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Comparative anatomy of zebrafish paired and median fin muscles: basis for functional, developmental, and macroevolutionary studies

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Abstract

In the last decades, *Danio rerio* became one of the most used model organisms in various evo-devo studies devoted to the fin skeletal anatomy and fin-limb transition. Surprisingly, there is not even a single paper about the detailed anatomy of the adult muscles of the five fin types of this species. To facilitate more integrative developmental, functional, genetic, and evolutionary studies of the appendicular musculoskeletal system of the zebrafish and to provide a basis for further comparisons with other fishes and tetrapods, we describe here the identity, overall configuration, and attachments of appendicular muscles in a way that can be easily understood and implemented by non-anatomist researchers. We show that the muscle pattern of the caudal fin is very different from patterns seen in other fins but is very consistent within teleosts. Our observations support the idea of the developmental and evolutionary distinction of the caudal fin and point out that the musculature of the adult zebrafish pectoral and pelvic fins is in general very similar. Both paired fins have superficial and deep layers of abductors and adductors going to all/most rays plus the dorsal and ventral arrectors going only to the first ray. Nevertheless, we noted three major differences between the pelvic and pectoral fins of adult zebrafishes: (i) the pectoral girdle lacks a retractor muscle, which is present in the pelvic girdle – the retractor ischii; (ii) the protractor of the pelvic girdle is an appendicular/trunk muscle, while that of the pectoral girdle is a branchiomic muscle; (iii) the first ray of the pectoral fin is moved by an additional arrector-3. The anal and dorsal fins consist of serially repeated units, each of which comprises one half-ray and three appendicular muscles (one erector, depressor, and inclinor) on each side of the body. The outermost rays are attachment points for the longitudinal protractor and retractor. Based on our results, we discuss whether the pectoral appendage might evolutionarily be closer to the head than to the pelvic appendage and whether the pelvic appendage might have been derived from the trunk/median fins. We discuss a hypothesis of paired fin origin that is a hybrid of the fin-fold and Gegenbaur's theories. Lastly, our data indicate that *D. rerio* is indeed an appropriate model organism for the appendicular musculature of teleosts in particular and, at least in the case of the paired fins, also of actinopterygians as a whole.

Key words: actinopterygians; appendicular muscles; comparative anatomy; fishes; median fins; paired fins; teleosts; zebrafish.

Introduction

Since 1981, when George Streisinger introduced the zebrafish *Danio rerio* (Family: Cyprinidae) as a system for the genetic analysis of vertebrate development (Streisinger et al. 1981; Stahl, 1995), this species has become one of the most commonly used model organisms in biology (Lieschke

& Currie, 2007; Diogo, 2008; Diogo et al. 2008b; Veldman & Lin, 2008; Howe et al. 2017). This includes numerous studies about fin skeletal development and morphology, evolution and evo-devo comparisons with other fishes, as well as general discussions on paired fins-limbs differences and similarities (Nakamura et al. 2016; Saxena & Cooper, 2016). Nevertheless, there is not a single paper that describes anatomical details of the adult muscles of the five fin types of this fish and that could be easily used by non-anatomist biologists as a basis for their comparative functional (e.g. physiological), developmental, and evolutionary studies.

For instance, the adult pectoral muscles of the zebrafish were first briefly described in a developmental work by

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Thorsen & Hale (2005), but these authors missed one of the seven muscles of the pectoral fin, the arrector-3. This muscle and the other six muscles of the zebrafish pectoral fin were later described and compared with the appendicular musculature of other teleosts (Diogo et al. 2008a) and of other fishes and tetrapods (Abdala & Diogo, 2010; Diogo & Abdala, 2010). Descriptions of these muscles were rather brief and none of the authors specified attachment sites of every pectoral muscle. Muscles of the pelvic fins were described in various actinopterygian fishes (Winterbottom, 1974; Molnar et al. 2017), including several derived species of the cyprinid family (Saxena & Chandy, 1966) – the family that includes the zebrafish – but they have never been described in adult zebrafishes. Furthermore, the muscles of the anal fin have never been described in adult zebrafishes, and the only description of the dorsal and caudal fin muscles are those given by Schneider & Sulner (2006) in a paper mainly focused on the fin innervation.

This scarce information on the zebrafish appendicular myology contrasts with the detailed descriptions of the skeleton of the five fin types, which have been described in detail at least in one paper (e.g. Cabbage & Mabee, 1996; Grandel & Schulte-Merker, 1998; Sanger & McCune, 2002; Bird & Mabee, 2003). To facilitate more integrative developmental, functional, genetic, and evolutionary studies of the appendicular musculoskeletal system of the zebrafish and to provide a basis for the comparison with other fishes and tetrapods, we describe here the identity, overall configuration, and attachments of each appendicular muscle of the adult zebrafish in a way that is easy to use and understand by researchers working in various biological fields. Apart from paving the way for such integrative works, we also provide a basis for broad comparative, evo-devo, and macroevolutionary works by comparing the adult zebrafish appendicular musculature with that of other teleosts, and other actinopterygians. In particular, we discuss whether the zebrafish is an appropriate model for the fin musculature of teleosts in general, as it is often assumed to be.

Methods

Five wild adult specimens of *D. rerio* fixed and stored in 70% ethanol were taken from the collection of the Diogo lab at Howard University College of Medicine and used for this study. All fins, including the left and right pectoral and pelvic fins, were dissected under magnification using a Nikon SMZ1500 stereomicroscope and photographed on a Nikon DS-Fi1 digital microscope camera. Within the five dissected fishes, no variation has been found in the myological features we analyzed. This fact indicates that these features are rather conservative and non-variable within adult specimens. This observation was further supported by the comparison of the wild fishes we dissected with available descriptions of the appendicular musculature of adult laboratory zebrafish specimens provided by other authors (see Discussion and Table 1). The myological nomenclature used in this study follows the methodology of our lab and provides an integrative and easy-to-use

terminology. It takes into account the most used terms within the literature and the information about muscle homology within different vertebrate taxa, making it appropriate to compare all fish groups as well as fishes and tetrapods (Diogo & Abdala, 2010). The terminology of the pectoral musculature in this work follows mainly Winterbottom (1974) and Diogo & Abdala (2010), that of the pelvic musculature follows Winterbottom (1974), and that of the caudal, dorsal, and anal muscles follows Schneider & Sulner (2006). To provide an anatomical basis for authors from different fields of biology, a list of synonyms of myological terms that have been used for different teleosts by other authors is given in Table 1.

Results

Pectoral fins

The musculature of each pectoral fin in the zebrafish is represented by seven muscles. Four of these muscles are positioned laterally and mainly ventrally (the abductor muscle mass, Fig. 1A) and three are lying medially and more dorsally (the adductor muscle mass, Fig. 1B). After skin removal, the most outer muscle can be seen: the flat and wide abductor superficialis (Fig. 1A). It is attached proximally to the lateral surface of the cleithrum and coracoid and distally to each pectoral fin ray, including the medial side of the first ray base. The lateral side of the first ray base is the distal attachment of the arrector ventralis, which originates from the scapula and cleithrum and is situated mainly deep and dorsal to the abductor superficialis. Ventral to the arrector ventralis lies the arrector-3, which originates from the cleithrum, scapula, and coracoid and inserts also on the first ray. Dorsal fibers of the arrector-3 are somewhat parallel to the edge of the coracoid and insert onto it anteroventrally, while medial and ventral fibers fan out and cover the major part of the coracoid; the most ventral fibers are oriented more dorsoventrally. Deep to the abductor superficialis lies the abductor profundus, which is blended with the abductor superficialis. It runs from the cleithrum and coracoid to all rays except the first one (Fig. 1A,B).

In a medial view, one can see the remaining three muscles (Fig. 1B). The adductor superficialis originates from the anterior edge of the ascending leg of the cleithrum and mesocoracoid arch, and the adductor profundus originates from the coracoid and mesocoracoid arch. Both muscles attach distally onto all fin rays except the first one. Deep to these two muscles lies the arrector dorsalis, which runs from the dorsomedial face of the cleithrum to the base of the first ray. It should be noted that apart from these seven muscles connecting the pectoral girdle to the pectoral fin, there is a muscle (protractor pectoralis) connecting the skull to the pectoral girdle at early stages of zebrafish development and that becomes much reduced or absent in adults (Diogo, 2008). This is a head (branchiomic) muscle derived from the cardiopharyngeal field (Diogo et al. 2015) that is often erroneously described as an appendicular muscle in fishes (see e.g., Diogo & Ziermann, 2015; Ziermann et al. 2017).

Table 1 List of appendicular muscles and their attachment sites in the zebrafish fins.

Muscle name	Distal attachment	Proximal attachment
Pectoral fins		
Arrector ventralis (pectoralis) (extensor pectoralis <i>sensu</i> Greene & Greene, 1914)	Ray 1	Cleithrum & scapula
Arrector-3 (pectoralis)	Ray 1	Cleithrum, scapula, coracoid
Arrector dorsalis (pectoralis)	Ray 1	Cleithrum
Abductor superficialis (pectoralis)	All rays	Cleithrum & coracoid
Abductor profundus (pectoralis)	All rays except 1	Coracoid & cleithrum
Adductor superficialis (pectoralis)	All rays except 1	Cleithrum & mesocoracoid arch
Adductor profundus (pectoralis)	All rays except 1	Coracoid & mesocoracoid arch
Pelvic fins		
Arrector ventralis pelvici	Ray 1	Pelvic girdle (basipterygium <i>sensu</i> Cabbage & Mabee, 1996)
Arrector dorsalis pelvici	Ray 1	Pelvic girdle (basipterygium <i>sensu</i> Cabbage & Mabee, 1996)
Abductor superficialis pelvici	All rays except 1 and 2	Tendon of protractor ischii & midline, contacting its counterpart (i.e. the same muscle on the other side of the body)
Abductor profundus pelvici	All rays	Pelvic girdle
Adductor superficialis	All rays except 1	Pelvic girdle
Adductor profundus pelvici	All rays	Pelvic girdle
Protractor ischii (infracarinalis anterior <i>sensu</i> Winterbottom, 1974)	Posterior (ischiac <i>sensu</i> Cabbage & Mabee, 1996) process of pelvic girdle	Pectoral girdle/trunk musculature
Retractor ischii (infracarinalis medius <i>sensu</i> Winterbottom, 1974)	Posterior region of pelvic girdle	Anterior region of anal fin
Dorsal fin		
Inclinatores dorsales	Lateral base of each ray	Skin & epaxial musculature
Erectores dorsales	Anterolateral base of each ray (lepidotrichia <i>sensu</i> Bird & Mabee, 2003)	Proximal pterygophores (radials <i>sensu</i> Bird & Mabee, 2003)
Depressores dorsales	Posterolateral base of each ray	Proximal pterygophores
Protractor dorsalis (supracarinalis anterior <i>sensu</i> Winterbottom, 1974)	Anterior base of ray 1	Posterior skull
Retractor dorsalis (supracarinalis posterior <i>sensu</i> Winterbottom, 1974)	Posterior base of last ray	Anterior region of caudal fin
Anal fin		
Inclinatores anales	Lateral base of each ray (no spike)	Hypaxialis
Erectores anales	Anterolateral base of each ray & spike	Haemal spines
Depressores anales	Posterolateral base of each ray (not to spike)	Haemal spines
Protractor analis (infracarinalis medius <i>sensu</i> Winterbottom, 1974)	Anterior base of first ray	Posterior pelvis
Retractor analis (infracarinalis posterior <i>sensu</i> Winterbottom, 1974)	Posterior base of last ray	Hypaxial musculature
Caudal fin		
Lateralis profundus dorsalis (dorsal slip of lateralis superficialis <i>sensu</i> Greene & Greene, 1914)	Rays 6–8	Myomeres, proximal caudal fin bones
Lateralis superficialis dorsalis	Ray 8	Midline
Lateralis superficialis ventralis	Ray 22	Myomeres, proximal caudal fin bones
Lateralis profundus ventralis (ventral slip of lateralis superficialis <i>sensu</i> Greene & Greene, 1914)	Rays 21–24	Midline, proximal caudal fin bones
Interradialis caudalis	Ray 5	Rays 6 & 7

(continued)

Table 1. (continued)

Muscle name	Distal attachment	Proximal attachment
Interfilamenti caudalis dorsalis (interradialis caudalis <i>sensu</i> Winterbottom, 1974)	Rays 7–12	Base of the dorsomedial fin rays
Interfilamenti caudalis ventralis (interradialis caudalis <i>sensu</i> Winterbottom, 1974)	Rays 16–22	Base of the ventromedial fin rays & dorsomedial ray 9
Flexor caudalis dorsalis superioris (flexor dorsalis <i>sensu</i> Winterbottom, 1974)	Rays 6–7	Vertebral column, proximal caudal fin bones
Flexor caudalis dorsalis inferioris (flexor dorsalis <i>sensu</i> Winterbottom, 1974)	Rays 7–8	Vertebral column, proximal caudal fin bones
Adductor caudalis ventralis (hypochordal longitudinalis <i>sensu</i> Winterbottom, 1974)	Rays 8–10	Vertebral column, proximal caudal fin bones
Flexor caudalis ventralis superior (flexor ventralis <i>sensu</i> Winterbottom, 1974)	Rays 15–23	Vertebral column, proximal caudal fin bones
Flexor caudalis ventralis inferior (flexor ventralis <i>sensu</i> Winterbottom, 1974)	Ray 24	Vertebral column, proximal caudal fin bones

Pelvic fins

The pelvic girdle is held within the body mainly by the protractor and retractor ischii (infracarinalis anterior and medial *sensu* Winterbottom, 1974) (Fig. 2A). The protractor ischii originates from the posteroventral region of the cleithrum and some fibers arise directly from the trunk lower (hypaxial) musculature. When they reach the pelvic girdle, the two protractor ischii muscles of the two sides of the body unite into the single protractor ischii tendon, which is attached posteriorly to the posterior process of the pelvic girdle (ischiac process *sensu* Cubbage & Mabee, 1996). The retractor ischii runs from the posterior region of the pelvic girdle to the anterior region of the anal fin.

The musculature that connects the pelvic girdle to the pelvic fin falls into two groups: the ventral (abductor) (Fig. 2A) and dorsal (adductor) muscles (Fig. 2B). Each group consists of three distinct muscles organized into two layers. The superficial ventral layer is composed of the abductor superficialis pelvius, which originates from the posterior part of the protractor ischii tendon and the midline, thus partially contacting its counterpart (Fig. 2A). The abductor superficialis pelvius is a very feeble and short muscle attached to all pelvic fin rays except rays 1 and 2. The deep ventral layer consists of the abductor profundus pelvius and arrector ventralis pelvius, which can be seen under the superficial layer without removing it. They both originate from the pelvic girdle (basipterygium *sensu* Bird & Mabee, 2003) and are mainly parallel to the fin rays. The abductor profundus pelvius lies more medially and is attached to the basis of all rays except the first one. The arrector ventralis pelvius is a narrow muscle that arises

more anteriorly and laterally and attaches only onto the first ray.

A somewhat similar pattern is present on the dorsal side with the adductor musculature (Fig. 2B): the arrector dorsalis pelvius, adductor superficialis pelvius, and adductor profundus pelvius originate from the anterior portion of the pelvic girdle and are mainly parallel to the rays. The arrector dorsalis pelvius is lateral to the other two muscles, attaching onto the base of the first ray, and the adductor superficialis pelvius inserts onto all other rays. The adductor profundus pelvius inserts on the bases of all rays, including the medial part of the base of the first ray.

Dorsal and anal fins

The muscles of these fins are described together because they share a similar pattern. Unlike other fins, the dorsal and anal fins consist of a continuous series of units decreasing in size posteriorly (Figs 3 and 4). Each muscular unit is composed of a half ray plus three muscles (inclinator, erector, and depressor), on each side of the body. The most anterior and posterior units have additional longitudinal non-paired muscles. In the case of the dorsal fin, they are the protractor dorsalis and retractor dorsalis, respectively (supracarinalis anterior and posterior *sensu* Winterbottom, 1974) (Fig. 3). The protractor dorsalis originates mainly from the posterior region of the skull and inserts onto the anterior base of the first dorsal ray. The retractor dorsalis runs from the anterior region of the anal fin to the posterior base of the last dorsal ray (ray 9 in the fish shown in Fig. 3; N.B. the number of dorsal rays is variable within zebrafishes). Similarly, the longitudinal muscles of the anal fin

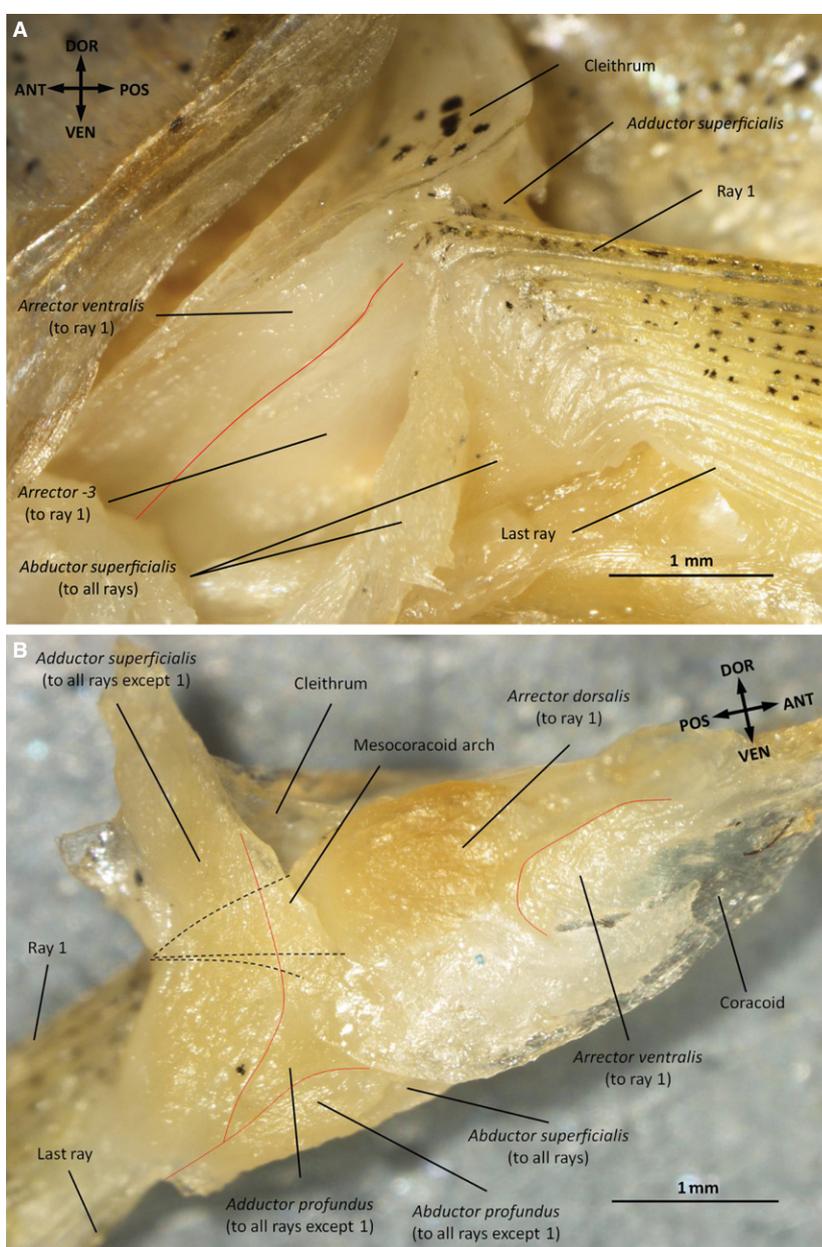


Fig. 1 Photographs of dissection of pectoral fins of *Danio rerio*. Lateral (A) and medial (B) views with the left pectoral fin and girdle reflected to show ventral and dorsal muscles.

are the protractor analis and retractor analis (infracarinalis medius and posterior *sensu* Winterbottom, 1974) (Fig. 4). The protractor analis is continuous with the retractor ischii anteriorly and inserts onto the anterior base of the first anal ray. The retractor analis attaches anteriorly to the posterior base of the last anal ray (ray 14 in the fish shown in Fig. 4) and then becomes continuous with the hypaxial musculature of the caudal fin.

Within the series of similar units, the superficial layer of the dorsal and anal fins is composed of paired (left and right) inclinator muscles (inclinatores dorsales and anales, respectively) (Figs 3 and 4). They connect the skin and trunk musculature to the lateral base of each ray. The deep layer of serial muscles includes paired erector and depressor

muscles (erectores and depressores dorsales and anales, respectively), which mainly originate from the radials and insert onto the anterior (erectores) and posterior (depressores) region of the base of each ray.

A distinguishing feature of the erectores anales is the presence of a tendon that runs along each muscle for one-third of its dorsoventral length towards its attachment site on the respective haemal spine. The presence of this peculiar tendon is thus particularly helpful to distinguish the erectores from the depressores of the anal fin (Fig. 4), and is also an important landmark to distinguish the anal and dorsal fins as no such tendon was seen in the erectores dorsales (Fig. 3). The anal spike, which is anterior to the first anal fin ray (Fig. 4), has an erector muscle connecting it to the

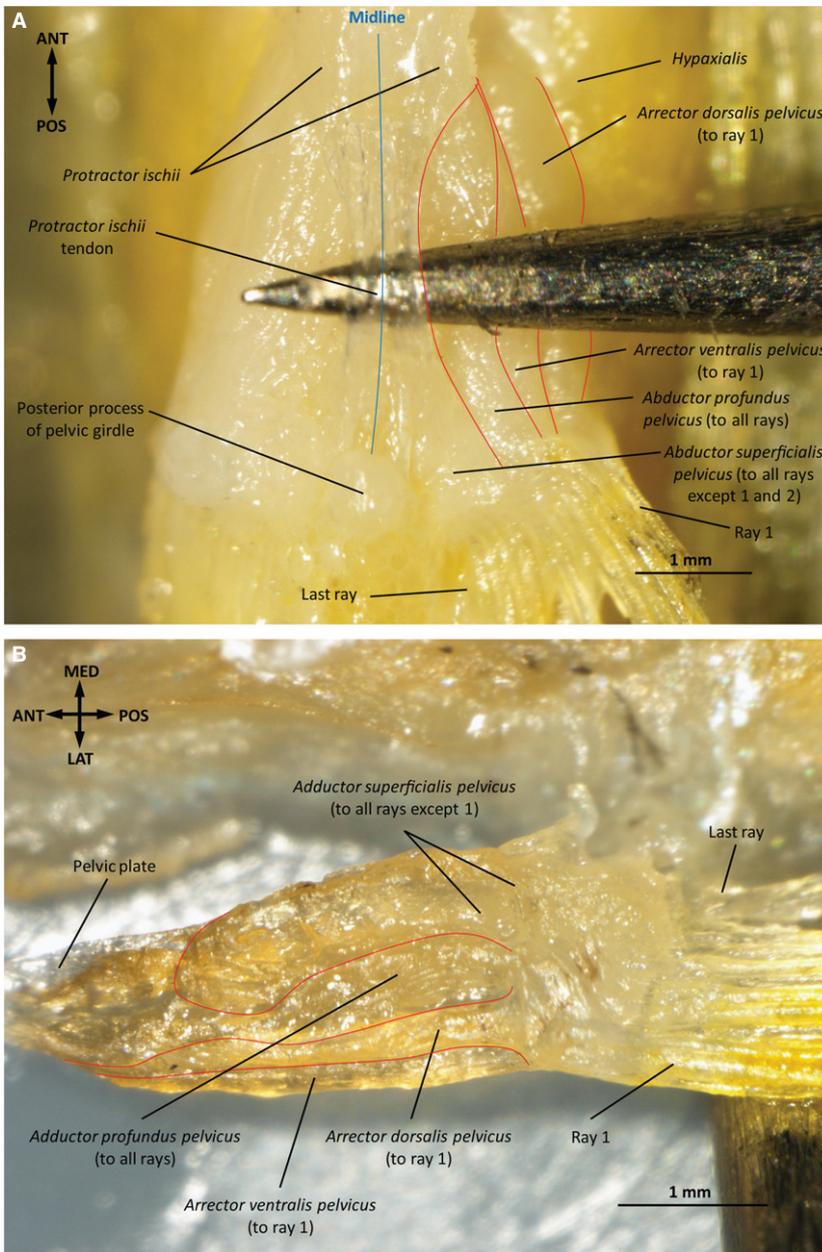


Fig. 2 Photographs of dissection of pelvic fins of *Danio rerio*. Ventral view (A) with both the pelvic fins and girdle and dorsal view (B) with the left pelvic fin and girdle reflected to show ventral and dorsal muscles.

hypaxial musculature, but it does not seem to have depressor and inclinator muscles. In the dorsal fin, the erector of the second ray lies deeper than other erectors and is hidden behind the depressor of the first ray (Fig. 3). Such positioning was specific for the second dorsal ray and can possibly be related to the large masses of the erectors of dorsal rays 1 and 3 and depressors of the first three dorsal rays.

Caudal fin

The musculature of the caudal fin in the adult zebrafish has a varying degree of separation. This variation was the only (minor) feature that was somewhat different in the dissected adult specimens. Muscles of the superficial layer

of the caudal fin are often fused with the overlying trunk musculature and do not have clearly defined borders (Fig. 5A). For instance, the lateralis profundus dorsalis and lateralis profundus ventralis originate from the myoseptum of the caudal musculature, proximal caudal fin bones, and the membrane connecting these bones. Posteriorly, these two muscles insert onto the caudal fin rays 6–8 and 21–24, respectively, of the fish shown in Fig. 5. The lateralis profundus dorsalis partially covers the small muscle interradialis caudalis, which connects the bases of rays 5, 6, and 7 (the first three long rays, Fig. 5A). This small muscle is only present on the dorsal side of this fin and illustrates an example of the dorsoventral asymmetry in the caudal fin musculature.

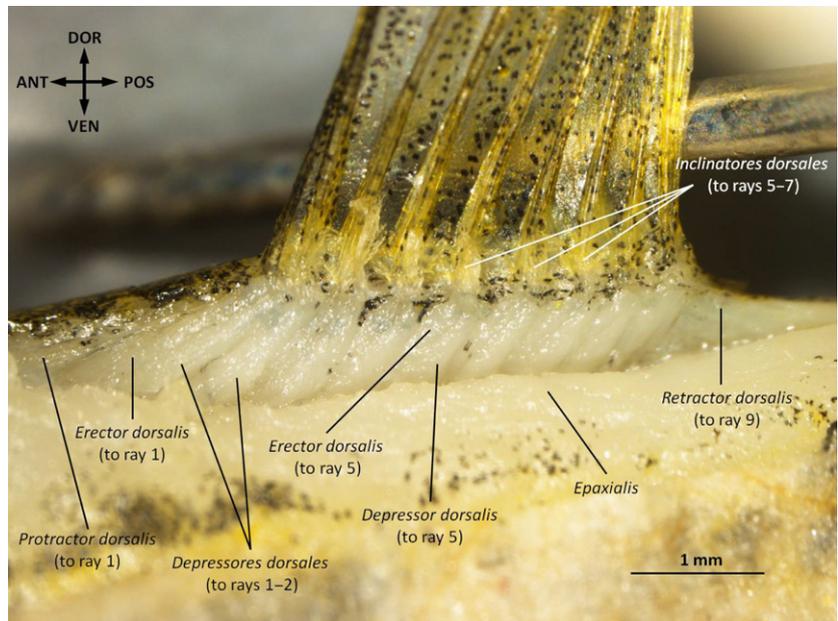


Fig. 3 Photograph of lateral view of the dorsal fin of *Danio rerio*.

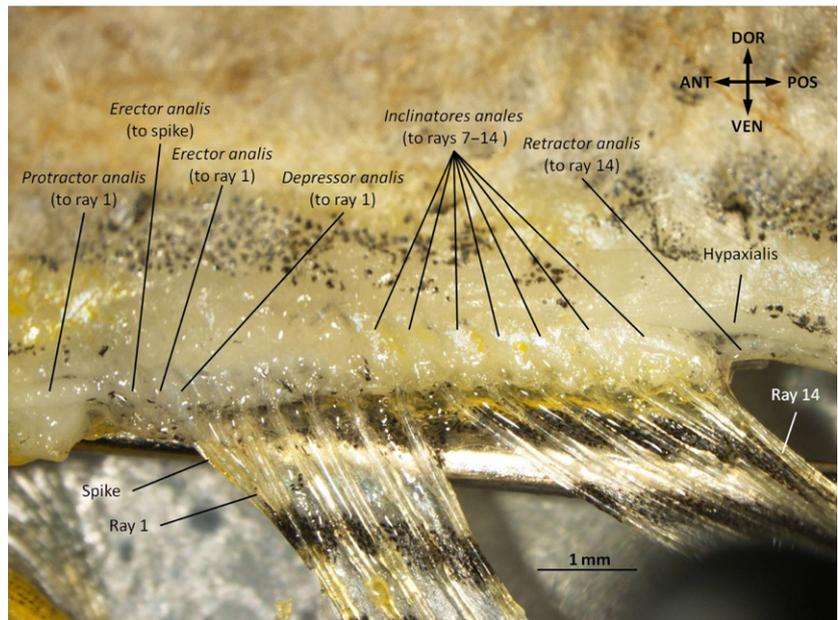


Fig. 4 Photograph of lateral view of the anal fin of *Danio rerio*.

The lateralis superficialis dorsalis and lateralis superficialis ventralis lie more medially, and cover only a small portion of the lateralis profundus dorsalis and ventralis (Fig. 5A). They originate from the dorsoventral midline of the caudal fin and run posteriorly to the dorsal ray 8 and ventral ray 22. Anteriorly, these muscles form a single complex with the trunk musculature but become more separated from other muscles posteriorly. At the bases of the fin rays, the lateralis superficialis dorsalis and ventralis overlie the muscles interfilamenti caudalis dorsalis and ventralis, respectively. The latter originate mainly from the ventrodorsal midline, fan out asymmetrically, and insert onto caudal rays 8–12 and 16–21, respectively (Fig. 5A). They mainly connect adjacent

rays but can also extend over more than one ray. The two most medial dorsal rays of the fin (13 and 14) as well as one medial ventral ray (15) do not have attachment sites for the interfilamenti caudalis dorsalis and ventralis. Notably, a small portion of medial fibers of the interfilamenti caudalis dorsalis comes from the ventral side of the caudal fin region and partially overlies the interfilamenti caudalis ventralis. Also notably, a portion of the interfilamenti caudalis ventralis originates from the base of the ray 9, which belongs to the dorsal part of the caudal fin.

The deep layer of the caudal muscles includes some flexor muscles and the adductor caudalis ventralis (Fig. 5B). These muscles originate from the vertebral column, proximal

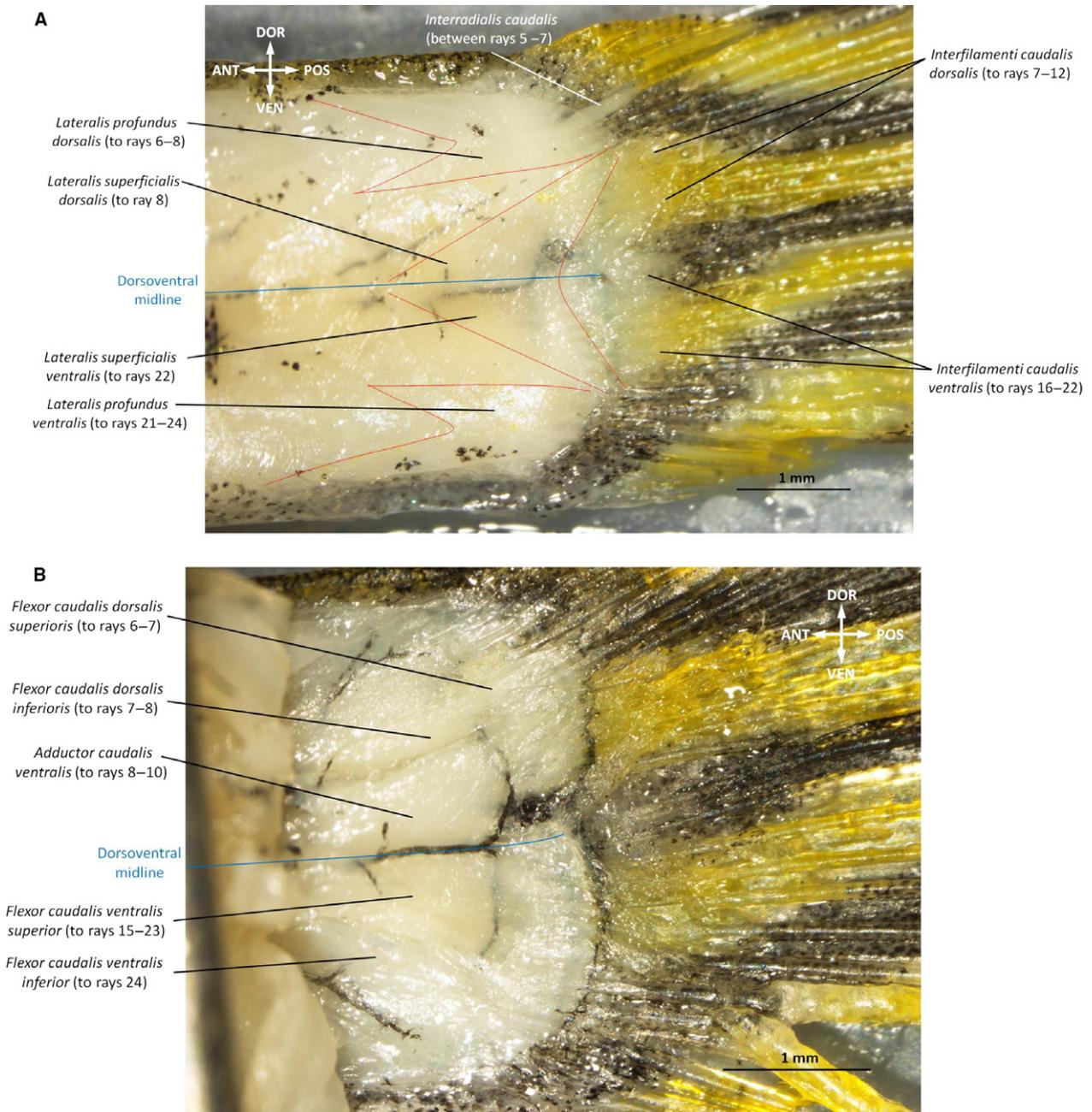


Fig. 5 Photographs of dissection of the caudal fin of *Danio rerio*. Superficial (A) and deep (B) layers of the caudal fin musculature on the left side. Scale bar: 1 mm.

caudal fin bones, and the membrane between them. The adductor caudalis ventralis runs mainly from the dorsoventral midline to the caudal fin rays 8–10. The flexor caudalis dorsalis superioris and inferioris mainly originate from the second last caudal vertebra and lie dorsomedially to the adductor caudalis ventralis, inserting onto the caudal fin rays 6–8. On the ventral side, the flexor caudalis ventralis superioris and inferioris originate from the last two vertebrae and the caudal bones and insert onto all the long ventral caudal rays (Fig. 5B).

Discussion

Similarities and differences between the musculature of paired fins

The musculature of the adult zebrafish pectoral fin is in general very similar to that of the pelvic fin. Both fins have three types of muscles, which are organized in layers: superficial and deep layers of abductors (i) and adductors (ii) for all/most rays, plus arrectors (iii) going only to the first ray of

each fin. This similarity had been noted in teleosts by previous authors (e.g. Winterbottom, 1974) and was recently discussed by Diogo et al. (2013, 2016), Diogo & Ziermann (2015), Miyashita & Diogo (2016), and Ziermann et al. (2017). The latter authors reviewed comparative, paleontological, and developmental data and suggested that the pectoral-pelvic musculature similarity seen in teleosts is a derived, homoplastic feature acquired independently in the evolutionary transition leading to these fishes, somewhat mirroring the homoplastic similarity seen in other derived gnathostome groups, such as tetrapods. For instance, in cartilaginous fishes, such as sharks, and in the living sister-group of all ray-finned fishes (actinopterygians), *Polypterus*, there is a single well-defined preaxial muscle in both the pelvic and pectoral appendages (Diogo & Ziermann, 2015; Wilhelm et al. 2015; Diogo et al. 2016; Molnar et al. 2017). As this single preaxial muscle is mainly a part of the ventral (abductor) musculature, it might correspond to the arrector ventralis of the pelvic and pectoral fins of teleosts, and therefore of the zebrafish. However, even if this is the case, it means that at least the arrector dorsalis of each fin arose independently within the evolutionary history of other actinopterygians. This means that derived actinopterygians such as teleosts display an increased number of muscles that are topologically similar between the pectoral and pelvic fins.

The main difference between the pectoral and pelvic musculature concerns the protractors and retractors of the girdles; namely, in the zebrafish as well as in most teleosts, there is not a well-defined retractor of the pectoral girdle that would topologically correspond to the retractor ischii of the pelvic girdle (Winterbottom, 1974; Diogo & Abdala, 2010). Moreover, the protractor ischii of the pelvic fin is an appendicular/trunk muscle, whereas the protractor of the pectoral girdle – the protractor pectoralis – is a head (branchiomic) muscle derived from the cardiopharyngeal field (Diogo et al. 2015; Lescroart et al. 2015). To explain why the protractor pectoralis (reduced in size or absent in adult zebrafishes) and many other muscles connect the pectoral girdle to the cranium in teleosts, one could point to the immediate proximity between the pectoral girdle and the skull. Following this argument, one could also note that pelvic fins lack such a possibility and that their remote posterior position leads to the pelvic girdle being fixed to the body via the hypaxial muscle mass attached to the pleural ribs. In other cyprinid fishes, this connection may be additionally strengthened by a ligament from the seventh pleural rib to the dorsal surface of the pelvic plate (Saxena & Chandy, 1966). Nevertheless, our comparative data indicate that this difference is not merely a question of topological position. Instead, the different developmental and evolutionary origin of the protractor pectoralis (head muscle) and protractor ischii (appendicular/trunk muscle) seem to reflect a profound, major difference between protractors of each girdle. A sharp distinction between these two muscles

can also be seen in anatomically generalized teleost such as *Elops* and *Oncorhynchus*. In these fishes, the protractor ischii is short and fused with the hypaxialis before reaching the pectoral girdle (Greene & Greene, 1914; Winterbottom, 1974). According to Winterbottom (1974), in basal teleosts this muscle is likely undifferentiated/more fused with the hypaxialis, i.e. in early teleosts the paired fins were probably not connected via a differentiated protractor ischii. However, more information is needed to determine the exact ancestral condition, and we plan to address precisely this subject in future works. These differences, together with the very different position and anatomy of the pelvic and pectoral girdles, support the idea recently defended by more and more authors that the pectoral appendage is at least partly derived from the head and thus it is very different originally/ancestrally from the pelvic appendage (see recent works by Diogo & Ziermann, 2015; Miyashita & Diogo, 2016; Nagashima et al. 2016; Ziermann et al. 2017).

Another evident difference in the muscular composition of the paired fins in the adult zebrafish is the presence of two arrector muscles on the ventral side of the pectoral fin (the arrector ventralis and arrector-3) and only one arrector muscle on the ventral side of the pelvic fin (arrector ventralis). The arrector-3 of the pectoral appendage has been frequently overlooked or considered as a bundle of the arrector ventralis or the fin abductor (e.g. by Brousseau, 1976a,b). However, the arrector-3 is found in various teleosts (Diogo & Abdala, 2010) and *Lepisosteus* (N. Siomava, pers. obs.), constituting either (i) a potential synapomorphy of neopterygians that was lost in Halecomorphi or (ii) synapomorphies of teleosts and of Ginglymodi. This muscular reinforcement of the first pectoral ray may possibly be related to the fact that in many teleosts the first pectoral and pelvic fin rays are functionally uncoupled from other rays, being for instance related to sound production or defensive tasks (e.g. Ladich, 2006; Hadjiaghai & Ladich, 2015).

The derived presence of three distinct arrectors in the pectoral fins of the zebrafish may also be related to the directive function of these fins in swimming. In many fishes, the pectoral fins act as the primary propulsors during rhythmic swimming (Webb, 1973; Blake, 1979; Drucker & Jensen, 1996a,b; Walker & Westneat, 1997; Hale, 2006), braking (Drucker & Lauder, 2003; Higham, 2005), and maneuvering (Drucker & Lauder, 2003). Being usually the longest and the thickest, the first ray of the pectoral fin experiences high resistance in water. In contrast, the pelvic fins of cyprinoid fishes seem to be less important for the equilibrium and power of locomotion (Harris, 1938). Harris (1938) proposed that these fins are mainly used to neutralize the lift force after stopping and to produce slow elevation and depression forces, which result in less resistance during movements. Therefore, in contrast to the leading pectoral fins, pelvic fins do not usually require additional reinforcement in usual conditions.

Similarities and differences between the musculature of the median fins

Similarly to the musculature of the paired fins, the anal and dorsal fins have three major types of muscles – which are called, in these median fins, erectors, depressors, and inclinators. In addition, the anal and dorsal fins have longitudinal protractors and retractors. They are somewhat similar to the protractor and retractor ischii of the pelvic appendage but they insert onto rays and not on a girdle, as the median fins have no girdle (Figs 2–4). The architecture of the dorsal and anal fins is very similar in general: with the exception of the protractors and retractors, both fins consist of serially repeated units of a ray and corresponding muscles, as explained above. Such a serial composition of units suggests that either *Hox* genes or another class of unidentified genes with a similar function contribute to their positioning (e.g. Mabee et al. 2002) and development. Interestingly, while dissecting and reviewing information about the dorsal fin in the zebrafish, we noticed some variation in the number of units. For example, Schneider & Sulner (2006) described 10 muscular units and 10 dorsal rays in their specimens, whereas the dorsal fins of our specimens were composed of nine units. The variation of units in the anal fin is unknown because there are no detailed studies describing or comparing this particular fin type among zebrafishes. All zebrafishes dissected for the present study had 14 units. While units of the dorsal fin significantly decreased in size posteriorly, in the anal fin they remained of a similar size. Another slight difference between the anal and dorsal fins is that the inclinators dorsales attach strongly to the skin and only small portions of their fibers are blended with the epaxial trunk muscles. After removing the skin during dissection, the inclinators dorsales were substantially destroyed, whereas the inclinators anales remained intact because they were mainly attached to the hypaxial trunk muscles. However, these differences are relatively minor, and there is little doubt that the anal and dorsal fins are basically a copy of each other, both anatomically (this study) and developmentally (e.g. Freitas et al. 2006).

This contrasts with what is seen in the caudal fin. Our observations show that the muscle pattern of the caudal fin is very different from the patterns seen in any other fin. They support the idea of its developmental and evolutionary uniqueness (Quint et al. 2002; Agathon et al. 2003). Thus, one can say that the caudal fin is in a way an ‘axial’ postcranial fin (i.e. it is associated with the posterior elements of the axial skeleton), and thus in a sense it is not a true appendicular structure like the other fins.

Can the muscles of the median fins correspond to those of the paired fins?

Recently, some authors have pointed out profound similarities in developmental and genetic mechanisms involved in

the formation of the paired and median fins (Freitas et al. 2006), with the exception of the median caudal fin, which as noted just above seems to be developmentally very different from other fins (Quint et al. 2002; Agathon et al. 2003). The sharing of similar mechanisms between the paired fins and the median dorsal and anal fins may support the fin-fold theory describing the origin of the paired fins from the median fin fold (Thacher, 1877; Mivart, 1879; Balfour, 1881). This theory was accepted by most authors during various decades but started to be increasingly called into question by recent authors (e.g. Gillis et al. 2009; Gillis & Hall, 2016). Some of these authors are coming back to Gegenbaur’s old – and for many decades discredited – idea that the paired appendages, or at least the pectoral ones, may be a derivative of the posterior pharyngeal region (Gegenbaur, 1859). Indeed, there are numerous evolutionary and functional reasons for the deep spatial relationship between the skull and pectoral girdle in early gnathostomes: the girdle forms the rear wall of the internal branchial chamber – a shield for the pericardial cavity and a secure insertion for the pectoral fins (Coates & Cohn, 1998; Matsuoka et al. 2005). Moreover, the pectoral appendage is closely associated with the head developmentally. Both these structures use strikingly similar developmental mechanisms, which include an *Shh*-dependence in both this appendage and in the pharyngeal arches in chondrichthyans, a commonality also seen in the pelvic appendage (Gillis et al. 2009).

A possible hybrid hypothesis that takes into account the fin-fold theory, Gegenbaur’s idea, and recent developmental, genetic, and comparative data would be that the pectoral appendage is at least partially derived from the head, whereas the pelvic appendage might be derived from the median fins. A similar idea has been recently advanced in the developmental work by Nagashima et al. (2016), who defended the idea that the pectoral appendage develops partially from the head region using a head ‘program’, whereas the pelvic appendage rather fully uses a trunk ‘program’ (Nagashima, H. pers. commun.).

So, how do the new anatomical data obtained in the present work relate to this crucial debate in the fields of comparative, developmental, and evolutionary biology? As noted above, the pelvic and pectoral appendages of teleosts do share some striking similarities, but (i) at least some of them seem to be derived (homoplasy, i.e. they were not present ancestrally), and (ii) there are some major differences between the musculature of these appendages, e.g. branchiomic muscles are only related to the pectoral appendage. These observations fit the hybrid scenario, asserting that at least part of the pectoral appendage (e.g. its girdle, as proposed by Nagashima et al. 2016) might be developmentally/evolutionarily related to the head. But could the muscles of the pelvic fin correspond, in some way, to those of the dorsal and anal fins thus supporting the idea that the pelvic fins are derived from the median fins,

as proposed by Freitas et al. (2006) and by the fin-fold theory?

At first sight, it may be difficult to see how pelvic muscles can be compared with the muscles of the dorsal and anal fins, composed of serially repetitive units. Nevertheless, we have already noted some similarities between the protractors and retractors of the anal and dorsal fins and the protractor and retractor ischii of the pelvic appendage. An additional potential similarity between the dorsal/anal fins and the pelvic fin is the excessive development of the most anterior (preaxial) erector in the dorsal fin/anal fin. Such an overdeveloped erector might thus be compared, both topologically and functionally, to the arrector ventralis of the pelvic fin (and also of the pectoral fin, for this matter). If this is the case, one could further speculate and suggest that perhaps during fish evolution the erectors of the more posterior units (from the second to the last) of the anal and dorsal fins could potentially have become fused with other muscles, e.g. with the depressors, to form a single muscle mass. An example of such a kind of fusion between the muscle units associated with most rays of the dorsal/anal fins can be seen in adult *Elops* and *Argyropelecus* (Winterbottom, 1974). In these fishes, the erectors and depressors of the posterior rays fuse together and form a single compound muscle. Alternatively, the erectors of the posterior rays may have become reduced and then absent, as they have in teleosts such as the seahorse *Hippocampus zosterae*. In this seahorse, the first ray retains three types of muscles (the erector, depressor, and inclinators muscles), but posterior rays have only two types – the depressors and inclinators (Consi et al. 2001). Following this line of reasoning, one could thus say that muscles of the posterior units of the dorsal and anal fins might have become undifferentiated during development/evolution and given rise to the abductor/adductor muscle masses of the pelvic and pectoral fins. In the early development of the pelvic and pectoral fin musculature of fishes such as sharks, the abductor and adductor muscle masses are formed from a series of individual muscles that clearly reflect a segmented pattern, which later in development becomes imperceptible (Jarvik, 1965; see also recent work of Ziermann et al. 2017). Moreover, the adductor and abductor masses of both the pelvic and pectoral appendages of adult chondrosteans fishes (N. Siomava, pers. obs.), which for some respects of muscle development are often considered to be paedomorphic, strongly resemble the clearly segmented, serial thin muscle bundles that can be seen in early developmental stages of fishes such as sharks (Ziermann et al. 2017). In fact, in adult chondrosteans there is a striking similarity between the configuration of the main abductor and adductor masses of the pectoral, pelvic, anal, and dorsal fins. It is thus possible that this was the plesiomorphic condition for those adult basal gnathostomes with these fins. Then, during evolution within extant fishes, the early individual muscles inserting exclusively onto the first ray could remain separate and form the adult

arrectors due to the functional uncoupling of this ray, particularly in the paired fins (Ladich, 2006, pp. 45–70; Hadjiaghai & Ladich, 2015). We plan to test these hypotheses in further comparative studies by including representatives of all major fish groups and detailed descriptions of the appendicular muscle development from early to adult stages in the zebrafishes and other fishes.

Is the zebrafish an appropriate model for the appendicular musculature of teleosts?

To pave the way for such future studies, either by us or by other authors, and to discuss whether the zebrafish is a good model for teleosts in general in comparative, developmental, functional, evolutionary, and evo-devo studies, we contrast here our results with descriptions of the fin muscles in other teleosts. The pectoral fin muscles of the zebrafish are similar to those of other teleosts, which usually have six muscles: two arrectors (dorsalis and ventralis), two abductors (superficialis and profundus), and two adductors (superficialis and profundus) (Winterbottom, 1974). The seventh pectoral fin muscle in the zebrafish – the arrector-3 – is often present in other teleosts, as noted above. In this sense, the overall configuration seen in the zebrafish is strikingly similar to that of numerous teleosts (Diogo & Abdala, 2010). Moreover, a recent review has shown that even the phylogenetically basal actinopterygian *Polypterus* has six pectoral fin muscles, of which five clearly correspond to muscles present in the zebrafish (Molnar et al. 2017). The postaxial muscle present in the *Polypterus* pectoral fin has no correspondence in the zebrafish, and the arrector dorsalis and arrector-3 of the zebrafish pectoral fin have no correspondence to the *Polypterus* muscles. These comparisons indicate that concerning the pectoral fin muscles the adult zebrafish is a very good model for teleosts in general and for actinopterygians (ray-finned) fishes as a whole.

The configuration of the six pelvic muscles in the zebrafish is also very similar to that found in other teleosts, including generalized forms such as *Elops* (Winterbottom, 1974). Interestingly, in certain Indian hill stream fishes of the cyprinid family – the family including zebrafishes –, the abductor superficialis can be complemented by an extra separate bundle, attached to the first ray (Saxena & Chandy, 1966). The reinforcement of the first pelvic fin ray with this extra muscle might be related to the high resistance in swift current. It resembles the reinforcement of the first ray in the pectoral fins with the arrector-3 and provides a further case of derived (homoplastic) similarity between these two types of fins. As recently noted in Molnar et al. (2017) and Diogo et al. (2016), of the six pelvic fin muscles present in the adult zebrafish, five might have been ancestrally present in actinopterygians, as they are found in the phylogenetically basal *Polypterus*. Similarly to what occurs concerning the muscle configuration in the pectoral fin, the postaxial pelvic fin muscle present in *Polypterus* has no

correspondence in the zebrafish, and the arrector dorsalis pelvici of the zebrafish has no correspondence in the *Polypterus* muscles (Molnar et al. 2017). Therefore, regarding the pelvic fin musculature the zebrafish is also a good model for both teleosts and actinopterygians as a whole.

The dorsal and anal fins are very similar anatomically to each other, and their musculature is in general conservative within teleosts, with only minor differences between some taxa. For instance, in *Elops* the erector muscles of the first two dorsal fin rays are absent and the first erector is thus attached to the third ray (Winterbottom, 1974). The same pattern can be seen in the anal fin of *Elops*: the first two rays are not associated with erectors. These observations support the idea that these two fins are deeply related/integrated developmentally and evolutionarily. In the seahorse *H. zosterae*, erectors are severely reduced and there is only a single pair of erector muscles attached to the anteriormost fin ray (Consi et al. 2001). In this case, the erection of the entire fin is achieved by pulling the first ray. All subsequent rays are pulled up via the inter-ray ligament, which mechanically couples all fin rays into one unit (Consi et al. 2001). Another type of muscular modification is a fusion of an erector and depressor into one muscle, which is usually attached medially to the ray and maintains both functions (Winterbottom, 1974). Such compound muscles are connected to the last two rays of the dorsal fin in *Elops* and to the last ray of the anal fin in *Argyrops* (Winterbottom, 1974). In contrast to these species, both the king salmon and zebrafish have more evenly distributed muscles. In the dorsal and anal fins of the salmon and zebrafish, the number of muscle series corresponds to the number of rays (present study, Figs 3 and 4; Greene & Greene, 1914). In this sense, the zebrafish is likely to be a very good model for teleost anal and dorsal fins. First, it has the same five types of muscles that are normally present in the dorsal and anal fins (inclinator, erectors, depressors, protractor, and retractor). Second, it might reflect an ancestral condition of having serial units for each ray. As there are no detailed comparative studies on the anal and dorsal fin muscles of gnathostomes as a whole, future studies are crucially needed to infer the ancestral condition for actinopterygians, bony fishes, and gnathostomes and to further these issues explore.

The caudal fin of the zebrafish is very similar to that of other teleosts, including generalized taxa such as the king salmon and *Elops* (Greene & Greene, 1914; Winterbottom, 1974; Schneider & Sulner, 2006). The superficial layer of the caudal fin consists of several muscles tightly consolidated with the hypaxial and epaxial trunk muscles, what makes it difficult to separate them and to define their sites of origin. Some fishes, e.g. the bluegill sunfish (*Lepomis macrochirus*), are known for the advanced articulation of their caudal fin. Thus, *L. macrochirus* can move each caudal fin ray independently (Flammang, 2014), but in spite of the high number of discrete caudal muscles, the overall structure of muscles in the tail fin resembles the deep layer in other teleosts,

particularly the zebrafish. Besides having a striking similarity, the zebrafish and other teleosts exhibit the dorsoventral asymmetry of the deep musculature (e.g. interradialis caudalis and adductor caudalis ventralis). This muscle asymmetry resembles the skeleton asymmetry seen in the caudal fin of various *Danio* species and contrasts with the symmetrical superficial muscle layer and the even ray distribution (Sanger & McCune, 2002). Altogether, these observations show that the adult zebrafish is a good model for the caudal fin musculature of teleosts. The highly conservative pattern of caudal muscles and their clear anatomical difference from those of other fins are in line with the idea that the caudal fin is a developmentally and evolutionarily distinct unit (Quint et al. 2002; Agathon et al. 2003) that is regulated via mechanisms different from those seen in the head and trunk (Flammang, 2014). Further studies should be undertaken to complement the very scarce comparative data on the caudal fin muscles in other fish groups and thus to infer whether the zebrafish can be used as a model for actinopterygians as a whole, as it can for the pectoral and pelvic muscles.

General remarks

In summary, it can be said that our comparative data show that *D. rerio* is a very good model for the appendicular muscles of teleosts. It is also an appropriate model to represent at least the paired fin muscles of actinopterygians as a whole. Therefore, the zebrafish should be further, and in fact increasingly, used for comparative, developmental, and macroevolutionary studies of all types of fins, including comparisons between fish paired appendages and tetrapod limbs. Moreover, the presence of the few paired fin muscles that are present in the zebrafish and that do not seem to have been ancestrally present in ray-finned fishes, i.e. the pectoral arrector-3 and arrector dorsalis as well as the pelvic arrector dorsalis, makes *D. rerio* a good model for comparative and macroevolutionary studies focused on locomotion and muscular segregation.

Author contributions

R.D. conceived the study. N.S. acquired the data, wrote the initial manuscript, and prepared all figures. R.D. worked on and revised the manuscript. The authors declare no conflicts of interest.

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