Locomotion pattern and trunk musculoskeletal architecture among Urodela

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Abstract

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We comparatively examined the trunk musculature and prezygaphyseal angle of mid-trunk vertebra in eight urodele species with different locomotory 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 prezygaphyseal angle for more flexible lateral locomotion. In contrast, the more terrestrial species have an increasingly vertical prezygaphyseal angle to provide stronger column support against gravity. Thus, we conclude trunk structure in urodeles differs clearly according to their locomotory modes.

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Introduction

Urodeles have been studied as model basal tetrapod animals because of their generalized body form and range of locomotory 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 differences in trunk muscles and trunk vertebrae among species with different locomotory 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 Antipenkov (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 vertebral body is defined by the direction and degree of movement (Slijper 1946; Bosczzyk 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 zygapophyseal angle. The objective was to quantify morphological variations in trunk musculature and trunk vertebrae 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*...
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 (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

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

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

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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 vertebral 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 layer 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.
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

![Cross-sections through the mid-trunk of](image)

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

| Table 3 | Muscle weight ratios (%) measured at mid-trunk (mean ± SEM) |
|---------|-----------------------------------------------------------|
| Species | Habitat | *M. dorsalis trunci* | *M. interspinalis* | *M. intertransversarius* | *M. subvertebralis* |
| Siren intermedia | Aquatic | 35.1 ± 0.3 ± | 34.0 ± 0.7 | 24.0 ± 0.2 | 9.2 ± 0.3 |
| Cynops ensicauda | Semi-aquatic | 40.6 ± 1.3 | 40.0 ± 0.7 | 26.0 ± 0.2 | 16.7 ± 0.6 |
| Hynobius lichenatus | Terrestrial | 47.3 ± 1.2 | 51.0 ± 0.3 | 32.0 ± 0.2 | 15.0 ± 1.6 |

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

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

| Species          | Habitat  | Prezygapophyseal angle |
|------------------|----------|------------------------|
| *Siren intermedia* | Aquatic  | 10.8 ± 0.7°C            |
| *Amphiuma tridactylum* | Aquatic  | 14.0 ± 2.6°C            |
| *Necturus maculosus* | Aquatic  | 12.6 ± 4.6°C            |
| *Andrias japonicus* | Aquatic  | 18.6 ± 4.0°C            |
| *Cynops pyrrhogaster* | Semi-aquatic | 17.5 ± 0.5°C        |
| *Hynobius nigrescens* | Terrestrial | 17.7 ± 2.4°C        |
| *Hynobius lichenatus* | Terrestrial | 19.1 ± 2.3°C        |
| *Ambystoma tigrinum* | Terrestrial | 21.6 ± 2.0°C        |

Different superscript letters indicate significant differences (ANOVA and Tukey’s test, *P* < 0.05).
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; Delvove 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 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.
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 restricts torsional load, whereas a more horizontal prezygapophyseal angle helps horizontal movement and restricts vertical movement (Slipper 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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