Phylogeny, Ecology, and Heart Position in Snakes

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ABSTRACT

The cardiovascular system of all animals is affected by gravitational pressure gradients, the intensity of which varies according to organismic features, behavior, and habitat occupied. A previous nonphylogenetic analysis of heart position in snakes—which often assume vertical postures—found the heart located 15%–25% of total body length from the head in terrestrial and arboreal species but 25%–45% in aquatic species. It was hypothesized that a more anterior heart in arboreal species served to reduce the hydrostatic blood pressure when these animals adopt vertical postures during climbing, whereas an anterior heart position would not be needed in aquatic habitats, where the effects of gravity are less pronounced. We analyzed a new data set of 155 species from five major families of Alethinophidia (one of the two major branches of snakes, the other being blind snakes, Scolecophidia) using both conventional and phylogenetically based statistical methods. General linear models regressing $\log_{10}$ snout-heart position on $\log_{10}$ snout-vent length (SVL), as well as dummy variables coding for habitat and/or clade, were compared using likelihood ratio tests and the Akaike Information Criterion. Heart distance to the tip of the snout scaled isometrically with SVL. In all instances, phylogenetic models that incorporated transformation of the branch lengths under an Ornstein-Uhlenbeck model of evolution (to mimic stabilizing selection) better fit the data as compared with their nonphylogenetic counterparts. The best-fit model predicting snake heart position included aspects of both habitat and clade and indicated that arboreal snakes in our study tend to have hearts placed more posteriorly, opposite the trend identified in previous studies. Phylogenetic signal in relative heart position was apparent both within and among clades. Our results suggest that overcoming gravitational pressure gradients in snakes most likely involves the combined action of several cardiovascular and behavioral adaptations in addition to alterations in relative heart location.

Introduction

With the exception of body length, which varies by two orders of magnitude, the snake *bauplan* is conserved across all taxa (Greene and McDiarmid 2005). All snakes are limbless, elongate, and lack a pectoral girdle; their anatomy is elegantly modified to fit a tubular body plan (Greene 1997; Cohn and Tickle 1999). Although they might be viewed as occupying a relatively small region of "morphospace" with respect to basic body plan, snakes have radiated extensively in both number of species (>3,100 named; Uetz et al. 2007) and behavioral ecology. Even within the apparent restrictions associated with a limbless and elongate lifestyle, snakes have evolved to occupy almost all ecological niches, including fully aquatic and pelagic sea snakes, arboreal species that rarely if ever come to the forest floor, snakes that glide, and completely fossorial burrowers (Greene 1997; Martins et al. 2008).

Despite its evolutionary success, the snake body plan is nevertheless subject to constraint at several levels. Gravity, in particular, may significantly affect the cardiovascular function of snakes, which are in essence long fluid-filled tubes (Lillywhite 1987). In a vertical column of fluid (e.g., arteries and veins), gravity creates a vertical pressure gradient that increases with the height of the tube (i.e., ρgh; where ρ is the density of blood, g is the acceleration due to gravity, and h is the vertical height of the fluid column above or below a reference plane; in this
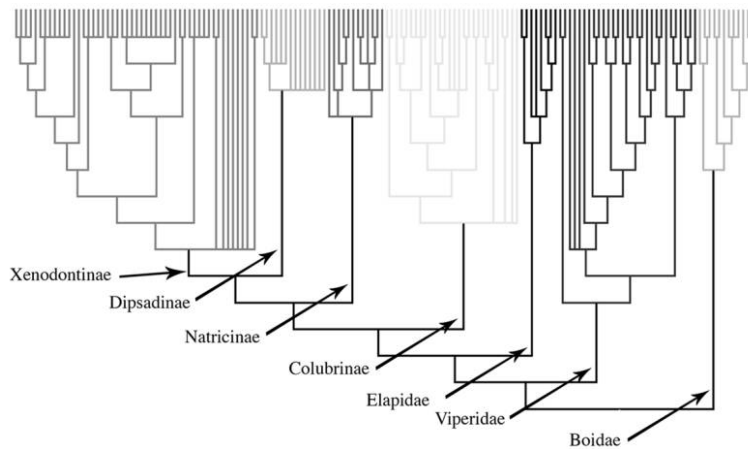
Figure 1. Phylogeny used for statistical analyses, with Pagel's (1992) arbitrary branch lengths. See Appendix B for details of tree construction and Appendix C for an electronic version (both appendixes are available in the online edition of *Physiological and Biochemical Zoology*).

case the heart). Gravitational pressure gradients have several physiological consequences. For example, increased gravitational pressure will distend the distal veins below the heart and may cause significant blood pooling and increased plasma leakage into the surrounding tissues. In addition, venous blood pooling will tend to reduce cardiac filling and cardiac output that if not compensated by an appropriate baroreceptor response can decrease arterial blood pressure and blood flow to the brain. Therefore, animals—particularly those that are upright or assume vertical postures—must find solutions to increased gravitational pressure gradients on the cardiovascular system. In the giraffe, for example, several mechanisms are known to work together to prevent edema in the lower extremities, including thick and impermeable capillary basement membranes, arterial-wall hypertrophy, and a prominent lymphatic system (Willamson et al. 1971; Nilsson et al. 1988; Hargens 1991).

For at least two reasons, snakes are an interesting group in which to study potential cardiovascular adaptations that counter gravitational pressure gradients. First, some species are completely terrestrial and therefore rarely encounter changes in gravity, whereas arboreal species frequently assume vertical postures while climbing, and aquatic species are less vulnerable to the effects of gravity. Second, the conservative body plan eliminates the confounding effects of appendages.

The utility of snakes as models to study the effects of gravity and adaptations of the cardiovascular system to gravitational pressure gradients has been previously reported (Lillywhite 1987, 1988; Seymour 1987). The most intriguing adaptive hypothesis to emerge from these studies is that heart position should correlate with behavioral ecology or habitat. Specifically, in nonaquatic snakes that frequently assume vertical postures, the height of the heart-head blood column should be reduced, thus reducing cardiac work. In contrast, aquatic species would have more centrally placed hearts because of the more dense and less gravity-stressing nature of the medium they occupy (water). Finally, terrestrial species would have a heart position

intermediate between aquatic and arboreal forms (Seymour and Lillywhite 1976; Lillywhite 1987, 1988; Seymour 1987; Lillywhite and Henderson 1993; Lillywhite et al. 1996a). Although this general adaptive hypothesis seems reasonable, it must be compared with alternative explanations to describe the evolutionary origin and maintenance of traits (e.g., see Gould and Lewontin 1979; Greene 1986; Garland et al. 1993; Garland and Adolph 1994; Garland and Carter 1994; Rose and Lauder 1996; Clobert et al. 1998; Orzack and Sober 2001; Blomberg and Garland 2002; Garland et al. 2005).

In this article, we analyze heart position in 155, primarily South American, snake species or subspecies to investigate the generality of the heart position/habitat hypothesis using both phylogenetic and nonphylogenetic statistical models. We account for phylogenetic "effects" (in the statistical sense) in two ways. The first is by use of a phylogenetic tree that is hierarchical (as shown in Fig. 1) versus one that has no hierarchical structure and appears as a "star" (one large polytomy). The hierarchical phylogeny, and alterations of it under an Ornstein-Uhlenbeck (OU) model of character evolution (see "Methods"), implies that related species will tend to be similar for the dependent variable of interest, in our case heart position. The second way that we allow for phylogenetic effects in statistical models is by coding major branches of the tree for our included species as different levels in a categorical factor (as shown in Fig. 1, we recognized seven different lineages or "clades"). By use of ln maximum likelihood ratio tests (LRTs), we can compare the fit of models that assume (1) a star phylogeny (implying no expected tendency for relatives to resemble each other), (2) the hierarchical phylogeny shown in Figure 1, or (3) the hierarchical phylogeny transformed under an OU model of evolution (see "Methods"). We also use LRTs to compare those three models with their counterparts that include the clade variable (which we can refer to as models 4, 5, and 6, respectively). Importantly, this approach is not the same as nested ANOVAs based on taxonomy rather than phylogeny per se (see review in Harvey and Pagel 1991). Those approaches could implement a star

phylogeny with categorical variables to represent membership in different named groups at one or more taxonomic levels (e.g., order, family, genus) but could not incorporate the full branching structure either among groups at a given level or within a group.

We also apply the above-listed six models with a separate categorical variable that codes for variation in an "ecological" factor—namely habitat (Table 1: terrestrial, fossorial, arboreal, semiaquatic)—and compare their fit with LRTs. Finally, we use the Akaike Information Criterion (AIC) to compare the fit of nonnested models that include the habitat variable, the clade variable, or both. These AIC comparisons allow for simultaneous consideration of the statistical effects of both phylogeny and ecology on relative heart position. Our overall statistical approach is widely applicable to many questions in comparative biology (see also Huey et al. 2009; Swanson and Garland 2009).

Methods

Heart Position, Habitat, and Body Size

We gathered data on snake heart position in 155 taxa representing seven major families and subfamilies from both new material and museum specimens (Table 1; see App. A in the online edition of *Physiological and Biochemical Zoology* for data). Snakes came primarily from a data set collected in Brazil by P. R. Manzani and D. V. Andrade (Manzani 1995; $N = 120$ species). The remaining species were measured by G. E. A Gartner ($N = 8$ species) and S. M. Secor ($N = 27$ species). We used the mean snout-heart length (SHL) when multiple individuals of a species were measured (App. A). In general, only adult snakes were used. Heart distance was measured by making a ventral incision from the neck until reaching the heart, then measuring the distance from the tip of the snout to the top of the atria. Snout-vent length (SVL) was measured from the tip of the rostral scale to the cloaca on the ventral side of the animal. Note that in Seymour (1987, p. 90) "the distances [were] measured between the head (eye), heart and tip of tail." This difference in measurements is, more likely than not, negligible from a hydrostatic standpoint, as it is unlikely that the evolution of the circulatory system is driven by such minimal differences in pressure.

Snakes were categorized with respect to general habitat usage using literature accounts—primarily field guides and various works on localized snake faunas (e.g., Wright and Wright

1957)—and the authors' own observations and experiences with many of the included species. Fossorial species actively burrow and are found underground or in litter, and most possess obvious morphological adaptations for burrowing (e.g., *Typhlops*). Semiaquatic species are commonly found in water, where they often feed or flee from predators but frequently take to the shore to bask, reproduce, etc. (e.g., *Nerodia*). Arboreal species are often long and gracile in appearance and are most frequently encountered in trees or low-lying shrubs (e.g., *Boiga*, *Corallus*). Terrestrial species lack any obvious morphological adaptations to the terrestrial realm and thus cannot be easily classified into any of the other groups (e.g., most elapids, *Pituophis*, *Drymarchon*). When possible, habitat categories were chosen to reflect those of a previous study (Seymour 1987). Thus, the terrestrial group not only includes obviously terrestrial animals found away from water or trees but also those snakes occasionally found swimming or climbing (as most snakes appear to be able to swim and climb to some extent). Unlike in Seymour (1987), our data set included only one species that might be considered aquatic (*Micrurus surinamensis*), so it was coded as semiaquatic for purposes of statistical analyses.

Phylogeny Construction

We constructed a composite tree using phylogenetic hypotheses from previously published studies. We began with higher-level relationships uniting the major lineages of snakes and nested less inclusive groups (lower-level relationships) within this framework. Our initial intent was to use the best available phylogenetic estimate at each hierarchical level rather than combining a number of phylogenies for a particular group. Most published trees, however, contained only a few taxa of interest for any particular group, and so we were often forced to use multiple trees—each of which may have employed different characters and methods in their analyses—to place particular taxa into our tree.

When a number of trees were available for a given group, we followed the methods of de Queiroz and Rodríguez-Robles (2006): maximum likelihood trees were preferred over those obtained by other methods (e.g., parsimony), and strict consensus trees were used when available. If a lower-level group had multiple trees available to choose from, then we used the number of characters and the number of taxa to differentiate

Table 1: Families or subfamilies included in the data set and their habitat distributions

	Xenodontinae	Dipsadinae	Natricinae	Colubrinae	Elapidae	Viperidae	Boidae	Total
Terrestrial	35	6	1	11	2	22	6	83
Fossorial	4	2	…	2	5	…	1	14
Arboreal	5	6	…	15	…	5	3	34
Semiaquatic	7	…	11	…	0	2	3	23
Aquatic	…	…	…	…	1[a]	…	…	1[a]
Total	51	14	12	28	8	29	13	155

[a] The aquatic *Micrurus surinamensis* was recoded as semiaquatic for purposes of statistical analyses.

Table 2: Allometry of snout-heart length in relation to snout-vent length

Model	Slope	SE	y-intercept	SE	r^2	ln Maximum Likelihood	AIC
Conventional:							
Least squares	1.027	.0489	−.729	.1375	.742	118.791	−231.6
Reduced major axis	1.193		−1.191				
Phylogenetic:							
Least squares	.926	.0351	−.405	.1204	.820	168.798	−331.6
Reduced major axis	1.023		−.679				
Regression with OU transform[a]	.937	.0362	−.447	.1061	.814	174.916	−341.8

[a] REML estimate of $d = 0.7306$.

among them. For instance, if two trees had similar numbers of taxa, we chose the tree with the greater number of characters (and vice versa).

In a few instances, the amount of clade support (i.e., bootstrap values) influenced our decision on tree selection. In those cases where no phylogenetic hypotheses could be found (particularly a problem for species-level relationships among the Xenodontinae) or where there was particularly weak nodal support, we collapsed clades to maintain a more conservative approach to our analysis.

Figure 1 shows the topology of the final tree and indicates the seven major clades identified in statistical analyses. Details of tree construction can be found in Appendix B in the online edition of *Physiological and Biochemical Zoology*. For statistical analyses, branch lengths were set by the arbitrary method of Pagel (1992), as shown in Figure 1, using the DOS PDTREE program (Garland et al. 1999; Garland and Ives 2000). Appendix C in the online edition of *Physiological and Biochemical Zoology* presents the tree in a standard electronic format.

Statistical Analyses

SVL and SHL were $\log_{10}$ transformed before analyses. To test and quantify phylogenetic signal, we used the methods of Blomberg et al. (2003).

We then described the simple allometry of snout-heart position in relation to SVL in the entire data set ($N = 155$) by use of ordinary least squares (OLS) linear regression and reduced major axis (RMA), with both conventional (i.e., nonphylogenetic or assuming a star phylogeny) and phylogenetic versions, using the DOS PDTREE program. PDTREE employs phylogenetically independent contrasts, which yields estimates that are the same as from a phylogenetic generalized least squares analysis (PGLS; Garland et al. 2005; Lavin et al. 2008). However, PDTREE provides certain statistics that are not currently available in most programs for PGLS. It is well known that OLS slopes will underestimate the true scaling relation when the independent variable (in this case, $\log_{10}$ SVL) contains "measurement error," and if the amount of such error is not known, then the RMA slope often gives a reasonable estimate (Rayner 1985; Warton et al. 2006; Ives et al. 2007). To obtain the likelihood of the alternative models, we used the Matlab Regressionv2.m program developed by A. R. Ives and T. Gar-

land Jr. (Lavin et al. 2008; for examples of applications, see Buchwalter et al. 2008; Jeffery et al. 2008; Warne and Charnov 2008; Huey et al. 2009; Swanson and Garland 2009).

Next, we examined the effects of body size (SVL), clade, and ecology using conventional multiple regressions with dummy variables that code for clade membership and habitat category (i.e., ANCOVA with parallel slopes). Formally, a "clade" is defined as a monophyletic group of organisms, including the ancestor and all descendant species. Practically, few if any comparative studies can include all members of a given clade because of extinctions and/or inaccessibility of living representatives. In this article, we use "clade" to refer to all of the species included in the available data set that are members of a formal clade (e.g., Colubrinae, Viperidae, Elapidae). For analyses, Re-
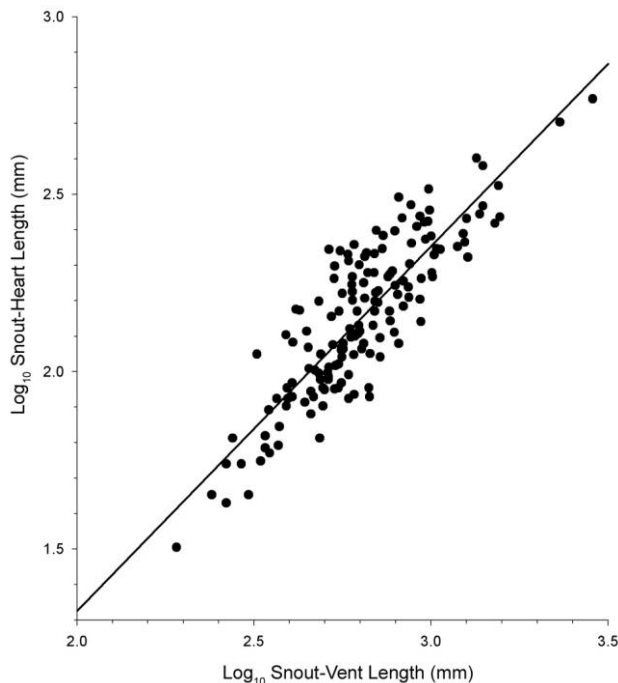

Figure 2. Isometry (slope not statistically different from 1.00) of snout-heart length in relation to snout-vent length for 155 species or subspecies of snakes. Line is conventional (nonphylogenetic) least squares linear regression (slope = 1.027, SE = 0.0489; see Table 2 for this and alternatives).

Table 3: Alternate regression models for predicting heart position of snakes

Model	Conventional (OLS)		Phylogeny (PGLS)		Phylogeny with OU Transform (RegOU)		Mean SE	SE of the Estimate	r^2 for Model	REML Estimate of d (OU Transform)
	ln ML	AIC	ln ML	AIC	ln ML	AIC				
SVL (simple allometry)	118.791	−231.6	168.798	−331.6	174.916	−341.8	6.210E−03	.07880	.8140	.7306
SVL + habitat	126.673	−241.3	178.459	−344.9	184.909	−355.8	5.567E−03	.07462	.8367	.7356
SVL + clade	187.324	−356.6	170.298	−322.6	193.673	−367.3	5.114E−03	.07151	.8602	.3024
SVL + clade + habitat	205.625	−387.3	180.134	−336.2	209.749	−393.5	4.234E−03	.06507	.8936	.1975
SVL + clade + "arboreal"	204.655	−389.3	179.974	−339.9	209.075	−396.2	4.210E−03	.06489	.8923	.2034

Note. ML = maximum likelihood. "Conventional (OLS)" indicates ordinary least squares (multiple) regression, which is mathematically equivalent to assuming a "star" phylogeny with no hierarchical structure. "Phylogeny" indicates generalized least squares analysis (PGLS), which is mathematically equivalent to phylogenetically independent contrasts. "Phylogeny with OU Transform (RegOU)" is a regression model in which the residuals are modeled as an Ornstein-Uhlenbeck process. RegOU models contain one more parameter than their OLS or PGLS counterparts, so whether a RegOU model fits the data significantly ($P < 0.05$) better can be tested by comparing twice the difference in ln likelihoods with the value 3.841 (the ninety-fifth percentile of the distribution of χ^2 with 1 df). Similar ln likelihood ratio tests can be used to compare models within the OLS, PGLS, or RegOU columns when one is a nested subset of the other (e.g., SVL + habitat vs. SVL but not SVL + habitat vs. SVL + clade). The AIC (see "Methods") can be used to compare any models, with smaller (more negative) values indicating a better fit. As a rule of thumb, models whose AIC is <2 units larger than the best model can also be said to have substantial support. See "Methods" and Lavin et al. (2008) for further explanation. For the models listed in the bottom row (SVL + clade + "arboreal"), the arboreal variable was always highly significant (all $P < 0.0001$), and the partial regression coefficient was always positive, indicating that heart position is more posterior than for nonarboreal species.

gressionv2.m automatically recoded clade as a series of six 0– 1 dummy variables.

Our simplest model used only SVL as an independent variable and hence was just a linear regression. Subsequent models added additional variables along with SVL (e.g., SVL + clade or SVL + habitat). Our most complex or "full" model included SVL, clade, and habitat. Based on inspection of the partial regression coefficients for that most complex model (see "Results"), it was apparent that the major effect of habitat was that arboreal animals were different from all three other habitat types. Therefore, we ran one additional model that considered SVL, clade, and only arboreal animals as a distinct category.

All analyses were then repeated using PGLS ANCOVA models with the Regressionv2.m program. Finally, we implemented phylogenetic ANCOVAs with a branch-length transformation parameter based on the OU model of evolution, termed "RegOU" (Lavin et al. 2008). The OU process has been suggested as a way to mimic the effects of stabilizing selection (e.g., see Felsenstein 1988; Garland et al. 1993; Blomberg et al. 2003; Halsey et al. 2006; Lavin et al. 2008; see also Martins and Hansen 1997). The statistical procedure begins with a user-specified phylogenetic tree (as shown in Fig. 1), then moves the internal nodes of the tree up and down, thus simultaneously stretching and contracting the branch lengths above and below the nodes. Small values of the OU transformation parameter (d) yield trees that are more starlike (i.e., long terminal branches and short internode branches near the root), whereas values of d greater than unity yield trees that are even more hierarchical than the original tree. A d value of exactly unity yields the original tree. The regression model in question is fitted using the entire range of stretched and compressed trees. The tree that yields the lowest mean squared error (residual variance) has the highest likelihood, and it is used to compute regression statistics. All of the multiple regression analyses were performed

in Matlab version 7.0 using Regressionv2.m (Lavin et al. 2008). For the conventional (nonphylogenetic) multiple regressions, analyses were also run in SPSS for Windows version 11.5 as a verification, and results were identical to those of Regressionv2.m. The fit of all alternate models considered was compared using the AIC, computed in the smaller-is-better form: AIC = ($-2 \times$ ln ML likelihood) + ($2 \times$ no. parameters).

AIC is particularly well suited to situations such as this, where numerous nonnested models are being compared (e.g., see Lavin et al. 2008). As a rule of thumb, models whose AIC is <2 units larger can also be said to have substantial support, whereas a difference of 4–7 indicates considerably less support, and a difference >10 indicates essentially no support (Burnham and Anderson 2002, p. 70). Where one model was a nested subset of the other, we compared them by ln maximum likelihood ratio tests (LRTs), 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 in the two models. If an LRT and/ or comparison of the AIC indicates that the phylogenetic version of the model is better than the nonphylogenetic version, then one can conclude that "phylogenetic signal"—the tendency for similar species to resemble one another (Blomberg and Garland 2002; Blomberg et al. 2003)—is present in the residuals of the nonphylogenetic model. From a more general perspective, if the clade variable is statistically significant in a model, then phylogenetic position is also important in predicting the dependent variable (i.e., heart position in this article) after controlling statistically for other independent variables in the model.

Results

SVL ($\log_{10}$ transformed) showed relatively low ($K = 0.265$; cf. Blomberg et al. 2003) but statistically significant ($P < 0.001$)

phylogenetic signal. Once corrected for its association with $\log_{10}$ SVL, $\log_{10}$ heart position showed higher signal ($K = 0.580$, $P < 0.001$).

Among all taxa, heart position scales isometrically with body size (Table 2; Fig. 2). The 95% confidence interval (CI; 0.931–1.124) about the OLS regression slope (1.027) includes unity, although, as must be the case, the RMA slope is higher (RMA slope = 1.193; RMA = OLS/r). The 95% CI (0.856–0.995) about the PGLS slope (0.926) excludes unity, but again, the RMA slope is higher (1.023). The phylogenetic regression with an OU transform (RegOU) is the best-fitting model (based on likelihood and AIC; Table 2) and has a slope of 0.937 with a 95% CI of 0.865–1.008.

Alternate models that include clade and/or habitat are presented in Table 3. Based on the AIC values (smaller is better), the relative fit of the models with various independent variables is the same for conventional OLS and RegOU models, improving in the following order: SVL (simple allometry), SVL + habitat, SVL + clade, SVL + clade + habitat, SVL + clade + "arboreal" (i.e., a single category of habitat vs. all others). However, for all models, the RegOU versions (which contain one more parameter) are significantly better than their OLS counterparts based on ln likelihood ratio (LR) tests, with the largest P value being 0.0041 for the model that includes SVL + clade + habitat. For both OLS and RegOU models, the best-fitting model, based on lowest AIC, includes SVL, clade, and a single dummy variable for arboreal snakes (rather than the set of three dummy variables to recognize all four habitat categories). This emphasizes that, for our data set, the major habitat effect on snake heart position is that arboreal snakes

have more posteriorly positioned hearts (see partial regression coefficients for the full models in Table 4).

Table 3 also presents PGLS models, which incorporate the phylogeny with untransformed branch lengths, as shown in Figure 1. In all cases, these models are significantly worse than their RegOU counterparts (which contain one additional parameter), based on LR tests (largest $P = 0.0005$). Therefore, we defer further consideration of these models to the "Discussion."

Discussion

We used a statistical approach that incorporates phylogenetic information to develop models that address whether ecological or historical factors (or a combination of the two) most affect relative heart position in snakes and to test for the presence of phylogenetic signal in this trait. We determined which version of a given model—OLS, PGLS, or RegOU—better fit the data by use of ln LRTs and by comparing AIC values.

Our general findings were that habitat, clade membership, and phylogenetic position within clades (and/or interclade relations) all accounted for some of the variation in relative heart position. The fact that the RegOU models fit better than the nonphylogenetic models even when clade is included as a factor (Table 3) means that the hierarchical structure within and/or among clades reflects some of the resemblance among related species (i.e., phylogenetic signal) in relative heart position. With respect to habitat, arboreal snakes had the most posteriorly placed hearts relative to SVL; with respect to clade, the Viperidae had the most posterior hearts (Fig. 3; Table 4).

Table 4: Full model including habitat and clade variables to predict $\log_{10}$ snout-heart length (mm), analyzed by conventional multiple regression and phylogenetically with an OU transform

Variable	Conventional (OLS)					Phylogenetic with OU Transform (RegOU)				
	Coefficient	SE	F	df	P	Coefficient	SE	F	df	P
y-intercept	−.6465	.0964	44.94			−.5746	.0968	35.26		
$\log_{10}$ SVL (mm)	.9694	.0331	858.82	1, 144	.0039	.9454	.0333	806.77	1, 144	<.0001
Arboreal	.0914	.0148	38.14	1, 144	<.0001	.0879	.0155	32.06	1, 144	<.0001
Fossorial	.0191	.0217	.77	1, 144	.3813	.0199	.0230	.75	1, 144	.3879
Semiaquatic	.0225	.0196	1.31	1, 144	.2537	.0156	.0227	.48	1, 144	.4895
Xenodontinae	−.0049	.0174	.08	1, 144	.7789	−.0094	.0224	.18	1, 144	.672
Dipsadinae	.0691	.0232	8.87	1, 144	.0034	.0564	.0307	3.37	1, 144	.0685
Natricinae	−.0448	.0297	2.28	1, 144	.1335	−.0483	.0366	1.74	1, 144	.1892
Boidae	.1226	.0235	27.14	1, 144	<.0001	.1314	.0303	18.81	1, 144	<.0001
Elapinae	.0718	.0301	5.70	1, 144	.0183	.0666	.0355	3.52	1, 144	.0627
Viperidae	.2153	.0187	132.27	1, 144	<.0001	.2079	.0229	82.49	1, 144	<.0001
Habitat			12.79	3, 144	<.0001			10.71	3, 144	<.0001
Clade			42.47	6, 144	<.0001			25.09	6, 144	<.0001

Note. For the habitat variable, terrestrial is arbitrarily chosen as the base group for comparison. For the clade variable, Colubridae is arbitrarily chosen as the base group for comparison. Thus, all coefficients and significance levels for the individual dummy variables within these categorical variables are relative to those base groups. Overall tests for habitat and clade are at the bottom. For the OLS model, rate of evolution (MSE) = 0.004438, SE of estimate (SEE) = 0.06620, model r^2 = 0.9160, ln maximum likelihood of model = 205.625, AIC = −387.250, AIC$_c$ = −385.053. For the RegOU model, MSE = 0.004234, SEE = 0.06507, model r^2 = 0.8936, REML estimate of OU transformation parameter (d) = 0.1975, ln maximum likelihood of model = 209.749, AIC = −393.499, AIC$_c$ = −390.917.

In all cases, the RegOU versions of models performed significantly better (based on LR tests) than their OLS or PGLS counterparts. The relative fit of the PGLS models, as shown in Table 3, reveals an interesting situation. The PGLS models incorporate the phylogeny with branch lengths as shown in Figure 1 and do not allow any branch-length transformations to improve the fit of the statistical model to the data. As compared with OLS models, their PGLS counterparts had much higher likelihoods when the clade variable was not in the model but lower likelihoods when it was included. This reflects a trade-off in the sense that the phylogenetic signal (the tendency for related species to resemble each other; Blomberg and Garland 2002) present in the residuals can be apportioned either generally throughout the tree or among the specified clades but not both, given the branch lengths shown in Figure 1. However, when the branch lengths are allowed to vary to optimize fit in the RegOU models, the nodes are pulled toward the root (estimated d value ~ 0.2, where $0 =$ a star and $1.0 =$ the original tree), thus making the tree more starlike than shown in Figure 1, and models that include clade are much better (difference in AIC $= 11.5$–40.4) than those that do not. In these RegOU models that include clade, phylogenetic signal thus exists both among clades and among species within clades (or in the form of related clades resembling each other).

The importance of estimating a branch-length transformation parameter simultaneously with estimating parameters in a phylogenetic regression model was first emphasized by Grafen (1989; see review in Lavin et al. 2008). Similarly, Garland et al. (1992) emphasized the importance of various diagnostics and possible transformations of branch lengths when implementing phylogenetically independent contrasts (see also Díaz-Uriarte and Garland 1998). These points are now well accepted in the comparative-method literature (e.g., Martins and Hansen 1997; Freckleton et al. 2002; Halsey et al. 2006; Duncan et al. 2007), and our study provides another clear example of how analyses can be improved by adding this flexibility. Moreover, debates about the importance of ecology versus phylogeny can be addressed statistically by comparison of a range of models in between a star and the original starter tree (e.g., Fig. 1) while simultaneously testing the effects of including ecological (e.g., habitat) and/or phylogenetic (clade membership) variables in alternate models (see also Huey et al. 2009; Swanson and Garland 2009).

In our study, the single best predictor of heart position was body size ($\log_{10}$ SVL), which is unsurprising given the large size range in the data set (Tables 2, 4; Figs. 2, 3). With body size in the model, clade membership (see Fig. 1 for the seven clades identified for analyses) appeared to be a significantly better predictor of heart position than habitat (four categories identified) for both nonphylogenetic (AIC values of -356.6 for clade vs. -241.3 for habitat) and RegOU models (-367.3 for clade vs. -355.8 for habitat). Moreover, those same AIC values indicate the clear superiority of the phylogenetic RegOU models as compared with the nonphylogenetic OLS models. Thus, relative heart position of snakes varies in relation to both differences among the major branches of the phylogenetic tree and
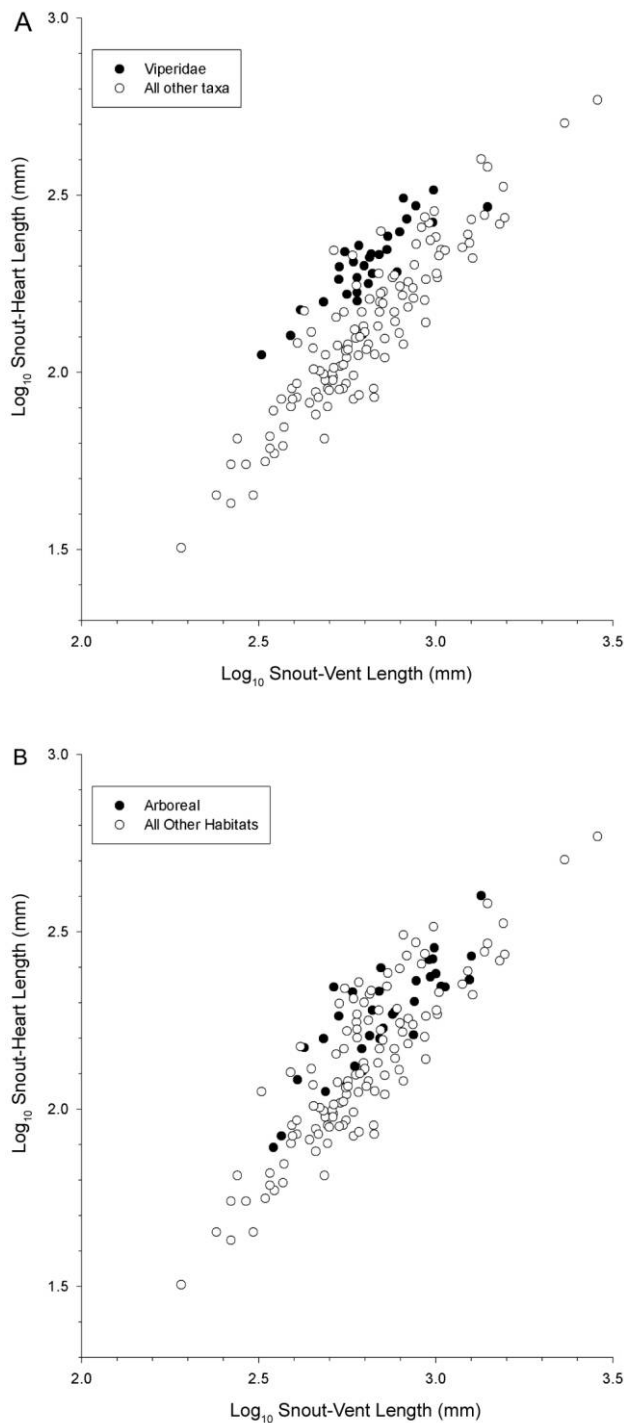

Figure 3. Snout-heart length in relation to snout-vent length for 155 taxa of snakes, indicating the clade that is most different (A, Viperidae) and the habitat classification (B, arboreal) that is most different from other snakes.

the detailed hierarchical structure of the tree. Stated another way, phylogenetic signal (sensu Blomberg and Garland 2002; Blomberg et al. 2003) is apparent both within and among clades (see also above). The best models, however, incorporated both clade and habitat on a hierarchical tree, and the single best
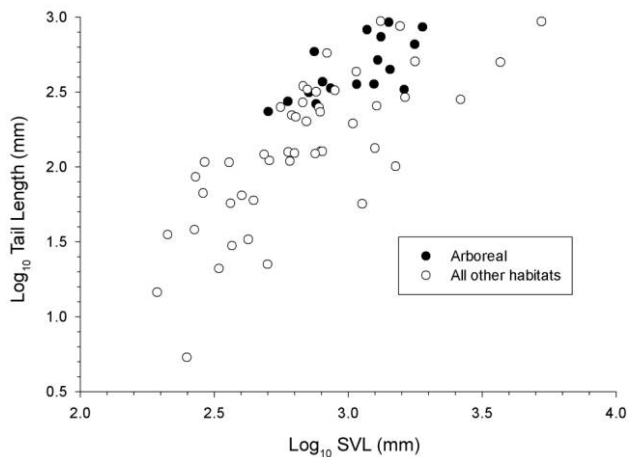
Figure 4. Tail length versus snout-vent length from a published data set for 65 South American taxa (Martins and Oliveira 1998). Note that arboreal species generally have longer tails (see text).

model included clade and only one aspect of habitat—inclusion in the arboreal category.

It is clear that both phylogeny and habitat are important in predicting heart position. For instance, in viperids, Seymour (1987) noted that the heart is generally shifted posteriorly (perhaps inherited from their common ancestor), but in arboreal members of the clade, the heart is positioned more anteriorly than in terrestrial members, which Seymour interpreted as an adaptation. Given their unusual heart positions, we also analyzed the viperids alone ($N = 29$ species), for which three habitat categories are represented (fossorial snakes are lacking). The ln likelihoods of the OLS, PGLS, and RegOU ANCOVA models were 45.0929, 44.8680, and 47.3945, respectively. Based on likelihood ratio tests, the RegOU model (estimated $d = 0.4630$) is significantly better than either the OLS ($P = 0.0319$) or PGLS model ($P = 0.0246$). The effect of habitat was not statistically significant in any of the models ($P > 0.5$ for the RegOU model).

For completeness, we also analyzed the Xenodontinae ($N = 51$) and Colubrinae ($N = 28$) alone. Results for Xenodontinae were similar to those for viperids; that is, RegOU was the best-fitting model, and habitat was not significant ($P > 0.5$ for the RegOU model). For Colubrinae, the conventional OLS model was best, and habitat was highly significant ($P < 0.0001$), with the effect attributable to arboreal species having hearts placed more posteriorly (partial regression coefficient = 0.1074, $P < 0.0001$). Thus, the habitat effect within Colubrinae alone is consistent with that shown in the overall analysis of all species (Table 4; Fig. 3).

Alternate Functional Hypotheses for Variation in Heart Position

The anterior heart position of arboreal snakes is hypothesized to reduce the cardiac work required to lift the blood to the head when in the vertical orientation (Seymour 1987; Lillywhite

1988). Alternatively, Badeer (1998) hypothesized that heart position is optimized for cardiac-filling pressures and is related to the compliance of the vessels above and below the heart. Briefly, this alternative view is based on the physical principles that determine the hydrostatic indifference point (HIP) in a vertically oriented vascular system (Wagner 1886; Clark et al. 1934; Gauer and Thron 1965). The HIP is a unique reference within the venous circulation where blood pressure is unaffected by vertical orientation (Gauer and Thron 1965; Buckner et al. 1999). HIP is determined by in vivo compliance of the veins above and below the heart. In the upright position, if the dependent veins (vessels below the heart) are highly compliant relative to the vessels above the heart, then the venous HIP will shift below heart level (Gauer and Thron 1965). The inferior location of the HIP results in a reduction in venous return and decreased cardiac-filling pressure (Buckner et al. 1999; Jarvis et al. 2007). Conversely, reducing the compliance (stiffening) of the dependent vessels can raise the HIP above heart level (Gauer and Thron 1965) and increase cardiac-filling pressures. The colocalization of the HIP and heart ensures that cardiac filling remains relatively stable despite changes in vertical orientation (Buckner et al. 1999). Based on these fundamental hemodynamic principles, Badeer (1998) hypothesized that the position of the heart in snakes should be correlated with the HIP.

Several studies have shown that arboreal snakes have less compliant caudal vessels compared with other terrestrial and aquatic species. In these species, the integument is tightly coupled to the underlying tissues, which will help prevent venous pooling in the upright position (Lillywhite 1993, 1996; Lillywhite et al. 1996b). Interestingly, this antigravity feature of the integument is similar to the "skin and fascial antigravity suit" seen in the legs of giraffes (Hargens et al. 1987), thus providing an apparent example of convergent evolution in function. In snakes, a more posterior location of the heart will also act in preventing blood from pooling below the heart when adopting an upright position (see the preceding discussion about HIP). Given that both decreased compliance (Lillywhite 1993, 1996; Lillywhite et al. 1996b) and posterior heart location (this study) are prevalent among arboreal species, this seems to indicate venous pooling and the associated risk of edema formation as an important gravitational stressor. Thus, previous adaptive scenarios placing the heart of arboreal snakes closer to the head may have overweighted the importance of ensuring an adequate blood supply to the head in detriment of the importance of venous return from the regions below the heart. Obviously, ensuring an adequate blood flow—above and below the heart— is important both on the arterial and venous side, and this is only made possible by the action of an orchestrated suite of cardiovascular and behavioral adaptations rather than to alterations in heart location alone. This, combined with differences in the intensity of the gravitational stress imposed by different habitats, may help to explain the absence of any expected correlation between heart location and habitat for aquatic and terrestrial species. Future studies should address this question in addition to determining the compliance and

Table 5: ANCOVA of snake heart position with $\log_{10}$ estimated total length as the covariate

Model	Conventional (OLS)		Phylogeny (PGLS)		Phylogeny with OU Transform (RegOU)		Mean Squared Error	SE of the Estimate	r^2 for Model	REML Estimate of d (OU Transform)
	ln ML	AIC	ln ML	AIC	ln ML	AIC				
Total length + clade + "arboreal"	204.655	−389.3	179.974	−339.9	209.075	−396.2	4.210E−03	.06489	.8923	.2034
P for clade	<.0001		.7890		<.0001					
P for arboreal	.0575		.3469		.0846					

Note. ML = maximum likelihood. See note to Table 3. The partial regression coefficient for the arboreal dummy variable was always positive, indicating that heart position is more posterior than for nonarboreal species.

the HIP of snakes from a variety of habitats, thus allowing direct tests of the hypothesis that heart position is correlated with HIP.

Variation in Tail Length as a Possible Confounding Factor

Seymour (1987) grouped snakes into six categories—arboreal, terrestrial, aquatic, semiaquatic, fossorial, and Viperidae—then analyzed heart position as a percent of total length (measured as eye to tail tip). ANOVA indicated highly significant group differences, with arboreal having the most anteriorly placed hearts (Table 6). In contrast, we analyzed SHL by ANCOVA with SVL as the covariate and found that arboreal snakes have more posteriorly placed hearts (Fig. 3; Table 4). One possible explanation for this discrepancy is that arboreal snakes in our data set tend to have relatively long tails (H. B. Lillywhite, personal communication). Indeed, some previous studies have found that arboreal snakes often do have relatively long tails (Goldsmith 1984; Guyer and Donnelly 1990; Lillywhite and Henderson 1993).

In an attempt to test this proposition, we did the following. First, we analyzed a separate, published data set for 65 South American taxa (Martins and Oliveira 1998) that also measured SVL (as in our study) rather than total length and also reported tail length as a percent of total length. Tail length as a percentage of total length was given as a range of values, so we used the upper end of the range to compute absolute tail length for their data set. We then computed SVL by subtraction. Figure 4 shows

that, for their data set, arboreal snakes do indeed tend to have relatively long tails. Conventional ANCOVA of their data yields the following equation:

$$\log_{10} \text{total length} = 0.04634 + (1.01654$$
$$\times \log_{10} \text{SVL}) + (0.06242 \times \text{arboreal}),$$

where "arboreal" is a dummy variable that is 1 for arboreal species and 0 for all others. Second, we used that equation to compute a $\log_{10}$ total length for all 155 species in our data set. Third, we analyzed the data with Regressionv2.m, mimicking the analyses presented in the bottom row of Table 3, but with $\log_{10}$ total length as the covariate rather than $\log_{10}$ SVL. In these analyses, the partial regression coefficient for the arboreal dummy variable was always positive, thus again indicating that arboreal snakes have hearts placed more posteriorly (unlike in the analyses of Seymour 1987), although the effect was not statistically significant (two-tailed $P = 0.0846$ for the best-fitting RegOU model; see Table 5).

Table 6 shows the mean values from Seymour (1987, Table 1) and from our study when snakes are grouped according to his categories. As a percent of estimated (see above) total length, our data set does not indicate arboreal species to have the most anteriorly placed hearts. Therefore, we conclude that the discrepancy between our analyses and those of Seymour (1987) cannot be explained entirely by the difference in the measure of body size used.

Table 6: Heart position as a percentage of body length

	Percentage of		
	Total Length (Seymour 1987)	Estimated Total Length (This Study)	Snout-Vent Length (This Study)
Arboreal	17.4 (2.2)	17.9 (4.1)	25.6 (5.8)
Terrestrial	18.8 (3.2)	15.6 (2.9)	19.4 (3.7)
Semiaquatic	22.7 (4.8)	15.7 (2.3)	19.4 (2.8)
Fossorial	23.6 (7.9)	17.6 (2.9)	21.8 (3.5)
Viperidae	33.3 (4.5)	25.2 (3.8)	31.9 (4.2)
Aquatic	33.4 (5.7)		

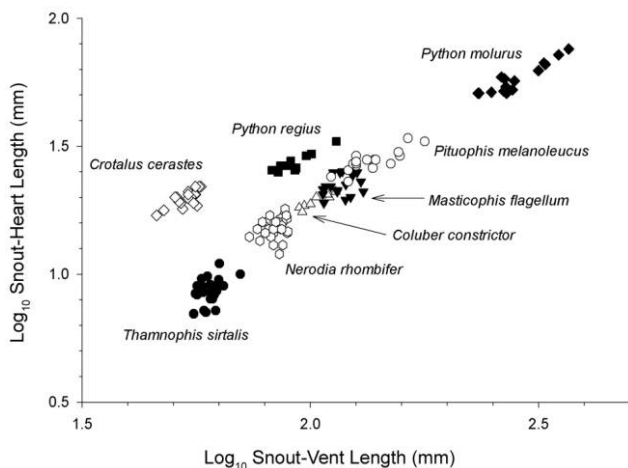Note. Data are means, with SDs in parentheses.

Figure 5. Illustration of the range of individual variation in snout-heart length in relation to snout-vent length for eight species in which we had at least 10 individuals per species. See "Discussion."

Caveats

A limitation of any comparative study based on data sets comprised of faunal surveys or literature reviews is that the various species are not measured under "common garden" conditions (Garland and Adolph 1991, 1994; Garland et al. 2005). Hence, it is possible that some of the differences we have observed among habitats or among clades might be reduced in magnitude if all animals had been raised under identical conditions or even housed under identical conditions for some weeks or months before measurement (see Garland and Adolph 1991 and references therein). On the other hand, it would not be possible to raise all species under identical conditions because, for example, they will not (voluntarily) eat exactly the same types of food. In addition, heart position can shift ontogenetically within a species (S. M. Secor, unpublished results), so

variation in age among species in our sample would affect species differences to some extent.

Most of the species included here are represented by a single individual, which obviously introduces error when comparing species. To get some indication of how this "noise" may have influenced our comparison, in Figure 5 we show the relation between $\log_{10}$ SHL and $\log_{10}$ SVL for the eight species from the Secor sample that were represented by 10 or more nonjuvenile individuals (total $N = 150$). As can be seen, the magnitude of the differences among some species in size-relative heart position is much larger than the range of variation observed within species. In general, noise introduced by small sample sizes should tend to reduce statistical power to detect effects of habitat, clade, etc. Given the several statistically significant effects we have detected (Table 3), this sampling variation was not large enough to obscure major results. An improvement for future analyses would be to fit regression models that explicitly account for the magnitude of within-species variation (Ives et al. 2007).

An additional caveat is that the taxa used in this study were different than those used in previous studies. In particular, only 12 of the 155 taxa used in this present study were also used in Seymour (1987; see Table 7). Moreover, 51 of our species (33%) were South American xenodontines, a taxon lacking from Seymour's (1987) sample, whereas we lacked any fully aquatic snakes, such as hydrophiid sea snakes, which accounted for 17% of Seymour's sample. On the other hand, of the 14 families and subfamilies included in one or the other of the two studies, nine were included in both (Table 7).

A final limitation is that it is very difficult to quantify and categorize behavior—especially in broadly defined regimes such as "habitat" (e.g., see Jayne 1982). Very few snakes live strictly in an arboreal regime or a terrestrial regime (sea snakes and blind snakes being notable exceptions) but instead tend to exist at the borders of any given behavioral spectrum (e.g., primarily

Table 7: Unique and shared taxa between Seymour (1987) and this study

Shared Taxa	Unique Taxa in This Study	Unique Taxa in Seymour (1987)	Shared Species
		Acrochordidae	
Boinae			*Agkistrodon piscivorus*
Colubrinae			*Arizona elegans*
Crotalinae			*Bothrops atrox*
	Dipsadinae		*Diadophis punctatus*
Elapinae			*Elaphe obsoleta*
Erycinae			*Lachesis muta*
		Homalopsinae	*Lampropeltis getulus*
		Hydrophiinae	*Masticophis flagellum*
Natricinae			*Nerodia sipedon*
Pythoninae			*Nerodia taxispilota*
Typhlopidae			*Pituophis melanoleucus*
Viperinae			*Thamnophis sirtalis*
	Xenodontinae		

Note. See "Discussion."

terrestrial but occasionally arboreal or primarily fossorial but occasionally terrestrial). Thus, categorization of habitat becomes particularly difficult for animals that can be classified as semiarboreal or semifossorial. For our purposes, any snake that frequently assumes vertical postures, even if commonly found in a terrestrial environment (e.g., *Pantherophis*), was considered arboreal, but one can see how tinkering with such a distinction could have important consequences for the results and conclusions of a study such as this. Note, however, that our data set does include some strictly arboreal species (e.g., *Corallus* sp., *Atheris squamiger*, *Bothriopsis bilineata*, *Oxybelis* sp., *Imantodes cenchoa*, etc.). Because we present all of the data analyzed here (App. A), it will be possible for future workers to try various recategorizations as field data become more available. In addition, future workers can incorporate additional ecological or behavioral predictors, such as diet (Hampton 2009).

Acknowledgments

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Appendix A from Gartner, Hicks, Manzani, Andrade, Abe, Wang, Secor, and Garland, "Phylogeny, Ecology, and Heart Position in Snakes"

(Physiol. Biochem. Zool., vol. 83, no. 1, p. 43)

Supplementary Data

In the data file, rows are arranged in phylogenetic order (starting from the left of the phylogeny, as shown in Fig. 1). HP and LP are heart and lung position, respectively, as a percentage of snout-vent length (SVL); LVL is the length of the vascularized lung tissue, SHL is absolute snout-heart length, and SLL is the absolute length from the snout to the beginning of the vascularized lung tissue. LSVL and LSHL are $\log_{10}$ SVL and $\log_{10}$ SHL, respectively.

Appendix B from Gartner, Hicks, Manzani, Andrade, Abe, Wang, Secor, and Garland, "Phylogeny, Ecology, and Heart Position in Snakes"

Phylogeny

The phylogenetic tree used for all analyses was constructed from published phylogenies for snakes using both high- (e.g., family) and low- (e.g., genus and species) level trees. Tree entry and manipulation were performed using Mesquite (ver. 1.12; Maddison and Maddison 2006; http://mesquiteproject.org). All 155 species of snakes listed in the Excel file database of Appendix A are represented in the tree. The final tree contained several polytomies for which we could not find published phylogenetic hypotheses or in which published phylogenies contained polytomies or weak nodal support.

We used Vidal and Hedges's (2002*b*) molecular study as the basis of our higher-levels relationships. The choice of this tree was arbitrary relative to Lee and Scanlon's (2002) tree, which used both morphological and molecular characters. The benefits of the Lee and Scanlon tree are generally higher bootstrap values, while the Vidal and Hedges tree used more taxa and many more characters. The following list describes the construction of several lower-level clades. The ordering does not reflect phylogenetic placement.

Boinae (within Boidae). The relationships are from Burbrink (2005, Fig. 4). This tree uses a combined approach using Kluge's (1991, 1993) morphological data and newer sequences from they cytochrome *b* gene. Burbrink's (2005) Figure 3 (lacking molecular data) resolves the polytomy among the anacondas (*Eunectes*), but we chose to maintain the polytomy for a more conservative approach to our analysis.

Erycinae (within Boidae). Relationships are from Burbrink (2005), although because we have only two taxa, they must be sister to one another.

Pythoninae (within Boidae). The relationships among the pythons comes from Kluge (1993).

Colubroidea (includes all other lineages shown in Fig. 1 in the text). The relationships are from the combined C-mos, 12S and 16S rRNA, and ND4 tree of Vidal and Hedges (2002*a*); and the maximum likelihood cyt *b*, 12S and 16S rRNA, and ND4 tree of Kelly et al. (2003). Both studies were used because the Kelly et al. study used the most informative characters while the Vidal and Hedges tree used the C-mos gene rather than the less phylogenetically informative cyt *b* (de Queiroz and Rodriguez-Robles 2006).

Crotalinae (within Viperidae). Relationships within the rattlesnakes and moccasins (*Crotalus* and *Agkistrodon*) follow Murphy et al. (2002). The South American palm vipers (*Bothrops* sensu lato) were a particularly difficult group. Our tree is based primarily on that of Parkinson et al. (2002), although particular species may have come from other phylogenetic hypotheses (e.g., Crother et al. 1992; Werman 1992; Vidal et al. 1997; Parkinson 1999; Salomão et al. 1999; Wüster et al. 2002, 2005).

Viperinae (within Viperidae). We have only two true vipers; thus they must be sister to one another. This is the sister group to the Crotalinae.

Elapidae. We have only two major groups of Elapids—the cobras (*Naja*) and the New World coral snakes (*Micrurus*). The relationships among the species of *Micrurus* come from Slowinski (1995) and Jorge Da Silva and Sites (2001).

Colubrinae. The relationships among this group were particularly difficult to resolve because of a lack of published phylogenetic hypotheses on the included taxa. We relied primarily on Creer (2001) but used Lopez and Maxon (1995) to distinguish the relationships between *Tantilla*, *Leptophis*, and *Chironius*.

Lampropelitni (within Colubrinae). The relationships between the North American king snakes, gopher snakes, and rat snakes came from Rodríguez-Robles and De Jesús-Escobar (1999, Fig. 4).

Masticophis and ***Coluber***. The relative relationships of these two genera with respect to the rest of the Colubrinae are from Creer (2001), although relationships among these genera are from Nagy et al. (2004).

Natricinae. The relationships among the water snakes and garter snakes come primarily from Alfaro and Arnold (2001, Fig. 4) and de Queiroz et al. (2002). The placement of *Thamnophis saurtius* is from de Queiroz and Lawson (1994).

Xenodontinae and Dipsadinae. We primarily used the hypotheses from Vidal et al. (2000, Fig. 2) over those of Cadle (1984*a*, 1984*b*). The former study contained more taxa and used individual characters rather than distances. We were forced to use the latter studies, however, for the placement of particular species or genera (e.g., *Waglerophis* from Cadle 1984*a*).

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Appendix C from Gartner, Hicks, Manzani, Andrade, Abe, Wang, Secor, and Garland, "Phylogeny, Ecology, and Heart Position in Snakes"

(Physiol. Biochem. Zool., vol. 83, no. 1, p. 43)

File of Phylogenetic Tree Used for All Analyses

This file (155P.BRK) of the phylogenetic tree (described in App. B and shown in Fig. 1) was produced by the DOS PDTREE.EXE program (Garland et al. 1993, 1999), available on request from Theodore Garland Jr. (http://www.biology.ucr.edu/people/faculty/Garland/PDAP.html). It is in the Newick Standard format (http://evolution.genetics.washington.edu/phylip/newicktree.html).

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