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The impact of diet, habitat use, and behaviour on head shape evolution in homalopsid snakes

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An organism's morphology is driven by selection on function while being constrained by phylogenetic and developmental factors as well as functional trade-offs. If selection on function is strong and solutions limited, then convergence is expected. In this paper we quantify head shape in a group of ecologically diverse snakes (homalopsid snakes) differing in habitat use and diet using three-dimensional geometric morphometric approaches. Using data on head shape we explore whether snakes eating different prey show different morphologies. Moreover, we test whether head shape is constrained by other factors such as habitat use, burrow use, or activity pattern. Our results demonstrate similar head shapes in species consuming similar prey. Snakes that capture elusive prey under water differ from those that capture and swallow prey like frogs or crustaceans. Moreover, habitat use, the use of burrows, and activity pattern also significantly impact head shape in this group of snakes. However, this signal appears to be partly confounded by the diet signal. For axes discriminating specifically between habitat use groups or animals that use burrows vs. those that do not shapes were in accordance with our predictions. Our results suggests an adaptive signal in the evolution of head shape in homalopsid snakes with diet, habitat use and the use of burrows all influencing the evolution of head shape in the group. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **118**, 634–647.

KEYWORDS: activity pattern – convergence – functional morphology – geometric morphometrics – prey capture – water snake.

INTRODUCTION

The morphology of an organism is often considered to be adaptive. Yet, morphology is also constrained by phylogenetic and developmental factors (Gould, 1977; Maynard Smith *et al.*, 1985; Stearns, 1986; Gans, 1989; Arnold, 1992; Hall, 1992) as well as potential functional trade-offs (e.g. Vanhooydonck, Van Damme & Aerts, 2001; Walker, 2007; Herrel *et al.*, 2009). Consequently, predicting the evolutionary outcome of the process of selection in a given environment is not always straightforward. For example, recent studies on the evolution of the jaw system in teleost fish (Wainwright *et al.*, 2004; Alfaro, Bolnick & Wainwright, 2005) have shown that in some clades different phenotypes can converge on the same area of functional space, resulting in so-called many-to-one mapping of morphology on function. This suggests that animals need not necessarily show morphological convergence even when confronted with similar functional or ecological demands. However, if selection on function is strong and if the morphological solutions are limited (i.e. one-to-one mapping of form to function) then conver-

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*Corresponding author. E-mail: aof3@duke.edu gence is expected (Herrel *et al.*, 2008). For example, the repeated and independent evolution of *Anolis* lizard ecomorphs on the Greater Antillean islands (Losos, 1990; Losos *et al.*, 1998; Mahler *et al.*, 2013) shows that evolution can indeed be predictable if functional constraints are strong.

Underwater capture of evasive prey has been suggested to impose strong constraints on head shape in snakes (Herrel *et al.*, 2008). Indeed, given the viscosity and density of water, animals that try to capture prey under water will have a tendency to push water in front of their heads, and generate a bow wave that may physically displace the prey unless compensatory suction is used (Van Damme & Aerts, 1997; Van Wassenbergh *et al.*, 2010). Moreover, bow waves generated by head displacement may trigger the lateral line system of fish, providing them with an early warning. Some aquatic snakes exploit the lateral line system in fish to their advantage to capture their prey (Catania, 2009). Because of the extreme reduction of the hyoid of snakes due to the specialization of the snake tongue for chemoreceptive purposes (McDowell, 1972; Schwenk, 1994), snakes most likely cannot use suction feeding. Although some snakes have partially overcome this constraint by using lateral strikes (Young, 1991; Herrel *et al.*, 2008), many also use frontally directed strikes (Drummond, 1983; Alfaro, 2002; Hibbits & Fitzgerald, 2005; Bilcke, Herrel & Van Damme, 2006). This behaviour has been suggested to place strong constraints on the evolution of head shape with frontal striking snakes typically having narrow and elongated heads (Herrel *et al.*, 2008; Van Wassenbergh *et al.*, 2010). However, the evolution of a narrow and elongate head may trade-off with head shapes that facilitate swallowing of large and bulky prey (i.e. prey that are heavy and/or prey that are relatively wide or tall for their length; Vincent *et al.*, 2006, 2009). Indeed, as most snakes are gape-limited predators, they rely on being able to spread their mandibles and quadrates allowing them to move their heads over large and bulky prey (Gans, 1961; Arnold, 1983; Greene, 1983; Forsman & Lindell, 1993; Cundall & Greene, 2000). As such, head shape in gape-limited predators like snakes, and especially aquatic snakes, is thought to reflect dietary adaptation (Savitzky, 1983).

Here, we explore whether head shape is related to diet, habitat use, burrow use, and activity pattern in a group of semi-aquatic snakes containing several dietary specialists: homalopsid snakes. Some homalopsid snakes have evolved a rather unique prey reduction behaviour allowing them to overcome gape limitations (Savitzky, 1983; Shine & Schwaner, 1985; Jayne, Voris & Ng, 2002; Voris & Murphy, 2002). Indeed, crustacean specialists such as *Gerarda prevostiana* are known to exploit recently molted soft-shelled crabs as prey from which they tear bite-sized pieces before swallowing (Jayne *et al.*, 2002). Moreover, similar behaviours have been suggested for other species that eat crustaceans (Savitzky, 1983). We here predict that crustacean specialists that have overcome gape limitations will have head shapes that are more similar to those of fish specialists as they reduce larger prey to smaller pieces that are more easily ingested. Streamlined heads are likely advantageous during locomotion in these largely aquatic species as it may reduce drag, and thus the cost of locomotion. For omnivorous homalopsid snakes that incorporate prey such as frogs into their diet, we predict that they will have wide and tall heads allowing them to accommodate the passage of diverse prey varying in size and shape. Conversely, fish specialists are predicted to have more narrow and elongated heads associated with the capture of prey under water. Although fish can also be large and bulky, most snakes that feed underwater typically eat more elongated and narrow prey, possibly due to the constraints imposed on head shape by underwater prey capture (Voris & Voris, 1983; Voris & Murphy, 2002).

Beyond diet, other factors are also known to influence head shape in vertebrates. For example, whether a species is nocturnal or diurnal may impact skull and head shape (e.g. Hall, 2009) as nocturnal species are thought to benefit from larger and more frontally directed eyes (Hall, 2009; Dumont *et al.*, 2015). The use of burrows has also been shown to impact head shape in vertebrates, with animals that use burrows typically having more streamlined heads (Teodecki *et al.*, 1998; Navas *et al.*, 2004; Barros, Herrel & Kohlsdorf, 2011; Herrel *et al.*, 2011; Vanhooydonck *et al.*, 2011). Among homalopsid snakes, several species are known to use burrows (Murphy, 2007) which may impact their head shape. Finally, whether snakes live in standing bodies of fresh water such as lakes or marshes or brackish mangrove habitats characterized by daily tidal movements might impact head shape as well. Snakes inhabiting mangroves may need to swim countercurrent to reach the exposed mud flats where they feed which may also impose constraints on head shape in relation to drag reduction (Murphy, 2007).

To test the factors that may impact the evolution of head shape we first quantify head shape using three-dimensional (3D) geometric morphometric approaches and establish the overall variation that exists within homalopsid snakes. We next test for differences in head shape, between: (1) species eating different prey, (2) species that use burrows vs. those that do not, (3) species that live in standing bodies of fresh water vs. those that live in tidal habitats such as mangroves, and (4) nocturnal vs. diurnal species. Specifically we predict narrower heads for piscivores and crustacean eaters than for omnivores; streamlined and elongated heads for species that use burrows and that live in tidal habitats; and differences in eye position in diurnal vs. nocturnal species. Finally, we test for the presence of phylogenetic signal in the data.

MATERIAL AND METHODS

SPECIMENS

We quantified head shape for 87 specimens of 14 species of homalopsid snakes from the collections of the Lee Kong Chian Natural History Museum, the Muséum National d'Histoire Naturelle, and the Field Museum of Natural History of Chicago (Table 1 and Supporting Information, Table S1). Our data set contains 26% of all known species and half of the known genera of homalopsid snakes. We follow the most recent taxonomy by Murphy & Voris (2014) in our designation of genus names. We follow the descriptions of the diet, habitat, burrow use, and activity pattern as summarized in Murphy (2007). Although sample sizes of some species are small, we believe that including species represented by one of two individuals (Table 1) is still informative. More individuals should, however, be added to better understand the within-species variation in these taxa.

GEOMETRIC MORPHOMETRICS

We used two different methods to obtain 3D coordinates of landmarks: microscribe digitizations and the digitization of landmarks of 3D surface scans. Microscribe digitizations were performed twice on each specimen using a Micro-Scribe1 G2 (Immersion) with a precision of ± 0.38 mm for specimens from the Lee Kong Chian Natural History Museum measured on site. Specimens were fixed in a clamp mounted on a ring-stand to allow access to the entire head with the microscribe. Once all landmark data were obtained, the two digitizations were averaged and rendered symmetrical (Kolamunnage & Kent, 2003) using the package 'Rmorph' (Baylac, 2012) in R (V. 3.2.2; R Core Team, 2014). For the 3D surface scans, Idav Landmark (Wiley *et al.*, 2005) was used to digitize the same landmarks. Surface scans were

Table 1. Summary table indicating the species included in the analysis, the number of individuals used, diet, habitat, the use of burrows, and the activity pattern of the different species

Species	*N*	Diet	Habitat	Burrows	Activity pattern
Bitia hydroides	5	Fish	Brackish water, estuaries, mangroves	Yes	Nocturnal
Cantoria violacea	4	Crustaceans	Brackish water, estuaries, mangroves	Yes	Nocturnal
Cerberus schneiderii	9	Fish	Brackish water, estuaries, mangroves	No	Nocturnal
Enhydris enhydris	7	Fish	Fresh water bodies	No	Diurnal
Erpeton tentaculatum	9	Fish	Fresh water bodies	No	Diurnal
Fordonia leucobalia	13	Crustaceans	Brackish water, estuaries, mangroves	Yes	Nocturnal
Gerarda prevostiana	5	Crustaceans	Brackish water, estuaries, mangroves	Yes	Nocturnal
Homalopsis buccata	9	Fish, anurans, crustaceans	Fresh water bodies	Yes	Nocturnal
Hypsiscopus plumbea	5	Fish, anurans, crustaceans	Fresh water bodies	No	Diurnal
Mintonophis pakistanicus	2	Fish, anurans, crustaceans	Fresh water bodies	Yes	Diurnal
Myrrophis chinensis	8	Fish, anurans, crustaceans	Fresh water bodies	No	Diurnal
Phytolopsis punctata	3	Unknown	Fresh water bodies	Yes	Diurnal
Subsessor bocourti	7	Fish, anurans, crustaceans	Fresh water bodies	No	Diurnal
Sumatranus albomaculatus	1	Unknown	Fresh water bodies	No	Nocturnal

obtained using a Breuckmann 3D surface scanner at the Muséum National d'Histoire Naturelle, Paris (white light fringe StereoSCAN^{3D} with a camera resolution of 1.4 megapixels). We used surface scans for animals in the collection of the MNHN and the FMNH obtained on loan.

Both scans and microscribe data allowed us to record the 3D coordinates of 23 landmarks on the head and 13 landmarks on the mandible describing the overall head shape (Fig. 1, Table 2). These landmarks consist of both anatomical landmarks as well as landmarks representing maxima of curvature. This was done separately for the head and the mandible. Note that these landmarks do not *per se* reflect internal bony structures but rather characterize overall external head shape. Next, a generalized Procrustes superimposition (Rohlf & Slice, 1990) was performed on the symmetrized point coordinates using the package 'Rmorph' (Baylac, 2012) in R (R Core Team, 2014).

A principal component analysis (PCA) on the Procrustes coordinates was performed to evaluate the distribution of species in morphospace (see Supporting Information, Figs. S1–S8). A multivariate mean shape was then calculated separately for each kind of diet, habitat, burrow use, and, activity pattern group using the tangent coordinates. This allows a multivariate visualization of the shape differences between the groups. The 3D visualization of the mean shape was computed using the function 'warp.-mesh' and 'shade3d' of the 'rgl' (Adler & Murdoch, 2012) and 'Morpho' (Schlager, 2013) libraries in R

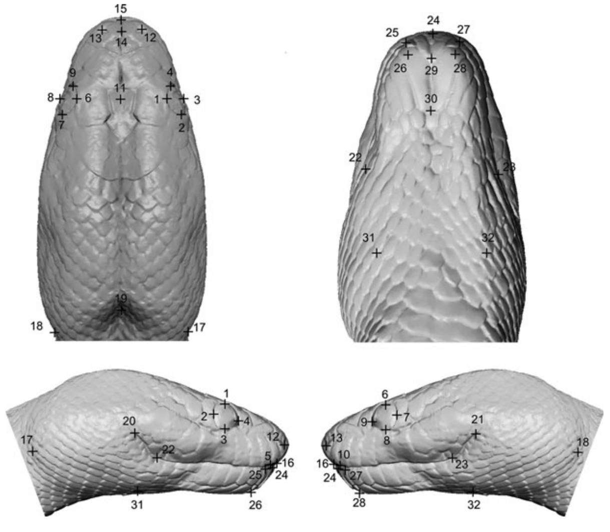

Figure 1. Three-dimensional surface scan of the head of a *Homalopsis buccata* (MNHN 1970.0518) illustrating the landmarks taken for the analysis of head shape. See Table 2 for a list and definitions of landmarks.

Table 2. Landmarks used to characterize shape variation in the head and mandible

Landmark	Definition
1	Most dorsal point of the eye, right side, head
2	Most posterior point of the eye, right side, head
3	Most ventral point of the eye, right side, head
4	Most anterior point of the eye, right side, head
5	Most antero-lateral part of the head, right side, head
6	Most dorsal point of the eye, left side, head
7	Most posterior point of the eye, left side, head
8	Most ventral point of the eye, left side, head
9	Most anterior point of the eye, left side, head
10	Most antero-lateral part of the head, left side, head
11	Dorsal point between 6 and 1 on the dorsal part of the head, middle, head
12	Point at the middle of the nostril, right side, head
13	Point at the middle of the nostril, left side, head
14	Dorsal point between 12 and 13 on the dorsal part of the head, middle, head
15	Most antero-dorsal point of the head, middle, head
16	Most antero-ventral point of the head, middle, head
17	Most postero-lateral point of the head, right side, head
18	Most postero-lateral point of the head, left side, head
19	Most dorso-posterior point of the head, middle, head
20	Most dorso-distal point of the mouth, right side, head and mandible
21	Most dorso-distal point of the mouth, left side, head and mandible
22	Most ventro-distal point of the mouth, right side, head and mandible
23	Most ventro-distal point of the mouth, left side, head and mandible
24	Most antero-dorsal point of the mandible, middle, mandible
25	Most dorso antero-lateral part of the mandible, right side, mandible
26	Most ventro antero-lateral part of the mandible, right side, mandible
27	Most dorso antero-lateral part of the mandible, left side, mandible
28	Most ventro antero-lateral part of the mandible, left side, mandible
29	Most antero-ventral point of the mandible, middle, mandible
30	Most distal point between the elongated scales, before the beginning of the small scales, middle, mandible
31	Most postero-lateral point under the point 20, right side, mandible
32	Most postero-lateral point under the point 21, left side, mandible

using thin-plate spline deformations of a 3D-scanned head of a *Homalopsis buccata* (MNHN 1970-0518). This specimen was selected because of the quality of the original scan.

Analyses

As species cannot be considered as independent data points disconnected from their evolutionary history, the use of comparative analysis has been advocated (Felsenstein, 1985). To estimate the phylogenetic signal in head and mandible shape, we used a randomization test following the method of Blomberg, Garland & Ives (2003) and the extended methods of Adams (2014). The phylogeny used is a composite phylogeny of the homalopsid relationships based on Voris *et al.* (2002), Alfaro *et al.* (2004, 2008) and Pyron, Burbrink & Wiens (2013) (Fig. 2). Branch lengths were computed using the Grafen (1989) with the 'compute.brlen' function of the 'Ape' library (Paradis *et al.*, 2012) in R (R Core Team, 2014). A multivariate K-statistic (Adams, 2014) was calculated based on the Procrustes coordinates using the 'geomorph' library (Adams & Otarola-Castillo, 2013) in R (R Core Team, 2014). Next, a univariate lambda (Ives, Midford & Garland, 2007) was calculated for the first five principal components of the mean shape of the cranium and mandible per species representing more than 90% of the variation (91.8% for the cranium and 91% for the mandible) using the 'phylosig' function in the 'phytools' library (Revell, 2012) in R (R Core Team, 2014).

Head shape differences

We used the first 11 principal components for the head and the first seven principal components for the mandible to quantitatively test for differences in shape between groups. These axes explained more than 90% of the overall shape variation of the head and the mandible. We used MANOVAs coupled to univariate ANOVAs in SPSS (IBM SPSS Statistics V.20) and Bonferroni post-hoc tests to explore whether snakes with different diets, habitats, and burrow use, and that differed in activity pattern showed differences in head and mandible shape.

RESULTS

Phylogenetic signal

The results of the multivariate K-statistic are not significant for the cranium (Kmult = 0.26, $P < 0.40$) or mandible data sets (Kmult = 0.27, $P < 0.30$). The univariate lambdas calculated for the first five axes, which explained more than 90% of the overall variation in shape (skull: 91.8%; mandible: 90%), were also non-significant ($P > 0.5$; Table 3) suggesting

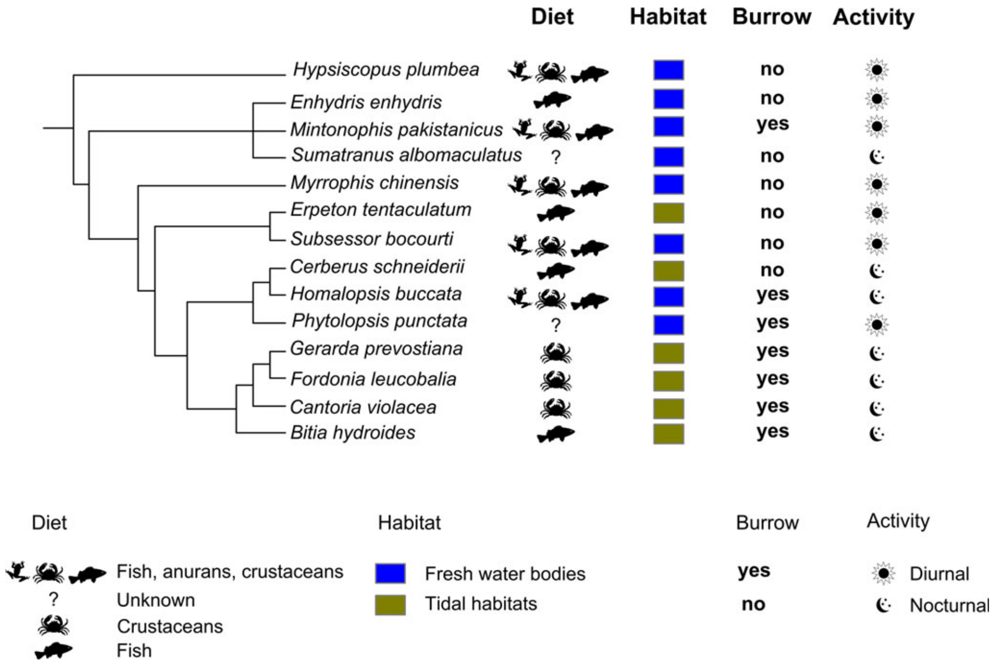

Figure 2. Composite phylogeny of the homalopsid relationships based on Voris *et al.* (2002), Alfaro *et al.* (2004, 2008) and Pyron *et al.* (2013). To the right is illustrated the diet, habitat, burrow type and activity pattern of the different species as summarized in Murphy (2007).

that there is no phylogenetic signal in the shape data.

The influence of diet on head shape

A MANOVA detected significant differences between animals with different diets (Wilks' lambda = 0.26; $F_{22,136} = 5.9$; $P < 0.001$). Subsequent univariate F-tests showed that differences were significant on axes two ($F_{2,78} = 4.11$; $P < 0.05$) and three ($F_{2,78} = 31.73$; $P < 0.001$; Supporting Information, Table S2). Post-hoc tests demonstrated that axis two discriminated between piscivores and crustacean eaters (Supporting Information, Table S3). Axis three discriminated between piscivores and all other groups (Supporting Information, Table S3). A MANOVA performed on the first seven principal components based on the shape data of the mandible showed significant difference between snakes depending on their diets (Wilks' lambda = 0.28; $F_{14,144} = 9.16$; $P < 0.001$). Differences were significant on the first ($F_{2,78} = 24.64$; $P < 0.001$), third ($F_{2,78} = 5.47$; $P < 0.01$), fourth ($F_{2,78} = 5.86$; $P < 0.005$), and fifth principal component ($F_{2,78} = 6.58$; $P < 0.005$). Post-hoc tests showed that axis one discriminated between crustacean eaters, piscivores, and omnivores (Supporting Information, Table S3). Axis three discriminated between piscivores and omnivores (Supporting Information, Table S3). The fourth axis discriminated between crustacean eaters and all other groups. Axis five discriminated omnivores from piscivores and crustacean eaters (Supporting Information, Table S3).

The cranium of crustacean specialists (Fig. 3) tends to be taller and relatively shorter and broader. In contrast, fish eaters have a narrower posterior cranium that is also more elongated. Omnivores show a flatter cranium with an intermediate morphology. The mandible shape of crustacean eaters (Fig. 3) tends to be short and broad with a large and square proximal part. In comparison, fish specialists display a mandible that narrows proximally. Omnivores display an intermediate morphology. Crustacean specialists and omnivores also tend to display a relatively shorter and broader mandible in comparison to fish specialists (Fig. 3).

Table 3. Pagel's lambda as calculated for the different PC axes

	Lambda	P
Cranium		
PC1	0.00007	1
PC2	0.00007	1
PC3	0.00007	1
PC4	0.00007	1
PC5	0.35	0.4
Mandible		
PC1	0.00007	1
PC2	0.00007	1
PC3	0.00007	1
PC4	0.00007	1
PC5	0.00007	1

The influence of habitat

A MANOVA detected significant differences in head shape (Wilks' lambda = 0.58; $F_{11,73} = 4.7$; $P < 0.001$). Only the third axis was significant in the subsequent ANOVAs ($F_{1,84} = 22.1$; $P < 0.001$). A MANOVA performed on the first seven principal components describing mandible shape also detected significant differences (Wilks' lambda = 0.63; $F_{7,77} = 4.7$; $P < 0.001$). The first ($F_{1,84} = 11.6$; $P < 0.001$) and third principal components ($F_{1,84} = 12.6$; $P < 0.001$) were significantly different (Supporting Information, Table S2).

The mean shape associated with snakes living in tidal habitats was characterized by a relatively broader and taller cranium. In comparison, those living in fresh water showed a flatter and more triangulated cranium which is posteriorly broader and have a narrower and more elongated snout (Fig. 4). Snakes that live in tidal habitats tend to display a mandible which is narrower proximally. Fresh water snakes, on the other hand, tend to display a broader proximal mandible (Fig. 4).

The influence of burrow use

A MANOVA detected significant differences between animals using burrows and those that do not (Wilks' lambda = 0.77; $F_{11,73} = 1.96$; $P < 0.05$). Only the third axis was significant in the ANOVAs ($F_{1,84} = 19.61$; $P < 0.001$). A MANOVA performed on the first seven principal components describing mandible shape also showed significant differences (Wilks' lambda = 0.79; $F_{7,77} = 2.89$; $P < 0.02$). The first ($F_{1,84} = 9.37$; $P < 0.005$) and seventh axis ($F_{1,84} = 5.84$; $P < 0.02$) were significantly different (Supporting Information, Table S2).

Species that use burrows display a relatively taller and broader cranium with a shorter and broader snout than species that do not use burrows (Fig. 5). Snakes that do not use burrows also tend to display a relatively narrower and more elongated mandible. In contrast, snakes that use burrows tend to display a broader and shorter mandible (Fig. 5).

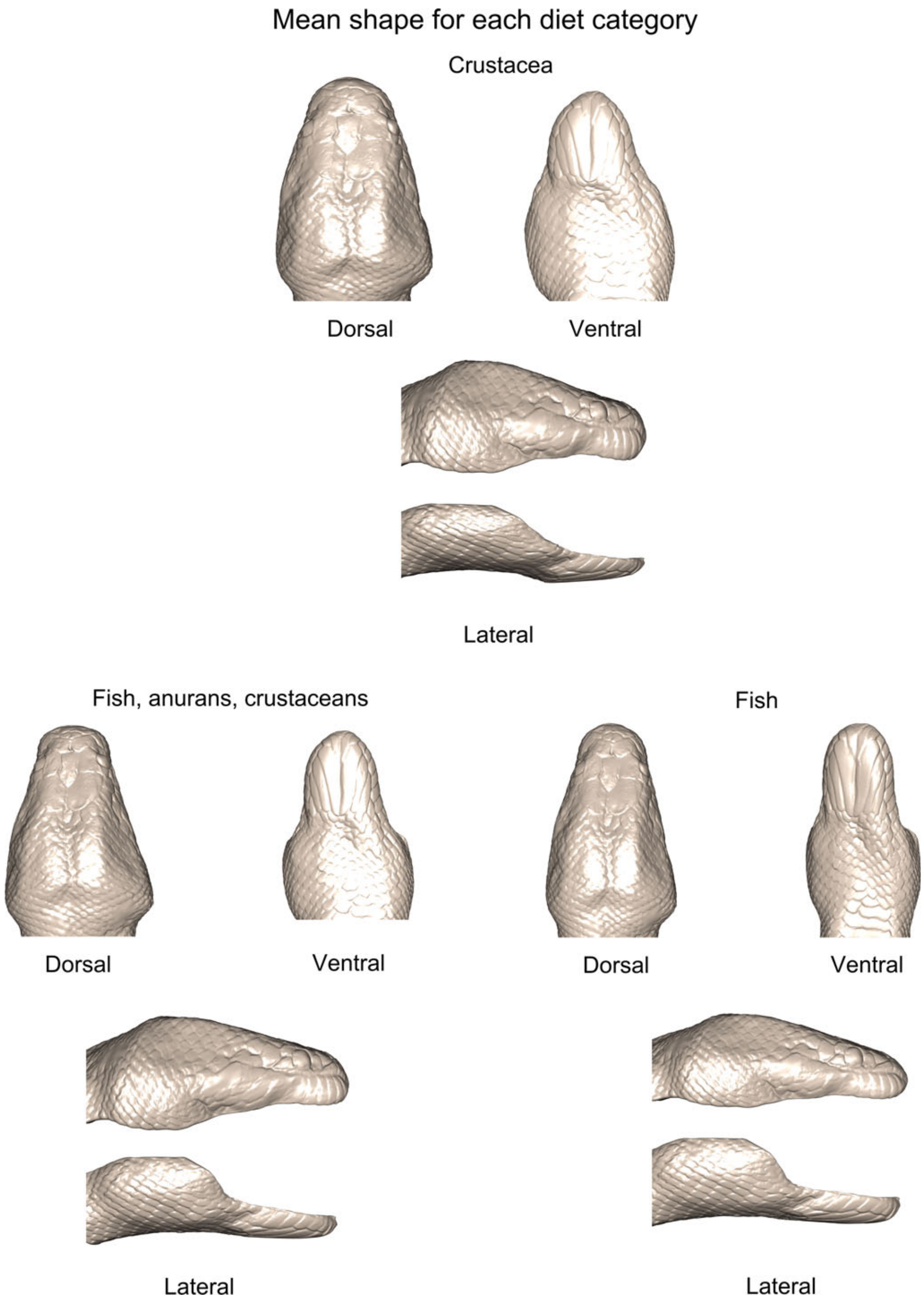

Figure 3. Mean shape of head of snakes with different diets.

THE INFLUENCE OF ACTIVITY PATTERN

A MANOVA detected significant differences in the shape of the cranium in snakes with different activity pattern (Wilks' lambda = 0.66; $F_{11,73} = 3.48$; $P < 0.001$). The second ($F_{1,84} = 7.2$; $P < 0.01$) and third principal components ($F_{1,84} = 16.92$; $P < 0.001$) were significantly different (Supporting Information, Table S2). The result of the MANOVA performed on the first seven principal components of the mandible was significant and showed differences (Wilks' lambda = 0.71; $F_{11,73} = 4.36$; $P < 0.001$). The first ($F_{1,84} = 18.29$; $P < 0.01$) and third axis ($F_{1,84} = 7.3$; $P < 0.01$) were significantly different (Supporting Information, Table S2).

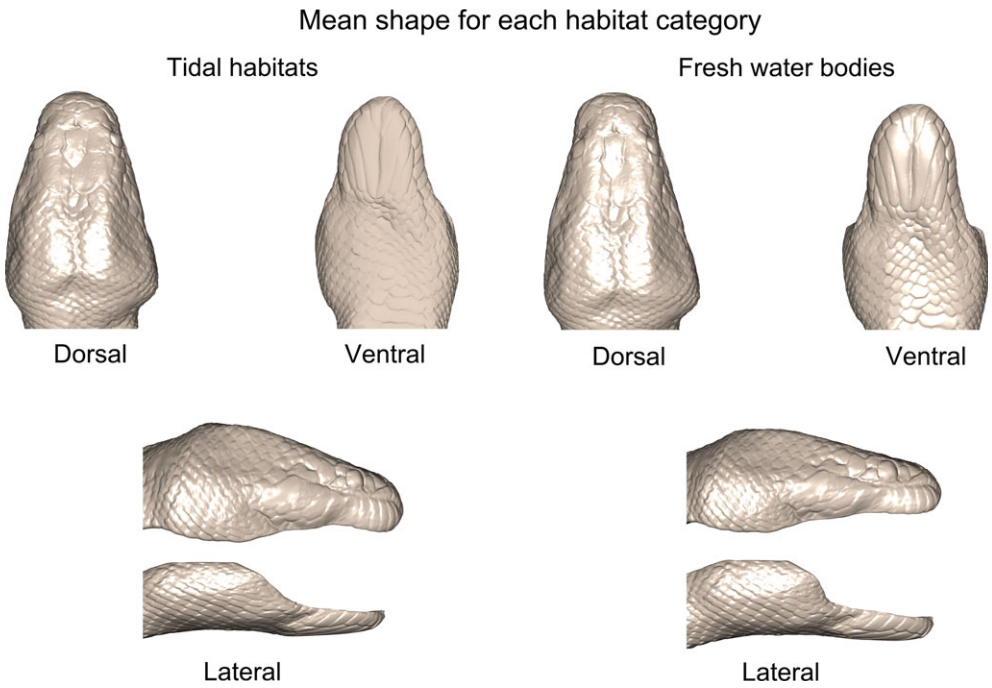

Figure 4. Mean shape of the head of snakes living in tidal brackish mangrove habitats vs. those that live in fresh water habitats.

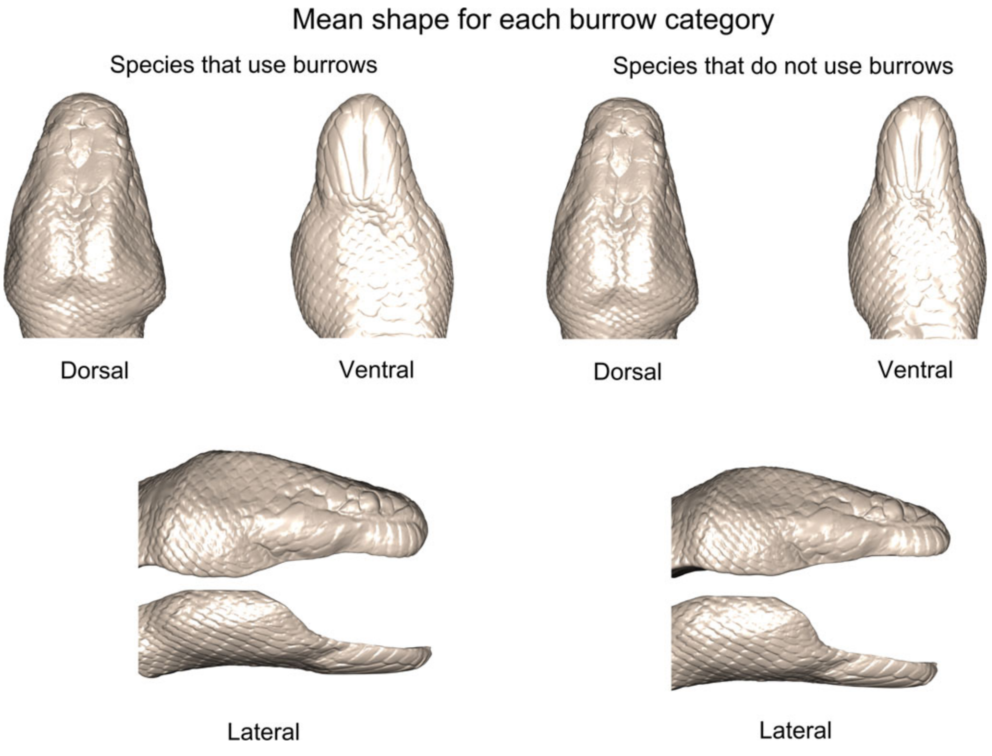

Figure 5. Mean shape of head of snakes that use burrows vs. those that do not.

The mean shape of nocturnal species corresponds to a cranium that is relatively taller and possess a shorter and broader snout. In contrast, diurnal species display a cranium that is relatively flatter and possesses a narrower and more elongated snout (Fig. 6). Nocturnal snakes tend to display a relatively curved mandible which is broader and shorter proximally. In comparison, diurnal species tend to display a relatively straight, more narrow and elongated mandible (Fig. 6). No obvious differences in eye position were observed.

DISCUSSION

Surprisingly few studies on head shape evolution in snakes have been conducted despite the suggested relevance of head shape to diet in gape-limited predators like snakes (Gans, 1961; Savitzky, 1983). Most studies exploring snake head shape have used linear descriptors in an attempt to explore growth allometries and sexual dimorphism (Arnold & Peterson, 1989; Vincent, Herrel & Irschick, 2004a,b; Vincent & Herrel, 2007; Henao-Duque & Ceballos, 2013; Borczyk, 2015) or to explore the role of head shape and head triangulation as a predator deterrent (Valkonen, Nokelainen & Mappes, 2011; Dalbosco *et al.*, 2012). Studies using geometric morphometrics are relatively rare and have mostly focused on head shape variation from a systematic perspective (Gentilli *et al.*, 2009; Mangiacotti *et al.*, 2014). However, some studies have explored the relationships between head shape and diet, and both diet and prey-capture behaviour have been suggested to impact head shape in snakes (e.g. Hibbits & Fitzgerald, 2005; Herrel *et al.*, 2008; Tjarks, 2009; Brecko *et al.*, 2011; Hampton, 2011).

The results of our study show that homalopsid snakes with different diets differ in the overall shape of the head and mandible. Specifically, our results show that dietary generalists that potentially include large and bulky prey into the diet and capture prey on land have wider and taller heads, as demonstrated previously for the skull in natricine snakes (Hampton, 2011). This is not surprising given that the time needed to swallow prey is directly related to head shape and specifically head width, and head depth (Vincent *et al.*, 2006; Hampton, 2011). Homalopsid snakes specializing in the underwater capture of elusive prey such as piscivores, have longer and narrower heads in accordance with results for natricine snakes (Hibbits & Fitzgerald, 2005; Herrel *et al.*, 2008). However, crustacean eaters that have circumvented constraints on maximal gape size through prey reduction strategies (Savitzky, 1983; Shine & Schwaner, 1985; Jayne *et al.*, 2002) did not have streamlined heads or mandibles as we predicted. In contrast, crustacean eaters had the widest

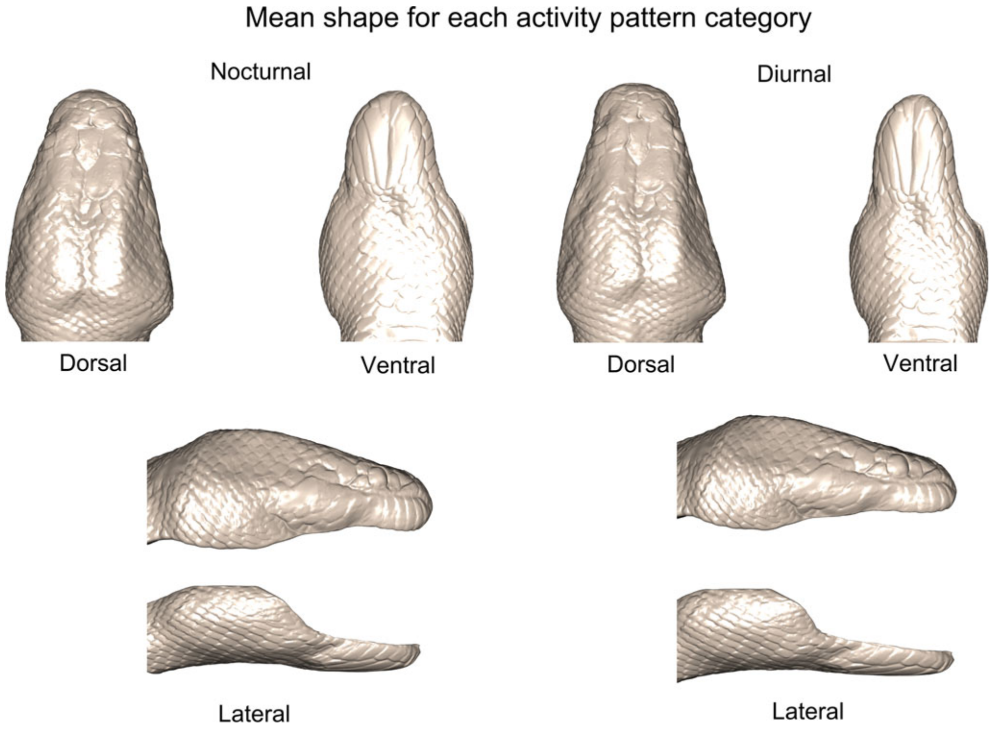

Figure 6. Mean shape of the head of snakes with different activity patterns (nocturnal vs. diurnal).

and tallest heads and mandibles. These tall heads and mandibles may provide these animals with the space and attachment area needed to house larger jaw muscles used during prey reduction. As our landmarks were placed to describe overall head shape, the presence of large jaw adductors should be captured by these landmarks. However, further studies looking at the musculature of these snakes are needed in order to corroborate this inference. Crustacean specialists also have modified skulls and teeth allowing them to effectively crush hard-shelled prey (Savitzky, 1983). One limitation of our study is that dietary data are derived from the literature and often based on small sample sizes or anecdotal information (see Murphy, 2007). Consequently, additional studies on the diet of these species would be of interest. One other shortcoming of the current analysis is that we were unable to take into account the strike behaviour of the species in our analysis. Among the species included in our study, some use lateral strikes (e.g., *Erpeton*; see Smith, Povel & Kardong, 2002; Catania, 2009) whereas others use both frontally and laterally directed strikes (e.g., *Cerberus*; Jayne, Voris & Heang, 1988; pers. obs.). Data, however, are available for only two species preventing any quantitative analyses. Additional studies quantifying prey-capture behaviour in homalopsid snakes are needed to better understand whether strike behaviour in itself also impacts head shape evolution as suggested for natricine snakes (Herrel *et al.*, 2008).

Beyond the differences between species eating different prey we also observed significant differences between species with different behaviours (i.e. burrow use and activity pattern) and species that use different habitats. For example, snakes that used burrows had different head shapes than those that do not. However, rather than having narrow elongated heads that would allow them to more easily move through burrows they had taller and wider heads. Only PC7 describing mandible shape was specific to the use of burrows (Supporting Information, Table S2). Animals that use burrows had more streamlined mandibles as seen in ventral view; those that do not had a wider posterior part of the mandible, rendering it less streamlined. This is in accordance with our *a priori* predictions and suggest that at least part of the head shape responds specifically to the demands associated with the use of burrows. Snakes inhabiting tidal habitats also generally had taller heads than those inhabiting fresh water bodies, contrary to our *a priori* predictions. Only PC6 describing cranial shape was specific to habitat use. Differences along this axis were, however, in accordance with our predictions (Supporting Information, Table S2). Species that inhabit standing freshwater habitats have a shorter and more triangulated cranium; in contrast those inhabiting tidal mangrove habitats have a more elongated and narrow cranium. Finally, differences between diurnal vs. nocturnal species were minimal with eye position being rather similar, in contrast to what has been observed for other vertebrates (Hall, 2009; Dumont *et al.*, 2015). None of the axes was specific to differences in activity pattern.

Given the fact that the overall results based on the multivariate shape visualizations counter our *a priori* predictions, it is possible that our predictions do not accurately reflect the biomechanical constraints associated with habitat use or the use of burrows by these snakes. However, axes specific to habitat or the use of burrows did show shape differences in accordance to our predictions. This suggests that the different behavioural and habitat use categories overlap and may not be independent. To explore this idea further we ran logistic regressions on our categorical data to test whether they are correlated. The results show that the different ecological groups are indeed inter-correlated (habitat ~ activity pattern: $P < 0.001$; activity pattern ~ use of burrows: $P < 0.02$; diet vs. all others: all $P < 0.001$) with the exception of habitat use and the use of burrows ($P = 0.069$). For example, among those species that use burrows, three are crustacean specialists and two are omnivores, the two diet groups known to be characterized by having wide and tall heads. Most of these are also nocturnal and live in brackish tidal habitats which may explain why these species, on average, have wide and tall heads despite the fact that they use burrows. Consequently, the signal carried by habitat use or behaviour may be confounded by the strong dietary signal detected. Additional studies exploring the ecology and behaviour of homalopsid snakes are essential to better understand the evolution of head shape in this fascinating and diverse group of snakes.

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SUPPORTING INFORMATION

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

Figure S1. Scatterplots of the first three principal components of the analysis performed on the species mean shapes of the cranium representing over 78% of the variation in the data set. The phylogeny has been plotted in the morphospace. (A) The distribution defined by the scatter plot of the first two axes tends to separate omnivorous snakes from crustacean eaters. Fish specialists tend to fall in the middle of the morphospace with a rather broad distribution. (B) The morphospace defined by the first and third axis tends to separate snakes with different diets with a small overlap between omnivores and piscivores. (C) The scatterplot of the second and third axis tends to separate all groups.

Figure S2. Scatterplots of the first three principal components of the analysis performed on the species mean shapes of the mandible representing over 77% of the variation in the data set. The phylogeny has been plotted in the morphospace. (A) The distribution defined by the scatter plot of the first two axes tends to separate piscivores from the two other groups. (B) The morphospace defined by the first and third axis tends to separate all groups. (C) The scatterplot of the second and third axis does not separate well snakes depending on their diet.

Figure S3. Scatterplots of the first three principal components of the analysis performed on the shapes of the cranium representing over 78% of the variation in the data set. The phylogeny has been plotted in the morphospace. (A) the distribution defined by the scatter plot of the first two axes tends to separate fresh water snakes from snakes living in tidal habitats on the first axis. (B) The morphospace defined by the first and third axis also tends to separate snakes with different habitats on the first axis. (C) The scatterplot of the second and third axis does not separate snakes occupying different habitats.

Figure S4. Scatterplots of the first three principal components of the analysis performed on the shapes of the mandible representing over 77% of the variation in the data set. The phylogeny has been plotted in the morphospace. (A) the distribution defined by the scatter plot of the first two axes does not separate snakes depending on the habitat occupied. (B) The morphospace defined by the first and third axis tends to separate fresh water snakes from snakes living in tidal habitats on the second axis. (C) The scatterplot of the second and third axis also tends to separate snakes depending on their habitats on the third axis.

Figure S5. Scatterplots of the first three principal components of the analysis performed on the shapes of the cranium representing over 78% of the variation in the data set. The phylogeny has been plotted in the morphospace. (A) The distribution defined by the scatter plot of the first two axes does not separate snakes that use burrows from snakes that do not use burrows. (B) The morphospace defined by the first and third axis tends also does not separate snakes using or not burrows. (C) The scatterplot of the second and third axis does not separate snakes using or not burrows clearly.

Figure S6. Scatterplots of the first three principal components of the analysis performed on the shapes of the mandible representing over 77% of the variation in the data set. The phylogeny has been plotted in the morphospace. (A) The distribution defined by the scatter plot of the first two axes does not separate snakes depending on the use of burrows. (B) The morphospace defined by the first and third axis does not separate snakes using burrows from snakes that do not use burrows. (C) The scatterplot of the second and third axis shows an overlap of snakes that use burrows with those that do not.

Figure S7. Scatterplots of the first three principal components of the analysis performed on the shapes of the cranium representing over 78% of the variation in the data set. The phylogeny has been plotted in the morphospace. (A) The distribution defined by the scatter plot of the first two axes tends to separate diurnal snakes from nocturnal snakes on the second axis. (B) The morphospace defined by the first and third axis tends does not separate snakes depending on their activity pattern. (C) The scatterplot of the second and third axis tends to separate snakes with different activity pattern on the second axis.

Figure S8. Scatterplots of the first three principal components of the analysis performed on the shapes of the mandible representing over 77% of the variation in the data set. The phylogeny has been plotted in the morphospace. (A) The distribution defined by the scatter plot of the first two axes does not separate snakes depending on their activity pattern. (B) The morphospace defined by the first and third axis tends to separate diurnal snakes from nocturnal ones. (C) The scatterplot of the second and third axis also tends to separate snakes with different activity pattern on the third axis.

Table S1. Specimen collection numbers of individuals used in our analysis.

Table S2. Results of the univariate ANOVAs.

Table S3. Results of the post-hoc tests testing for differences between dietary groups.