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# Anatomy of the pectoral and forelimb muscles of wildtype and green fluorescent protein-transgenic axolotls and comparison with other tetrapods including humans: a basis for regenerative, evolutionary and developmental studies

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## Abstract

The axolotl *Ambystoma mexicanum* is one of the most used model organisms in evolutionary, developmental and regenerative studies, particularly because it can reconstitute a fully functional and complete forelimb/hindlimb. Surprisingly, there is no publication that describes all the pectoral and forelimb muscles of this species or provides a comparative framework between these muscles and those of other model organisms and of modern humans. In the present paper we describe and illustrate all these muscles in *A. mexicanum* and provide the first report about the myology of adults of a model organism that is based on analyses and dissections of both wildtype animals and transgenic animals that express green fluorescent protein (GFP) in muscle fibers. On the one hand, the inclusion of GFP-transgenic animals allows us to show the muscles as more commonly seen, and thus easier to understand, by current developmental and regenerative biologists. On the other hand, by including wildtype and GFP-transgenic animals and by visualizing these latter animals with and without a simultaneous transmission laser light, we were able to obtain a more complete and clearer understanding of the exact limit of the fleshy and tendinous parts of the muscles and their specific connections with the skeletal elements. This in turn allowed us to settle some controversies in previous anatomical and comparative studies. As most developmental, regenerative and evolutionary biologists are interested in comparing their observations of *A. mexicanum* with observations in other model organisms, and ultimately in using this information to increase the understanding of human evolution and medicine, we also provide tables showing the homologies between the pectoral and forelimb muscles of axolotls, of model organisms such as mice, frogs and chicken, and of *Homo sapiens*. An example illustrating the outcomes of using our methodology and of our observations is that they revealed that, contrary to what is often stated in the literature, *A. mexicanum* has a muscle coracoradialis that has both a well developed proximal fleshy belly and a distal long and thin tendon, supporting the idea that this muscle very likely corresponds to at least part of the amniote biceps brachii. Our observations also: (i) confirmed that the flexores digitorum minimi, interphalangeus digiti 3, pronator quadratus and palmaris profundus 1 are present as distinct muscles in *A. mexicanum*, supporting the idea that the latter muscle does not correspond to the pronator accessorius of reptiles; (ii) confirmed that the so-called extensor antebrachii radialis is present as a distinct muscle in this species and, importantly, indicated that this muscle corresponds to the supinator of other tetrapods; (iii) showed that, contrary to some other urodeles, including some other *Ambystoma* species, there is no distinct muscle epitrochleoanconeus in *A. mexicanum* and; (iv) showed that the ulnar and radial bundles of the abductor et extensor digiti 1 correspond to the abductor pollicis longus and extensor pollicis longus of other tetrapods, respectively.

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**Key words:** *Ambystoma*; anatomy; axolotl; evolution; homologies; model organisms; muscles; tetrapods; urodele amphibians.

## Introduction

The neotenuous axolotl *Ambystoma mexicanum* (Amphibia: Urodela) is one of the most used model organisms in evolutionary, developmental and regenerative studies, being a particularly powerful regenerative model because it can reconstitute a fully functional and complete forelimb/hindlimb (Kragl et al. 2009; see also the reviews of, e.g. Carlson, 2003, 2007; Nacu & Tanaka, 2011; Stocum & Cameron, 2011; Agata & Inoue, 2012). Amputation anywhere between the shoulder and the hand triggers the formation of a progenitor cell zone (blastema) that regenerates the epidermis, dermis, muscle, nerve, blood vessels and skeletal elements of the regenerated forelimb (e.g. Kragl et al. 2009).

It is therefore surprising that there is not a single paper or monograph that describes all the pectoral and forelimb muscles of *A. mexicanum* or provides a comparative framework between the muscles found in this species and those found in other tetrapod model organisms and in modern humans as an anatomical basis for regenerative, developmental and evolutionary works. A few myological studies have focused on other species of *Ambystoma* (e.g. Ribbing, 1907; Anthony & Vallois: *Ambystoma tigrinum*; Blount, 1935; : *Ambystoma punctatum*; Diogo, 2007; Diogo & Abdala, 2007, 2010; Diogo et al. 2009; Abdala & Diogo, 2010; : *Ambystoma ordinarium*), but to our knowledge the only published paper that describes in detail some of the forelimb muscles of *A. mexicanum* is that of Grim & Carlson (1974). Although that paper is undoubtedly an excellent piece of anatomical work and clearly contributed to knowledge about this species' myology, we felt the need to undertake the present study for four main reasons.

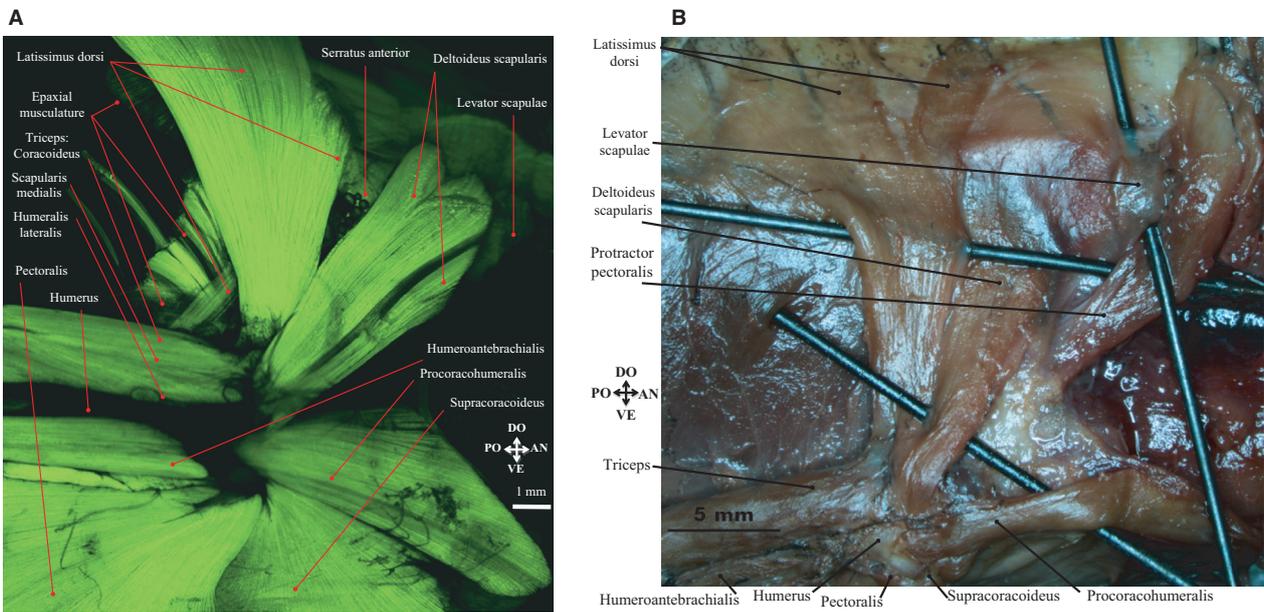
The first and more important reason for re-investigating the myology of *Ambystoma mexicanum* is that Grim & Carlson's study only analyzed the forearm and hand muscles; the justification being that their paper was mainly a basis for regenerative studies. However, as will be seen below, a great part of the current studies on axolotl regeneration also include at least the arm muscles, and even when the amputation is at the level of the arm, some muscles that are connected to the most medial portion of the pectoral girdle (e.g. coracoradialis) are also regenerated and anatomically affected after injury. Therefore, apart from their importance for evolutionary, developmental and comparative works (e.g. Diogo & Abdala, 2010), the arm and pectoral muscles are also crucial for regenerative studies. The second reason is that, as will also be explained below, there are some differences between our observations of the forearm and hand muscles of *A. mexicanum* and the descriptions of Grim & Carlson (1974). This is related to a third reason, which is that our study provides, for the first time, a report

about the myology of adults of a model organism that is based on dissections and analyses of both wildtype animals and transgenic animals that express green fluorescent protein (GFP) in muscle fibers (Fig. 1A,B; see Materials and methods). One of the main purposes of including GFP-transgenic animals in the study is that this allows us to show the muscles in photographs and in a way that is more commonly seen and easier to understand for developmental and regenerative biologists that are less accustomed to the type of anatomical drawings (which are excellent) provided by Grim & Carlson (1974). The other, related, main purpose is that by including both wildtype and GFP-transgenic animals and visualizing the latter animals with and without a simultaneous transmission laser light, one can obtain a more complete and clearer understanding of the exact limit of the fleshy (shown as fluorescent green in GFPs) and tendinous (examined in dissections of both wildtypes and GFPs and/or using the transmission light) parts of the muscles and their specific connections with the skeletal elements (Fig. 2A,B). It is precisely the use of these different methods that allowed us to settle some controversies seen in previous anatomical and comparative studies and revealed some of the differences with the anatomical descriptions of the adult myology reported by Grim & Carlson (1974; see below). The fourth reason is that most developmental, regenerative and evolutionary biologists that work with *A. mexicanum* are interested in comparing their observations with those made in other model organisms, and ultimately to discuss that information in the context of human evolution and medicine. Therefore, we felt the need to provide detailed tables showing the muscle homologies between axolotls and other vertebrates, including model organisms such as mice, frogs and chicken as well as our own species, *Homo sapiens*.

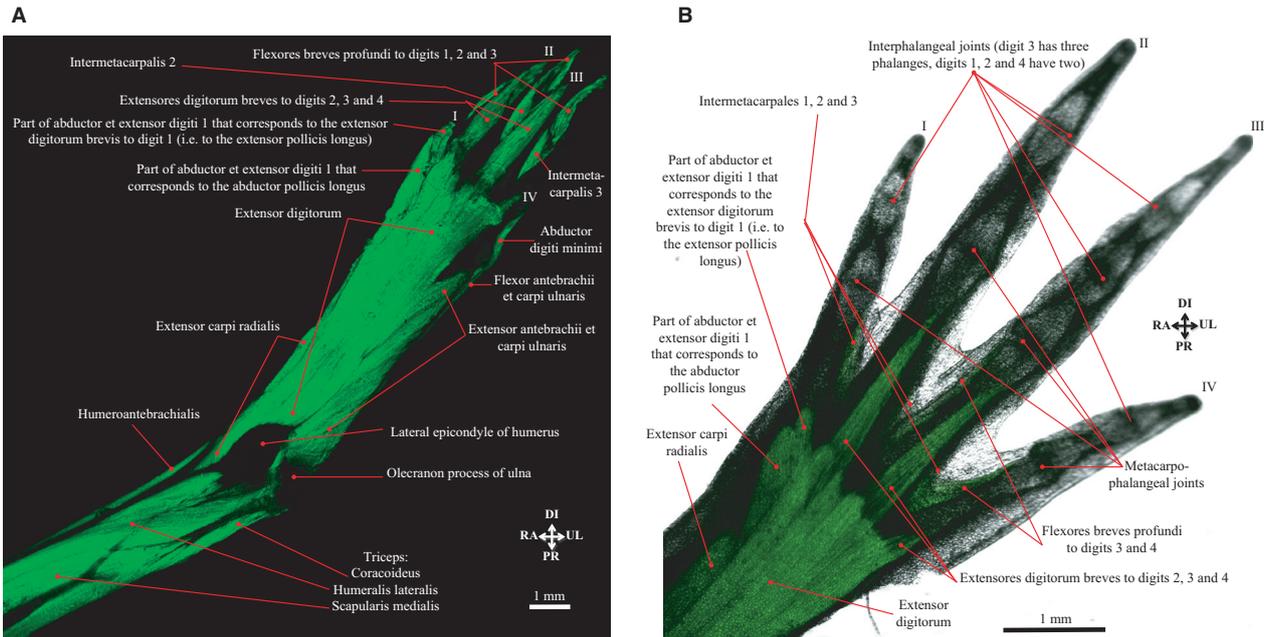
The main goal of this paper is therefore to establish a basis for future evolutionary and particularly regenerative and developmental studies on the forelimb of axolotls and other tetrapods, by (i) providing brief, simple descriptions of each pectoral and forelimb muscle of *A. mexicanum* that can be understood by both anatomists and non-anatomists, in particular with the aid of photographs of GFP-transgenic animals and (ii) providing a succinct comparison between our observations and those of other authors and a short, updated discussion and tables of the muscle homologies between this species and other tetrapod taxa, including model organisms and modern humans.

## Materials and methods

A total of 17 *Ambystoma mexicanum* forelimbs were examined for this study. Except for two adult wildtype specimens (HU AM1, right



**Fig. 1** (A) Lateral view of the left pectoral and arm muscles of an adult GFP-transgenic axolotl (CRTD AM11); the protractor pectoralis (not a pectoral or a forelimb muscle) was removed (horizontal flop automatically done with the LAS-AF software, v 2.6). (B) Same view of the right pectoral and arm muscles of an adult wildtype axolotl. In this figure and the next figures, AN, PO, DO, VE, RA, UL, DI and PR means anterior, posterior, dorsal, ventral, radial, ulnar, distal and proximal, respectively.



**Fig. 2** (A) Dorsal view of the forelimb muscles of the left limb of an adult GFP-transgenic axolotl (CRTD AM11; horizontal flop automatically done with the LAS-AF software, v 2.6). (B) Same view of the forearm and hand muscles of the right limb of a 3-month-old GFP-transgenic axolotl (CRTD AM11) but also using simultaneously transmitted laser light, a method that the authors used to analyze the relationship between muscles and skeletal structures in the GFP transgenic animals, together with manual dissections (see Materials and methods). In this figure and the next Figures, 1, 2, 3 and 4 means digits 1, 2, 3 and 4, respectively.

forelimb examined; HU AM2, both forelimbs examined) that were from the Anatomy Department of Howard University, all the *A. mexicanum* specimens analyzed and dissected were from the CRTD (Center for Regenerative Therapies Dresden), including an adult

wildtype (CRTD AM0, both forelimbs examined) and 11 transgenic animals that express GFP in muscle fibers (Homo-Cardiac-Alpha-Actin: CRTD AM11 to 20, adults, left forelimb examined; CRTD AM10, 3 months' post-fertilization, both forelimbs examined). No

animal was purposely sacrificed for this anatomical study: the two forelimbs of CRTD AM10 were amputated for regenerative studies and thus the original limbs were fixed in 4% paraformaldehyde (PFA), while the other animals were examined just after being sacrificed for reasons related to research/work of other individuals (e.g. colony keeping, experiments). The imaging of the GFP-transgenic animals was performed with a Leica TCS LSI confocal microscope at the Light Microscopy Facility of the CRTD-BIOTEC. Images were acquired using a  $\times$  zoom objective, the GFP fluorescence being excited with the 488-nm laser line and fluorescence being collected between 500 and 520 nm with the standard PMT. To check the relationship between muscle fibers, tendons and skeletal structures in the GFP transgenic animals we not only undertook manual dissections but also used simultaneously transmitted laser light that was detected with a T-PMT to create a transmitted light image (e.g. Fig. 2B, compare with Fig. 2A); overlay of both channels was created using LAS-AF software (v 2.6). The nomenclature of the muscles mainly follows Diogo & Abdala (2010; see also Diogo et al. 2009 and Abdala & Diogo, 2010); so, for example, hypobranchial muscles such as the omohyoideus and branchial muscles such as the trapezius are not considered in the present paper. When we refer to the anterior, posterior, dorsal and ventral regions of the body, we do so in the sense the terms are used for pronograde tetrapods (e.g. the forelimb is anterior to the hindlimb, and in each limb the extensor muscles are dorsal to the flexor muscles). It should be noted that, apart from *A. mexicanum*, we have dissected, in the past, specimens from all the major groups of tetrapod and of non-tetrapod vertebrates; a list of all the dissected specimens is given in Diogo & Abdala (2010).

## Results

The results of our anatomical analyses are summarized in Tables 1 and 2 shown in Figs 1–7. Therefore in this section we will provide a brief description of all the pectoral and forelimb muscles of *A. mexicanum* based on these analyses, following the order shown in Tables 1 and 2.

### Pectoral muscles derived from the postcranial axial musculature

The *serratus anterior* (Fig. 1) runs from the epaxial musculature to the dorsal and mesial portion of the suprascapular cartilage, some of its fibers being continuous with those of the levator scapulae Table 1. The *levator scapulae* (or 'opercularis'; Fig. 1) runs from the region of the operculum to the suprascapular cartilage, being continuous with the origin of the deltoideus scapularis.

### Appendicular muscles of the pectoral girdle and arm

The *pectoralis* (Fig. 1) originates from the coracoid near the midline (it does not meet its counterpart at the midline) and from the rectus abdominis and sternum, and inserts onto the proximal portion of the humerus Table 1. The *supracoracoideus* (or 'coracohumeralis'; Figs 1 and 4) runs from the superficial ventral surface of the coracoid to the proximal portion of humerus, near the insertion of the

pectoralis, the posterior portion of the former muscle being deep (dorsal) to the anterior portion of the latter muscle. The *deltoideus scapularis* (or 'dorsalis scapulae'; Fig. 1) extends from the lateral and dorsal surfaces of the suprascapular cartilage to the ventral margin of the proximal portion of the humerus. The *procoracohumeralis* (or 'procoracohumeralis longus'; Figs 1 and 4) runs from the procoracoid cartilage to the proximal portion of the humerus; the 'procoracohumeralis brevis' *sensu*, e.g. Romer (1922, 1924, 1944) and Howell (1935) is not present as a distinct muscle. The *subcoracoscapularis* (or 'scapulohumeralis brevis'; Fig. 3) extends from the anterior portion of the medial surface of the scapular base (the muscle thus cannot be seen in a lateral view of the pectoral girdle) to a prominent medial process of the humerus at about one-third of the proximodistal length of this bone. The *latissimus dorsi* (Fig. 1) is divided into a broader, posterior bundle and a smaller, anterior bundle, which run from the dorsal fascia and insert onto the proximal portion of the humerus. The *triceps brachii* (sometimes erroneously designated as 'anconeus'; Figs 1, 2 and 7) is divided into four bundles, all being blended distally to insert onto the olecranon process of the ulna: the triceps coracoideus originates from a prominent posterior process of the coracoid and is medial to the triceps scapularis medialis, which originates from the scapula and from connective tissue surrounding the shoulder joint capsule (both these bundles are superficial to the triceps humeralis lateralis and triceps humeralis medialis); the triceps humeralis lateralis originates from the lateral surface of the proximal portion of the humerus, while the triceps humeralis medialis originates from about one-third of the proximo-distal length of the humerus. The *humeroantebrachialis* (or 'brachialis'; Figs 1–5) connects the proximal portions of the humerus and radius. The *coracoradialis* (or 'sterno-radialis'; Fig. 4) has a well developed fleshy belly that originates from the ventral surface of the coracoid deep (dorsal) to the superficial (ventral) layers of the pectoralis and supracoracoideus and that gives rise to a long and thin tendon. This tendon is proximally indirectly connected to the proximal humerus by tendinous bands and by the attachments of the superficial pectoral muscles, and then extends distally to insert onto the proximal portion of the radius just next to the insertion of the humeroantebrachialis. The *coracobrachialis* (Figs 3 and 4) is differentiated into two bundles that originate from the posteroventral and posterolateral margin of the coracoid: the coracobrachialis longus inserts onto the distal humerus and the elbow joint, while the coracobrachialis brevis inserts onto the medial side of approximately the proximal one-third of the humerus.

### Appendicular muscles of the ventral forearm

The *palmaris profundus 1* (or 'pronator profundus'; Figs 5 and 6) is deep to the contrahentium caput longum and runs

**Table 1** Scheme illustrating the hypotheses regarding the homologies of the pectoral and arm muscles of adults of *Ambystoma mexicanum* and representative tetrapod taxa, including model organisms such as frogs, chickens and rat, as well as modern humans; in both this table and Table 2 the cells highlighted in grey are those that differ from the tables provided in Diogo et al. (2009), Diogo & Abdala (2010) and Abdala & Diogo (2010) – see text for more details. The nomenclature of the muscles follows that used in the text. The black arrows indicate the hypotheses that are most strongly supported by the evidence available; the grey arrows indicate alternative hypotheses that are supported by some data, but overall are not as strongly supported by the evidence as are the hypotheses indicated by the black arrows. Bas., Basiscapularis; Pan. car., Panniculus carnosus; sup., superioris; ur., urodeles.

<i>Ambystoma mexicanum</i>	<i>Bufo arenarum</i>	<i>Timon lepidus</i>	<i>Gallus domesticus</i>	<i>Rattus norvegicus</i>	<i>Homo sapiens</i>
<b>Axial: Pectoral Girdle</b>					
Serratus anterior	Serratus anterior	Serratus anterior	Serratus anterior	Serratus anterior	Serratus anterior
–	→ 'Rhomboideus'	–	'Rhomboideus'	Rhomboideus major + minor + occipitalis	Rhomboideus major + minor
Levator scapulae	Levator scapulae sup.	Levator scapulae	–	Levator scapulae + Levator clavicularae	Levator scapulae
–	→ Opercularis	–	–	–	–
–	–	Sternocoracoideus	Sternocoracoideus	Subclavius	Subclavius
–	–	Costocoracoideus	–	–	–
<b>Appendicular: Pectoral Girdle and Arm</b>					
Pectoralis	Pectoralis	Pectoralis	Pectoralis	Pectoralis major + minor + Pan. Car. (part)	Pectoralis major + minor
Supracoracoideus	Supracoracoideus	Supracoracoideus	Supracoracoideus	Infraspinatus + Supraspinatus	Infraspinatus + Supraspinatus
Deltoideus scapularis	Deltoideus scapularis	Deltoideus scapularis	Deltoideus scapularis	Deltoideus scapularis + Teres minor	Deltoideus (part) + Teres minor
–	–	–	→ Scapulo-humeralis posterior	–	–
Procoracohumeralis	Procoracohumeralis	Deltoideus clavicularis	Deltoideus clavicularis	Deltoideus clavicularis + acromialis	Deltoideus (part)
–	–	–	→ Humeroradialis	–	–
–	–	Scapulo-humeralis anterior	Scapulo-humeralis anterior	–	–
Subcoracoscapularis	Subcoracoscapularis	Subcoracoscapularis	Subcoracoscapularis	Subscapularis + Teres major	Subscapularis + Teres major
Latissimus dorsi	Latissimus dorsi	Latissimus dorsi	Latissimus dorsi	Latissimus dorsi	Latissimus dorsi
Triceps brachii	Triceps brachii	Triceps brachii	Triceps brachii	Triceps brachii + Dorsoepitrochlearis	Triceps brachii
Humeroantibrachialis	Humeroantibrachialis	Brachialis	Brachialis	Brachialis	Brachialis
Coracoradialis	Coracoradialis	–	–	–	–
–	–	→ Biceps brachii	Biceps brachii	Biceps brachii	Biceps brachii
Coracobrachialis	Coracobrachialis	→ Coracobrachialis	Coracobrachialis	Coracobrachialis	Coracobrachialis

**Table 2** Scheme illustrating the hypotheses regarding the homologies of the forearm and hand muscles of adults of *Ambystoma mexicanum* and representative tetrapod taxa, including model organisms such as frogs, chickens and rat, as well as modern humans (see caption of Table 1).

<i>Ambystoma mexicanum</i>	<i>Bufo arenarum</i>	<i>Timon lepidus</i>	<i>Gallus domesticus</i>	<i>Rattus norvegicus</i>	<i>Homo sapiens</i>
<b>Appendicular: Ventral Forearm</b>					
Palmaris profundus 1	—	—	—	—	—
Pronator quadratus	Pronator quadratus	Pronator quadratus Pronator accessorius	Pronator quadratus	Pronator quadratus	Pronator quadratus
—	—	—	—	—	—
Contraheptium caput longum	Contraheptium caput longum	—	—	—	—
Flexor accessorius lateralis	Flexor accessorius	—	—	—	—
Flexor accessorius medialis	—	—	—	—	—
Flexor digitorum communis	Flexor digitorum communis	Flexor digitorum longus	Flexor digitorum longus	Flexor digitorum pr. + sup. (part)	Flexor digitorum pr. + sup. (part) + Fl. p. l.
—	—	— (but 'Pa.l.' found in some lep.)	—	Palmaris longus	Palmaris longus
Flexor an. et carpi ulnaris	Flexor carpi ulnaris	Flexor carpi ulnaris	Flexor carpi ulnaris	Flexor carpi ulnaris	Flexor carpi ulnaris
—	Epitrochleoanconeus	Epitrochleoanconeus	Epitrochleoanconeus	Epitrochleoanconeus	—
Flexor an. et carpi radialis	Flexor carpi radialis	Flexor carpi radialis	Flexor carpi radialis	Flexor carpi radialis	Flexor carpi radialis
—	Pronator teres	Pronator teres	Pronator teres	Pronator teres	Pronator teres
<b>Appendicular: Ventral and Dorsal Hand</b>					
Flexores breves superficiales	Flexores breves superficiales	Flexores breves superficiales	Flexores breves superficiales	Flexor digitorum sup. (part) + Pa.br.	Flexor digitorum sup. (part) + Pa.br.
— (but Lumb. found in other ur.)	Lumbricales	Lumbricales	—	Lumbricales	Lumbricales
Contraheptes digitorum	Contraheptes digitorum	Contraheptes digitorum	Contraheptes digitorum	Contraheptes digitorum + Adductor pollicis	Adductor pollicis + 'Volaris primus of Henle'
Flexores breves profundi	Flexores breves profundi	Flexores breves profundi	Flexores breves profundi	Flexores breves profundi + Flexor po.br. + Flexor di.mi.br. + Op.di.mi.	Flexor brevis profundus 2 + Flexor po.br. + Op.pol. + Flexor di.mi.br. + Op.di.mi. + Interossei (part)
Flexores digitorum minimi	—	—	—	—	—
Interphalangeus digiti 3	—	—	—	—	—
—	Abductor pollicis brevis	Abductor pollicis brevis	Abductor pollicis brevis	Abductor pollicis brevis	Abductor pollicis brevis
Abductor digiti minimi	Abductor digiti minimi	Abductor digiti minimi	Abductor digiti minimi	Abductor digiti minimi	Abductor digiti minimi
Intermetacarpales	Intermetacarpales	Intermetacarpales	Intermetacarpales	Intermetacarpales	Interossei (part)
—	Dorsometacarpales	Dorsometacarpales	Dorsometacarpales	—	—

Table 2 (continued)

<i>Ambystoma mexicanum</i>	<i>Bufo arenarum</i>	<i>Timon lepidus</i>	<i>Gallus domesticus</i>	<i>Rattus norvegicus</i>	<i>Homo sapiens</i>
Appendicular: Dorsal Forearm					
Extensor carpi radialis	Extensor an. et carpi radialis	Extensor an. et carpi radialis	Extensor an. et carpi radialis	Extensor carpi radialis lo. + br. + Brachior.	Extensor carpi radialis lo. + br. + Brachior.
Supinator	–	Supinator	Supinator	Supinator	Supinator
Extensor an. et carpi ulnaris	Extensor carpi ulnaris	Extensor an. et carpi ulnaris	Extensor carpi ulnaris	Extensor carpi ulnaris	Extensor carpi ulnaris
– (but Anc. found in some ur.)	Anconeus	– (but Anc. found in some lep.)	Anconeus	Anconeus	Anconeus
Extensor digitorum	Extensor digitorum	Extensor digitorum	Extensor digitorum	Extensor digitorum	Extensor digitorum
Extensores digitorum breves	Extensores digitorum breves	Extensores digitorum breves	Extensores digitorum breves	Ex. d. m. + Ex. d. q. + Ex. i. + Ex. p. l.	Ex. d. m. + Ex. i. + Ex. p. l.
Abductor et extensor digiti 1	Abductor pollicis longus	Abductor pollicis longus	Abductor pollicis longus	Abductor pollicis longus	Abductor pollicis longus + Ex. p. b.

an., antebrachii; Anc., Anconeus; Brachior., brachioradialis; br., brevis; di. mi. br., digiti minimi brevis; Ex. d. m., Extensor digiti minimi; Ex. d. q., Extensor digiti quarti; Ex. i., Extensor indicis; Ex. p. b., Extensor pollicis brevis; Ex. p. l., Extensor pollicis longus; Fl. p. l., Flexor pollicis longus; lep., lepidosaurs; Lumb., lepidosaurs; Op. di. mi., Opponens digiti minimi; Pa. br., palmaris brevis; Pa. l., palmaris longus; po. br., pollicis brevis; pol., pollicis; pr., profundus; sup., superficialis; ur., urodeles.

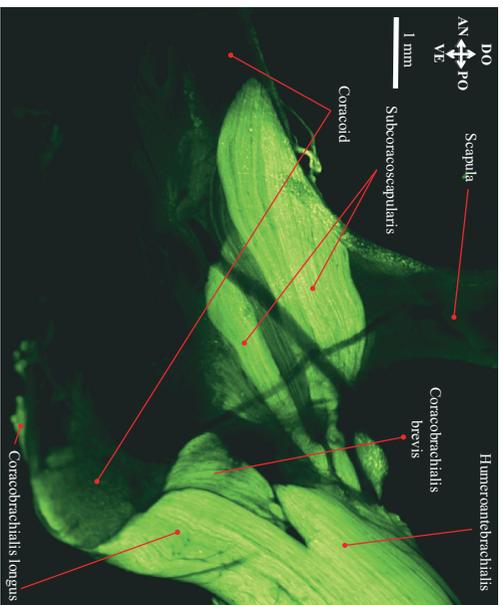


Fig. 3 Medial view of the left pectoral and arm muscles of an adult GFP-transgenic axolotl (CRTD AM11) after removing all the pectoral muscles except the subcoracoscapularis (horizontal flop automatically done with the LAS-AF software, v 2.6).

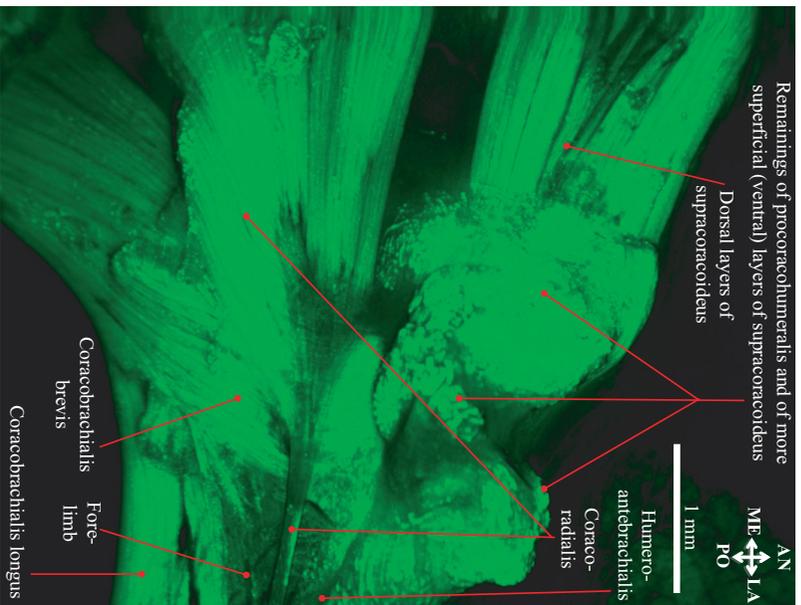
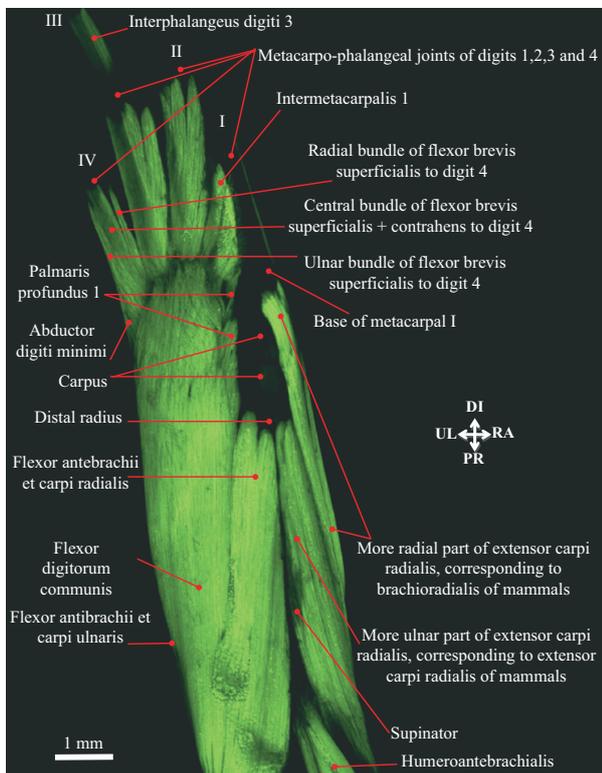


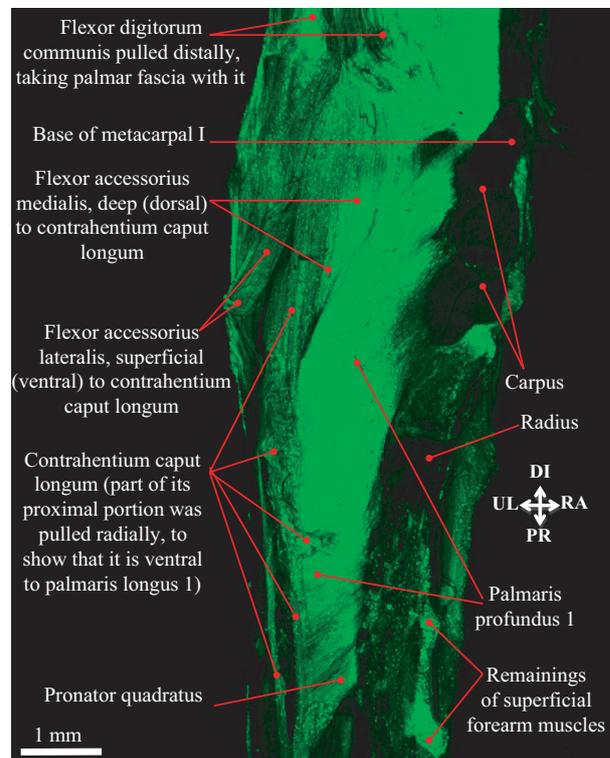
Fig. 4 Ventral view of the left pectoral and arm muscles of an adult GFP-transgenic axolotl (CRTD AM11) after removing the most superficial (ventral) layers of the pectoralis and of the supratoracoides in order to show fleshy belly and tendon of the coracobrachialis.

from the ulna, intermedium and ulnare to the carpus and base of metacarpal I (Table 2). The pronator quadratus (or 'interosseous antebrachii'; Fig. 6) lies deep to the palmaris



**Fig. 5** Ventral view of the forearm and hand muscles of the left limb of an adult GFP-transgenic axolotl (CRTD AM11; horizontal flop automatically done with the LAS-AF software, v 2.6).

profundus 1 and thus to the other ventral forearm muscles, running from the ulna to the radius, extending a little bit more proximally than the proximal extremity of the palmaris profundus 1. The *contrahentium caput longum* (or 'ulnocarpalis'; Fig. 6) lies deep (dorsal) to the flexor antebrachii et carpi ulnaris and to the flexor digitorum communis and originates from the proximal portion of the ulna and inserts onto the carpus through a tendon, being continuous with the contrahentes digitorum. The *flexor accessorius lateralis* (or 'palmaris profundus 3'; Fig. 6) extends from the distal portion of the ulna and the ulnare to the dorsal surface of the palmar fascia, passing superficially (ventrally) to the *contrahentium caput longum*. The *flexor accessorius medialis* (or 'palmaris profundus 2'; Fig. 6) connects the distal portion of the ulna and the intermedium to the dorsal surface of the palmar fascia, originating deep (dorsal) to the *contrahentium caput longum*. The *flexor digitorum communis* (or 'palmaris superficialis'; Figs 5 and 6) is the most superficial (ventral) muscle of the ventral forearm, originating from the medial epicondyle of the humerus, being connected to the palmar fascia, and sending a thin tendon to the distal phalanx of each of the four digits. The *flexor antebrachii et carpi ulnaris* (Figs 2, 5 and 7) lies on the ulnar side of, and is partially covered ventrally by, the flexor digitorum communis, running from the med-

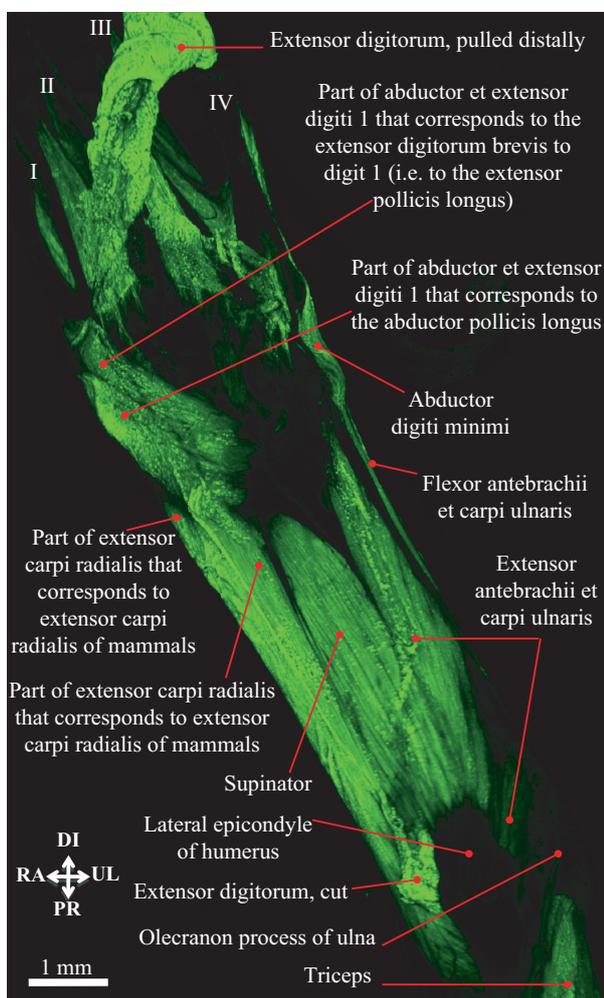


**Fig. 6** Ventral view of the deeper forearm muscles of the left limb of an adult GFP-transgenic axolotl (CRTD AM15; horizontal flop automatically done with the LAS-AF software, v 2.6) after removing the flexor antebrachii et carpi radialis, the flexor digitorum communis, and the flexor antebrachii et carpi ulnaris.

ial epicondyle of the humerus to the ulna and ulnare. The *flexor antebrachii et carpi radialis* (Fig. 5) is divided into a longer, more superficial (ventral) and proximo-distally oriented bundle that runs from the medial epicondyle of the humerus to the radius and radiale and that very likely corresponds to the flexor carpi radialis of other tetrapods, and a shorter, deeper (dorsal) and more oblique bundle that runs from the medial epicondyle of the humerus to about half of the length of the radius, but which instead of inserting onto the radius directly, is mainly associated distally with the longer bundle. That is why the shorter bundle cannot be considered to be a distinct muscle pronator teres, i.e. it likely corresponds to the pronator teres of other tetrapods (see Table 2 and Discussion) but is not present as a separate, distinct muscle in *A. mexicanum*.

### Appendicular muscles of the hand

The *flexores breves superficiales* (Fig. 5) are the most ventral intrinsic muscles of the hand, running from the aponeurosis and tendons of the flexor digitorum communis and the palmar fascia to the metacarpophalangeal joint of each digit; all digits receive an ulnar, a radial and a central bundle from these muscles, the latter bundle being blended



**Fig. 7** Dorsal view of the deeper forearm muscles of the left limb of an adult GFP-transgenic axolotl (CRTD AM14; horizontal flop automatically done with the LAS-AF software, v 2.6) after removing the extensor digitorum.

with the *contrahens* going to the same digit (Table 2). The *contrahentes digitorum* (or 'flexores breves medii'; Fig. 5) constitute the second layer of intrinsic hand muscles and run from the carpus (being deep – dorsal – to, but also somewhat associated with, the tendons of the flexor digitorum communis) to the metacarpophalangeal joints of digits 1, 2, 3 and 4. The *flexores breves profundi* (Fig. 2) form the third layer, running from the carpals at the base of their respective digits to the distal portion of the metacarpals of digits 1, 2, 3 and 4, being mainly attached to the ulnar and radial sides of each metacarpal. The *flexores digitorum minimi* are two short, thin muscles that extend from the central part of the ventral surface of metacarpals I and IV to the metacarpophalangeal joints of digits 1 and 4, respectively, together with the flexores breves superficiales and the *contrahentes digitorum*. The *interphalangeus digiti 3* (Fig. 5) runs from the ventral margin of the base of the proximal phalanx of digit 3 to the proximal interphalangeal joint of

this digit. The *abductor digiti minimi* (or 'extensor lateralis digiti IV'; Figs 2, 5 and 7) extends from the ulnare to the ulnar side of the base of metacarpal IV, being blended proximally to the tendon of the flexor carpi ulnaris and distally to the ulnar bundle of the flexor brevis profundus of digit 4. There are three *intermetacarpales* (Figs 2 and 5), which run distally and radially from the radial sides of metacarpals II, III and IV to the ulnar sides of metacarpals I, II and IV, respectively.

### Appendicular muscles of the dorsal forearm

The *extensor carpi radialis* (Figs 2, 5 and 7) is well differentiated from the supinator, being in turn divided into two bundles: the more radial one runs from the distal one-half of the radial side of the humerus to the radial side of the distal radius, carpus, and base of metacarpal I, and very likely corresponds to the brachioradialis of mammals; the more ulnar one runs from the lateral epicondyle of the humerus to the radial side of the distal radius and radiale and very likely corresponds to the extensor carpi radialis of mammals (see Table 2 and Discussion). The *supinator* (or 'extensor antebrachii radialis'; Figs 5 and 7) originates from the lateral epicondyle of the humerus and is distally separated from the extensor carpi radialis by the whole ventral side of the radius, because it inserts onto the medial surface of the distal one-third of the radius (Table 2). The *extensor antebrachii et carpi ulnaris* (Figs 2 and 7) extends from the lateral epicondyle of the humerus to the ulna and ulnare; the antebrachial part of this muscle can be recognized because there are a few fibers that go almost transversally from the lateral epicondyle of the humerus to the proximal portion of the ulna, but distally these fibers are blended and continuous with the main body of the muscle and thus do not form a separate, distinct anconeus (see Table 2 and Discussion). The *extensor digitorum* (or 'humero-dorsalis'/'humero-metacarpalis'; Figs 2, 5 and 7) is the most superficial (dorsal) of the dorsal forearm muscles, originating from the lateral epicondyle of the humerus and sending thin tendons to the metacarpals I, II, III and IV. There are three *extensores digitorum breves* (Fig. 2), which lie deep (ventral) to the main body of the extensor digitorum, originate from carpal bones, and send thin tendons to the distal phalanx of digits 2, 3 and 4; the fleshy part of the extensor digitorum brevis to digit 4 extends only to the proximal portion of the respective metacarpal, whereas the fleshy parts of the *extensores digitorum breves* to digits 2 and 3 almost reach the distal portion of the respective metacarpals. The *abductor et extensor digiti 1* (or 'supinator manus'; Figs 2 and 7) originates from carpal bones and from the distal portions of the radius and ulna, and has two bundles: the most radial one inserts onto the radial side of the base of metacarpal I and very likely corresponds to the abductor pollicis longus of other tetrapods; the most ulnar one sends a thin tendon to the distal phalanx of digit 1 and thus very likely corresponds

to the extensor pollicis longus of other tetrapods (see Table 2 and Discussion).

## Discussion

In this section we will first provide a brief comparison between our observations of *A. mexicanum* and those provided in the most relevant previous myological studies about this and other species of *Ambystoma*, and then provide a short, updated discussion of the muscle homologies with other tetrapods, including *H. sapiens* and model organisms such as frogs, chickens and rats.

The descriptions of the forearm and hand muscles of *A. mexicanum* provided by Grim & Carlson (1974) are supported overall by our observations but there are some differences that are worth mentioning. For instance, Grim & Carlson state that the fibers of hand muscles such as the flexores breves superficiales and the flexores digitorum minimi extend to the proximal phalanges. However, our dissections of wildtype animals and particularly the dissections of, and use of, simultaneous transmitted laser light to examine the GFP animals revealed that no forelimb muscle other than the interphalangeus digit 3 has fleshy fibers extending distally to the metacarpophalangeal joint (Figs 2B and 5). In this specific aspect, our observations are thus more similar to those of Blount (1935) in *A. punctatum*, who states that the flexores breves superficiales only reach, distally, the metacarpophalangeal joints. Grim & Carlson also state that the extensores digitorum breves to digits 2 and 3 go only halfway of the metacarpals, but with the techniques used in the present study one can clearly see that these muscles actually reach the distal portion of the metacarpals (Fig. 2B). A third difference is that Grim & Carlson state that the extensor carpi radialis inserts onto the radiale and radius, but our analyses reveal that the most lateral bundle of this muscle extends, distally, all the way to the base of metacarpal I (Fig. 5). Similarly, Grim & Carlson state that the flexor antebrachii et carpi ulnaris runs from the medial epicondyle of the humerus to the ulnare, but our analyses revealed that the muscle also inserts onto the ulna, also supporting the observations of Blount (1935) in *A. punctatum*. Lastly, the report of Grim & Carlson is confusing because their figures and description suggest that it is the radial bundle of the abductor et extensor digiti 1 that sends a tendon to the distal phalanx, but our study pointed out that it is instead the ulnar bundle that gives rise to this tendon (this also makes more sense in a phylogenetic context, because this ulnar bundle corresponds to the extensor pollicis longus of other tetrapods: see Table 2 and below).

There are some minor differences concerning the descriptions of *A. punctatum* by Blount (1935) and our observations of *A. mexicanum*, but these may well reflect interspecific differences. For instance, Blount states that the coracobrachialis longus inserts onto the distal portion of

the humerus and that the palmaris profundus 1 only inserts onto metacarpal I, but both our analyses and the study of Grim & Carlson (1974) point out that in *A. mexicanum* there is an insertion of the former muscle onto the humerus and elbow joint, and of the latter muscle onto the carpus and base of metacarpal I. Similarly, Blount states that the extensor carpi radialis inserts onto the radius and radiale, but in our *A. mexicanum* specimens the muscle also extends to the base of metacarpal I. Three muscles are present in the specimens of *A. mexicanum* analyzed by us and by Grim & Carlson (1974) that were not reported by Blount in *A. punctatum*: the two flexores digitorum minimi, which are very small and deep intrinsic muscles of the hand that were also reported by Ribbing (1907) in *A. tigrinum*, and the supinator, which in various urodele taxa is not differentiated from the extensor carpi radialis, forming the extensor antebrachii et carpi ulnaris (e.g. Ribbing, 1907; Diogo & Abdala, 2010; Abdala & Diogo, 2010). However, it should be noted that Ribbing (1907) did not describe the abductor digiti minimi in *A. tigrinum* and other urodeles, although this muscle was found in the present work and has been consistently found in urodeles by other authors, including McMurrich (1903), Blount (1935), Grim & Carlson (1974), Diogo & Abdala (2010) and Abdala & Diogo (2010). An important discrepancy between our analyses of *A. mexicanum* and the descriptions of *A. punctatum* provided by Blount (1935) and that is very likely *not* due to an interspecific difference is that Blount does not refer to a coracoradialis; this issue will be discussed in the paragraphs below, which concern the comparative anatomy and homologies of the pectoral and forelimb muscles of axolotls and other tetrapods.

Previous work done by the first author of the present paper and other colleagues have provided a detailed discussion of the homologies between urodeles and other tetrapods, mainly based on the newt *Taricha torosa* and on *Ambystoma ordinarium* (Diogo et al. 2009; Diogo & Abdala, 2010; Abdala & Diogo, 2010). The hypotheses proposed in those works regarding the homologies and evolution of a muscle were based on a detailed analysis of all the lines of evidence either obtained from dissections of wildtype animals or gleaned from the literature (e.g. innervation, relation with other muscular structures, relation with hard tissues, configuration/orientation of the fibers, development, function, phylogeny, presence/absence/configuration in fossils, etc.). Therefore, for the purpose of this paper (see Introduction), we will mainly summarize the homologies between axolotls and other tetrapods in Tables 1 and 2 and in the text will thus focus our attention on the cases highlighted in grey in these Tables, which are the ones that differ from the data provided in the previous work, because (i) the present study is based on observations of *A. mexicanum*, and not *A. ordinarium*; (ii) the new information reported in the present paper is based on our observations of both wildtype and GFP-transgenic axolotls, which allow

us to provide a more complete and updated comparison with other tetrapods and discussion of the tetrapod muscle homologies; and also (iii) there is a needed correction of a typographic error, i.e. in the printed tables of those previous works the abductor digiti minimi was listed in birds and not in crocodylians, whereas their texts explained that this muscle is present in the latter and not in the former.

One of the main differences between Tables 1 and 2 and the tables provided by Diogo & Abdala (2010) and Abdala & Diogo (2010) is that the latter authors did not report a true pronator quadratus in *A. punctatum* and thus named the palmaris profundus 1 as 'pronator quadratus'. The palmaris profundus 1 (or 'pronator profundus') and the pronator quadratus (or 'interosseous antebrachii') derive from the same anlage, and both are often present as distinct structures in urodeles (e.g. McMurrich, 1903; Ribbing, 1907; Blount, 1935; Straus, 1942; Grim & Carlson, 1974; present work). According to authors such as Straus (1942) the two muscles are never differentiated in anurans; Ribbing (1907) states that the 'abductor pollicis' muscle that Gaupp (1896) reported in anurans corresponds in fact to the palmaris profundus 1, and not to the abductor pollicis brevis, *sensu* the present work (Table 2). According to McMurrich (1903) and Straus (1942), a different muscle derived from the same anlage, the pronator accessorius, is only fully differentiated from the pronator quadratus in reptiles, i.e. this reptilian muscle does not correspond to the palmaris profundus 1 of urodeles. Our observations and comparisons support this idea because, as can be seen in Fig. 6, the palmaris profundus 1 of urodeles usually runs from the ulna to the carpus and/or metacarpal I in urodeles, whereas the pronator accessorius of reptiles usually runs from the proximal margin of the ulna or even the medial epicondyle of the humerus to the radius. Therefore, the plesiomorphic condition for tetrapods is likely one in which there was a single muscle, the pronator quadratus, that then became independently differentiated into a pronator quadratus (often running from ulna to radius) and a palmaris profundus I (often running from ulna to carpus and/or metacarpal I) in urodeles, and into a pronator quadratus (often designated as 'pronator profundus', and usually running from ulna to radius and/or carpus) and a pronator accessorius (often running from ulna and/or medial epicondyle of humerus to radius) in reptiles. An undivided muscle pronator quadratus is thus present in anurans and mammals (Table 2).

The flexores digitorum minimi and an interphalangeus digiti 3 are very small muscles that have only been described in some urodeles by a few authors (e.g. Eisler, 1895; McMurrich, 1903; Ribbing, 1907; Blount, 1935), and that were reported by Grim & Carlson (1974) in *A. mexicanum*. Most authors have not reported these muscles in the urodele taxa studied by them, very likely because these muscles are difficult to detect in wildtype animals, and these discrepancies raised doubt about their actual presence and taxonomic distribution within urodeles; consequently the muscles were

not listed in the tables of Diogo & Abdala (2010) and Abdala & Diogo (2010). The analysis and dissections of GFP animals allowed us to confirm the presence of these muscles in *A. mexicanum*. For instance, Fig. 5 clearly shows that some of the fibers of the intrinsic muscles of the hand are situated distal to all the other fibers of these muscles and form a well defined, distinct muscle interphalangeus 3.

The tables of Diogo & Abdala (2010) and Abdala & Diogo (2010) show that in *A. ordinarium* the flexor antebrachii et carpi ulnaris is differentiated into two muscles, the flexor carpi ulnaris and the epitrochleoanconeus, the latter muscle running from the medial epicondyle of the humerus to the olecranon process of the ulna. As explained by those authors, this muscle is often neglected in myological studies of amphibians, so it is usually difficult to know whether the muscle is present in the species described in those studies. Grim & Carlson (1974) did not describe an epitrochleoanconeus ('flexor antebrachii ulnaris') in *A. mexicanum*. Our observations and comparisons show that in the members of this species this structure is effectively not present as a distinct muscle.

Most authors also do not describe a distinct 'extensor antebrachii radialis' in urodeles, and Diogo & Abdala (2010) and Abdala & Diogo (2010) also did not find this muscle in *A. ordinarium*. Grim & Carlson (1974) reported this muscle in *A. mexicanum*. Our methods, analyses and comparisons allowed us to confirm the presence of this muscle in this species and to show it clearly in both a ventral and a dorsal view of the forearm (Figs 5 and 7). In addition, they strongly support the idea that this muscle corresponds to the supinator of other tetrapods (Table 2) because, as the supinator, this is a deep dorsal muscle that lies between the extensor carpi radialis and the extensor carpi ulnaris, and that, contrary to these two muscles, does not extend to the carpus (inserting, in *A. mexicanum*, only onto the ulnar side of the radius). This homology hypothesis is also corroborated by our observations of the extensor carpi radialis, which includes two bundles. One, more radial, originates not only from the lateral epicondyle of the humerus but also from the region of the humerus that lies proximally to this epicondyle, and thus seems to correspond to the brachioradialis of other tetrapods (Table 2). The other bundle is more ulnar and originates only from the lateral epicondyle of the humerus, thus corresponding to the extensor carpi radialis of other tetrapods (Table 2; for more details, see Diogo & Abdala, 2010).

Based on their own dissections of the abductor et extensor digiti 1 and on a review of the literature, Diogo & Abdala (2010) and Abdala & Diogo (2010) stated that this muscle could include the extensor digitorum brevis to digit 1, but that this was still a controversial issue because there are often discrepancies concerning not only the homologies but even the exact configuration of this muscle in the amphibian literature. For instance, as explained above, regarding this particular aspect, the paper of Grim &

Carlson (1974), which is otherwise outstanding, is confusing because their figures and description suggest that it is the radial bundle of the abductor et extensor digiti 1 that goes to the distal phalanx, thus raising doubts about the identity and homologies of the bundles of this muscle (see their Fig. 4). Our study showed that it is in fact the ulnar bundle of the muscle that gives rise to this tendon, thus confirming that it is this ulnar bundle that corresponds to the extensor digitorum brevis to digit 1 and thus to the extensor pollicis longus of other tetrapods. The radial bundle thus corresponds to the abductor pollicis longus of other tetrapod taxa (Table 2). This is also supported by the fact that the radial bundle originates from the radius and ulna and inserts onto the base of metacarpal I (Figs 2 and 7), which are the typical attachments of the abductor pollicis longus in numerous tetrapods; the extensores digitorum breves do not normally originate from forearm bones or insert onto the metacarpals (Diogo & Abdala, 2010).

The last paragraphs of this section concern one of the most controversial issues, if not the most controversial, within the comparative anatomy and evolution of the tetrapod forelimb muscles: the homologies of the amphibian coracoradialis and of the amniote biceps brachii. Some authors suggested that the amphibian coracoradialis is homologous with pectoral muscles found in amniotes. For instance, Carus (1827) suggested that it corresponds to the pectoralis minor of modern humans, and stated that in some mammals there is a muscle that is topologically very similar to the coracoradialis, going all the way from the midline of the pectoral region to the radius, being similar to the so-called small flexor of the forearm of birds. However, most authors state that the coracoradialis is only present as well developed muscle with a fleshy belly in anurans (e.g. Remes, 2008). This muscle is also named 'sternoradialis' because in anurans it often extends all the way from the ventral surface of the sternum, from which originates its broad fleshy belly, to the radius, to which its long distal tendon is attached (e.g. Ecker, 1889; Duellman & Trueb, 1986). The fact that the coracoradialis was often regarded as a peculiar pectoral muscle of anurans, together with the fact that its fleshy belly is innervated by a pectoral nerve, i.e. the supracoracoid nerve (e.g. Anthony & Vallois, 1914), led many authors to consider that the coracoradialis did not contribute to the biceps brachii of amniotes. For instance, Romer (1944) stated that the long and short heads of the biceps brachii correspond respectively to part of the humeroantibrachialis and to part of the coracobrachialis of amphibians.

However, other authors emphasized the similarities between the coracoradialis of amphibians and the biceps brachii of amniotes, and suggested that the two heads of the latter muscle actually correspond to parts of the former muscle. This was for instance the view of Humphry (1872), who also stated that at least some urodeles (e.g. *Cryptobranchus japonicus*) do have a well developed coracoradialis.

Anthony & Vallois (1914) also reported a coracoradialis in *Cryptobranchus japonicus* and in other urodeles such as *A. tigrinum*, *Proteus anguinus*, *Triton pleurodeles*, and *Menobranchus lateralis*, but they stated that, contrary to anurans, this coracoradialis is not really a distinct muscle but instead a deep (dorsal) bundle of the supracoracoideus. The most consensual view in the past decades (e.g. Blount, 1935) and at the present moment (e.g. Kardong, 2002; Diogo & Abdala, 2010; Abdala & Diogo, 2010) is that the coracoradialis is usually not present as a distinct, well defined, fleshy muscle in urodeles. To our knowledge, the muscle has for instance never been described in *A. mexicanum*. Therefore, ours is the first study that clearly shows that the muscle is present in this species, and that its anatomy is actually strikingly similar to that found in anurans, i.e. it has a fleshy belly (which expresses GFP fibers: Fig. 4) that originates from the ventromedial surface of the pectoral girdle (namely of the coracoid), deep to the most ventral pectoral muscles, and that then just proximal to the proximal extremity of the humerus gives rise to a long and thin tendon (Fig. 4) that goes all the way to attach on the proximal portion of the radius, just next to the attachment of the humeroantibrachialis muscle. These observations, together with the descriptions of a few authors such as Humphry (1872) and Anthony & Vallois (1914) strongly suggest that the muscle is found in various species of urodeles, and thus that it was very likely present in the last common ancestor of anurans and urodeles.

Moreover, our observations and comparisons show that the coracoradialis of *A. mexicanum* is also strikingly similar to the biceps brachii of phylogenetically plesiomorphic living amniotes such as *Sphenodon*, which has a fleshy origin from the medial part of the superficial surface of the coracoid, becomes tendinous in the region of the shoulder joint and deltopectoral crest, and then inserts via a long and thin tendon onto the ulna and also to the radius, just next to, and blended with, the attachment of the brachialis inferior. This strongly indicates that at least part of the biceps brachii of amniotes is effectively derived from the coracoradialis, and that the brachialis inferior of amniotes very likely corresponds directly to the humeroantibrachialis of amphibians (Table 2). Howell & Straus (1932) suggested that a 'primitive tetrapod coraco-antibrachial flexor' may have given rise to both the long and short head of the biceps brachii of amniotes, the origin of the long head having migrated to the supraglenoid border, whereas the short head retained its coracoid origin, possibly strengthened by additional fibers from the coracobrachialis. Fürbringer (1876) had previously defended a similar idea, i.e. that the long head of the biceps brachii derived from a 'primitive coraco-antibrachialis', whereas the short head is derived from part of the coracobrachialis.

The idea defended in the present paper, which is shown in Table 1, is that the overall topology strongly supports the hypothesis of homology between the coracoradialis

and at least part of the biceps brachii (namely, its long head, the short head probably being at least partially derived, or receives a contribution, from the coracobrachialis) because the muscle is strikingly similar to the biceps brachii of plesiomorphic amniotes, as explained above. The innervation apparently contradicts this hypothesis of homology, because the coracoradialis receives innervation from a pectoral nerve (the supracoracoid nerve). However, as recently emphasized in Diogo & Abdala's (2010) monograph, there is often some variability in the innervation of the muscles of vertebrates, e.g. the mammalian dorsoepitrochlearis is an arm muscle that is usually innervated by the radial nerve, but due to its association with the latissimus dorsi it sometimes receives innervation from pectoral nerves. Importantly, the relationship between the dorsoepitrochlearis and the latissimus dorsi is very similar to that between the long distal tendon of the coracoradialis and the proximal fleshy pectoral belly of the muscle, which is often said to be actually part of the pectoral muscle supracoracoideus (see above). Therefore, it cannot be discarded that the plesiomorphic condition for tetrapods is to have an arm muscle biceps brachii and that only in amphibians was there then a proximal connection with a pectoral muscle forming the typical amphibian coracoradialis muscle, which is accordingly at least partially innervated by pectoral nerves. Be that as it may, in terms of cladistic parsimony it is more likely that the coracoradialis corresponds to at least part of the biceps brachii of amniotes than that this muscle is completely absent in amniotes. This is because the first hypothesis does not require the loss or gain of any structure (0 steps; i.e. the amniote biceps brachii corresponds to the coracoradialis and likely also to part of the coracobrachialis of amphibians, the remains of this latter muscle corresponding to the amniote coracobrachialis, and the amniote brachialis corresponding directly to the amphibian humeroantibrachialis). Otherwise, one would have to accept that the coracoradialis was lost in the node leading to amniotes and that, exactly at the same node, there was a gain of a new muscle, the biceps brachii, that strikingly resembles the lost muscle in many aspects (two steps). In summary, taking into account all evidence (i.e. topology strongly supports the first hypothesis, cladistic parsimony as well, and innervation contradicts it but not in an insurmountable way), in Table 1 we demonstrate that the amniote biceps brachii most likely corresponds to the coracoradialis (its long head) and to part of the coracobrachialis (its short head), but also use a grey arrow to show that it cannot be completely discarded that the long head of the biceps could derive instead from part of the humeroantibrachialis. Therefore, for the moment, and until this controversy is completely solved, we prefer to continue using the name humeroantibrachialis for the amphibian muscle, which if our hypothesis is further corroborated would thus correspond directly with the brachialis of amniotes and should therefore be accordingly designated as brachialis.

A last note concerns the regeneration of the coracoradialis. The results of our regenerative work on *A. mexicanum* will be published in detail elsewhere, but for the purpose of the present paper it is worth mentioning that in four of the 10 regenerated adult limbs studied in our work the coracoradialis has fleshy fibers at the level of the arm and/or forearm, contrary to the situation found in the original amputated limbs. In a previous study about the regeneration of nerves in *Ambystoma*, Piatt (1957) also stated that, in contrast to the original amputated limbs, in a few of the regenerated limbs there was a fleshy coracoradialis at the level of the arm/forearm. This is relevant for the present work for two main reasons. The first is that, if at least part of the amniote biceps brachii corresponds to the amphibian coracoradialis, this would mean that at some point in evolution the tendinous part of the coracoradialis of amphibians that lies in the forearm and arm regions had to be or to become associated with fleshy fibers, because the biceps brachii of amniotes usually has fleshy fibers at these regions. As Alberch (1989) has shown, there is often a parallel between the anatomical anomalies/variations occurring in biological organisms (naturally, or after experimentation) and the normal phenotype found in their closest relatives, i.e. evolution is highly constrained and similar phenotypes are therefore often created. Along this line of thought, the occurrence of an abnormal regeneration of fleshy fibers associated with the tendinous portion of the coracoradialis that lies at the level of the forearm/arm in urodeles makes it more feasible to envisage that this portion of the coracoradialis corresponds to at least part of the fleshy biceps brachii of amniotes. The second reason for mentioning our regeneration results here is that the observations that in at least some regenerated limbs of *A. mexicanum* the regenerated coracoradialis displays some significant, and enigmatic, differences with respect to the original muscle present in the original amputated limbs show how important it is to include not only the hand and forearm, but also the arm and pectoral muscles in regenerative studies of the urodele limbs.

## Conclusions

In the present paper we describe and illustrate the pectoral and forearm muscles of *A. mexicanum* and provide the first report on the myology of adults of a model organism that is based on analyses and dissections of both wildtype and GFP-transgenic animals. The inclusion of these two types of animals allowed us to obtain a more complete and clearer understanding of the muscle anatomy and therefore to find some discrepancies with previous works and to settle some controversies seen in previous anatomical and comparative studies. Our observations and comparisons confirmed that the flexores digitorum minimi, interphalangeus digiti 3, pronator quadratus and palmaris profundus 1 are present as distinct muscles in *A. mexicanum*, and support the idea that the latter muscle does not correspond to the pronator accessori-

us of reptiles. They also confirmed that the so-called extensor antebrachii radialis is present as a distinct muscle in this species and, importantly, indicate that this muscle corresponds to the supinator of other tetrapods. They also show that, contrary to some other urodele and even *Ambystoma* species, there is no distinct muscle epitrochleoanconeus in *A. mexicanum*. Importantly, they also show that the ulnar bundle and the radial bundle of the abductor et extensor digiti 1 directly correspond to the abductor pollicis longus and extensor pollicis longus of other tetrapods. One of the most important outcomes of our methodology and observations is that, contrary to what is often stated in the literature, *A. mexicanum* has a muscle coracoradialis that has both a well developed proximal fleshy belly and a distal long and thin tendon, supporting the idea that this muscle might well correspond to at least part of the biceps brachii of other tetrapods.

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## References

- Abdala V, Diogo R** (2010) Comparative anatomy, homologies and evolution of the pectoral and forelimb musculature of tetrapods with special attention to extant limbed amphibians and reptiles. *J Anat* **217**, 536–573.
- Agata K, Inoue T** (2012) Survey of the differences between regenerative and non-regenerative animals. *Dev Growth Differ* **54**, 143–152.
- Alberch P** (1989) The logic of monsters: evidence for internal constraint in development and evolution. *Geobios Mem Spec* **12**, 21–57.
- Anthony R, Vallois H** (1914) Sur la signification des elements ventraux de la ceinture scapulaire chez les batraciens. *Bibl Anat* **24**, 218–276.
- Blount IWH** (1935) The anatomy of normal and reduplicated limbs in Amphibia, with special reference to musculature and vascularization. *J Exp Zool* **69**, 407–457.
- Carlson BM** (2003) Muscle regeneration in amphibians and mammals: passing the torch. *Dev Dyn* **226**, 167–181.
- Carlson BM** (2007) *Principles of Regenerative Biology*. Amsterdam: Elsevier.
- Carus CG** (1827) *An Introduction To The Comparative Anatomy Of Animals: Comp. With Constant Reference To Physiology, And Elucidated By Twenty Copper-plates, Volume 1*. London: Longman.
- Diogo R** (2007) *On the Origin and Evolution of Higher-Clades: Osteology, Myology, Phylogeny and Macroevolution of Bony Fishes and the Rise of Tetrapods*. Enfield: Science Publishers.
- Diogo R, Abdala V** (2007) Comparative anatomy, homologies and evolution of the pectoral muscles of bony fish and tetrapods: a new insight. *J Morphol* **268**, 504–517.
- Diogo R, Abdala V** (2010) *Muscles of Vertebrates – Comparative Anatomy, Evolution, Homologies and Development*. Oxford: Taylor & Francis.
- Diogo R, Abdala V, Aziz MA, et al.** (2009) From fish to modern humans – comparative anatomy, homologies and evolution of the pectoral and forelimb musculature. *J Anat* **214**, 694–716.
- Duellman WE, Trueb L** (1986) *The Biology of Amphibians*. New York: McGraw-Hill Book Company.
- Ecker A** (1889) *Anatomy of the Frog*. Oxford: Clarendon Press.
- Eisler P** (1895) Die flexores digitorum. *Anat Anz* **10**, 135–145.
- Fürbringer M** (1876) Zur vergleichenden Anatomie der Schultermuskeln – 3 Teil, Capitel IV: Saurier und Crocodile. *Gegenbaurs Morphol Jahrb* **1**, 636–816.
- Gaupp E** (1896) *A. Ecker's und R. Wiedersheim's Anatomie des Frosches, part 1*. Braunschweig: Friedrich Vieweg und Sohn.
- Grim M, Carlson BM** (1974) A comparison of morphogenesis of muscles of the forearm and hand during ontogenesis and regeneration in the axolotl (*Ambystoma mexicanum*). I. Anatomical description of muscles of the forearm and hand. *Z Anat Entwicklungsgesch* **145**, 137–148.
- Howell AB** (1935) Morphogenesis of the shoulder architecture, Part III - Amphibia. *Q Rev Biol* **10**, 397–431.
- Howell AB, Straus WL** (1932) The brachial flexor muscles in primates. *Proc US Natl Mus* **80**, 1–31.
- Humphry GM** (1872) The disposition of muscles in Vertebrate animals. *J Anat Physiol* **6**, 293–376.
- Kardong KV** (2002) *Vertebrates: Comparative Anatomy, Function, Evolution* (3rd edn). New York: McGraw-Hill.
- Kragl M, Knapp D, Nacu E, et al.** (2009) Cells keep a memory of their tissue origin during axolotl limb regeneration. *Nature* **460**, 60–65.
- McMurrich JP** (1903) The phylogeny of the palmar musculature. *Am J Anat* **2**, 463–500.
- Nacu E, Tanaka EM** (2011) Limb regeneration: a new development? *Annu Rev Cell Dev Biol* **27**, 409–440.
- Piatt J** (1957) Studies on the problem of nerve pattern, III, Innervation of the regenerated forelimb in *Ambystoma*. *J Exp Zool* **136**, 229–247.
- Remes K** (2008) *Evolution of the pectoral girdle and forelimb in Sauropodomorpha (Dinosauria, Saurischia): Osteology, myology and function*. Unpublished PhD Thesis, LMU München.
- Ribbing L** (1907) Die distale Armmuskulatur der Amphibien, Reptilien und Säugetiere. *Zool Jb* **23**, 587–680.
- Romer AS** (1922) The locomotor apparatus of certain primitive and mammal-like reptiles. *Bull Am Mus Nat Hist* **46**, 517–606.
- Romer AS** (1924) Pectoral limb musculature and shoulder-girdle structure in fish and tetrapods. *Anat Rec* **27**, 119–143.
- Romer AS** (1944) The development of tetrapod limb musculature – the shoulder region of *Lacerta*. *J Morphol* **74**, 1–41.
- Stocum DL, Cameron JA** (2011) Looking proximally and distally: 100 years of limb regeneration and beyond. *Dev Dyn* **240**, 943–968.
- Straus WL** (1942) The homologies of the forearm flexors: urodeles, lizards, mammals. *Am J Anat* **70**, 281–316.