I, Philip Nicodemo Jr., hereby submit this original work as part of the requirements for the degree of Master of Science in Biological Sciences.

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Longitudinal variation in the axial muscles of snakes

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Abstract

The axial muscles of snakes are notable for having long tendons within individual segments that span several vertebrae. Consequently, muscles that extend anteriorly have a constraint on their length as their origins are located closer to the skull. However, this and other aspects of longitudinal variation in axial muscle morphology are poorly documented either within or between species of snakes. For the anterior half of the trunk in 25 phylogenetically and morphologically diverse species of snakes, we compared patterns of segmentation and morphology of the *m. spinalis* (SP) muscle, which is one of the largest epaxial muscles in snakes that is used in most types of locomotion and while constricting prey. Among the species studied, mid-body segments of the SP muscle spanned from 9-46 vertebrae, whereas the most anterior segments spanned from 7-17 vertebrae. In all species examined, the anterior decreases in total span of SP segments resulted primarily from reduced length of the long anterior anterior tendon rather than contractile tissue. Furthermore, reductions in segmental length occurred at more posterior locations than were necessary based on lengths of the mid-body segments. Several modifications in SP segments were observed that resulted in reduced total span, some of which varied among taxa. The number of vertebrae anterior to the origin of the most anterior SP segment attachment was fairly uniform among snakes (usually 5-8) and may facilitate identifying a posterior boundary for a cervical region in snakes.
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INTRODUCTION

Segmented axial structures are a key attribute of the vertebrate body plan as well as that of several lineages of invertebrate animals (Schilling, 2011; Tautz, 2004). In early vertebrate development, embryonic tissues are segmented during somitogenesis, ultimately resulting in adult forms with segmentally organized bones, muscles, nerves, and blood vessels. Within vertebrates the segmental organization of axial muscles is most conspicuous in the myomeres of fish. The sheet-like myosepta that delineate myomeres of fishes are so thin that they do not contribute appreciably to the longitudinal span of muscle segments. Instead, the convoluted W-like shape of all of the contractile tissue within each myotome collectively spans several vertebrae, even though the lengths of individual muscle fibers between successive myosepta often only approximate the length of a single vertebra (Gemballa and Röder, 2004; Greene and Greene, 1913; Jayne and Lauder, 1994). Although many extant amphibians such as salamanders have myosepta, they also have some additional distinct layers of axial muscle that are not in fish myomeres (Azizi et al, 2002). Numerous axial muscles of amniotic vertebrates often have distinct cord-like tendons rather than the conspicuous myosepta of fishes and amphibians, but segmentation is often still evident (Gasc, 1981).

Compared to other amniotic vertebrates, snakes have extraordinarily long axial muscles in which the contractile tissue commonly spans 3-6 vertebrae, and tendons may span from one to more than 30 vertebrae, depending on the particular muscle and species (Gasc, 1981; Jayne, 1982; Mosauer, 1935). Substantial comparative data on the lengths of axial muscle segments of snakes are available from locations roughly midway between the head and the base of the tail (Auffenberg, 1961; Gasc, 1974; Gasc, 1981; Jayne, 1982;
Mosauer, 1935). By contrast, for the anterior trunk of snakes, little information on the anatomy of axial muscles is known beyond the work of Pregill (1977), who focused mostly on the qualitative features of several axial muscles, particularly those unique to this region near the skull. Clearly the numbers of vertebrae spanned by axial muscle segments must decrease to fit within the reduced numbers of vertebrae as the sites of muscle attachment approach the anterior and posterior ends of the vertebral column. However, quantitative data on this likely source of longitudinal variation in snake axial muscles are sparse.

We investigated the longitudinal variation in the morphology of *m. spinalis* (SP) portion of the *m. semispinalis-spinalis* (SSP-SP) for the following reasons. The length of the SP is highly variable between species, and is correlated to some aspects of phylogeny and habitat (Gasc, 1974; Jayne, 1982; Mosauer, 1935). The SSP-SP in snakes is one of the three largest epaxial muscles along with the *m. longissimus dorsi* (LD) and the *m. iliocostalis* (IL) muscles (Jayne and Riley 2007; Ruben, 1977a). A practical reason for choosing the SP is that unlike the LD, IL and SSP, the *m. spinalis* portion of the SSP-SP has anterior and posterior tendons that attach directly to the vertebrae in all snake taxa, whereas the LD, IL and SSP may have tendons that connect to other muscles or to diffuse connective tissue, depending on the species (Gasc, 1989; Mosauer, 1935). Electromyography has verified that the SSP-SP has important functions for both locomotion and constriction of prey (Gasc et al., 1989; Jayne, 1988a, b; Moon, 2000a, b), and the anatomical location of the SSP-SP suggests that it is also important for behaviors such as bridging gaps, prey transport and striking. (Jayne and Riley, 2007; Kley and Brainerd, 2002; Young, 2010). Determining the most anterior extent of where the SP terminates may also provide insights into the growing body of evidence for the presence of
a cervical region in snakes (Tsuihiji et al., 2006; Tsuihiji et al., 2012; Woltering et al., 2009).

Therefore, for several ecologically and phylogenetically diverse species of snakes, we documented longitudinal variation of SP muscle segments with the following main goals. First, we quantified the extent and location of anterior reductions in SP length that occur along the axial skeleton. A plausible null hypothesis is that the length of a muscle segment does not shorten until it is mathematically necessary to do so, as would be the case 19 vertebrae posterior to the skull in a species with a mid-body SP span of 20 vertebrae. Second, we quantified whether or not the proportions of contractile tissue to tendon within a segment vary. One plausible expectation is that both of these tissues decrease proportionately as SP length decreases. Alternatively, if the anterior reduction in length occurs similarly to most of the previously reported SP interspecific variation in length at mid-body, then the length of tendon per segment may decrease with little change in the length of contractile tissue. Finally, we determined whether or not qualitatively different morphologies were involved in the anterior reduction in SP length among diverse taxa with different SP morphology. Many of the previous studies comparing the axial muscles of snakes have documented considerable qualitative variation between phylogenetically distant groups such as the Colubroidea and Henophidia (Gasc, 1974; Jayne, 1982; Mosauer, 1935).

**MATERIALS AND METHODS**

We dissected *m. spinalis* (SP) muscle segments in 25 species representing 23 genera, and 6 families (Table 1). The families Colubridae, Viperidae, and Elapidae belong to the vast (> 2,500 extant species) monophyletic superfamily the Colubroidea (Pyron et al.,
The Acrochordidae is the sister group to the Colubroidea (Sanders et al., 2010). The Boidae and Pythonidae are part of the superfamily the Henophidia, which collectively comprises the sister group to Acrochordidae + Colubroidea (Sanders et al., 2010).

Rather than attempting to obtain a random sample of snake taxa, we deliberately chose species that collectively had a large range and variance in the SP lengths at mid-body. The primary goal of this study was not to correlate morphology with habitat, but we used various monographs (Cogger, 1992; Ernst and Ernst, 2003; Pitman, 1974; Pope, 1935) to categorize habitat because this factor does have some correlations with mid-body SP length in colubroid snakes (Jayne, 1982). For example, we preferentially selected several species such as arboreal colubroids known to have or likely to have long SP segments at mid-body. Longer SP segments seemed likely to require greater amounts shortening from posterior to anterior and thus were of greater interest for our goal of documenting longitudinal variation. The phylogenetic diversity of the arboreal taxa in our study was sufficient to include several different independent evolutionary origins of arboreality (Hampton, 2011; Jayne, 1982; Pizzatto et al., 2007). We included some highly aquatic taxa mainly to obtain colubroid species with short SP length. We included some henophidian and acrochordid species, in part because they have some qualitative differences in the morphology of the homologous muscle compared to the colubroid snakes (Gasc, 1981; Jayne, 182; Mosauer, 1935). We dissected mostly colubroid species because they have a much greater diversity of SP length than other lineages of snakes. All of the boid, pythonid, and acrochordid species constrict their prey, but the only colubroid species in our sample that do so were Pantherophis guttatus, Boiga irregularis, and Chrysopelea paradisi (Greene and Burghardt, 1978; Ernst and Ernst, 2003; Pope, 1935).
Most of the specimens dissected were from the Field Museum of Natural History (FMNH), and the remainder was from private collections. All specimens had been fixed in formalin and stored in a solution of 70% ethanol, and we used a stereo microscope with 6.7-40X magnification to facilitate the dissections. For most species we determined the number of pre-cloacal vertebrae by counting ventral scales (Alexander and Gans, 1966), but for the highly aquatic species with reduced ventral scales, the numbers of pre-cloacal vertebrae were estimated from mean values in the literature (Alexander and Gans, 1966; Voris, 1975).

We used the following two criteria to delineate what constituted a single SP segment, as well as what differentiated a SP segment from some of the specialized axial muscles that are near or are associated with the skull. First, the anterior portion of the SP contractile tissue attached to a single tendon which had a 1:1 ratio with underlying vertebrae in all of the species studied, even though individual SP segments usually arose from more than one vertebra (Fig. 1). Thus, we used this anterior tendon to delineate a single SP segment. Second, all of the SP muscle segments that we describe were part of a complete m. semispinalis-spinalis (SSP-SP) complex. In colubroid species a complete SSP-SP complex had two distinct heads (m. semispinalis and m. spinalis) (Fig. 1B), whereas in henophidain and acrochordid species a complete SSP-SP complex lacked distinct heads but still had both a dorsal site of attachment to the neural spine of a vertebra and a ventral site of attachment to the postzygapophysis of a vertebra. Anterior to the SP segments that we dissected, modifications to the SSP-SP complex were associated with specialized craniovertebral muscles (Pregill, 1977; Tsuihiji et al., 2012). Descriptions of
these complex epaxial muscles associated with the skull were beyond the scope of our study.

We dissected a total of approximately 500 SP muscle segments to assess longitudinal variation within and between species. For each snake, we first dissected a SP segment for which the anterior insertion was near mid-body (approximately 50% snout vent length (SVL)). For this and all other segments dissected, we recorded the numbers of vertebrae spanned by the entire muscle segment (including the vertebrae of origin and insertion), the anterior tendon, and the contractile tissue as well as the number of vertebrae giving rise to contractile tissue (Table 1, origin) that formed an entire segment (Fig. 1A). We subsequently dissected SP muscle segments originating from consecutive locations every 10 vertebrae anterior to the prior dissection. Beginning at a location posterior to the skull approximately 10 vertebrae plus the number of vertebrae spanned by the mid-body SP segment, we dissected all of the remaining SP segments anterior to this point. Finally, we recorded the numbers of vertebrae anterior to the most anterior vertebrae of origin of the most anterior SP segment (Table 1, no SP).

We used least squares linear regressions to test whether the values of length for the entire SP segment at mid-body had significant predictive value for the total change in length that occurred from mid-body to the most anterior segment. To clarify the longitudinal location of where reduction in the numbers of vertebrae spanned by SP segments occurred, we calculated the percentages of the total length reduction that occurred anterior to the "vertebra of constraint" (located posterior to the skull by a number of vertebrae equal to the total mid-body SP length) as well as the corresponding percentages for of total SP length reduction that occurred anterior to locations at 15, 30 and 45 vertebrae
posterior to the skull. Although the selection of these latter three locations was somewhat arbitrary, it permitted comparisons for intervals with equal numbers of vertebrae unlike the comparisons of the amount of shortening at the variable location based on the vertebra of constraint.

RESULTS
General Features of SP Anatomy

The general anatomical features of the SP conformed closely to previous descriptions (Gasc, 1974; Gasc, 1981; Mosauer, 1935) (Fig. 1). In the Henophidia and Acrochordidae, the morphology of the SP (hereafter referred to as the “henophidian” arrangement) different from colubroids, because SSP-SP muscle fibers lacked two distinct heads and originated posteriorly from a tendinous arch that attached dorsally to the neural spine and ventrally to the postzygapophysis of each vertebra (Fig. 1A). The dorsal portion of the tendinous arch was considered the posterior tendon of origin for the SP fibers when determining the total muscle length. The segmental length of the posterior tendon in the species with the henophidian arrangement usually spanned only one or two vertebrae. Dorsally, the SSP-SP muscle fibers originated posteriorly from multiple slips that are attached to 5-7 adjacent posterior tendons. Consequently, a single vertebra had contractile tissue from several adjacent muscle segments in which the most superficial contractile tissue belonged to the most anterior SSP-SP segment arising from that vertebra. The SSP-SP fibers were nearly parallel to the long axis of the snake as they coursed anteriorly and attached to a broad anterior tendon. The anterior tendon coursed anterodorsally, medially and tapered before it inserted on the posterolateral border of each neural spine. In the
henophidian arrangement an additional ventral portion contributed to an intermuscular septum between the SSP-SP and the *m. longissimus dorsi* (not illustrated in Fig. 1A).

In colubroids, the SSP-SP fibers diverged posteriorly to form two distinct heads, a more dorsal SP, and a more ventral SSP (Fig. 1B-D). The SP originated posteriorly via slips of muscle fibers that were attached to the lateral surface of the posterior tendons of the *m. multifidus*. The posterior tendon of colubroids usually spanned from one to three vertebrae. The SP fibers usually originated from two to three adjacent vertebrae (Table 1). Therefore, a single vertebra usually had fibers from more than one adjacent muscle segment (Fig. 1B). From the medially located origin, the SP muscle fibers coursed laterally and slightly anteroventrally as they usually spanned three to five vertebrae, and ultimately the SP fibers converged with those of the SSP. The SSP fibers coursed medially and anterodorsally either directly from the postzygapophysis of each vertebra or from the medial tendon of the more ventrally and laterally located *m. longissimus dorsi*, depending on the species. The SSP-SP fibers were usually fused anteriorly for one to two vertebrae, and ultimately attached to an anterior tendon that coursed anterodorsally and slightly medially and inserted onto the posterolateral border of the neural spine of each vertebra.

Three species of arboreal colubroids, (*Imantodes cenchoa, Trimeresurus albolabris*, and *T. mucrosquamatus*) had an unusual anterior tendon morphology, for which the contractile tissue of an individual SP segment had a single tendon that branched anteriorly into two to three tendons that inserted on the neural spines of multiple adjacent vertebrae. This morphology was observed several times within each individual of each species.
Longitudinal Changes in Segmental Length

Among the species studied, segments of the SP muscle spanned from 9-46 vertebrae at mid-body, whereas the most anterior segments spanned from 7-17 vertebrae (Table 1). All six of the species in which the total length of SP segments was reduced by more than 20 vertebrae were slender arboreal colubroids (Table 1). Rather than changing linearly with longitudinal position, the rate of the anterior length reduction usually increased from posterior to anterior (see slopes in Figs. 2-5).

Significantly more reduction in total segmental length (Fig. 6A) and in anterior tendon length (Fig. 6B) occurred with increased total length of SP mid-body segments. By contrast, changes in the number of vertebrae spanned by the SP contractile tissue did not change significantly with increased total length of SP segments at mid-body (Fig. 6C). Thus, most of the changes in the total segmental length were the result of changes in the length of anterior tendon, rather than changes in the length of contractile tissue. An additional consequence of these longitudinal changes was that the proportion of an SP segment composed of contractile tissue increased anteriorly in all species.

In all of the species examined the reductions in the total numbers of vertebrae spanned by SP segments occurred at more posterior locations than were necessary based on the mid-body lengths of the segments (Figs. 2-5; Table 1). In all the species of boids, pythonids, acrochordids, and Hydrophis, all of the reduction in SP length occurred posterior to the vertebra of constraint (Table 1, Ant. Red.). By contrast, in the terrestrial and arboreal colubroids, substantial reduction in SP length occurred both posterior (14-60%) and anterior (40-86%) to the vertebra of constraint, with a significantly more reduction in length occurring anteriorly (paired $t = 8.21, P < 0.001, n = 18$). The percentage of reduction in SP
length that occurred anterior to vertebrae 15, 30 and 45 did not change significantly with SP length at mid-body for the entire sample of snakes (all $P > 0.2$), but when the species that were fully aquatic or with the henophidian arrangement were excluded, these two quantities had a significant negative relationship (all $P < 0.0009$) (Fig. 7B-D).

Although species with the henophidian arrangement usually had little longitudinal reduction in the numbers of vertebrae spanned by SP segments, the absolute lengths (mm) of the most anterior SP segments averaged only 56.4% (SE = 5.4) of the absolute length of the mid-body SP segments. This was consistent with previous data showing that the lengths of vertebrae decrease anteriorly in many henophidian taxa (Gasc, 1976; Hoffstetter and Gasc, 1969).

**Mechanisms of Segmental Length Reduction**

Near mid-body, successive SP segments usually had similar length and shape, and segments were simply translated anteriorly by a single vertebra so that the more anterior segment of a pair of adjacent segments had attachment sites that were all one vertebra anterior to the homologous locations of the neighboring muscle segment. However, at locations closer to the skull we observed three distinct deviations from this pattern that could result in SP segments spanning fewer vertebrae.

The most common deviation observed was an anterior translation of the most posterior site of origin by more than one vertebra, while the attachment site of the anterior tendon was only translated anteriorly by a single vertebra (Fig. 1B). This condition also usually included a reduced number of muscular slips giving rise to the more anterior SP segment within a pair of successive segments (Fig. 1B). All of the vertebrae in these
regions still had contractile tissue from at least one segment because the number of “skipped” origins between adjacent segments never exceeded the number of muscular slips contributing to a single SP segment.

Another anterior modification, which was only observed in colubroid species, was the apparent fusion of contractile tissue of multiple adjacent SP segments to a single tendon of insertion (Fig. 1C). In some colubroids, the numbers of muscular slips in the anteriormost segments would triple or quadruple compared to segments, and these integer multiples of the number of slips compared with mid-body segments strongly suggested the number of segments that may have fused. Commonly, if a single fused SP segment spanned N vertebrae and had 5 slips, then the length of the next anterior segment was approximately N-5. These fused segments also usually had a deep central tendon with a posterior portion that was surrounded by the muscle fibers and hence not superficially visible (Fig. 1C, arrow), whereas the more anterior portion of the anterior tendon was more superficial and lacked any additional surrounding contractile tissue from the SP segment. Consequently, the anterior edge of the SP contractile tissue of a fused segment (Fig. 1C, vertebra 5) was often much more than one vertebrae anterior to the corresponding structure of the next most posterior SP segment (Fig. 1C, vertebra 10). Fused SP segments occurred in most colubroids examined, and they were usually more common closer to the head of the snake. Segments with fused contractile tissue occurred repeatedly within some individuals, especially in species with longer SP muscle segments.

In some colubroid species, only the most anterior SP segment had the fused morphology, and in these cases the fused segment was not associated with a subsequent anterior reduction in SP segment length. Thus, despite the presence of fused morphology,
these species were not considered to have fused SP segments as a mode of reducing segment length (Table 1). The anteriormost SP segments in species with the henophidian arrangement were distinct from the trunk because their anterior tendons usually had fibers of the *m. spinalis capitus* originating from them. These segments overlapped substantially and appeared to have SSP-SP fibers that intermingled, but because these segments had discrete anterior tendons they were not considered fused.

A third anterior modification associated with shortening SP segments was the fusion of anterior tendons of multiple SP segments to a single vertebra of insertion (Fig. 1D). Although the posterior sites of origin were translated in this condition, the anterior sites of insertion were not. Successively anterior SP segments associated with anterior tendons that were fused to a single tendon were often shortened by two vertebrae (Fig. 1D). Fused anterior tendons were only observed in the anterior trunk of four species of highly arboreal colubrid snakes in the genera *Ahaetulla, Dendrelaphis, Oxybelis,* and *Uromacer* (Table 1). The numbers of segments, and thus the numbers of tendons fused varied between species. *Ahaetulla* and *Dendrelaphis* both had two to four tendons fused, whereas *Oxybelis* and *Uromacer* only had two tendons fused. Furthermore, the number of times fused tendons occurred within an individual species varied. *Ahaetulla* and *Dendrelaphis* both had two to three occurrences of fused tendons, whereas *Oxybelis* and *Uromacer* both had only one occurrence of fused tendons. When fused tendons occurred more than once, they always occurred in consecutive segments in the anterior trunk.
DISCUSSION

Within the species investigated, SP muscle lengths decreased substantially for more anterior locations, and in some species the extent of this longitudinal variation nearly encompassed all of the previously reported interspecific variation in mid-body SP length (Jayne, 1982). Regardless of the extent of reduction that occurred intraspecifically, four characteristics were observed relating to SP segment modification. (1) Reductions occurred on either side of the vertebra of constraint in most species, however all species had reductions that occurred posterior to the vertebra of constraint. (2) Different mechanisms of length reduction were observed among taxa. (3) The proportions of tendon and contractile tissue decreased in many species, but often did not substantially change in species with the henophidian arrangement. (4) The span of the most anterior segment, as well as the numbers of vertebrae anterior to this segment was remarkably similar across species.

First, reductions occurred on either side of the vertebra of constraint in most species, and the proportion of reduction that occurred on either side varied between species (Table 1, Fig. 2-5). Thus, no species maintained SP muscle lengths until the theoretical most anterior limit, and reductions began posterior to the vertebra of constraint in all species. Interestingly, species with shorter muscles segments, and therefore the least constraint on SP length, had all SP reduction occur posterior to the vertebra of constraint (Table 1). By contrast, species with longer muscle segments had percent SP reductions that occurred on either side of the vertebra of constraint, with significantly more reduction occurring anteriorly (Fig. 7). Thus, species with shorter muscles generally have greater homogeneity in SP muscle lengths over larger regions of the body, whereas species with
longer muscle segments generally have anterior regions with highly variable lengths of SP muscle segments (Figs 2-5).

Secondly, several mechanisms of reduction were observed, some of which varied between taxa. Skipping a site of origin was the most common mode, and was the only mechanism of reduction observed in species with the henophidian arrangement, whereas all modes of reduction were present in the colubroid species examined (Table 1). Given the comparatively large number of overlapping, simultaneously active SP segments during many locomotor modes, skipped origins probably have a negligible effect on the overall functionality of trunk (Jayne, 1988a,b).

Other presumably derived modes of reduction, which involve fusion of several adjacent segments, may be functionally significant because several adjacent muscle segments are exerting force to a single site of insertion, often on or near the skull (Ruben, 1977b). In the species that have several adjacent segments of contractile tissue fused, the muscle architecture has a pinnate fiber arrangement where the fibers of several segments are attached to a single, central tendon that courses into the muscle belly (Fig. 1C). Thus, this arrangement may be an effective method of packaging an increased number of muscle fibers acting on a single tendon without substantially increasing the anatomical cross-sectional area. Although fused mechanisms of reduction were common among the colubroid species, fusion of anterior tendons was only observed in four species of arboreal colubrid species (*Ahaetulla, Dendrelaphis, Oxybelis*, and *Uromacer*). The colubrid genera with fused tendons are distantly related, suggesting this morphology was possibly acquired convergently (Pyron et al., 2011).
The specific events that occur during myogenesis which results in skips or fusions of SP tissue are unclear. However, other tissues in the body that are segmentally arranged, such as ventral scale rows and ribs, can also exhibit abnormalities that disrupt a 1:1 segmental correspondence with the vertebrae. These abnormalities appear related to thermal influence during embryogenesis, with lower temperatures producing more abnormalities such as occurrence of two ribs per vertebra, or half scales (Löwenborg et al. 2011). Perhaps the evolution of the different SP modes of reduction, which also deviate from a 1:1 correspondence with the vertebrae, were related to features of the thermal environment which influenced events during embryogenesis.

Thirdly in most species the ratio of tendon to contractile tissue decreased anteriorly, whereas in species with the henophidian arrangement, the ratio often did not substantially change. Thus, much of the longitudinal intraspecific variation in SP length resulted from differences in the length of tendon. Tendon length also accounts for the interspecific variation in SP length at mid-body (Jayne, 1982). Reduction in the proportion of tendon in the anterior trunk SP segments may be functionally significant, especially since several behaviors, such as constriction or striking, occur anteriorly. Intriguingly a similar constraint exists for SP length for segments approaching the tail tip, however few studies have explored anatomical or functional aspects of this region. Snakes also utilize their tails for several behaviors, therefore how muscle segments are modified also may be functionally important in this region. Preliminary dissections revealed that similar mechanisms exist in the tail which results in SP length change (Pers. Observation).

Tendon is relatively stiff compared to contractile tissue therefore, muscle-tendon units that are primarily composed of tendon should be relatively inflexible compared to a
muscle segment composed mostly of contractile tissue (Roberts and Azizi, 2011). This difference in flexibility is related to the fact that tendon is relatively stiff compared to contractile tissue, and therefore changes in length occur primarily in the contractile portion during events that cause strain. Thus segments that are mostly tendon are able to undergo relatively smaller changes in length when compared to muscle segments of identical span, but mostly contractile tissue. The anterior trunk may therefore be more flexible than regions in the body that have muscle segments composed of more tendinous tissue (Jayne 1982). This could potentially explain why some species that have muscles with long tendons at mid-body, such as *Boiga* or *Chrysopelea*, are also accomplished at constricting, which is a behavior which presumably requires a relatively flexible anterior trunk (Jayne, 1982).

Furthermore, muscle segments exposed to identical strains, but differ in the ratio of tendon to muscle, should theoretically differ in active force output (Rassier et al., 1999). This is related to the fact in a muscle segment that is largely composed of tendon, most of the change in length occurs within the contractile tissue, and therefore the contractile tissue undergoes a relatively larger change in length when compared to a segment of identical length but with less tendon. Thus, when exposed to identical strains, the segment with more tendon would be displaced on a length-tension curve to a position that results in a larger reduction in active force output compared to the segment with less tendon. During lateral undulation, axial muscles at a particular longitudinal position undergo cyclic strain cycles, and the strains experienced by the muscles should be directly related to the amplitude and length of the kinematic wave. Since the ratio of tendon to muscle varies longitudinally, lateral bends with identical amplitudes and wavelengths, but at different
longitudinal positions can have substantially different consequences for the strains imposed on the muscle fibers, and therefore the active force producing capabilities of these segments (Rassier et al., 1999).

Species with the henophidian arrangement often had remarkable homogeneity in the numbers of vertebrae spanned by SP muscle segments and minimal changes in the proportions of tendon and contractile tissue. However, the absolute lengths of SP segments were reduced anteriorly, and therefore muscle length and vertebral length are correlated in these species (Gasc, 1976; Hoffstetter and Gasc, 1969). Thus, in species with the henophidian arrangement, the anterior trunk should theoretically be more flexible compared to other regions of the body because there are more joints per unit of length. Therefore this portion of the body can folded into relatively tighter curvatures and may be functionally important because all species with the henophidian arrangement constrict their prey mostly with the anterior trunk. Longitudinal differences in vertebral length appear related to modifications that occur during postembryonic growth in henophidians (Head and Polly, 2007; Polly et al., 2001). Although, both henophidians and colubroids appear to have different anatomical modifications to the anterior trunk, interestingly both could result in increased flexibility in this region.

Finally, there was remarkable similarity in the length, and numbers of vertebrae anterior to the most anterior SP segment, and these lengths are consistent with recently proposed cervical boundaries in snakes by both molecular and other anatomical investigations (Tsuihiji et al., 2006; Tsuihiji et al., 2012; Woltering et al., 2009). The span of the anteriormost SP segment exhibited little variability, with the majority being in the range of vertebra 7-12. The numbers of vertebrae anterior to the anteriormost vertebra of
origin also had remarkably similar values that ranged from 2-8. These vertebral localities are in close proximity to the posterior extent of several other muscles, such as the *m. retractor quadrati, m. rectus capitus anterior* and *m. semispinalis capitus*, that have been suggested to indicate an approximate cervical boundary in snakes (Tsuihiji et al., 2006; Tsuihiji et al., 2012). Furthermore, the numbers of vertebrae anterior to the anteriormost SP site of origin are particularly consistent with molecular findings that have delineated anterior expression boundaries for Hox genes that are associated with a cervical identity and suggest the presence of a cervical region in snakes (Woltering et al., 2009).

Thus, if the span of the most anterior SP segment was used to potentially define a cervical boundary in snakes, it would support the previously hypothesized boundaries, as well as the claim that this boundary would be relatively short compared to the entire pre-cloacal region in snakes (Hoffstetter and Gasc, 1969; Tsuihiji et al., 2006; Tsuihiji et al., 2012). Despite the lack of an anatomically conspicuous neck in snakes, Hox expression domains in snakes retain a pattern that closely approximates that of lizards in the cervical region, which have an anatomically defined neck region (Di-Poi et al., 2010; Woltering et al., 2009). These findings are in contrast to other proposed hypotheses that suggest the elongate, limbless body form characteristic of snakes evolved primarily from lengthening of the cervical region, or that snakes completely lack this region (Caldwell, 2000; Cohn and Tickle, 1999).
LITERATURE CITED


TABLE 1. Spinalis morphology and modes of reduction of the study species

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen (FMNH)</th>
<th>Habitat</th>
<th>Numbers of Vertebrae</th>
<th>Mode</th>
<th>Ant. Red.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boa constrictor</strong></td>
<td>Pers</td>
<td>Ter</td>
<td>Pre-clo. SP 50%SVL,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SP Anterior Seg. No SP</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Origin Cont. Origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Corallus hortulanus</strong></td>
<td>Pers</td>
<td>Arb</td>
<td>240 15 7 5 10 7 5 5</td>
<td>S</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Pythonidae</strong></td>
<td></td>
<td></td>
<td>277 12 6 5 9 6 7 2</td>
<td>S</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Python reticulatus</strong></td>
<td>53282</td>
<td>Ter</td>
<td>329 13 6 6 12 9 10 2</td>
<td>S</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Morelia viridis</strong></td>
<td>215856</td>
<td>Arb</td>
<td>231 12 6 6 10 5.5 6 4</td>
<td>S</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Acrochordidae</strong></td>
<td></td>
<td></td>
<td>213614 203 9 3 5 8 4 5 3</td>
<td>S</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Colubridae</strong></td>
<td></td>
<td></td>
<td>35190 178 23 5 2 11 6.5 6 5 S,F</td>
<td>58%</td>
<td></td>
</tr>
<tr>
<td><strong>Coluber constrictor</strong></td>
<td></td>
<td></td>
<td>127 21 5 2 10 7 5 5 S,F</td>
<td>55%</td>
<td></td>
</tr>
<tr>
<td><strong>Pantherophis guttatus</strong></td>
<td>Pers</td>
<td>Ter</td>
<td>224 17 5 3 10 8 5 5 S,F</td>
<td>86%</td>
<td></td>
</tr>
<tr>
<td><strong>Ahaetulla prasina</strong></td>
<td>Pers</td>
<td>Arb</td>
<td>215 36 6 3 11 6 7 5 S,F,T</td>
<td>68%</td>
<td></td>
</tr>
<tr>
<td><strong>Boiga irregularis</strong></td>
<td>Pers</td>
<td>Arb</td>
<td>262 30 5 2 13 7 5 8 S,F</td>
<td>71%</td>
<td></td>
</tr>
<tr>
<td><strong>Chrysopelea paradisi</strong></td>
<td>Pers</td>
<td>Arb</td>
<td>225 24 4 2 9 4 4 5 S,F</td>
<td>73%</td>
<td></td>
</tr>
<tr>
<td><strong>Dendrelaphis pictus</strong></td>
<td>180034</td>
<td>Arb</td>
<td>174 34 3 2 14 8 9 5 S,T</td>
<td>65%</td>
<td></td>
</tr>
<tr>
<td><strong>Dipsas variegatus</strong></td>
<td>217217</td>
<td>Arb</td>
<td>179 28 6 2 7 3 2 5 S,F</td>
<td>71%</td>
<td></td>
</tr>
<tr>
<td><strong>Imantodes cenchoa</strong></td>
<td>21882</td>
<td>Arb</td>
<td>248 46 7 3 17 13 12 5 S,F</td>
<td>69%</td>
<td></td>
</tr>
<tr>
<td><strong>Opheodrys aestivus</strong></td>
<td>60568</td>
<td>Arb</td>
<td>155 19 3 2 8 4 4 4 S,F</td>
<td>64%</td>
<td></td>
</tr>
<tr>
<td><strong>Oxybelis fulgidus</strong></td>
<td>130675</td>
<td>Arb</td>
<td>198 32 4 2 12 7 6 6 S,F,T</td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td><strong>Uromacer catesbyi</strong></td>
<td>18367</td>
<td>Arb</td>
<td>167 25 4 2 14 9 9 5 S,T</td>
<td>55%</td>
<td></td>
</tr>
<tr>
<td><strong>Viperidae</strong></td>
<td></td>
<td></td>
<td>171272 178 22 3.5 2 12 7 7 5 S</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td><strong>Bothrops alternatus</strong></td>
<td>12367</td>
<td>Arb</td>
<td>157 18 5 3 9 6 5 4 S</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td><strong>Trimeresurus albolabris</strong></td>
<td>6712</td>
<td>Arb</td>
<td>161 25 4 2 9 4 4 5 S,F</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td><strong>T. mucrosquamatus</strong></td>
<td>120792</td>
<td>Arb</td>
<td>209 36 7 2 15 9 7 8 S,F</td>
<td>67%</td>
<td></td>
</tr>
<tr>
<td><strong>Elapidae</strong></td>
<td></td>
<td></td>
<td>161424 188 25 4 2 9 7 6 3 S,F</td>
<td>69%</td>
<td></td>
</tr>
<tr>
<td><strong>Naja naja</strong></td>
<td>22595</td>
<td>Arb</td>
<td>215 34 5.5 2 12 7 5 7 S,F</td>
<td>64%</td>
<td></td>
</tr>
<tr>
<td><strong>Dendroaspis viridis</strong></td>
<td>164988</td>
<td>Aq</td>
<td>220 10 3 3 9 4 3 6 S</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><strong>Hydrophis brooki</strong></td>
<td>202094</td>
<td>Aq</td>
<td>197 11 3 3 10 4 3 6 S</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

Pers indicates personal specimens. Ter, Arb, and Aq indicate terrestrial, arboreal, and aquatic habitats respectively. Pre-clo. indicate the numbers of body vertebrae anterior to the tail. The numbers of vertebrae are given for the total segmental length (Tot.), contractile portion (Cont.), and origin (Origin) of the spinalis (SP) for the segment at mid-body and the anteriormost segment. No SP indicates the numbers of vertebrae anterior to the most anterior spinalis origin. For the mode of length reduction S, F, and T indicate skipped origins, fused contractile tissue, and fused tendons, respectively. Ant. Red. is the percentage of the total anterior reduction in numbers of vertebrae spanned by an SP segment that occurred anterior to the vertebra of constraint.
Fig. 1. Anatomy of SP muscle segments. A: A henophidian (*Boa constrictor*) muscle segment. Distinct SP and SSP heads are present in colubroids (B-D). B: Skipped vertebra of origin in *Pantherophis guttatus*. C: Fusion of multiple adjacent segments of contractile tissue to a single tendon of insertion in *Trimeresurus mucrosquamatus*. The arrow indicates the most posterior extent of the central tendon within the muscle belly. D: Fusion of multiple adjacent anterior tendons to a single vertebra of insertion in *Ahaetulla prasina*. Anatomical drawings are from right, lateral views, and numbers between vertical lines indicate the numbers of vertebrae posterior to the skull. Dashes between vertical lines indicate multiple vertebrae were excluded from the drawing. To simplify the illustration, all vertebral lengths are the same. Abbreviations: SP, *m. spinalis*; SSP, *m. semispinalis*; AT, anterior tendon; PT, posterior tendon.
Fig. 2

Total Segmental Length (Vertebrae)

A. granulatus

B. hortulanus

C. viridis

P. reticulatus

B. constrictor
Fig. 2. Longitudinal variation in the segmental length of the SP muscle in species with the henophidian arrangement. A: *Acrochordus granulatus* B: *Corallus hortulanus* C: *Morelia viridis* D: *Python reticulatus* E: *Boa constrictor*. Triangles, circles, and squares represent arboreal, terrestrial, and aquatic species, respectively. Black, dark gray, and light gray symbols represent the lengths of the total SP segment, contractile portion, and the anterior tendon, respectively. White symbols correspond to a vertebra posterior to the skull that is equal to the number of vertebrae spanned by the SP segment at 50% SVL. Abbreviations: SP, *m. spinalis*. 
Fig. 3
Fig. 3. Longitudinal variation in the segmental length of the SP muscle in species of Colubrinae.


The colors and symbols are as in Fig. 2. Abbreviations: SP, m. spinalis.
Fig. 4. Longitudinal variation in the segmental length of the SP muscle in species of Natricinae (A) and Dipsadinae (B-D). A: *Nerodia fasciata* B: *Uromacer catesbyi* C: *Dipsas variegatus* D: *Imantodes cenchos*. The colors and symbols are as in Fig. 2. Abbreviations: SP, *m. spinalis*. 
Fig. 5

A. B. schlegeli
B. B. alternatus
C. T. albolabris
D. T. mucrosquamatus
E. H. brookii
F. H. melanoma
G. N. naja
H. D. viridis

Total Segmental Length (Vertebrae)
Segment of Origin (Vertebrae)
Fig. 5. Longitudinal variation in the segmental length of the spinalis SP muscle in species of Viperidae (A-D) and Elapidae (E-H). A: Bothriechis schlegeli B: Bothrops alternatus C: Trimeresurus albolabris D: Trimeresurus mucedorus E: Hydrophis brooki F: Hydrophis melanosoma G: Naja naja H: Dendroaspis viridis. The colors and symbols are as in Fig. 2. Abbreviations: SP, m. spinalis.
Fig. 6

A Total

- Henophidian Type
- Colubridae
- Viperidae
- Elapidae

\[ R^2 = 0.95 \]
\[ p < 0.0001 \]

B Tendon

\[ R^2 = 0.98 \]
\[ p < 0.0001 \]

C Contractile

\[ R^2 = 0.14 \]
\[ p > 0.05 \]
Fig. 6. Relationships between the total longitudinal changes in segmental length and mid-body SP length of A: The entire SP segment B: Anterior tendon and C: Contractile tissue for all species (n=25). Black circles, triangles, and squares represent species within Colubridae, Elapidae, and Viperidae, respectively. The white circles represent Boidae, Pythonidae, and Acrochordidae, all of which have the henophidian arrangement. Individual species names and familial grouping are provided in Table 1. Abbreviations: SP, m. spinalis.
Fig. 7

A

Ant. Constraint

% SP Anterior Reduction (% Mid-body SP Length)

B

V15

C

V30

D

V45

% SP Anterior Reduction (% Mid-body SP Length)

Mid-Body Segmental Length (Vertebrae)

- Henophidian Type
- Colubridae
- Viperidae
- Elapidae
Fig. 7. Relationships between the % SP reduction and SP length occurring anterior to the A: Ant. Constraint, which is the vertebra posterior to the skull which is equal to the mid-body SP length B: V15 C: V30 and D: V45 for all species (n=25). The colors and symbols are as in Fig. 6. Individual species names and familial groupings are provided in Table 1. Abbreviations: SP, *m.spinalis*; Ant. Constraint, vertebra of constraint; V, vertebra.