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The Ontogeny of *Pseudis platensis* (Anura, Hylidae): Heterochrony and the Effects of Larval Development on Postmetamorphic Life

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ABSTRACT Recent studies have described the giant tadpole, delayed metamorphic transformations, and absence of postmetamorphic growth of the skeleton of Pseudis Platensis. These features address questions about derived patterns of life cycles and the role of the heterochrony during the metamorphosis in anurans. Using anatomical methods, we provide new data on the development of reproductive, digestive and integument systems, and age inference obtained from ontogenetic series of *Pseudis platensis*. Our results indicate that at the end of metamorphosis, the adult skin is completely differentiated, including the calcified dermal layer; the testis has seminiferous tubules with spermatogonia, spermatocytes, and spermatids; ovarian sacs present previtellogenic ova; and the adult digestive tract is fully formed. The froglets differ from adults only in being unable to reproduce. The entire life cycle of *P. platensis* can occur in 4 years. In the first year, larval development, growth to adult size, and gonad differentiation are completed. Long larval development rather than size of the tadpoles seems to be involved in the absence of juvenile stages. J. Morphol. 271:496–510, 2010.

KEY WORDS: *Pseudis platensis*; metamorphosis; life cycle; anatomy; tadpole

INTRODUCTION

The concept of heterochrony is a persistent component of discussions about the interaction of evolution and development (Smith, 2001). Alberch and Alberch (1981) used heterochrony to describe those permutations in timing of differentiation events, and those changes in rates of development through which morphological changes and novelties originate during phyletic evolution. Size, shape, time, sexual maturity, and developmental trajectories are parameters to study heterochrony in a taxon compared with its ancestor. Smith (2001, 2002) differentiated "growth heterochrony" and "sequence heterochrony." Growth heterochrony focuses the relative timing of developmental events to changes in size and shape, following the models proposed by Gould (1977) and Alberch et al. (1979). Sequence heterochrony is based on a developmental trajectory that is conceptualized as a series of discrete events from which the heterochrony is demonstrated when the sequence position of an event changes relative to other events in that sequence (Smith, 2001, 2002). The direction of the heterochronic change in the descendant species demands a phylogenetic hypothesis from which evolutionary interpretations could be proposed. In this context, comparative data on the relative timing of developmental events affecting size, shape, and ontogenetic trajectories permit to detect growth heterochrony and sequence heterochrony in closely related taxa even when the ancestor ontogeny is not available.

Comparative studies of anuran ontogeny, e.g., the frog *Pseudis platensis*, have provided data on heterochrony in the life cycle of this species (Fabrezi and Quinzio, 2008; Fabrezi and Goldberg, 2009; Fabrezi et al., 2009). Growth heterochrony is detected in rates of body growth during larval and postmetamorphic periods (Fig. 1A,B). *Pseudis platensis* has a giant tadpole, long larval developmental time, and minimal postmetamorphic growth compared with other frogs having large tadpoles. Dissociated metamorphic events that include later offset of larval somatic morphologies (e.g., vent tube and tail that are present when mouth transformations have already finished) revealed sequence heterochrony. Moreover, when tadpole of this species metamorphoses the young frog is nearly the size of an adult and at that time the skeleton, including the sound-conducting apparatus, is fully formed (Fig. 1C,D).

On the basis of an analysis of heterochronic events during metamorphosis, Fabrezi and Quinzio (2008) proposed two derived patterns of metamor-

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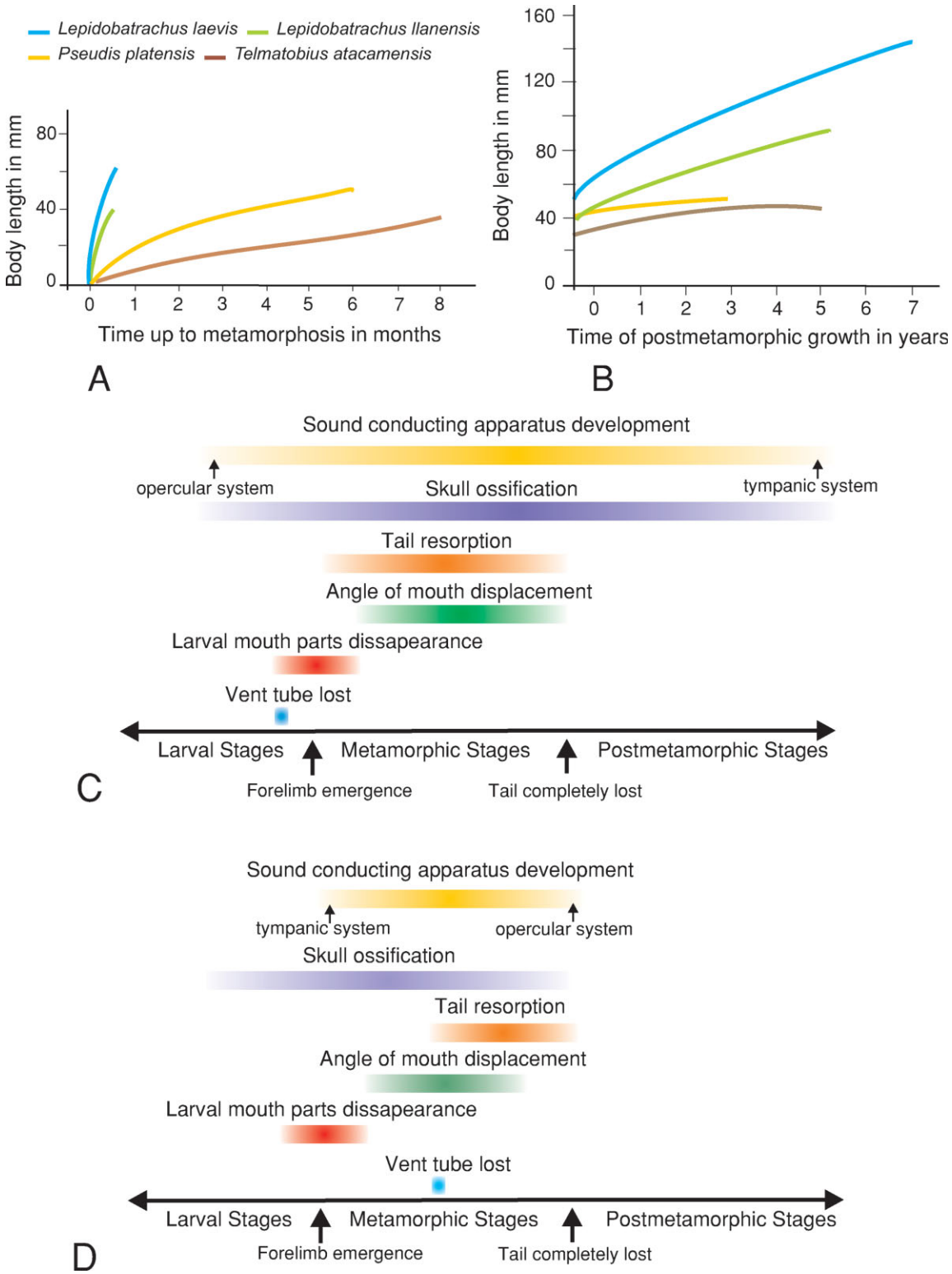

Fig. 1. **A**: Patterns of growth during larval development in four anuran species that have large tadpoles. Acceleration is the simple perturbation that results in a peramorphic pattern relative to size at the end of metamorphosis in *Lepidobatrachus* spp. In *Pseudis platensis* and *Telmatobius atacamensis*, the later offset of growth (hypermorphosis) with a lower developmental rate respect to *Lepidobatrachus* spp. are the perturbations involved in large size of metamorphosing individuals. **B**: Patterns of growth during postmetamorphic stages for the same anuran species. *Lepidobatrachus* spp. present high-growth rates and latter offset of growth. In *T. atacamensis* and *P. platensis* decelerated growth results in adults slightly larger than recently metamorphosed individuals. The offset of body growth in *P. platensis* is predisplaced. **C**: A summary of the relative timing of developmental changes during the metamorphosis for most anurans that have been described following standard staging tables for larval development. **D**: Summary of the relative timing of developmental changes during the metamorphosis for *P. platensis* from previous studies (Fabrezi and Quinzio, 2008; Fabrezi and Goldberg, 2009; Fabrezi et al., 2009). Dissociation and changes in the onset and offset of some traits are revealed comparing developmental sequences. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

phosis in anurans: 1) precocious metamorphosis (with a fast developmental rate) in which adult features are already present in young tadpoles (*Lepidobatrachus* spp.); and 2) delayed metamorphosis (with a slow developmental rate), as occurs in *P. platensis*, in which larval somatic morphologies are still present when most adult features are well differentiated.

The monophyletic genus *Pseudis* from the lowlands of South America is a member of the Dendropsophini clade of the family Hylidae (Faivovich et al., 2005; Aguiar et al., 2007). The genus *Pseudis* has 11 species with small to medium-sized aquatic adults that have completely webbed feet with a distinct paddle-like morphology that is unique among the neobatracians and convergent with pipids (Goldberg and Fabrezi, 2008). A molecular phylogenetic hypothesis of the relationships of *Pseudis* species (Aguiar et al., 2007), recognized *P. bolbodactyla*, *P. fusca*, *P. paradoxa*, *P. platensis*, and *P. tocantins* as a terminal clade. These species have giant tadpoles, and their adults are larger than those of the other *Pseudis* species.

Within the Dendropsophini clade, which includes the genus *Sphaenorhynchus* (Faivovich et al., 2005), some individuals of *S. bromelicola* may reach sexual maturity while still retaining some larval somatic morphology, such as a long tail (Bokermann, 1974). The possibility of a shift of the timing of sexual maturity in the group inspired us to examine the topic relative to sexual maturity that could occur during the delayed metamorphosis of *P. platensis*.

This study provides data on the ontogeny of the reproductive organs, digestive system, and the integument of *Pseudis*. Comparisons of ontogenetic trajectories of different characters with data obtained from other hylid species (*Scinax* spp. and *Phyllomedusa* spp.) reveal new evidence of heterochrony, provide new insights into the delayed metamorphosis that could be extensive in the genus *Pseudis*, and contribute to the discussion on the relative timing of sexual maturity.

MATERIALS AND METHODS

We studied larval and postmetamorphic specimens of *Pseudis platensis* [Gallardo, 1961]. Specimens were fixed in 10% formalin and adults were preserved in 70° alcohol. All specimens were collected from 1997 to 2007 in ephemeral ponds along National Route 81 (23°10′–14′S, 63°21′–39′W) in San Martin Department, Salta (Argentina). Specimens accessioned as lots are deposited in the Herpetological Collection of the Museo de Ciencias Naturales (MCN), Universidad Nacional de Salta (Argentina), with the following collection data: MCN 597 (February 1997, larvae); MCN 812, MCN 1141 (December 1998, postmetamorphic specimens); MCN 972 (December 2004, larvae); MCN 988, MCN 1060, MCN 1114 (February 2005, larvae); MCN 1086 (February 2005, postmetamorphic specimens); MCN 1012 (December 2005, larvae); MCN 1120 (December 2005, larvae and postmetamorphic specimens); MCN 1171 (March 2006, larvae); MCN 1110 (November 2006, larvae and postmetamorphic specimens); and MCN 1197 (April 2007, larvae). Larval specimens of *Scinax acuminatus* (MCN 800), *Phyllomedusa azurea* (MCN 1160), and *P. sauvagii* (MCN 1309), and metamorphosed individuals of *Scinax nasicus* (MCN 1317) were used for comparisons. Larval development was staged according to Fabrezi et al. (2009) for *P. platensis* and according to Gosner (1960) for other species.

Data were obtained from several sources: 1) manual dissection of larval and postmetamorphic specimens to describe changes in the digestive tract and gonads. We studied developmental series of *Pseudis platensis* that consisted of 20 premetamorphic tadpoles between Stages I–IV (MCN 988; MCN 1060), 20 metamorphic tadpoles between Stages V–XII (MCN 1110; MCN 1197), and six sexually mature specimens (MCN 1141; MCN 1086). Further, larvae of *Scinax fuscovarius* (MCN 1191, six specimens), *Phyllomedusa azurea* (MCN 1160, nine specimens), and *P. sauvagii* (MCN 1309, five specimens) between Stages 36 and 43 were also analyzed. 2) Histological sections of the integument were made to study ontogenetic transformations in the transition from larva to adult. For this purpose, we cut pieces of the skin from the dorsum, lateral, and ventral body wall, and limbs from nine *P. platensis* tadpoles at Stages I (MCN 1012), II (MCN 1038; 1114), III (MCN 988), IV (MCN 1171), VII, VIII, X, and XIII (MCN 1197). 3) The age of sexually mature specimens of *P. platensis* was estimated by counting the number of lines of arrested growth (LAGs) in transverse section of phalangeal bones of toe IV. Specimens were selected from the lots MCN 1110 (three males and three females), and MCN 1120 (two males and one female). 4) Histological changes during gonad development were described from *Pseudis platensis* tadpoles at larval Stages I (MCN 1171, one specimen); II (MCN 1060; MCN 1171, MCN 1197, three specimens), III (MCN 597; MCN 1197, two specimens), IV (MCN 972; MCN 1110, two specimens), V (MCN 1197, one specimen), VIII (MCN 1110; MCN 1197, two specimens), IX (MCN 1110, one specimen), and one adult male (MCN 1110). Data obtained from *P. platensis* were compared with those of larval specimens of *Scinax acuminatus* (MCN 800, two specimens at Stages 39 and 43), *Phyllomedusa azurea* (MCN 1160, two specimens at Stages 36 and 43), and *Phyllomedusa sauvagii* (MCN 1309, two specimens at Stages 38 and 43). 5) Cleared and double-stained postmetamorphic specimens of *P. platensis* (MCN 812, one adult; MCN 1197, one metamorphic specimen at Stage XI) and *Scinax nasicus* (MCN 1317, one adult and one metamorphic specimen at Stage 46) to determine the postmetamorphic growth to adulthood.

For histological sections, pieces of integument, gonads, and toe IV were separated from preserved specimens (toe IV was decalcified), dehydrated, embedded in paraffin, and sectioned at 6 μm. Sections were stained with hematoxylin and eosin following the protocol by Martoja and Martoja-Pierson (1970). The analysis of LAGs was performed according to the technique detailed in Hemelaar (1986). Double-stained specimens with Alcian Blue and Alizarin Red S for cartilage and bone visualization, respectively, were obtained following the method of Wassersug (1976).

To document ovarian differentiation, we followed the stages of Ogielska and Kotusz (2004). Testis development was staged according to Gramapurohit et al. (2000).

Descriptions, illustrations, and photographs were made with either a Nikon SMZ1000 stereo dissection microscope or a Leica DM EP compound microscope equipped with an 8.1 megapixel digital camera. All measurements were made with dial calipers (0.02 mm) and are given in millimeters.

RESULTS

Integument

Integument at Stage I presents the typical larval configuration with two layers of flattened epidermal cells, the skein cells and the apical cells (Fig. 2A). The outermost cells are the apical cells which are flattened and have microvilli, the inner cells are the skein cells that are cuboidal and character-

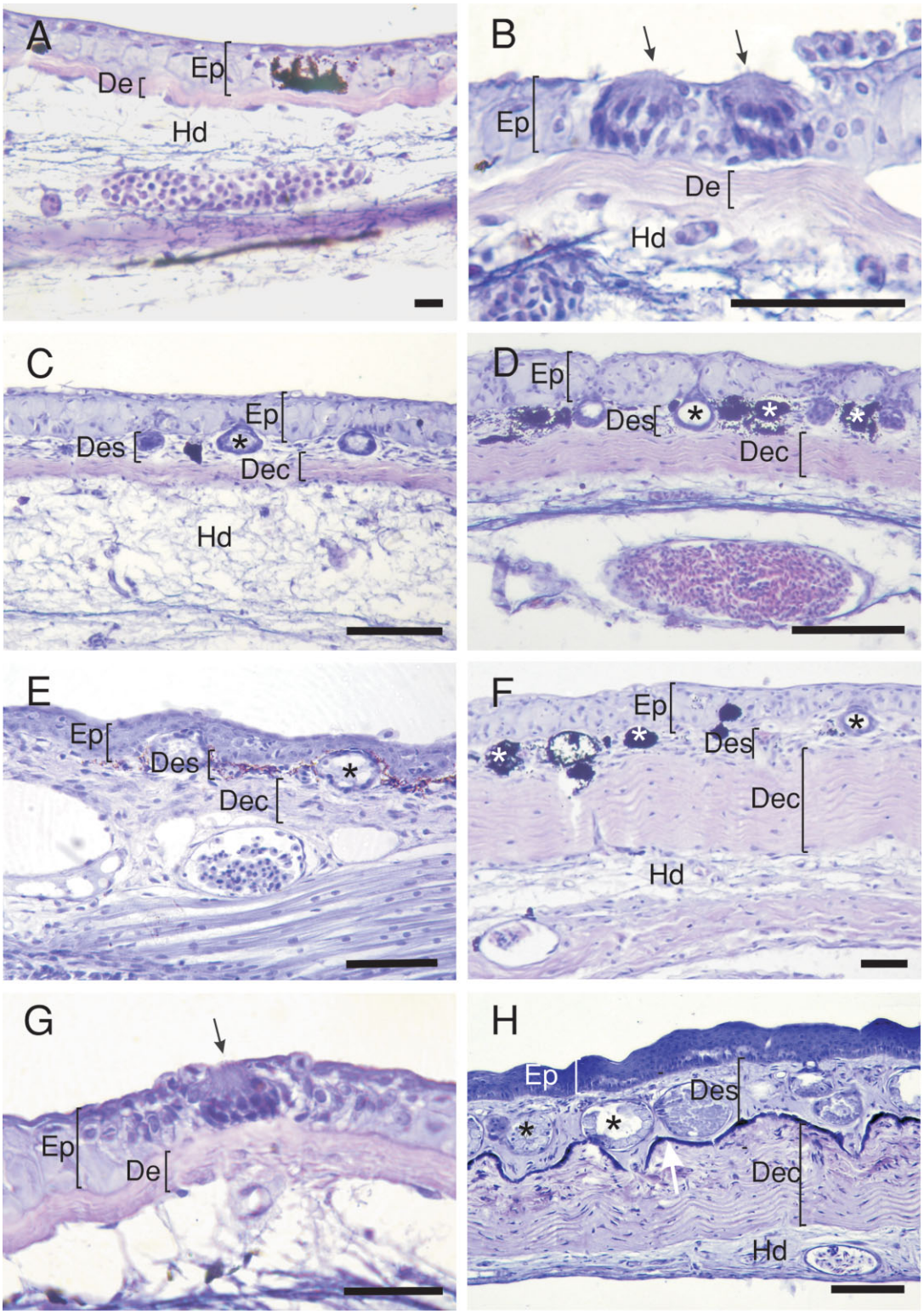

Fig. 2. Cross-sections of the integument during larval development and metamorphosis in *Pseudis platensis*. Developmental stages follows Fabrezi et al. (2009). **A**: Stage I, dorsal integument is constituted by only two layers, epidermis and dermis; below the dermis the hypodermis is observed. **B**: Stage I, dorsal integument where black arrows point neuromasts immersed in the epidermis. **C**: Stage V, dorsal integument. Developing glands (black asterisk) are in the dermis, the stratum compactum and the stratum spongiosum are distinguishable. **D**: Stage VII, dorsal integument. In the stratum spongiosum, the developing glands already possess excretory conducts and melanocytes are numerous (white asterisk). **E**: Stage IV, limb integument has acquired the adult configuration before metamorphosis. **F**: Stage VIII, dorsal integument. The stratum compactum is fully developed. **G**: Stage IX, tail integument remains larval configuration; neuromasts without any structural alterations are still present. **H**: Stage XII, dorsal integument, the adult configuration is reached; between the stratum spongiosum and stratum compactum the calcified layer is observed (white arrow). De, dermis; Dec, stratum compactum; Des, stratum spongiosum; Ep, epidermis; Hd, hypodermis. Scale bar = 0.01 mm in (A, C, D, F, H) and 0.05 mm in (B, E, G). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ized by large lobulated nuclei and clear cytoplasm. Within the epidermal cells, few isolated melanocytes were recognized. Directly associated with the epidermal basement membrane is a very thin dermis, formed only by the stratum compactum with dense collagen fibers. Below the dermis is a thick layer of connective tissue, which connects the integument to the body wall. This layer remains throughout larval development. The skin of fore limb and hind limb shows the same features as the larval body skin, but it has a thinner dermis. At this stage, the lateral line system presents well differentiated neuromasts on the surface of the epidermis. In cross-section, the neuromasts are pear-shaped. Their sensory cells have large, oval, proximally positioned nuclei, and kinocilia projecting out of the epidermis (Fig. 2B).

Changes related to the acquisition of a complex integument in the transition from larva to adult occur during metamorphosis, although some modifications take place earlier. At Stage IV, the basal cells appear beneath skein cells in the epidermis and there is an increase in the number of melanocytes; a slight thickening of the stratum compactum also occurs.

At the onset of metamorphosis, transformations of the body skin begin in the dorsal integument. The basal cells of the epidermis begin to proliferate and organize in layers. In the dermis, the stratum spongiosum becomes conspicuous between the basement membrane and the stratum compactum, with mucous and serous glands developing and melanocytes immersed in a connective tissue matrix (Fig. 2C). The stratum compactum shows no differences with respect to the larval period. At this stage, the integument of the ventral body and tail retains its larval features.

In subsequent stages of metamorphosis, the stratum spongiosum of the dorsal integument includes abundant melanocytes and glands with developing excretory ducts, whereas the stratum compactum shows a marked thickening relative to previous stages (Fig. 2D).

The limb integument retains the larval configuration up to the end of Stage IV. The transformation from larval to adult limb integument occurs synchronously for fore and hind limbs, before the dorsal integument changes. These transformations are completed before forelimb protrusion at the beginning of metamorphosis (Fig. 2E).

By the middle of metamorphosis (Stage VIII) most of the dorsal integument has acquired the adult structure (Fig. 2F). It is during this stage that the ventral integument shows some modifications; the stratum compactum begins to thicken, with few glands and melanocytes visible in the stratum spongiosum which remains thin. The neuromasts on the trunk of the tadpole disappear.

The tail integument keeps its larval structure throughout metamorphosis until the tail has been resorbed completely (Fig. 2G). In cross-sections of tail skin at Stages IX and X, neuromasts do not present any structural alteration, but stitches of the lateral lines arranged along the caudal musculature and dorsal fin are covered by skin (Fabrezi et al., 2009). These neuromasts are lost simultaneously with the regression of the tail (Stage XI).

At the end of metamorphosis (Stage XII), the epidermis consists of five or more layers of stratified basal, germinative, and granular cells although there is no evidence of a keratinized epithelium (Fig. 2H). The stratum spongiosum is well developed with numerous melanocytes, as well as serous and mucous glands. In the stratum compactum, collagenous columns and lamellae are also present. In both, the ventral and the dorsal integument, we found an intermediate basophil layer between the stratum compactum and the stratum spongiosum, which is usually designated as Eberth-Katschenko layer (Azevedo et al., 2005). The hypodermis consists of connective tissue with blood vessels and neural fibers included.

Digestive Tract

Between Stages I–V, tadpoles have the characteristic long intestine which is nearly uniform in diameter with a thin and almost transparent wall (Fig. 3A,B). The proximal region (foregut) of the intestine is located on the right side of the abdominal cavity and forms an inverted U-loop which encloses the long pancreas. This region of the intestine increases slightly in diameter in older tadpoles (from Stage IV) and is identified as the "manicotto glandulare." The intestine turns and forms a double spiral where the inner curves represent the most posterior portions of the intestine that opens in the vent tube. The center of this spiralled mass of intestine is positioned on the left side of the abdominal cavity. The liver has three lobes that extend on the rostral portion of the gut, and the large gall bladder lies distal to the manicotto glandulare. Lungs are inflated and lie dorsolateral to the liver and intestine coils.

As soon as the metamorphosis has begun, abrupt changes in the volume of the intestine occur which appears almost completely covered by the hepatic lobes. The double spiralled intestine decreases greatly in diameter and its walls thicken further (Fig. 3C–F). The foregut dilates, becomes shorter, straight, thicker, and distinct from the posterior intestine (Fig. 3C,D). At this time, the stomach and duodenum are insinuated. As metamorphosis progresses, the stomach acquires an anterior constriction that is the boundary with the esophagous (Fig. 3E,F). There is no constriction between the duodenum and the ileum but differences in the diameter and the coils denote these regions. The intestine ends posteriorly into the cloaca. During

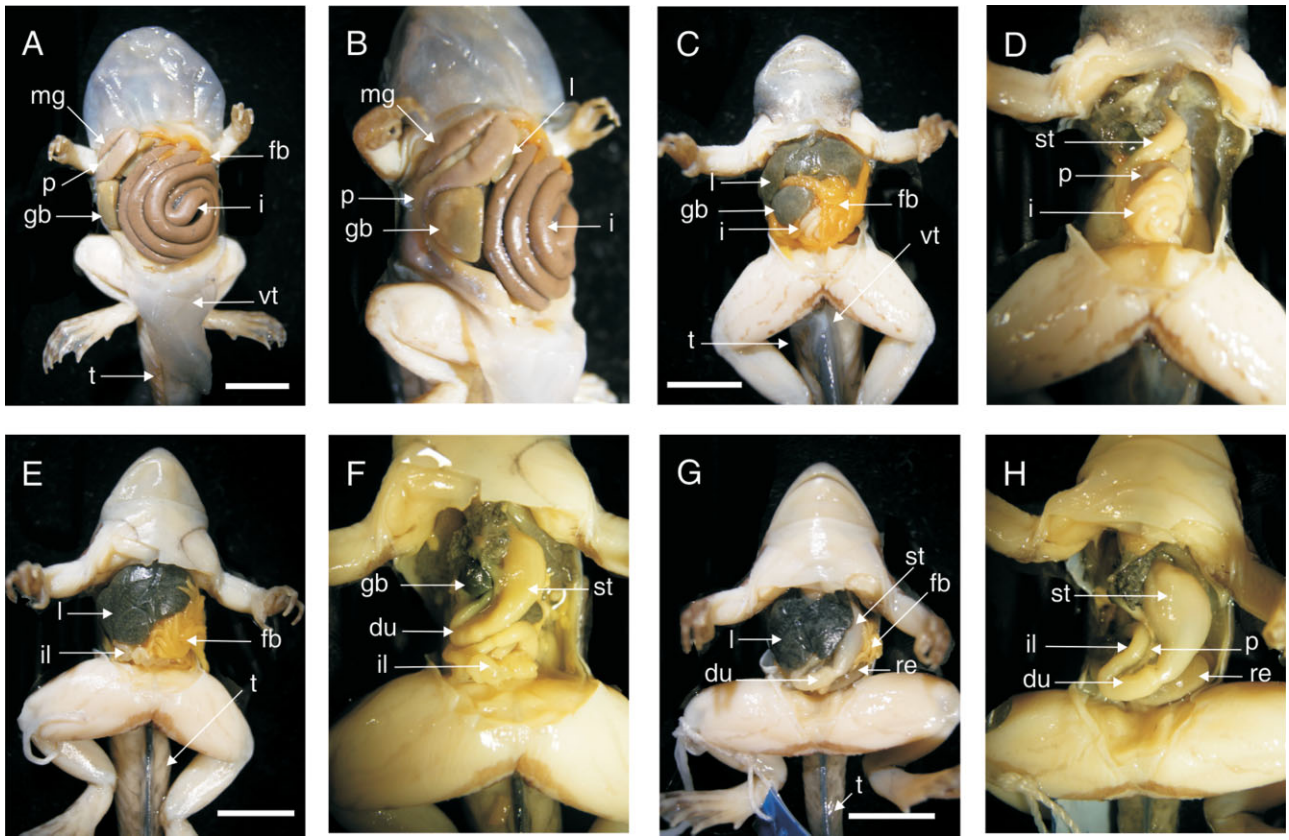

Fig. 3. Gut changes in metamorphosing tadpoles of *Pseudis platensis*. **A**: Stage V. Ventral view with skin, and muscles of the body wall removed. The large larval intestine occupies the abdominal cavity. **B**: Lateral view of the same specimen as in (A). The foregut forms a U-loop that embraces the pancreas. **C**: Stage VII. Ventral view with skin, and muscles of the body wall removed. Growth of hepatic lobes and fat bodies cover the intestine. **D**: The same specimen as in (C), liver and fat bodies removed. The spiralled intestine decreases in diameter and the anterior portion enlarges, becomes thicker and distinct from the posterior intestine. **E**: Stage VIII. Ventral view with skin, and muscles of the body wall removed. **F**: The same specimen as in (E), liver and fat bodies removed. The intestine presents regional differentiation of stomach, duodenum, and ileum. **G**: Stage X. Ventral view with skin, and muscles of the body wall removed. The digestive tract is fully differentiated. There is a constriction separating stomach from duodenum. **H**: The same specimen as in (G), liver and fat bodies removed. The stomach and the terminal segment of the ileum (rectum) are not empty insinuating that at this stage the digestive tract is functional. du, duodenum; fb, fat body; gb, gall bladder; I, larval intestine; il, ileum; l, liver; mg, manicotto glandulare; p, pancreas; re, rectum; st, stomach; t, tail; vt, vent tube. Scale bar = 10 mm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

these metamorphic stages, we found no evidence of feeding.

Before the end of the metamorphosis, when the tail is still present, the digestive tract is fully differentiated. The stomach has a distal constriction (pyloric) delimiting it from the duodenum (Fig. 3G,H). The diameter of the ileum increases distally to form the rectum which opens into the cloaca. Specimens with a tail stub (Stages X and XI) are able to feed like adult frogs.

Gonad Differentiation

Undifferentiated gonads. In Stage I, the gonads are undifferentiated, and appear as whitish, thin, paired cords which diverge proximally. The gonadal chords are located ventrally between the mesonephros (Fig. 4A). The undifferentiated organs contain both somatic tissue and primordial germ cells (Fig. 5A).

In Stage II, undifferentiated gonads remain as thin cords (Fig. 4B), but now contain only primordial germinal cells in active mitotic division (Fig. 5A). In Stages III and IV, some specimens still possess undifferentiated gonad that remains like cylindrical, paired, sparsely-pigmented cords.

Ovarian differentiation. The first signs of gonadal differentiation are evident (in histological sections) at late Stage I by the appearance of a central lumen and primordial germ cells in active mitosis. During Stage II, ovaries grow in size and can be identified by their external lobulation. In transverse sections, the central lumen becomes bordered by an epithelium, with germinal cells surrounding it (Fig. 5B).

At the end of Stage III, the ovaries are larger. They are now hollow, whitish, and folded structures; the ovarian sacs are well defined (Fig. 4D). Primary and secondary oogonia are arranged in

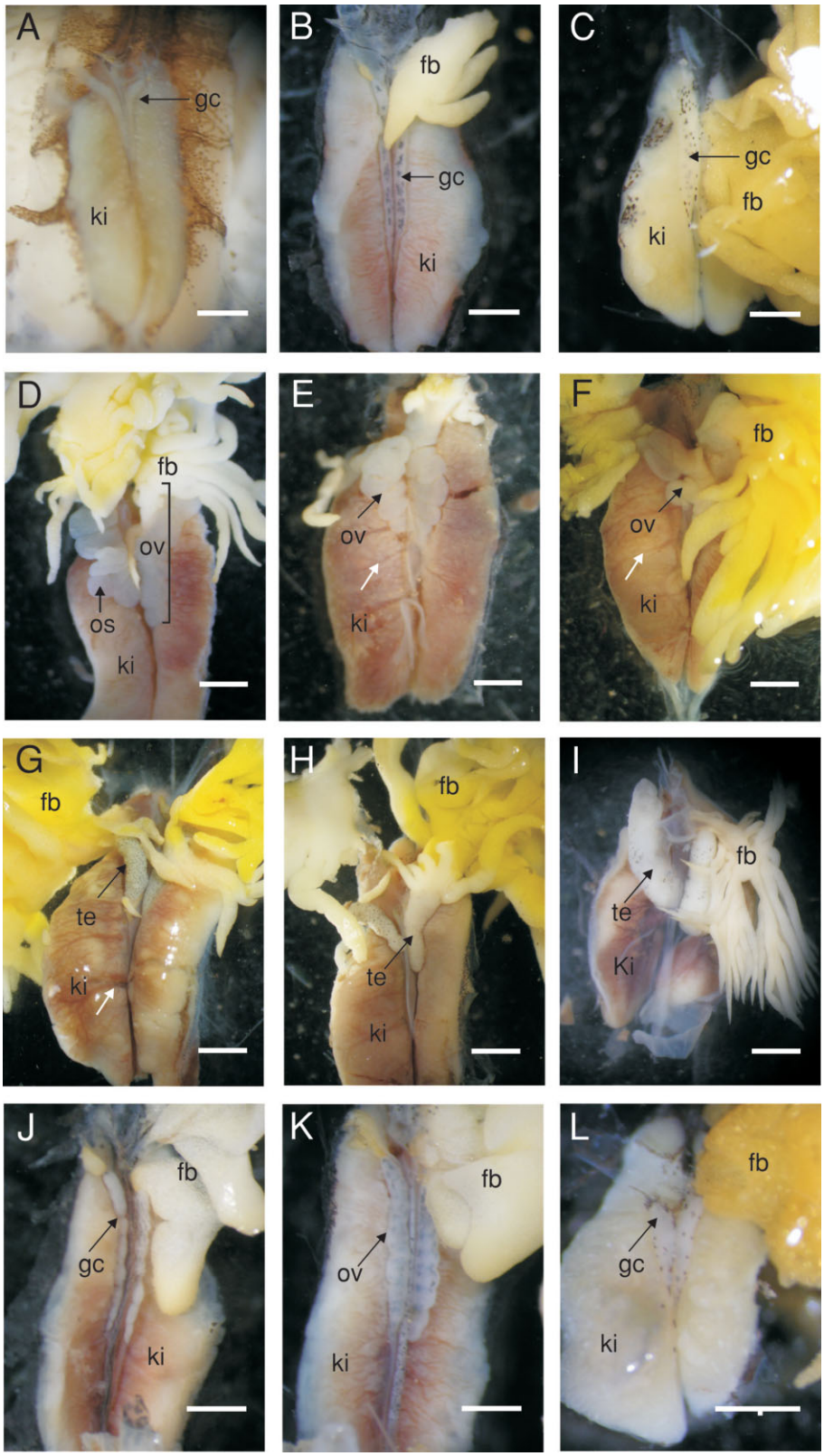

Fig. 4. External morphology of gonads during larval development in *Pseudis platensis* (staged following Fabrezi et al., 2009), *Scinax acuminatus* and *Phyllomedusa sauvagii* (staged following Gosner, 1960). **A**: *P. platensis*, Stage I. Undifferentiated gonads. **B**: *P. platensis*, Stage II. Undifferentiated gonads. **C**: *P. platensis*, Stage III. Undifferentiated gonads. **D**: *P. platensis*, Stage III. Differentiated ovaries. The ovary is characterized by its external lobulation; note the development of fat bodies. **E**: *P. platensis*, Stage VIII. Differentiated ovaries. Vascularization of the mesonephros is indicated by a white arrow. **F**: *P. platensis*, Stage XII. Differentiated ovaries. **G**: *P. platensis*, Stage IV. Testes before the metamorphosis. **H**: *P. platensis*, Stage IX. Testes at middle metamorphosis. **I**: *P. platensis*. Adult testes. **J**: *P. sauvagii*, Stage 38. Undifferentiated gonads before metamorphosis. **K**: *P. sauvagii*, Stage 43. Differentiated ovary at middle metamorphosis. **L**: *S. acuminatus*, Stage 43. Undifferentiated gonads at middle metamorphosis. fb, fat body; gc, gonadal cord; ki, kidney; os, ovary sac; ov, ovary; te, testis. Scale bar 2 mm in (A–I) and 1 mm in (J–L). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

nests and are located in the periphery of the ovary. The oogonia have large nuclei with chromatin uniformly distributed throughout the cytoplasm. Oocytes in diplotene are present at this stage; they are globular cells with large, spherical, and central nuclei with noncondensed chromatin, and many

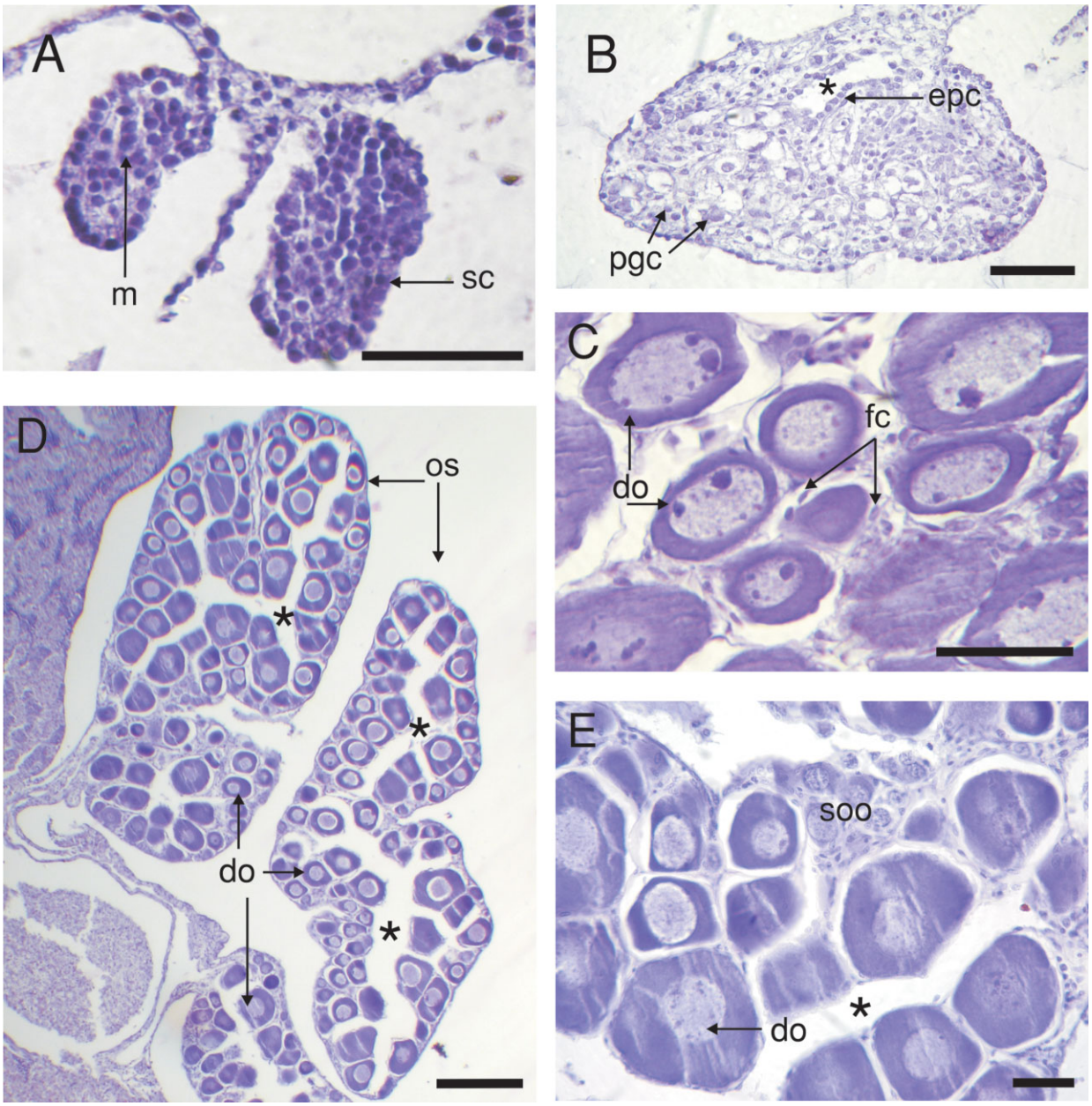

Fig. 5. Transverse sections showing the sequence of events during ovary differentiation through larval development and metamorphosis in *Pseudis platensis*. **A**: Stage I. Undifferentiated organs formed only by somatic and medullary cells. **B**: Stage II. Differentiated ovary with its central lumen (asterisk) bordered by epithelial cells and primordial germ cells in mitotic division placed in the cortex. **C**: Stage III. Diplotene oocytes are bordered by follicular cells (black arrows). **D**: Stage V. The ovarian sacs have numerous diplotene oocytes reducing the lumen. **E**: Stage VIII. Diplotene oocytes are larger than in previous stages and knots of secondary oogonia are present in the cortex. do, diplotene oocytes; epc, epithelial cells; fc, follicular cells; m, medullary cells; os, ovary sac; pgc, primordial germ cells, sc, somatic cells; soo, secondary oogonia. Scale bar = 0.02 mm in (B, C, E, G); 0.04 mm in (A, D, F, H). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

micronucleoli (Fig. 5C). Each oocyte is enveloped by follicular cells, marking the formation of the first growth phase (Fig. 5C). Such oocytes are located toward the central portion of the ovary.

During Stage IV, vascularization of the mesonephros becomes conspicuous (Fig. 4D–F). The ovary grows in size due to the increasing number and size of diplotene oocytes (Fig. 5D). At the beginning of metamorphosis (Stage V), oogonia form isolated knots in low numbers in the periphery of the ovary (Fig. 5E). Diplotene oocytes continue to grow in size and number (Fig. 5E).

Testis differentiation. During Stage IV, differentiated testes are present and differ from the ova-

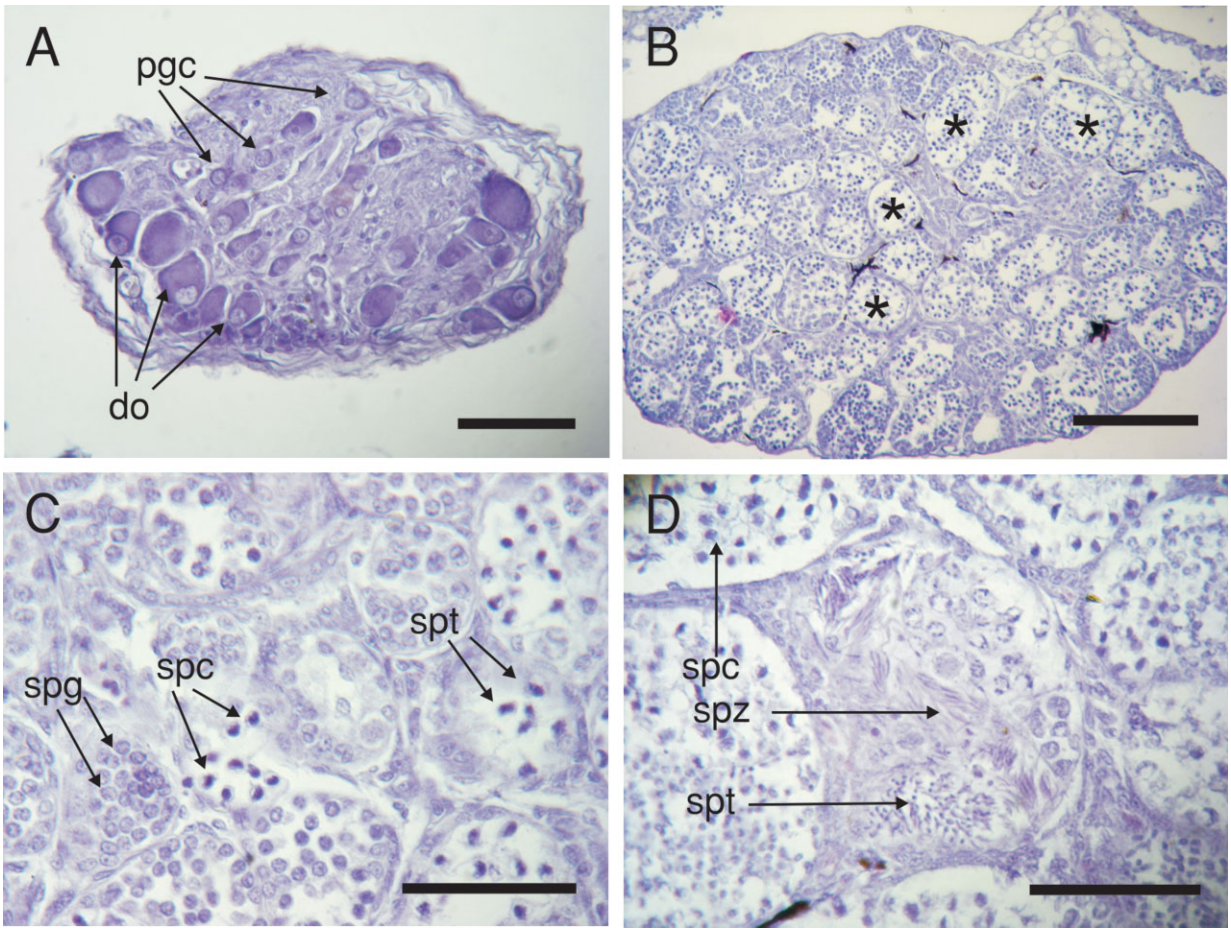

Fig. 6. Transverse sections during testis differentiation in *Pseudis platensis*. **A**: Stage VIII. Diplotene oocytes in degeneration at the middle of metamorphosis. **B**: Stage IX. The medulla has developed seminiferous tubules (black stars). **C**: Detail of (B). Seminiferous tubules in which spermatogonia, spermatocytes, and some early spermatids are differentiated. **D**: Adult testis. All stages of spermatozoid differentiation are present in the seminiferous tubules. do, diplotene oocyte; pgc, primordial germ cells; spt, spermatid; spc, spermatocyte; spg, spermatogonia; spz, spermatozoid. Scale bar = 0.05 mm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ries in being cylindrical, pigmented masses, without lobulation, and smaller than ovaries at comparable stage (Fig. 4G).

During metamorphic Stage VIII, testes have degenerating oocytes at their periphery and some germ cells are present centrally (Fig. 6A); no central lumen is observed. At the beginning of Stage IX, the medulla of the gonad presents numerous seminiferous tubules (Fig. 6B), each contains primary and secondary spermatogonia and a few spermatocytes (Fig. 6C). Spermatogonia are easily recognizable because they are the largest cells, each with an irregular nucleus and dispersed chromatin in a strongly punctate pattern. Adjacent to the walls of the lobules, deposits of melanin begin to appear. At the end of this stage, the testes are longer and the external pigmentation is more conspicuous; they diverge proximally and are wider in their proximal section (Fig. 4H). They have well-defined seminiferous tubules with several groups of spermatocytes; each group contains spermatocytes in the same developmental stage, and early spermatids. At the end of metamorphosis, each testis is made up of several seminiferous tubules with spermatogonia, spermatocytes, and spermatids in the primary rounded stage.

Adult males have white testes with little pigmentation (Fig. 4I) and seminiferous tubules with spermatozoids (Fig. 6D).

Postmetamorphic Growth and Aging

Size variation during ontogeny showed that metamorphic specimens have means of snout-vent length close to that of sexually mature specimens; so postmetamorphic growth is minimal in *Pseudis platensis* (Fig. 7).

To estimate the age of sexually mature specimens, from skeletochronological analyses, we found two specimens (male, SVL = 34.78 mm, female, SVL = 36.28 mm) without LAGs. The absence of LAGs could be interpreted alternatively: 1) these speci-

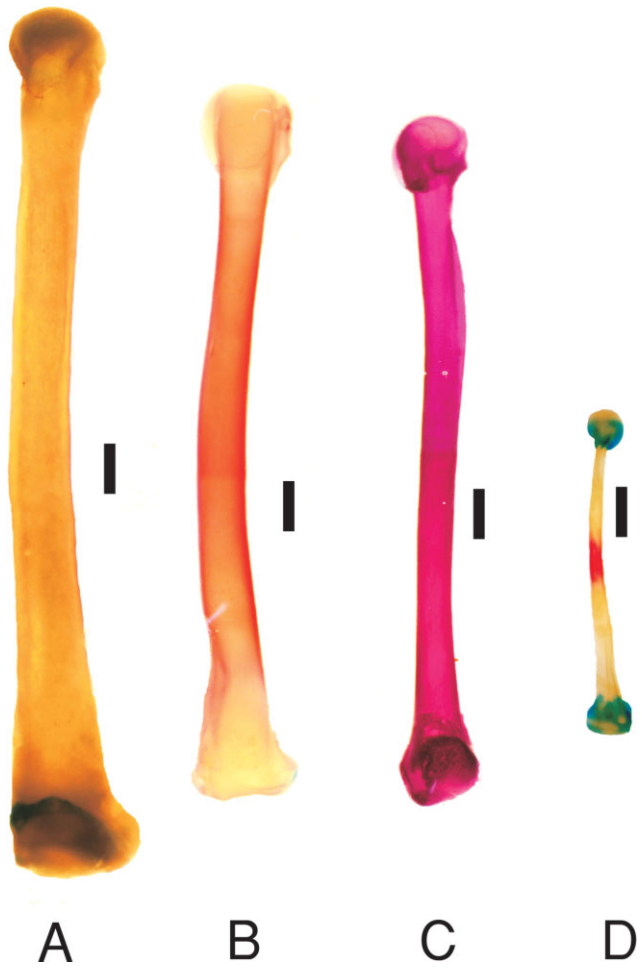

Fig. 7. Comparisons of femur length between *Pseudis platensis* and *Scinax nasicus* adult and metamorphic specimens. **A**: *P. platensis*, adult. **B**: *P. platensis*, advanced metamorphic (Stage XI) specimen. Increase of femur length is minimal. **C**: *S. nasicus*, adult. **D**: *S. nasicus*, metamorphosed specimen. Scale bar is equal 1 mm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

mens are recently metamorphosed and immediately reach sexual maturity (e.g., vocal sacs, vitellogenic ova); or 2) there was endosteal resorption, which resulted in underestimation of age (Castanet et al., 1993). The other results revealed: one LAG (male SVL = 45.24 mm; female SVL = 38.68 mm); two LAGs (male SVL = 36.58 mm; female SVL = 46.02 mm); and three LAGs (male SVL = 44.62 mm; female SVL = 37.30 mm). These few data suggest that 1) sexually mature specimens, could live al least, 3 years after metamorphosis; 2) the postmetamorphic growth rate is low; and 3) the sexual maturity could be attained before the first year.

DISCUSSION

Integument

The histological transformations of the body integument that occur during the metamorphosis are similar to those observed in other anuran species (Duellman and Trueb, 1986; Yoshizato, 1992; Utoh et al., 2000; Brown and Cai, 2007; Fig. 8). Tamakoshi et al. (1998) studied the dissociation of metamorphic transformations in the larval integument of *Lithobates catesbeianus* (formerly *Rana catesbeiana*, Frost et al., 2006) and noted a shift in the timing of the histological changes in the skin, which begins on the lateral sides, progresses toward the dorsal region, and ends in the limb integument. This pattern is different from that observed here for *P. platensis*. Given that the patterns of spatio-temporal changes in the larval integument are recorded for only a few taxa (e.g., *Lithobates* and *Pseudis*), the observed differences suggest that the time pattern of the metamorphosis of the integument vary between species. Metamorphosis of the integument in *P. platensis* shows a spatio-temporal pattern that begins at the limbs, continues to the dorsum by the middle of metamorphosis, and ends on lateral and ventral body skin on completion of metamorphosis.

Another feature related to the acquisition of an adult integument is the appearance of the calcified dermal layer (Azevedo et al., 2005). It is present primarily in terrestrial taxa, but occurs also in the more aquatic clades, such as *Pseudis*, *Lithobates catesbeianus*, and *Hoplobatrachus occipitalis* (Toledo and Jared, 1993). In *P. platensis* and *L. catesbeianus* (Tamakoshi et al., 1998), the calcified dermal layer develops during metamorphic stages, earlier than in terrestrial and aquatic ceratophryids (e.g., *Telmatobius atacamensis*, *Lepidobatrachus spp*., and *Ceratophrys cranwelli*) in which the calcified dermal layer appears during juvenile stages (Quinzio, unpublished data).

Digestive Tract

Tadpoles of *Pseudis* are suspension feeders that graze mostly on phytoplankton and supplement their diets with zooplankton and seeds of phanerogams (Arias et al., 2002; Downie et al., 2009). Ulloa Kreisel (2003) and Downie et al. (2009) described as *Pseudis* tadpoles grew, both gut length and diameter increased considerably; however, the ratio between gut and body length remained nearly the same (15:1 in *P. platensis*; 17–18:1 in *P. paradoxa*) before the metamorphosis. Anatomical regionalization of the larval gut is not evident although histological features of the proximal segment (U-loop) revealed the distinctive glandular layer named manicotto glandulare (Ulloa Kreisel, 2003). The manicotto gladulare represents a storage-tube that lacks digestive functions and it is assumed as a plesiomorphic trait for tadpoles (Viertel and Richter, 1999).

As in most anurans, the metamorphic changes in the gut are abrupt at the beginning of metamorphosis and, concomitant with the loss of larval

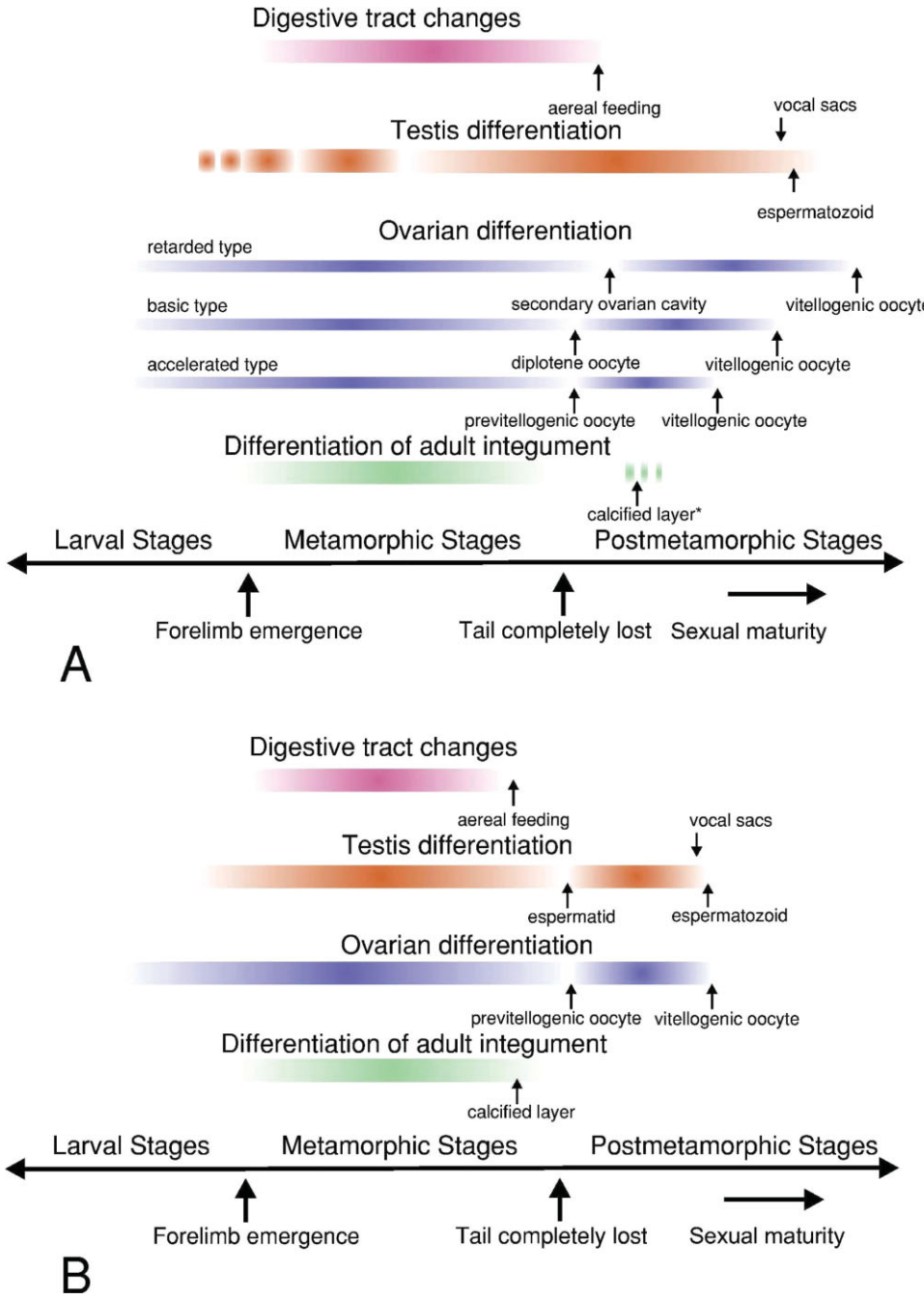

Fig. 8. **A**: Summary of the relative timing of developmental changes during the metamorphosis in integument, digestive tract, and gonadal differentiation for most anurans. The graph is based on data of ovarian differentiation in hylids from this study and data from literature discussed in the text. The asterisk in calcified layer means that *Lithobates catesbeianus* should be excluded. **B**: Summary of the relative timing of developmental changes during the metamorphosis in the integument, the digestive tract, and gonads of *Pseudis platensis*. The graph is based on data from this study. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

oral structures, the diameter of the intestine is drastically reduced. This transformation occurs simultaneously along the intestine. During the metamorphosis, differentiation of the stomach, duodenum, ileum, and rectum progresses from rostral to caudal (see Fig. 3). Metamorphosis of the digestive system in *P. platensis* is in accordance with the pattern exhibited by most other anurans except for the delayed loss of the vent tube, and the formation of the cloacal opening which occur during the middle metamorphosis (Fabrezi et al., 2009).

The anatomical and histological transformations along the larval intestine in *Pseudis platensis* end at the tailed larval Stage X. At this stage, most of the metamorphic changes of the jaws, hyoid and the tongue occur earlier than in other anurans which complete those transformations only after the metamorphosis (see Fig. 1). At Stage X, the intestine is functional in *P. platensis*. In contrast, Downie et al. (2009) described guts were essentially empty in specimens laboratory-reared of *P. paradoxa* at the end of metamorphosis.

For most anurans, the adult feeding starts after of metamorphosis when anatomical transformations and the feeding mechanism (e.g., tongue protrusion) are established. In *P. platensis*, tailed individuals have completed these changes and are able to feed as adults suggesting the tail does not represent a constraint for feeding and prey capture. Adults use floating vegetation as a substrate for prey capture, where they prey on insect (such as dipterans, hemipterans, lepidopterans, and coleopterans) and sometimes other anurans (Duré and Kehr, 2001).

Gonad Differentiation

Our findings show that gonad differentiation in *P. platensis* occurs in a manner similar to that of most other anurans (e.g., Lopez, 1989; Gramapurohit et al., 2000; Ogielska and Kotusz, 2004), but with an accelerated pattern of differentiation (see Fig. 8).

Ogielska and Kotusz (2004) described 10 stages of anuran ovary development, and distinguished three types of developmental rate relative to the differentiation of germ cells (basic, retarded, and accelerated). In the basic and retarded types, the ovary reaches the differentiation of a secondary ovarian cavity, and diplotene oocytes at the end of metamorphosis, respectively (Ogielska and Kotusz, 2004; see Fig. 8). In *P. platensis*, the secondary ovarian cavity differentiated in earlier tadpoles (during limb bud appearance). Ovary development progresses with an accelerated rate having previtellogenic oocytes at the beginning of metamorphosis (Ogielska and Kotusz, 2004). Downie et al. (2009) described ovaries with large previtellogenic oocytes in later tadpoles of *Pseudis paradoxa*. Besides, the accelerated ovarian development has been recorded at the end of the metamorphosis for *Pelophylax lessonae* and *P. ridibundus* (formerly *Rana*, Frost et al., 2006), and *Clinotarsus curtipes* (formerly *Rana curtipes*; Frost et al., 2006) as it was mentioned by Ogielska and Kotusz (2004) and Gramapurohit et al. (2000).

Our comparison with other hylid frogs (*Scinax acuminatus*, *Phyllomedusa sauvagii*, and *Phyllomedusa azurea*) shows that these taxa present the retarded or basic types of ovarian development (see Fig. 9). During the metamorphosis, *S. acuminatus* has an undifferentiated organ with primary gonadal cavity and primordial germ cells (retarded type); *P. sauvagii* presents ovaries with secondary oogonia (basic type), and *P. azurea* have diplotene oocytes (basic type). The basic type has been also reported for *Hyla arborea* (Ogielska and Kotusz, 2004). Among Hylidae, the accelerated type of ovarian development has only been described in *Pseudis platensis* and *P. paradoxa* (Downie et al., 2009).

Ogielska and Kotusz (2004) mentioned that the differentiation of oocytes in developing ovaries has its own timing, independent of somatic development. However, large tadpoles and long larval developmental time are features shared by taxa having an accelerated rate of ovarian development, such as *Pseudis* spp., *Pelophylax* spp., and *Clinotarsus curtipes* (Gramapurohit et al., 2000; Ogielska and Kotusz, 2004; Downie et al., 2009).

Previous studies dealing with the differentiation of the anuran testes are few. *Pseudis platensis* exhibits a pattern of gonadal differentiation in which the testes differentiate initially into ovaries irrespective of the genetic sex, and later the ovaries in genetic males transform into testes (Gramapurohit et al., 2000). The onset of testicular formation in *P. platensis* is associated with the degeneration of oocytes at the beginning of metamorphosis, as in *Rhacophorus arboreus* (Tanimura and Iwasawa, 1989). In other species, such as *Lithobates catesbeianus* (Chavadej et al., 2000), *Clinotarsus curtipes*, and *Pelophylax lessonae* (Gramapurohit et al., 2000), ovarian degeneration and testicular development occurs earlier. During metamorphic stages of *Phyllomedusa sauvagii* and *Scinax acuminatus*, differentiated testes were histomorphologically indistinct. In contrast, Downie et al. (2009) described spermatogenesis and well-formed seminiferous tubules as early as Gosner Stage 31 in *Pseudis paradoxa*, and concluded that males are essentially mature at metamorphosis representing a unique case among amphibians.

Our descriptions of testis development in *P. platensis* indicate that: 1) testis differentiation starts at the beginning of metamorphosis; and 2) testis development is accelerated, i.e., by the end of metamorphosis clearly defined seminiferous tubules with spermatogonia, spermatocytes, and spermatids are present. In other nonhylid taxa, spermatids are found in testes later in ontogeny, such as in postmetamorphic juvenile stages (Lamotte et al., 1973; Chavadej et al., 2000; Gramapurohit et al., 2000; El Jamil et al., 2008; Fig. 8).

Timing of Sexual Maturity in *Pseudis platensis*

Many authors, e.g., Reilly et al. (1997), Ogielska and Kotusz (2004), and Rot-Nikcevic and Wassersug (2004), noted that gonad development may be

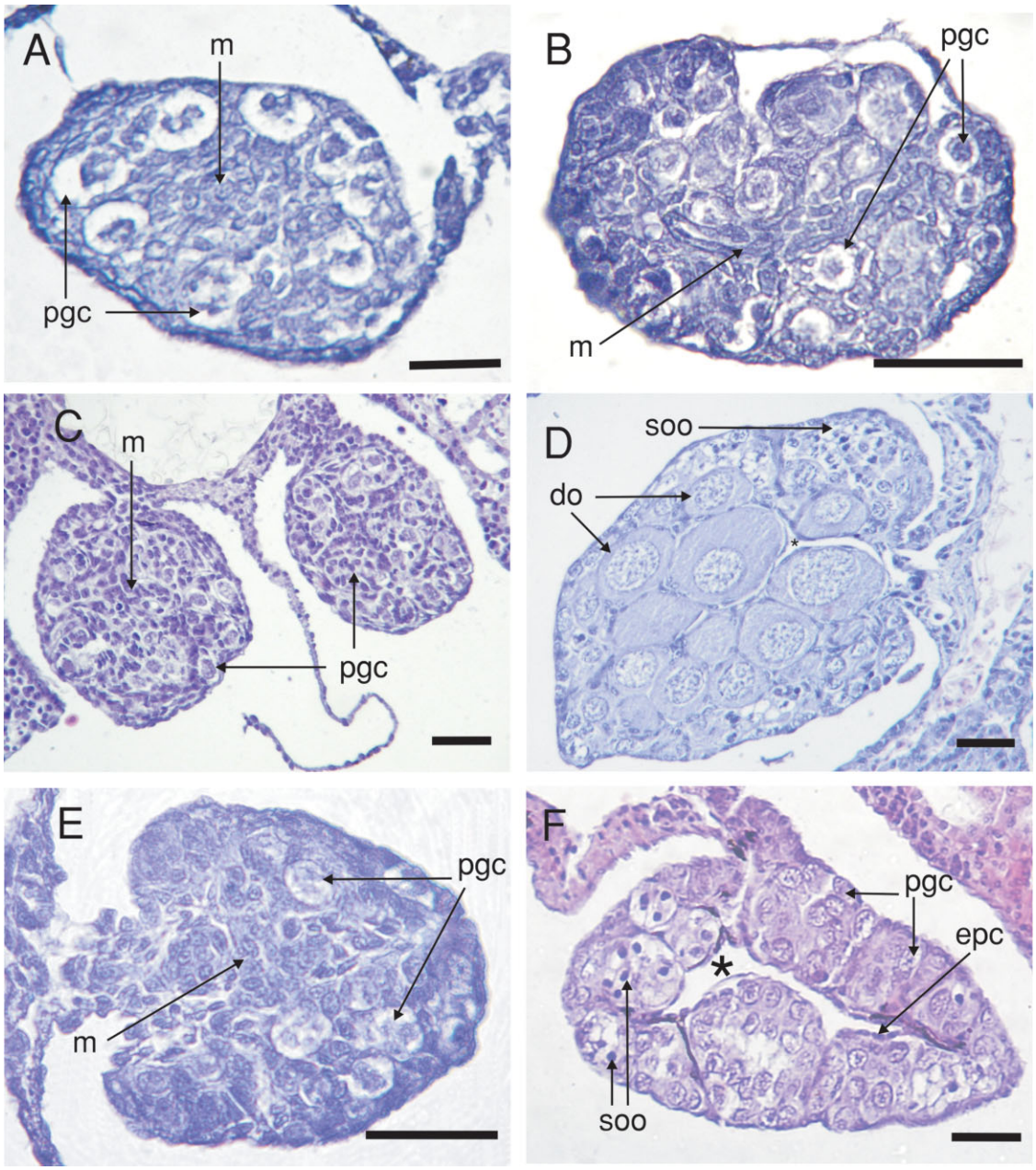

Fig. 9. Transverse sections of developing gonads in premetamorphic and metamorphic specimens of hylids staged following Gosner (1960). **A**: *Scinax acuminatus*, Stage 39. Undifferentiated gonad in which only primordial germ cells and medullary cells are present. **B**: *S. acuminatus*, Stage 43. The gonad remains still undifferentiated, the priomordial germ cells are in mitotic division. **C**: *Phyllomedusa azurea*, Stage 36. Undifferentiated gonads. **D**: *P. azurea*, Stage 43. Developing ovary with secondary oogonia and diplotene oocytes in its first growth face. **E**: *Phyllomedusa sauvagii*, Stage 36. Undifferentiated gonad. **F**: *P. sauvagii*, Stage 43. Primary and secondary oogonia are present, the central lumen is bordered by epithelial cells. do, diplotene oocytes; epc; epithelial cells; m, medullary cells; pgc, primordial germ cells; soo, secondary oogonia. Scale bar = 0.025 mm in (A); 0.05 mm in (B, C, D). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

decoupled from somatic development and/or metamorphosis. Paedomorphosis (as was described in the urodele *Ambystoma*; Reilly et al., 1997), represents a heterochronic event in which the disappearance of some somatic larval features is decelerated. When the timing of onset of sexual maturity is conserved compared from the ancestral ontogeny, reproduction retaining larval features is identified as neoteny (Gould, 1977). Larval reproduction has never been reported in anurans; however, at least occasionally some individuals of *Sphaenorhynchus bromelicola* may reach sexual maturity (vitellogenic oocytes, spermatozoids, and vocal sacs) while still retaining tails (Bokermann,

1974). In *Pseudis* spp. (this study; Downie et al., 2009), the gonads reach advanced stages of differentiation while the tail is still present, but reproduction seems improbable without sexual maturity (vitellogenesis and secondary sexual characters).

In a study of exceptionally old (8 years old) giant tadpoles of *Xenopus laevis* that lacked thyroid glands, Rot-Nikcevic and Wassersug (2004) found mature gonads with eggs and sperm, whereas normal tadpoles of the same stage had undifferentiated gonads. In this case, prolonged period of larval development may have been a factor permitting gonad maturation. However, these giant tadpoles were unable to metamorphose and reproduce. The disproportionate growth of tissues and organs appeared to be the factor precluding successful metamorphosis (Rot-Nikcevic and Wassersug, 2004). Normal giant tadpoles, such as *P. platensis*, were described in the extinct pipoid *Paleobatrachus* (Roček et al., 2006). In this taxon, the increase of body size did not appear to inhibit metamorphosis. Giant larvae in *Paleobatrachus* may have evolved under favorable growing conditions of warm, permanent, semitropical bodies of water with few predators (Roček et al., 2006).

For anurans, a long larval development and large tadpole have been associated with early differentiation of the gonads (Emerson, 1988; Ogielska and Kotusz, 2004; Rot-Nikcevic and Wassersug, 2004). Low-environmental temperatures and a shift in photoperiod length, as well as slow rates of differentiation and growth, could influence the production of giant tadpoles (Emerson, 1988). However, *Pseudis* inhabits semitropical environments with warm bodies of water where species with large tadpoles are rare. Some ceratophryids of these environments (e.g., *Chacophrys*, *Lepidobatrachus*) that have rapid larval development (2 weeks) and large tadpoles, present retarded patterns of gonad differentiation, and substantial postmetamorphic growth (Fabrezi and Quinzio, 2008). Thus, a long period of larval development rather than size at metamorphosis could be related to an accelerated pattern of gonad differentiation. This fact suggests that the onset and rate of ovary and testis differentiation are independent of somatic development (larval or adult-like).

Some morphological data suggest that sexual maturity in *P. platensis* could occur as soon as the metamorphosis is complete. Fabrezi and Goldberg (2009) described in *Pseudis platensis* the developmental pathway of the sound-conducting apparatus, which is already fully formed during metamorphosis. In contrast to most anurans, the sound-conducting apparatus had an inverse sequence of development in which the operculum was the last element to be differentiated at the end of metamorphosis. Smirnov and Vorobyeva (1988) suggested that the opercular system appears first and the tympanic system usually progresses through postmetamorphic development, whereas its morphological differentiation may be delayed until sexual maturity as one of the functions of the tympanic system is the perception of specific reproductive information.

The Life Cycle in *Pseudis*

Species of *Pseudis* are extreme examples of taxa that have tadpoles which are larger than the adults. Certainly, with a total length of the tadpoles exceeding 200 mm, which is three times that of the adult, they are unique among vertebrates (Emerson, 1988). The tadpole total length includes the tail which grows allometrically to the larval body size (Fabrezi et al., 2009). Growth heterochrony demonstrated that postmetamorphic growth is decelerated (Fig. 1B). Adult specimens may only be identified by secondary sexual characters and/or presence of vitellogenic oocytes, traits that appear after metamorphosis is complete.

Data on age for hylids are limited to a few miniature species (Platz and Lathrop, 1993; Maglia et al., 2007). In *Pseudacris maculata* and *P. triseriata*, the oldest males are 3 years old, and the younger mature males are 2 years old (Platz and Lathrop, 1993). However, in *Acris crepitans* the complete population turnover occurs in 16 months and postmetamorphic growth involves increases of size and rapid attainment of reproductive maturity (Maglia et al., 2007). Larval development in *P. platensis* occurs, at least, in 6 months (Fabrezi et al., 2009), and skeletochronological data on sexually mature specimens revealed they could reach 3 years of age, which could indicate postmetamorphic stages would comprise 75% of the life span.

Figure 8 summarizes ontogenetic trajectories emerging from the exploration of metamorphic transformations in integument, viscera, and gonadal differentiation during larval development in *Pseudis platensis* and compares it with data for other species reported in the literature. Events of the ontogenetic trajectory of *P. platensis* when compared with that of related taxa indicate heterochronic patterns implying that, in *P. platensis*, during the long larval development typical patterns of metamorphic changes and postmetamorphic development overlap and therefore postmetamorphic juvenile stages seem to be absent.

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