

Locomotion pattern and trunk musculoskeletal architecture among Urodela

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Introduction

Urodeles have been studied as model basal tetrapod animals because of their generalized body form and range of locomotive modes (Deban and Schilling 2009). Order Urodela is composed of ten families, which include aquatic, semi-aquatic and terrestrial species. Because urodeles use their trunk for undulatory locomotion in water and on ground (Deban and Schilling 2009), a morphological study of their trunk structure is necessary to understand the locomotion system of basal tetrapods. In the present study, we focus on the morphological

Abstract

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We comparatively examined the trunk musculature and prezygapophyseal angle of mid-trunk vertebra in eight urodele species with different locomotive modes (aquatic *Siren intermedia*, *Amphiuma tridactylum*, *Necturus maculosus* and *Andrias japonicus*; semi-aquatic *Cynops pyrrhogaster*, *Cynops ensicauda*; and terrestrial *Hynobius nigrescens*, *Hynobius lichenatus* and *Ambystoma tigrinum*). We found that the more terrestrial species were characterized by larger dorsal and abdominal muscle weight ratios compared with those of the more aquatic species, whereas muscle ratios of the lateral hypaxial musculature were larger in the more aquatic species. The lateral hypaxial muscles were thicker in the more aquatic species, whereas the *M. rectus abdominis* was more differentiated in the more terrestrial species. Our results suggest that larger lateral hypaxial muscles function for lateral bending during underwater locomotion in aquatic species. Larger dorsalis and abdominal muscles facilitate resistance against sagittal extension of the trunk, stabilization and support of the ventral contour line against gravity in terrestrial species. The more aquatic species possessed a more horizontal prezygapophyseal angle for more flexible lateral locomotion. In contrast, the more terrestrial species have an increasingly vertical prezygapophyseal angle to provide stronger column support against gravity. Thus, we conclude trunk structure in urodeles differs clearly according to their locomotive modes.

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differences in trunk muscles and trunk vertebrae among species with different locomotive modes.

In water, urodeles swim in an undulatory manner using their axial muscles, with their limbs pressed against the body (Gray 1944), generating travelling waves (Bennett *et al.* 2001). On the ground, they bend their bodies laterally in a cyclic manner generating standing waves during walking (Ashley-Ross 1995; Bennett *et al.* 2001). Thus, urodeles need to sustain their own weight and maintain their posture against gravity (Liem *et al.* 2001). The limbs of urodeles are used as anchors to increase stride length (Barclay 1946). The activity

pattern of hypaxial muscles during swimming is different from that during walking in *Dicamptodon ensatus* (Carrier 1993) and *Ambystoma tigrinum* (Bennett et al. 2001). The overall fibre-type distribution pattern of paravertebral musculature is very similar between the two species of *Ambystoma*, *A. tigrinum* and *A. maculatum*, but the former possesses larger muscles than those of the latter, possibly reflecting differences in digging behaviour (Schilling and Daban 2010). The myosepta of the swimming salamander *Siren lacertina* have been suggested to increase strain amplification of the muscle fibres by reducing mediolateral bulging of the myomeres and redirecting the bulging to the dorsoventral direction (Azizi et al. 2002).

Only a few studies, however, have investigated the morphological differences in trunk musculature among urodeles with different locomotive modes. Trunk musculature has been quantified by examining cross-sections, and the relationships between trunk muscles and ecological habitats were investigated by Simons and Brainerd (1999). They suggested that thinner hypaxial muscles are associated with the more terrestrial species because a reduction in relative thickness of body wall and more dorsal placement of epaxial musculature are suitable and needed for terrestrial behaviour. *M. intertransversarius* and *M. interspinalis* play a central role in stabilization on the vertebral column during swimming and walking (Deban and Schilling 2009); however, the mass of these muscles has not been quantified in urodeles. Because the *M. intertransversarius* and *M. interspinalis* lie between the successive vertebrae and ribs, quantifying these muscles is difficult using a cross-sectional approach. Thus, we quantified trunk muscle mass through macroscopic dissections and compared these muscles among three amphibians with different locomotive modes.

The morphology of Urodele trunk vertebrae varies between different locomotive modes. Hilton (1948) and Antipenkova (1994) published short descriptions of the vertebrae in urodeles. Comparative analyses of vertebral morphology in various urodeles have been performed (Mivart 1870; Worthington and Wake 1972; Ratnikov and Litvinchuk 2007). It was reported that the zygapophyseal angle of the vertebra adjusts the direction and degree of movement (Slijper 1946; Boszczyk et al. 2001; Hua 2003). Because the degree of the zygapophyseal angle decides on the depth of articulation of the vertebra, zygapophyseal angle is expected to reflect the strength of the vertebral column. Thus, quantifying the zygapophyseal angle is necessary to infer the function of vertebral morphology.

In this study, functional–morphological differences in trunk structure among urodele species with different locomotive modes were examined by weighing each trunk muscle and measuring vertebral prezygapophyseal angle. The objective was to quantify morphological variations in trunk musculature and trunk vertebra of urodeles of different ecotypes, and test the relationship between locomotive mode and trunk morphology.

Materials and Methods

Shape and muscle weight ratios of trunk muscles

Three species of adult salamanders representing three families and three different habitats (aquatic, semi-aquatic and terrestrial) were used in this study (Table 1). The examined specimens were deposited at The University Museum, The University of Tokyo. The specimens were fixed in a straight body position in 10% formalin and were preserved in 70% ethanol solution.

Trunk muscles were divided as presented in Fig. 1, following Maurer (1892, 1911), Francis (1934) and Naylor (1978). The following trunk muscles were examined in this study: *M. dorsalis trunci*, *M. interspinalis*, *M. intertransversarius*, *M. subvertebralis*, *M. obliquus externus*, *M. obliquus externus superficialis*, *M. obliquus externus profundus*, *M. obliquus internus*, *M. transversus abdominis*, *M. rectus profundus*, *M. rectus lateralis* and *M. rectus abdominis*. Each trunk muscle was dissected on the left side of the body using tweezers to determine trunk muscle weight, and the specimens were observed from the lateral view. Specimens were kept wet with water during dissection to avoid drying and causing shrink. Each trunk muscle was weighed using an AUW220 electronic balance (Shimadzu Co., Ltd., Kyoto, Japan), and the weight ratio of each muscle to the weight of all measured trunk muscles was calculated.

For comparing weight ratio of each muscle for three species, the values for six muscles were classified into three groups according to position, running direction and function as follows: *M. obliquus externus superficialis* and *M. obliquus externus profundus*; *M. obliquus internus* and *M. transversus abdominis*; and *M. rectus profundus* and *M. rectus abdominis*. The number of layers of lateral hypaxial muscles and the presence/absence of *M. rectus profundus* differ among species. To compare the weight ratios of trunk muscles, the number of the groups of trunk muscles should be equal among species. Bennett et al. (2001) mentioned that in *Ambystoma tigrinum*, all four layers of lateral hypaxial musculature are active simultaneously to bend the body towards the active side during swimming. During walking, the *M. transversus abdominis*

Table 1 Specimens used for measuring and observing muscle in this study

Species	Habitat	Snout-vent length (mm)
<i>Siren intermedia</i>	Aquatic	261
		230
		242
<i>Cynops ensicauda</i>	Semi-aquatic	052
		051
		054
<i>Hynobius lichenatus</i>	Terrestrial	054
		055
		053

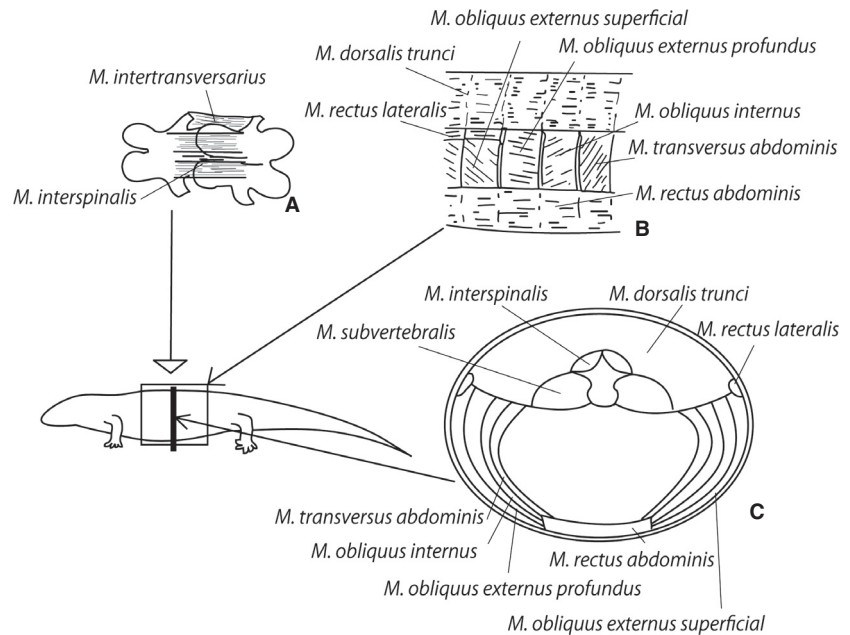


Fig. 1—**A.** Dorsal view of perivertebral musculature after removal of *M. dorsalis trunci*. —**B.** Lateral view of trunk musculature. —**C.** Cross-sectional view.

and the *M. obliquus internus* are active together on the extending side of the body at the same time as the *M. obliquus externus superficialis* and *M. obliquus externus profundus* are active together on the flexing side of the body. Then, two inner layers and two outer layers were grouped since the locomotive unit is all four layers or is divided into inner and outer two layers. *M. rectus profundus* is present only in *Hynobius lichenatus*. This muscle lies deep to *M. rectus abdominis* and extends anteroposteriorly along the mid-sagittal line as does the latter muscle. Though the function of *M. rectus profundus* has not been clarified, it may have similar function as those of *M. rectus abdominis* because of their positions and running direction. In this study, therefore, *M. rectus profundus* was grouped together with *M. rectus abdominis* for comparison of muscle weight ratios.

Subsequently, cross-sections were obtained from the mid-trunk region, between the pectoral and pelvic girdles, on the right side of the body. Lateral view and cross-section images were observed and recorded using a microscope with a single-lens reflex camera and adapter (Micronet NY1S, Saitama, Japan).

Prezygapophyseal angle of mid-trunk vertebrae

Eight species of adult salamanders representing seven families and three different habitats (aquatic, semi-aquatic and terrestrial) were used (Table 2). The mid-trunk vertebrae were scanned using a micro-CT (R_mCT[®]; Rigaku Co., Tokyo, Japan) at the Department of Oral and Maxillofacial Radiology, Nihon University School of Dentistry, Tokyo, Japan, and I-View-R (Rigaku Co.) was used for image processing. The prezygapophyseal angle was measured on scanned images

Table 2 Specimens used for measuring prezygapophyseal angle of vertebra in this study

Species	Habitat	Snout-vent length* (mm)
<i>Siren intermedia</i>	Aquatic	261
		230
		242
<i>Amphiuma tridactylum</i>	Aquatic	408
		434
<i>Necturus maculosus</i>	Aquatic	471
		175
		172
<i>Andrias japonicus</i>	Aquatic	168
		420
		408
<i>Cynops pyrrhogaster</i>	Semi-aquatic	642
		053
		048
<i>Hynobius nigrescens</i>	Terrestrial	045
		070
		072
<i>Hynobius lichenatus</i>	Terrestrial	073
		054
		055
<i>Ambystoma tigrinum</i>	Terrestrial	053
		111
		103
		086

(Fig. 2). Here, this angle is defined as the one between the articular surface on the prezygapophysis and the horizontal plane measured on images taken perpendicular to the long axis of the vertebra. The prezygapophyseal angle was also

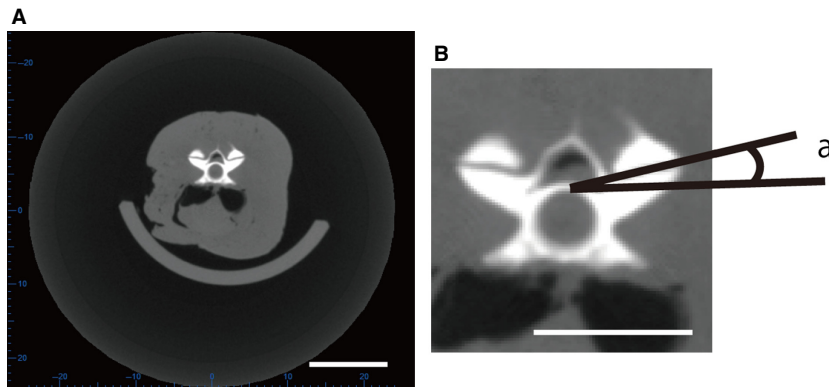


Fig. 2—Frontal view of mid-trunk vertebra. —**A**. μ -CT scanned image of vertebra of *Siren intermedia*. —**B**. Frontal view of mid-trunk vertebra of *Siren intermedia* for measuring prezygapophyseal angle. —**a**. Prezygapophyseal angle. Scale bar = 1 cm.

measured on skeletal specimens of large individual of *Amphiuma tridactylum* and *Andrias japonicus*. Homogeneity of variances and means of each item between species were confirmed by ANOVA and Tukey's test.

Statistical analyses were performed to confirm significant differences in the muscle weight ratios and prezygapophyseal angle among species. Homogeneity of variances and means between species were confirmed by analysis of variance (ANOVA). When significant differences were identified by ANOVA, the differences between species were detected using Tukey's test.

Results

Observation of trunk muscles in lateral view

The lateral views of the trunk muscles for the three species are shown in Fig. 3. *M. dorsalis trunci* occupied the bulk of the epaxial muscle mass in all three species, and the muscle fibres ran in a longitudinal direction between successive myosepta. *M. interspinalis* connecting to the vertebrae was dorsolaterally situated. Muscle fibres of *M. interspinalis* ran in the longitudinal direction between the vertebral spines. *M. intertransversarius* passed between the transverse processes of the vertebrae, and the direction of the muscle fibres was longitudinal. Only *Cynops ensicauda* possessed *M. rectus lateralis*. *M. rectus lateralis* was found along the trunk on the lateral hypaxial muscles, which ran longitudinally between the surfaces of the rib edges.

M. obliquus externus in *Siren intermedia* and *Hynobius lichenatus* comprised the most superficial layers of the lateral hypaxial muscles. *M. obliquus externus* ran between myosepta from the craniodorsal to caudoventral direction. *C. ensicauda* possessed differentiated *M. obliquus externus superficialis* and *M. obliquus externus profundus* instead of a single, undifferentiated *M. obliquus externus*. *M. obliquus externus superficialis* was the most superficial layer of the *C. ensicauda* lateral hypaxial muscles. The fibres extended from a craniodorsal to a caudoventral direction between myosepta. In this species, *M. obliquus externus profundus* lied deep to *M. obliquus externus superficialis*. *M. obliquus externus profundus* had

the origin and insertion similar to *M. obliquus externus superficialis* but had the direction of the fibres more longitudinal than in the latter.

M. obliquus internus and *M. transversus abdominis* composed the inner lateral hypaxial layers. *S. intermedia* and *C. ensicauda* possessed *M. obliquus internus* and *M. transversus abdominis* as inner lateral hypaxial layers, whereas *H. lichenatus* had only *M. transversus abdominis* as an inner lateral hypaxial layer. *M. obliquus internus* ran from the cranioventral to caudodorsal direction between myosepta. *M. transversus abdominis* attached on peritonea extends from the shoulder to the pelvic regions. *M. transversus abdominis* ran from the cranioventral to caudodorsal direction, and its fibre angle was more vertical than that of *M. obliquus internus*.

M. rectus abdominis of all three species formed the most ventral part of the body wall, with muscle fibres running in a sagittal direction. This muscle extended from the anterior edge of the pelvis to the sternal cartilage. *M. rectus abdominis* was differentiated from lateral hypaxial muscles in *S. intermedia*, but not in *C. ensicauda* or *H. lichenatus*. *H. lichenatus* was equipped with *M. rectus profundus*, which lied deep to *M. rectus abdominis*.

Cross-sectional observations

The relative area occupied by each muscle in the cross-section of the trunk in the three species of salamanders is shown in Fig. 4. The number of muscle layers and characteristics of each muscle differed among species. Three layers (*M. obliquus externus*, *M. obliquus internus* and *M. transversus abdominis*) composed the lateral hypaxial musculature of *S. intermedia*. In this species, the *M. rectus abdominis* was not differentiated from the lateral hypaxial muscles whereas the lateral hypaxial muscles were relatively thick. The lateral hypaxial muscles of *C. ensicauda* consisted of four layers: *M. obliquus externus superficialis*, *M. obliquus externus profundus*, *M. obliquus internus* and *M. transversus abdominis*. The lateral hypaxial muscles of *C. ensicauda* were comparatively thin. *C. ensicauda* possessed *M. rectus lateralis*, and *M. subvertebralis* of *C. ensicauda* was connected with *M. transversus abdominis*. *M. rectus abdominis*

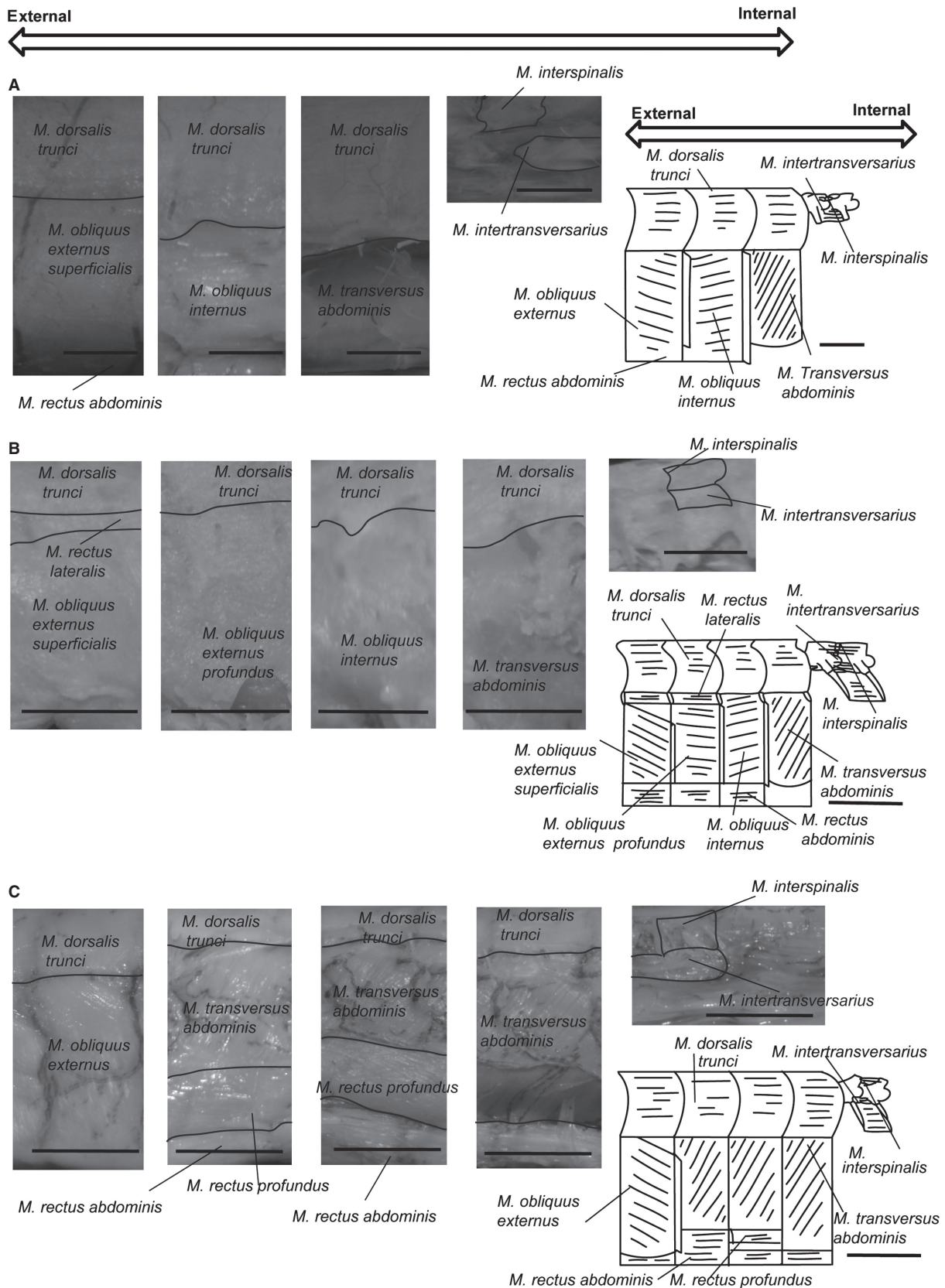


Fig. 3—Lateral view of the trunk muscles of—**A.** *Siren intermedia*,—**B.** *Cynops ensicauda*,—**C.** *Hynobius lichenatus*. Scale bar = 5 mm.

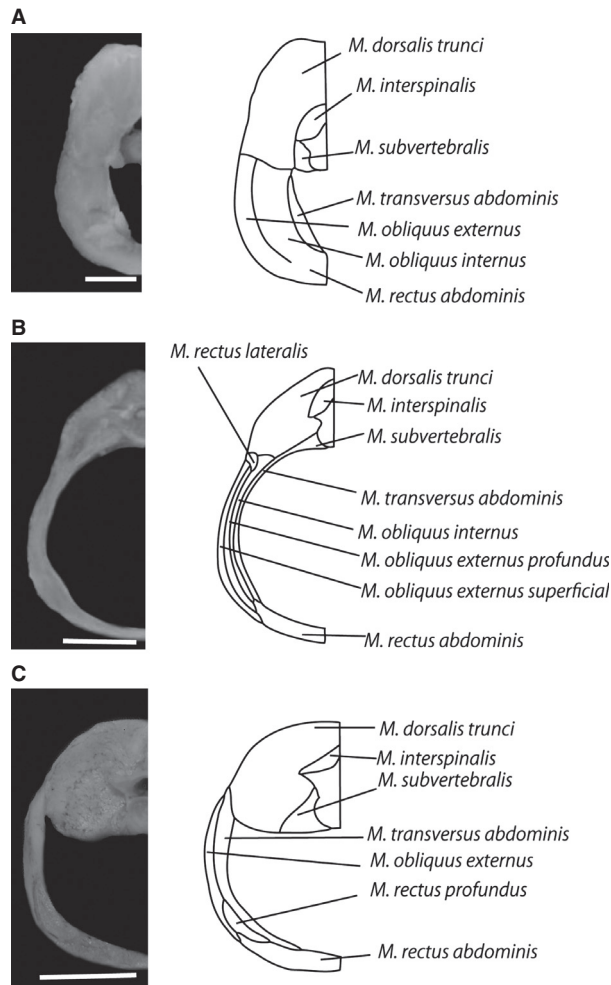


Fig. 4—Cross-sections through the mid-trunk of—**A.** *Siren intermedia*, —**B.** *Cynops ensicauda*, —**C.** *Hynobius lichenatus*. Scale bar = 5 mm.

of *C. ensicauda* was differentiated from the lateral hypaxial musculature. Two layers composed the lateral hypaxial muscles of *Hynobius lichenatus*: *M. obliquus externus* and *M. transversus abdominis*. *H. lichenatus* had thinner lateral hypaxial muscles. *H. lichenatus* possessed *M. rectus profundus*, which was differentiated from *M. rectus abdominis*.

Muscle weights

The ratios of the weight of each trunk muscle to the total weight of all measured muscles are represented in Table 3 and Fig. 5. Considerable variations were observed in these ratios among salamanders.

Significant differences among species occurred for the weight ratio of *M. dorsalis trunci*. This ratio was lower in the more aquatic species than in the more terrestrial species: whereas the weight ratio of *M. dorsalis trunci* was lower for

Table 3 Muscle weight ratios (%) measured at mid-trunk (mean ± SEM)

Species	Habitat	<i>M. dorsalis trunci</i>	<i>M. interspinalis</i>	<i>M. intertransversarius</i>	<i>M. subvertebralis</i>	<i>M. obliquus externus</i> , <i>M. obliquus externus superficial</i> + <i>M. obliquus externus profundus</i>	<i>M. transversus abdominis</i> , <i>M. obliquus internus</i> + <i>M. transversus abdominis</i>	<i>M. rectus abdominis</i> , <i>M. rectus profundus</i> + <i>M. rectus abdominis</i>
<i>Siren intermedia</i>	Aquatic	35.1 ± 0.3 ^c	3.4 ± 0.5 ^b	2.4 ± 0.1 ^b	6.1 ± 0.3 ^c	24.6 ± 0.5 ^a	25.7 ± 0.4 ^a	2.5 ± 0.3 ^c
<i>Cynops ensicauda</i>	Semi-aquatic	40.6 ± 1.3 ^b	4.0 ± 0.2 ^b	2.6 ± 0.1 ^b	11.4 ± 0.5 ^b	11.5 ± 1.0 ^b	16.7 ± 0.6 ^b	9.6 ± 0.6 ^b
<i>Hynobius Lichenatus</i>	Terrestrial	47.3 ± 1.2 ^a	5.1 ± 0.3 ^a	3.2 ± 0.2 ^a	14.2 ± 0.7 ^a	7.1 ± 0.2 ^c	8.0 ± 0.5 ^c	15.0 ± 1.6 ^a

Different superscript letters indicate significant differences (ANOVA and Tukey's test, *P* < 0.05).

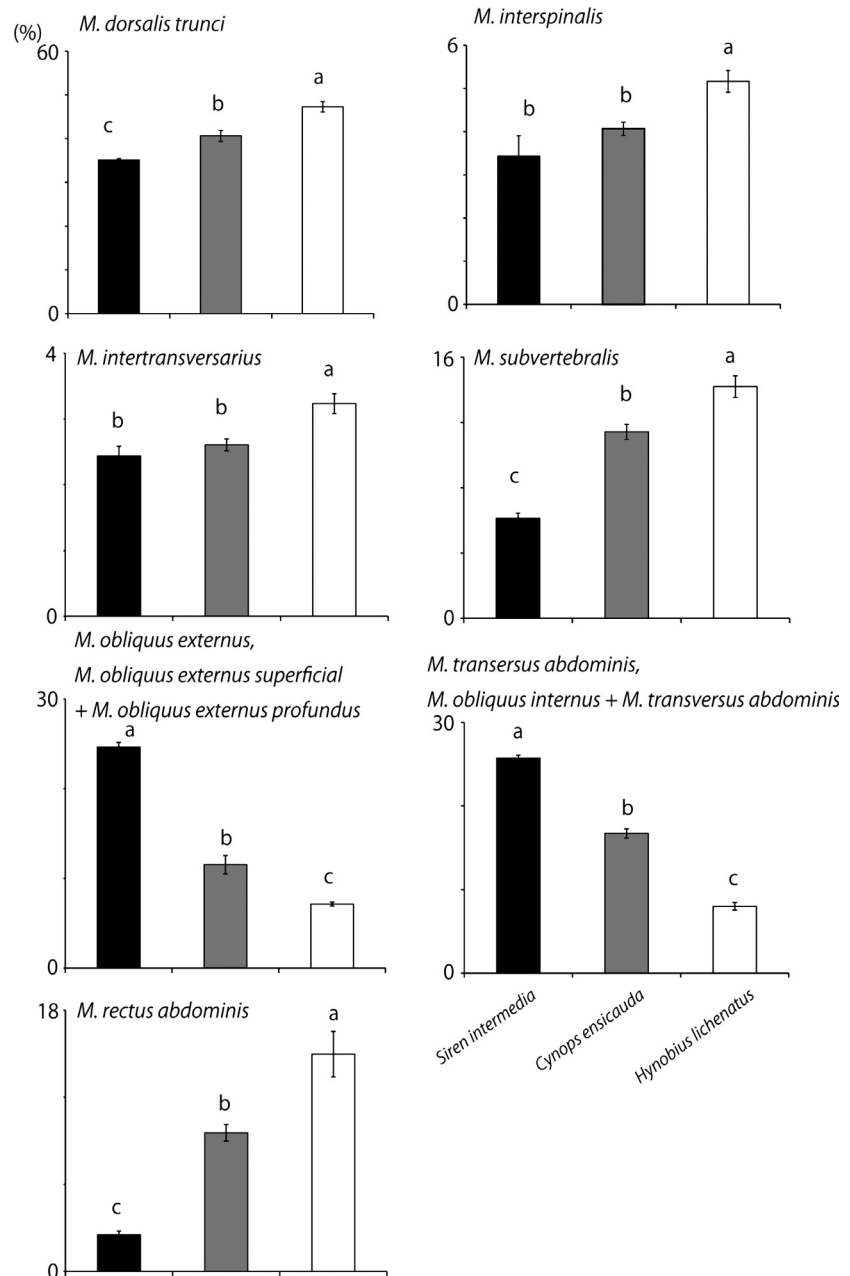


Fig. 5—Muscle weight ratios. Different superscript letters indicate significant differences (ANOVA and Tukey's test, $P > 0.05$). Black bar: aquatic species, grey bar: semi-aquatic species, white bar: terrestrial species.

S. intermedia at 35.1%, it is 40.6% for *C. ensicauda* and 47.3% for *H. lichenatus*. Although no significant differences were observed between *S. intermedia* and *C. ensicauda* for the weight ratio of *M. interspinalis*, the more aquatic species tended to have smaller weight ratio of this muscle. Terrestrial species had larger weight ratio of *M. intertransversarius*, whereas it is small (<3%) in *C. ensicauda* and *S. intermedia*. Significant differences among species were observed for the weight ratio of *M. subvertebralis*, with the more terrestrial species having a larger weight ratio. The value of aquatic species was 6.1% and that of *H. lichenatus* was 14.2%. Sig-

nificant differences in the weight ratio of *M. obliquus externus* were observed among species. That is, the weight ratio of *M. obliquus externus* for the aquatic species was significantly larger than that for the semi-aquatic and terrestrial species, with the significantly highest value of more than 24% found in *S. intermedia*. Significant differences in the weight ratio of *M. transversus abdominis* were observed among species with larger weight ratio revealed in the more aquatic species. This ratio of *M. transversus abdominis* was >25% for *S. intermedia*. Significant differences were also revealed in the weight ratio of *M. rectus abdominis*, with the

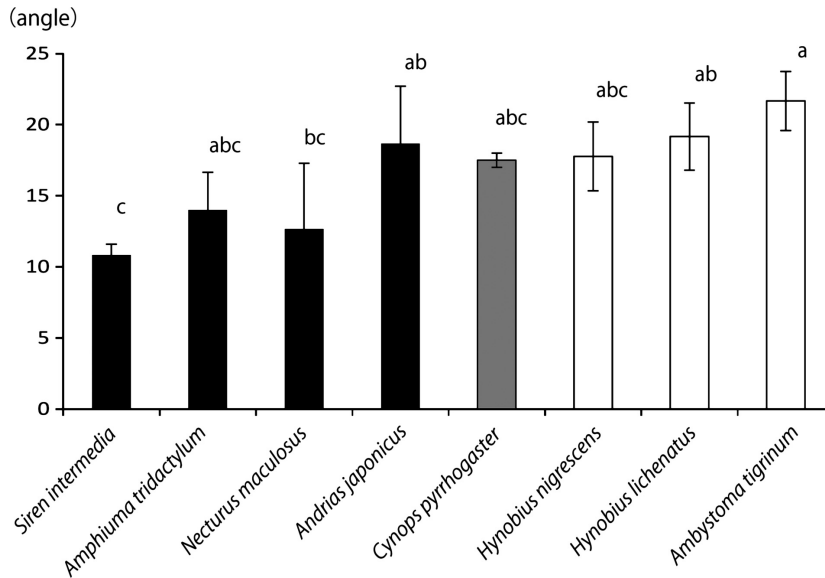


Fig. 6—Prezygapophyseal angle of mid-trunk vertebra. Different superscript letters indicate significant differences (ANOVA and Tukey's test, $P > 0.05$). Black bar: aquatic species, grey bar: semi-aquatic species, white bar: terrestrial species.

more terrestrial species possessing larger ratio. This weight ratio was 15% for *H. lichenatus*, which was the largest weight ratio among the examined species.

Measurement of mid-trunk vertebrae

The results of prezygapophyseal angle measurements of mid-trunk vertebrae are presented in Fig. 6 and Table 4. Interspecific differences were observed in these values. The more aquatic species tended to have a smaller prezygapophyseal angle than that of the more terrestrial species, but the differences were not statistically significant. The prezygapophyseal angle of *S. intermedia* was the smallest at 10.8°. Prezygapophyseal angles were <15° in the aquatic species, except for that of *A. japonicus*. The prezygapophyseal angles of the terrestrial species were >17.7°.

Discussion

Observation of trunk muscles

In this study, it was revealed that the morphology of trunk muscles in the lateral region differed considerably between species (Fig. 3). In addition, trunk muscles of mid-trunk region showed differences in relative cross-sectional area (Fig. 4). The cross-section analysis revealed that thicker hypaxial muscles were found in the fully aquatic, swimming salamander and thinner hypaxial musculature was found in the more terrestrial species (Fig. 4). This result was similar to that of a previous study by Simons and Brainerd (1999) that compared *S. lacertina*, *A. tridactylum*, *Cryptobranchus alleganiensis* and *A. tigrinum*. We confirmed that *S. intermedia* have relatively thick lateral hypaxial muscles as observed by Simons and Brainerd 1999. It is postulated that the main functional

Table 4 Prezygapophyseal angle of mid-trunk vertebra (mean ± SEM)

Species	Habitat	Prezygapophyseal angle
<i>Siren intermedia</i>	Aquatic	10.8 ± 0.7 ^c
<i>Amphiuma tridactylum</i>	Aquatic	14.0 ± 2.6 ^{abc}
<i>Necturus maculosus</i>	Aquatic	12.6 ± 4.6 ^{bc}
<i>Andrias japonicus</i>	Aquatic	18.6 ± 4.0 ^{ab}
<i>Cynops pyrrhogaster</i>	Semi-aquatic	17.5 ± 0.5 ^{abc}
<i>Hynobius nigrescens</i>	Terrestrial	17.7 ± 2.4 ^{abc}
<i>Hynobius lichenatus</i>	Terrestrial	19.1 ± 2.3 ^{ab}
<i>Ambystoma tigrinum</i>	Terrestrial	21.6 ± 2.0 ^a

Different superscript letters indicate significant differences (ANOVA and Tukey's test, $P < 0.05$).

role of the lateral hypaxial muscles in locomotion was altered into controlling limb, as terrestrial species came to possess more robust limbs, and that the limbs took over the role of locomotion, resulting in a decrease in the thickness of lateral hypaxial muscles (Romer 1970). *M. rectus abdominis* was not clearly differentiated from the lateral hypaxial muscles in *S. intermedia*, whereas the *M. rectus abdominis* for *C. ensicauda* and *H. lichenatus* was clearly independent from the lateral hypaxial muscles (Figs 3 and 4). Naylor (1978) reported that *M. rectus abdominis* is not differentiated from the lateral hypaxial muscles (*M. obliquus internus* and *M. obliquus externus*) for *S. lacertina*. In our study, *M. rectus abdominis* in *S. intermedia* was similar to that in *S. lacertina* (Naylor 1978), in which it merged into the lateral hypaxial muscles.

Differentiated muscles have more specialized function than muscles with simplified structures (Liem *et al.* 2001). Therefore, the more terrestrial species may possess a more specialized function of *M. rectus abdominis*, which helps

maintain position of the trunk and sustain weight. As long-trunked species require more muscular force along the ventral contour line (Preuschoft *et al.* 2007), we postulate that *M. rectus abdominis*, which lies in the most ventral trunk position, is larger in the more terrestrial species. This is consistent with the fact that *M. rectus abdominis* plays a central role counteracting sagittal extension of the trunk caused by the epaxial muscles (Deban and Schilling 2009; Schilling 2011) and by gravity due to the elongated trunk in salamanders (Preuschoft *et al.* 2007). Although the number of hypaxial muscle layers differed among species, strong correlation between such a number and locomotive modes was not found (Figs 3 and 4). This finding was consistent with that of Simons and Brainerd (1999) who reported that ecology and predominant locomotive mode is not clearly associated with the number of hypaxial muscle layers. Further investigation is needed in the future to test whether the number of hypaxial muscle layers reflects phylogenetic inertia or ecological adaptations. That is, the differences in the morphology observed among species may reflect not only functional and habitual differences but also phylogenetic differences. In this study, we observed muscle morphology in three species of Urodela. Therefore, we cannot completely exclude the possibility that the observed differences may be partly due to phylogenetic or taxonomic differences.

Muscle weight ratios

Because the evolutionary transition from water to land was primarily accompanied by decreased inertia and drag and increased gravitational loading, postural work for limb and trunk muscles increased (Schilling 2011). Salamanders use most axial muscles for lateral bending, modulating body stiffness and/or providing local stabilization during swimming (Schilling 2011). Axial musculature also stabilizes the body against gravitational force during terrestrial locomotion (Schilling 2011). We found that the more terrestrial species possessed larger *M. dorsalis trunci* (Fig. 5). This propensity among salamanders is somewhat parallel to the general macroevolutionary tendency of the increased dorsal placement of epaxial musculature during the terrestrial transition from teleosts to lizards (Romer 1970). The *M. dorsalis trunci* produces lateral bending during swimming in salamanders (Frolich and Biewener 1992; Delvolve *et al.* 1997; Deban and Schilling 2009). In addition, the activation pattern suggests that *M. dorsalis trunci* has a role in lateral bending during walking (Deban and Schilling 2009). Furthermore, this muscle prevents sagging and torsion and may increase trunk stiffness against gravity during terrestrial walking (Deban and Schilling 2009). In addition, a stabilizing function of epaxial muscles was proposed by O'Reilly *et al.* (2000). Although *M. dorsalis trunci* is used in lateral bending and stabilization during swimming and walking (Deban and Schilling 2009), the more terrestrial species may utilize *M. dorsalis trunci* to stabilize

against gravitational forces. Therefore, larger *M. dorsalis trunci* may prevent the effect of gravitational forces in addition to producing lateral bending in the more terrestrial species.

A larger *M. interspinalis* was found, in the more terrestrial species (Fig. 5). An experimental study suggested that this muscle functions in vertebral stabilization rather than lateral bending during swimming (Deban and Schilling 2009). The *M. interspinalis* generates force during shorting and lengthening of the sides of the trunk and stabilizes the intervertebral joint to ensure structural linkage of vertebrae during walking (Deban and Schilling 2009). The vertebral column of terrestrial vertebrates acts as a beam to support the body weight against gravitational forces and transfers weight to the girdle and appendages (Liem *et al.* 2001). We assume that more terrestrial species require a stronger connection between successive vertebrae to stabilize vertebrae against gravity. Therefore, as more terrestrial species employ more *M. interspinalis* on the ground, this muscle is larger in species with this locomotive mode. A larger *M. intertransversarius* weight ratio was found in more terrestrial species (Fig. 5). Because *M. intertransversarius* connects successive transversus processes of vertebrae (Naylor 1978), we postulate that *M. intertransversarius* functions to ensure spinal integrity in a similar manner as *M. interspinalis*. The more terrestrial species is considered as utilizing *M. interspinalis* to stabilize the spine against gravity; thus, the more terrestrial species is expected to exhibit a larger *M. intertransversarius* weight ratio. Larger *M. subvertebralis* weight ratio was also found in the more terrestrial species (Fig. 5). The activity pattern of *M. subvertebralis* during swimming and walking suggests that this muscle functions during lateral bending and/or sagittal flexion (Schilling and Daban 2010). We suggest that the more terrestrial species are equipped with larger *M. subvertebralis*, as this muscle stabilizes the spine, which is a function needed more in a terrestrial environment.

The more aquatic species were characterized by larger lateral hypaxial muscles (*M. obliquus externus*, *M. obliquus externus superficialis*, *M. obliquus externus profundus*, *M. transversus abdominis*, *M. obliquus internus* and *M. transversus abdominis*) (Fig. 4). The lateral hypaxial muscles act during swimming to bend the body synergistically (Bennett *et al.* 2001). The trunk muscles produce lateral bending actively during aquatic locomotion in species with less developed limbs, whereas lateral bending is produced passively by extrinsic limb muscle actions on the trunk via the limb girdles in salamanders (Schilling 2011). Lateral hypaxial muscles act for torsion control during walking (Carrier 1993; Bennett *et al.* 2001). Although lateral hypaxial muscles are employed during swimming and walking, aquatic species seem to rely more on lateral bending for forward movement. Probably because of this, the ratio of lateral hypaxial muscles among trunk muscles is larger in aquatic species. We suggest that the most important factor determining the relative mass of lateral hypaxial muscles is the frequency of use for lateral bending. A smaller weight ratio of *M. rectus abdominis* was found in the more aquatic species

(Fig. 4). Elongated bodies require more muscle force along the ventral contour line (Preuschoft *et al.* 2007); thus, a larger weight ratio of *M. rectus abdominis* is necessary for terrestrial species. Deban and Schilling (2009) suggested that the *M. rectus abdominis* functions to prevent sagittal extension of the trunk caused by the action of the epaxial muscles in salamanders. Because the epaxial muscles (*M. dorsalis trunci*, *M. interspinalis* and *M. intertransversarius*) were also larger in the more terrestrial species (Fig. 5), sagittal extension of the trunk may be larger in these species.

Prezygapophyseal angle of the mid-trunk vertebrae

A larger (more vertical) prezygapophyseal angle was observed in the more terrestrial species (Fig. 6 and Table 4). The zygapophyseal joints control the range of movement along the vertebral column, adjusting direction and range of motion (Boszczyk *et al.* 2001; Hua 2003; Pierce *et al.* 2011).

A more vertical prezygapophyseal angle confines sagittal movement and resists torsional load, whereas a more horizontal prezygapophyseal angle helps horizontal movement and restricts vertical movement (Slijper 1946; Boszczyk *et al.* 2001; Hua 2003; Pierce *et al.* 2011). Notably, vertebra with a more horizontal prezygapophyseal angle, which was typically found in the more aquatic species, connects weakly with the postzygapophysis of the next vertebra and possibly enables more flexible undulatory movement of the trunk. Aquatic species are not required to maintain posture in water because of buoyancy; therefore, strong connections between vertebrae are not required in water. The more vertical prezygapophyseal angle found in terrestrial species allows deep and tight connections between vertebrae from the base of the prezygapophysis. Then, a more vertical prezygapophyseal angle may enable the vertebral column to act as supporting beam against gravity. The prezygapophyseal angle of the aquatic *A. japonicus* was more vertical than that of the semi-aquatic *C. pyrrhogaster* and the terrestrial *H. nigrescens*. We suggest that *A. japonicus*, which is considerably larger than other species, requires more vertical prezygapophyseal angles to maintain their large body.

To conclude, larger lateral hypaxial muscles and more horizontal prezygapophyseal angle of vertebra found in the more aquatic species are related to flexible undulatory locomotion. In contrast, the more terrestrial species are equipped with larger dorsalis and abdominal muscles and a greater vertical prezygapophyseal angle, all contributing to counter gravity during locomotion.

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References

- Antipenkova, T. P. 1994. Axial skeleton. The Siberian Newt (*Salamandrella keyserlingii* Dybowski, 1870). Zoogeography, Systematics, Morphology, pp. 153–158. Nauka, Moscow.
- Ashley-Ross, M. A. 1995. Patterns of hindlimb motor output during walking in the salamander *Dicamptodon tenebrosus*, with comparisons to other tetrapods. – *Journal of Comparative Physiology A* 177: 273–285.
- Azizi, E., Gullis, G. B. and Brainerd, E. L. 2002. Morphology and mechanics of myosepta in a swimming salamander (*Siren lacertina*). – *Comparative Biochemistry and Physiology: Part A, Molecular & Integrative Physiology* 133: 967–978.
- Barclay, O. R. 1946. The mechanics of amphibian locomotion. – *Journal of Experimental Biology* 23: 177–203.
- Bennett, W. O., Simons, R. S. and Brainerd, E. L. 2001. Twisting and bending: the functional role of salamander lateral hypaxial musculature during locomotion. – *Journal of Experimental Biology* 204: 1979–1989.
- Boszczyk, B. M., Boszczyk, A. A. and Putz, R. 2001. Comparative and functional anatomy of the mammalian lumbar spine. – *Anatomical Record* 247: 542–555.
- Carrier, D. R. 1993. Action of the hypaxial muscles during walking and swimming in the salamander *Dicamptodon ensatus*. – *Journal of Experimental Biology* 180: 75–83.
- Deban, S. M. and Schilling, N. 2009. Activity of trunk muscles during aquatic and terrestrial locomotion in *Ambystoma maculatum*. – *Journal of Experimental Biology* 212: 2949–2959.
- Delveve, I., Bem, T. and Cabelguen, J. M. 1997. Epaxial and limb muscle activity during swimming and terrestrial stepping in the adult newt, *Pleurodeles waltl*. – *Journal of Neurophysiology* 78: 638–650.
- Francis, E. T. B. 1934. The Anatomy of the Salamander. Oxford University Press, London.
- Frolich, L. M. and Biewener, A. A. 1992. Kinematic and electromyographic analysis of the functional role of the body axis during terrestrial and aquatic locomotion in the salamander *Ambystoma tigrinum*. – *Journal of Experimental Biology* 162: 107–130.
- Gray, J. 1944. Studies on the mechanics of the tetrapod skeleton. – *Journal of Experimental Biology* 20: 88–116.
- Hilton, W. A. 1948. The vertebrae of Salamanders. – *Journal of Entomology and Zoology* 40: 47–65.
- Hua, S. 2003. Locomotion in marine mesosuchians (Crocodylia): the contribution of the “locomotion profiles”. – *Neues Jahrbuch für Geologie und Palaontologie – Abhandlungen*, 227: 139–152.
- Liem, K. F., Walker, W. F., Bemis, W. E. and Grande, L. 2001. Functional Anatomy of the Vertebrates: An Evolutionary Perspective. Harcourt College Press, Philadelphia, PA.

- Maurer, F. 1892. Der aufbau und die Entwicklung der ventralen Rumpfmuskulatur bei den urodelen Amphibien und deren Beziehungen zu den gleichen Muskeln der Selachier und Teleostier. – *Morphologisches Jahrbuch* 18: 76–179.
- Maurer, F. 1911. Die ventrale Rumpfmuskulatur von Menobranchius, Menopoma und Amphiuma. – *Jenaische Zeitschrift für Medizin und Naturwissenschaft* 47: 1–42.
- Mivart, G. 1870. On the axial skeleton of the Urodela. – *Proceedings of the Zoological Society of London* 1870: 260–278.
- Naylor, B. G. 1978. The Systematics of Fossil and Recent Salamanders (Amphibia: Caudata) –with Special Reference to the Vertebral Column and Trunk Musculature [dissertation]. University of Alberta Press, Edmonton.
- O'Reilly, J. C., Summers, A. P. and Ritter, D. A. 2000. The evolution of the functional role of trunk muscles during locomotion in adult amphibians. – *American Zoologist* 40: 123–135.
- Pierce, S. E., Clack, J. A. and Hutchinson, J. R. 2011. Comparative axial morphology in pinnipeds and its correlation with aquatic locomotory behaviour. – *Journal of Anatomy* 219: 502–514.
- Preuschoft, H., Schulte, D., Distler, C., Witzel, U. and Hohn, B. 2007. Body shape and locomotion in monitor lizards. – *Mertensiella* 16: 59–78.
- Ratnikov, V. Y. and Litvinchuk, S. N. 2007. Comparative morphology of trunk and sacral vertebrae of tailed amphibians of Russia and adjacent countries. – *Russian Journal of Herpetology* 14: 177–190.
- Romer, A. S. 1970. *The Vertebrate Body*, 4th edn. WB Saunders, Philadelphia, PA.
- Schilling, N. 2011. Evolution of the axial system in craniates: morphology and function of the perivertebral musculature. – *Frontiers in Zoology* 8: 4–22.
- Schilling, N. and Daban, S. M. 2010. Fiber-type distribution of the Perivertebral Musculature in *Ambystoma*. – *Journal of Morphology* 271: 200–214.
- Simons, R. S. and Brainerd, E. L. 1999. Morphological variation of hypaxial musculature in salamanders (Lissamphibia: Caudata). – *Journal of Morphology* 241: 153–164.
- Slijper, E. J. 1946. Comparative biologic–anatomical investigations on the vertebral column and spinal musculature of mammals. – *Verhandelingen der Koninklijke Nederlandsche Akademie van Wetenschappen, Amsterdam II* 42: 1–128.
- Worthington, R. D. and Wake, D. B. 1972. Patterns of regional variation in the vertebral column of terrestrial salamanders. – *Journal of Morphology* 137: 257–278.