

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/269109330>

Development, Metamorphosis, Morphology and Diversity: The Evolution of Chordate muscles and the Origin of Vertebrates

Article in *Developmental Dynamics* · December 2014

DOI: 10.1002/dvdy.24245

CITATIONS

22

READS

1,584

2 authors:



Rui Diogo

Howard University

351 PUBLICATIONS 4,723 CITATIONS

[SEE PROFILE](#)



Janine M. Ziermann

Howard University

116 PUBLICATIONS 877 CITATIONS

[SEE PROFILE](#)

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



Diversity and Evolution of Gymnotiformes [View project](#)



Comparative vertebrate musculoskeletal anatomy [View project](#)

Development, Metamorphosis, Morphology, and Diversity: The Evolution of Chordate Muscles and the Origin of Vertebrates

Rui Diogo* and Janine M. Ziermann*

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

Recent findings that urochordates are the closest sister-group of vertebrates have dramatically changed our understanding of chordate evolution and vertebrate origins. To continue to deepen our understanding of chordate evolution and diversity, in particular the morphological and taxonomical diversity of the vertebrate clade, one must explore the origin, development, and comparative anatomy of not only hard tissues, but also soft tissues such as muscles. Building on a recent overview of the discovery of a cardiopharyngeal field in urochordates and the profound implications for reconstructing the origin and early evolution of vertebrates, in this study we focus on the broader comparative and developmental anatomy of chordate cephalic muscles and their relation to life history, and to developmental, morphological and taxonomical diversity. We combine our recent findings on cephalochordates, urochordates, and vertebrates with a literature review and suggest that developmental changes related to metamorphosis and/or heterochrony (e.g., peramorphosis) played a crucial role in the early evolution of chordates and vertebrates. Recent studies reviewed here supported de Beer's "law of diversity" that peramorphic animals (e.g., ascidians, lampreys) are taxonomically and morphologically less diverse than nonperamorphic animals (e.g., gnathostomes), probably because their "too specialized" development and adult anatomy constrain further developmental and evolutionary innovations. *Developmental Dynamics* 00:000–000, 2015. © 2015 Wiley Periodicals, Inc.

Key words: amphioxus; cephalochordates; urochordates; cyclostomes; gnathostomes; life history; heterochrony; paedomorphosis; peramorphosis

Submitted 14 October 2014; First Decision 29 November 2014; Accepted 1 December 2014; Published online 5 December 2014

Introduction

Most classic works or evo-devo studies published in the last decades on the evolution of chordates and origin of vertebrates included detailed accounts of the musculature of cephalochordates, urochordates, and vertebrates. For instance, the defenders of a segmentation origin of the vertebrate head argue that the anterior somites of an amphioxus-like animal gave rise to vertebrate head cavities and eye and jaw muscles (Holland et al., 2008; Holland, 2015). However, they often do not take into account that apart from the somites, both the amphioxus (cephalochordate) larvae and adults have a complex orobranchial musculature that is in many ways similar, developmentally and anatomically, to the vertebrate branchiomic musculature (Fig. 1; see below). Now is a particularly appropriate time for a multi-disciplinary review of the origin and evolution of the vertebrate head, and the head muscles in particular. Evo-devo has become a central area in biological sciences, combining fields as diverse as genetics and developmental, evolutionary, and comparative biology and precipitating an exponential increase in the interest in

muscle development (Diogo et al., 2008; Diogo and Abdala, 2010). One result of this increased interest was the discovery of a cardiopharyngeal field which gives rise to the heart and to a subset of head muscles in vertebrates and is also present in urochordates, now broadly accepted to be the closest extant relatives of vertebrates (recently reviewed by Diogo et al., 2015). In the current study, we focus instead on subjects that are deeply related to the main subject of this *Developmental Dynamics Special Issue*: the broader comparative and developmental anatomy of the cephalic muscles of chordates and its relation to life history and development—namely to metamorphosis and heterochrony—as well as with morphological and taxonomical diversity.

Recent Findings About the Origin and Evolution of the Vertebrate Head and its Muscles

Data obtained in the last decades about the origin and early evolution of the vertebrate head and head muscles can be divided into three major categories: those that support ideas developed in

*Correspondence to: Rui Diogo, Department of Anatomy, Howard University College of Medicine, 520 W Street NW, Washington, DC, 20059.
E-mail: rui.diogo@howard.edu

Article is online at: <http://onlinelibrary.wiley.com/doi/10.1002/dvdy.24245/abstract>
© 2015 Wiley Periodicals, Inc.

the last decades of the 20th century (e.g., Gans and Northcutt, 1983), those that revive earlier ideas, and those that present new, and often surprising, scenarios. As an example of the first category, paleontological studies support the idea that a head skeleton composed of cartilage and calcified tissues derived from neural crest and sclerotomal mesoderm is an ancestral vertebrate feature (e.g., Valentine, 2004). However, these studies also revealed specific evolutionary changes that differ markedly from previous assumptions; for example, the first gnathostomes (e.g., placoderms) probably possessed not only calcified endochondral bones, but also dermal bones (e.g., maxillary) similar to those found only in extant bony fish (osteichthyans) (Zhu et al., 2013). Moreover, the specific phenotypic changes involving the evolution of the head skeleton are still heatedly debated. For instance, Mallat's (2008) neoclassical hypothesis for the origin of the vertebrate jaw is more conservative in assuming that the upper lip and its muscles in sharks are homologous with those of lampreys, while the heterotopic hypothesis by Kuratani et al. (2013) assumes that the upper lip was lost in gnathostomes and acquired de novo in some gnathostome groups, such as sharks.

The second category of data includes a surprising revival of ideas defended by classical authors such as Goodrich, Garstang, Gegenbaur, Edgeworth, and even Darwin. Some of these ideas were widely accepted in the late 19th and/or early 20th centuries, but they were largely abandoned during the second half of the 20th century. They include (1) the sister-group relationship between urochordates and vertebrates (Delsuc et al., 2006), previously defended by authors such as Garstang (1928) and Darwin (1871); (2) Gegenbaur's (1878) hypothesis that the pectoral appendage (girdle + fin) originated as an integral part of the head (Gillis et al., 2009) and, therefore, that the pectoral appendage evolved independently of, and may not be serially homologous to, the pelvic appendage (Diogo et al., 2013; Diogo and Tanaka, 2014; Diogo and Ziermann, 2014); and (3) Edgeworth's (1935) hypothesis that the cucullaris derivatives (e.g., trapezius) derive from the branchiomic musculature and/or follow a head program (e.g., Piotrowski and Nusslein-Volhard, 2000; Diogo and Abdala, 2010; Sambasivan et al., 2011; Minchin et al., 2013).

The third category comprises new and mostly unexpected scenarios. For instance, contrary to what was usually accepted in the 1980s, cranial neural crest cells, while giving rise to numerous skeletal elements of the head and serving as precursors for connective tissue and tendons, do *not* form muscles (Noden, 1983, 1986; Noden and Francis-West, 2006). Instead, the mesoderm-derived muscle progenitors fuse together to form myofibers within cranial neural crest-derived connective tissue in a precisely coordinated manner. Muscles of a certain arch are usually associated with connective tissue, and through this tissue also with skeletal elements, of the same arch (Köntges and Lumsden, 1996). As noted above, another remarkable discovery was that of a cardiopharyngeal field and specifically of data suggesting that some branchiomic muscles are more closely related to certain heart muscles than to other branchiomic muscles (Lescroart et al., 2010; Lescroart and Meilhac, 2012; reviewed by Diogo et al., 2015). Another surprising finding is that the first (mandibular) arch probably was not part of the original series of branchial arches. Instead, the most rostral branchial arch of basal chordates such as cephalochordates and "prevertebrate" fossils such as *Halkouella* is thought to correspond to the second (hyoid) branchial arch of vertebrates (Mallatt, 2008). According to this idea, the first arch was incorporated into the branchial arches only in more

derived chordates, which explains why it is the only arch in vertebrates in which *Hox* genes are not expressed and do not pattern arch formation (Mallatt, 2008; see below). Based on a comprehensive review of the recent literature, Miyashita's (2015; personal communication) "Mandibular Confinement Theory" specifically proposes that the gnathostome jaw evolved by means of developmental spatial confinement of ancestral anterior (oral) chordate structures; due to this confinement these structures acquired/co-opted, in vertebrates, the genetic/patterning programs that operate in the more posterior arches, thus forming a mandibular arch resembling these arches (Miyashita, 2015; personal communication). Also falling into the third category is the surprising evidence that appendicularians are the most basal extant urochordates, although the phylogeny of urochordates is still controversial and not all authors accept such a basal position of appendicularians (e.g., Valentine, 2004; Lemaire, 2011) (Fig. 1). Together with the revival of the Ambulacraria (e.g., Gee, 2001) and support for a urochordate-vertebrate clade, these data significantly change the phylogenetic framework used for decades to discuss the origin and early evolution of the vertebrate head and its muscles. Based on the cladogram shown in Figure 1, the last common ancestor (LCA) of chordates, urochordates and vertebrates was probably pelagic, mobile, and symmetrical, as are adult appendicularians (e.g., Vargas and Aboitiz, 2005). Therefore, at least some of the features of the peculiar sessile ascidian adults and of asymmetric cephalochordate larvae, which lack an atrium, very likely result from addition of ontogenetic stages late (peramorphosis) and early in development, respectively. The pelagic adult LCA of chordates might have evolved from larvae similar to those that give rise to the benthic ambulacrarian adults, and the notochord and expanded branchial region of chordates may be adaptations for swimming and feeding (Valentine, 2004). This new information, together with molecular evidence supporting the monophyly of cyclostomes (lampreys + hagfish; Heimberg et al., 2010) and the results of recent myological cyclostome studies, profoundly changes the reconstruction of the LCA of vertebrates, the LCA of cyclostomes, and the LCA of gnathostomes, and thus had profound implications for our understanding on the development and evolution of vertebrates, as will be explained in the Sections below.

Surprising Recent Discoveries in Urochordates

For a long time, it was accepted that one of the main differences between vertebrates and invertebrates is that vertebrates possess complex sense organs and associated cranial ganglia, while non-vertebrate chordates have poorly specialized sense organs and no neurogenic placodes (e.g., Gans and Northcutt, 1983). However, studies performed in the last three decades, particularly on urochordates, strongly contradict this scenario. Apart from the discovery of a cardiopharyngeal field in urochordates, the results of these studies have shown that urochordates also have placodes and neural crest-like cells, as recently summarized by Graham and Shimeld (2013) and Hall and Gillis (2013) (Fig. 1). The points made by these latter authors are briefly summarized below.

For example, some fossil and neontological data suggest that the ascidian atrium, which begins as an invagination in the posterior part of the larval brain and develops at metamorphosis into an outflow chamber connected to the atrial siphon, is

homologous with the vertebrate otic placode (Jefferies, 1986). Recent evo-devo studies supported Jefferies' conclusion, e.g., by revealing that gene expression in ascidian oral and atrial siphon primordia is similar to that in vertebrate placodes. Generic placode markers of the *Sir* and *Eya* gene families are expressed by both oral and atrial siphon primordia in the ascidian *Ciona* (Mazet et al., 2005); in addition, oral siphon primordium also expresses the anterior placode markers *Pitx* and *Dlx* and atrial siphon primordium expresses the posterior placode markers *FoxI* and *Pax2/5/8* (Graham and Shimeld, 2013). Some of the genes associated with the atrial primordia are also expressed in the developing pharyngeal slits of vertebrates, cephalochordates, and hemichordates. Based on this and other evidence, including recent studies on appendicularian urochordates and cephalochordates, Graham and Shimeld (2013) provided a compelling line of reasoning to support homology between urochordate siphon primordia and vertebrate placodes (for more details, see recent review of Diogo et al., 2015).

Development and Evolutionary Origin of Jaw Muscles and Other Head Muscles

For many decades, it has been commonly accepted that branchiomic muscles were acquired at the origin of vertebrates (e.g., Gans and Northcutt, 1983). However, recent works, as well as some older and unfortunately often ignored studies, indicate that branchiomic muscles related to the pharynx were present in the LCA of chordates (Fig. 1). More than 100 years ago, it was reported that larvae of the giant cephalochordate *Epigonichthys* develop complex orobranchial musculature, but almost no investigation of the orobranchial musculature of this clade has been completed since (reviewed in Yasui et al., 2014). In the cephalochordate amphioxus, the larval mouth and unpaired primary gills develop five groups of orobranchial muscles as the larval mouth enlarges posteriorly, the oral musculature developing without segmental patterning (Yasui et al., 2014). During metamorphosis, the orobranchial musculature disappears completely and the adult oral, velar and pterygial (= subatrial, or transverse) muscles develop independently. Yasui et al. (2014) suggested that the cephalochordate orobranchial muscles are probably a larval adaptation to prevent harmful intake, but they noted that the larval orobranchial muscles are perhaps more similar anatomically to the vertebrate branchiomic muscles than are adult cephalochordate oral, velar, and pterygial muscles. They also noted that vestigial muscles appear transiently with secondary gill formation, suggesting an ancestral state of bilateral muscular gills and a segmental pattern of branchiomic muscles in chordates. Six years after the study by Gans and Northcutt (1983), Gans (1989) did recognize that the muscles of the atrial region of cephalochordates might correspond to the vertebrate branchiomic muscles. He explained that cephalochordates and vertebrates have two patterns of motor innervation: one involves somatic motor neurons located within the basal plate of the spinal cord (somatic muscles); the other is seen in the cranial end of cephalochordates, where somatic motor axons leave the neural tube by means of a dorsal cranial root that proceeds ventrally to innervate the striated pterygial muscles of the atrial floor. Therefore, according to Gans (1989), and contrary to Gans and Northcutt (1983), the pterygial musculature of amphioxus could be homologous with the branchiomic muscles of vertebrates,

which could well have arisen by an invasion of paraxial mesoderm to surround the pharynx laterally and ventrally, instead of by muscularization of hypomeric tissues (see also Northcutt, 2005).

At first glance, the proposed homology between vertebrate and urochordate cardiopharyngeal muscles and cephalochordate muscles might seem counterintuitive: one would expect the urochordate oral siphon and the cephalochordate oral/velar muscles, rather than the urochordate atrial siphon and the cephalochordate atrial muscles, to correspond to, for example, the mandibular (first arch) muscles of vertebrates. In ascidians, water flows into the body through the oral siphon and is then expelled out of the body through the atrial siphon; therefore, it is the oral siphon that most likely corresponds to the mouth of vertebrates (Gans, 1989). However, recent studies have shown that the ascidian atrial siphon muscles derive from the cardiopharyngeal field, as do the branchiomic muscles of vertebrates, but that the ascidian oral siphon muscles do not derive from this field (e.g., Stolfi et al., 2010). This fact seems to lend support to the idea that the mandibular arch was not part of the plesiomorphic branchial arch series of chordates (Mallatt, 2008). In cephalochordates and urochordates, the oral/velar region lacks a skeleton, and the branchial bars are positioned a short distance behind the velum in a region that seems to correspond to the second branchial (hyoid) arch of vertebrates; e.g., Mallatt and Chen, 2003. That is, it makes sense that in early chordate evolution the oral/velar muscles were not part of the branchiomic muscle group, and that they only became integrated into this group in vertebrates, with the later co-option/homeotic shift of at least some oral structures and their muscles to form the first branchial (mandibular) arch. Of interest, in basal vertebrates such as lampreys *Tbx1/10* is expressed first in the mesodermal core of the branchial arches and pharyngeal muscles and the region of the otic vesicle, which seemingly corresponds to the atrium of nonvertebrates, and only later in development becomes expressed in the labial/oral and velar muscles (Sauka-Spengler et al., 2002). If in this case, there is a parallel between ontogeny and phylogeny, these data would, therefore, also support the hypothesis that the inclusion of the velar/oral muscles in the branchiomic muscle group was a derived (later) event within chordate evolution.

However, there is at least one alternative scenario: that the urochordate oral siphon muscles do not correspond to any of the branchiomic muscle groups present in extant vertebrates and that the urochordate atrial siphon also includes at least some muscles that correspond to/are precursors of the vertebrate first arch muscles. However, and interestingly, some oral/velar muscles of adult cephalochordates are innervated by neurons from a region of the brain that is putatively homologous with the region that gives rise to the facial motor neurons innervating the muscles of the second (hyoid) arch in vertebrates (Northcutt, 2005). Further studies are needed to investigate whether the cephalochordate oral/velar musculature corresponds to the oral siphon musculature (Fig. 1) or instead/also includes part of the atrial musculature of urochordates.

Development and Evolution of the Head, Neck, and Trunk in Chordates

While serially arranged gill openings in the pharynx associated with musculature are found in enteropneust-type hemichordates,

this musculature is very different developmentally, anatomically and histologically from the branchiomeric musculature of chordates (Yasui et al., 2014). In fact, the pharynx of the hemichordate *Saccoglossus* does not express *Tbx1*. According to Gillis et al. (2012), *Tbx1*-expressing pharyngeal mesoderm probably originated along the chordate stem, and the acquisition of cranial paraxial mesoderm within the pharyngeal region is probably a chordate synapomorphy. *Tbx1/10* is expressed in the pharyngeal mesoderm of cephalochordates and the atrial muscles of urochordates and *Tbx1* in the branchiomeric muscles of vertebrates, while *AmphiPax3/7* is expressed in the anterior and posterior somites of amphioxus (cephalochordate) and *Pax3* in all somitic muscles of vertebrates (Mahadevan et al., 2004). This distribution of gene expression indicates that the pterygial and oral/velar muscles of basal chordates and the branchiomeric muscles of vertebrates do not derive from the anterior somites, and thus that the LCA of chordates already had a separation between somitic muscles (“*Pax3*”) and branchiomeric muscles (“*Tbx1*”). However, *Tbx1/10* is expressed in the atrial siphon muscles and also in so-called “body wall muscles” of urochordates (Stolfi et al., 2010) and in the pharyngeal mesoderm and the ventral part of some somites of amphioxus (Mahadevan et al., 2004), meaning that this separation probably was not as well-defined in early chordates as it is in extant vertebrates.

However, although more defined, the separation between branchiomeric and somitic muscles, and between the head and the trunk in general, remains uncertain. An illustrative example is the cucullaris, one of the best-studied yet most puzzling vertebrate muscles. Its amniote derivatives, the trapezius and sternocleidomastoideus have played a central role in the origin and evolution of the vertebrate head and neck. These muscles share characteristics of at least four different muscle types: somitic epibranchial, somitic hypobranchial migratory, somitic limb nonmigratory (“primaxial”), and somitic limb migratory (“abaxial”). Topologically, the cucullaris resembles the epibranchial muscles of lampreys (e.g., Kusakabe et al., 2011), yet its developmental migration is similar to that of somitic hypobranchial migratory muscles (e.g., Matsouka et al., 2005). Additionally, the trapezius receives contributions from both “primaxial” and “abaxial” cells (e.g., Shearman and Burke, 2009). However, long-term fate mapping studies have shown that muscles that are consensually accepted as branchiomeric, derived not only from posterior (e.g., laryngeal) but also from more anterior (e.g., the hyoid muscle interhyoideus) arches receive a partial contribution from somites (Piekarski and Olsson, 2007). These studies further complicate the distinction between head/neck and trunk, but in turn also show that the fact that the trapezius receives some somitic contribution does not contradict its original branchiomeric origin. Actually, the balance of available developmental, molecular, and anatomical data strongly supports the idea that the cucullaris and its derivatives are branchial, and thus branchiomeric, muscles. The cucullaris originates anatomically from the postero-dorsal region of the branchial musculature and is usually innervated by the 11th (accessory) cranial nerve (e.g., Edgeworth, 1935; Diogo and Abdala, 2010; Ziermann and Diogo, 2013, 2014). Also, the levatores arcuum branchialium of bony fish, consensually considered to be branchial muscles, were clearly derived from the undivided cucullaris of plesiomorphic gnathostomes (Ziermann et al., 2014). Finally, neural crest cells from a caudal branchial arch travel with trapezius myoblasts and form tendinous and skeletal cells within its zone of attachment (e.g., Noden and Schneider, 2006).

As recently reviewed by Diogo et al. (2015), the strongest support for the branchiomeric identity of the cucullaris and its derivatives comes, however, from recent gene expression studies in mammals: *Tbx1* is expressed in/its lack affects the branchiomeric (e.g., laryngeal, first, and second arch) muscles and also in the trapezius, while *Pax3* is expressed in/its lack affects all of the somitic (i.e., limb, diaphragm, tongue, infrahyoid, and trunk) muscles, but not the trapezius (Sambasivan et al., 2011). In turn, these data also emphasize the heterogeneity of the vertebrate neck, which, therefore, includes branchiomeric (e.g., trapezius and sternocleidomastoideus), hypobranchial (e.g., tongue and infrahyoid), and trunk (somitic epaxial; e.g., deep neck and back) muscles (for more details, see Diogo et al., 2015).

Despite its profound implications for evolutionary and developmental biology and human medicine in general, heterogeneity in the vertebrate head and neck is poorly documented in textbooks, academic and medical curricula, and even many specialized research publications. In fact, the human head includes at least five different groups of muscles that *do not* form a phylogenetically monophyletic group nor a single developmental unit, and that are in turn divided into sub-groups that are themselves developmentally more related to other muscle tissues (e.g., specific heart regions) than with other sub-groups of their respective head/neck group (Diogo et al., 2015): (1) first (mandibular) arch branchiomeric muscles; (2) second (hyoid) arch branchiomeric muscles; (3) branchiomeric muscles of the most posterior branchial arches, including the pharyngeal and laryngeal muscles and the cucullaris derivatives trapezius and sternocleidomastoideus, which migrate caudally and ventrally to attach onto the sternum and pectoral girdle; (4) extraocular muscles; and (5) hypobranchial muscles, which derive from the ventral part of the somites and, therefore, are not branchiomeric muscles (Edgeworth, 1935; Diogo and Abdala, 2010; Lours-Calet et al., 2014). In addition, Cyclostomata, Selachii, and Holocephali (Fig. 1) possess a sixth group of head muscles, designated epibranchial muscles, which derive from the anterior portion of the somites (Edgeworth, 1935). Even within the same arch, muscles can follow different genetic programs; for instance, in zebrafish, *Ret* signaling is necessary for the development of only a few specific mandibular and hyoid muscles associated with the movements of the opercle (bony plates supporting the gill covers) (Knight et al., 2011). Likewise, *C-met* is crucial for the development and migration of the mammalian muscles of facial expression, derived from the second (hyoid) arch, but not for the other second arch muscles (Prunotto et al., 2004).

Some authors suggest that the extraocular muscles are not branchiomeric muscles at all: that the former derive from the prechordal mesoderm and the latter from the head mesoderm situated posterior to it (Sambasivan et al., 2011). The idea that extraocular muscles are not branchiomeric muscles, which is supported by cell lineage studies in mice (e.g., Harel and Tzaho, 2013), would further reinforce the heterogeneous character of the vertebrate head muscles. However, recent clonal studies on mice suggest that the extraocular muscles are branchiomeric derivatives related to the mandibular and right ventricle muscles (Lescroart et al., 2010; Lescroart and Meilhac, 2012), and various recent genetic and experimental developmental studies also support these results. For instance, cell labeling studies in lampreys showed that some mandibular mesodermal cells migrate near the eye, although it is not clear whether the labeled cells develop into eye muscles (Kuratani et al., 2004). In addition, detailed embryological studies

undertaken in the later 19th century and earlier 20th century have indicated that the mandibular head cavity of sharks gives rise to both eye and mandibular muscles and that these muscles derive from the same primordium in many other vertebrate groups (Edgeworth, 1935). In either case, the extraocular muscles provide a good example of the remarkable complexity of the evolutionary and developmental relationships of vertebrate head muscles. Future studies should focus on the specific homologies of each of the six muscle groups listed above across vertebrates and particularly on their homologies and evolutionary history within chordates as a whole, based on detailed comparisons with both urochordates and cephalochordates. These studies and comparisons should concentrate not only on anatomical and developmental traits, but also on genetic traits, and should take into account the possibility that, as is the case with anatomical and developmental traits, at least some genetic traits (e.g., gene expression of *Tbx1*, *Pax3* or *Pitx2*) might have been acquired independently (i.e., homoplasically) and/or have different functions in different chordate taxa (see, e.g., Hall, 2007, 2013). In addition, future studies should investigate if there is, or not, a parallel between the developmental-genetic specification of head/neck muscles/muscle groups and skeletal structures/groups; for instance, are there groups/anatomical complexes in the adult head/neck skeleton that specifically correspond to the adult hypobranchial and/or epibranchial muscle groups/complexes?

Metamorphosis, Life History, Development, and Chordate Early Evolution

As pioneered by de Beer's (1940) *Embryos and Ancestors*, the origin and early evolution of vertebrates has attracted broader theoretical interests such as the relationship between ontogeny and phylogeny. An accepted view in the early 20th century positioned a neotenic chordate as the LCA of vertebrates (e.g., Garstang, 1928; reviewed in Gee, 1996). Specifically, an elongate, motile chordate larva that metamorphosed into a sessile adult was considered as a plesiomorphic condition for both urochordates and deuterostomes (Fig. 1). This scenario suggested a swimming capacity and elongated body of adults as derived conditions in cephalochordates and vertebrates due to retardation of somatic development relative to sexual maturity with respect to the ancestor (neoteny). Neoteny, or "juvenilization," is one type of paedomorphosis. It is defined as reduced rate in morphological differentiation (McNamara, 1986) or in other words, it is the retention of traits that are ancestrally specific to ontogenetically younger individuals (i.e., the deletion of terminal somatic developmental stages). Another type of paedomorphosis is progenesis (precocious sexual maturation), where sexual development is accelerated relative to somatic growth than in ancestors (de Beer, 1940; Gould, 1977; McNamara, 1986, 1990). According to the evolutionary scenario most accepted at de Beer's (1940) time, the paedomorphic events occurred during the transitions between the LCA of chordates, the LCA of vertebrates, and the LCA of gnathostomes were considered more likely than opposite peramorphic (often also named gerontomorphic) events, where ancestral adult characters appear in descendant juveniles due to additions of terminal somatic developmental stages (McNamara, 1990). This is because the latter (peramorphic) transitions would include (1) the restriction of the typical adult swimming elongated body adapted to suspension feeding of the LCA of vertebrates to the

lamprey larva; and (2) the addition of new, derived terminal ontogenetic stages that resulted in the metamorphosis of the structures of the lamprey larva into structures that are mainly related to parasitism, which is the mode of live of many adult lampreys. Therefore, the resemblance between the elongate, motile adult in the LCA of gnathostomes and the motile lamprey larvae was usually seen as the result of neotenic events that occurred during the transitions from the LCAs of vertebrates and of gnathostomes, and *not* as the result of peramorphic events that occurred during the transitions from the LCAs of vertebrates and of cyclostomes.

However, as noted above, most authors now accept that urochordates (and not cephalochordates) are the closest living relatives of vertebrates (Fig. 1), and many authors defend that appendicularians (and not ascidians) are the most basal extant urochordates. Because cephalochordates also have a motile adult stage, this is likely a condition for the adult LCA of urochordates + vertebrates. This would suggest that sessile adults in extant ascidians represent a derived condition by means of terminal additions (peramorphosis) during the transitions from the LCAs of urochordates to that of ascidians. Be that as it may, what seems to be clear is that the adult members of the LCA of vertebrates had an elongated motile body similar to that of the adult members of basal gnathostomes and of amphioxus (= lancelet, cephalochordate). Adult amphioxus and lamprey larvae were often compared by early anatomists. Similarities include a pharynx with pharyngeal slits, a notochord, a dorsal hollow nerve cord and a series of somites that extend anterior to the otic vesicle. Like amphioxus, the lamprey larvae are filter feeders and lack jaws (Holland et al., 2008). However, amphioxus larvae are highly asymmetric, probably due to derived adaptations associated with the feeding mechanism, and, thus, to the addition of early ontogenetic stages (Presley et al., 1996). In contrast, lamprey larvae also possess some apparently plesiomorphies like an endostyle and their early embryonic pattern resembles that of hagfishes, while their oral apparatus, including upper and lower lips, resembles those of some adult fossil gnathostome species more than the adult lamprey does (Kuratani et al., 2002).

Recent molecular and developmental data have contradicted the physiological and/or morphological (incl. paleontological) data supporting the paraphyly of cyclostomes (hagfishes + lampreys: Fig. 1), for instance by providing examples of similar ontogenetic skeletal features and their developmental mechanisms shared uniquely by hagfish embryos and lamprey larvae (but not by adults of these two groups), as well as by these embryos and larvae and both the embryos and adults of gnathostomes (e.g., Oisi et al., 2013a,b). By doing so, they further supported the idea that during the transitions leading to extant cyclostomes there was: (1) a restriction, by means of developmental acceleration, of the typical features associated to suspension feeding (supposedly seen in adult members of the LCA of vertebrates) to early ontogenetic stages; and (2) the addition of derived terminal ontogenetic stages (peramorphosis) in the origin of cyclostomes and then independently in hagfishes and particularly in lampreys, resulting in the highly peculiar adult features of each of these two groups.

The adult lamprey cannot simply be regarded as a hypermorphic state of a hypothetical lamprey larva-like adult LCA of cyclostomes. This is partly because lamprey adults have structures under-developed in larvae but shared with hagfish. For example, the lingual apparatus in adult lampreys (also present in adult hagfish) appears after metamorphosis (Yalden, 1985). That

is why the more likely scenario is that some of the peculiar adult features of hagfishes and lampreys were acquired independently, while others were acquired during the transitions leading to the LCA of cyclostomes. Together with the data mentioned in the previous paragraph, these data thus seem to indicate that metamorphosis might have been acquired independently in cephalochordates (mainly due to early ontogenetic somatic additions), lampreys (mainly due to terminal ontogenetic somatic additions; N.B., hagfishes have no true metamorphosis), and some gnathostomes (e.g., actinopterygians and amphibians, in which terminal somatic additions are usually related to transitions to a terrestrial lifestyle). In fact, although in chordates metamorphosis is seemingly always regulated by thyroid hormones, it shows very different patterns in different chordate groups, e.g., in ascidians all larval tissues are drastically remodeled into the adult animal, in amphioxus the highly asymmetric larva transforms into a relatively symmetric adult, and in some flatfish the symmetric larvae becomes an asymmetric adult (Paris et al., 2008).

Metamorphosis is a process in which a larval form is remodeled into an adult form that differs from the former one in morphology and ecology. Metamorphosis occurs in urochordates (e.g., ascidians), cephalochordates, lampreys, and some ray-finned fishes (Actinopterygii) and lissamphibians (Paris and Laudet, 2008; Paris et al., 2008, 2010). It is usually accepted that metamorphosis evolved several times in chordates (e.g., Heyland et al., 2005). However, a common character for all these chordate taxa is that metamorphosis is always tightly linked to changes in thyroid hormone levels (Paris and Laudet, 2008). This led some authors to propose that metamorphosis might actually represent a homologous feature within these taxa (Paris and Laudet, 2008; Paris et al., 2008). However, the morphological remodeling process is drastically different in each of the metamorphic chordate taxa. For instance, the remodeling is particularly drastic in frogs: a free swimming larva with its own feeding mechanism and without legs reorganizes the cranium and internal organs, develops limbs, and loses its tail (e.g., Rose, 2005). In lampreys, structures remodeled during metamorphosis include the eyes, the gut epithelium, the mouth, and the larval kidney (replaced by a juvenile kidney), and the endostyle (a thyroid gland in adults) (Youson, 1980, 1997). Hagfishes have a direct development, but their post-embryonic development is not yet fully studied (Jørgensen, 1998). Thyroid hormones are also involved in the metamorphosis of the cephalochordate amphioxus, in which the bilaterally asymmetric motile larva transforms into a benthic and more bilaterally symmetric juvenile by shifting the left mouth antero-ventrally and by duplicating and shifting the pharyngeal slits from the right side to the left side (e.g., Willey, 1894; Stokes and Holland, 1995; Paris et al., 2008). Within urochordates, ascidians undergo a metamorphosis, whereas members of the Appendicularia keep a larval body plan in adult stages and of Thaliacea seem to be direct developers as they skip an obvious larval stage (Paris and Laudet, 2008).

Moreover, it is clearly more parsimonious to accept that metamorphosis evolved independently, than to consider that it was present in the LCA of vertebrates and then secondarily lost. This is because these two options require, respectively, a minimum of three steps (acquisition in lampreys, actinopterygian fish, and batrachians [urodeles + anurans]) vs. six or seven steps (loss in hagfishes, chondrichthyans, coelacanth, dipnoans, caecilians, and amniotes, and eventual acquisition in the LCA of vertebrates if not present in the LCA of urochordates + vertebrates). There-

fore, based on phylogenetic parsimony, one can hypothesize that the involvement of thyroid hormones in metamorphosis might be a homoplasious process. Furthermore, the degree of changes during metamorphosis differs in and between different clades, and the developmental patterns can be highly changed (e.g., presence of both metamorphosis and direct development in urochordates, urodeles, and anurans). Direct development can be interpreted as a suppression of the larval stage. However, in the direct developing frog *Eleutherodactylus coqui*, it was shown that thyroid hormones are still important during embryonic development (e.g., for skin, muscle, and gut development) and the comparison of cranial muscle development in *E. coqui* and in metamorphosing frogs supports the idea of a cryptic metamorphosis (not obvious metamorphosis in the egg) (e.g., Elinson, 2013; Ziermann and Diogo, 2014).

Muscles, Metamorphosis, Diversity, and Chordate Evolution

The overall combination of the data summarized in the Section above would support the idea that in general the adult members of basal gnathostome groups share more similarities with lamprey larvae and hagfish embryos than with lamprey and hagfish adults due to peramorphic events that occurred in the evolutionary history of cyclostomes and not to neotenic events leading to the LCA of gnathostomes. But this hypothesis was never tested on muscle anatomy studies, which as stated above have been in general neglected in analyses on the origin and early evolution of vertebrates, and in particular on the comparative anatomical analyses of lampreys, hagfishes, and gnathostomes. Recently, we have presented for the first time a synthetic examination of data from us and others about the cephalic muscles of all major groups of vertebrates, including hagfish and lampreys, which thus allow us to have a more comprehensive understanding of the relationships between development, metamorphosis, heterochrony, and morphological and taxonomical diversity within chordates (Ziermann et al., 2014). In Figures 2 and 3, we briefly summarize our hypotheses on the homology and evolution of the larval and adult cephalic muscles in cyclostomes and gnathostomes based on that synthesis and on an extensive review of the literature regarding muscle genetics, development, innervation, attachments, and overall topology and relationship to other structures such as cartilages.

According to the data compiled by us, we inferred that the LCA of extant vertebrates had an undifferentiated intermandibularis muscle sheet, labial muscles, and some other mandibular muscles, a hyoid muscle (at least a constrictor hyoideus), branchial muscles (at least some constrictores branchiales), and undifferentiated epi-branchial and hypobranchial muscle sheets (Ziermann et al., 2014). As the adductores branchiales are absent in hagfishes and bony fishes, it is more parsimonious to assume that they were independently acquired in lampreys and chondrichthyans (two steps) than to assume that they were acquired in the LCA of extant vertebrates and then lost in hagfishes and bony fishes (three steps). Importantly, the comparison of the muscles of cyclostomes and gnathostomes (Figs. 2 and 3) supports that the lamprey larvae is a better model for the cranial muscles of adults of the LCA of gnathostomes and the LCA of vertebrates, than is the adult lamprey. A major argument supporting this idea is that the inferred adult muscles of the LCA of vertebrates, which were inferred based

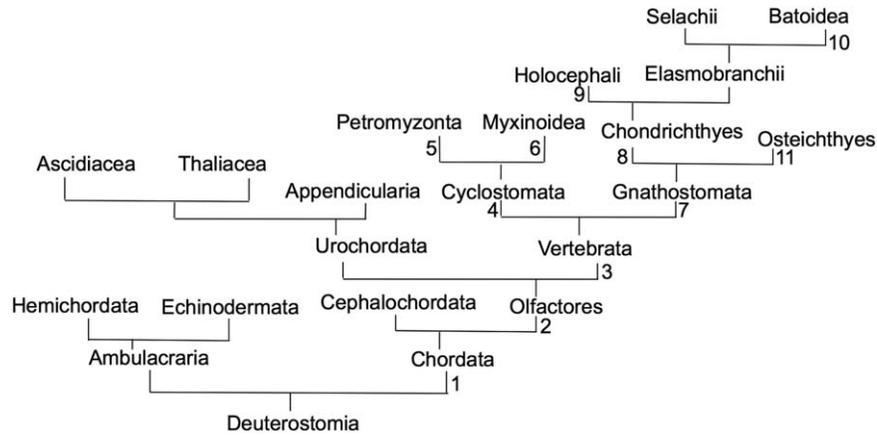


Fig. 1. Phylogenetic tree showing some of the synapomorphies of key chordate subgroups based on our data and literature review (for more details, see text): **(1)** Presence of complete notochord in adults; presence of segmented longitudinal (somatic) muscles and of branchiomic muscles (possibly not all of them, i.e., the inclusion of oral/velar muscles into the branchiomic series might have occurred during vertebrate evolution). **(2)** Neural crest-like cells and placodes. **(3)** Neural crest; cranium; differentiation of epibranchial and hypobranchial muscles. **(4)** Substantial increase of number of first arch muscles in adults, up to 26 and 24 in total in lampreys and hagfishes, respectively (vs. fewer than 10 in adult chondrichthyans, for example), probably related to peramorphosis, at least in lampreys. **(5)** Loss of second arch muscles and marked increase in number of muscles of more posterior arches (up to 76 total in adults), probably also related to peramorphosis. **(6)** Substantial decrease in number of muscles of more posterior arches (3 or fewer in total). **(7)** Differentiation between hypaxial and epaxial musculature; paired appendages and fin muscles; jaws. **(8)** Almost complete loss of calcified dermal and endochondral bones. **(9)** Cucullaris divided into bundles. **(10)** Loss of epibranchial muscles; cucullaris divided into levatores arcuum branchialium attaching onto branchial arches and protractor pectoralis attaching onto the pectoral girdle (the protractor pectoralis later gave rise, in amniotes, to the neck muscles trapezius and sternocleidomastoideus).

exclusively on our observations and on comparisons between *adult* taxa (Ziermann et al., 2014), is strikingly similar to the condition that is actually present in the lamprey larva (Fig. 3). We inferred at least one constrictor hyoideus in the vertebrate LCA; lamprey larvae have a likely homologue, the constrictor hyoideus (“prebranchialis”), whereas adult lampreys do not seem to have muscles that can be clearly recognized as hyoid muscles (Fig. 3). We also inferred at least one labial muscle and an undifferentiated intermandibularis muscle sheet (in the sense that this sheet it is not separated or divided in several parts) in the vertebrate LCA, and lamprey larvae have in fact two muscles within the labial and intermandibularis groups, whereas lamprey adults have many more (Figs. 2 and 3). Our inference postulated at least one velar and/or dorsal mandibular muscle in the vertebrate LCA; the lamprey larvae have in fact two of these muscles, while adult lampreys do not seem to have muscles that can be clearly recognized as part of this specific group (Figs. 2 and 3; see also below). Furthermore, we inferred at least some constrictores branchiales, and possibly some adductores branchiales as well, in the vertebrate LCA; lamprey larvae have in fact adductores and constrictores branchiales, whereas adult lampreys have a much more complex configuration with an impressive number and many different subgroups of true branchial muscles (Figs. 2 and 3). Based on our inference the LCA of vertebrates had an undifferentiated epibranchial muscle sheet; lamprey larvae have three epibranchial muscles, whereas lamprey adults have five. Regarding the nasal, lingual, and/or dental muscles, we inferred that a few of these muscles could have been present in this LCA; lamprey larvae have six muscles, while lamprey adults have many more. Lastly, we inferred the presence of one undifferentiated hypobranchial muscle sheet in the vertebrate LCA, and this is the condition found in lamprey larvae; this is actually the only case where the condition inferred for this LCA is the same to that also found in adult lampreys.

As expected, there are more differences between the lamprey larva and the adult members of the LCA of gnathostomes than between the lamprey larva and the adults of the vertebrate LCA. However, despite the gnathostome peculiar synapomorphies, the lamprey larva has many potentially homologous muscles with those inferred for the adult members of the LCA of extant gnathostomes. One of the two major exceptions (i.e., differences between lamprey larvae and adults of the gnathostome LCA) are: (1) no nasal, lingual, and/or dental muscles are inferred for the gnathostome LCA; and (2) the adult gnathostome LCA likely had an adductor mandibulae A2 (Figs. 2 and 3; Ziermann et al., 2014). However, it is possible that all these muscles derive from the same mandibular anlage, i.e., the dorso-medial mandibular sensu Edgeworth (1935) (see Diogo and Abdala, 2010). The other major difference between adults of the LCA of gnathostomes and lamprey larvae is that the former have a cucullaris, which according to the idea defended in the present study is part of the branchial musculature. It should, however, be noted that, as explained above, according to some authors the cucullaris may have derived from “infraotic” epibranchial muscles, which are present in the lamprey larvae.

Apart from the direct comparison between the muscles of larval lamprey and of adults of other vertebrate taxa, support for the hypothesis that lampreys are peramorphic vertebrates also comes from a broader analysis of the number of muscles found in adults of both cyclostome and non-cyclostome taxa (Figs. 2 and 3; Ziermann et al., 2014). The number of mandibular muscles in adults of *Myxine glutinosa*, *Petromyzon marinus*, *Hydrolagus collicii*, *Squalus acanthias*, and *Leucoraja erinacea* is respectively 24, 26, 7, 5, and 6; for the hyoid muscles is 4, 0, 4, 3, and 6; for the branchial muscles is 3, 76, 16, 27, and 28, and for the hypobranchial muscles is 3, 1, 2, 3, and 4 (Ziermann et al., 2014). The total number of adult muscles in *P. marinus* is 103, whereas the total

number of cranial muscles in adults of the other taxa ranges between 29 and 44. The mandibular muscle count in adult cyclostomes is, therefore, much higher than in adult chondrichthyans and osteichthyans, which indicates a significant increase of the number of these muscles during cyclostome evolution. Regarding the muscles of lamprey larvae, in total we could identify 44 larval cephalic muscles. Twelve (10 mandibular + 1 hyoid + 1 epibranchial) of larval cephalic muscles seem to be only present in larvae, disappearing during metamorphosis; the other 32 muscles persist until adulthood. Interestingly, the persisting muscles are all hypobranchial, epibranchial, and true branchial. The muscle count per group in lamprey larvae is: mandibular, hyoid, true branchial, epibranchial, hypobranchial: 10, 1, 28, 4, 1; these numbers are, therefore, clearly much closer to the count in the adult gnathostome taxa shown in Figure 2 than the total, striking number of adult cephalic muscles found in *P. marinus* (103).

Although in lamprey larvae all five groups of cephalic muscles (mandibular, hyoid, branchial, epibranchial, and hypobranchial) are well separated from each other, most of these groups are in such a close contact to each other that it seems that they became secondarily fused to each other in adults (e.g., the hypobranchial muscle hypobranchialis with the epibranchial muscle subocularis: Fig. 2C,D). This contrasts with the situation found in adult chondrichthyans, in which these muscle groups are in general more clearly separated from each other (Fig. 2E–H; Ziermann et al., 2014). Lamprey larvae have lower and upper lips, which resemble the oral apparatus of some adult fossil vertebrate taxa such as heterostracans and osteostracans more than the adult lamprey does (e.g., Kuratani et al., 2002). These lips are associated with labial muscles that are more similar to adult gnathostome muscles than are the adult lamprey muscles. In addition, the *engrailed* immunoactivity in the lamprey larval muscle velothyroideus (which is missing in adult lampreys) lead to the suggestion that this muscle might be homologous to the adult and embryonic muscles levator arcus palatini and dilatator operculi of teleosts (Holland et al., 1993). These two latter teleost muscles are included in the mandibular constrictor dorsalis group (sensu Edgeworth, 1935) and are, therefore, in the same group as the levator arcus palatini of the adult members of the LCA of gnathostomes. The visceral muscles of adult lampreys differentiate from blastema as those in lamprey larvae degenerate during metamorphosis, and it is still not clear whether the larval muscles are precursors of adult muscles or not. Therefore, identification of possible correspondence, even topological, between the larval and adult lamprey muscles is very difficult because of the dramatic rebuilding processes during metamorphosis (e.g., Marinelli and Strenger, 1954). In the case one accepts the proposed homology by Holland et al. (1993), this would suggest that the velum of lampreys and the gnathostome palatoquadrate could be homologous to each other, or at least, homogenic (e.g., Miyashita, 2012). During lamprey metamorphosis, the velum and endostyle are both reduced; instead, a lingual apparatus develops in the location corresponding to the larval muscle velothyroideus. Therefore, if the homology proposed by Holland et al. (1993) would be accepted, this would provide a further case where the muscles of lamprey larvae have a better correspondence to the muscles present in the adult members of the LCA of gnathostomes than have the adult lamprey muscles.

In summary, one can say that both the larval chondrocrania (Oisi et al., 2013a,b) and the larval muscles of cyclostomes resemble more the adult plesiomorphic vertebrate and gnathostome

condition than do the adult cyclostome structures. As noted above, in the development of cephalic muscles in the amphioxus *Branchiostoma japonicum* (cephalochordate), the asymmetric larval mouth and unpaired primary pharyngeal slits are associated with asymmetric orobranchial muscles (Yasui et al., 2014). The iterated branchial (pharyngeal) muscles match the dorsal myomeric pattern before metamorphosis. The oral and pharyngeal regions are then dramatically remodeled during metamorphosis. Meanwhile, the orobranchial musculature disappears and the adult muscles in the oral hood, velum, and the pterygial coeloms develop independently, as well as some branchial muscles that were associated with the secondary pharyngeal arch/gill formation but that then also disappeared. Yasui et al. (2014) suggested that in some aspects the amphioxus' larval branchial system, rather than that of the adult, could be a better correspondence to the vertebrate system, but then recognized that the amphioxus larva very likely represents a derived feature acquired during the evolutionary history of cephalochordates. This latter idea is also supported by our comparisons.

First, the branchial muscles exist mainly (i.e., with exception to the vestigial muscles associated with the secondary gill formation) when the amphioxus displays a unilateral body pattern that is markedly distinct from the vertebrate bilateral pattern, and their function seems to be specialized for preventing harmful intake in larval life. Because there is no antagonistic system against the larval orobranchial musculature that functions as obturator and levator, once constricted, the pharynx recovers its dilatation very slowly, and thus the muscle system does not serve as a rhythmical pump nor functions in active predation. These features support instead the hypothesis that the larval orobranchial musculature is a derived feature for larval adaptation. That is, the highly asymmetric amphioxus larva is probably the result of additions of somatic stages early in cephalochordate ontogeny, and thus the adult members of this group are very likely a better model for the adult condition of the LCA of vertebrates in this regard. Second, the transient appearance of branchial muscles in association with the secondary gill formation during metamorphosis suggests that the larval unilaterality of extant amphioxus has been derived from a bilateral LCA of chordates that possessed a bilateral muscular branchial system similar to that found in the LCA of vertebrates. Third, the developmental contrast between the labial/velar/pterygial and somatic pattern after the metamorphosis of amphioxus is similar to that found in the head of the lamprey larvae. Lastly, the postmetamorphic, symmetric amphioxus labial, velar, and pterygial muscles are more similar to the labial, velar, and other branchiomeric muscles of cyclostome larvae/embryos than are the highly asymmetric larval amphioxus cephalic muscles. Notably, the adult pterygial amphioxus muscles develop ventrally in the pharynx and are innervated by peripheral nerves that are similar to the branchiomeric nerves in vertebrates, as explained above (Fritsch and Northcutt, 1993; Yasui et al., 2014). In summary, these developmental data do support that: (1) metamorphosis might have been acquired independently in cephalochordates (mainly due to early ontogenetic somatic additions) and lampreys (mainly due to terminal ontogenetic somatic additions); and (2) in general adult members of basal gnathostome groups share more similarities with lamprey larvae than with lamprey and hagfish adults, due to peramorphic events occurred in the evolutionary history of cyclostomes and not due to neotenic events leading to the LCA of gnathostomes. This conforms to de Beer's (1940) prediction that, contrary to what was often believed

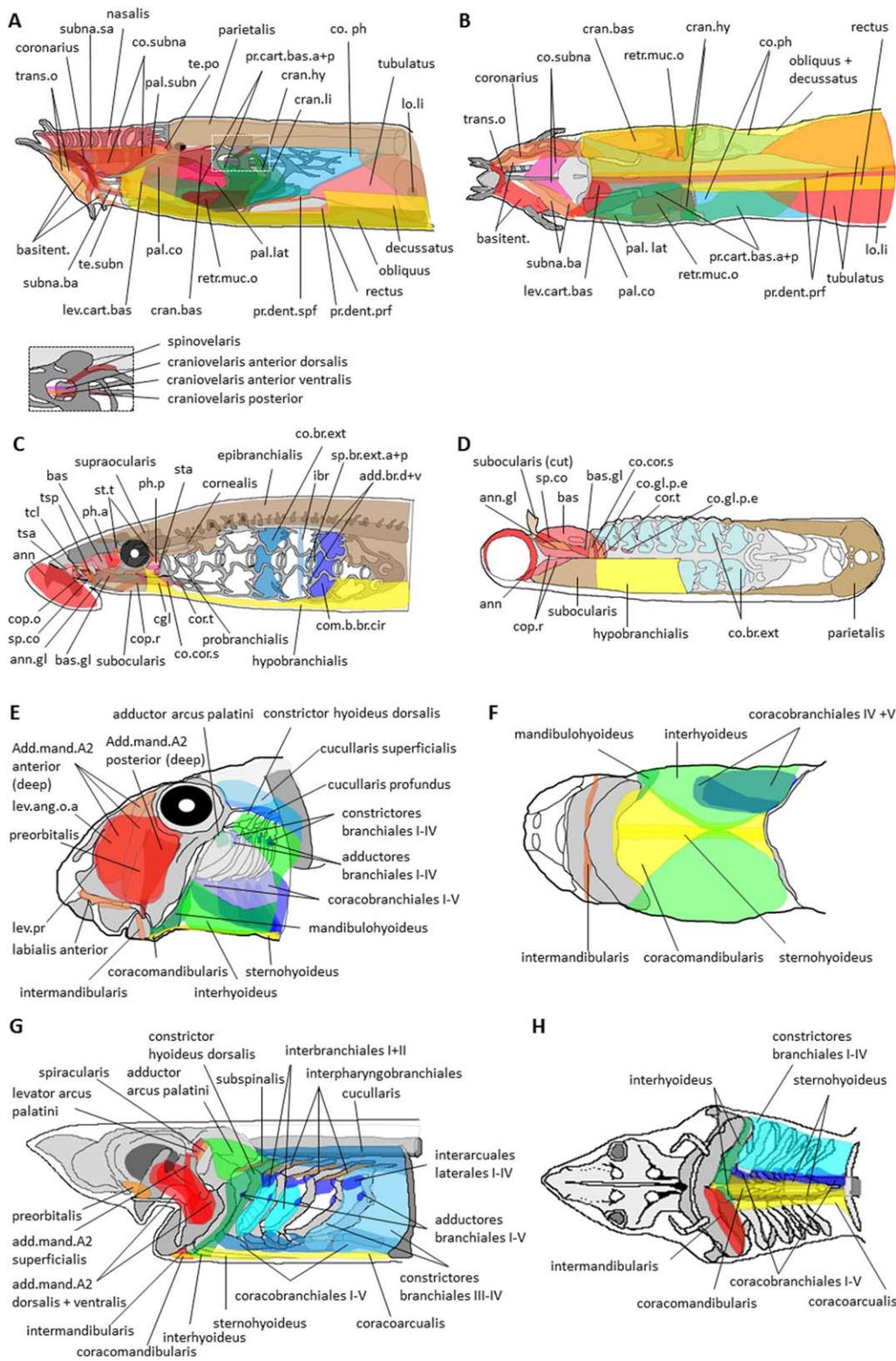


Fig. 2. Our hypothesis, based on the data reviewed here, about the homologies of cephalic muscle groups in Cyclostomata and Chondrichthyes: Mandibular (red, orange, pink); Hyoid (green), True branchial (blue), Epibranchial (brown), and Hypobranchial (green) muscles. **A-H:** Left lateral and ventral view of *Myxine glutinosa* (**A,B**), *Petromyzon marinus* (**C,D**), *Hydrolagus colliei* (**E,F**), and *Squalus acanthias* (**G,H**). Chondrocranium of *M. glutinosa* and *P. marinus* redrawn from Marinelli and Strenger (1954, 1956). Chondrocranium of *H. colliei* after dissections of J. M. Ziermann and of *S. acanthias* redrawn from Livingstone (2014). Specimens not to scale. Not all muscles shown on both sides in ventral view. Not all branchial arch muscles are shown in some views. A-D are adapted from Ziermann et al. (2014). A: Parietalis and decussatus cut to enable view to deeper layers. White box – velar muscles in window below the animal. B: Basitentacularis cut on right side. C: All branchial arch muscles (blue) are present in each segment but not shown in each segment. D: The hypobranchial extends backward but was cut to show the anterior basket. E: Coracobranchiales I-III not shown. F: Coracobranchiales I-III not shown. G: Constrictores branchiales I + II and interbranchiales III+IV not shown. add.br.d+v, adductores branchiales dorsales + ventrales; add.mand.A2, adductor mandibulae A2; ann, annularis; ann.gl, annuloglossus; bas, basilaris; bas.gl, basilariglossus; basitent., basitentacularis; cgl, cornuoglossus; co.br.ext., constrictores branchiales externi; co.cor.s, constrictor cornualis superficialis; co.gl.p.e, constrictor glossae profundus externus; co.ph, constrictor pharyngis; co.subna, cornuosubnasalis; com.b.br.cir, compressores bursae branchiales circulares; cop.r, copuloglossus rectus; cop.o, copuloglossus obliquus; cor.t, cornuotaenalis; cran.bas, craniobasalis; cran.hy, craniohyoideus; cran.li, craniolingualis; ibr, interbranchiales; lev.ang.o.a, levator anguli oris anterior; lev.cart.bas, levator cartilagine basalis; lev.pr, levator prelabialis; lo.li, longitudinalis linguae; pal.co, palatocoronarius; pal.lat, palatinalis lateralis; pal.subn, palatosubnasalis; ph.a, pharyngicus anterior; ph.p, pharyngicus posterior; pr.cart.bas.a+p, protractor cartilagine basalis anterior and posterior; pr.dent.prf, protractor dentium profundus; pr.dent.spf, protractor dentium superficialis; retr.muc.o, retractor mucosae oris; sp.br.ext.a+p, sphincters branchiales anteriores and posteriores; sp.co, spinosocopularis; sta, styloapicalis; st.t, styloectalis; subna.ba, subnasobasalis; subna.sa, subnasonasalis; tcl, tectolateralis; tcl, tectolateralis; te.po, tentacularis posterior; te.subn, tentaculosubnasalis; trans.o, transversus oris; tsa, tectospinosus anterior; tsp, tectospinosus posterior.

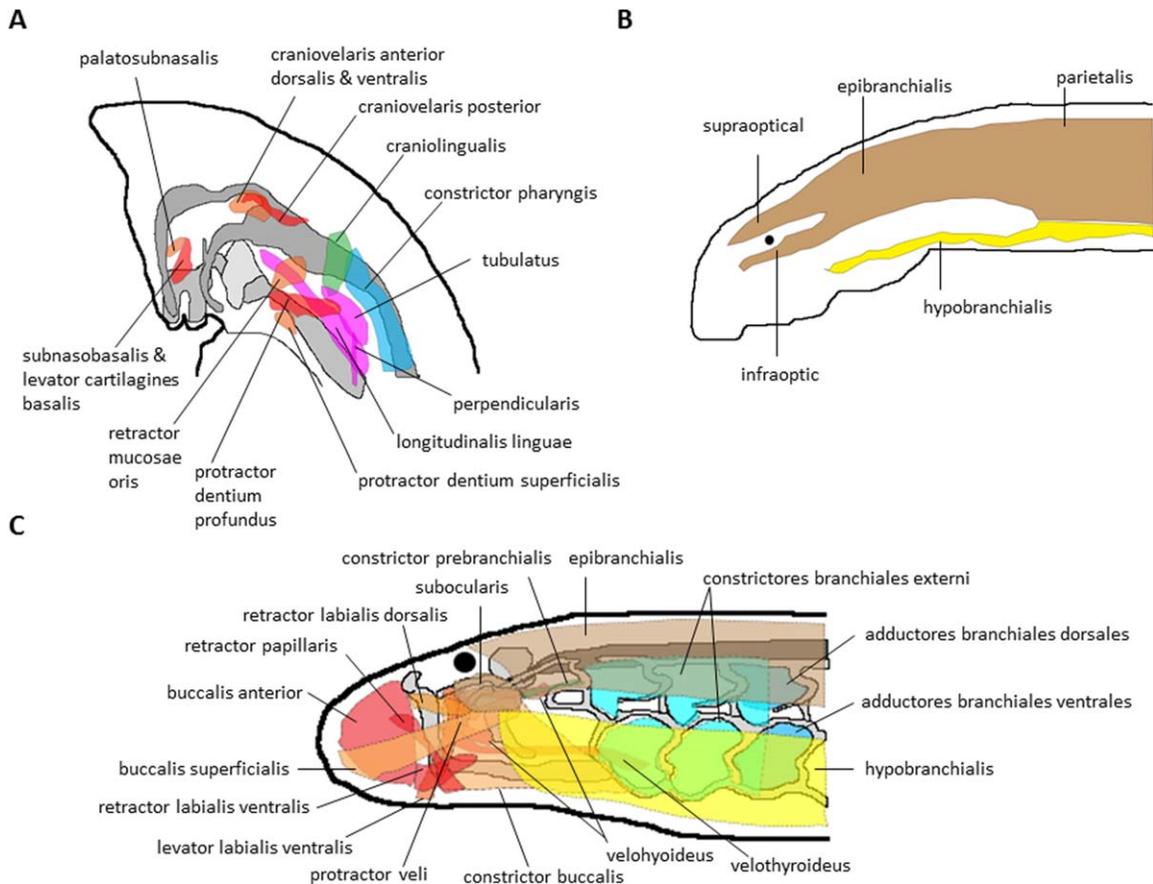


Fig. 3. Our hypothesis, based on the data reviewed here, about the homologies of cephalic muscle groups of embryos/larvae of cyclostomes: Mandibular (red, orange, pink); Hyoid (green), True branchial (blue), Epibranchial (brown), and Hypobranchial (green) muscles. Left lateral views. **A:** *Myxine glutinosa* embryo redrawn from Miyashita (2012). Somites (removed for clarity) extend to just behind the otic capsule (m. parietalis and m. obliquus appear not fully differentiated at this stage; Oisi et al., 2013a,b). **B:** *Petromyzon*: Ammocoete larva (redrawn from Tulenko et al., 2013) **C:** Ammocoete larva (redrawn from Miyashita, 2012); not all branchial muscles shown; constrictores branchiales externi sheet cut to reveal underlying adductores branchiales. Specimens not to scale.

during the various decades that Haeckel's recapitulation was in vogue, peramorphic events often lead to the origin of taxa displaying a relatively limited taxonomical diversity. This is because while in paedomorphosis the deletion of terminal ontogenetic stages often leads to more generalized forms that may in turn evolve in completely new ways, in peramorphosis, the addition of terminal stages often results in very specialized forms adapted to very specific environments. This is actually seen in lampreys, in which most adults have a parasitic life style and a remarkably specialized cranial musculature comprising a total of 103 muscles. Accordingly, lampreys as well as hagfishes have a remarkably poor taxonomic diversity, with only 38 and 60 extant species (Hardisty, 2006; Nelson, 2006), accordingly, in contrast to the roughly 60,000 living gnathostome species.

General Remarks and Future Directions

In the light of the recent studies reviewed here, the dichotomy between head and trunk appears less clear than previously thought. Structures often considered in textbooks to be part of the trunk, such as the cucullaris and its derivatives are actually developmentally and evolutionarily head structures. In turn, some structures in the adult head are derived from trunk primordia (e.g., the tongue muscles from ventral somites), emphasizing

the striking heterogeneity and evolutionary complexity of the vertebrate head. Recent data also undermine the clear distinction previously made between vertebrates and other animals. Urochordates have a cardiopharyngeal field as well as neural crest-like cells and very likely have placodes. Another crucial outcome of the data reviewed here is that contrary to previous assumptions and scenarios still often seen in textbooks and even in specialized papers, neoteny does *not* seem to have played a major role (in contrast to, e.g., the addition of early/late ontogenetic stages) in the major transitions leading to the origin of chordates, cephalochordates, urochordates, vertebrates, cyclostomes, and gnathostomes. In fact, metamorphosis was probably acquired independently in cephalochordates (mainly due to early ontogenetic somatic additions resulting in a peculiar asymmetric larva) and lampreys (mainly due to terminal ontogenetic somatic additions, i.e., of peramorphosis, resulting in a highly peculiar parasitic adult). The adults of the LCA adult of vertebrates were very likely pelagic and motile, as are for instance the adult appendicularian urochordates; therefore, the sessile adult urochordates (e.g., ascidians) are probably also the result of peramorphosis. The lamprey larvae is, therefore, a better model for the cranial muscles of adults of the LCAs of gnathostomes and particularly of vertebrates, than is the adult lamprey, due to peramorphic events that have occurred in the evolutionary history

of cyclostomes, and not to neotenic events leading to the LCA of gnathostomes.

Acknowledgments

We thank Shigeru Kuratani and Tetsuto Miyashita in particular for discussions on issues reviewed in this study. Research by J.M.Z. and R.D. has been supported by a start-up package from the Howard University College of Medicine.

References

- Darwin C. 1871. The descent of man and selection in relation to sex. London: J Murray.
- De Beer G. 1940. Embryos and ancestors. Oxford: Clarendon Press.
- Delsuc F, Brinkmann H, Chourrout D, Philippe H. 2006. Tunicates and not cephalochordates are the closest living relatives of vertebrates. *Nature* 439:965–968.
- Diogo R, Abdala V. 2010. Muscles of vertebrates - comparative anatomy, evolution, homologies and development. Enfield, New Hampshire: CRC Press; Science Publisher. 482 p.
- Diogo R, Hinitz Y, Hughes SM. 2008. Development of mandibular, hyoid and hypobranchial muscles in the zebrafish: homologies and evolution of these muscles within bony fishes and tetrapods. *BMC Dev Biol* 8:1–22.
- Diogo R, Linde-Medina M, Abdala V, Ashley-Ross M. 2013. New, puzzling insights from comparative myological studies on the old and unsolved forelimb/hindlimb enigma. *Biol Rev* 88:196–214.
- Diogo R, Tanaka EM. 2014. Development of fore- and hindlimb muscles in GFP-Transgenic axolotls: morphogenesis, the tetrapod Bauplan, and new insights on the forelimb-hindlimb enigma. *J Exp Zool B Mol Dev Evol* 322:106–127.
- Diogo R, Ziermann JM. 2014. Development of fore- and hindlimb muscles in frogs: morphogenesis, homeotic transformations, digit reduction, and the forelimb-hindlimb enigma. *J Exp Zool B Mol Dev Evol* 322:86–105.
- Diogo R, Kelly RG, Christiaen L, Levine M, Ziermann JM, Molnar JL, Noden DM, Tzahor E. 2015. A new heart for a new head in vertebrate cardiopharyngeal evolution. *Nature* 520:466–473.
- Edgeworth FH. 1935. The cranial muscles of vertebrates. London: Cambridge at the University Press.
- 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. p 259–276.
- Fritzsch B, Northcutt RG. 1993. Cranial and spinal nerve organization in amphioxus and lampreys: evidence for an ancestral cranial pattern. *Cells Tissues Organs* 148:96–109.
- Gans C. 1989. Stages in the origin of vertebrates: analysis by means of scenarios. *Biol Rev Camb Philos Soc* 64:221–268.
- Gans C, Northcutt RG. 1983. Neural crest and the origin of vertebrates: a new head. *Science* 220:268–274.
- Garstang W. 1928. Memoirs: the morphology of Tunicata, and its bearing on the phylogeny of the chordata. *Q J Microsc Sci* 2:51–187.
- Gee H. 1996. Before the backbone: views on the origin of the vertebrates. New York: Springer.
- Gee H. 2001. Deuterostome phylogeny: the context for the origin and evolution of chordates. In: Ahlberg PE, editor. Major events in early vertebrate evolution: palaeontology, phylogeny, genetics and development. London: Taylor & Francis. p 1–14.
- Gegenbaur C. 1878. Elements of comparative anatomy. London: Macmillan & Co.
- Gillis JA, Dahn RD, Shubin NH. 2009. Shared developmental mechanisms pattern the gill arch and paired fin skeletons in vertebrates. *Proc Natl Acad Sci U S A* 106:5720–5724.
- Gillis JA, Fritzenwanker JH, Lowe CJ. 2012. A stem-deuterostome origin of the vertebrate pharyngeal transcriptional network. *Proc Biol Sci* 279:237–346.
- Gould SJ. 1977. Ontogeny and phylogeny. Cambridge: Harvard University Press.
- Graham A, Shimeld SM. 2013. The origin and evolution of the ectodermal placodes. *J Anat* 222:32–40.
- Hall BK. 2007. Homoplasy and homology: dichotomy or continuum? *J Hum Evol* 52:473–479.
- Hall BK. 2013. Homology, homoplasy, novelty, and behavior. *Dev Psychobiol* 55:4–12.
- Hall BK, Gillis JA. 2013. Incremental evolution of the neural crest, neural crest cells and neural crest-derived skeletal tissues. *J Anat* 222:19–31.
- Hardisty MW. 2006. Lampreys: life without jaws. Ceredigion: Forrest Text.
- Harel I, Tzahor E. 2013. Head muscle development. In: McLoon LK, Andrade FH, editors. Craniofacial muscles, a new framework for understanding the effector side of craniofacial muscle control. New York: Springer. p 11–28.
- Heimberg AM, Cowper-Sal-lari R, Sémon M, Donoghue PCJ, Peterson KJ. 2010. microRNAs reveal the interrelationships of hagfish, lampreys, and gnathostomes and the nature of the ancestral vertebrate. *Proc Natl Acad Sci U S A* 107:19379–19383.
- Heyland A, Hodin J, Reitzel AM. 2005. Hormone signaling in evolution and development: a non-model system approach. *Bioessays* 27:64–75.
- Holland LZ. 2015. Genomics, evolution and development of amphioxus and tunicates: the goldilocks principle. *J Exp Zool B Mol Dev Evol* 324:342–352.
- Holland LZ, Holland ND, Gilland E. 2008. Amphioxus and the evolution of head segmentation. *Integr Comp Biol* 48:630–646.
- Holland ND, Holland LZ, Honma Y, Fujii T. 1993. Engrailed expression during development of a lamprey, *Lampetra japonica*: a possible clue to homologies between agnathan and gnathostome muscles of the mandibular arch. *Dev Growth Differ* 35:153–160.
- Jefferies RPS. 1986. The ancestry of the vertebrates. London: British Museum (Natural History).
- Jørgensen JM. 1998. The biology of hagfishes. (1st ed)., London: Chapman & Hall. 578 p.
- Knight RD, Mebus K, d'Angelo A, Yokoya K, Heanue T, Tübingen 2000 Screen Consortium, Roehl H. 2011. Ret signalling integrates a craniofacial muscle module during development. *Development* 138:2015–2024.
- Köntges G, Lumsden A. 1996. Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. *Development* 122:3229–3242.
- Kuratani S, Adachi N, Wada N, Oisi Y, Sugahara F. 2013. Developmental and evolutionary significance of the mandibular arch and prechordal/premandibular cranium in vertebrates: revising the heterotopy scenario of gnathostome jaw evolution. *J Anat* 222:41–55.
- Kuratani S, Kuraku S, Murakami Y. 2002. Lamprey as an evo-devo model: lessons from comparative embryology and molecular phylogenetics. *Genesis* 34:175–183.
- Kuratani S, Murakami Y, Nobusada Y, Kusakabe R, Hirano S. 2004. Developmental fate of the mandibular mesoderm in the lamprey, *Lethenteron japonicum*: comparative morphology and development of the gnathostome jaw with special reference to the nature of the trabecula cranii. *J Exp Zool B Mol Dev Evol* 302:458–468.
- Kusakabe R, Kuraku S, Kuratani S. 2011. Expression and interaction of muscle-related genes in the lamprey imply the evolutionary scenario for vertebrate skeletal muscle, in association with the acquisition of the neck and fins. *Dev Biol* 350:217–227.
- Lemaire P. 2011. Evolutionary crossroads in developmental biology: the tunicates. *Development* 138:2143–2152.
- Lesacroart F, Meilhac SM. 2012. Cell lineages, growth and repair of the mouse heart. *Results Probl Cell Differ* 55:263–289.
- Lesacroart F, Kelly RG, Le Garrec JF, Nicolas JF, Meilhac SM, Buckingham M. 2010. Clonal analysis reveals common lineage relationships between head muscles and second heart field derivatives in the mouse embryo. *Development* 137:3269–3279.
- Livingstone I. 2014. I.Livingstone@BIODIDAC http://biodidac.bio.uottawa.ca/thumbnails/filedet.htm/File_name/chon027b/File_type/cdr & http://biodidac.bio.uottawa.ca/thumbnails/filedet.htm/File_name/chon015b/File_type/gif
- Lours-Calet C, Alvares LE, El-Hanfy AS, Gandesha S, Walters EH, Sobreira DR, Wotton KR, Jorge EC, Lawson JA, Kelsey Lewis A,

- Tada M, Sharpe C, Kardon G, Dietrich S. 2014. Evolutionarily conserved morphogenetic movements at the vertebrate head-trunk interface coordinate the transport and assembly of hypopharyngeal structures. *Dev Biol* 390:231–246.
- Mahadevan NR, Horton AC, Gibson-Brown JJ. 2004. Developmental expression of the amphioxus *Tbx1/10* gene illuminates the evolution of vertebrate branchial arches and sclerotome. *Dev Genes Evol* 214:559–566.
- Mallatt J. 2008. The origin of the vertebrate jaw: neoclassical ideas versus newer, development-based ideas. *Zool Sci* 25:990–998.
- Mallatt J, Chen JY. 2003. Fossil sister group of craniates: predicted and found. *J Morphol* 258:1–31.
- Marinelli W, Strenger A. 1954. Vergleichende Anatomie und Morphologie der Wirbeltiere. I Lieferung. *Lampetra fluviatilis* (L.). 1–80.
- Marinelli W, Strenger A. 1956. Vergleichende Anatomie und Morphologie der Wirbeltiere. II Lieferung. *Myxine glutinosa* (L.). 81–172.
- Matsuoka T, Ahlberg PE, Kessarar N, Iannarelli P, Dennehy U, Richardson WD, McMahon AP, Koentges G. 2005. Neural crest origins of the neck and shoulder. *Nature* 436:347–355.
- Mazet F, Hutt JA, Milloz J, Millard J, Graham A, Shimeld SM. 2005. Molecular evidence from *Ciona intestinalis* for the evolutionary origin of vertebrate cranial placodes. *Dev Biol* 282:494–508.
- McNamara KJ. 1986. A guide to the nomenclature of heterochrony. *J Paleo* 60:4–13.
- McNamara KJ. 1990. The role of heterochrony in evolutionary trends. In: McNamara KJ, editor. *Evolutionary trends*. London: Belhaven Press. p 59–74.
- Minchin JEN, Williams VC, Hinits Y, Low S, Tandon P, Fan C-F, Rawls JF, Hughes SM. 2013. Oesophageal and sternohyal muscle fibres are novel Pax3-dependent migratory somite derivatives essential for ingestion. *Development* 140:2972–2984.
- Miyashita T. 2012. Comparative analysis of the anatomy of the Myxinoidea and the ancestry of early vertebrate lineages. Thesis. University of Alberta. Edmonton, Alberta.
- Miyashita T. 2015. Fishing for jaws in early vertebrate evolution: a new hypothesis of mandibular confinement. *Biol Rev*. doi: 10.1111/brv.12187.
- Nelson JS. 2006. *Fishes of the world*. 4th ed. Hoboken, NJ: Wiley. xix, 601 p.
- Noden DM. 1983. The embryonic origins of avian cephalic and cervical muscles and associated connective tissues. *Am J Anat* 168:257–276.
- Noden DM. 1986. Patterning of avian craniofacial muscles. *Dev Biol* 116:347–356.
- Noden DM, Francis-West P. 2006. The differentiation and morphogenesis of craniofacial muscles. *Dev Dyn* 235:1194–1218.
- Noden DM, Schneider RA. 2006. Neural crest cells and the community of plan for craniofacial development: historical debates and current perspectives. In: Saint-Jeannet J, editor. *Neural crest induction & differentiation*. Advances in experimental medicine and biology, Volume 589. Georgetown: Landes Bioscience. p 1–31.
- Northcutt RG. 2005. The new head hypothesis revisited. *J Exp Zool Mol Dev Evol* 304B:274–297.
- Oisi Y, Ota KG, Kuraku S, Fujimoto S, Kuratani S. 2013a. Craniofacial development of hagfishes and the evolution of vertebrates. *Nature* 493:175–180.
- Oisi Y, Ota KG, Fujimoto S, Kuratani S. 2013b. Development of the chondrocranium in hagfishes, with special reference to the early evolution of vertebrates. *Zool J Linn Soc* 30:944–961.
- Paris M, Escriva H, Schubert M, Brunet F, Brtko J, Ciesielski F, Roeklin D, Vivat-Hannah V, Jamin EL, Cravedi J-P, Scanlan TS, Renaud J-P, Holland ND, Laudet V. 2008. Amphioxus postembryonic development reveals the homology of chordate metamorphosis. *Curr Biol* 18:825–830.
- Paris M, Laudet V. 2008. The history of a developmental stage: metamorphosis in chordates. *Genesis* 46:657–672.
- Paris M, Hillenweck A, Bertrand S, Delous G, Escriva H, Zalko D, Cravedi J-P, Laudet V. 2010. Active metabolism of thyroid hormone during metamorphosis of amphioxus. *Integr Comp Biol* 50: 63–74.
- Piekarski N, Olsson L. 2007. Muscular derivatives of the cranial-most somites revealed by long-term fate mapping in the Mexican axolotl (*Ambystoma mexicanum*). *Evo Devo* 9:566–578.
- Piotrowski T, Nüsslein-Volhard C. 2000. The endoderm plays an important role in patterning the segmented pharyngeal region in zebrafish (*Danio rerio*). *Dev Biol* 225:339–356.
- Presley R, Horder TJ, Slipka J. 1996. Lancelet development as evidence of ancestral chordate structure. *Israel J Zool* 42:S97–S116.
- Prunotto C, Crepaldi T, Forni PE, Leraci A, Kelly RG, Tajbakhsh S, Buckingham M, Ponzetto C. 2004. Analysis of *Mlc-lacZ* Met mutants highlights the essential function of Met for migratory precursors of hypaxial muscles and reveals a role for Met in the development of hyoid arch-derived facial muscles. *Dev Dyn* 231: 582–591.
- Rose CS. 2005. Integrating ecology and developmental biology to explain the timing of frog metamorphosis. *Trends Ecol Evol* 20: 129–135.
- Sambasivan R, Kuratani S, Tajbakhsh S. 2011. An eye on the head: the development and evolution of craniofacial muscles. *Development* 138:2401–2415.
- Sauka-Spengler T, Le Mentec C, Lepage M, Mazan S. 2002. Embryonic expression of *Tbx1* a DiGeorge syndrome candidate gene, in the lamprey *Lampetra fluviatilis*. *Gene Expr Patterns* 2: 99–103.
- Shearman RM, Burke AC. 2009. The lateral somitic frontier in ontogeny and phylogeny. *J Exp Biol Mol Dev Evol* 312B:602–613.
- Stokes MD, Holland ND. 1995. Ciliary hovering in larval lancelets (= Amphioxus). *Biol Bull* 188:231–233.
- Stolfi A, Gainous TB, Young JJ, Mori A, Levine M, Christiaen L. 2010. Early chordate origins of the vertebrate second heart field. *Science* 329:565–568.
- Tulenok FJ, McCauley DW, MacKenzie EL, Mazan S, Kuratani S, Sugahara F, Kusakabe R, Burke AC. 2013. Body wall development in lamprey and a new perspective on the origin of vertebrate paired fins. *Proc Natl Acad Sci U S A* 110:11899–11904.
- Valentine JW. 2004. *On the origin of Phyla*. Chicago: University of Chicago Press.
- Vargas AO, F Aboitiz. 2005. How ancient is the adult swimming capacity in the lineage leading to euarchontates?. *Evo Devo* 7: 171–174.
- Willely A. 1894. *Amphioxus and the ancestry of the vertebrates*. New York: MacMillan & Co.
- Yalden DW. 1985. Feeding mechanisms as evidence for cyclostome monophyly. *Zool J Linn Soc* 84:291–300.
- Yasui K, Kaji T, Morov AR, Yonemura S. 2014. Development of oral and branchial muscles in lancelet larvae of *Branchiostoma japonicum*. *J Morphol* 275:465–477.
- Youson JH. 1980. Morphology and physiology of lamprey metamorphosis. *Can J Fisheries Aquatic Sci* 37:1687–1710.
- Youson JH. 1997. Is lamprey metamorphosis regulated by thyroid hormones? *Am Zool* 37:441–460.
- 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, Diogo R. 2014. Cranial muscle development in frogs with different developmental modes: direct development vs. biphasic development. *J Morphol* 275:398–413.
- Ziermann JM, Miyashita T, Diogo R. 2014. Cephalic muscles of Cyclostomes (hagfishes and lampreys) and Chondrichthyes (sharks, rays, and holocephalans): comparative anatomy and early evolution of the vertebrate head muscles. *Zool J Linn Soc* 172:771–802.
- Zhu M, Yu X, Ahlberg PE, Choo B, Lu J, Qiao T, Qu Q, Zhao W, Jia L, Blom H, Zhu YA. 2013. A Silurian placoderm with osteichthyan-like marginal jaw bones. *Nature* 502:188–193.