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Article in *The Anatomical Record Advances in Integrative Anatomy and Evolutionary Biology* · March 2015

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Muscles of Chondrichthyan Paired Appendages: Comparison With Osteichthyans, Deconstruction of the Fore–Hindlimb Serial Homology Dogma, and New Insights on the Evolution of the Vertebrate Neck

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ABSTRACT

Here we present the first study comparing all the paired appendage muscles of representatives of each major extant gnathostome group. We address a crucial and enigmatic question in evolutionary and comparative anatomy: Why are the pelvic and pectoral appendages of gnathostomes, and particularly of tetrapods, in general so similar to each other? We argue that an integrative analysis of the new myological data and the information from the literature contradicts the idea that the forelimbs and hindlimbs are serial homologues. The data show that many of the strikingly similar fore- and hindlimb muscles of extant tetrapods evolved independently in each appendage because the ancestors of extant gnathostomes and osteichthyans only had an adductor and an abductor in each fin. Therefore, these data contradict the idea that at least some muscles present in the tetrapod fore- and hindlimbs were already present in some form in the first fishes with pectoral and pelvic appendages, as the result of an ancestral duplication of the paired appendages leading to a true serial homology. The origin of the pectoral girdle was instead likely related to head evolution, as illustrated by the cucullaris of gnathostomes such as chondrichthyans inserting onto both the branchial arches and pectoral girdle. Only later in evolution the cucullaris became differentiated into the levatores arcuum branchialium and protractor pectoralis, which gave rise to the amniote neck muscles trapezius and sternocleidomastoideus. These changes therefore contributed to an evolutionary trend toward a greater anatomical and functional independence of the pectoral girdle from head movements. *Anat Rec*, 00:000–000, 2014. © 2014 Wiley Periodicals, Inc.

Key words: anatomy; chondrichthyans; fins; limbs; muscles; paired appendages; serial homology

The idea that the structures of the fore- and hindlimbs are serial homologues was first proposed by authors such as Vicq d'Azyr (1774), Oken (1843), and Owen (1849, 1866). This idea is still accepted in most textbooks and scientific papers. However, a careful examination of the original works of these authors reveals that their fore- and hindlimb comparisons were almost exclusively based on bones, and almost no attention was paid to soft tissues such as muscles, nerves and

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Author contributions: RD and JMZ designed the research, dissected the specimens, and wrote the manuscript.

Received 17 February 2014; Accepted 30 July 2014.

DOI 10.1002/ar.23047

Published online 10 September 2012 in Wiley Online Library (wileyonlinelibrary.com).

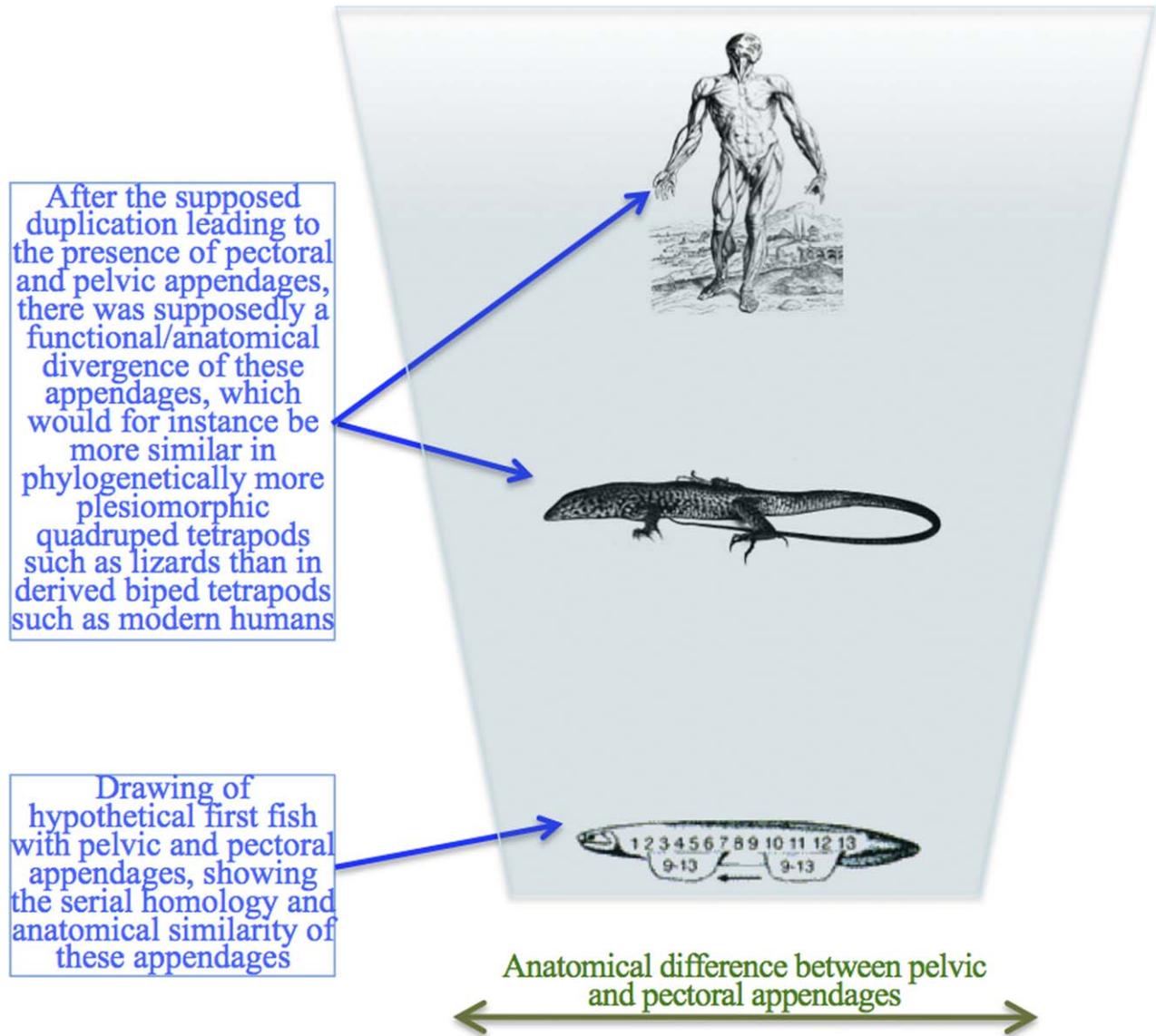


Fig. 1. Simplified scheme illustrating the “serial homology followed by functional/anatomical divergence” hypothesis often shown in textbooks and followed in more technical papers, particularly within the fields of developmental biology and evo-devo. The picture of the hypothetical fish is modified from Shubin et al.’s (1997) scheme showing the origin and evolution of paired appendages. According to that scheme, establishment of serially homologous appendages was proposed to result from gene cooption during the evolution of Paleozoic vertebrates. That is, *Hox* genes were initially involved in specifying regional identities along the primary body axis, particularly in caudal

segments, and then during the origin of jawed fish there was a cooption of similar nested patterns of *HoxD* expression in the development of both sets of paired appendages (numbers shown within the fish body). According to this scheme the cooption may have happened in both appendages simultaneously, or *Hox* expression could have been initially present in a pelvic appendage and been coopted in the development of an existing pectoral outgrowth (arrow below fish body; the pictures of the other taxa are modified from Diogo and Abdala, 2010 and references therein).

blood vessels. Moreover, in most cases the use of the term “limb serial homology” clearly referred to what is currently viewed as evolutionary parallelism or convergence, that is, as homoplasy, and not to serial homology. A true, ancestral morphological or developmental serial homology of these appendages would in fact imply that these appendages were the result of an ancestral duplication and were therefore originally similar and then diverged anatomically/functionally (Fig. 1).

The historical context about the development of the fore-hindlimb serial homology hypothesis was discussed in some detail by Diogo et al. (2013) and Diogo and Molnar (2014). In a short and simplified summary, it can be said that for earlier non-evolutionary comparative anatomists the striking similarity of several individual structures of the fore- and hindlimb was not a major problem, because they could argue that the design of animals followed an “archetype,” created by a supernatural or a vital power. For instance, within the *Naturphilosophie*

paradigm followed by authors such as Oken, the term “serial homology” did not necessarily imply an anatomical duplication of a primitive “limb archetype.” The romantic definition of an “archetype,” which was partially followed by Owen, instead often referred to all the possible different, peculiar phenotypes that a structure or organ could assume, thus having some resemblance with the current definition of a “norm of reaction” (see, e.g., Russell, 1916; Richards, 2002). This is illustrated in *On the Nature of Limbs* (Owen, 1849), in which the examples of striking similarity between the fore- and hindlimb refer mainly to tetrapods with highly derived limbs, horses and plesiosaurs. When referring to these examples Owen, who was influenced by the German *Naturphilosophie* (Richards, 2002), actually uses the word parallelism more often than “serial homology.” Moreover, when Owen discusses phylogenetically more plesiomorphic taxa, for example, chondrichthyans, he states that those taxa actually confuse the notion of “archetype” and “serial homology.” That is, he was in fact always referring to derived, peculiar cases of homoplasy, not to the concept of serial homology that is commonly accepted nowadays (see, e.g., Roth, 1994; Wagner, 1994; Wilmer, 2003; Diogo et al., 2013).

For early evolutionary comparative anatomists the similarity between the fore- and hindlimb (“fore-hindlimb enigma” sensu Diogo et al., 2013) became a particularly important subject of discussion, and there were two major, different ways to try to address this issue (see, e.g., Humphry, 1872a,b; Quain et al., 1894; McMurrich, 1905; Bardeen, 1906; and references therein). One was to follow the romantic idea that the vertebrate body is perfectly segmented (e.g., Goodrich, 1930) and thus to postulate that the hindlimb is nothing more than a second forelimb, which was in turn often viewed as a derivative of a posterior branchial arch (e.g., Gegenbaur, 1878). The other was to argue that all the hard and soft tissue structures that are present in the fore- and hindlimb of basal tetrapods were already present in some form in plesiomorphic gnathostome fish, as the result of an unique, ancestral duplication of the paired appendages. An illustrative example is Humphry’s (1872b) study, in which various vertebrate animals were compared and in which it was defended that not only dipnans but also fish such as sharks have for instance a “latissimus dorsi” muscle or a “pectoralis” muscle similar to those of extant tetrapods. Since the publication of these classical studies, a large body of evidence has been published on the subject and has contradicted (1) the view that the limbs are the result of supranatural or vital phenomena, and (2) the romantic idea of perfect segmentation of the vertebrate body (Diogo et al., 2013).

However, strikingly, authors continued to cite these and other classical studies to “show” that the structures of the pelvic and pectoral appendages, including the ones of the tetrapod fore- and hindlimb, were serial homologues. Instead of questioning this idea, researchers instead preferred to focus on more specific details about the origin and evolution of the paired appendages within—and thus accepting *a priori*—the serial homology paradigm. More recent developmental studies have provided some evidence that apparently contradicted Goodrich’s and Gegenbaur’s hypotheses about the origin of the paired appendages, and suggest that pectoral and pelvic fins actually evolved from continuous stripes of

competency for appendage formation located ventrally and laterally along the embryonic flank (see, e.g., Don et al., 2013; but see Gillis et al. 2009, and Discussion below). A continuation of this theory proposed that the paired appendages evolved with a shift in the zone of competency to the lateral plate mesoderm in conjunction with the establishment of the lateral somitic frontier, which allowed for the formation of fin/limb buds with internal supporting skeletons (Don et al., 2013).

The idea that the pectoral and pelvic appendages are serial homologues is generally associated to the notion that these appendages were originally similar to each other, and that there was a subsequent functional/anatomical divergence between them (Diogo et al., 2013). For instance, Don et al. (2013) explain that the ancestral *Tbx4/5* cluster of vertebrates probably underwent a duplication event and that now *Tbx4* is related to the hindlimb and *Tbx5* with the forelimb of tetrapods; they then state that “pectoral fins evolved first and then duplicated to form pelvic fins” (see their Fig. 3). This illustrates the confusion, in evo-devo studies, between (1) a duplication of the *Tbx4/5* cluster and subsequent cooption for the genetic pathways associated with the ontogeny of the different paired appendages, and (2) the morphological duplication of the appendages themselves and of their individual structures (e.g., of their muscles and bones). That similar genes operate to facilitate an outgrowth that gives rise to different limbs in the same animal does not necessarily mean that these limbs are serial homologues; similar genes and gene cascades/networks may have been simply recruited homoplasically as organizers of limb development (see, e.g., Wilmer, 2003).

In fact, the detailed analysis of recent developmental, genetic, paleontological, and functional studies also raises serious questions about the serial homology hypothesis. An extensive review of these lines of evidence was provided by Diogo et al. (2013), who strongly contradicted that hypothesis based on a review of the literature and on the results of a long-term study of the muscles of osteichthyans (see, e.g., Diogo, 2007; Diogo and Abdala, 2007, 2010; Diogo and Wood, 2011, 2012; Diogo et al., 2009, 2012; Diogo and Tanaka, 2012). More recently, that long-term project was expanded to include both developmental and regenerative studies of tetrapod taxa such as salamanders and frogs, which also provided new insights on the fore-hindlimb enigma and further evidence contradicting the fore-hindlimb serial homology idea (Diogo and Tanaka, 2014; Diogo and Ziermann, 2014; Diogo et al., 2014a,b). However, so far this long-term project had not included detailed dissections of the muscles of the paired appendages of chondrichthyans, which are crucial to test the hypothesis that at least some muscles that are present in the fore- and hindlimb of basal tetrapods were already present in the first fish with pectoral and pelvic appendages, as the result of an unique, ancestral morphological duplication of the paired appendages (see above). This is because if for example a certain appendicular muscle of tetrapods is not present in chondrichthyans (the sister-group of extant osteichthyans) and in osteichthyans such as sarcopterygian fish (the sister-groups of tetrapods; e.g., dipnans, *Latimeria*) and/or cladistian actinopterygians (the sister-group of all other extant actinopterygians; e.g., *Polypterus*), it would be very difficult to argue that the muscle was present in the last common ancestor (LCA) of

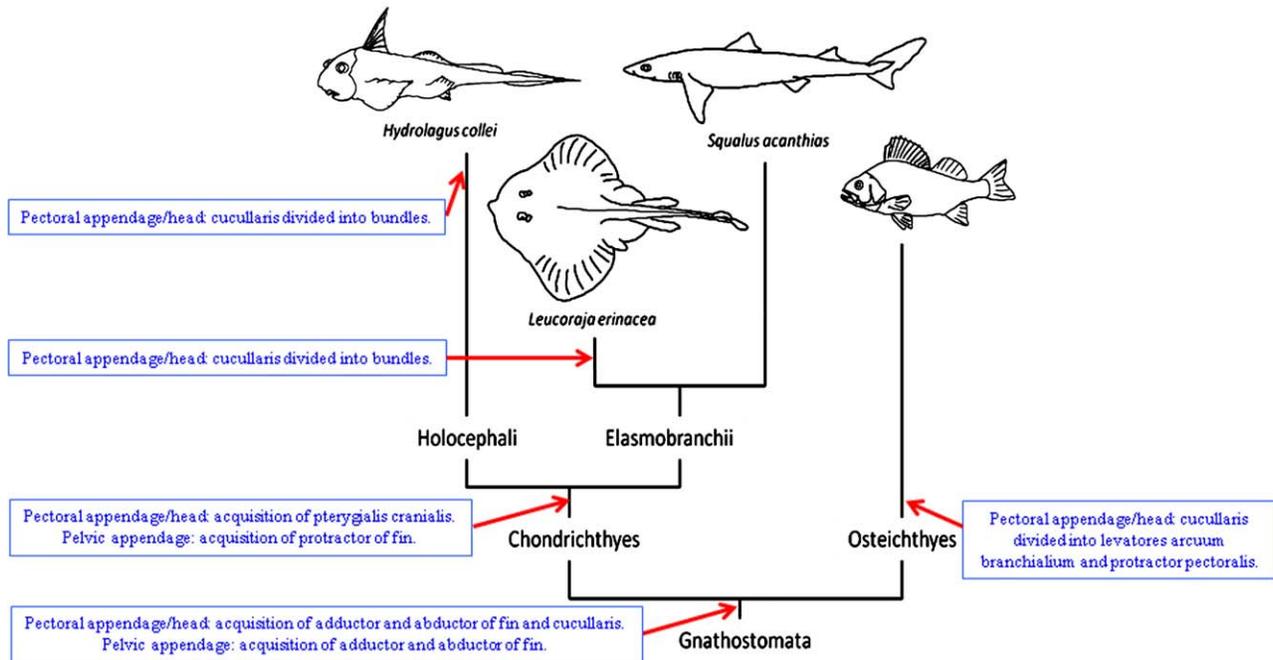


Fig. 2. Simplified cladogram of the species used in this study. The Osteichthyes are shown to complete the cladogram and also because they are included in the discussions of the text. The major evolutionary changes that occurred in each of the groups shown, following the results obtained in the present work, are also shown (for more details about these evolutionary changes, see Discussion).

chondrichthyans and osteichthyans, that is, of extant gnathostomes.

In the present work we therefore provide, for the first time, the results of our dissections of the muscles of the paired appendages of representatives of the three major chondrichthyan extant clades (Holocephali and the Elasmobranchii clades Selachii, or sharks, and Batoidea, or rays and skates). This article is therefore the culmination of our long-term comparative study, which now includes dissections of all the muscles of the head and of the paired appendages of all major extant gnathostome groups. There have been various anatomical studies of chondrichthyans and more recently also various developmental studies of these gnathostomes, particularly due to the interest of these fish for evo-devo and evolutionary biology because they are crucial to understand the origin and early evolution of vertebrate and gnathostome structures. However, those studies have mainly focused on hard tissues and/or on the cephalic region, and the few studies that have focused on appendicular muscles have normally used their own nomenclature and did not really provide comparisons with older, classical anatomical and evolutionary works and/or with works focused on other gnathostomes, and therefore lacked a broader historical, comparative and evolutionary framework (see, e.g., review by Diogo and Abdala, 2010). That is why in this article we combine the results of our recent dissections of chondrichthyans and an extensive review of the literature, of both recent anatomical and developmental papers and of older, classical works, in order to provide such a broad framework, which is crucial and will pave the way for further developmental and evolutionary discussions. Apart from providing new, updated textual and visual descriptions of the muscles of the pelvic and

pectoral girdles and fins of chondrichthyans, the new data obtained from our dissections will therefore also allow us to discuss which muscles were likely present in the LCA of extant gnathostomes, of extant chondrichthyans and of extant osteichthyans, and thus to specifically test the hypothesis (proposed by authors such as Humphry, 1872b: see above) that at least some hindlimb and/or forelimb muscles of tetrapods were already present in early gnathostomes.

MATERIALS AND METHODS

We dissected the muscles of the paired appendages of representatives of the three major chondrichthyan extant clades (Fig. 2): the holocephalan *Hydrolagus collei* (Holocephali; spotted ratfish; three adult female specimens, bought from Ward's Natural Science, Rochester, NY) and the elasmobranchs *Squalus acanthias* (Selachii; dogfish shark, three juvenile male specimens sent by Michelle Bailey, Mount Desert Island Biological Laboratory—MDIBL, Salisbury Cove, Maine) and *Leucoraja erinacea* (Batoidea; little skate, three juvenile female specimens, sent by Michelle Bailey, MDIBL, and by Nicole Theodosiou, Union College Schenectady, NY). The results were compared with descriptions provided by other authors in the literature and to the results of our dissections of representatives of all major groups of osteichthyans. Photographs and drawings showing the muscles of other gnathostomes were published in the monographs and papers published so far as part of our long-term project (see Introduction), particularly in Diogo and Abdala (2010). It is important to note that in this latter book and other previous publications, Diogo et al. have proposed the building of an unifying muscle

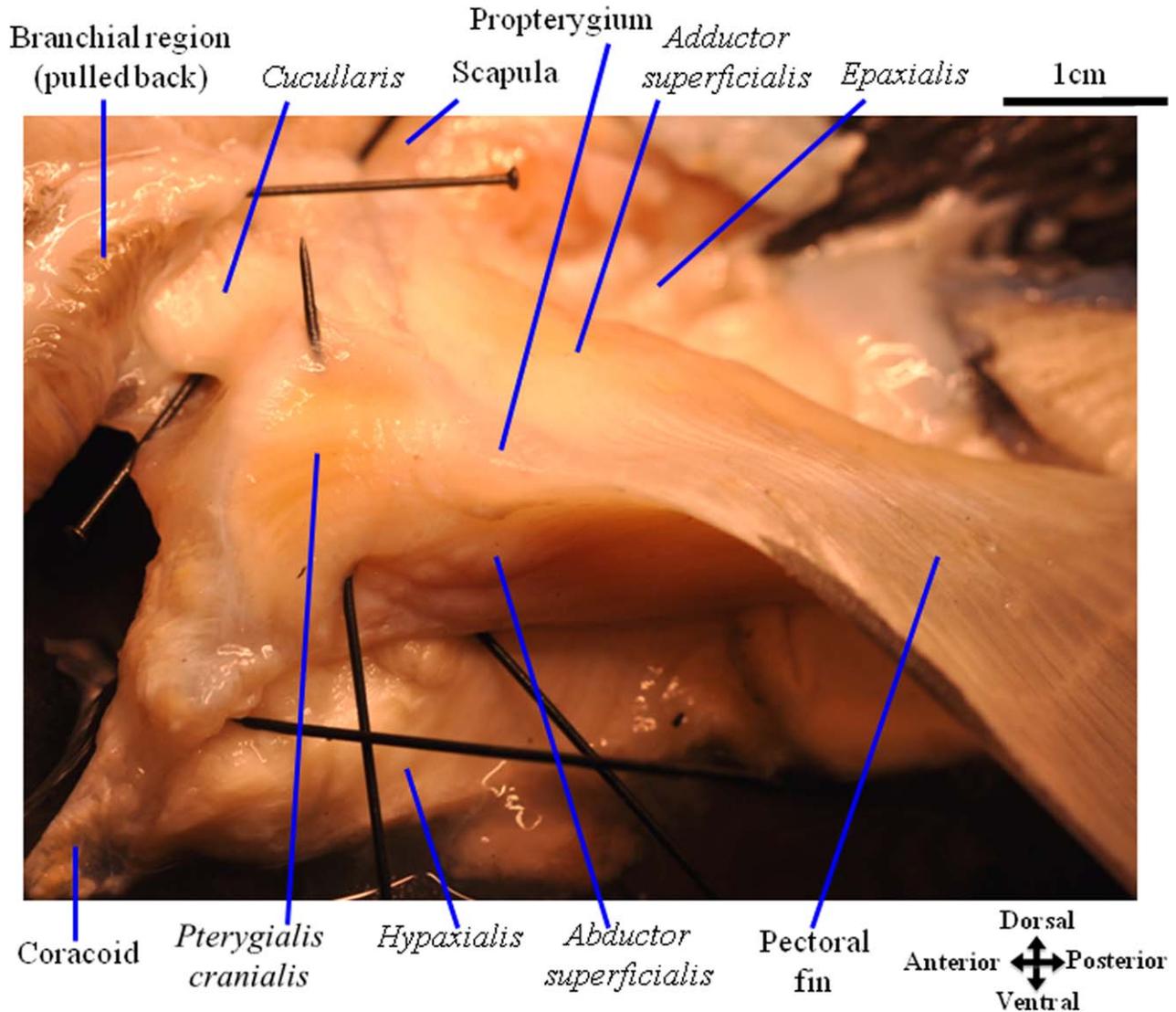


Fig. 3. Lateral view of the left pectoral girdle and fin muscles of the shark *Squalus acanthias*.

nomenclature for the clade Vertebrata as a whole, which takes into account the nomenclatures used in human anatomy and other animals. In the present article we follow the same unifying nomenclature, that is, a certain muscle of a *same paired appendage in different taxa* is always designated by the same name. To compare the information provided in this article with that provided by other authors, other names commonly used by other authors are given in the descriptions of the Results section.

RESULTS

The results of our analyses are shown in detail in Figs. 3–9; therefore in this section we will provide a brief textual description of these results, which is divided into three subsections. It should be noted that we did not find any differences concerning the configuration and attachments of the muscles of different specimens dissected from a same species.

Muscles of Paired Appendages of *Squalus acanthias*

The *adductor* muscle of the pectoral fin (Fig. 3; “extensor or levator or pterygii dorsalis” sensu, e.g., Walker, 1954, and Jarvik, 1965) arises from the pectoral girdle (mainly scapular region) to the dorsal surfaces of the radial cartilages (Figs. 3 and 4). The name *adductor* follows the nomenclature of Diogo and Abdala (2010), as it corresponds to the *adductor* of osteichthyan fish. Some of its fibers also contact the propterygium, metapterygium and mesopterygium, because the muscle has a superficial bundle (*adductor superficialis*; Fig. 3) but also a deep bundle (*adductor profundus*) that attaches onto a region of the pectoral fin that is more proximal than the insertion of the superficial bundle. The *abductor* of the pectoral fin (Fig. 3; “flexor or depressor or pterygii ventralis” sensu, e.g., Walker, 1954; Jarvik, 1965) spreads mainly from the coracoid bar to the

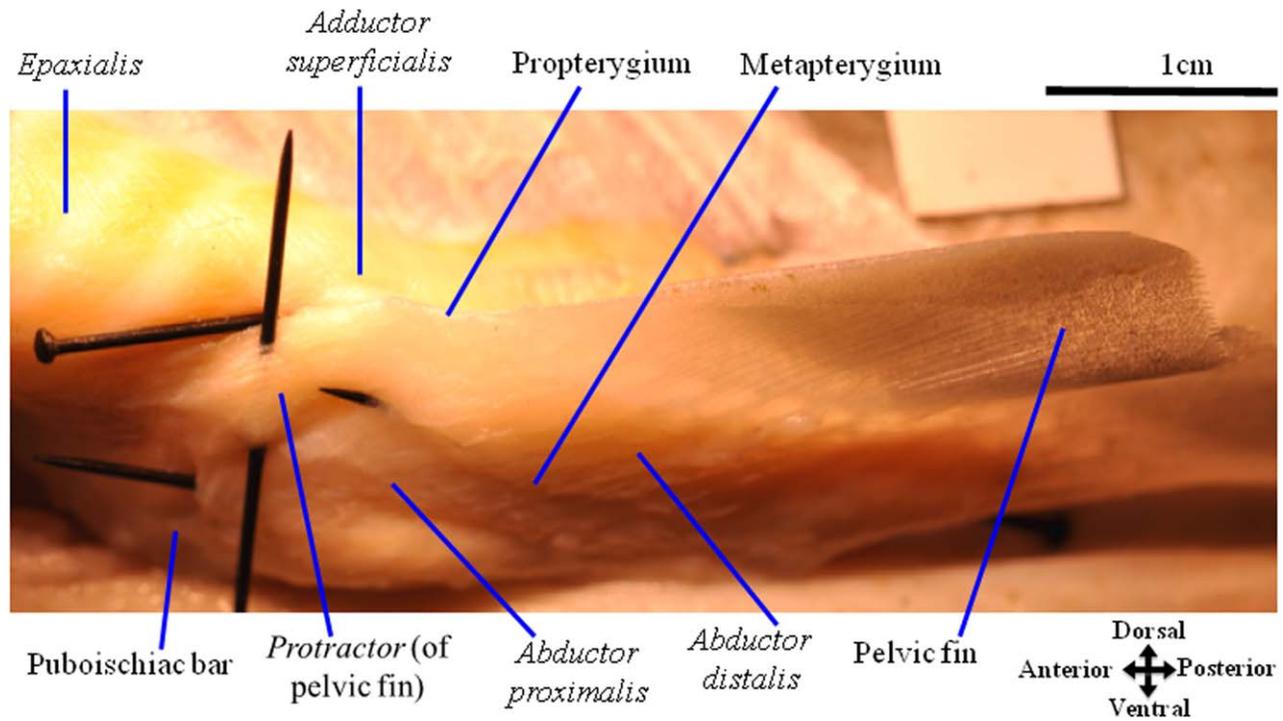


Fig. 4. Lateral view of the left pelvic girdle and fin muscles of the shark *Squalus acanthias*.

ventral surfaces of the radial cartilages, also having a superficial bundle (*abductor superficialis*; Fig. 3) and a deep bundle (*abductor profundus*) that goes from the pro-, meta- and meso-ptyerygium to an insertion point that is proximal to the insertion of the superficial bundle. The *ptyerygialis cranialis* (Fig. 3; sensu, e.g., Marinelli and Strenger, 1959), which is essentially a protractor of the pectoral fin, although it was designated as “adductor” by Jarvik (1965), is clearly distinct from the *abductor* of the pectoral fin, from which it seems to derive, running from the pectoral girdle (both scapular region and coracoid bar) to the propterygium [the ontogeny of this protractor muscle is shown by Jarvik (1965), who also designated it as a “modified anterior radial muscle”]. The *epaxialis* and *hypaxialis* (Fig. 3) are not differentiated into individual muscles (e.g., “pectoralis,” “latissimus dorsi”), contrary to what was suggested by Humphry (1872a,b; see Introduction). For the context of the present work, it is worthy to also describe the muscle *cucullaris* (Fig. 3; “trapezius” sensu Marion, 1905), which is a head (branchial) muscle (usually innervated by cranial nerve X in chondrichthyans: e.g., Edgeworth, 1935; see also Diogo and Abdala, 2010) but is partially inserted on the pectoral girdle. In the *Squalus acanthias* specimens dissected by us, the *cucullaris* is a continuous muscle originating from the fascia of the epibranchial and/or epaxial musculature and inserting onto both the pectoral girdle and the dorsal surface of at least all branchial arches.

The *levatores arcuum branchialium* were not found by us, and the *cucullaris* actually seems to correspond to the *protractor pectoralis* plus the *levatores arcuum branchialium* of osteichthyan fish, and that is why we use the name *cucullaris* to designate this muscle (see Discussion).

Regarding the muscles of the pelvic appendage, the *adductor* of the pelvic fin (Fig. 4; “extensor or levator” sensu, e.g., Walker, 1954; Jarvik, 1965) arises mainly from posterior trunk myomeres, particularly its superficial bundle (*adductor superficialis*; Fig. 4). Its deep bundle (*adductor profundus*) originates mainly from the iliac process and metapterygium and inserts less distally than the superficial bundle, both bundles going to the dorsal surfaces of the radial cartilages; therefore, the *adductor* of the pelvic fin is anatomically somewhat similar to the *adductor* of the pectoral fin. However, the *abductor* of the pelvic fin (Fig. 4; “flexor or depressor” sensu, e.g., Walker, 1954, and Jarvik, 1965) has instead proximal and distal bundles (*abductor proximalis*, *abductor distalis*; Fig. 4), being therefore anatomically different from the *abductor* of the pectoral fin. Its proximal bundle runs from the puboischiac bar to the metapterygium, while its distal bundle runs from the metapterygium to the ventral surfaces of the radial cartilages. The *protractor* of the pelvic fin (Fig. 4) is anatomically similar to the *ptyerygialis cranialis* of the pectoral appendage but somewhat less differentiated, that is, it is more deeply blended to the *abductor* of the pelvic fin from which it seems to derive (i.e., probably why Jarvik, 1965, did not label this muscle in the pelvic fin shown in his Fig. 9E, even though the muscle is somewhat visible in that figure). The *protractor* of the pelvic fin runs from the pelvic girdle to the propterygium of this fin.

Muscles of Paired Appendages of *Leucoraja erinacea*

The *abductor* of the pectoral fin (Fig. 5) runs from the pectoral girdle to the ventral surfaces of the radial

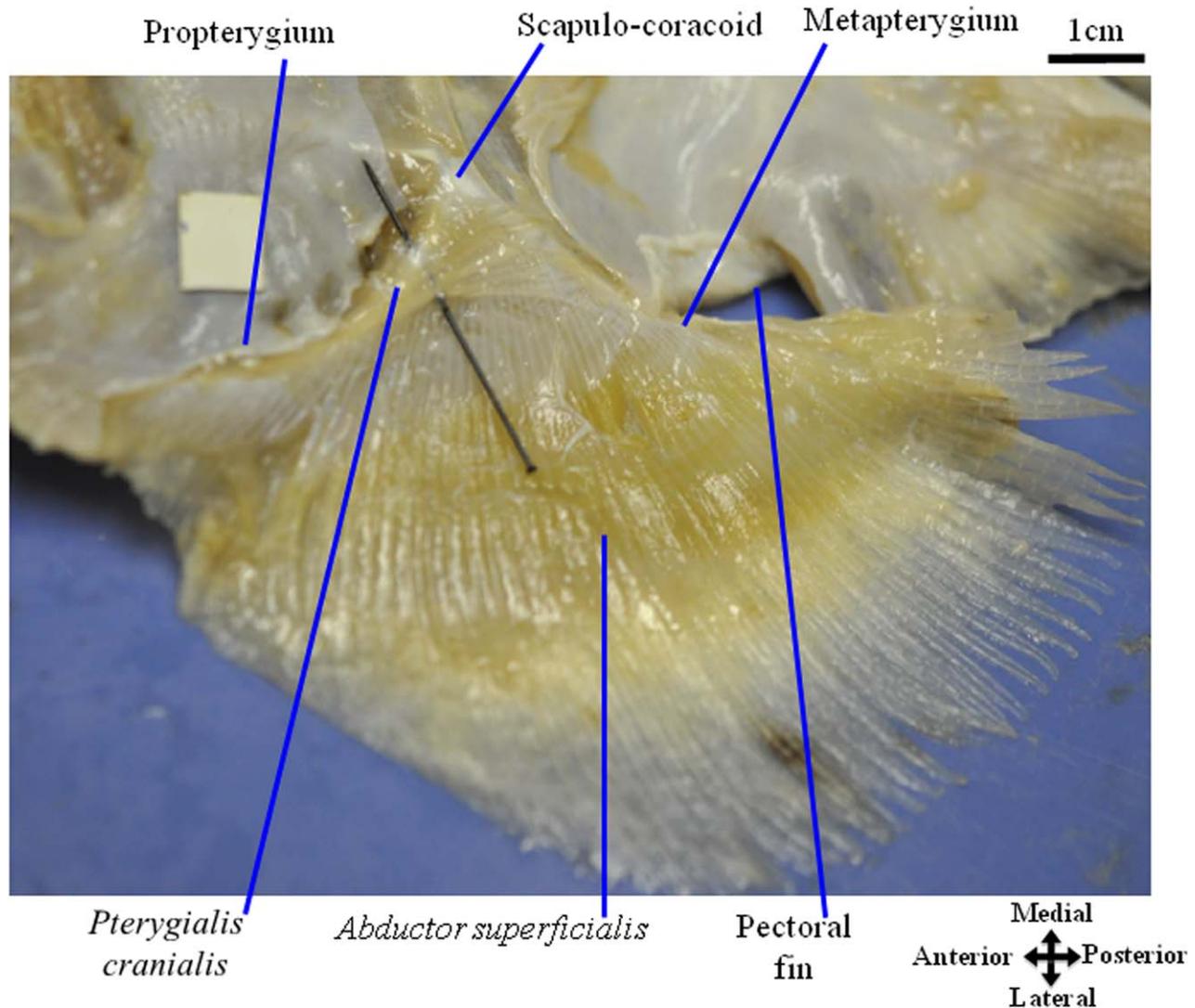


Fig. 5. Vento-lateral view of the pectoral girdle and fin muscles of the little skate *Leucoraja erinacea*.

cartilages, having a superficial bundle and a deep bundle that originates from the propterygium and meta- pterygium and inserts at a point proximal to the insertion of the superficial bundle (Figs. 5–7). There is a distinct muscle that corresponds topologically, and seems to be homologous, to the *pterygialis cranialis* of sharks, running (mainly anteriorly, not posteriorly as in sharks, due to the peculiar position of the propterygium of *Leucoraja*) from the pectoral girdle to the propterygium (Fig. 5). The *adductor* of the pectoral fin (Fig. 6) runs from the pectoral girdle to the dorsal surfaces of the radial cartilages, some fibers also contacting the propterygium and metapterygium, because the muscle has superficial and deep bundles similar those found in *Squalus acanthias*. There is also a muscle *levator 4* of the pectoral fin (Fig. 6), which is somewhat similar to the *levator 3* of the pectoral fin of *Hydrolagus* because it originates from the fascia of, and is superficial to, the epaxial/hypaxial musculature and mainly inserts onto the metapterygium, but is much broader and more

vertical than the relatively thin and oblique (antero-ven- trally directed) *levator 3* of *Hydrolagus* (see below). On the *adductor* (dorsal) side of the pectoral fin of *Leucoraja* there is a mesial muscular band running from the pectoral girdle to the metapterygium that is similar to that described in sharks by Jarvik (1965) but that is more differentiated in *Leucoraja* than in the *Squalus* speci- mens dissected by us. In *Leucoraja* there is also a similar muscular band on the *adductor* side of the pectoral fin running from the pectoral girdle to the the proptery- gium, but both, this structure and the mesial muscular band attaching onto the metapterygium, are much less differentiated than is the *pterygialis cranialis* of the *abductor* side of the pectoral fin, that is, these muscular bands are seemingly simply bundles of the *abductor superficialis* of this fin. Apart from the *levator 4*, in *Leucoraja* the only structures related to the epaxial/hypaxial musculature and to the pectoral appendage that seem to constitute separate muscles are the *retractor mesio- ventralis pectoralis* (from skull to epaxial musculature,

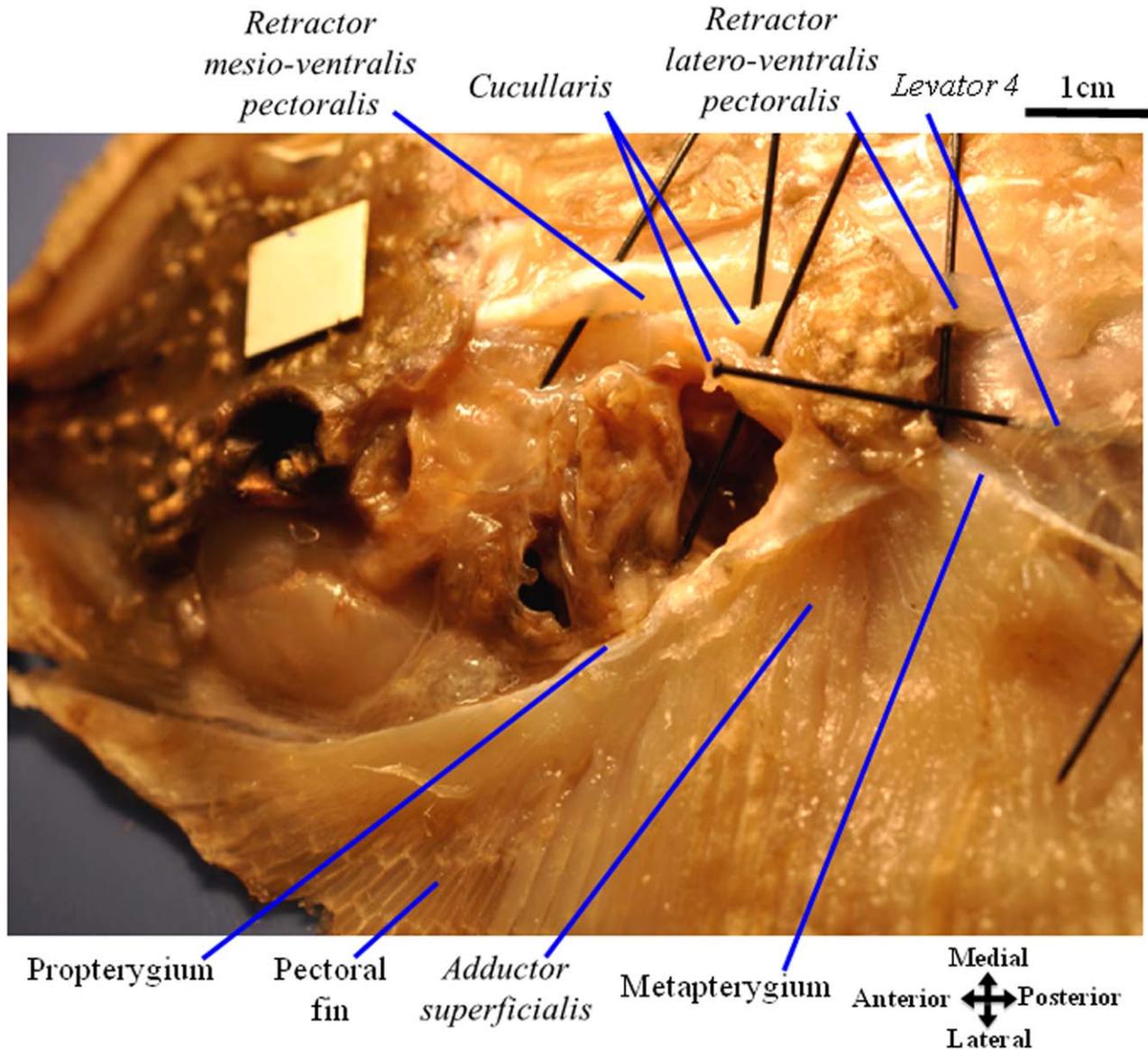


Fig. 6. Dorso-lateral view of the pectoral girdle and fin muscles of the little skate *Leucoraja erinacea*.

being loosely connected to medial side of pectoral girdle) and the *retractor latero-ventralis pectoralis* (from epaxial musculature to lateral surface of the pectoral girdle) (Fig. 6). The head muscle *cucullaris* (Fig. 6) is divided into a medial bundle running from the first vertebra to the suprascapula, and a lateral bundle (not two lateral bundles as described by Marion, 1905) that inserts onto at least some of the most posterior branchial arches and also onto the pectoral girdle.

Concerning the muscles of the pelvic appendage, the *abductor* of the pelvic fin (Fig. 7; “flexor or depressor” sensu, e.g., Macesic and Kajiura, 2010) has two distinct bundles, that is, a proximal bundle running from the pelvic girdle and hypaxial musculature to the metapterygium and a distal bundle running from the metapterygium to the ventral surfaces of the radial cartilages; that is, this muscle is anatomically somewhat similar to

the *abductor* of the pelvic fin of *Squalus*. The *adductor* of the pelvic fin (Fig. 7; “extensor or levator” sensu, e.g., Macesic and Kajiura, 2010) is also anatomically similar to that of *Squalus*. It arises mainly from posterior trunk myomeres, particularly its superficial bundle, its deep bundle arising largely from the metapterygium and inserting less distally than the superficial bundle, both bundles going to the dorsal surfaces of the radial cartilages. Apart from these two muscles of the pelvic fin, *Leucoraja* also has four ventral and four dorsal muscles attaching onto the propterygium, which are probably derived structures related to the peculiar type of locomotion of benthic batoids such as *L. erinacea*, as suggested by Macesic and Kajiura (2010). At least some of these additional muscles, particularly some of the ventral ones, seem to correspond to parts of the pelvic fin *protractor* of sharks. The four additional ventral muscles

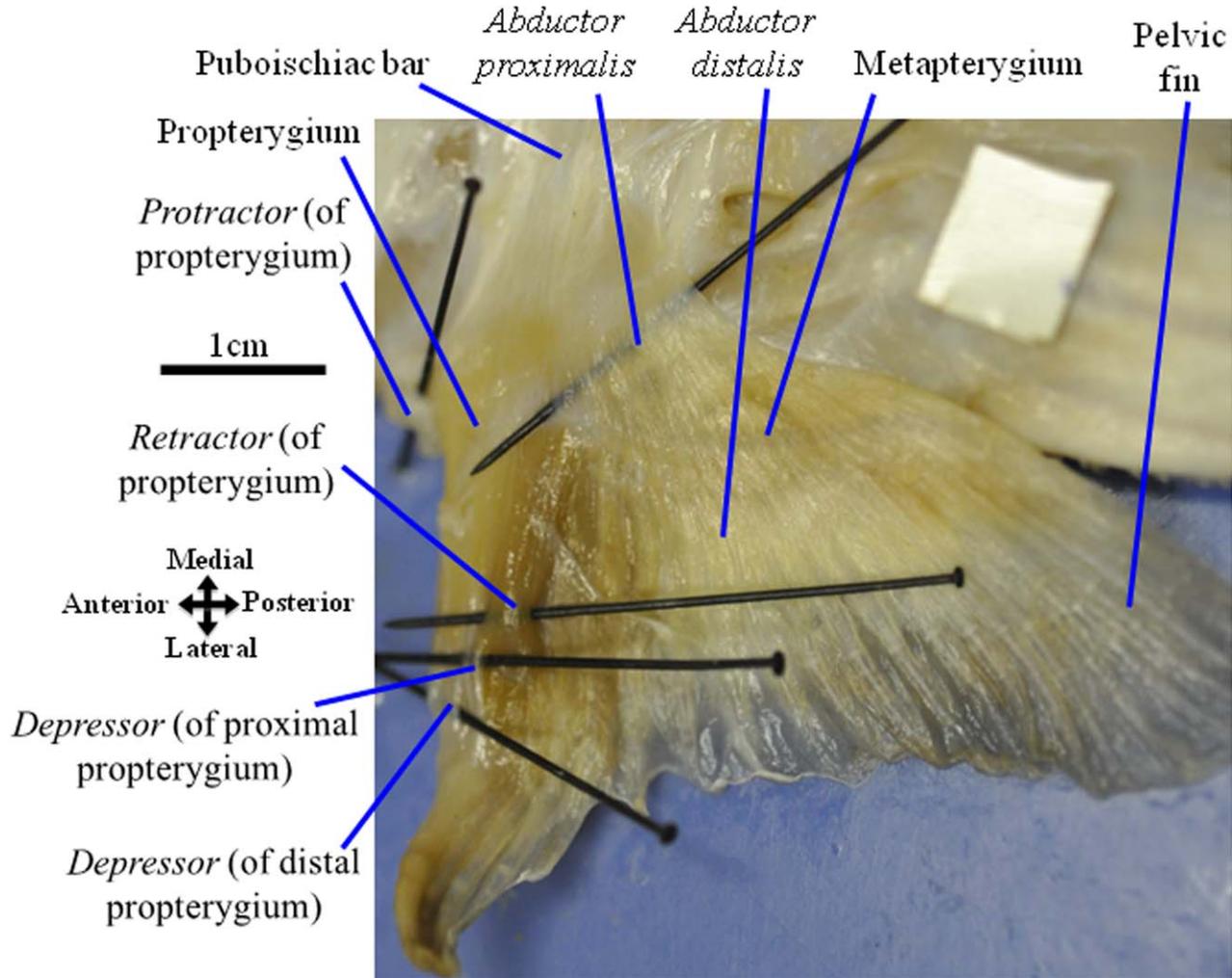


Fig. 7. Vento-lateral view of the pelvic girdle and fin muscles of the little skate *Leucoraja erinacea*.

are the ventral *protractor* of the proximal propterygium (mainly from body musculature to proximal portion of propterygium), the *depressor* of the proximal propterygium (from pelvic girdle to proximal portion of propterygium), the *depressor* of the distal propterygium (from pelvic girdle to distal portion of propterygium) and the *retractor* of the propterygium (from pelvic girdle to propterygium) (Fig. 7). The four additional dorsal muscles are the dorsal *protractor* of the proximal propterygium (mainly from body musculature to proximal portion of propterygium), the *flexor* of the proximal propterygium (from pelvic girdle to proximal portion of propterygium), the *flexor* of the distal propterygium (from pelvic girdle to distal portion of propterygium) and the dorsal *retractor* of the propterygium (from pelvic girdle to propterygium).

Muscles of Paired Appendages of Hydrolagus coliei

The *adductor* of the pectoral fin (Fig. 8) runs from the pectoral girdle (mainly scapular region) to the dorsal

surfaces of the radial cartilages, some fibers also contacting the propterygium and metapterygium, because the muscle has superficial and deep bundles similar to those of sharks (Figs. 8 and 9). The *levator 3* (Fig. 8) was designated by Didier (1987, 1995) as “adductor superficialis” but is very likely not homologous to the adductor superficialis of osteichthyans because it runs primarily from the fascia of the epaxial musculature to the dorsal surface of the propterygium. That is, it elevates and adducts the pectoral fin but also rotates it laterally (in the sense of human terminology, that is, it displaces the lateral portion of the pectoral fin dorsally). The origin of the *levator 2* (Fig. 8) is ventro-medial to that of the *levator 3*, that is, the muscle arises from the fascia of the hypaxial musculature to insert onto the dorsal surface of the metapterygium and the more medial radial cartilages; so besides elevating and adducting the pectoral fin it also rotates it ‘medially’ (in the sense that it pulls the medial portion of the pectoral fin dorsally). The *abductor* of the pectoral fin (Fig. 8) runs mainly from the coracoid bar to the ventral surfaces of the radial cartilages, also having a superficial bundle and a deep

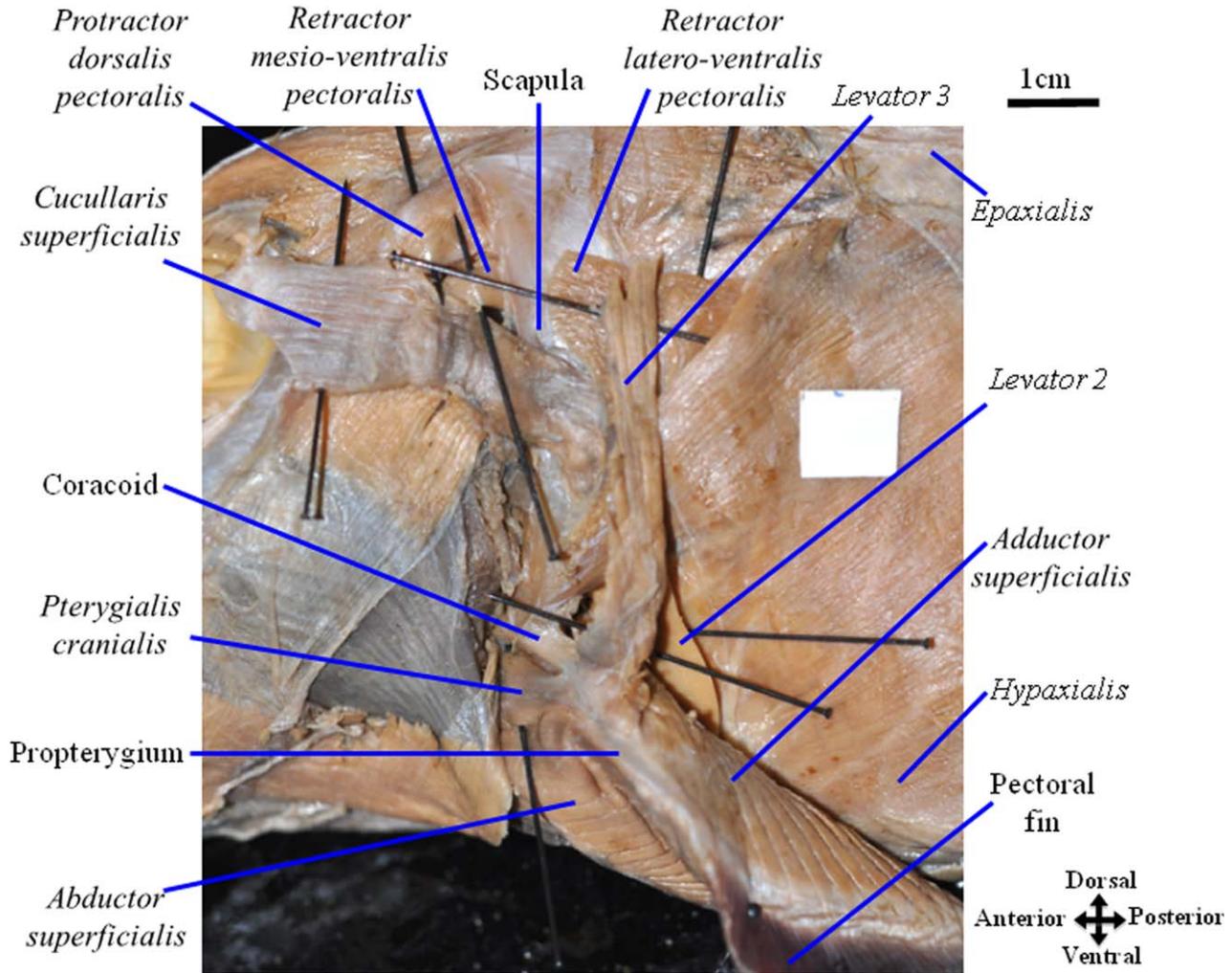


Fig. 8. Lateral view of the left pectoral girdle and fin muscles of the holocephalan fish *Hydrolagus coliei*.

bundle that are similar to those of sharks. The *pterygialis cranialis* is anatomically similar to that of sharks but is more blended to the *abductor* of the pectoral fin than is the case in sharks. It runs from the pectoral girdle (both scapular region and coracoid bar) to the propterygium of the pectoral fin. Apart from the *levators 2 and 3*, the epaxial and hypaxial musculature is divided into various muscles. The *retractor latero-ventralis pectoralis* (Fig. 8) is constituted by a single bundle (as described by Anderson, 2008, and contrary to Didier, 1987, who described two bundles) running from the epaxial musculature to the lateral surface of the pectoral girdle. The *retractor mesio-ventralis pectoralis* (Fig. 8) is deep to the *cucullaris superficialis* and runs from the epaxial musculature to the medial side of the pectoral girdle and mainly to the back of the chondrocranium. The *protractor dorsalis pectoralis* (Fig. 8) arises from the postorbital ridge of the chondrocranium and inserts onto the scapular process of the pectoral girdle and has a single bundle as described by Anderson (2008; not two as described by Didier, 1987). It should be noted that the “retractor dorsalis pectoralis” and “latero-ventralis” sensu Didier

(1987) are simply part of the body musculature going to the pectoral girdle and chondrocranium, and not separate, distinct muscles; this fact is also suggested by Didier (1995), who listed the “retractor dorsalis pectoralis” as part of the epaxial musculature. The head muscles *cucullaris superficialis* and *cucullaris profundus* (Fig. 8; “trapezius superficialis and profundus” sensu Didier, 1987) run from the postorbital ridge and surrounding structures to the pectoral girdle and to at least some branchial arches, respectively.

With respect to the pelvic appendage, the *adductor* of the pelvic fin (Fig. 9) arises from the fascia of the body musculature, particularly its superficial bundle, its deep bundle arising mainly from the basipterygium and going less distal than the superficial bundle, both bundles attaching onto the dorsal surfaces of the radial cartilages. There is a *levator 5* of the pelvic fin (Fig. 9) running medially to the *adductor* of the fin and connecting the fascia of the body musculature to the basipterygium and medial radial cartilages, so its function seems to be somewhat analogue to that of the *levator 2* of the pectoral fin. The *abductor* of the pelvic fin (Fig. 9) has a

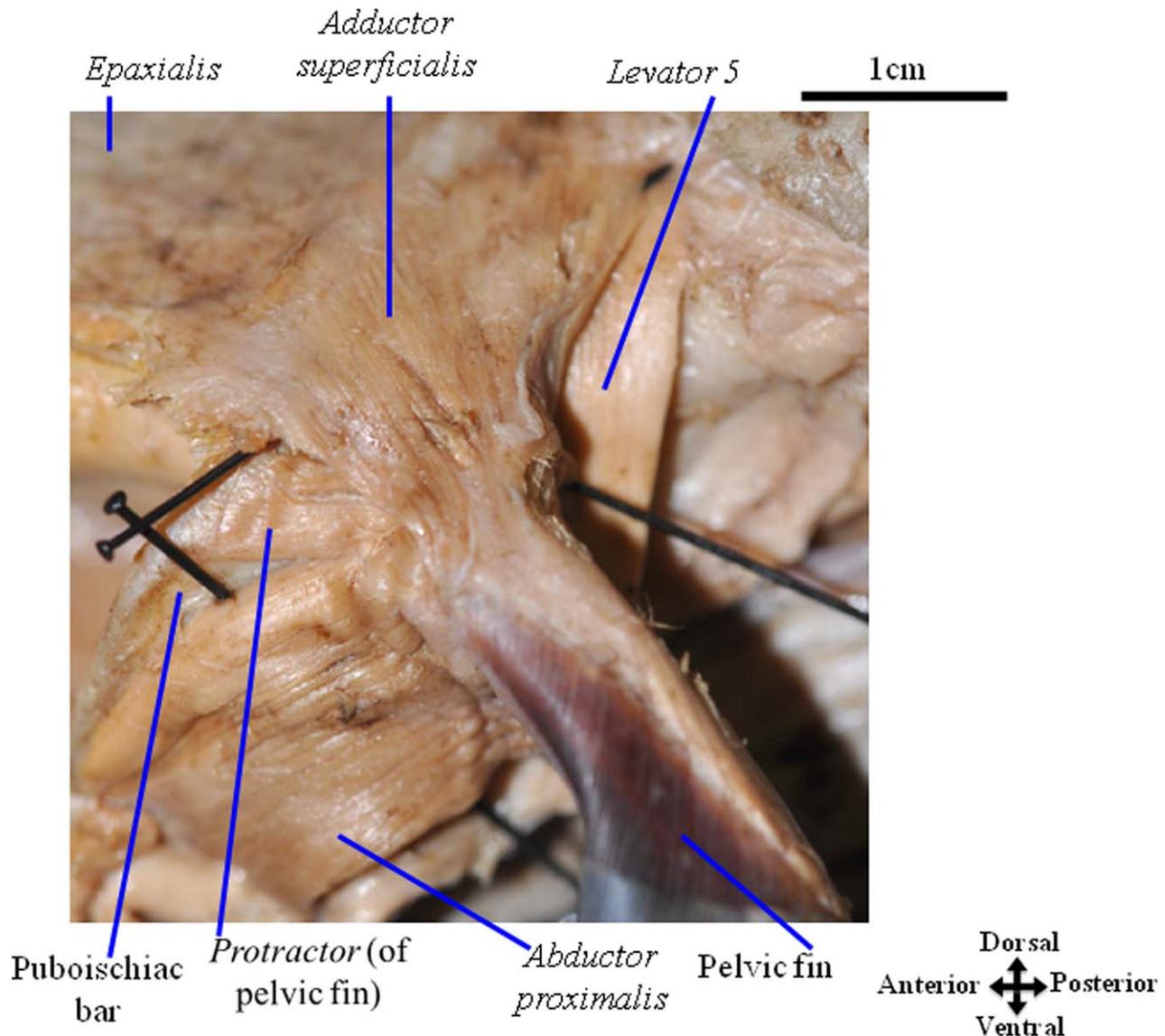


Fig. 9. Lateral view of the left pelvic girdle and fin muscles of the holocephalan fish *Hydrolagus colliciei*.

proximal bundle running from the puboischiac bar to the basipterygium and a distal bundle extending from the basipterygium to the ventral surfaces of the radial cartilages. The *protractor* of the pelvic fin (Fig. 9) arises from the pelvic girdle and inserts into the propterygium, being similar to that of sharks.

DISCUSSION

The Plesiomorphic Configuration for Chondrichthyans and the Evolution of the Cucullaris

The comparison between the muscles attached onto the pelvic and pectoral appendages (PEL, PEC) of the representatives of the three major chondrichthyan extant clades (Holocephali, Selachii, and Batoidea: see

Introduction) dissected by us and described in the literature allow us to provide the first detailed discussion about which is the plesiomorphic configuration of the pelvic and pectoral muscles in chondrichthyans (Fig. 2) and also about the similarity between these muscles in these fish. In general, in chondrichthyans the *adductor* of the pelvic fin is somewhat similar anatomically and functionally to the *adductor* of the pectoral fin, because both muscles predominantly extend the fins and have similar superficial and deep bundles, the deep bundle (*adductor profundus*) running mainly from the principal cartilages of the fins (propterygium, metapterygium and/or mesopterygium) to a region of the radial cartilages of the fins that is more proximal than the insertion of the superficial bundle (*adductor superficialis*). The *ptyrgialis cranialis* of the pectoral appendage and the *protractor* of the pelvic fin are also similar anatomically and

functionally, because both muscles predominantly protract the fin and run from the girdles to the propterygium, being seemingly differentiated from the *abductor superficialis*. The *abductor* of the pectoral fin of chondrichthyans has superficial and deep bundles that are similar to those of the *adductor* of the pelvic and pectoral fins, but the *abductor* of the pelvic fin has instead proximal and distal bundles, the proximal mainly inserting onto the metapterygium and the distal mainly originating from this latter bone. This stresses the fact that there are significant anatomical differences between not only the hard tissues (e.g., Coates and Cohn, 1998; Diogo et al., 2013) but also the soft tissues of the pelvic and pectoral appendages even in phylogenetically plesiomorphic gnathostomes such as chondrichthyans.

It should be noted that we are not defending that fish such as sharks should be seen as ideal models of the first fish that had both pelvic and pectoral appendages. As can be seen in Fig. 10, the oldest fossil fish discovered so far with both these appendages (e.g., *Parayunnapepis*; see Zhu et al., 2012); are remarkably different morphologically from sharks, for instance. Sharks display numerous derived, peculiar anatomical features (autapomorphies). However, as recently stressed by Diogo et al. (2014), one should not take the extremist—and now often politically correct—view that, because of that, sharks and other chondrichthyans cannot tell us much about the plesiomorphic condition of, for example, the soft tissues of the first gnathostomes. In fact, based on strict cladistic phylogenetic studies, with respect to, for example, their head muscles, sharks are indeed one of the best living models to investigate the configuration of the muscles of the last common ancestor of gnathostomes (Diogo and Abdala, 2010). This also seems to apply to the appendicular muscles, as will be explained below.

In fact, Coates and Cohn (1998) defended that the close similarity between the hard tissues of the pelvic and pectoral appendages seen in non-chondrichthyan extant fish—i.e., in osteichthyans—is a derived, specialized feature within gnathostomes. Regarding the soft tissues, the differences between the pelvic and pectoral appendages of chondrichthyans and of other gnathostomes is also illustrated by the partial attachment of the head muscle *cucullaris* to the pectoral girdle; no similar muscle is found on the pelvic region. The understanding of the evolution of the *cucullaris* in gnathostomes is of crucial importance to comprehend the origin and evolution of the paired fins of these vertebrates. The developmental origin of the *cucullaris*, its osteichthyan derivative protractor pectoralis and amniote derivatives trapezius and sternocleidomastoid has been subject of much debate (see review of Diogo and Abdala, 2010). Our dissections of chondrichthyans and comparisons with other vertebrates (e.g., Diogo and Abdala, 2010), our recent developmental studies of tetrapods (e.g., Ziermann and Diogo, 2013, 2014; Diogo and Ziermann, 2014), and our updated review of the literature allow us to solve the controversy regarding a body *versus* head origin of the *cucullaris*. In adult sharks such as *Squalus* there is a single, continuous muscle *cucullaris* inserting onto both the dorsal surface of the branchial arches and the posterior surface of the pectoral girdle. Edgeworth (1935) has shown that this condition is seen from the first embryonic stages until the adult stages of sharks,

the *cucullaris* developing from the dorsal portion of the branchial muscle plates. In adult holocephalans such as *Hydrolagus* the *cucullaris* has two bundles, one (superficial) inserting onto the pectoral girdle and the other (deep) inserting onto the dorsal portion of the branchial arches. This is furthermore supported by the development in holocephalans where the *cucullaris* develops from the dorsal portion of the branchial muscle plates, forming first a single, continuous muscle, and then separating during ontogeny into the deep and superficial bundles (Edgeworth, 1935). This can be also observed in batoids with the slight difference that the *cucullaris* develops only from the dorsal portion of the last branchial muscle plate before dividing into bundles that attach onto the pectoral girdle and branchial arches, in taxa such as *Leucoraja* (Edgeworth 1935; see also, e.g., Miyake et al., 1992). According to Edgeworth in chondrichthyans the *cucullaris* is mainly innervated by CNX; in osteichthyans there is usually (1) a protractor pectoralis (or its amniote derivatives, e.g., trapezius and sternocleidomastoideus) that derives ontogenetically exclusively from the last branchial muscle plate and inserts exclusively onto the pectoral girdle (in bony fish and most tetrapods); and (2) levatores arcuum branchialium developing from the dorsal portion of the branchial muscle plates and attaching exclusively onto the dorsal surface of the branchial arches (in bony fish and amphibians, these muscles being absent in amniotes). Our recent developmental studies strongly support the idea that the protractor pectoralis and its amniote derivatives trapezius and sternocleidomastoideus develop anatomically exclusively from the last branchial plate (e.g., Ziermann and Diogo, 2013, 2014).

We did not find the levatores arcuum branchialium muscles in any of the chondrichthyan specimens dissected by us and almost all authors agree that these are not present as distinct muscles in chondrichthyans. However, Kesteven (1942) did describe these muscles as very thin structures in sharks such as *Mustelus* and *Orectolobus* and batoids such as *Dasyatis* and holocephalans such as *Callorhynchus* and *Hydrolagus*, and as even thinner and seemingly vestigial structures in sharks such as *Squalus* and batoids such as *Leucoraja*, going to the dorsal surface of five branchial arches. Didier (1987, 1995) does not describe these muscles in any of the numerous holocephalan taxa dissected by her, and states that some of the levatores arcuum branchialium sensu Kesteven (1942) actually correspond to part of the *cucullaris profundus* while others correspond to part of the epibranchial muscle *subspinalis* of holocephalans. But it should be noted that Kesteven (1942) described the subspinalis, the *cucullaris* and the levatores arcuum branchialium in chondrichthyans, so the synonymies proposed by Didier are questionable. However, the fact that to our knowledge no other author (apart from Kesteven, 1942) has described distinct, fleshy muscles levatores arcuum branchialium in chondrichthyans, together with the fact that even Kesteven recognized that the structures that he designated under the name levatores arcuum branchialium in these fish are mainly innervated by “spinal” nerves, while those in osteichthyans are mainly innervated by nerves CNX and/or CNIX, also put in question the homology of the muscles of these two major gnathostome taxa proposed by Kesteven. In fact, our dissections and direct comparisons with Kesteven’s

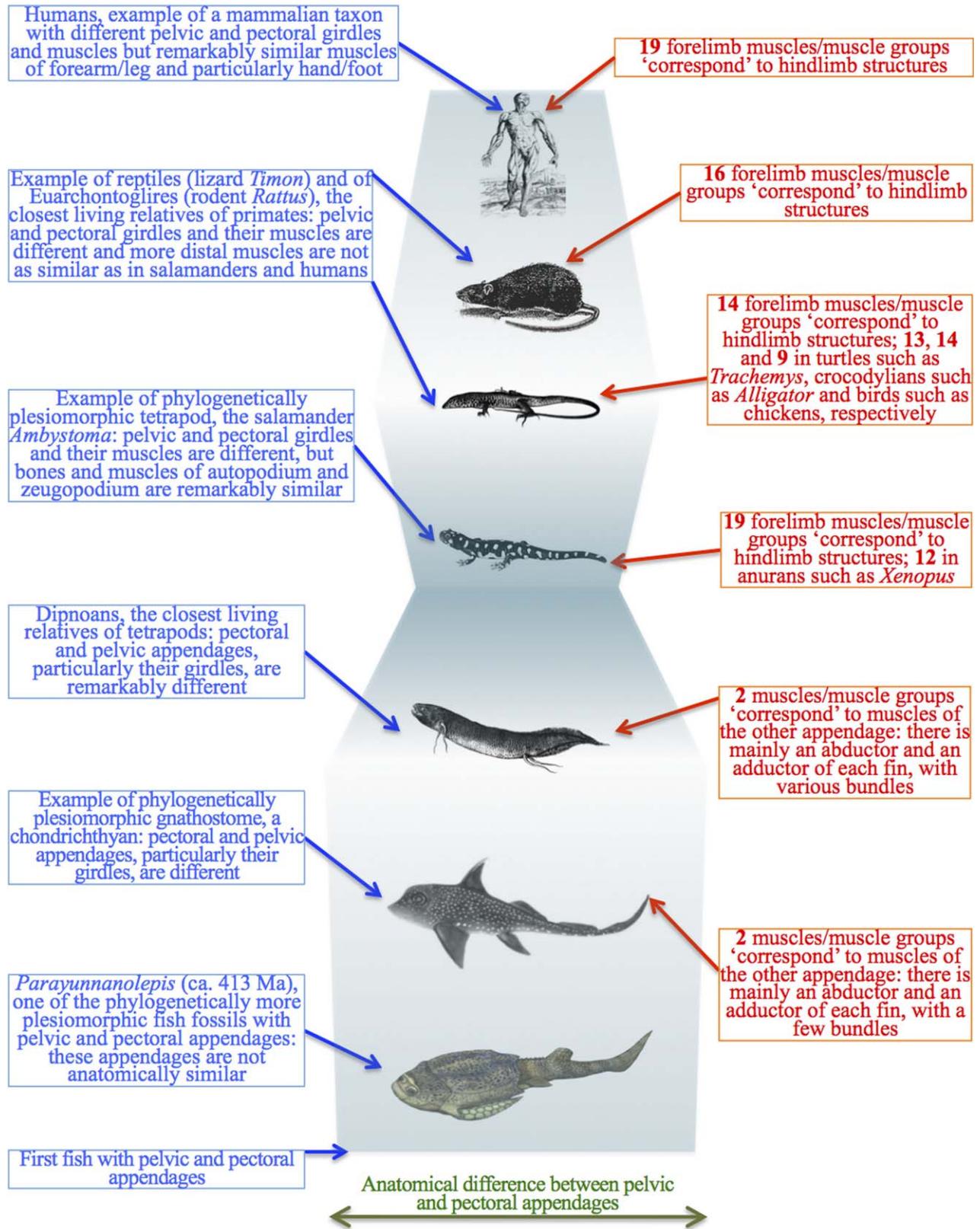


Fig. 10. The evolutionary history of the pelvic and pectoral appendages was much more complex than the “serial homology followed by functional/anatomical divergence” hypothesis shown in Fig. 1 suggests. This is because it was the result of a complex interplay between ontogenetic, functional, topological and phylogenetic constraints leading to cases of anatomical divergence followed by cases

of anatomical convergence (“similarity bottlenecks”). This is exemplified in this simplified scheme of the evolutionary muscle transitions leading to modern humans (picture of antiarch *Parayunnanolepis* modified from Zhu et al., 2012; pictures of other taxa modified from Diogo and Abdala, 2010, and references therein; number of muscles from Diogo et al., 2013, and Diogo and Molnar, 2014).

(1942) descriptions point out that the levatores arcuum branchialium muscles described by Kesteven are very likely just part of the *cucullaris* sensu the present work, or alternatively, part of the epibranchial musculature, as suggested by Didier (1995), but clearly do not seem to correspond to the true levatores arcuum branchialium of osteichthyans.

Because Edgeworth (1935) regarded the dipnoans as the most plesiomorphic group of gnathostomes, he considered the condition found in dipnoans and thus in other osteichthyan (bony) fish (i.e., to have levatores arcuum branchialium plus a protractor pectoralis) as the plesiomorphic gnathostome condition. However, a review of all the data available clearly indicates that the plesiomorphic gnathostome condition is to have a single branchial muscle *cucullaris* innervated by cranial nerves and inserting onto both the branchial arches and the pectoral girdle, as found in both the early development, and in adults, of sharks, batoids and holocephalans. The fact that the *cucullaris* only divides into bundles later during the ontogeny of batoids and holocephalans, together with the fact that the *cucullaris* bundles of adult batoids are different anatomically to those of adult holocephalans, clearly indicates that the division of the *cucullaris* into bundles is a derived condition for chondrichthyans. The derived and homoplastic division of the *cucullaris* in batoids and holocephalans into bundles going exclusively to the pectoral girdle seems to be the result of an evolutionary trend seen in gnathostomes. That is, in the first gnathostomes the pectoral girdle usually was, basically (at least functionally and often also anatomically), part of the head. Subsequently, during later phases of gnathostome evolution the pectoral girdle often tended to become functionally and/or anatomically more separated from the head, and thus it makes evolutionary and functional sense to not have a continuous muscle inserting onto both the branchial arches and the pectoral girdle. The fact that the *cucullaris* of chondrichthyans is always associated with the elevation of at least some branchial arches might also explain why in these gnathostomes the levatores arcuum branchialium are not differentiated as distinct muscles, contrary to bony fish, in which the protractor pectoralis does not attach to the branchial arches and in which the levatores arcuum branchialium are accordingly usually well developed (see section below).

As explained in the Results section, the epaxial and hypaxial muscles are not divided into distinct muscles attached to the paired appendages in sharks (e.g., pectoralis, latissimus dorsi) as suggested by Humphry (1872a,b). In fact, even sharks with peculiar locomotor behaviors (e.g., *Hemiscyllium ocellatum*, which has a walking/crawling locomotion), have basically the same kind of muscle configuration seen in *Squalus*, with just very few minor changes (Goto et al., 1999). However, batoids and holocephalans do have some muscles that are apparently derived from the epaxial and/or hypaxial musculature, including the *protractor dorsalis pectoralis* (e.g., in *Hydrolagus*), the *retractor latero-ventralis pectoralis* and *retractor mesio-ventralis pectoralis* (in, e.g., *Leucoraja* and *Hydrolagus*), the *levators 2 and 3* of the pectoral fin (e.g., in *Hydrolagus*), the *levator 4* of the pectoral fin (e.g., in *Leucoraja*), and the *levator 5* of the pelvic fin (e.g., in *Hydrolagus*). Authors such as Anderson (2008) suggested that the *protractor dorsalis pector-*

alis, *retractor latero-ventralis pectoralis* and *retractor mesio-ventralis pectoralis* of holocephalans are branchial muscles innervated by cranial nerves IX and X. However, anatomically these muscles do not seem to be branchial muscles. Moreover Didier (1995) stated that only the *protractor dorsalis pectoralis* might be a branchial muscle innervated by nerves CNIX and/or CNX, and that the other muscles are clearly body muscles. The *protractor dorsalis pectoralis* could therefore in theory be a branchial muscle, or eventually be instead an epibranchial muscle, because it lies in the epibranchial region, just deep to the *cucullaris* and connecting, as does the *cucullaris*, the cephalic region to the pectoral girdle. But Edgeworth (1935) studied in detail the development of the head muscles of holocephalans and stated that the only epibranchial muscles of these fish are the interpharyngobranchiales and subspinalis, so the *protractor dorsalis pectoralis* does not seem to be an epibranchial muscle. According to Edgeworth (1935), within gnathostomes an epibranchial musculature is only present in the Selachii and Holocephali. Kesteven (1942) also described epibranchial muscles (namely, the interpharyngobranchiales, the subspinalis being absent) in batoids such as *Dasyatis* (the muscles being reduced to fibrous structures in *Leucoraja*) but more recent works, including those focused precisely on batoids (e.g., Miyake et al., 1992) state that batoids do not have epibranchial muscles. A characteristic feature of the epibranchial muscles is that they are innervated by spinal nerves. Therefore, if the *protractor dorsalis pectoralis* is effectively innervated by nerves CNIX and/or CNX, as described by Didier (1995) and Anderson (2008), this muscle is very likely not part of the epibranchial musculature. However, Edgeworth (1935) also does not list this muscle as a branchial muscle in holocephalans, nor does Miyake et al. (1992). Moreover, our review of the literature reveals that most authors refer to an innervation of the muscle by nerves CNIX and/or CNX basically because they consider it a branchial muscle *a priori*, and not because they really studied its innervation in detail. Therefore, based on all the data available, and before more detailed studies of the development and innervation of this muscle are done, the most likely hypothesis is to consider that the *protractor dorsalis pectoralis*, *retractor latero-ventralis pectoralis* and *retractor mesio-ventralis pectoralis* are derived from the body muscles. Be that as it may, only the *retractor latero-ventralis pectoralis* and *retractor mesio-ventralis pectoralis* seem to be consistently found in batoids and holocephalans (our study, see also, e.g., Shann 1920, 1924, and below), so the *protractor dorsalis pectoralis*, as well as the *levators 2, 3, 4, and 5*, do not seem to have been present in the LCA of extant chondrichthyans (i.e., of elasmobranchs and holocephalans).

In summary, it can be said that the plesiomorphic condition for chondrichthyans is that the pectoral appendage is associated with the head muscle *cucullaris* (inserted onto both the pectoral girdle and at least some branchial arches), the trunk muscles *retractor latero-ventralis pectoralis* and *retractor mesio-ventralis pectoralis*, and the *abductor* and *adductor* muscles of the pectoral fin (both divided into superficial and deep bundles), as well as the *pterygialis cranialis* (Fig. 2). Regarding the PEL, the plesiomorphic chondrichthyan condition is to have *abductor* and *adductor* muscles of the pelvic fin

(the latter divided into superficial and deep bundles and the former divided, very likely, into proximal and distal bundles) as well as a *protractor* muscle of this fin (Fig. 2).

The Plesiomorphic Configuration for Gnathostomes and the Fore–Hindlimb Serial Homology Dogma

The *pterygialis cranialis* muscle of the pectoral fin and the *protractor* muscle of the pelvic fin of chondrichthyans are somewhat similar to the “arrector” muscles of osteichthyan fish such as the Neopterygii (including teleosts, *Lepisosteus* and *Amia*) (Diogo and Abdala, 2010). In particular, the *pterygialis cranialis* of the pectoral fin is very similar to the *arrector ventralis* of neopterygians, being a derivative of the ventro-lateral (abductor) muscle mass that runs laterally to the other muscles derived from this muscle mass to attach onto the first ray of the pectoral fin and/or closely related fin structures. However, the “arrector” muscles were almost surely absent in the LCA of extant osteichthyans, because both the sarcopterygians and the two phylogenetically most basal actinopterygian clades (the cladistians and chondrosteans) do not have these muscles (Diogo and Abdala, 2010). The “*dilatator anterior* and *dilatator posterior*” described in *Polypterus* by Shann (1924) correspond to parts of the *abductor* and *adductor* masses of *Polypterus sensu* Diogo and Abdala (2010) respectively, and not to the *pterygialis cranialis* of chondrichthyans. That is, it is more parsimonious to consider that these similar muscles were acquired independently (two evolutionary steps) in the node leading to chondrichthyans (the *pterygialis cranialis*) and in the node leading to the Neopterygii (the *arrector ventralis*) than to consider that they were secondarily lost (three evolutionary steps) in sarcopterygians, cladistians, and chondrosteans. This means that each of the paired fins of the first fish that had both pelvic and pectoral fins were very likely moved only by two muscles, that is, an *adductor* and an *abductor* of the fin (Fig. 2). In fact, further evidence supports the idea that muscles such as the *pterygialis cranialis* and the *arrector ventralis* were independently acquired. For instance, in fish such as *Polypterus* there is a thin bundle of the *abductor* mass that goes to the first ray of the pectoral fin (see, e.g., Fig. 8.1A of Diogo and Abdala, 2010). This bundle therefore corresponds topologically to the *arrector ventralis* of neopterygians and the *pterygialis cranialis* of chondrichthyans. However, the bundle is not fully differentiated into a distinct muscle in *Polypterus*, and seems to not be differentiated/present at all in chondrosteans, indicating that similar divisions of the *adductor* and *abductor* masses might in fact have been acquired independently, with different degrees of separation/differentiation, in different clades.

As explained above, plesiomorphically chondrichthyans have three distinct muscles related to the movements of the pectoral girdle: the head muscle *cucullaris* and the trunk muscles *retractor latero-ventralis pectoralis* and *retractor mesio-ventralis pectoralis*. Based on the ideas of authors such as Humphry (1872a,b) that at least some of the specific muscles of the pectoral and/or pelvic appendages found in chondrichthyans are homologous to the muscles of the forelimb and/or

hindlimb of tetrapods (see Introduction), Shann (1920, 1924) stated that the *retractor latero-ventralis pectoralis* and the *retractor mesio-ventralis pectoralis* of holocephalans are homologous to muscles with the same names in sharks and respectively to the “serratus” and the “obliquus/rectus abdominis” of dipnoans and tetrapods. However, we did not find a *retractor latero-ventralis pectoralis* nor a *retractor mesio-ventralis pectoralis* in sharks, and these muscles are also not described by most authors that have studied the muscles of these fish in some detail (e.g., Marion, 1905; Kesteven, 1942; Walker, 1954; Jarvik, 1965; Anderson, 2008). In fact, a careful reading of Shann (1920, 1924) and comparison with Humphry’s (1872a,b) descriptions and the results of our own dissections of all major extant gnathostomes groups, reveals that, as recognized by Shann himself, the only living fish taxon in which these muscles are truly differentiated is the Holocephali. That is, Shann (1920, 1924) recognized that in the other living fish in which he listed these muscles, including sharks, he was in fact referring to parts of the epaxial/hypaxial musculature that do not form distinct, separate muscles. As explained above, we did find the *retractor latero-ventralis pectoralis* and the *retractor mesio-ventralis pectoralis* in batoids; however, we did not find these muscles in most osteichthyans dissected by us in the past (see Diogo and Abdala, 2010). Therefore, the plesiomorphic condition for the first gnathostomes that had both pelvic and pectoral appendages clearly seems to be that there were almost no distinct muscles differentiated from the body musculature that attached to the paired girdles and that were specifically related to the movements of these girdles. However, many muscles associated with the movements of these girdles were then independently acquired during gnathostome evolution.

In summary, it can be said that the plesiomorphic condition for both gnathostomes and osteichthyans is that the pectoral appendage is associated with very few muscles, including the head muscle *cucullaris* (originally inserted onto both the pectoral girdle and at least some branchial arches, and then differentiated, in osteichthyans, into a *protractor pectoralis* attached onto the pectoral girdle and *levatores arcuum branchialium* inserted onto branchial arches), and the *abductor* and *adductor* muscles of the pectoral fin (both divided into superficial and deep bundles) (Fig. 2). Regarding the pelvic appendage, the plesiomorphic gnathostome and osteichthyan condition is to have *abductor* and *adductor* muscles of the pelvic fin (both divided, very likely, into superficial and deep bundles) (Fig. 2). This conclusion therefore further contradicts the fore-hindlimb serial homology hypothesis, because it directly contradicts the idea, defended by authors such as Humphry (1872b), that some muscles that are present in the fore- and hindlimb of basal tetrapods were already present in some form in plesiomorphic gnathostome fish, as the result of an unique, ancestral duplication of the paired appendages (see Introduction). That is, the present study strongly supports the scenario shown in Fig. 10, that is, that all the numerous, and in many cases strikingly similar, muscles of the tetrapod fore- and hindlimb were undoubtedly acquired *independently (homoplasi-cally)* at some stage during the evolutionary transitions that occurred from early gnathostomes to tetrapods. Consequently, under a historical (phylogenetic)

definition of homology these fore- and hindlimb structures can in no way be considered serial homologues. One could however argue that they may be homologues under the morphological or developmental definitions of homology (see Wagner, 1994), but this view is also contradicted by the integration of the results of the present work on chondrichthyans and of our previous works on osteichthyans (Fig. 10). As explained in the Introduction, ancestral morphological serial homology of these appendages would in fact also normally imply that these appendages were originally similar and then diverged anatomically/functionally, as shown in Fig. 1. But this is not the case (Fig. 10), because in the more plesiomorphic fish found so far with pectoral and pelvic appendages the hard tissues of these appendages are anatomically very different (e.g., Coates and Cohn, 1998; Zhu et al., 2012). Both the hard and soft tissues of the proximal region of the paired appendages (particularly the girdles) actually remained, in general, markedly different throughout all gnathostome clades (Coates and Cohn, 1998; Diogo et al., 2013; this work). For instance, within all tetrapod clades studied by Diogo and Molnar (2014), including anatomically plesiomorphic taxa such as urodeles (caudates), there is not even a pelvic-thigh muscle with a clear “topological equivalent” in the pectoral region and arm. This is seemingly due to a strong phylogenetic constraint, that is the pelvic and pectoral girdles of fish were anatomically markedly different from the start, and the girdles of tetrapods are consequently also quite different anatomically. In contrast, the more peculiar, derived distal regions of the tetrapod fore- and hindlimb, particularly the autopodium (hand/foot), include at least various new bones, and a developmental plan that is quite different from those of the fish paired appendages (Diogo et al., 2013). That is, the evolution of the autopodium represents a major “evolutionary novelty” and thus there were less phylogenetic constraints involved, and the developmental constraints/factors resulting from further cooption of some similar genes in the ontogeny of the fore- and hindlimb thus lead to a more marked similarity between the distal regions of these limbs in basal tetrapods (“similarity bottleneck”: Fig. 10). But, importantly, the cooption of similar genes during the “fins-limbs transitions” is however not sufficient to explain the striking similarity between the forearm-hand and leg-foot muscles of, and thus the other “similarity bottlenecks” leading to, phylogenetically and anatomically derived tetrapods such as our bipedal species, *Homo sapiens* (Fig. 10). As explained by Diogo et al. (2013) and Diogo and Molnar (2014), those derived “similarity bottlenecks” were instead the result of a complex evolution also including topological and functional constraints.

General Remarks: The Separation Between the Head and Trunk in Vertebrates

The present study shows that plesiomorphically the paired appendages only had two muscles to move the fins (*abductor* and *adductor*) and, therefore, that all the similar muscles of the zeugopodia and autopodia of the tetrapod fore- and hindlimbs were necessarily the result of homoplastic evolution. That it, it contradicts the fore-hindlimb serial homology hypothesis because it contradicts the idea that some muscles that are present in the

more proximal regions of the tetrapod fore- and hindlimbs (e.g., *latissimus dorsi*, *serratus anterior*) were already present in some form in plesiomorphic gnathostome fish, as the result of an unique, ancestral duplication of the paired appendages. In fact, the present study stresses the fact that the soft tissues associated with the pectoral and pelvic girdles were very different from the beginning of gnathostome evolution, further contradicting the serial homology hypothesis. This is because a major difference between the soft tissues associated with the pelvic and pectoral girdles is also clearly seen in non-tetrapod gnathostomes, including chondrichthyans, in which the movements of the pectoral girdle are associated with the *cucullaris* muscle, while there is no similar muscle associated to the pelvic girdle. A major difference between the pectoral and pelvic appendages is actually that the origin of the pectoral girdle was likely deeply related anatomically and functionally to the evolution of the head. This difference is illustrated by the fact that in gnathostomes such as chondrichthyans the *cucullaris* is a continuous muscle inserting onto both the branchial arches and the pectoral girdle. In this sense, this study provides new insights not only about the fore-hindlimb enigma, but also about the evolution of the vertebrate neck, by identifying three major transitions concerning the evolution of the muscles lying between the head and the pectoral girdle: (1) plesiomorphically in gnathostomes there is a single, continuous muscle (*cucullaris*) running from the cephalic region to the branchial arches and pectoral girdle; (2) in osteichthyan (bony) fish the pectoral girdle is still anatomically and functionally deeply associated to the head, but the *cucullaris* differentiated into the muscle protractor pectoralis (attaching only onto the pectoral girdle) and the muscles levatores arcuum branchialium (attaching only onto the branchial arches), thus allowing more mobility between the head and pectoral girdle; (3) in tetrapods the pectoral girdle is anatomically and functionally more independent from the head movements than in bony fish, and in amniotes the protractor pectoralis gave rise to the neck muscles trapezius and sternocleidomastoideus. According to Coates and Cohn (1998), there are in fact numerous evolutionary and functional reasons for the deep spatial relation between the pectoral girdle and the skull in early gnathostomes: the girdle forms the rear wall of the internal gill chamber, a shield for the pericardial cavity, and a secure insertion for the pectoral fins. Recent developmental studies of chondrichthyans are also pointing out not only that the pectoral girdle of early gnathostomes was anatomically and functionally deeply associated to the head, but also that the developmental mechanisms leading to the formation of this girdle are strikingly similar to those leading to the formation of the branchial arches (e.g., Gillis et al., 2009).

Another insight regarding the separation between the head and trunk in derived vertebrates comes from the study of the evolutionary history of the *retractor mesioventralis pectoralis*. As explained above, this muscle is present in at least some chondrichthyans, and has been also described as, and confirmed to be in developmental studies, a distinct muscle in various osteichthyan fishes (e.g., Shann, 1925), including the zebrafish (e.g., Windner et al., 2011). In a study in the zebrafish, Windner et al. (2011) provided interesting information about the

migratory (abaxial) origin of this muscle from the hypaxial musculature. In the zebrafish this muscle lies posterior to the pectoral fin and attaches to the cleithrum, the first-formed element of the teleost pectoral girdle. The founder cells of this muscle, which Windner et al. (2011) designated as “posterior hypaxial muscle” and is often designated as “ventral-most hypaxial muscle,” “inferior obliquus muscle,” or “anterior hypaxial muscle” in teleosts, arise from somites 5 and 6 and express the same marker genes of cell migration (*lhx1* and *met*) as pectoral fin muscle precursors. As noted by these authors, the muscle probably has a transient importance during early larval suction feeding, in co-action with the hypobranchial sternohyoideus muscle, which become one of the neck muscles of tetrapods. As noted above, according to Shann (1925) the *retractor mesio-ventralis pectoralis* of fish gave rise to the rectus and/or oblique abdominal muscles of tetrapods, an hypothesis supported by our comparative studies between fish and tetrapods. In amphibians these abdominal muscles also attach onto the pectoral girdle and are also continuous with the sternohyoideus and other infrahyoid (neck) muscles, in a condition similar to that found in the zebrafish. However, in a further example of the evolutionary trend towards the separation of the head from the trunk within vertebrate evolution, these abdominal muscles became increasingly separated from both the pectoral girdle and the infrahyoid muscles during amniote evolution. This derived condition is for instance found in our own species *H. sapiens*, in which the only infrahyoid muscle that is attached to the pectoral girdle is the thin omohyoideus. This derived condition therefore allows these abdominal muscles to be contracted without interfering with the movements of the head, and the infrahyoid muscles to be contracted without any major interference with the main postcranial axial or limb movements.

It is therefore hoped that the new data and the discussion provided in the present article will help deconstructing the fore-hindlimb serial homology dogma and also promote future and more integrative anatomical, functional and developmental studies on the puzzling and fascinating evolutionary history of the paired appendages and of the vertebrate neck under a new paradigm that is more complex, but also more realistic.

ACKNOWLEDGEMENTS

RD was supported by a Howard University College of Medicine start-up package.

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