

Ecological and phylogenetic variability in the spinalis muscle of snakes

J. L. TINGLE* , G. E. A. GARTNER*¹, B. C. JAYNE† & T. GARLAND JR*

*Department of Evolution, Ecology, and Organismal Biology, University of California, Riverside, CA, USA

†Department of Biological Sciences, ML006, University of Cincinnati, Cincinnati, OH, USA

Keywords:

adaptation;
comparative methods;
constriction;
ecomorphology;
grade shifts;
habitat;
morphology;
phylogeny;
sidewinding;
vertebrae.

Abstract

Understanding the origin and maintenance of functionally important subordinate traits is a major goal of evolutionary physiologists and ecomorphologists. Within the confines of a limbless body plan, snakes are diverse in terms of body size and ecology, but we know little about the functional traits that underlie this diversity. We used a phylogenetically diverse group of 131 snake species to examine associations between habitat use, sidewinding locomotion and constriction behaviour with the number of body vertebrae spanned by a single segment of the spinalis muscle, with total numbers of body vertebrae used as a covariate in statistical analyses. We compared models with combinations of these predictors to determine which best fit the data among all species and for the advanced snakes only ($N = 114$). We used both ordinary least-squares models and phylogenetic models in which the residuals were modelled as evolving by the Ornstein–Uhlenbeck process. Snakes with greater numbers of vertebrae tended to have spinalis muscles that spanned more vertebrae. Habitat effects dominated models for analyses of all species and advanced snakes only, with the spinalis length spanning more vertebrae in arboreal species and fewer vertebrae in aquatic and burrowing species. Sidewinding specialists had shorter muscle lengths than nonspecialists. The relationship between prey constriction and spinalis length was less clear. Differences among clades were also strong when considering all species, but not for advanced snakes alone. Overall, these results suggest that muscle morphology may have played a key role in the adaptive radiation of snakes.

Introduction

Tetrapods lacking limbs have evolved repeatedly and radiated into a variety of fossorial, terrestrial, aquatic and arboreal niches. Accordingly, functional variation in elongate, limbless taxa has received considerable attention in the fields of evolutionary and organismal biology (Gans, 1960; Nussbaum & Naylor, 1982; Mehta *et al.*, 2010; Ward & Mehta, 2010; Brischoux & Shine, 2011; Ward *et al.*, 2015), but relatively few studies have

used an ecomorphological approach with limbless vertebrates (Vitt & Vangilder, 1983; Guyer & Donnelly, 1990; Martins *et al.*, 2001; Pizzatto *et al.*, 2007; Alencar, 2010). Even though limbless vertebrates must rely on their axial musculoskeletal systems for locomotion in their environment, broad-based comparisons of their axial musculoskeletal system are limited (Jayne, 1982).

Compared to any other clade of limbless terrestrial vertebrates, snakes are much more diverse, with over 3600 extant species that have filled a wide variety of niches (Uetz *et al.*, 2007; Pyron & Burbrink, 2012). Consequently, they are an ideal clade for correlating phylogeny, ecology and behaviour with morphology. Of their morphological features, axial muscles are of particular interest due to their important roles in locomotion and prey handling as well as the marked variation in their lengths. One of the largest of these

Correspondence: Theodore Garland, Jr., Department of Evolution, Ecology, and Organismal Biology, University of California, 900 University Ave., Riverside, CA 92521, USA.
Tel.: (951) 827 3524; fax: (951) 827 4286;
e-mail: tgarland@ucr.edu

¹Present address: Life Sciences Department, Santa Monica College, Santa Monica, CA 90405, USA

muscles, the semispinalis–spinalis (Gasc, 1974, 1981; Ruben, 1977), contributes to lateral or dorsal flexion of the vertebral column depending on the mode of locomotion (Jayne, 1988a,b; Moon, 1999). The lengths of individual semispinalis–spinalis muscle–tendon units show remarkable interspecific variation, spanning from 10 to more than 40 vertebrae (Jayne, 1982). Most of this variation results from variable lengths of tendon, as contractile tissue often spans only four to six vertebrae (Mosauer, 1932; Gasc, 1974, 1981; Jayne, 1982).

Some of the variation in axial muscle morphology stems from gross differences among major clades, such as between ‘advanced snakes’ (Caenophidia) and the boas and pythons (traditionally grouped taxonomically as the Henophidia) (Mosauer, 1932; Gasc, 1974; Jayne, 1982). For example, Ruben (1977) compared the fast-moving, actively foraging and nonconstricting Coachwhip (*Masticophis flagellum* Shaw 1802, a caenophidian) to the Rosy Boa (*Lichanura trivirgata* Cope 1861), which is a slow-moving ambush predator that constricts its prey. He suggested that the Coachwhip’s longer muscle–tendon units enhance speed, whereas the Rosy Boa’s shorter muscle–tendon units could enhance flexibility and increase force production in the tight axial bends used during prey constriction due to their greater proportion of contractile tissue. Hence, rapid locomotor speed might preclude effective constriction and vice versa. However, for such widely divergent pairs of species, phylogeny could confound attempts to relate interspecific morphological variation to performance (Garland & Adolph, 1994), prompting Jayne’s (1982) study of morphological variation in a much larger sample of species.

Jayne (1982) determined the total number of vertebrae spanned by the contractile tissue and tendons of the spinalis portion of the semispinalis–spinalis complex for a taxonomically and ecologically diverse sample of 94 snake species. He found that all boas and pythons and some other species that also constrict often had large numbers of body (preloacal) vertebrae and short spinalis muscle–tendon units, which could increase flexibility and force production during constriction. Furthermore, he found that habitat has an apparent effect on morphology. For example, highly arboreal caenophidian species often had the longest axial muscle–tendon units, perhaps due to a mechanical advantage from long, stiff tendons that could facilitate bridging long gaps between branches. Subsequent studies of gap-bridging performance have been consistent with this suggestion (Lillywhite *et al.*, 2000; Hoefer & Jayne, 2013). Additional tests of arboreal locomotor performance have found that heavily muscled species with short axial muscle–tendon units rely more on gripping branches when they climb, whereas light-bodied species with longer muscle–tendon units rely more on sliding and balancing and often attain faster speeds (Byrnes & Jayne, 2014; Jayne & Byrnes, 2015). By

contrast, many burrowing and aquatic specialists had short axial muscle–tendon units, and three sidewinding vipers had the shortest muscle–tendon units of all vipers in Jayne’s (1982) sample. Hence, locomotor specialization likely correlates with muscle morphology.

Jayne’s (1982) study occurred before the advent of quantitative phylogenetic comparative methods that address issues of potential statistical nonindependence among species (Felsenstein, 1985; Garland *et al.*, 2005; Rezende & Diniz-Filho, 2012). The present study expands upon Jayne’s (1982) work by analysing his data in the context of phylogenetic information and statistical methods not then available, with the addition of some species that fill important phylogenetic or ecological gaps in the data set. In addition, we simultaneously consider the statistical effects of habitat, constricting behaviour, sidewinding and major clade by the use of multiple regression models, whereas Jayne (1982) performed separate analyses comparing families and habitat categories. We compare statistical models incorporating a hierarchical phylogeny and alterations of it under an Ornstein–Uhlenbeck (OU) model of character evolution. We test for phylogenetic signal – the tendency for related species to resemble one another (Blomberg & Garland, 2002; Blomberg *et al.*, 2003) – in two ways, by examining the presence of grade shifts (differences in the mean value of the trait of interest between or among clades) and/or evidence that a statistical model fitted with a hierarchical phylogenetic tree fits the data better than does a star phylogeny. Additionally, we use these models to determine whether phylogenetic relatedness, number of body vertebrae, constriction, habitat, specialization for sidewinding locomotion or some combination best predicts the number of vertebrae spanned by the spinalis muscle–tendon unit. Our general approach of simultaneously examining multiple factors that may have affected the evolutionary history of traits is one widely applicable to many questions in comparative biology (Brainerd & Patek, 1998; Huey *et al.*, 2009; Swanson & Garland, 2009; Gartner *et al.*, 2010; Nunn, 2011; Smith *et al.*, 2011).

Materials and methods

Muscle length, vertebral number, constriction, sidewinding and habitat

We gathered anatomical data on the total number of vertebrae spanned by all of the contractile tissues and tendons within a single, mid-body segment of the spinalis muscle in addition to number of preloacal vertebral for 131 species of snakes, representing 13 major clades (families or subfamilies) from both published data and new measurements of museum specimens (Table 1). Data on muscle–tendon unit lengths and vertebral numbers came primarily from a previously

Table 1 Independent variable coding. Coding of clades ($N = 13$) and habitats ($N = 5$) used for statistical analyses. Numbers in parentheses refer to the number of constricting taxa in each category, and numbers in brackets refer to the number of sidewinding taxa in each category.

Taxa	Clade	Aquatic and Semi-Aquatic	Arboreal	Burrowing	Terrestrial	Total
<i>Scolecophidia</i>	Typhlopidae	0	0	1	0	1
' <i>Henophidia</i> '	Tropidophiidae	0	0	0	1 (1)	1 (1)
	Boidae	0	1 (1)	1 (1)	7 (7)	9 (9)
	Pythonidae etc.	0	0	3 (3)	3 (3)	6 (6)
<i>Caenophidia</i>	Acrochordidae	2 (2)	0	0	0	2 (2)
	Pareidae	0	0	0	1	1
	Viperidae	0	3	0	11 [3]	14 [3]
	Homalopsidae	17	0	0	0	17
	Elapidae	13	1	7	20	41
	<i>Pseudaspis</i>	0	0	1 (1)	0	1 (1)
	Atractaspididae	0	0	5	1 (1)	6 (1)
	<i>Nerodia</i> + <i>Regina</i>	2	0	0	0	2
	Natricinae + Dipsadinae	0	2	1	4 (1)	7 (1)
	Colubrinae	0	7 (1)	5 (2)	11 (8)	23 (11)
Total		34 (2)	14 (2)	24 (7)	59 (21) [3]	131 (32) [3]

published study by Jayne (1982, $N = 99$). The remaining data ($N = 36$) were collected by G.E.A.G. and B.C.J. New taxa were selected to fill in clades or habitat types poorly represented in the previous study. In our data set, $N = 1$ for all species, with the following exceptions: *Crotalus adamanteus* Beauvois 1799 ($N = 2$), *Pituophis catenifer* Blainville 1835 ($N = 2$), *Pituophis melanoleucus* Daudin 1803 ($N = 3$), *Coluber constrictor* Linnaeus 1758 ($N = 5$) and *Nerodia fasciata* Linnaeus 1766 ($N = 5$). We used mean values for all species with $N > 1$. Because of the small sample size for most species, we could not use methods that account for within-species variation (Ives *et al.*, 2007), but species for which several specimens have been dissected show very little intraspecific variation in muscle–tendon unit lengths (Jayne, 1982).

We performed dissections to isolate an individual segment of the spinalis muscle–tendon unit near mid-body and to determine its total longitudinal length as the number of vertebrae spanned, including the vertebrae of origin and insertion. We counted ventral scales as a proxy for vertebral number because they are known to be heritable within snake populations (Dohm & Garland, 1993) and correspond directly with precaudal vertebrae in most snakes (Alexander & Gans, 1966; Kerfoot, 1970). For species whose vertebral numbers cannot be estimated from ventral-scale counts, we used average values from the literature (e.g. Voris, 1975 for most Hydrophiid sea snakes) or X-rays to count vertebral numbers.

We categorized species as constricting or nonconstricting following Jayne (1982). We also categorized species as sidewinding specialists or not. Many snake species will perform sidewinding or something similar under certain conditions, but we limited the final choice of coding for the presence of sidewinding to three specialized species (*Crotalus cerastes* Hallowell

1854, *Cerastes cerastes* Linnaeus 1758 and *Eristicophis macmahonii* Alcock & Finn 1897), which were the only taxa in our sample that use sidewinding as their primary mode of locomotion in their natural habitat.

We categorized habitat (e.g. see Gartner *et al.*, 2010; Brischoux *et al.*, 2011) from the literature (e.g. field guides and works on local snakes faunas) as well as the authors' own observation and experiences with many of the taxa included in this study (e.g. B.C.J. with Homalopsidae). Habitat categories included:

Burrowing/Fossorial: Fossorial taxa actively burrow and are found primarily underground or in leaf litter. Many species possess obvious behavioural and external morphological adaptations for burrowing, such as reduced eyes and an under-slung jaw.

Arboreal: Arboreal taxa spend most of their time in the branches of trees or shrubs.

Aquatic and semi-aquatic: Aquatic and semi-aquatic species spend all or most of their time in water, and they therefore likely face selective pressure for swimming ability.

Terrestrial: Terrestrial species live primarily on the ground. Although they lack any obvious morphological or behavioural adaptations to the terrestrial realm, they also cannot be easily classified into any of the other groups. The terrestrial group therefore includes animals that may, on occasion, swim or climb (as most snakes have some abilities to climb or swim).

Quantifying and categorizing behavioural ecology is difficult, particularly in such broadly defined regimens as habitat (Al-Kahtani *et al.*, 2004; Gartner *et al.*, 2010; Canoville *et al.*, 2016). Like many other animals, snakes rarely reside in exclusively one type of habitat, although notable exceptions include the viviparous sea snakes and blind snakes (*Scolecophidia*). Some species occur in multiple habitats (e.g. *Notechis scutatus* Peters

1861 in semi-aquatic or xeric habitats, Bonnet *et al.*, 2002). In such situations, we selected the most typical habitat for a particular species or, if specified, the population or habitat of the specimen used in the analyses. Hence, we present all of the data and categorizations used so that future workers can add to the database and/or re-categorize and reanalyse if and when improved information becomes available.

Phylogeny

We used a time-calibrated phylogeny from Tonini *et al.* (2016) as the basis for our analysis. This phylogeny contained all of our species except for one, *Chilorhinophis carpenteri*. As it contained a congener, *Chilorhinophis butleri*, we simply made a substitution (see Appendices S1 and S2 for the tree in two file formats).

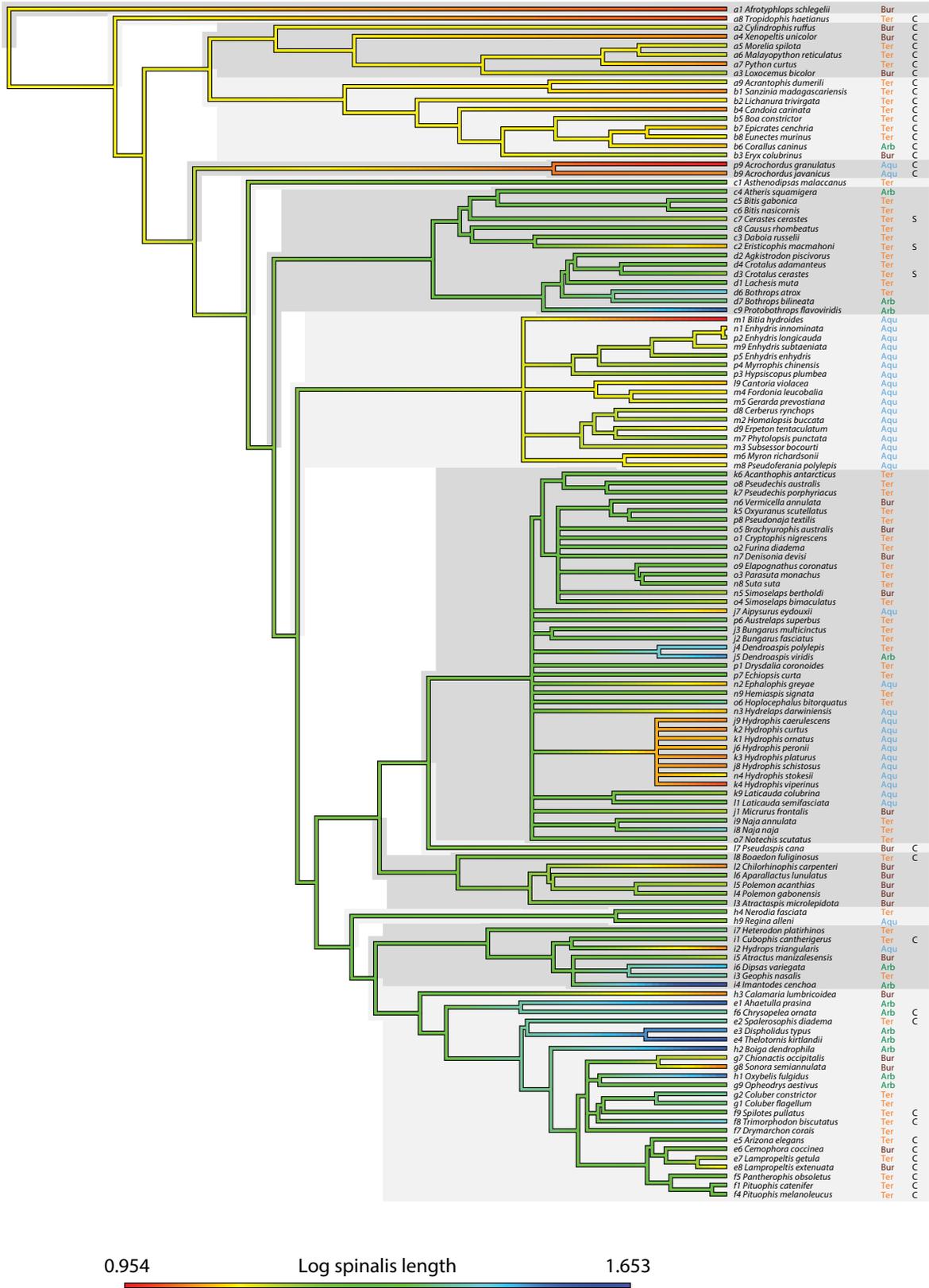
Statistical analyses

We used number of body vertebrae as a covariate in all statistical analyses. Previous studies have shown that snout-vent length (SVL) and number of body vertebrae are generally positively correlated, with the strength of correlation varying among clades, and approaching $r = 0.9$ in some (Lindell, 1994; Head & Polly, 2007). We chose to use body vertebrae rather than SVL as a covariate because the former is constant throughout ontogeny and was available for all species in our data set. In support of this choice, the number of body vertebrae was a statistically significant predictor of spinalis muscle length in the full models that incorporated habitat, clade, sidewinding and constriction.

Total numbers of body vertebrae and the vertebrae spanned by a single segment of the spinalis muscle–tendon unit were \log_{10} -transformed prior to analyses. The effects of \log_{10} total body vertebrae, clade, habitat, sidewinding specialization and constriction on \log_{10} total muscle–tendon unit length (number of vertebrae spanned) were analysed by multiple regressions, with the latter four variables coded as a series of dummy variables (equivalent to analysis of covariance [ANCOVA] with parallel slopes). Although our data set does not include all members of any given clade, we use ‘clade’ operationally to refer to sets of species in our data set that represent distinct branches on our tree (Fig. 1).

All models were estimated using the Matlab Regressionv2 program (Lavin *et al.*, 2008; empirical examples in Huey *et al.*, 2009; Gartner *et al.*, 2010; Brischox *et al.*, 2011; Oufiero *et al.*, 2011). Regression and ANCOVA models were estimated with conventional (nonphylogenetic) ordinary least squares (OLS) and with phylogenetic models incorporating a branch-length transformation based on an Ornstein–Uhlenbeck (OU) model of evolution for residual muscle length variation (henceforth, RegOU: Lavin *et al.*, 2008) – a process suggested as a way to mimic the effects of stabilizing selection (Felsenstein, 1988; Garland *et al.*, 1993; Gartner *et al.*, 2010). The strength of the OU process is estimated via the parameter d (Lavin *et al.*, 2008). A value of one indicates the tree is unchanged from the original, a value > 1 indicates that the nodes have been ‘pushed’ closer towards the tips of the tree, resulting in a more strongly hierarchical phylogeny, and a value < 1 indicates a tree that is less strongly hierarchical than the original (Blomberg *et al.*, 2003; Lavin *et al.*, 2008). Values of $d > 0$ indicate that the residuals of the statistical model have phylogenetic signal. A test for whether the amount of phylogenetic signal is statistically significant can be accomplished by comparing a phylogenetic model with its nonphylogenetic counterpart using a likelihood ratio test (LRT), where twice the difference in \ln likelihoods is assumed to be distributed asymptotically as a χ^2 distribution with degrees of freedom equal to the difference in the number of parameters estimated in the two models (d.f. = 1 for addition of the d parameter for all LRTs used in this study). If phylogenetic versions of models fit the data significantly better than conventional, nonphylogenetic versions by a LRT, then the amount of phylogenetic signal present in the residuals is statistically significant. Phylogenetic signal is also indicated by models that include a statistically significant ‘clade’ variable (e.g. see Gartner *et al.*, 2010). In addition, we computed the K statistic of Blomberg *et al.* (2003) and used randomization tests based on the MSE to test for phylogenetic signal in muscle length and in the number of body vertebrae (both \log -transformed). We hypothesized that all of the independent variables considered would have an effect on muscle–tendon unit length. For some independent variables, such as constriction and arboreality, one-tailed tests could have been employed, but for

Fig. 1 Phylogeny of all 131 species used in the analyses, taken primarily from Tonini *et al.* (2016). Alternating grey shading indicates clade divisions ($N = 13$) as used for statistical analyses, with four of the clades represented by a single species and one represented by only two species in our data set. The term ‘caenophidians’ refers to ‘advanced snakes’, beginning with *Acrochordus* in our sample and extending to the bottom of the figure. Coding for habitat (Aqu = aquatic; Arb = arboreal; Bur = burrowing; Ter = terrestrial), constriction (‘C’) and specialization for sidewinding (‘S’) are indicated to the right of the species names. The two-character codes correspond to taxon names in the tree PDI file (Appendix S1). Branch colours indicate hypothetical ancestral values of \log spinalis muscle length (in units of number of vertebrae spanned). Values at internal nodes were reconstructed via a rerooting procedure (fastAnc in phytools: Revell, 2012) that yields the maximum-likelihood estimates of ancestral states under a Brownian motion model of character evolution (the same results can be obtained by a squared-change parsimony algorithm: Maddison, 1991; Schluter *et al.*, 1997; Garland *et al.*, 1999). Values along branches were then interpolated with contMap (Revell, 2013).



simplicity all significance tests were two-tailed. However, hypothesis testing is complicated by the fact that two different sets of models were fitted. Therefore, we also used a model-fitting approach in which we compared 34 models – 17 using conventional ordinary least squares (OLS, which effectively assumes a ‘star’ phylogeny with contemporaneous tips) and 17 RegOU models with Tonini *et al.*'s (2016) time-calibrated phylogeny. These sets of 17 models always included body vertebrae as a covariate (see above) and then encompassed all possible combinations of the other four independent variables, except for one model which included no independent variables. The model with no independent variables assumes that the trait changes through time via a process like Brownian motion (OLS) or the OU process (RegOU), but with no relationship to our independent variables, and thus serves as a type of null model relative to all models that contain independent variables.

The fit of alternate models was compared using AICc (Akaike's Information Criterion [AIC]) with a second-order correction for small sample sizes (Burnham & Anderson, 2002). Smaller values of AICc indicate a better fit of the model to the data. In general, the use of AICc is suggested when the ratio $n/K < 40$, where K = number of parameters in the global or full model (Burnham & Anderson, 2002, 2004). However, because AICc converges to AIC as n gets large, AICc can be employed regardless of sample size. As a general rule, smaller Δ AICc values indicate better-supported models; however, no general heuristic or statistical test using solely Δ AICc scores separates well-supported models from poor models (Burnham & Anderson, 2002; Burnham *et al.*, 2011). Therefore, as an additional qualitative measure of model fit, we present Akaike weights (w_i , where the probability of a given model is equal to the likelihood of model i divided by the sum of the likelihoods across all models). We also present evidence ratios (ER), which are the ratios of likelihoods of any two models i and j (larger values for evidence ratios are indicative of a less-supported model (Burnham *et al.*, 2011)). All models in the results section are compared relative to our top model (i.e. Δ AICc = 0, ER = 1). We also summed the weights across models containing each independent variable to compare the cumulative weight of each independent variable, which equal the probabilities of those variables to be included in the best model if we were to re-collect the data using the same methods (Burnham & Anderson, 2002; Dlugosz *et al.*, 2013).

Previous research suggests that the axial musculature of the boas and pythons is relatively invariant (Jayne, 1982). Therefore, to examine patterns of morphological variation in axial musculature both within and among major groups of snakes, we have duplicated our analyses for a subset of species that includes only the Caenophidia (the advanced snakes, *sensu* Vidal & Hedges, 2002, $N = 114$).

Results

As shown in Fig. 1, log spinalis muscle–tendon unit length (in units of number of vertebrae spanned) has both increased and decreased multiple times during snake evolution, even considering our small sample of the ~3600 described species of living snakes. In addition, most of the caenophidians have longer muscle–tendon units than the more basal snakes depicted at the top of Fig. 1.

Considering all 131 species, the K statistic of Blomberg *et al.* (2003) was 0.847 for log muscle length and 0.288 for log body vertebrae. Randomization tests based on the MSE indicated that phylogenetic signal was statistically significant for both traits ($P < 0.0005$ and $P = 0.014$, respectively). Similarly (see Appendix S3), the lnML for the RegOU model (estimated $d = 0.652$) with no independent variables (87.336) as compared with that for the corresponding OLS model (63.078) indicates a highly significant LRT.

For the full sample of 131 species, habitat, clade, sidewinding and number of body vertebrae were all important predictors of muscle length (Table 2). This finding was supported by two statistical approaches (F tests on the full models [Table 3]; model comparisons based on information-theoretic criteria [Appendix S3]). Constriction showed no relationship to spinalis length in the full model, but it did appear in some of the top models for the model comparison approach. Caenophidians (‘advanced snakes’) had more variable muscle–tendon unit lengths than noncaenophidians did, with muscle–tendon units reaching significantly greater lengths (Fig. 2A). Arboreal snakes had long muscle–tendon units compared to all other habitat groups, whereas aquatic and burrowing snakes usually had short muscle–tendon units compared to snakes in other habitat categories (Fig. 2B). Constrictors tended towards shorter muscle–tendon units than nonconstrictors did, but this result was not significant in the full model (Fig. 2C; Table 3). Sidewinding specialists (*Crotalus cerastes*, *Cerastes cerastes* and *Eristicophis macmahoni*, all in Viperidae) had relatively short muscle–tendon units as compared with other vipers (Fig. 2D).

Considering all models for the full data set (Appendix S3), the cumulative weight for each independent variable was, in order of importance: habitat = 1.000, clade = 0.999, sidewinding = 0.966, constriction = 0.522. Within the caenophidians ($N = 114$ species), the cumulative weights for the independent variables were as follows: habitat = 1.000, sidewinding = 0.993, constriction = 0.922, clade = 0.002. The large difference in the weight for the clade variable could result from two possible patterns: either caenophidian clades do not show differences among them, aside from variation related to ecological differences, whereas the noncaenophidian clades show more variation; or most of the variation associated with clade occurs between the caenophidians and the

Table 2 Model comparison for all species and for Caenophidia only. Alternate regression models predicting total muscle length (muscle segment plus anterior and posterior tendons) among all taxa ($N = 131$ species), and for caenophidians only ($N = 114$ species). Models are listed in ascending order of Δ AICc (the best model is at the top). d = OU transformation parameter; lnML = maximum log likelihood; Δ AICc = difference in model AICc score from the 'top' model; w_i = Akaike weighted probability; ER = evidence ratio. 'RegOU' indicates a phylogenetic regression model in which the residuals are modelled as an Ornstein–Uhlenbeck process, which can be viewed as mimicking stabilizing selection superimposed on the expected covariances of residuals as derived from the phylogenetic tree (Lavin *et al.*, 2008). All other models are ordinary least squares ('OLS') multiple regressions, which are mathematically equivalent to analysis on a 'star' phylogeny with no hierarchical structure. Here, we have included only the most supported models. See Online Supplementary Materials for complete information on all models.

All species ($N = 131$)										
Model	RegOU or OLS	d	r^2	lnML	AICc	Δ AICc	expdel1	W_i	ER	
Sidewinding + Constriction + Habitat + Clade + Log ₁₀ Body Vertebrae	OLS		0.7616	156.992	-263.507	0.000	1.000	0.416	1.0	
Sidewinding + Habitat + Clade + Log ₁₀ Body Vertebrae	OLS		0.7560	155.475	-263.314	0.193	0.908	0.377	1.1	
Sidewinding + Constriction + Habitat + Clade + Log ₁₀ Body Vertebrae	RegOU	0.094	0.7194	156.905	-260.440	3.067	0.216	0.090	4.6	
Sidewinding + Habitat + Clade + Log ₁₀ Body Vertebrae	RegOU	0.110	0.7104	155.379	-260.281	3.226	0.199	0.083	5.0	
Habitat + Clade + Log ₁₀ Body Vertebrae	OLS		0.7380	150.819	-256.792	6.715	0.035	0.014	28.7	
Constriction + Habitat + Clade + Log ₁₀ Body Vertebrae	OLS		0.7432	152.113	-256.589	6.918	0.031	0.013	31.8	
Habitat + Clade + Log ₁₀ Body Vertebrae	RegOU	0.167	0.6726	150.746	-253.855	9.652	0.008	0.003	124.7	
Constriction + Habitat + Clade + Log ₁₀ Body Vertebrae	RegOU	0.168	0.6760	152.048	-253.618	9.889	0.007	0.003	140.4	
Sidewinding + Constriction + Habitat + Log ₁₀ Body Vertebrae	RegOU	0.551	0.5194	135.180	-250.873	12.634	0.002	0.001	553.9	
Caenophidia only ($N = 114$)										
Model	RegOU or OLS	d	r^2	lnML	AICc	Δ AICc	expdel1	W_i	ER	
Sidewinding + Constriction + Habitat + Log ₁₀ Body Vertebrae	OLS		0.7560	136.528	-255.684	0.000	1.000	0.017	1.0	
Sidewinding + Constriction + Habitat + Log ₁₀ Body Vertebrae	RegOU	0.000	0.7545	136.417	-253.104	2.580	0.275	0.005	3.6	
Sidewinding + Habitat + Log ₁₀ Body Vertebrae	OLS		0.7399	132.886	-250.715	4.969	0.083	0.001	12.0	
Sidewinding + Habitat + Log ₁₀ Body Vertebrae	RegOU	0.000	0.7383	132.769	-248.167	7.517	0.023	0.000	42.9	
Constriction + Habitat + Log ₁₀ Body Vertebrae	OLS		0.7279	130.317	-245.577	10.107	0.006	0.000	156.6	
Constriction + Habitat + Log ₁₀ Body Vertebrae	RegOU	0.000	0.7265	130.248	-243.124	12.560	0.002	0.000	533.8	

noncaenophidians in our data set. Because noncaenophidians are relatively homogenous in their spinalis length, whereas caenophidians show much greater variation (Fig. 2A), we conclude that variation between caenophidians and noncaenophidians explains why the clade variable weighs heavily in the full data set but not the caenophidian-only data set.

Likelihood ratio tests (LRTs) comparing OLS and RegOU models with the same independent variables indicated no significant difference for any of the best models, all of which included clade (Table 2). Thus, after accounting for differences among clades, and for the effects of other independent variables, we did not detect phylogenetic signal in the residuals of our statistical models.

Discussion

Our results demonstrate statistically significant associations between the length of a key locomotor muscle–tendon unit and indicators of behavioural ecology, thus

suggesting that this subordinate trait has evolved as a result of natural selection during the radiation of snakes. More specifically, our analysis upholds Jayne's (1982) conclusion that arboreal snakes have longer spinalis muscle–tendon units, whereas aquatic and burrowing snakes have shorter spinalis muscle–tendon units (Fig. 2B, Table 3). Additionally, we found strong statistical evidence to corroborate Jayne's observation that sidewinding specialists have shorter muscle–tendon units (Fig. 2D, Table 3). However, our analysis only partially supported Jayne's (1982) conclusion that prey constriction is associated with shorter spinalis muscle–tendon units (in Caenophidia alone, but not for all 131 species). In contrast with the earlier study, which found no statistically significant simple correlation between total number of body vertebrae and spinalis length ($N = 107$, $r = 0.187$, $P = 0.054$), we found that the partial regression coefficients for the log of the number of vertebrae were highly significant in the full models (Table 3). Overall, our findings indicate that both functional demands and phylogenetic history have shaped

Table 3 Full models for all species and for Caenophidia only. Partial regression coefficients and *F* tests of categorical variables for the full models predicting \log_{10} number of vertebrae spanned by one segment (muscle and anterior and posterior tendons) of the spinalis muscle, which includes all independent variables considered (\log_{10} total number of body vertebrae, habitat, clade, sidewinding and constriction), analysed by conventional (OLS) multiple regression and phylogenetically with an OU transform for all species and for Caenophidia only (see Table 2 for model comparisons). Variables statistically significant in both models based on partial *F* tests are in boldface. Colubrinae and terrestrial categories were chosen as base groups from comparison among all other levels of 'clade' and 'habitat', respectively, in the model; hence, there are no coefficients for these categories. These were chosen because a terrestrial colubrid snake best represents the 'general snake condition' – one without any obvious structural or behavioural modifications for life in a particular habitat. Main effects of clade and habitat variables are presented at the bottom of the table. All *P* values are for two-tailed tests.

All species (<i>N</i> = 131)										
Variable	OLS				RegOU					
	<i>B</i>	SE	<i>F</i>	<i>P</i>	<i>B</i>	SE	<i>F</i>	<i>P</i>	d.f. (d)	
y-intercept	0.77	0.21	12.85	< 0.001	0.59	0.21	7.88	< 0.01	1,111	
Log₁₀ body vertebrae	0.25	0.09	6.96	< 0.01	0.33	0.09	12.63	< 0.001	1,111	
Typhlopidae	-0.22	0.08	6.91	< 0.01	-0.23	0.09	6.85	0.01	1,111	
Tropidophiidae	-0.27	0.08	10.85	< 0.01	-0.28	0.09	10.21	< 0.01	1,111	
Boidae	-0.19	0.03	30.06	< 0.001	-0.20	0.04	23.91	< 0.001	1,111	
Pythonidae, etc.	-0.13	0.04	10.10	< 0.01	-0.14	0.05	9.08	< 0.01	1,111	
Acrochordidae	-0.07	0.07	1.04	0.31	-0.10	0.07	1.77	0.19	1,111	
Pareidae	-0.02	0.08	0.04	0.84	-0.02	0.09	0.03	0.86	1,111	
Viperidae	0.00	0.03	0.01	0.92	0.01	0.04	0.02	0.89	1,111	
Homalopsidae	0.03	0.04	0.70	0.40	0.01	0.05	0.05	0.82	1,111	
Elapidae	-0.01	0.03	0.09	0.76	-0.02	0.03	0.23	0.63	1,111	
<i>Pseudaspis</i>	0.00	0.08	0.00	0.98	-0.02	0.09	0.03	0.86	1,111	
Atractaspididae	-0.04	0.04	0.77	0.38	-0.05	0.05	1.31	0.25	1,111	
<i>Nerodia + Regina</i>	0.08	0.06	1.55	0.22	0.08	0.07	1.33	0.25	1,111	
Natricinae + Dipsadinae	0.04	0.04	1.39	0.24	0.04	0.04	0.98	0.32	1,111	
Aquatic	-0.22	0.03	71.62	< 0.001	-0.21	0.03	44.79	< 0.001	1,111	
Arboreal	0.14	0.03	27.71	< 0.001	0.13	0.03	21.91	< 0.001	1,111	
Burrowing	-0.11	0.02	18.96	< 0.001	-0.10	0.02	15.62	< 0.001	1,111	
Constriction	-0.05	0.03	2.60	0.11	-0.05	0.04	1.62	0.21	1,111	
Sidewinding	-0.15	0.05	8.58	< 0.01	-0.15	0.05	7.59	< 0.01	1,111	
Clade			4.11	< 0.001			3.38	< 0.001	13,111	
Habitat			39.90	< 0.001			28.76	< 0.001	3,111	

Caenophidia (<i>N</i> = 114)										
Variable	<i>B</i>	SE	<i>F</i>	<i>P</i>	<i>d</i> = 6.94e-21					
					<i>B</i>	SE	<i>F</i>	<i>P</i>	d.f. (d)	
y-intercept	0.80	0.21	13.98	< 0.001	0.79	0.21	13.72	< 0.001	1,98	
Log₁₀ body vertebrae	0.24	0.09	6.31	0.01	0.24	0.09	6.49	0.01	1,98	
Acrochordidae	-0.06	0.06	1.05	0.31	-0.06	0.06	1.05	0.31	1,98	
Pareidae	-0.03	0.08	0.11	0.74	-0.03	0.08	0.11	0.74	1,98	
Viperidae	-0.01	0.03	0.05	0.82	-0.01	0.03	0.05	0.82	1,98	
Homalopsidae	0.03	0.04	0.67	0.42	0.03	0.04	0.67	0.42	1,98	
Elapidae	-0.01	0.03	0.15	0.70	-0.01	0.03	0.15	0.70	1,98	
<i>Pseudaspis</i>	0.04	0.08	0.23	0.63	0.04	0.08	0.23	0.63	1,98	
Atractaspididae	-0.01	0.04	0.06	0.81	-0.01	0.04	0.06	0.81	1,98	
<i>Nerodia + Regina</i>	0.07	0.06	1.40	0.24	0.07	0.06	1.41	0.24	1,98	
Natricinae + Dipsadinae	0.04	0.03	1.23	0.27	0.04	0.03	1.24	0.27	1,98	
Aquatic	-0.23	0.03	83.30	< 0.001	-0.23	0.03	82.86	< 0.001	1,98	
Arboreal	0.15	0.03	29.50	< 0.001	0.15	0.03	29.33	< 0.001	1,98	
Burrowing	-0.15	0.03	32.37	< 0.001	-0.15	0.03	32.22	< 0.001	1,98	
Constriction	-0.06	0.03	3.22	0.08	-0.06	0.03	3.24	0.07	1,98	
Sidewinding	-0.15	0.21	13.98	< 0.001	-0.15	0.05	9.21	< 0.01	1,98	
Clade			0.94	0.49			0.94	0.49	9,98	
Habitat			49.27	< 0.001			49.03	< 0.001	3,98	

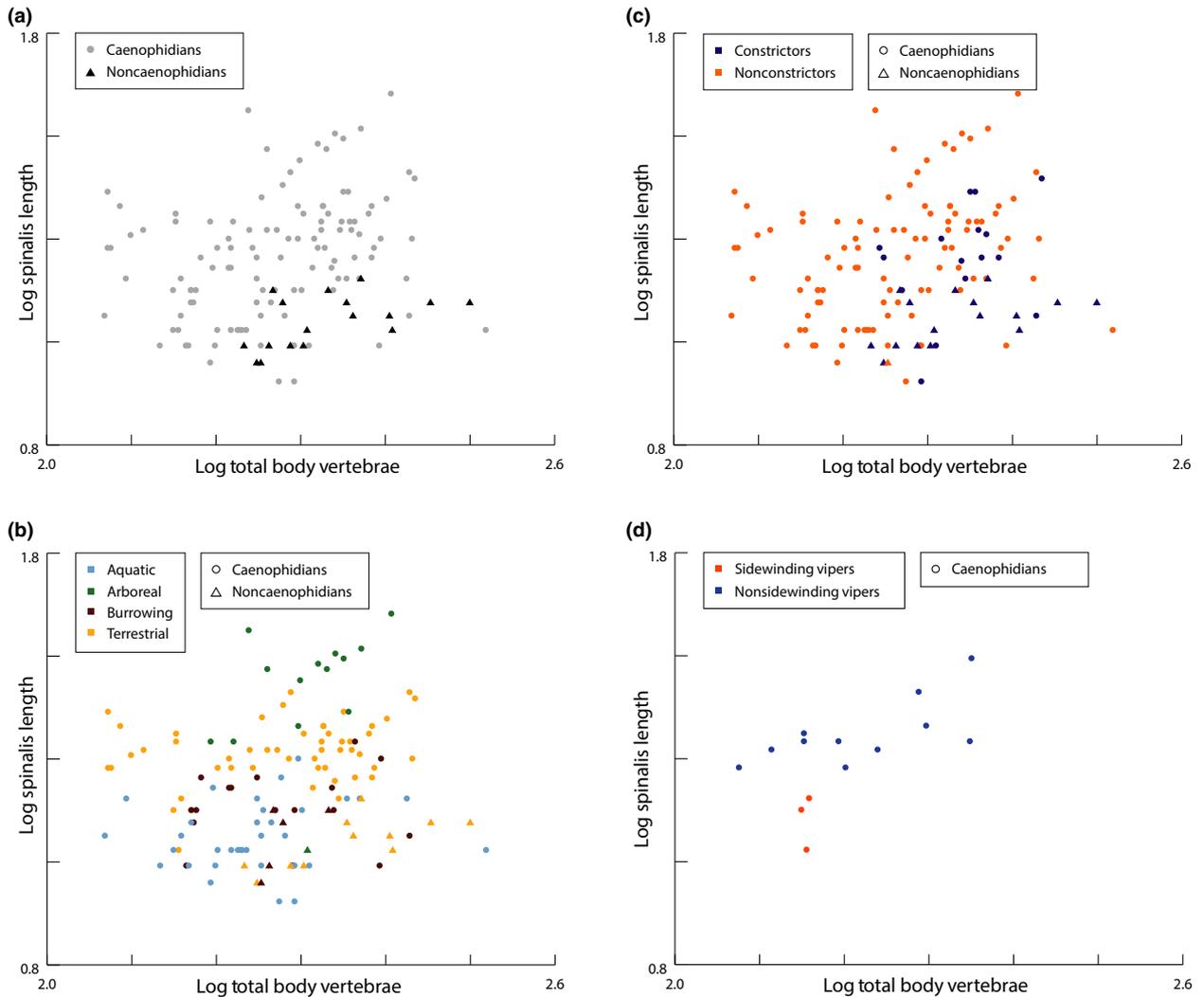


Fig. 2 Log–log plots of vertebrae spanned by one segment of the spinalis muscle (including both anterior and posterior tendons) in relation to total numbers of body vertebrae for 131 snake species. Panel A illustrates differences between caenophidians (‘advanced snakes’) and noncaenophidians. Caenophidians have highly variable muscle lengths, whereas all species in the noncaenophidian clades have relatively short muscles. Panel B illustrates differences among habitat types. Panel C illustrates the shorter muscles in constrictors relative to nonconstrictors. Panel D shows data for vipers only, illustrating the shorter muscles in sidewinding specialists relative to other vipers. Statistical analyses (see Results) indicate that clade, habitat, constricting and sidewinding are all important predictors of muscle length.

the muscle–tendon morphology of present-day snakes, and our multiple regression analyses made these relationships much more evident than did the previous study that relied on separate analyses of phylogenetic and habitat groupings.

We can compare the weights of variables summed across models to distinguish their relative importance (Appendix S3). Habitat has a weight of 1.000 for all species and for Caenophidia only, meaning that there is a 100% chance it would appear in the best model if we were to repeat data collection and analysis. Sidewinding

was very important in both data sets, with weights of 0.966 and 0.993. Constriction showed slight importance in the full data set, with a weight of 0.522, and it showed much greater importance for Caenophidia only, with a weight of 0.922. Clade has a very different weight depending on the data set used: 0.999 for all species, but only 0.002 for just caenophidians. This huge difference indicates that caenophidians are very different from the noncaenophidians in our data set, but that clade has little association with muscle length within Caenophidia.

Our results lend support for an adaptive hypothesis that the evolution of habitat usage has strongly influenced the morphology of the spinalis muscle–tendon unit in snakes. In agreement with Jayne’s (1982) study, arboreal snakes had significantly longer muscle–tendon units than terrestrial snakes did, primarily through an increase in anterior tendon length, a trend that likely has a functional basis. As it lengthens, the anterior tendon increases the lever arm through which the muscle works, which in turn reduces the possibility of buckling due to increased torque while bridging larger gaps in trees (Jayne & Riley, 2007). Furthermore, long tendons could allow a greater number of segments to produce force in all of the serially homologous muscle–tendon units that cross an individual joint (Jayne & Riley, 2007). Indeed, a recent electromyographic study of a specialized arboreal colubrid with spinalis length of 30 vertebrae has confirmed that 30 adjacent ipsilateral spinalis segments are simultaneously active to prevent buckling of the body where it crosses the edge of a gap while cantilevering (Jayne & Jorgensen, In Press). In addition, a study of 31 snake species from five families, and another comparing eight arboreal species with eight terrestrial relatives, found that arboreal snakes could extend a significantly greater percentage of their body between gaps (Lillywhite *et al.*, 2000; Rozar, 2010). Thus, arboreal snakes crossing gaps in shrubs and trees may face selection for increased spinalis lengths among other morphological specializations to improve cantilevering performance.

Aquatic and burrowing snakes generally have fewer vertebrae than terrestrial or arboreal species (Marx & Rabb, 1972; Lindell, 1994; Polly *et al.*, 2001), and in our study, they had shorter spinalis muscle–tendon units as well (Table 3). Additionally, burrowing snakes are often substantially smaller than terrestrial or arboreal species and have fewer vertebrae per unit length than members of other groups (Lindell, 1994; Polly *et al.*, 2001). Similar to the theoretical arguments for how shorter muscle–tendon units in constricting snakes could facilitate creating tight bends with high attendant forces, both of these capacities would seem well suited for burrowing given the tight confines within burrows and the very high forces used by some squamates while moving through soil (Navas *et al.*, 2004). We had no strong *a priori* reason to expect shorter muscle–tendon units in aquatic snakes. However, the aquatic medium is distinct for the uniform support that it provides along the entire length of the body, which is the antithesis of the discontinuous points of support that are encountered so frequently by arboreal snakes moving across multiple branches. Some aquatic species also may face selection for enhanced burrowing performance, as the shorelines and intertidal areas in South-East Asia, where marine snakes are most diverse (Voris *et al.*, 2002), commonly have thick mud. Several clades, including the Homalopsidae, Acrochordidae and

Hydrophiinae, have a number of species that actively burrow in mud (Jayne *et al.*, 1988). Whether burrowing behaviour represents an ancestral character that gave rise to the shorter spinalis of these groups merits investigation.

We found that specialization for sidewinding locomotion also significantly predicts spinalis length. The vipers *Crotalus cerastes*, *Cerastes cerastes* and *Eristicophis macmahoni* all live in sandy desert regions and progress primarily by sidewinding (Gans & Mendelssohn, 1971), and they all have shorter spinalis segments than do the other vipers in this study. Functional demands of sidewinding may differ from those of lateral undulation, concertina and rectilinear locomotion because sidewinding involves bilateral activation of the spinalis muscles where the body is lifted from the ground (Jayne, 1988b). Thus, sidewinding involves a unique axial motor pattern associated with displacement in two planes rather than just one, unlike other well-known forms of terrestrial locomotion in snakes. This aspect of the movement could require more flexibility than is typical for other vipers, and as shorter spinalis segments have a higher ratio of contractile tissue to stiff tendinous tissue, they could increase a snake’s capacity for sidewinding.

The relationship between spinalis segment length and constriction is less clear. We did not find a statistically significant effect of constriction on spinalis length in any of our full models (Table 3). Some authors have hypothesized that shorter spinalis segments might better meet the functional demands of constriction because the muscle length change associated with making tight coils must be accommodated by contractile tissue rather than stiff tendons, and as previously mentioned, shorter muscle–tendon units have a higher percentage of contractile tissue. The Boidae, Pythonidae and Acrochordidae have a much higher percentage of contractile tissue per segment than does any Colubrid family (Jayne, 1982), a morphological difference that could result from widespread constriction within Boidae or from historical coincidence. Among terrestrial caenophidians, on the other hand, constricting species do not have significantly higher percentages of contractile tissue than nonconstricting species do (Jayne, 1982), and at least one species of slender, highly arboreal vine snake (*Langaha madagascariensis*) constricts its prey despite our finding that arboreality correlates with increased tendon length (Tingle, 2012). This ambiguity raises the possibility that even if the spinalis is important for constriction in some species, other muscles could make up for suboptimal spinalis length species that constrict but have long spinalis segments. Furthermore, constriction often involves the more anterior region of the snake, which raises the possibility that longitudinal variation in spinalis length could allow the anterior portion to be effective at constriction, whereas the more posterior regions still retain reasonably long

tendons. Indeed, in several species that have been examined, the anterior spinalis segments spanned fewer vertebrae than the more posterior spinalis segments did (Nicodemo, 2012).

Conclusion

This study has demonstrated that snakes show great diversity in the length of an axial muscle–tendon unit. Moreover, spinalis length strongly relates to habitat and behaviour, suggesting adaptation of the trunk morphology for the various functions that the trunk must carry out in animals with no limbs. Although we and other authors (e.g. Ruben, 1977; Jayne, 1982) have put forth hypotheses for the functional advantage of longer vs. shorter muscles for various activities, experiments should test whether differing spinalis lengths actually confer performance benefits. For example, a comparison of sidewinding in specialized and unspecialized species across a diversity of spinalis lengths would show what, if any, aspect of sidewinding performance improves with shorter muscles.

Variation in spinalis length highlights that animals with superficially similar bodies may have very different underlying musculature, which in turn facilitates diverse habits. Although we expect exaggerated differences in the trunk musculature of snakes because their limblessness places much greater importance on the trunk for most of their activities, these differences could exist for limbed vertebrates as well, especially in somewhat elongate species.

We have confirmed that many results of a well-designed study carried out prior to the availability of phylogenetic comparative methods (Jayne, 1982) hold up under closer scrutiny, but with some differences. Most notably, the relationship between short spinalis muscles and prey constriction seems more ambiguous in the light of this new analysis, warranting a closer examination of the muscular mechanisms of this behaviour.

Acknowledgments

We thank M. Chappell and D. Reznick of UC Riverside for comments on early versions of this manuscript, as well the editors and reviewers of the *Journal of Evolutionary Biology* for their helpful feedback on the submitted version. We are also grateful to H. K. Voris and A. Resetar of the Field Museum for facilitating our access to specimens and providing a wealth of information regarding marine snakes. The study was partially supported by a grant from the National Science Foundation IOS 0813497 to B.C.J. Partial funding for G.E.A.G. came from the Department of Evolution, Ecology and Organismal Biology at UC Riverside. The project was completed in partial fulfilment of the Ph.D. for G.E.A.G.

References

- Alencar, L. 2010. *Ecomorphology in neotropical snakes: A study with the tribe Pseudoboini*. Master's thesis. Universidade de São Paulo, São Paulo.
- Alexander, A.A. & Gans, C. 1966. The pattern of dermal-vertebral correlation in snakes and amphisbaenians. *Zool. Meded.* **41**: 171–190.
- Al-Kahtani, M.A., Zuleta, C., Caviedes-Vidal, E. & Garland, T. 2004. Kidney mass and relative medullary thickness of rodents in relation to habitat, body size, and phylogeny. *Physiol. Biochem. Zool.* **77**: 346–365.
- Blomberg, S.P. & Garland, T. 2002. Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. *J. Evol. Biol.* **15**: 899–910.
- Blomberg, S.P., Garland, T. & Ives, A.R. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.
- Bonnet, X., Pearson, D., Ladyman, M., Lourdis, O. & Bradshaw, D. 2002. “Heaven” for serpents? A mark-recapture study of tiger snakes (*Notechis scutatus*) on Carnac Island, Western Australia. *Austral Ecol.* **27**: 442–450.
- Brainerd, E.L. & Patek, S.N. 1998. Vertebral column morphology, C-start curvature, and the evolution of mechanical defenses in Tetraodontiform fishes. *Copeia* **1998**: 971.
- Brischoux, F. & Shine, R. 2011. Morphological adaptations to marine life in snakes. *J. Morphol.* **272**: 566–572.
- Brischoux, F., Gartner, G.E.A., Garland, T. & Bonnet, X. 2011. Is aquatic life correlated with an increased hematocrit in snakes? *PLoS One* **6**: e17077.
- Burnham, K.P. & Anderson, D.R. 2002. *Model selection and Multi-Model Inference: A Practical Information-Theoretic Approach*, 2nd edn. Springer, New York.
- Burnham, K.P. & Anderson, D.R. 2004. Multimodel inference: understanding AIC and BIC in model selection. *Sociol. Methods Res.* **33**: 261–304.
- Burnham, K.P., Anderson, D.R. & Huyvaert, K.P. 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behav. Ecol. Sociobiol.* **65**: 23–35.
- Byrnes, G. & Jayne, B.C. 2014. Gripping during climbing of arboreal snakes may be safe but not economical. *Biol. Lett.* **10**: 20140434.
- Canoville, A., de Buffrénil, V. & Laurin, M. 2016. Microanatomical diversity of amniote ribs: an exploratory quantitative study. *Biol. J. Linn. Soc.* **118**: 706–733.
- Dlugosz, E.M., Chappell, M.A., Meek, T.H., Szafranska, P.A., Zub, K., Konarzewski, M. *et al.* 2013. Phylogenetic analysis of mammalian maximal oxygen consumption during exercise. *J. Exp. Biol.* **216**: 4712–4721.
- Dohm, M.R. & Garland, T. 1993. Quantitative genetics of scale counts in the Garter Snake *Thamnophis sirtalis*. *Copeia* **1993**: 987.
- Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* **125**: 1–15.
- Felsenstein, J. 1988. Phylogenies and quantitative characters. *Annu. Rev. Ecol. Syst.* **19**: 445–471.
- Gans, C. 1960. A taxonomic revision of the Trogonophinae, and a functional interpretation of the amphisbaenid adaptive pattern. *Bull. Am. Mus. Nat. Hist.* **119**: 129–204.
- Gans, C. & Mendelssohn, H. 1971. Sidewinding and jumping progression of vipers. In: *Toxins of Animal and Plant Origin*

- (A. De Vries & E. Kochva, eds), pp. 17–38. Gordan and Breach, Science Publishers, Inc., New York.
- Garland, T. & Adolph, S.C. 1994. Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.* **67**: 797–828.
- Garland, T., Dickerman, A.W., Janis, C.M. & Jones, J.A. 1993. Phylogenetic analysis of covariance by computer simulation. *Syst. Biol.* **42**: 265.
- Garland, T., Midford, P.E. & Ives, A.R. 1999. An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. *Am. Zool.* **39**: 374–388.
- Garland, T., Bennet, A.F. & Rezende, E.L. 2005. Phylogenetic approaches in comparative physiology. *J. Exp. Biol.* **208**: 3015–3035.
- Gartner, G.E.A., Hicks, J.W., Manzani, P.R., Andrade, D.V., Abe, A.S., Wang, T. *et al.* 2010. Phylogeny, ecology, and heart position in snakes. *Physiol. Biochem. Zool.* **83**: 43–54.
- Gasc, J.-P. 1974. L'interprétation fonctionnelle de l'appareil musculo-squelettique de l'axe vertébral chez les serpents (Reptilia). *Mém Muséum Natl. Hist. Nat. Sér. Zool.* **83**: 1–182.
- Gasc, J.-P. 1981. Axial musculature. In: *Biology of the Reptilia* (C. Gans & T.S. Parsons, eds), pp. 355–435. Academic Press, London.
- Guyer, C. & Donnelly, M.A. 1990. Length-mass relationships among an assemblage of tropical snakes in Costa Rica. *J. Trop. Ecol.* **6**: 65–76.
- Head, J.J. & Polly, P.D. 2007. Dissociation of somatic growth from segmentation drives gigantism in snakes. *Biol. Lett.* **3**: 296–298.
- Hoefler, K.M. & Jayne, B.C. 2013. Three-dimensional locations of destinations have species-dependent effects on the choice of paths and the gap-bridging performance of arboreal snakes. *J. Exp. Zool. Part Ecol. Genet. Physiol.* **319**: 124–137.
- Huey, R.B., Deutsch, C.A., Tewksbury, J.J., Vitt, L.J., Hertz, P.E., Alvarez Perez, H.J. *et al.* 2009. Why tropical forest lizards are vulnerable to climate warming. *Proc. R. Soc. B Biol. Sci.* **276**: 1939–1948.
- Ives, A.R., Midford, P.E. & Garland, T. 2007. Within-species variation and measurement error in phylogenetic comparative methods. *Syst. Biol.* **56**: 252–270.
- Jayne, B.C. 1982. Comparative morphology of the semispinalis-spinalis muscle of snakes and correlations with locomotion and constriction. *J. Morphol.* **17**: 83–96.
- Jayne, B.C. 1988a. Muscular mechanisms of snake locomotion: an electromyographic study of lateral undulation of the Florida Banded Water Snake (*Nerodia fasciata*) and the Yellow Rat Snake (*Elaphe obsoleta*). *J. Morphol.* **197**: 159–181.
- Jayne, B.C. 1988b. Muscular mechanisms of snake locomotion: an electromyographic study of the sidewinding and concertina modes of *Crotalus cerastes*, *Nerodia fasciata* and *Elaphe obsoleta*. *J. Exp. Biol.* **140**: 1–33.
- Jayne, B.C. & Byrnes, G. 2015. The effects of slope and branch structure on the locomotion of a specialized arboreal colubrid snake (*Boiga irregularis*). *J. Exp. Zool. Part Ecol. Genet. Physiol.* **323**: 309–321.
- Jayne, B.C. & Jorgensen, R.M. In Press. Three-dimensional trajectories affect the epaxial muscle activity of arboreal snakes crossing gaps. *J. Exp. Biol.* <https://doi.org/10.1242/jeb.164640>.
- Jayne, B.C. & Riley, M.A. 2007. Scaling of the axial morphology and gap-bridging ability of the brown tree snake, *Boiga irregularis*. *J. Exp. Biol.* **210**: 1148–1160.
- Jayne, B.C., Voris, H.K. & Heang, K.B. 1988. Diet, feeding behavior, growth and numbers of a population of *Cerberus rynchops* (Serpentes: Homalopsinae) in Malaysia. *Fieldiana Zool.* **50**: 1015.
- Kerfoot, W.C. 1970. The effect of functional changes upon the variability of lizard and snake body scale numbers. *Copeia* **1970**: 252.
- Lavin, S.R., Karasov, W.H., Ives, A.R., Middleton, K.M. & Garland, T. 2008. Morphometrics of the avian small intestine compared with that of nonflying mammals: a phylogenetic approach. *Physiol. Biochem. Zool.* **81**: 526–550.
- Lillywhite, H.B., LaFrentz, J.R., Lin, Y.C. & Tu, M.C. 2000. The cantilever abilities of snakes. *J. Herpetol.* **34**: 523.
- Lindell, L.E. 1994. The evolution of vertebral number and body size in snakes. *Funct. Ecol.* **8**: 708.
- Maddison, W.P. 1991. Square-change parsimony reconstructions of ancestral states for continuous-valued characters on a phylogenetic tree. *Syst. Biol.* **40**: 304–314.
- Martins, M., Araujo, M.S., Sawaya, R.J. & Nunes, R. 2001. Diversity and evolution of macrohabitat use, body size and morphology in a monophyletic group of Neotropical Pitvipers (*Bothrops*). *J. Zool.* **254**: 529–538.
- Marx, H. & Rabb, G.B. 1972. Phyletic analysis of fifty characters of advanced snakes. *Fieldiana Zool.* **63**: 1–321.
- Mehta, R.S., Ward, A.B., Alfaro, M.E. & Wainwright, P.C. 2010. Elongation of the body in eels. *Integr. Comp. Biol.* **50**: 1091–1105.
- Moon, B.R. 1999. Testing an inference of function from structure: snake vertebrae do the twist. *J. Morphol.* **241**: 217–225.
- Mosauer, W. 1932. On the locomotion of snakes. *Science* **76**: 583–585.
- Navas, C.A., Antoniazzi, M.M., Carvalho, J.E., Chaui-Berlink, J.G., James, R.S., Jared, C. *et al.* 2004. Morphological and physiological specialization for digging in amphisbaenians, an ancient lineage of fossorial vertebrates. *J. Exp. Biol.* **207**: 2433–2441.
- Nicodemo, P. 2012. *Longitudinal variation in the axial muscles of snakes*. University of Cincinnati, Master's thesis. Cincinnati, Ohio.
- Nunn, C.L. 2011. *The Comparative Approach in Evolutionary Anthropology and Biology*. University of Chicago Press, Chicago, Illinois.
- Nussbaum, R.A. & Naylor, B.G. 1982. Variation in the trunk musculature of caecilians (Amphibia: Gymnophiona). *J. Zool.* **198**: 383–398.
- Oufiero, C., Gartner, G.E.A., Adolph, S.C. & Garland, T.J. 2011. Latitudinal and climatic variation in body size and dorsal scale counts in *Sceloporus* lizards: a phylogenetic perspective. *Evolution* **65**: 3590–3607.
- Pizzatto, L., Marques, O.A.V. & Martins, M. 2007. Ecomorphology of Boine snakes, with emphasis on South American forms. In: *Biology of the Boas and Pythons* (R.W. Henderson & R. Powell, eds), pp. 35–48. Eagle Mountain Publishing LC, Eagle Mountain, Utah.
- Polly, P.D., Head, J.J. & Cohn, M.J. 2001. Testing modularity and dissociation: the evolution of regional proportions in

- snakes. In: *Beyond Heterochrony: The Evolution of Development* (M.L. Zelditch, ed), pp. 305–335. Wiley-Liss Inc., New York.
- Pyron, R.A. & Burbrink, F.T. 2012. Extinction, ecological opportunity, and the origins of global snake diversity. *Evolution* **66**: 163–178.
- Revell, L.J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**: 217–223.
- Revell, L.J. 2013. Two new graphical methods for mapping trait evolution on phylogenies. *Methods Ecol. Evol.* **4**: 754–759.
- Rezende, E.L. & Diniz-Filho, J.A.F. 2012. Phylogenetic analyses: comparing species to infer adaptations and physiological mechanisms. In: *Comprehensive Physiology* (R. Terjung, ed), pp. 640–674. John Wiley & Sons Inc, Hoboken, NJ, USA.
- Rozar, R.C. 2010. *The role of morphology in the locomotor performance of arboreal snakes*. Doctoral dissertation. University of Miami, Miami, Florida.
- Ruben, J.A. 1977. Morphological correlates of predatory modes in the Coachwhip (*Masticophis flagellum*) and Rosy Boa (*Lichanura roseofusca*). *Herpetologica* **33**: 1–6.
- Schluter, D., Price, T., Mooers, A.O. & Ludwig, D. 1997. Likelihood of ancestor states in adaptive radiation. *Evolution* **51**: 1699.
- Smith, K.L., Harmon, L.J., Shoo, L.P. & Melville, J. 2011. Evidence of constrained phenotypic evolution in a cryptic species complex of Agamid lizards. *Evolution* **65**: 976–992.
- Swanson, D.L. & Garland, T. 2009. The evolution of high summit metabolism and cold tolerance in birds and its impact on present-day distributions. *Evolution* **63**: 184–194.
- Tingle, J.L. 2012. Field observations on the behavioral ecology of the Madagascan Leaf-Nosed Snake, *Langaha madagascariensis*. *Herpetol. Conserv. Biol.* **7**: 442–448.
- Tonini, J.F.R., Beard, K.H., Ferreira, R.B., Jetz, W. & Pyron, R.A. 2016. Fully-sampled phylogenies of squamates reveal evolutionary patterns in threat status. *Biol. Conserv.* **204**: 23–31.
- Uetz, P., Goll, J. & Hallermann, J. 2007. Die TIGR-reptiliendatenbank. *Elaphe* **15**: 22–25.
- Vidal, N. & Hedges, S.B. 2002. Higher-level relationships of caenophidian snakes inferred from four nuclear and mitochondrial genes. *C. R. Biol.* **325**: 987–995.
- Vitt, L.J. & Vangilder, L.D. 1983. Ecology of a snake community in northeastern Brazil. *Amphib.-Reptil.* **4**: 273–296.
- Voris, H.K. 1975. Dermal scale-vertebra relationships in Sea Snakes (Hydrophiidae). *Copeia* **1975**: 746.
- Voris, H.K., Alfaro, M.E., Karns, D.R., Starnes, G.L., Thompson, E. & Murphy, J.C. 2002. Phylogenetic relationships of the Oriental-Australian rear-fanged water snakes (Colubridae: Homalopsinae) based on mitochondrial DNA sequences. *Copeia* **2002**: 906–915.
- Ward, A.B. & Mehta, R.S. 2010. Axial elongation in fishes: using morphological approaches to elucidate developmental mechanisms in studying body shape. *Integr. Comp. Biol.* **50**: 1106–1119.
- Ward, A.B., Costa, A., Monroe, S.L., Aluck, R.J. & Mehta, R.S. 2015. Locomotion in elongate fishes: a contact sport. *Zoology* **118**: 312–319.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1 Phylogenetic tree file, PDI.

Appendix S2 Phylogenetic tree file, DSC.

Appendix S3 Full model comparison.

Appendix S4 Raw data.

Appendix S5 Data file for analysis of all species.

Appendix S6 Data file for analysis of Caenophidia only.

Data deposited at Dryad: <https://doi.org/10.5061/dryad.bc507>.

Received 21 May 2017; revised 3 August 2017; accepted 18 August 2017