

Jack-of-all-trades master of all? Snake vertebrae have a generalist inner organization

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Abstract Snakes are a very speciose group of squamates that adapted to various habitats and ecological niches. Their ecological diversity is of particular interest and functional demands associated with their various styles of locomotion are expected to result in anatomical specializations. In order to explore the potential adaptation of snakes to their environment we here analyze variation in vertebral structure at the micro-anatomical level in species with different locomotor adaptations. Vertebrae, being a major element of the snake body, are expected to display adaptations to the physical constraints associated with the different locomotor modes and environments. Our results revealed a rather homogenous vertebral microanatomy in contrast to what has been observed for other squamates and amniotes more generally. We here suggest that

the near-absence of microanatomical specializations in snake vertebrae might be correlated to their rather homogeneous overall morphology and reduced range of morphological diversity, as compared to lizards. Thus, snakes appear to retain a generalist inner morphology that allows them to move efficiently in different environments. Only a few ecologically highly specialized taxa appear to display some microanatomical specializations that remain to be studied in greater detail.

Keywords Snakes · Vertebrae · Microanatomy · Locomotor adaptation · Generalist

Introduction

Many biological studies have focused on the extent to which organisms are morphologically adapted to their environment, specifically focusing on the relationship between biological form and function (e.g., Aerts et al. 2000; Irschick and Garland 2001). Locomotor adaptations are essential for survival as locomotion plays a crucial role in many biological functions including the capture of prey, competing with possible rivals, and escaping predators (Garland and Losos 1994; Irschick and Garland 2001; Aubret 2004). As a consequence, specializations (notably in skeletal shape and proportions) linked to locomotor demands are often observed, such as the specializations of the forelimb to flying, swimming, running, burrowing that can be observed across vertebrate taxa and that illustrate the power of adaptation by natural selection.

Lizards have become a model system for ecomorphological studies because of their diversity in limb, body, and tail shape (Pianka and King 2004; McElroy and Reilly 2009) and the clear co-evolution of morphology and ecology (Losos 1994; Losos et al. 1997; Herrel et al. 2008). However, many squamate lineages contain taxa displaying some degree of limb reduction, culminating in snakes, one of the most speciose and ecologically

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diverse clades of squamates. Snakes are characterized by an extremely elongate body associated with a large number of vertebrae, the absence of legs and girdles (although some taxa retain vestigial pelvic and hind limb elements), and the absence of regionalization resulting from the absence of girdles (Cohn and Tickle 1999). Locomotion strongly differs from that of four-legged taxa whose propulsion and support is generally essentially ensured by the limbs (Aerts et al. 2000; McElroy and Reilly 2009). Indeed, snakes can use their entire body to generate propulsion when in contact with the substrate. Despite the uniform body shape, snakes show diverse forms of locomotion: lateral undulation, rectilinear, concertina, slide-pushing, and sidewinding. Importantly, most snakes are capable of using all of these locomotor modes, the choice of locomotor mode depending on the physical properties of the substrate (Jayne 1986; Gans 1986; 1994).

Vertebrae comprise the most important part of the snake skeleton and are strongly involved in snake locomotion (e.g., Johnson 1955; Gasc 1976, 1977; Jayne 1986; Moon 1999). Thus, adaptations of snake vertebrae to locomotor mode and locomotor environment can be expected if locomotion imposes mechanical constraints on vertebral structure, shape, and function. Snakes show a wide variety of locomotor ecologies including many burrowing, aquatic, and arboreal forms that likely differ in the physical demands placed upon the locomotor skeleton.

Here, we decide to investigate the bone microanatomy as this level of organization should reflect the different physical constraints of locomotion in these different ecological contexts (see e.g., Turner 1998; Ruimerman et al. 2005; Liu et al. 2009). Indeed, bone microanatomical features are considered a powerful tool to gain insights into the mode of life, and notably into the functional constraints imposed on organisms (e.g., Canoville and Laurin 2010; Houssaye 2013), and vertebral microanatomy is known to reflect locomotor specializations within amniotes (Dumont et al. 2013; Hayashi et al. 2013; Houssaye 2013). A preliminary analysis of the ecological signal of the vertebral microanatomical features within extant squamates (Houssaye et al. 2010) suggested that fossorial taxa have denser vertebrae than terrestrial ones, with those of aquatic taxa being of intermediate density. However, despite this ecological trend, a significant phylogenetic signal in the data was detected with snake vertebrae being notably denser than those of lizards, leading us to examine these patterns in greater detail in snakes.

The principal objective of this study was thus to test whether vertebral microanatomical features are related to habitat use in snakes and, more generally, to describe the different patterns of snake vertebral microanatomy and to discuss their relationship to phylogenetic, ecological, and structural constraints. In accordance with prior studies (Houssaye et al. 2010), we predict a decreasing gradient of vertebral compactness from fossorial to aquatic and terrestrial taxa. Moreover, we expect arboreal species to display microanatomical adaptations that reduce

overall body mass. We also test whether the positive correlation between size and bone trabecular tightness observed within squamates was retained within this clade. Absence of correlation between locomotor ecology and vertebral microstructure would suggest a jack-of-all-trades master of all morphology allowing snakes to switch between diverse locomotor modes.

Material and methods

The material consists of dorsal vertebrae of 54 snake species (48 genera; see Table 1) encompassing the diversity of snakes from both phylogenetic and ecological perspectives (see Fig. 1). Vertebrae were preferentially taken at about one third of the preloacal length. The taxonomy follows the reptile database (see <http://www.reptile-database.org/>). Both longitudinal (in the mid-sagittal plane) and transverse (in the neutral transverse plane; see Buffrénil et al. 2008) thin sections (i.e., in the two reference planes) were analyzed for the present paper.

About half of the sections analyzed correspond to histological thin sections, made using standard techniques (see Buffrénil et al. 2008; Table 1). The others were based on microtomographic investigations, allowing a non-destructive imaging of the three-dimensional outer and inner structure of the samples. Both conventional and synchrotron X-ray microtomography (see Table 1) were used: laboratory microtomography (1) using a high-resolution computed tomography (GEphoenix | X-ray v | tome | xs 180 and 240; resolution between 6.0 and 33.9 μm ; reconstructions performed using datox/res software) at the Steinmann-Institut, University of Bonn (Germany); (2) at the University of Poitiers (France), using a X8050-16 Viscom model (resolution between 16.7 and 32.3 μm ; reconstructions performed using Feldkamp algorithm with DigiCT software, version 1.15 [Digisens SA, France]) at the laboratory Etudes-Recherches-Matériaux (ERM, Poitiers, France; www.erm-poitiers.fr); and (3) at the University of Montpellier (France), using a SkyScan 1076 scanner (resolution: 9.4 μm , reconstructions performed with NRecon software [SkyScan, Belgium]); and (4) third-generation synchrotron microtomography (Tafforeau et al. 2006) at the European Synchrotron Radiation Facility (ESRF, Grenoble, France), on beamline ID 19 (resolution between 5.0 and 14.9 μm , reconstructions performed using filtered back-projection algorithm with the ESRF PyHST software). Image segmentation and visualization were performed using Amira 4.1.1. (Mercury Computer Systems, Chelmsford, MA), Avizo 6.3. (VSG, Burlington MA, USA) and VGStudioMax 2.0. (Volume Graphics Inc., Heidelberg, Germany).

Institutional abbreviations AH: Anthony Herrel personal collections, MCZ: Museum of Comparative Zoology, Harvard University, Cambridge, USA; MNHN: Muséum National

Table 1 List of the material analyzed with corresponding indices

Family	Taxon	Ha	Ha+M	Collection reference	μ CT Resol (μ m)	CL	Cls	Cts	TNCT	TNCL	SNC
Leptotyphlopidae	<i>Leptotyphlops bicolor</i>	F	F	MNHN 1993 3431 ^a	5.0	0.3	68.9	93.2	5	4	60.6
Anomalepididae	<i>Typhlophis squamosus</i>	F	F	MNHN 1995.2042 ^a	5.0	0.5	–	98.4	3	–	46.8
Typhlopidae	<i>Typhlops punctatus</i>	F	F	ZFMK 56090 ^b	12.6	2.9	66.7	81.2	5	46	59.8
Aniliidae	<i>Anilius scytale</i>	F	F	MNHN 1996 2701 ^b	9.3	3.6	82	78.2	13	20	33.5
					–	3.6	66.8	–	–	26	–
				MNHN 1997 2106 ^a	10.1	1.6	85.7	95.8	13	22	37.4
Cylindrophiidae	<i>Cylindrophis ruffus</i>	F	F	MNHN 1998 0201	–	2.9	80.6	96.1	6	25	24.1
	<i>Cylindrophis maculatus</i>	F	F	ZFMK 16 549 ^b	5.6	2.0	63.9	89.4	3	16	37.6
Tropidophiidae	<i>Trachyboa boulengeri</i>	F	F	AH S0001 ^b	9.4	3.9	82.9	83.1	11	101	14.9
Xenopeltidae	<i>Xenopeltis unicolor</i>	F	F	MNHN 1990 5174	–	2.1	72.6	85.4	5	13	38.1
Pythonidae	<i>Bothrochilus boa</i>	G	C	ZFMK 5203 ^b	25.7	5.2	73.0	86.9	20	68	22.4
	<i>Python reticulatus</i>	G	C	MNHN AC 1931 70	–	16.5	72.6	–	–	154	–
				MNHN AC 1931 69	–	11.6	73.2	–	–	59	–
				MNHN AC 2002 18	–	5.9	72.9	69.5	7	46	7.7
					–	5.9	–	70.4	10	–	7.1
				MNHN SQ-Vert 11	–	12.3	72	–	–	56	–
				MNHN SQ-Vert 12	–	–	–	84.9	5	–	7.6
				MNHN SQ-Vert 13	–	–	–	70.5	34	–	10.8
	<i>Python curtus</i>	G	HB	ZFMK 81 777 ^b	33.7	3.6	92.5	99.6	5	16	34.1
	<i>Morelia carinata</i>	Ar	E	AH S0002 ^b	9.4	3.9	66.6	72.1	6	30	25.1
	<i>Morelia viridis</i>	Ar	E	MNHN SQ-Vert 10	–	2.4	63.7	79	12	13	23.7
Boidae	<i>Eryx jaculus</i>	F	F	MNHN AC 2005 58	–	3.9	71.9	78.8	31	34	19.1
				MNHN SQ-Vert 7	–	4.2	79.4	–	–	20	–
				MNHN SQ-Vert 8	–	4.4	76.2	–	–	27	–
	<i>Calabaria reinhardti</i>	F	F	ZFMK 89190 ^b	26.7	3.8	80.0	81.9	28	39	18.4
	<i>Acrantophis madagascariensis</i>	G	HB	ZFMK 86 469 ^b	24.6	5.5	89.2	88.7	10	49	13.3
	<i>Sanzinia madagascariensis</i>	Ar	C	ZFMK 70 428 ^b	30.2	7.7	72.5	67.7	62	147	8
	<i>Boa constrictor</i>	G	HB	ZFMK 54844 ^b	33.9	6.6	77.6	79.2	16	56	12.7
	<i>Corallus hortulanus</i>	Ar	E	AH S0003 ^b	9.4	2.4	53.2	40.7	6	30	28.3
	<i>Epicrates cenchria</i>	Ar	C	ZFMK 86470 ^b	30.6	3.5	95.7	96.2	11	24	28.3
	<i>Eunectes murinus</i>	SA	SA	MNHN AC 1893 197	–	9	69.1	73.8	20	77	7.9
				MNHN AC 1940 353	–	14.3	69.1	–	–	64	–
				MNHN SQ-Vert 9	–	15.8	58	78.4	16	131	19.4
Acrochordidae	<i>Acrochordus javanicus</i>	EA	EA	MNHN SQ-Vert 14	–	8.4	66.8	77.5	12	35	15.9
				AH S0004 ^b	9.4	4.3	48.1	56	4	76	19.9
Pareatidae	<i>Pareas carinatus</i>	Ar	E	MNHN 2000 4272	–	–	–	76.6	4	–	36
Viperidae	<i>Bitis arietans</i>	G	HB	MNHN AC 1885 246	–	8.5	84.8	77.4	19	106	3.3
				MNHN AC 1977 13	–	9.2	84.3	–	–	66	–
				MNHN SQ-Vert 19	–	9.1	73.7	–	–	50	–
	<i>Bothrops lanceolatus</i>	G	C	MNHN AC 1887 934	–	7.1	71	65.8	12	50	8
					–	7.1	–	67.4	12	–	9
	<i>Agkistrodon contortrix</i>	G	HB	AH S0005 ^b	9.4	5.3	42.8	63.7	35	77	28.8
	<i>Agkistrodon piscivorus</i>	SA	SA	MNHN 1990 3854	–	7.5	68	64.3	11	63	19.7
Grayiinae	<i>Grayia ornata</i>	EA	EA	AH S0006 ^b	9.4	6.3	60.8	–	–	113	–
Colubrinae	<i>Chrysopelea ornata</i>	Ar	C	MCZ R 177291 ^a	14.9	1.3	84.9	98.5	4	14	56.7
	<i>Leptophis mexicanus</i>	Ar	E	AH S0007 ^b	9.4	4.6	64.6	86.8	4	73	39
	<i>Salvadora grahamiae</i>	G	C	AH S0008 ^b	9.4	3.3	55.1	70	4	35	33.8
	<i>Orthriophis taeniurus</i>	G	E	ZFMK 5215 ^b	27.8	6.6	80.1	72.8	8	47	23.3

Table 1 (continued)

Family	Taxon	Ha	Ha+M	Collection reference	μ CT Resol (μ m)	CL	Cls	Cts	TNCT	TNCL	SNC
	<i>Elaphe quatuorlineata</i>	G	C	ZFMK 5218 ^b	26.7	5.9	77.0	76.6	26	59	17.6
	<i>Pantherophis guttatus</i>	G	C	MNHN SQ-Vert 15	–	1.1	42.5	64.3	5	6	58.3
					–	1.1	53.5	–	–	7	–
					–	1.2	61.2	–	–	8	–
	<i>Rhinocheilus lecontei</i>	F	F	AH S0009 ^b	9.4	3.2	71.3	86.4	9	56	30.1
Natricinae	<i>Xenochrophis piscator</i>	SA	SA	ZFMK 74 287 ^b	9.2	3.9	55.8	80.8	6	28	43.2
	<i>Afronatrix anoscopus</i>	SA	SA	ZFMK 65488 ^b	9.1	4.1	66.8	81.1	13	63	29.6
	<i>Natriciteres fuliginoides</i>	SA	SA	AH S0010 ^b	9.4	2.7	67.1	89.1	11	64	29.1
	<i>Amphiesma stolatum</i>	SA	SA	ZFMK 18169 ^b	8.3	4.5	58.4	95.5	4	35	54.7
	<i>Thamnophis sauritus</i>	G	E	AH S0011 ^b	9.4	3.7	72.3	88.2	6	29	36.6
	<i>Natrix natrix</i>	SA	SA	MNHN AC 1874 535	–	5.3	73	–	–	39	–
				ZFMK 64057 ^b	6.0	3.7	65.4	93	9	73	35.4
	<i>Natrix tessellata</i>	SA	SA	ZFMK 24680 ^b	25.7	4.0	70.9	84.1	13	45	25.9
Homalopsidae	<i>Enhydria plumbea</i>	SA	EA	ZFMK 44891	–	2.9	70	79.9	5	23	29.5
	<i>Erpeton tentaculatum</i>	SA	EA	AH S0012 ^a	7.5	1.8	93.0	94.8	9	44	40.4
	<i>Enhydria bocourti</i>	SA	EA	MNHN 1999 8361	–	3.8	78.9	87.7	9	30	21.8
Atractaspididae	<i>Atractaspis microlepidota</i>	F	F	MNHN 1999 8559	–	–	–	70.6	4	–	50
Elapidae	<i>Micrurus lemniscatus</i>	G	E	MNHN 1997.2353 ^a	7.6	0.6	76.2	88.0	4	30	48.2
	<i>Naja nivea</i>	G	C	AH S0013 ^b	9.4	5.3	51.1	74.9	9	73	31.7
	<i>Ophiophagus hannah</i>	G	C	MNHN SQ-Vert 17	–	11.9	65.8	64.4	5	66	15.9
				MNHN AC 2002–42 ^b	32.3	12.5	75.2	72.1	26	85	14.9
	<i>Dendroaspis jamesoni</i>	Ar	E	MNHN SQ-Vert 16	–	6.0	76.6	72.4	6	24	28.3
					–	5.8	67.7	–	–	33	–
	<i>Bungarus fasciatus</i>	G	C	ZFMK 61719 ^b	24.6	5.9	89.7	87.5	5	60	14.5
	<i>Hydrophis</i> sp.	EA	EA	MNHN SQ-Vert 18	–	–	–	84	3	–	23.6
				MNHN AC 1887 897	–	4.4	88.8	84.2	5	31	18.2
	<i>Pelamis platura</i>	EA	EA	ZFMK 36436	–	2.3	54.9	–	–	27	–
				AH S0014 ^b	9.4	4.2	65.7	82.9	7	37	25
	<i>Laticauda laticaudata</i>	EA	EA	ZFMK 36425	–	3.1	72.5	87.2	3	20	30.2
					–	2.9	66.2	74.5	3	16	22.9

Ha categories based on habitat, Ar arboreal, EA essentially or fully aquatic, F fossorial and semi-fossorial, G terrestrial or generalist, SA semi-aquatic, Ha+M categories based on habitat and morphology, C common morphology, E elongated, HB heavy bodied, CL centrum length, Cls global compactness of the centrum in longitudinal section, Cts global compactness in transverse section, TNCT total number of cavities in transverse section, TNCL total number of cavities in longitudinal section, SNC size of the neural canal

^aResolution is given for specimens for which synchrotron X-ray microtomography was used

^bResolution is given for specimens for which conventional X-ray microtomography was used

d'Histoire Naturelle, Paris, France; ZFMK: Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany.

Quantitative analyses

All measurements except “centrum length” (CL; see below), were taken directly on the sections using ImageJ (Abramoff et al. 2004). The measurements taken were:

(a) The length of the centrum between the condylar and cotylar rims (CL) which is used as an indicator of specimen

size. This index was also used as a size estimate for the transverse sections when longitudinal and transverse sections come from either the same vertebra or from consecutive vertebrae in the same specimen, assuming that centrum length is similar between consecutive vertebrae. This index was measured under the microscope for classical sections and via image visualization software for virtual sections;

(b) The global compactness in transverse section (Cts), calculated as the total sectional area minus the area occupied by cavities and the neural canal multiplied by 100 and

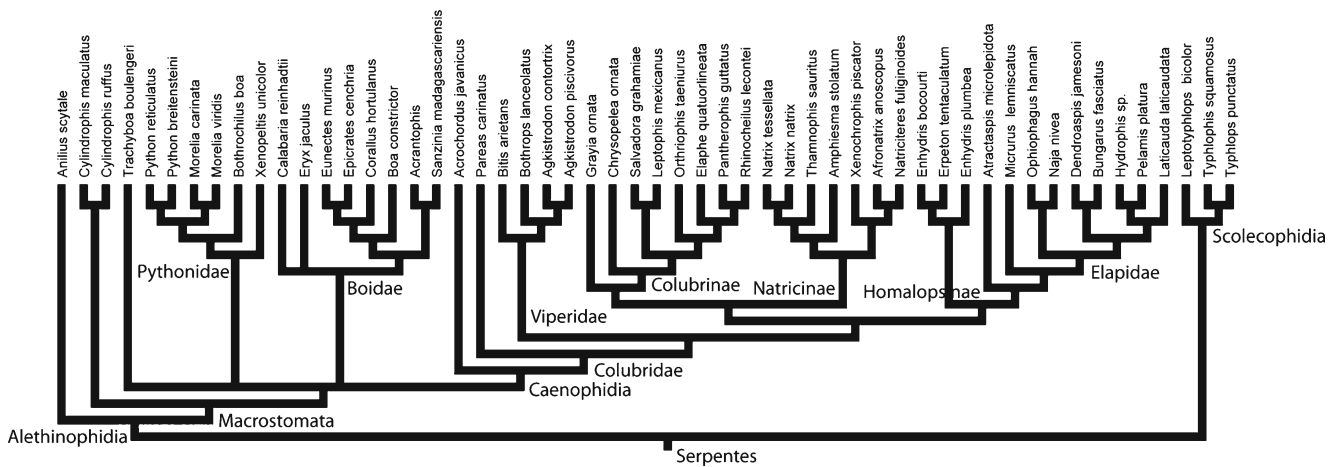


Fig. 1 Consensus phylogenetic tree (essentially from Rawlings et al. 2008; Lawson et al. 2005; Lee and Scanlon 2002)

- divided by the total area minus the area occupied by the neural canal;
- The global compactness of the centrum in longitudinal section (CIs), calculated as the total area of the centrum minus the area occupied by cavities multiplied by 100 and divided by the total area of the centrum;
 - The total number of cavities in transverse section (TNCT);
 - The total number of cavities in longitudinal section (TNCL);
 - The area occupied by the neural canal (SNC), calculated as the area occupied by the neural canal multiplied by 100 and divided by the total sectional area;

All data were transformed prior to analyses to meet assumptions of normality and homoscedasticity required for parametric analyses as follows: (a) $\log(\text{CL}+1)$, (b) $\text{Arcsin}(\text{CIs}/100)$, (c) $\text{Arcsin}(\text{Cts}/100)$, (d) $1/\sqrt{\text{TNCT}}$, (e) $\sqrt{\text{TNCL}}$, (f) $\text{Arcsin}(\sqrt{\text{SNC}/100})$.

We investigated the amount of phylogenetic signal for the different parameters analyzed. Statistical tests were performed using a consensus phylogeny derived from several published phylogenies that represents a current best estimate of relationships based on both molecular and morphological data (essentially Rawlings et al. 2008; Lawson et al. 2005; Lee and Scanlon 2002; Fig. 1). We calculated the K statistic following Blomberg et al. (2003), which compares the observed phylogenetic signal in a trait (based only on the reference tree structure) to the signal under a Brownian motion model of trait evolution. A K value lower than 1 implies less similarity between relatives than expected under Brownian motion. We then performed randomization tests to test the phylogenetic signal of each parameter.

Analyses were first performed independently on the transverse and longitudinal sections respectively, and then a third analysis combined data from both longitudinal and transverse

sections in those taxa for which both were available. Species means were used when several specimens were available for the same species.

We tested the influence of size (using CL as our estimate of size) on the various microanatomical parameters using linear regression analyses. When a phylogenetic signal was detected, we calculated independent contrasts on the transformed original data and forced regressions through the origin (Garland et al. 1992). In order to test whether vertebral microanatomical features were different for species living in different habitats, species were classified into five habitat categories: fossorial and semi-fossorial, terrestrial and generalist, arboreal, semi-aquatic, aquatic. ANOVAs, ANCOVAs (when a size effect was detected), and phylogenetic ANCOVAs (when both size and phylogenetic effects were detected) were performed. A second analysis was performed based on only three habitat categories, the terrestrial, generalist, and arboreal taxa being grouped together. A third analysis was performed still taking into consideration the fossorial and aquatic habitats but discriminating the other taxa based on the elongation of their body rather than habitat. Species were thus classified into six categories: fossorial and semi-fossorial, generalist morphology, heavy bodied, elongated, semi-aquatic, and aquatic. ANOVAs and ANCOVAs were performed using R (R Development Core Team); phylogenetic ANCOVAs were performed using the PDSIMUL and PDANOVA routines implemented in PDAP (Garland et al. 1993). In the PDSIMUL program, we used Brownian motion as our model for evolutionary change and ran 1,000 unbounded simulations to create an empirical null distribution against which the F value from the original data could be compared.

We decided not to use variance partitioning methods (see Cubo et al. 2008) to account for the different components acting on bone microanatomy, as their approach has been recently criticized (Rohlf 2006; Dumont et al. 2013).

Results

Qualitative observations

Vertebral microanatomy in snakes generally shows the typical pseudo-tubular organization described for modern squamates. This consists of a peripheral layer of primary periosteal bone and an internal layer of secondary (lamellar or parallel-fibered) bone surrounding the neural canal, connected by few trabeculae (Houssaye et al. 2010). Various trends are nevertheless observed. The distribution, number, and size of cavities strongly varies, and important variations in compactness occur (Fig. 2). However, no morphotypes can be distinguished and all microanatomical patterns analyzed show continuous variation rather than discrete forms. Thus, whereas the extremes are clearly distinct (despite a similar basic microanatomical organization), no clear microanatomical categories

were observed (Fig. 2). A trend towards bone mass increase via osteosclerosis (cf. Houssaye 2009) is observed in some aquatic (especially *Enhydryis plumbea*, *Hydrophis*, and *Erpeton tentaculatum*) and fossorial (*Anilius scytale* and *Cylindrophis ruffus*) snakes, but also in the heavy-bodied *Python curtus* and *Acrantophis madagascariensis*, in the arboreal forms *Epicrates cenchria* and *Chrysopelea ornata*, and in the terrestrial *Bungarus fasciatus*. Conversely, some specimens display the opposite trend. This is the case for the arboreal snake *Corallus hortulanus*, for one specimen of the aquatic snake *Acrochordus javanicus*, and for the terrestrial snakes *Agkistrodon contortrix* and *Pantherophis guttatus*.

Quantitative analyses

The K statistics calculated are all much lower than 1 ($0.18 < K < 0.46$). However, the randomization tests indicate a significant

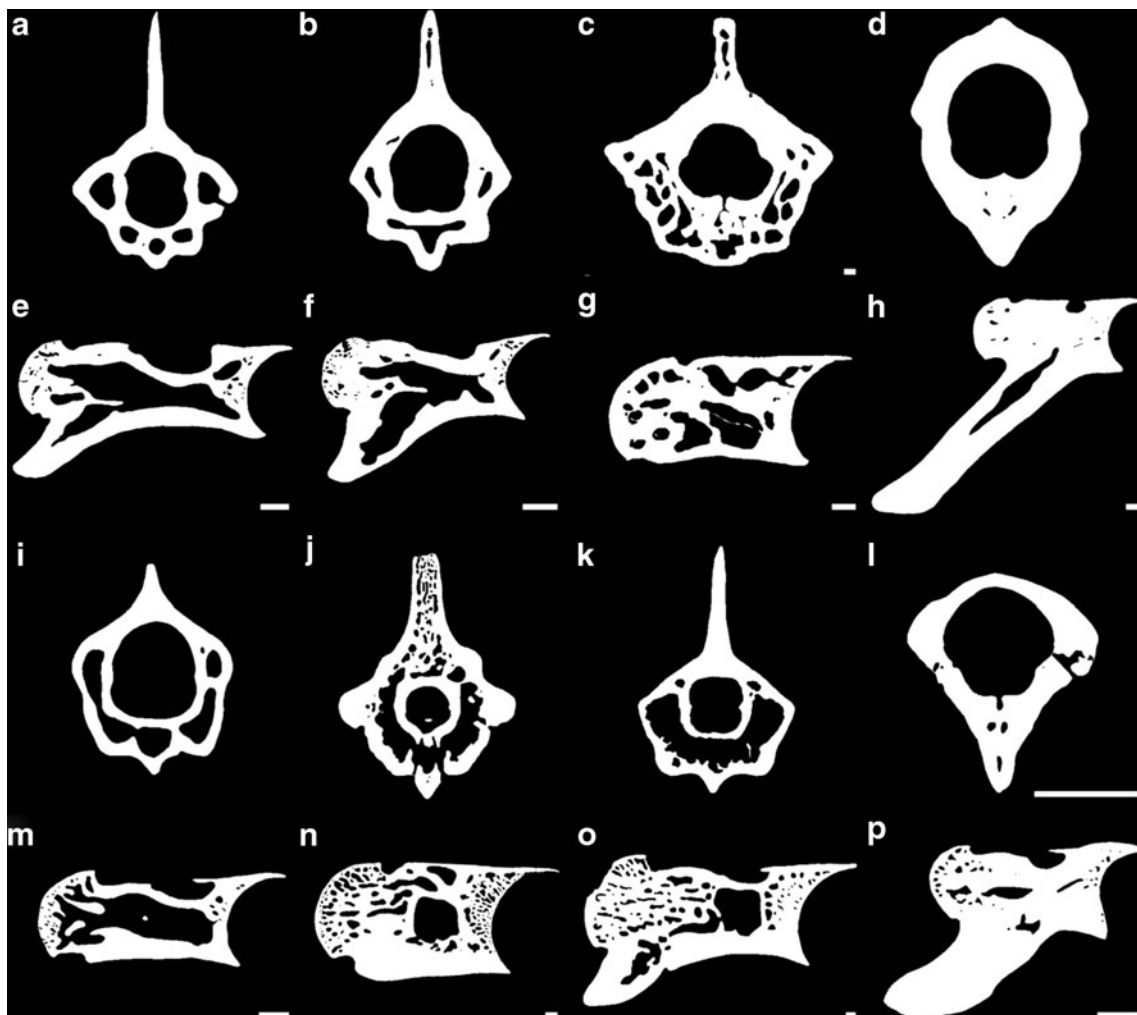


Fig. 2 Schematic drawing illustrating the various microanatomical patterns observed in *a–d*, *i–l* neutral transverse sections (NTS) of the vertebrae and *e–h*, *m–p* mid-sagittal sections (MSS) of the centra. White bone; black cavities. (*A,E*) *Pelamis platura* AH 0014; (*B,F*) *Natriciteres fuliginoides* AH 0011; (*C,G*) *Eryx jaculus* MNHN AC 2005 58; (*D,H*)

Python curtus ZFMK 81 777; (*I,M*) *Salvadora grahamiae* AH 0009; (*J,N*) *Sanzinia madagascariensis* ZFMK 70 428; (*K,O*) *Ophiophagus hannah* MNHN SQ-Vert 17; (*L,P*) *Erpeton tentaculatum* AH S0012. Scale bars 1 mm and 500 μ m in NTS and MSS, respectively

phylogenetic signal for SNC ($p=0.002$ and $p=0.008$). SNC is thus the only parameter showing phylogenetic signal, although the latter is rather small.

Linear regressions showed an impact of size on all parameters (Cts, $r=-0.46$, $p=8.0e-04$; TNCT, $r=-0.54$, $p=3.9e-05$; SNC, $r=-0.70$, $p=7.8e-09$; TNCL, $r=0.72$, $p=2.2e-9$) except CIs ($r=0.011$; $p=0.94$).

Analyses of (co)variance testing for differences in vertebral microanatomical features depending only on the habitat revealed no significant differences between groups (Table 2). This remained for analyses using only three habitat categories, and when using combined habitat and morphological data as dependent variables (Table 2). Only SNC showed significant variation between groups when considering both ecological and morphological features; however, this variation was not significant when phylogeny was taken into consideration ($F_{\text{trad}} < F_{\text{phyl}}$; Table 2).

Some taxa do not show any cavity in their neural spine, which does not seem correlated to ecology or body shape (logistic regression; p values >0.13 for the three types of categories defined above) but rather to size (logistic regression; $p < 0.01$). The possible link between the proportion of large cavities and habitat or morphological specializations was also investigated, but no trend could be identified.

Discussion

According to the constructional morphology model (Seilacher 1970; Gould 2002; Cubo 2004), biological features and, among them, bone microanatomical features are considered as the outcome of phylogenetic, adaptive, and architectural components, renamed as historical, functional, and structural constraints by Gould (2002) (Cubo et al. 2008). It appears, however, very difficult to determine the relative “weight” of these components in explaining bone microanatomical and histological features (e.g., Cubo et al. 2008), and thus to determine the causes of the various patterns observed. Our analyses enable us to discuss the impact of these different components on the microanatomical features of snake vertebrae.

Phylogenetic signal

A phylogenetic signal was only observed for the parameter SNC and appears rather slight. This suggests that the microanatomical pattern in snake vertebrae, at least based on the descriptive parameters used here, does not seem to be driven by historical constraints. Yet, some previous analyses showed a clear phylogenetic signal in vertebral microanatomy and significant differences between lizards and snakes (Houssaye et al. 2010; Dumont et al. 2013). Thus, the absence of such signal may be a snake-specific feature due to either strong morphological constraint at the origin of the group and little further variation, or due to rampant morphological divergence in each clade.

Size

Most parameters (except CIs) show a correlation with size. This is notably the case for SNC and TNCL. The positive correlation between the number of cavities (raw data) and size, especially in longitudinal sections, was already observed in extant and fossil squamates (Houssaye et al. 2010; Houssaye and Bardet 2012) and mammals (Dumont et al. 2013). This important correlation of microanatomical parameters with size suggests strong structural constraints on bone microanatomy dependent on overall size. Why size drives the observed patterns in squamates and mammals remains currently poorly understood and merits further investigation.

Ecological signal

Two types of microanatomical specializations (bone mass increase and a spongy organization) are observed in the vertebrae of some fossil squamates (Late Cretaceous varanoid lizards and hind-limbed snakes; see, e.g., Houssaye and Bardet 2012; Houssaye 2013). However, such specializations have not been described for extant forms. An ecological signal was previously suggested to be present in extant squamate vertebrae, with different degrees of vertebral compactness being observed between squamates occupying different

Table 2 Table showing the F and p values obtained for the various analyses of (co)variance

Parameter	Cts	TNCT	SNC	SNC	CIs	TNCL
Type of analysis	ANCOVA	ANCOVA	ANCOVA	Phylogenetic ANCOVA	ANOVA	ANCOVA
Habitat 5 categories	$F_{4,52}=0.31$ $p=0.58$	$F_{4,52}=0.27$ $p=0.61$	$F_{4,52}=0.49$ $p=0.49$	$F_{4,52}=6.97$ $p=0.94$	$F_{4,65}=0.545$ $p=0.46$	$F_{4,64}=1.13$ $p=0.29$
Habitat 3 categories	$F_{2,53}=1.97$ $p=0.17$	$F_{2,53}=0.20$ $p=0.66$	$F_{2,53}=0.31$ $p=0.58$	$F_{2,53}=10.32$ $p=0.87$	$F_{2,66}=0.03$ $p=0.88$	$F_{2,65}=1.78$ $p=0.19$
Habitat & Morphology	$F_{5,51}=0.65$ $p=0.42$	$F_{5,51}=0.60$ $p=0.44$	$F_{5,51}=4.64$ $p=0.036$	$F_{5,51}=5.86$ $p=0.98$	$F_{5,64}=1.05$ $p=0.31$	$F_{5,63}=0.59$ $p=0.45$

habitats. Yet, this result was not statistically significant when controlling for phylogeny (Houssaye et al. 2010). This previous study nevertheless suggested denser vertebrae in fossorial than in terrestrial species (with climbers and arboreal taxa taken together), and vertebrae of intermediate density in aquatic taxa. The present study revealed no correlation at all with habitat and snake vertebral microanatomy appears rather homogenous.

Lizards display a great diversity in limb and body morphology (Losos 1994; Losos et al. 1997; Vanhooydonck and Van Damme 2001; Herrel et al. 2008; McElroy and Reilly 2009) as well as specific morphological adaptive features (prehensile tail, adhesive pads, webbed feet, fused toes; Luke 1986; Irschick et al. 1996; Bickel and Losos 2002) associated with substrate usage and the ecological context of locomotion in general. Conversely, the general morphology of snakes appears to be much more homogeneous. Without limbs or girdles, the functional demands imposed by locomotion in different habitats change (Walton et al. 1990; McElroy and Reilly 2009). Specifically, gravitational constraints that are accentuated at the level of the limbs are now spread over the entire body. As such, no single element likely experiences the same level of reaction and/or gravitational forces during locomotion.

With the exception of a correlation between habitat use and overall body shape (Mattison 2008) only relatively few morphological adaptations of the axial skeleton in snakes can be detected. Despite the fact that snakes display different locomotor modes, these are not taxon-specific and a single individual often uses several locomotor modes depending on the structural features of its environment. The fact that most snakes must switch from one locomotor mode to another because of substrate changes probably explains why most species are not strongly specialized. Indeed, if locomotor mode needs to be modulated instantaneously in response to substrate type and/or inclination change, a rigid specialization to one peculiar locomotor mode is not to be expected (van Damme et al. 2003). As such, one can consider snakes as having a generalist morphology allowing them to be versatile in a variety of environments which may help explain their evolutionary success.

It would be wrong, however, to consider that snakes do not display any morphological adaptations to their milieu at all. Whereas it was, for example, demonstrated that terrestrial snakes, in general, cantilever almost as well as arboreal species, aquatic species, on the contrary (e.g., *Acrochordus*, *Laticauda*), have almost no cantilever ability (Lillywhite et al. 2000). Thus, if terrestrial and semi-aquatic forms also have advantages in having cantilever abilities allowing them to occasionally climb or to negotiate holes on the terrestrial substrate, this is likely not the case for aquatic species (Lillywhite et al. 2000). Arboreal species generally display more elongated and narrower vertebrae with shorter

zygapophyses and longer epaxial muscles and associated tendons than ground-dwelling relatives (Lillywhite et al. 2000). However, the absence of these morphological adaptations in terrestrial snakes does not prevent them from having good cantilever abilities. Nevertheless, the sum of both slight adaptations linked to an arboreal and to an aquatic life seems sufficient to explain the absence of cantilever abilities in aquatic snakes. If snakes do not appear to be strongly adapted to one specific milieu, adaptations, though slight, still occur and may reduce the number of habitats in which they can move efficiently.

Locomotor abilities have shown to be highly plastic in vertebrates, enabling organisms to match their phenotypes to local conditions (Aubret 2004). This plasticity, combined with the need for most snakes to locomote in different environments probably selected for a generalized morphology minimizing costs and maximizing benefits in the different habitats used. If one habitat is preferred to another, specializations to this milieu would be beneficial but only if they do not trade-off with locomotor demands in the other environments used, as has been demonstrated for sea snakes (Aubret et al. 2007). Despite physical and behavioral adaptations for breathing, diving, and achieving osmotic balance (Sanders et al. 2012), sea snakes are generally still able to move rather efficiently on land and are even capable of climbing (e.g., Bonnet et al. 2005). Whereas functional requirements differ between swimming and terrestrial locomotion (Jayne 1982), rather balanced phenotypes are observed. The strong behavioral and locomotor flexibility in snakes depending on habitat type (Aubret 2004) thus likely participate to their rather low degree of specialization.

Specific specializations

Despite our general observation that snakes are rather unspecialized in their vertebral microanatomy, a few appear specialized to one specific lifestyle only and show trends similar to those previously observed in extant squamates. Bone mass increase is observed in some aquatic snakes. This specialization is generally associated with slow swimming at the bottom of shallow water (Houssaye 2009). *Erpeton tentaculatum* is an ambush predator and generally remains suspended or anchored to vegetation while hunting (Smith et al. 2002); *Hydrophis* and *Enhydriis bocourti* forage into crevices for food and thus need to spend considerable amounts of time under water (Murphy 2012). These taxa are largely confined to a single milieu and probably exhibit only a single locomotor mode for the predominant part of their life which may explain their microanatomical specialization. Conversely, *Laticauda* forages in deeper water but also moves across land, which probably explains the absence of bone mass increase in this taxon. This specialization is also absent in surface swimmers like *Pelamis*. Even though these forms almost exclusively

forage in one milieu, they apparently do not need to control their buoyancy or do so by means other than an increase in vertebral density. Most fossorial snakes (e.g., *Anilius*, *Cylindrophis*, *Trachyboa*) also display a high inner compactness, which can probably be explained by the functional needs associated with burrowing although this remains to be explored further.

Bone mass increase is also observed in some heavy bodied strikers, like *Python curtus*, *Acrantophis madagascariensis*, and *Bitis arietans*. This could potentially be explained by the need to have a high body mass to anchor the body during striking and would lead to the prediction that regional differences in vertebral density might be present in these snakes as it is the posterior two-thirds of the body that anchor the snake. *Agkistrodon piscivorous* is also a striker but, as it swims at the surface, an increase in bone mass would negatively impact its buoyancy. Moreover, it is a generalist species foraging both on land and in water and it eats a diversity of small, slow prey and even carrion (Vincent et al. 2005). However, the high degree of compactness observed in the terrestrial snakes *Bungarus* and the long-bodied *Micrurus* and, especially, in the arboreal forms *Epicrates* and *Chrysopelea* is more difficult to explain. Conversely, *Corallus*, *A. contortrix*, *Pantherophis*, and one of the two specimens of *Acrochordus* display a relatively low compactness. The *Pantherophis* specimens were very young specimens raised in captivity, which might explain this peculiarity. However, the others were wild-caught adult specimens with distinct ecologies and morphologies and this feature remains difficult to explain.

Conclusion

Ecomorphological theory predicts a match between an organism's locomotor abilities and its environment. However, the degree of matching is highly taxon-dependent (Aerts et al. 2000). Whereas lizards show a high degree of body shape diversity and a wide range of locomotor specializations (Aerts et al. 2000), snake body shape, despite important differences in size and proportion, is rather homogeneous. This study revealed a general absence of microanatomical specialization in snake vertebrae, except in some extremely ecologically specialized taxa (bottom-water foragers, heavy-bodied strikers) and in a few species for which no clear ecological or functional factor could be identified. This absence probably relies on the fact that most snakes generally use different habitats and locomotor modes. Our analysis also revealed that vertebral microanatomy does not seem to carry a strong phylogenetic signal within snakes. However, some parameters highlight a correlation with size and thus suggest that the variation observed at the microanatomical level within snake vertebrae essentially reflects structural constraints. The important effect of size on the microanatomical

parameters suggests that size should always be taken into consideration in comparative studies.

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