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Musculoskeletal anatomy of the pelvic fin of *Polypterus*: implications for phylogenetic distribution and homology of pre- and postaxial pelvic appendicular muscles

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Abstract

As a member of the most basal clade of extant ray-finned fishes (actinopterygians) and of one of the most basal clades of osteichthyans (bony fishes + tetrapods), *Polypterus* can provide insights into the ancestral anatomy of both ray-finned and lobe-finned fishes, including those that gave rise to tetrapods. The pectoral fin of *Polypterus* has been well described but, surprisingly, neither the bones nor the muscles of the pelvic fin are well known. We stained and dissected the pelvic fin of *Polypterus senegalus* and *Polypterus delhezi* to offer a detailed description of its musculoskeletal anatomy. In addition to the previously described adductor and abductor muscles, we found preaxial and postaxial muscles similar to those in the pectoral fin of members of this genus. The presence of pre- and postaxial muscles in both the pectoral and pelvic fins of *Polypterus*, combined with recent descriptions of similar muscles in the lobe-finned fishes *Latimeria* and *Neoceratodus*, suggests that they were present in the most recent common ancestor of bony fishes. These results have crucial implications for the evolution of appendicular muscles in both fish and tetrapods.

Key words: Actinopterygii; bichir; Cladistia; locomotion; water–land transition.

Introduction

The musculoskeletal anatomy of the pelvic fin in bichirs – genus *Polypterus* – is a key piece of evidence for reconstructing the evolution of complex pelvic appendicular musculature in gnathostomes as a whole and osteichthyans (bony fishes + tetrapods) in particular, and for understanding the evolutionary and functional relationships between the pelvic and pectoral fins. The bichirs (11 species) and the reedfish (*Erpetoichthys*) are the only extant members of the clade Cladistia, the most basal living clade within actinopterygians (ray-finned fishes), which are the sister-group of sarcopterygians (lobe-finned fishes and tetrapods). The phylogenetic position of *Polypterus* makes it a key taxon for resolving the ancestral condition not only of actinopterygians but also of osteichthyans as a whole, including those sarcopterygians that made the transition from fins to limbs. Members of the genus *Polypterus* are

capable of terrestrial locomotion and exhibit developmental plasticity in both pectoral fin anatomy and locomotor function, potentially making these fish a good model for studying the water–land transition in tetrapods (Standen et al. 2014).

For these reasons, the pectoral appendage of *Polypterus* has been the subject of numerous musculoskeletal studies in the past (e.g. Pollard, 1892; Klaatsch, 1896; Braus, 1900; Shann, 1924; Jessen, 1972; Diogo & Abdala, 2007, 2010; Wilhelm et al. 2015). Wilhelm et al. (2015) recently reviewed these studies and, based on that revision and their own examination of the pectoral appendage of *Polypterus senegalus* using contrast-enhanced micro-CT staining, they reported seven distinct muscles inserting onto the pectoral fin: the superficial and deep abductors and adductors, one preaxial muscle ('zonoproperterygialis') and a postaxial complex divided into two muscles ('coracomertapterygialis I–II'; Wilhelm et al. 2015).

However, the structure and function of the pelvic fin in *Polypterus* are much less-studied than those of the pectoral fin. Although the pelvic fin does not contribute substantially to forward propulsion in most fish, it plays an active, important role in locomotion in at least some osteichthyans (Standen, 2008, 2010). For example, in the teleostean

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rainbow trout the pelvic fins are actively oscillated, damping body oscillation and altering flow direction downstream of the fins (Standen, 2010). The pelvic fin even performs tetrapod-like movements during locomotion in some fish, such as the lungfish *Protopterus* (King et al. 2011) and the actinopterygian *Cryptotora* (Flammang et al. 2016). A number of batoids (rays) and bottom-dwelling sharks such as Orectolobiforms use their pelvic fins in a walking or 'punting' motion against the substrate (e.g. Pridmore, 1995; Wilga & Lauder, 2004; Macesic & Kajiura, 2010). Over the water-land transition in tetrapods, the pelvic fin acquired a role of primary importance in locomotion, and in modern tetrapods such as salamanders the pelvic limb provides most of the propulsive force for locomotion (e.g. Kawano & Blob, 2013).

Only a few studies have described the pelvic musculature of *Polypterus*. In his monograph on the dipnoan *Neoceratodus*, Braus (1900) described two muscles in the pelvic fin musculature of *Polypterus*, one dorsal (adductor) and one ventral (abductor). However, he noted that his description differed somewhat from that of von Davidoff (1879) and that the muscle fibers were difficult to distinguish macroscopically. No attempts to describe the pelvic musculature of *Polypterus* in detail have been made since the beginning of the 20th century. This lack of knowledge about the pelvic musculature makes it difficult to discuss the evolution and homologies of pelvic appendicular muscles and bones across gnathostomes, including the divergence of chondrichthyans and osteichthyans and, within osteichthyans, the origin of actinopterygians and derived teleosts. It also impedes comprehensive analysis of the evolution of functional morphology and behavior among gnathostomes, including the evolution of terrestrial locomotion.

In view of the scant information available, we decided to re-examine the pelvic musculoskeletal structures of

Polypterus and compare them with its pectoral structures and with the paired appendicular structures of other gnathostomes.

Methods

Three adult specimens of *P. senegalus* (snout-vent length 75–90 mm) and one specimen *Polypterus delhezi* (62 mm), acquired from commercial suppliers, were dissected under magnification (Fig. 1A–C). One fin of *P. senegalus* was dehydrated in 90% ethanol and stained with Alcian blue and Alizarin red to visualize bones and cartilage (Fig. 2A,B) and with Alcian blue only (Fig. 2C).

Results

Skeleton

The pelvic appendicular skeletal structures of *Polypterus* have been described previously (e.g. Braus, 1900; Goodrich, 1901 – *Polypterus bichir*) and are figured, but not described in detail, in works such as Bartsch et al. (1997; *P. senegalus*). However, because no detailed developmental study has been performed for the pelvic appendage in this taxon, the identities and homologies of these structures are not entirely clear. Our observations largely agree with those of previous authors. Embedded within the hypaxial muscle of the body wall, with hypaxial muscle fiber insertion laterally and rostrally, is an elongate cartilaginous plate (pelvic plate) with a small triangular piece of cartilage attached to its proximal end (Fig. 3A). This plate is variously referred to as the pelvic cartilage (Goodrich, 1901), the basal bone (*basales Knochenstück* or *Basalstück*; Braus, 1900) or the pelvic plate (Patterson, 1982; Gardiner, 1984) in the literature. We use the term 'pelvic plate' to be consistent with the more recent literature and discuss possible homologies

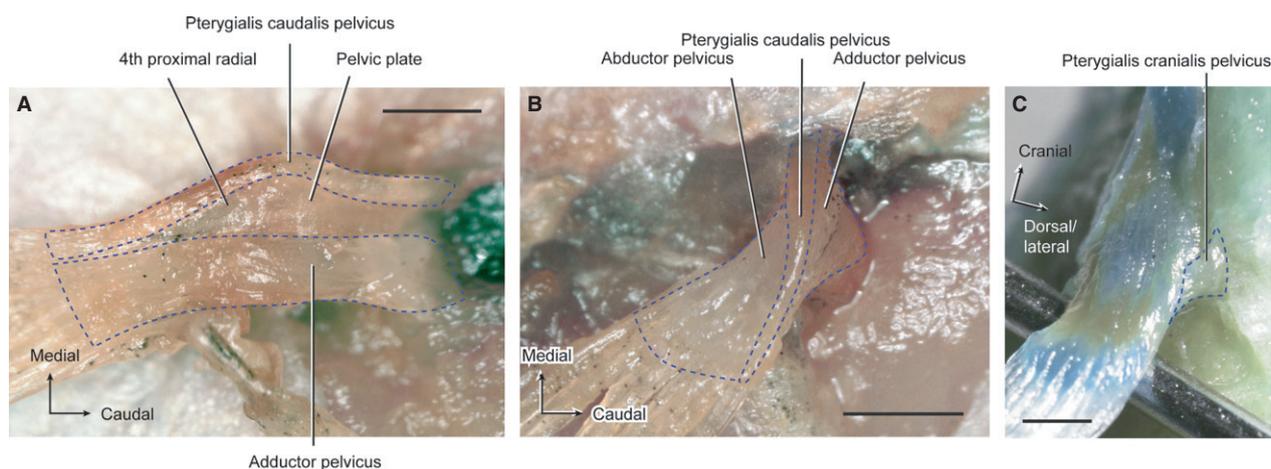


Fig. 1 Photographs of dissection of pelvic fins of *Polypterus senegalus*. (A) Ventral view with right pelvic fin and girdle reflected to show dorsal muscles. (B) Ventral view with right pelvic fin reflected and rotated to show pterygialis caudalis muscle. (C) Ventrolateral view of left pelvic fin (cartilage stained with Alcian blue) to show pterygialis cranialis muscle. Scale bar: 1 mm.

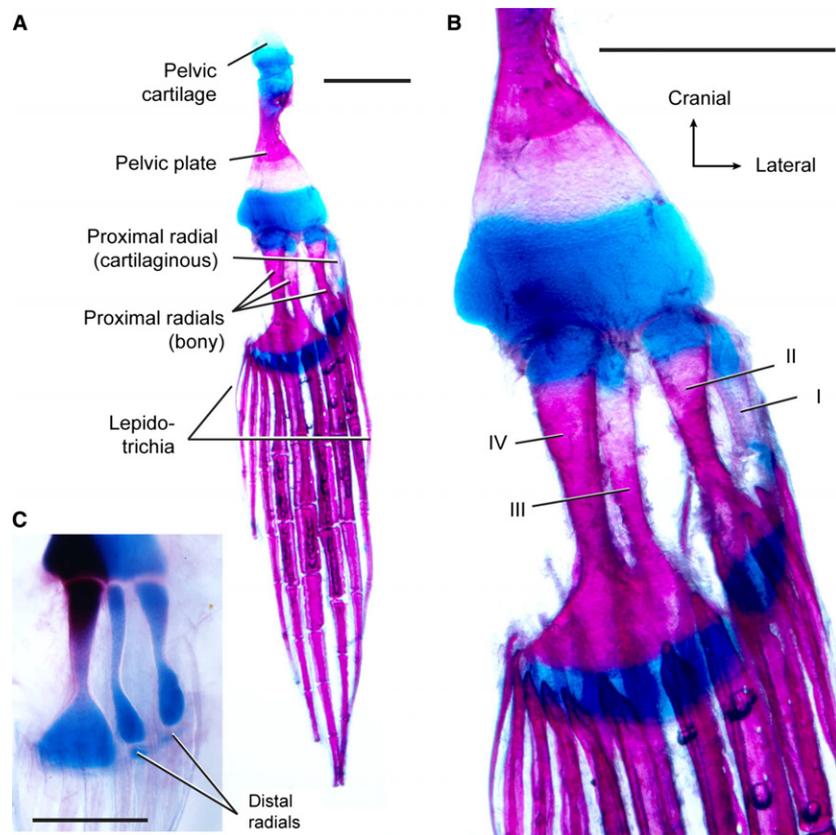


Fig. 2 Photographs of skeletal elements of pelvic fins of *Polypterus senegalus*. (A) Skeletal elements stained to show cartilage (blue) and bone (pink). (B) Close-up of (A) to show proximal radials in detail. (C) Middle portion of fin stained for cartilage only (blue) to show distal radials. Scale bar: 1 mm.

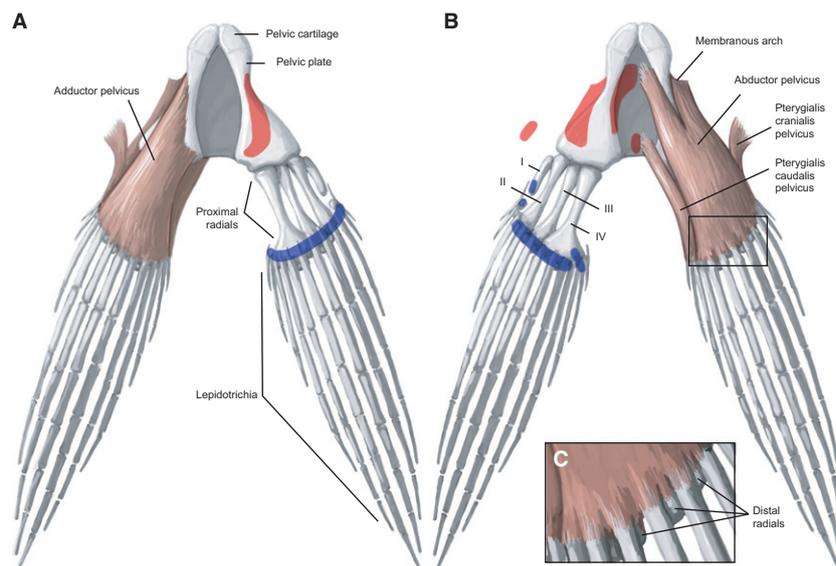


Fig. 3 Musculoskeletal anatomy of the pelvic appendage of *Polypterus* in (A) dorsal and (B) ventral views. Left fin shows musculature and right fin shows muscle origins (red) and insertions (blue). Inset (C) is an enlargement of (B) to show attachment of muscle to distal radials and lepidotrichia.

in the next section. The pelvic plate articulates proximally with its counterpart from the opposite side and distally with four rod-like proximal radials. The preaxial proximal radial is cartilaginous, but the other three larger radials are bony (Fig. 2A). There are several cartilaginous, roughly polygonal distal radials measuring 15–20% of the axial length of the proximal radials. These elements were clearly visible in our

specimen stained with Alcian blue only, but they were difficult to distinguish in the unstained specimens because they are embedded in fibrocartilaginous matrix and in the specimens stained with Alizarin because they lie between the dorsal and ventral lepidotrichia. In the Alcian blue-stained specimen there was one distal radial associated with proximal radial II and one with proximal radial III (Fig. 2C). The

same configuration was figured by Goodrich (1901), whereas three distal radials were figured by Rosen et al. (1981). The variation in number of radials may be due to ontogenetic changes; in the closely related *Acipenser* the distal radials do not form until the animals reach approximately 60 mm in length (Hilton et al. 2011). The distal ends of the distal radials are associated with the lepidotrichia (Figs 2B and 3C).

Musculature

We refer to the main dorsal and ventral muscles of the actinopterygian pelvic fin as 'adductor pelvificus' and 'abductor pelvificus,' respectively, because these muscles are homologous with the 'abductor' and 'adductor' muscles of the pelvic fin in other gnathostome fishes (Diogo & Ziermann, 2015). The abductor pelvificus, which corresponds to the pterygialis communialis ventralis of Braus (1900), is the largest muscle on the ventral surface of the fin (Fig. 2B). It is a flat, parallel-fibered muscle with two heads. The medial head originates from the medial side of the ventral aspect of the pelvic plate and from the membrane that connects the pelvic plate to its partner on the other side of the body, and it contacts its counterpart at the midline. The lateral head originates from the lateral side of the ventral aspect of the pelvic plate, the fascia covering the adjacent hypaxial muscles, and the membranous arch that connects the lateral edge of the pelvic plate to the hypaxial muscles. It inserts on all five distal radials and on the bases of the lepidotrichia.

The pterygialis caudalis pelvificus is a narrower muscle (about 1/4 the breadth of the abductor pelvificus and of similar thickness) that lies just medial to the abductor pelvificus (Figs 1A,B and 3B). This muscle was not described by Braus (1900), probably because it is difficult to separate from the abductor pelvificus. However, the two muscles are separated by connective tissue along their lengths and have slightly different fiber directions, as revealed by our dissections. The fibers of the pterygialis caudalis have a more oblique orientation, converging toward the long axis of the fin, whereas those of the abductor pelvificus are aligned with the long axis of the fin. The pterygialis caudalis pelvificus originates from the membrane between the two pelvic plates, where it meets its counterpart at the midline. It inserts on the distal end of the 4th proximal radial, the 5th distal radial, the joint capsule between the two, and the bases of the postaxial lepidotrichia.

The adductor pelvificus, which corresponds to the pterygialis communialis dorsalis of Braus (1900), is the counterpart of the abductor pelvificus on the dorsal surface of the fin (Figs 2A,B and 3A). The adductor pelvificus has a single head that originates from the medial side of the distal half of the dorsal aspect of the pelvic plate. Its fibers fan out to cover the proximal radials and insert on all five distal radials and on the bases of the lepidotrichia. The lateral edge of the

abductor pelvificus and the medial edge of the pterygialis caudalis are visible from the dorsal aspect on either side of this muscle.

The pterygialis cranialis pelvificus is a very thin, flat muscle with fibers running in a cranio-lateral to caudo-medial direction (Figs 2C and 3B). It is slightly broader than the pterygialis caudalis but only 5–10% of its thickness. It originates from the fascia covering the hypaxial muscles and the adjacent skin at the lateral edge of fin-fold and inserts on the preaxial edge of the 1st proximal radial.

Discussion

Distribution of pre- and postaxial muscles in gnathostomes

Diogo & Abdala (2010) hypothesized that osteichthyans plesiomorphically had only two main muscle masses in the pectoral fin, an abductor and an adductor. This would mean that the complex musculature of the limbs of tetrapods and the fins of fish such as coelacanths and teleosts was independently acquired. One of the main pieces of evidence for this hypothesis was the supposition that the phylogenetically basal actinopterygian *Polypterus* has only two fully differentiated muscles (an adductor and an abductor, each divided into superficial and deep parts) in the pectoral fin. However, Diogo & Abdala (2010) did show that at least the abductor mass was also partially differentiated into a preaxial bundle. Wilhelm et al. (2015) considered Diogo & Abdala's (2010) 'preaxial bundle' to be a separate muscle, which they named 'zonoprotapterygialis'. As noted above, the authors further reported two postaxial muscles of the pectoral fin, which they named 'coracometa-ptyerygialis I and II'.

Within the pelvic fin, pre- and postaxial muscles are present in many gnathostome clades (Tables 1 and 2), although these muscles are not necessarily homologous (see next section). Chondrichthyans typically have three pelvic appendicular muscles, including a preaxial muscle, often designated protractor pelvificus (Diogo & Ziermann, 2015). Teleosts usually have six pelvic muscles that originate on the pelvis and insert on the fin rays: superficial and deep abductors and adductors, and dorsal and ventral arrectors pelvificus (preaxial muscles *sensu* this paper; Yamanoue et al. 2010). Some teleosts also have one postaxial muscle, the extensor proprius (Stiassny & Moore, 1992). The taxonomic distribution of this muscle is poorly known; it appears to be present in cyprinids, characins, osmerids, argentinids, salmonids, toadfish, and many percomorphs but absent in many other teleostean groups (Stiassny & Moore, 1992 and references therein). Additional muscles are present in some teleosts (radialis ventralis, abductor accessorius, arrector tertius), but they are relatively rare (Winterbottom, 1973). The coelacanth *Latimeria* has a remarkably similar pattern to that of teleosts, but with one preaxial muscle and one

Table 1 Muscles of the pelvic appendage of *Polypterus* and inferred actions based on anatomy and comparison with other taxa from the literature (see text for references).

Muscle	Origin	Insertion	Inferred action(s)
Abductor pelvici	Medial head: medial side of ventral aspect of pelvic plate and membrane between left and right pelvic plates Lateral head: lateral side of ventral aspect of pelvic plate, fascia covering hypaxial muscles, and membranous arch between pelvis and hypaxial muscles	Distal radials and bases of lepidotrichia	Abduct fin
Adductor pelvici	Distal half of dorsal aspect of pelvic plate	Distal radials and bases of lepidotrichia	Adduct fin
Pterygialis cranialis pelvici (preaxial muscle)	Fascia covering hypaxial muscles and skin at lateral edge of fin-fold	Preaxial edge of 1st proximal radial	Protract fin
Pterygialis caudalis pelvici (postaxial muscle)	Distal edge of membrane between left and right pelvic plates	4th proximal radial, 5th distal radial, capsule of joint between them, and lepidotrichia	Retract fin and abduct trailing edge

Table 2 Preaxial pelvic muscles in gnathostomes (not necessarily homologous – see Discussion).

Taxon	Clade	Name used in original work	Origin	Insertion	Dorsal/ventral position
<i>Squalus acanthias</i> *	Chondrichthyes	Pterygialis cranialis pelvici (or protractor pelvici)	Pelvic girdle	Propterygium	Ventral
<i>Neoceratodus forsteri</i> ^{†‡}	Sarcopterygii: Dipnoi	Pterygialis cranialis pelvici (or part of superficial ventromesial abductor)	Ventrolateral face of pelvis	Proximal lateral face of pelvic fin	Ventral (but derived from both ventral/dorsal muscle masses ¹⁾)
<i>Latimeria chalumnae</i> ^{‡§}	Sarcopterygii: Actinistia	Pterygialis cranialis pelvici (or abducteur pelvien)	Mesial hypophysis of pelvis	Six first preaxial lepidotrichia	Ventral
<i>Polypterus senegalus</i>	Actinopterygii: Cladistia	Pterygialis cranialis pelvici	Fascia and skin at lateral edge of fin-fold	Preaxial edge of 1st (preaxial) proximal radial	Ventral
<i>Perca fluviatilis</i> [¶]	Actinopterygii: Teleosti	Arrector dorsalis pelvici	Dorsolateral face of the pelvis	Dorsolateral half of the first (preaxial) fin radial	Dorsal
		Arrector ventralis pelvici	Ventrolateral face of the pelvis	Ventrolateral half of the first (preaxial) fin radial	Ventral

*Diogo & Ziermann (2015).

[†]Boisvert et al. (2013).[‡]Diogo et al. (2016).[§]Millot & Anthony (1958).[¶]Winterbottom (1973).

postaxial muscle (Tables 2 and 3), as well as a number of intrinsic pelvic fin muscles that do not cross the joint between the pelvic girdle and fin (Millot & Anthony, 1958). Dipnoans have a variable number of pelvic appendicular muscles: *Protopterus* has reduced pelvic appendages with only two muscles – adductor and abductor – that cross the hip joint (King & Hale, 2014). *Neoceratodus*, however, which is more basal phylogenetically in that it is the sister group of other extant lungfish, has nine muscles, including preaxial and postaxial ones (Young et al. 1989; Boisvert

et al. 2013; Tables 2 and 3). The functions of these muscles, where known, are discussed in the final section of the manuscript.

Homology of pelvic skeletal structures

First, regarding the pectoral fin of *Polypterus*, three specialized basal bones are present: the propterygium, mesopterygium, and metapterygium. The metapterygium is thought to be plesiomorphic for gnathostomes (but lost in teleosts),

Table 3 Postaxial pelvic muscles in gnathostomes (not necessarily homologous – see Discussion).

Taxon	Clade	Name used in original work	Origin	Insertion	Dorsal/ventral position
<i>Squalus acanthias</i> *	Chondrichthyes	- (not present)	–	–	–
<i>Neoceratodus forsteri</i> ^{†‡}	Sarcopterygii: Dipnoi	Pterygialis caudalis pelvicus (or superficial ventrolateral adductor + superficial ventromesial adductor)	Median posteroventral margin of the pelvis and adductor muscle mass from other side	First (proximal) axial element and radials	Dorsal (but derived from both ventral/dorsal muscle masses ¹⁾)
<i>Latimeria chalumnae</i> ^{‡§}	Sarcopterygii: Actinistia	Pterygialis caudalis pelvicus (or adducteur pelvien)	Dorsal side of pelvis	Fascia of pronator 5 and bases of 9–10th postaxial lepidotrichia	Dorsal
<i>Polypterus senegalus</i>	Actinopterygii: Cladistia	Pterygialis caudalis pelvicus	Distal edge of membrane between pelvic plates	4th proximal radial, 5th distal radial, lepidotrichia	Ventral
<i>Perca fluviatilis</i> [¶]	Actinopterygii: Teleostei	Extensor proprius	Anterolateral surface of the pelvic girdle	Base of the medial (postaxial) pelvic radials	Dorsal

*Diogo & Ziermann (2015).

[†]Boisvert et al. (2013).[‡]Diogo et al. (2016).[§]Millot & Anthony (1958).[¶]Winterbottom (1973).

and homologous with the sarcopterygian humerus (Shubin, 1995). In the pelvic appendage of fish, the term 'basipterygium' is often used to refer to the topological equivalent(s) of the pectoral propterygium, mesopterygium, and/or metapterygium. The homologies of these bones in the pelvic appendage are controversial, but, as we argue below, the balance of evidence suggests that the pelvic plate of *Polypterus* is homologous with the pelvic girdle of sarcopterygians, chondrichthyans, and probably teleosts, and that the pelvic metapterygium (i.e. the homologue of the metapterygial axis of the sarcopterygian pelvic fin) was either lost or incorporated into the girdle.

Due to its resemblance – both in shape and topology – to the pelvic metapterygium of chondrichthyans, several authors in the late 1800s suggested that the pelvic plate in *Polypterus*, which they called the 'basipterygium' or 'metapterygium,' is homologous with the basals in other fish (e.g. von Davidoff, 1879; Thompson, 1886; Wiederheim, 1897). According to these authors, the small proximal cartilages (Fig. 2A) represent a very reduced pelvic girdle. Rosen et al. (1981) later suggested that the metapterygium was incorporated into the pelvic girdle and that the pelvic plate thus represents both structures, and this view was followed by some other authors (e.g. Ahlberg, 1989; Coates, 1994). Developmental evidence from the closely related genera *Acipenser* and *Polyodon* (both members of the most basal extant non-cladistian actinopterygian clade, the Chondrostei) lends some support to the hypothesis that the pelvic plate is at least partially metapterygial. In these fish, the proximal portion of the pelvic appendage

condenses from a series of fused basipterygial/proximal radial elements (Mabee & Noordsy, 2004; Hilton et al. 2011), forming a structure visually similar to the distal portion of the pelvic plate in *Polypterus*.

Developmental evidence that the entire pelvic plate is metapterygial is somewhat weaker. For example, there is no equivalent of the elongate proximal portion of the pelvic plate of *Polypterus* in *Acipenser*, merely a small proximal projection surrounded, in larger individuals, by perichondral bone (Hilton et al. 2011). Moreover, the pelvic adductor and abductor originate from the pelvic plate in *Polypterus* (Table 1), and the adductors and abductors in other gnathostome fishes, which are generally considered to be homologous among these taxa (e.g. Diogo & Abdala, 2010; Diogo & Ziermann, 2015), originate from the pelvic girdle (Tables 2 and 3). As Goodrich (1901) asks, 'if we are to believe that the girdle has disappeared and been functionally replaced by bones derived from an ingrowth of the already differentiated fin skeleton, we may well ask [...] during this important change what has become of the muscles attached to the girdle for the moving of the fin? Have they disappeared also, and been replaced by others, or have they shifted their base of attachment on to the basals?' While it is not uncommon for muscle attachments to change over the course of evolution, especially when a bony element is lost or reduced (e.g. Diogo & Abdala, 2010; Diogo et al. 2015), it seems more likely that the pelvic girdle in *Polypterus* persists as part or all of the pelvic plate.

In addition to the pelvic plate, the ancestral actinopterygian pelvic appendage is thought to have comprised two

series of radials, proximal and distal (Gardiner, 1984). This pattern is present in *Polypterus*: these elements seem to correspond to the 'middle' and 'distal' radials of *Acipenser* (Hilton et al. 2011) and *Polyodon* (Mabee & Noordsy, 2004). Teleosts have only one row of radials in the pelvic fin (Yamanoue et al. 2010).

Homology of pre- and postaxial pelvic muscles

Are the pre- and postaxial muscles in the pelvic fin of *Polypterus* homologous with similar muscles in other osteichthyans? Hypotheses of muscle homology ('primary homology' *sensu de Pinna, 1991*) generally refer to bony attachments, fiber orientation, innervation, and developmental origin (reviewed in Diogo & Abdala, 2010). In terms of attachments, the preaxial muscles are similar in all taxa we considered except *Polypterus* and possibly *Squalus*, while the postaxial muscles are similar in all taxa except *Squalus*, which lacks this muscle (Tables 2 and 3). Preaxial muscles originate from the pelvic girdle in all taxa except *Polypterus*, in which the muscle originates from the fascia of body wall muscles and the skin at the lateral edge of the fin-fold. However, as noted above, the origins of these muscles may have shifted with the loss or reduction of the pelvic girdle. The preaxial muscles insert onto the first preaxial radial or fin ray in all taxa except *Squalus*, where it inserts on the propterygium, which may or may not be homologous with the preaxial radials in osteichthyans. Postaxial muscles, where present, originate from the pelvic girdle in all taxa (in *Polypterus*, the muscle originates from a membrane connecting the left and right pelvic plates rather than from the skeletal portion of the girdle). These muscles insert on the postaxial radials and/or lepidotrichia. In *Neoceratodus*, the postaxial superficial ventrolateral and ventromesial adductors insert both on the postaxial radials and on the first axial element (metapterygium).

Structurally, the pre- and postaxial pelvic muscles are similar across fish taxa in that they are slender, parallel-fibered muscles without visible internal or external tendons. There are also some differences: the postaxial muscles in *Neoceratodus* are distinctive in having dual insertions, whereas teleosts have two preaxial pelvic muscles (arrector dorsalis pelvici and arrector ventralis pelvici).

Appendicular muscles in all gnathostomes begin as dorsal and ventral muscle masses, which subsequently divide (e.g. Diogo & Ziermann, 2015). Therefore, homologous muscles can be expected to arise from the same initial muscle mass and appear at similar stages of development. Among the species listed in Tables 2 and 3, direct evidence of developmental origin of pelvic appendicular muscles is available only for *Neoceratodus forsteri* (Boisvert et al. 2013). According to Boisvert et al. (2013) the preaxial muscle and one of the postaxial muscles in *Neoceratodus* are both dorsal and ventral, while the second postaxial muscle is ventral. However, in terms of their main adult topological divisions, the

pelvic preaxial and postaxial masses are consistently ventral and dorsal, respectively, in the taxa listed in Tables 2 and 3, supporting the hypothesis that they are homologous across gnathostomes. The major exception is the arrector dorsalis pelvici of teleosts, which is preaxial in position but usually lies mainly on the dorsal side of the pelvic appendage (Tables 2 and 3). Therefore, this muscle may derive via secondary differentiation from the preaxial fibers of the dorsal (adductor) muscle mass, or it may derive from the ventral (abductor) muscle mass and have secondarily migrated dorsally. In *Polypterus*, it was not possible to determine whether the preaxial muscle was part of the dorsal or ventral musculature. The postaxial muscle, because it is closely apposed to the abductor pelvici and lies on the ventral side of the fin, is probably part of the ventral musculature.

To test these hypotheses of homology ('secondary homology' *sensu de Pinna, 1991*) we would need to perform a comprehensive phylogenetic analysis. In a preliminary sense, based solely on the results and broader comparisons provided in the present work, the evolutionary pattern in the pelvic appendage appears to be very similar to that of the pectoral appendage. Namely, whereas pelvic preaxial muscles were present in all gnathostome groups in our sample, pelvic postaxial muscles are consistently found only in osteichthyans (Fig. 4). However, the architecture and attachments of these muscles in teleosts show enough differences from those of other gnathostomes that they are probably neomorphic. For example, teleosts have two preaxial muscles rather than one, and these muscles arise from the

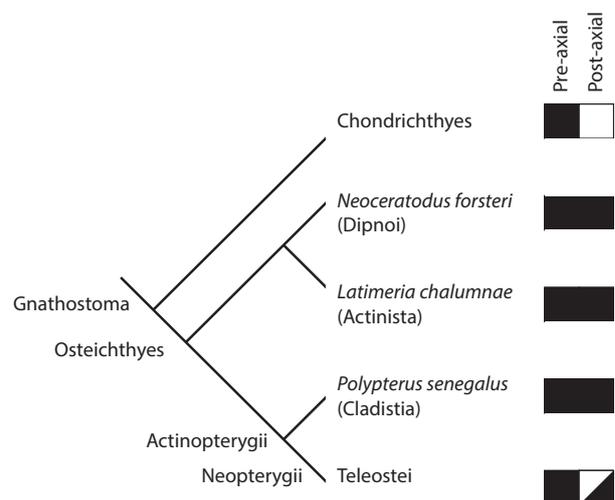


Fig. 4 Presence/absence of pre- and postaxial muscles in selected gnathostome groups mapped onto a simplified cladogram from Brinkmann et al. (2004) and Inoue et al. (2003). Black = present, white = absent, black/white = present in some taxa. Muscle information for *Polypterus* pelvic appendage was taken from the present study, and all other muscle information was taken from the literature (see text for references).

medial portion of the girdle and pass diagonally across the fin to reach the preaxial border (e.g. Winterbottom, 1973). Future detailed developmental comparative studies are needed to test these hypotheses. Specifically, these studies would show whether the adult topological positions of the preaxial and postaxial muscles listed in Tables 2 and 3 correspond to their developmental origins.

Pelvic fin muscle function and the water–land transition

Additional muscles such as the pterygialis cranialis pelvici and pterygialis caudalis pelvici may help to produce complex three-dimensional pelvic fin movements that aid locomotion. Although the locomotor function of pelvic fins during steady swimming is often considered to be limited (Standen, 2008), at least some fish employ active, dynamic, and/or three-dimensional movements of the pelvic fins during swimming. For example, the pelvic fin of *Onchorhynchus*, one of the teleosts that does possess the postaxial muscle extensor proprius (Winterbottom, 1973), performs both mediolateral and anteroposterior active movements during steady swimming (Standen, 2008). This muscle is thought to allow the fish to move downward (Stiassny & Moore, 1992). The pelvic fins of sarcopterygian fish often have unusually complex structures and functions. In addition to the muscles that cross the hip joint, the pelvic fin of the lungfish *Protopterus* has a series of ‘circumradial’ muscles that wrap around the axis of the fin (King & Hale, 2014). *Protopterus* is able to use its pelvic fins to produce tetrapod-like walking and bounding gaits during submerged locomotion, and these gaits involve fin movement in both the dorso-ventral and rostro-caudal planes (King et al. 2011). The pelvic fins of *Latimeria* ‘move in sync with the pectoral fins to help create thrust’ (Fricke et al. 1987). In neopterygians, the two preaxial muscles (arrector dorsalis and ventralis) are mostly related to movement of first fin ray (Diogo, 2004; Diogo & Abdala, 2010). This ray often becomes specialized; for example, in catfishes it is used for stridulation (sound production) and defense (Diogo & Vandewalle, 2003). In aquatic locomotion, the control of the leading – and to a lesser extent the trailing – edge of the fin is very important in hydrodynamics, together with the pitch and camber of the fin (Lauder & Drucker, 2004). It is likely that the pre- and postaxial muscles contribute to this control in fish such as *Polypterus*. Based on their attachments and fiber directions, the preaxial muscle may function to protract the fin and the postaxial muscle to retract the fin and abduct its trailing edge (Table 1). However, to date functional evidence has only been gathered for the preaxial pectoral muscle in sharks (Wilga & Lauder, 2004). During terrestrial locomotion of the mudskipper *Periophthalmus*, which has elaborate pelvic musculature, the preaxial muscle protractor pelvici is thought to play an important role in stabilizing the girdles and assisting in

vertical force production during ‘skipping,’ while the postaxial muscle levator pelvis raises the girdle off the ground (Harris, 1960). Terrestrial locomotion in *Polypterus* is mainly accomplished by movements of the pectoral fin and body (Standen et al. 2014), so it is unlikely that the pelvic fin muscles play a major role in this activity. Electromyography (EMG) studies of the pelvic pre- and postaxial muscles of *Polypterus* would be required to identify definitively the functions of these muscles and their roles in locomotion, including terrestrial locomotion.

Our observations also have implications for the early evolution of tetrapod limb musculature. The pelvic girdle and proximal fin elements in extant sarcopterygians are homologous with the pelvis, femur, tibia, and fibula in tetrapods, and these skeletal structures are very similar between lungfish and salamanders early in development (Boisvert et al. 2013). However, the transformation from pelvic fin to hindlimb in the ancestors of tetrapods is not nearly as well-known as that from pectoral fin to forelimb (Boisvert, 2005). If, as our analysis suggests, pre- and postaxial pelvic appendicular muscles are ancestral for osteichthyans, then the most recent common ancestor of extant sarcopterygians probably possessed these muscles as well, and they may be homologous with muscles or muscle groups in extant tetrapods.

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Author contributions

R.D. conceived the study. J.M., P.J., B.E., and R.D. acquired and analyzed the data. J.M. wrote the manuscript. P.J., B.E., and R.D. revised the manuscript.

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