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

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ABSTRACT. Clarification of the trunk structure in Urodela is important in understanding the locomotive evolution of basal tetrapods. The components of the muscular trunk wall among Urodela using different modes of locomotion were compared. Since the whole trunk may be used for swimming and the effect of limbs may be small in the more aquatic species, they showed smaller differences in the trunk muscles among anterior, middle and posterior sections of the trunk. By contrast, in the more terrestrial species, the dorsal and abdominal muscles are larger in the middle section than those in the anterior and posterior sections. High compressive stresses occur in the supporting limbs and their insertion at the trunk on the ventral side, and spread from the forelimbs along the back to the supporting hindlimbs on the dorsal side. Tensile stresses occur in the middle ventral part. The components of the trunk muscles among the three sections may reflect differences in stresses occurring in the trunk of the more terrestrial species. The findings also suggest that in the middle section, larger dorsal muscles for stiffening the back to maintain posture and larger abdominal muscles are responsible for balancing the body weight while it is supported by the limbs in the more terrestrial species.
KEY WORDS: axial muscle, Caudata, locomotion, salamander, Urodela

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Urodele amphibians are often chosen as models to infer the evolution of locomotion of early tetrapods, because they possess a “generalized” body form and amphibious lifestyle [5]. Urodela use their body in an undulatory manner for locomotion both in water and on ground [5]. For understanding the locomotive evolution of basal tetrapods, clarification of the trunk structure in urodeles is essential.

The relationship between trunk muscles and ecological habitats was previously investigated by examining cross-sections of mid-trunk [18]. Those authors suggested that terrestrial species have thinner hypaxial muscles than aquatic species. Omura *et al*. [13, 14] quantified the weight ratios at the middle of the trunk. They divided the trunk muscles into three components, namely dorsal muscles, lateral hypaxial muscles and abdominal muscle. They showed that more terrestrial species possess larger dorsal muscles and a larger abdominal muscle which is separated from lateral hypaxial muscles by fascia, whereas aquatic species possess larger and thicker lateral hypaxial muscles and a smaller abdominal muscle which is not clearly separated from lateral hypaxial muscles by fascia. It was suggested that the more terrestrial species use their enlarged dorsal and abdominal muscles to stabilize the body and to sustain the animal’s own weight against gravity, whereas the aquatic species use larger lateral hypaxial muscles as powerful locomotor apparatus for lateral bending during swimming [13, 14].

These studies regarded the trunk as one unit during quantification. However, the internal structure of the trunk may differ according to positions, because muscle usage and structure differ according to the environment. Thus, a quantification of the differences between the components of trunk muscles among the locomotor types is required. The methods of coping with buoyancy and gravity considerably differ between aquatic and terrestrial species [11]. The degree of limb development also differs among aquatic, semi-aquatic and terrestrial species, and these factors may affect the structure of the trunk. Because the components of trunk musculature differ according to the effects of gravity and the distance from the limbs, observations of only the mid-trunk region and comparison of the whole weight of each trunk muscle are not sufficient to understand the functional significance of trunk structure. Trunk muscles should be affected and vary in size depending on their position, the use made of the trunk and the external forces acting against the trunk [17]. Furthermore, the relative length of the trunk differs among urodele species, although they all have elongated bodies. During swimming, elongated tetrapods perform anguilliform locomotion [2, 6, 8, 19]. Gary [7] showed that sirenid salamanders swim by propagating undulatory waves posteriorly along the body. In contrast, different stresses occur depending on trunk position in terrestrial tetrapods with

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elongated bodies [9]. Monitor lizards, which have elongated bodies similar to salamanders, are under the influences of downward gravity and sustain body weight by ventral muscles when they rest quadrupedally on their 2 pairs of limbs [16]. Here, the types of stress (tension or compression) and the amount of stress vary with body position [16].

A previous study examined the fiber types of trunk muscles in 2 terrestrial salamanders, *Ambystoma tigrinum* and *A. maculatum* [17]. The distribution of fiber types of perivertebral muscles along the body axis was similar in the 2 terrestrial species. However, only the distribution of fiber type was examined in that study, and the positional difference of all trunk muscles was not included. Thus, the relationship between the patterns of variation in muscle position and habitats was unclear. In this study, we compared the differences of components of trunk musculature among different modes of locomotion among Urodela with different habitats to examine the relationships between locomotive mode and trunk morphology.

Table 1. Specimens used in this study

Species	Habitat	SVL* (mm)
Siren intermedia	Aquatic	261
		230
		242
Amphiuma tridactylum	Aquatic	408
		434
		471
Cynops pyrrhogaster	Semi-aquatic	53
		48
		45
Cynops ensicauda	Semi-aquatic	52
		51
		54
Hynobius nigrescens	Terrestrial	70
		72
		73
Ambystoma tigrinum	Terrestrial	111
		103
		86

*Snout-vent length.

MATERIALS AND METHODS

Six species of adult salamanders representing 5 families and 3 different habitats (aquatic, semi-aquatic and terrestrial) were used in this study (Table 1). The specimens were individual samples from the collection belonging to Dr. Omura. The specimens were fixed in a straight body position in 10% formalin and were maintained in 70% ethanol solution.

Cross-sections including one vertebra were cut from the anterior, middle and posterior parts of the fixed specimens using a scalpel (Fig. 1). The anterior section was cut posterior to the front limbs. The posterior section was cut from the front of the pelvic girdle. The middle section was cut from a position halfway between the anterior and posterior sections. Cross-sectional images were recorded using a single-lens reflex camera. Trunk muscles were treated as 3 groups of muscles, namely the dorsal muscles, lateral hypaxial muscles and abdominal muscles, from the functions and positions according to Naylor [12] and Omura *et al*. [13, 14]. Dorsal muscles, lateral hypaxial muscles and abdominal muscles were examined from the cross-sectional images (Fig. 2). The areas of these muscle groups were measured on the digital image of the cross-section using Photoshop CS5. The area ratio of each muscle group to the area of all trunk muscles in one section was calculated to determine the component rate of each muscle group.

Statistical analyses were performed to test significant differences in the muscle area ratios among Urodela of different lifestyles: aquatic, semi-aquatic and terrestrial. Homogeneity of variances and means between species were examined by analysis of variance (ANOVA). When significant differences were detected by ANOVA, these differences were identified by using Tukey's test.

RESULTS

The differences of trunk muscles among the locomotor modes are shown in Table 2 and Fig. 2. Within aquatic species, the differences of trunk muscles were smaller than those within terrestrial species. In *S. intermedia*, the dorsal muscles accounted for approximately 53% of the total musculature on all sections. *S. intermedia* had almost 43% lateral hypaxial muscles and approximately 3% abdominal muscle on all sections. In *A. tridactylum*, no significant differences were observed among the three sections in dorsal muscles and lateral hypaxial muscles. In addition, although significant differences between the anterior and middle sections were observed in the abdominal muscle, the range in area ratios of the abdominal muscle in all sections was narrow (3.2–3.9%). In *C. pyrrhogaster*, there was no significant difference among the 3 sections of the dorsal muscles. The lateral hypaxial muscles were significantly larger in the middle section than in the anterior and posterior sections, and the abdominal muscle was smaller in the anterior section of the trunk than in the middle and posterior sections. In *C. ensicauda*, lateral hypaxial muscles were larger in the anterior and posterior sections of the trunk (formed over 37%) than in the middle section. In *H. nigrescens*, the abdominal muscle was significantly smaller in the anterior and posterior sections of the trunk than in the middle section. The dorsal muscles were larger in the middle section (over 67%) than in the anterior and posterior sections. In addition, significantly larger lateral hypaxial muscles were present in the anterior and posterior sections (over 30%) than in the middle section. Finally, the abdominal muscle was found to be larger in the middle section (over 15%) than in the anterior and posterior sections in *H. nigrescens*. *A. tigrinum* had significantly larger dorsal muscles in the middle section (over 37%) than in the anterior and posterior sections. Lateral hypaxial muscles were larger in the anterior and posterior sections than in the middle section in *A. tigrinum*. The abdominal muscle was larger (over 12%) in the middle section than in the anterior and posterior sections of the trunk.

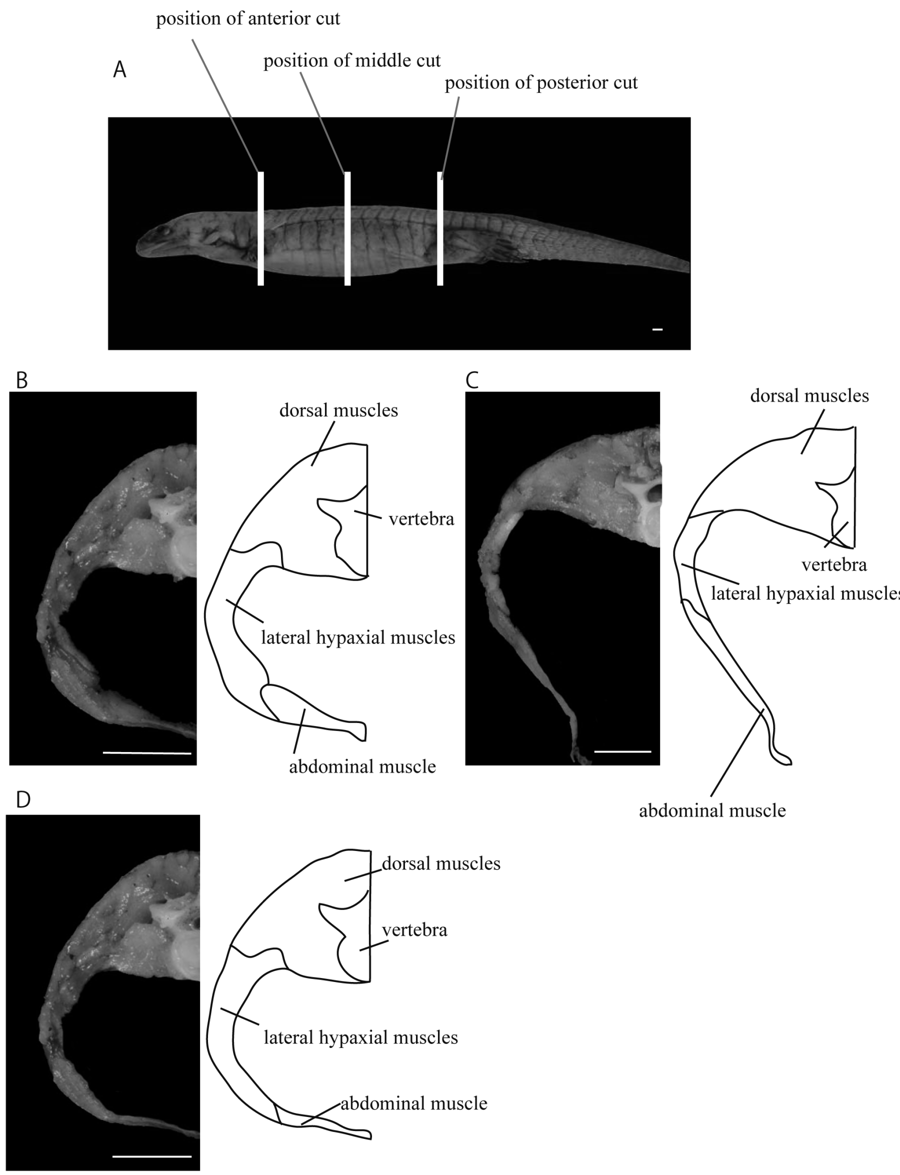

Fig. 1. A: Lateral view of skinned *Ambystoma tigrinum* B: Cross-sectional view of anterior part of trunk, C: Cross-sectional view of middle part of trunk, C: Cross-sectional view of posterior part of trunk. Scale bar=5 mm.

DISCUSSION

In water, Urodela have to cope with the density and viscosity of water. When they swim, trunk muscles produce a traveling wave of lateral flexions [6]. From an electromyographical study, we know that “activation travels from anterior to posterior part of trunk [6]”. In contrast, on ground, Urodela have to keep their posture by sustaining their own weight, because of the effect of gravity. Furthermore, when they walk on ground, ground reaction forces arise as a consequence of gravity and locomotion in the limbs [4]. The animal’s body has to resist ground reaction forces [4]. Therefore, tensile as well as compressive stresses derived from bending of the trunk are larger in terrestrial than in aquatic forms [16].

In this study, the differences in the components of trunk muscles among the 3 sections of the trunk were smaller in the more aquatic species than those in the more terrestrial species. In the more terrestrial species, the middle section possessed larger dorsal and abdominal muscles than the anterior and posterior sections.

Aquatic species have elongated bodies and less-developed limbs for anguilliform swimming, that means by flexing their body stem in a medio-lateral plane. Tail possessing amphibians swim using a posterior traveling wave along their bodies [5, 6]. This suggests that aquatic species may use

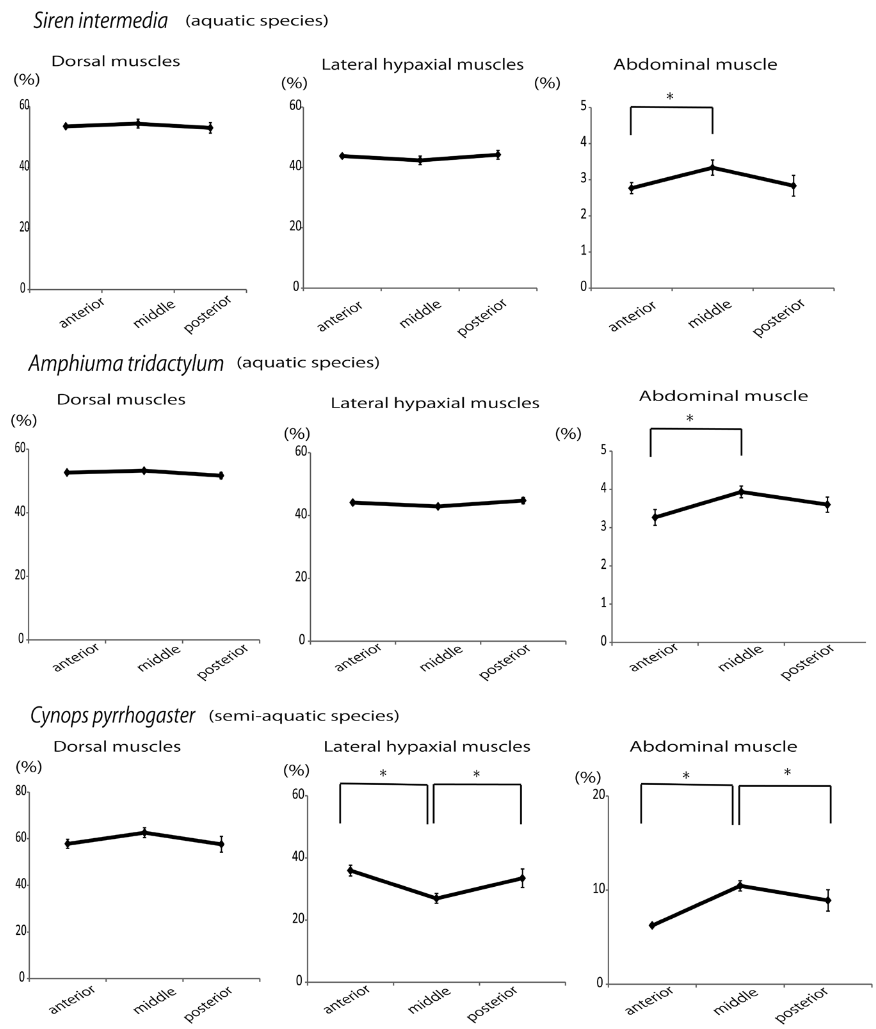

Fig. 2-1. The major components of the trunk musculature among 3 lifestyles: aquatic, semi-aquatic and terrestrial (ANOVA and Turkey' s test, *P*>0.05).

their whole trunk for swimming. Although aquatic or semi-aquatic species also utilize their limbs for aquatic walking on the substrata [1, 3], ground reaction forces during aquatic walking are very probably smaller than those during terrestrial walking. *Amphiuma* and *Siren* possess greatly reduced limbs [10], and their limbs are suggested to be functionally ineffectual [15]. In accordance with this, we have found that among the 3 sections of the trunk, minor differences occur in the more aquatic species compared to those in the more terrestrial species.

By contrast, on the ground, the more terrestrial species need to resist downward gravity. Stresses differ according to the sections of the trunk in terrestrial species [16] (Fig. 3). In the dorsal muscles, maintaining posture requires tension above the shoulder and pelvic region. The length of segments in which the tensile forces are required can differ according to locomotor habitats, limb lengths or distance between shoulder and pelvic joint. The middle of the trunk tends to be bent dorsally concave by gravity, and the section can be shorter or longer.

In the middle section, tensile stress occurs on the ventral side [16] (Fig. 3B), and the abdominal muscle prevents dorsally concave against gravity. Dorsal muscles stabilize the body in a dorso-ventral plane [15]. The dorsal muscles must be active when the tail is lifted from the ground. This may cause dorsal and abdominal muscles in the middle section to be larger than the anterior and posterior sections.

In the anterior and posterior sections, above the shoulder and pelvic regions, are subject to tensile stress (Fig. 3A and 3C). Another study has shown that compressive stress occurs between the supporting forelimbs on the ventral side [9]. Since stronger pectoral and pelvic girdles are subject to higher stress, the responsibility of resisting the effect of gravity and stress may be smaller than that in the middle section. This would explain why our findings showed that the dorsal and abdominal muscles in the anterior and posterior

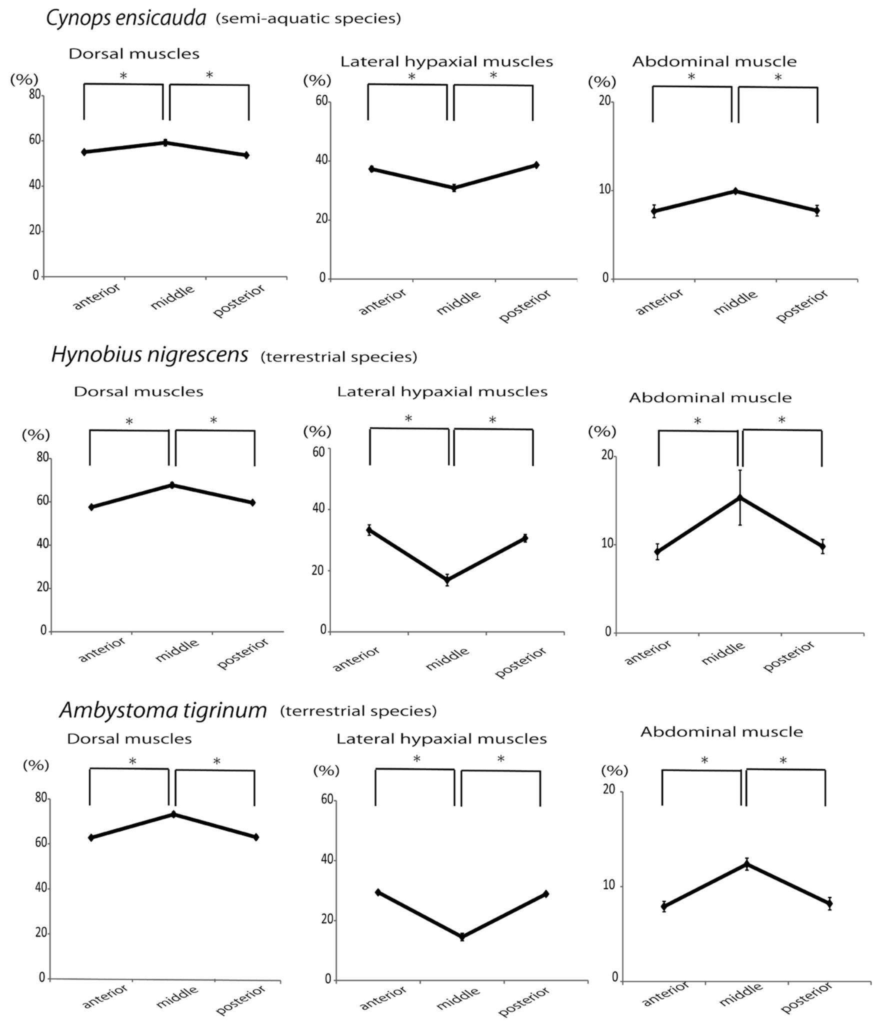

Fig. 2-2. The major components of the trunk musculature among 3 lifestyles: aquatic, semi-aquatic and terrestrial (ANOVA and Turkey' s test, $P>0.05$).

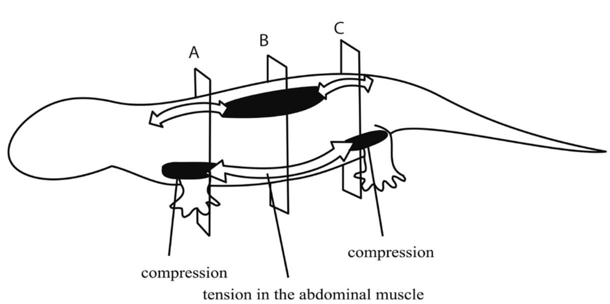

Fig. 3. Resting quadruped on 2 pairs of limbs, with distribution of stresses derived from body weight. White arrows show tension. Black distributions represent compression. Modified from Preuschoft *et al*. [16].

sections were smaller than those in the middle section of the more terrestrial species.

In conclusion, the differences between the major components of the trunk musculature obviously differ according to habitats and locomotion in urodeles. Our findings clarify the relationships between locomotor mode and trunk structure in basal tetrapods.

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Table 2. Muscle area ratios (%) measured at three parts in trunk (mean ± S.E.M.)

Species	Position of trunk		Muscle area ratio
Siren intermedia	dorsal muscles	anterior	53.4 ± 0.7
		middle	53.2 ± 0.4
		posterior	51.6 ± 0.9
	lateral hypaxial muscles	anterior	43.7 ± 0.7
		middle	42.3 ± 1.4
		posterior	44.2 ± 1.4
	abdominal muscle	anterior	2.7 ± 0.1
		middle	3.3 ± 0.2
		posterior	2.8 ± 0.2
Amphiuma tridactylum	dorsal muscles	anterior	52.6 ± 0.5
		middle	53.2 ± 0.4
		posterior	51.6 ± 0.9
	lateral hypaxial muscles	anterior	44.1 ± 0.7
		middle	42.8 ± 0.5
		posterior	44.7 ± 0.9
	abdominal muscle	anterior	3.2 ± 0.2
		middle	3.9 ± 0.1
		posterior	3.6 ± 0.2
Cynops pyrrhogaster	dorsal muscles	anterior	57.8 ± 1.9
		middle	62.5 ± 2.1
		posterior	57.6 ± 3.3
	lateral hypaxial muscles	anterior	35.9 ± 1.7
		middle	26.9 ± 1.5
		posterior	33.4 ± 2.9
	abdominal muscle	anterior	6.2 ± 0.1
		middle	10.4 ± 0.5
		posterior	8.9 ± 1.1
Cynops ensicauda	dorsal muscles	anterior	55.0 ± 0.3
		middle	59.2 ± 1.4
		posterior	53.6 ± 0.3
	lateral hypaxial muscles	anterior	37.3 ± 0.9
		middle	30.8 ± 1.1
		posterior	38.6 ± 0.4
	abdominal muscle	anterior	7.6 ± 0.7
		middle	9.9 ± 0.2
		posterior	7.7 ± 0.6
Hynobius nigrescens	dorsal muscles	anterior	57.5 ± 0.9
		middle	67.7 ± 1.2
		posterior	59.6 ± 0.5
	lateral hypaxial muscles	anterior	33.2 ± 1.7
		middle	16.9 ± 1.9
		posterior	30.6 ± 1.2
	abdominal muscle	anterior	9.2 ± 0.9
		middle	15.3 ± 3.1
		posterior	9.8 ± 0.8
Ambystoma tigrinum	dorsal muscles	anterior	62.7 ± 0.3
		middle	73.1 ± 1.0
		posterior	62.9 ± 0.9
	lateral hypaxial muscles	anterior	29.3 ± 0.5
		middle	14.5 ± 1.2
		posterior	28.8 ± 0.6
	abdominal muscle	anterior	7.9 ± 0.5
		middle	12.3 ± 0.6
		posterior	8.2 ± 0.6

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