

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/259054365>

Cranial Muscle Development in Frogs with Different Developmental Modes: Direct Development versus Biphasic Development

Article in *Journal of Morphology* · April 2014

DOI: 10.1002/jmor.20223

CITATIONS

36

READS

1,061

2 authors:



Janine M. Ziermann

Howard University

116 PUBLICATIONS 877 CITATIONS

[SEE PROFILE](#)



Rui Diogo

Howard University

351 PUBLICATIONS 4,723 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Morphological variations of the lower limb in human fetuses. [View project](#)



Diversity, Diversification and Evolutionary History of the Electric Eel [View project](#)

Cranial Muscle Development in Frogs with Different Developmental Modes: Direct Development versus Biphasic Development

Janine M. Ziermann* and Rui Diogo

Department of Anatomy, Howard University College of Medicine, 520 W St. NW, Washington, District of Columbia 20059

ABSTRACT Normal development in anurans includes a free swimming larva that goes through metamorphosis to develop into the adult frog. We have investigated cranial muscle development and adult cranial muscle morphology in three different anuran species. *Xenopus laevis* is obligate aquatic throughout lifetime, *Rana (Lithobates) pipiens* has an aquatic larvae and a terrestrial adult form, and *Eleutherodactylus coqui* has direct developing juveniles that hatch from eggs deposited on leaves (terrestrial). The adult morphology shows hardly any differences between the investigated species. Cranial muscle development of *E. coqui* shows many similarities and only few differences to the development of *Rana (Lithobates)* and *Xenopus*. The differences are missing muscles of the branchial arches (which disappear during metamorphosis of biphasic anurans) and a few heterochronic changes. The development of the mandibular arch (adductor mandibulae) and hyoid arch (depressor mandibulae) muscles is similar to that observed in *Xenopus* and *Rana (Lithobates)*, although the first appearance of these muscles displays a mid-metamorphic pattern in *E. coqui*. We show that the mix of characters observed in *E. coqui* indicates that the larval stage is not completely lost even without a free swimming larval stage. Cryptic metamorphosis is the process in which morphological changes in the larva/embryo take place that are not as obvious as in normal metamorphosing anurans with a clear biphasic lifestyle. During cryptic metamorphosis, a normal adult frog develops, indicating that the majority of developmental mechanisms towards the functional adult cranial muscles are preserved. *J. Morphol.* 000:000–000, 2013. © 2013 Wiley Periodicals, Inc.

KEY WORDS: direct development; evolution; cranial muscles; amphibian; anura

INTRODUCTION

Most amphibians have a biphasic life cycle characterized by a free swimming larva and a terrestrial adult. This represents the ancestral mode for extant species (Duellman and Trueb, 1994; Hanken et al., 1997a). The complex process of metamorphosis connects the phases (Duellmann and Trueb, 1994; Hanken, 1999; Denver et al., 2009; Shi, 2013). Metamorphosis is characterized by massive transformations (e.g., Denver et al., 2002;

Brown and Cai, 2007; Heimeier and Shi, 2010). The loss and remodeling of many larval features and the development of new components lead to the adult configuration (e.g., Alley and Omerza, 1999; Shi, 2013). The whole process is under neuroendocrine control from pituitary, adrenocortical and thyroid hormones (Kikuyama et al., 1993; Tata, 2006; Denver et al., 2002, 2009; Denver, 2013).

The most extreme change in the ancestral biphasic developmental mode in amphibians is the evolution of direct development. Direct development is not only marked by the lack of a free swimming larva, but also by an early development of adult structures like limbs and the reduced appearance or lack of larval characters such as horny mouthparts, operculum, gills, gill slits, and coiled intestine (e.g., Townsend and Stewart, 1985; Elinson, 2013 and citations within). The appearance of direct developing species in all orders of amphibians led to the conclusion that the larval stage might be an “insertion” into the life history, during the evolution of these tetrapods (e.g., Callery et al., 2001; Fig. 1). However, this topic is still controversially discussed. Besides the decreased requirement for aquatic breeding sites, direct development also tends to negate larval constraints on adult morphology (Schlosser and Roth, 1997b; Meza-Joya et al., 2013). However, it was also argued that tadpoles and adults are tightly connected via a variable ontogeny and that

JMZ: staining *E. coqui*; investigating development; dissection of all adults; main writer; RD: discussion, manuscript.

Contract grant sponsor: Howard University College of Medicine (Rui Diogo's start-up package).

*Correspondence to: Janine M. Ziermann; Department of Anatomy, Howard University College of Medicine, 520 W St. NW, Room 1111, Washington, DC 20059. E-mail: jziermann@yahoo.de

Received 17 June 2013; Revised 30 September 2013; Accepted 3 October 2013.

Published online 00 Month 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jmor.20223

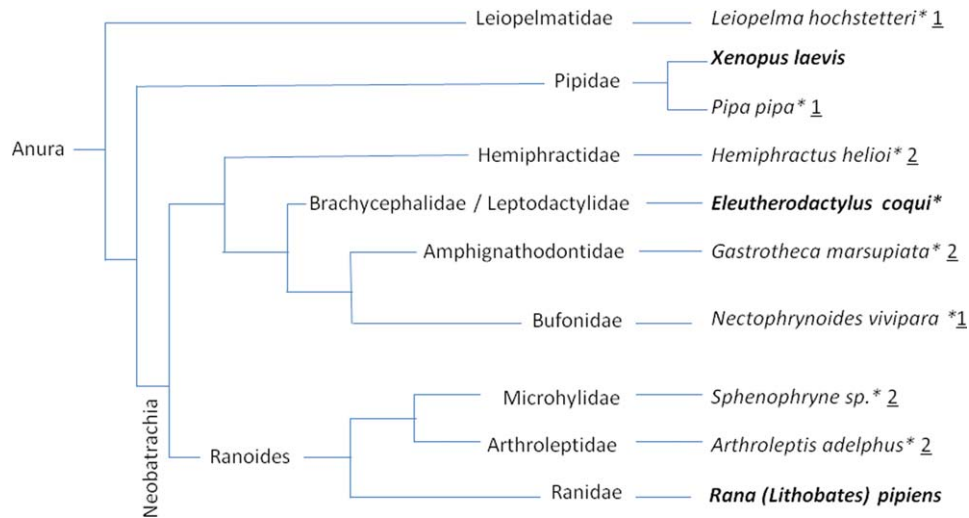


Fig. 1. Simplified cladogram (modified from Frost et al., 2006) showing species investigated here (bold) and the occurrence of direct developing frogs (*) in several anuran families (1 = Kerney et al., 2007; 2 = Frost et al., 2006; Anura, 2013).

without the process of metamorphosis the outcome in adult morphology is constrained (Altig, 2006).

The leopard frog, *Rana (Lithobates) pipiens* (Ranidae) and the African clawed frog, *Xenopus laevis* (Pipidae) undergo metamorphosis with the former being terrestrial and the latter being aquatic, as adults (Fig. 1). The morphology of several *Rana (Lithobates)* species (Ecker, 1864; Edgeworth, 1935; de Jongh, 1968) and of *X. laevis* is well investigated (e.g., Paterson, 1939; Weisz 1945a, b; Sedra and Michael, 1957; Ziermann and Olsson, 2007a,b), and both are often used as model species for anurans or even amphibians. The changes of muscle fibers during metamorphosis were investigated in *Rana (Lithobates) pipiens* (e.g., adductor jaw muscles: Alley, 1989; Alley and Omerza, 1999) and in *Xenopus* (e.g., dorsal body muscles: Chanoine and Hardy, 2003).

The Puerto Rican direct-developing frog *Eleutherodactylus coqui* (Leptodactylidae) is an intensively studied direct developing frog (Fig. 1; Desnitskiy, 2004; Elinson, 2013). It has internal fertilization and the male broods the eggs on land (Townsend et al., 1981, 1984; Elinson, 1987). The eggs of *E. coqui* are about 3.5 mm (Elinson et al., 1990) and have a 20× higher volume than the ones from *Xenopus laevis* (Buchholz et al., 2007). Both, the increase in egg size due to yolk (del Pino and Elinson, 1983; Elinson and del Pino, 1985), and the direct development are major changes of terrestrial breeding frogs (Elinson, 1987). In the end, a fully developed and independent juvenile hatches from the jelly capsule, with a short tail that is lost soon after hatching.

Due to its special development, *E. coqui* has been the subject of numerous studies, including investigations of cranial nerve and some muscles

(Schlosser and Roth, 1997b; Schlosser, 2003), neural crest cell migration (Olsson et al., 2002), mesoderm formation (Ninomiya et al., 2001), thyroid gland development (Jennings and Hanken, 1998), cranial ontogeny (Hanken et al., 1992; Kerney et al., 2010), jaw muscle development (Hanken et al., 1997b) and other aspects (e.g., Fang and Elinson, 1996, 1999; Elinson and Fang, 1998; Callery and Elinson, 2000a,b; Elinson, 2007, 2013). Hanken and colleagues (1992) described the development of the cranium in *E. coqui*. Several cartilages that are typically present in anuran larvae, such as the suprarostrale and cornua trabeculae, are absent and many regions of the skull appear in a postmetamorphic morphology during ontogeny. The lower jaw (Meckel's cartilage), palatoquadrate and hyobranchial skeleton develop from a midmetamorphic configuration as can be found in metamorphosing frogs such as *Bombina orientalis* (Hanken et al., 1992). These structures are remodeled at later ontogenetic stages.

Here, we present the first detailed description of the cranial muscle development in the direct developing frog *E. coqui* and compare the patterns with those found in *X. laevis* and *R. pipiens*, both with a free swimming larval stage. Comparison of those transformations in the head in frogs with alternative developmental modes gives insight into the evolution of complex morphological structures (e.g., Wake and Hanken, 1996). We compare the development and morphology of the cranial muscles in these frogs and review the literature in order to find out how striking the differences are between them and to provide insights into the evolution and developmental constraints of cranial muscles in anurans.

MATERIAL AND METHODS

Larvae and adults from *Eleutherodactylus coqui*, Thomas, 1966, *Xenopus laevis* (Daudin, 1802), and *Rana (Lithobates) pipiens* (Schreber, 1782), are from Richard Elinson's lab (Elinson, Pittsburgh, Duquesne University). Additional adults of *R. pipiens* were sent by Connecticut Valley Biological. All larval stainings were done at Richard Elinson's Lab, while observation of the development and the dissection of the adults were done in Rui Diogo's lab. Larvae were studied using a microscope (Nikon AZ100, Nikon Instruments Inc.) and photographed with an attached camera (Nikon DS-Fi1; software NIS-Elements D4.00.03, NIS-Elements D4.00.03). We investigated 20 embryos and larvae of *R. pipiens* in the stages S16 to S25 (staging after Shumway, 1940), 26 embryos/larvae of *X. laevis* in the stages NF33/34 to NF49 (staging after Nieuwkoop and Faber, 1967), and 20 embryos/hatchlings of *E. coqui* in the stages TS5 to TS15 to posthatch (staging after Townsend and Stewart, 1985).

For the immunohistochemistry we followed for half of the investigated *E. coqui* the method as described by Elinson and Fang (1998). The embryos were fixed in Dent's fixative (80% methanol and 20% DMSO—dimethyl sulfoxide; Dent et al., 1989), and stored at -20°C . The larvae's pigment was bleached with 10% H_2O_2 in 67% Dent's for 1–3 days when necessary. The larvae were stained for muscle with 12/101 antibody (monoclonal, Hybridoma Bank; diluted 1:10 in 10 mg/ml BSA, 0.1% Triton X-100 in PBS) (Kintner and Brockes, 1985). The primary antibody was visualized using a HRP-labeled goat anti-mouse secondary antibody (HRP—horse radish peroxidase). All other embryos/larvae investigated here were stained in Richard Elinson's Lab as described above.

Adults were anesthetized in 1% MS222 and the larvae in 0.1% MS222. When no retraction reflex could be observed after pinching the foot of the adult frogs, they were frozen. The dissection of the adults was done at low magnification with a dissecting microscope (Nikon SMZ-2B). The specimens were photographed in several steps during the dissection using a Nikon digital camera (D90). The terminology of adult muscle mainly follows Diogo and Abdala (2010). This terminology takes into account information about the homologies, evolution and development of the cephalic muscles in representatives of all major groups of vertebrates, including fish. The more specific names of the anuran larval muscles followed Haas (2001, 2003) if not stated otherwise. A list of commonly used synonyms for the muscle terminology followed here is given in Table 1. This table also relates larval and adult muscles and their terminology as used here to other studies on cranial muscles in anurans. In order to be still comparable with developmental studies undertaken by other authors we decided to keep the commonly used larval cranial muscle terms in this table while using Diogo and Abdala's (2010) terminology for the adult cranial muscles, which will be used in further investigations and discussions of cranial muscle evolution in vertebrates.

RESULTS

Development

Xenopus cranial muscle development has been previously described in some detail (Sedra and Michael, 1957; Nieuwkoop and Faber, 1967; Weisz, 1945a,b; Ziermann and Olsson, 2007a). Here, we give a summary of the developmental pattern of all investigated species, including *X. laevis*, since all were treated with the same method and we can therefore exclude differences caused by the methodology. The muscles present in each species during development and in the adult frogs are shown in Table 2.

***Xenopus laevis*.** The first muscles to develop are members of the mandibular and hyoid arch.

The anlagen of the m. levator mandibulae longus and the lateral (orbitohyoideus, Quadratohyoangularis) and ventral (interhyoideus) hyoid muscles can be distinguished by stage NF36 (Figs. 2A, 3A). The m. sternohyoideus is visible from the earliest stage onwards (Fig. 2A–G). In stage NF40 the first signs of the mm. levatores arcuum branchialium (lev. arc. br.) are visible and the m. geniohyoideus starts its development from its origin towards its insertion onto the lower jaw (Figs. 2B, 3B–D). In NF41 the m. dilatator laryngis can be seen from its origin close to the posterior end of the m. lev. arc. br. IV turning ventro-medially (Fig. 2C). The mm. constrictores branchialium are also visible from this stage onwards (Fig. 2C–E). The m. lev. arc. br. I–IV form a continuous muscle band caudal to the eye from NF43 to NF47 (Fig. 2D, E). The m. interhyoideus develops from its origin to insert in a median raphe with its contralateral muscle (NF43; Fig. 2D). In NF45 the m. protractor pectoralis can be distinguished from the posterior fibers of the m. lev. arc. br. IV (Fig. 2D). The m. intermandibularis posterior develops from its origin at Meckel's cartilage towards its insertion with the contralateral muscle (Fig. 3D). The posterior fibers overlap the anterior fibers of the m. interhyoideus ventrally (Fig. 3D). The m. subarcualis obliquus II is first visible by stage NF45. By stage NF47 the reorganization of the cranial skeleton begins, accompanied by the reduction of the ventral branchial arch muscles (constrictores branchialium, subarcualis obliquus; subarcuales recti I, II–IV). The mm. lev. arc. br. I–IV loosen (Fig. 2F) and are replaced by the single petrohyoideus muscle in adults. The majority of the mandibular and hyoid muscles at this stage are located anterior to the eye. Already by stage NF49 it is obvious that the elongation of the jaw leads to a posterior shift of the origins of the afore mentioned muscle groups (Fig. 2G).

***Rana (Lithobates) pipiens*.** By stage S20 faint anlagen of the mandibular arch muscles ventral of the eye are visible (Fig. 4A). Shortly thereafter the anlage is elongated and the faint hyoid anlage posterior to it appears (S21; Fig. 4B). In stage S21.5 the levatores mandibulae longus et internus, the m. orbitohyoideus, the m. interhyoideus and two mm. constrictores branchialium (II+III) can be distinguished (Fig. 4C). The m. interhyoideus develops from its origin at the ceratohyal towards its insertion with its contralateral muscle (Fig. 5B–E). The m. geniohyoideus and the m. mandibulolabialis are first visible late in S22 (Fig. 5C). The former muscle develops from its origin towards its insertion at the lower jaw (Fig. 5F). The m. levator mandibulae externus is visible from S22.5 onwards (Fig. 4D). The m. orbitohyoideus is well developed and the m. suspensoriohyoideus can be identified at the posterior border of this muscle (Fig. 4D). The m. angularis muscles develop medial to the orbitohyoideus (Fig. 4D). All

TABLE 1. *Synonyms*

Terminology used here		Synonyms
Larval muscles	Adult muscles	
Mandibular arch muscles		
Intermandibularis anterior	Intermandibularis anterior	submentalis (Ecker, 1864; Iordansky, 1992; Haas, 2001; Carroll, 2007)
Intermandibularis posterior	Intermandibularis posterior	intermandibularis (Iordansky, 1992; Haas, 2001)
Lev. mand. externus	Adductor mandibulae A2	m. add. mand. externus + posterior (subexternus) (Luther, 1914; Carroll and Holmes, 1980); lev. mand. externus + anterior subexternus (Edgeworth, 1935); levator mandibulae externus (Sedra and Michael, 1957; Schlosser and Roth, 1997b); lev. mand. posterior externus + subexternus (Duellman and Trueb, 1994); add. mand. externus (Iordansky, 1996; Johnston, 2011); lev. mand. externus superficialis + externus profundus (Haas, 2001); lev. mand. externus (Ziermann and Olsson, 2007a); add. mand. A2 subexternus (Diogo and Abdala, 2010)
Lev. mand. articularis	Add. mand. A2-PVM	add. mand. anterior/posterior articularis (Luther, 1914); lev. mand. anterior articularis (Edgeworth, 1935); m. lev. mand. posterior (Sedra and Michael, 1957); add. mand. posterior articularis (Carroll and Holmes, 1980); lev. mand. posterior articularis (Duellman and Trueb, 1994); add. mand. posterior (Iordansky, 1996; Johnston, 2011)
Lev. mand. lateralis	Add. mand. A2 lateralis	add. mand. posterior lateralis (Luther, 1914; Carroll and Holmes, 1980); lev. mand. anterior lateralis (Edgeworth, 1935); lev. mand. posterior lateralis (Duellman and Trueb, 1994); add. mand. lateralis (Iordansky, 1996; Johnston, 2011); lev. mand. anterior lateralis (Schlosser and Roth, 1997b); add. mand. A2 externus (Diogo and Abdala, 2010)
Lev. mand. longus (sup. + prof.)	Adductor mandibulae A3' (superficial PSE)	temporalis (Gaupp, 1896); add. mand. posterior longus (sup. + prof.) (Luther, 1914; Carroll and Holmes, 1980); lev. mand. posterior (superficialis + profundus) (Edgeworth, 1935); lev. mand. anterior (pars lateralis + intermedia) (Sedra and Michael, 1957); lev. mand. posterior longus (Duellman and Trueb, 1994); add. mand. temporalis (Iordansky, 1996); add. mand. A2 longus (Diogo and Abdala, 2010); add. mand. longus (Johnston, 2011)
Lev. mand. internus	Adductor mandibulae A3'' (deep PSE)	pteryogoideus (Gaupp, 1896; Luther, 1914); lev. mand. anterior (Edgeworth, 1935); M. levator mandibulae anterior, pars medialis (Sedra and Michael 1957); add. mand. internus (Carroll and Holmes, 1980; Johnston, 2011); lev. mand. anterior longus (Duellman and Trueb, 1994); pseudotemporalis (Iordansky, 1996; Diogo and Abdala, 2010)
Hyoid arch muscles		
Orbitohyoideus	Depressor mandibulae	levator hyoideus (Paterson, 1939) depressor maxillae (Ecker, 1864)
Suspensoriohyoideus		levator hyoideus (Edgeworth, 1930)
Branchial arch muscles		
Lev. arc. br. I-IV	Petrohyoidei I-IV	constrictores arcuum branchialium (Sedra and Michael, 1957); petrohyoideus anterior (=I) + posterior (=II-IV) (Ecker, 1964)
Protractor pectoralis	Protractor pectoralis	cuticularis (Edgeworth, 1935; Schlosser and Roth, 1997b)
Hypobranchial muscles		
Sternohyoideus	Sternohyoideus	rectus cervicis (Ziermann and Olsson, 2007a)
–	Genioglossus	genioglossus basalis + medialis (Duellman and Trueb, 1994)

four mm. levatores arcuum branchialium develop with a clear gap between them (Fig. 4D, F). By stage S24 the ventral branchial arch muscles are developed: mm. subarcuales recti I, II-IV, m. subarcualis obliquus II. However, the muscles are not strongly stained until the next stage (Fig. 5G). During S24 the angularis muscles become more clear and can be divided into m. hyoangularis, m. suspensorio angularis and m. quadrato angularis (Fig. 5E). The m. intermandibularis posterior runs from its origin at Meckel's cartilage towards the midline (Fig. 5E), where it will connect with the

contralateral muscle by S25 (Fig. 5F). There is a clear gap between m. intermandibularis and m. interhyoideus. By S25 the protractor pectoralis splits from the m. lev. arc. br. IV (Fig. 5F) and the m. dilatator and constrictor laryngis are faintly stained (Fig. 5F).

Eleutherodactylus coqui. Small external gills are visible from TS5 onwards (Fig. 6A), but they disappear around stage TS9. The first muscles to be visible are the mandibular arch levators (m. adductor mandibulae A3 = levator mandibulae longus + internus), the hyoid arch

TABLE 2. Muscles in the anurans *Xenopus laevis*, *Rana pipiens*, *Eleutherodactylus coqui*, larval and adult

Terminology used here		<i>X. laevis</i>		<i>R. pipiens</i>		<i>E. coqui</i>	
Larval muscles	Adult muscles	larval	adult	larval	adult	embryo to TS9	adult from TS10
Mandibular arch muscles							
Intermandibularis anterior	Intermandibularis anterior	–	p	–	p	p	p
Intermandibularis posterior	Intermandibularis posterior	p	p	p	p	p	p
Lev. mand. externus	Adductor mandibulae A2	p	p	p	p	p	p
Lev. mand. articularis	Add. mand. A2-PVM	p	p	p	p	p	p
Lev. mand. lateralis	Add. mand. A2 lateralis	–	p	–	p	p	p
Lev. mand. longus (sup. + prof.)	Adductor mandibulae A3' (superficial PSE)	p(2)	p	P(2)	p	p	p
Lev. mand. internus	Adductor mandibulae A3'' (deep PSE)	p	p	p	p	p	p
Hyoid arch muscles							
Interhyoideus anterior	Interhyoideus anterior	p	p	p	p	p	p
Orbitohyoideus	Depressor mandibulae	–	p	p	p	p	p
Suspensoriohyoideus		–	–	p	–	p	–
Quadratoangularis		p	–	p	–	p	–
Hyoangularis		–	–	p	–	p	–
Suspensorioangularis		–	–	p	–	–	–
Branchial arch muscles							
<i>Sensu stricto</i>							
Lev. arc. br. I-IV	Petrohyoideus	p	p(1)	p	p(4)	–	p(4)
Protractor pectoralis	Protractor pectoralis	p	p	p	p	p	p
–	Interscapularis	–	p	–	p	–	p
Subarcualis rectus I	–	p	–	p	–	–	–
Subarcualis rectus II-IV	–	p	–	p	–	–	–
Subarcualis obliquus II	–	p	–	p	–	–	–
Constrictores branchialium II, III, IV	–	p	–	p	–	–	–
Laryngeal muscles							
Constrictor laryngis	Constrictor laryngis	p	p	p	p	?	p
Dilatators laryngis	Dilatators laryngis	p	p	p	p	?	p
Hypobranchial muscles							
Geniohyoideus	Geniohyoideus (lat + med)	p	p	p	p	p	p
Sternohyoideus	Sternohyoideus	p	p	p	p	p	p
–	Omohyoideus	–	p	–	p	–	p
–	Genioglossus	–	–	–	p	–	p
–	Hyoglossus	–	–	–	p	–	p

Adult includes muscles that develop during metamorphosis. In the case of *E. coqui* that means from TS9 onwards (when the reorganization in the head can be observed). Lev. arc. br. = Levatores arcuum branchialium; Lev. mand. = Levator mandibulae muscle; PVM = postero-ventro-mesial; PSE = Pseudotemporalis; p = present; ? could not be seen. (#) = number of muscles, i.e. Lev. mand. longus (sup. + prof.) – p(2) = both longus sup. and prof. are present, Petrohyoideus – p(4) = petrohyoideus I, II, III, and IV are present.

muscles (with a lateral anlage and a ventral interhyoideus), and the hypobranchial muscles sternohyoideus and geniohyoideus (TS7; Figs. 6A, 7A). All muscles develop postero-ventrally to the eye. By stage TS8 the m. adductor mandibulae A2–PVM (postero-ventro-mesial) becomes visible ventrally to the m. adductor mandibulae A3 (Fig. 6B faint, 6C clear). Also in this stage appear the petrohyoidei muscles (Fig. 6B). The mm. intermandibularis anterior and posterior appear during TS9 (Fig. 7C). They develop from lateral to medial where the m. intermandibularis anterior meets its contralateral muscle in a median raphe (Fig. 7D). The m. intermandibularis posterior seems to have, throughout development, a small gap between its counterparts. The m. geniohyoideus has nearly reached its insertion and starts splitting into a medial and a lateral part (Fig. 7C). The m. pro-

tractor pectoralis is stained just behind the last m. petrohyoideus muscle (Fig. 6C). The rotation of the mandibular adductor starts at TS10 and is caused by an elongation of the lower jaw that moves the insertion sites of the mandibular adductors caudally, while the origins stay more or less fixed. The hyoid arch muscles can be further differentiated into m. suspensoriohyoideus, m. quadratoangularis, and m. hyoangularis by stage TS11. The m. hyoglossus is developed by stage TS11 (Fig. 6D) and the m. adductor mandibulae A2 can be seen faintly, before becoming clearer by stage TS12 (Fig. 6E). The lateral fibers of the m. sternohyoideus give rise to the m. omohyoideus (Fig. 6E, F). The m. petrohyoidei I-IV are covered by other muscles and are not visible anymore from a lateral view (Fig. 6E). During TS13 the m. adductor mandibulae rotates further, while the lower jaw grows

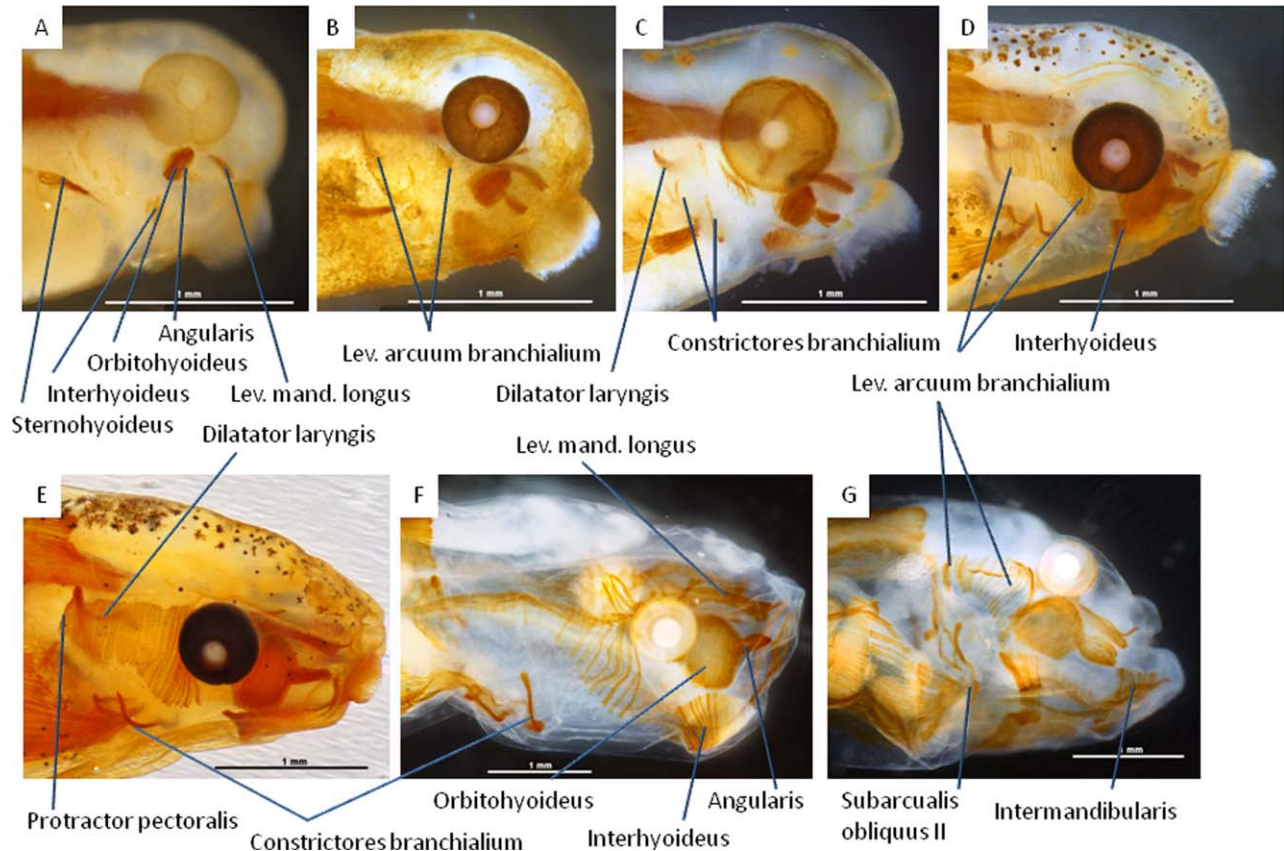


Fig. 2. *Xenopus laevis*, cranial muscle development, lateral view. Muscles stained with 12/101 antibody. A: NF36 (NF = stage after Nieuwkoop and Faber, 1967). B: NF40. C: NF41. D: NF43. E: NF45. F: NF47. G: NF49. Mandibular and hyoid arch muscles start to develop anterior of the eye, but due to the elongation of the jaw they seem to turn ventral around the eye. Angularis = Quadratohyoangularis; Lev. = Levator. Lev. mand. = Levator mandibulae. Scale bar = 1 mm.

(compare Fig. 6E with F, Fig. 7E with F); the m. suspensoriohyoideus fuses with the m. orbitohyoideus posteriorly, while the angularis muscles fuse anteriorly with the m. orbitohyoideus. In adopting the future position of the m. depressor mandibulae, those three muscles together continue to rotate and end up in a right angle to the jaw articulation by stage TS15 (Fig. 6G). The m. protractor pectoralis is covered by the posterior sheet of the m. depressor mandibulae in TS14. The m. adductor mandibulae A2 lateralis is the last adductor muscle to appear in our staining (Fig. 6F). At TS15 most of the muscles have reached their adult orientation. After hatching, the muscles continue growing without further morphological alteration.

Adult Morphology

The cranial muscles of all investigated species, during development and in the adults, are shown in Table 2. Because the adult morphology of *R. pipiens* was described in some detail by Ecker (1864) and the one of *X. laevis* by Sedra and Michael (1957), we focus here mainly on the morphology of *E. coqui* and the comparison with the other two species. The

following descriptions are for all three investigated species if not otherwise stated.

Mandibular and Hyoid arch muscles. The adult adductor mandibulae complex develops from the larval levator mandibulae (Tables 1, 2). In *E. coqui* four adductor mandibulae muscles can be distinguished: the A2, A3'', the A3'+A2-PVM, and A2 lateralis (Fig. 8E, F). The A3'+A2-PVM has developed through the fusion of the deep A2-PVM and the A3'. In *R. pipiens* and *X. laevis* all adductor mandibulae muscles can be separated (Fig. 8B). The mandibular branch of the trigeminal nerve (N. V3) passes between A3'+A2-PVM and A3'' rostrad and turns ventral at about the middle of width of the A3' in all species investigated (Fig. 8F, G). The m. depressor mandibulae is well developed (Fig. 8A, C, E) and has an anterior part where the fibers originate from the annulus tympanicus, a medial part with the fibers originating from the squamosum and a posterior part with fibers from a dorsal fascia (Fig. 9A). The ventral mandibular muscles, that is, mm. intermandibularis anterior and posterior, and the ventral hyoid muscle m. interhyoideus form one thin sheet, with the latter being the most caudal part of it. In

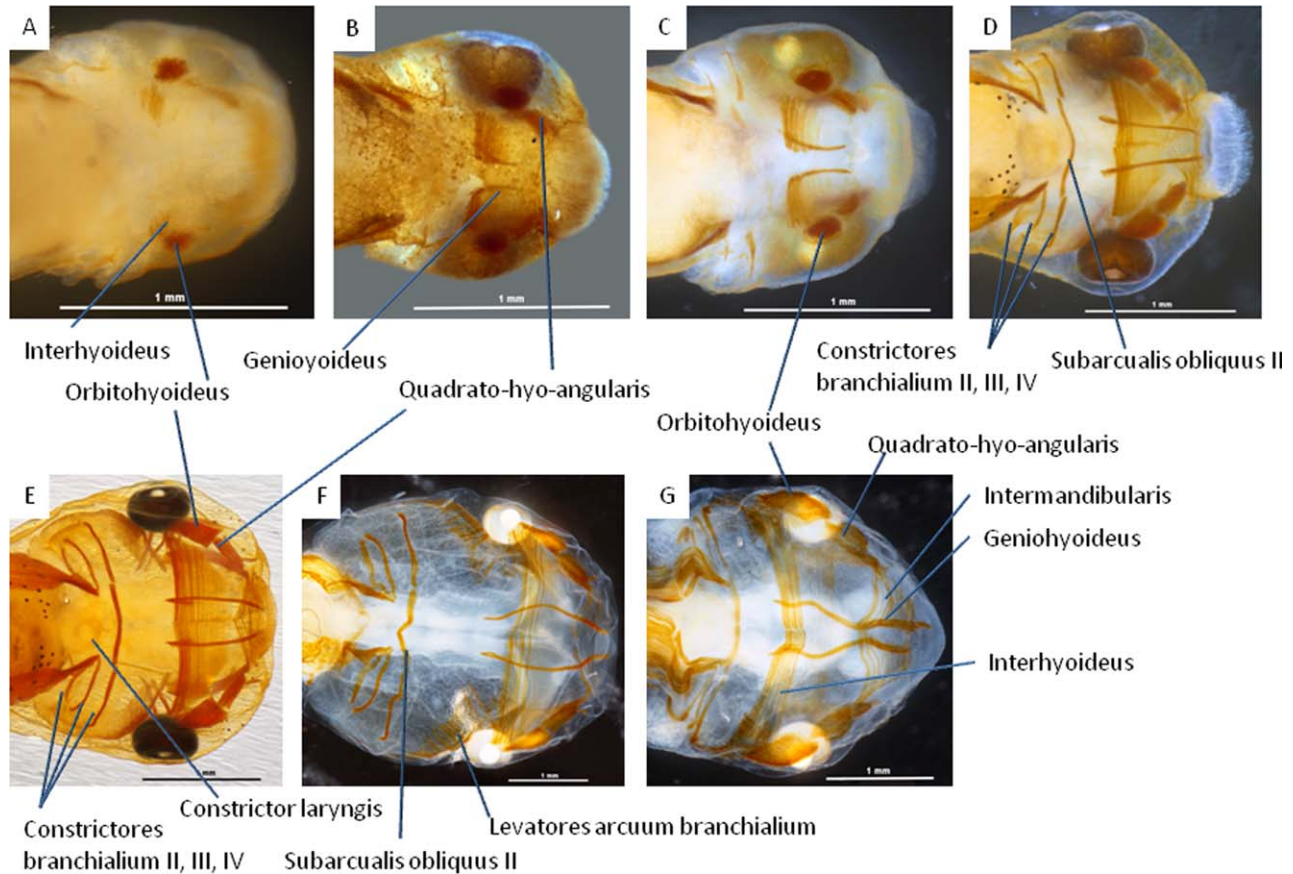


Fig. 3. *Xenopus laevis*, cranial muscle development, ventral view. Muscles stained with 12/101 antibody. A: NF36 (NF = stage after Nieuwkoop and Faber, 1967). B: NF40. C: NF41. D: NF43. E: NF45. F: NF47. G: NF49. Several muscles develop from their origin towards their insertion: e.g., geniohyoideus and interhyoideus (B-D). Scale bar = 1 mm.

R. pipiens the m. interhyoideus shows an unusual flap protruding postero-laterally to the m. depressor mandibulae (Fig. 8C).

Branchial arch muscles. While *R. pipiens* and *E. coqui* have four petrohyoidei muscles, *X. laevis* has only one. The muscles are clearly visible in *R. pipiens* (Fig. 9B), but very thin in *E. coqui*. All originate from the crista parotica of the otic capsule and run steeply ventrad. The m. petrohyoideus I inserts as a flat sheet of ventral fibers at the anterolateral process of the hyoid, the other three muscles insert next to each other from anterior to posterior on the posterior hyoid process. In *Xenopus* the single muscle connects with a flat broad head to its contralateral muscle (Fig. 9C).

The m. protractor pectoralis originates medially from the petrohyoidei muscles at the otic capsule and inserts on the scapula (Fig. 9D). The m. interscapularis connects medially the suprascapula with the scapula. The m. constrictor laryngis surrounds the larynx and consists of two parts that arise from a ventral raphe and a median raphe, respectively, and inserts onto the arytenoid cartilage. The m.

dilatator laryngis inserts laterally to the constrictor laryngis, onto the arytenoid cartilage.

Hypobranchial muscles. The m. geniohyoideus splits into a lateral and a medial portion. *Xenopus laevis* has no tongue and the genioglossus and hyoglossus muscles are absent, while they are both well developed in *R. pipiens* and *E. coqui*. The m. sternohyoideus originates dorsally from the sternum and reaches rostrad to insert onto the hyoid. In its course it lies between the two parts of the m. geniohyoideus. The m. omohyoideus runs from the sternum to the hyoid and attaches laterally to the m. sternohyoideus.

DISCUSSION

We describe for the first time in detail the development of all cranial muscles in *E. coqui* including not only the previously described mandibular and hyoid arch muscles (Hanken et al., 1992b), but also branchial arch muscles sensu stricto as well as laryngeal and hypobranchial muscles. Furthermore, we present developmental data supporting the presence of a cryptic metamorphosis in

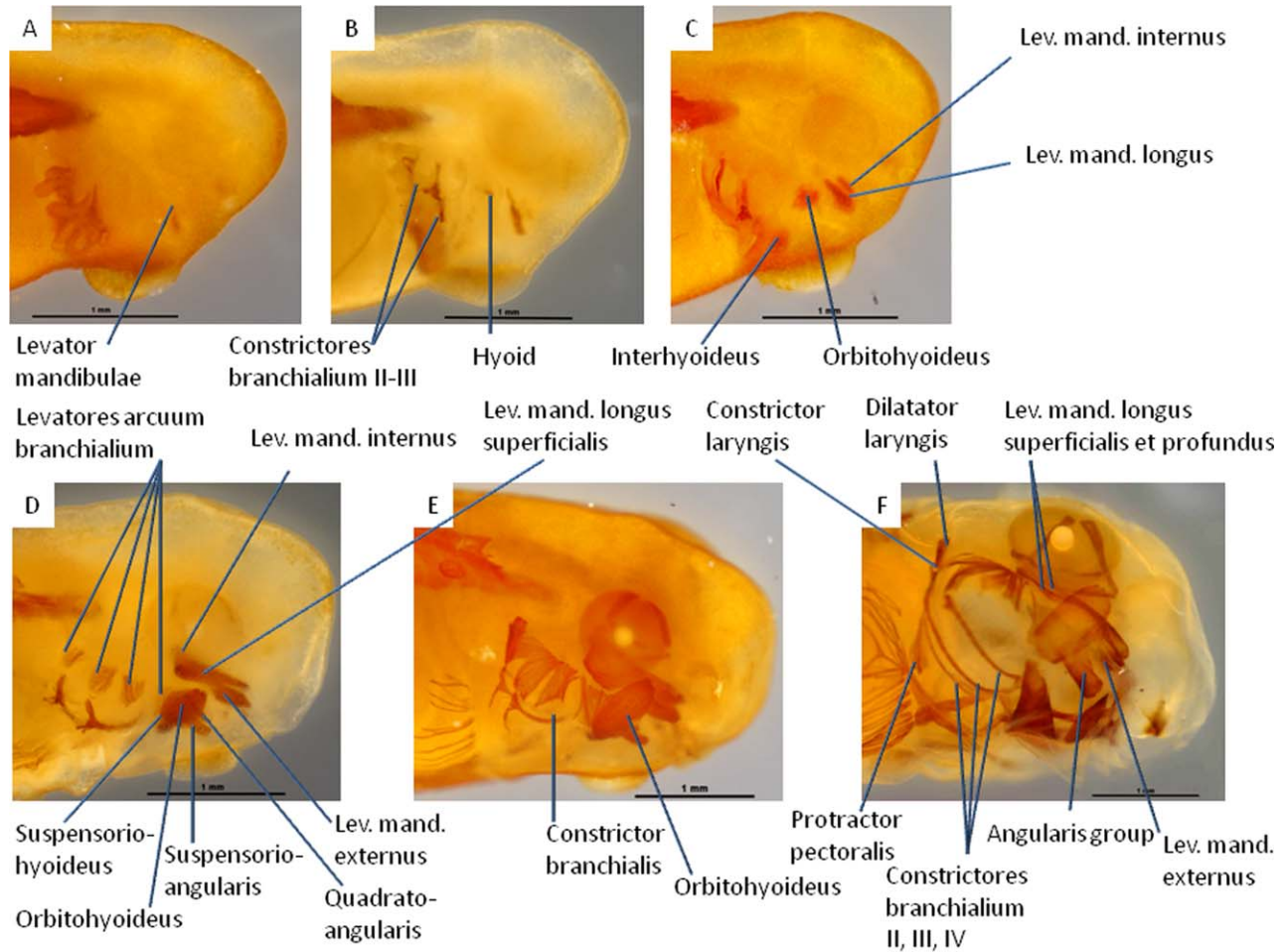


Fig. 4. *Rana pipiens* cranial muscle development, lateral view. Muscles stained with 12/101 antibody. A: S20 (S = stage after Shumway, 1940). B: S21. C: S21.5. D: S22.5. E: S24. F: S25; ventrolateral view. Muscles of the mandibular arch develop first (A), being closely followed by the hyoid arch muscles (B) and the lateral branchial arch muscles (D). Angularis group = suspensorio-, quadrato-, hyo-angularis; Hyoid = Anlage of hyoid arch muscles; Lev. mand. = Levator mandibulae. Scale bar = 1 mm.

E. coqui. As already described for *Ambystoma mexicanum* (salamander; Ziermann and Diogo, 2013), the fusion of muscles leads to a smaller number of muscles after metamorphosis [*Rana (Lithobates)*, *Xenopus*] or during the course of embryonic development (*E. coqui*). This contradicts the commonly accepted view that development results in a differentiation of muscles and thus tends to increase the total number of muscles.

Adult morphology

The dissection of the muscles of the adult frogs did not reveal major differences that could be related to mode of development when comparing the direct developing frog *Eleutherodactylus coqui* with the biphasic frogs *Xenopus laevis* and *Rana (Lithobates) pipiens*. The only major differences we found concern the tongue muscles, which are missing in the tongue-less *Xenopus*, and the presence of a single petrohyoideus muscle in *Xenopus*. *Xeno-*

pus laevis has no tongue and therefore the genio-glossus and hyoglossus muscles are usually absent, while they are both well developed in *R. pipiens* and *E. coqui*. However, it should be noted that Horton (1982) described traces of the m. hyoglossus and m. genioglossus fused to the ventral surface of the floor of the mouth in adults of *Xenopus muelleri* and *X. capensis*. That is, there is seemingly some variability within the form of these muscles in adult *Xenopus*, despite the fact that all adult members of this genus lack a tongue.

There are four petrohyoidei muscles in *R. pipiens* and *E. coqui*, while there is only one in *X. laevis*. They seem to be derived from the branchial arch levators and appear in variable numbers of three to four in anurans (Duellman and Trueb, 1994). Sedra and Michael (1957) stated that the appearance of the single muscle petrohyoideus of *Xenopus* develops from the m. transversus ventralis IV and the m. levator arcuum branchialium IV,

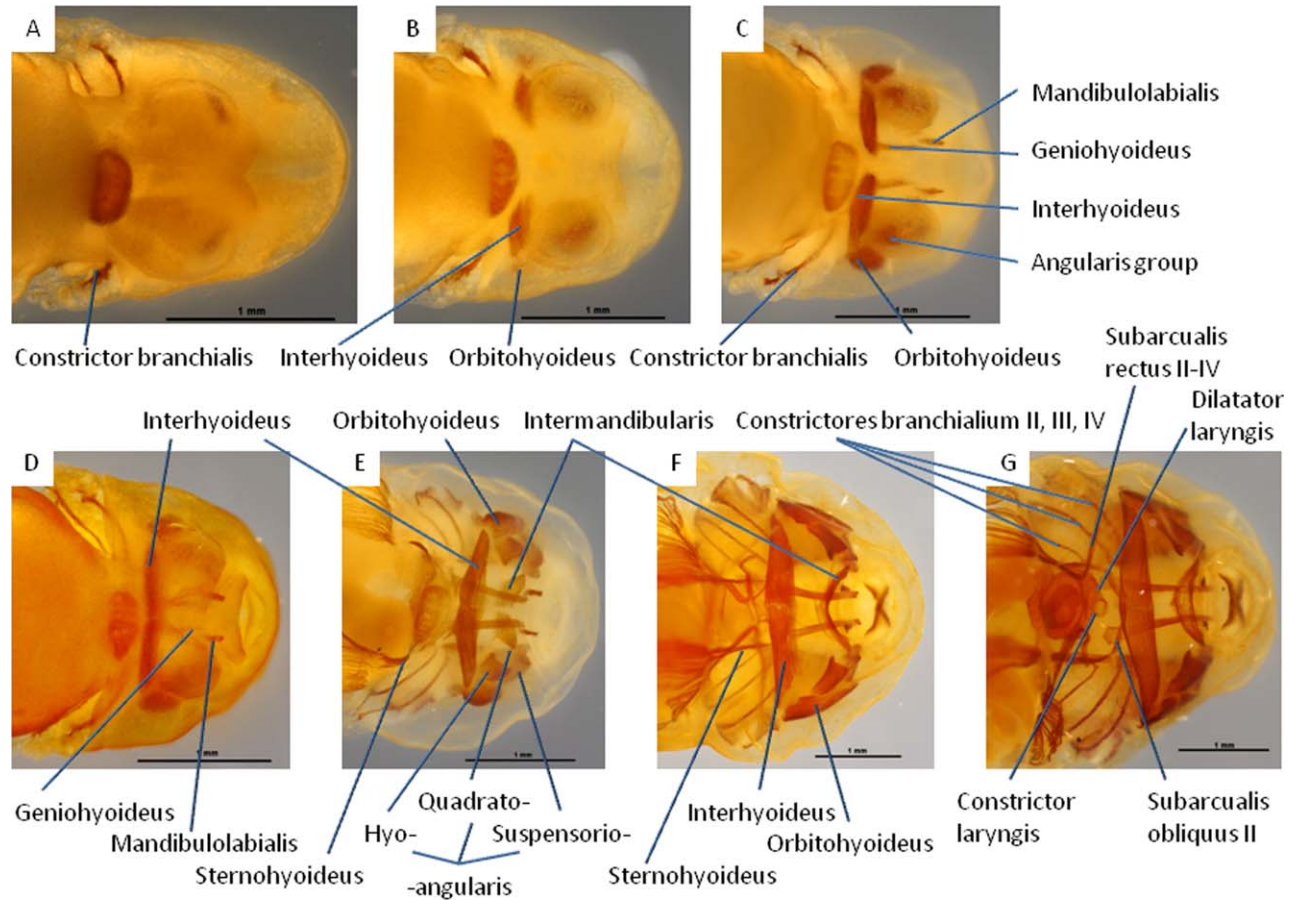


Fig. 5. *Rana pipiens* cranial muscle development, ventral view. Muscles stained with 12/101 antibody. A: S21 (S = stage after Shumway, 1940). B: S22. C: S22. D: S22.5. E: S24. F: S25. G: S25+. Several muscles develop from their origin towards their insertion: e.g., geniohyoideus, intermandibularis and interhyoideus (B-F). Angularis group = suspensorio-, quadrato-, hyo-angularis. Scale bar = 1 mm.

which would indicate that the m. petrohyoideus of *Xenopus* is homologous to the m. petrohyoideus IV of other anurans. However, our observations and comparisons indicate that the single m. petrohyoideus found in *Xenopus* is actually the result of a fusion of the m. petrohyoidei II-IV. This hypothesis is supported, for example, by the broad insertion of the muscle ventrally to the laryngeal region; but further investigations are necessary to test this hypothesis.

Development

The proposed separation of *E. coqui*'s development into two phases corresponds to the observations of the need of thyroid hormone from TS9 onwards for normal development (Callery and Elinson, 2000b; Callery et al., 2001). Thyroid hormone regulates metamorphosis in biphasic frogs (e.g., Tata, 2006; Brown and Cai, 2007). Therefore, TS9 is thought to represent the start of a period that is called "cryptic metamorphosis" (see also Callery and Elinson, 2000b; Callery et al., 2001; Kulkarni et al., 2010; Elinson and del Pino, 2012;

Elinson, 2013). This is supported by our developmental data where during stage TS9 the last larval characters develop, as for example the appearance of the m. intermandibularis anterior and the m. protractor pectoralis (NF45 in *X. laevis*, S25 in *R. pipiens*), followed by the start of the reorganization/reorientation (cryptic metamorphosis) in TS10 (NF47 in *X. laevis*).

Despite skipping a free swimming larval stage, *E. coqui* develops some larval characters (e.g., external gills), although those structures are reduced in their appearance (Desnitskiy, 2004; Elinson, 2013). While the loss of some larval characters is in theory not directly related to functional constraints on the development of adult structures (e.g., Meza-Joya et al., 2013), the retention of other larval characters in adults may reflect phylogenetic and/or functional constraints (Schlosser and Roth, 1997b). Alternatively, it was argued that tadpoles and adults are tightly connected by the process of metamorphosis and without this process the outcome in adult morphology would be constrained (Altig, 2006).

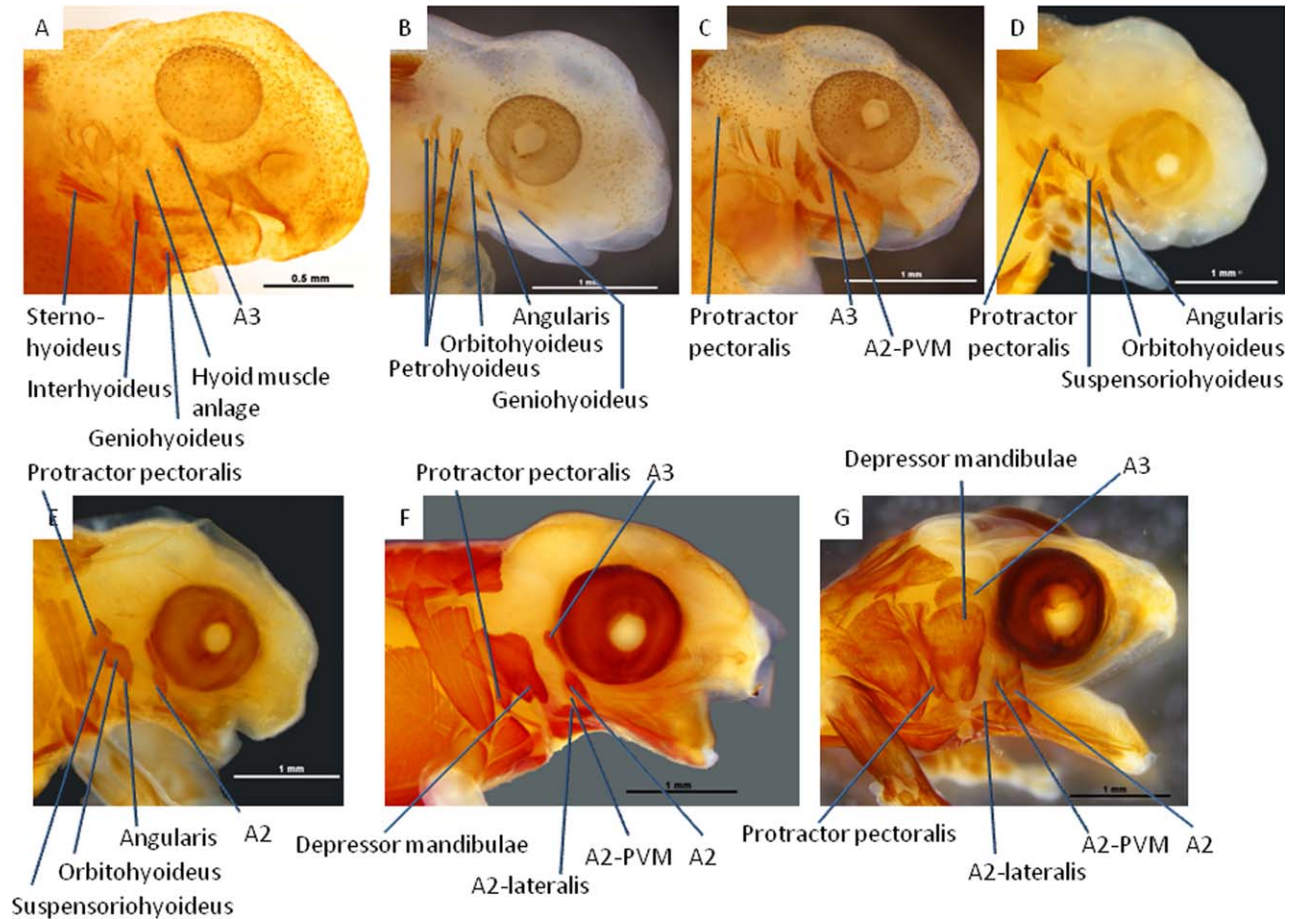


Fig. 6. *Eleutherodactylus coqui* cranial muscle development, lateral view. Muscles stained with 12/101 antibody. A: TS7 (TS = stage after Townsend and Stewart, 1985). B: TS8. C: TS9. D: TS11. E: TS12. F: TS14. G: TS15+Posthatch. All muscles have almost their adult configuration. From TS9 (C) onwards changes can be observed, which are usually seen during metamorphosis in anurans with a free swimming larvae: e.g., rotation of the adductor mandibulae muscles. Angularis group = quadrato- and hyo-angularis. A3 = adductor mandibulae A3 (= PSE = pseudotemporalis = levator mandibulae longus + internus), A2-PVM = adductor mandibulae A2 postero-ventro-mesial. Scale bar = 1 mm.

As shown for the hyoid arch muscles (Hanken et al., 1997b; Fig. 6A, D, F) and for most cranial muscles in *E. coqui*, there are patterns during development that are also typical for metamorphosing frogs. Some muscles develop from their region of origin towards their insertion (e.g., geniohyoideus, intermandibularis, interhyoideus) as is also described for several larval muscles in anurans as *X. laevis* (Ziermann and Olsson, 2007a), *R. pipiens*, and salamanders (Ziermann, 2008; Ziermann and Diogo, 2013). We also observed an outside-in pattern with the lateral muscles developing before the medial ones. Furthermore, the muscles develop in an anterior to posterior wave; in general this means that the mandibular arch muscles develop before all other muscles, followed by hyoid and branchial arch muscles. This was formerly described for other vertebrate taxa (e.g., anurans and caudates: Ziermann, 2008; Ziermann and Diogo, 2013; Australian lungfish: Ericsson,

2003; zebrafish: Schilling and Kimmel, 1997; quail: McClearn and Noden, 1988).

In *E. coqui* this developmental sequence is however not as clear. While the anlagen of mandibular and hyoid arch muscles are indeed visible before that of the branchial arch muscles (mm. petrohyoidei), hypobranchial muscles such as the m. geniohyoideus develop much earlier than they do in the development of other frogs (e.g., Ziermann, 2008). We could not observe the development of the laryngeal muscles, probably due to lack of penetration and/or because these muscles were covered by other muscles.

Callery and Elinson (2000b) indicated that the border between the embryonic and the metamorphic phase is blurred as a result of heterochronic shifts in character development (Callery et al., 2001). Other structures in *E. coqui* are also accelerated in their development, as for example the limb buds (Hanken et al., 2001) and the spinal

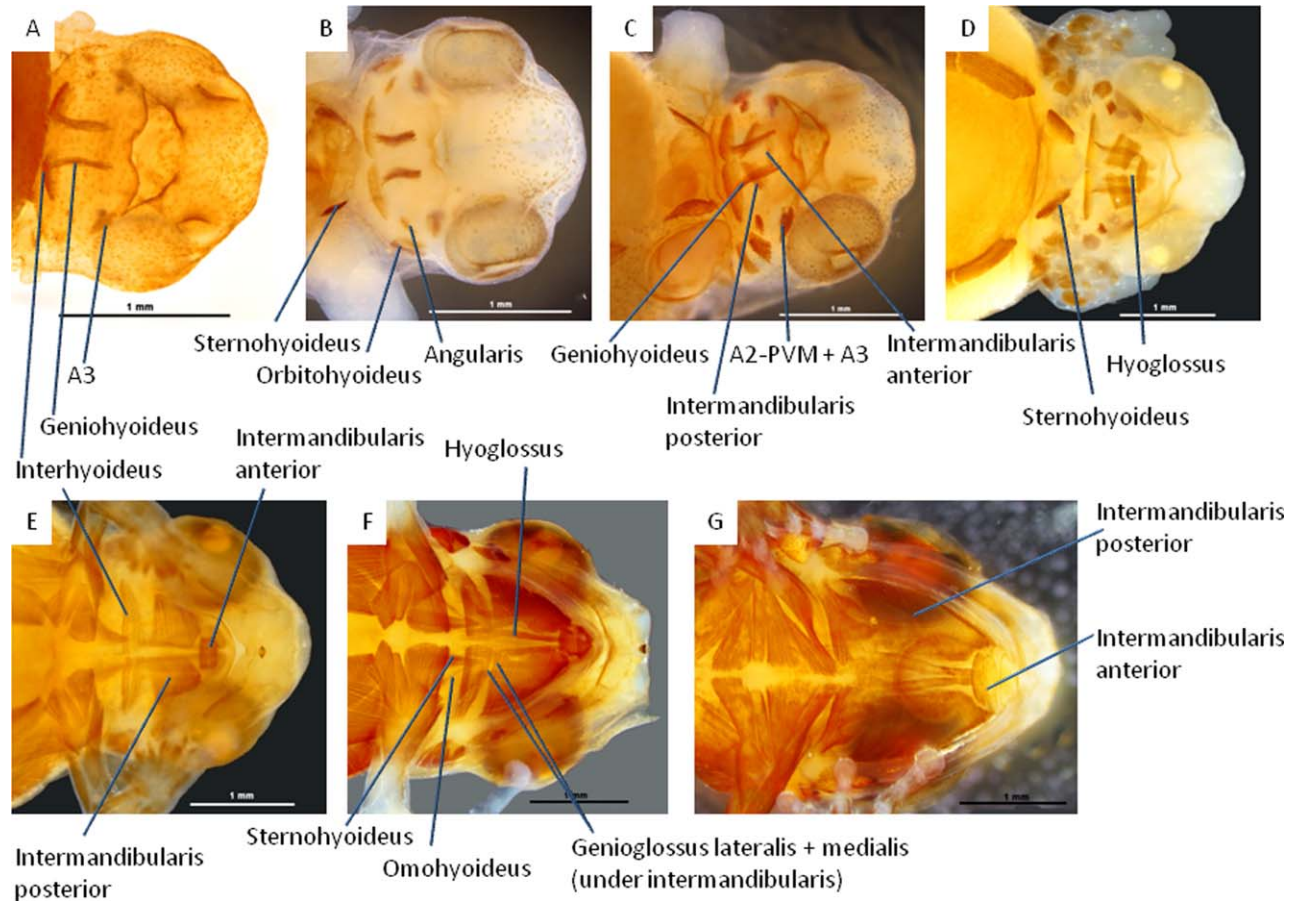


Fig. 7. *Eleutherodactylus coqui* cranial muscle development, lateral view. Muscles stained with 12/101 antibody. A: TS7 (TS = stage after Townsend and Stewart, 1985). B: TS8. C: TS9. Geniohyoideus splits into lateral and medial part. D: TS11. E: TS12. F: TS14. G: TS15+Posthatch. All muscles have basically their adult configuration. Angularis group = quadrato- and hyo-angularis. A3 = adductor mandibulae A3 (= PSE = pseudotemporalis = levator mandibulae longus + internus), A2-PVM = adductor mandibulae A2 postero-ventro-mesial. Scale bar = 1 mm.

cord (Schlosser, 2003). In the direct developing *Eleutherodactylus johnstonei* it was shown that the development of the vertebral column resembles that of a biphasic anuran (Meza-Joya et al., 2013). However, in the same species there are heterochronic changes as, for example, a delay in centra chondrogenesis and the onset of ossification, compared to that of metamorphic species.

The modifications observed in cranial muscle development in *E. coqui* mirror changes in the development of the palatoquadrate and Meckel's cartilage (Hanken et al., 1992) and in the orientation of the cranial nerves (Schlosser and Roth, 1997b) in anurans. The Meckel's cartilage, the palatoquadrate and the hypobranchial skeleton appear in a midmetamorphic morphology from the onset of their development in TS8 (Hanken et al., 1992) as do the lateral (mandibular and hyoid) muscles that are situated between their future origin and insertion (see also Hanken et al., 1997b; Schlosser and Roth, 1997b). In *R. pipiens* all muscles develop laterally ventral to the eye, while

the mandibular adductors ("levators") develop medially to the eye in *X. laevis*. Furthermore, the m. intermandibularis anterior and the m. levator mandibulae lateralis (or m. adductor mandibulae A2 lateralis) develop earlier in *E. coqui* than in *X. laevis* and *R. pipiens* (see Table 2). The development of the m. intermandibularis posterior seems to be delayed in *R. pipiens* compared to *X. laevis*. This could be caused by the development of the huge cement gland that covers the region in which this muscle develops. In *E. coqui* the petrohyoidei muscles develop directly without a previous development of branchial arch levators or constrictors. Those differences are clearly related to a fast developmental mode. When *E. coqui* hatches, most of the muscles have already achieved their adult orientation, while in *Rana (Lithobates)* and *Xenopus* the transformations during metamorphosis appear long after hatching (e.g., Sedra and Michael, 1957).

The most remarkable similarity between the taxa examined concerns the development of the

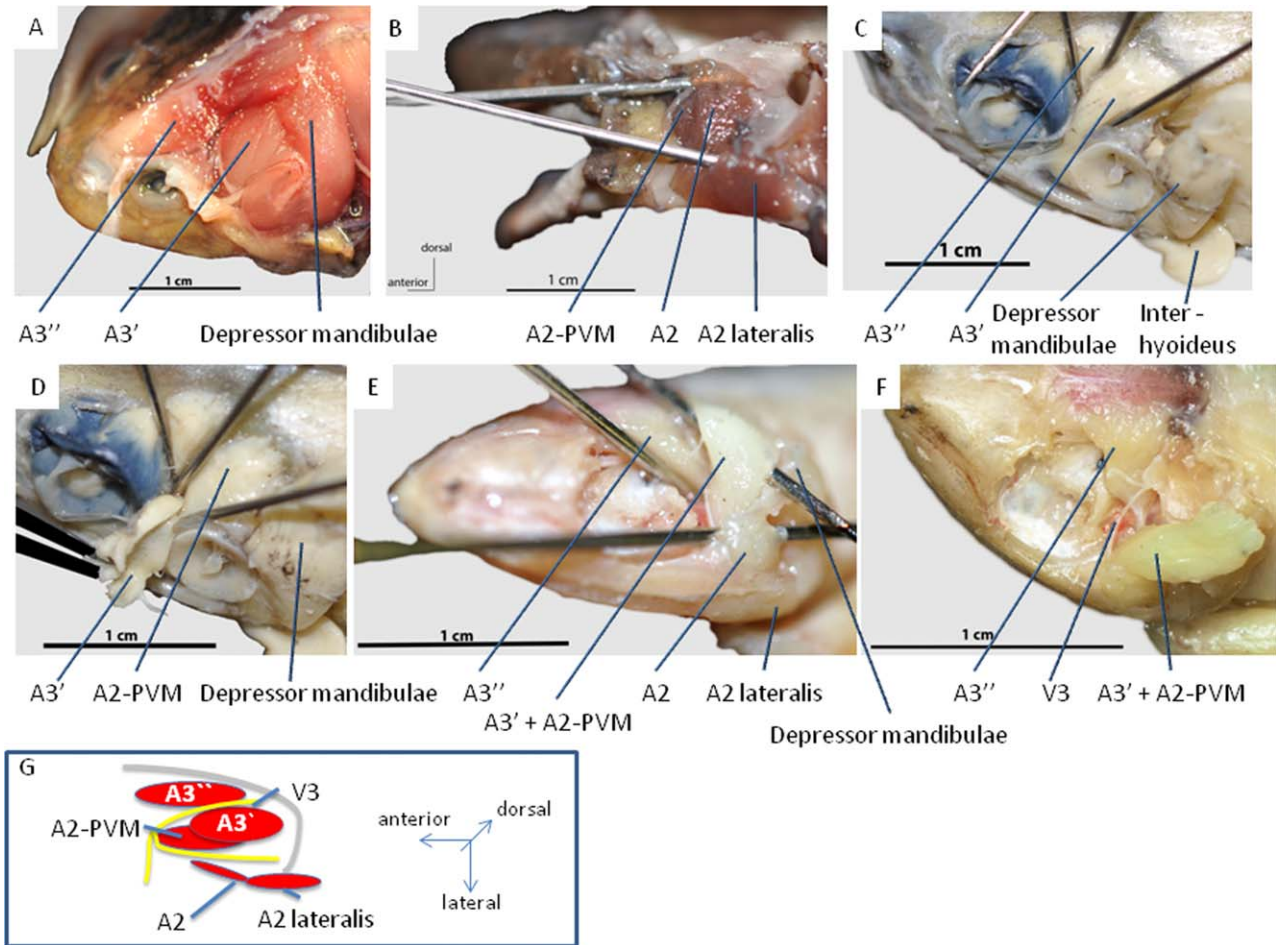


Fig. 8. Adductor mandibulae muscles and depressor mandibulae in the adult frogs of *Xenopus laevis* (A, B), *Rana pipiens* (C, D), and *Eleutherodactylus coqui* (E, F). Anterior is to the left. The adductor mandibulae muscles are essentially similar in all three species, with the exception that in *E. coqui* the A3' and A2-PVM cannot be separated close to their origin (E, F). G: Schematic dorsal view of the course of the mandibular branch of the trigeminal nerve (N. V3) through the mandibular adductors of *Xenopus laevis*. The V3 runs rostrad between the A3' and A3'' in all species investigated. A2 = Adductor mandibulae A2; A3 = Adductor mandibulae A3; A3' = superficial; A3'' = deep; PVM = postero-ventro-mesial; V3 = mandibular branch of the trigeminal nerve (N. V3). Scale bar = 1 cm.

lateral hyoid arch muscles. As in metamorphosing frogs, *E. coqui* develops distinctive portions such as the m. orbitohyoideus, m. suspensoriohyoideus, m. quadratoangularis, and m. hyoangularis (see also Hanken et al., 1997b). The m. suspensorioangularis does not develop in *E. coqui* and *X. laevis*. All lateral hyoid arch muscles fuse during development and form the adult m. depressor mandibulae in all frogs. Similarities between the taxa examined can also be found in gene expression patterns. Studies on the expression of *bmp4*, *sox9* and *runx2* revealed the repatterning of cranial cartilages as well as transient suprarostral precursors in the frontonasal processes, which never form as distinct cartilages in *E. coqui* (Kerney et al., 2010). Another resemblance is that the cranial nerves and their major rami, and the differentiation and innervation of cranial muscles, develop in a comparable sequence in *E. coqui* and *Discogloss-*

us pictus (Schlosser and Roth, 1997a,b). The description by Schlosser and Roth (1997b) showed that several nerves and their branches develop in a way as if they would innervate larval muscles like the mm. constrictores branchialium, mm. subarcuales recti, and m. transversus ventralis in other frogs. However, these muscles never develop in *E. coqui* so this might be a relict of the species' evolution (Schlosser and Roth, 1997b). These branchial muscles degenerate during the climax of metamorphosis in biphasic frogs (Edgeworth, 1935). The gene expression patterns specific for larval cartilages, and the nerves that innervate the muscles, that never develop in *E. coqui* shows that some mechanisms during development are needed to develop both the myological and the osteological features of a normal adult morphology. Another interpretation could be that these rudimentary structures are retained without an

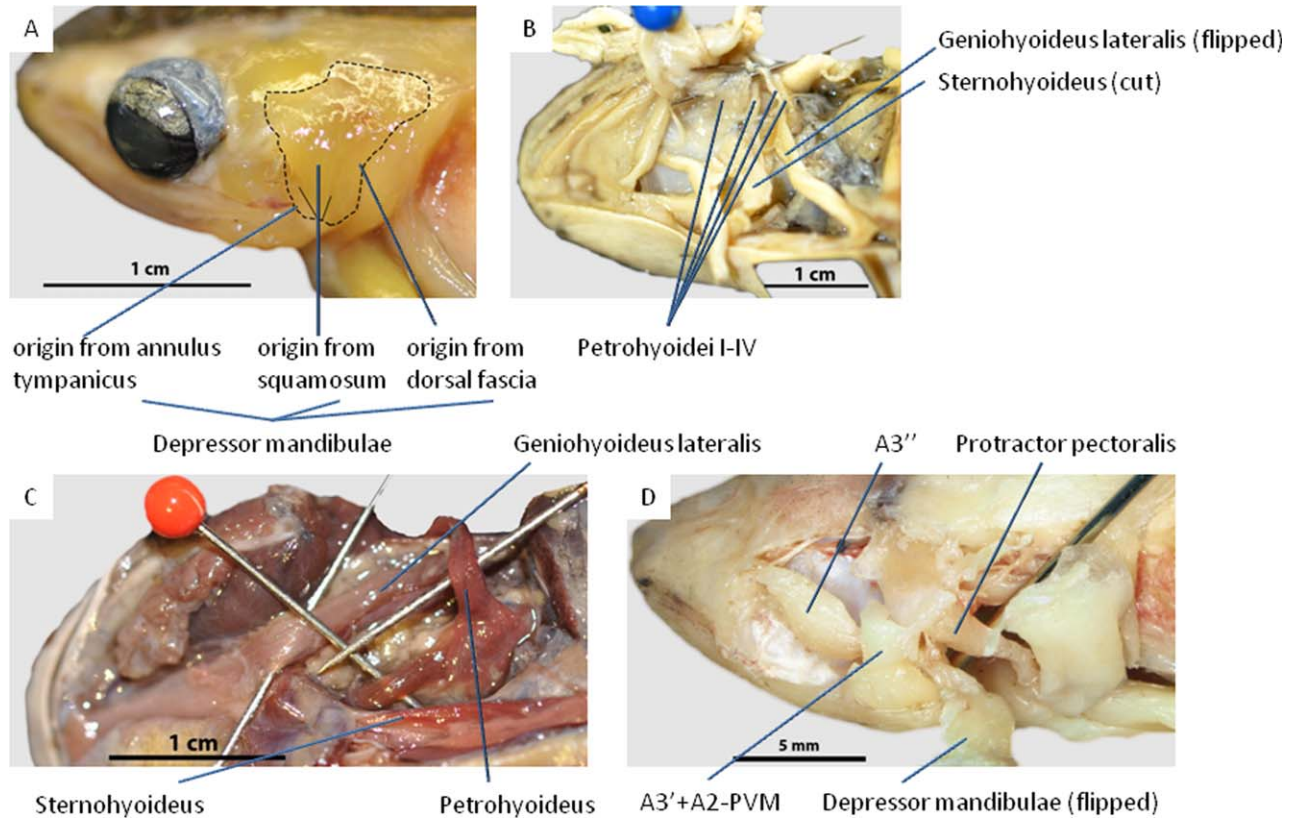


Fig. 9.. A: *Eleutherodactylus coqui*, portions of the depressor mandibulae. B: *Rana pipiens*, ventral view. Geniohyoideus cut close to its insertion and flipped backwards. Sternohyoideus cut. Four petrohyoidei muscles clearly visible. C: *Xenopus laevis*, ventral view. A single petrohyoideus muscle inserts ventrally to the larynx, being connected to its contralateral muscle. D: *Eleutherodactylus coqui* lateral view. Protractor pectoralis originating from the otic capsule and inserting at the pectoral girdle.

associated mechanism of development needed to retain the features of adult morphology.

Evolution of Direct Development – Cryptic Metamorphosis

The developmental phases in *E. coqui* are dependent on thyroid hormone from TS9 onwards (Callery and Elinson, 2000b). The sum of intermediate developing characters (e.g., Hanken et al., 1997b; Schlosser and Roth, 1997b; present study) and the results on hormone control of the development from Kulkarni et al. (2010) and Callery and Elinson (2000b; Callery et al., 2001) support the idea that metamorphosis occurs before hatching from the jelly capsules. Callery and Elinson (2000b) call this time between TS9 and hatching “cryptic metamorphosis” (see also Kulkarni et al., 2010; Elinson and del Pino, 2012; Elinson, 2013). In several experiments it was shown that genes involved in the formation of larval structures are present and expressed but the tissue fails to respond as it usually does in biphasic frogs (Fang and Elinson, 1996, 1999; Schlosser, 2002; reviewed in Desnitskiy, 2004; Buchholz et al., 2007; Kerney et al., 2010). All the above mentioned similarities

such as the several precursors of the depressor mandibulae, the nerve development to muscles that never develop, and the midmetamorphic appearance of the cranial cartilages and muscles with subsequent remodeling (Hanken et al., 1992, 1997b; Schlosser and Roth, 1997b; Kerney et al., 2010; present study) are examples of a shortened and modified cryptic metamorphosis.

Changes in the life history mode (e.g., paedomorphosis, direct development) are probably related to changes in the neuroendocrine mechanism (Hanken, 1992; Denver et al., 2002). Feeding frogs with tails (Elinson et al., 1999) and early induction of limb muscles (Tata et al., 1991) can be achieved by the manipulation of thyroid hormone levels during development. These experiments indicate that the change of the hormonal control of mechanisms that regulate metamorphosis could be a basis for the evolution of direct development in *Eleutherodactylus* (Jennings and Hanken, 1998). However, it was shown that the development of *E. coqui* is under neuroendocrine control (Callery and Elinson, 2000b; Kulkarni et al., 2010), similar to that found in biphasic anurans (e.g., Denver, 2009). But the assumption that the thyroid activation is accelerated is

problematic, because the application of thyroid hormone to tadpoles leads to changes but not to normal metamorphosis, which is also due to temporal and spatial differences in the tissue response to thyroid hormone (Elinson, 2013).

Hanken et al. (1992) suggested that the limitation of trophic supply forced an early availability of the feeding mechanism to explain the abbreviation during ontogeny. However, this is unlikely because the froglets of *E. coqui* hatch with a huge yolk supply that will last several days post hatching; an abbreviation of development due to a lack of sufficient food is therefore not necessary (e.g., Schlosser and Roth, 1997b; Buchholz et al., 2007; Singamsetty and Elinson, 2010). There are more attempts to explain the evolution of direct or abbreviated development, as for example the need for large eggs to escape competition and/or predator pressures in the aquatic environment (e.g., Lutz, 1948) or that there is a selection for an early escape mechanism (jumping) from egg-predators (Schlosser and Roth, 1997b).

Furthermore, Schlosser and Roth (1997b) suggested that the early appearance of metamorphic characters occurs in a mosaic fashion. The muscle development of the hyobranchial region and the mandibular arch seems to be more precocious than the cartilage development of the hyoid and branchial arches. These and some other heterochronic changes lead to the conclusion that the metamorphic events are not necessarily tightly coupled (Schlosser and Roth, 1997b); this pattern was also found in direct developing salamanders (Wake and Hanken, 1996). In their analyses of heterochrony plots, Schlosser and Roth (1997b) described an interesting reversal of the polarity in the dissociation between limb and cranial development. That is, in the beginning the limb development is early with respect to cranial development, while later (from TS9 onwards) the cranial development is predisplaced relative to the limb development. Furthermore the limb development is independent of thyroid hormone in *E. coqui* (Elinson, 2013).

These observations show that limb and cranial development are modules that can change the speed in which processes occur independent from each other and the acceleration/delay should be therefore only limited by internal restrictions in either head or limb. The connective tissues, muscles and nerves are clearly dependent on the developmental timing of both parts – as seen in the protractor pectoralis. This muscle develops early but because it grows towards the pectoral girdle, a “delay” in the scapula development is not a problem as long as the region of insertion is defined when the muscle cells arrive, which is the case in our observations (see Fig. 6).

As shown above, although *E. coqui* is designated as a direct developing frog, it actually still shows metamorphic characters, but the long larval

phase, with hardly any changes in the head, is skipped in *E. coqui*. It seems clear that the loss and modification of the appearance of larval structures, and the heterochronic changes in the appearance of adult structures, reflect a complex mix of mechanisms leading to the embryonic transformations that enable a “direct development” in anurans, and probably also in caudates and caecilians. In particular, as shown here, a mix of characters such as that found in *E. coqui* indicates that the larval stage is not completely lost even without a free swimming larval stage. Therefore the term “cryptic metamorphosis” (Callery and Elinson, 2000b; “differential metamorphosis” used by Wake and Hanken, 1996) might be more suitable. Direct developing salamanders also continue to show larval characters during late ontogeny (e.g., *Plethodon cinereus* in Kerney et al. 2011).

We therefore propose that not only head and limb development, but also the free swimming larval phase, should be considered as modules. This would explain why both head and limb development are accelerated with respect to the time spent as free swimming larvae. Furthermore, head and limb development are morphological modules, while the “free swimming larvae” is a life history trait. This indicates that the morphological modules are independent from the life history trait, but enable the occurrence of a free living larval stage in metamorphosing anurans/species. The module “free swimming larvae” itself is simply deleted and the developmental processes are done in the egg (Callery and Elinson, 2000b). This skipping of the larval stage leads to the disappearance of larval muscles that would be needed in a feeding larva but that are lost during metamorphosis. We assume that the shortening of the larval phase, the disappearance of this phase, and the reduction and ultimate loss of larval structures, are parallel processes during evolution. Elinson (2013) furthermore suggested that the loss of larval structures, including the free swimming phase, eliminated the function of thyroid hormone to regulate the cell death and resorption of tadpole-specific tissues. Therefore, the hormone is only needed to support the development of adult structures. Investigations in frogs with large and yolk-rich eggs (Ninomiya et al., 2001; Elinson and Beckham, 2002) have suggested that the transformations during early anuran development could be parallel to the events that led to the emergence of the large yolk-rich eggs of amniotes (Desnitskiy, 2004).

ACKNOWLEDGMENTS

The authors thank Richard Elinson for providing adults and stained larvae from all species investigated here, and for critical comments on

the manuscript. We thank the reviewers for their critical comments on an earlier version of the manuscript. The 12/101 developed by Jeremy P. Brockes was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242.

LITERATURE CITED

- Alley KE. 1989. Myofiber turnover is used to retrofit frog jaw muscles during metamorphosis. *Amer J Anat* 184:1–12.
- Alley KE, Omerza FF. 1998. Neuromuscular remodeling and myofiber turnover in *Rana pipiens*' jaw muscles. *Cell Tissues Organs* 164:46–58.
- Altig R. 2006. Tadpoles evolved and frogs are the default. *Herpetologica* 62:1–10.
- Anura. 2013. Encyclopaedia Britannica Online. Available at: <http://www.britannica.com/EBchecked/topic/29023/Anura/275978/Direct-development-from-egg-to-froglet>, Accessed on August 23, 2013.
- Brown DD, Cai L. 2007. Review: Amphibian metamorphosis. *Dev Biol* 306:20–33.
- Buchholz DR, Singamsetty S, Karadge U, Williamson S, Langer CE, Elinson RP. 2007. Nutritional endoderm in a direct developing frog: A potential parallel to the evolution of the amniote egg. *Dev Dyn* 236:1259–1272.
- Callery EM, Elinson RP. 2000a. Opercular development and ontogenetic re-organization in a direct-developing frog. *Dev Genes Evol* 210:377–381.
- Callery EM, Elinson RP. 2000b. Thyroid hormone-dependent metamorphosis in a direct developing frog. *PNAS* 97:2615–2620.
- Callery EM, Fang H, Elinson RP. 2001. Frogs without polliwogs: Evolution of anuran direct development. *BioEssays* 23: 233–241.
- Carroll RL. 2007. The Palaeozoic Ancestry of Salamanders, Frogs and Caecilians. *Zool J Linn Soc* 150:1–140.
- Carroll RL, Holmes R. 1980. The skull and jaw musculature as guides to the ancestry of salamanders. *Zoo J Linn Soc* 68:1–40.
- Chanoine C, Hardy S. 2003. *Xenopus* muscle development: From primary to secondary myogenesis. *Dev Dyn* 226:12–23.
- de Jongh HJ. 1968. Functional morphology of the jaw apparatus of larval and metamorphosing *Rana temporaria* L. *Netherlands J Zool* 18:1–103.
- del Pino EM, Elinson RP. 1983. A novel development pattern for frogs: Gastrulation produces an embryonic disc. *Nature* 306:589–591.
- Dent JA, Polson AG, Klymkowsky MW. 1989. A whole-mount immunocytochemical analysis of the expression of the intermediate filament protein vimentin in *Xenopus*. *Development* 105:61–74.
- Denver RJ. 2009. Stress hormones mediate environment–genotype interactions during amphibian development. *Gen Comp Endocr* 164:20–31.
- Denver RJ. 2013. Neuroendocrinology of amphibian metamorphosis. In: Shi Y-B, editor. *Current Topics in Developmental Biology*. Burlington: Academic Press. pp 195–228.
- Denver RJ, Boorse GC, Glennemeier KA. 2002. Endocrinology of complex life cycles amphibians. In: Pfaff AAD, Etgen A, Fahrbach S, Moss R, Rubin R, editors, *Hormones, Brain and Behavior*, Vol. 2. Academic Press: San Diego, CA, pp. 469–513.
- Denver RJ, Hu F, Scanlan TS, Fulrow JD. 2009. Thyroid hormone receptor subtype specificity for hormone-dependent neurogenesis in *Xenopus laevis*. *Dev Biol* 326:155–168.
- Desnitskiy AG. 2004. Evolutionary transformations of ontogenesis in anuran amphibians. *Russian J Dev Biol* 35:125–130.
- Diogo R, Abdala V. 2010. Muscles of vertebrates - comparative anatomy, evolution, homologies and development. Enfield, New Hampshire: CRC Press; Science Publisher. 482 p.
- Duellman WE, Trueb L. 1994. *Biology of Amphibians*. Baltimore and London: Johns Hopkins University Press.
- Ecker A. 1864. *Die Anatomie des Frosches*. Braunschweig: Verlag Friedrich Vieweg und Sohn.
- Edgeworth FH. 1930. On the masticatory and hyoid muscles of larvae of *Xenopus laevis*. *J Anat* 64:184–188.
- Edgeworth FH. 1935. *The Cranial Muscles of Vertebrates*. London: Cambridge at the University Press.
- Elinson RP. 1987. Fertilization and aqueous development of the Puerto Rican terrestrial-breeding frog, *Eleutherodactylus coqui*. *J Morphol* 193:217–224.
- Elinson RP. 2007. Muscle development in a biphasic animal: The frog. *Dev Dyn* 236:2444–2453.
- Elinson RP. 2013. Metamorphosis in a frog that does not have a tadpole. In: Shi Y-B, editor. *Current Topics in Developmental Biology*. Burlington: Academic Press. pp 259–276.
- Elinson RP, Beckham Y. 2002. Development in frogs with large eggs and the origin of amniotes. *Zoology* 105:1–13.
- Elinson RP, del Pino EM. 1985. Cleavage and gastrulation in the egg-brooding, marsupial frog, *Gastrotheca riobambae*. *J Embryol Exp Morphol* 90:223–232.
- Elinson RP, del Pino EM. 2012. Developmental diversity of Amphibians. *WIREs Dev Biol* 1:345–369.
- Elinson RP, Fang H. 1998. Secondary coverage of the yolk sac by the body wall in the direct developing frog, *Eleutherodactylus coqui*: an unusual process for amphibian embryos. *Dev Genes Evol* 208:457–466.
- Elinson RP, Del Pino EM, Townsend DS, C. CF, Eichhorn P. 1990. A practical guide to the developmental biology of terrestrial-breeding frogs. *Biol Bull* 179:163–177.
- Elinson RP, Remo B, Brown DD. 1999. Novel structural elements identified during tail resorption in *Xenopus laevis* metamorphosis: lessons from tailed frogs. *Dev Biol* 215:243–252.
- Ericsson R. 2003. *A Comparative Study of Head Development in Mexican Axolotl and Australian Lungfish: Cell Migration, Cell Fate and Morphogenesis*. Uppsala: Faculty of Science and Technology. 47 p.
- Fang H, Elinson RP. 1996. Patterns of distal-less gene expression and inductive interactions in the head of the direct developing frog *Eleutherodactylus coqui*. *Dev Biol* 179:160–172.
- Fang H, Elinson RP. 1999. Evolutionary alteration in anterior patterning: otx2 expression in the direct developing frog *Eleutherodactylus coqui*. *Dev Biol* 205:233–239.
- Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, de Sa' RO, Channing A, Wilkinson M, Donnellan SC, Raxworthy CJ, Campbell JA, Blotto BL, Moler P, Drewes RC, Nussbaum RA, Lynch JD, Green DM, Wheeler WC. 2006. The amphibian tree of life. *Bull Am Mus Nat Hist* 297:370.
- Gaupp E. 1896. *Anatomie des Frosches*. 1. Abteilung: Lehre vom Skelet und vom Muskelsystem Vieweg, Braunschweig.
- Haas A. 2001. Mandibular arch musculature of anuran tadpoles; with comments on homologies of amphibian jaw muscles. *J Morphol* 247:1–33.
- Haas A. 2003. Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics* 19:23–89.
- Hanken J. 1992. Mini Review. Life history and morphological evolution. *J Evol Biol* 5:549–557.
- Hanken J. 1999. Larvae in amphibian development and evolution. In: Hall BK, Wake MH, editors. *The Origin and Evolution of Larval Forms*. San Diego and London: Academic Press, pp. 61–108.
- Hanken J, Klymkowsky MW, Summers CH, Seufert DW, Ingebrigsten N. 1992. Cranial ontogeny in the direct-developing frog, *Eleutherodactylus coqui* (Anura: Leptodactylidae), analysed using whole-mount immunohistochemistry. *J Morphol* 211:95–118.
- Hanken J, Jennings DH, Olsson L. 1997a. Mechanistic basis of life-history evolution in anuran amphibians: Direct development. *Amer Zool* 37:160–171.

- Hanken J, Klymkowsky MW, Alley KE, Jennings DH. 1997b. Jaw muscle development as evidence for embryonic repatterning in direct-developing frogs. *Proc R Soc Lond B* 264: 1349–1354.
- Hanken J, Carl TF, Richardson MK, Olsson L, Schlosser G, Osabutey CK, Klymkowsky MW. 2001. Limb development in a “nonmodel” vertebrate, the direct-developing frog *Eleutherodactylus coqui*. *J Exp Zool (Mol Dev Evol)* 291:375–388.
- Heimeier RA, Shi Y-B. 2010. Amphibian metamorphosis as a model for studying endocrine disruption on vertebrate development: Effect of bisphenol A on thyroid hormone action. *Gen Comp Endocr* 168:181–189.
- Horton P. 1982. Diversity and systematic significance of anuran tongue musculature. *Copeia* 3:595–602.
- Iordansky NN. 1992. Jaw muscles of the Urodela and Anura: some features of development, functions, and homology. *Zool Jb Anat* 122:225–232.
- Iordansky NN. 1996. Evolution of the musculature of the jaw apparatus in the amphibia. *Adv Amphibian Res Former Soviet Union* 1:3–26.
- Jennings DH, Hanken J. 1998. Mechanistic basis of life history evolution in anuran amphibians: Thyroid gland development in the direct-developing frog, *Eleutherodactylus coqui*. *Gen Comp Endoc* 111:225–232.
- Johnston P. 2011. Cranial muscles of the anurans *Leiopelma hochstetteri* and *Ascapus truei* and the homologies of the mandibular adductors in Lissamphibia and other Gnathostomes. *J Morphol* 272:1492–1512.
- Kerney R, Meegaskumbura M, Manamendra Arachchi K, Hanken J. 2007. Cranial ontogeny in *Philautus silus* (Anura: Ranidae: Rhacophorinae) reveals few similarities with other direct developing anurans. *J Morphol* 268:715–725.
- Kerney RR, Gross JB, Hanken J. 2010. Early cranial patterning in the direct developing frog *Eleutherodactylus coqui* revealed through gene expression. *Evol Dev* 12:373–382.
- Kerney R, Blackburn DC, Müller H, Hanken J. 2011. Do larval traits re-evolve? Evidence from the embryogenesis of a direct developing salamander, *Plethodon cinereus*. *Evolution* 66: 252–262.
- Kintner CR, Brockes JP. 1985. Monoclonal antibodies to the cells of a regenerating limb. *J Embryol Exp Morphol* 89:37–55.
- Kikuyama S, Kawamura K, Tanaka S, Yamamoto K. 1993. Aspects of amphibian metamorphosis: Hormonal control. *Internat Rev Cytol* 145:105–148.
- Kulkarni SS, Singamsetty S, Buchholz DR. 2010. Corticotropin-releasing factor regulates the development in the direct developing frog, *Eleutherodactylus coqui*. *Gen Comp Endocr* 169: 225–230.
- Luther A. 1914. Über die vom N. trigeminus versorgte Muskulatur der Amphibien mit einem vergleichenden Ausblick über den Adductor mandibulae der Gnathostomen, und einem Beitrag zum Verständnis der Organisation der Anurenlarven. *Acta Soc Sci Fenn* 7:1–151.
- Lutz B. 1948. Ontogenetic evolution in frogs. *Evolution* 2:29–39.
- McClearn D, Noden DM. 1988. Ontogeny of architectural complexity in embryonic quail visceral arch muscles. *Amer J Anat* 183:277–293.
- Meza-Joya FL, Ramos-Palares EP, Ramirez-Pinilla MP. 2013. Ontogeny of the vertebral column of *Eleutherodactylus johnstonei* (Anura: Eleutherodactylidae) reveals heterochronies relative to metamorphic frogs. *Anat Rec* 296:1019–1030.
- Nieuwkoop PD, Faber J. 1967. Normal table of *Xenopus laevis* (Daudin). North Holland Publishing Co. Amsterdam, 252 p.
- Ninomiya H, Zhang Q, Elinson RP. 2001. Mesoderm formation in *Eleutherodactylus coqui*: Body patterning in a frog with a large egg. *Dev Biol* 236:109–123.
- Olsson L, Moury DJ, Carl TF, Hastad O, Hanken J. 2002. Cranial neural crest-cell migration in the direct-developing frog, *Eleutherodactylus coqui*: molecular heterogeneity within and among migratory streams. *Zoology* 105:3–13.
- Paterson NF. 1939. The Head of *Xenopus laevis*. *Q J microsc Sci* 81:161–234.
- Schilling TF, Kimmel CB. 1997. Musculoskeletal patterning in the pharyngeal segments of the zebrafish embryo. *Development* 124:2945–2960.
- Schlosser G. 2002. Development and evolution of lateral line placodes in amphibians II. Evolutionary diversifications. *Zoology* 105:119–146.
- Schlosser G. 2003. Mosaic evolution of neural development in anurans: Acceleration of spinal cord development in the direct developing frog *Eleutherodactylus coqui*. *Anat Embryol* 206:215–227.
- Schlosser G, Roth G. 1997a. Evolution of nerve development in frogs I: The development of the peripheral nervous system in *Discoglossus pictus* (Discoglossidae). *Brain Behav Evol* 50:62–93.
- Schlosser G, Roth G. 1997b. Evolution of nerve development in frogs II: Modified development of the peripheral nervous system in the direct-developing frog *Eleutherodactylus coqui* (Leptodactylidae). *Brain Behav Evol* 50:94–128.
- Sedra SN, Michael IM. 1957. The development of the skull, visceral arches, larynx and visceral muscles of the south african clawed toad, *Xenopus laevis* (Daudin) during the process of Metamorphosis (from Stage 55 to Stage 66). Amsterdam: Verhandelingen der Koninklijke Nederlandse Akademie van Wetenschappen, AFD. Natuurkunde. pp 5–80.
- Shumway W. 1940. Stages in the normal development of *Rana pipiens*. I. External form. *Anat Rec* 78:139–146.
- Shi Y-B. 2013. Animal Metamorphosis. (Vol. 103). Access Online via Elsevier. pp 456.
- Singamsetty S, Elinson RP. 2010. Novel regulation of yolk utilization by thyroid hormone in embryos of the direct developing frog *Eleutherodactylus coqui*. *Evol Dev* 12:437–448.
- Tata JR. 2006. Amphibian metamorphosis as a model for the developmental actions of thyroid hormone. *Mol Cell Endocr* 246:10–20.
- Tata JR, Kawahara A, Baker BS. 1991. Prolactin inhibits both thyroid hormone-induced morphogenesis and cell death in cultured amphibian larval tissues. *Dev Biol* 146:72–80.
- Townsend DS, Stewart MM. 1985. Direct development in *Eleutherodactylus coqui* (Anura: Leptodactylidae): A staging table. *Copeia* 1985:423–356.
- Townsend DS, Stewart MM, Pough FH, Brussard PF. 1981. Internal fertilization in an oviparous frog. *Science* 212:469–471.
- Townsend DS, Stewart MM, Pough FH. 1984. Male parental care and its adaptive significance in a neotropical frog. *Anim Behav* 32:421–431.
- Wake DB, Hanken J. 1996. Direct development in the lungless salamanders: What are the consequences for developmental biology, evolution and phylogenesis? *Internat J Devel Biol* 40: 859–869.
- Weisz PB. 1945a. The development and morphology of the larva of the south african clawed toad, *Xenopus laevis*: I. The third-form tadpole. *J Morph* 77:163–192.
- Weisz PB. 1945b. The development and morphology of the larva of the south african clawed toad, *Xenopus laevis*: II. The hatching and the first- and second-form tadpole. *J Morph* 77: 193–217.
- Ziermann JM. 2008. Evolutionäre Entwicklung larvaler Cranialmuskulatur der Anura und der Einfluss von Sequenzheterochronien [PhD]. Jena: Friedrich Schiller University Jena. 347 p.
- Ziermann JM, Diogo R. 2013. Cranial muscle development in the model organism *Ambystoma mexicanum*: implications for tetrapod and vertebrate comparative and evolutionary morphology and notes on ontogeny and phylogeny. *Anat Rec* 296: 1031–1048.
- Ziermann JM, Olsson L. 2007a. Patterns of spatial and temporal cranial muscle development in the african clawed frog, *Xenopus laevis* (Anura: Pipidae). *J Morphol* 268:791–804.
- Ziermann JM, Olsson L. 2007b. A new staging table for stages relevant to cranial muscle development in the African Clawed Frog, *Xenopus laevis* (Anura: Pipidae). *J Morphol* 268:791–804.