

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/236643975>

Cranial Muscle Development in the Model Organism *Ambystoma mexicanum* : Implications for Tetrapod and Vertebrate Comparative and Evolutionary Morphology and Notes on Ontogeny and Ph...

Article in *The Anatomical Record Advances in Integrative Anatomy and Evolutionary Biology* · July 2013

DOI: 10.1002/ar.22713 · Source: PubMed

CITATIONS

39

READS

355

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:



history and philosophy of sciences, bias and racism [View project](#)



Comparative vertebrate musculoskeletal anatomy [View project](#)

Cranial Muscle Development in the Model Organism *Ambystoma mexicanum*: Implications for Tetrapod and Vertebrate Comparative and Evolutionary Morphology and Notes on Ontogeny and Phylogeny

JANINE M. ZIERMANN* AND RUI DIOGO

Department of Anatomy, Howard University College of Medicine, Washington DC

ABSTRACT

There is still confusion about the homology of several cranial muscles in salamanders with those of other vertebrates. This is true, in part, because of the fact that many muscles present in early ontogeny of amphibians disappear during development and specifically during metamorphosis. Resolving this confusion is important for the understanding of the comparative and evolutionary morphology of vertebrates and tetrapods because amphibians are the phylogenetically most plesiomorphic tetrapods, concerning for example their myology, and include two often used model organisms, *Xenopus laevis* (anuran) and *Ambystoma mexicanum* (caudate). Here we provide the first detailed report of the cranial muscle development in axolotl from early ontogenetic stages to the adult stage. We describe different and complementary types of general muscle morphogenetic gradients in the head: from anterior to posterior, from lateral to medial, and from origin to insertion. Furthermore, even during the development of neotenic salamanders such as axolotls, various larval muscles become indistinct, contradicting the commonly accepted view that during ontogeny the tendency is mostly toward the differentiation of muscles. We provide an updated comparison between these muscles and the muscles of other vertebrates, a discussion of the homologies and evolution, and show that the order in which the muscles appear during axolotl ontogeny is in general similar to their appearance in phylogeny (e.g. differentiation of adductor mandibulae muscles from one anlage to four muscles), with only a few remarkable exceptions, as for example the dilator laryngis that appears evolutionary later but in the development before the intermandibularis. Anat Rec, 00:000–000, 2013. © 2013 Wiley Periodicals, Inc.

Key words: homology; development; evolution; *Ambystoma mexicanum*; amphibians; tetrapods; vertebrates; cranial muscles

Grant sponsor: Rui Diogo's start-up package from the Howard University College of Medicine.

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

Received 13 December 2012; Accepted 26 March 2013.

DOI 10.1002/ar.22713

Published online in Wiley Online Library (wileyonlinelibrary.com).

INTRODUCTION

Besides extensive studies on the morphology of amphibians and some comparative myological works (Lubosch, 1914; Edgeworth, 1935; Iordansky, 1996; Haas, 2001, 2003), the homologies between the muscles of amphibians and other tetrapods is still a subject that raises much controversy and that interests researchers from various different fields of biology. This is because amphibians are the phylogenetically most plesiomorphic tetrapods, at least concerning their myology, and include two of the most used model organisms, *Xenopus laevis* (anuran frog) and *Ambystoma mexicanum* (caudate axolotl). Apart from anurans and caudates (salamanders), the clade Amphibia also includes the not so studied caecilians. Clarifying the homologies between the hard and soft tissues of amphibians and other vertebrates is therefore an important task for the understanding of the comparative and evolutionary morphology of vertebrates as a whole and of the origin and evolution of tetrapods in particular (Diogo and Abdala, 2010). *A. mexicanum* is considered to be the most useful model organism to discuss the early evolutionary history of tetrapods because this is a salamander species and thus is anatomically more plesiomorphic than the frog *X. laevis* (e.g., Schmidt et al., 2013). Moreover, it is also a crucial model for regenerative and evolutionary developmental biology (e.g., Diogo and Tanaka, 2012).

In amphibians several types of metamorphosis are known that show more or less changes from a larval to an adult stage (Lynn, 1961). The morphology of the jaw apparatus in salamanders is relatively stable during ontogeny until adulthood (Iordansky, 1992). Therefore, it is easier to compare the muscles in larvae and adult salamanders as compared with anurans where massive changes in the cranium occur during development (Haas, 1996). The salamander family Ambystomatidae includes several species that are neotenic, which means that they fail to undergo a full metamorphosis. The head morphology of some members of this family was described over the past century (Luther, 1914; Edgeworth, 1935; Piatt, 1938), and the development of larval cranial muscles was also investigated but to a lesser extent (e.g. Edgeworth, 1935; Ericsson and Olsson, 2004; Piekarski and Olsson, 2007; Ziermann, 2008).

The Mexican Axolotl (*A. mexicanum*, Shaw and Noder, 1798) is a neotenic salamander species of which the adults display many larval characters (e.g. external gills, tail fin, no eye lids, branchial arch levators). This salamander has become an important model organism in developmental, regenerative, comparative and evolutionary works, but paradoxically there is not a single article explicitly dedicated to the study of the development of the mandibular, hyoid, branchial, and hypobranchial muscles of this species. Most studies focus only on a few muscles that are of interest for providing discussions about specific subjects such as functional morphology (Lauder and Reilly, 1988), the evolution of novelties (Schmidt et al., 2013), the role of the neural crest in cranial development (Ericsson et al., 2004), or the origin of cranial muscles derived from somites (Piekarski and Olsson, 2007).

In this article, we provide the first detailed report on the development and adult configuration of the

mandibular, hyoid, branchial, and hypobranchial muscles of *A. mexicanum*, from early ontogenetic stages to the adult stage. We provide an updated comparison between these muscles and the muscles of other tetrapods and other vertebrates, as well as a discussion of their homologies. We focus particularly on the controversial homologies of the depressor mandibulae, levator hyoideus, branchiohyoideus, and of the protractor pectoralis, which have crucial implications for the understanding of amphibian and tetrapod biology and of the origin of reptiles and mammals.

MATERIAL AND METHODS

Embryos and larvae for the histological sectioning and staining with Azan and Bunke were provided from Piekarski (Jena, Germany). Twenty-five embryos and hatchlings with 12/101 stained muscles were provided by R. Elinson (AMP1; Pittsburgh). All those embryos and hatchlings were used to investigate the development and morphology of the cranial muscles. They were studied under a microscope (Nikon AZ100) and photographed with an attached camera (Nikon DS-Fi1; software NIS-Elements D4.00.03). Staging followed Bordzilovskaya et al. (1989) and Nye et al. (2003). The embedding of the embryos and hatchlings, the sectioning and the following staining with either Azan or Bunke followed the protocols from Ziermann and Olsson (2007). Specimens were dehydrated in an ethanol series, embedded in paraffin or Technovit 8100 (Heraeus Kulzer, Germany) dependent on their size. The embryos and larvae were sectioned at 7 or 8 μm (paraffin) or 5 μm (Technovit) on a Microm HM360 microtome. Paraffin sections were stained with Heidenhain's Azan technique and Technovit sections with Bunke staining (Böck, 1989). For immunocytochemistry we followed the procedure as described by Elinson and Fang (1998). The embryos were fixed in Dent's fixative (80% methanol and 20% dimethyl sulfoxide, DMSO; Dent et al., 1989), and stored at -20°C . The embryo's pigment was bleached with 10% H_2O_2 in 67% Dent's for 1 to 3 days if necessary. They were stained for muscle with 12/101 antibody (Kintner and Brockes, 1985). The primary antibody was visualized using a HRP-labeled goat antimouse secondary antibody (horse radish peroxidase, HRP).

Two adult specimens (AMP4, *Ambystoma* Genetic Stock Center, Lexington, KY) were dissected and compared with the descriptions of adults provided in the literature to confirm the adult morphology. The dissection was done under low magnification with a dissecting microscope (Nikon SMZ-2B). The specimens were photographed at several steps of the dissection with a Nikon digital camera (D90). The labeling of both the embryonic and adult structures was done in Microsoft PowerPoint. As this article focus on a broader comparative morphology investigation throughout vertebrates the muscle terminology mainly follows Diogo and Abdala (2010); the depressor and levator branchiarum muscles were named after Ericsson and Olsson (2004). The nomenclature of adult bones mainly follows Carroll (2007). Larval cartilages were named as in Haas (1996, 2001, 2003), while the developmental stages of muscle development (Table 1) follow Ziermann and Olsson (2007) and Ziermann (2008). A list of muscle synonyms used by other authors is given in Table 2.

TABLE 1. Updated and completed scheme showing the development of cranial muscles in *Ambystoma mexicanum*

| Stages/Muscles | 34 | 37 | 37 | 38 | 38 | 38 | 38 | 38/39 | 38/39 | 38/39 | 39 | 40 | 42 | 42 | 42 | 43 | 43 | 44 | 45 | Adult |
|-------------------------------------|----|----|----|-----|-----|-----|----|-------|-------|-------|-----|----|----|----|----|----|----|----|----|-------|
| Mandibular muscles | | | | | | | | | | | | | | | | | | | | |
| intermandibularis anterior | | | | | | | | i | ii | iii | iv | | | | | | | | | |
| intermandibularis posterior | i | i | | ii | ii | ii | ii | ii | ii | iii | iv | | | | | | | | | |
| pseudotemporalis, sup. (A3') | i | i | i | ii | ii | ii | ii | ii | ii | iii | iv | | | | | | | | | iv |
| pseudotemporalis, deep (A3'') | | | | | | | | | | ii | iii | iv | | | | | | | | |
| <i>levator bulbi</i> | i | i | | | | | | | | | | | | | | | | | | |
| adductor mandibulae A2 | | | | i | ii | ii | ii | ii | iii | iv | | | | | | | | | | |
| adductor mandibulae A2-PVM | | | | i | ii | ii | ii | ii | iii | iv | | | | | | | | | | |
| Hyoid muscles | | | | | | | | | | | | | | | | | | | | |
| interhyoideus anterior | | i | | ii | ii | ii | ii | ii | iii | iv | | | | | | | | | | |
| interhyoideus posterior | | | | | | i | ii | ii | iii | iv | | | | | | | | | | |
| depressor mandibulae | i | i | i | ii | ii | ii | ii | ii | iii | iv | | | | | | | | | | iv |
| levator hyoideus | i | i | i | ii | ii | ii | ii | ii | iii | iv | | | | | | | | | | |
| branchiohyoideus | | | | i | ii | ii | ii | ii | iii | iv | | | | | | | | | | |
| Branchial muscles | | | | | | | | | | | | | | | | | | | | |
| levator arcuum branchialium I | | i | ii | iii | iv | | | | | | | | | | | | | | | |
| levator arcuum branchialium II | | i | ii | iii | iv | | | | | | | | | | | | | | | |
| levator arcuum branchialium III | | i | ii | iii | iv | | | | | | | | | | | | | | | |
| levator arcuum branchialium IV | | i | ii | iii | iv | | | | | | | | | | | | | | | |
| <i>protractor pectoralis</i> | | | | | | | | | | iii | iv | | | | | | | | | |
| subarcualis rectus I | | | | i | ii | iii | iv | | | | | | | | | | | | | |
| subarcualis rectus II-IV | | | | i | ii | iii | iv | | | | | | | | | | | | | |
| subarcualis obliquus II | | | | i | ii | iii | iv | | | | | | | | | | | | | |
| subarcualis obliquus III | | | | i | ii | iii | iv | | | | | | | | | | | | | |
| levatores and depressores br. I-III | | | | ii | iii | iv | | | | | | | | | | | | | | |
| transversus ventralis IV | | | | | | | | | ii | iii | iv | | | | | | | | | |
| Laryngeal muscles | | | | | | | | | | | | | | | | | | | | |
| <i>laryngeus</i> | | | | | | | | | | | | | | | | | | | ? | ? |
| constrictor laryngis | | | | | | | | | | ii | iii | iv | | | | | | | | ? |
| dilatator laryngis | | | | i | ii | iii | iv | | | | | | | | | | | | | ? |
| Hypobranchial muscles | | | | | | | | | | | | | | | | | | | | |
| geniohyoideus | | i | ii | iii | iv | | | | | | | | | | | | | | | |
| sternohyoideus | | i | ii | iii | iv | | | | | | | | | | | | | | | |
| omohyoideus | | | | | | | | | | | | | | | | | | | | iv |

Staging following Bordzilovskaya et al. (1989) and Nye et al. (2003). I = anlage (myoblasts); II = myocytes visible; III = fiber development starts; IV = functional muscle (modified from Ziermann, 2008). Thicker arrows indicating development from another muscle. The small arrows showing the observed contribution of the levator hyoideus to the branchiohyoideus and depressor mandibulae. The magenta cells refer to the fact that in adults some muscles are integrated into each other. This is the case with the levator hyoideus and the deep pseudotemporalis (A3'') which are not present as distinct muscle structures because they are integrated in the adult depressor mandibulae and the adult pseudotemporalis, respectively. In stage 34 and 37 the long blue boxes (merged cells) indicate a common anlage. When anlage or muscle can be seen individually the cells are separated. Red font highlights muscles that were not included in Ziermann's (2008) detailed developmental table but that were found in the present work in the adult axolotl. "sup." means superficial, "br." means branchiarum, and "?" refers to muscles that we could not identify in the adult axolotls dissected for the present project but that are often present in salamanders and in larval *Ambystoma mexicanum* as reported by Ziermann (2008); the only exception is the laryngeus (see Table 5 below).

RESULTS

Mandibular Muscles

During stage 34 an elongated cluster is visible lateral to the brain reaching the ventral portion of the head.

The ventral part is the anlage of the intermandibularis posterior muscle, while the dorsal part is the adductor mandibulae anlage (see Table 3). By stage 36 both anlagen can be clearly divided (Figs. 1A and 2A). At the beginning of stage 38 all mandibular arch muscles, except

TABLE 2. Synonyms used for the adult mandibular, hyoid, branchial, and hypobranchial arch muscles

| <i>A. mexicanum</i> | Synonyms used in Caudates |
|--|---|
| Mandibular muscles | |
| Intermandibularis anterior | Submentalis (Iordansky, 1992) |
| Intermandibularis posterior | – |
| Adductor mandibulae A2 | Levator mandibulae externus (Edgeworth, 1935; Piatt, 1938; Larsen and Guthrie, 1975; Carroll and Holmes, 1980; Ericsson and Olsson, 2004; Piekarski and Olsson, 2007; Ziermann, 2008); adductor mandibulae externus (Iordansky, 1992; Lauder and Shaffer, 1985; Carroll and Holmes, 1980) |
| Adductor mandibulae A2-PVM | Levator mandibulae posterior (Edgeworth, 1935; Piatt, 1938); adductor mandibulae posterior (Piatt, 1938; Carroll and Holmes, 1980; Iordansky, 1992); levator mandibulae articularis (Ziermann, 2008) |
| Pseudotemporalis, superficial (A3') | Superficial levator mandibulae anterior (Edgeworth, 1935; Piekarski and Olsson, 2007); levator mandibulae anterior superficialis (Piatt, 1938, 1939); part of adductor mandibulae internus (Carroll and Holmes 1980; Lauder and Shaffer, 1985); levator mandibulae longus (Haas, 2001; Ericsson and Olsson, 2004; Ziermann, 2008); adductor mandibulae A3' (Diogo 2007, 2008) |
| Pseudotemporalis, deep (A3'') ^a | Deep levator mandibulae anterior (Edgeworth, 1935; Piatt, 1938; Piekarski and Olsson, 2007); part of adductor mandibulae internus (Carroll and Holmes, 1980; Lauder and Shaffer, 1985); levator mandibulae internus or pseudopterygoideus (Haas, 2001; Ericsson and Olsson, 2004; Ziermann, 2008); adductor mandibulae A3'' (Diogo, 2007, 2008) |
| Levator bulbi | – |
| Hyoid muscles | |
| Interhyoideus anterior + posterior | Interhyoideus anterior + posterior (sensu Piatt, 1938; Bauer, 1992, 1997; Ericsson and Olsson, 2004) |
| Depressor mandibulae | Depressor mandibulae anterior (Diogo 2007, 2008; Diogo et al., 2008a,b) |
| Branchiohyoideus | Branchiohyoideus externus (Piekarski and Olsson, 2007) |
| Levator hyoideus ^a | Depressor mandibulae posterior sensu Diogo 2007, 2008 and Diogo et al., 2008a,b) |
| Ceratomandibularis ^a | Branchiomandibularis (Edgeworth, 1935); ceratohyoideus externus (Piatt, 1938); subhyoideus (Duellman and Trueb, 1986) |
| Branchial muscles | |
| True branchial muscles s.st. | – |
| Protractor pectoralis | Cucullaris or cucullaris major (Edgeworth, 1935; Carroll, 2007; Piekarski and Olsson, 2007) |
| Constrictor laryngis ^a | – |
| Dilatator laryngis ^a | – |
| Laryngeus ^a | Laryngeus ventralis (Edgeworth, 1935) |
| Hypobranchial muscles | |
| Geniohyoideus | – |
| Sternohyoideus | Rectus cervicis (Carroll, 2007); rectus cervicis (Ziermann, 2008) |
| Omohyoideus | Pectoriscapularis (Edgeworth, 1935); abdomino-hyoideus (Piatt, 1938) |

^aDeep pseudotemporalis and levator hyoideus are present as distinct muscles in some ontogenetic stages of *A. mexicanum* but are completely integrated in the pseudotemporalis and depressor mandibulae of the adult, respectively (see text and Tables 1, 3, and 4); the ceratomandibularis is not present as a distinct muscle in all observed developmental stages of *A. mexicanum* (see text and Table 4); the constrictor laryngis and dilatator laryngis are clearly usually present in *A. mexicanum* but the laryngeus was not identified in the present work either because it is missing in this species or because it is a very small and deep laryngeal muscle (see text and Table 5). "s.st." means sensu stricto.

the intermandibularis anterior, are visible as muscle cell bands (Table 1). In stage 40 the fiber development is clearly visible and by the end of stage 42 the mandibular arch muscles are fully developed.

The intermandibularis anterior is the only muscle that is always a bit delayed in its development compared with most other cranial muscles (Table 1; Fig. 2D). It is visible by the end of stage 38, stretching anteriorly between the cartilages of Meckel and with a clear gap to the intermandibularis posterior. The intermandibularis anterior connects with the contralateral muscle in a median raphe. In the adult this muscle band stretches between the most anterior medial sides of the dentary (Fig. 3E). The intermandibularis posterior develops from its origin at Meckel's cartilage to its insertion where it meets the contralateral muscle in a median raphe (Fig. 2). During the early development are the fibers postero-medially oriented. By stage 40 (Fig. 2E) the fibers have

turned to their final latero-medial orientation and connect with the contralateral muscle. In the adults this muscles covers the anterior ventral half of the head, running from the dentary toward the median raphe and forming a continuous sheet with the interhyoideus (Fig. 3C).

In stage 36 (Fig. 1A) a small anlage of lateral mandibular muscles is visible just posterior to the eye anlage. This stretches during the following stages and by stage 39 (Fig. 1D) it can be distinguished between the anterior developing pseudotemporalis and the posterior superficial developing adductor mandibulae (A2) (Fig. 1D). The deep adductor mandibulae A2-PVM (posteroventromedial) is not visible until stage 43 in our antibody stained sample (Fig. 4C). However, in histological sections this muscle can be distinguished from the adductor mandibulae A2 and the pseudotemporalis from stage 38 onwards (Table 1). The A2-PVM is blended with both the A2 and

TABLE 3. Updated and completed scheme illustrating the authors' hypotheses regarding the homologies of the mandibular muscles of adults of representative sarcopterygian taxa (modified from Diogo and Abdala, 2010)

| | <i>Latimeria</i> (7 muscles) | <i>Lepidosiren</i> (5 muscles) | <i>Amblystoma</i> (6 muscles) | <i>Timon</i> (11 muscles) | <i>Ornithorhynchus</i> (9 muscles) | <i>Rattus</i> (9 muscles) | <i>Cynocephalus</i> (8 muscles) | <i>Tupaia</i> (9 muscles) | <i>Homo</i> (8 muscles) |
|---------------------|---------------------------------|-----------------------------------|----------------------------------|------------------------------|---------------------------------------|------------------------------|------------------------------------|------------------------------|----------------------------|
| VENTRAL | Interm. posterior | Interm. | Interm. posterior | Interm. posterior | Mylohyoideus | Mylohyoideus | Mylohyoideus | Mylohyoideus | Mylohyoideus |
| | Interm. anterior | --- | Interm. anterior | Interm. anterior | Digastricus anterior | Digastricus anterior | Digastricus anterior | Digastricus anterior | Digastricus anterior |
| | Ad. man. A2 | Ad. man. A2 | Ad. man. A2 | Ad. man. A2 | Masseter | Masseter | Masseter | Masseter | Masseter |
| | --- | --- | --- | --- | Detrahens man. | --- | --- | --- | --- |
| | --- | --- | --- | --- | Temporalis | Temporalis | Temporalis | Temporalis | Temporalis |
| | --- | --- | --- | --- | Pterygoideus lateralis | Pterygoideus lateralis | Pterygoideus lateralis | Pterygoideus lateralis | Pterygoideus lateralis |
| | --- | Ad. man. A2-PVM | Ad. man. A2-PVM | Ad. man. A2-PVM | --- | --- | --- | --- | --- |
| | --- | Retractor ang. oris | --- | Le. anguli oris mandibularis | --- | --- | --- | --- | --- |
| | Ad. mand. A3' | Ad. man. A3' | Pseudotemporalis | Pseudotemporalis | --- | --- | --- | --- | --- |
| | Ad. mand. A3" | --- | --- [but present so.am.] | --- [but present so.rep.] | --- | --- | --- | --- | --- |
| ADDUCTOR MANDIBULAE | --- | --- | --- [but present so.am.] | Pterygomandibularis | --- | --- | --- | --- | --- |
| | --- | --- | --- | --- | Pterygoideus medialis | Pterygoideus medialis | Pterygoideus medialis | Pterygoideus medialis | Pterygoideus medialis |
| | --- | --- | --- | Tensor tympani | Tensor tympani | Tensor tympani | Tensor tympani | Tensor tympani | Tensor tympani |
| | --- | --- | --- | Tensor veli palatini | Tensor veli palatini | Tensor veli palatini | Tensor veli palatini | Tensor veli palatini | Tensor veli palatini |
| | Ad. mand. A4o | --- | --- | Ad. man. A4o | --- | --- | --- | --- | --- |
| | Le. arcus palatini | --- | Le. pterygoidei | Le. pterygoidei | --- | --- | --- | --- | --- |
| | --- | --- | Protractor pterygoidei | Protractor pterygoidei | --- | --- | --- | --- | --- |
| | --- | --- | Le. bulbi | Le. bulbi | --- | --- | --- | --- | --- |
| | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| DORSAL | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | --- | --- | --- | --- | --- | --- | --- | --- | --- |

The nomenclature of the muscles follows that used in the text. Data from evidence provided by our own dissections and comparisons and by a review of the literature. The black arrows indicate the hypotheses that are most strongly supported by the evidence available; the grey arrows indicate alternative hypotheses that are supported by some of the data, but overall they are not as strongly supported by the evidence available as are the hypotheses indicated by black arrows. Ventral, dorsal = Ventral musculature and dorsal constrictor musculature sensu Edgeworth, 1935; ad. = adductor; interm. = intermandibularis; le. = levator; man. = mandibulae; so.am. = in some amphibians; so.rep. = in some reptiles.

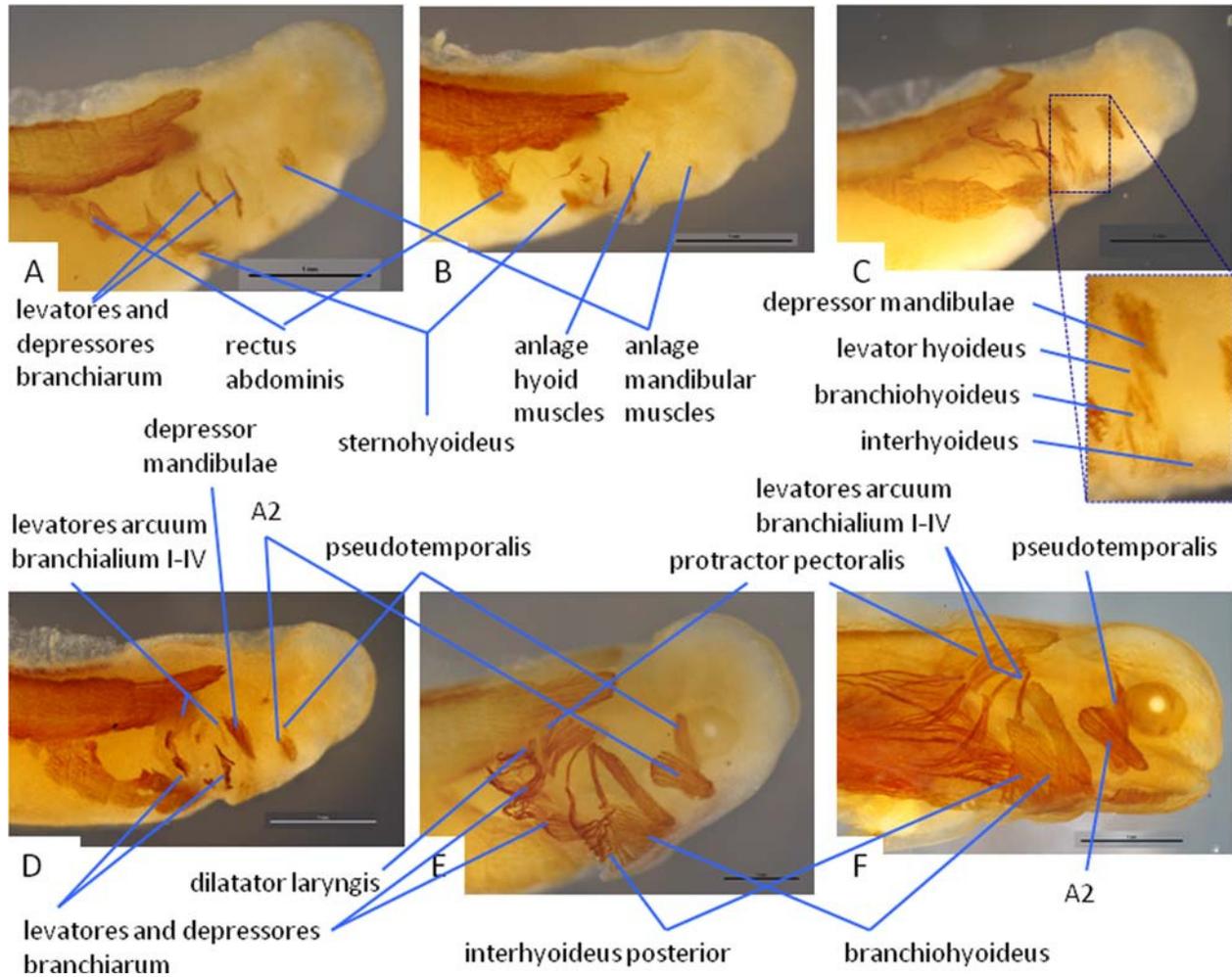


Fig. 1. Lateral views of different stages during development of *Ambystoma mexicanum*. Muscles stained with 12/101 antibody. The muscle differentiation can be observed from anterior (mandibular arch; A) toward posterior (branchial arches; D). The lateral anlage of the

mandibular and hyoid arch splits up in several lateral muscles, while the branchial arch muscles develop more or less one by one. For details see text. Stages: A = 36, B = 36, C = 37, D = 39, E = 40, F = 43. Scale bar = 1 mm.

the pseudotemporalis in the adult, its posterior fibers being particularly blended with the fibers of the A2 (Fig. 1F). Between A2 and A2-PVM runs the mandibular branch of the trigeminal nerve (cranial nerve V3) (Figs. 3B, 4B). The anterior fibers of the A2-PVM in both, the larvae and the adult, look like a part of the pseudotemporalis. In larvae the A2-PVM originates from the palatoquadrate (commissura quadrato-cranialis anterior) and inserts onto Meckel's cartilage. The adductor mandibulae A2 originates in larvae from the palatoquadrate and the orbital cartilage and in the adult from the squamosal (Fig. 4A). During development the A2 changes its orientation from vertical (from dorsal to ventral posterior to the eye) toward diagonal (from caudodorsal to anteroventral). Its insertion is dorsal at Meckel's cartilage (larvae) or dentary (adult) (Fig. 4B,C).

In larvae a small deeper part of the anlage that gives rise to the pseudotemporalis superficialis (corresponding the A3' of fish) gives rise to the pseudotemporalis profundus (corresponding to the A3'' of fish); this anlage is visible from stage 39 onwards (Table 1). During larval

development the pseudotemporalis profundus will become completely integrated in the main body of the pseudotemporalis forming a mainly undifferentiated muscle in the adult. The origin of the pseudotemporalis superficialis moves dorsal during development (Fig. 1D,F): in larvae it originates dorsolaterally from the palatoquadrate (orbital cartilage) and in adults it originates from the midline of the parietal and caudal from the first vertebrae (Figs. 3A and 4A). In larvae, the pseudotemporalis profundus originates ventrolaterally from the palatoquadrate, being blended with from medial fibers of the pseudotemporalis; both portions insert at Meckel's cartilage. In the adult the undivided pseudotemporalis inserts caudodorsally at the dentary.

The levator bulbi is a muscle that is related to the eye and that we only could identify in the adults (Fig. 4A). Superiorly to this muscle runs the maxillary branch of the trigeminal nerve, i.e. cranial nerve V2 (Fig. 4A; see Discussion). Medial to this nerve V2 lies a branch that runs to the levator bulbi, probably innervating it. However, we were not able to confirm if this branch is part

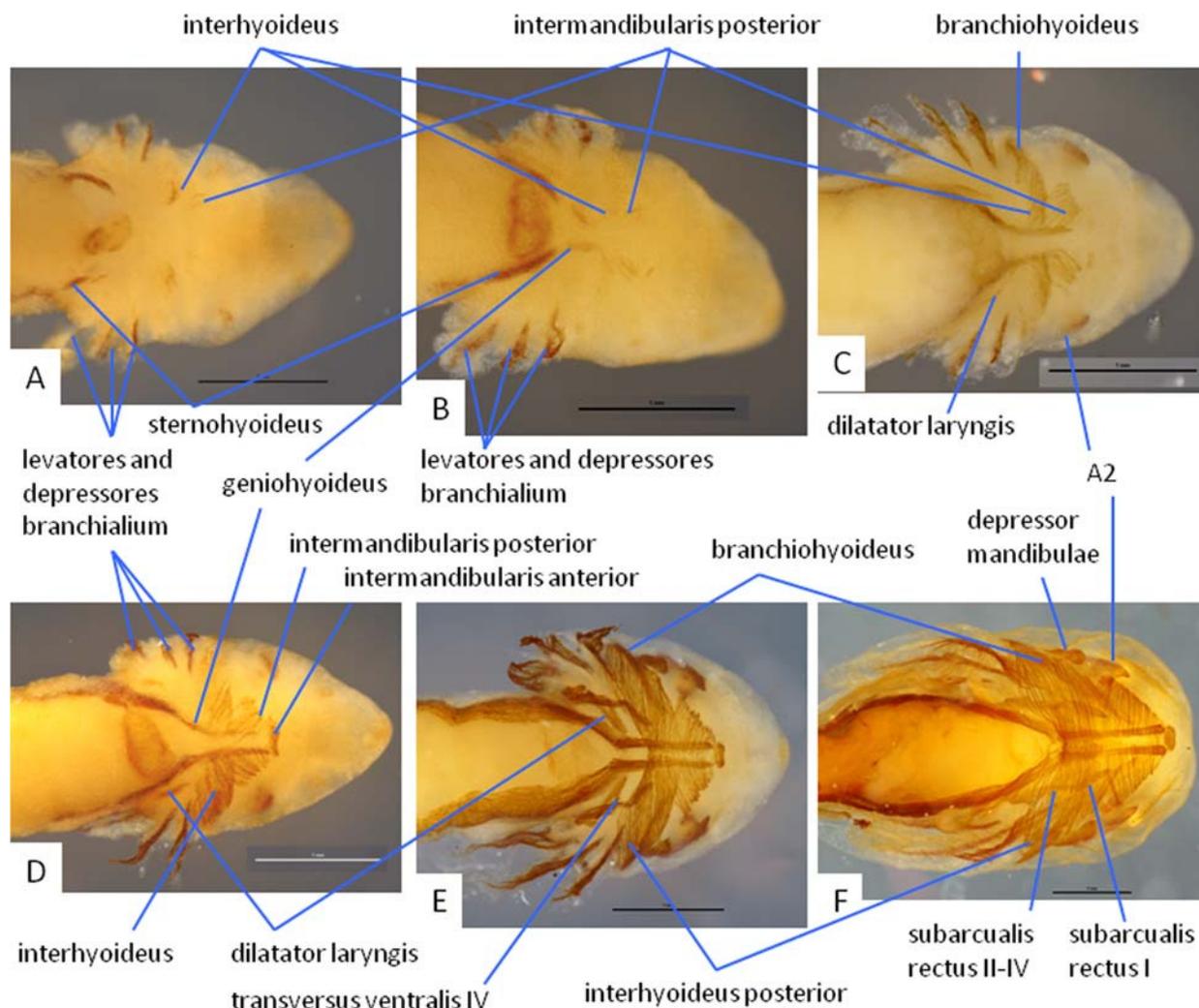


Fig. 2. Ventral views of different stages during development of *Ambystoma mexicanum*. Same specimens as shown in Fig. 1. Many muscles develop from their region of origin toward their future insertion site, which can be best observed in ventral muscles such as the geniohyoideus (Fig. 1B–F). The ventral muscles intermandibularis

posterior and interhyoideus (including interhyoideus posterior) are clearly separated early in development (Fig. 1A–D), but can hardly be distinguished anymore in advanced larvae (Fig. 1E–F). For details see text. Scale bar = 1 mm.

of V2, as it seems to be, or instead the abducens nerve (VI) (see Discussion). The levator bulbi is usually considered to be derived from a dorsal mandibular anlage and an innervation by V2 would support this idea, although the orientation of its fibers in the adult suggests that it could perhaps derive also/exclusively from the anlage of the pseudotemporalis (see Table 3 and Discussion). This is because during development the pseudotemporalis lays close to the posterior edge of the eye and the orientation of the fibers of the deeper layer of the dorsal part of the pseudotemporalis in the adult also appears more to be somewhat similar to that of the fibers of the levator bulbi.

Hyoid Muscles

In a histological section of stage 34 the anlage of the hyoid arch muscles is visible lateral to the buccal cavity

(Table 1). The ventral part of this anlage stretches caudally, later during development forming the anlage of the interhyoideus posterior at stage 38 (Fig. 2D–F). The dorsomedial part (see Table 4) becomes the anlage of the branchiohyoideus, levator hyoideus, and depressor mandibulae (Fig. 1C), this anlage being distinct at stage 38 (Table 1; Fig. 1D–F). Muscle cells are visible in all hyoid muscles at stage 38, in the interhyoideus posterior at stages 38–39; during stage 40 the fiber development increases and by stage 42 all hyoid arch muscles are visible.

The interhyoideus originates from the ceratohyal and meets the contralateral muscle in a median raphe (Fig. 3C). It develops from its origin to its insertion and the anterior part before the posterior part (Fig. 2). An anterior thin part (interhyoideus anterior) and a posterior thicker part (interhyoideus posterior) can be distinguished even if the border between both parts is not always clear (Fig.

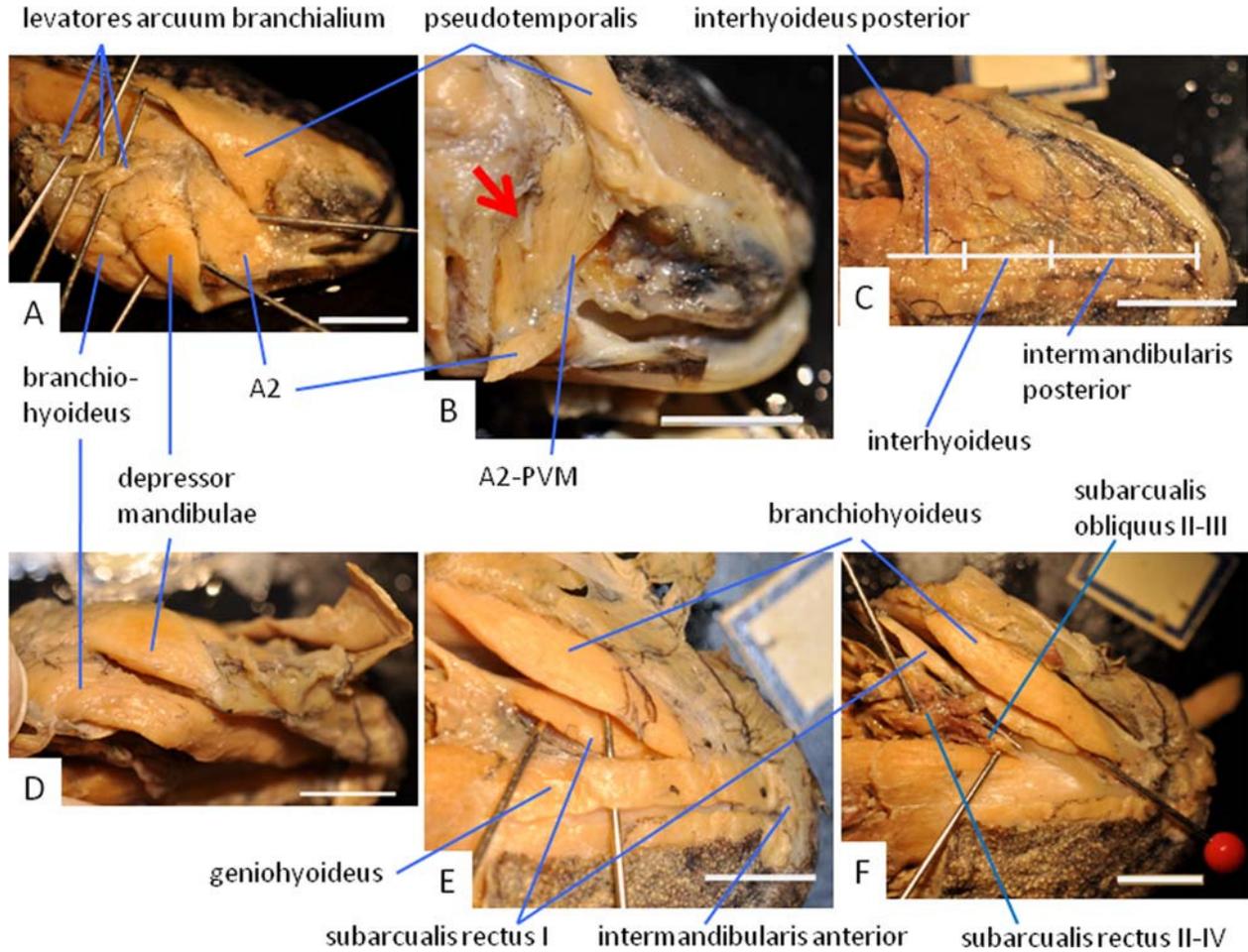


Fig. 3. Dissection of cranial muscles of an adult *Ambystoma mexicanum*. Anterior is on the right in all pictures. **A, B:** right lateral view. **A)** superficial muscles, **B)** to show the A2-PVM, the pseudotemporalis and A2 are flipped dorsally and ventrally, respectively. The red arrow is pointing towards the mandibular branch of the trigeminal nerve (n. V3). **C, E, F:** Ventral views. **C)** All superficial ventral muscles are fused

into one thin sheet and can only be divided by their origins at the mandible (intermandibularis posterior) and the ceratohyal (interhyoideus). **E)** and **F)** deeper ventral muscles. **D:** Ventro-lateral view to show the branchiohyoideus originating laterally and curving posterior to the jaw articulation to insert along the ceratohyal. Scale bar = 1 cm.

2E,F). In larvae, the fibers of the interhyoideus posterior stretch lateral to the depressor mandibulae, ventral to the opercular fold and posteroventrolaterally to the ceratobranchial I. In adults the interhyoideus posterior is mostly restricted to an attachment onto the ceratohyal and only a few fibers still originate laterally at the ceratobranchial and in the opercular fold. The interhyoideus posterior is the thickest portion of the ventral muscle sheet covering the adult head.

In larvae the dorsomedial anlage of the hyoid muscles stretches giving rise to three muscles (Fig. 1C). The branchiohyoideus seems to develop from the caudal ventralmost part of this dorsomedial anlage (Fig. 1C), inserting only onto the ceratohyal (Fig. 2C). Interestingly, during the larval development both origin and insertion are changed. The origin shifts toward the lateral part of the ceratobranchial I (Fig. 1E,F) while the insertion will be ventral from the whole length of the ceratohyal, ventral to the origin of the interhyoideus (Fig. 2E,F). The anlage of the branchiohyoideus is visible at stage 37 (Table 1). Shortly thereafter muscle cells are

visible, followed by fiber development and at the end of stage 42 the muscle is fully developed.

The depressor mandibulae and the levator hyoideus originate from the otic capsule. From the developmental investigation it seems that the ventral fibers of the levator hyoideus continue into the depressor mandibulae, which attaches to the Meckel's cartilage in larvae and to the dentary in the adult (Fig. 1C). The depressor mandibulae extends anteriorly and ends caudal to the anlage of the Meckel's cartilage (stage 38-39). In later stages (43 onwards) there seems to be a turn in the fiber orientation of the depressor mandibulae (Figs. 1F and 4C): the dorsal fibers have a more vertical orientation while the ventral fibers are more diagonal. This could indicate that the dorsomedial fibers still represent the levator hyoideus and the dorsolateral and ventral fibers represent the part of the depressor mandibulae that derives directly from the original depressor mandibulae anlage. By definition, an origin from the otic capsule and an insertion onto the ceratohyal corresponds to the configuration of the levator hyoideus. The dorsomedial fibers of

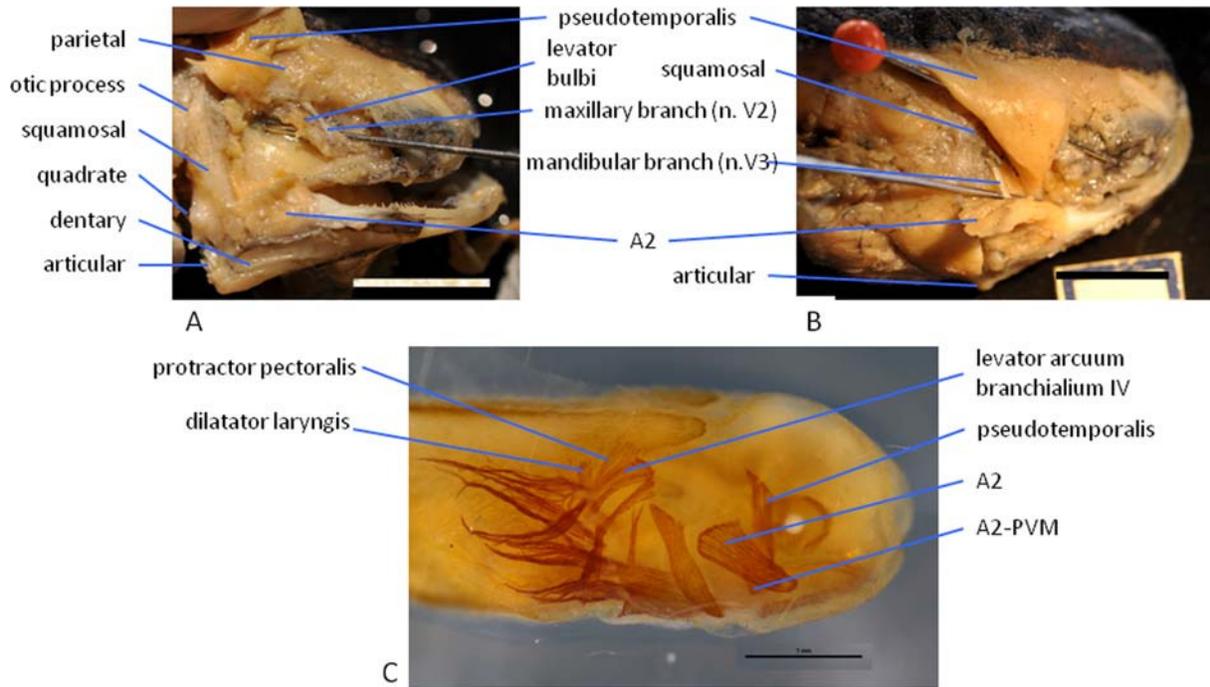


Fig. 4. Lateral views of adult (A, B) and advanced larvae (C). A, B: branches of the trigeminus nerve between the A2 and the A2-PVM (n. V3) and dorsal to the levator bulbi (n. V2, see Discussion). C: Muscles stained with 12/101, stage 44. The protractor pectoralis separates from the posterior fibers of the levator arcuum branchialium IV.

the levator hyoideus do reach the hyoid arch in early stages of development (Fig. 1C), but during development this muscle shifts its insertion completely to the mandible and is hardly, or not at all, distinguished from the depressor mandibulae in later stages (Fig. 1E,F). In stage 44 some medial fibers of the levator hyoideus/depressor mandibulae complex still reach in a steep angle the ceratohyal, which makes this part recognizable as the levator hyoideus. The lateral fibers attach to Meckel's cartilage and are probably a mix of the lateral fibers of the levator hyoideus and the fibers of the depressor mandibulae (see Discussion).

Branchial Muscles

The branchial arch muscles can be divided into the true branchial muscles *sensu stricto*, the protractor pectoralis, and the laryngeal muscles (see Table 5). The anlagen of the levatores arcuum branchialium (LAB) I and II can be identified in stage 37 (Table 1). The LAB III and IV are visible by stage 38. Muscle cells are first visible in LAB I (stage 38), followed by LAB II–IV (stage 38–39) (Fig. 1D). The fiber development in LAB I–IV starts by stage 40, and by stage 42 the LAB I–IV are fully developed (Fig. 1F). They originate from the fascia cephalodorsalis covering the otic capsule and lateral to the epaxial muscles, each inserting onto its respective ceratobranchial. The LAB I–IV develop slightly before the true ventral branchial muscles *sensu stricto* (Fig. 1D, cf. with Fig. 2D,E). These include the anlagen of the subarcualis rectus I, subarcualis rectus II–IV and subarcuali obliqui II and III, which are visible at stage 38

(Table 1). Shortly thereafter there are muscle cells visible in the subarcualis rectus I, followed by subarcualis obliquus II and then subarcualis obliquus III, while at stage 39 the subarcualis rectus II–IV also has visible muscle cells. The subarcualis rectus I attaches from stage 42 onwards onto the ceratohyal. The subarcualis rectus II–IV only reaches toward the ceratobranchial III in stage 40. Fiber development of the subarcualis rectus I starts in stage 40, followed by development of subarcualis obliquus II and III and then of subarcualis rectus II–IV. The anlagen of the levatores and depressores branchiarum are visible at stage 36 (Table 1; Fig. 1A), the first muscle cells being observed at stage 39 and fiber development at stage 42. Fully developed muscles are seen at stage 44. The transversus ventralis IV is visible from stage 40 onwards (Fig. 2E), its fiber development starting at stage 42. Therefore, at the end of stage 42 all true ventral branchial muscles *sensu stricto* are fully developed, although with the 12/101 antibody staining we were not able to identify the subarcualis obliqui muscles that we could identify in the histological sections (see above and Table 1).

The subarcualis rectus I runs from the ceratobranchial I to the ceratohyal in larvae and in the adults. The subarcualis rectus II–IV originates from the ceratobranchial IV and inserts medially onto the ceratobranchial I and ventrally onto the ceratobranchialia II and III. The subarcualis obliquus II and III originate from the ceratobranchialia II and III, respectively. Toward their insertion their fibers fuse, inserting together onto the basibranchial. The levatores and depressores branchiarum I–III originate from the commissurae terminales I, II,

TABLE 5. Updated and completed scheme illustrating the authors' hypotheses regarding the homologies of the branchial, pharyngeal and laryngeal muscles of adults of representative sarcopterygian taxa (see caption of Table 3 and text).

| | <i>Laimeria</i> (4 mus.-not st.) | <i>Lepidosiren</i> (3 mus.-not st.) | <i>Ambystoma</i> (4 mus.-not st. ^a) | <i>Timon</i> (4 mus.-not br. st.) | <i>Ornithorhynchus</i> (11 mus.-not br. st.) | <i>Rattus</i> (18 mus.-not br. st.) | <i>Cynocephalus</i> (17 mus.-not br. st.) | <i>Tapaca</i> (16 mus.-not br. st.) | <i>Homo</i> (15 mus.-not br. st.) |
|---------------------|-------------------------------------|--|--|--------------------------------------|--|--|--|--|--|
| TRUE BRANCHIAL MUS. | Fu. mus.br. ap. | Fu. mus. br. ap. | Fu. mus.br. ap. | Hyobranchialis *Ceratohyoideus* | Ceratohyoideus Subarcualis rectus III Acromiotrapeziius Spinotrapeziius Dorsocutaneous | Stylopharyngeus Ceratohyoideus | Stylopharyngeus Ceratohyoideus | Stylopharyngeus Ceratohyoideus | Stylopharyngeus |
| | | Pro. pectoralis | Pro. pectoralis | Trapezius | Acromiotrapeziius Spinotrapeziius | Acromiotrapeziius Spinotrapeziius | Acromiotrapeziius Spinotrapeziius | Trapezius | Trapezius |
| PHARYNGEAL MUS. | | | | Sternocleidomastoideus | Cleidomastoideus Sternomastoideus | Cleidomastoideus Sternomastoideus | Cleidomastoideus Sternomastoideus | Cleido-occipitalis Cleidomastoideus Sternomastoideus | Sternocleidomastoideus |
| | | | | Co. pharyngis | Co. pharyngis | Co. pharyngis medius | Co. pharyngis medius | Co. pharyngis medius | Co. pharyngis medius |
| | | | | Cricothyroid | Cricothyroid | Co. pharyngis inferior Cricothyroides | Co. pharyngis inferior Cricothyroides | Co. pharyngis inferior Cricothyroides | Co. pharyngis inferior Cricothyroides |
| | | | | | | Co. pharyngis superior Pterygopharyngeus* | Co. pharyngis superior Pterygopharyngeus | Co. pharyngis superior | Co. pharyngis superior |
| | | | | Palatopharyngeus | Palatopharyngeus | Palatopharyngeus | Palatopharyngeus | Palatopharyngeus | Palatopharyngeus |
| | | | | | | Medialis veli palatini Le. veli palatini | Medialis veli palatini Le. veli palatini | Medialis veli palatini Le. veli palatini | Medialis veli palatini Le. veli palatini |
| | | | | | | Salpingopharyngeus | Salpingopharyngeus | Salpingopharyngeus | Salpingopharyngeus |
| | | ?Present? | Co. laryngis | Co. laryngis | | | | | |
| | | | | Laryngeus* | Thyrocricoarytenoideus | Thyroarytenoideus | Thyroarytenoideus | Thyroarytenoideus | Thyroarytenoideus (Vocalis is part of t.a.) |
| | | | | | Arytenoideus | Criccoarytenoideus lateralis Arytenoideus | Criccoarytenoideus lateralis Arytenoideus | Criccoarytenoideus lateralis Arytenoideus | Criccoarytenoideus lateralis Arytenoideus |
| LARYNGEAL MUS. | | Dilatator laryngis | Dilatator laryngis* | Criccoarytenoideus post. | Criccoarytenoideus post. | Criccoarytenoideus post. | Criccoarytenoideus post. | Criccoarytenoideus post. | |

^aLaryngeus, dilatator laryngis and constrictor laryngis, which are present in some *Ambystoma* species, could not be identified in the adult axolotl studied for the present work; the constrictor laryngis and dilatator laryngis are clearly usually present in *A. mexicanum* (see Table 1) but the laryngeus was not identified in the present work either because it is missing in this species or because it is a very small and deep laryngeal muscle. ab. = absent; ap. = apparatus; br. = branchial; co. = constrictor; fu. = functional; le. = levator; mus. = muscles; post. = posterior; pre. = present; pro. = protractor; st = sensu stricto; t.a., thyrohyoideus.

and III and extend to the external gills (Fig. 4C). The transversus ventralis IV originates ventrally from the proximal end of the ceratobranchial IV and extends medially to connect with its contralateral muscle in a medial raphe. In the adults the transversus ventralis IV is a thin muscle sheet originating along the whole ceratobranchial IV. In stage 40 the posterior fibers of the levator arcuum branchialium IV become more separated from the anterior fibers of this muscle and start to differentiate into the protractor pectoralis muscle (Fig. 1E,F; see Discussion). In the adult axolotls the protractor pectoralis is broad and very thin, originating caudal to the head from the fascia cephalodorsalis and attaching onto the scapula.

Regarding the laryngeal muscles, the dilatator laryngis starts as a band of mesodermal cells lateroventrally to the oesophagus. Muscle cells become obvious first in the dilatator laryngis (stages 38-39, Fig. 2D) and then in the constrictor laryngis (stage 39) (Table 1). During stage 39 the dilatator laryngis ends in connective tissue situated ventrolaterally to the oesophagus (Fig. 2D), while the constrictor laryngis only becomes to be distinct at stage 42. The constrictor oesophagi, which is not a laryngeal nor a head muscle by definition (see Table 5), lies near the laryngeal muscles and is visible at stage 38 as ring of mesodermal cell around the oesophagus. The fiber development in the constrictor oesophagi and dilatator laryngis is clearly visible at stage 42, and shortly thereafter starts also in the constrictor laryngis. The dilatator laryngis is fully developed by stage 42, while the constrictor oesophagi is by stage 43 and the constrictor laryngis by stage 45 (Table 1). The dilatator laryngis originates from the fascia cephalodorsalis lateral to the epaxial muscles in close proximity to the protractor pectoralis and the levator arcum branchialium IV (Fig. 1E). It turns ventral and attaches laterally onto the larynx (Fig. 2E). The constrictor laryngis originates from a raphe ventral to the larynx and dorsal to the transversus ventralis IV and inserts ventrally to the dilatator laryngis onto the larynx. The constrictor laryngis could only be observed in the larval histological sections, while the dilatator laryngis could be observed in both the sections and 12/101 antibody staining; the laryngeus, which is present in at least some salamanders and some specimens of the genus *Ambystoma*, could not be identified in the sections nor in the antibody staining (see Tables 1 and 5). The dilatator laryngis, constrictor laryngis, and laryngeus were not observed in the adult specimens.

Hypobranchial Muscles

The hypobranchial muscles are usually considered to be mainly derived from the body musculature, followed by a migration to the ventral region of the head, and are divided into an anterior “geniohyoideus” group and a posterior “rectus cervicis” group (see Table 6). In stage 37 first the sternohyoideus (from “rectus cervicis” group) and then the geniohyoideus (from the “geniohyoideus” group) are visible (Table 1, Fig. 2). In stage 38 the anlage of the geniohyoideus is ventral to the ceratobranchial I anlage, is a band of muscle cells visible dorsally to the interhyoideus anterior at stages 38-39, and reaches the caudal parts of the intermandibularis posterior by stage 40. At stage 38 the muscle cells of the sternohyoideus are visible, and by stage 40 starts the fiber development of both these muscles, which are fully

developed at stage 42. The geniohyoideus develops from its origin at the basibranchial to its insertion onto Meckel’s cartilage and/or dentary, close to the origin of the intermandibularis anterior (Fig. 2). In adults the muscle is wider and closes the gap existing between both contralateral muscles, medially. The sternohyoideus is blended posteriorly to the rectus abdominis, develops lateroventrally (Figs. 1 and 2) toward its insertion onto the hypobranchial, dorsal onto the basibranchial and medial onto the rostral end of ceratobranchial I. The omohyoideus in the adult is deeply blended with the sternohyoideus, from which it derives ontogenetically, extending posterolaterally to attach onto the pectoral girdle (Tables 1 and 6). Other hypobranchial muscles such as the tongue muscles hyoglossus and genioglossus (see Table 6) are missing in both the larvae and the adult.

DISCUSSION

A. mexicanum is considered to be the best model organism to discuss the early evolutionary history of tetrapods. The axolotl larval muscles identified in the present work are in general similar to the descriptions of other salamanders (e.g., Edgeworth, 1935) and to the partial myological descriptions of *A. mexicanum* (e.g., Ericsson and Olsson, 2004; Ericsson et al., 2004; Schmidt et al., 2013). However, our descriptions are more detailed and include information collected from a wide range of methods, which is important because there are, for instance, differences in the observation of timing of muscle appearance using histological sections and antibody staining with the 12/101 antibody (i.e. this antibody stains against myosin, a protein that appears after the anlage of the muscle is visible for a while using other techniques).

There are only a few major topological changes during the development from larvae to the adult *A. mexicanum*. This is expected because the aquatic adults and the larvae have a similar feeding style as the larvae (Lauder and Shaffer, 1985; Lauder and Reilly, 1988). *Ambystoma* species do not go through a complete metamorphosis, i.e. they are neotenic, which explains the occurrence of larval characters in adults such as the presence of branchial arch levators and external gills, and the missing tongue and tongue muscles. Most muscles simply gain muscle mass and change slightly their orientation due to the growth of the bony elements, which leads to an increase in unidirectional pressure forces in adult axolotls (Lauder and Reilly, 1988). For example, the adductor mandibulae muscles, which have in feeding larvae a diagonal orientation between the palatoquadrate and the posterior end of Meckel’s cartilage, have their insertions shifted backwards due to the backward elongation of the lower jaw, leading to a more vertical orientation of those muscles in the adult (Fig. 3). Below we provide a brief discussion of each of the major muscle groups, focusing particularly on those that have more or less clear homologies with the muscles of other vertebrates and/or that are of special importance for the understanding of the early evolutionary history of tetrapods. Tables 3 to 6 provide updated schemes illustrating the authors’ hypotheses regarding the homologies of the mandibular, hyoid, branchial, and hypobranchial muscles of adults of representative sarcopterygian taxa including mammals such as humans.

TABLE 6. Updated and completed scheme illustrating the authors' hypotheses regarding the homologies of the hypobranchial muscles of adults of representative sarcopterygian taxa (see caption of Table 3 and text)

| | <i>Latimeria</i> (2 mus.) | <i>Lepidosiren</i> (2 mus.) | <i>Ambystoma</i> (6 mus. - not in to.)* | <i>Timon</i> (5 mus. - not in. to.) | <i>Ornithorhynchus</i> (6 mus. - not in. to.) | <i>Rattus</i> (8 mus. - not in. to.) | <i>Cynocephalus</i> (7 mus. - not in. to.) | <i>Tupaia</i> (8 mus. - not in. to.) | <i>Homo</i> (9 mus. - not in. to.) |
|--------------------|------------------------------|--------------------------------|--|--|--|---|---|---|---------------------------------------|
| 'GENIOHYOIDEUS' | Coracomandibularis | Coracomandibularis | Geniohyoideus | Geniohyoideus | Geniohyoideus | Geniohyoideus | Geniohyoideus | Geniohyoideus | Geniohyoideus |
| | --- | --- | Genioglossus* | Genioglossus | Genioglossus | Genioglossus | Genioglossus | Genioglossus | Genioglossus |
| | --- | --- | --- | Intrinsic.mus. tongue | Intrinsic.mus. tongue | Intrinsic.mus. tongue | Intrinsic.mus. tongue | Intrinsic.mus. tongue | Intrinsic.mus. tongue |
| | --- | --- | Hyoglossus* | Hyoglossus | Hyoglossus | Hyoglossus | Hyoglossus | Hyoglossus | Hyoglossus |
| | --- | --- | --- | --- | --- | Styloglossus | Styloglossus | Styloglossus | Styloglossus |
| 'RECTUS-CERVICIS?' | --- | --- | Interradialis* | --- | --- | --- | Palatoglossus | --- | Palatoglossus |
| | Sternohyoideus | Sternohyoideus | Sternohyoideus | Sternohyoideus | Sternohyoideus | Sternohyoideus | Sternohyoideus | Sternohyoideus | Sternohyoideus |
| | --- | --- | Omohyoideus | Omohyoideus | Omohyoideus | Omohyoideus | Omohyoideus | Omohyoideus | Omohyoideus |
| | --- | --- | --- | Sternothyroideus* | Sternothyroideus* | Sternothyroideus | Sternothyroideus | Sternothyroideus | Sternothyroideus |
| | --- | --- | --- | --- | --- | Thyrohyoideus | --- | Thyrohyoideus | Thyrohyoideus |

*Genioglossus, hyoglossus, and interradialis, which are present in some salamanders, including some *Ambystoma* species, are not present in the adult and embryos of *A. mexicanum* studied in this work; this is because the members of this species do not develop a tongue.
 'GENIOHYOIDEUS,' 'RECTUS CERVICIS' = 'geniohyoideus' and "rectus cervicis" groups sensu Edgeworth, 1935; in. to. =intrinsic muscles of the tongue; mus. =muscles.

Mandibular Muscles

In some adult amphibians and reptiles the pseudotemporalis is divided into superficial and deep structures that clearly seem to correspond directly to the adductor mandibulae A3' and adductor mandibulae A3'' of fish, respectively (Tables 1; see, e.g., Iordansky, 2010; Diogo and Abdala, 2010). Those two pseudotemporalis portions have been described in several *Ambystoma* species (e.g., Larsen and Guthrie, 1975; Carroll and Holmes, 1980; Iordansky, 1992; Haas, 2001). They are often recognized due to a different fiber orientation and in some cases cannot be separated from each other. Interestingly, the deep bundle seems to derive first from the superficial bundle, and then becomes again integrated into it, forming a single, undivided pseudotemporalis muscle in the adult (Table 1). The relatively short jaw present in axolotl larvae could cause this pattern; with the steep angle of the pseudomandibularis profundus a stronger force to open the mouth can be achieved. This is not necessary in adults, where all the adductor mandibulae muscles have a similar vertical fiber orientation. In summary, both the adductor mandibulae A3' and A3'' are seemingly included, and usually inseparable, in the pseudotemporalis of adult members of extant tetrapod non-mammalian species such as *A. mexicanum*. According to Haas (2001) the deep and superficial pseudotemporalis bundles in axolotl larvae (his "levator mandibulae longus" and "levator mandibulae internus or pseudopterygoideus," respectively; Table 2) correspond to the "levator mandibulae internus" of anurans and to the "levator mandibulae internus" plus "pterygoideus" of caecilians.

The muscle "pterygoideus" that is present in some caudates, sensu Haas (2001), is usually not present as a separate muscle in *Ambystoma*, and seems to be homologous to the "pterygoideus" of caecilians and, thus, to the pterygomandibularis of reptiles, being probably derived from the pseudotemporalis profundus, i.e. from the A3'' of fish (Table 3). Diogo and Abdala (2010) already discussed the problematic use of the name "pterygoideus" to designate this muscle. Iordansky (2010) suggested that the pterygomandibularis of caecilians and of reptiles is directly homologous to the A3'' of fish and the pseudotemporalis profundus of anurans and caudates. However, he then confusingly states that the pterygomandibularis developed from the deep pseudotemporalis/A3'' and thus was acquired homoplastically in caecilians and reptiles. Haas (2001) noted that Iordansky (1996) did not mention the presence of a true "pterygoideus" muscle in a few caudates, i.e. seemingly in addition to the pseudotemporalis profundus. This also supports the observations and comparisons made in the present work, according to which the pterygomandibularis derives from, but is not directly homologous to, the A3'' of fish and thus the pseudotemporalis profundus. As some caudates seem to have such a true "pterygoideus" (pterygomandibularis sensu the present work), it is quite possible that this muscle was present in the last common ancestor (LCA) of tetrapods and then lost in anurans and most caudates, although it is also possible that it was independently acquired in caecilians, in some caudates, and in reptiles (at least three evolutionary steps in both hypotheses).

The A2-PVM of salamanders was suggested to be ontogenetically derived from the A3' and/or A3'' of fishes (Piatt, 1938) or from the fish A2 (Ericsson and Olsson,

2004; Diogo and Abdala, 2010; Table 3). The homologies of the ventral mandibular muscles intermandibularis anterior and posterior are relatively well established (Table 3). However, the homology of the levator bulbi has been a subject of much controversy (see Diogo and Abdala, 2010). It was suggested that this muscle is derived from the adductor mandibulae anlage (Edgeworth, 1935) or from the levator arcus palatini of fish, which is in turn derived from the dorsal mandibular anlage (Brock, 1938; Iordansky, 1996; Diogo and Abdala, 2010; Table 3). Diogo and Abdala (2010) proposed that in the LCA of amphibians + amniotes the dorsal mandibular muscles were already divided into (1) a levator bulbi and (2) an undivided "levator platini."

Our study supports the hypothesis that the levator bulbi is derived from the dorsal mandibular anlage, but also indicates that it might be partially derived from the pseudotemporalis. This is because the position and orientation of the fibers of the levator bulbi are somewhat similar to those of the dorsal portion of the pseudotemporalis profundus of adult axolotls. An origination from the pseudotemporalis would support the idea defended by Edgeworth (1935). However, our study of the nerves of adult axolotls contradicts it. The levator bulbi found in caudates and anurans, and the levator quadrati found in caecilians, are usually innervated by the trigeminal nerve (Ramaswami, 1942; Fox, 1959; Kleinteich and Haas, 2007). Coghill (1902) reported that in *A. tigrinum* the levator bulbi is innervated by the ophthalmic branch of the trigeminal nerve (V1) as well as by the abducens nerve (VI), which normally innervates the eye muscles. That is, the levator bulbi of caudates and anurans could potentially be a complex structure derived from both the anlage of the eye muscles and the anlage of the mandibular muscles, as indicated by our morphological analyses. Francis (1934) and Gaupp (1899) stated the levator bulbi of salamander and frogs is innervated by the maxillary (V2), and not the ophthalmic (V1), branch of the trigeminal nerve. In the adults dissected by us V1 lies superficially (as shown by Piatt, 1939) but parallel to V2 (Fig. 4), and just medial to the main body of V2 is a branch that is far from the mandibular branch (V3) and that goes to the levator bulbi. But we could not confirm that this branch is really part of V2, and not the abducens nerve (VI) or part of it, because this latter nerve usually runs close to V2 and often fuses with it (see, e.g., Ramaswami, 1942). It is, however, clear that in the axolotl adults dissected by us the levator bulbi is not innervated by V1 or V3. Our observations thus give some support to the hypothesis that this muscle derives from the dorsal mandibular anlage (e.g., Diogo and Abdala, 2010), because all the other mandibular muscles of axolotls are exclusively innervated by V3. However, it should be noted that the levator arcus palatini of fish, including coelacanth, is derived from the dorsal mandibular anlage and is usually said to be innervated by V2 but also/or by V3 (e.g., Schilling and Kimmel, 1997; Diogo and Abdala, 2010). It should be noted that the levator bulbi lies deep (so more difficult penetration) and develops late (duration of breeding of the animals) which unfortunately gave us not enough samples of the older stages to resolve the issue of the levator bulbi here. Further detailed studies are clearly needed, and currently planned in our lab, to clarify the evolutionary and developmental origin and innervation of the levator bulbi.

Hyoid Muscles

The ventral muscle sheet in adult axolotl resembles closely the organization for adult *Necturus*, which is also a neotenic salamander (Bauer, 1992). The interhyoideus posterior develops from a separate anlage (Ericsson and Olsson, 2004) and might be not only derived from the hyoid region but possibly also from anterior somites (Piekarski and Olsson, 2007). During metamorphosis in non-neotenic salamanders the first ceratobranchial remodels and at least the dorsal part of it degenerates (Bauer, 1992). As a result the origin is shifted towards other structures as for example the mandible in *Salamandra salamandra* (Francis, 1934). In the adult axolotl the interhyoideus posterior still shows the larval characterization with fibers toward the ceratobranchial I and into the opercular fold, which are clearly neotenic characters. The interhyoideus gave rise to numerous mammalian muscles of facial expression (Diogo and Abdala, 2010; Table 4).

The homologies of the derivatives of the dorsomedial hyoid anlage have been controversial, but are crucial to understand tetrapod origin and evolution because this anlage gives rise to muscles important for the functional morphology of both the hyoid and masticatory apparatuses, of the inner ear and of facial expressions in mammals (Diogo and Abdala, 2010; Table 4). The depressor mandibulae is a single bundle in adult *A. mexicanum*. In other *Ambystoma* and in other salamander species the muscle has anterior and posterior portions (e.g., Diogo, 2008a; Table 4). Diogo (2008a) suggested that the depressor mandibulae anterior largely corresponds to the depressor mandibulae of dipnoans because several salamander species have fibers of the depressor mandibulae posterior that correspond to the levator hyoideus of dipnoans, which also attaches to the mandible. Forey (1986) described that both the levator hyoideus and the depressor mandibulae of extant dipnoans develop from the same ontogenetic anlage. Our study clearly shows that in *A. mexicanum* the larval levator hyoideus becomes completely incorporated in the depressor mandibulae during development, changing its insertion towards the posterior end of the mandible. In *Lissotriton helveticus* and *Ichthyosaura alpestris*, both non-neotenic taxa, some fibers of the larval depressor mandibulae change their orientation to become part of the larval branchiohyoideus (Ziermann, 2008). In caecilians the levator hyoideus is an independent muscle in larvae (e.g., Edgeworth, 1935; Kleinteich and Haas, 2007, 2011) and in some (perhaps in all) adult caecilians it is fused with the depressor mandibulae (Edgeworth, 1935). Both muscles are usually separated in anuran larvae; with the depressor mandibulae often subdivided into suspensorio-, quadrato-, and hyo-angularis, and the levator hyoideus possibly subdivided into suspensorio- and orbito-hyoideus (e.g., Edgeworth, 1935; Olsson et al., 2001; Kleinteich and Haas, 2007; Carroll, 2007).

The problems related to the identification of homologues between the branchiohyoideus and depressores branchiales of caudates and the muscles of anurans were discussed by various researchers (e.g., Haas, 1996; Cannatella, 1999). One hypothesis, mainly based on muscle origins and insertions, is that the salamander branchiohyoideus is homologous to the constrictor branchialis I of frogs (Ziermann, 2008). Schlosser and Roth

(1995) showed that the innervation of the four constrictores branchiales of anurans is through the main branch of the n. glossopharyngeus (N. IX) and through three branchial branches of the n. vagus (N. X). However, the branchiohyoideus is innervated by the n. facialis (N. VII), and therefore it is not likely that this muscle is a homologue of the constrictor branchialis I of anurans (e.g., Diogo and Abdala, 2010). It has also been proposed that the branchiohyoideus could be a caudal portion of the depressor mandibulae (Lightoller, 1939).

The new data obtained in this work indicate that the branchiohyoideus develops from the ventromedial fibers of the depressor mandibulae in *A. mexicanum*, supporting the observations done in *Ichthyosaura alpestris* and *Lissotriton helveticus* by Ziermann (2008). The branchiohyoideus and the other hyoid muscles appear at about the same ontogenetic stages in salamanders (Edgeworth, 1935; Ericsson and Olsson, 2004), which makes it difficult to conclude whether the branchiohyoideus is ontogenetically a derivative from the ventral or the dorsomedial hyoid musculature. The results from Piatt's (1938) study of *A. punctatum* indicate a dorsomedial origin, as does our study of *A. mexicanum*. Interestingly, our study shows that during larval development both the origin and the insertion of the branchiohyoideus are changed and the adult muscle runs from the ceratobranchial I to the ceratohyoideus. Those changes are one of the main reasons that has confused the homology of this muscle and that has erroneously suggested that this could be a branchial muscle.

We therefore support the hypothesis that the "hyomandibularis" (or "subhyoideus" sensu Carroll, 2007) of caecilians corresponds to both the ceratomandibularis ("branchiomandibularis") and branchiohyoideus of caudates (Table 4) (for more details about this hypothesis, see, e.g., Edgeworth, 1935; Carroll, 2007; Kleinteich and Haas 2007, 2011; Diogo and Abdala, 2010). The ceratomandibularis and branchiohyoideus seem both to derive ontogenetically from the same anlage (e.g. Piatt, 1938; Bauer, 1997). In fully metamorphosed caudates the branchiohyoideus is not present as an independent muscle, nor is the ceratomandibularis, which is possibly fused with the depressor mandibulae in some species (Edgeworth, 1935; Eaton, 1936; Bauer, 1997). However, the ceratomandibularis may be present in adult obligate neotene salamanders (e.g., *Ambystoma originarium*: Table 4; Bauer, 1997). The ceratomandibularis or the branchiohyoideus might be missing in adult anurans as an independent structure (Edgeworth, 1935; Jarvik 1963, 1980).

Branchial Muscles

The dilatator laryngis is present in the axolotl larvae studied by us, in full-grown larvae of *A. maculatum* (Piatt, 1939), and in axolotl adults (e.g., Piekarski and Olsson, 2007). These latter authors showed that the dilatator laryngis is at least partially derived from anterior somites. This of course does not mean that the dilatator laryngis is not a head muscle and specifically a branchial muscle (because these authors also showed that the hyoid muscle interhyoideus and the branchial muscles levatores arcuum branchialium are also partially derived from somites). They did, however, not provide detailed information about any somitic contribution

to the other branchial muscles (Table 1). The levator arcuum branchialium IV is anterior to the protractor pectoralis and the dilatator laryngis. In the figures from Piekarski and Olsson (2007) it is clearly shown that the dilatator laryngis crosses the protractor pectoralis dorsally while the protractor pectoralis extends postero-ventrally to attach onto the pectoral girdle. Although the protractor pectoralis and the levator arcuum branchialium IV were shown in close proximity in Piekarski and Olsson (2007), these authors did not refer to a common ontogenetic origin of these two muscles.

The protractor pectoralis (“cucullaris” sensu Edgeworth, 1935) derives at least partially from somites (Piekarski and Olsson, 2007) and from a caudal levator arcus branchialis of plesiomorphic vertebrates and secondarily inserts to the pectoral girdle (Edgeworth, 1935). The evolution of the protractor pectoralis has been the subject of various recent studies, mainly due to its implications for the origin and evolution of the neck in vertebrates (e.g., Diogo and Abdala, 2010; Ericsson et al., 2012). Importantly, our observations clearly show that the protractor pectoralis in *A. mexicanum* derives ontogenetically from the anlage of the levator arcuum branchialium IV (e.g., Figs. 2, 4, and Table 1). The protractor pectoralis is present in adult anurans and caudates, but absent in adult caecilians (Edgeworth, 1935). This is likely related to the loss of the pectoral girdle during evolution towards extant caecilians (e.g., Carroll, 2007). The protractor pectoralis gave rise to the trapezius and sternocleidomastoideus of mammals, in which the only muscle that derives from the anlage of the true branchial muscles sensu stricto is the stylopharyngeus (Table 5).

The constrictor laryngis and dilatator laryngis (Tables 1 and 5) are antagonistic muscles in amphibians (e.g., Duellman and Trueb, 1986), and form, together with the laryngeus, the laryngeal muscles sensu Diogo and Abdala (2010; Table 5). The laryngeus is absent in anurans, but present in caecilian and most caudates (Edgeworth, 1935; Table 5). The laryngeus dorsalis is absent in *Ambystoma* so only the laryngeus ventralis can be found in species of this genus (Edgeworth, 1935). The dilatator laryngis and constrictor laryngis are present in axolotl larvae (Table 1; Piatt, 1939), but we could not identify these muscles in the adult, although Duellman and Trueb (1986) state that the constrictor laryngis is present in adults of all neotenic salamanders. We could also not identify the laryngeus in any specimen, but it is not clear if this is because the muscle is missing or because this is a small and very deep muscle that is usually very difficult to detect (see, e.g., Diogo and Abdala, 2010). Notably, we did not find any cartilages in the laryngeal region, an observation in line with Tucker (1993) but contradicted by Sasaki (2006), who states that lateral cartilages may be observed in certain amphibians such as axolotls in which they form bars for the attachment of the dilatator laryngis. Piatt (1939) shows all three muscles in a full grown larvae of *Ambystoma maculatum*.

Hypobranchial Muscles

Diogo and Abdala (2010 and citations within) discussed the homologies of the hypobranchial muscles. Edgeworth (1935) divides the hypobranchial muscles

into a “geniohyoideus” group and a “rectus cervicis” group (Table 6). The coracomandibularis of extant sarcopterygian fish is included in the former group and the sternohyoideus in the latter group sensu Edgeworth (1935). Our study shows that the tongue and tongue muscles (genioglossus, hyoglossus, and interradialis in amphibians) are absent in *A. mexicanum* (Table 6). A hypobranchial muscle interradialis, probably derived from the genioglossus, is present in at least some *Ambystoma* (Piatt, 1938; Table 6). The “hyoglossus” of some caudates, however, might at least partially derive from the sternohyoideus (e.g., Edgeworth, 1935) and is absent as an independent structure in caecilians following Duellman and Trueb (1986).

The omohyoideus in caudates seems to be homologous to that of anurans (Edgeworth, 1935; Diogo and Abdala, 2010) and is also present in the adult axolotl according to our new study, although it is partially blended with the sternohyoideus (Table 1). The pectoral girdle is absent in caecilians and so is the omohyoideus (Diogo and Abdala, 2010), but not the sternohyoideus (e.g., Edgeworth, 1935; Kleinteich and Haas, 2007).

CONCLUDING REMARKS

The confusion about the homology and evolution cranial muscles in amphibians comes partly from the fact that many larval muscles disappear/are fused during development and particularly during metamorphosis. Even in a neotenic species such as *A. mexicanum*, lacking a full metamorphosis and showing many larval characters (Fig. 3), some muscles become completely indistinct during ontogeny: for instance, the pseudotemporalis profundus and the levator hyoideus become completely integrated in the pseudotemporalis superficialis and in the depressor mandibulae, respectively (Table 1). Furthermore, muscles that are often considered as distinct, such as the intermandibularis posterior and the interhyoideus, can in reality only be distinguished by their attachments in adults (Fig. 1C). This developmental trend contradicts the commonly accepted view that during ontogeny the tendency is almost always toward the differentiation, and not the undifferentiation, of muscles. Recent studies in modern humans have provided similar data and also contradicted this latter view (Diogo and Wood, 2012).

We found three main morphogenetic trends in the ontogeny of the axolotl cranial muscles. First, the order of the cranial muscles development mainly goes from anterior to posterior (Table 1). The muscle anlagen can be observed in histological stained embryos from stage 34 onwards; the first anlage to be observed in the 12/101 stained specimens is also the mandibular one (Fig. 1A, stage 36), followed immediately by the hyoid anlage (Fig. 1B, stage 36), and then by the branchial muscles (Fig. 1C, stage 37) including the laryngeal muscles (Fig. 1D, stage 39). Second, the larval cranial muscle development shows an outside-in pattern with more lateral muscles developing in general before more medial muscles (Fig. 1). Thirdly, several muscles develop from their origin toward their insertion (e.g., the development of the geniohyoideus; Fig. 2).

With the detailed data about the muscle development, it is possible to compare the order in which the muscles appeared during axolotl ontogeny and during phylogeny.

According to Diogo et al. (2008b), in the case of the zebrafish head muscles there is in general a parallelism between ontogeny and phylogeny, with however a few differences regarding, e.g., the early ontogenetic appearance of muscles that evolved late in phylogeny but that play a particularly important role in the feeding mechanisms of the zebrafish. In the axolotl studied in the present work, there is also in general such a parallelism.

Regarding the mandibular muscles, from stage 34 were two anlagen distinguished: the ventral one of the intermandibularis, and a dorso-medial one including the A2 (A2/A2-PVM) and the A3 (pseudotemporalis superficialis/profundus, mainly corresponding to A3'/A3''). In stage 37 those dorso-medial muscles are already differentiated and three main events follow: (1) the differentiation of the intermandibularis anterior from the intermandibularis muscle mass (late stage 38); (2) the differentiation of the pseudotemporalis profundus from the main A3 muscle mass (stage 39); and (3) the differentiation of the levator bulbi (Table 1). These events do follow the phylogenetic order of events, because a division into intermandibularis anterior and posterior is present in early gnathostomes, while a division between A3' and A3'' (which mainly give rise to the pseudotemporalis superficialis and to the pseudotemporalis profundus, respectively) is only present in osteichthyans and the levator bulbi is only present in tetrapods (Diogo and Abdala, 2010).

Concerning the hyoid muscles, from stage 34 where the anlagen of the interhyoideus and levator hyoideus/depressor mandibulae are already differentiated, there are two main events: (1) the differentiation of the branchiohyoideus (early stage 38); and (2) the differentiation of the interhyoideus posterior (later stage 38; Table 1). This order of events does not follow the order of phylogenetic events, because the division of the interhyoideus into bundles is already present in early gnathostomes, while the branchiohyoideus is only present in amphibians (Diogo and Abdala, 2010).

The true branchial muscles *sensu stricto* are already present in early vertebrates such as lampreys, while the protractor pectoralis appears later, in gnathostomes, and the laryngeal muscles seemingly later, in osteichthyans, although there are not many data available about these latter muscles in fish (Diogo and Abdala, 2010). We do not have detailed data about the timing of differentiation of the protractor pectoralis in *A. mexicanum*, but it is clear that this muscle, as well as the laryngeal muscles dilatator laryngis and constrictor laryngis, also appear later in development than the true branchial muscles *sensu stricto* (Table 1).

Regarding the hypobranchial muscles, the sternohyoideus appears at early stage 37 and the geniohyoideus at late stage 37 (Table 1). Both the sternohyoideus and coracomandibularis were present in the last common ancestor (LCA) of gnathostomes, and the geniohyoideus mainly derives from the coracomandibularis, although the geniohyoideus itself appeared only in tetrapods (Table 6).

If we compare the ontogeny and phylogeny of the cranial muscles as a whole, we can list the major sequence of ontogenetic differentiations and the respective times of phylogenetic origin, as: (1) sternohyoideus (early 37; LCA of gnathostomes); (2) geniohyoideus (late 37; tetrapods, but mainly corresponds to coracomandibularis which

evolved at LCA of gnathostomes); (3) branchiohyoideus (early 38; amphibians); (4) dilatator laryngis (mid 38; early osteichthyans); (5) and 6) interhyoideus posterior and intermandibularis anterior (late 38; early gnathostomes); (7) pseudotemporalis profundus (39; mainly corresponds to A3'' which evolved in LCA of osteichthyans); (8) constrictor laryngis (early osteichthyans); (9) levator bulbi (tetrapods). Within these nine ontogenetic events, only the differentiation of branchiohyoideus (3) and of dilatator laryngis (4), directly contradict the order of phylogenetic events. These numbers are similar to those obtained by Diogo et al., (2008b) in the zebrafish and point out that in at least the case of the zebrafish and of the axolotl, the ontogeny of cranial muscle ontogeny parallels, in general, the order in which these muscles evolved in phylogeny, with just a few, but probably functionally and ontogenetically important (see Diogo et al., 2010), exceptions.

We hope that this detailed description of the model organism *A. mexicanum* paves the way for future comparative evolutionary developmental works. In this regard, we plan to investigate the larval development and adult morphology of the cranial muscles of representatives of other caudate taxa as well as of anuran and caecilian taxa. This will allow us to address crucial questions regarding the origin, homology and evolution of the muscles of the amphibia, the evolutionary history of the tetrapods as a whole and, importantly, the origin of the ground-plan of reptiles and mammals.

ACKNOWLEDGEMENTS

The authors are particularly thankful to Nadine Piekarski for providing *Ambystoma mexicanum* embryos and larvae for histological staining for previous parts of this project, and to Richard Elinson for providing 12/101 stained embryos and larvae of this species. The authors also thank the anonymous reviewers for their suggestions to improve this article.

LITERATURE CITED

- Bauer WJ. 1992. A contribution to the morphology of the m. interhyoideus posterior (VII) of urodele Amphibia. *Zool Jb Anat* 122: 129–139.
- Bauer WJ. 1997. A contribution to the morphology of visceral jaw-opening muscles of urodeles (Amphibia: Caudata). *J Morphol* 233: 77–97.
- Böck P. 1989. *Romeis Mikroskopische Technik*. Urban and Schwarzenberg, München, Wien; Baltimore.
- Bordzilovskaya NP, Dettlaff TA, Duhon ST, Malacinski GM. 1989. Developmental-stage series of axolotl embryos. In: Armstrong JB, Malacinski GM, editors. *Developmental biology of the Axolotl*. New York: Oxford University Press. p 201–219.
- Brock GT. 1938. The cranial muscles of the *Gecko*—a general account with a comparison of muscles in other gnathostomes. *Proc Zool Soc Lond Ser B* 108:735–761.
- Cannatella DC. 1999. 4. Architecture: cranial and axial musculoskeleton. In: McDiarmid RW, Altig R, editors. *Tadpoles—the biology of Anuran Larvae*. Chicago: The University of Chicago Press. p 52–81.
- 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. *Zool J Linn Soc* 68:1–40.
- Coghill GE. 1902. The cranial nerves of *Amblystoma tigrinum*. *J Comp Neurol* 12:205–289.

- 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.
- Diogo R. 2007. Origin and evolution of higher clades: osteology, myology, phylogeny and macroevolution of bony fishes and the rise of tetrapods. Enfield: Science Publishers.
- Diogo R. 2008. Comparative anatomy, homologies and evolution of mandibular, hyoid and hypobranchial muscles of bony fish and tetrapods: a new insight. *Anim Behav* 6:1–50.
- Diogo R, Abdala V, Lonergan N, Wood BA. 2008a. From fish to modern humans—comparative anatomy, homologies and evolution of the head and neck musculature. *J Anat* 213:391–424.
- Diogo R, Hinits Y, Hughes S. 2008b. Development of mandibular, hyoid and hypobranchial muscles in the zebrafish, with comments on the homologies and evolution of these muscles within bony fish and tetrapods. *BMC Dev Biol* 8:24–46.
- Diogo R, Abdala V. 2010. Muscles of vertebrates—comparative anatomy, evolution, homologies and development. Enfield, New Hampshire: CRC Press.
- Diogo R, Tanaka EN. 2012. Anatomy of the pectoral and forelimb muscles of wildtype and GFP-transgenic axolotls and comparison with other tetrapods including humans: a basis for regenerative, evolutionary and developmental studies. *J Anat* 221:622–635.
- Diogo R, Wood BA. 2012. Violation of Dollo's law: evidence of muscle reversions in primate phylogeny and their implications for the understanding of the ontogeny, evolution and anatomical variations of modern humans. *Evolution* 66:3267–3276.
- Duellman WE, Trueb L. 1986. The biology of amphibians. New York: McGraw-Hill Book Company.
- Edgeworth FH. 1935. The cranial muscles of vertebrates. Cambridge, London: University Press.
- Ericsson R, Cerny R, Falck P, Olsson L. 2004. The role of cranial neural crest cells in visceral arch muscle positioning and morphogenesis in the Mexican Axolotl, *Ambystoma mexicanum*. *Dev Dyn* 231: 237–247.
- Ericsson R, Olsson L. 2004. Patterns of spatial and temporal visceral arch muscle development in the Mexican Axolotl (*Ambystoma mexicanum*). *J Morphol* 261:131–140.
- Ericsson R, Knight R, Johanson Z. 2012. Evolution and development of the vertebrate neck. *J Anat* 222: 67–78.
- 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.
- Forey PL. 1986. Relationships of lungfishes. *J Morphol (Suppl)* 1: 75–91.
- Fox H. 1959. A study of the development of the head and pharynx of the larval urodele *Hynobius* and its bearing on the evolution of the vertebrate head. *Philos Trans R Soc London* 242:151–204.
- Francis ETB. 1934. IV The muscles. In: Francis ETB, editor. The anatomy of the salamander. Oxford. The Clarendon Press. p 48–75.
- Gaupp E. 1899. Ecker's and R. Wiedersheim's anatomie des frosches. Vol. 2. Braunshweig: Vieweg. p 128–159.
- Haas A. 1996. Das larvale Cranium von *Gastrotheca riobambae* und seine Metamorphose (Amphibia, Anura, Hylidae). *Verh Naturwiss Ver Hamburg* 36:33–162.
- 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.
- 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 Amph Res Former Sov Union* 1: 3–26.
- Iordansky NN. 2010. Pterygoideus muscles and other jaw adductors in amphibians and reptiles. *Biol Bull* 37:905–914.
- Jarvik E. 1963. The composition of the intermandibular division of the head in fishes and tetrapods and the diphyletic origin of the tetrapod tongue. *Kungl Sven Vetensk Handl* 9:1–74.
- Jarvik E. 1980. Basic structure and evolution of vertebrates. London: Academic Press.
- Kintner CR, Brockes JP. 1985. Monoclonal antibodies identify blastemal cells derived from dedifferentiating muscle in newt limb regeneration. *Nature* 308:67–69.
- Kleinteich T, Haas A. 2007. Cranial musculature in the larva of the caecilian, *Ichthyophis kohlaensis* (Lissamphibia: Gymnophiona). *J Morphol* 268:74–88.
- Kleinteich T, Haas A. 2011. The hyal and ventral branchial muscles in caecilian and salamander larvae: homologies and evolution. *J Morphol* 272:598–613.
- Larsen JH, Guthrie DJ. 1975. The feeding system of terrestrial tiger salamanders (*Ambystoma tigrinum melanostictum* Baird). *J Morphol* 147:137–154.
- Lauder GV, Shaffer HB. 1985. Functional morphology of the feeding mechanism in aquatic ambystomatid salamanders. *J Morphol* 185:297–326.
- Lauder GV, Reilly SM. 1988. Functional design of the feeding mechanism in salamanders: causal bases of ontogenetic changes in function. *J Exp Biol* 134:219–233.
- Lightoller GHS. 1939. Probable homologues. A study of the comparative anatomy of the mandibular and hyoid arches and their musculature. Part I. Comparative myology. *Trans Zool Soc London* 24: 349–402.
- Lubosch W. 1914. Vergleichende Anatomie der Kaumuskeln der Wirbeltiere, in fünf Teilen. Erster Teil: Die Kaumuskeln der Amphibien. *Jen. Z. Naturwiss* 53:51–188.
- 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.
- Lynn WG. 1961. Types of amphibian metamorphosis. *Am Zool* 1: 151–161.
- Nye HLD, Cameron JA, Chernoff EAG, Stocum DL. 2003. Extending the table of stages of normal development of Axolotl: Limb development. *Dev Dyn* 226:555–560.
- Olsson L, Falck P, Lopez K, Cobb J, Hanken J. 2001. Cranial neural crest cells contribute to connective tissue in cranial muscles in the anuran amphibian, *Bombina orientalis*. *Dev Biol* 237: 354–367.
- Piatt J. 1938. Morphogenesis of the cranial muscles of *Amblystoma punctatum*. *J Morphol* 63:531–587.
- Piatt J. 1939. Correct terminology in salamander myology. I. Intrinsic gill musculature. *Copeia* 4:220–224.
- Piekarski N, Olsson L. 2007. Muscular derivatives of the cranial-most somites revealed by long-term fate mapping in the mexican axolotl (*Ambystoma mexicanum*). *Evol Dev* 9:566–578.
- Ramaswami LS. 1942. The segmentation of the head of *Ichthyophis glutinosus* (Linné). *Zool Soc Lond* 112:105–112.
- Sasaki CT. 2006. Review: Part 1. Oral cavity, pharynx and esophagus. Anatomy and development and physiology of the larynx. *GI Motility online* (2006) doi:10.1038/gimo7.
- Schilling TF, Kimmel CB. 1997. Musculoskeletal patterning in the pharyngeal segments of the zebrafish embryo. *Development* 124: 2945–2960.
- Schlosser G, Roth G. 1995. Nerves in tadpoles of *Discoglossus pictus*: distribution of cranial and rostral spinal nerves in tadpoles of the frog *Discoglossus pictus* (Discoglossidae). *J Morphol* 226: 189–212.
- Schmidt J, Piekarski N, Olsson L. 2013. Cranial muscles in amphibians: development, novelties and the role of cranial neural crest cells. *J Anat* 222:134–146.
- Tucker HM. 1993. The larynx. 2nd ed. New York: Thieme.
- Ziermann JM. 2008. Evolutionäre Entwicklung larvaler Cranialmuskulatur der Anura und der Einfluss von Sequenzheterochronien. Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum. PhD Thesis. Jena: Friedrich Schiller University. p 347.
- Ziermann JM, Olsson L. 2007. Patterns of spatial and temporal cranial muscle development in the African Clawed Frog, *Xenopus laevis* (Anura: Pipidae). *J Morphol* 268:791–804.