

学位論文

Adaptation for aquatic and terrestrial environments

by locomotive trunk structure in Urodela

両棲類有尾目の体幹部運動器形態による水陸環境適応

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Adaptation for aquatic and terrestrial environments
by locomotive trunk structure in Urodela

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

In Devonian Period, vertebrates had a significant evolutionary change when the first tetrapod stepped on to the land. During the transition from fish to tetrapod, the structure of their body had to be largely modified for adaptation from water to ground. Body weight of aquatic species may be effectively zero since they are buoyed up by the water, and there is viscosity in water. In contrast, on land, the body is usually held up by developed limbs, and the skeleton and the internal organs have to structurally be modified against the downwards pull of gravity. Aquatic species adapt for the stress of lateral stretching and bending and viscosity of water during swimming whereas terrestrial tetrapod is affected by gravity as main force (Benton, 2005).

The Devonian taxa such as *Metaxygnathus*, *Elginerpeton*, and *Obruchevichthys* fragmentarily shows the close to the evolutionary transitional forms from sarcopterygian fishes to basal tetrapod (Ahlberg, 1995; Robert, 1995; Benton, 2005). *Acanthostega* and *Ichthyostega* are the most completely known Devonian tetrapod. *Acanthostega* and *Ichthyostega* are thought to remain a fish body outline (Benton, 2005). The late Devonian tetrapod used their limbs more in swimming than walking, and they were still aquatic (Benton, 2005). *Pederpes* is the earliest-known tetrapod to the beginning of terrestrial locomotion in the early Carboniferous Period (Clack, 2002). After the Carboniferous, a number of early Permian temnospondyls, were equipped with terrestrially-adapted forms such as *Eryops* which possesses robust limbs and

more massive skeleton than its earlier relatives (Benton, 2005). According to the evolutionary transition from water to ground, they developed limbs and modified vertebra to resist gravity. The primitive amphibian possessed vertebra with undeveloped zygapophysis (Romer, 1970), since the primitive amphibian is thought to spend their life more in water than on land. The structure of vertebra in *Ichthyostega* slightly developed from that of *Crossopterygii*, and it is suggested that vertebral column of *Ichthyostega* did not suitable for sustaining their weight on ground (Romer, 1970). But, the inheritor of *Ichthyostega* was equipped with specialized vertebra with the functional structure of articulation (Romer, 1970). The strong structure of vertebral column constructed by articulated vertebra enables tetrapod to sustain own weight (Romer, 1970; Liem et al., 2001).

From these morphological transitions from fish to terrestrial tetrapod, their locomotive mode is roughly mentioned (Pierce et al., 2013). Certainly the fossil information is helpful, surveying and comparing the structure of living species are necessary to clarify the evolutionary morphological changes of fish to tetrapod. The Living species contribute to reconstruct the structure of fossil species. Tsuihiji (2004) described the ligament system in the neck of the living ratite bird, *Rhea Americana*, which is extant ratite bird for hypothetical reconstruction of the proposed ligament system in *Camarasaurus* and *Apatosaurus* which are extinct dinosaur. Fujiwara (2009) surveyed the orientation of the olecranon as an indicator of the angle of elbow

joint in various extant species and estimated forelimb posture in extinct quadruped species such as *Desmostylus* and *Paleoparadoxia*.

The living Urodela include the species which morphologically resemble the extinct early tetrapod. Urodela possess elongated body and limbs of which lengths are not so different between forelimbs and hind limbs. These features are commonly confirmed in the fossil amphibians as Labyrinthodontia and Lepospondyli. The degree of specialization of external form in Urodela is the lowest among extinct amphibians. Urodela consist of aquatic, semi-aquatic and terrestrial species (Kentwood, 2007). Aquatic species usually possess undeveloped limbs and girdles, and the more terrestrial species are equipped with more developed limbs than that of aquatic species. Therefore, extant Urodela can be considered as model of the early tetrapod to construct the theory of landing of the vertebrates with limbs against gravity.

Urodela species use their trunk undulatory both in swimming and walking (Deban and Schilling, 2009). Aquatic Urodela should be equipped with the trunk structure which is suitable for doing the lateral bending more flexibly against water viscosity. By contrast, terrestrial Urodela have to possess the trunk structure for terrestrial walking and keeping posture and sustaining own weight against gravity, and resisting torsion from ground reaction forces (Carrier, 1993). Then, the structure of trunk of Urodela may vary according to their habitats and

locomotive mode. The locomotive function is morphologically determined by the muscular and skeletal system. Muscles produce locomotive power and help to support the body (Liem et al., 2001). Myomeres remain segmented in trunk muscle in Urodela amphibian for lateral bending. The trunk muscle of fish is constructed from epaxial portion of myomeres and hypaxial portion of myomeres which are segmented. Epaxial muscles are separated whereas hypaxial muscles are segmented and occupied by layer structures (Liem et al., 2001, Naylor, 1978). *M. rectus abdominis* occurs in Urodela (Liem et al., 2001, Naylor, 1978) as one of the derived muscles supporting body. It is suggested that early tetrapod modified their trunk muscles since the structure of trunk muscles differ between living fish and Urodela. To clarify the morphological transitional changes of trunk muscles in early tetrapod, revealing aquatic and terrestrial adaptations of the trunk muscles in living Urodela is needed. The axial skeleton forms the framework of the body and plays a role in the support and movement of the body (Liem et al., 2001). The axial skeleton of Urodela becomes strengthen to support the body against the gravity unlike the fish (Hildebrand and Goslow, 2001). Though amphibians including Urodela have zygapophysis of vertebra which does not exist in typical living fish to strengthen the spine and control its flexibility (Hildebrand and Goslow, 2001), the morphological variations of zygapophysis among aquatic, semi-aquatic and terrestrial species have not been clarified.

To deal with the adaptive way for aquatic and terrestrial environments in Urodela, ontogenetic

changes should be morphologically examined. Urodela include the species which changes their habitats from water to ground by metamorphosis. The life history is thought to be inherited from the common ancestor of living amphibians (Hanken, 1999). Fossil demonstrates that the aquatic larvae show the morphological characteristics of ancient amphibians (Kenwood, 2007). Urodela larvae possess several distinctive characteristics. These include flattened tail fins, external gills, and open-gill slits (Iwasawa and Yamashita, 1991; Kenwood, 2007). Urodela larvae change their aquatic feature at metamorphosis for terrestrial life except paedomorphic species. Urodela larvae do the locomotion of anguilliform and lateral bending of trunk and tail (Hoff et al., 1989; Wassersug, 1989). By contrast, after metamorphosis, juveniles walk on land. To morphologically clarify the adaptive way from water to land in the larvae may reveal the evolutionary strategy of the landing in the early tetrapod. Ontogenetic changes of trunk structure of various species of Urodela have been described in external figure (Iwasawa and Kera 1980), however, the trunk muscle has not been ontogenetically quantified.

The purpose of this study is clarifying the adaptive way for land from water in early tetrapod by characterizing and quantifying morphological variations in trunk structure in salamanders of different ecotypes representing aquatic, semi-aquatic and terrestrial species. First, I compared trunk structure among salamanders of three different habitats by observing and quantifying whole trunk musculature (Chapter 1) and quantifying trunk vertebra (Chapter 2) to clarify the

relationship among trunk structure and habitats and locomotion modes. Based on the result from chapter 1, positional differences of trunk musculature were explored in Chapter 3 to detail the adaptational strategy from water and ground by trunk muscle structure. In Chapter 4, ontogenetic changes of trunk musculature of salamander were surveyed. In the general discussion, the theory was established regarding morphological adaptations of the trunk structure as a locomotive system.

CHAPTER 1

Strategy of environmental adaptations for aquatic and terrestrial environments of trunk muscles in Urodela

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Introduction

Trunk muscle of Urodela has been morphologically studied since trunk is used in undulatory locomotion both in water and ground in Urodela. The activity pattern of hypaxial muscles is measured in *Dicamptodon* (Carrier, 1993) and *Ambystoma tigrinum* (Bennet et al., 2001), and they showed that the pattern during swimming is different from that of during walking. The fiber-type distribution pattern of perivertebral muscles of *Ambystoma maculatum* and *Ambystoma tigrinum* has been reported (Schilling and Deban, 2010) that these tendencies are similar between the two species, but *A. tigrinum* has possessed relatively larger muscles than *A. maculatum*, which may be effected by its digging behavior.

Despite these studies, the morphological differences of trunk musculature among Urodela with different locomotion modes have been investigated in a few. The relationships between ecological habitats and structure of trunk muscles were investigated by quantifying cross-sectional area in mid-trunk by Simons and Brainerd (1999). They suggested that the terrestrial species possess thinner hypaxial muscles since terrestrial behavior is accompanied with reduction on relative body thickness and more dorsal placement of epaxial muscles.

Though the way of quantifying muscle is measuring cross-sectional area in the study, there are some muscles in trunk which are difficult to be quantified by cross-sectional approaches. In electromyogram study, *M. intertransversarius* and *M. interspinalis* play a role in stabilization during swimming and walking (Deban and Schilling, 2009). But these muscles have not been quantified in Urodela by cross-sectional approaches since these muscles are located between vertebrae and ribs. In contrast, weighting muscle is thought to clarify the work of muscle. Cross sectional area determines the force which can be produced by muscle, while the length of the muscle determines the distance through it can be contracted. Then, it is implied that cross sectional area multiplied by muscle length is the work which can be performed by the muscle. Cross sectional area multiplied by length is equal to the volume of the muscle, and volume is proportional to weight. In this study, therefore, muscles were weighed to get the product of its work.

In this chapter, the way of adaptation of trunk muscles for aquatic and terrestrial environment was investigated by observing and weighing trunk muscles.

Materials and methods

Shape and muscle weight ratios of Trunk muscles

Eight species of adult salamanders representing six families and three different habitats

(aquatic, semi-aquatic, and terrestrial) were used in this study (Table 1). These specimens were deposited in The University Museum, The University of Tokyo. Specimens were fixed in straight body position by 10 % formalin and have been maintained in 70 % ethanol solution.

Each trunk muscle was divided as shown in Fig.1. Maurer (1892), Maurer (1911), Francis (1934) and Naylor (1978) were used as references for dividing muscles. 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 using tweezers on the left side of the body in all three species to determine trunk muscle weight, and specimens were observed from the lateral view. During dissection, specimens were kept wet by moistening with water to avoid drying and causing measurement error. Each trunk muscle was weighed using an electronic balance AUW220 Shimadzu co ltd, Kyoto, Japan. The weight ratio of each muscle to the weight of all measured trunk muscles was calculated. For comparing each muscle weight ratio of three species, values for six muscles were grouped in three groups according to these position, running direction and function as follows : *M. obliquus externus superficialis* and *M. obliquus externus profundus*; *M. obliquus internus* and *M. transversus abdominis*; *M. rectus profundus* and *M. rectus abdominis*. Subsequently, cross-sections were

obtained from the middle of the trunk between the pectoral and pelvic girdles on the right side of the body. Images of the lateral view and cross-sections were observed using a microscope with a single-lens reflex camera and adapter (Micronet NY1S, Saitama, Japan).

Statistical tests were done to confirm whether significant difference of muscle weight ratio is shown among species. Homogeneity of variances and means between species were confirmed by ANOVA. When significant differences were identified by ANOVA, differences between species were estimated using Tukey's test.

Results

Observation of lateral view trunk muscles

Fig.2 shows the lateral view of trunk muscles of eight species. Trunk muscles were segmented in myosepta. *M. dorsalis trunci* occupied the bulk of dorsal mass in all species, and the fibers of *M. dorsalis trunci* ran in a longitudinal direction between successive myosepta. *M. interspinalis* ran between spine of vertebra and connected adjacent vertebra in all species. *M. intertransversarius* was located between transverse process, and the direction of muscle fibers was longitudinal. *M. rectus lateralis* was shown in *C. pyrrhogaster*, *C. ensicauda*, and *A. tigrinum* while other species were not equipped with *M. rectus lateralis*. *M. rectus lateralis* was situated on the most external lateral hypaxial muscle along the trunk and the muscle ran

longitudinally between the surface of the edges of ribs.

The separation of lateral hypaxial muscles was different among species. *S. intermedia*, *N. maculosus*, *H. nigrescens*, and *H. lichenatus* possessed *M. obliquus externus* as most superficial layers of the lateral hypaxial muscles. The muscle ran between myosepta craniodorsally to caudoventrally. *A. tridactylum*, *C. pyrrhogaster*, *C. ensicauda*, and *A. tigrinum* possessed *M. obliquus externus superficialis* and *M. obliquus externus profundus* instead of single *M. obliquus externus*. *M. obliquus externus superficialis* was the most superficial layer of lateral hypaxial muscles, and *M. obliquus externus profundus* lay under *M. obliquus externus superficialis*. Though fibers of these obliquus muscles ran caudoventrally between myosepta, the direction of the fibers of *M. obliquus externus superficialis* was more longitudinal than that of *M. obliquus externus profundus*.

The inner lateral hypaxial layers were composed by *M. obliquus internus* and *M. transversus abdominis*. *H. nigrescens* and *H. lichenatus* did not have *M. obliquus internus*, and possessed only *M. transversus abdominis* as an inner lateral hypaxial layer. Other species were equipped with two inner lateral hypaxial layers. The fibers of *M. obliquus internus* ran caudodorsally in myosepta. *M. transversus abdominis* ran caudodorsally, and the fiber angle was more longitudinal than that of *M. obliquus internus*. *M. transversus abdominis* verged on peritonea extends from the shoulder to the pelvic region.

M. rectus abdominis of all species constructed the most ventral part of the body wall, with muscle fibers running in a sagittal direction, and ran from the anterior edge on the pelvis to the sternal cartilage. *A. tridactylum*, *S. intermedia* and *N. maculosus* possessed *M. rectus abdominis* which was not separated from lateral hypaxial muscles. *H. nigrescens* and *H. lichenatus* was equipped with *M. rectus profundus*, which was underneath of *M. rectus abdominis* and ran in sagittal direction.

Cross-sectional observations

Fig.3 shows the relative contribution of the cross-sectional areas of the trunk muscle. The number of lateral hypaxial muscle layers was different among species. Two layers (*M. obliquus externus* and *M. transversus abdominis*) composed the lateral hypaxial muscles of *H. nigrescens* and *H. lichenatus*. The lateral hypaxial muscles of *S. intermedia* and *N. maculosus* were constructed from the three layers (*M. obliquus externus*, *M. obliquus internus*, and *M. transversus abdominis*). The four layers (*M. obliquus externus superficialis*, *M. obliquus externus profundus*, *M. obliquus internus*, and *M. transversus abdominis*) composed the lateral hypaxial muscles of *A. tridactylum*, *C. pyrrhogaster*, *C. ensicauda* and *A. tigrinum*. Thicker lateral hypaxial muscles were shown in *S. intermedia*, *A. tridactylum* and *N. maculosus*.

Relatively thinner lateral hypaxial muscles were observed in other five species. Though *M. rectus abdominis* of *S. intermedia*, *A. tridactylum*, *N. maculosus* was not differentiated from the lateral hypaxial muscles, the *M. rectus abdominis* of *C. pyrrhogaster*, *C. ensicauda*, *H. nigrescens*, *H. lichenatus* and *A. tigrinum* was separated from the lateral hypaxial muscles. *H. nigrescens* and *H. lichenatus* possessed *M. rectus profundus*. *M. rectus lateralis* was seen in *C. pyrrhogaster*, *C. ensicauda* and *A. tigrinum*.

Muscle weight ratios

Muscle weight ratios are shown in Table 2 and Fig.4. Considerable variations were found in the muscle ratio among species. The *M. dorsalis trunci* weight ratio was smaller in the more aquatic species. *M. dorsalis trunci* weight ratio of *S. intermedia*, *A. tridactylum*, *N. maculosus* was lower than 36 %. In contrast, *M. dorsalis trunci* weight ratio for *H. nigrescens*, *H. lichenatus* and *A. tigrinum* was more than 45%. *M. interspinalis* occupied a smaller percentage in the more aquatic species than in the more terrestrial species. In *S. intermedia*, *A. tridactylum* and *N. maculosus*, *M. interspinalis* made up less than 4 % of total muscle weight. *H. lichenatus* and *A. tigrinum* possessed significantly larger *M. interspinalis* weight ratio which is over 5 % than in the other species. The more terrestrial species had larger weight ratio of *M. intertransversarius*, whereas the weight ratio of *M. intertransversarius* for the other species

were fewer than 3 %. The more terrestrial species possessed larger weight ratio of *M. subvertebralis*. The value of *H. nigrescens*, *H. lichenatus*, and *A. tigrinum* was over 14 %. Significant differences in the weight ratio of *M. obliquus externus* were shown among species. *M. obliquus externus* weight ratio for aquatic species was significantly larger than that for the semi-aquatic and terrestrial species. *M. obliquus externus* weight ratio was over 20 % for aquatic species. Larger weight ratio of *M. transversus abdominis* was revealed in the more aquatic species significantly. The weight ratio of *M. transversus abdominis* was over 25 % for aquatic species. The more terrestrial species had larger weight ratio of *M. rectus abdominis*. Aquatic species possessed under 5 % of *M. rectus abdominis* weight ratio, and semi-aquatic species had almost 9 % of *M. rectus abdominis* weight ratio, and terrestrial species involved over 12 % of *M. rectus abdominis* weight ratio.

Discussion

Observation of trunk muscles

Interspecific differences of trunk muscle were shown in lateral views (Fig.2) and cross-sectional views (Fig.3). From the cross sectional analysis, thicker hypaxial muscles were found in the fully aquatic species and thinner hypaxial muscles were shown in the more terrestrial species (Fig.3). The present result was similar to that of previous comparing study of

S. lacertina, *A. tridactylum*, *Cryptobranchus alleganiensis*, and *A. tigrinum* that aquatic species possessed thicker lateral hypaxial muscles (Simons and Brainerd, 1999). This tendency within Urodela is parallel to the general macroevolutionary tendency of the increased dorsal placement of epaxial muscles according to terrestrial transition from teleosts to lizards (Romer, 1970). It is suggested that the locomotive function of the lateral hypaxial muscles transfer into limbs, resulting in a decrease in lateral hypaxial muscles in the more terrestrial species with more robust limbs. Though the number of lateral hypaxial layers differed among species (Fig.2, 3), strong correlation between number of lateral hypaxial muscles and locomotion mode was not found. These findings were consistent with those of the comparing study of Simons and Brainerd (1999), which showed that the number of lateral hypaxial layers is not strongly associated with predominant locomotive mode and ecological habitats.

M. rectus abdominis of *C. pyrrhogaster*, *C. ensicauda*, *H. nigrescens*, *H. lichenatus*, and *A. tigrinum* was clearly independent from the lateral hypaxial muscles though in *S. intermedia*, *A. tridactylum* and *N. maculosus*, *M. rectus abdominis* was not separated from the lateral hypaxial muscles (Fig.2). In general, differentiated muscles have more specialized function than muscles with simplified structure (Liem et al., 2001). Therefore, it is suggested that the more terrestrial species need a more specialized function of *M. rectus abdominis*, which helps Urodela to maintain posture and sustain weight. Since species with elongated trunk require more muscular

force along the ventral contour line (Preuschoft et al., 2007), *M. rectus abdominis* which lies in the most ventral position was larger in the more terrestrial species. *M. rectus abdominis* plays a main role in counteracting sagittal extension of the trunk caused by the epaxial muscles (Deban and Schilling, 2009; Schilling, 2011) and by gravity (Preuschoft et al., 2007). Then, the more terrestrial species may need separated *M. rectus abdominis*.

Muscle weight ratios

During aquatic swimming, the salamanders use most axial muscles for lateral bending, modulating body stiffness, and/or stabilization of trunk (Schilling, 2011). In contrast, during terrestrial locomotion, axial muscles also stabilize the body against gravitational force (Schilling, 2011). In this study, larger *M. dorsalis trunci* was shown in the more terrestrial species. During swimming and walking, the *M. dorsalis trunci* bend laterally (Frolich and Biewner, 1992; Delvolve et al., 1997; Deban and Schilling, 2009). In addition, during walking, *M. dorsalis trunci* prevents trunk from sagging and torsion and would also increase trunk stiffness against gravity (Deban and Schilling, 2009). O'Reilly et al. (2000) also proposed a stabilizing function of epaxial muscles. Although *M. dorsalis trunci* is used in lateral bending and stabilization of trunk during both swimming and walking, the more terrestrial species may exploit more *M. dorsalis trunci* against gravity for stabilizing. The more terrestrial species possessed larger *M.*

dorsalis trunci in this study, since *M. dorsalis trunci* may ward off the effect of gravitational forces in addition to lateral bending in the more terrestrial species.

The larger *M. interspinalis* was found in the more terrestrial species (Fig. 4). It is suggested that the function of this muscle is vertical stabilization of body rather than lateral bending (Deban and Schilling, 2009; Schilling and Deban, 2010). During walking, *M. interspinalis* produces force for the movement of the trunk and stabilizes the intervertebral joint to strengthen joints of vertebrae (Deban and Shilling, 2009). The vertebral column acts as a beam that supports body weight against gravitational forces and transfer weight to the girdle and appendages in terrestrial species (Liem et al., 2001). Therefore, it is considerable that *M. interspinalis* is larger in the more terrestrial species since the more terrestrial species may employ more *M. interspinalis* on ground.

M. intertransversarius was larger in more terrestrial species (Fig. 4). *M. intertransversarius* is between transversus process of vertebra. Though the function of *M. intertransversarius* has not been clarified, considering its position, *M. intertransversarius* acts as ensuring spinal integrity in a similar function as *M. interspinalis*. The more terrestrial species exhibited larger *M. intertransversarius* weight ratio (Table 2, Fig. 4) since the more terrestrial species use *M. interspinalis* to stabilize the spine on ground. Larger *M. subvertebralis* was shown in the more terrestrial species (Fig. 4). The activity of *M. subvertebralis* from electromyogram study

suggested that this muscle functions as subunits that can stabilize and mobilize the trunk as well as modulate of body stiffness (Schilling and Deban, 2010). Because of the role of its stabilization, the more terrestrial species are equipped with *M. subvertebralis*.

Larger lateral hypaxial muscles (*M. obliquus externus*, *M. obliquus externus superficialis*, *M. obliquus externus profundus*, *M. obliquus internus*, *M. transversus abdominis*) were shown in the more aquatic species (Fig. 4). The lateral hypaxial muscles control torsion and stabilize body (Carrier, 1996; Bennet et al., 2001; Deban and Schilling, 2009). Lateral bending was suggested to be actively produced by trunk muscles to facilitate the placement of the feet; in contrast, lateral bending may be produced passively by extrinsic limbs muscle actions on the trunk via the limb girdle (Schilling, 2011). Though lateral hypaxial muscles are used during both swimming and walking, aquatic species seem to more depend on lateral bending for the lateral hypaxial muscles. Since aquatic species need to resist water viscosity during swimming, and aquatic species have less developed limbs, the ratio of lateral hypaxial muscles is larger in aquatic species. It is suggested that the more aquatic species have larger lateral hypaxial muscles for powerful lateral undulatory swimming though lateral hypaxial muscles are also utilized for stabilization and torsion control on ground.

The more terrestrial species was equipped with larger *M. rectus abdominis* (Fig. 4). Larger weight ratio of *M. rectus abdominis* may be necessary for terrestrial locomotion since elongated

body requires more muscle force along ventral contour line (Preuschoft et al., 2007). *M. rectus abdominis* plays a role in preventing sagittal extension of the trunk caused by the action of the epaxial muscles (Deban and Schilling, 2009) and by the gravity (Preuschoft et al., 2007). Since the epaxial muscles were larger in the more terrestrial species in this study (Fig. 4), and gravitational forces are born to more terrestrial species, I suggest that the more terrestrial species possessed larger *M. rectus abdominis*.

CHAPTER 2

Relationships between prezygapophyseal angle of vertebra
and their habitat in Urodela

CHAPTER 2: Relationships between prezygapophyseal angle of vertebra and their habitat in

Urodela

Introduction

Trunk vertebra of Urodela is suggested to morphologically vary between different locomotion modes. Short descriptions of vertebra in Urodela have been done by Hilton (1948), Teege (1957), and Antipenkova (1994). Mivart, (1870), Teege (1957), Worthington and Wake (1972), and Ratnikov and Litvinchuk (2007) have undertaken comparative analyses of vertebral morphology of various species. It has been reported that the zygapophyseal angle of vertebra adjusts the direction of movement and the degree of movement (Slijper, 1946, Boszczyk et al., 2001, Hua. 2003). Since the degree of zygapophyseal angle depends on the length of articulation of the vertebra, zygapophyseal angle is expected to reflect the strength of the vertebral column. A more vertical prezygapophyseal angle restricts sagittal movement of trunk and helps trunk to resist torsional load, while a more horizontal prezygapophyseal angle restricts horizontal movements and allows resisting ventral motion (Sliper, 1946; Boszczyk et al., 2001; Hua, 2003; Pierce et al., 2011). Though vertebral structure has been studied in many authors, zygapophyseal angle of Urodela has not been quantified, and the relationships between zygapophyseal angle and locomotion mode in Urodela have not been clarified. In this chapter,

in order to explore the relationships between skeletal structure and locomotion modes in Urodela, prezygapophyseal angle of vertebra was quantified.

Materials and Methods

Eight species of adult salamanders representing seven families and three different habitats (aquatic, semi-aquatic, and terrestrial) were used in this study (Table 3). Mid-trunk vertebra was scanned by micro-CT (R_mCT[®], Rigaku, Tokyo, Japan) at Department of Oral and Maxillofacial Radiology, Nihon University School of Dentistry, Tokyo, Japan. I-View-R (Rigaku Co.,Tokyo, Japan) was used for image processing. Prezygapophyseal angle was measured from scanning image (Fig. 5). Bone specimens were made by large specimens (*Amphiuma tridactylum* and *Andrias japonicus*), and prezygapophyseal angles were measured from bone specimens. To clarify the differences of habitats of species, averages of prezygapophyseal angles in each habitat were calculated. Homogeneity of variances and means of each item between species and averages in each habitat group were confirmed by ANOVA. When significant differences were identified by ANOVA, differences between species were estimated using Tukey's test.

Results

The results of measurements of prezygapophyseal angle of mid-trunk vertebra are shown in Table 4 and Figure 6. Interspecific differences were shown in prezygapophyseal angle. More aquatic species tended to possess smaller prezygapophyseal angle than that of more terrestrial species while significant difference were not clear interspecifically. Prezygapophyseal angle of *S. intermedia* was the smallest angle that was 10.8°. In aquatic species, prezygapophyseal angles were smaller than 15° except of that of *A. japonicus*. Prezygapophyseal angles of terrestrial species were larger than 18°.

The averages of prezygapophyseal angles in each habitat are represented in Figure 7 and Table 5. Aquatic group possessed smaller prezygapophyseal angle than in terrestrial group significantly. Prezygapophyseal angle in terrestrial group was 19.5°; in contrast, aquatic group possessed 14.0° of prezygapophyseal angle. Semi-aquatic group which prezygapophyseal angle was 17.5° did not show significant differences with aquatic species, and with terrestrial species.

Discussion

In the more aquatic species, a more horizontal prezygapophyseal angle was observed (Table 4, 5, Fig. 6, 7). The zygapophyseal joints control the range of movement along the vertebral column, and adjust the direction of movement and range of motion (Boszczyk et al., 2001; Hua 2003; Pierce et al., 2011). A more horizontal prezygapophyseal angle restricts movements to the

horizontal plane and helps to resist ventral shear, whereas a more vertical prezygapophyseal angle restricts movement to the sagittal plane and helps to resist torsional loads (Sliper, 1946; Boszczyk et al., 2001; Hua, 2003; Pierce et al., 2011). Since the base of more horizontal prezygapophysis cannot connect with the base of postzygapophysis of adjacent anterior vertebra, this structure enables their trunk to locomote undulatory more flexibly in more aquatic species. If torsional load or vertical load is applied to the articulate of vertebra of aquatic species, vertebral column cannot maintain the connection and the joint structure may be broken. In water, since aquatic species need not maintain their posture because of buoyancy, strong connections between vertebrae are not required in water, and the flexibility of vertebral column prevails rather than the strength of vertebral column. By contrast, the more vertical prezygapophyseal angle shown in the more terrestrial species allows deep and tight connections between vertebrae from the base of the prezygapophysis. A more vertical prezygapophyseal angle may enable the vertebral column to act as a stronger supporting beam against gravity and resist torsion since the base of prezygapophysis connects these of adjacent vertebra against loading stress. The prezygapophyseal angle of aquatic *A. japonicus* was more vertical than that of the semi-aquatic *C. pyrrhogaster* and the terrestrial *H. nigrescens*. It is suggested that *A. japonicus* which possess larger body size than other species requires more vertical prezygapophyseal angles to maintain their large body. In addition, since *A. japonicus* is equipped with large body, the length of

anteroposterior projection of the zygapophyses of *A. japonicus* is long. The length of anteropostirior projection of the zygapophyses has been shown to have a relationship with the degree of movement at the joint, for example, longer distance permits a greater range of motion (Pierce et al.,2012). As longer zygapophyseal length enables *A. japonicus* to swim undulatory with larger range, *A. japonicus* may need more vertical prezygapophyseal angle of vertebra for preventing the joint from separating against stronger and larger lateral bending. Further study is needed about the relationship between zygapophyseal length and locomotion and about the effect of the size of specimens on the structure of vertebra.

CHAPTER 3

Positional strategy of trunk muscles among aquatic, semi-aquatic,
and terrestrial species

CHAPTER 3: Positional strategy of trunk muscles among aquatic, semi-aquatic, and terrestrial species

Introduction

The structure of trunk as locomotor apparatus of early tetrapod may transition from water to ground according to their changing habitats. Whole trunk muscle structure of modern Urodela which resemble from early tetrapod morphologically was different quantitatively and qualitatively among aquatic, semi-aquatic, and terrestrial species as shown in chapter 1. In chapter 1, it is showed that the more aquatic species possessed larger lateral hypaxial muscles for undulatory swimming, and the more terrestrial species were equipped with larger dorsalis muscles and abdominal muscle for keeping posture and sustaining own weight. The differences between aquatic and terrestrial habitats are in buoyancy and in gravity. The degree of develop of limbs differs among aquatic, semi-aquatic and terrestrial species. It is suggested that composition rate of trunk muscles differs according to position of trunk by the distance from limbs and the effect of gravity. To clarify detailed environmental adaptation of the trunk muscles, the average of weight ratios of trunk muscles is not enough. The study of positional differences of the trunk muscles is needed.

The positional differences of the trunk structure may be affected by the usage of trunk, and the stress for trunk. Though all Urodela have elongated body, the length of trunk and the degree of

development of limbs vary among species. Anguilliform locomotion is the mode of undulatory swimming used by elongate vertebrates (Gray, 1933; Andrew, 1977; Wardle et al., 1995; Gary, 1996). Sirenids salamanders swim by propagating undulatory waves posteriorly along the body (Gary, 1997). Then, anguilliform swimmers may use their whole trunk. In contrast, in terrestrial quadrupedal vertebrate with elongated body, stress differently occurs by trunk positions. Higher compressive stress occurs near the limbs (Bianca et al., 2013). Monitor lizard with elongated body like salamander resting quadrupedally on two pairs of limbs is owed stresses for vertical and ventral direction (Preuschoft et al., 2007). Preuschoft et al. (2007) showed that types of stress (tension or compression) and the amount of stress varied among body positions.

The past study of positional differences of trunk muscle is only about fiber type distribution of perivertebral muscles of the two terrestrial species, *Ambystoma tigrinum* and *Ambystoma maculatum* (Schilling and Deban, 2010). They found that no major changes along the body axis of the composition or distribution of fiber types of perivertebral muscle appeared in either species. Though there were no positional differences of fiber type distribution of perivertebral muscles among both terrestrial species (*Ambystoma tigrinum* and *Ambystoma maculatum*) (Schilling and Deban 2010), positional differences of all trunk muscles have not been quantified. Moreover, the pattern of positional differences among species with different habitats has remained unclear. It is considerable that the patterns of positional differences vary according to

habitats and locomotion patterns since their usage of trunk seem to be decided by environment. To establish the theory of environmental adaptation for land from water in early tetrapod, comparing positional differences of trunk muscles among aquatic, semi-aquatic and terrestrial species is needed. In this chapter, positional differences of trunk musculature were compared among Urodela of different habitats to clarify the way of environmental adaptation of trunk muscles by positions.

Material and method

Six species of adult salamanders representing five families and three different habitats (aquatic, semi-aquatic, and terrestrial) were used in this study (Table 6). These 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 maintained in 70% ethanol solution.

Cross-sections were obtained from the anterior, middle and posterior positions (Fig. 8). Anterior part was got in behind pectoral girdle. Middle part was between the pectoral and pelvic girdles. Posterior part was forward the pelvic girdle. Cross-sectional images were recorded using a single-lens reflex camera. Dorsalis muscles, lateral hypaxial muscles and abdominal muscle were examined (Fig.8). Muscle group areas were measured on the image of cross-section by using graphic software, Photoshop CS5. The area ratio of each muscle group to

the area of all trunk muscles in one section was calculated.

Statistical analyses were performed to confirm significant differences in the muscle area ratios among three positions. 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

The patterns of positional differences of area ratios of trunk muscle group differed among species (Table 7, Fig. 9). Positional differences of the trunk parts were smaller within aquatic species (Table 7, Fig. 9). In *S. intermedia*, dorsalis muscles were around 53 % in all parts. *S. intermedia* possessed almost 43 % of lateral hypaxial muscles, and around 3 % of abdominal muscle in all parts. No significant differences were shown among three parts in dorsalis muscles and lateral hypaxial muscles in *A. tridactylum*. Though significant differences between anterior and middle part were shown in abdominal muscle in *A. tridactylum*, abdominal muscle was from 3.2 to 3.9 % in *A. tridactylum*. Significant differences among three parts were not shown in dorsalis muscles in *C. pyrrhogaster* (Table 7, Fig. 9). In middle part, *C. pyrrhogaster* was equipped with larger lateral hypaxial muscles than in anterior and posterior parts. Anterior part of trunk of *C. pyrrhogaster* had significantly smaller abdominal muscle than in middle and

posterior parts. Larger dorsalis muscles were shown in middle part than in anterior and posterior parts which were under 56 % in *C. ensicauda*. In *C. ensicauda*, larger lateral hypaxial muscles which were over 37 % lay in anterior and posterior parts than in middle part (Table 7, Fig. 9). Abdominal muscle was significantly smaller in anterior and posterior parts than in middle part in *C. ensicauda*. *H. nigrescens* possessed larger dorsalis muscles in middle part which was over 67 % than in anterior and posterior parts. Larger lateral hypaxial muscles were observed in anterior and posterior parts which were over 30 % than in middle parts in *H. nigrescens* significantly (Table 7, Fig. 9). Larger abdominal muscle was shown in middle part which was over 15 % than in anterior and posterior parts in *H. nigrescens*. *A. tigrinum* was equipped with larger dorsalis muscles in middle part which was over 73 % than in anterior and posterior parts significantly. Lateral hypaxial muscles were larger in anterior and posterior parts than in middle part in *A. tigrinum* (Table 7, Fig. 9). Larger abdominal muscle was shown in middle part which was over 12 % than anterior and posterior parts in *A. tigrinum*.

Discussion

The patterns of positional differences of the area ratios of trunk muscles differed among species in this study. In the more aquatic species, positional differences were smaller than in the more terrestrial species (Table 7, Fig. 9). In contrast, in the more terrestrial species, middle part

of trunk was equipped with larger dorsal and abdominal muscles than in anterior and posterior parts (Table 7, Fig. 9). The tendency is thought to reflect their habitats and locomotion mode.

Aquatic species used in this study possess elongated body and less-developed limbs, and they are anguilliform swimmers which use their trunk undulatory. Urodela swim by posteriorly traveling wave (Deban and Schilling, 2009). Gary (1996) mentioned that sirenid salamander swims by propagating undulatory waves along the body. Then, it is suggested that aquatic species may use their whole trunk for lateral bending. Aquatic or semi-aquatic species of salamanders also utilize limb-based locomotion, aquatic-walking, against the substrata in water (Brand, 1996; Azizi and Horton, 2004) in addition to main trunk-based locomotion. Though ground reaction forces occur (Carrier, 1993) and the stress produced from limb insertion to trunk (Preuschoft et al., 2007) during terrestrial walking (Carrier, 1993), ground reaction forces may be decreased or effectively zero by buoyancy during aquatic walking. O'Reilly et al (2000) mentioned that limbs with elongate salamanders such as aquatic *Siren* and *Amphiuma* are functionally ineffectual. *Siren* lack hind limbs and their front limbs are reduced, and *Amphiuma* are equipped with greatly reduced limbs (Kentwood, 2007). Therefore, it is postulated that minor positional differences of trunk of aquatic species were shown in this study (Fig. 9) since whole trunk may be used for anguilliform swimming and the effect of limbs for trunk structure may be functionally small.

By contrast, in terrestrial species, occurring stress differs according to trunk position (Preuschoft et al., 2007) (Fig. 10). The more terrestrial species possess larger limbs for sustaining and moving body and larger pectoral and pelvic girdles for transferring weight to the limbs and receiving the thrusts of the limbs (Liem et al., 2001). In the anterior and posterior parts near the limbs in the terrestrial species, dorsal side is owed tensile stress (Preuschoft et al., 2007) (Fig. 10 A, C). Though muscles of dorsal side in anterior and posterior parts are needed for owning tensile stress, the effect of compressive stress in middle-dorsal part may be larger than that of tensile stress for that near the limbs. Since limbs have the function of sustaining own weight, the duty for stabilizing of body in dorsal side may be smaller in anterior and posterior parts which are near the limbs. Then, smaller dorsalis muscle in anterior and posterior parts were shown than in middle-dorsal part in the more terrestrial species in this study. In ventral side of anterior and posterior parts, compression stress occurs (Preuschoft et al., 2007) (Fig. 10 A, C). Highest compressive stress occurs at insertions of the limbs, and it spreads dorsally across the midline of the trunk in elongated body species on ground (Bianca et al., 2013). Near the limbs, Urodela possess pectoral and pelvic girdles. The girdles of terrestrial vertebrate including Urodela transfer body weight to the limbs and receive the thrusts of the legs (Liem et al., 2001). Since girdles receive compression stress from limbs, compressive stress may decrease for ventral side in anterior and posterior part of trunk. Therefore, abdominal

muscle in ventral side in anterior and posterior parts was smaller than that of in middle part in this study since the stress in anterior and posterior parts of ventral side is smaller than that of in middle part of ventral side.

By contrast, in the middle part of trunk, compressive stress occurs in dorsal side (Preuschoft et al., 2007) (Fig. 9 B). Dorsal concave occurs by own weight in the middle part of trunk since middle part of trunk is suspended as a beam between limbs. One of the functions of dorsalis muscles is stabilizing the body (O'Reilly et al., 2000). Since dorsalis muscles is needed in middle part of trunk for preventing trunk from dorsal concave against gravity, dorsalis muscles were larger in middle part than in anterior and posterior parts in this study (Fig. 8). In middle-ventral part of the trunk, tensile stress occurs in abdominal muscle to support own weight (Preuschoft et al., 2007) (Fig. 9 B). Terrestrial species have to pull up their trunk from ground and bear own body against gravity. In the middle part, ventral side of trunk has to sustain own weight by itself without support of the limbs which elevate the body from ground. In the middle part of the trunk, since larger abdominal muscle may act as a main supporter of body weight, abdominal muscle was larger in middle part of trunk than in anterior and posterior parts (Fig. 8).

Early tetrapod modified their trunk structure by landing event. In this chapter, the positional strategy of trunk muscles which is required for landing from water was morphologically

revealed. Aquatic species possessed less-differences trunk muscles along the elongated body for anguilliform swimming; whereas the structure of muscle of trunk of the more terrestrial species differ among positions for terrestrial locomotion against gravity.

CHAPTER 4

Ontogenetic changes of trunk muscles in the terrestrial Urodela,

Hynobius nigrescens

CHAPTER 4: Ontogenetic changes of trunk muscles in the terrestrial Urodela, *Hynobius nigrescens*

Introduction

Ancient amphibians reached land from water. Urodela contain the species which ontogenetically change their habitats from water to ground (Duellman and Trueb, 1994) as the evolutionary landing of ancient amphibians. According to the ontogenetic transition of habitats, they change locomotive mode. Urodela larvae do the swimming locomotion of anguilliform and lateral bending of both trunk and tail in water (Hoff et al., 1989; Wassersug, 1989). In contrast, after completion of metamorphosis, juveniles walk on ground. To clarify the way of adaptation from water to ground in early tetrapod by trunk which acts as main part of locomotive structure, revealing ontogenetic morphological changes of trunk muscle in Urodela is needed. Though environmental adaptation for aquatic and terrestrial habitats of trunk muscles in Urodela is phylogenetically clarified in chapter 1, the way of transitional adaptation from water to ground has not been ontogenetically quantified.

By ontogenetic transition of habitats from water to ground, the trunk as locomotive structure may change for swimming to walking in Urodela. Developmental changes of external figure have been studied in various species in Urodela (Iwasawa and Kera, 1980). In Japanese species, *Cynops pyrrhogaster* (Oyama, 1930), *Andrias japonicus* (Kudo, 1938), *Hynobius nigrescens*

(Usui and Hamazaki, 1939; Iwasawa and Yamasita, 1991), and *Onychodactylus japonicus* (Iwasawa and Kera, 1980) have been described. About inner structure, developmental changes of trunk musculature of Urodela have been examined by Maurer (1892) and Fujimoto (1960). Fujimoto (1960) described ontogenetic changes of trunk muscles in *Hynobius nebulosus*. Fujimoto (1960) showed *M. obliquus internus* occurs ventrally from the inner ridge of growth girdle of ventral trunk muscle in *H. nebulosus*. *M. obliquus externus* is formed along the end of ventral surface of *M. obliquus internus* dorsally, and *M. rectus abdominis* appears ventrally. After these muscles developed, *M. subvertebralis*, and *M. transversus abdominis* appeared. Regardless of these studies of ontogenetic changes of trunk structure of Urodela, the theory of the way of ontogenetic adaptation for water to ground ontogenetically has not been established. Furthermore, trunk muscles have not been functional-morphologically quantified.

In this chapter, therefore, ontogenetic changes of trunk structure of *Hynobius nigrescens* was observed and quantified. The aim of this chapter is clarifying the way of adaptation of locomotive structure for water to ground in early tetrapod by ontogenetically revealing the morphological strategy of the environmental adaptation of trunk muscles in living Urodela.

Material and Methods

Egg batch of *Hynobius nigrescens* was collected in Niigata prefecture on April, 2012. Egg

batch was put on aquarium with aeration. After hatching, larvae were maintained until their completion of metamorphosis to obtain larvae of different developmental stages. Three samples were randomly captured in the six developmental stages (Table 8). The earliest stage used in this study was st 38 which is gill formation III when the gill buds and balancers elongate. The next developmental stage used in this study was st 50 which is digital differentiation III when the balancers disappear and first and second finger primordial develop patently. The third developmental stage used in this study stage was st 58 which is digital differentiation VI, when the fourth toe is clearly recognized. The fourth developmental stage used in this study was st 63A which is full-grown larva I when the membrane between each toe disappears. The fifth developmental stage used in this study in this study was st66 which is disappearance of fin II when dorsal fin as far back as hind limbs regresses, and small gill pieces remain present. The last developmental stage used in this study was st68 which is completion of metamorphosis, when the gills and tail fin completely disappear and the eyeball protrudes. These developmental stages were followed by Iwasawa and Yamashita (1991). Samples were fixed in a straight body position in 10% formalin and were maintained in 70% ethanol solution. These specimens were deposited at The University Museum, The University of Tokyo.

Trunk muscles were laterally observed by dissection. The following groups of trunk muscles were examined in this study: dorsalis muscles, lateral hypaxial muscles, and abdominal muscle.

During dissection, specimens were kept wet by moistening with water to avoid drying and causing measurement error. Each trunk muscle group was weighed using an electronic balance AUW220 (Shimadzu co ltd, Kyoto, Japan). The weight ratio of each muscle group to the weight of all measured trunk muscles was calculated.

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

Result

Observation of trunk muscles

Trunk muscles were developed and changed morphologically according to growth (Fig. 11). In st 38, immediate hatching, they possessed single thick dorsalis muscle and single thick *M. ventralis* (Fig.11 A). In st 50, when first and second finger primordial develop patently, thin *M. transversus abdominis* of which fibers ran caudodorsally was developed from *M. ventralis* and ventrally enlarged (Fig.11 B). The dorsalis muscles were segmented by myosepta as also observed in st 38. When hind limbs were revealed and forelimbs were developed on the st 58, thin *M. obliquus externus* of which fibers ran caudoventrally was shown from the edge of

abdominal contour line of *M. transversus abdominis*. From the ventral edge of *M. obliquus externus*, the direction of fibers of *M. obliquus externus* became parallel to the sagittal line (Fig.11 C). In st 63 A, when the membrane between each toe disappears, *M. obliquus externus* dorsally developed to the level of lateral line (Fig.11 D). Thin *M. rectus abdominis* occurred in the ventral edge of trunk (Fig.11 D). In st 66 when fin II disappears, *M. rectus abdominis* expanded and increase its thickness, and was obviously separated from the fibers of lateral hypaxial layers (Fig.11 E). In st 68, after metamorphosis and landing, *M. rectus abdominis* became thicker and enlarged (Fig.11 F).

Muscle weight ratios of trunk

Muscle group weight ratios are represented in Table 9 and Fig. 12. Ontogenetic changes were found in the muscle group ratio among stages. Muscle weights ratio of dorsalis muscles increased according to growth from 52% in st 38 to 61% in st 68 significantly (Table 9, Fig. 12). Lateral hypaxial muscles decreased the weight ratio by growth (Table 9, Fig. 12). Though in st 38, 50, 58, they possessed over 40% of the weight ratio of lateral hypaxial muscles, they had under 30% of the weight ratio of the lateral hypaxial muscles after st 66. The weight ratio of abdominal muscle increased according to growth significantly (Table 9, Fig. 12). In st 38 and 50, they did not possess abdominal muscle and the percentage of abdominal muscle was zero. From

st 58 to 68, muscle weight ratio of abdominal muscle increased from 3.0 to 16.5 %.

Discussion

Observation of trunk muscles

Trunk muscles ontogenetically developed (Fig. 11). In the beginning of growth, the number of lateral hypaxial layer was only one, and the layer was thick (Fig. 11 A). Typical fish possesses thick trunk muscle divided into epaxial and hypaxial parts, by myosepta, but does not show layer structure (Liem et al., 2001). Since locomotion mode of laevae of *H. nigrescens* is swimming and they does not possess limbs in the beginning of growth, they had single thick ventral muscle as lateral hypaxial muscle for undulatory swimming like typical fish. In later stages of development, the thickness of *M. ventralis* decreased and thin layer of *M. transversus abdominis* was developed from the *M. ventralis* (Fig.11 B). In the st 58, thin layer of *M. obliquus externus* appeared as one of the lateral hypaxial muscles (Fig. 11 C). The number of layer of the lateral hypaxial muscles increased from one to two, and the two lateral hypaxial muscles were thinner than *M. ventralis* which appears in the early stages of development (Fig. 11 C). When they land on ground, they need to resist torsion in addition to lateral bending. Since resisting torsion may need two crossed lateral hypaxial layers, the number of the lateral hypaxial muscles increased from one to two by growth. Furthermore, body wall may be

strengthened like chipboard by crossed direction of running fibers in the two lateral hypaxial layers. In the st 58 when the fourth toe is clearly recognized, the direction of fibers of lateral hypaxial muscle became longitudinal in the ventral edge of trunk (Fig.11 C). After st 58, *M. rectus abdominis* grew and enlarged (Fig.11 C, D, E, F). The occurrence of *M. rectus abdominis* in *Hynobius nebulosus* points out that *M. rectus abdominis* develops for ventral median line from the ventral edges of *M. obliquus externus* and *M. obliquus internus* by transferring the fibers of *M. obliquus externus* and *M. obliquus internus* for *M. rectus abdominis* when the development of *M. obliquus externus* is started (Fujimoto, 1960). It is postulated that in *H. nigresces*, *M. rectus abdominis* is developed from *M. obliquus externus* in this study.

Since *M. rectus abdominis* plays a role in keeping posture (Deban and Schilling, 2009) and sustaining own weight (Preuschoft et al., 2007), *M. rectus abdominis* arises as the basic functional system for terrestrial locomotion.

It is suggested that the basic structure of trunk muscles in swimming typical fish does not have *M. rectus abdominis* and is constructed by epaxial and hypaxial muscles for lateral bending (Liem et al., 2001) since the frequency of using of *M. rectus abdominis* may be smaller in swimming than in terrestrial walking. The phenomenon that *M. rectus abdominis* is developed when the larvae still live in water shows that early tetrapod may prepare *M. rectus abdominis* even in water for landing.

Dorsalis muscles had a segmented structure through st 38 to st 68 in lateral view (Fig. 12).

Dorsalis muscles of Urodela may be segmented by myosepta through in the developmental stages from larvae to adult since Naylor (1978) observed the similar segmented structure in both sub-adult and adult. It is suggested that the segmented structure of dorsalis muscles may be seen in developmental stages through larvae to juvenile in this study since the segmented dorsalis muscles play a role in lateral bending and stabilization of body (Frolich and Biewner, 1992; Delvolve et al., 1997; Deban and Scilling, 2009).

Hynobius nebulosus which changes their habitats from water to ground ontogenetically though *H. nigrescens* possessed three layers: *M. obliquus externus*, *M. obliquus internus* and *M. transversus abdominis*, as lateral hypaxial muscles (Fujimoto, 1960). The order of development of hypaxial trunk muscles of *H. nebulosus* is that the first is *M. ventralis*, the second is *M. obliquus internus* from ventral muscle, the third is *M. obliquus externus* and *M. rectus abdominis*, and the last is *M. transversus abdominis* (Fujimoto, 1960). Though *M. obliquus internus* did not appear and *M. transversus abominis* appeared before the appearance of *M. obliquus externus* and *M. rectus abdominis* in *H. nigrescens*, the hypaxial muscles in *H. nebulosus* are developed as following order: 1) ventral muscle, 2) inner lateral hypaxial layer from ventral muscle, 3) outer lateral hypaxial layer and *M. rectus abdominis*. The developmental order of hypaxial muscles of *H. nebulosus* (Fujimoto, 1960) is similar with that of *H. nigrescens*

in this study.

Muscle weight ratios of trunk

Ontogenetic changes of the ratios of weight of muscle group were shown (Table 9, Fig. 12). According to growth, weight ratios of dorsalis muscles and abdominal muscle increased, in contrast, weight ratios of lateral hypaxial muscles decreased. Epaxial muscles are used in lateral bending during swimming and walking (Frorich and Biewner, 1992; Devolve et al., 1997; Deban and Schilling, 2009). In addition, dorsalis muscles also function as stabilizer of trunk (O'Reilly et al., 2000). *M. dorsalis trunci* which is the largest epaxial muscle prevents trunk from sagging and torsion and also increases trunk stiffness against gravity during walking (Deban and Schilling, 2009). Since the need for stabilization of trunk and resisting gravity on ground appears after metamorphosed and landed, weight ratio of dorsalis muscles increased for land.

According to growth, the weight ratio of lateral hypaxial muscles decreased (Table 9, Fig. 12). The functions of lateral hypaxial muscles are controlling torsion and stabilizing body (Carrier, 1996; Bennet et al., 2001; Deban and Schilling, 2009) and lateral bending (Schilling, 2011). Since lateral hypaxial muscles are needed for undulatory swimming, aquatic swimming larvae may possess larger lateral hypaxial muscles than in terrestrial juvenile. After limbs are developed, and when they transition from water to ground by metamorphosis, the locomotive

need of lateral hypaxial muscles may decrease and transfer into limbs. Then, lateral hypaxial muscles may decrease according to growth in this study. They mainly do the undulatory swimming in addition to aquatic walking after they are equipped with limbs. Though larger lateral hypaxial muscles are needed for undulatory swimming in water, modifying trunk muscles gradually for the preparation of landing has to be occurred. The decreasing weight ratio of lateral hypaxials and the increasing weight ratios of dorsalis and abdominal muscles in developmental stages (Fig. 12) may be consistent with phylogenetic preadaptation for land.

The weight ratio of abdominal muscle increased after it existed according to growth (Table 9, Fig.13). Abdominal muscle has functions to prevent sagittal extension of the trunk by the action of the epaxial muscles (Deban and Schilling, 2009) and to sustain own weight against gravity (Preuschoft et al., 2007). Since abdominal muscle may be necessary for terrestrial life, abdominal muscle appeared and increased after the developmental stages of appearance of the interdigital process in the hind limb anlage for the preparation of landing.

On the middle stages of development, they swim in water with limbs. In the beginning of the development, they may use dorsalis muscles and lateral hypaxial muscles for lateral bending for swimming. Though the duty of abdominal muscle may be smaller in water than on ground, *M. rectus adbominis* plays a role in preventing sagittal extension of trunk in water (Deban and Schilling, 2009). Larvae in the middle stages of development may use decreasing lateral

hypaxial muscles and increasing dorsalis muscles and abdominal muscle for both swimming and aquatic walking. After metamorphosed, they adapt to the terrestrial life against gravity by larger dorsalis muscles and abdominal muscle. It is suggested that in early tetrapod, they may reduce lateral hypaxial muscles and multiply dorsalis muscles and abdominal muscle for landing.

GENERAL DISCUSSION

GENERAL DISCUSSION

Phylogenetic differences and ontogenetic changes of trunk muscles

To clarify the way of adaptation for water and ground by locomotive structure in early tetrapods, the theory of environmental adaptation of trunk structure of modern Urodela was established in this study. Interspecific and ontogenetic differences of trunk muscles were shown in chapters 1, 3 and 4. In phylogenetically comparing study, the more aquatic species possessed unseparated *M. rectus abdominis* and larger and thicker lateral hypaxial muscles for powerful undulatory swimming, in contrast, the more terrestrial species were equipped with larger dorsalis muscles and larger separated abdominal muscle for stabilizing body and sustaining own weight against gravity (Chapter 1). In ontogenetic adaptation from water to ground in *H. nigrescens*, swimming larvae in the early developmental stages had larger and thicker lateral hypaxial muscles for lateral bending (chapter 4). By contrast in the late developmental stages, juvenile possessed larger dorsalis muscles and abdominal muscle for keeping posture and carrying body against gravity (chapter 4). The evolutionary adaptation to aquatic and terrestrial environments corresponds to that of the developmental changes in Urodela. Then, the strategy of trunk muscles of Urodela that they needs larger and thicker lateral hypaxial muscles for swimming, and larger dorsalis muscles and larger and separated abdominal muscle for terrestrial

walking can be found. The tendency of the way of environmental adaptation of trunk muscles in Urodela is consistent with that in the other vertebrates when the morphological strategy is compared between aquatic and terrestrial species (Romer, 1970). The strategy of trunk muscles may be common among vertebrates in the adaptation for aquatic and terrestrial environments.

In this study, phylogenetic and ontogenetic variations of trunk structure show the adaptive strategy for aquatic and terrestrial environments (chapter 1 and chapter 4). Ernst Heinrich Haeckel's fundamental biological law described parallels between ontogenetic changes and evolutionary history or phylogeny (Reviewed by Rieppel, 1988).

The relationships between the separation of *M. rectus abdominis* and the usage of *M. rectus abdominis* in terrestrial habitat

The *M. rectus abdominis* was not separated in aquatic species, in contrast the separated *M. rectus abdominis* was shown in semi-aquatic and terrestrial species, and the weight ratio of *M. rectus abdominis* was larger in the more terrestrial species (chapter 1). In the ontogenetic changes, *M. rectus abdominis* appeared and increased in the late of developmental stages for landing (chapter 4). Since *M. rectus abdominis* plays a role in preventing sagittal extension of the trunk caused by the action of the epaxial muscles (Deban and Schilling, 2009) and by the gravity (Preuschoft et al., 2007), the separation and existence of *M. rectus abdominis* may be

required for terrestrial life. *M. rectus abdominis* ran from the anterior edge on the pelvis to the sternal cartilage and covered ventral side of trunk. The fibers of the muscle ran in a longitudinal direction in Urodela (chapter 1). *M. rectus abdominis* was larger in the middle part of trunk than in the anterior and posterior trunk for being own tensile stress and for sustaining own weight (chapter 3). From this position and running direction of *M. rectus abdominis*, the muscle may sustain own weight dorsally. Species with elongate trunk keep their posture by *M. rectus abdominis* in ventral contour line and vertebral column (Preuschoft et al., 2007). Since lateral hypaxial muscles ran caudoventrally or caudodorsally (chapter 1), the strength and work of lateral hypaxial muscles may not adequately sustain own weight dorsally. In typical fishes, their trunk is constructed from epaxial and hypaxial muscles, and the muscles lie in the lateral side of the trunk (Liem et al., 2001). It may be the reason for the form of muscles in typical fishes that the position of muscle may be suitable for lateral bending for swimming and they need not sustain own weight because of buoyancy. Therefore, it is suggested that *M. rectus abdominis* may be obtained when early tetrapod landed from water.

Though *M. rectus abdominis* of aquatic species was unseparated from lateral hypaxial muscles, the direction of fibers of *M. rectus abdominis* was longitudinal in this study (chapter 1). Since the living aquatic Urodela secondarily adapted to aquatic life, they may be equipped with *M. rectus abdominis* as remnant of ancestral character. Aquatic species use their limbs for

stabilization of body on substrata against water flow and walk on substrata in water (Chien, 1935). Though the essential role of *M. rectus abdominis* has not been examined in aquatic life, the activity of *M. rectus abdominis* was observed in swimming and in aquatic trotting in electromyogram study (Deban and Schilling, 2009). Deban and schilling (2009) suggested that the function of *M. rectus abdominis* in swimming is preventing sagittal extension of trunk. Though the duty of *M. rectus abdominis* is smaller in water than on ground, aquatic species may not completely lose *M. rectus abdominis*.

The relationships between prezygapophyseal angle and habitats of Urodela

The more aquatic species possessed more horizontal prezygapophyseal angle of vertebra and the more terrestrial species were equipped with more vertical prezygapophyseal angle of vertebra in this study (chapter 2). The averages of prezygapophyseal angles among species of different habitats were calculated in chapter 2, and the result showed that aquatic species had 14.0° of prezygapophyseal angle, semi-aquatic species possessed 17.5°, and 19.5° was shown in terrestrial species on average. A more horizontal prezygapophyseal angle restricts movements to the horizontal plane and helps to resist ventral shear, though a more vertical prezygapophyseal angle restricts movements to the sagittal plane and helps to resist torsional loads (Sliper, 1946; Boszczyk et al., 2001; Hua, 2003; Pierce et al., 2011). If prezygapophyseal angle was

completely horizontal, the connection and the joint structure might be easily broken. In contrast, if prezygapophyseal angle was completely vertical, vertebral column could not move laterally. Aquatic species swim by lateral undulation of trunk (Gary, 1997; Bennet et al., 2001). Their trunk bends laterally with large amplitudes to push water to forward movement. It is suggested that 14.0° is the best angle for more flexible lateral bending for swimming and strength of joint structure in aquatic species. In contrast, since terrestrial species possess more developed limbs and they walk in using limbs (Simons and Brainerd, 1999), the need of lateral bending of trunk in terrestrial walking may be smaller than in aquatic species. But, since various terrestrial species mate and lay eggs in water in breeding season (Matsui, 1996), they have to functionally conserve the ability of flexible lateral bending for swimming. The prezygapophyseal angle of 19.5° may enable terrestrial species to mainly resist gravity in addition to bend their trunk laterally for swimming. Semi-aquatic species possessed prezygapophyseal angle of 17.5° on average (chapter 2). Semi-aquatic species representing *C. pyrrhogaster* and *C. ensicauda* spend their life both in aquatic and terrestrial environments. The need for resisting gravity may be smaller in semi-aquatic species than in terrestrial species whereas that for lateral bending fluently may be smaller than in aquatic species. Then, the prezygapophyseal angle of 17.5° in semi-aquatic species may enable semi-aquatic species both to swim and resist gravity efficiently.

The locomotion mode of early tetrapod

In this study, the environmental adaptive strategy of trunk muscles demonstrated that the more aquatic living Urodela with undeveloped limbs possess larger lateral hypaxial muscles for powerful swimming, whereas the more terrestrial living Urodela have larger dorsalis and abdominal muscles for sustaining own weight against gravity (chapters 1 and 4). It is suggested that early tetrapod may change the structure of trunk muscles as revealed in living Urodela in this study. Early tetrapod may obtain larger dorsalis and abdominal muscles for landing.

Though vertebral architecture in the earliest stem tetrapods representing *Ichthyostega*, *Acanthostega*, and *Pederpes*, was examined by Pierce et al. (2013), the transitional changes of zygapophyseal angle of early tetrapod from water to ground is not quantified. The more aquatic species had more horizontal vertebrate for lateral bending flexibly, by contrast, the more vertical prezygapophyseal angle was shown in the more terrestrial species for resisting torsion and strengthening vertebral column in this study of living Urodela. It is suggested that early tetrapod may increase their prezygapophyseal angle for landing from in water.

Aquatic species use their elongated body for undulatory swimming by traveling waves along the body, whereas terrestrial species are owed stress differed by the position of trunk against gravity. Therefore, aquatic species showed smaller positional differences in the form of the

trunk muscle than in the more terrestrial species. When early tetrapod landed from water, positional differences may increase for adaptation to land.

In the metamorphological changes of *H. nigrescens*, *M. rectus abdominis* is developed in the later stage, and *H. nigrescens* is equipped with *M. rectus abdominis* when they live still in water (chapter 4). As morphological preadaptation for land, early tetrapod may possess *M. rectus abdominis* when they are still in aquatic, and landing early tetrapod became equipped with larger *M. rectus abdominis* than before landing.

SUMMARY

Early tetrapod landed from water to ground 370 million years ago. According to this event, their locomotion mode was changed and their limbs were developed. To detail the evolutionary changes of the locomotive structure during the development of limbs, knowledge of morphology of the living Urodela amphibian is needed. Since Urodela use their trunk for undulatory locomotion both in water and on ground, trunk structure is noticeable to reveal the way of environmental adaptation. In this study, the way of adaptation for aquatic and terrestrial environments by locomotive trunk structure in Urodela was clarified to establish the theory of evolution of landing of early tetrapod.

Adult salamanders representing three different habitats (the aquatic *Siren. intermedia*, *Amphiuma tridactylum*, *Necturus maculosus* and *Andrias japonicus*, the semi-aquatic *Cynops phyllorhogaster*, *Cynops ensicauda*, and the terrestrial *Hynobius nigrescens*, *Hynobius lichenatus* and *Ambystoma tigrinum*) were used for comparing study.

Firstly, interspecific variations of structure of trunk muscle were observed and quantified. Aquatic species possessed thicker lateral hypaxial muscles, and undifferentiated *M. rectus abdominis*. Semi-aquatic species and terrestrial species had thinner lateral hypaxial muscles and separated *M. rectus abdominis*. Since semi-aquatic and terrestrial species sustain their own weight against gravity, their *M. rectus adbominis* was morphologically separated for keeping their ventral contour line from underneath. Futhermore, weight ratio of each trunk muscle was calculated. The more aquatic species possessed larger lateral hypaxial muscles. Larger

dorsalis muscles and *M. rectus abdominis* were equipped with the more terrestrial species. It is suggested that the more aquatic species use their lateral hypaxial muscles for powerful undulatory swimming against water viscosity. By contrast, the more terrestrial species may keep posture and sustain own weight by using their larger dorsalis muscles and abdominal muscle.

Secondly, the interspecific variations of structure of trunk vertebra were quantified. More aquatic species possessed vertebra with more horizontal prezygapophyseal angle for flexible undulatory swimming. More vertical prezygapophyseal angle of vertebra tended to lie in more terrestrial species. Vertebra with vertical prezygapophyseal angle may enable terrestrial species to possess strong beam of vertebral column to sustain own weight, and resist torsion against gravity.

Thirdly, positional differences representing anterior, middle, and posterior parts, of the trunk muscles were quantified. More aquatic species had smaller positional differences of muscle structure since their anguilliform swimming mode is conducted by propagating undulatory waves along the body. Dorsalis muscles and abdominal muscle are significantly larger in middle part than in anterior and posterior parts for sustaining own weight effectively in more terrestrial species.

Fourthly, ontogenetic changes of muscle morphology was observed and examined from hatching to landing. *Hynobius nigrescens* which changes habitat from in water to on ground ontogenetically was used. In the beginning of the development of *H. nigrescens*, lateral hypaxial muscle was composed of only one thicker *M. ventralis*, whereas *M. rectus abdominis* did not appear. The thicker single lateral hypaxial muscle is actually suitable for swimming. In contrast, by continued growth, the number of lateral hypaxial muscles increased

from one to two, and *M. rectus abdominis* appeared. It is suggested that they may adapt to terrestrial life by developing *M. rectus abdominis*. By continued growth, lateral hypaxial muscles weight ratio decreased, whereas dorsalis muscles and *M. rectus abdominis* weight ratios increased for preparation for resisting gravity.

In this study, the way of adaptation for aquatic and terrestrial environments by trunk structure in Urodela was clarified. The adaptive strategy for aquatic and terrestrial environments by trunk muscles shown in comparative interspecific observations corresponds to that in the ontogenetic changes from in water to on ground of trunk muscles in *H. nigrescens*. In terrestrial environment, *M. rectus abdominis* is needed for resisting the effect of gravity since its longitudinal running direction of bundles and its location from the anterior edge of the pubis to the level of the sternal cartilage are suitable for sustaining own weight. The aquatic species had 14.0° of prezygapophyseal angle on average for lateral bending more flexibly and avoiding dislocating vertebral joint. Prezygapophyseal angle was 17.5° on average in the semi-aquatic species for both lateral bending and resisting gravity effectively. The terrestrial species possessed 19.5° of prezygapophyseal angle for mainly resisting gravity.

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REFERENCES

- Ahlberg PE (1995) *Elginerpeton pancheni* and the earliest tetrapod clade. *Nature* 373: 420-425.
- Andrew RB (1977) The muscular control of vertebrate swimming movements. *Biol Rev* 52: 181-218.
- Antipenkova TP (1994) Axial skeleton. In: *The Siberian Newt (Salamandrella keyserlingii* Dybowski, 1870). *Zoogeography, Systematics, Morphology*. Nauka, Moscow. pp.153-158.
- Azizi E, Hopton JM (2004) Pattern of axial and appendicular movements during aquatic walking in the salamander *Siren lacertina*. *Zool* 107: 111-120.
- Bennett WO, Simons RS, Brainerd EL (2001) Twisting and bending: the functional role of salamander lateral hypaxial musculature during locomotion. *J Exp Bio* 204: 1979-1989.
- Benton M J (2005) *Vertebrate Paleontology*. 5th ed. Blackwell Press, Victoria.
- Bianca HS, Holger P, Ulrich W, Claudia DH (2013) Biomechanics and functional preconditions for terrestrial lifestyle in basal tetrapods, with special consideration of *Tiktaalik roseae*, *Histor Biol: Int J Paleobiol* 25: 1-15.
- Boszczyk BM, Boszczyk AA, Putz R (2001) Comparative and functional anatomy of the mammalian lumbar spine. *Anat Rec* 247: 542-555.
- Brand LR (1996) Variations in salamander trackways resulting from substrate differences. *J Paleontol* 70: 1004-1010.

- Carrier DR (1993) Action of the hypaxial muscles during walking and swimming in the salamander *Dicamptodon ensatus*. *J Exp Biol* 180: 75-83.
- Clack JA (2002) An early tetrapod from 'Romer's gap'. *Nature* 418: 72-76.
- Deban SM, Schilling N (2009) Activity of trunk muscles during aquatic and terrestrial locomotion in *Ambystoma maculatum*. *J Exp Biol* 212: 2949-2959.
- Delvolve I, Bem T, Cabelguen JM (1997) Epaxial and limb muscle activity during swimming and terrestrial stepping in the adult newt, *Pleurodeles waltl*. *J Neurophysiol* 78: 638-650.
- Duellman WE, Trueb L (1986) *Biology of Amphibians*. Johns Hopkins University Press, New York.
- Francis ETB (1934) *The Anatomy of the Salamander*. Oxford University Press, London.
- Frolich LM, Biewener AA (1992) Kinematic and electromyographic analysis of the functional role of the body axis during terrestrial and aquatic locomotion in the salamander *Ambystoma tigrinum*. *J Exp Biol* 162: 107-130.
- Fujimoto T (1960) Development of the abdominal musculature of *Hynobius nebulosus*. *J Okayama Med Assoc* 611: 2205-2220.
- Fujiwara S (2009) Olecranon orientation as an indicator of elbow joint angle in the stance phase, and estimation of forelimb posture in extinct quadruped animals. *J Morph* 270:1107-1121.
- Gary BG (1996) Undulatory locomotion in elongate aquatic vertebrates: anguilliform swimming.

- Amer Zool 36: 656-665.
- Gary BG (1997) Anguilliform locomotion in an elongate salamander (*Siren intermedia*): effect of speed on axial undulatory movements. J Exp Biol 200: 767-784.
- Gray J (1933) The movement of fish with special reference to the eel. J Exp Biol 10: 88-104.
- Gray J (1944) Studies on the mechanics of the tetrapod skeleton. J Exp Biol 20: 88-116.
- Kentwood DW (2007) The Ecology and Behavior of Amphibians. Univ Chicago Press, Chicago.
- Hanken J (1999) Larvae in amphibian development and evolution. In: In the Origin and Evolution of Larvae Forms. Academic Press, San Diego. pp 61-108.
- Hildebrand M, Goslow GE (2001) Analysis of Vertebrate Structure. 5th ed. Hamilton Press, New York.
- Hilton WA (1948) The vertebrae of salamanders. J Entomol Zool 40: 47-65.
- Hoff KS, Hug N, King VA, Wassersug RJ (1989) The kinematics of larval salamander swimming (Ambystomatidae: Caudata). Can J Zool 67: 2756-2761.
- Hua S (2003) Locomotion in marine mesosuchians (Crocodylia): the contribution of the "locomotion profiles". Neu Jahrb Geol Paläontol Abhand 227: 139-152.
- Iwasawa H, Kera Y (1980) Normal stages of development of the Japanese lungless salamander, *Onychodactylus japonicus* (Houttuyn). Jpn J Herpetol 8: 73-89.
- Iwasawa H, Yamashita K (1991) Normal stages of development of Hynobiid Salamander,

- Hynobius nigrescens* Stejneger. Jpn J Herpetol 14: 39-62.
- Kentwood DW (2007) The ecology and behavior of amphibians. The University of Chicago Press, Chicago.
- Kudo T (1938) Normentafel zur Entwicklungsgeschichte des japanischen Riesensalamanders (*Megalobatrachus japonicus* Temminck). Normen Ent Wirbel 16: 98.
- Liem KF, Walker WF, Bemis WE, Grande L (2001) Functional Anatomy of the Vertebrates: An Evolutionary Perspective. Harcourt College Press, Philadelphia.
- Matsui M (1996) Natural History of the Amphibia. University of Tokyo Press, Tokyo.
- 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. Morphol Jahrb 18: 76-179.
- Maurer F (1911) Die ventrale Rumpfmuskulatur von *Menobanchus*, *Menopoma* und *Amphiuma*. Jena Z Naturwiss 47: 1-42.
- Mivart G (1870) On the axial skeleton of the Urodela. Proc. Zool. Soc. London. 1870: 260-278.
- Naylor BG (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.
- Noble GK (1931) The Biology of the Amphibia. McGraw-Hill Press, New York.

- O'Reilly JC, Summers AP, Ritter DA (2000) The evolution of the functional role of trunk muscles during locomotion in adult amphibians. *Am Zool* 40: 123-135.
- Oyama J (1930) Normal stages of development of Japanese newt. *Zool Sci* 42: 465-473.
- Pierce SE, Clack J.A and Hutchinson JR (2011) Comparative axial morphology in pinnipeds and its correlation with aquatic locomotory behaviour. *J Anat* 219: 502–514.
- Pierce SE, Ahlberg PE, Hutchinson JR, Molnar JL, Sanchez S, Tafforeau, Clack JA (2013) Vertebral architecture in the earliest stem tetrapods. *Nature* 494: 226-230.
- Preuschoft H, Schulte D, Distler C, Witzel U, and Hohn B (2007) Body shape and locomotion in monitor lizards. *Mertensiella* 16:59-78.
- Ratnikov VY, Litvinchuk SN (2007) Comparative morphology of trunk and sacral vertebrae of tailed amphibians of Russia and adjacent countries. *Russ J Herpetol.* 14: 177-190.
- Rieppel CO (1988) *Fundamentals of Comparative Biology*. Birkhäuser Verlag, Basel and Boston.
- Robert C (1995) Between fish and amphibian. *Nature* 373: 389-390.
- Romer AS (1970) *The Vertebrate Body*. 4th ed, WB Saunders, Philadelphia.
- Schilling N (2011) Evolution of the axial system in craniates: morphology and function of the perivertebral musculature. *Front Zool* 8: 4-22.
- Schilling N, Daban SM (2010) Fiber-type distribution of the perivertebral musculature in

Ambystoma. J Morph 271: 200-214.

Simons RS, Brainerd EL (1999) Morphological variation of hypaxial musculature in salamanders (Lissamphibia: Caudata). J Morph 241: 153-164.

Slijper EJ (1946) Comparative biologic-anatomical investigations on the vertebral column and spinal musculature of mammals. Verh. Kon. Nederl. Akad. Wetensch. Amsterdam II 42: 1-128.

Teege MJ (1957) Studien zur Entwicklung und Gestalt der Urodelenwirbel. Z Wiss Zool 160: 95-164.

Tsuihiji T (2004) The ligament system in the neck of *Rhea americana* and its implication for the bifurcated neural spines of sauropod dinosaur. J Vert Paleontol 24: 165-172.

Usui M, Hamasaki M (1939) Normal stages of development of *Hynobius nigrescens*. Zool Sci 51: 195-206.

Wardle CS, Videler JJ, Altringham JD (1995) Turning into fish swimming waves: body form, swimming mode and muscle function. J Exp Biol 198: 1629-1636.

Wassersug RJ (1989) Locomotion in amphibian larvae (or "Why aren't tadpoles built like fishes?"). Amer Zool 29: 65-84.

Worthington RD, Wake DB (1972) Patterns of regional variation in the vertebral column of terrestrial salamanders. J Morph 137: 257-278.