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Brain Allometry Across Macroevolutionary Scales in Squamates Suggests a Conserved Pattern in Snakes

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ABSTRACT

Despite historical interest in brain size evolution in vertebrates, few studies have assessed variation in brain size in squamate reptiles such as snakes and lizards. Here, we analyzed the pattern of brain allometry at macroevolutionary scale in snakes and lizards, using body mass and snout vent length as measures of body size. We also assessed potential energetic trade-offs associated with relative brain size changes in Crotalinae vipers. Body mass showed a conserved pattern of brain allometry across taxa of snakes, but not in lizards. Body length favored changes of brain allometry in both snakes and lizards, but less variability was observed in snakes. Moreover, we did not find evidence for trade-offs between brain size and the size of other organs in Crotalinae. Thus, despite the contribution of body elongation to changes in relative brain size in squamate reptiles, snakes present low variation in brain allometry across taxa. Although the mechanisms driving this conserved pattern are unknown, we hypothesize that the snake body plan plays an important role in balancing the energetic demands of brain and body size increase at macroevolutionary scales. We encourage future research on the evolution of brain and body size in snakes to test this hypothesis.

1. Introduction

The relationship between brain and body size in vertebrates constitutes an evolutionary conundrum. While an evolutionary increase in body size can both promote and constraint the evolution of brain size (Rensch 1948; Voje et al., 2014; Tsuboi et al., 2016), larger brains are energetically costly, likely imposing constraints on the evolution of body size (Mink et al., 1981; Niven and Laughlin, 2008; Herculano-Houzel, 2011; Karbowski, 2014). This relationship between brain and body size has been traditionally analyzed using allometry, which represents the study of structural changes related to changes of body size (Huxley, 1924, 1932; Gould, 1966). This approach provides a mathematical framework to describe the association between brain and body size as a linear relationship given by the equation $\log(y) = \log(a) + b \times \log(x)$. Here, a corresponds to the intercept (i.e., a measure of the relative brain size), and b to the slope of the relationship, which represents the extent of change in brain size in relation to changes in body size (Huxley, 1924, 1932; Striedter, 2005; Kaas, 2009; Pelabon et al., 2014).

At macroevolutionary scales, the patterns of brain allometry can be split into two categories. Some studies have reported an increasing slope across taxonomic hierarchies, likely reflecting an increase in the extent of change in brain size with taxonomic hierarchies, and thus indicating evolutionary change in brain size in relation to the changes in body size among species within taxonomic hierarchies (Gould, 1975; Pagel and Harvey, 1988, 1989; Finarelli and Flynn, 2009; Franklin et al., 2014). By contrast, other studies have found a conserved slope across taxonomic hierarchies (Yopak et al., 2010; Tsuboi et al., 2018; Burger et al., 2019; Font et al., 2019), likely reflecting constraints in brain size evolution across taxonomic hierarchies. However, most of these reports have focused on traditional vertebrate taxa such as birds (Franklin et al., 2014; Ksepka et al., 2020), mammals (Pagel and Harvey, 1988, 1989; Finarelli and Flynn, 2009; Burger et al., 2019) and fishes (Bauchot et al.,

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1977; Yopak et al., 2010; Yopak, 2012; Tsuboi et al., 2016; Salas et al., 2017). Few studies have explored the brain allometric parameters in squamate reptile clades, and even less research has been done in snakes (Platel, 1979; Tsuboi et al., 2018; De Meester et al., 2019; Font et al., 2019). Here, we provide one of the first macroevolutionary analyses of allometry of brain size in snakes and lizards.

Regarding the energetic consequences of the brain and body size relationship, hypotheses such as the Expensive Tissue Hypothesis (ETH) (Aiello and Wheeler, 1995), the Energetic Trade-Off Hypothesis (ETOH) (Isler and van Schaik, 2006) and the Expensive Brain Hypothesis (EBH) (Isler and van Schaik, 2009) postulate that the evolution of brain size reflects the interplay between selection for larger brains and the mitigation of the energetic costs of brain size increase. Specifically, the ETH indicates that the costs of large brains in hominids are mitigated by a reduction in the gut size, accompanied by an improvement in dietary quality (Aiello and Wheeler, 1995). The ETOH predicts reductions in tissues, and also in physiological and life history traits (Isler and van Schaik, 2006). The EBH unifies the concepts of ETH and ETOH and incorporate the increases in energetic turnover, as compensatory strategies for mitigating brain size increase (Isler and van Schaik, 2009). For example, vertebrates with relative large brains incur high energetic costs that result in a reduction in size of other organs and tissues. This includes, a reduction in digestive tissues in some primates (Aiello and Wheeler, 1995; Barrickman and Lin, 2010; Hartwig et al., 2011), fishes (Kaufmann et al., 2003; Kotrschal et al., 2013b; Tsuboi et al., 2015) and frogs (Liao et al., 2016), adipose tissue in mammals (Navarrete et al., 2011), pectoral muscle mass in birds (Isler and van Schaik, 2006), and gonads in bats (Picknick et al., 2006). Even a reduction of offspring production in birds and fishes (Isler and van Schaik, 2006; Kotrschal et al., 2013b), as well as an increase in oxygen consumption in electric fishes (Sukhum et al., 2016) has been observed. To our knowledge, however, no study have explored these energetic tradeoffs in snakes and lizards.

Snakes and lizards provide an opportunity to assess patterns in brain-body size relationship across macroevolutionary scales. They are phylogenetically closely-related suborders, but present interesting contrasts in their overall body plan. And differences in the body plan are expected to have a strong influence in the allometric patterns among taxa (Smaers et al., 2012; Font et al., 2019; Rogell et al., 2020). In addition, snakes have a similar body design across extant taxonomic hierarchies, facilitating comparisons of patterns of brain and body size evolution under a common and simple body plan (i.e., tubular, elongated and limbless design). By contrast, lizards present variation in limb development, ranging from limbless to limbed clades (Vitt and Caldwell, 2014; Wiens et al., 2006). Additionally, the energy budget for brain of reptiles is comparable to that of endotherms (Mink et al., 1981), but their global energetic budget for other physiological processes is lower (White et al., 2006). To our knowledge, only two studies have assessed brain size allometry in snakes and lizards at macroevolutionary scale. Specifically, Platel (1979) showed that the brain of basal Henophidia snakes changed at higher rates following changes in body size than that of advanced snakes in Caenophidia, but this study only included two henophidian species and 12 caenophidians (Platel, 1979). More recently, De Meester et al. (2019) showed that lizards present large brain size for a given body size, likely as a consequence of limb development. Interestingly, snakes have a disproportionally elongated body, such that snakes with similar body mass to lizards present longer bodies. This body elongation entails an elongation of the spinal cord, with potential consequences for variation in brain size. For instance, body elongation in primates is known to promote both neuronal and whole brain changes related to spinal cord length (Burish et al., 2010).

We expand on the research on brain allometry in squamate reptiles using a combination of fresh (from this study) and published (from Platel, 1979; Zippel et al., 1998; De Meester et al., 2019) samples. We also evaluate potential energetic trade-offs involved in the brain-body size relationship of snakes, following the theoretical framework of ETH and ETOH (Aiello and Wheeler, 1995; Isler and van Schaik, 2006). By combining these data, we explore: i) what are the patterns of brain allometry at higher macroevolutionary scales among and within snakes and lizards?, and ii) are there energetic trade-offs compensating brain size increase in snakes? For the first question, we assessed allometric parameters at the level of Infraorder, Superfamily, Family and Subfamily in snakes and lizards, using measurements of both body mass and body length, and contrasting limbed and limbless taxa. For the second, we evaluated interspecific correlations between brain size and the size of other organs in pit-viper species of Crotalinae (Viperidae).

2. Materials and Methods

2.1. *Sampling design and snake body size*

We sampled adult individuals of seven pit-viper snake species (Crotalinae), one boid (Boidae), one coral snake (Elapidae) and two colubrids (Colubridae) collected in Panama during 2016 and 2017. All the samples, except for *Cerrophidion sasai*, were collected during the rainy season, which could reduce the effect of environmental variation on body size, body condition and brain size. Individuals were euthanized within 24 h of sampling, using an intraperitoneal injection of 2% bromoethanol solution. We then obtained body mass for each individual, using two digital scales: a HCB 123 (Adam Equipment®) for small individuals and a H-110 Digital Hanging Scale (American Weigh Scales Inc.) for larger individuals. Both scales are 0.001 g of precision, but the former has a high upper limit. We also estimated the snout vent length (SVL) for each individual using a measuring tape. We then dissected each individual, and stored the head and trunk in 10% formaldehyde and 70% ethanol, respectively. We complemented our field data with data (i.e., adult brain mass, body mass and SVL) of other snake species from the literature (Platel, 1979; Shine 1994; Zippel et al., 1998; Santos and Pleguezuelos, 2003; Luiselli et al., 2005; Ramesh and Bhupathy, 2010; Feldman and Meiri, 2013; Penning et al., 2015; De Meester et al., 2019). We also included, in the Crotalinae sample, a few individuals stored and preserved at the herpetological collections at the Universidad Autónoma de Chiriquí. We removed the viper species *Agkistrodon piscivorus* from Platel, 1979) because the reported brain mass (0.64 g) and body mass of (728 g), falls outside the possible brain mass for a snake with such body size. Thus, these values likely represent measurement errors. To contrast patterns of brain and body size allometry between snakes of lizards, we obtained recently published data on brain and body mass in lizards as well as SVL (Feldman et al., 2015; De Meester et al., 2019). Data on body mass and SVL of snakes and lizards, and brain mass of lizards are available in Table S1.

2.2. *Snake dissection*

To perform brain dissections, we first cleaned each skull and decalcified them by overnight immersion in Decalcifier System I (composition: <12% formic acid, <2% methanol, <6% formaldehyde of total volume) (Leica Biosystems). We then dissected intact brains which included olfactory bulbs and tracts, brain hemispheres, tectum opticum, cerebellum, medulla oblongata, and hypophysis. However, for the current analyses, we only focus on overall brain mass, which we quantified immediately after dissection of the skull, using a digital scale (HCB 123; Adam Equipment®), 0.001 g of precision. Data for brain mass for snakes are in Table S1. We also quantified the mass of other organs such as liver, heart, trachea, gut, esophagus, gall bladder, pancreas, kidneys, ovaries (when possible) and intestines. We weighted each organ three times to reduce measurement errors. In addition, we subtracted the mass of prey remains and feces from the gastrointestinal tract from the body mass obtained in the field. Thus, we used a body mass measurement free of food or fecal content in our analysis. We also dissected the trunk's skin (with scales) and obtained three replicates of both the mass of the trunk`s skin and the musculoskeletal and osseous tissue of the trunk

(MSOTT). We also obtained the mass of visceral fat. We used a digital portable scale (H-110 Digital Hanging Scale, American Weigh Scales Inc), 0.001 g of precision, to estimate the MSOTT and trunk's skin for large individuals. Data on the trunk's organs are available in Table S2. This study was performed following the guidelines and regulations approved by the IACUC protocol at INDICASAT-AIP, Ciudad del Saber, Panama (IACUC 16-002 to Luis C. Elizondo Lara).

2.3. Data Analysis

We used the mean brain and body mass of adult individuals for each species, or individual data points when only one individual per species was available. Then, we used decimal logarithm ($\log_{10}$) to transform these data before allometric analyses. The allometric parameters, slope and intercept, were extracted using the General Least Square (GLS) regression as suggested in previous work (Kelly and Price, 2004; Hansen and Bartoszek, 2012; Voje et al., 2014; Kilmer and Rodríguez, 2016), but without considering phylogenetic dependence due to small sample size in snakes. We analyzed the data using the software Microsoft Excel and Past 3 (Natural History Museum, University of Oslo). To compare macroevolutionary variation in allometric parameters between snakes and lizards, we estimated an index of evolutionary variation (IEV) in the allometric parameters. This index consists of the coefficient of variation ($CV = SD/M$), which represents the ratio of the standard deviation (SD) to the mean (M), divided by the root age of each clade. For Serpentes, we used 167 my (Caldwell et al., 2015) as root age, which represents the oldest known snakes from the Middle Jurassic-Lower Cretaceous. For Sauria, we used 202.1 my, as suggested for Gekkota (Zheng and Wiens, 2016), our most basal saurian clade, which is very close in millions of years to the divergence of Squamata (Zheng and Wiens, 2016; Simões et al., 2020). We included the root age of each clade in this analysis to account for variation in evolutionary time among clades, considering that we had overall low sample size. We also performed a Phylogenetic General Least Square (PGLS) regression to account for phylogenetic dependence in the Infraorder Alletinophidia, which contained the largest sample size of snakes. For this analysis, we used a time-calibrated tree for Serpentes downloaded from the timetree website (Kumar et al., 2017) and pruned the tree to the species used in this study. These analyses were performed using the package caper (Orme et al., 2018) in the R statistical environment (R Core Team, 2017). Plotting of the data was performed in the R statistical environment (R Core Team, 2017). Given the potential effect of body length on brain size (see introduction), we compared the allometric relationships between body mass and SVL, as well as the allometric relationships between brain size and SVL for snakes and lizards (including limbless and limb-reduced taxa), and between the residuals of these relationships to correct for the effect of body length.

To determine potential energetic trade-offs between brain mass and the mass of other organs in our freshly collected Crotalinae species, we first estimated allometric parameters for each organs, by performing GLS regressions between body and organ size ($\log_{10}$ transformed). We did not performed a PGLS with these data because of the small sample size (seven Crotalinae species). Then, we applied a multiple regression between $\log_{10}$ brain size and the residuals of the organ-body size relationship for each organ, which corrects for body size effects (but see Rogell et al., 2020). The residuals for each organ were extracted from one model for each organ or tissue. Negative associations indicate energetic trade-offs compensating brain size increase (Aiello and Wheeler, 1995; Isler and van Schaik, 2006).

3. Results

3.1. Brain allometry in snakes and lizards

We found similar brain allometry (when using body mass) across high macroevolutionary hierarchies in snakes, but not in lizards. In snakes, both allometric parameters were highly similar across taxa, and corresponded to a slope ranging from 0.49 to 0.54 and an intercept ranging from -1.86 to -2.07 (Table 1). These relationships showed a strong association between brain and body size across taxa and were statistically significant. The only exception was the subfamily Colubrinae (Colubridae), which contained the smallest sample size (Table 1). Among taxa, the superfamily Henophidia showed the lowest values for both slope and intercept. The families Viperidae and Colubridae showed similar values, and the subfamilies Colubrinae and Crotalinae, the superfamily Caenophidia and the Infraorder Alletinophidia showed the highest values (Table 1). Our PGLS regression showed a similar slope across Alletinophidia (0.55), suggesting a minor effect of phylogenetic dependence on the evolution of brain size allometry in snakes (Fig. 1).

In contrast to snakes, the families and infraorders of lizards (Sauria) showed a wide range of allometric parameters across taxa, with the slope ranging from 0.35 to 0.68, and the intercept ranging from -2.04 to -1.34 (Table 2, Fig. 2). The entire Suborder Sauria showed a slope of 0.61 and an intercept of -1.82 (Table 2). Across the entire Order Squamate (combining snakes, lizards and one amphisbaenian from Platel (1979)), the slope corresponded to 0.53 and the intercept to -1.78 ($n = 187$). The combined sample of limbless and limb-reduced lizards showed a slope of 0.61 and an intercept of -2.13 (r: 0.98, $p <$ 0.01, n= 14). In Fig. 3 we present a comparison of allometric slopes for snakes, lizards and limbless and limb-reduced lizards (Fig. 3).

Furthermore, the coefficient of variation and the IEV across taxonomic hierarchies for both parameters were lower for snakes (CV: 0.043, IEV: 0.000263 for slope; CV: -0.045, IEV: -0.000274; for intercept; $n = 4$; Table 3) than lizards (CV: 0.193, IEV: 0.000959 for slope; CV: -0.117, IEV: -0.000579 for intercept; $n = 11$; Table 3).

The relationships between body mass and SVL in our sample showed positive allometry for high macroevolutionary hierarchies in snakes, with slopes ranging from 1.23 to 2.47 and intercepts ranging from -4.87 to -1.15 (Table S3). Most of these relationships were statistically significant (Table S3). Lizards showed a similar pattern, with slopes ranging from 1.95 to 4.12 (although most were between 2.08 and 2.84) and intercepts between -7.41 and -3.07 (Table S4). Both snakes and lizards showed a positive brain allometry across taxonomic hierarchies when related to SVL (Tables S5 and S6). A conserved pattern of brain allometry parameters was observed for most snake taxa when controlling for SVL, except for Henophidia and Colubrinae, which were non-significant, possibly due to low sample size (Table S7). For lizards, we observed high variability across macroevolutionary hierarchies in allometric parameters, but only half of the relationships were statistically significant (Table S8). For the combined sample of limbless and limb-reduced lizards the slope was 0.70 and intercept -9.27E-07 (r: 0.91, $p < 0.01$, $n = 14$).

Table 1

Allometric parameters for the relationships body mass–brain mass in taxa of Serpentes (Squamata) with different macroevolutionary hierarchies using General Least Square regression. Asterisks denote statistical significance (** < 0.01 and * < 0.05).

Taxa	*b*	*a*	r	*p*	n
Suborder Serpentes					
Infraorder					
Alletinophidia	0.54	−2.07	0.97	**	27
Superfamily					
Henophidia	0.49	−1.86	0.96	*	4
Caenophidia	0.54	−2.07	0.96	**	23
Family					
Colubridae	0.51	−1.98	0.94	**	10
Subfamily					
Colubrinae	0.54	−2.05	0.79	n.s.	5
Viperidae	0.51	−2.02	0.98	**	11
Crotalinae	0.54	−2.1	0.99	**	8

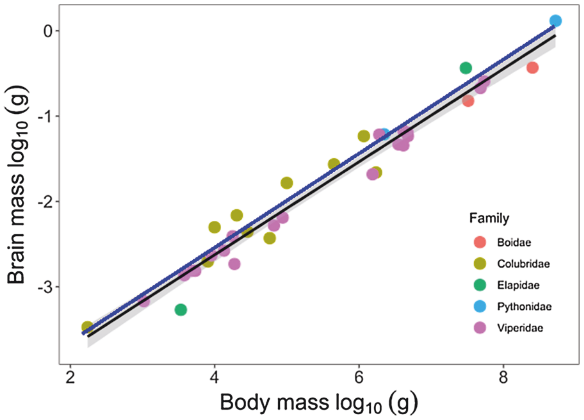

Fig. 1. Brain allometry in Alletinophidian snakes using General Least Square regression (y= -2.07 + 0.54x). Trend lines represent the slope of the GLS (black) (0.54) and PGLS (blue) (0.55).

Table 2
Allometric parameters for the relationships body mass–brain mass in different taxa of Sauria (Squamata) with different macroevolutionary hierarchies using General Least Square regression. Asterisks denote statistical significance (** < 0.01 and * < 0.05).

Taxa	*b*	*a*	r	*p*	n
Suborder Sauria	0.61	−1.82	0.95	**	157
Infraorder					
Anguimorpha	0.64	−1.95	0.93	**	9
Family					
Varanidae	0.56	−1.66	0.96	**	6
Gekkota	0.57	−1.81	0.9	**	36
Carphodactylidae	0.41	−1.55	0.98	**	3
Diplodactylidae	0.56	−1.82	0.93	**	8
Gekkonidae	0.67	−1.84	0.97	**	20
Pygopodidae	0.46	−2.04	0.99	0.08	3
Iguania	0.49	−1.58	0.94	**	47
Agamidae	0.51	−1.59	0.96	**	30
Dactyloidae	0.35	−1.34	0.55	0.15	8
Lacertoidea	0.68	−1.85	0.95	**	16
Lacertidae	0.58	−1.75	0.97	**	11
Teiidae	0.66	−1.75	0.94	0.058	4
Scincoidea	0.63	−1.93	0.97	**	47
Scincidae	0.62	−1.93	0.96	**	44

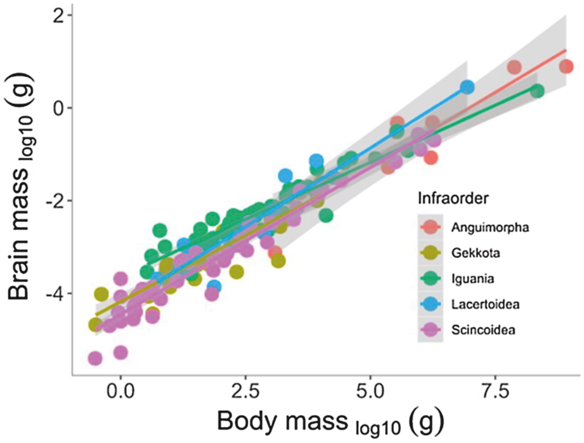

Fig. 2. Brain allometry in infraorders of the suborder Sauria (y = -1.82 + 0.61x) using a General Least Square regression.

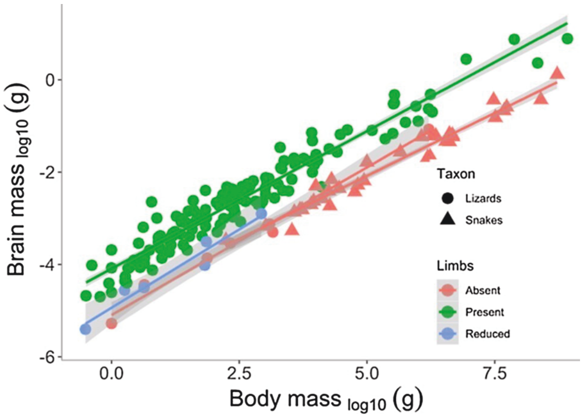

Fig. 3. Allometric slopes for snakes of Alletinophidia and lizards of Sauria presenting limb development, limb reduction, and limbless condition.

Table 3
Variation in allometric parameters and the index of evolutionary variation for Serpentes and Sauria. Root age corresponds to millions of years for Serpentes (Caldwell et al., 2015) and Gekkota, which is the most basal lizard clade in our sample (Zheng and Wiens, 2016; Simões et al., 2020). IEV corresponds to index of evolutionary variation.

	Serpentes		Sauria	
	b	*a*	*b*	*a*
SD	0.0224	0.0895	0.1047	0.2051
Mean	0.51	−1.95	0.54	−1.75
N	4	4	11	11
CV	0.043	−0.045	0.193	−0.117
Root age	167	167	202.1	202.1
IEV	0.000263	−0.000274	0.000959	−0.000579

3.2. *Energetic trade-offs in Crotalinae*

We did not find evidence for trade-offs between brain size and the size of any of the organs in Crotalinae (Table 4). However, the size of organs approached isometry, with some organs showing weak trends of both negative and positive allometry, such in the case of the stomach (*b*: 0.82), the trachea (*b*: 1.16), and the MSOTT (*b*: 1.13) (Table 4).

4. Discussion

We found low variation in brain allometric parameters (*a* and *b*) across macroevolutionary hierarchies in snakes in comparison to lizards. This pattern did not seem to be influenced by the age of either clade nor by phylogenetic dependence among species of snakes, or the inclusion of

Table 4
Correlational and allometric analysis of the main organs of the trunk in Crotalinae snakes (Squamata: Serpentes: Alletinophidia: Viperidae). Asterisks denote statistical significance (** < 0.01 and * < 0.05).

Organ	N	Correlational parameters		Allometric parameters		
		r	r^2	*b*	*a*	r
Skin	7(16)	0.08	0.007	1.01	−0.9	0.97**
Trachea	7(15)	0.04	0.002	1.16	−2.51	0.98**
Heart	7(17)	0.07	0.005	0.94	−2.28	0.96**
Liver	7(17)	0.08	0.007	0.92	−1.43	0.93**
Esophagus	7(16)	0.05	0.003	1.05	−2.21	0.95**
Stomach	7(17)	0.04	0.001	0.82	−1.23	0.95**
Gall Bladder	7(13)	0.05	0.002	1.10	−2.86	0.93**
Pancreas	7(14)	0.02	0.0005	1.09	−3.17	0.97**
Intestines	7(17)	0.03	0.001	1.01	−1.62	0.97**
Kidneys	7(17)	0.06	0.004	1.05	−2.38	0.96**
Ovaries	7(12)	0.06	0.004	0.93	−2.35	0.48
MSOTT	7(15)	0.08	0.006	1.13	−0.77	0.98**

body length in our analyses. The only exceptions were Henophidia and Colubrinae, which showed non-significant relationships, likely due to small sample size. In addition, we did not find evidence for trade-offs between brain size and the size of other organs in the snake subfamily Crotalinae. These findings contrasts with previous studies reporting substantial variation in allometric parameters at macroevolutionary scales in ectotherms, such as fishes, amphibians and even reptiles, including dinosaurs (Jerison, 1969; Bauchot et al., 1977; Yopak, 2012; Salas et al., 2017). Other studies have also reported low brain allometric variation across vertebrates (Yopak et al., 2010; Tsuboi et al., 2018; Burger et al., 2019; Font et al., 2019), but to our knowledge, few studies have focused on snakes (Platel, 1979). Thus, despite limitations in sample size, our results suggest that basal henophidian and derived caenophidian snakes have a uniquely conserved pattern of brain allometry at macroevolutionary scales among vertebrates. This conserved pattern of brain allometry in alletinophidian clades also contrasts with previous analyses, which found higher brain size variation, for a given body size, in henophidian than in caenophidian snakes (Platel, 1979).

If the conserved pattern for snakes holds with the addition of new data, it may reflects a macroevolutionary equilibrium resulting from the balance of the energetic requirements of brain and body size increase (Kleiber, 1947; Mink et al., 1981; Isler and Van Schaik, 2006, 2009; Niven and Laughlin 2008), despite rapid ecological and body size diversification in snakes. This possibility appears to be supported by the absence of negative relationships between brain size and the size of the other organs in Crotalinae, and the isometric trends observed for most of the organs in this group. In fact, we only found slight deviations from isometry in a few organs such as stomach, trachea and the MSOTT, suggesting an overall absence of energetic trade-offs associated with brain size increase. It is unclear to us if diet shifts from small to large preys following the increase of body size in Crotalinae, similar to other snake clades (Vitt and Caldwell, 2014), is associated with an increase in energetic turnover to mitigate energetic constraints of brain size or whole body size (Isler and van Schaik, 2006, 2009), but this is certainly a possibility that deserves further research.

The specific mechanisms driving this conserved pattern of macroevolutionary brain allometry in snakes are not yet clear, but it is likely that changes in either brain or body size (or both) are involved (Deacon, 1990; Montgomery et al., 2010). We propose that perhaps the snake body plan, a tubular and elongated shape together with the loss of limbs, may play an important role. Previous studies indicate that body size imposes constraints on brain size increase (Tsuboi et al., 2016; De Meester et al., 2019), but the snake body plan may be more energetically efficient than the body plan in other taxa, likely facilitating the balance of the energetic constraints between both brain and body size increase at macroevolutionary scales. This could be supported by the fact that the basic snake body plan – including the loss of limbs – has remained almost invariable across clades since their divergence from lizards approximately 170 Myr ago (Caldwell et al., 2015; Zheng and Wiens, 2016). Furthermore, snakes present lower energetic costs of body maintenance than lizards, possibly because of their simple body plan (Zhang et al., 2018). This might also explain the repeated evolution of this body design in modern lizards (Wiens et al., 2006).

Limb development is an important factor driving brain size evolution in squamate reptiles (Platel, 1979; De Meester et al., 2019). In lizards, limb development appears to be associated with higher variation in brain allometric parameters (Platel 1979). For instance, the infraorders Gekkota and Iguania showed low slopes, and a very low intercept in the case of Iguania. Moreover, some families with pronounced limb development within infraorders with high brain allometric parameters also showed a reduction in both slope and intercept, such as Varanidae from Anguimorpha and Lacertidae from Lacertoidea. Interestingly, the brain allometric parameters for the entire sample of limbless or limb reduced lizards were similar to the sample of the Scincidae family and the entire suborder Sauria, which suggests that brain allometry in these lizards is less variable, possibly associated with their body plan (Platel 1979). However, the allometric parameters controlling for the effect of SVL appeared more variable in limbless lizards, also suggesting a more complex brain allometric pattern across lizards. Thus, the snake body plan might be an important factor maintaining conserved the brain allometric parameters across taxonomic scales.

Although our sampling effort included, to our knowledge, all currently available brain mass data for snakes and lizards, additional data are crucial to explore these patterns further. In particular, future studies must include a wider range of body sizes in snakes of the superfamilies Henophidia and Caenophidia, and the Infraorder Scolecophidia, as well as families of limbless or limb-reduced lizards such as the ancestral Dibamidae and Pygopodidae (Wallach et al., 2014; Uetz et al., 2020). Future studies should also consider the effect of body length on brain allometry, given that the spinal cord is known to influence brain mass and organization in primates (Burish et al., 2010). Thus, given that weight and length are different physical entities with potentially different consequences for brain evolution, their complementary effects deserves further attention in snakes, as well as in other taxa. Finally, expanding current available data will also help solve the nested pattern of snake species in our sampling, as well as improving our phylogenetic statistical approach for both lizards and snakes.

5. Conclusions

We found remarkable differences in macroevolutionary patterns of brain allometry in snakes and lizards. Brain allometric parameters were highly variable in lizards across taxa. By contrast, in snakes, they showed a conserved pattern at multiple taxonomic scales, without evidence for trade-offs between brain size and the size of other organs in Crotalinae. Body length favored brain allometry in both snakes and lizards, but less variability was observed in snakes. While these findings are preliminary, given the limited availability of data, they suggest the possibility of a macroevolutionary equilibrium in brain size allometry in snakes. Although the mechanisms driving this pattern are unknown, we suggest that the snake body plan, which has remained nearly invariable since the evolution of the clade, may play an important rolein balancing energetic constraints of brain and body size increase at macroevolutionary scales. We hope that these results will encourage future research on the evolution of brain and body size in lizards and snakes – a fascinating, yet understudied branch of the tree of live.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

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