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Morphological evolution in relationship to sidewinding, arboreality and precipitation in snakes of the family Viperidae

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Compared with other squamates, snakes have received relatively little ecomorphological investigation. We examined morphometric and meristic characters of vipers, in which both sidewinding locomotion and arboreality have evolved multiple times. We used phylogenetic comparative methods that account for intraspecific variation (measurement error models) to determine how morphology varied in relationship to body size, sidewinding, arboreality and mean annual precipitation (which we chose over other climate variables through model comparison). Some traits scaled isometrically; however, head dimensions were negatively allometric. Although we expected sidewinding specialists to have different body proportions and more vertebrae than non-sidewinding species, they did not differ significantly for any trait after correction for multiple comparisons. This result suggests that the mechanisms enabling sidewinding involve musculoskeletal morphology and/or motor control, that viper morphology is inherently conducive to sidewinding ('pre-adapted') or that behaviour has evolved faster than morphology. With body size as a covariate, arboreal vipers had long tails, narrow bodies and lateral compression, consistent with previous findings for other arboreal snakes, plus reduced posterior body tapering. Species from wetter environments tended to have longer tails, wider heads and reduced anterior tapering. This study adds to the growing evidence that, despite superficial simplicity, snakes have evolved various morphological specializations in relationship to behaviour and ecology.

ADDITIONAL KEYWORDS: adaptation – allometry – biomechanics – climate – convergent evolution – ecomorphology – habitat – locomotion – pleomerism – scaling.

INTRODUCTION

Locomotor behaviours and related morphologies should often be under relatively strong selection (Garland & Losos, 1994; Dickinson *et al.*, 2000; Irschick *et al.*, 2008). Therefore, locomotion is ideal for studies of ecological morphology (or 'ecomorphology'), a field that examines how the physical form of an animal relates to its external environment, generally with the goal of finding evidence for evolutionary adaptation (Van der Klaauw, 1948). In addition to locomotion, many other factors can influence the morphology of an animal, such as habitat specialization and body size. Moreover, the factors that influence the evolutionary trajectory of morphology can interact. For example, a small-bodied lineage that evolves to specialize on arboreal habitats might do so in ways that differ greatly from those that occur in a large-bodied lineage, as demonstrated by differential use of leaping vs. swinging in small and large arboreal primates (Fleagle & Mittermeier, 1980). Furthermore, specialization for certain types of habitats can be facilitated by the evolution of different modes of locomotion (e.g. digging facilitates use of underground habitats).

The body plan of an organism constrains the type(s) of locomotion it can perform. For example, limbless terrestrial vertebrates interact with the environment in a fundamentally different way from their limbed relatives. Lacking hands and feet to provide traction, they rely entirely on the trunk musculature and vertebral column for propulsion (Gasc, 1974; Jayne, 1988a, b). Although a limbless body plan might impose constraints, elongation with concurrent limb reduction has evolved > 25 times in squamate reptiles (Gans, 1975; Wiens *et al.*, 2006). Many limbless tetrapods are

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primarily burrowers, but snakes have radiated into a wide variety of niches on six continents and have developed many ways of moving without limbs. They effectively use subterranean, terrestrial, arboreal and aquatic environments (Greene, 1997), and they can use many distinct types of movement (Jayne, 2020). Most snakes, like all limbless tetrapods, generally use lateral undulation on land (Gans, 1962). However, some environments elicit other types of locomotion that not all species can perform adequately, including a specialized gait termed 'sidewinding' (Gans, 1962; Tingle, 2020).

In the present study, we examined whether body size, sidewinding locomotion, arboreality and/or climate (specifically, mean annual precipitation) could predict interspecific variation in body shape and scalation in vipers. Sidewinding locomotion and arboreal habitats both seem likely to pose special demands on the body. By including precipitation in our study, we can tease apart whether any morphological differences relate more to ecological specialization (arboreality and/or sidewinding) or to broader-scale environmental characteristics.

Sidewinding locomotion has evolved in several distantly related viper species and in a few other snake species (Tingle, 2020). This type of locomotion might allow desert-dwelling species to deal with the difficulties of shifting sand and/or to move quickly so that they can minimize the amount of time spent in dangerous open areas (e.g. see Cowles, 1920, 1956; Gans & Mendelssohn, 1971). During sidewinding, the snake alternately lifts some regions of its body up and forwards while other regions remain in static contact with the ground. Given the relative uncommonness of this mode of locomotion, we expected that sidewinding species would show clear evidence of morphological differences from non-sidewinding snakes, i.e. coadaptation of morphology with sidewinding behaviour. However, only one previous study has tested this hypothesis directly, finding shorter spinalis muscles in sidewinding species (Tingle *et al.*, 2017).

Arboreality has evolved many more times than has sidewinding, including several independent origins in vipers (Alencar *et al.*, 2017; Harrington *et al.*, 2018). Animals living in trees face special challenges, such as the need to grip branches and maintain balance on narrow surfaces (Cartmill, 1985). Although snakes do not have limbs or claws that could be modified for climbing, some species that regularly move through trees have been shown to exhibit elements of trunk and tail morphology that are presumed to aid their movement, such as slender, laterally compressed bodies, relatively long tails and long spinalis muscles (Henderson & Binder, 1980; Jayne, 1982; Vitt & Vangilder, 1983; Guyer & Donnelly, 1990; Lillywhite & Henderson, 1993; Martins *et al.*, 2001; Pizzatto *et al.*, 2007a, b; França *et al.*, 2008; Alencar, 2010; Tingle *et al.*, 2017). Arboreal vipers show some convergence with arboreal snakes in other clades, including slender bodies and relatively long tails (Alencar *et al.*, 2017), but it is unknown whether they show specialization with regard to other body shape traits or scalation.

Here, we combined tests of specific hypotheses with exploratory data analysis to study the evolution of body shape in vipers. Given that ours is the first study to quantify body shape in sidewinding species, we chose to explore many body measurements and indices representing body shape. We had ideas for some important traits to examine based on previous observations and (bio)mechanical principles, as follows: (1) anecdotally, sidewinding species might have relatively wide, flat bodies (Gray, 1968: pp. 180–181), which would increase contact with the ground, possibly reducing slippage; (2) sidewinding species might have relatively short tails, because the tail seems not to contribute to force production during sidewinding (Jayne, 1988b); and (3) more vertebrae would increase flexibility (Jayne, 1982), which could be important for forming tight bends in the body during sidewinding.

Unlike sidewinding, arboreality has been the focus of several previous studies; therefore, we had clearer expectations with respect to the morphology of arboreal species. As noted above, Alencar *et al.* (2017) found slender bodies and relatively long tails in arboreal vipers. We expected that arboreal vipers might show several additional specializations to their habitat, unexplored by Alencar and colleagues. In particular, we tested the following hypotheses: (1) that arboreal vipers would be laterally compressed in cross-section, convergent with arboreal snakes in other clades (Lillywhite & Henderson, 1993; Pizzatto *et al.*, 2007a,b); (2) that the bodies of arboreal species would taper more sharply in the front, thus shifting their centre of mass backwards (Peters, 1960; Cadle & Greene, 1993), which could aid in gap-bridging; and (3) that arboreal species would have more vertebrae, which would increase flexibility, facilitating complex motions.

To determine whether differences in body shape were related to specialized behaviour (sidewinding or use of arboreal habitats) or to some other environmental characteristic, we also examined whether body shape varied with climate. For example, Scanlon (2001) suggested that sidewinding snakes are generally smaller than other species in their clades. However, such a trend could result from limited food ability in deserts rather than from the demands of sidewinding locomotion. By examining the potential influence of one or more variables related to climate, we could tease apart these potential causes of morphological differences. Given that body size and phylogenetic

history also influence morphology, we additionally examined scaling relationships and phylogenetic signal in the traits that we measured.

MATERIAL AND METHODS

Our study included 86 geographically diverse species from across the viper phylogeny. For each species in the study, we measured the following parameters with either digital callipers or measuring tape: snout–vent length (SVL); tail length; neck width immediately behind the quadrates; head width at the corners of the mouth; ventral head length, measured from the tip of the snout to the anterior edge of the first true ventral scale as defined by Dowling (1951); dorsal head length, measured from the tip of the snout along the body axis to a line connecting the distal ends of the two quadrates at jaw articulation (this measurement is likely to be correlated with mandible length); body width, height and girth (circumference) at 25, 50 and 75% of SVL; number of ventral scales, according to the convention put forth by Dowling (1951); number of subcaudal scales; and number of dorsal scale rows. We collected measurements from museum specimens, because it would be unfeasible to collect measurements from a large sample size of live vipers and impossible for us to obtain access to live specimens for many of these species. We did not use body measurements affected by large gut contents, gravidity, damage or obvious deformation in our analyses. We measured several individuals of most species (mean, 8.3; range, 1–51) for a total sample of 716 adult specimens. Although we collected measurements for some juvenile specimens, we conducted analyses on data from adult specimens only (we determined adulthood based on specimens having reached an SVL consistent with published adult size ranges for their species). Raw data, including museum catalogue numbers for specimens, can be found in the Supporting Information (File S1). In some cases, catalogues contained out-of-date species identifications; therefore, we changed species identities according to the most current literature (details in Supporting Information, File S2).

From these measurements, we calculated several indices describing body shape. The average body width-to-height ratio from measurements at 25, 50 and 75% SVL describe whether the body is flattened or laterally compressed in cross-section. We calculated an index to describe anterior tapering by dividing the body width measurement at 50% SVL by the body width measurement at 25% SVL and an index to describe posterior tapering by dividing the body width measurement at 50% SVL by the body width measurement at 75% SVL.

We coded both sidewinding and arboreality as zero/one indicator (a.k.a.) dummy variables, in which one indicated specialization. We followed the coding by Tingle (2020) for sidewinding specialization, with two exceptions: *Echis pyramidum* and *Bitis schneideri*. Tingle (2020) categorized *Echis carinatus* as the only specialized sidewinder in its genus based mainly on the work of Gans & Mendelssohn (1971). However, *E. carinatus* has been split into multiple species, and Gans and Mendelssohn's '*Echis carinatus* subspecies' from Kenya is now considered to be *E. pyramidum* (Pook *et al.*, 2009); therefore, we categorized *E. pyramidum* as a sidewinder. Tingle (2020) placed *B. schneideri* in the second rather than the most specialized category for sidewinding owing to differences among populations: although some populations of *B. schneideri* use sidewinding as a primary mode of locomotion, other populations seem never to use sidewinding despite living in sandy habitats. For this study, we have decided to count *B. schneideri* among the sidewinding species. We used various accounts in the literature to determine specialization for arboreality (Pitman, 1938; Campbell & Lamar, 1989; Gloyd & Conant, 1990; Campbell, 1998; Grismer, 2002; Orlov *et al.*, 2002; Spawls *et al.*, 2002; Mallow *et al.*, 2003; Stebbins, 2003; Vogel, 2006). Specialization for sidewinding locomotion is estimated to have five independent origins in vipers (Tingle, 2020), all of which are included in our dataset (Fig. 1). Arboreality probably has seven independent origins in vipers (Harrington *et al.*, 2018), of which we have captured four (Fig. 1).

In addition to sidewinding and arboreality, we examined the relationship between climate and morphology. We used Global Positioning System (GPS) coordinates associated with specimens in our sample to obtain climate data from the WorldClim database (Fick & Hijmans, 2017). For some specimens, coordinates were obtained directly from VertNet.org. For others, VertNet.org did not list coordinates, but it did list locality information, allowing us to obtain approximate GPS coordinates. Some specimens did not have locality information or had locality information that was not precise enough to approximate GPS coordinates meaningfully. For those specimens, we left latitude and longitude, in addition to climate data, as not assessed (NA). For two species (*Bothrops barnetti* and *Montivipera latifii*), none of the specimens we measured had locality data; therefore, we obtained range information from the International Union for Conservation of Nature and Reptile Database websites and chose GPS coordinates representing a point in the middle of their ranges.

Climate variables obtained from WorldClim included the following: average maximum temperature of the hottest month (T_{max}; in degrees Celsius), average minimum temperature of the coldest month (T_{min}; in degrees Celsius), mean annual precipitation (which

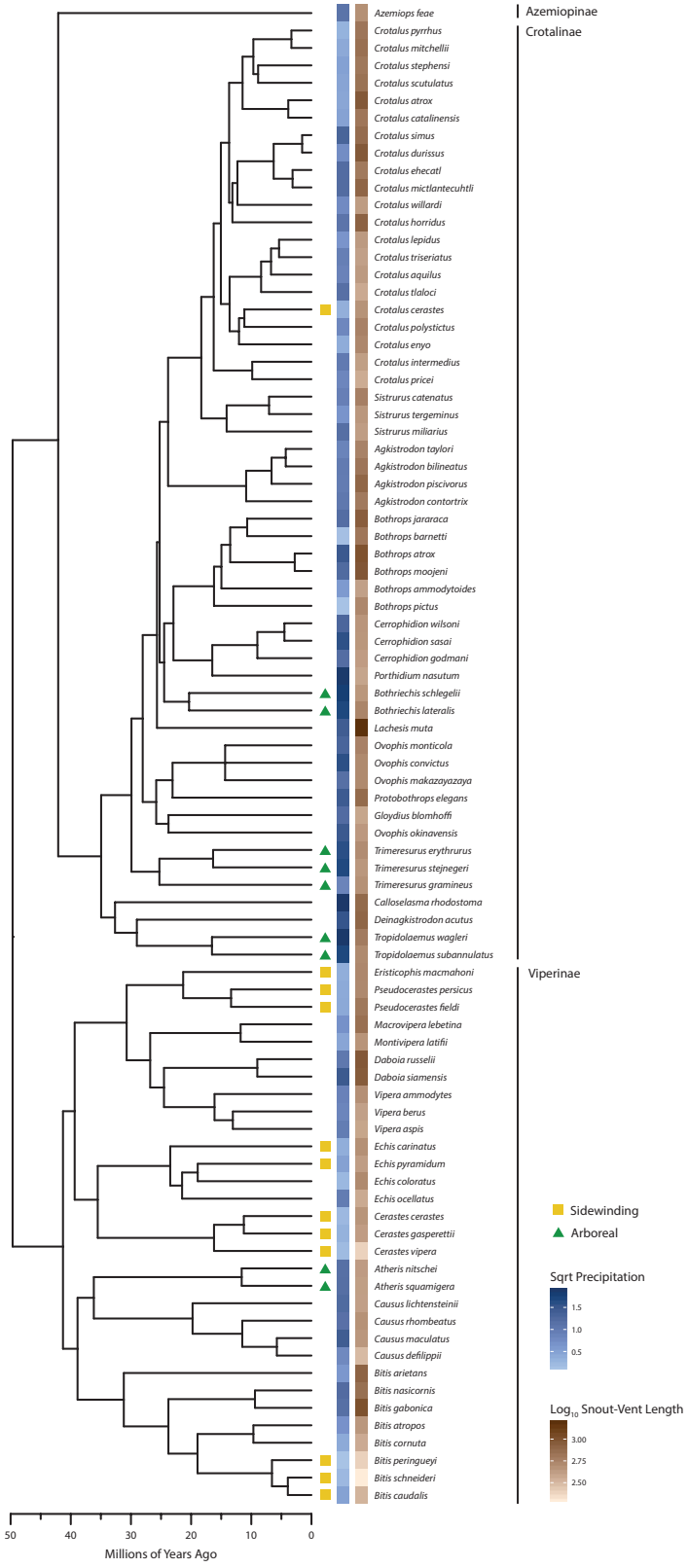

Figure 1. Phylogeny used for statistical analyses, with tip labels for locomotor specialization, the square root (sqrt) of precipitation (in metres) and $\log_{10}$ snout–vent length (in millimetres). Note that both sidewinding and arboreality are estimated to have evolved multiple times. Branch lengths are proportional to estimated divergence times. Data for this figure were visualized using the R package GGTREE (Yu *et al.*, 2017).

we recorded in metres) and precipitation seasonality (coefficient of variation). We square-root transformed precipitation to reduce negative skewness. We also calculated two indices representing climate: the aridity index of Emberger (1942, 1955), [precipitation (in millimetres) × 100]/[(T_{max} + T_{min})(T_{max} – T_{min})], which has subsequently been used in several other ecophysiological studies within and among species (e.g. Tieleman *et al.*, 2003; Oufiero *et al.*, 2011; Wishingrad & Thomson, 2020); and the desert index of McNab & Morrison (1963), T_{max}/precipitation (in centimetres), subsequently used by MacMillen & Garland (1989).

Before analysis, we $\log_{10}$-transformed linear measurements and scale counts, but not body shape indices, for individual specimens. We then aggregated climatic and morphological data by species, calculating a mean and a standard error for each trait of interest so that we could account for within-species variation (which includes measurement error) in our analyses. The Supporting Information (Files S3 and S4) provides the species means and standard errors and, because those files use two-character codes ('PDI codes') to identify species, Supporting Information (File S5) maps the PDI codes to species names. Ives *et al.* (2007) showed that when it is not accounted for, within-species variation can lead to bias and excessive uncertainty in parameter estimates. Several other studies have also emphasized the importance of accounting for within-species variation (Felsenstein, 2008; Freckleton, 2011; Hardy & Pavoine, 2012; Johnson *et al.*, 2014).

Several species in our sample were represented by a single adult individual (*Agkistrodon taylori*, *Bothrops barnetti*, *Crotalus ehecatl*, *Crotalus mictlantecuhtli*, *Crotalus simus*, *Crotalus triseriatus*, *Daboia siamensis*, *Ovophis convictus* and *Trimeresurus gramineus*), meaning that we could not calculate a standard error directly for these species. Therefore, we regressed standard error on sample size for each trait of interest and used the slope and intercept to calculate the expected standard error for $N = 1$. In general, species with larger sample sizes have lower standard errors for a given trait. Accordingly, our approach for dealing with singleton species assigns relatively high errors to those species, accounting for our uncertainty in the true trait means for those species. Methods accounting for within-species variation give lower weight to species with greater errors. Thus, our method appropriately gives less weight to the species for which we measured only one individual. Additionally, for some species, all specimens were collected from the same locality and therefore had the same GPS coordinates. As a result, they had a standard error of zero for climate variables, which is clearly inappropriate. Therefore, we replaced those zeros with the expected standard error for $N = 1$.

As a result of their shared evolutionary history, species typically do not represent statistically independent samples (Felsenstein, 1985; Harvey & Pagel, 1991; Rezende & Diniz-Filho, 2012). Therefore, we conducted phylogenetic regressions to explore whether morphological traits relate to sidewinding, arboreality and climate. We used a type of Ornstein–Uhlenbeck model of trait evolution that incorporates intraspecific variation, using the MATLAB program MEREGPHYSIGV2.M (Ives *et al.*, 2007; Johnson *et al.*, 2014). We chose MEREGPHYSIGV2 to conduct our analyses because it can handle measurement error in addition to multiple independent variables, including categorical variables, and it can perform simulations to provide confidence intervals for model coefficients. The program is available from T.G. upon request.

Predictors for SVL included sidewinding, arboreality and the square root of precipitation. For other traits, predictors included sidewinding, arboreality, the square root of precipitation and SVL. Although sidewinding species all occur in arid or semi-arid environments, the correlation between sidewinding and precipitation was only –0.52, which is low enough to eliminate concerns regarding multicollinearity (Slinker & Glantz, 1985). We chose the square root of precipitation over the other possible climate variables by model selection. MEREGPHYSIGV2 can handle four predictor variables; therefore, we compared models including sidewinding plus arboreality plus SVL plus one of our potential climate variables or the absolute value of latitude. In all cases, the model with precipitation had higher log-likelihood than the other options (for full model comparison, see Supporting Information, File S6). Therefore, we present the results of models including precipitation.

We used the partial regression coefficient and associated 95% confidence interval for SVL from simulations performed in MEREGPHYSIGV2 to examine scaling relationships. We corrected for multiple comparisons with adaptive False Discovery Rate (FDR), implemented in SAS Procedure MULTTEST. Based on analysis of the 59 P-values, those ≤ 0.02 would still be considered significant.

We used the phylogeny from the study by Alencar *et al.* (2016) as the basis of our analyses, adding 11 additional species: *Ovophis convictus*, *Ovophis makazayazaya*, *Cerrophidion wilsoni*, *Crotalus tlaloci*, *Crotalus ehecatl*, *Crotalus mictlantecuhtli*, *Crotalus stephensi*, *Crotalus pyrrhus*, *Sistrurus tergeminus*, *Daboia russelii* and *Causus maculatus* (for details on species placement, see Supporting Information, File S7). Figure 1 shows our final tree, and the Supporting Information (Files S8 and S9) provides the tree in dsc and nexus formats.

For comparison with previous studies of ecomorphological traits, we estimated the phylogenetic signal for each trait using the program PHYSIG_LL.m in MATLAB (Blomberg *et al.*, 2003), available from T.G. upon request. Before conducting

the analysis for phylogenetic signal, we corrected traits for body size according to the methods of Blomberg *et al.* (2003), by regressing log(trait) on log(SVL) using phylogenetic generalized least squares in REGRESSIONv2.M and using that slope to calculate log(trait/SVL^{slope}).

RESULTS

All traits showed statistically significant phylogenetic signal, with the *K* values for subcaudal scale count (1.221) and dorsal scale row count (1.262) being substantially larger than one (Table 1). Thus, related species tended to resemble each other, generally less than expected under purely Brownian motion evolution, but occasionally more so.

Tail length and mid-body width, height and girth scaled isometrically with SVL (i.e. slopes not significantly different from one for $\log_{10}$-transformed variables); however, neck width and all head measurements scaled with negative allometry (Table 2; Fig. 2). All three scale counts increased significantly with body size (Table 2; Fig. 2).

Snout–vent length did not vary in relationship to locomotor specialization or precipitation (Table 2).

Table 1. Phylogenetic signal of snout–vent length and size-corrected traits estimated using PHYSIG_LL in MATLAB (Blomberg *et al.*, 2003)

Trait	Expected MSE_0/MSE	Observed MSE_0/MSE	*K*	MSE	MSE_{star}	*P*-value	Maximum likelihood	Maximum $likelihood_{star}$
SVL, $\log_{10}$-transformed	2.117	1.375	0.650	0.0871	0.1189	< 0.001	0.650	–29.957
Tail length, size corrected	2.117	1.906	0.901	0.0604	0.1020	< 0.001	–0.838	–0.838
Neck width, size corrected	2.117	1.357	0.641	0.0295	0.0400	< 0.001	29.981	16.856
Head width, size corrected	2.117	1.345	0.635	0.0264	0.0355	< 0.001	34.725	22.024
Head length (ventral), size corrected	2.117	1.277	0.603	0.0182	0.0206	< 0.001	50.822	45.502
Head length (dorsal), size corrected	2.117	1.171	0.553	0.0123	0.0144	< 0.001	67.557	60.794
Mid-body width, size corrected	2.117	1.580	0.746	0.0411	0.0599	< 0.001	15.681	–0.511
Mid-body height, size corrected	2.117	1.195	0.628	0.0407	0.0487	< 0.001	16.105	8.432
Mid-body girth, size corrected	2.117	1.557	0.736	0.0325	0.0449	< 0.001	25.816	11.870
Ventral scale count, size corrected	2.117	1.563	0.739	0.0076	0.0118	< 0.001	88.564	69.431
Subcaudal scale count, size corrected	2.117	2.585	1.221	0.0566	0.1335	< 0.001	1.943	–34.947
Dorsal row count, size corrected	2.117	2.671	1.262	0.0104	0.0275	< 0.001	74.644	33.026
Body width/height, size corrected	2.117	1.124	0.531	0.0227	0.0255	< 0.001	41.198	36.268
Anterior tapering, size corrected	2.117	0.868	0.410	0.0121	0.0102	0.015	68.269	75.613
Posterior tapering, size corrected	2.117	1.279	0.604	0.0073	0.0087	< 0.001	89.856	82.315

We $\log_{10}$-transformed snout–vent length (SVL) before analysis. Following Blomberg *et al.* (2003), we corrected all other traits for body size by regressing the trait on SVL using phylogenetic generalized least squares (no branch length transformations), then using the estimated slope to calculate log(trait/SVL^{slope}). MSE_0/MSE represents the ratio of the mean squared error of the species data on a star phylogeny to the mean squared error of the species data calculated using the variance–covariance matrix from the phylogenetic tree. The expected MSE_0/MSE under Brownian motion depends on the size and shape of the phylogenetic tree. The *K* statistic (observed MSE_0/MSE divided by expected MSE_0/MSE) indicates the amount of phylogenetic signal. Values less than one indicate less phylogenetic signal than expected under Brownian motion evolution along the specified tree, whereas $K > 1$ indicates more than expected. Significance levels (*P*-values) are based on randomization tests as described by Blomberg *et al.* (2003), which test the null hypothesis of no phylogenetic signal.

Adjusting for variation in SVL and after correcting for multiple comparisons, sidewinding specialists did not differ significantly from non-sidewinding species for any measured trait (Table 2). Arboreal snakes, however, had relatively long tails, narrow mid-body width, a low body width-to-height ratio and a low posterior tapering index in comparison to other species. They also had high subcaudal scale counts, and this effect remained when we replaced SVL with tail length as a predictor variable in the model, indicating that arboreal vipers have high subcaudal counts even relative to their tail length (results not shown). Ventral scale counts did not differ between arboreal and terrestrial species. Precipitation was positively related to head width, but negatively related to anterior tapering index (Table 2).

DISCUSSION

Phylogenetic signal

All traits showed statistically significant phylogenetic signal (Table 1). Values for the K statistic ranged from 0.410 to 1.262, with a mean of 0.724. All of these values are within the range reported by blomberg *et al.* (2003) for morphological traits, and the mean is similar to their value of 0.71.

Allometry and scaling

All head measurements scaled with negative allometry, in agreement with a previous study that reported head length allometry in 30 snake species from four families (Phillips & Shine, 2006). Allometric scaling of head dimensions could have functional ramifications for prey ingestion, because snakes are gape-limited predators whose ability to ingest large prey increases with relative head size (Pough & Groves, 1983; Forsman & Lindell, 1993). Negative interspecific allometry in head dimensions could allow smaller species to eat disproportionately large prey for their body size. However, macrostomatan snakes have achieved their prodigious gape through complex morphological changes, and several traits can contribute to differences in gape size among species, including the properties of multiple skeletal elements in addition to soft tissues (Gans, 1961; Arnold, 1983; Cundall & Irish, 2008; Hampton & Moon, 2013). Therefore, although head size allometry might have implications for gape size allometry, the two probably do not correspond exactly.

Geometric similarity predicts that any linear scale dimension should vary in direct proportion to body length. If the scales are the same size relative to body size, then the number of scales should not vary with body size (i.e. geometric similarity equates to an allometric slope of zero for scale counts). However, one would not expect geometric similarity for ventral or subcaudal scale counts in snakes because they correspond 1:1 with the vertebrae in vipers (Charas, 1669; Tyson, 1682; Alexander & Gans, 1966), and several studies have shown that larger snake species tend to have more vertebrae (Lindell, 1994; Head & Polly, 2007; Lee *et al.*, 2016). We found that all three scale counts increased with body size, meaning that they show significant positive allometry, i.e. slopes greater than zero (Table 2; Fig. 2). We are not aware of any previous studies that examine allometry of dorsal scale row counts in snakes. Opposite to our findings for snakes, Oufiero *et al.* (2011) found negative allometry for dorsal scale row count in *Sceloporus* lizards.

The adaptive significance of positive allometry in scale counts, if any, remains obscure. This allometry could result from developmental differences among species that do not necessarily reflect selection on the number of body segments per se. Ventral and subcaudal scales with their corresponding vertebrae arise from the segmentation of embryos into somites (Richardson *et al.*, 1998). The number and size of somites correspond to the rate of axial growth (Tam, 1981). Thus, any change in axial growth rates, which are themselves likely often to be under selection, could lead to changes in the numbers of scales, vertebrae and associated muscle segments.

No apparent morphological adaptations in sidewinding vipers

We did not find evidence of morphological specialization in sidewinding vipers. This apparent lack of morphological differentiation between sidewinding specialists and terrestrial generalists might be explained in various ways, which are not mutually exclusive. First, our analyses might have lacked statistical power. This seems unlikely, given that our dataset contains five independent origins of sidewinding specialization and only four independent origins of specialization for arboreality, yet we detected several morphological differences between arboreal and terrestrial vipers. Second, the viper body plan might be pre-adapted (*sensu* Futuyma & Kirkpatrick, 2017) for sidewinding, in which case their morphology need not change (much) after the sidewinding behaviour evolves. For example, Gray (1968: pp. 180–181) observed that sidewinding species might tend to have stout, flattened bodies and large, wide heads, but these traits generally characterize the family Viperidae (Pough & Groves, 1983; Feldman & Meiri, 2013). Although some non-vipers can approximate sidewinding in certain conditions, the highly specialized sidewinders are all vipers (Tingle, 2020). Third, body shape might not affect sidewinding

Table 2. Phylogenetic regression models accounting for measurement error (within-species variation; Johnson *et al.*, 2014)

Parameter	Sidewinding					Arboreality					sqrt(Precipitation)					log(SVL)				
	$B_{estimate}$	B_{lower}	B_{mean}	B_{upper}	*P*-value	$B_{estimate}$	B_{lower}	B_{mean}	B_{upper}	*P*-value	$B_{estimate}$	B_{lower}	B_{mean}	B_{upper}	*P*-value	$B_{estimate}$	B_{lower}	B_{mean}	B_{upper}	*P*-value
Snout–vent length, $d = 0.698$	−0.071	−0.183	−0.070	0.041	0.216	−0.106	−0.228	−0.105	0.015	0.083	0.079	−0.004	0.078	0.159	0.065	–	–	–	–	–
Tail length, $d = 0.656$	0.003	−0.090	0.002	0.095	0.965	**0.156**	**0.050**	**0.154**	**0.256**	**0.005**	**0.116**	**0.070**	**0.116**	**0.161**	**< 0.001**	1.049	1.005	1.049	1.092	0.027*†
Neck width, $d = 1.906$	0.001	−0.069	0.000	0.070	1.000	−0.088	−0.185	−0.087	0.018	0.104	0.000	−0.032	0.000	0.030	0.995	**0.880**	**0.849**	**0.880**	**0.911**	**< 0.001**†
Head width, $d = 1.198$	0.050	−0.017	0.050	0.119	0.149	0.003	−0.079	0.004	0.088	0.939	**0.048**	**0.017**	**0.049**	**0.079**	**0.005**	**0.835**	**0.804**	**0.835**	**0.865**	**< 0.001**†
Head length (ventral), $d = 1.016$	0.004	−0.056	0.005	0.062	0.824	−0.020	−0.088	−0.020	0.045	0.564	0.009	−0.025	0.009	0.040	0.512	**0.800**	**0.766**	**0.800**	**0.834**	**0.001**†
Head length (dorsal), $d = 1.037$	0.001	−0.045	0.000	0.045	0.989	−0.001	−0.057	−0.001	0.053	0.978	0.021	0.003	0.021	0.041	0.028*	**0.784**	**0.763**	**0.784**	**0.804**	**< 0.001**†
Mid-body width, $d = 1.292$	0.027	−0.054	0.028	0.109	0.496	**−0.156**	**−0.258**	**−0.156**	**−0.054**	**0.004**	−0.016	−0.060	−0.015	0.027	0.514	0.991	0.949	0.991	1.034	0.650†
Mid-body height, $d = 1.410$	0.025	−0.064	0.024	0.114	0.608	−0.054	−0.171	−0.056	0.061	0.341	−0.007	−0.050	−0.007	0.038	0.726	1.006	0.962	1.006	1.049	0.786†
Mid-body girth, $d = 1.395$	0.028	−0.054	0.027	0.102	0.468	−0.087	−0.188	−0.088	0.016	0.097	−0.018	−0.053	−0.018	0.017	0.321	1.030	0.994	1.030	1.066	0.096†
Ventral scale count, $d = 1.781$	−0.014	−0.050	−0.014	0.024	0.460	0.026	−0.029	0.026	0.083	0.353	−0.005	−0.028	−0.005	0.017	0.608	**0.259**	**0.240**	**0.259**	**0.279**	**< 0.001**
Subcaudal scale count, $d = 1.282$	−0.039	−0.133	−0.039	0.050	0.398	**0.190**	**0.069**	**0.188**	**0.307**	**0.001**	0.013	−0.031	0.014	0.060	0.563	**0.239**	**0.193**	**0.239**	**0.285**	**< 0.001**
Dorsal row count, $d = 2$	−0.030	−0.073	−0.029	0.014	0.180	0.012	−0.051	0.014	0.080	0.698	−0.012	−0.027	−0.012	0.002	0.689	**0.210**	**0.195**	**0.210**	**0.224**	**< 0.001**
Body width/height, $d = 0.604$	−0.045	−0.185	−0.043	0.094	0.539	**−0.212**	**−0.366**	**−0.214**	**−0.069**	**0.006**	−0.061	−0.151	−0.061	0.031	0.181	0.039	−0.049	0.040	0.124	0.337
Anterior tapering, $d = 0.466$	0.065	−0.067	0.065	0.187	0.309	0.102	−0.036	0.104	0.249	0.157	**−0.096**	**−0.173**	**−0.096**	**−0.017**	**0.012**	**0.165**	**0.091**	**0.164**	**0.240**	**< 0.001**

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Table 2. Continued

Parameter	Sidewinding					Arboreality					sqrt(Precipitation)					log(SVL)				
	$B_{estimate}$	B_{lower}	B_{mean}	B_{upper}	*P*-value	$B_{estimate}$	B_{lower}	B_{mean}	B_{upper}	*P*-value	$B_{estimate}$	B_{lower}	B_{mean}	B_{upper}	*P*-value	$B_{estimate}$	B_{lower}	B_{mean}	B_{upper}	*P*-value
Posterior tapering, $d = 0.625$	−0.026	−0.113	−0.026	0.061	0.571	**−0.146**	**−0.250**	**−0.144**	**−0.038**	**0.005**	0.028	−0.020	0.029	0.077	0.235	**−0.110**	**−0.156**	**−0.109**	**−0.061**	**< 0.001**

Body measurements, scale counts and body shape indices were analysed in relationship to sidewinding locomotion (dummy variable, in which one indicates sidewinding), arboreality (dummy variable, in which one indicates arboreal) and the square root of precipitation [sqrt(Precipitation)]. We also included the $\log_{10}$ of snout–vent length (SVL) as a predictor variable for all other measurements and scale counts. Before conducting the analysis, we $\log_{10}$-transformed measurements and scale counts for individual specimens, then computed means and standard errors for each species. Estimated effects are partial regression coefficients ($B_{estimate}$) along with their 95% confidence intervals and *P*-values based on parametric bootstrapping (B_{lower} and B_{upper}). B_{mean} is the mean coefficient for the simulated datasets) (Johnson *et al.*, 2014). The parameter *d* represents the Ornstein–Uhlenbeck branch length transformation: a value of one indicates the original branch lengths; a value of zero indicates a star phylogeny; values between zero and one indicate a tree that is less hierarchical than the original tree; and values greater than one indicated a more hierarchical tree than the original (by a more hierarchical tree, we mean one in which the internal nodes are nodes pushed up towards tips and away from the root). Bold text indicates cases where sidewinding, arboreality or precipitation showed a significant relationship with a given morphological trait or when a trait scaled with allometry rather than isometry.

†For linear measurements, we used one rather than zero as the null expectation under isometry.

*Values of $P > 0.02$ were non-significant after correction for multiple comparisons by the adaptive FDR procedure.

locomotion. Finally, specialization for sidewinding might have evolved recently enough that the morphology has not yet caught up to the behaviour. Comparative evidence is consistent with the idea that behavioural traits might generally evolve more rapidly than do morphological traits (Blomberg *et al.*, 2003). Thus, organisms often evolve increased expression of a behaviour or even new behaviours with little, if any, accompanying change in overt morphology, which causes an ecomorphological mismatch until the morphology evolves to support the altered behaviour better (Lister, 2014; Diogo, 2017).

Apparent morphological adaptations to arboreality

Arboreal vipers in our study had relatively long tails (Table 2; Fig. 2), consistent with a previous study on arboreal vipers (Alencar *et al.*, 2017). Long tails characterize arboreal snakes in many clades (Vitt & Vangilder, 1983; Martins *et al.*, 2001; Pizzatto *et al.*, 2007a, b; Alencar, 2010; Lawing *et al.*, 2012; Sheehy *et al.*, 2016). When they cross gaps, snakes often resist the torque that they experience at the edge of the perch by putting part of their body or tail underneath the perch or even by wrapping the tail around it (Jayne & Riley, 2007; Byrnes & Jayne, 2012). Juveniles of one arboreal viper (*Trimeresurus albolabris*) can perform defensive strikes > 50% of their body length, holding onto branches with their prehensile tails (Herrel *et al.*, 2011). Thus, the tail can play an important role in preventing snakes from pitching downwards during cantilevering manoeuvres and strikes by providing a counterweight for the body and/or by grasping a perch in the case of prehensile tails. In cases where the tail acts as a counterweight, longer tails are presumably more effective than shorter ones because they presumably have greater total mass and because they increase the length of the moment arm for the counter-torque that prevents the body from pitching downwards. Another plausible explanation for long tails in arboreal snakes relates to non-locomotor demands of arboreality. Sheehy *et al.* (2016) suggested that a relatively long tail, which is composed of stiffer tissues than the body and therefore prevents blood pooling better, could help to offset cardiovascular stress resulting from gravity when the snake is climbing in an upright position.

Arboreal vipers had significantly lower mid-body width than did terrestrial generalists (Table 2; Fig. 2). This result matches the findings of previous studies, including one study on vipers and several on other snake clades, that found higher elongation ratios (length divided by width) (Vitt & Vangilder, 1983; Martins *et al.*, 2001; Pizzatto *et al.*, 2007a; Alencar *et al.*, 2017) or lower body mass relative to length (Guyer &

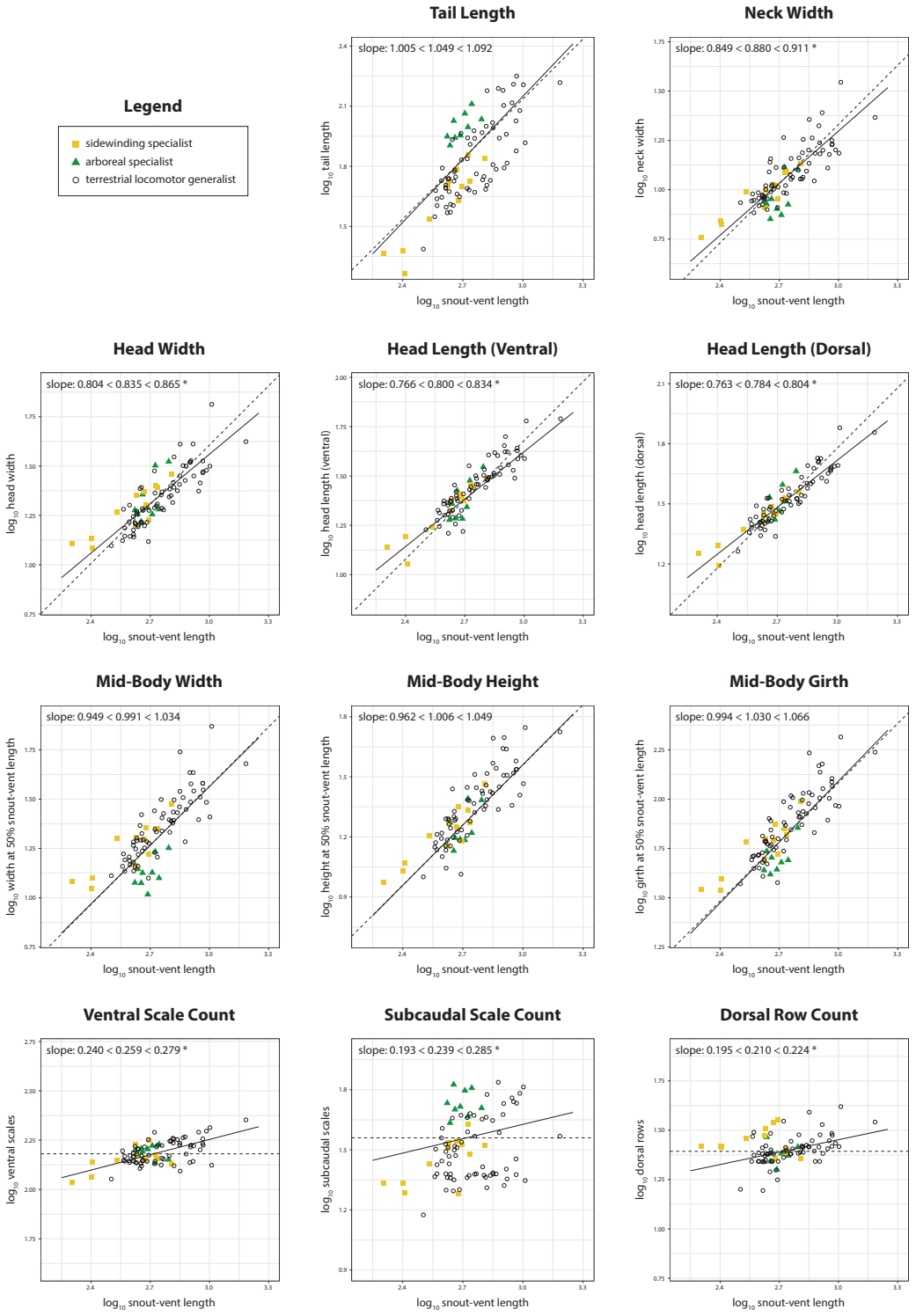

Figure 2. Scaling and effects of locomotor and habitat specialization. Linear measurements were recorded in millimetres, and all traits and were $\log_{10}$-transformed before the analysis. Dotted black lines have a slope equal to isometry (one for linear measurements and zero for scale counts) and go through the mean value of (x,y). Continuous black line segments have a slope equal to the estimated partial regression coefficient for the snout–vent length from the models shown in Table 2 and pass through the phylogenetic mean of (x,y), computed in the MATLAB program REGRESSIONv2.M (Lavin *et al.*, 2008). The estimated slopes along with 95% confidence intervals (from parametric bootstrapping in the MATLAB

Donnelly, 1990; Feldman & Meiri, 2013) in arboreal species. Slender bodies might aid arboreal snakes in several ways, probably leading to strong selection. Gravity acting on the mass of unsupported parts of the body causes torque, which can lead to downward bending (Byrnes & Jayne, 2012; Hoefer & Jayne, 2013). Thus, we expect that more massive snakes will experience greater torque while cantilevering across gaps (shown experimentally by Hoefer & Jayne, 2013), which might limit them to crossing relatively shorter gaps than a more slender snake of a similar size might cross. Juvenile rat snakes (*Pantherophis obsoletus*), which have lower body mass relative to their body length than adults, had significantly higher cantilever performance in one experiment (Lillywhite *et al.*, 2000). We expect that if the slenderer viper species in our study were to be tested in a similar experiment, they would show better cantilevering performance than the stockier species.

An alternative explanation for why arboreal snakes have more slender bodies hinges on the idea that stockier snakes of a given length might have a disadvantage not in gap-bridging performance, but in the diameter of branches that will support their weight without bending (Lillywhite & Henderson, 1993). Branches of very small diameters predominate in some arboreal habitats (Mattingly & Jayne, 2004), and the greater tendency for these narrower branches to bend under applied weight has consequences for locomotion (Byrnes & Jayne, 2010). Slender bodies are likely to allow arboreal snakes to move effectively along branches with smaller diameters than heavy snakes would be able to negotiate without excessive bending or breaking (Lillywhite & Henderson, 1993). This advantage should persist whether the snakes crawl along a single narrow twig or whether they distribute their weight over many narrow twigs. Hoefer & Jayne (2013) pointed out that brown tree snakes (*Boiga irregularis*) in Guam were repeatedly found with their weight supported by tiny stems measuring only 2–3 mm in diameter, despite the relatively large size of the snakes considered in terms of body length. A slender form could also improve crypsis in environments where slender branches predominate (Lillywhite & Henderson, 1993). The rough green snake (*Opheodrys aestivus*) spends most of its time on branches well under 1 cm in diameter (Goldsmith, 1984), and two arboreal snakes of the genus *Uromacer* use branches of ≤ 1 cm in diameter, with the smaller species using branches averaging only 5 mm across (Henderson *et al.*, 1981).

Lateral compression characterizes arboreal species in several snake clades (Pizzatto *et al.*, 2007a, b). Our study is the first to show evidence for lateral compression in arboreal vipers (Table 2; Fig. 3). Some species can also actively compress themselves laterally during climbing; Lillywhite *et al.* (2000) observed this phenomenon in some boas. Jayne *et al.* (2015) also commented on this ability of snakes to change their cross-sectional shape. External lateral compression can relate to vertebral differences in arboreal species, such as the higher length-to-width ratio of the vertebrae (Lawing *et al.*, 2012) and the lesser lateral projection of the prezygapophyses (Johnson, 1955). Additionally, some species, especially among the boas and vipers, have this body shape because the ribs are directed markedly downward (Johnson, 1955). Lillywhite & Henderson (1993) suggested that lateral compression would increase the ability of arboreal snakes to grasp certain objects 'due to the increased contact area for static friction'; however, this hypothesis assumes that the snakes wrap laterally around objects and that increased body surface area would improve grasping ability. We could find no supporting evidence for either assumption in the literature. If we consider the body of a snake projecting from a branch across a gap as a beam attached at one end to a wall, we can explain lateral compression with Euler–Bernoulli beam theory. This theory describes the vertical deflection of a beam, which depends in part on the material properties and cross-sectional shape of the beam. A laterally compressed cross-section leads to a stiffer beam and therefore reduces bending, which is a desirable outcome for a snake that needs to cross a gap. This explanation relies on the assumption that snakes behave in a reasonably similar manner to beams, an assumption that we have not tested.

We found that arboreal viper species do not taper more in the front of the body than terrestrial species do, but they taper significantly less posteriorly, with some arboreal species having wider measurements towards the back of the body than at mid-body (Table 2; Fig. 3). Thus, arboreal species have generally shifted their centre of mass towards the back of the body. Apparently, ours is the first study to test for such a morphological configuration in snakes. Cadle & Greene (1993) listed 'center of gravity shifted posteriorly' as a characteristic of arboreal snakes, and Peters (1960) observed several modifications for arboreality, including an 'abrupt narrowing of the body immediately posterior to the head' in snakes of the subfamily Dipsadinae. However, neither these

program MEREGPHYSIGV2.M; Johnson *et al.*, 2014) are labelled on the plots. All traits that depart significantly from isometry after correcting for multiple comparisons are indicated with an asterisk. Sidewinding specialists do not differ from non-sidewinding species, but arboreal specialists differ from terrestrial species for several traits (Table 2).

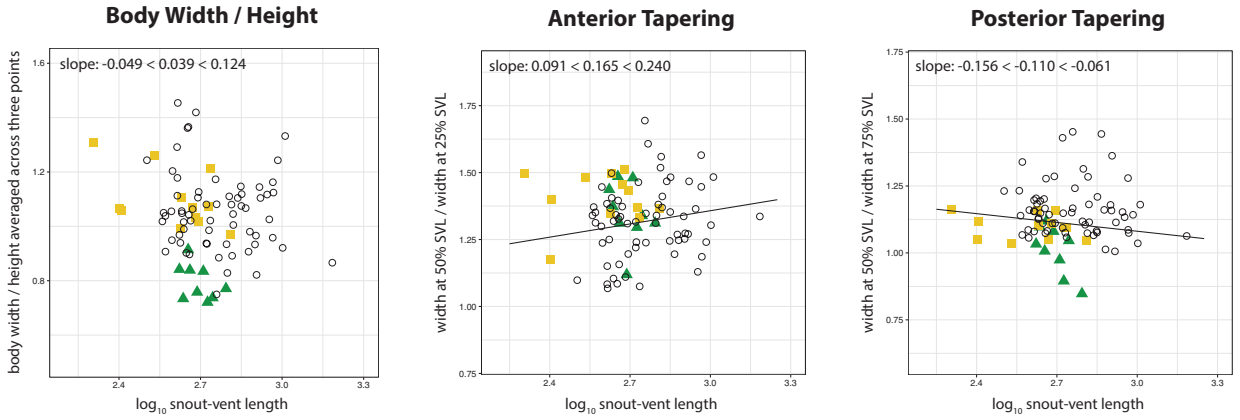

Figure 3. Variation of body shape indices with respect to locomotor and habitat specialization. These body shape indices are all ratios calculated from raw measurements, and they are not $\log_{10}$-transformed. As in Figures 1 and 2, yellow squares indicate sidewinding specialists, green triangles arboreal specialists and black circles terrestrial locomotor generalists. Continuous black line segments are shown for the two indices that show a significant correlation with snout–vent length (SVL). These line segments have a slope equal to the estimated partial regression coefficient for SVL from the models shown in Table 2 and pass through the phylogenetic mean of (x,y), computed in the MATLAB program REGRESSIONv2.M (Lavin *et al.*, 2008). The estimated slopes along with 95% confidence intervals (from parametric bootstrapping in the MATLAB program MEREGPHYSIGv2.M; Johnson *et al.*, 2014) are labelled on the plots. Sidewinding specialists do not differ from terrestrial generalists for any body shape indices. Arboreal specialists have a lower body width-to-height ratio than terrestrial generalists, indicating a laterally compressed shape in cross-section, and they show less tapering towards the back of their bodies.

authors nor the references they cite provide empirical evidence for these observations. Therefore, it is unclear whether a more posterior centre of gravity characterizes arboreal snakes in other clades and, if so, whether other arboreal snakes achieve this shift via increased tapering of the anterior body or decreased tapering of the posterior body. Intuitively, a caudally shifted centre of mass would benefit arboreal snakes as they extend the front part of their bodies unsupported across gaps; therefore, we would expect to see similar tapering patterns in other clades.

We did not find evidence for increased numbers of body vertebrae in arboreal vipers, but we did find increased numbers of tail vertebrae, even when controlling for their relatively long tails. Previous studies have shown mixed results with respect to the relationship between arboreality and number of body vertebrae. Jayne (1982) found that arboreal snakes had relatively more body vertebrae, whereas Lindell (1994) found no difference between arboreal and terrestrial species. Hampton (2011) found that arboreal vipers had more total vertebrae (body plus tail vertebrae) relative to their total body length, but it is unclear whether this trend resulted from increases in the number of both body and tail vertebrae or whether higher tail vertebrae alone might have driven this trend. A previous study showed that vipers with prehensile tails have more tail vertebrae, and all arboreal viper clades have evolved prehensile tails (Hampton, 2011).

Relationship between precipitation and morphology

Viper species from locations with higher precipitation had longer tails. One previous study found evidence for longer tails in snake species that live in leaf litter (Guyer & Donnelly, 1990). In principle, the leaf-litter niche should be tied to precipitation; low-precipitation biomes, such as deserts and grasslands, do not have leaf litter, whereas high-precipitation biomes, such as temperate deciduous forests and rainforests, do. Unfortunately, we do not have information on substrate use for most of the species in our sample, and the potential function of long tails in leaf-litter species remains untested. One testable hypothesis relates to defensive signalling. Many snake species vibrate the tail when disturbed (Greene, 1988), which generates noise most effectively in dry plant matter and might therefore be more beneficial to leaf-litter species than to species dwelling on other substrates, such as rocks or sand. Additionally, several viper species use their tails

to lure prey (Heatwole & Davison, 1976), and a few species can even constrict prey with the tail (Greene, 1977; Murphy, 1977). Future studies could investigate whether these behaviours are more common in some habitats than in others.

An alternative explanation involves the effect of environmental variation on developing embryos. Some studies have shown that temperature during gestation or incubation can affect the number of body vertebrae and other phenotypic traits in snakes (e.g. Fox, 1948; Osgood, 1978; Lourdais *et al.*, 2004), although Arnold & Peterson (2002) found that manipulating temperature during gestation of the garter snake *Thamnophis elegans* led to differences in the number of scale abnormalities but not in the number of scales. Thus, direct environmental effects on development are possible. However, the present study does not take a common garden approach (i.e. all animals raised in common conditions), as is true for all broad-based comparative studies (cf. Garland & Adolph, 1991, 1994; Rezende & Diniz-Filho, 2012); therefore, it is not possible to infer whether developmental effects might be involved in the relationship between tail length and precipitation.

In addition to longer tails, vipers from wetter environments also had significantly wider heads. Given that larger heads enable snakes to swallow larger prey (Pough & Groves, 1983; Shine, 1991; Forsman & Lindell, 1993), we speculate that differences in head width in wetter vs. drier habitats reflect differences in prey availability. One study on a Mediterranean viper species found that individuals from drier environments include a higher proportion of lizards in their diet, whereas those from wetter environments mainly consume mammals (Santos *et al.*, 2008), a pattern that corresponds to an increased abundance of small mammals in the wetter parts of the region (Barbosa & Benzal, 1996). In general, even small mammals are larger in body size than lizards (e.g. Pough, 1980). Interspecific dietary differences might track climate-related patterns of prey relative abundance in a similar manner, which might lead to selection on head morphology in gape-limited predators, such as snakes. Previous studies examining head size and prey type in snakes have provided mixed results. In pit vipers of the genus *Bothrops*, juveniles, but not adults, have significantly larger heads in species specializing on mammals (Martins *et al.*, 2002). Results of one study suggest that boids specializing on mammals might have longer heads, in comparison to generalists (Pizzatto *et al.*, 2007b). As discussed above, external head dimensions do not provide a full picture of gape size, because many traits contribute to gape (Gans, 1961; Arnold, 1983; Cundall & Irish, 2008; Hampton & Moon, 2013). Detailed studies of the skull and soft tissue anatomy, in addition to functional studies, could therefore provide further insight into the relationships between environmental characteristics, diet and swallowing performance.

Conclusions and future directions

Although vipers have superficially simple morphology, they have evolved along several axes with respect to ecology and behaviour. Here, we show that several body shape and scalation traits vary in relationship to specialization for arboreal habits and/or precipitation. Contrary to our expectations, sidewinding species did not exhibit statistically significant specialization in body shape or the number of vertebrae. Given that many viper species, including some close relatives of sidewinding specialists, do not sidewind even under duress on low-traction surfaces (Marvi *et al.*, 2014), some factor is likely to prevent them from expressing this behaviour. If external body characteristics do not enable or prevent sidewinding, perhaps it relies on particular configurations of the underlying musculoskeletal system, such as reduced spinalis muscle lengths (Tingle *et al.*, 2017), or aspects of motor control. The statistically supported differences between arboreal and non-arboreal vipers, mostly consistent with patterns found in other snake clades, suggest that we have chosen functionally relevant measurements and that these cases provide examples of coadaptation (correlated responses to multivariate natural selection) between behaviour/habitat selection and morphology.

The present study provides several leads for future work on adaptive ecomorphological variation in snakes. In particular, functional studies could test various hypotheses regarding the reasons for those patterns. For example, do slender bodies aid crypsis in arboreal snakes? Do long tails improve climbing or cantilever performance? What prevents some species from sidewinding proficiently? Previous studies have provided insight into the relationships between habitat, morphology, functional performance and diversification in various clades (for some examples, see: Gomes *et al.*, 2009; Price *et al.*, 2011, 2012; Crumière *et al.*, 2016; Alencar *et al.*, 2017; Ceccarelli *et al.*, 2019; Yuan *et al.*, 2019). Given that snakes move in fundamentally different ways from aquatic vertebrates or limbed terrestrial vertebrates, answers to outstanding questions about snake locomotion would improve our general understanding of the relationships between locomotor adaptation and functional diversification.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

File S1. Raw measurement data, including museum catalogue numbers, for all specimens.
File S2. Details on differences between species identities given in museum catalogues vs. those used in our study.
File S3. Data file containing species means.
File S4. Data file containing species standard errors.
File S5. File mapping species names to the two-character codes ('PDI codes') used in Files S3 and S4.
File S6. Model comparison for climate variables.
File S7. Details on placement of species that we added to the phylogeny from the study by Alencar (2016).
File S8. Phylogeny used for analyses in dsc format.
File S9. Phylogeny used for analyses in nexus format.