheric end, even after flattening and commencement of digit differentiation, without evidence of a primary axis, although the forelimb autopodium is slightly pointed.

Stage III.—Tadpoles at this stage are in Gosner Stages 35–37 (Table 1). Body size (mean = 33.01 mm SVL) and total length (mean = 97.60 mm TL) varied from 23.54–67.12 mm and 48.84–130.00 mm, respectively (N = 59, Fig. 2). Tadpoles at this stage are present from December to April. This stage shows progressive differentiation of digits, accompanied by an increase in their size (Fig. 7D, H). Toe IV elongation is modestly noticeable, the autopodia conserve paddle-like morphology. Digit differ-

entiation and elongation proceeds with the appearance of interdigital grooves within the confines of interdigital tissues and of small interdigital invaginations at the distal end of the bud (Fig. 7D). Hind-limb interdigital membrane looks opaque and thick. Finger IV is evident and finger elongation occurs as an outgrowth from the interdigital tissues to become totally free (Fig. 7H).

Stage IV.—Tadpoles at this stage are in Gosner Stages 38–41 (Table 1). Body size (mean = 43.90 mm SVL) and total length (mean = 133.26 mm TL) varied from 33.70–62.24 mm and 110.40–177.20 mm, respectively (N = 61, Fig. 2). Tadpoles at this stage are present from December to April. Vent tube length has increased (Fig. 3C–D). Toes and interdigital tissues elongate together, which results in a fully webbed foot with a transparent and thin interdigital membrane (Fig. 7E). Inner metatarsal tubercle appears as a ventral projection, preaxial to Toe I. The tubercle increases in size until reaching its final prominent and elliptical shape (Fig. 7J). Subarticular tubercles are very small, almost indistinguishable (Fig. 7J). At the
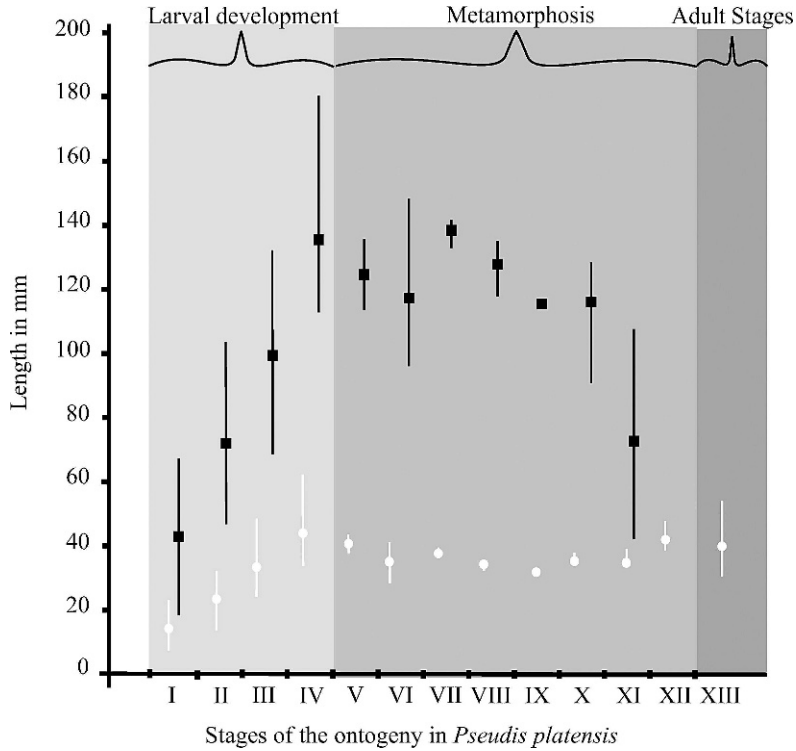
FIG. 2. Size variation during ontogeny in *Pseudis platensis*. Maximum, minimum, and mean of snout–vent length (white) and total length (black).
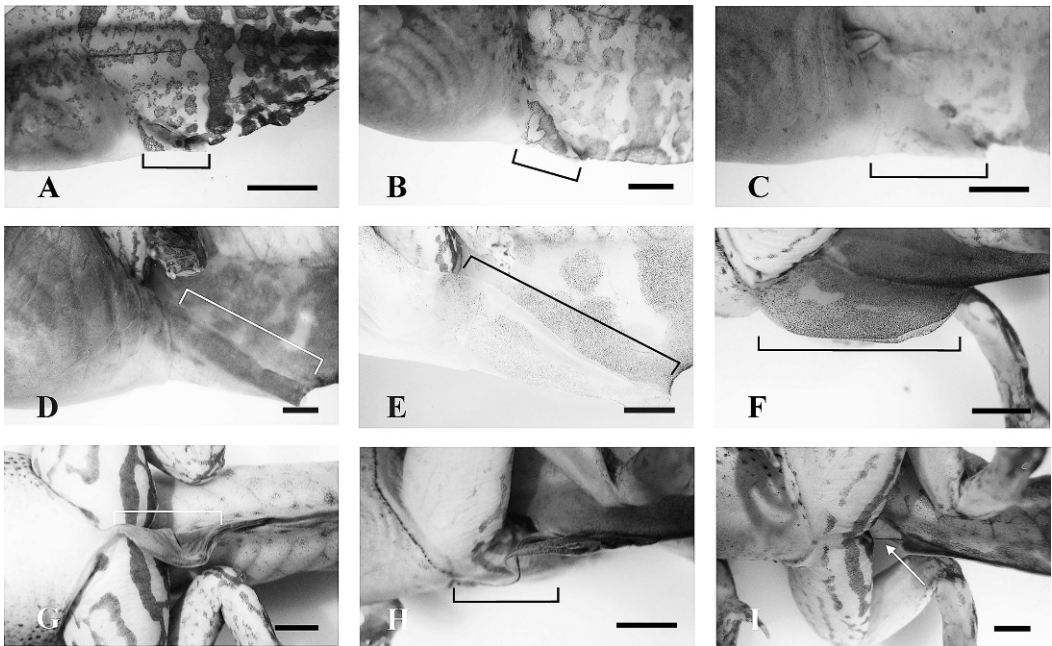

FIG. 3. Vent tube variation during larval development in *Pseudis platensis*. (A) Stage I, vent tube funnel-shaped. (B) Stage II. (C) Stage III. (D) Stage IV. (E) Stage V, the vent tube reaches its largest size. (F) Stage VI, the vent tube collapses. (G) Stage VII, the vent tube is shorter. (H) Stage VIII, the vent tube is almost vestigial but remains joined with ventral fin. (I) Stage X, the vent tube is lost, and cloacal opening is visible. Parenthesis indicates the length of vent tube. Scale bar = 5 mm.
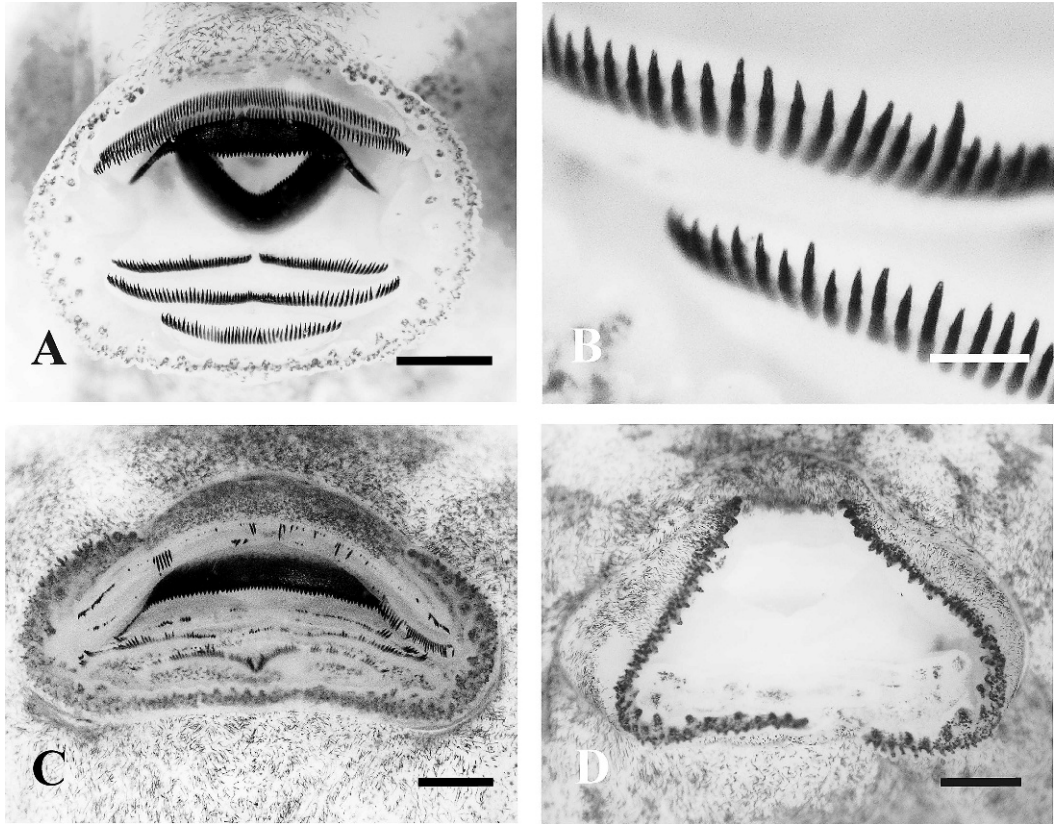
FIG. 4. Oral disc and sequence of lost of larval mouth parts in *Pseudis platensis*. (A) Stage IV. (B) Stage IV, teeth rows of lower lip. (C) Stage V, only upper jaw sheath and some vestigial keratodonts are present. (D) Stage VI, reduced marginal papillae are still bordering oral disc. Scale bar in A, C, D = 1 mm. Scale bar in B = 0.2 mm.

end of the stage, forelimb autopodium presents inner and outer metacarpal tubercles, whose enlargement is evident, and small subarticular tubercles (Fig. 7K). Base of Finger II becomes wider than that of other fingers. Finger II changes its position and places opposite to Fingers III–V (Fig. 7I, K).

Stage V.—Metamorphosis begins at this stage and could represent an early Gosner Stage 42 (Fig. 1B; Table 1). Body size (mean = 40.79 mm SVL) and total length (mean = 122.79 mm TL) varied from 37.78–43.80 mm and 111.88–133.70 mm, respectively (N = 2, Fig. 2). Tadpoles at this stage were present during April. Forelimb protrusion is asynchronous (N = 5), with the right limb as the first one to emerge through a circular hole in the lateroventral region of the body. Before protrusion of the left limb, the spiracle enlarges, becoming wider and thinner, and the skin surrounding the limb becomes weak and thinner. When the limb has fully emerged, the spiracle has disappeared. Forelimbs continue their growth outside but covered by their own skin, different from body skin. Keel of

dorsal fin is displaced posteriorly on the trunk. Vent tube becomes longer, acquires a conical shape, and thickness skin increases (Fig. 3E). The developing nictitating membrane is recognizable as a fold of tissue at anteroventral corner of eye (Fig. 8A). Loss of larval mouth parts begins. Teeth rows of upper lip are the first to disappear, followed by the distal row of lower lip. At the end of this stage lower jaw sheath is absent (Fig. 4C). All lines of lateral line system are present, but the number of stitches is reduced in some lines (Fig. 5D–F). The right ventral line is interrupted by forelimb protrusion. Changes in color pattern occur with the clear dark brownish striped pattern of earlier tadpoles becoming diffuse and more intensively pigmented in older tadpoles.

Stage VI.—This stage could be equivalent to a late Gosner Stage 42 (Fig. 1B). Body size (mean = 35.28 mm SVL) and total length (mean = 114.92 mm TL) varied from 28.46–41.12 mm and 94.02–145.70 mm, respectively (N = 16, Fig. 2). Tadpoles at this stage are present from March to April. The holes from which
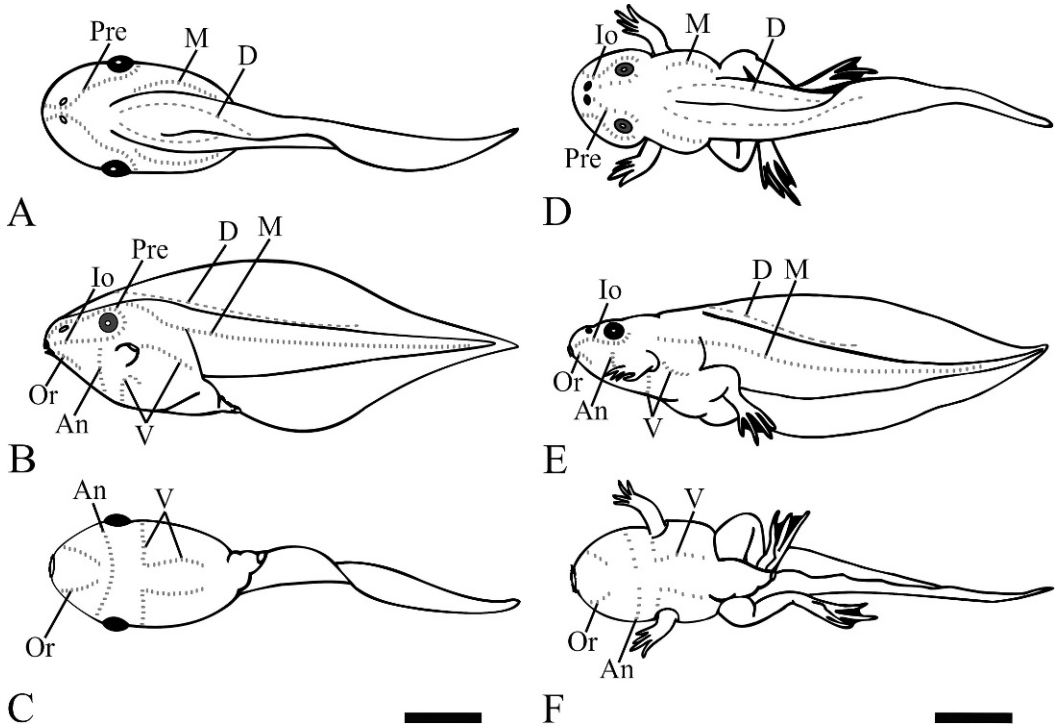
FIG. 5. Topography and changes of lateral line system in *Pseudis platensis*. Dorsal, lateral, and ventral views. (A–C) Stage IV, the system is fully formed. (D–F) Stage V, the metamorphosis of lateral line system has started. Dotted lines represent neuromasts lines. Anterior lateral line system formed by preorbital line (Pre); infraorbital line (Io); oral line (Or); angular line (An). The posterior lateral line system is composed by: dorsal line (D); medial line (M); and ventral line (V). Scale bar = 10 mm.

the forelimbs emerged become wider ventrally (Fig. 8B). The large vent tube stops growing, and a constriction separates it from abdominal skin (Fig. 3E). Nictitating membrane grows and extends up to posterior margin of eye (Fig. 8B). Labial keratodonts and upper jaw sheath gradually shed, disappearing by the end of the stage. The morphology of the oral disc is preserved by the soft mouth parts (tooth ridges and labial ridges) and marginal papillae, which begin to reduce in size (Fig. 4D). Lines of anterior lateral line system exhibit a progressive reduction in their extension and an evident regression of their neuromasts.

Stage VII.—This stage is equivalent to Gosner Stage 43 (Fig. 1B). Body size (mean = 37.53 mm SVL) and total length (mean = 135.40 mm TL) vary from 36.32–38.74 mm and 131.10–139.70 mm, respectively (N = 2, Fig. 2). Tadpoles at this stage are present from March to April. Emergence of forelimbs is total. The line where forelimb skin and body tegument meet is dark and forms a scarf that remains up to adult stages (Fig. 8C). The tegument in the dorsum presents a uniform green coloration that differs from that of tail and hind limbs. Vent tube is

now smaller than in the previous stage, collapses (both walls adhere to each other) and seems to be closed (Fig. 3G). Lower eyelid is well differentiated (Fig. 8C). Marginal and submarginal papillae are completely absent. Oral disc begins to elongate transversally (Fig. 8C). At the end of this stage, angle of mouth is located between nostril and the anterior margin of eye. Snout is slightly pointed in dorsal and lateral views. Distance between nostril and eye reduces. A developing tongue appears. Preorbital, oral, and angular lines disappear.

Stage VIII.—This stage is equivalent to an early Gosner Stage 44 (Fig. 1A, B). Body size (mean = 34.56 mm SVL) and total length (mean = 125.70 mm TL) varied from 32.14–35.56 mm and 132.88–155.44 mm, respectively (N = 6, Fig. 2). Tadpoles at this stage are present from March to April. Vent tube is reduced to a skin fold, separated from abdominal skin by a notch (Fig. 3H). Angle of mouth is at the midpoint of the eye (Fig. 8D). The tongue is completely developed. Dorsal, medial and ventral lines of posterior lateral line system are less evident. Their neuromasts seem to be in regression (Fig. 6D, E). Stitches of the lines along caudal
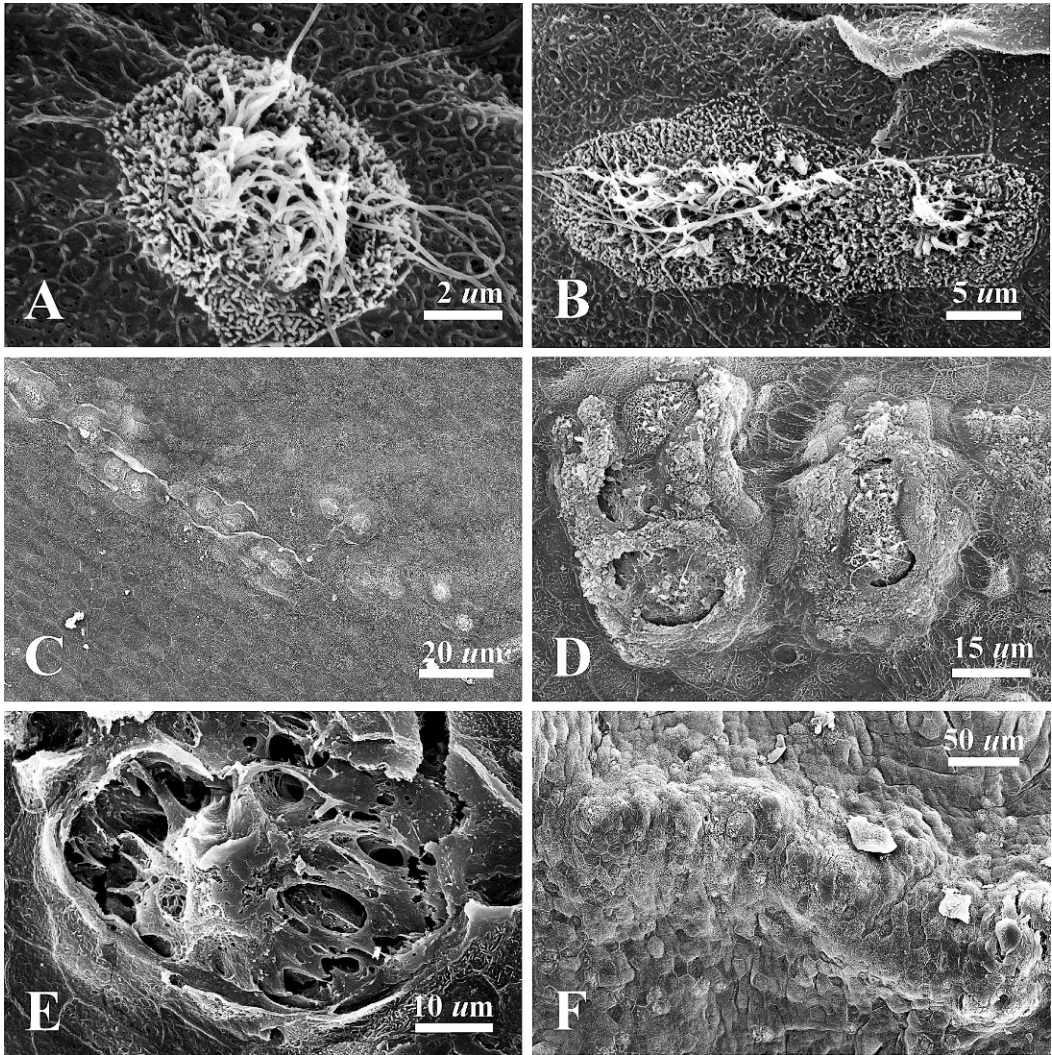
FIG. 6. Scanning electron micrograph of neuromasts and stitches in *Pseudis platensis* showing their morphology and changes. (A–C) Stage IV, (D–F) Stage VIII. (A) Single neuromast of medial line. Oval-shaped in surface view and about 7 μm in minor axis and 9 μm in major axis, with kinocilia (long cilia), indicating approximated number of sensory cells per neuromast. (B) Accessory neuromasts of medial line aligned along their long axis. (C) Medial line stitch with numerous neuromasts. (D) Neuromasts within ventral line stitches at beginning of degeneration. (E) Neuromast of medial line in advanced state of degeneration. (F) Medial line stitches covered by skin on tail suggesting there are not functional.

musculature and dorsal fin are still present but covered by skin suggesting that they are not functional (Fig. 6F). Tail conserves well-developed muscular mass and dorsal and ventral fins (Fig. 1A). Caudal fin and musculature acquire a dark and uniform coloration.

Stage IX.—This stage is equivalent to a late Gosner Stage 44 (Fig. 1B). Body size (mean = 31.69 mm SVL) and total length (mean = 113.54 mm TL) varied from 31.38–32.00 mm and 112.84–114.24 mm, respectively (N = 2, Fig. 2). Tadpoles at this stage are present during April. Angle of mouth is between the midpoint and the posterior margin of eye (Fig. 8E). Upper eyelid is well defined (Fig. 8E). Infraorbital and ventral lines have completely disappeared.

Stage X.—This stage is equivalent to an early Gosner Stage 45 (Fig. 1B). Body size (mean = 35.23 mm SVL) and total length (mean = 113.97 TL) varied from 33.24–37.80 mm and 88.68–126.38 mm, respectively (N = 6, Fig. 2). Tadpoles at this stage are present during April. Angle of mouth is placed at the posterior margin of eye (Fig. 8F). Tail muscular mass
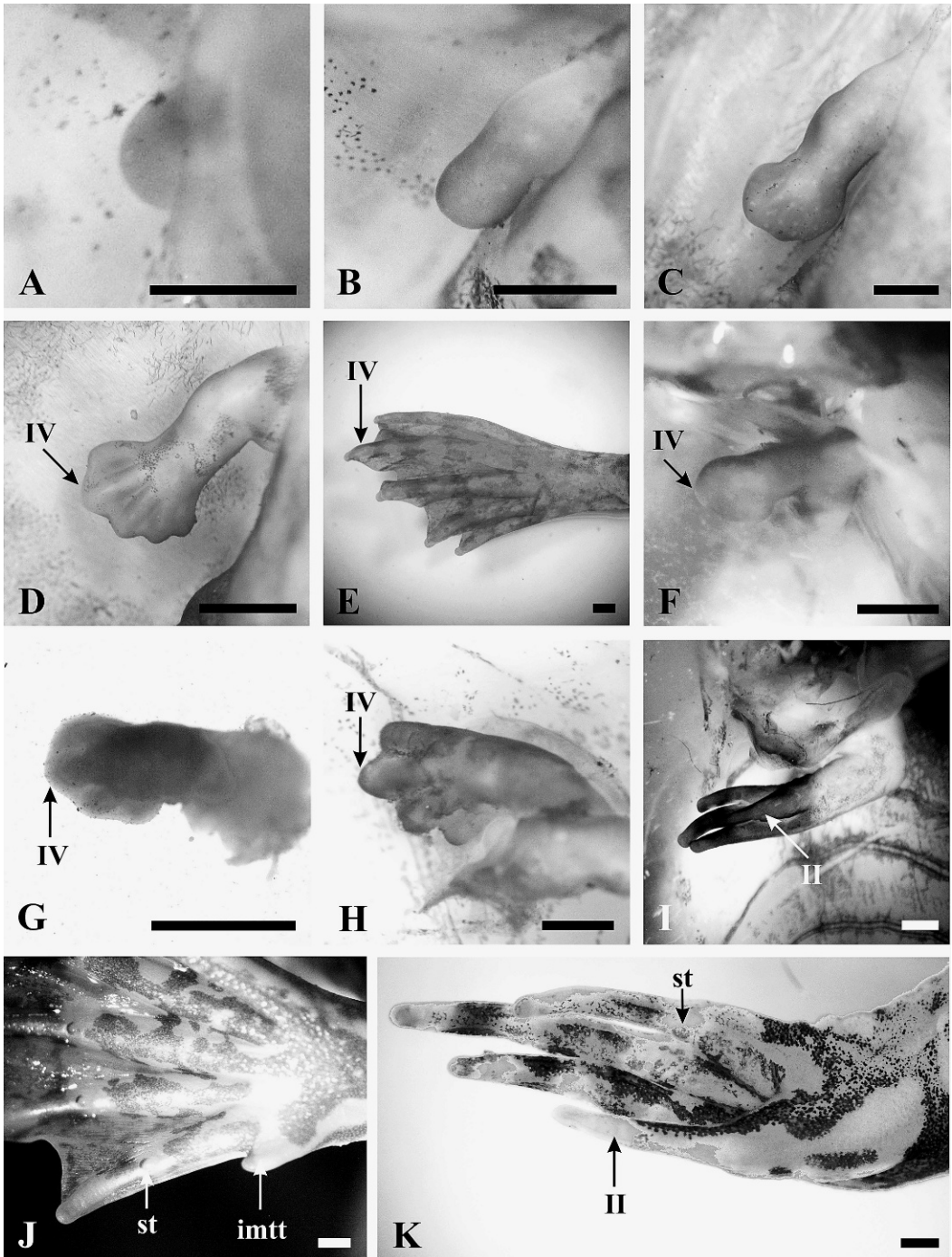
Fɪɢ. 7.   Hind-limb and forelimb development in *Pseudis platensis*. (A–E) Hind limbs in dorsal view. (F–H) Forelimbs in dorsal view. (A) Beginning of Stage I. (B) Stage II, there is no evidence of primary axis. (C) Stage II, autopodium keeps its paddle-like morphology. (D) Stage III, toes differentiation and elongation progress within interdigital tissues. (E) Stage IV; autopodium displays extensive interdigital membranes reaching toe tips. (F) Beginning of Stage II, the primary axis is scarcely evident. (G) End of Stage II, finger differentiation proceeds within margins of interdigital tissues. Finger IV elongation is more evident than in hind limb. (H) Stage III, finger growth happens beyond interdigital tissues. (I) Stage IV, forelimb autopodium possess free digits, without

keeps its morphology and length while dorsal and ventral fins begin to disappear. Vestiges of vent tube that remain joined to ventral fin disintegrate and disappear together with ventral fin, leaving the cloacal opening totally exposed (Fig. 3I).

Stage XI.—This stage is equivalent to a late Gosner Stage 45 (Fig. 1B). Body size (mean = 34.75 mm SVL) and total length (mean = 71.84 mm TL) varied from 32.88–39.12 mm and 41.50–107.00 mm, respectively ($N = 9$, Fig. 2). Tadpoles at this stage are present during April. A progressive degeneration of the tail is observed, which becomes simultaneously shorter and thinner until it is reduced to a stub. Neuromasts of dorsal and medial lines are lost simultaneously with the tail shortening. Angle of mouth is caudal to the posterior margin of eye (Fig. 8G). At the end of this stage, the tympanic membrane is well differentiated (Fig. 8G).

Stage XII.—This stage is equivalent to Gosner 46 (Figs. 1B, 8H). Body size (mean = 42.10 mm SVL) varied from 38.40–47.80 mm ($N = 4$, Fig. 2). Specimens at this stage are present during April. Tail has disappeared completely.

Stage XIII.—Adult stage. Body size (mean = 37.42 mm SVL) varied from 30.46–44.40 mm ($N = 12$, Fig. 2). Males having vocal sacs (mean = 36.15 mm SVL, $N = 7$) and females with mature oocytes (mean = 39.19 mm SVL, $N = 5$) are calling from November to March.

DISCUSSION

Tadpole Development.—*Pseudis platensis* presents an isochronic onset of both fore- and hind-limb buds. Bininda-Emonds et al. (2007) proposed that the relative timing of fore- versus hind-limb development in vertebrates shows a strong phylogenetic component where the plesiomorphic condition would be that of predisplaced forelimbs. They found variation in Lissamphibians, although they studied a small anuran sample (*Xenopus laevis* and *Eleutherodactylus coqui*) that have predisplaced hind limbs. Our data in *P. platensis* revealed isochrony in the timing of the onset of limb buds that agree with patterns observed in *Discoglossus pictus* and *E. coqui* (Schlosser, 2001).

Hind-limb development in *P. platensis* shows some special features shared with pipoids and that differ from most anurans. Goldberg and Fabrezi (2008) have reported a paddle-like bud morphology, without evidence of elongation of Toe IV; toes development within the confines of interdigital tissues, which are retained up to the adult stage; and isometry in digit growth in *Pseudis* spp. and pipoids. These features could be related to aquatic habits, although not all aquatic anurans present them. Goldberg and Fabrezi (2008) interpreted the convergence of hind-limb development and configuration between unrelated taxa (*Pseudis* and pipoids) as a reappearance in *Pseudis* of an ancestral condition. In contrast, the forelimbs of *P. platensis* exhibit a developmental pattern similar to most anurans.

Topography of the lateral line system has been described in no more than 100 anuran species (Lanoo, 1987; Chou and Lin, 1997; Quinzio et al., 2006; Fabrezi and Quinzio, 2008; among others). Lannoo (1987) depicted a generalized pattern similar to that found in *P. platensis*. However, in this species there are differences from that pattern, in the ventral line (long extension resulting from its lateral and ventral position, and the left line is discontinuous), location of the dorsal line (on dorsal fin), and absence of neuromasts supraotic. Neuromasts of *P. platensis* are arranged in longitudinal stitches that are perpendicular to the direction of lateral lines in the entire system, except in the dorsal line where the stitches are parallel to the lines. This pattern has been reported in *X. laevis* (Shelton, 1970). Lannoo (1987) described stitches with two or three neuromasts for most species; six or more in *X. laevis* and *Phrynomantis annectens*, 18 in *Rhinophrynus dorsalis*, and suggested five or more neuromasts for stitch as typical condition for suspension feeder larvae. *Pseudis platensis* having stitches with 12–20 neuromasts, shares this condition.

Little is known about temporal and spatial sequences of degenerative process in components of the lateral line system. The few available studies, limited to *Rana temporaria* and *Hyperolius michelli*, demonstrated that this process begins simultaneously in all components of the system (neuromasts, nerves, and nucleus on alar plate of romboencephalon) during the metamorphic climax (Wahnschaffe et al., 1987). In *P. platensis*, all neuromasts belonging to the lateral line system disappear at the end of metamorphosis with evident asynchrony. Two different and independent

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interdigital tissues, with Finger II in a different plane with respect to other digits. (J) Ventral view of adult hind-limb autopodium, with a prominent inner metatarsal tubercle and small subarticular tubercles. (K) Lateral view of adult forelimb autopodium, with very small subarticular tubercles. Abbreviations: IV, Toe/Finger IV; II, Finger II; imtt, inner metatarsal tubercle; st, subarticular tubercles. Scale bar = 1 mm.
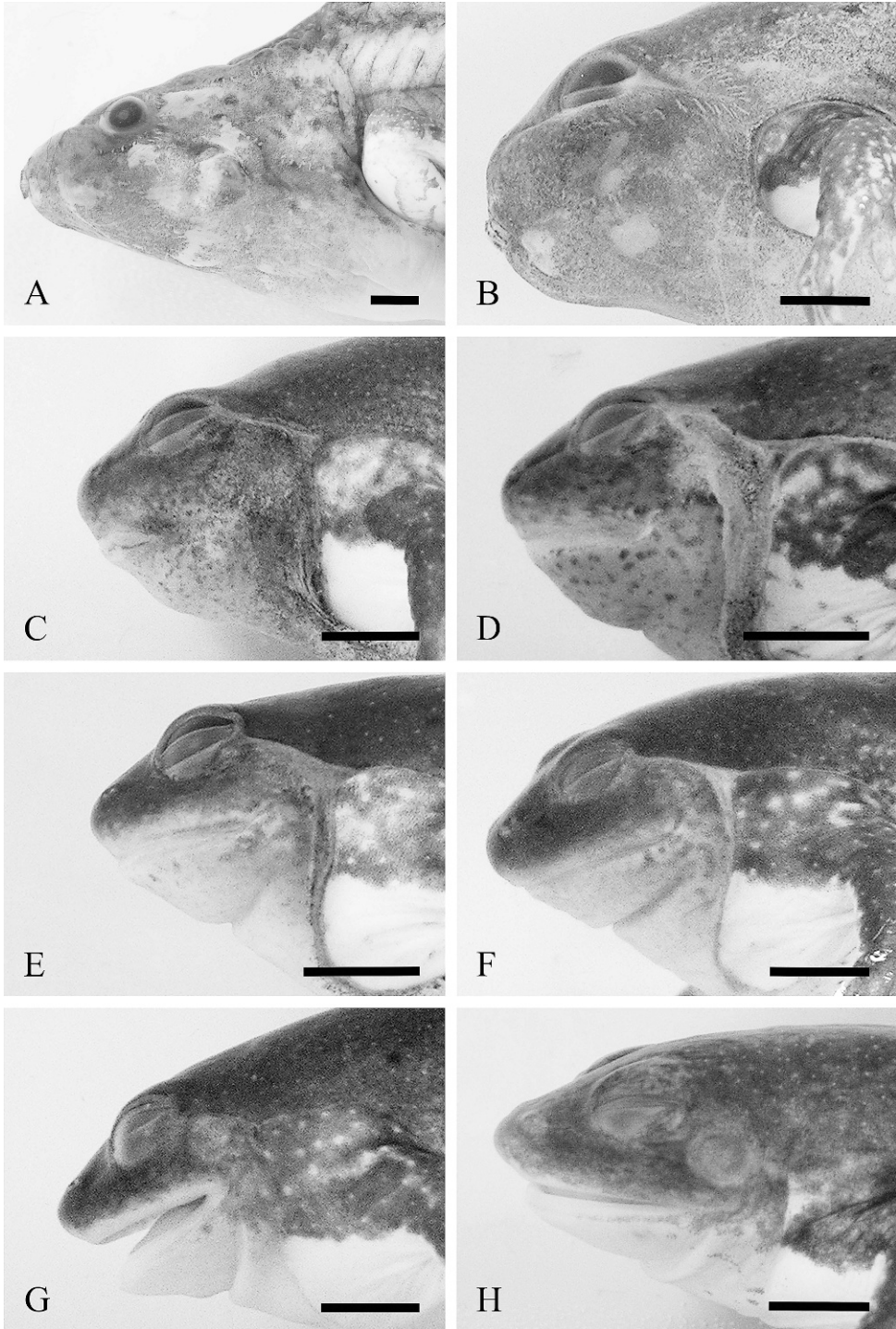
FIG. 8. Metamorphic changes in the head of *Pseudis platensis*. (A) Stage V, oral disc with larval structures and nictitating membrane in anteroventral corner of eye. (B) Stage VI, angle of mouth anterior to nostril, the nictitating membrane reaches the posterior margin of eye, and the forelimb skin is different from body skin. (C) Stage VII, angle of mouth between nostril and the anterior margin of eye and the lower eyelid becomes distinct. (D) Stage VIII, angle of mouth at medial level of eye. (E) Stage IX, angle of mouth between midpoint and
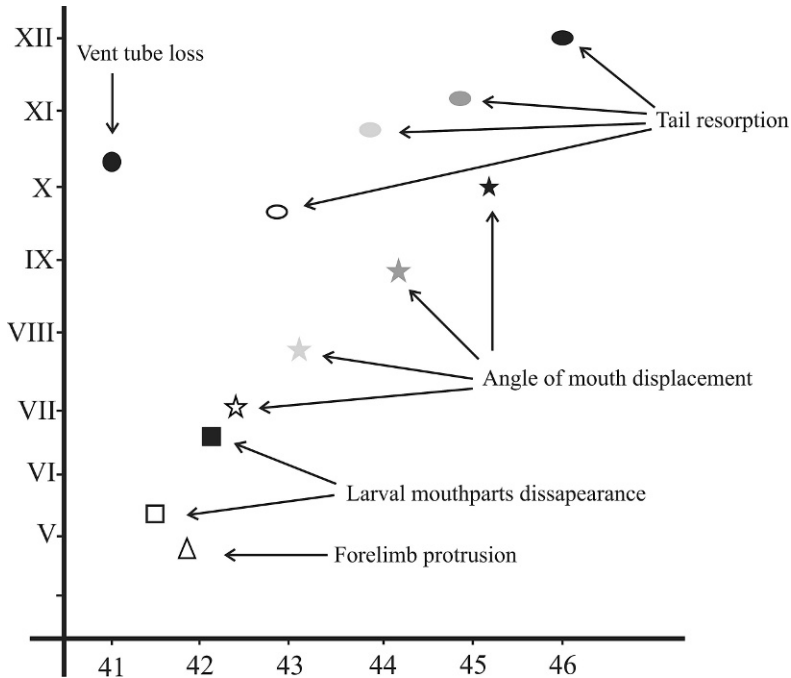
Fɪɢ. 9.   Plot comparing sequences of development at later larval stages, in most anurans and *Pseudis platensis*. Onset of transformation is represented by white symbol, offset is denoted by black symbols, and intermediate steps are depicted with grey symbols. X-axis represents stages by Gosner (1960) for most anurans, and Y-axis corresponds to stages described for *P. platensis*. This plot reveals a pattern of dissociation of developmental events between most anurans and *P. platensis*.

pathways are observed: (1) the progressive degeneration of neuromasts belonging to anterior and posterior lateral line system on the head and trunk from the beginning of metamorphosis (Fig. 6D, E); and (2) the persistence up to late metamorphic stages (X–XI) of nonfunctional neuromasts on tail musculature and caudal fin which are lost with the tail (Fig. 6F).

For most anurans, the end of prometamorphosis involves an initial separation of the vent tube from the abdomen and its complete disappearance at Stages 38–41 (Gosner, 1960; Hall et al., 1997). Therefore, the vent tube is a structure typical of the tadpole, and its disappearance may be the first conspicuous sign of the digestive tract metamorphosis. In *P. platensis*, however, the vent tube persists up to late metamorphosis, indicating a postdisplaced offset. Compared to more typical tadpoles, this produces a unique morphology for metamorphosing individuals, with the ultimate disap-

pearance of the vent tube exceptionally gradual and late (Figs. 3D–H, 9).

In most anurans, loss of larval oral structures takes place before forelimb protrusion and together with vent tube loss at Gosner Stages 40–41 (Gosner, 1960; Hall et al., 1997; Grosjean, 2005; Vera Candioti et al., 2005) but in *P. platensis*, the loss is delayed to the beginning of metamorphosis (Stages V–VII, Fig. 9). The sequence of degeneration of larval mouth parts is similar to that described for other anurans (Thibaudeau and Altig, 1988; Hall et al., 1997; Vera Candioti et al., 2005). For most anurans, the displacement of the mouth angle posterior to the caudal margin of the eye coincides with the complete resorption of the tail (Gosner, 1960). In *P. platensis*, mouth elongation is already finished when tail resorption starts and the vent tube has just been lost (Table 1, Fig. 9).

Metamorphosis in most anurans occurs in a short period of time involving rapid and

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posterior margin of eye. (F) Stage X, angle of mouth is placed at the posterior margin of eye. (G) Stage XI, angle of mouth posterior the eye, the tympanic membrane is formed, and the upper eyelid is distinct. (H) Stage XII, angle of mouth as is observed in adult. Scale bar = 5 mm.

coordinated transformations and may be characterized by five well-defined morphologies of metamorphosing individuals (e.g., Gosner Stages 42–46) (Fig. 9). The end of metamorphosis is defined as total absence of the tail that takes place as soon as the angle of the mouth is caudal to the margin of the eye. In *P. platensis*, both events are dissociated, and the vent tube is conserved in metamorphic stages. Despite the hypermorphic tadpole, *P. platensis* displays dissociation of developmental events, some of which are decelerated and imply paedomorphosis (Reilly et al., 1997). Such heterochronic dissociation of events at metamorphosis is uncommon among anurans. Nodzenski and Inger (1990) reported that larval oral discs, beaks, and denticles are retained much longer during metamorphosis in tadpoles of the bufonid *Ansonia longiditata*, and the ranid species *Meristogenys* (*Meristogenys orphnocnemis*, *Meristogenys phaeomerus*, and *Meristogenys poecilus*). These authors related the changes in the generalized pattern of metamorphosis with larval specializations of species that have torrent-dwelling tadpoles. Fabrezi and Quinzio (2008) described in ceratophriyne tadpoles the early beginning of metamorphic transformations (gut, skin, remodelation of skull cartilages) that are most evident in the highly specialized carnivorous tadpole of *Lepidobatrachus*. However, the pattern of dissociation of metamorphic changes during the long larval development of *Pseudis* would seem to be more related to their aquatic adult stage because at the end metamorphosis, the habitat is unchanged (E. Lehr, pers. comm.).

Tadpole Size and Growth.—Emerson (1988) analyzed the maximum adult snout–vent length of 60 frog species in relation to the maximum total length of their tadpoles and found relatively few species with tadpoles much larger than the adult. Among the latter, Emerson (1988) determined that *P. paradoxa* has the largest tadpole of any species for which there are data. In addition to its large larva, *P. paradoxa* is unusual compared to other species with large tadpoles, such as *Rana catesbeiana* in terms of the depth of its tail, length of its tail, and extension of tail keel onto the head. Figure 2 shows the pattern of larval growth for *P. platensis* that involves a strong increase of tail length at older prometamorphic stages (Stage IV), after body length (snout–vent) has already reached metamorphic size.

In the Chaco where *P. platensis* lives, the active period is largely a function of higher temperatures and rainfall (November to April). Adults are abundant and call even at midday, and adults and tadpoles share ephemeral ponds. These ponds are large, semipermanent,

muddy and become covered by floating vegetation providing numerous shelters to avoid predators. Older tadpoles (Stages V–XII) are found only at early autumn (late March to April), together with young tadpoles, suggesting that all larval development can be completed in half a year (Fabrezi and Quinzio, 2008). This represents the longest larval development of any anuran species in the Chaco region (Cei, 1981). This suggests that hypermorphosis (a pattern of peramorphosis, Reilly et al., 1997) is, in part, the consequence of an extended growth during larval development of *P. platensis*. As was mentioned by Roçek et al. (2006), as in *Paleobatrachus* larvae, some environmental factors make possible conditions for the giant tadpoles of *Pseudis* such as seasonally warm and wet climate, and the formation of large temporary ponds where the water is trapped by the soil rich in clay during the rainy season. Thus, three environmental conditions, long-lasting pond, warm and wet season, and shelters seem to be factors that facilitate the large tadpole with long larval development.

Wassersug and Sperry (1977) explored the biomechanical and ecological aspects of the locomotion in larvae and metamorphic specimens in *P. triseriata* and found the following: (1) the swimming ability is positively correlated with the tail length before the metamorphosis; (2) individuals with a midmetamorphic morphology (with exposed forelimbs and tail still present) show diminished swimming ability and are inferior jumpers to frogs that have completed metamorphosis; and (3) the rapidity of transformation and certain morphological and behavioral peculiarities of anuran development may be adaptive responses to the high risk of predation during metamorphosis. Some of these findings are useful to discuss what happens with *P. platensis* development. The increase of tail length in old tadpoles could be related to an improvement of the swimming ability. This ability could be retained up to the middle of metamorphosis, when the tail is still long, because the metamorphosis does not, implies a change of habitat. The delayed loss of the tail and the acquisition of jumping ability after finishing other metamorphic transformations (e.g., skull metamorphosis) would seem to be related to the complete development of the ilio-sacral articulation and the fusion of the coccyx and hipochord in the urostyle that take place when the tail is reduced to stub (Fabrezi and Goldberg, 2009). The persistence of the tail during metamorphosis could be related with the capability of metamorphic individuals to swim and avoid predators. Thus, the prolonged metamorphosis in *P. platensis* is not constrained by the locomotive disadvantage of the meta-

morphic morphologies as in *P. triseriata* (Wassersug and Sperry, 1977).

Tadpole Size, Adult Size, and Aging.—Species of *Pseudis* have the longest tadpoles of any anuran genus for which there are data and can metamorphose at a length very close to that of sexual maturity (Cei, 1981, Emerson, 1988). Fabrezi and Quinzio (2008) studied patterns of larval growth in species having large tadpoles and demonstrated that at similar body sizes, *P. platensis* tadpoles exhibit longer tails than other species (e.g., *Lepidobtrachus* spp., *Chacophrys pierottii*). Like *P. platensis*, metamorphosed ceratophryines have large body sizes, but unlike it, adults of ceratophryines show marked postmetamorphic growth (Fabrezi and Quinzio, 2008). Sexually mature specimens of *P. platensis* show a mean snout–vent length quite similar to that of recently metamorphosed individuals (Fig. 2), even when their estimated age from bone growth rings imply two or three years old (Fabrezi and Quinzio, 2008).

Heterochrony during larval development is frequent among urodeles. Among them, complete somatic metamorphosis is the plesiomorphic condition for *Ambystoma* spp., and retention of larval somatic morphology in adults is derived (Reilly et al., 1997). In anurans, individuals with sexual maturity and larval somatic morphology were only reported for the anuran *Sphaenorhynchus bromelicola* (Bokermann, 1974). In this species, a group of tailed male tadpoles exhibited vocal sacs and regular spermatogenesis, whereas the tailed female tadpoles had well-developed oocytes. Bokermann (1974) described these ''precocious tadpoles'' as larger than normal tadpoles when they started metamorphosis, and they had longer tails. The precocious tadpoles also were 25% larger than normal tadpoles when the tail was completely lost.

The role of thyroid hormone inducing the metamorphosis has been demonstrated in several studies (Shi and Hayes, 1994; Schreiber et al., 2001; Shi et al., 2001). The occasional occurrence of larval gigantism among some anuran taxa (e.g., *Rana*, *Pelobates*, *Xenopus*), where growth continues without differentiation well before metamorphosis, appears associated with a disruption in thyroid hormone production (Rot-Nikcevic and Wassersug, 2004; Roçek et al., 2006). This is not the case of the gigantism of the tadpoles of *P. platensis*. Furthermore the disproportionate growth of the *P. platensis* tadpole does not preclude metamorphosis.

Neoteny, via paedomorphosis by retardation of somatic development, defined by Gould (1977), seems to be improbable among anurans (Rot-Nikcevic and Wassersug, 2004). However, at least occasionally, some individuals of *S. bromelicola* may reach sexual maturity while still retaining some larval somatic morphology (Bokermann, 1974) and, thus, approaching spontaneous neoteny. Curiously, *Pseudis* and *Spahenorhynchus* are placed in the Dendropsophini clade of Hylinae (Faivovich et al., 2005). Thus, it would be interesting to explore ontogenetic variation in size and shape within this clade because it includes dwarf species (e.g., *Dendropsophus*), species with occasional approximate partial neoteny (e.g., *Sphaenorhynchus*), and *Pseudis*, with its giant tadpoles, and delayed caudal metamorphosis. This clade could represent a model system for studying heterochrony expressed in different directions in the biphasic anuran life cycles.

In conclusion, ontogeny of *P. platensis* exhibits features suggesting larval development for this species has evolved in an unusual direction: (1) its tadpole is giant; (2) compared to other anurans that reproduce and breed in ephemeral ponds of tropical environments, its larval development is the longest; (3) dissociated events during larval development produce a delayed metamorphosis. The vent tube is conserved in metamorphosing individuals, and mouth transformations during metamorphosis finishes earlier than loss of the tail; and (4) the metamorphosis almost defines the adult size.

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