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Morphological integration and adaptation in the snake feeding system: a comparative phylogenetic study

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Abstract

A long-standing hypothesis for the adaptive radiation of macrostomatan snakes is that their enlarged gape – compared to both lizards and basal snakes- enables them to consume 'large' prey. At first glance, this hypothesis seems plausible, or even likely, given the wealth of studies showing a tight match between maximum consumed prey mass and head size in snakes. However, this hypothesis has never been tested within a comparative framework. We address this issue here by testing this hypothesis in 12 monophyletic clades of macrostomatan snakes using recently published phylogenies, published maximum consumed prey mass data and morphological measurements taken from a large sample of museum specimens. Our nonphylogenetically corrected analysis shows that head width – independent of body size – is significantly related to mean maximum consumed prey mass among these clades, and this relationship becomes even more significant when phylogeny is taken into account. Therefore, these data do support the hypothesis that head shape is adapted to prey size in snakes. Additionally, we calculated a phylogenetically corrected morphological variance–covariance matrix to examine the role of morphological integration during head shape evolution in snakes. This matrix shows that head width strongly covaries with both jaw length and out-lever length of the lower jaw. As a result, selection on head width will likely be associated with concomitant changes in jaw length and lower jaw out-lever length in snakes.

Introduction

The origin, maintenance and diversification of organismal form and function have been attributed primarily to adaptation to the external environment by natural selection (Williams, 1966; Endler, 1986). Yet, recent authors have argued cogently that evolutionary and functional biologists must also consider phenotypic integration of genetically or functionally integrated units when attempting to understand how organismal form evolves (Wagner & Schwenk, 2000; Pigliucci, 2003). According to Pigliucci (2003), phenotypic integration can be defined broadly as 'the pattern of functional, developmental and/or genetic correlation among different traits in a given organism,' and can act to either enhance the adaptive process or constrain future evolution (see Wagner & Schwenk, 2000 for an overview). Wagner, for example, posited that the higher the degree of functional or genetic covariation (i.e. integration) among traits, the greater the possibility trade-offs may occur between external and internal selection (Wagner, 1995; Wagner & Altenberg, 1996). Furthermore, recent authors have called repeatedly for studies assessing the roles of both

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external selection to the environment and internal selection for maintaining coherence among functionally integrated units within an explicit phylogenetic context (Wagner & Schwenk 2000; Pigliucci, 2003). In this way, evolutionary biologists can more clearly understand the interplay among adaptation to the external environment, phenotypic integration and 'phylogenetic effects' (similarity due to common ancestry) and their cumulative influence on organismal form. Unfortunately, few studies to date have answered this call (but see Ackermann & Cheverud, 2000; Hulsey & Wainwright, 2002).

The snake feeding apparatus is an ideal system to address the roles of adaptation to the external environment, phenotypic integration and common ancestry on organismal form due to the spectacular dietary diversity exhibited by snakes (Greene, 1997), as well as their highly specialized cranial morphologies (Cundall & Greene, 2000). Specifically, snakes are known to consume a wide variety of prey types, including such morphologically and functionally diverse forms as ant larvae, centipedes, spiders, earthworms, slugs, snails, crabs, eggs, fish, frogs, mammals, birds, lizards, turtles and even other snakes (Greene, 1997). Moreover, the major extant radiations of snakes (i.e. Scolecophidia, Anilioidea and Macrostomata) have evolved cranial morphologies that differ markedly compared to both nonophidian squamates (i.e. 'lizards'), and to one another. Our understanding of the phylogenetic interrelationships of snakes has improved steadily in recent years (e.g. Tchernov *et al.*, 2000; Lee & Scanlon, 2002; Slowinski & Lawson, 2002; Vidal & Hedges, 2002a,b,2004; Wilcox *et al.*, 2002; Kelly *et al.*, 2003; Townsend *et al.*, 2004; Vidal & David, 2004), now enabling researchers to study the relationships between morphology and diet within a more explicit phylogenetic context (e.g. Alfaro & Arnold, 2001; Martins *et al.*, 2002).

The majority of research on the snake feeding apparatus has been stimulated by the hypothesis that adaptations to ingest large prey have played a central role in the evolutionary and ecological success of snakes (Gans, 1961; Greene, 1997; Cundall & Greene, 2000). Certainly, the most species rich and phenotypically diverse snakes belong to a single clade, Macrostomata (enlarged gape snakes), named so for their ability to consume prey that are markedly larger than their own head (Gans, 1961; Greene, 1983; Rodriguez-Robles *et al.*, 1999). Previous authors have therefore hypothesized that the skeletal elements leading to this increased gape size should co-evolve as an integrated functional unit in tandem with maximum prey size (see Cundall & Greene, 2000 for an overview). However, no studies to date have tested whether head dimensions co-vary with one another in multivariate morphological space or with maximum prey size after taking phylogeny into account within snakes.

In this study, we examined the influences of both morphological integration and maximum consumed prey mass on the evolution of the macrostomatan feeding apparatus, taking common ancestry into account. We examined these two issues by generating standardized independent contrasts for mean head and body dimensions and mean maximum consumed prey size for 12 monophyletic clades of macrostomatan snakes. These data were subsequently used to calculate a phylogenetically corrected and size-adjusted morphological variance–covariance matrix to test which residual head dimensions co-vary in multivariate space. Second, we used these data to test, which head dimensions most strongly predict consumed prey mass using stepwise regression. These data provide the first phylogenetic test of the hypothesis that morphological divergence within Macrostomata is functionally linked to maximum consumed prey size, as well as a quantitative estimate of the morphological covariance of head dimensions within snakes.

Methods

Subjects and morphological measurements

Sexual dimorphism in body size and head shape, and ontogenetic variation in head dimensions are well-documented phenomena in snakes (e.g. Arnold, 1993; Shine, 1994). Therefore, we only considered adult males in this study. Gender was determined either by hemipenial probing (Fitch, 1987), or for species in which this technique is unreliable we only used specimens with externally protruding hemipenes. To minimize the potential for Types I and II errors when comparing large numbers of species with small intraspecific sample sizes (Harmon & Losos, 2005), we attempted to measure as many specimens per species as possible. As a result, we measured a minimum of 3 and a maximum of 108 specimens per species (see Appendix S1 for individual species sample sizes) from a total of 12 major clades of macrostomatan snakes (Fig. 1). In total, we measured 1087 specimens from 201 species. The phylogenetic interrelationships of these species were estimated from recent phylogenetic analyses (e.g., Tchernov *et al.*, 2000: Alfaro & Arnold, 2001; Lee & Scanlon, 2002; Slowinski & Lawson, 2002; Kelly *et al.*, 2003; Townsend *et al.*, 2004). To minimize geographical differences in head shape, specimens from the same geographic areas were used when possible.

We recorded the following morphological measurements (in mm) for each specimen: snout-vent length (SVL), maximum head width, maximum head height, head length (measured from the posterior edge of the parietal scale to the tip of the snout; for species lacking a parietal scale, the posterior edge of the skull was detected by firmly pressing the tip of the caliper against the top of the head in order to locate the caudal border of the exoccipitals), jaw length (straight-line distance from the retroarticular process to the anterior tip of the dentary) and the out-lever of the lower jaw (straight-line distance

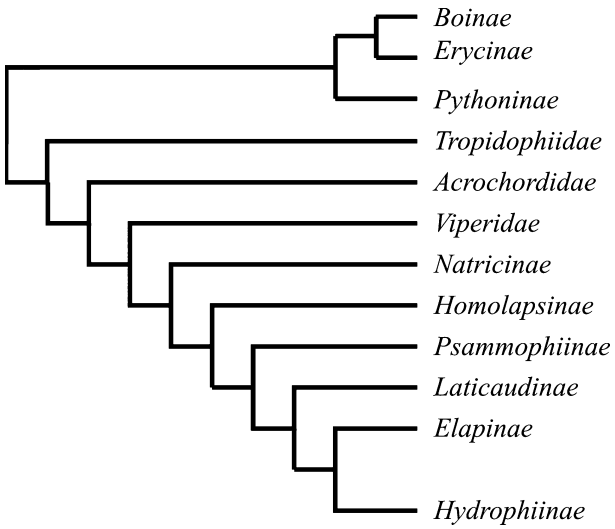

Fig. 1 Phylogenetic relationships among the snake clades analysed in this study. This is a composite phylogeny generated from the published phylogenies of Lee & Scanlon (2002), Slowinski & Lawson (2002), Kelly *et al.* (2003) and Townsend *et al.* (2004).

from the quadratomandibular joint to the anterior tip of the dentary). We determined SVL by laying dental floss along the mid-ventral surface of the snake and then measuring the floss using a meter stick. All head measurements were taken using Mitutoyo digital calipers (± 0.1 mm). Head volume was estimated as the volume of water (mL) displaced in a graduated cylinder by a snake's submerged head. To ensure that this measurement was comparable among individuals, the posterior-most supralabial scale was used as a stopping point for submersion.

We recorded these particular six head measurements because they have clear functional implications during feeding. Specifically, head volume is a measure of overall head size in snakes, which takes into account all of the cranial muscle masses as well as the masses of the skull bones. Therefore, one may predict that larger muscles are needed to walk a heavier skull over prey. By contrast, head length, head width, head height, jaw length and lower jaw out-lever length are all believed to contribute to maximum gape size in snakes (Frazzetta, 1966; Vincent *et al.*, 2004). Head width is also associated with the space available for food passage through the buccal cavity, and the length of the out-lever of the lower jaw is presumably related to both jaw opening/closing velocity and maximum gape size.

Dietary data

To test whether these morphological variables and maximum consumed prey size are significantly related after taking into account phylogenetic history, we surveyed the literature to record data on maximum prey size (mass) for males for each of the 12 clades studied. We used maximum prey mass instead of average prey mass because gape-limitation in snakes only constrains the upper size range of prey that can be successfully ingested (see Gans, 1961; Arnold, 1993; Forsman & Lindell, 1993). Therefore, previous authors have hypothesized that maximum prey mass was the primary selective force driving head size/shape evolution within snakes (Gans, 1961; Forsman & Lindell, 1993). We chose prey mass (g) instead of prey circumference as our estimate of 'prey size' because mass is the most widely reported measure in snake dietary studies (see Arnold, 1993). Unfortunately, we were not able to exactly match the species or sexes sampled for the morphological and dietary data sets. Therefore, we attempted to sample a wide range of maximum consumed prey sizes within each clade; hence, our morphological and dietary data sets represent estimates of both the mean maximum consumed prey mass and morphological dimensions per clade. However, we were forced to estimate maximum consumed prey mass for one species of Tropidophiidae (*Tropodophis melanurus*), based on the average body masses of the heaviest prey item in the diet of this species (birds; Greene, 1983). This estimation was performed because the only other species in this clade with a reported maximum prey mass (*Trachyboa boulengeri*) consumes very small prey. As a result, using data for *T. boulengeri* we would likely underestimate the mean maximum consumed prey mass for this clade. Furthermore, we were only able to obtain relatively small bodied male specimens for most *Acrochordus* species. We therefore size-matched prey mass data for male *Acrochordus arafure* to the average body size of the specimens used in this study (Houston & Shine, 1993; Appendix S1). As our aim was not to exhaustively sample the dietary literature for each clade, but rather to estimate the mean maximum prey mass for each clade, species that consumed similar prey masses compared to species already sampled (within 0.5 g) were excluded from our sample (Table 1).

Additionally, please note that while these estimates of mean maximum consumed prey mass may be either over or underestimating this value for each clade (e.g. pythons), these results are robust to estimation errors. We tested the robustness of these data by incrementally increasing and decreasing the mean values for each mass data point by 5%, 20%, 25% and 50% and reran the multiple regressions (see below). Overall, we found that changing the mean value up to 25% altered the magnitude of the beta coefficients, but not the significant relationships between head variables and diet. Furthermore, most regressions (2/3) remained significant with even a 50% change in mean maximum prey mass for a given clade.

Statistical analysis

We used SPSS (Version 11.5; SPSS Inc., Chicago, IL, USA) on a PC computer for all statistical analyses. We

Table 1 Published maximum consumed prey mass for species within the 12 monophyletic clades sampled in this study. Species with highly similar max prey sizes (within 0.5 g) compared to other species already sampled were excluded. Because we only had access to small bodied specimens of *Acrochordus arafurae*, we used the maximum prey mass reported for similar SVL's reported in Houston & Shine (1993). Please note that while some species can consume larger prey than the values reported here, the significant morphological-dietary relationships found in this study are robust to even large errors in maximum consumed prey mass data (see Dietary data section).

Species	Max prey size (g)	Source
Acrochordidae		
Acrochrodus arafurae	3	Houston & Shine (1993)
Boinae		
Eunectes murinus	5700	Rivas & Owens (2000)
Epicrates sp.	210	Wiley (2003)
Charina bottae	6	Rodriguez-Robles *et al.* (1999)
Boa constrictor	1850	Quick *et al.* (2005)
Elapinae		
Dendroaspis polylepis	75	Branch *et al.* (1995)
Naja melanoleuca	168	Luiselli *et al.* (2002)
Austrelaps superbus	22.6	Shine (1977)
Hemiaspis signata	46.3	Shine (1977)
Notechis scutatus	37.7	Shine (1977)
Pseudechis porphyriacus	250	Shine (1977,1991)
Pseudonaja textiles	40.6	Shine (1977)
Erycinae		
Calabaria reinhardtii	33	Luiselli *et al.* (1998)
Homolapsiinae		
Bitia hydroides	0.4	Jayne *et al.* (1995)
Fordonia leucobalia	9	Voris & Murphy (2002) Karns *et al.* (2002)
Cantoria violacea	5.1	Voris & Murphy (2002) Karns *et al.* (2002)
Cerebus rynchops	8.9	Voris & Murphy (2002) and Karns *et al.* (2002)
Enhydris plumbea	5.85	Jayne *et al.* (1995)
Hydrophiinae		
Emydocephalus annulatus	0.00008	Shine *et al.* (2004)
Lapemis curtus	3	Lobo *et al.* (2005)
Enhydrina schistose	90	Voris & Moffet (1981)
Laticaudinae		
Laticauda semifasciata	131	Su *et al.* (2005)
Natricinae		
N. cyclopion	2.02	Mushinsky *et al.* (1982)
N. erythrogaster	7.89	Mushinsky *et al.* (1982)
N. fasciata	1.9	Mushinsky *et al.* (1982)
N. rhombifer	3.84	Mushinsky *et al.* (1982)
Pythoninae		
Moreila spilota	3000	Slip & Shine (1988) and Shine (1991)
Python regius	70	Luiselli *et al.* (1998)
Python reticulatus	8000	Shine *et al.* (1998a))
Python sebae	5000	Luiselli *et al.* (1998)
Psammophiinae		
Psammophis phillipsi	6	Luiselli *et al.* (1998)
Viperidae		
Agkistrodon piscivorus	52.4	Vincent *et al.* (2004)
Bitis caudalis	15	Shine *et al.* (1998b))
Bothrops moojeni	41	Noguerira *et al.* (2003)
Crotalus enyo	8.5	Taylor (2001)
Vipera berus	75	Forsman & Lindell (1993)
Tropidophiidae		
Trachyboa boulengeri	62	Greene (1983)
*Tropodophis melanurus**	40	Greene (1983)

*Indicates that maximum prey mass was estimated from the average body mass of the heaviest prey item reported in the diet.

$\log_{10}$-transformed all variables to meet the assumption of homoscedascity for regression analyses (Sokal & Rohlf, 1981; Kachigan, 1991), and tested all transformed data for normality using Lillifores tests. The mean $\log_{10}$-transformed morphological and dietary values for each clade were used in all statistical tests.

Morphological variance–covariance matrix

Nonphylogenetically corrected matrix

To examine how head dimensions co-vary with one another independently of size, we calculated a size-adjusted phenotypic variance–covariance matrix. This size-adjusted matrix was calculated by regressing all mean $\log_{10}$ head variables (y-axis) against the mean $\log_{10}$ SVL (x-axis) using ordinary least-squares regressions in order to generate residual values (see Sokal & Rohlf, 1981 for justification of this technique). These 'size-adjusted' contrast values were subsequently used as input into a principal components analysis in order to calculate the variance–covariance matrix (see Houle *et al.*, 2002 and references therein for justification of this technique).

Phylogenetically corrected matrix

To take common ancestry into account in this analysis, we also calculated a phylogenetically corrected variance–covariance matrix. To calculate this second matrix, we generated phylogenetically corrected standardized contrasts for all morphological variables. Contrasts were generated by using the phylogeny depicted in Fig. 1 (see Phylogenetic analysis below for details), with the mean $\log_{10}$ morphological variables per clade as input into the PDTREE program (Garland *et al.*, 1993). To remove the effects of size in this analysis, we regressed all standardized head variable contrasts (y-axis) against the standardized SVL contrast (x-axis) using ordinary least-squares regressions, forced through the origin, in order to generate residual values. Size-adjusted contrast values were subsequently used as input into a principal components analysis in a similar manner as was done in the nonphylogenetic analysis.

The broken stick method was used to determine which axes explained a significant amount of variation in the data in both the nonphylogenetically corrected and phylogenetically corrected analyses (see Frontier, 1971; Jackson, 1993).

Morphology and diet

To examine the relationships between mean head dimensions and mean maximum consumed prey mass among clades independently of body size, we first performed a nonphylogenetically corrected multiple stepwise regression (backwards & forwards models) with $\log_{10}$-transformed maximum consumed prey mass as the dependent variable and all head dimensions and SVL (body size) as the independent variables. Additionally, we included the significant PCA axes (see Results) in the nonphylogenetically corrected matrix as independent variables in this analysis to test whether prey mass is causally linked to the covariation among size-adjusted morphological traits. Partial regression coefficients express the correlation between two variables under the condition that all concomitantly measured variables are held constant at their mean values (Kachigan, 1991). Therefore, the partial regression coefficients in this model will yield the relationships between maximum consumed prey mass and absolute head dimensions independent of body size, in a fashion analogous to residual analysis but without the loss of a degree of freedom (see Darlington & Smulders, 2001).

In a second analysis, we took phylogeny into account because similarities among species often arise because of their shared evolutionary histories, precluding species from being considered as independent data points (Felsenstein, 1985; Harvey & Pagel, 1991; Garland *et al.*, 1993). Moreover, when species are used as independent data points, degrees of freedom are often inflated when using standard tabular *F*-distributions (see Vanhooydonck & Van Damme, 1999 and references therein for an overview). Thus, we used the composite phylogeny depicted in Fig. 1 as input into the PDTREE software program (Garland *et al.*, 1993). We set all branch lengths to unity (see Martins & Garland, 1991) because divergence times for most clades remain unknown, and inspected diagnostic statistics in the PDTREE program (Garland *et al.*, 1993) to verify that these branch lengths were adequate for all traits.

To examine the relationships between head dimensions and maximum consumed prey mass within a phylogenetic framework, we calculated standardized independent contrasts (Garland *et al.*, 1993) for all traits in the PDTREE program. These standardized independent contrasts were subsequently used as input into a multiple stepwise regression, forced through the origin, in the same manner as was done in the nonphylogenetically corrected analysis. Additionally, to test whether the phylogenetically corrected/size-adjusted covariance matrix was significantly related to prey mass, the significant axes from the PCA were included as independent variables in this multiple regression.

Results

The PCA performed on the size-adjusted nonphylogenetically corrected head variables yielded two significant axes, together explaining 90.6% of the total variation in the head shape data (Table 2). All head variables except residual head volume loaded highly on PC 1 (based on eigenvalues; see Table 2). However, head width, jaw length, and out-lever length of the lower jaw all had similar magnitudes (>0.90) and directions (positive) of co-variation on PC 1; thus, these three head shape variables appear to co-vary most strongly with one another before correction for phylogentic history. Similarly, head length and height also appear to co-vary with one another given their similar magnitudes and directions (positive) on PC 1 before correction for common ancestry (Table 2). By contrast, head length was the only variable to load highly and positively on PC 2.

In the phylogenetically corrected PCA, the size-adjusted standardized head variable contrasts also yielded two significant axes, together explaining 78.7% of the total variation in the head shape data (Table 3). However, head width, jaw length and out-lever length of the lower jaw were the only variables to load positively and highly on PC 1; thus, these three head shape variables co-vary strongly with one another both before and after correction for phylogenetic history. Further, head length was once again the only variable to load highly and positively on PC 2. Nonetheless, although both head volume and head height loaded weakly on both PC 1 and 2, the directions and relative magnitudes of their covariance are similar on both axes in the phylogenetically corrected analysis (Table 4). Therefore, head volume and height also appear to co-vary (albeit weakly) with one another after correction for common ancestry.

In the nonphylogenetically corrected multiple regression, head width was the only morphological variable

Table 2 Phenotypic variance–covariance matrix for all 'size-adjusted' nonphylogenetically corrected head dimensions measured in this study. Variables loading most strongly on each factor are indicated in bold.

Variable	PC1	PC2
Residual head volume	0.752	−0.555
Residual head width	**0.942**	−0.250
Residual head length	**0.855**	**0.460**
Residual head height	**0.826**	−0.360
Residual jaw length	**0.911**	0.310
Residual out-lever	**0.925**	0.294
Eigenvalue	4.55	0.894
% Variation explained	75.8	14.8

Table 3 Phenotypic variance–covariance matrix for all 'size-adjusted' phylogenetically corrected head dimensions measured in this study. Variables loading most strongly on each factor are indicated in bold.

Variable	PC1	PC2
Residual head volume	0.539	−0.586
Residual head width	**0.942**	−0.253
Residual head length	0.480	**0.668**
Residual head height	0.618	−0.567
Residual jaw length	**0.896**	0.266
Residual out-lever	**0.854**	0.404
Eigenvalue	3.32	1.41
% Variation explained	55.3	23.4

Table 4 Results from a phylogenetically corrected multiple regression testing which head and body dimensions or their covariance (PC 1 and PC2) significantly explain the variation in mean maximum prey mass among 12 monophyletic clades of snakes. Highly similar results were found for both the nonphylogenetically corrected and phylogenetically corrected multiple regressions, in that only head width was significantly related to mean maximum prey mass. Therefore, we have only reported the nonsignificant results for the phylogenetically corrected regressions.

Variable	B	*P*
SVL	0.363	NS
Head volume	0.161	NS
Head length	0.061	NS
Head width	**0.672**	**<0.05**
Jaw length	−0.09	NS
Out-lever	0.05	NS
PC1	−0.46	NS
PC2	0.212	NS

Bold type indicates SVL, $P = .303$; Head volume, $P = .656$; Head length, $P = .867$; Head width, $P = .024$; Head height, $P = .582$; Jaw length, $P = .789$; Out-lever, $P = .873$; PC 1, $P = .182$; PC 2 = .186

significantly related to mean maximum consumed prey mass ($\beta = 0.61$, $r^2 = 0.38$, $P < 0.05$; Fig. 2a). Similar results were obtained for the phylogenetic analysis, in that the standardized head width contrast was the only variable to remain significantly related to standardized max prey mass contrast ($\beta = 0.67$, $r^2 = 0.45$, $P < 0.05$; Fig. 2b; see Table 4 for pertinent regression statistics). Therefore, maximum prey mass is significantly related to max head width in snakes both before and after phylogenetic correction for common ancestry.

Discussion

Previous authors have hypothesized that head size and shape in macrostomatan snakes are adapted for consuming 'large' prey in terms of either mass or circumference (Gans, 1961; Greene, 1983; Rodriguez-Robles *et al.*, 1999). Indeed, some authors have even suggested that the functional capacity to consume prey larger than their head was a key innovation leading to the adaptive radiation of macrostomatan snakes (Gans, 1961; Greene, 1983). This hypothesis is qualitatively plausible given that macrostomatans are by far the most speciose and widely distributed snakes, and that they consume the largest diversity of prey sizes, shapes and types amongst extant clades of snakes (see Greene, 1997 for an overview). Yet, the hypothesis that maximum ingestible prey size has driven phenotypic divergence within Macrostomata has never been tested within an explicit phylogenetic context. Here we tested this hypothesis using recently published phylogenies, published maximum consumed prey mass data and head/body measurements taken from a large sample of museum specimens. Our nonphylogenetically corrected analysis showed that head width was significantly related to mean maximum consumed prey mass among these

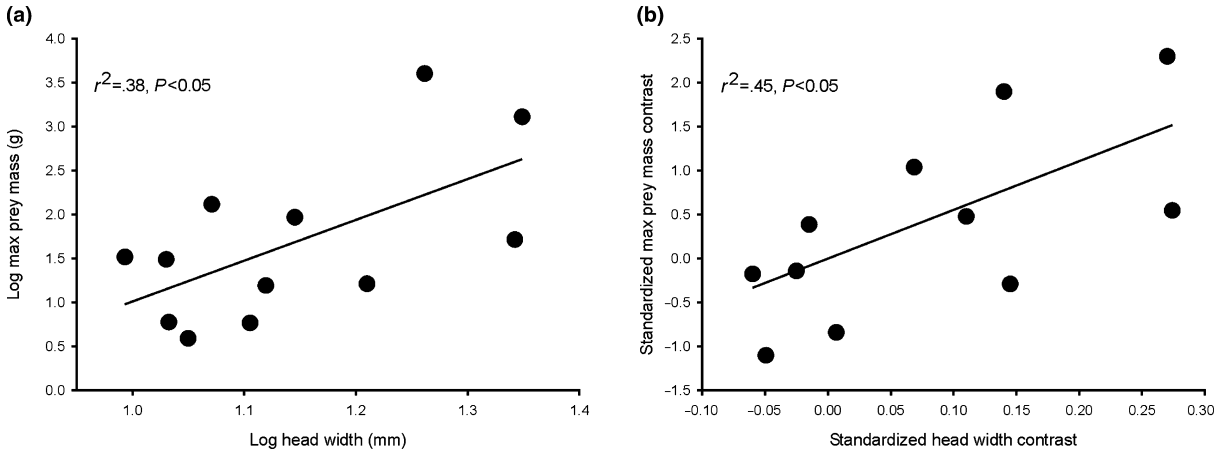

Fig. 2 (a) Plot of $\log_{10}$-transformed head width (*x*-axis) vs. average maximum consumed prey mass. (b) Plot of the standardized head width contrast (*x*-axis) vs. the standardized prey mass contrast (*y*-axis) with the slope forced through the origin. Head width is significantly related to maximum consumed prey mass both before and after phylogenetic correction.

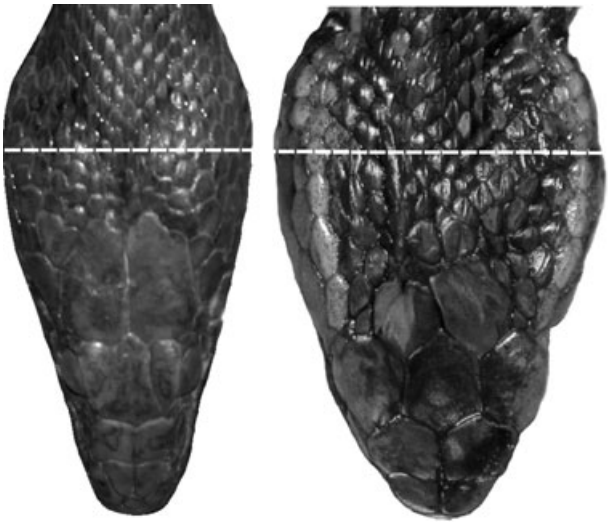

Fig. 3 Digital images of a natricine (*Thamnophis rufipunctatus*; left) and a viper (*Agkistrodon piscivorus*; right) scaled to one another. Dotted lines indicate the widest point of the head.

clades, and this relationship became even more significant after taking phylogeny into account. Therefore, the evolution of macrophagy in snakes is tightly coupled with an evolutionary increase in head width.

A relevant question is thus, how is head width functionally linked to prey mass in snakes? In this study, maximum head width was associated primarily with the widest space available for food passage through the buccal cavity, typically located just anterior to the attachment of the quadrates to the supratemporal bones (Fig. 3). Thus, snakes with wider heads in this sample of macrostomatans had larger areas available for food passage. Nonetheless, it should also be noted that the stretch capacities of the intermandibular soft tissues are also functionally important during intraoral prey transport in snakes (Groombridge, 1979; Cundall, 1987; Young, 1998). This finding is interesting because a recent study demonstrated that wider and taller prey significantly influenced maxillary and quadratric displacements, as well as the degree of intermandibular separation in a water snake (Vincent *et al.*, 2006). By contrast, these data clearly show that the maximum prey size a snake can consume is largely a function of the area available for food passage, and this result holds across macrostomatans (Fig. 2a, b). Hence, the extensive kinesis found within the macrostomatan feeding apparatus appears to better enable the upper and lower jaws to conform to a prey's overall shape (Vincent *et al.*, 2006), whereas head width determines the maximum area available for food passage through the buccal cavity (shown here; Fig. 3). Although population level studies examining how relative head width relates to prey mass are rare, studies examining phenotypic plasticity in the head dimensions of snakes have shown that residual head width responds most strongly to differing food amounts (prey mass) over ontogeny compared to residual head and jaw lengths (Queral-Regil & King, 1998; Bonnet *et al.*, 2001). Therefore, the macroevolutionary patterns reported here do closely match the results found at the within species level.

Coupled with this evolutionary increase in head width, however, are the concomitant changes in jaw length and lower jaw out-lever length (Tables 2 and 3). In other words, these three head dimensions appear to be linked with one another both evolutionary and functionally, given their high phenotypic and likely genetic, covariance. Indeed, previous authors have suggested this very scenario leading to the increased gape width found in macrostomatans (see Cundall & Greene, 2000 for an overview), but this study is the first to support this prediction after taking phylogeny into account. We suggest that field-based selection studies examining the relationships between head shape and prey dimensions could shed significant light on the evolutionary consequences of this tight morphological integration within the macrostomatan feeding apparatus.

Even so, previous authors have hypothesized that there are several potential selection pressures on macrostomatan feeding apparatus in addition to maximum ingestible prey size: habitat use, locomotion, prey capture in functionally challenging environments and the intraoral transport of differently shaped prey (see Cundall, 1987; Lindell, 1994; Martins *et al.*, 2002; Sanders *et al.*, 2004). Sexual selection is believed to only rarely influence head shape in snakes (see Shine, 1994). Although nonadaptive factors such as phylogenetic inertia and/or developmental constraints may also play a role, recent work has shown that prey shape directly influences feeding kinematics and performance in snakes (Vincent *et al.*, 2006). Moreover, a recent phylogenetic comparative study on aquatically feeding natricines revealed that head shape is also directly influenced by prey capture environment (i.e. aquatic feeding specialist snakes have narrow heads adapted for feeding underwater; Hibbits & Fitzgerald, 2005). Therefore, the hypothesis that prey size should tightly match head size/shape within snakes only accounts for the functional challenges of ingestion, not prey capture in difficult environments (e.g. crevices, water), habitat use (e.g. burrowing), or the influence of prey shape during ingestion in snakes (Jackson *et al.*, 2004; Vincent *et al.*, 2006). Further work is thus needed to evaluate the role of each of these putative selection pressures in driving divergence in cranial shape within snakes.

In order to understand the evolutionary and functional relationships between feeding morphology and diet in snakes, we suggest that further studies should focus on the relationships between head shape and prey shape, as well as between head shape and prey capture behaviour in functionally difficult environments (e.g. aquatic medium, see Young, 1991; Vincent *et al.*, 2005 for

quantitative predictions for how the aquatic medium may influence head shape evolution in snakes), within an explicit phylogenetic context (e.g. Hibbits & Fitzgerald, 2005). Unfortunately, phylogenetic comparative studies testing the former relationship are not currently possible because few studies have reported prey shape measurements in snakes (but see Vincent *et al*., 2005). Nevertheless, given that phylogenetically corrected head width only explained 45% of the variation in maximum consumed prey mass among these 12 clades, these data clearly demonstrate the need of future authors to report prey measurements (prey length, max width, height, etc.) other than, and in addition to, mass and/or circumference. Additionally, more specific morphological measurements such as quadrate, maxilla and palatopterygoid lengths would be very helpful when examining these relationships. Such data are vital to understanding the variety of factors that may influence head shape evolution within Macrostomata.

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Supplementary Material

The following supplementary material is available for this article online:

Appendix S1. Mean morphological values for the species examined here.

This material is available as part of the online article from http://www.blackwell-synergy.com

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