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1 **Inside the head of snakes: influence of size, phylogeny, and sensory ecology on endocranium**
2 **morphology.**

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14 **Abstract**

15 Environmental properties, and the behavioral habits of species impact sensory cues available for
16 foraging, predator avoidance and inter/intraspecific communication. Consequently, relationships
17 have been discovered between the sensory ecology and brain morphology in many groups of
18 vertebrates. However, these types of studies have remained scarce on snake. Here, we investigate
19 the link between endocranial shape and the sensory-related ecology of snakes by comparing 36
20 species of snakes for which we gathered six sensory-ecology characteristics. We use μ CT scanning
21 and 3D geometric morphometrics to compare their endocranium in a phylogenetically informed
22 context. Our results demonstrate that size is a major driver of endocranial shape, with smaller
23 species tending to maximize endocranial volume using a more bulbous shape, while larger species
24 share an elongate endocranial morphology. Phylogeny plays a secondary role with more derived
25 snakes diverging the most in endocranial shape, compared to other species. The activity period
26 influences the shape of the olfactory and optic tract, while the foraging habitat impacts the shape
27 of the cerebellum and cranial nerve regions: structures involved in orientation, equilibrium, and
28 sensory information. However, we found that endocranial morphology alone is not sufficient to
29 predict the activity period of a species without prior knowledge of its phylogenetic relationship.

30 Our results thus demonstrate the value of utilizing endocranial shape as complementary
31 information to size and volume in neurobiological studies.

32 **Key words:** 3D geometric morphometrics, ecological morphology, allometry, snake endocranium,
33 activity period, foraging habitat

34 **Declarations**

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36 **Conflicts of interest/Competing interests:** The authors declare no conflict of interest.

37 **Availability of data and material (data transparency):** The original landmarks coordinates for
38 all specimens will be deposited in a Dryad Repository or added in the Supplementary Material and
39 the 3D scans will be uploaded in MorphoSource.

40 **Code availability (software application or custom code):** Available in Supplementary Material.

41 **Authors' contributions:** MS conceived the ideas, designed the methodology and collected the
42 data. MS and RC analyzed the data. MS led the writing of the manuscript. All authors contributed
43 to the interpretation and discussion of the results, and to the editing of the manuscript.

44 **Ethics approval:** Not applicable to this study.

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48

49 **Introduction**

50 Endocranial shape has been extensively used as a proxy for the brain shape, to compare or
51 infer the ecology of both extant and extinct species (for a review, see Balanoff & Bever, 2017);
52 including habitat (Allemand et al. 2017), sensory abilities (Lautenschlager et al. 2012; Holloway
53 et al. 2013; Carril et al. 2016) and behavior (Balanoff et al. 2016; Bertrand et al. 2019; Macrì et al.
54 2019). However, endocranial morphology could also be used to make more precise inferences
55 regarding the sensory ecology of elusive (Iwaniuk et al. 2020) or fossil species, if we were able to
56 recognize a direct link between sensory-relevant aspects of the ecology of extant species and the
57 shape of their endocranium. An extensive comparative study of the brain shape of fish has
58 demonstrated a relationship between brain shape and precise details of the ecology such as
59 microhabitat use and diet (Kotrschal et al. 1998) supporting the hypothesis that brain shape is
60 adaptive. Snakes, like fish, have a compartmentalized brain (Naumann et al. 2015), with clearly
61 identifiable sensory-associated structures such as the olfactory bulbs and optic tectum, and because
62 the brain sits tightly within the endocranial cavity, the latter is a good proxy for brain shape (Starck
63 1979; Nieuwenhuys et al. 1998, pers. obs.). The main goal of this study is to determine if there are
64 potential ecological drivers of endocranial shape in snakes, by statistically testing for relationships
65 between sensory ecology and endocranial morphology. We focus on snake' species that use
66 aquatic habitats to varying extents as water requires specific sensory adaptations, especially for
67 secondarily aquatic animals. We worked on a phylogenetically large sample of species to ensure
68 our results reflect adaptive changes due to shared ecological constraints.

69 Snakes have an arsenal of sensory modalities that they can use. They are known to use
70 vomerolfaction using their forked tongue to sample the environment (Daghfous et al. 2012), but
71 they can also process olfactory and gustative cues, even though they rely more on the first
72 modality. Despite their lack of visual acuity and despite popular belief, snakes heavily rely on
73 visual cues to sense their environment (Czaplicki and Porter 1974; Drummond 1985; Hart et al.
74 2012), but they also use thermal cues (Newman and Hartline 1982; De Cock Buning 1983;
75 Krochmal et al. 2004; Ebert and Westhoff 2006; Ebert et al. 2007), chemical cues (Shine et al.
76 2004a; Young et al. 2008; Smargiassi et al. 2012), airborne and/or waterborne acoustic cues
77 (Randall and Matocq 1997; Young 2003, 2007; Friedel et al. 2008), water motion through
78 mechanoreception (Povel and Van Der Kooij 1997; Westhoff et al. 2005; Catania et al. 2010;

79 Crowe-Riddell et al. 2016) and even phototaxis (Crowe-Riddell et al. 2019) (albeit the latter may
80 not be mediated in the brain). There is some evidence that snakes can also adapt their sensory
81 modalities depending on properties of their environment, behavior or diet, if those impact the
82 availability or reliability of the sensory cues (Burghardt and Ford 1993; Vincent et al. 2005; Cooper
83 2008; Schwenk 2008; Hart et al. 2012; Crowe-Riddell et al. 2016; Kutsuma et al. 2018). Our main
84 hypothesis is that the morphology of the endocranium, and its sensory structures is related to their
85 ecology of species as the latter seems to impact their sensory modalities. Studies quantifying the
86 sensory modalities used by snakes are scarce (Burghardt and Ford 1993) so we cannot directly test
87 how the preferred senses of species are related to the shape of the associated sensory area.
88 Therefore, we defined six ecological and behavioral factors that could impact the sensory
89 modalities of snakes and consequently lead to change in their endocranium shape: diet type,
90 foraging strategy, foraging habitat, main habitat, activity period, and dimensionality of the trophic
91 interaction (Table 1).

92 Dietary preferences in fish, and especially the importance of piscivory, correlate with the size
93 and shape of the brain, and more specifically the sensory-related parts of the brain such as the
94 olfactory bulbs and optic lobes (Kotrschal et al. 1998). In birds, diet and brain regions are also
95 related, but whereas in fish this seems to be based on perception/detection of a prey, in birds it is
96 more related to the complexity of the food manipulation (Gutiérrez-Ibáñez et al. 2010). Snakes not
97 only have a very diverse dietary range, but they also use their head to manipulate and swallow
98 their prey (Moon et al. 2019). Some crustacean-eating snakes such as *Fordonia leucobalia*,
99 *Cantoria violacea* and *Gerarda prevostiana* even show complex manipulation behavior (Jayne et
100 al. 2018). We distinguished 5 diet types depending on the media in which prey live and the
101 potential manipulation cost: generalists are species of snake that eat both aquatic and non-aquatic
102 prey, and four specialists are species that eat exclusively certain types of aquatic or semi-aquatic
103 prey (i.e., fish, crustacean, fish and crustaceans, fish and amphibians).

104 The foraging strategy is also known to impact the preferred sensory cues used by snakes to
105 locate their prey; ambush predators tends to rely more upon vision (Czaplicki and Porter 1974) or
106 mechanoreception (Westhoff et al. 2005; Catania et al. 2010) whereas active foragers track their
107 prey using chemical cues (Cooper 2008; Smargiassi et al. 2012). We divided foraging strategy in
108 3 categories: species actively chasing prey, ambushing, or doing both alternatively.

109 The properties of the environment in which an animal behaves influence the availability and
110 predictability of the sensory cues (Thewissen and Nummela 2008; Stevens 2013). For instance,
111 the chemical diffusion under water is slower but more traceable than in air. The light level and
112 spectrum decrease with depth under water, while sound is more efficiently propagated in water
113 than in air. Different media also retain cues of different nature; mud and slime retain more
114 hydrophobic odorant; water carries hydrophilic molecules and air small volatile compounds. We
115 divided the environment of species in two categories: foraging habitat and main habitat. We
116 defined the main habitat depending on the substrate in which the species spends most of its time
117 and divided it in 3 categories: aquatic species are the ones rarely found outside of water, semi-
118 aquatic species are found both on land and under water, and mud/fossorial species. The foraging
119 habitat is the medium in which species forage most of the time (i.e., land, water, or both). Some
120 species can forage in one media but rest in another, which would require more adaptability of the
121 sensory modalities of these species, whereas species that only are foraging in one media should
122 demonstrate more specialization.







123 The activity pattern (i.e., nocturnal, diurnal, cathemeral) is another aspect of the ecology of
124 species that impacts their sensory preferences. Nocturnality and low light environments (scotopic)
125 strongly impact the brain shape of vertebrates (Barton et al. 1995; Kaas 2017) in two alternative
126 strategies. Some species of birds or fish demonstrate a reduction of the visual system that is often
127 associated with reduction in size of the visual apparatus and the increase of another sensory
128 pathway (e.g. olfaction or mechanoreception); or alternatively show an increase of parts or the
129 whole visual apparatus to allow more light reception (Kotrschal et al. 1998; Kaas 2017). Diurnal
130 snakes are expected to rely more heavily on visual cues than cathemeral species that might use a
131 combination of sensory information.








132 Dimensionality and complexity of the foraging habitat have been correlated with shape and
133 size variations of some brain regions (i.e. cerebellum) in fish, birds and some squamates (Kotrschal
134 et al. 1998; Kaas 2017; Yopak et al. 2017; Macrì et al. 2019). Instead of considering habitat
135 dimensionality, we considered the dimensionality of the trophic interaction (Pawar et al. 2012),
136 which is the number of dimensions the predator is using to detect prey (e.g. 2D for grazers and 3D
137 for flying insect catchers). In a foraging context, the predator must quickly gather and process
138 reliable sensory information and adjust his behavior consequently, which might require specific

139 adaptation for snakes foraging in 3D environments. We considered snakes foraging in open water
140 to have a 3D interaction with their prey, and substrate-surface foraging species to have 2D trophic
141 interactions (e.g. crevice-foraging, coral reef probing or bottom-dwelling fish specialists).

142 These six ecological and behavioral characteristics have been demonstrated to be related to
143 the endocranial and/or brain shape in other groups of vertebrates. Previous work have highlighted
144 a link between brain or endocasts and evolutionary history in birds, fish and some squamates
145 (Kotrschal et al. 1998; Allemand et al. 2017; Yopak et al. 2017). However, this link becomes
146 weaker when the phylogenetic distance becomes small and when species are closely related, but
147 ecological influences become stronger (Kotrschal et al. 1998). To our knowledge, no work
148 attempted to gather all this information to create a complete ecological identity for a large number
149 of species and relate it to the endocranial shape. Because we do not only focus on the whole shape
150 of the endocranium, but also on its different regions, we summarized (see Table 1) our predictive
151 factors (with definitions), the way they could impact sensory cues, and the endocranial area that
152 might be impacted. For this study, we compared the endocranium of 36 snake species that are
153 geographically, phylogenetically, and ecologically diverse and show different degrees of aquatic
154 habits. We used μ -CT (computed tomography) scans of museum specimens; we characterize the
155 endocranium shape using a 3D geometric morphometric approach. This method allows us to
156 transform volume into a size and shape component and offers a more comprehensive approach to
157 investigate the morphology of the endocranium (Kawabe et al. 2013; Marugán-Lobón et al. 2016).
158 We used phylogenetic comparative methods to test for the relationship between sensory ecology
159 and shape, and a reclassification algorithm to assess whether the endocranium shape could be used
160 to infer the sensory ecology of elusive or fossil snake species.

161 **Table 1:** Ecological characteristics of interest and hypothetical impacts on the sensory modalities and endocranial shape of snakes.

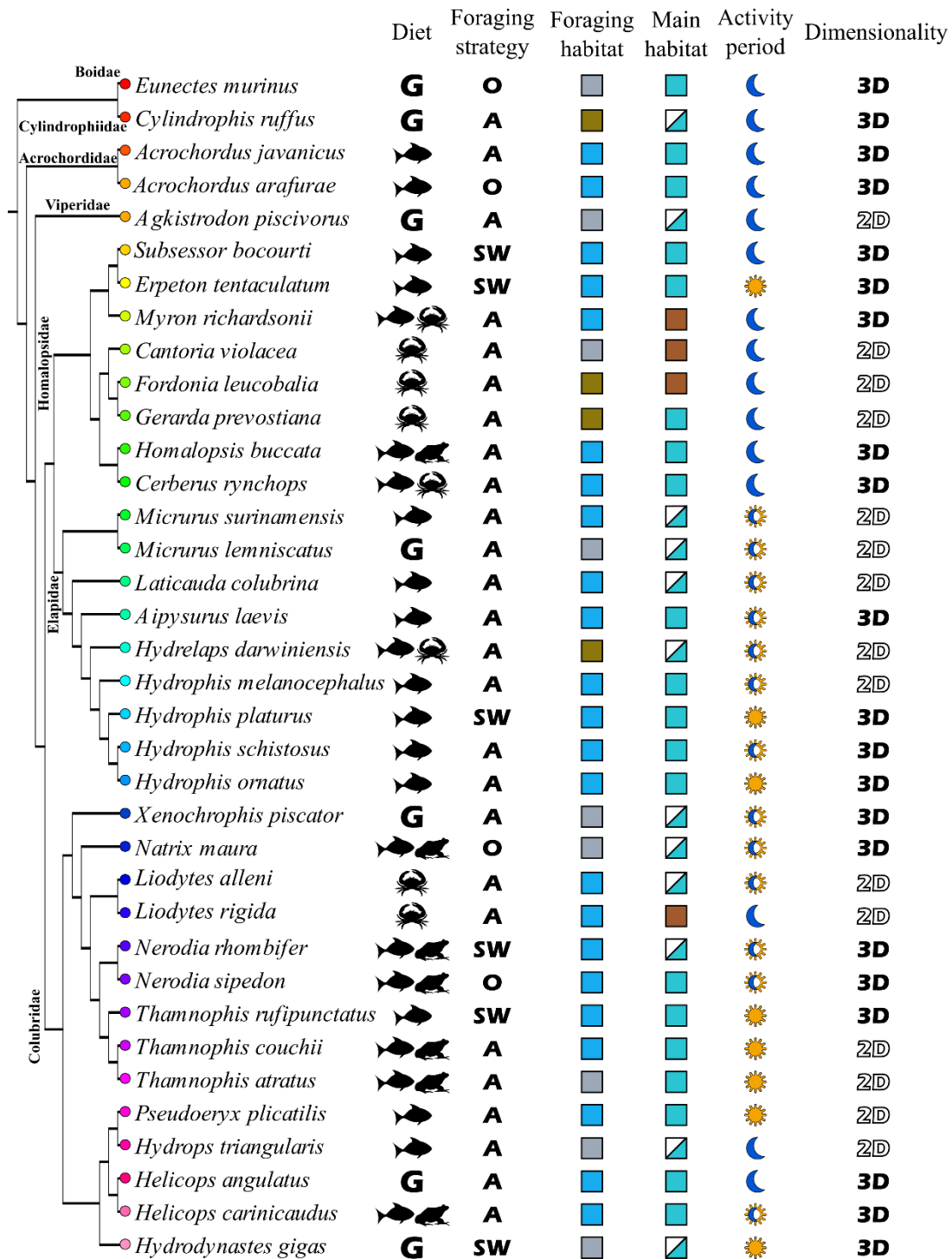
Ecological Factor	Categories	Comment	Impact on sensory modalities	Potentially impacted endocranial area
Diet type	Fish specialist 	Some species of snakes are specialized in specific fish species (e.g., <i>L. colubrina</i>)	Fish specialists need to accurately detect and recognize their specific prey either through olfactory or visual cues (Kutsuma et al. 2018).	
	Crustacean specialist 	Can be either fully aquatic or semi-aquatic Some species specialized in freshly molted or hard crustaceans	Crustacean specialists rely on chemical cues to detect their prey (Mark Waters and Burghardt 2005) and show complex manipulation (Jayne et al. 2002; Noonloy et al. 2018) which may be reflected in their olfactory tract and cerebellum.	➤ optic tract ➤ olfactory tract
	Fish and crustaceans 	Mostly semi-aquatic species	Fish and amphibians are fast moving preys that are generally detected by snakes using vision and/or mechanoreception (Czaplicki and Porter 1974; Camilleri and Shine 1990; Catania et al. 2010) while fish and crustacean eaters can rely on chemo- and mechanoreception, but also on vision.	➤ cerebral hemispheres ➤ cerebellum
	Fish and amphibians 	Mostly semi-aquatic species		➤ cranial nerves
	Generalist G	More opportunistic species Can catch either aquatic or non-aquatic prey Usually more terrestrial species	Generalist snakes are expected to show no specialization related to diet as they must detect and catch preys in two different media and are opportunistic.	
Foraging strategy	Active A	Tracking a prey	Rely on persistent cues such as chemical and/or visual cues.	➤ optic tract
	Sit-and-wait SW	Fast triggering cue in a close range	Rely more on mechanoreception or visual cues.	➤ olfactory tract ➤ cerebral hemispheres
	Opportunistic O	Using one or the other technique	Same as generalists.	➤ cranial nerves (trigeminal)
Foraging habitat	Water 	- Odorant: hydrophilic molecules, low diffusion speed, high predictability of the source position - Visual cues: light level and spectrum decrease with depth and turbidity, high refractive index - Acoustic cues and mechanoreception: high velocity and impedance	Fully aquatic species rely more on chemical cues if active forager and acoustic/mechanoreception if sit-and-wait (Camilleri and Shine 1990). They use visual cues to locate preys (Kutsuma et al. 2018) and strike at moving objects (Czaplicki and Porter 1974; Catania et al. 2010). Depending on associated dimensionality, aquatic snakes might need to process sensory cues in 3D.	➤ olfactory tract ➤ cerebral hemispheres ➤ cerebellum
	Land 	- Odorant: volatile molecules, high diffusion speed, impaired predictability - Visual cues: good during daylight if no obstruction, low refractive index - Acoustic cues and mechanoreception: low velocity and impedance	Mostly terrestrial snakes probably rely on vision and vibrational or airborne acoustic cues during foraging as these are more accurate and traceable on land (Young et al. 2008).	➤ optic tract ➤ cranial nerves (trigeminal & VIII)

	Both 	Difference of refractive index when changing media	Requires adaptability in the sensory modalities because of the difference in the signal nature and propagation in different media.	
Main habitat	Aquatic 	See “water” in Foraging habitat	Some aquatic species use vision to find mate (Shine 2005). Sea snakes show a diversification of the visual pigments suggesting that vision is important in their ecology (Simões et al. 2020). Depending on associated dimensionality, might need to process sensory cues in 3D.	➤ olfactory tract ➤ cerebral hemispheres
	Semi-aquatic 	Change of refractive index when changing media	Some semi-aquatic snakes evolved accommodation mechanisms suggesting that vision is important on both land and in water (Schaeffel and de Queiroz 1990; Schaeffel and Mathis 1991).	➤ cerebellum ➤ optic tract
	Mud 	Negative impact on vision, long persistence time of chemical cues, acoustic or mechanical cues probably less reliable	Mud snakes must rely on vomerolfaction or olfaction, and their optic tract might have regressed compared to the aquatic and semi-aquatic species.	➤ cranial nerves (trigeminal & VIII) and otic area ➤
Activity period	Diurnal 	Foraging during day light	Diurnal snakes have larger eyes (Liu et al. 2012; Da Silva et al. 2017) suggesting that they might heavily rely on vision.	➤ optic tract
	Nocturnal 	Foraging in low light environment	Two alternative strategies: - enlargement of visual system to capture more light - reduction of the visual system and reliance on chemical and/or mechanical cues.	➤ olfactory tract ➤ cerebral hemispheres
	Cathemeral 	Sporadic activity during the day or night	Requires the use of various cues depending on the light level.	
Prey-predator interaction dimensionality	3D	Foraging in open water	Requires precise stereo sensory information and a more complex signal processing which may be reflected in their cerebellum.	➤ cerebral hemispheres
	2D	Foraging along a substrate/surface	Simpler sensory cues to process	➤ cerebellum (Macrì et al. 2019)

163 **Material and Methods**

164 *Endocranium acquisition*

165 We compared 36 species that cover both the phylogenetic and ecological diversity of snakes, from
166 fully aquatic, highly specialized to generalist species (Fig. 1). We included 2-6 adult specimens
167 per species (see list in Supplementary Material 1) from several museum collections (AMNH,
168 FMNH, CAS). In total, the skulls of 98 specimens were scanned using the X-ray μ CT-scanner
169 (2010 GE phoenix v|tome|x s240 high-resolution microfocus computed tomography system,
170 General Electric, Fairfield, CT, USA) at the Microscopy and Imaging Facility at the AMNH (New
171 York, NY, USA). Scans were performed with a voltage between 100-150kV and current between
172 130-160 μ A for a voxel size between 15.6-57.4 μ m. The 3D reconstruction was performed using
173 the software Phoenix datos|x2 and the subsequent segmentation was done using VGStudioMax v.
174 3.0 (Volume Graphics GmbH, Heidelberg, Germany). Previous studies used virtual/digital brain
175 endocasts which are obtained by manually filling the endocranial space (Olori 2010; Balanoff et
176 al. 2016; Carril et al. 2016; Allemand et al. 2017). This technique is widely used but raises many
177 questions regarding the repeatability and biological accuracy of the obtained 3D objects, especially
178 concerning the foramina and fenestrae (Balanoff et al. 2015). To avoid any bias or extrapolation,
179 our landmarks were placed directly on the internal surface of the reconstructed skull (i.e. the
180 endocranium) using the software MorphoDig 1.2 (Lebrun 2017). Some areas related to sensory
181 structures of the brain are easily identifiable in the endocranium and were used to test our
182 hypotheses, namely the olfactory tract, the cerebral hemispheres, the optic tract, the cerebellum,
183 and the area where the cranial nerves meet the brain, that we named cranial nerve area
184 (Supplementary Material 2b.). This area is also shaped by the presence of the inner ear and can
185 give us a proxy for the shape of the otic capsule. We included the infundibulum and pituitary gland
186 in our analyses despite these structures are not involve in the sensory system but rather in hormone
187 secretions (Fig. 2).



188

189 **Fig. 1: Phylogeny of the 36 included snake species** (reduced phylogeny from Pyron & Burbrink,
 190 2014) along with ecological and behavioral characteristics: diet type: generalist **G**, piscivorous
 191 , fish and amphibians , crustaceans , fish and crustaceans ; foraging strategy:
 192 active **A**, sit-and-wait **SW**, opportunistic **O**; foraging habitat: land , water , both ; main
 193 habitat: aquatic , mud , semi-aquatic ; activity period: diurnal , nocturnal , cathemeral
 194 ; dimensionality: **2D** or **3D**

195

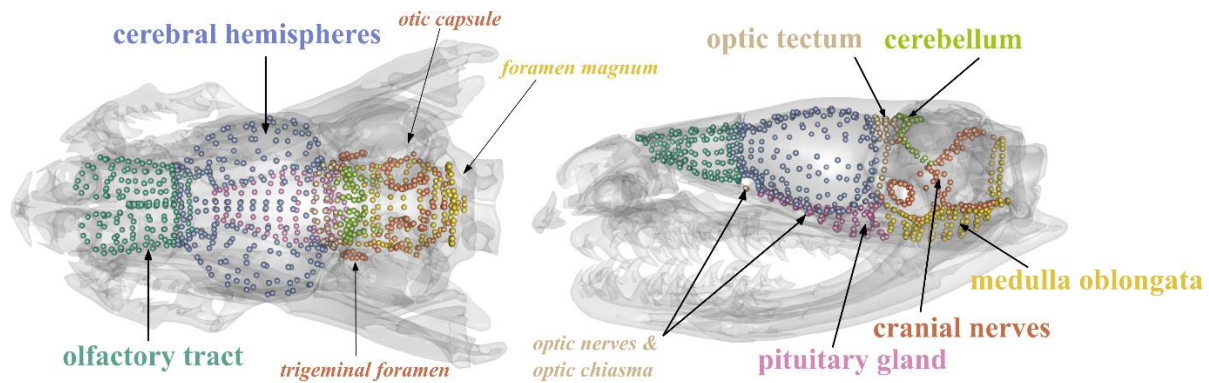
Geometric morphometrics

196 We created a template of the endocranium using a total of 848 landmarks: 73 anatomical
197 landmarks, 425 curve semi-landmarks and 350 surface semi-landmarks (Fig. 2, see Supplementary
198 Material 2). We placed the anatomical landmarks and curve semi-landmarks by hand on each
199 specimen. Then, we used the ‘Morpho’ package (Schlager 2015) to project and relax the surface
200 semi-landmarks of the template on each specimen. Finally, the curve and surface semi-landmarks
201 were allowed to slide on each specimen while minimizing the bending energy between the
202 specimen and the mean shape landmark configuration (Gunz and Mitteroecker 2013). To obtain a
203 mean shape for each species, we performed a Procrustes superimposition (GPA) of the specimens
204 of each species separately and we symmetrized the configurations using the function *procSym* of
205 the ‘Morpho’ package. We resized the obtained mean shape per species using their mean centroid
206 size and obtained our array of species configuration. Finally, we performed another Procrustes
207 superimposition on the species mean shapes using the function *gpagen* of the ‘geomorph’ package
208 (Adams et al. 2020). We used Procrustes coordinates as the shape variable to test our hypotheses.
209 Additionally, we performed a Principal Component Analysis (PCA) using the *plotTangentSpace*
210 function in ‘geomorph’ to test our hypothesis on a subset of variables, as inferential tests are
211 sensitive to the number of variables. We chose to test two subsets: 90% of the overall shape
212 variability (PC90: first 9 Principal Components) and the meaningful PC using the function
213 *getMeaningfulPCs* in the ‘Morpho’ package (mPC: 2 first PCs; Supplementary Material 3). For
214 the endocranial areas analyses, we used subsets of the mean species array and performed a GPA
215 using *gpagen* as we did for the whole endocranium (Fig. 2, Supplementary Material 2b.). We
216 performed the same statistical analysis on each area as we did for the whole endocranium.

217

Sensory-related traits

218 We defined each species characteristics based on the literature and on personal observations for
219 some less documented species (Fig. 1, see Supplementary Material 1 for a complete reference
220 source of these data). We summarized in Table 1 the list of the ecological and behavioral
221 characteristics we considered, along with the associated impact on the sensory modalities and the
222 area of the endocranium that could hypothetically be impacted.



223

224 **Fig. 2: Template of the endocranium of snake and its areas.** Skull of a specimen of *Cantoria*
 225 *violacea* (CAS11909) in dorsal (left) and lateral (right) view rendered partly transparent to show
 226 the 848 landmarks and semi-landmarks used. Colors correspond to brain areas (details in
 227 Supplementary Material 2b.). Important structures mentioned in this manuscript are indicated in
 228 *italic and colored according to the area they belong to*

229 *Analyses*

230 We estimated the phylogenetic signal in the endocranium using the multivariate K-statistic
 231 (Blomberg et al. 2003; Adams 2014a) implemented in the ‘geomorph’ package (Adams et al. 2020)
 232 using 1000 random permutations. We tested for the phylogenetic signal in both the Procrustes
 233 coordinates and on each PC (Supplementary Material 3). We found a phylogenetic signal in the
 234 endocranium (Table 2) and in 5/9 PCs ($P < 0.001$), but not PC1 ($P = 0.08$) (Supplementary Material
 235 3). We ran phylogenetic ANCOVA to test the effect of our predictive variables on the shape of the
 236 endocranium and its sensory areas using the function *procD.pgls* in ‘geomorph’ (Adams 2014b).
 237 To avoid over-parametrization of the models, each relevant factor was tested separately with size
 238 as covariate for each endocranial structure following hypotheses in Table 1. Only factors showing
 239 a significant signal were kept in the final models. We used the log-corrected centroid size as a
 240 covariate to test for evolutionary allometry. We assessed the statistical significance of the
 241 predictive variables by performing 1000 permutations of the phenotypic data at the tip of our
 242 branches. We used the same procedure for the analyses of each endocranial area. Size was removed
 243 from the ANCOVA when its distribution was not normal even after transformation, and allometry
 244 was tested separately from the predictive factors. We used the function *shape.predictor* and
 245 *mshape* from ‘geomorph’ to respectively extract the shapes associated with the allometry and other
 246 significant factors. Finally, we tested whether the shape of the endocranium or its sensory areas
 247 could be used to infer the sensory ecology of species for which we have little to no ecological,

248 behavioral, or phylogenetic information (e.g., elusive, or fossil species). We used a k-nearest
249 neighbour algorithm (k-NN) combined with a leave-one-out cross validation, using the function
250 *knn.cv* from ‘class’ package (Ripley and Venables 2020), to assess the reliability of sensory-
251 ecology classification of species based on shape data. To classify a data point of interest, this
252 pattern recognition method uses its k-nearest neighbours in terms of Euclidean distance and
253 performs a majority vote to determine which class the point belongs to. k-NN associated with a
254 cross-validation is considered to be one of the most powerful machine learning algorithms in terms
255 of predictive power and accuracy. Yet, as with many other methods, k-NN suffers from the curse
256 of dimensionality, thus we used our PC90 subsets (>90% of the shape variability for each
257 structure). The optimum k was determined for each test as follow: 1) $k > 1$ to avoid overfitting, 2)
258 k must be inferior to the number of species in the smallest class to avoid underfitting (i.e., $k < 8$ for
259 activity classification and $k < 4$ for foraging habitat), 3) k with the maximum classification
260 accuracy. We choose not to correct for phylogenetic relationship to assess whether an accurate
261 classification of fossil would be possible without knowing its relationship with extant species. The
262 significant phylogenetic signals, along with our main results show that endocranial shape variation
263 is, at least partly, structured by phylogenetic relationship between species (Fig. 3). Thus, closely
264 related species are often the nearest neighbours in terms of the Euclidean distance, so if $k=1$, the
265 chances of the nearest neighbour being close because of phylogeny and not ecology is high.
266 Therefore, we choose $k > 1$ to alleviate this potential issue. We used the *knn.cv* function from the
267 ‘class’ package (Ripley and Venables 2020). All geometric morphometric, statistical analyses and
268 visualizations were performed in R version 3.4.4 (R Core Team, 2018) (R code and data available
269 in Supplementary Material), except the landmark acquisition performed in MorphoDig (Lebrun
270 2017). All statistical results are available in Table 2.

271 **Results**

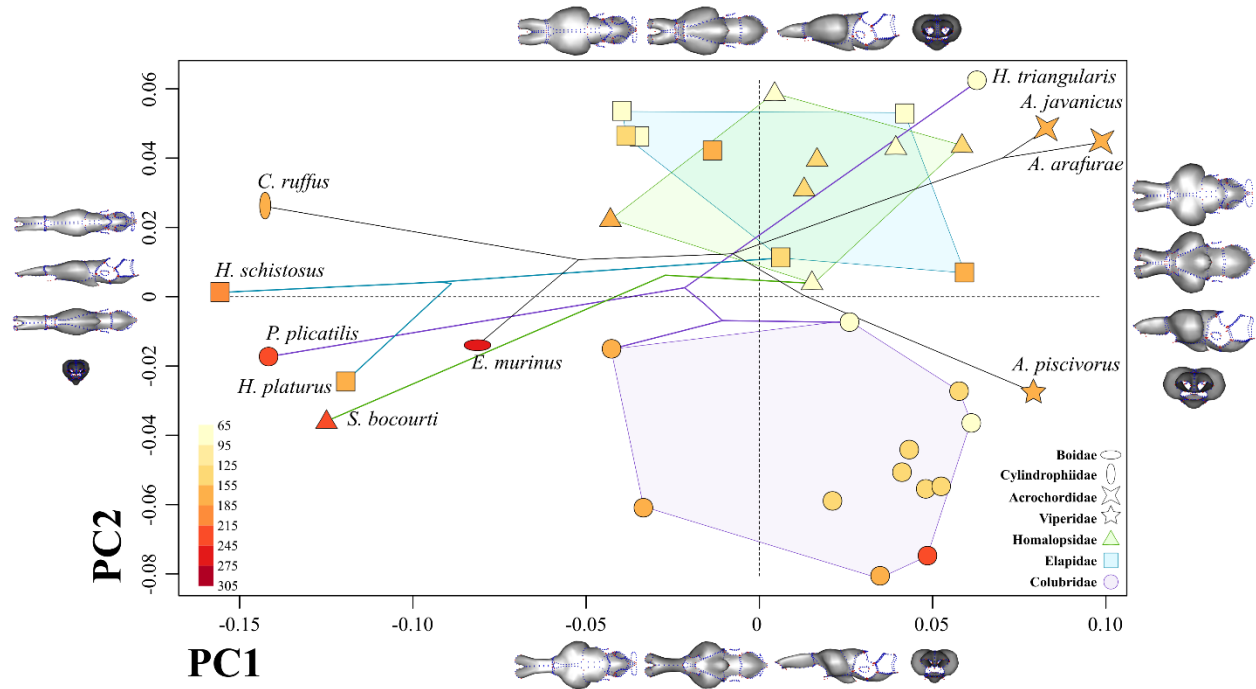
272 *Phylogeny and allometry*

273 Evolutionary allometry accounts for 34% of the overall variability and respectively 40% and 49%
274 of the PC90 and mPC subsets (Table 2). These results are illustrated in Figure 3, in which size
275 drives the variation along PC1, which accounts for 45.3% of the overall shape variability. Larger
276 species gather on the negative side of the axis (PC1-), and smaller species on the positive side

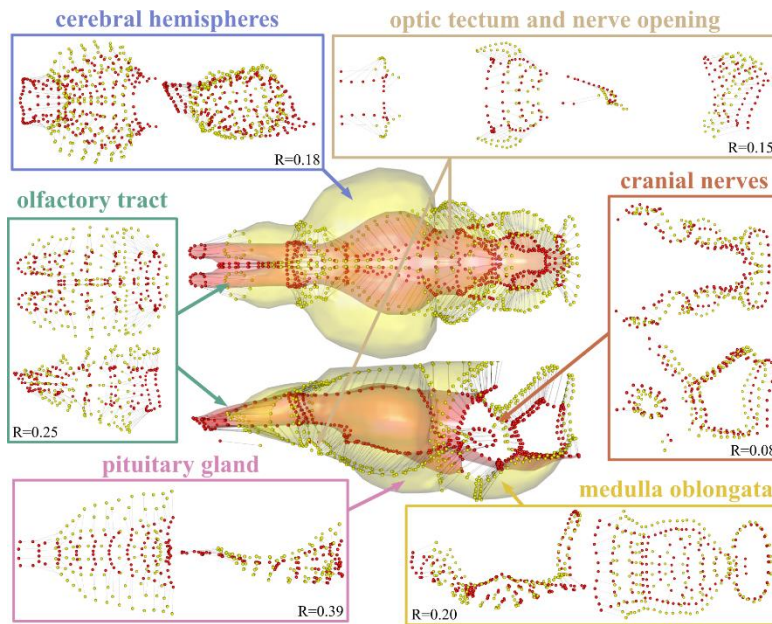
277 (PC1+). Larger endocrania are characterized by antero-posteriorly elongation while small
 278 endocrania are short, bulky, and laterally expanded (PC1+ and PC1- shapes in Fig. 3 and Fig. 4).
 279 The variation along PC2, which accounts for 17.8% of the variability, is driven by phylogenetic
 280 differences between smaller species: on the positive side (PC2+) gather the Homalopsidae,
 281 Elapidae and Acrochordidae, while the Colubridae and the only Viperidae occupy the negative
 282 side of PC2 (PC2-) (Fig. 3). All endocranial areas showed a significant phylogenetic signal (Kmult
 283 [0.32-0.42]; $P < 0.015$) (Table 2). Allometries were present to different extents in all the
 284 endocranial areas, except the cerebellum (Table 2) for which size was removed from the
 285 subsequent analyses. The allometric trends for the individual areas follow the allometric pattern
 286 described in the whole endocranium but some areas vary more (e.g., pituitary gland) or less (e.g.,
 287 cranial nerves) depending on their R^2 coefficient (Fig. 4). Globally, the rear part of the
 288 endocranium, where the cranial nerves insert in the brainstem, shows less allometry, especially the
 289 foramen magnum (Fig. 4).

290 **Table 2:** Summary of statistics: phylogenetic signal (K , P -value), number of principal components
 291 that carry more than 90% of the variability (PC90), number of meaningful PCs (mPC), results
 292 from the phylogenetic ANCOVAs (D-PGLS) tested on the Procrustes coordinates, 90% of the
 293 variability and the meaningful PC based on 1000 permutations. For more clarity, only the
 294 significant results are indicated.

Region	Physignal	PC90	mPC	Factor	D-PGLS coordinates			D-PGLS PC90			D-PGLS mPC		
					R	F	P	R	F	P	R	F	P
Endocranium	K= 0.38 P<0.001	1-9	2	Size Activity	0.34 0.07	18.95 2.11	0.001 0.041	0.4	23.97	0.001	0.49	34.47	0.001
Olfactory tract	K= 0.35 P<0.001	1-7	1	Size Activity	0.25 0.13	13.22 3.39	0.001 0.002	0.29 0.13	15.93 3.59	0.001 0.003	0.42 0.14	30.78 5.13	0.001 0.013
Optic tectum and nerves	K=0.45 P<0.001	1-5	1	Size Activity	0.15 0.13	6.52 2.94	0.001 0.02	0.17 0.14	7.72 3.25	0.001 0.025	0.15	6.8	0.019
Cerebellum	K=0.42 P<0.001	1-7	0	Foraging Habitat	0.13	2.58	0.034	0.013	2.47	0.05			
Cerebral hemispheres	K=0.43 P= 0.002	1-5	1-2	Size	0.18	8.26	0.001	0.21	9.43	0.001	0.23	10.97	0.001
Cranial nerves	K=0.41 P<0.001	1-13	1	Size	0.08	3.32	0.013	0.09	3.74	0.013	0.18	8.16	0.017
Pituitary gland	K=0.32 P=0.015	1-5	1	Size	0.39	21.65	0.001	0.43	25.9	0.001	0.52	36.85	0.001
Medulla oblongata	K=0.34 P=0.004	1-9	1	Size	0.20	8.72	0.001	0.23	10.41	0.001	0.49	33.53	0.001



295
 296 **Fig. 3: Main axes of shape variation in the endocranium.** Scatter plot of the principal
 297 components one and two (PC1 & PC2) representing respectively 45.3% and 17.8% of the
 298 endocranial shape variance among the 36 snake species. Each dot represents one species, the
 299 symbols correspond to the species' family (legend bottom right corner) and the color corresponds
 300 to the centroid size of their endocranium (color scale in mm bottom-left corner). Colored polygons
 301 correspond to species grouping with their family and the colored lines indicate the phylogenetic
 302 link between outlier species and the rest of their family, black lines show the link between families
 303 with only 1 or 2 species. These links were generated using the function *phylomorphospace* in
 304 'phytools' (Revell 2012). On each PC extreme are positioned dorsal, lateral, ventral, and frontal
 305 views of the corresponding reconstructed endocranium



306

307 **Fig. 4: Evolutionary allometry in the endocranium and its areas.** Yellow: smaller species, red:
 308 larger species. Central position: allometry in the whole endocranium. Each panel shows the
 309 allometric shape variation for each area. The R coefficients are indicated in each panel. Individual
 310 patterns of allometry for each area follows the general allometry pattern observed in the whole
 311 endocranium

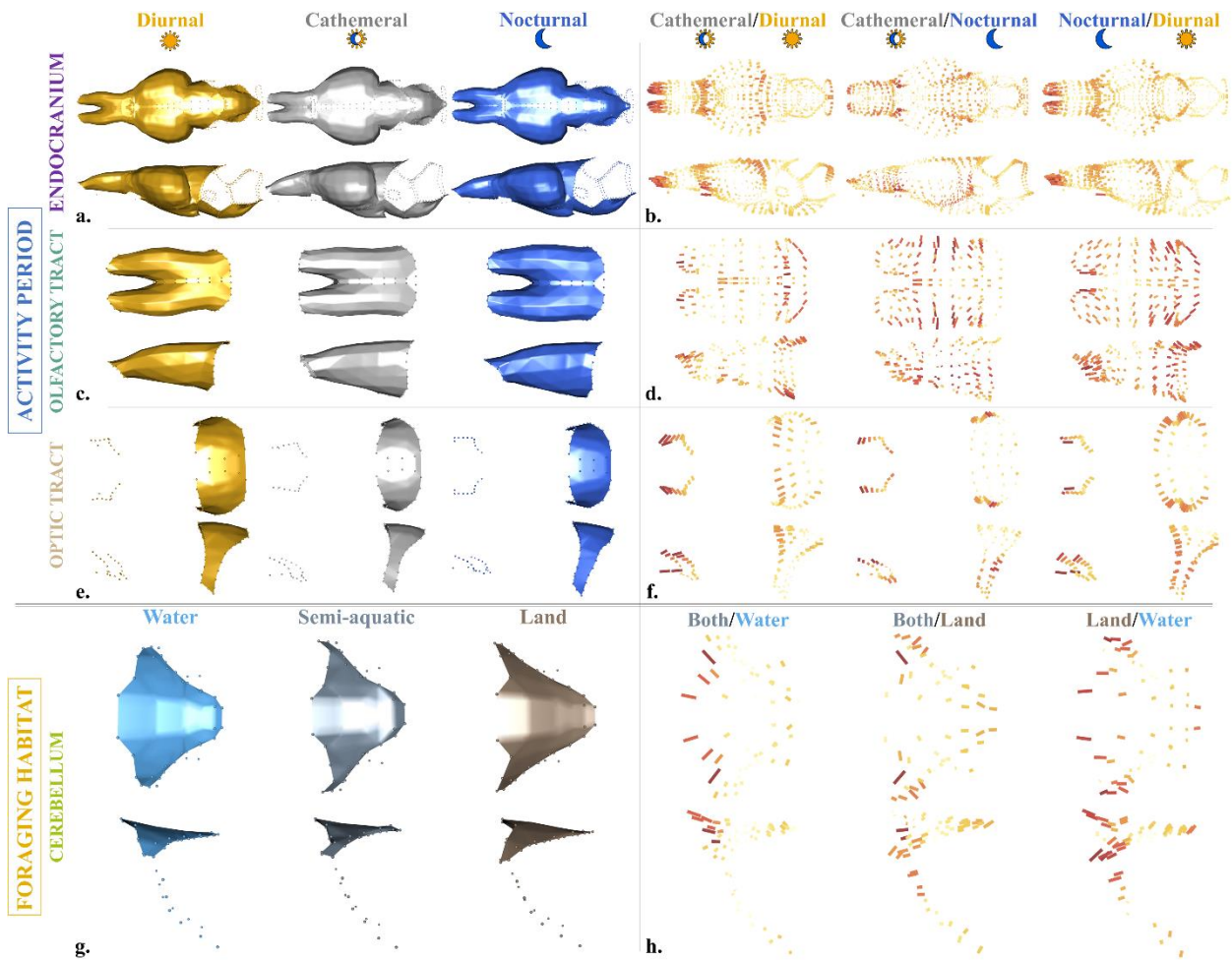
312 *Activity period*

313 The activity period is also significantly related to the endocranium shape but in smaller
 314 proportion than size, and it is not significant in the subsets (Table 2). The main shape variations
 315 between groups are concentrated in the areas responsible for olfaction and vision (Fig. 5b.).
 316 Coherently, the activity period is significantly related to the shape of the olfactory and optic tracts,
 317 their 90% variability subsets and the meaningful PC of the olfactory tract (i.e., PC1) (Table 2).
 318 Diurnal species have more elongated and bifurcated olfactory bulbs and their whole olfactory tract
 319 is the slenderest (Fig. 5c.), they also have the widest and longest, posteriorly extended, optic tract
 320 (Fig. 5e-f.). The endocranium of cathemeral species has an intermediate profile but shows less
 321 difference with the nocturnal species (Fig. 5b.). Cathemeral species have the shortest bifurcation
 322 of the olfactory bulbs (Fig. 5c-d.). Their optic tectum is of intermediate proportion compared to
 323 the diurnal and nocturnal species, with shorter distances with diurnal species in the middle part of
 324 the olfactory tract, while showing less variation in the most distal parts when compared to the
 325 nocturnal group (Fig. 5f.) but the anterior part of the optic tract (i.e., optic nerve opening) is the
 326 narrowest (Fig. 5e.). Nocturnal species have the shortest and bulkiest olfactory tract, but the

327 septum is slightly more posteriorly positioned than the cathemeral species (Fig. 5c-d.). The
 328 nocturnal species have a thin and rather slender optic tract (Fig. 5e.).

329 *Foraging habitat*

330 The shape of the cerebellum and its 90% variability subset are significantly associated with
 331 the foraging habitat of species (Table 2). The cerebellum of aquatic predators is more anteriorly
 332 and dorsally expanded but more laterally compressed, whereas the mainly terrestrial foragers have
 333 a more laterally expanded cerebellum on its anterior part, but its posterior part is more elongated
 334 and slenderer, while the semi-aquatic foragers show an intermediate shape (Fig. 5 g-f.).












335 g.
 336 **Fig. 5: Summary of the morphological variations of the endocranial structures related to the**
 337 **activity period of species (a-f) or foraging habitat (g-h) of species. a, c, e, g: Mean shapes for**
 338 **each group in dorsal (top) and lateral (bottom) views. b, d, f, h: Pairwise shape differences**
 339 **between groups represented by distance vectors. Vector size and color depending on the distance**

340 *between two corresponding landmarks, from short, pale yellow to long, dark red. Color palette*
 341 *generated using the 'scico' package (Pedersen and Cramer 2020)*

342 *Reclassification in ecological groups*

343 The reclassification accuracy of the activity period of species based on their olfactory tract and
 344 optic tract shape is respectively 52.8% and 63.9% (Table 3). The best reclassifications for the
 345 activity period are the cathemeral and nocturnal species based on their optic tract, but the diurnal
 346 species are always misclassified (Table 3). For both areas, diurnal species are mostly misclassified
 347 in the cathemeral group (62.5-75%), but little to no cathemeral species are misclassified as diurnal
 348 (0-8%). We did not use the k-NN algorithm here as it cannot be performed on the Procrustes
 349 coordinates and as our PC subsets for the endocranium did not show a significant signal for the
 350 activity period. Foraging habitat reclassification accuracy based on cerebellar shape is also low,
 351 with no correct classification of the terrestrial and semi-aquatic foragers but a high score for
 352 aquatic predators (Table 3).

353 **Table 3:** *Reclassification accuracy of the ecology of species based on 90% of the morphological*
 354 *variability of each structure (PC90) using a k-NN algorithm. Below the name of the structure are*
 355 *indicated k (the number of considered neighbors) and the overall reclassification accuracy. Bold*
 356 *indicates the percentages of correct reclassification per group.*

ACTIVITY PERIOD						FORAGING HABITAT				
OLFACTORY TRACT			OPTIC TRACT			CEREBELLUM				
k=5 52.8%			k=4 63.9%			k=4 58.3%				
						Water	Both	Land		
	12.5	75	12.5	0	62.5	37.5	91	4.5	4.5	Water
	8	54	38	0	85	15	89	0	11	Both
	13	13	73	7	13	80	25	75	0	Land

357 **Discussion**

358 *Size, Phylogeny and Endocranium*

359 The repartition of species along the main axes of shape variation (Fig. 3) can be described by three
 360 clusters: 1) large species from different snake families, 2) the Elapidae and Homalopsidae and 3)

361 the Colubridae. The main driver of endocranial shape variation is size which explains about 34%
362 of the overall variability, and half the variation of the two first components, with the minima of
363 PC1 and PC2 being driven by larger species (Fig. 3). Head dimensions are highly correlated with
364 the centroid size of the endocranium (Supplementary Material 4): species with larger heads have
365 a more elongated and slenderer endocranium, while small-headed snakes have a shorter and
366 bulkier endocranium. This pattern of globular shape and lateral extension in small species is also
367 consistent with mammals (“squirrels”: Bertrand *et al.*, 2019, marsupials (Weisbecker *et al.* 2021),
368 and birds: Kawabe *et al.*, 2013; Marugán-Lobón *et al.*, 2016). The shape difference provides
369 smaller species with a relative endocranial volume almost 4 times greater than the larger species,
370 when they are at the same scale (i.e., centroid size=1). Such a difference in relative volume will
371 certainly provide an advantage for the encephalization of species with small heads, and therefore
372 potentially improve their cognitive abilities (Iwaniuk 2017). Additionally, a more globular shape
373 allows the reduction in length of the neuronal connections which has been demonstrated to increase
374 information processing speed (Sepulcre *et al.* 2010; Balanoff and Bever 2017). Some of our
375 preliminary results indicate that the globular shape of the small species is similar to the shape of
376 the endocranium of juvenile specimens from both small and large species of snakes. More data are
377 needed to confirm the generality of these observations, but it seems that the evolutionary allometric
378 variation originates from a retention of juvenile features (i.e., paedomorphism) in adult specimens
379 of small species, while larger species develop an elongated endocranium.

380 Phylogenetic relationships also contribute to the morphology of the endocranium but to a
381 lesser extent than size. Except for the larger species, the other species tend to cluster by family
382 along PC2, forming two main clusters: a cluster grouping the Elapidae, Homalopsidae and
383 Acrochordidae (PC2+), and another with the Colubridae (PC2-) (Fig. 3). Colubrids appear to have
384 followed their own evolutionary path regarding endocranial shape. They show the largest
385 respective size of the optic tectum compared to other shapes (Fig. 3) which is not surprising as
386 they seem to heavily rely on visual cues to detect prey (Franz 1977; Schaeffel and de Queiroz
387 1990; Alfaro 2002). Some species have even developed accommodation mechanisms to adapt their
388 visual acuity to both air and water media (Schaeffel and de Queiroz 1990; Schaeffel and Mathis
389 1991). Elapid, Homalopsid and Acrochordid snakes also use visual cues but these are not sufficient
390 to elicit a strike and are usually coupled with either mechanical (i.e. pressure variation or tactile)
391 or chemical cues (Kropach 1975; Voris *et al.* 1978; Heatwole 1999; Shine *et al.* 2004b; Vincent *et*

392 al. 2005; Catania et al. 2010). Evidence shows that the ancestral state of the visual system of snake
393 was adapted to low-light conditions probably associated with a nocturnal lifestyle (Schott et al.
394 2018). This is corroborated in our phylogeny with the nocturnal species representing more basal
395 lineages, while the more distal taxa (i.e., colubrids) tend to be more cathemeral or diurnal. In
396 addition, previous studies demonstrated genetic and molecular specializations of their visual
397 system (Simões et al. 2016; Schott et al. 2018). Colubrids are also characterized by a long and
398 fused olfactory tract that connects to medially expended cerebral hemispheres through a bulbous
399 region. Both structures are involved in transmitting and processing odorant cues, which are crucial
400 in prey-predator interactions and social behaviors (Halpern and Kubie 1984). While none of the
401 feeding-related factors shows any significant relationship with the global shape of the
402 endocranium, the specific shape observed in Colubrids could be explained by other activities such
403 social behaviors (Skinner and Miller 2020).

404 The large shape variation in the endocranium that we report here is not fully explained by size
405 or sensory ecology, suggesting that other behavioral or cognitive abilities may also be influencing
406 endocranium morphology. Although of great interest to us, it is currently impossible to draw a
407 direct link between endocranial shape and the evolution of elaborated behaviors in snakes, due to
408 the scarcity of data on snakes' cognitive abilities, and the phylogenetic bias of published studies
409 toward colubrids and viperids. We encourage further work to explore this potential, once it
410 becomes possible to better characterize and quantifying snake behavior in a broad comparative
411 context. It should then be able to draw stronger links between behavior and endocranium
412 morphology, and make reliable inferences not only about the ecology, but also the cognitive
413 abilities of extinct species based on their endocasts.

414 *Activity pattern, Endocranium, Olfactory and Optic tracts*

415 Although the shape of the endocranium is significantly related to the activity pattern of species,
416 most of this variation is confined to the sensory areas dedicated to olfaction and vision. Diurnal
417 and nocturnal species are the most different, while cathemeral species share similarities with both
418 groups but are overall closer to the nocturnal species. In our predictions, we proposed two
419 alternative strategies for species living in low light environments, either an enlargement of the
420 visual system or a reduction accompanied by a compensation with another sense, such as olfaction.

421 Our results demonstrate that the optic tectum in nocturnal species is slightly reduced compared to
422 diurnal species, while their olfactory tract is shorter but stouter. A similar pattern was demonstrated
423 in fish in which the most interspecifically variable areas were associated with vision, olfaction,
424 and taste (North American Shiners), and some scotopic species exhibit an enlargement of the
425 olfactory bulbs (e.g. benthic sharks, nocturnal eel-like *Calamoichthys*) (Kotrschal et al. 1998). We
426 also found a widening of the optic tectum and a shortening of the distance between nerve entry
427 and optic tectum in diurnal snake species compared to the other two groups. The hypothesis of
428 specialization of the optic tract shape in diurnal species is in accordance with previous studies on
429 genetic and molecular specialization of vision in this group (Simões et al. 2016; Schott et al. 2018).
430 As previously mentioned, it seems the visual system of snakes was originally suited for a scotopic
431 environment (Schott et al. 2018) which might explain the lack of significant difference between
432 cathemeral and nocturnal species, while diurnal species developed a more derived visual system
433 (e.g. visual adaptation in diurnal and aquatic species: Hibbard & Lavergne, 1972; Schaeffel & de
434 Queiroz, 1990; Da Silva *et al.*, 2017).

435 *Foraging habitat and Cerebellum*

436 In non-mammalian vertebrates, the cerebellum is involved in locomotor abilities such as
437 coordination of movements or agility, and is also the regulatory center for sensory inputs, and
438 probably involved in some higher cognitive functions such as memory and emotions (Yopak et al.
439 2017). A comparative study in squamates demonstrated a link between locomotor mode and the
440 cerebellum shape and organization (Macrì et al. 2019). Yet, they did not test for the habitat and
441 considered the 11 snake species they tested as “multi-habitat”. Given the amount of overlap they
442 highlighted in the shape of the different brain structures in snakes, and considering they defined
443 the locomotor mode of species partly based on their “habitat use”, it seemed coherent and
444 complementary to test for this parameter in our study, especially as species in our dataset mainly
445 use one type of locomotion on both land and under water (i.e., undulatory movements). However,
446 moving on land or under water requires different locomotor abilities and sensory inputs which is
447 reflected in the cerebellum. Foraging habitat contributes only partly to the shape of the cerebellum
448 (Table 2). It would be interesting to complete our results with more anatomical and histological
449 data, as in Macrì *et al.* (2019), focusing on aquatic and semi-aquatic species. We should then be
450 able to draw more general conclusions about the contribution of locomotion and habitat in driving

451 cerebellar shape in snakes. It should also be noted that the habitat in which a species spends most
452 of its time (i.e., its main habitat) is not statistically significant in our study while the foraging
453 habitat is, suggesting that the predator-prey interaction imposes more constraints on the sensory
454 adaptation of species. More precise definitions of “habitat use” in combination with locomotion
455 purposes (e.g., predation, thermoregulation, reproduction) should be considered as they probably
456 affect the brain differently and might allow more refined conclusion on the relationship between
457 the sensory ecology of species and their brain morphology.

458 *Can endocranium shape predict sensory ecology in snakes?*

459 One of the goals of this study was to determine if we could make accurate inferences on
460 the sensory ecology of species based on the shape of the endocranium and/or its areas. We had
461 three candidate structures, namely the optic tract and olfactory tract for the activity period and the
462 cerebellum for the foraging habitat. We chose a method that is powerful and rigorous but cannot
463 be applied to Procrustes coordinates which forced us to exclude some structures for which subsets
464 revealed they were not significantly associated with sensory ecology (i.e., endocranium). Overall,
465 we found that accurately predicting the activity pattern of species based on the shape of their visual
466 or olfactory related areas, without a priori knowledge on their phylogenetic relationship was not
467 possible. The low reclassification accuracy based on the olfactory tract could be due to the
468 dominance of allometry (Table 2). These results could be related to the imbalance of the groups in
469 our dataset (e.g., the small species sample, where some ecological groups were only represented
470 by 4 species), or it might indicate that, overall, inferring the sensory ecology of species without
471 knowing their phylogenetic relationships with other species is virtually impossible based on
472 ordinate data. These hypotheses should be tested on a broader range of species and ecologies. In
473 addition, using Procrustes coordinates rather than principal component could lead to better
474 classifications of sensory ecology as the mathematical organization of the variance resulting for a
475 PCA may hide biologically relevant information.

476 **Conclusion**

477 The aim of this study was to investigate the relationship between the sensory ecology of snakes
478 and the shape of the endocranium and its different sensory-related areas. Our results align with
479 those of previous studies on other vertebrates, but we also identify specificities concerning snakes.

480 First, size is a major component of shape disparity in the entire endocranium, and especially for
481 the olfactory bulbs and cerebral hemispheres. Phylogeny is another key component influencing
482 endocranial shape, with a major divergence of colubrids from the “basal” families. Finally, the
483 shape of the endocranium, especially the olfactory and optic tract, is related to the activity period,
484 but it does not accurately predict the sensory ecology of species without prior knowledge of their
485 phylogeny. The shape of the cerebellum is also related to the foraging habitat. Overall, our study
486 demonstrates that the use of shape information brings novel insights into the sensory adaptations
487 of snakes. The study of the morphology of brain related structures is also expected to be
488 complementary to volumetric information and histological studies, and will further characterize
489 the relationship between the endocranium, the brain, and the sensory ecology of species. However,
490 endocranial morphology alone cannot be used to infer the sensory ecology of species.

491 We hope our results will encourage further work on brain anatomy, morphology, and behavior of
492 snakes, as these animals remain largely underrepresented in neurobiological studies, as strikingly
493 demonstrated in the most recent and extensive book on the evolution of nervous systems in
494 vertebrates (Kaas 2017). The ecological and behavioral diversity of snakes, along with their
495 underestimated cognitive abilities associated and more basic brain structure, makes them a
496 valuable model to understand the evolution of more complex brains as suggested in (Naumann et
497 al. 2015).

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509

510 **References**

- 511 Adams DC (2014a) A generalized K statistic for estimating phylogenetic signal from shape and
512 other high-dimensional multivariate data. *Syst Biol* 63:685–697.
513 <https://doi.org/10.1093/sysbio/syu030>
- 514 Adams DC (2014b) A method for assessing phylogenetic least squares models for shape and
515 other high-dimensional multivariate data. *Evolution* 68:2675–2688.
516 <https://doi.org/10.1111/evo.12463>
- 517 Adams DC, Collyer ML, Kaliontzopoulou A (2020) Package “geomorph.” 1–140
- 518 Alfaro ME (2002) Forward attack modes of aquatic feeding garter snakes. *Funct Ecol* 16:204–
519 215. <https://doi.org/10.1046/j.1365-2435.2002.00620.x>
- 520 Allemand R, Boistel R, Daghfous G, et al (2017) Comparative morphology of snake (Squamata)
521 endocasts: evidence of phylogenetic and ecological signals. *J Anat* 231:849–868.
522 <https://doi.org/10.1111/joa.12692>
- 523 Balanoff AM, Bever GS (2017) The role of endocasts in the study of brain evolution. In: Kaas
524 JH (ed) *Evolution of Nervous Systems*. Academic Press, pp 223–241
- 525 Balanoff AM, Bever GS, Colbert MW, et al (2015) Best practices for digitally constructing
526 endocranial casts: examples from birds and their dinosaurian relatives. *J Anat* 229:173–190.
527 <https://doi.org/10.1111/joa.12378>
- 528 Balanoff AM, Smaers JB, Turner AH (2016) Brain modularity across the theropod–bird
529 transition: testing the influence of flight on neuroanatomical variation. *J Anat* 229:204–214.
530 <https://doi.org/10.1111/joa.12403>
- 531 Barton RA, Purvis A, Harvey PH (1995) Evolutionary radiation of visual and olfactory brain
532 system in primates, bats and insectivores. *Philos Trans R Soc B Biol Sci* 348:381–392.
533 <https://doi.org/10.1098/rstb.1995.0076>
- 534 Bertrand OC, San Martin-Flores G, Silcox MT (2019) Endocranial shape variation in the
535 squirrel-related clade and their fossil relatives using 3D geometric morphometrics:
536 contributions of locomotion and phylogeny to brain shape. *J Zool* 308:197–211.
537 <https://doi.org/10.1111/jzo.12665>
- 538 Blomberg SP, Garland TJ, Ives AR (2003) Testing for phylogenetic signal in comparative data:
539 behavioral traits are more labile. *Evolution* 57:717–745. <https://doi.org/10.1111/j.0014-3820.2003.tb00285.x>
- 541 Burghardt GM, Ford NB (1993) Perceptual mechanisms and the behavioral ecology of snakes.
542 In: *Snakes: Ecology and behavior*. The Blackburn Press, Caldwell, New Jersey, USA, pp
543 117–164
- 544 Camilleri C, Shine R (1990) Sexual dimorphism and dietary divergence : differences in trophic
545 morphology between male and female snakes. *Copeia* 1990:649–658.
546 <https://doi.org/10.2307/1446430>
- 547 Carril J, Tambussi CP, Degrange FJ, et al (2016) Comparative brain morphology of Neotropical
548 parrots (Aves, Psittaciformes) inferred from virtual 3D endocasts. *J Anat* 229:239–251.

549 <https://doi.org/10.1111/joa.12325>

550 Catania KC, Leitch DB, Gauthier D (2010) Function of the appendages in tentacled snakes
551 (*Erpeton tentaculatus*). *J Exp Biol* 213:359–367. <https://doi.org/10.1242/jeb.039685>

552 Cooper WEJ (2008) Tandem evolution of diet and chemosensory responses in snakes.
553 *Amphibia-Reptilia* 29:393–398

554 Crowe-Riddell JM, Simões BF, Partridge JC, et al (2019) Phototactic tails: evolution and
555 molecular basis of a novel sensory trait in sea snakes. *Mol Ecol* 1–16.
556 <https://doi.org/10.1111/mec.15022>

557 Crowe-Riddell JM, Snelling EP, Watson AP, et al (2016) The evolution of scale sensilla in the
558 transition from land to sea in elapid snakes. *Open Biol* 6:160054.
559 <https://doi.org/10.1098/rsob.160054>

560 Czaplicki JA, Porter RH (1974) Visual cues mediating the selection of goldfish (*Carassius*
561 *auratus*) by two species of *Natrix*. *J Herpetol* 8:129–134

562 Da Silva MAO, Heegaard S, Wang T, et al (2017) Morphology of the snake spectacle reflects its
563 evolutionary adaptation and development. *BMC Vet Res* 13:1–8.
564 <https://doi.org/10.1186/s12917-017-1193-2>

565 Daghfous G, Smargiassi M, Libourel PA, et al (2012) The function of oscillatory tongue-flicks in
566 snakes: insights from kinematics of tongue-flicking in the banded water snake (*Nerodia*
567 *fasciata*). *Chem Senses* 37:883–896. <https://doi.org/10.1093/chemse/bjs072>

568 De Cock Buning T (1983) Thermal sensitivity as a specialization for prey capture and feeding in
569 snakes. *Am Zool* 23:363–375

570 Drummond HM (1985) The role of vision in the predatory behaviour of natricine snakes. *Anim*
571 *Behav* 33:206–215. [https://doi.org/10.1016/S0003-3472\(85\)80134-2](https://doi.org/10.1016/S0003-3472(85)80134-2)

572 Ebert J, Müller S, Westhoff G (2007) Behavioural examination of the infrared sensitivity of ball
573 pythons. *J Zool* 272:340–347. <https://doi.org/10.1111/j.1469-7998.2006.00275.x>

574 Ebert J, Westhoff G (2006) Behavioural examination of the infrared sensitivity of rattlesnakes
575 (*Crotalus atrox*). *J Comp Physiol A Neuroethol Sensory, Neural, Behav Physiol* 192:941–
576 947. <https://doi.org/10.1007/s00359-006-0131-8>

577 Franz R (1977) Observations on the food, feeding behavior, and parasites of the striped swamp
578 snake, *Regina alleni*. *Herpetologica* 33:91–94

579 Friedel P, Young BA, Van Hemmen JL (2008) Auditory localization of ground-borne vibrations
580 in snakes. *Phys Rev Lett* 100:. <https://doi.org/10.1103/PhysRevLett.100.048701>

581 Gunz P, Mitteroecker P (2013) Semilandmarks: A method for quantifying curves and surfaces.
582 *Hystrix* 24:103–109. <https://doi.org/10.4404/hystrix-24.1-6292>

583 Gutiérrez-Ibáñez C, Iwaniuk AN, Wylie DR (2010) The independent evolution of the
584 enlargement of the principal sensory nucleus of the trigeminal nerve in three different
585 groups of birds. *Brain Behav Evol* 74:280–294. <https://doi.org/10.1159/000270904>

586 Halpern M, Kubie JL (1984) The role of the ophidian vomeronasal system in species-typical
587 behavior. *Trends Neurosci* 7:472–477. [https://doi.org/10.1016/S0166-2236\(84\)80258-1](https://doi.org/10.1016/S0166-2236(84)80258-1)

588 Hart NS, Coimbra JP, Collin SP, Westhoff G (2012) Photoreceptor types, visual pigments, and
589 topographic specializations in the retinas of Hydrophiid sea snakes. *J Comp Neurol*
590 520:1246–1261. <https://doi.org/10.1002/cne.22784>

591 Heatwole H (1999) Sea snakes. University of New South Wales Press Ltd, Kensington, NSW,
592 Australia, Australia

593 Hibbard E, Lavergne J (1972) Morphology of the retina of the sea-snake, *Pelamis platurus*. *J*
594 *Anat* 112:125–136

595 Holloway WL, Claeson KM, O’keefe FR (2013) A virtual phytosaur endocast and its
596 implications for sensory system evolution in archosaurs. *J Vertebr Paleontol* 33:848–857.
597 <https://doi.org/10.1080/02724634.2013.747532>

598 Iwaniuk AN (2017) Functional Correlates of Brain and Brain Region Sizes in Nonmammalian
599 Vertebrates. In: Kaas JH (ed) *Evolution of Nervous Systems*. Academic Press, pp 335–348

600 Iwaniuk AN, Mardon K, Leseberg NP, Weisbecker V (2020) The endocast of the Night Parrot
601 (*Pezoporus occidentalis*) reveals insights into its sensory ecology and the evolution of
602 nocturnality in birds. *Sci Rep* 10:9258:1–9. <https://doi.org/10.1038/s41598-020-65156-0>

603 Jayne BC, Voris HK, Ng PKL (2018) How big is too big? Using crustacean-eating snakes
604 (Homalopsidae) to test how anatomy and behaviour affect prey size and feeding
605 performance. *Biol J Linn Soc* 123:636–650. <https://doi.org/10.1093/biolinnean/bly007>

606 Jayne BC, Voris HK, Ng PKL (2002) Snake circumvents constraints on prey size. *Nature*
607 418:143

608 Kaas JH (2017) *Evolution of Nervous Systems - Second Edition*. Academic Press

609 Kawabe S, Shimokawa T, Miki H, et al (2013) Variation in avian brain shape: Relationship with
610 size and orbital shape. *J Anat* 223:495–508. <https://doi.org/10.1111/joa.12109>

611 Kotschal K, van Staaden MJ, Huber R (1998) Fish brains: evolution and environmental
612 relationships. *Rev Fish Biol Fish* 8:373–408. <https://doi.org/10.1023/A>

613 Krochmal AR, Bakken GS, LaDuc TJ (2004) Heat in evolution’s kitchen: Evolutionary
614 perspectives on the functions and origin of the facial pit of pitvipers (Viperidae: Crotalinae).
615 *J Exp Biol* 207:4231–4238. <https://doi.org/10.1242/jeb.01278>

616 Kropach CN (1975) The yellow-bellied sea snake, *Pelamis*, in the eastern Pacific. In: Dunson
617 WA (ed) *The biology of sea snakes*. University Park Press, Baltimore, pp 185–213

618 Kutsuma R, Sasai T, Kishida T (2018) How snakes find prey underwater: Sea Snakes use visual
619 and chemical cues for foraging. *Zoolog Sci* 35:483–486. <https://doi.org/10.2108/zs180059>

620 Lautenschlager S, Rayfield EJ, Altangerel P, et al (2012) The Endocranial Anatomy of
621 Therizinosauria and Its Implications for Sensory and Cognitive Function. *PLoS One* 7:.
622 <https://doi.org/10.1371/journal.pone.0052289>

623 Lebrun R (2017) ISE-MeshTools v1.3.4. pp 1–101

624 Liu Y, Ding L, Lei J, et al (2012) Eye size variation reflects habitat and daily activity patterns in
625 colubrid snakes. *J Morphol* 273:883–893. <https://doi.org/10.1002/jmor.20028>

626 Macrì S, Savriama Y, Khan I, Di-Poi N (2019) Comparative analysis of squamate brains unveils

627 multi-level variation in cerebellar architecture associated with locomotor specialization. *Nat*
628 *Commun* 10:5560. <https://doi.org/10.1038/s41467-019-13405-w>

629 Mark Waters R, Burghardt GM (2005) The interaction of food motivation and experience in the
630 ontogeny of chemoreception in crayfish snakes. *Anim Behav* 69:363–374.
631 <https://doi.org/10.1016/j.anbehav.2004.03.014>

632 Marugán-Lobón J, Watanabe A, Kawabe S (2016) Studying avian encephalization with
633 geometric morphometrics. *J Anat* 229:191–203. <https://doi.org/10.1111/joa.12476>

634 Moon BR, Penning DA, Segall M, Herrel A (2019) Feeding in snakes: form, function, and
635 evolution of the feeding system. In: Bels V, Whishaw IQ (eds) *Feeding in Vertebrates:*
636 *Evolution, Morphology, Behavior, Biomechanics*. Springer Nature Switzerland, pp 528–574

637 Naumann RK, Ondracek JM, Reiter S, et al (2015) The reptilian brain. *Curr Biol* 25:R317–R321.
638 <https://doi.org/10.1016/j.cub.2015.02.049>

639 Newman EA, Hartline PH (1982) The Infrared “Vision” of Snakes. *Sci Am* 246:116–127.
640 <https://doi.org/10.1038/scientificamerican0382-116>

641 Nieuwenhuys R, ten Donkelaar HJ, Nicholson C (1998) *The Central Nervous System of*
642 *Vertebrates*. Springer, Berlin

643 Noonloy T, Kunya K, Chanhom L, et al (2018) Crab-ripping: an unusual feeding behavior
644 newly recorded in freshwater snakes. *Bull Chicago Herpetol Soc* 53:53–56

645 Olori JC (2010) Digital endocasts of the cranial cavity and osseous labyrinth of the burrowing
646 snake *Uropeltis woodmasoni* (Alethinophidia: Uropeltidae). *Copeia* 2010:14–26.
647 <https://doi.org/10.1643/CH-09-082>

648 Pawar S, Dell AI, Savage VM (2012) Dimensionality of consumer search space drives trophic
649 interaction strengths. *Nature* 486:485–489. <https://doi.org/10.1038/nature11131>

650 Pedersen TL, Crameri F (2020) Package “scico.” 1–7

651 Povel DE, Van Der Kooij J (1997) Scale sensillae of the file snake (Serpentes: Acrochordidae)
652 and some other aquatic and burrowing snakes. *Netherlands J Zool* 47:443–456.
653 <https://doi.org/10.1017/CBO9781107415324.004>

654 Pyron RA, Burbrink FT (2014) Early origin of viviparity and multiple reversions to oviparity in
655 squamate reptiles. *Ecol Lett* 17:13–21. <https://doi.org/10.1111/ele.12168>

656 Randall JA, Matocq MD (1997) Why do kangaroo rats (*Dipodomys spectabilis*) footdrum at
657 snakes? *Behav Ecol* 8:404–413. <https://doi.org/10.1093/beheco/8.4.404>

658 Revell LJ (2012) phytools: An R package for phylogenetic comparative biology (and other
659 things). 217–223

660 Ripley B, Venables W (2020) Package ‘class.’ 1–19

661 Schaeffel F, de Queiroz A (1990) Alternative mechanisms of enhanced underwater vision in the
662 garter snakes *Thamnophis melanogaster* and *T. couchii*. *Copeia* 1990:50–58

663 Schaeffel F, Mathis U (1991) Underwater vision in semi-aquatic European snakes.
664 *Naturwissenschaften* 78:373–375. <https://doi.org/10.1007/BF01131614>

665 Schlager S (2015) Package “Morpho.” *Stat. Shape Deform. Anal. Methods, Implement. Appl.*

- 667 Schott RK, Van Nynatten A, Card DC, et al (2018) Shifts in selective pressures on snake
668 phototransduction genes associated with photoreceptor transmutation and dim-light
669 ancestry. *Mol Biol Evol* 35:1376–1389. <https://doi.org/10.1093/molbev/msy025/4904158>
- 670 Schwenk K (2008) Comparative anatomy and physiology of chemical senses in nonavian aquatic
671 reptiles. In: Thewissen JGM, Nummela S (eds) *Sensory Evolution on the Threshold:
672 Adaptations in Secondarily Aquatic Vertebrates*. University of California Press, pp 65–81
- 673 Sepulcre J, Liu H, Talukdar T, et al (2010) The organization of local and distant functional
674 connectivity in the human brain. *PLoS Comput Biol* 6:1–15.
675 <https://doi.org/10.1371/journal.pcbi.1000808>
- 676 Shine R (2005) All at sea: Aquatic life modifies mate-recognition modalities in sea snakes
677 (*Emydocephalus annulatus*, Hydrophiidae). *Behav Ecol Sociobiol* 57:591–598.
678 <https://doi.org/10.1007/s00265-004-0897-z>
- 679 Shine R, Bonnet X, Elphick MJ, Barrott EG (2004a) A novel foraging mode in snakes: browsing
680 by the sea snake *Emydocephalus annulatus* (Serpentes, Hydrophiidae). *Funct Ecol* 18:16–
681 24. <https://doi.org/10.1046/j.0269-8463.2004.00803.x>
- 682 Shine R, Brown GP, Elphick MJ (2004b) Field experiments on foraging in free-ranging water
683 snakes *Enhydryis polylepis* (Homalopsinae). *Anim Behav* 68:1313–1324.
684 <https://doi.org/10.1016/j.anbehav.2004.03.004>
- 685 Simões BF, Gower DJ, Rasmussen AR, et al (2020) Spectral diversification and trans-species
686 allelic polymorphism during the land-to-sea transition in snakes. *Curr Biol* 1–8.
687 <https://doi.org/10.1016/j.cub.2020.04.061>
- 688 Simões BF, Sampaio FL, Douglas RH, et al (2016) Visual pigments, ocular filters and the
689 evolution of snake vision. *Mol Biol Evol* 33:2483–2495.
690 <https://doi.org/10.1093/molbev/msw148>
- 691 Skinner M, Miller N (2020) Aggregation and social interaction in garter snakes (*Thamnophis
692 sirtalis sirtalis*). *Behav Ecol Sociobiol* 74:. <https://doi.org/10.1007/s00265-020-2827-0>
- 693 Smargiassi M, Daghfous G, Leroy B, et al (2012) Chemical basis of prey recognition in
694 Thamnophiine snakes: the unexpected new roles of parvalbumins. *PLoS One* 7:7–14.
695 <https://doi.org/10.1371/journal.pone.0039560>
- 696 Starck D (1979) Cranio-cerebral relations in recent reptiles. In: *Biology of the Reptilia, 9
697 Neurology A*. Academic Press Inc. Ltd, London, United Kingdom, pp 1–38
- 698 Stevens M (2013) *Sensory Ecology, Behavior, and Evolution*. Oxford University Press, Oxford,
699 United Kingdom
- 700 Thewissen JGM, Nummela S (2008) *Sensory Evolution on the Threshold: Adaptations in
701 Secondarily Aquatic Vertebrates*. University of California Press, London, United Kingdom
- 702 Vincent SE, Shine R, Brown GP (2005) Does foraging mode influence sensory modalities for
703 prey detection in male and female filesnakes, *Acrochordus arafurae*? *Anim Behav* 70:715–
704 721. <https://doi.org/10.1016/j.anbehav.2005.01.002>
- 705 Voris HK, Voris HH, Liat LB (1978) The food and feeding behavior of a marine snake,

706 *Enhydrina schistosa* (Hydrophiidae). Copeia 1978:134–146

707 Weisbecker V, Rowe T, Wroe S, et al (2021) Global elongation and high shape flexibility as an
708 evolutionary hypothesis of accommodating mammalian brains into skulls. Evolution (N Y)
709 75:625–640. <https://doi.org/10.1111/evo.14163>

710 Westhoff G, Fry BG, Bleckmann H (2005) Sea snakes (*Lapemis curtus*) are sensitive to low-
711 amplitude water motions. Zoology 108:195–200. <https://doi.org/10.1016/j.zool.2005.07.001>

712 Yopak KE, Pagan JMP, Wylie D (2017) The Cerebellum of Nonmammalian Vertebrates. In:
713 Evolution of Nervous Systems, Second Edi. Elsevier, pp 373–385

714 Young BA (2003) Snake bioacoustics: toward a richer understanding of the behavioral ecology
715 of snakes. Q Rev Biol 78:303–325. <https://doi.org/10.1360/zd-2013-43-6-1064>

716 Young BA (2007) Response of the yellow anaconda (*Eunectes notaeus*) to aquatic acoustic
717 stimuli. In: Henderson RW, Powell R (eds) Biology of the boas and pythons. Eagle
718 Mountain Publishing, Eagle Mountain, Utah, pp 199–205

719 Young BA, Aguiar A, Lillywhite HB (2008) Foraging cues used by insular Florida
720 cottonmouths, *Agkistrodon piscivorus conanti*. South Am J Herpetol 3:135–144.
721 [https://doi.org/10.2994/1808-9798\(2008\)3\[135:fcubif\]2.0.co;2](https://doi.org/10.2994/1808-9798(2008)3[135:fcubif]2.0.co;2)

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