

Heterochrony in Growth and Development in Anurans from the Chaco of South America

Marissa Fabrezi

Received: 1 April 2011 / Accepted: 20 June 2011
© Springer Science+Business Media, LLC 2011

Abstract Heterochrony refers to those permutations in timing of differentiation events, and those changes in rates of growth and development through which morphological changes and novelties originate during phyletic evolution. This research analyzes morphological variation during the ontogeny of 18 different anuran species that inhabit semi-arid environments of the Chaco in South America. I use field data, collection samples, and anatomical methods to compare larval growth, and sequences of ontogenetic events. Most species present a similar pattern of larval development, with a size at metamorphosis related to the duration of larval period, and disappearance and transformations of larval features that occur in a short period between forelimb emergence and tail loss. Among these 18 species, *Pseudis paradoxa* has giant tadpole and long larval development that are the results of deviations of rates of growth. In this species events of differentiation that usually occur at postmetamorphic stages have an offset when tail is still present. Tadpoles of *Lepidobatrachus* spp. reach large sizes at metamorphosis by accelerate developmental rates and exhibit an early onset of metamorphic features. The uniqueness of the ontogeny of *Lepidobatrachus* indicates that evolution of anuran larval development may occasionally involve mid-metamorphic morphologies conserving a free feeding tadpole and reduction of the morphological-ecological differences between tadpoles and adults.

Keywords Tadpoles · Development · Metamorphosis · Growth · Anurans · Morphology

M. Fabrezi (✉)
Instituto de Bio y Geociencias—Consejo Nacional de
Investigaciones Científicas y Técnicas, Mendoza 2, 4400 Salta,
Republic of Argentine
e-mail: mfabrezi@conicet.gov.ar

Introduction

Amphibians with a biphasic life history pattern possess a free-living larval stage; their ontogeny is often characterized by the development of a suite of larval specializations that are lost (or transformed) at metamorphosis (Dunker et al. 2000). Additional changes during metamorphosis could result in an increase of the morphological-ecological differences between larvae and adults which would have maximum consequences in the anuran clade (Schoch and Fröbisch 2006; Handrigan and Wassersug 2007).

At present, there is an important volume of literature on anuran tadpole morphology at comparable stages to characterize larval diversity and construct hypotheses of relationships based on tadpole morphology (Sokol 1975; Altig and Johnston 1989, Wassersug and Heyer 1988; Haas 2003). The recent paper by Roelants et al. (2011) analyzed the anuran radiations and the evolution of tadpole morphospace and found the slowdown in the morphospace expansion was caused not only by a drop in evolutionary rate after the basal anuran radiation but also by an overall increase in homoplasy in the characters that did evolve during later radiations that may have enhanced tadpole diversity by creating unique combinations of homoplastic traits, but the lack of innovative character changes prevented the exploration of fundamental regions in morphospace.

Despite of tadpole morphology seems to be conservative, knowledge about morphological variation during larval development is referred to a few developmental standard tables (e.g. Taylor and Kollros 1946; Nieuwkoop and Faber 1956; Gosner 1960) from which some generalizations would neglect the interspecific variation in larval development and larval growth, including the metamorphic stages, that could have consequence in anuran diversification.

The metamorphosis, as a series of complex developmental events, is a period of the life cycle that delimits the tadpole anuran phenotype (non reproductive stage) from the adult (reproductive stage) anuran phenotype (Fig. 1). During metamorphosis, larval traits (e.g. vent tube, larval mouth parts, spiracle, and tail) disappear, and others typical of the adult are transformed from larval precursors (cartilaginous jaws, gut). Further, typically adult features (e.g. ossifications) appear before the metamorphosis and progress to end at adult stages.

Morphological changes during metamorphosis are numerous, quite simultaneous, and occur fast (in a short period of time compared with length of larval or/and postmetamorphic life). Downie et al. (2004) found some patterns of body growth and tail loss related to metamorphic duration. They explored the metamorphic duration in 14 selected anurans over a narrow temperature range (25.5–28°) to test factors that could be influencing metamorphic duration. Their results show that extension of larval development, including metamorphosis duration, could be modified or altered by environmental factors (such as food availability, predation, climate conditions, etc.) acting on developmental programs with their own historical properties. Thus, species living upon similar natural environments would be expected to display more shared traits of larval development with related species but also some features common for all of these species.

I review the morphological variation during larval development in selected anurans, including data on larval growth, sequences of morphological changes, and timing in which the larval development occurs in wild conditions. Descriptions are based on 18 species belonging to five anuran clades (Fig. 2) that inhabit semi-arid environments of the Chaco in South America. These species have biphasic life cycles, and breed simultaneously in ephemeral ponds. As they belong to different anuran clades, and live in similar geographical and ecological ranges, comparisons of their larval development allow detect developmental and historical constraints, and heterochronic variation. I interpret the dissociation of larval growth and larval development under the concepts of growth heterochrony and sequence heterochrony (Smith 2001, 2002). Finally, I discuss the evolutionary effects of heterochrony in the whole ontogeny of *Lepidobatrachus* spp.

Specimens and Methods

This study included larval and postmetamorphic specimens of five anuran families: Ceratophryidae [*Ceratophrys cranwelli* Barrio, 1980; *Chacophrys pierottii* (Vellard, 1948); *Lepidobatrachus laevis* Budgett, 1899; *L. llanensis* Reig and Cei, 1963)]; Hylidae [*Phyllomedusa azurea* Cope, 1862; *P. sauvagii* Boulenger, 1882; *Pseudis paradoxa*

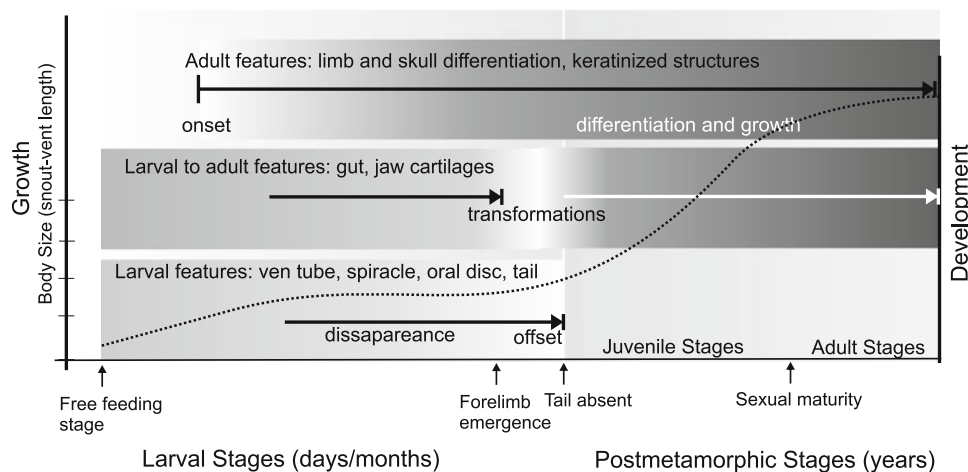


Fig. 1 Synthesis of the biphasic life cycle in anurans. The ontogeny comprises growth and development. The ideal curve of body growth (dotted line) is represented by increment of snout-vent length: growth of the larva occurs simultaneously with limb development; the larval growth stops at forelimb emergence and continues after complete tail disappearance to reach the adult size. Development includes: (a) events acting on larval body plan, with some features that are lost and others that are transformed at advanced stages of larval development; and (b) events involving development of features of the adult body plan. During larval development, all these events are hormone dependent and may vary modifying developmental sequences (Hayes 1997; Stolow et al. 1997; Brown et al. 2005)

whereas external factors (i.e. temperature, competition, diet (Kupferberg 1997), predation (Wassersug and Sperry 1977), cannibalism (Pfenning et al. 1993) may affect its duration. Denver (1997) concluded: (1) species that breed in permanent and predictable habitats generally have longer larval periods, while those that breed in unpredictable and ephemeral habitats have rapid growth and development; and (2) the length of larval periods is determined by genetics. Permanence of the ancestral pond has apparently set a rigid upper limit to the length of the larval period, while rates of growth and development have set the lower limit (Denver 1997). During long evolutionary times, variation in developmental programs resulted from reciprocal influences between external and internal factors

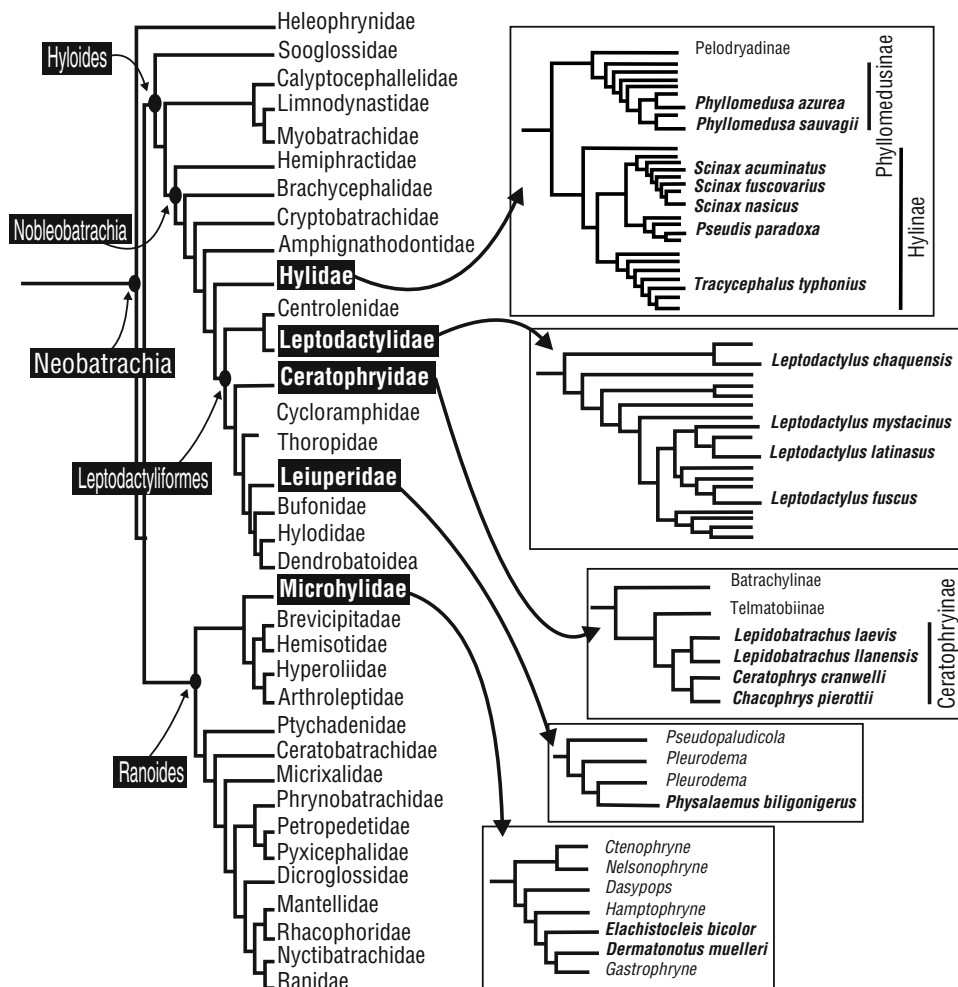


Fig. 2 The neobatrachian relationships (Frost et al. 2006; Grant et al. 2006) without Ceuthomantidae, Craugastoridae, Eleutherodactylidae, Nasikabatrachidae, and Strabomantidae (Frost 2011). Sokol (1975) proposed an anuran phylogeny based in four basic free-living tadpole morphotypes (Orton 1953, 1957) in which morphotypes I and III represent most basal anurans. Tadpoles in neobatrachian have keratinized mouthparts adapted to scrape food particles from substrate (morphotype IV) with the exception of Microhylidae, which tadpoles lacking keratinized mouthparts that primarily feed on suspended

(planktonic) food particles (morphotype II). Species examined are placed in Hylidae, Leptodactylidae, Ceratophryidae, Leiuperidae, and Microhylidae. Relationships within hylids are simplified from Faivovich et al. (2005), within leptodactylids from Ponssa (2008), and within ceratophryids, leiuperids, and microhylids from Grant et al. (2006). Other hypotheses based on morphological data (Fabrezi 2006; Fabrezi and Quinzio 2008) described *Ceratophrys* or *Chacophrys* as basal taxon within ceratophryines

(Linnaeus, 1758); *Scinax acuminatus* (Cope, 1862); *S. fuscovarius* (Lutz, 1925); *S. nasicus* (Cope, 1862); *Trachycephalus typhonius* (Linnaeus, 1758); Leiuperidae [*Physalaemus biligonigerus* (Cope, 1861)]; Leptodactylidae [*Leptodactylus chaquensis* Cei, 1950; *L. fuscus* (Schneider, 1799); *L. latinasus* Jiménez de la Espada, 1875; *L. mystacinus* (Burmeister, 1861)]; and Microhylidae [*Dermatonotus muelleri* (Boettger, 1885); *Elachistocleis bicolor* (Guérin-Méneville, 1838)].

Specimens of these species and field data were obtained in different places of the Chaco region of South America. A brief description of the Chaco is given in the “Appendix” with additional information on the localities studied. Secretaria/Ministerio de Medio Ambiente y Desarrollo

Sustentable, Gobierno de la Provincia de Salta, República Argentina provided collecting permits.

Specimens were euthanized in an aqueous solution of chloretone, and fixed in neutral-buffered formalin (10%) in the field, and adults were preserved in 70° alcohol. Specimens accessioned as lots are deposited in the Herpetological Collection of the Museo de Ciencias Naturales (MCN), Universidad Nacional de Salta (Argentina). Collection data of studied specimens are given in the “Appendix”.

For each species, larval series were staged following the standard table of Gosner (1960) from stages in which all five toes are separated (Stage 36) up to stages when tail is completely lost (Stage 46).

To document growth variation during the ontogeny, measurements of snout-vent length in larvae and post-metamorphic specimens, and total length in larvae (snout-tip tail length) were made with dial callipers (0.02 mm). These measurements are given in millimeters. As larval growth increases up to late prometamorphic stages (larval Stages 39–41), I considered the snout-vent length when forelimbs emerge is the maximum size that the fully metamorphosed individual may reach.

Duration of the larval period was estimated from field data; for each species the appearance of clutches in water at a temporary pond was considered as the starting point, and the time in which metamorphic (four legged) specimens were found as the ending point. For *Leptodactylus fuscus*, *L. latinasus*, and *L. mystacinus* field data on larval development duration are tentative since in the studied areas these three species call in holes in the ground where fertilization and early development could take place approximately 4–8 days before it rains. After rain, tadpoles at Gosner's stages (31–36) are found in recently formed ponds. Growth to reach metamorphic stages occurs during 25 days in *L. latinasus*, and during 60 days in *L. fuscus* and *L. mystacinus*. Solano (1987) reported that larval development for *Leptodactylus fuscus* occurs in 75 days in Brazil.

Variation of external morphology during the ontogeny was described by observations of the complete series of development using a stereomicroscope. Changes in the digestive tract, muscles, and gonads were analyzed by manual dissection of larval and postmetamorphic specimens. The study of skeletal variation was conducted using cleared and double-stained specimens following the method of Wassersug (1976), in which cartilage is stained blue with Alcian Blue and bone is stained red with Alizarine Red S. Additional anatomical preparations to study the innervation were obtained by the procedures described by Filipinski and Wilson (1984), Filipinski (1985), Nishikawa (1987) to get whole mounts of cleared and stained nerves with Sudan Black B. Anatomical terminology is based on the amphibian anatomical ontology (www.amphibanat.org).

Developmental events set as reference, based on Gosner table (1960), to compare among different species are the following: (1) appearance of internal metatarsal tubercle (old tadpoles, Stage 38), (2) forelimb emergence (beginning of metamorphosis, Stage 42), and (3) complete tail resorption (end of metamorphosis, Stage 46). Sequences of morphological variation during larval development were separated following three criteria:

- (1) Development and disappearance of features present only at larval stages: Typical larval features, such as keratinized mouth parts, spiracle, vent tube, and tail

exhibit interespecific variation which is used to characterize taxa at different levels (e.g. genera, species). Usually, description of tadpoles focuses on larval Stages 35–37 of Gosner's table (1960) because some typical larval features may change at later stages. These late changes are considered in this study.

- (2) Development and transformation of structures during tailed stages: Differences between larval and adult body plan involve structural transformations in tegument and feeding structures, including cartilages of jaws, hyobranchial skeleton, and gut. Gosner (1960) characterized stages of the metamorphosis following changes in the position of the mouth commissure which denotes the internal changes in jaw cartilages. These changes correspond to mouth commissure between the nostril and midpoint of the eye (Stage 43); mouth commissure between the midpoint and the posterior margin of the eye (Stage 44); and mouth commissure at the posterior margin of the eye (Stage 45).
- (3) Differentiation and development of structures necessary for anural stages: Gosner Stages 26–37 are defined by hind limb differentiation and correlate with differentiation of primary cartilages that occurs in proximo-distal and postaxial-preaxial direction to complete the pentadactyl hind-autopodium. Simultaneously, differentiation of the forelimbs happens within the branchial chamber following similar pattern. After differentiation of limb cartilages, perichondrial ossification progresses in diaphyses in proximo-distal direction, concomitant with limb growth. Mineralization of epiphyses, carpal/tarsal elements, and prepollical/prehallical elements takes place when growth stops or is decelerated (Fabrezi 1992, 1993, 2001; Fabrezi and Alberch 1996; Fabrezi and Barg 2001; Fabrezi and Goldberg 2009). Literature data for skull ossification in non-pipoid anuran are based on: de Sá (1988), Wiens (1989), Wild (1997, 1999), Pugener and Maglia (1997, 2007), Maglia and Pugener (1998), Haas (1999), Perotti (2001), Sheil and Alamillo (2005), Maglia et al. (2007), Fabrezi and Quinzio (2008), and Fabrezi and Goldberg (2009).

Results

Patterns of Growth

Growth of the tadpoles increases concomitant hind limb differentiation up to forelimb emergence. Table 1 listed the

Table 1 Interspecific variation of tadpole size (body length and total length), duration of larval development, and adult size

Species	Tadpole (Stages 39–42)		Larval development duration (in days)	Adult Snout-vent length (in mm)
	Snout-vent length(in mm) X ± SD	Total length(in mm) X ± SD		
<i>Ceratophrys cranwelli</i>	27.90 ± 2.92 (N = 13)	62.61 ± 5.60 (N = 13)	20–24	120
<i>Chacophrys pierottii</i>	42.70 ± 2.27 (N = 17)	101.66 ± 6.40 (N = 17)	15–18	55
<i>Lepidobatrachus laevis</i>	46.60 ± 2.91 (N = 17)	93.51 ± 8.86 (N = 17)	15–18	110–120
<i>Lepidobatrachus llanensis</i>	38.20 ± 3.97 (N = 13)	86.11 ± 7.15 (N = 13)	15–18	65–100
<i>Pseudis platensis</i>	40.39 ± 4.69 (N = 19)	126.03 ± 12.45 (N = 19)	>180	35–55
<i>Phyllomedusa azurea</i>	20.41 ± 0.98 (N = 18)	54.02 ± 3.01 (N = 18)	48–54	40
<i>Phyllomedusa sauvagii</i>	25.99 ± 0.91 (N = 10)	67.45 ± 4.24 (N = 10)	57–63	70
<i>Scinax acuminatus</i>	15.16 ± 1.03 (N = 16)	45.79 ± 2.33 (N = 16)	43–46	45
<i>Scinax fuscovarius</i>	15.25 ± 1.33 (N = 6)	42.58 ± 3.39 (N = 6)	37–42	41–44
<i>Scinax nasicus</i>	12.21 ± 0.64 (N = 20)	36.53 ± 1.85 (N = 20)	36–40	27–35
<i>Trachycephalus typhonius</i>	17.20 ± 1.38 (N = 22)	45.08 ± 3.39 (N = 22)	40–42	70
<i>Leptodactylus chaquensis</i>	22.83 ± 0.58 (N = 6)	62.39 ± 1.73 (N = 6)	58–60	85
<i>Leptodactylus fuscus</i>	21.81 ± 1.36 (N = 8)	52.42 ± 4.27 (N = 8)	65–70	50–55
<i>Leptodactylus latinasus</i>	13.03 ± 0.75 (N = 10)	32.56 ± 3.40 (N = 10)	29–32	30–40
<i>Leptodactylus mystacinus</i>	22.15 ± 1.82 (N = 9)	55.07 ± 4.16 (N = 9)	65–70	50–60
<i>Physalaemus biligonigerus</i>	12.07 ± 0.84 (N = 12)	28.17 ± 2.59 (N = 12)	20–24	35–40
<i>Dermatonotus muelleri</i>	14.75 ± 0.81 (N = 19)	31.6 ± 0.99 (N = 19)	25–30	40–50
<i>Elachistocleis bicolor</i>	9.36 ± 0.89 (N = 7)	23.22 ± 1.86 (N = 7)	18–20	40–45

Data of adult size were taken from Cei (1981)

average sizes and standard deviations of the tadpoles before the beginning the metamorphosis being this body length the size of the just metamorphosed individuals for each species (Fig. 3a). Body length for most species varies between 9 and 30 mm; in *Pseudis paradoxa*, *Chacophrys pierottii*, *Lepidobatrachus laevis*, and *L. llanensis* it reaches sizes upper 36 mm being *P. paradoxa* and *L. laevis* the species with larger body sizes.

Before the metamorphosis, total length of tadpoles is almost twice the body length in *Lepidobatrachus laevis* indicating tail is as long as body size (Table 1, Fig. 3a). In the other extreme, *Pseudis paradoxa* presents a total length in which tail length represents approximately 66% being the species with the longest tail (Table 1, Fig. 3a).

Larval development for most of the studied species extends from 20 to 75 days (Table 1), with some relation between size at the metamorphosis and duration of larval development suggesting similar growth rates (Fig. 3b). In *Elachistocleis bicolor* larval development happens in 20 days, it metamorphoses at the smallest size of the sample. *Chacophrys pierottii*, *Lepidobatrachus* spp. display accelerated patterns of larval growth, with large metamorphic individuals completing larval development in short periods. *Ceratophrys cranwelli* presents also an accelerated growth rate. At the other end of the spectrum, *Pseudis paradoxa* has the longest larval development (at least 180 days) with a large size at metamorphosis but revealing a low growth rate.

Among the studied species, adult size (Table 1) indicates postmetamorphic growth differs and seems to be clearly independent from metamorphic size.

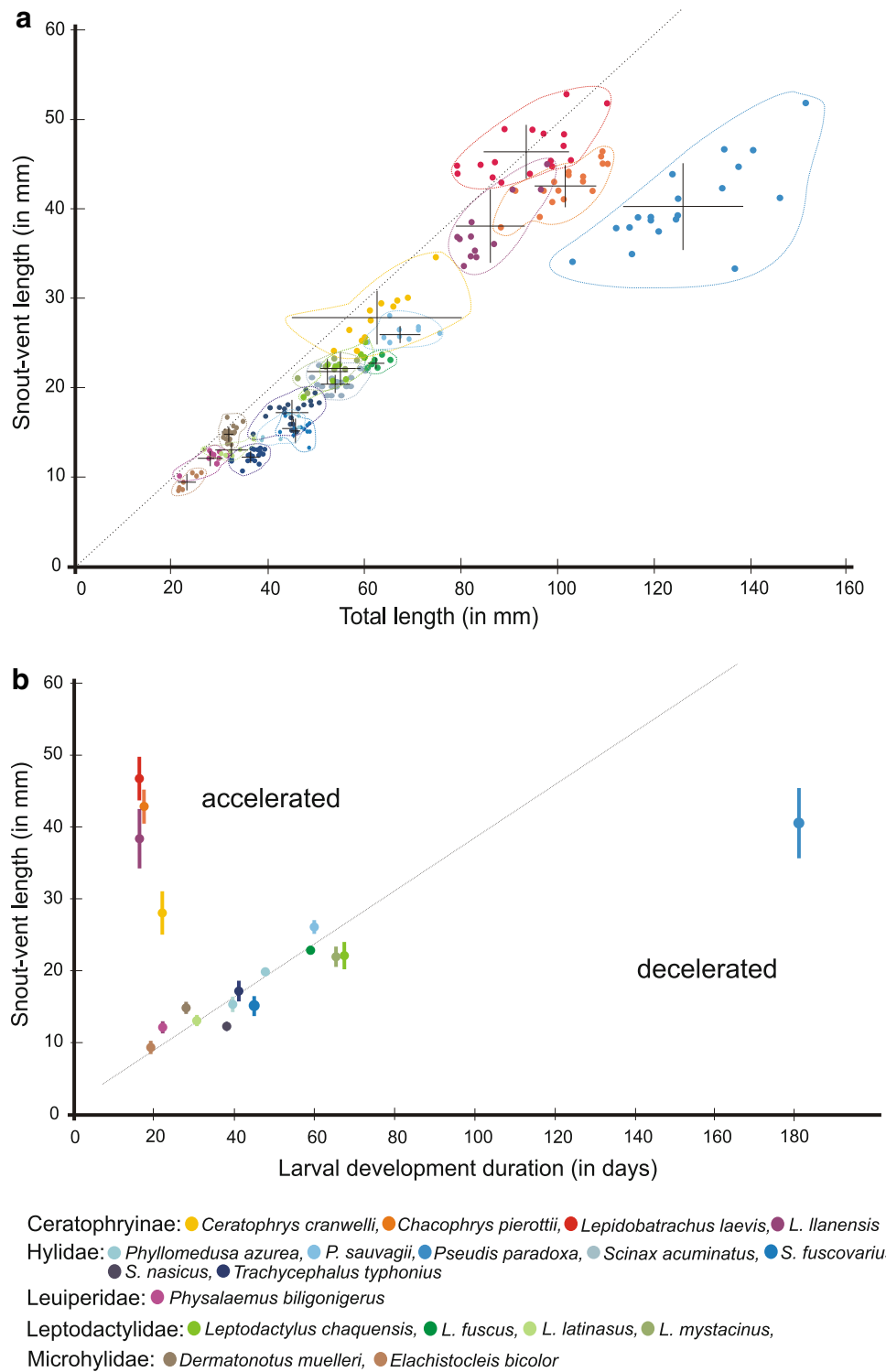
Patterns of Development

Disappearance of Features Present Only at Larval Stages

Figure 4 depicts observed variation in the timing of disappearance of larval features. Most of the studied species present a sinistral spiracle that disappears when left forearm protrudes through it. In *Dermatonotus muelleri* and *Elachistocleis bicolor* the single medio-posterior spiracle starts to migrate in cephalic direction as soon as forelimbs emerge. Its progressive disappearance finishes when the opening is fully incorporated into the ventral wall. In *Lepidobatrachus* spp. each branchial chamber is covered by lateral tegument that projects posteriorly forming a flap that ends in a wide opening (Fig. 5a). Thus, the developing forelimb grows behind the branchial basket exposed to external conditions as the hind limbs do. The flap shortens up (Fig. 5b, c) until it fuses with the skin surrounding the forelimb, this event coincides with the loss of the larval mouth parts (Fig. 5d, e).

In late tadpoles the vent tube presents morphological variation, which was described in Quinzio et al. (2006), Fabrezi and Quinzio (2008), and Fabrezi et al. (2009). The abdominal wall forms a large posterior funnel-shaped tube,

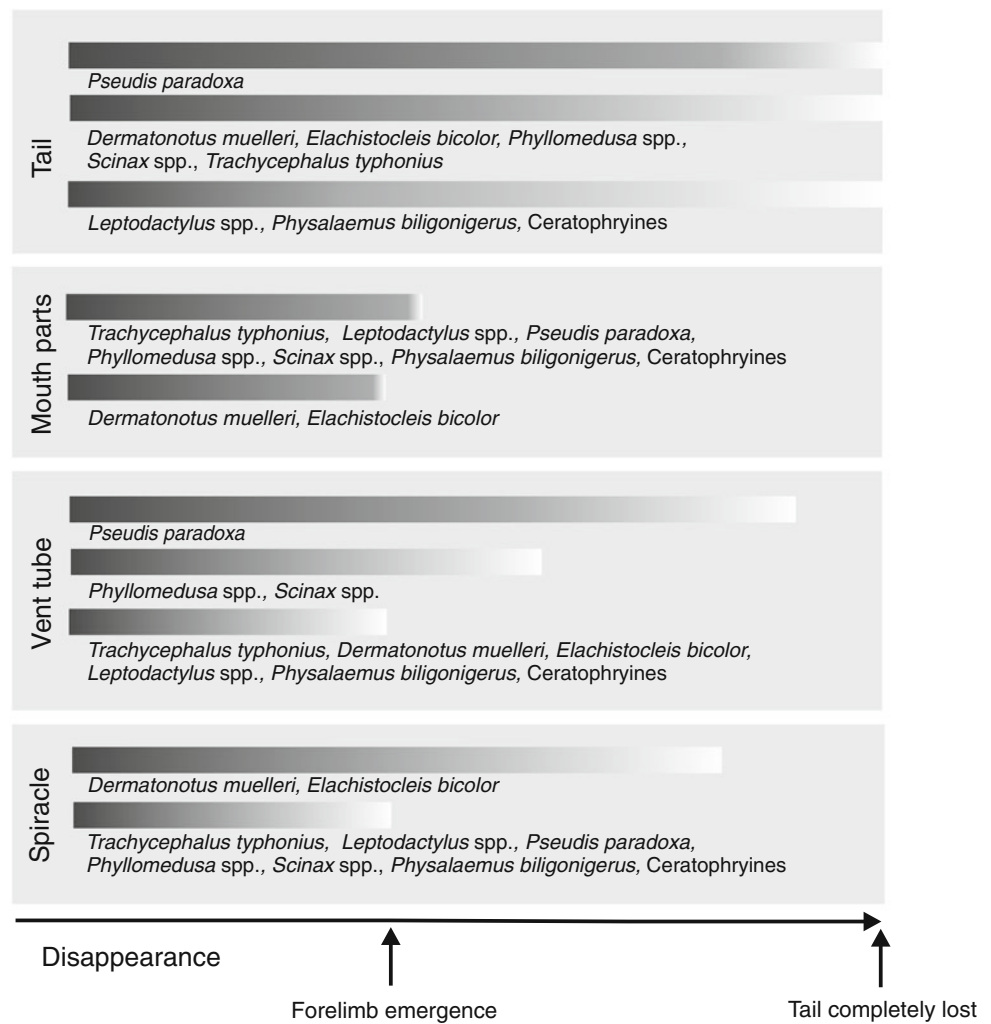
Fig. 3 Tadpole size variation among the studied species. **a** The plot depicts snout-vent length versus total length (that comprises snout-vent length plus tail length) of old tadpoles (Gosner Stages 39–42) and shows tadpoles of *Chacophrys pierottii*, *Lepidobatrachus* spp. and *P. paradoxa* are larger than most species. The dotted line indicates where total length is 50% the body length. In *Pseudis paradoxa* total length is twice longer than snout-vent length. **b** The plot shows snout-vent length (average and standard deviation) at metamorphosis versus larval development duration. The dotted line indicates the growth rate for most species. Accelerated rates occur in *Ceratophrys cranwellii*, *Chacophrys pierottii*, *Lepidobatrachus* spp. and decelerated rate in *P. paradoxa* resulting in both cases in large tadpoles



located medially, and associated with the ventral fin in *Phyllomedusa* spp. (Fig. 5f, g, h), *Scinax* spp., and *Pseudis paradoxa*. In *Dermatonotus muelleri* and *Elachistocleis bicolor* the vent tube is also funnel-shaped but very short and partially covered by the spiracle. Before its disappearance, this tube collapses, detaches from the ventral

wall but remains joined with the ventral fin. When this vent tube is lost, there is a gap between the cloaca and the ventral fin. In *Trachycephalus typhonius* (Fig. 5i, j, k), *Ceratophrys cranwellii*, *Chacophrys pierottii*, *Leptodactylus* spp., and *Physalaemus biligonigerus* the vent tube is cylindrical, short and separated by a fold from the

Fig. 4 Comparisons of relative timing of disappearance of larval external features in the anuran species of the Chaco



abdominal wall. In *Lepidobatrachus* spp. it is short, wider, and its aperture is enlarged along the sagittal plane. Before being incorporated to form the cloaca, the tube becomes gradually shorter.

As soon as both forelimbs emerge, mouth parts disappear rapidly in the following sequence: teeth rows, followed by jaw sheaths, and finally the soft mouth parts (e.g. tooth ridges and labial ridges) and marginal papillae (Hall et al. 2002; Fabrezi et al. 2009). Exceptions are found in microhylids and in *Lepidobatrachus* spp. In microhylids (*Dermatonotus muelleri* and *Elachistocleis bicolor*) oral structures are absent, and changes in larval mouth imply a reduction of the lateral alae of upper lip overlapping lower lip, and occur before forelimb emergence. In *Lepidobatrachus* spp. larval mouth presents only vestigial sheaths and complete single rows of marginal papillae bordering the upper and lower lips. Loss of these structures is fast, occurs simultaneously, and the first sign is the breaking and shedding of upper and lower sheaths.

Events of tail reduction start simultaneously with disappearance of larval mouth parts except for *Pseudis*

paradoxa (Fabrezi et al. 2009). Tail reduction is evident when dorsal and ventral fins become low, after which tail musculature starts to be shortened. Before disappearance, atrophied musculature mass with vestiges of fins without larval pattern of pigmentation decreases in size and may be dehiscent. In ceratophryines, *Leptodactylus* spp., and *Physalaemus biligonigerus*, tail shortening occurs simultaneously with early changes in larval mouth, whereas in *Dermatonotus muelleri*, *Elachistocleis bicolor*, *Trachycephalus typhonius*, *Phyllomedusa* spp., *Scinax* spp., and *Pseudis paradoxa* events of tail reduction become evident when mouth commissure has reached or surpassed mid-point of eye.

Structural Changes Between Larval and Postmetamorphic Stages

The larval lower jaw is formed by Meckel's cartilages and infrarostrals (Fig. 6). The Meckeli' cartilages are L-shaped, and oriented transversally. Each cartilage presents on its extremes a convex surface to articulate with the pars

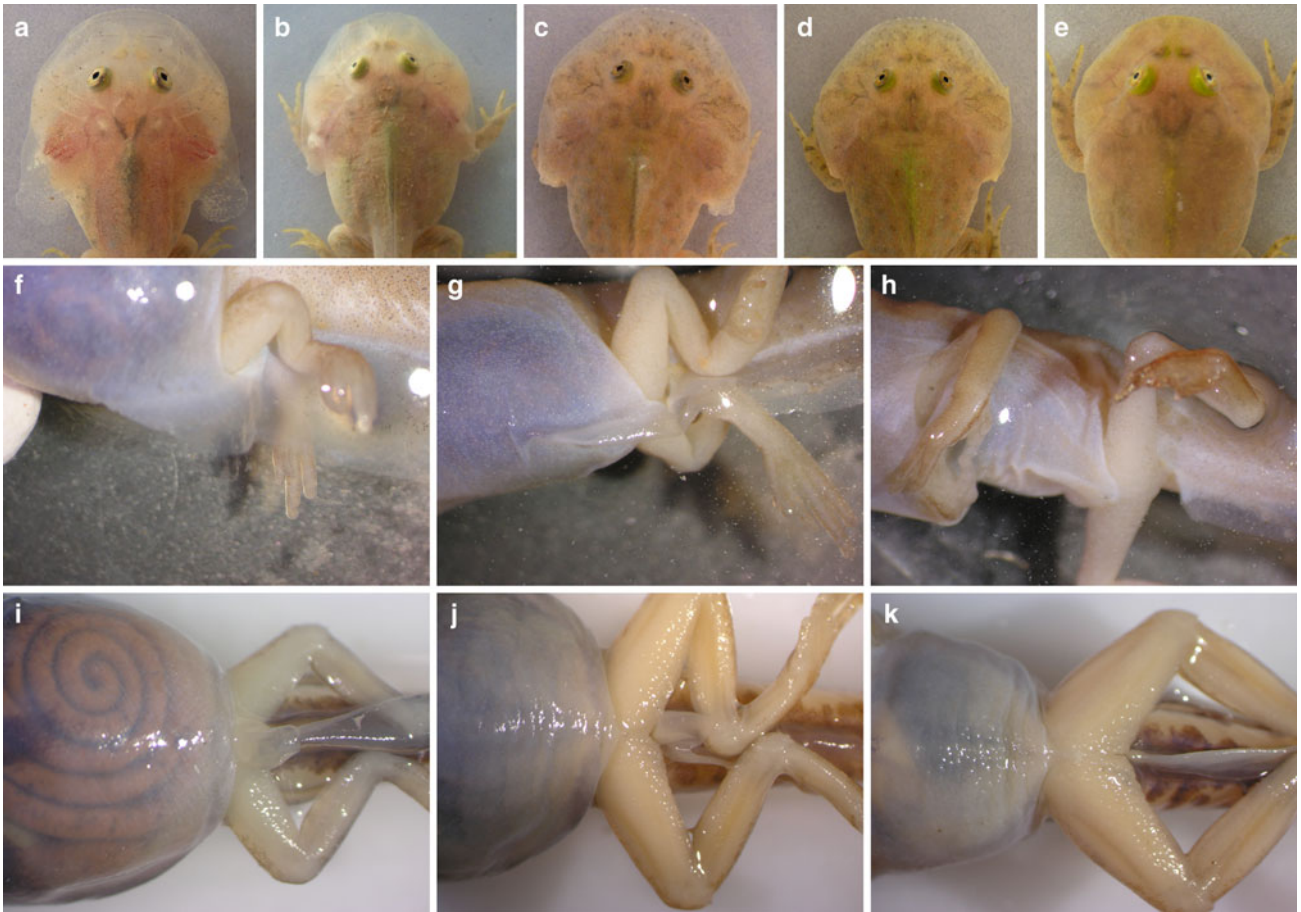


Fig. 5 a–e Sequences of changes in the spiracular skin in *Lepidobatrachus laevis* (Gosner Stages 40–43). f–h Sequences of disappearance of the vent tube in *Phyllomedusa sauvagii* (Gosner Stages

42–44). i–k Sequences of disappearance of the vent tube in *Trachycephalus thyphonius* (Gosner Stages 39–41)

articularis of palatoquadrate, and a concave surface to articulate with the infrarostral. Each infrarostral is oblong, curve and joins to the other by the copula mandibularis forming a U-shaped structure that delimit ventral portion of the mouth. In *Dermatonotus muelleri* and *Elachistocleis bicolor*, infrarostrals are different since they form a thin cartilaginous band that fuses laterally with the pointed end of the Meckel's cartilages (Fig. 6). Articulation of palatoquadrate-Meckel's cartilage is positioned anterior to the anterior half of the cornua trabeculae in most tadpoles, or in front of the cornua trabeculae in *D. muelleri* and *E. bicolor*, whereas tadpoles of *Lepidobatrachus* spp. present longer Meckel's cartilages and infrarostrals and then, the articulations of lower jaw locate backwards, almost at level of the trabecular planum.

Changes in the cartilages of the lower jaw imply that larval infrarostral and Meckel's cartilages lengthen with a concomitant posterior displacement of pars articularis of palatoquadrate (Fig. 6). When the tail is completely lost, the lower jaw articulation may be positioned at the level of: the midpoint of the eye (*Elachistocleis bicolor* and

Dermatonotus muelleri), between midpoint and posterior margin of the eye (*Physalaemus biligonigerus*, *Scinax* spp., *Phyllomedusa* spp., *Trachycephalus typhonius*, *Leptodactylus* spp.), posterior to the eye (*Pseudis paradoxa*), at level of the cranio-vertebral joint (*Chacophrys pierottii*; *Ceratophrys cranwelli*), or posterior to the cranio-vertebral joint (*Lepidobatrachus* spp.).

Organization of larval jaws in *Lepidobatrachus* suggests an early repatterning in the first visceral arch involving muscles and innervation (Fig. 7a, b). As result, the tadpole is suctorial and the larval oral cavity is huge, allowing it to swallow large preys that are usually living animals, like the adults.

The larval intestine is long and thin, forming a double spiral where the inner coils represent the posterior portion that opens through the vent tube (Fig. 8a). For most species, at the same time in which forelimbs protrude and the tadpole stops feeding, the double spiralled intestine decreases in diameter and its walls thicken further. Foregut dilates, becomes more straight, thicker, and distinct from the posterior intestine, insinuating the stomach and

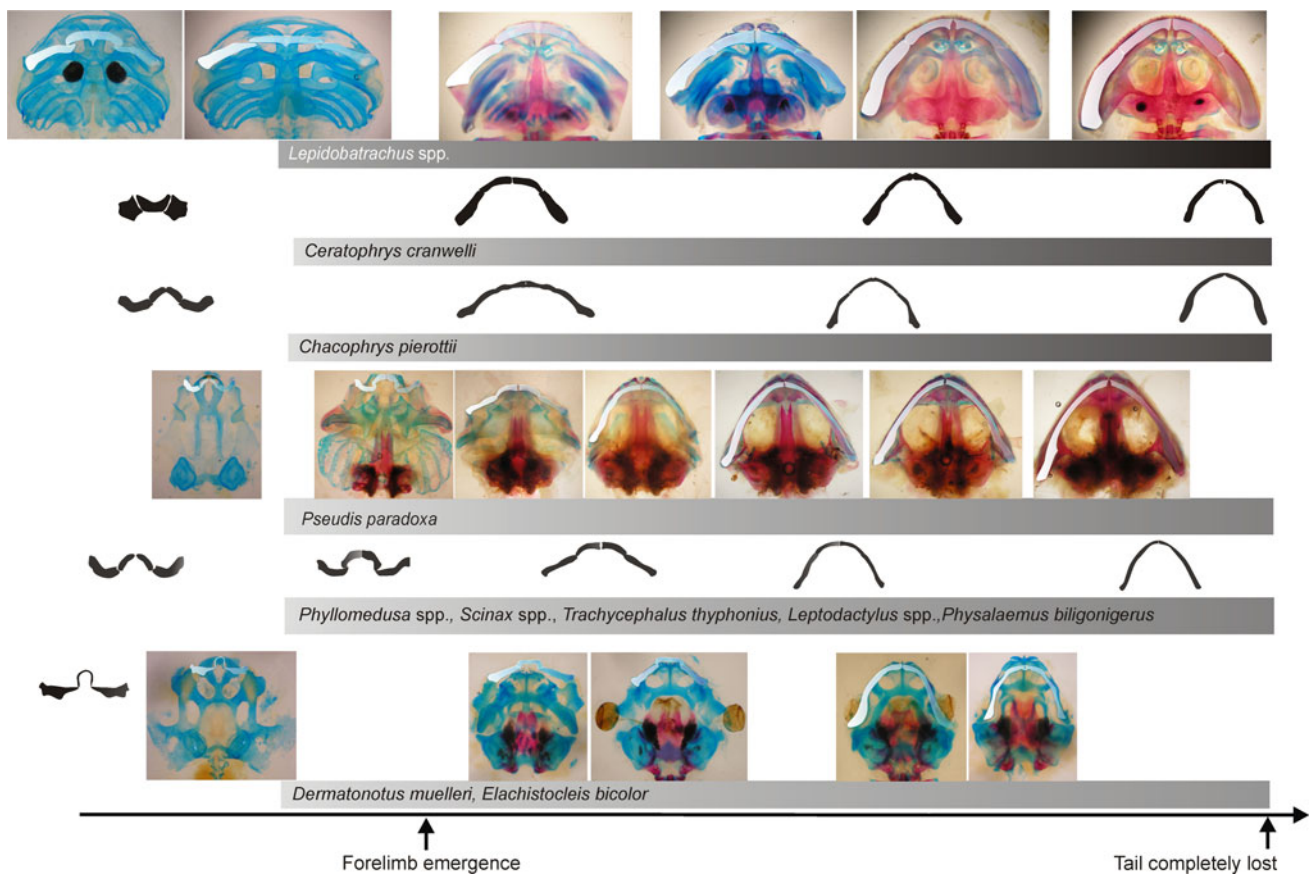


Fig. 6 Ontogenetic changes of the lower jaw cartilages. Larval lower jaws are formed by the medial infraostrals and the lateral Meckel’s cartilages delimiting a small mouth opening with the upper suprarostrals. However, larval jaws in *Lepidobatrachus* spp. resemble the metamorphosing jaws of all species. During metamorphosis, jaw

cartilages lengthen in all species with some variation. In microhylids, lower jaw cartilages are short at the end of metamorphosis and define small mouth gap; differently in *Lepidobatrachus* spp. the mouth gap is the biggest at the end of metamorphosis

duodenum (Fig. 8b). Differentiation progresses in the following sequence: the stomach acquires an anterior constriction that is the boundary with the esophagus; the distal constriction (pyloric) delimiting it from the duodenum is defined; and the diameter of the ileum increases distally to form the rectum which opens into the cloaca. Digestive tract is fully differentiated when tail is reduced to a stub. The stomach is already insinuated before emergence of forelimbs in *Ceratophrys cranwelli*, *Dermatonotus muelleri*, and *Elachistocleis bicolor*. Furthermore, tadpoles of *Ceratophrys cranwelli* have shorter intestines than other tadpoles for having a carnivorous diet. Tadpoles of *Lepidobatrachus* spp. possess a digestive tract in which a well differentiated stomach, pyloric constriction, duodenum, ileum, and rectum with a dilated cecum do not change during metamorphosis (Fig. 8c, d).

Differentiation of Adult Features

Among the studied species, *Pseudis paradoxa* is the only taxon in which epiphyses and carpal/tarsal elements are

mineralized at the time the tail is reduced to a stub (Fig. 9).

Some aspects of external limb features are related to limb development. After differentiation of hind limb cartilages, distal elements of the prehallux protrude the skin representing the internal metatarsal tubercle which defines Gosner Stage 38. In the studied hylids, leptodactylids, and microhylids two or more discrete distal skeletal elements form the prehallux, and one hypertrophic distal element is present in *Physalaemus biligonigerus* and ceratophryines (Fabrezi 2001). In ceratophryines the adult inner metatarsal tubercle is strongly keratinized and constitutes the spade organ typical of terrestrial anurans with digging capability. Keratinization of the inner metatarsal tubercle exhibits variation within ceratophryines (Fig. 10). In *Lepidobatrachus llanensis* it occurs earlier, almost simultaneously with the growth of the skeletal piece, and the keratinized inner metatarsal tubercle is already present before metamorphosis. In this species, keratinized finger claws also appear simultaneously with the inner metatarsal tubercle. In *L. laevis* and *Chacophrys pierottii* keratinization of the

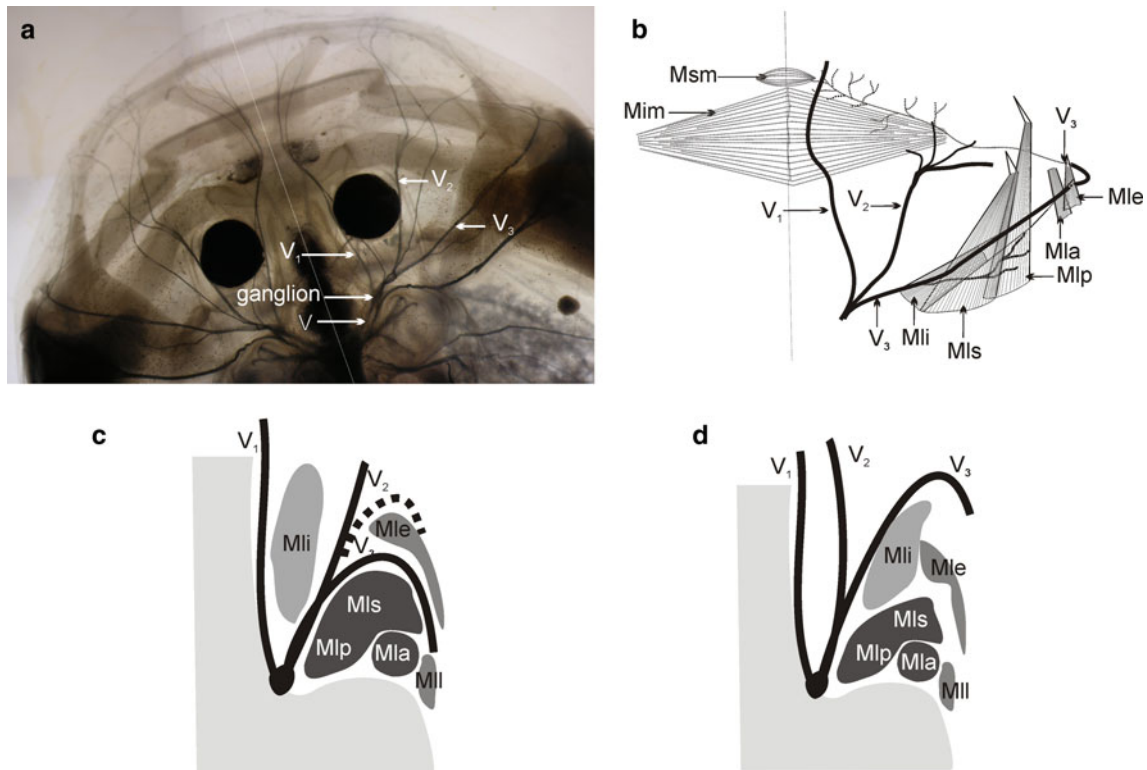


Fig. 7 **a** Dorsal view of the head of cleared and Sudan Black-stained whole-mount tadpole of *Lepidobatrachus laevis* (Gosner Stage 36) to observe peripheral cranial nerves. The dotted line represents the sagittal axis of the head. The trigeminal nerve (V) is formed by sensorial and motor fibers. The nerve emerges in a single root and forms the trigeminal ganglion which is placed adjacent the prootic foramen. From the ganglion, the trigeminal ramii separate into the ramus ophthalmicus profundus (V_1) and the ramus maxillo-mandibularis that immediately divides in the maxillary (V_2) and mandibularis (V_3) ramii. **b** Scheme of the mandibular muscles in dorsal view of the tadpole in (**a**). The dotted line represents the sagittal axis of the head. The ramus mandibularis (V_3) orients anterolaterally to run towards the set of muscles levatorae mandibulae and continue turning ventrally to innervate the muscles intermandibularis and submental. **c** Scheme of the generalized anuran tadpole condition with the relative position of trigeminal divisions (V_1 , V_2 , and V_3) respect to muscles levatorae mandibulae. Haas (2001) reviewed the homology

of amphibians jaw musculature and proposed new terminology and hypothesis of homology based on muscle insertion and origin, fiber orientation, and relative position of trigeminal divisions (V_1 , V_2 , and V_3). This interpretation is suitable for larval and adults anurans since relations of the nerve divisions and muscles in larvae are maintained during metamorphosis. **d** Scheme of the *Lepidobatrachus* spp. condition with the relative position of trigeminal divisions (V_1 , V_2 , and V_3) respect to muscles levatorae mandibulae. This condition is different from all anurans and resulted from a posterolateral displacement of jaw suspension. In tadpoles and adults of *Lepidobatrachus* spp., trigeminal ramii are positioned anteriorly to the set of muscles levatorae mandibulae. Abbreviations: *Mim* muscle intermandibularis, *Mla* muscle levator mandibulae articularis, *Mle* muscle levator mandibulae externus, *Mli* muscle levator mandibulae internus, *Mlp* muscle levator mandibulae longus profundus, *Mls* muscle levator mandibulae longus superficialis, *Msm* muscle submental

inner metatarsal tubercle develops at the beginning of metamorphosis whereas in *Ceratophrys cranwelli* it occurs during postmetamorphic stages.

Parasphenoid, frontoparietals, prootics, and exoccipitals are the first skull ossifications to be differentiated (Fig. 9). They are present in tadpoles with pentadactyl hind limbs. Nasals, septomaxillae, premaxillae, and maxillae appear after the cartilages of the olfactory capsules are developed and before resorption of cornua trabeculae. In four legged stages, immediately after cartilages of the lower jaw start to lengthen, angulosplenic and dentary are present. The squamosal develops adjacent to the external border of the processus muscularis of palatoquadrate, when resorption of this processus has

started. Pterygoids become differentiated early before the pars articularis of palatoquadrate reaches the otic capsule. In ceratophryines and *Pseudis paradoxa*, quadratojugals, prevomers, and palatines are present when tail is still present. In *Dermatonotus muelleri*, quadratojugals are present but incipient at later stages of tail reduction. With exception of *Pseudis paradoxa*, skull ossification progresses during postmetamorphic growth to complete the development of some bones (prevomers, palatines, quadratojugals) or differentiation of others (sphenotmoid, mentomeckelians). Different from all studied species, the set of skull bones, teeth, articulations, and sutures are completely differentiated when tail is almost disappeared in *Pseudis paradoxa*.

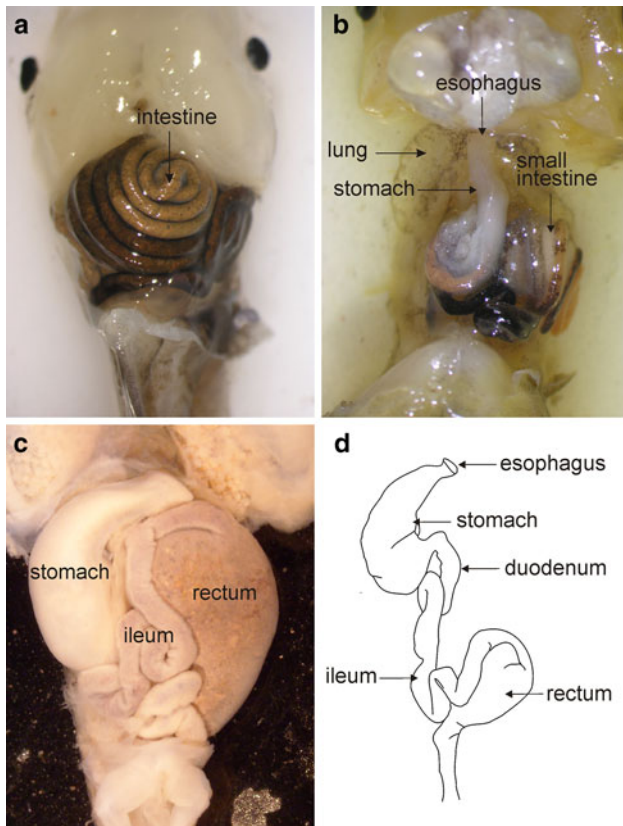


Fig. 8 Gut changes in tadpoles. **a** Tadpole of *Dermatonotus muelleri* (Gosner Stage 36), ventral view with skin, and muscles of the body wall removed. The long larval intestine occupies the abdominal cavity. **b** Tadpole of *Dermatonotus muelleri* (Gosner Stage 41), ventral view with skin, muscles of the body wall, and liver removed. The spiralled intestine decreases in diameter and the anterior portion enlarges, becomes thicker and distinct from the posterior intestine. The intestine presents regional differentiation of stomach, duodenum, and ileum. **c** Tadpole of *Lepidobatrachus laevis* (Gosner Stage 36), ventral view with skin, and muscles of the body wall removed. There is a constriction separating stomach from duodenum. The stomach and the terminal segment of the ileum (rectum) presents a dilated caecum. **d** Scheme of the digestive tract in *Lepidobatrachus* tadpoles at the beginning of the metamorphosis in which gross morphology is similar to that of the adult

Discussion

Growth Heterochrony

Wilbur and Collins (1973) proposed that the ability to dissociate growth rate and developmental rate can occur through the larval period after a minimum metamorphic size is attained to explain intraspecific variation in development and growth in amphibian larvae. Wilbur-Collins model predicts that if the growth rate of the larva increases, developmental rate is retarded to capitalize on the improved growth conditions. If growth rate decreases, developmental rate is predicted to increase to escape a deteriorating environment. Based on Wilbur-Collins model, several

experimental studies have demonstrated the role of some ecological factors (i.e. diet, food quality, temperature, density, predation, desiccation) to influence and modify amphibian larval development (Fig. 1).

In natural populations, size at the metamorphosis is a parameter of larval development that seems to have been evolved concomitant with ecological factors fixing limits of variation for each species in which large sizes in wild conditions are not frequent (Emerson 1988; Rot-Nikcevic and Wassersug 2004; Roček et al. 2006).

Larval size and larval development duration suggest similar rates of growth for most studied species. Species with accelerated growth show variation since larval development in *Lepidobatrachus* spp. and *Chacophrys pierottii* needs 2 weeks and their tadpoles are larger than tadpoles of *Ceratophrys cranwelli* which larval development occurs in 3 weeks (Fig. 3b, Fabrezi and Quinzio 2008). Differently, the whole larval development in *Pseudis paradoxa* demands at least 6 months with a lower developmental rate than the other species (Fig. 3b, Fabrezi et al. 2009).

For the anuran assemblage studied, acceleration and deceleration in rates of larval growth are examples of growth heterochrony (Smith 2002). 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). The studied species have explosive reproduction, start larval development simultaneously, with *Pseudis paradoxa* and *Lepidobatrachus laevis* having huge tadpoles, and both could be considered hypermorphic or giant respect of tadpoles of most anurans; however, accelerated rate of growth indicates only peramorphosis in *L. laevis*.

For anuran species, huge tadpoles and long larval development were associated to template climate and permanent ponds that provide good environmental conditions to guarantee metamorphosis (Emerson 1988) and permanent semitropical lakes, but with few predators (Roček et al. 2006). Differently, accelerated growth rates were related to unpredictable environmental conditions in which explosion of resources suggest that increased protein in diet enhances either growth or development (Kupferberg 1997). Among ceratophryines, with explosive reproductions in the earlier ephemeral pools, *Chacophrys pierottii* has an omnivorous tadpole, *Ceratophrys cranwelli* has a carnivorous tadpole, and *Lepidobatrachus* spp. have a suctorial tadpole able to swallow large prey, including co-specific tadpoles. Large tadpoles resulting from fast growth would be expected in *Ceratophrys* and *Lepidobatrachus* rather than in *Chacophrys*. However, ecological factors (e.g. food availability, diet, temperature and risk of desiccation) that have proved effects on larval growth in experimental conditions would have had different

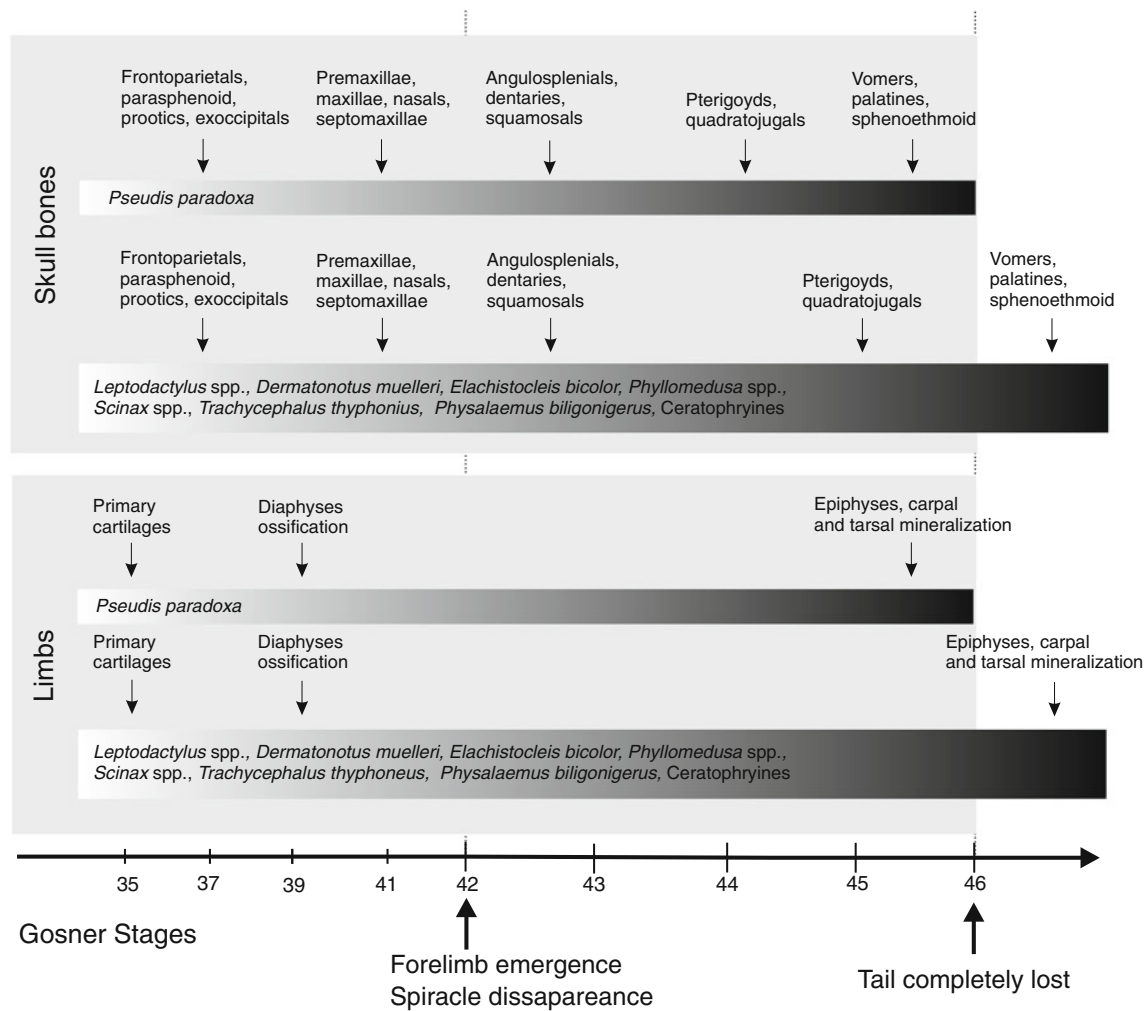


Fig. 9 Comparisons of the ontogeny of adult features during larval development exemplified by limb and skull ossification since bone in frogs is an exclusively postembryonic tissue (Hanken and Hall

1984). Among the studied species, skull and limb ossification progresses in a general pattern but finishes early before tail loss in *Pseudis paradoxa*

influences and determined growth rates and timing of larval development for each species of ceratophryines.

Different interspecific patterns of growth exhibited by tadpoles demonstrate that size, as a signal of peramorphosis/paedomorphosis, should be carefully considered in comparative analyses since heterochrony involves timing of growth and development which are necessary to interpret size variation.

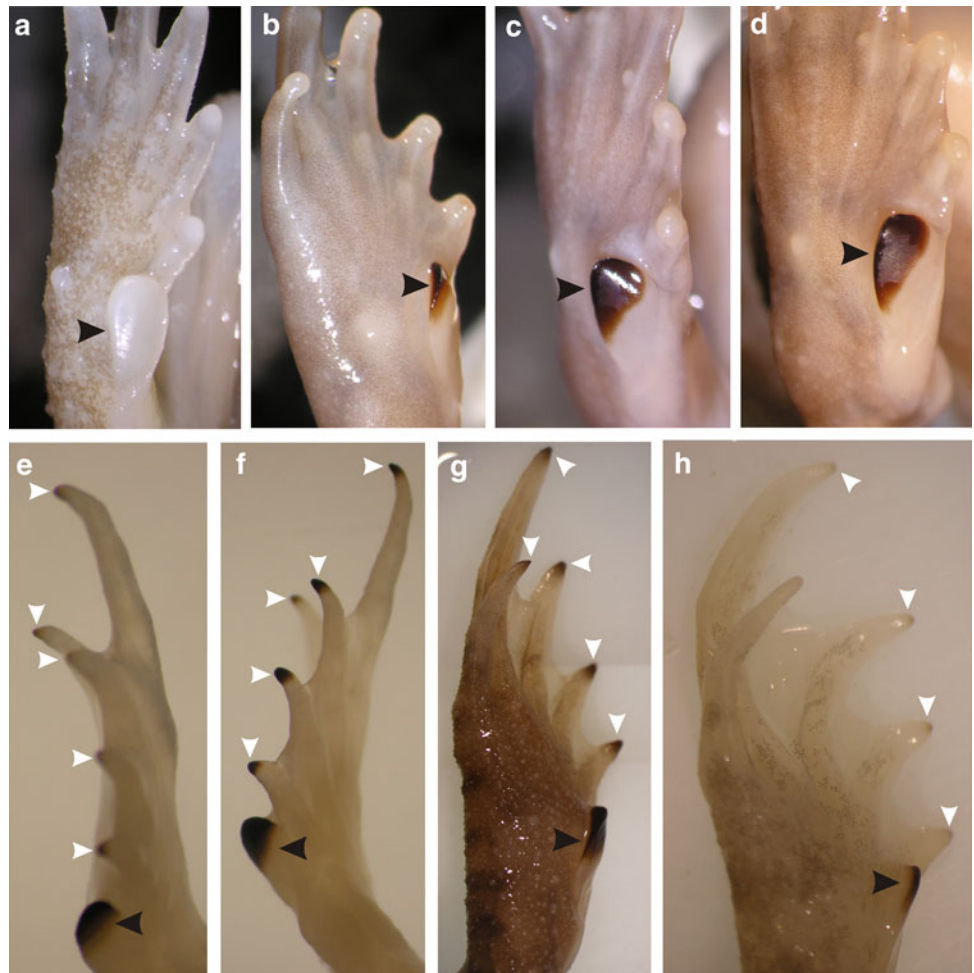
Sequence Heterochrony

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). For most of the studied species, growth and development present certain

parallelism, even during the metamorphosis. This parallelism implies that growth and developmental sequences occur in a generalized pattern in which growth and developmental show scarce variation among species under similar environmental conditions (Fig. 11a). In *Ceratophrys* and *Chacophrys*, the accelerated growth rates do not imply dissociation or important alterations in developmental sequences, whereas in *Pseudis paradoxa* and *Lepidobatrachus* spp. deviated growth rates appear concomitant with dissociation and/or alteration in developmental sequences (Fig. 11b, c).

Among the studied species (Fig. 4), the timing of disappearance of larval traits seems to be associated to: (a) the condition of the spiracle and its relation with the protruding forelimbs: the medial spiracle, as in *Dermatonotus muelleri* and *Elachistocleis bicolor*, disappears later than the double spiracle of

Fig. 10 Keratinization in hind limbs. **a** The internal metatarsal tubercle is not keratinized at the end of the metamorphosis in *Ceratophrys cranwelli* (Gosner Stage 46). Keratinization of the internal metatarsal tubercle in *Chacophrys pierottii*: **b** when forelimb are emerging (Gosner Stage 42); **c** when tail is reduced (Gosner Stage 44); and **d** when tail is completely lost (Gosner Stage 46). Keratinization of the internal metatarsal tubercle and claws in *Lepidobatrachus llanensis*: **e** immediately after fingers are completely separated and the internal metatarsal tubercle is differentiated (Gosner Stage 38); **f** before reduction of skin flaps covering the branchial chambers (Gosner Stage 42); and **g** when tail is completely lost (Gosner Stage 46). **h** Keratinization of the internal metatarsal tubercle and claws in *Lepidobatrachus laevis* before reduction of skin flaps covering the branchial chambers (Gosner Stage 42). *Black arrows* indicate internal metatarsal tubercles and *white arrows* point claws



Lepidobatrachus or the sinistral spiracle of the remaining species. (b) the complexity of the larval mouth; species with reduced (i.e. *Lepidobatrachus* spp.) or absent keratinized mouth parts (i.e. *Dermatonotus muelleri* and *Elac-histocleis bicolor*) exhibit an abrupt change in larval mouth; (c) the length of the vent tube (Fig. 5); species having short and small vent tubes lose it before forelimb emergence whereas species with large funnel-shaped vent tube (*Phyllomedusa* spp., *Scinax* spp, *Pseudis paradoxa*) conserve it up to mid or more advanced metamorphic stages. (d) The length of the tail: the shortness of the tail in *Pseudis paradoxa* (the longest tail with respect of snout-vent length) is delayed up to later metamorphic stages (Fabrezi et al. 2009) (Fig. 3a).

Disappearance of larval features may exhibit certain variation but the end of the larval stage is always denoted by the complete loss of the tail. This event coincides with the fusion of hypochord and coccyx to form the urostyle and the fully completion of the pattern of muscles to terrestrial locomotion (Pugener and Maglia 2009; Prikryl et al. 2009).

For most anurans, events of transformations from larval to adult structures (gut, visceral arches and associated

muscles, skin, among others) seem to be coordinated to occur simultaneously and rapidly, during advanced larval stages (Figs. 6, 8). Thyroid hormone levels are low during early larval development and peak at metamorphic climax stimulating jaw and head remodelling (Hanken and Summers 1988; Hanken et al. 1989); transformation of the gut (Schreiber et al. 2001), and changes from larval to adult skin (Yoshizato 1992; Tamakoshi et al. 1998), among others. Steroids, including sex steroids, also may be involved in metamorphosis (Hayes 1997). A pre-displacement of adult like morphologies, as was described in *Lepidobatrachus* spp. (see also Fabrezi and Quinzio 2008 for skin features) is unique for anurans and could be originated in changes in the hormone system.

Wassersug and Hoff (1982) explored the reorientation of the suspensorium at metamorphosis among anurans. These authors proposed that evolution has increased the difference between larvae and adults, which shows an inverse relationship between the angle of jaw suspension in the larva and the angle of jaw suspension in the adult. Frogs recognized as basal tend to have the least difference in this feature between larvae and adults (gradual

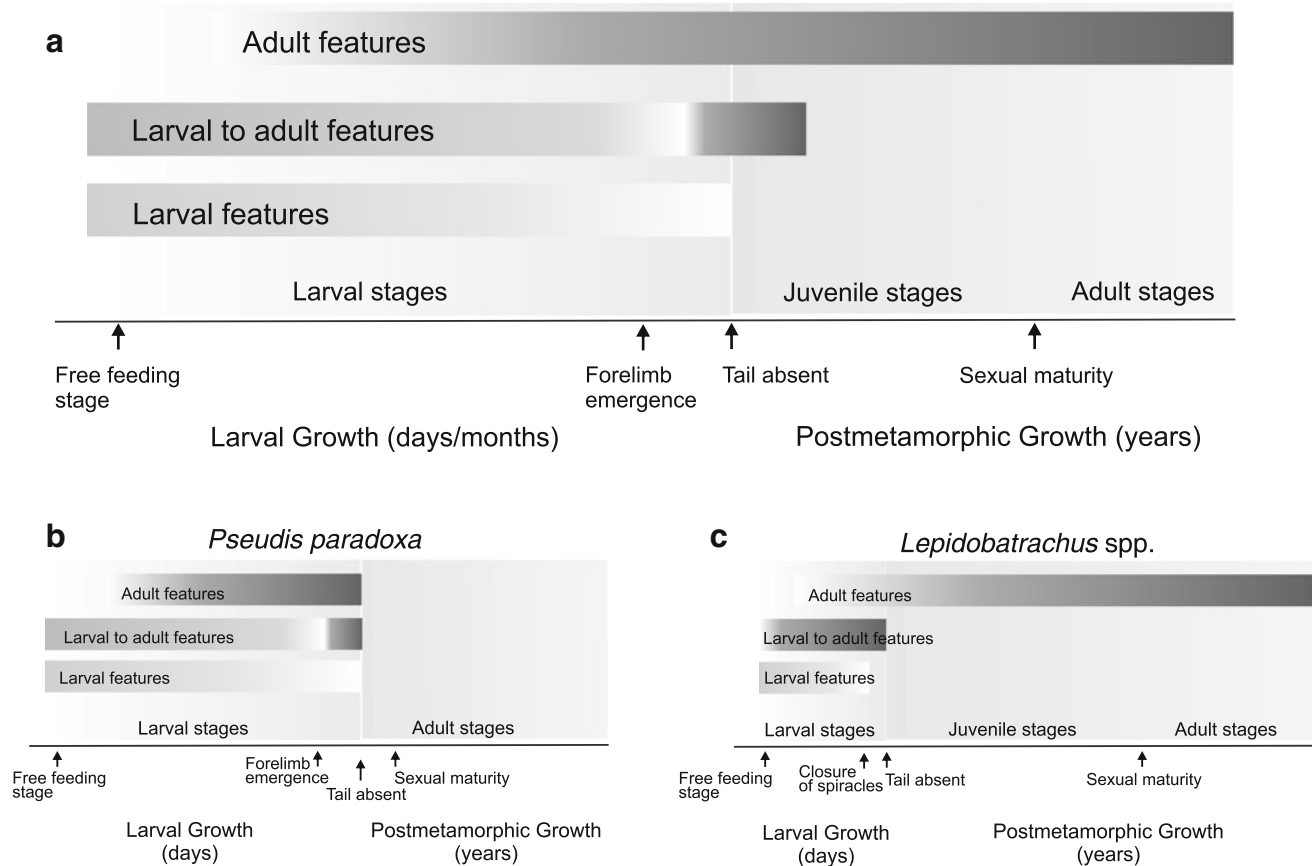


Fig. 11 Ontogenetic patterns of anurans living in the Chaco of South America. **a** Larval development for most species. Disappearance of larval features and morphological transformations from larval to adult plans occur quite simultaneously during a short period between the

metamorphosis), whereas advanced frogs have the greatest difference (abrupt metamorphosis). Following the criteria proposed by Wassersug and Hoff (1982), metamorphosis of jaws in microhylid species is gradual whereas in ceratophryines it is abrupt (Fig. 7). Yeh (2002) stated that changes in skull shapes among anurans where the result of an increased growth of upper and lower jaws reflects more ontogenetic changes. Among ceratophryines, elongation of jaw templates displays more ontogenetic changes than in other species being a distinctive trait independently of the morphology of larval jaw (Fabrezi 2006). However, larval jaws in *Lepidobatrachus* spp. are clearly different from larval jaws of other anurans and even when lower jaw morphology could be comparable with morphology of the anuran jaw at mid-metamorphic stages (Hanken 1993), the location of the muscles levatorae mandibulae and the trajectory of the mandibular ramus of the nervus trigeminus indicates an early repatterning of the mandibular arch that is unique among anurans (Fig. 7).

Intestine is another organ that is remodelled during metamorphosis. Tadpole's intestine has a much longer but

forelimb emergence and tail loss. Development of structures of the adult body plan, as well as growth after metamorphosis, has an offset independent of the size at metamorphosis. **b** Pattern of the ontogeny in *Pseudis paradoxa*. **c** Pattern of the ontogeny in *Lepidobatrachus* spp

simpler structure that reflects changes in the physiological functions between herbivorous tadpoles and carnivorous frogs. In *Lepidobatrachus* spp. morphological transformations during larval development are not significant since tadpoles have a short alimentary tract with an adult-like stomach (Ruibal and Thomas 1988; Carroll et al. 1991; Ulloa Kreisel 2002).

Differentiation and development of adult traits seem to occur in a more conservative sequence. In amphibians, bone elongation progresses at the epiphyses which remain cartilaginous during growth (Farnum 2007). Endochondral ossification and/or mineralization of epiphyses indicate growth is arrested. The epiphyses, carpalia, and tarsalia are still cartilaginous in postmetamorphic juvenile stages with the exception of *Pseudis paradoxa* in which the complete ossification/mineralization of limbs (epiphyses, carpalia, and tarsalia) indicates growth is arrested at the end of metamorphosis (Fabrezi and Goldberg 2009).

Even though some variation in the sequence of skull bones differentiation has been reported (Trueb 1994), there is a general pattern summarized as follows: ossification of

skull starts with neurocranium bones, proceeds towards snout bones, continues with lower and upper jaw bones to end with palatal bones. Trueb and Alberch (1985) and Yeh (2002) related body size with skull ossification and set different patterns of paedomorphosis in which small size and absence and/or underdevelopment of some bones (vomeres, palatines, quadratojugals) have evolved independently in anuran lineages. Trueb and Alberch (1985) noted that heterochrony, size, and degree of ossification are independent variables, since degree of ossification, which is a measure of the amount of bone-matrix deposition, and calcification are related allometrically to body size; however, the rate of osteoblastic proliferation and bone-matrix deposition can be independent of both size and heterochronic events such as retardation of developmental rates.

Among the studied species, *Elachistocleis bicolor* and *Dermatonotus muelleri* have reduced squamosals and vomeres, and quadratojugals are absent in *E. bicolor*. The remaining species have skulls with complete set of bones, and the ceratophryines are characterized by hyperossified skulls with exostosed maxillae, squamosals, and frontoparietals. Except for *Pseudis paradoxa* in which skull bones are fully formed (e.g. complete teeth in premaxillae, maxillae, and vomeres, sphenothmoid, and tympanic ear), and articulations and sutures are already similar to the adult skull at the end of the metamorphosis (Fabrezi and Goldberg 2009), the other studied species finish their metamorphoses with some bones (e.g. vomeres, palatines, sphenothmoid) incompletely developed or not differentiated. These data confirm the independence of size, development, and rate of ossification but show evidence that absolute age could be related with the whole skull ossification in *Pseudis paradoxa*.

Certain invariance of the pattern of limb development, and skull bones differentiation, as well as gonadal development (Ogielska and Kotosz 2004; Fabrezi et al. 2010) confirm the idea that those systems exclusively of the adult plan have their own developmental program independent of other events of larval development.

Heterochrony in *Pseudis*

In *Pseudis paradoxa* (Fig. 11b), long larval development with low growth rate produces huge tadpoles, with a metamorphosis in which delayed loss of larval structures (e.g. vent tube, tail) coincides with the offset of metamorphic transformations (e.g. skin, digestive tract, remodelling of mandibular, hyoid, and branchial cartilages and muscles), and the offset of differentiation of typical adult elements (tympanic ear, the complete set of skull bones, limb ossification) (Fabrezi et al. 2009, 2010; Fabrezi and Goldberg 2009). The froglets present gonads with advanced development and maturation (ovaries with

previtellogenic oocytes and testes with spermatids) but the absence of secondary sexual characters indicates that they are non-reproductive stages (Downie et al. 2009; Fabrezi et al. 2010). For this species, during the long larval development typical patterns of metamorphic changes and postmetamorphic development overlap and therefore postmetamorphic juvenile stages seem to be absent (Fabrezi and Goldberg 2009; Fabrezi et al. 2010). In this ontogeny, growth heterochrony and sequence heterochrony during larval development converge at the end of metamorphosis in individuals in which somatic development stops.

Heterochrony in Ceratophryines

Ceratophryinae are associated with terrestrial habits in arid to semiarid lowland environments in tropical to semi-tropical latitudes, and they are more diverse in the semi-arid environments of the Chaco being *Chacophrys* and *Lepidobatrachus* the only anuran genera restricted to this region (see “Appendix”). The ceratophryines share a set of numerous morphological features (Haas 2003; Fabrezi and Emerson 2003; Fabrezi 2006; Quinzio et al. 2006; Fabrezi and Quinzio 2008; Fabrezi and Lobo 2009). Besides, they also share an accelerated growth of their larvae. The fast larval development of ceratophryines with large tadpoles could suggest an evolutionary response to the dryness of the environments in which they live. This situation is comparable with data on *Spea* larvae, in which fast growth and fast development occur in wild populations in semi-arid environments of North America. *Spea* larvae are facultative carnivores that express alternate resource-use phenotypes that are adaptive for either detritus or shrimp (Pfennig et al. 1993; Ledón-Rettig et al. 2009, 2010).

Dissociation of developmental events concomitant with fast larval growth occurs only in *Lepidobatrachus* spp. (Fig. 11c) in which the tadpole is distinctive, with exposed forelimbs, keratinized claws and internal metatarsal tubercle in *L. llanensis*, big mouth with vestigial keratinized jaw sheaths, well differentiated stomach, and adult-like skin with double layered dermis (stratum spongiosum and compactum, Fabrezi and Quinzio 2008). These features are typical of metamorphosing frogs. Furthermore, most of the distinctive characters of *Lepidobatrachus* tadpoles are related with feeding. Tadpoles of *Lepidobatrachus* are suctorial (O’Reilly et al. 2002) and able to swallow large prey. Roelants et al. (2011) found several anuran clades, whose tadpoles are classified as Orton’s morphotype IV (Anomocoela, Nobleobatrachia, Natatanura, and Afrobatrachia) occupy contiguous or even overlapping regions in morphospace. Together, these clades are estimated to contain over 80% of modern anuran species, implying that the majority of extant tadpoles are

concentrated in a relatively small part of morphospace. However, among Nobleobatrachia, *Lepidobatrachus* is the exception for its proximity to the morphotype I (Xenoanura) and III (*Ascaphus* and Costata) domains caused by the apparent reversal of various cranial and branchial features lost earlier in the evolution of morphotype IV tadpoles and the evolution of traits that are superficially similar to those of xenoanuran tadpoles (including a wide slit-like mouth, paired spiracles, and reduction of keratinized mouthparts).

Hanken (1992, 1993) interpreted the bizarre morphology of the *Lepidobatrachus* tadpole as a phenomenon involving large-scale modifications of the larva in which features characteristic of the adult that develop at metamorphosis in the ancestral life history, are advanced into the larval period (i.e. precocious metamorphosis). Fabrezi and Quinzio (2008) explained the fast developmental rate and the precocious metamorphic morphologies in *Lepidobatrachus* tadpoles define a peramorphic larval body plan suggesting that the free feeding stage in *Lepidobatrachus* spp. could be equivalent to metamorphic larval stages (between forelimb emergence and tail completely loss) of most anurans (Fig. 11c).

Both adult and larval morphologies of *Lepidobatrachus* are divergent from its relatives (*Chacophrys* and *Ceratophrys*). *Lepidobatrachus* is aquatic with some distinctive traits (flattened stout body covered with loose skin, very broad head, prominent dorsally located eyes, small tympanum, short legs, webbed toes with keratinized claws, subarticular tubercles reduced or absent, a functional lateral line system in *L. laevis*, discontinuous ceratohyalia, single dermal ossification dorsally located in the hyoid, reduction or loss of hyoid and tongue muscles, small tongue) deviated from the pattern of terrestrial and aquatic ceratophryids (i.e. *Telmatobius*) (Fabrezi and Lobo 2009).

Fast growth and early acquisition of adult-like features in *Lepidobatrachus* imply that there are no changes in its life style from tadpole to adult. Tadpoles and post metamorphic individuals remain in the aquatic habitat, feed underwater, being voracious predators on insects and other tadpoles, including co-specific ones. They are not good jumpers and move with some difficulties outside of the water suggesting they can disperse only when ponds are interconnected during periods of high precipitation; after pool desiccation they stay buried in the mud. This fact could be an explanation for the stability of their populations over long periods of time.

It would be expected that anurans of the semi arid environments of the Chaco region breed in places in which larval growth and larval development may be attained in conditions favourable to metamorphose. However, metamorphosed *Lepidobatrachus* are the exception in which fast larval development and growth may result in specializations to avoid desiccation, but metamorphosed individuals

depend on ephemeral ponds to grow, feed, and reproduce, with life spans of at least 6 years (Fabrezi and Quinzio 2008). Even when the aquatic adults of *Lepidobatrachus* share some specializations to live in arid environments with other ceratophryines, other features, such as the lateral line system in the adult of *Lepidobatrachus laevis*, reveal that the aquatic life style has evolved in the genus from or towards the closed relative terrestrial *Chacophrys* or *Ceratophrys*.

Conclusions

In the morphological evolution of anuran larvae many of the character changes during the basal radiation reflect adaptive differentiation into universal ecological/trophic niches (originally benthic/nektonic substrate feeding in morphotype III and IV tadpoles); these niches seems timeless, and there is no indication that aquatic habitats imposed radically different selective pressures on the tadpole body plan during successive radiation periods (Roetlants et al. 2011). Larval development of the studied species occurs in ephemeral or semipermanent ponds in which larval development may vary by changes in growth and development within limits for most species. Growth heterochrony may occur without producing alterations in development (*Ceratophrys*, *Chacophrys*) but it seems to be necessary or pre-required to produce distinctive dissociated patterns of larval development in *Pseudis* and *Lepidobatrachus*. In *Pseudis paradoxa* (Fig. 11b) the larval development is paedomorphic: individuals reach large sizes when tail is lost (hypermorphosis) by they grow for a long time with low growth rate (deceleration) respect of the other species; disappearance of larval features and morphological transformations from larval to adult plans occur quite simultaneously during the period between the forelimb emergence and tail loss. The offset of growth and development of structures of the adult body plan coincides with tail loss. Differently, the larval development in *Lepidobatrachus* spp. (Fig. 11c) is peramorphic: individuals reach large size when tail is lost (hypermorphosis) by high growth rate (acceleration); transformations from larval to adult plans are quite absent suggesting an early onset of metamorphic or adult-like morphologies. The uniqueness of the *Lepidobatrachus* free feeding tadpole implies that growth heterochrony and sequence heterochrony in larval development have influenced the adult body plan. This fact suggests evolution of larval morphospace, as was interpreted by Roetlants et al. (2011) could be extrapolated to the whole ontogeny for *Lepidobatrachus*. Further studies focusing on molecular genetics of different ceratophryine populations, and research on hormonal regulation at early development are

still necessary to understand the morphological evolution of the ontogeny *Lepidobatrachus*.

Acknowledgments I am grateful to Natalia von Ellenrieder for many suggestions on the English written. Two anonymous reviewers contributed with many comments to improve the manuscript. Javier Goldberg, Silvia Quinzio, and Fernando Hongn provided many useful suggestions and comments. Fernando Hongn, Gladys Gonzo, Javier Goldberg, Silvia Quinzio, Roberto Bernal, and Soledad Valdecantos collaborated with field work. This research was supported Consejo Nacional de Investigaciones Científicas y Técnicas PIP 0239.

Appendix

Localities Studied

Localities in which larval development was studied are placed in the Chaco region that comprises a plain of approximately 1.141.000 km² in the south-central South America (from 16° 55' S–33° 52' and 67° 50'W– 57° 52'W). It is an arid and subtropical region of low forests and savannas traversed by only two permanent rivers: Pilcomayo and Bermejo that flow southeastward across the plain from their Andean headwaters to Paraguay River. The Chaco is subject to climates that vary from tropical in the north to warm-temperate in the south. The mean of annual temperature varies between 26° and 16° with an average of 22°C. The highest recorded temperatures for the continent occur in the Chaco with absolute maximums that may reach 54°C. There is marked seasonality, with warm summers with precipitations ranging with a West-East gradient from 300 to 1,000 mm. Evaporation losses (1,500–900 mm) sharply reduce the effective precipitation and give the Chaco an arid nature that is absent only in the permanent swamps and forests along Paraguay River. Specimens and field data presented in this research were obtained from three localities:

- Locality 1 Totoralejos, Córdoba, Argentina: 29°38'28.00''S, 64°50'58.37''W. Taxa found in this locality at the same time (late December 2004; January 2006): Ceratophryidae (*Chacophrys pierottii*, *Lepidobatrachus llanensis*), Leptodactylidae (*Leptodactylus mystacinus*)
- Locality 2 Provincial Route 5, between 24°44'8.00''S, 64°12'8.23''W–24°26'9.17''S, 63°59'17.62''W. Taxa found in this locality during the four rainy seasons (November–April 1994–1997, 2007): Bufonidae (*Rhinella arenarum*, *R. schneideri*), Ceratophryidae (*Ceratophrys cranwelli*, *Lepidobatrachus llanensis*), Cycloramphidae (*Odonotophrynus lavillai*), Hylidae (*Phyllomedusa sauvagii*, *Scinax fuscovarius*, *S. nasicus*, *Trachycephalus typhonius*), Leiuperidae (*Physalemus*

biligonigerus, *P. cuqui*; *Pleurodema tucumanum*), Leptodactylidae (*Leptodactylus chaquensis*, *L. bufonius*, *L. fuscus*, *L. latinasus*, *L. mystacinus*), Microhylidae (*Dermatonotus muelleri*, *Elachistocleis bicolor*)

- Locality 3 National Route 81, between 23°12'34.68''S, 63°34'27.48''W–23°14'49.96''S, 63°21'11.64 W. Species found in this locality during several rainy seasons (November–April, 1996–2010): Bufonidae (*Rhinella granulosa*, *R. schneideri*), Ceratophryidae (*Ceratophrys cranwelli*, *Chacophrys pierottii*, *Lepidobatrachus laevis*, *L. llanensis*), Hylidae (*Dendropsophus nanus*, *Hypsiboas raniceps*, *Phyllomedusa azurea*, *P. sauvagii*, *Pseudis paradoxa*, *Scinax acuminatus*, *S. fuscovarius*, *S. nasicus*, *Trachycephalus typhonius*), Leiuperidae (*Physalemus biligonigerus*, *P. cuqui*); Leptodactylidae (*Leptodactylus chaquensis*, *L. bufonius*, *L. fuscus*, *L. latinasus*, *L. mystacinus*), Microhylidae (*Dermatonotus muelleri*, *Elachistocleis bicolor*)

Geographical Ranges of Studied Species

Frost (2011) summarized the distribution of the studied species as follows:

Ceratophrys cranwelli: Chacoan region, Argentina, Bolivia, Brazil, and Paraguay

Chacophrys pierottii: Chacoan region, Argentina, Paraguay, and Bolivia

Dermatonotus muelleri: Northern Argentina, eastern Bolivia; Paraguay; Brazil

Elachistocleis bicolor: Central Argentina and Uruguay, Paraguay, Bolivia and Amazonian Brazil.

Lepidobatrachus laevis: Chacoan region, Argentina, Paraguay, and Bolivia

Lepidobatrachus llanensis: Chacoan region, Argentina, Paraguay, and Bolivia

Leptodactylus chaquensis: Chacoan region, Argentina, Bolivia, Paraguay and northern Uruguay, and southern Brazil.

Leptodactylus fuscus: Savannas from Panama throughout South America, east of the Andes, Brazil, Bolivia, Paraguay, and Argentina.

Leptodactylus latinasus: Chacoan region, Argentina, Bolivia, and Paraguay and Uruguay, and southern Brazil

Leptodactylus mystacinus: Bolivia, Brazil, Uruguay, Paraguay to central Argentina.

Phyllomedusa azurea: Chacoan region, Argentina, Paraguay, and Bolivia and pantanal and cerrado regions, Brazil with an isolated population in southern Brazil.

Phyllomedusa sauvagii: Chacoan region, Argentina, Bolivia, Brazil, and Paraguay

Physalaemus biligonigerus: Northern and central Argentina; Bolivia; Paraguay; Uruguay; southern Brazil.

*Pseudis paradoxa**: Lower Río Magdalena Valley as well as Arauca and Meta in Colombia; Guianas from northeastern Venezuela east through Guyana, Surinam, and French Guiana to the mouth of the Amazon, thence west in the Amazon Valley to northern Bolivia and southeastern Peru; Trinidad. * Populations in the northern of Argentina were recognized as *Pseudis paradoxa* by Garda et al. (2010).

Scinax acuminatus: Chacoan region, Argentina, Bolivia, Brazil, and Paraguay

Scinax fuscovarius: Argentina, Bolivia, Brazil, and Paraguay, between 150 and 1,800 m elevation.

Scinax nasicus: Chacoan region, Argentina, Bolivia, Brazil, and Paraguay and central Argentina, Uruguay, and southern Brazil

Trachycephalus typhonius: Lowlands of tropical Mexico, Central America, Trinidad and Tobago, Amazon Basin, Paraguay, northern Argentina.

Specimens Examined

Analyses of external morphology, measurements, and whole mounts of skeleton and peripheral nerves were obtained from the following list of specimens that are deposited in the Museo de Ciencias Naturales, Universidad Nacional de Salta, Argentina (MCN).

Ceratophryidae

Chacophrys pierottii

Locality 1 MCN 1027 (60 specimens, larval stages 26–31, January, 16 2006); MCN 1028 (40 specimens, larval stages 31–36, January, 22, 2006), MCN 1029 (56 specimens, larval stages 35–46, January, 28, 2006), MCN 987 (nine males, three females, December, 29, 2004), MCN 1045 (one female, January, 16, 2006)

Lepidobatrachus llanensis

Locality 1 MCN 986 (six males, 3 females, December 29, 2004), MCN 1122 (14 specimens, larval stages 39–46, January 28, 2006), MCN 1178 (36 specimens, larval stages 32–38, January 16, 2006)

Locality 2 MCN s/n, (14 specimens, larval stages 31–37, November 15, 1996), MCN s/n (3 specimens, larval stages 39–41, November 21, 1996)

Locality 3 MCN 567 (55 specimens, larval stages 33–39, December 1, 1998), MCN 932 (43 specimens, larval stages 31–37, November 25, 2000), MCN 665 (70 specimens, larval stages 36–41, January 5, 2000), MCN 1118 (45 specimens, larval stages 36–46, December 18, 2005), MCN 1319/1321 (36 specimens, larval stages 39–46, December 9, 2009)

Lepidobatrachus laevis

Locality 3 MCN 936 (one juvenile, November 2, 1998), MCN 663 (15 specimens, larval stages 36–41, December 1, 1998), MCN 647 (25 specimens, larval stages 33–40, January 5, 2000), MCN 666 (seven specimens, larval stages 38–43 and one froglet, January 5, 2000); MCN 668 (one froglet, January 5, 2000), MCN 695/696 (three juvenile specimen, January 5, 2000), MCN 934 (one juvenile, November 25, 2000), MCN 931 (three adults, January 20, 2001); MCN 935 (one juvenile, January 2003); MCN 815 (one adult and three juveniles, December 15, 2003), MCN 814 (six specimens, larval stages 36–38, December 28, 2003), MCN 817 (three juveniles, December 28, 2003), MCN 989 (one female, April 15, 2005), MCN 1175 (35 specimens, larval stages 34–46, December 18, 2005), MCN 1174 (female, March 10, 2006), MCN 1341 (two specimens, larval stages 39, December 27, 2008), MCN 1324 (seven froglets, February 7, 2009), MCN 1320/1322 (56 specimens, stages 39–46, December 8, 2009)

Ceratophrys cranwelli

Locality 2 MCN 414 (38 specimens, larval stages 28–33, December 1, 1995), MCN 413 (22 specimens, larval stages 37–46, December 16, 1995); MCN 415 (24 specimens, larval stages 26–31, January 18, 1996), MCN 420 (13 specimens, larval stages 36–40, January 26, 1996), MCN 425 (seven specimens, larval stages 42–46, February 2, 1996), MCN 1345 (58 specimens, larval stages 26–36, December 1, 2007), MCN s/n (25 specimens, larval stages 39–44, December 12, 2007)

- Locality 3 MCN 1190 (23 specimens, larval stages 33–38, December 18, 2005); MCN 1034 (27 specimens, larval stages 37–43, March 23, 2006), MCN 1318 (14 specimens, larval stages 34–37, December 8, 2009)

Hylidae

Phyllomedusa azurea

- Locality 3 MCN 1054 (44 specimens, larval stages 34–40, February 10, 2005); MCN 1177 (78 specimens larval stages 33–44, April 1, 2006); MCN 1309 (66 specimens, larval stages 36–44, March 13, 2007); MCN 1326 (80 specimens larval stages 33–41, February 27, 2010); MCN 1346 (54 specimens, larval stages 37–46, April 14, 2010)

Phyllomedusa sauvagii

- Locality 3 MCN 1159 (76 specimens, larval stages 31–39, December 29, 2004); MCN 1077 (69 specimens, larval stages 33–42, February 17, 2005); MCN 1309 (103 specimens, larval stages 37–43, March 13, 2007); MCN 1348 (46 specimens larval stages 32–41, February 27, 2010); MCN 1330 (45 specimens, larval stages 38–46, April 04, 2010)

Pseudis paradoxa

- Locality 3 MCN 988 (35 specimens, larval stages 33–38, February 10, 2005); MCN 1171 (33 specimens, larval stages 33–42, March 10, 2006), MCN 1196 (54 specimens, larval stages 33–43, March 13, 2007); MCN 1197 (59 specimens, larval stages 33–46, April 4, 2007)

Scinax acuminatus

- Locality 3 MCN 1145 (48 specimens, larval stages 38–42, December 12, 2004); MCN 1339 (78 specimens, larval stages, 38–44, December 27, 2009); MCN 1347 (45 specimens, larval stages 37–46, April 14, 2010)

Scinax fuscovarius

- Locality 3 MCN 1134 (33 specimens, larval stages 34–38, November 2, 2001); MCN 1105 (43 specimens,

larval stages 37–41, November 29, 2004); MCN 1144 (38 specimens, larval stages 38–44, December 29, 2004); MCN 1191 (60 specimens, larval stages 32–42, December 18, 2005); MCN 1315 (47 specimens, larval stages 38–46, January 05, 2008)

Scinax nasicus

- Locality 3 MCN 1350 (48 specimens, larval stages, 36–43, December 28, 2005); MCN 1349 (15 specimens, larval stages 37–44, December 30, 2006); MCN 1317 (53 specimens, larval stages 38–46, January 5, 2008)

Trachycephalus typhonius:

- Locality 3 MCN 1127 (49 specimens, larval stages 37–43, January 2, 2001); MCN 1337 (58 larval specimens, larval stages 37–42, December 27, 2007), MCN 1333 (33 specimens, larval stages 38–46, January 05, 2008)

Leiuperidae

Physalaemus biligonigerus

- Locality 3 MCN 1092 (45 specimens, larval stages 36–43, December 29, 2004); MCN 1097 (58 specimens, larval stages 37–46, February 17, 2005); MCN 1078 (35 specimens, larval stages 39–46, February 17, 2005), MCN 1079 (33 specimens, larval stages 38–42, March 12, 2005)

Leptodactylidae

Leptodactylus chaquensis

- Locality 2 MCN 1343 (56 specimens larval stages 35–37, December 1, 2007), MCN s/n (35 specimens, larval stages 40–42, December 12, 2007)

- Locality 3 MCN 1148 (78 specimens, larval stages 35–38, January 17, 2000); MCN 1335 (85 specimens, larval stages 39–44; 45, December 27, 2007)

Leptodactylus fuscus

- Locality 2 MCN 1344 (33 specimens, larval stages 39–43, December 1, 2007)

Locality 3 MCN 816 (27 specimens, larval stages 38–42, December 28, 2003); MCN 1089 (45 specimens, larval stages 39–43, December 29, 2004); 1336 (73 specimens, larval stages 39–44, December 27, 2007)

Leptodactylus mystacinus

Locality 3 MCN 1351 (15 specimens, larval stages 39–46, December 30, 2006); MCN 1342 (25 specimens, larval stages 39–42, December 27, 2007)

Leptodactylus latinasus

Locality 3 MCN 1126 (43 specimens, larval stages 37–42, February 10, 2005), MCN 1352 (140 specimens, larval stages 38–46, December 27, 2007)

Microhylidae

Dermatonotus muelleri

Locality 3 MCN 1016 (60 specimens, larval stages 34–39, March 4, 2004); MCN 946 (55 specimens, larval stages 30–37, December 11, 2004); MCN 1094 (38 specimens, larval stages 38–43, December 29, 2004); MCN 997 (45 specimens, larval stages 31–37, February 9, 2005); MCN 1080 (23 specimens, larval stages 38–44, February 17, 2005), MCN s/n (100 specimens, larval stages 26–31, December 12, 2007), MCN 1340 (123 specimens, larval stages 35–39, December 27, 2007), MCN 1332 (75 specimens, larval stages 37–46, January 5, 2008)

Elachistocleis bicolor

Locality 3 MCN 1164 (15 specimens, larval stages 37–42, January 17, 2000); MCN 1062 (22 specimens, larval stages 33–40, February 17, 2005); MCN 1081 (12 specimens, larval stages 32–37, March 12, 2005); MCN 1340 (5 specimens, larval stages 40–43, December 27, 2007)

References

- Alberch, P., Gould, S. J., Oster, G. F., & Wake, D. B. (1979). Size and shape in ontogeny and phylogeny. *Paleobiology*, *5*, 296–317.
- Altig, R. I., & Johnston, G. F. (1989). Guilds of anuran larvae: Relationships among developmental modes, morphologies, and habitats. *Herpetological Monographs*, *3*, 81–109.
- Brown, D. D., Cai, L., Das, B., Marsh-Armstrong, N., Schreiber, A. M., & Juste, R. (2005). Thyroid hormone controls multiple independent programs required for limb development in *Xenopus laevis* metamorphosis. *PNAS*, *102*, 12455–12458.
- Carroll, E. J., Jr, Seneviratne, A. M., & Ruibal, R. R. (1991). Gastric pepsin in an anuran larva. *Development Growth and Differentiation*, *33*, 499–507.
- Cei, J. M. (1981). Amphibians of Argentina. *Monitore Zoologico Italiano, Monographia*, *2*, 1–609.
- de Sá, R. O. (1988). Chondrocranium and ossification sequence of *Hyla lanciformis*. *Journal of Morphology*, *195*, 345–355.
- Denver, R. J. (1997). Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. *American Zoologist*, *37*, 172–184.
- Downie, J. R., Bryce, R., & Smith, J. (2004). Metamorphic duration: An under-studied variable in frog life histories. *Biological Journal of the Linnean Society*, *83*, 261–272.
- Downie, J. R., Sams, K., & Walsh, P. T. (2009). The paradoxical frog *Pseudis paradoxa*: Larval anatomical characteristics, including gonadal maturation. *Herpetological Journal*, *19*, 1–10.
- Dunker, N., Wake, M. H., & Olson, W. (2000). Embryonic and larval development in the caecilian *Ichthyophis kohtauensis* (Amphibia, Gymnophiona): A staging table. *Journal of Morphology*, *243*, 3–34.
- Emerson, S. B. (1988). The giant tadpole of *Pseudis paradoxa*. *Biological Journal of the Linnean Society*, *34*, 93–104.
- Fabrezi, M. (1992). El carpo de los anuros. *Alytes*, *10*, 1–29.
- Fabrezi, M. (1993). The anuran tarsus. *Alytes*, *11*, 47–63.
- Fabrezi, M. (2001). A survey of prepollex and prehallux variation in anuran limbs. *Zoological Journal of the Linnean Society*, *131*, 227–248.
- Fabrezi, M. (2006). Morphological evolution of Ceratophryinae (Anura, Neobatrachia). *Journal of Zoological Systematics and Evolutionary Research*, *44*, 153–166.
- Fabrezi, M., & Alberch, P. (1996). The carpal elements of anurans. *Herpetologica*, *52*, 188–204.
- Fabrezi, M., & Barg, M. (2001). Patterns of carpal development among anuran amphibians. *Journal of Morphology*, *249*, 210–220.
- Fabrezi, M., & Emerson, S. B. (2003). Parallelism and convergence in anuran fangs. *Journal of Zoology*, *260*, 41–51.
- Fabrezi, M., & Goldberg, J. (2009). Heterochrony during skeletal development of *Pseudis platensis* (Anura, Hylidae) and the early offset of skeleton development and growth. *Journal of Morphology*, *270*, 205–220.
- Fabrezi, M., & Lobo, F. J. (2009). Hyoid skeleton, related muscles, and morphological novelties in the frog *Lepidobatrachus* (Anura, Ceratophryidae). *Anatomical Record*, *292*, 1700–1712.
- Fabrezi, M., & Quinzio, S. I. (2008). Morphological evolution in Ceratophryinae frogs (Anura, Neobatrachia): the effects of heterochronic changes during larval development and metamorphosis. *Zoological Journal of the Linnean Society*, *154*, 752–780.
- Fabrezi, M., Quinzio, S. I., & Goldberg, J. (2009). The giant tadpole and delayed metamorphosis of *Pseudis platensis* Gallardo, 1961 (Anura, Hylidae). *Journal of Herpetology*, *43*, 228–243.
- Fabrezi, M., Quinzio, S. I., & Goldberg, J. (2010). The ontogeny of *Pseudis platensis* (Anura, Hylidae): Heterochrony and the effects of larval development in the postmetamorphic life. *Journal of Morphology*, *271*, 496–510.
- Faivovich, J., Haddad, C. F., Garcia, P. C. A., Frost, D. R., Campbell, J. A., & Wheeler, W. C. (2005). Systematic review of the frog family Hylidae, with special reference to Hylinae: Phylogenetic analysis and taxonomic revision. *Bulletin American Museum of Natural History*, *294*, 1–240.

- Farnum, C. E. (2007). Postnatal growth of fins and limbs through endochondral ossification. In B. K. Hall (Ed.), *Fins into limbs. Evolution, development, and transformation* (pp. 118–151). Chicago: Chicago University Press.
- Filipski, G. T. (1985). Staining nerves in whole cleared amphibians and reptiles using Sudan Black B. *Copeia*, 1985, 500–502.
- Filipski, G. T., & Wilson, M. H. V. (1984). Sudan Black B as a nerve stain for whole cleared fishes. *Copeia*, 1984, 204–208.
- Frost, D. R. (2011). Amphibian species of the World: An online reference. Version 5.5 (January 31, 2011). Electronic Database accessible at <http://research.amnh.org/vz/herpetology/amphibia/American> Museum of Natural History, New York, USA.
- Frost, D. R., Grant, T., Faivovich, J., Bain, R. H., Haas, A., Haddad, C. F. B., et al. (2006). The amphibian tree of life. *Bulletin of American Museum of Natural History*, 297, 1–370.
- Garda, A. A., Santana, D. J., & de Avelar São Pedro, V. (2010). Taxonomic characterization of Paradoxical frogs (Anura, Hylidae, Pseudae): Geographic distribution, external morphology, and morphometry. *Zootaxa*, 2666, 1–28.
- Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16, 183–190.
- Gould, S. J. (1977). *Ontogeny and phylogeny*. Cambridge: The Belknap Press of Harvard University Press.
- Grant, T., Frost, D. R., Cladwell, J. P., Gagliardo, R., Haddad, C. F. B., Kok, P. J. R., et al. (2006). Phylogenetic systematics of dartpoison frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). *Bulletin of American Museum of Natural History*, 299, 1–262.
- Haas, A. (1999). Larval and metamorphic development in the fast developing frog *Pyxicephalus adspersus* (Anura, Ranidae). *Zoomorphology*, 119, 23–35.
- Haas, A. (2001). Mandibular arch musculature of anuran tadpoles, with comments on homologies of amphibian jaw muscles. *Journal of Morphology*, 247, 1–33.
- Haas, A. (2003). Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics*, 19, 23–89.
- Hall, J. A., Larsen, J. H., Jr, & Fitzner, R. E. (2002). Morphology of the Premetamorphic larva of the spadefoot toad, *Scaphiopus intermontanus* (Anura: Pelobatidae), with an emphasis on the lateral line system and mouthparts. *Journal of Morphology*, 252, 114–130.
- Handrigan, G. R., & Wassersug, R. J. (2007). The anuran Bauplan: A review of the adaptive, developmental, and genetic underpinnings of frog and tadpole morphology. *Biological Reviews*, 82, 1–25.
- Hanken, J. (1992). Life history and morphological evolution. *Journal of Evolutionary Biology*, 5, 549–557.
- Hanken, J. (1993). Model systems versus outgroups: Alternative approaches to the study of head development and evolution. *American Zoologist*, 33, 448–456.
- Hanken, J., & Hall, B. K. (1984). Variation and timing of the cranial ossification sequence of the oriental fire-bellied toad, *Bombina orientalis* (Amphibia, Discoglossidae). *Journal of Morphology*, 182, 245–255.
- Hanken, J., & Summers, C. H. (1988). Skull development during anuran metamorphosis: III. Role of thyroid hormone in chondrogenesis. *Journal of Experimental Zoology*, 246, 156–170.
- Hanken, J., Summers, C. H., & Hall, B. K. (1989). Morphological integration in the cranium during anuran metamorphosis. *Experientia*, 45, 872–875.
- Hayes, T. B. (1997). Steroids as potential modulators of thyroid hormone activity in anuran metamorphosis. *American Zoologist*, 37, 185–194.
- Kupferberg, S. J. (1997). The role of larval diet in anuran metamorphosis. *American Zoologist*, 37, 146–159.
- Ledón-Rettig, C. C., Pfennig, D. W., & Crespi, E. J. (2010). Diet and hormonal manipulation reveal cryptic genetic variation: Implications for the evolution of novel feeding strategies. *Proceedings of the Royal Society B*, 277, 3569–3578.
- Ledón-Rettig, C. C., Pfennig, D. W., & Crespi, E. J. (2009). Stress hormones and the fitness consequences associated with the transition to a novel diet in larval amphibians. *Journal of Experimental Biology*, 212, 3743–3750.
- Maglia, A. M., & Pugener, L. A. (1998). Skeletal development and adult osteology of *Bombina orientalis* (Anura: Bombinatoridae). *Herpetologica*, 54, 344–363.
- Maglia, A. M., Pugener, L. A., & Mueller, J. M. (2007). Skeletal morphology and postmetamorphic ontogeny of *Acris crepitans* (Anura: Hylidae): A case of miniaturization in frogs. *Journal of Morphology*, 268, 194–223.
- Nieuwkoop, P. D., & Faber, J. (1956). *Normal table of Xenopus laevis (Daudin)*. Amsterdam: North Holland Publ. Co.
- Nishikawa, K. C. (1987). Staining peripheral nerves with Sudan Black B: Progressive vs. regressive methods. *Copeia*, 1987, 489–491.
- O'Reilly, J. C., Deban, S. M., & Nishikawa, K. C. (2002). Derived life history characteristics constrain the evolution of aquatic feeding behaviour in adult amphibians. In P. Aerts, K. D'auot, A. Herrel, & R. Van Damme (Eds.), *Topics in functional and ecological vertebrate morphology* (pp. 153–190). Maastricht: Shaker Publishing.
- Ogielska, M., & Kotusz, A. (2004). Pattern of ovary differentiation with reference to somatic development in anuran amphibians. *Journal of Morphology*, 259, 41–54.
- Orton, G. I. (1953). The systematics of vertebrate larvae. *Systematic Zoology*, 2, 63–75.
- Orton, G. I. (1957). The bearing of larval evolution on some problems in frog classification. *Systematic Zoology*, 6, 79–86.
- Perotti, M. G. (2001). Skeletal development of *Leptodactylus chaquensis* (Anura: Leptodactylidae). *Herpetologica*, 57, 318–335.
- Pfennig, D. W., Hudson, K. R., & Sherman, P. W. (1993). Kin recognition and cannibalism in spadefoot toad tadpoles. *Animal Behavior*, 46, 87–94.
- Ponssa, M. L. (2008). Cladistic analysis and osteological descriptions of the frog species in the *Leptodactylus fuscus* species group (Anura, Leptodactylidae). *Journal of Zoological Systematics and Evolutionary Research*, 46, 249–266.
- Příkryl, T., Aerts, P., Havelková, P., Herrel, A., & Roček, Z. (2009). Pelvic and thigh musculature in frogs (Anura) and origin of anuran jumping locomotion. *Journal of Anatomy*, 214, 100–139.
- Pugener, L. A., & Maglia, A. M. (1997). Osteology and skeletal development of *Discoglossus sardus* (Anura: Discoglossidae). *Journal of Morphology*, 233, 267–286.
- Pugener, L. A., & Maglia, A. M. (2007). Skeletal morphology and development of the olfactory region of *Spea* (Anura: Scaphiopodidae). *Journal of Anatomy*, 211, 754–768.
- Pugener, L. A., & Maglia, A. M. (2009). Developmental evolution of the anuran sacro-urostylic complex. *South American Journal of Herpetology*, 4, 193–209.
- Quinzio, S. I., Fabrezi, M., & Faivovich, J. (2006). Redescription of the tadpole of *Chacophrys pierottii* (Vellard, 1948) (Anura: Ceratophryidae). *South American Journal of Herpetology*, 1, 202–209.
- Roček, Z., Böttcher, R., & Wassersug, R. J. (2006). Gigantism in the tadpoles of the Neogene frog *Paleobatrachus*. *Paleobiology*, 32(4), 666–675.
- Roelants, K., Haas, A., & Bossuyt, F. (2011). Anuran radiations and the evolution of tadpole morphospace. *PNAS*, 108, 8731–8736.
- Rot-Nikčević, I., & Wassersug, R. J. (2004). Arrested development in *Xenopus laevis* tadpoles: How size constrains metamorphosis. *Journal of Experimental Biology*, 207, 1133–1145.
- Ruibal, R. R., & Thomas, E. (1988). The obligate carnivorous larvae of the frog *Lepidobatrachus laevis* (Leptodactylidae). *Copeia*, 1988, 591–604.

- Schoch, R. R., & Fröbisch, (2006). Metamorphosis and neoteny: Alternative pathways in an extinct amphibian clade. *Evolution*, *60*, 1467–1475.
- Schreiber, A. M., Das, B., Huang, H., Marsh-Armstrong, N., & Brown, D. D. (2001). Diverse developmental programs of *Xenopus laevis* metamorphosis are inhibited by a dominant negative thyroid hormone receptor. *PNAS*, *98*, 10739–10744.
- Sheil, C. A., & Alamillo, H. (2005). Osteology and skeletal development of *Phyllomedusa vaillanti* (Anura: Hylidae: Phyllomedusinae) and a comparison of this arboreal species with a terrestrial member of the genus. *Journal of Morphology*, *265*, 343–368.
- Smith, K. (2001). Heterochrony revisited: The evolution of developmental sequences. *Biological Journal for the Linnean Society*, *73*, 169–186.
- Smith, K. (2002). Sequence heterochrony and the evolution of development. *Journal of Morphology*, *252*, 82–97.
- Sokol, O. (1975). The phylogeny of anuran larvae: A new look. *Copeia*, *1975*, 1–23.
- Solano, H. (1987). Algunos aspectos de la biología reproductiva del sapito silabador *Leptodactylus fuscus* (Schneider) (Amphibia: Leptodactylidae). *Amphibia-Reptilia*, *8*, 111–128.
- Stolow, M. A., Ishizuya-Oka, A., Su, Y., & Shi, Y.-B. (1997). Gene regulation by thyroid hormone during amphibian metamorphosis: Implications on the role of cell–cell and cell–extracellular matrix interactions. *American Zoologist*, *37*, 195–207.
- Tamakoshi, T., Oofusa, K., & Yoshizato, K. (1998). Visualization of the initiation and sequential expansion of the metamorphic conversion of anuran larval skin into the precursor of adult type. *Development Growth and Differentiation*, *40*, 105–112.
- Taylor, A. C., & Kollros, (1946). Stages in the normal development of *Rana pipiens* larvae. *Anatomical Record*, *94*, 7–23.
- Trueb, L. (1994). Patterns of cranial diversity among Lissamphibia. In J. Hanken & B. K. Hall (Eds.), *The skull, Vol. 2: Patterns of structural and systematic diversity* (pp. 255–343). Chicago: The University of Chicago Press.
- Trueb, L., & Alberch, P. (1985). Miniaturization and the anuran skull: A case study of heterochrony. In H.-R. Duncker & G. Fleisher (Eds.), *Vertebrate morphology* (pp. 113–121). New York: Gustav Fischer Verlag.
- Ulloa Kreisel, Z. E. (2002). Características morfológicas del tubo digestivo en larvas carnívoras de *Lepidobatrachus laevis* (Anura: Leptodactylidae). *Acta Zoologica Lilloana*, *46*, 31–38.
- Wassersug, R. J. (1976). A procedure for differential staining of cartilage and bone in whole formalin fixed vertebrates. *Stain Technology*, *51*, 131–134.
- Wassersug, R. J., & Heyer, W. R. (1988). A survey of internal oral features of Leptodactyloid larvae (Amphibia: Anura). *Smithsonian Contributions to Zoology*, *457*, 1–96.
- Wassersug, R. J., & Hoff, K. (1982). Developmental changes in the orientation of the anuran jaw suspension: A preliminary exploration into the evolution of anuran metamorphosis. *Evolutionary Biology*, *15*, 223–246.
- Wassersug, R. J., & Sperry, D. G. (1977). The relationships of locomotion to differential predation on *Pseudacris triseriata* (Anura, Hylidae). *Ecology*, *58*, 830–839.
- Wiens, J. J. (1989). Ontogeny of the skeleton of *Spea bombifrons* (Anura: Pelobatidae). *Journal of Morphology*, *202*, 29–51.
- Wilbur, H. M., & Collins, J. P. (1973). Ecological aspects of amphibian metamorphosis. *Science*, *182*, 1305–1314.
- Wild, E. R. (1997). Description of the adult skeleton and developmental osteology of the hyperossified horned frog, *Ceratophrys cornuta* (Anura: Leptodactylidae). *Journal of Morphology*, *232*, 169–206.
- Wild, E. R. (1999). Description of the chondrocranium and osteogenesis of the chacoan burrowing frog, *Chacophrys pierottii* (Anura: Leptodactylidae). *Journal of Morphology*, *242*, 229–246.
- Yeh, J. (2002). The effect of miniaturized body size on skeletal morphology in frogs. *Evolution*, *56*, 2628–2641.
- Yoshizato, K. (1992). Death and transformation of larval cells during the metamorphosis of Anura. *Development Growth and Differentiation*, *34*, 607–612.