

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA COMPARADA

Ecomorphological evolution of South-American Colubroidea snakes (Squamata: Serpentes)
Evolução ecomorfológica das serpentes Colubroidea sul-americanas (Squamata: Serpentes)

Gabriel Spanghero Vicente Ferreira

Dissertação apresentada à Faculdade de Filosofia,
Ciências e Letras de Ribeirão Preto, Universidade de
São Paulo, como parte dos requisitos para obtenção do
título de Mestre em Ciências, obtido no
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Orientador: Prof. Dr. Tiana Kohlsdorf

Co-orientador: Prof. Dr. Wilfried Klein

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“Ecomorphological evolution of South-American Colubroidea snakes (Squamata: Serpentes)”

Gabriel Spanghero Vicente Ferreira

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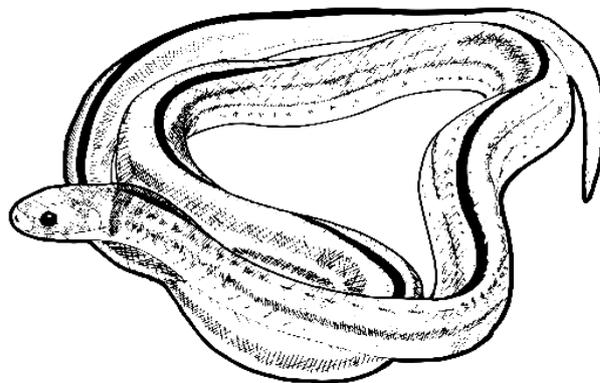
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Tantilla melanocephala

Abstract

This dissertation explored ecomorphological patterns in South American Colubrid snakes, testing for associations between body shape and ecological parameters. Comparative phylogenetic tools were used to answer macroevolutionary questions related to body shape evolution in colubrid snakes that differ in microhabitat usage and activity period. Major assumptions presume associations between form (morphology) and function (locomotion and thermoregulation in different environmental settings), premises corroborated by previous functional studies using live snakes. The dissertation comprises two chapters that used morphological measurements obtained in 61 species of snakes from the Colubridae and Dipsadidae families and ecological information available in the literature.

The first chapter rescued evidence of functional morphology related to locomotion on different habitat structures already proposed by other studies to support predictions of convergent patterns on slenderness and tail length associated with different degrees of arboreality in Colubroidea. We compared patterns of slenderness and tail length patterns to evaluate if both traits evolved under similar environmental pressures related to arboreality. To test our predictions, we constructed a dataset of morphological measurements - snout vent length (SVL), mid-body circumference (MBC) and tail length - and another dataset of ecological information (index of arboreality). Associations between relative mid-body circumference (a proxy of slenderness) and relative tail length with different degrees of arboreality were tested using two comparative phylogenetic tools: 1) phylogenetic generalized least square (PGLS) and 2) evolutionary models fitting. The morphological evolution of the group included specialization in the use of arboreal microhabitats associated with an increase in body elongation, however, highly slenderer arboreal species did not necessarily exhibit longer tails. We proposed that, even if both characteristics evolved in association with the arboreal lifestyle in general, tail length is apparently similar among species that occupies any degree of arboreal substrate, while slenderness possibly evolved associated with the frequency of time spent in arboreal substrate.

The second chapter rescued the theory of heat exchange dynamics related to variation in body shape to investigate associations between Surface Area to Volume ratio (S/V) and differential use of thermal environments. We aimed to investigate if species that explore similar thermal environments (derived from similar microhabitats), such as fossorial, terrestrial and arboreal, or that are active at similar diel periods (diurnal or nocturnal) evolved similar Surface Area to Volume ratio, which likely differs from that observed among species that explore dissimilar microhabitats or diverge in the activity period. We constructed one morphological dataset and an ecological data base for each species. Methods used in applied stereology and geometry were imported here to compile the morphological data of S/V, and available

information from literature was used to construct the ecological data base. Ecomorphological associations between S/V and different ecological categories were tested using PGLS and phylogenetic ANOVAs. No differences in S/V patterns were identified between species that use distinct microhabitats or differ in activity periods. Possibly, snakes have developed physiological and behavioral traits that allow them to explore a variety of different thermal environments. In agreement with previous studies, this study indicates that snakes in general did not evolved specific morphological patterns associated with thermal variables

Key words: arboreality, colubrid, ecomorphology, Surface Area to Volume and slenderness

Resumo

Esta dissertação explorou padrões ecomorfológicos em serpentes colubrídeos sul-americanas, testando associações entre a forma corpórea e parâmetros ecológicos. Ferramentas de métodos comparativo filogenético foram utilizadas para responder as questões macroevolutivas relacionadas à evolução da forma corpórea em serpentes que diferem no uso de microhabitats e no período de atividade. Os principais pressupostos assumem associações entre forma (morfologia) e função (locomoção e termorregulação em diferentes ambientes), premissas corroboradas por estudos funcionais anteriores com serpentes vivas. A dissertação é composta por dois capítulos, os quais utilizaram medidas morfológicas obtidas em 61 espécies de serpentes das famílias Colubridae e Dipsadidae e informações ecológicas disponíveis na literatura.

O primeiro capítulo resgatou evidências de morfologia funcional relacionada à locomoção em diferentes estruturas de habitat já propostas por outros estudos de modo a suportar previsões de padrões convergentes de alongamento e comprimento de cauda associados a diferentes graus de arborealidade em Colubroidea. Comparamos os padrões de alongamento e os padrões de comprimento da cauda para avaliar se ambas as características evoluíram sob pressões ambientais semelhantes relacionadas à arborealidade. Para testar nossas previsões, construímos uma base de dados composta por medidas morfológicas – comprimento rostro-cloacal (CRC), circunferência do meio do corpo (CMC) e comprimento da cauda. Assim como outra base de dados composta por informações ecológicas (índice de arborealidade). As associações entre a circunferência média relativa do corpo (um *proxy* de alongamento) e o comprimento relativo da cauda com diferentes graus de arborealidade foram testadas usando duas ferramentas de métodos comparativo filogenético: 1) mínimos quadrados generalizados filogenéticos (PGLS – sigla em inglês) e 2) modelos evolutivos. A evolução morfológica do grupo compreendeu a especialização no uso de microhabitats arbóreos associada ao aumento no alongamento corporal, todavia as espécies arbóreas delgadas não necessariamente exibiram caudas mais longas. Foi proposto que,

mesmo que ambas as características estejam relacionadas ao estilo de vida arbóreo em geral, o comprimento da cauda é aparentemente similar entre as espécies arborícolas e independente do grau de arborealidade. Enquanto que, o alongamento possivelmente evoluiu associado à frequência do tempo gasto em substrato arbóreo.

O segundo capítulo resgatou a teoria da dinâmica de troca de calor relacionada à variação na forma corporal para investigar associações entre a relação Área de Superfície para Volume (S/V) e uso diferencial de ambientes térmicos. Nosso objetivo foi investigar se as espécies que exploram ambientes térmicos semelhantes (derivados de microhabitats semelhantes), como fossorial, terrestre e arbóreo, ou que são ativas em períodos diurnos semelhantes (diurno ou noturno) evoluíram proporções de S/V similares, que provavelmente diferem daquelas observadas entre espécies que exploram microhabitats dissimilares ou divergem no período de atividade. Construímos uma base de dados morfológicos e uma base de dados ecológicos para cada espécie. Métodos usados em estereologia aplicada e geometria foram resgatados para compilar os dados morfológicos de S/V, e informações disponíveis da literatura foram usadas para construir a base de dados ecológica. Associações ecomorfológicas entre S/V e diferentes categorias ecológicas foram testadas usando PGLS e ANOVAs filogenéticas. Não foi identificado diferenças nos padrões de S/V entre as espécies que usam microhabitats distintos ou diferem nos períodos de atividade. Possivelmente, as serpentes desenvolveram traços fisiológicos e comportamentais que permitem explorar uma variedade de ambientes térmicos distintos. Em concordância com estudos prévios, este estudo indica que, serpentes em geral não evoluíram com padrões morfológicos específicos associados às variáveis térmicas

Palavras chaves: arborealidade, colubrídeo, ecomorfologia, razão Área de Superfície por Volume e alongamento

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Theoretical Background

This Theoretical Background concisely introduces major concepts and associated topics of the present dissertation. Due to the high diversity of preceding concepts used in the chapters, this introduction is structured in sections that discuss some of the main topics addressed along the two chapters of this dissertation. The chapters are structured in the format of article manuscripts, with associated introductions focused on specific topics of each chapter. The sections encompassed in the current Theoretical Background, however, address these concepts using a broader approach and some historical context, which likely highlight core aspects for understanding the specific chapters. We also intent to show, even if summarized, what the Master's candidate has learned during the project development. Moreover, this Theoretical Background aims to facilitate the study of these concepts by new students in the future.

Evolution

Phenotypic diversity in all living beings, being morphological, physiological or behavioral, is recognized as fundamentally resulting from evolutionary processes (Futuyma and Kirkpatrick 2017). In the first synthesis of the evolutionary thinking, popularized by Charles Darwin, the environment had already played a protagonist role in evolutionary processes. Darwin implemented the concept of natural selection as the primary environmental force responsible for directly driving phenotypic changes throughout generations as a consequence of differential trait selection and relative adaptation of the organisms (Darwin 1859; Gould 2002). However, the understanding on how environmental pressures originate novel phenotypes has been updated since the 1980, with expansion of the initial idea that environmental pressures act only as a selective agent is actually just one of the processes that occurs in nature (Meyer 1987; Streans 1989; Hendry 2016). Recent evidence sustains the perspective of the environment playing also a role as an inducer of novel phenotypes, instead of only selector. Even though, despite the variety in ways which environment is related to the process originating the diversity of phenotypes in nature, its protagonist role driving evolution remains as the most important factor related to change of form and function in species (West-Eberhard 2003).

Macroevolution

Over wide *tempo* and space, major lineage diversifications are entangled with massive temporal or spatial changes in the environment, often eliciting radiations (Cracraft 1973; Mayr 1982; Jablonski 2005). This is the case of coniferous plants, which radiated and grew in

abundance after two million years of intense raining in the Planet (Gianolla et al. 2018). However, not all diversification processes necessarily occur as a consequence of large-scale environmental changes. It is known now that the most common processes of lineage diversification have been associated with the conquest of new environments previously unoccupied by the ancestors (Simpson, 1949, 1953; Yoder et al., 2010). Ecological Opportunity is one of the most important concepts used primarily in Ecology and Evolutionary Biology, which explains lineage diversifications in basically all extinct and extant clades (Ehrlich and Raven 1964; Cox 1977). Despite variation in the way it is used, this concept (Ecological Opportunity) helps biologists to understand how ancient populations with restricted habitat can posteriorly occupy a wide range of novel habitats and consequently originate novel phenotypes and ultimately, give rise to new species. Ecological Opportunity depends not only on the existence of an available niche, but also presumes pre-established conditions in the organism that enable full exploration of the new niche colonized. At first glance, the ancestral population must have the minimum condition to start using new environments, which allows them to survive and persist through time; these conditions could be related either with morphological traits that allow them to move on the new habitat or the physiological capability to deal with new environmental characteristics, as temperature or humidity. After large temporal scale, depending on the characteristics of some populations, few may be selected along with traits that permit them to better explore that environment in a different way. This process of phenotypic change associated with different environments is complex and slow, but often explains the origin of similar phenotypic patterns among species phylogenetically distant (Losos and Mahler 2010; Stroud and Losos 2016).

Morphological Evolution

Processes and patterns of morphological evolution might be among the oldest questions in Evolutionary Biology. Morphological diversity spreading within a lineage and through time can be explained by several sources. Populational genetics, heritability, mutation, developmental plasticity and environmental signals have been understood to participate in different processes that lead to morphological changes between organisms (Wagner 2011; Martin et al. 2015). Although how these new phenotypes are established through time and space could be attributed to either random events like neutral evolution or genetic drift, still have major influence of a proper match between a particular phenotype and the environment in most of the cases. Environmental parameters may favor the persistence of a particular genetic pool, associated to specific phenotypes, along several generations (Wagner 2011; Freeman 2014; Futuyma and Kirkpatrick 2017).

In several lineages, organisms that live in similar environments also show similar morphological patterns. Morphological similarity may evolve through different processes, namely convergence and parallelism. What biologically differs these concepts is fundamentally the developmental process underlying evolution of phenotypic similarity. Parallelism is defined as the process where two or more lineages present similar phenotypes that originate throughout the same developmental processes. Convergence, on the other hand, refers to superficial similar phenotypes originated by distinct developmental processes (Meyer 1999; Wake 1999). Although, the choice of which term to use in a determined situation is more often based on phylogenetic distance between the organisms and related with the trait presence in the ancestral (Parra-Olea and Wake 2001; Arendt and Reznick 2008). The presence of a trait in two species that do not share a recent common ancestor is considered convergence, or the presence of a trait in two species when the feature is not found in their most recent common ancestor but is present in a more distant is parallelism (Sanderson and Hufford 1996; Hall 2007).

Both concepts share the idea that environmental conditions might influence morphological evolution leading to similar, or convergent, patterns (I will use the words “convergence” and “convergent” hereafter when referring to similar phenotypes regardless of the associated developmental processes). In Tetrapoda, numerous examples illustrate convergent morphology associated with the use of specific environments. Body elongation, limb reduction and increased insulant fat are good examples of morphology convergence in separate lineages of aquatic mammals (English 1976; Reeb et al. 2007; Fish et al. 2008). Studies from paleontological and neontological studies suggest that along the evolutionary history of the lineage of all aquatic mammals while its gradual use of aquatic environment was accompanied with such morphological changes (Fish et al. 2008; Liwanag et al. 2012). In Squamata, evolution of snake-like phenotypes comprise addition of vertebrae correlated with the use of underground microhabitats in fossorial lizards (Woltering 2012). In frogs, morphological correlation with the use of particular microhabitats is also seen in a global scale (Citadini et al. 2018). Semiaquatic species present large leg muscles and extensive foot webbing, arboreal species have large finger and toe tips, and burrowing frogs present large metatarsal tubercles and short legs (Moen 2019). Moreover, evidence suggesting morphological similarities among animals that live in similar environmental conditions and equivalent geographical distributions have become so extensive that at some point it become a separated field of study in biology called Ecomorphology.

Ecomorphology

Environmental associations of morphological evolutionary patterns has been well documented since early Evolutionary Biology, but only during the 1940, the field named Ecological Morphology or Ecomorphology has been established (van der Klaauw 1948; Wright 2017). Among the first studies revealing mirrored aspects of morphology and environment that popularized the term, I highlight Karr and James (1975), which demonstrated the existence of patterns in morphological features associated with specific ecological aspects in birds. Ecomorphology frequently refers to morphological convergent patterns, and studies often reveal that species sharing ecological similarities repeatedly comprise more similar morphological traits in comparison with species ecologically different (Wainwright and Reilly, 1994; Muschick and Indermaur, 2012; Trontelj et al., 2012). Because of the evolutionary understanding by that time (1970), convergent morphological patterns associated with ecological aspects had been first seen as evidence of adaptations. However, evolutionary biologists critically discussed the impacts of a generalized use of the adaptationist program, especially due to the lack of evidence about how morphological traits would directly affect fitness (Gould and Lewontin 1979; Mayr 1983). Only in during 1980 a novel approach was synthesized in the field of Evolutionary Ecology, offering a framework that partitions adaptation gradients by correlating morphology with function and then function with fitness. In that work, Stevan J. Arnold expressed a fully theoretical and empirical pathway to test if a morphological variable can be interpreted as an adaptation (Arnold 1983), later recognized as the ‘Arnold’s Paradigm’). Arnold’s paper showed that only by selecting good morphological candidates and further measuring their functionality with performance tests, then quantifying fitness in the field (i.e., quantifying reproduction of parental and survivorship of the kin until the same age of the parental), one might appropriately infer adaptation. However, as himself expressed in his article, the feasibility of all of the stages proposed is not always possible in practice, particularly because of the longitudinal estimates of fitness on the field.

Despite the challenges of implementing a full Arnold’s Paradigm in a single study, his idea became the spinal cord of most ecomorphological studies. Extensive literature on morphological associations with environmental variables has been published since the dissemination of the paradigm, commonly being sustained by empirical data and supporting the paradigm (Janzen 1993; Dudley 1996; Herrel et al. 2005; López-Fernández et al. 2012; De Meyer et al. 2019). Since then, researchers often also combine data from separate studies to generate integrated understanding of a given lineage. Information on functional morphology derived from one study can now be directly correlated with environmental aspects to infer evolutionary

associations of form-function with ecology. Also, by previous demonstration of morphological relationships with function and performance, researchers are able to import information gathered by separated studies that investigated associations between morphology and ecology (Feilich and López-Fernández 2019).

A popular word in ecomorphology is “ecomorphs”, which refers to a typical morphology associated with a particular ecological category, when it reflects a pattern observed in several species. This term has been disseminated since early and famous studies developed with *Anolis* lizards from the Great Antille Islands (Williams 1972, 1983). In these studies, Williams described morphological patterns associated with the used of specific microhabitats among several species of the genus. He suggested structured patterns in *Anolis* lizards that associate limb lengths with the height preferred occupied in the trees. These species were grouped into different morphological categories associated with the microhabitat structure (Figure 1). Furthermore, studies also described ecomorphs associated not only with tree height but also with branch diameter, and described relationships between ecomorphological patterns and performance (Losos 1990a; Irschick and Losos 1998, 1999).

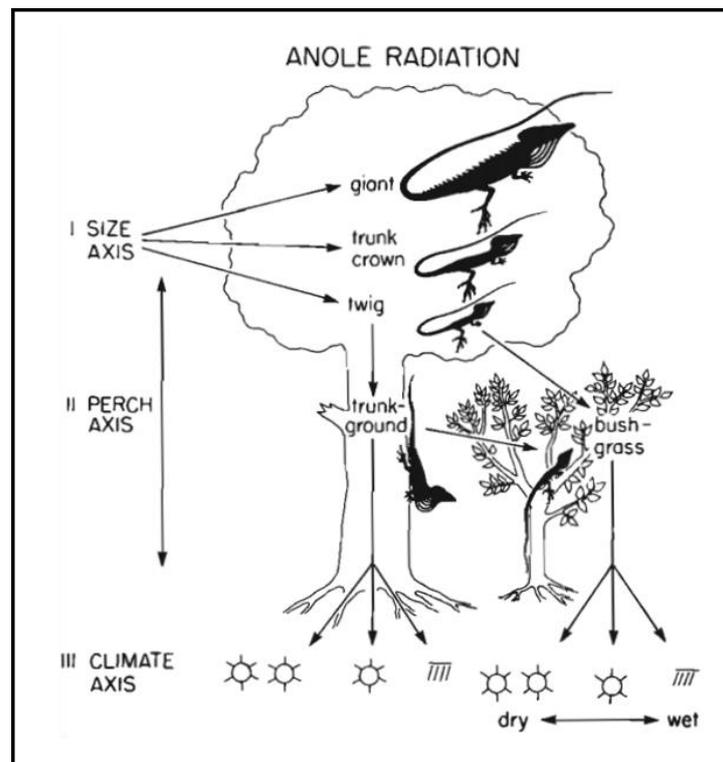


Figure 1 Image retrieved from: Williams, EE. 1983. Ecomorphs, faunas, island size, and diverse end points in island radiations of *Anolis*. Lizard ecology: studies of a model organism. **Representation of different morphologies and its associated microhabitat categories.**

In ecomorphology, there are more than one way of thinking in an evolutionary-ecology rationale and to direct a question to be answered. The so-called “morphometry-driven” approach focuses on measuring variation in shape and examining patterns of shape evolution (Swartz et al. 2003; Zelditch et al. 2012); it can be used simply to investigate differences in morphology among organisms. It can be considered as ecomorphology when measures of shape and form are examined in the context of interactions between organisms and the environment (Feilich and López-Fernández 2019). The approach called “function-driven” focuses on measuring how morphological variation relates to differences in function and performance (Wainwright and Reilly 1994). Again, it can be considered ecomorphology when the function of interest reflects how organisms interact with their environment (Feilich and López-Fernández 2019), being commonly applied in studies of functional morphology. Finally, the “ecology-driven” approach (Swartz et al. 2003; Feilich and López-Fernández 2019) focuses on measuring or describing morphology as a surrogate for ecological attributes. Implicitly or explicitly, the ecology-driven approach assumes *a priori* that morphology reflects an expected function or performance (e.g., long legs swim faster) and its relation with a particular environment (longer legs reflect the use of aquatic habitat). These studies use morphological variation to either describe ecological diversity given the absence of or inability to obtain primarily ecological information (Feilich and López-Fernández 2019). Again, these are oversimplified divisions among research approaches that complement each other, eliciting the connection from separate studies within a single and integrated perspective (see Feilich and López-Fernández 2019 for more details about the assumptions of each approach)

I highlight the “morphometry-driven” and “ecology-driven” approaches, as they are the ones that better describe the work reported in this dissertation. Both approaches have complementary significance and assumptions. The morphometry-driven approach focuses on how morphology varies in the intraspecific scale, and has two guiding directions: the geometric-morphometric-based approach and the functionally-relevant one (Gerry et al. 2011; Holzman et al. 2012). Both approaches focus on morphological variation, although they differ in how this variation is informative. While the geometric-morphometric-based approach depends solely on the variation to actually indicate further steps, subsequently leading to ecological and functional interpretations, the functionally-relevant approach is primarily dependent on a functional *a priori* knowledge about the variant form (McGill et al. 2006; Feilich and López-Fernández 2019). In this context, morphometry-driven approaches can be linked with ecology-driven studies when ecological information is also part of the scientific question and relate to functionality of the morphological trait.

Morphological Measurements

The morphological measurement deserves special attention, as it can be either the focus or the correlate variable in ecomorphological studies. Definition of a specific trait is quite complex, especially in comparative biology (Bookstein 1982; Bock 1994). A morphological trait can be either quantitative (e.g. SVL, limb length) or qualitative (e.g. presence or absence of dorsal spine). Here I highlight the caveats of quantitative or continuous traits. In morphometrics, morphology is the empirical combination of geometry and biological meaning, with a geometrical meaning pointing out the position of the trait in a morpho-space and the biological meaning determining its homology among other species (Bookstein 1982). In such context, the homology of traits is not necessarily the decedent's traits that are derived from an common ancestral, but one of the systematic paths to accurate measuring equivalent parts of the organism under investigation, which enables further comparison (Benson et al. 1982; Brigandt 2003). Systematization of morphological measures allows comparisons within a study or between studies with a particular clade. For example, front limb length is usually measured using external linear measures of each segment that correspond to the humerus and radius/ulna, still not directly measuring the bones (Kulyomina et al. 2019; Lowie et al. 2019). Although, some studies focus on particular individualized elements that could precisely rescue the homology characterization of the traits (Wagner 2016).

Due to the complexity of morphological traits, two types of measures can be used to capture the form, and choice depends on the interest of the question. Linear measures and (geometric measures) landmarks can be used to infer trait shape and relative proportions (Figure 2), although these result in distinct data treatments afterwards (James and McCulloch 1992). Linear measures can be analyzed by either univariate and multivariate whereas landmarks are necessarily multivariate (Zelditch et al. 2012). But as mentioned, these approaches vary in accord to the scientific question; if the researcher is interest in the overall head shape, that has several measures such as length, high, deep, a multivariate analysis might be able to recognize all of these measures as a whole and verify patterns all together (Bookstein 1982, 2016; Zelditch et al. 2012).

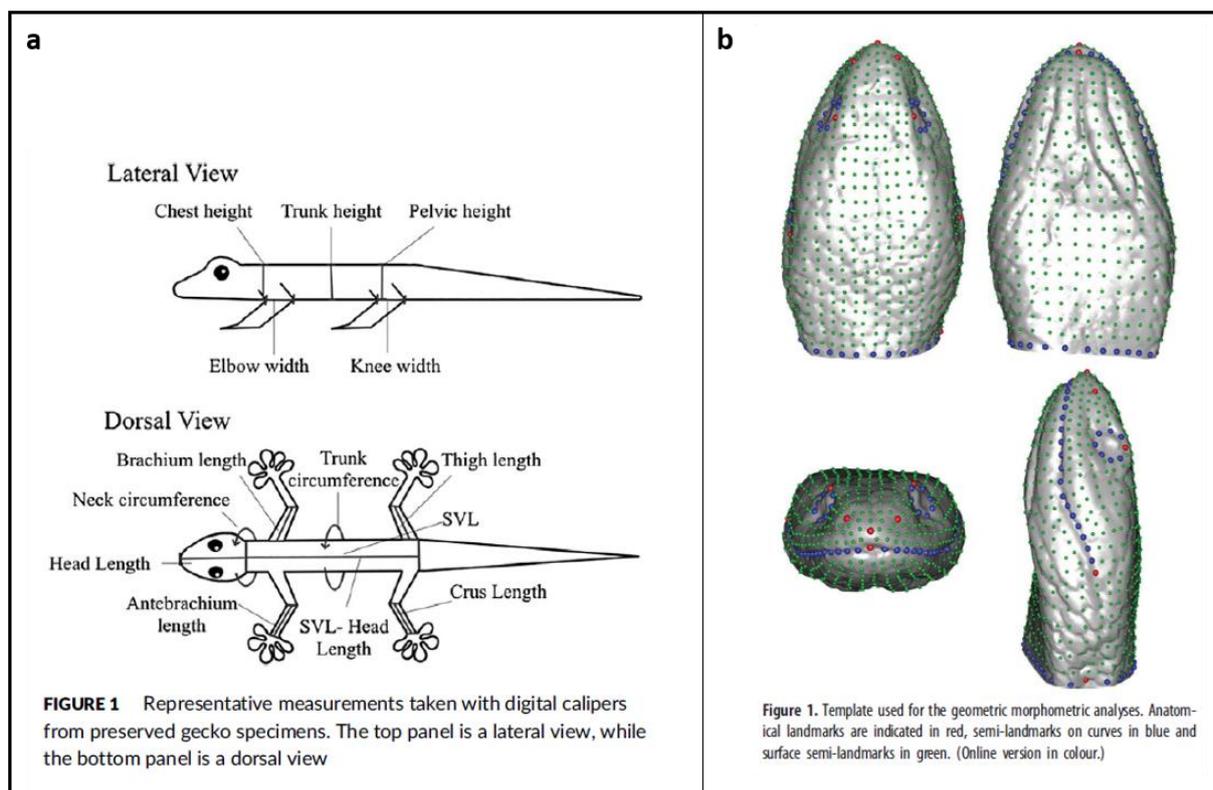


Figure 2 Images retrieved from: a) (Linear measures) Kulyomina Y, Moen DS, Irschick DJ. 2019. The relationship between habitat use and body shape in geckos. *Journal of Morphology* b) (geometric measures) Segall M, Cornette R, Fabre AC, Godoy-Diana R, Herrel A. 2016. Does aquatic foraging impact head shape evolution in snakes? *Proceedings of the Royal Society B: Biological Sciences*.

Ecology and Natura History data

In order to investigate evolutionary relationship between morphology and the environment, criteria used to choose the type of environmental information is extremely important, even though it is not always straightforward. There are two points to be considered here, first is the quality and liability of ecological information and the second is which kind of information to be used. Both points will dictate the results of the analyses and consequently our biological interpretation. If the quality of the data incorporated in macroevolutionary studies is poor, by no means due to absence of biological coherence, results will probably provide wrong interpretations (Meloro et al. 2013; Messier et al. 2017; Barr 2018). Ecological information also depends on the scientific question and how the type of ecological information actually describes the environmental parameter that the question is trying to address. This is central because the environmental parameter of interest, and must, in theory, be related to the functional meaning of the morphology (Ricklefs and Miles 1994). When the environmental parameter fails to express

associations with morphology, correlations tests between them will be vague in the biological meaning (Feilich and López-Fernández 2019). How the animal interacts with the environment has to be seen in nature before macroevolutionary hypotheses can be tested (Bock 1994).

Phylogenetic Comparative Methods

Comparative studies often seek regularities among organisms that require explanations and foster subsequent questions and ideas. Since initial studies focusing on phenotypic differences among species, the Comparative Method represents the most general technique used to investigate questions regarding patterns of evolutionary change under interspecific approach (Price and Schmitz 2016; Harmon 2018). Comparative methods, however, changed substantially in the past couple of decades. The major changes are attributed to the development of specific evolutionary and statistical models. Techniques innovated how evolutionary questions could be answered by making it possible to appropriately implement statistical processes while incorporating the connection among species in a formal hypothesis-test in evolutionary analyses (Felsenstein 1985; Harvey and Pagel 1991). Recent updates on comparative methods derive significantly from studies with squamates, and now represent a unifying and shared tool applied to any phenotypic variable, such as morphology, physiology and biochemistry (Mangum and Hochachka 1998; Martins et al. 2001; Garland et al. 2005; Stuginski et al. 2018). In comparative studies, discussion regarding the adaptative significance between phenotypic variables and the environment is another factor in evidence. As mentioned before, this issue has to be treated with caution. In order to avoid misinterpretation, it has been consensual that observation of patterns between a phenotype and the environment should be view as correlations or associations, instead of adaptation (Arnold 1983; Losos 2011). Adaptation presumes inference on functional morphology and fitness that allows wide generalization of the adaptationist significance of the variable of interest (Tamborini 2020).

Phylogenetic Comparative Methods (PCM) have a long history. During the 80s, Comparative Methods in Comparative Biology were improved by incorporation of phylogenetic information (Felsenstein 1985). From 1980 to 2000, a wide variety of studies have been published (Cheverud et al. 1985; Felsenstein 1985; Grafen 1989; Hansen 1997; Butler and King 2004; Revell 2009) and nurtured the field with innovation mainly on the analytical aspects of evolutionary biology (For more details about the historical timeline and discoveries, see Chapter 1 from *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology Evolutionary Models* by Lazlo Zsolt Garamszegi). The central aspect of that time, which remains in current discussions, is the possibility of running standard statistical analyses that

consider species as non-independent points by incorporating distances that reflect shared ancestral (Figure 3). The most popular techniques are the Independent Contrasts, developed by Felsenstein (Felsenstein 1985), and the Phylogenetic Generalized Least Square or PGLS (Grafen 1989; Hansen 1997). Independent contrasts deal with evolutionary relationship by recognizing that the differences ('contrasts') between closely related species or clades only correspond to independent data points in statistical analyses if divided by divergence time (or other measurement of phylogenetic distance). Similarly, the PGLS identifies from phylogeny the amount of expected correlation among species based on their shared evolutionary history, and weights it in the generalized least squares regression calculation by incorporating phylogenetic distance into the variance-covariance matrix of the data (Grafen 1989). Despite being slightly different methods, the results of PGLS in their initial form are ultimately the same of those derived from independent contrasts (Blomberg et al. 2012).

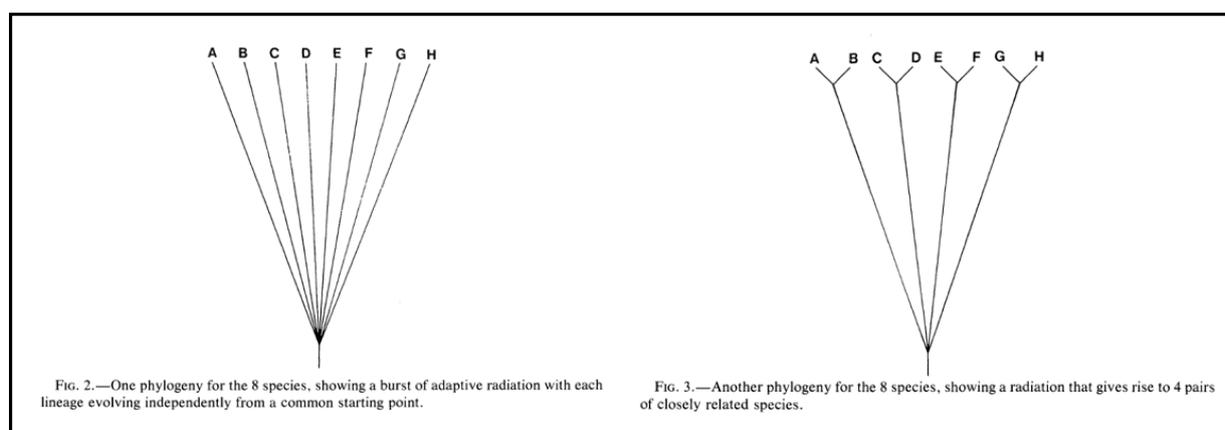


Figure 3 Edited image retrieved from: Felsenstein J. 1985. Phylogenies and the comparative method. *The American Naturalist*.

From a macroevolutionary perspective, trait evolution can be fitted into different “models”, and each model might explain patterns of change based on the observed values (Hansen 1997; Cooper et al. 2016). These models can either be inferred to explain the patterns of change in a given trait, but may also be incorporated into a linear regression analysis that assumes a given model *a priori* (Hansen et al. 2008; Revell 2012). When best model fit is the analysis goal, the model can predict if a trait has different optimum values, if it changed with different rates depending on the lineage, and also it can infer a value for a selective “pull” force (Butler and King 2004; Beaulieu et al. 2012). Calculations of these models are based on maximum likelihood scores and ancestral state reconstructions (Butler and King 2004; Revell et al. 2008).

Brownian Motion (BM) is the evolutionary model that assumes a “random walk” of the trait mean values that can be established in different directions and distances over time. The statistical process of Brownian motion was first defined in physics as the type of movement of particles from a given fluid (Felsenstein 1985; Harmon 2018). When using BM, one assumes that changes in a trait value are mostly explained by phylogenetic relationships among species and reflect cladogenetic events, trait evolution is drawn by normal distribution with mean zero and variance proportional to time (Felsenstein 1985, 1988). The BM infers values for a parameter of evolutionary rate, referred to as sigma squared. This parameter determines how fast traits randomly change through time, despite assuming a single rate of trait change in all lineages. From one generation to the next, a trait value might change due to processes including selection, genetic drift and random mutations (Hansen and Martins 1996). If movements are independent and identically distributed and have an additive effect through time (Figure 4b), according to the central limit theorem the evolutionary trajectory fits a Brownian motion process (if the movements have a multiplicative effect through time, the log of the trait value evolved under Brownian motion) (Butler and King 2004; Butler et al. 2008). Another mode, named Ornstein–Uhlenbeck (OU), better describes processes where movements tend to express the direction of a particular trait value (See Figure 4 for differences in trait change under BM and OU models).

The OU model started to be used by phylogenetic comparative methods when proposed by Felsenstein (Felsenstein 1985), but became popular after development of methods and theory by Butler and King 2004 with the *OUCH* package for R environment. Overall, OU models are phenomenological models of optimum movement rather than quantitative genetics models of adaptation (Garamszegi 2014). Nonetheless, OU models can be included in linear regression analyses, similar to BM, despite assuming that the trait would have a single mean optimum (θ). This approach adds an effect from phylogenetic branch lengths, affecting the expected Brownian Motion. The main innovation of this model is the use on evolutionary model fitting (Butler and King 2004; Beaulieu et al. 2012a; Cooper et al. 2016). In model fitting, it is possible to determine possible selective pressures that may be correlated with differences in trait averages. Specific analytical tools allow estimates of ancestral states for the trait of interest and also the expected selective pressure (assigned as an environmental predictor, such as habitat type or activity period), then calculating evolutionary correlations. Moreover, such analyses have the capacity of rescuing more than one mean optimum (θ) of trait value (Figure 4c) and different evolutionary rates (σ) depending on the lineage in the phylogeny and the selective “pull” (α) for the particular selective force of the model (Felsenstein 1988; Butler and King 2004; Beaulieu et al. 2012).

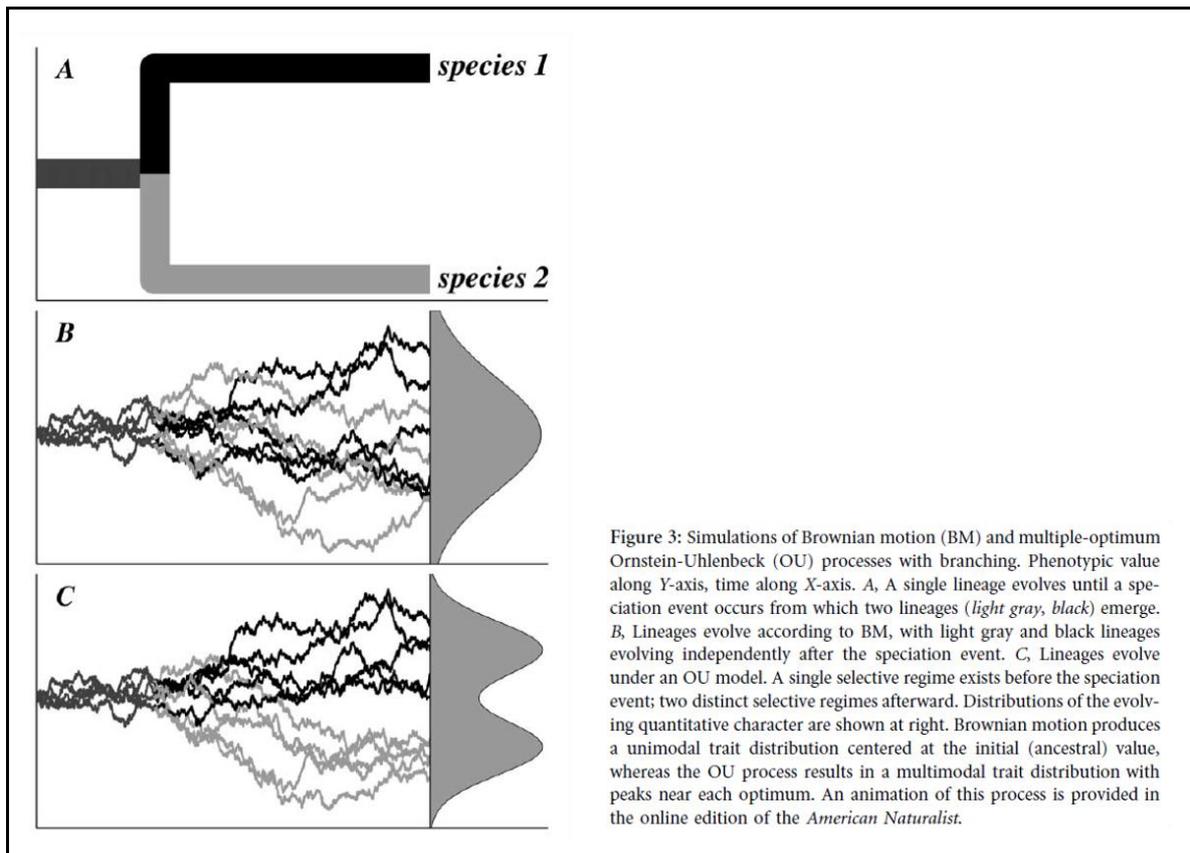


Figure 4 Edited image retrieved from: Butler MA, King AA. 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *The American Naturalist*.

Thermal Environment as Phenotypic Evolutionary Drivers

Morphological evolution may occur in association to different selective pressures. Specific morphological profiles might be under selection if variation in state reflects variation in performance and ultimately in fitness (Arnold 1983, 1992). This is actually a very straightforward rationale. Even though, what enables morphology to perform the expected function? Physiology and muscular biochemistry are interpreted as being intermediates between morphology and performance (Hochachka and Somero 2002). Biochemical reactions in each organism are established under specific temperature ranges, which is particularly relevant in vertebrate ectotherms (Angilletta et al. 2002; Sunday et al. 2012).

During the evolutionary trajectory of a given lineage, phenotypes evolve in association with climatic conditions that directly influence environmental parameters, and specific traits that enhance performance of particular functions may be favored in each setting. Several traits may evolve in association with the thermal environment, and these include mostly physiological and behavioral aspects but also morphological traits (Bicego et al. 2007; Tattersall et al. 2017),

especially because form and function are entangled. Physiology might evolve in association with changes in thermal environment when populations use novel habitats, which likely modifies the range of temperatures suitable for a given function (temperature optimal), e.g., body optimal temperature necessary to function evolved in association with conquest of novel habitat in more northern latitudes (Berger et al. 2014). Changes in the optimal temperature for specific functions is more often observed in vertebrate ectotherms that are thermoconformers, as their body optimal frequently remains closer to air temperatures (Figure 5) (Huey et al. 2009; Chevin et al. 2010). Although, behavioral adjustments may actually preclude physiological or morphological changes in animals that thermoconform and also in those that thermoregulate because of its elevated flexibility (Sartorius et al. 2002; Muñoz and Bodensteiner 2019). Some lineages of Australian birds comprise species occurring in colder regions that display the back rest (tuck bill into the plumage) behavior and sitting much more often, allowing them to slow the heat loss (Pavlovic et al. 2019). Behavioral adjustments are often view as thermoregulatory adaptations that grant maintenance of body temperature near the optimum range for specific tasks, being usually at higher temperatures than the environment (Huey 1974, 1976; Muñoz and Losos 2018).

The time involved in increasing or decreasing body temperatures determines heating and cooling rates depends on several parameters including morphological traits (Bakken et al. 1985; Dzialowski and O'Connor 2001). Insulation of the most distal layer on the body and Surface Area to Volume ratio (S/V) also have great impact on the rates of heat exchange in an animal. Animals with high proportion of Surface Area to Volume (S/V) tend to gain and lose heat faster than those having lower surface area in relation to their volume (which have increased thermal inertia). This is essentially a consequence of how much contact the surface of the animal has with the air, proportionally to its volume, which is basically the size of space inside the animal that is going to receive heat (Spotila et al. 1973; Bakken et al. 1985; Stevenson 1985). Implications of heat exchange rates may encompass trade-off costs related to predator avoidance activities, food acquisition and sociality, which ultimately contribute for energetic balance. This model captures most aspects of the thermoregulation equation, and ultimately predicts cost-benefits, although predictability may be affected by any change in physiological or morphological parameters that modify a given variable (Huey 1976).

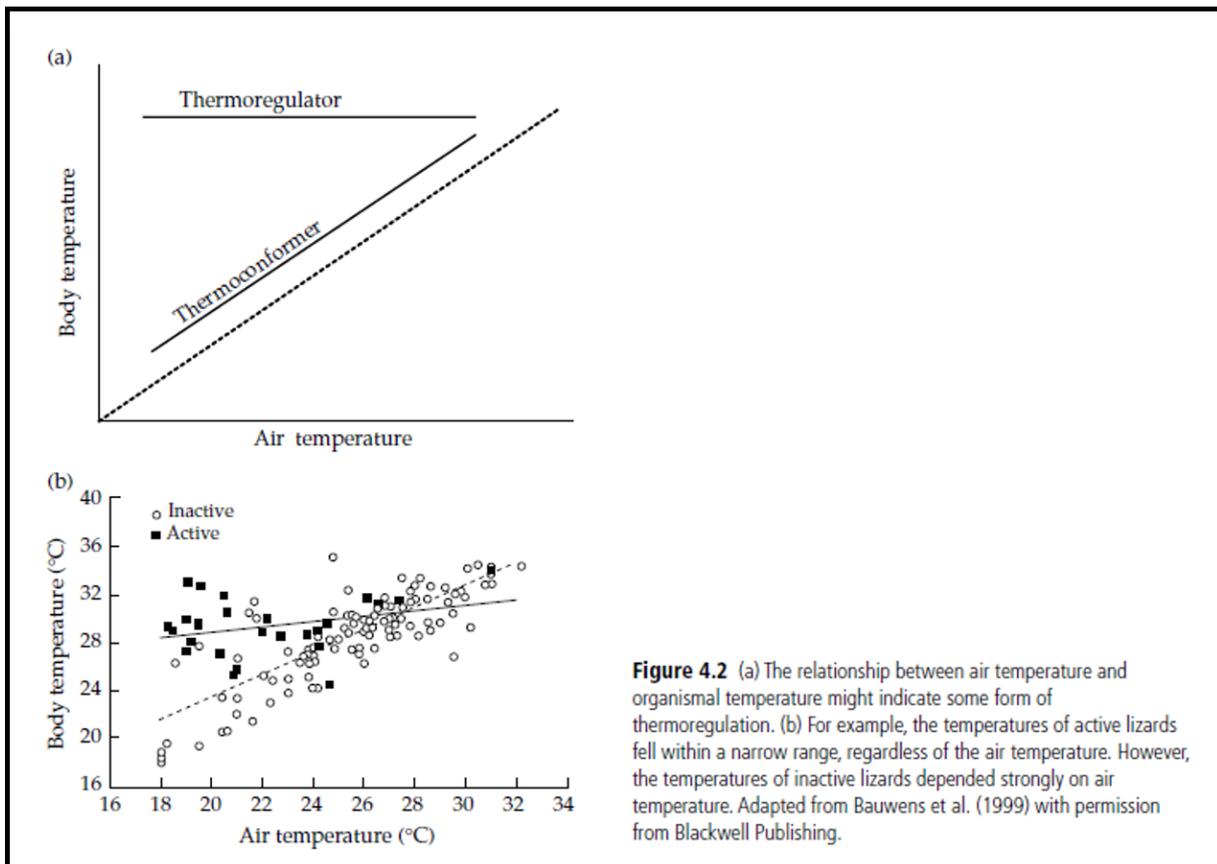


Figure 5 Edited images retrieved: Angilletta Jr MJ, Angilletta, MJ. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press. **Representation of body temperature in response to air temperature n thermoregulatory and thermoconformation ectotherms.**

Relationships between S/V and body size (usually inferred from body length) often comprise a negative correlation because of the differential dimensionality of both variables, consequently its disproportional relationship with the object length. In isometric changes (increase in size without change in shape) surface is proportional to square of length and volume is proportional to cubes of length (Figure 6) (Vogel 1988). Body weight or length has been frequently used as a proxy to S/V despite their imprecision in rescue the direct ration between Surface Area and Volume (Tattersall et al. 2017). Estimates based solely on volume might also be compromising as these likely disregard shape variations (non-isometric change) that ultimately affect Surface Area and consequently influence the Surface Area to Volume ratio. Still, studies have used body mass or length as a proxy to S/V when investigating morphological evolution of species in different thermal environments. These interests are mostly illustrated by discussions on the Bergmann's rule, which predict body mass negatively correlated to environmental temperatures (Zamora-Camacho et al. 2014; Torres-Romero et al. 2016).

According to this hypothesis, larger and massive animals are more probable to occur at geographic distributions comprising low temperatures, such as the higher latitudes or elevated regions, and smaller animals be more often associated to tropical regions (Blackburn et al. 1999). Evidence supports these predictions for endothermic lineages, such as mammals and some birds, but patterns are less conspicuous for ectotherms (Ashton and Feldman 2003). Among squamates, for example, opposite trends have been described depending on the continent, and some lineages comprise larger animals associated to warmer places (Olalla-Tárraga et al. 2009; Slavenko et al. 2019).

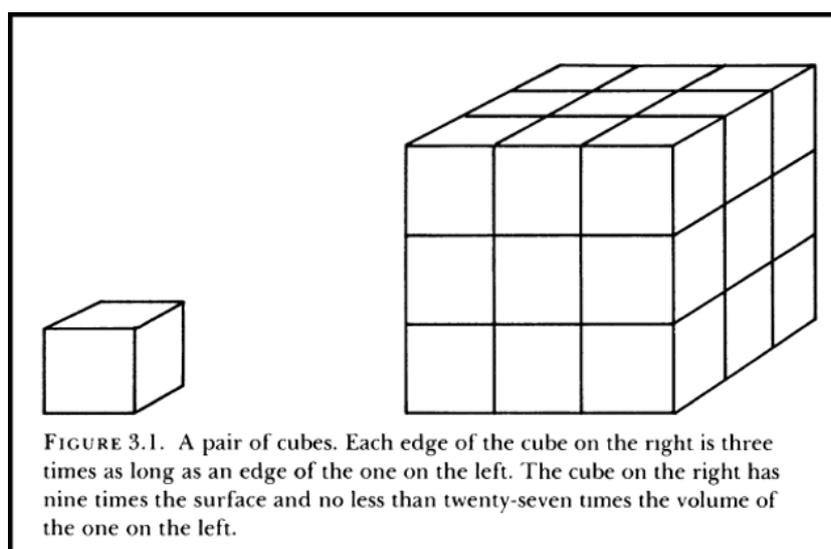


Figure 6 Images retrieved from: Morrison P. 1989. *Life's Devices: The Physical World of Animals and Plants*.

In addition to the absence of a pattern for thermally-associated morphological evolution associated to latitudinal gradients under interspecific approaches, effects of thermal heterogeneity related to microhabitat and period of activity remain obscure in the Serpentes lineage. In localities where the air temperature is relatively similar along a given geographic region, microhabitat aspects may comprise particular thermal characteristics related to overall structure (Mayr 1955; Whitaker and Shine 2002; Medina et al. 2007; Feldman and Meiri 2013; Woods et al. 2015; Pincebourde et al. 2016). Microhabitats near to rivers and streams, for example, usually have cooler and constant temperature, while fully terrestrial comprise more variation (Navas 1996). The same is observed at underground tunnels where fossorial species are found, which usually are more homogeneous environments (López et al. 1998). Arboreal microhabitats also display particular conditions, and studies using operative temperature suggest that, for small animals, the diameter of the tree branch may affect rate exchange and maximal

temperatures reached by these animals (Bakken 1989; Dzialowski 2005). Also, because of the distance to the substrate, arboreal habitats are less affected from the heat retained in the ground (Pincebourde et al. 2016), which may affect animals that are active at early night because the heat retained from the ground may smooth the rate of cooling as temperature decreases (Dorcas and Peterson 1998). Accordingly, arboreal ectotherms may be more susceptible to increased heat exchanges with the environment, a relationship likely affected by morphological patterns such as body shape and size.

Snake Evolution

Snakes have long been one of the most striking animals that fashioned researchers because of their elongated and limbless phenotype. Snake origins and systematic relationships with other lineages place the Serpentes clade within Squamata (Figueroa et al. 2016). Evidence suggests that Serpentes originated about 110 Mya, during the end of Early Cretaceous, specifically in the Albian stage (Pyron et al. 2011, 2013). This clade is highly diverse and rich in number of species (more than 3000 species; see Pyron and Burbrink 2012; Hsiang et al. 2015), with representatives around the globe except the poles. Snakes are distributed in terrestrial environments that include open habitats, deserts, forests and anthropomorphic altered habitats, and may specialize in fossorial habitats, arboreal habitats comprising species that never move on the ground, and even specialize in aquatic lifestyle, including some semi-aquatic species that leave the water just to transit from a water body to another (Greene, 1997).

The Serpentes lineage also comprises remarkable morphological diversity, despite encompassing only limbless-elongated representatives. Head shape in snakes vary from ‘chunky’ (e.g. *Imantodes* species) to extremely angulated (e.g. *Ahaetulla* species); while all snakes are cylindrical, some species exhibit trunks that are compressed lateral or dorsoventrally (Greene 1997; Jayne et al. 2015). Some Scolecophidians and basal snakes have rudimentary pelvic girdles and external vestigial hind limbs (e.g.: Boidae and Pythonidae), although none have traces of pectoral girdle or frontal limbs (Green 1997). Body size in snakes can be extremely variable, being smallest species known on average 100 mm² (Leptotyphlopidae family in Barbados Island), while the largest snakes belong to the Pythonidae family and can be over 7 meters long (Hedges 2008), being distributed in several parts of the Asian continent (Murphy et al. 1997).

Some snake lineages have more recent origins, and recent studies suggest for example that the Caenophidian lineage diverged 35 Mya, in the Oligocene, originating the Colubroidea and Elapoidea superfamilies (Zaher et al. 2019). Colubroidea is one of the most recent clades within Serpentes, having originated in the Asia continent (Pyron and Burbrink 2012). Still,

species from this lineage are among the mostly diversified in ecology and morphology, with a less conservative ecology than older clades such as Boidae and Pythonidae (Bars-closel et al. 2017). Some species from Colubridae and Dipsadidae families from South America are highly specialized in specific microhabitats, activity of periods, and diet, with species so specialized that only eat snails at night, for example (Alencar et al. 2013; de Sousa et al. 2014; Harrington et al. 2018). A considerable proportion of South American colubrid is partially specialized in arboreal microhabitats (Harrington et al. 2018). Species vary in how they use the arboreal microhabitats, and some even use trees only for resting or thermoregulating, avoiding terrestrial predation, while others forage and rest mostly on the trees, consequently spending more time above ground (Lillywhite 2014).

Snakes that are specialized in arboreal microhabitats or that often move along shrubs and trees usually display morphological particularities (Martins et al. 2001). Longer tail lengths have been extensively reported in arboreal snakes, although slenderness has been addressed less frequently (Lillywhite et al. 2000; Lillywhite 2014; Sheehy et al. 2016). These traits have been primarily informally evaluated in relation to the use of arboreal microhabitats, but in 1990 functional studies started to demonstrate how these relate to locomotion in arboreal microhabitats using more sophisticated techniques (Seymour 1987; Lillywhite 1993; Astley and Jayne 2007). Tail length has been measured in different arboreal species from several families, and results identifying longer tails demonstrated that this aspect prevents blood oedema during vertical position (Lillywhite 1993; Lillywhite et al. 2012). Regarding to functional implications of specific phenotypes, arboreal snake species display slenderer bodies that apparently enhance biomechanics of locomotion along tree branches, as observed in the cantilever behavior (Figure 7) (Jayne and Herrmann 2011). Furthermore, similar ecomorphological patterns in snakes have been reported in several arboreal species, but remains relegated in specific lineages of limited geographic distribution, which might comprise particular evolutionary trajectories that deserve attention.

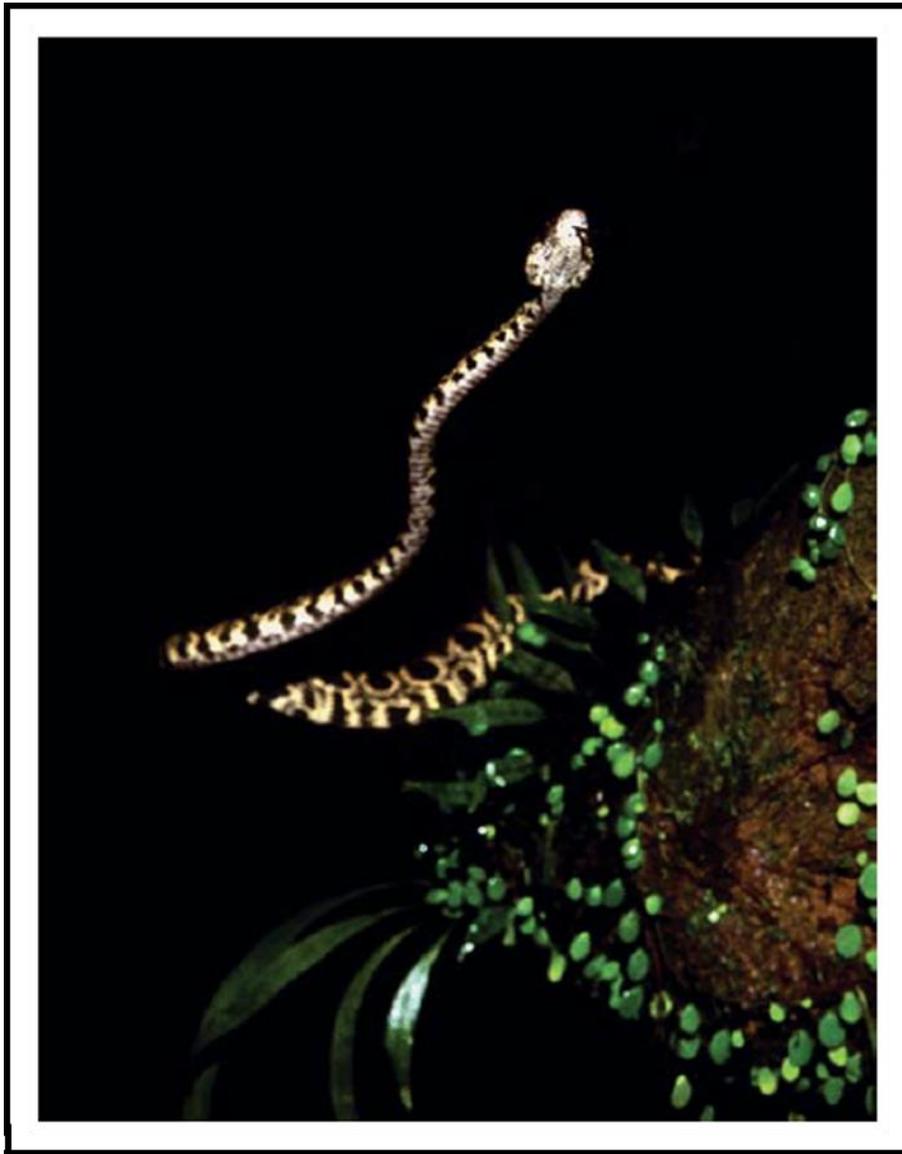


Figure 7 Image retrieved from: Lillywhite H B. 2019. How Snakes Work: Structure, Function and Behavior. Amphib. Reptile Conservation. **Cantilevering behavior (extension of the body on the air, usually to move over a gap).**

Chapter 1

Does body shape evolve in association with arboreal life-style? Ecomorphological associations in Neotropical lineages of Colubridae and Dipsadidae (Serpentes, Squamata)

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Abstract

Evolution of specialized microhabitat usage is often coupled with phenotypic changes that likely optimize traits for demands from particular environmental settings. Associations between snake morphology and arboreality are often addressed as a straightforward pattern, but such ecomorphological associations seem to be more complex when context-restricted lineages are evaluated. In this study we used linear morphometrics and advanced comparative phylogenetic methods to test for associations between morphological patterns (slenderness and tail length) and microhabitat usage in snake species from the Neotropical families Colubridae and Dipsadidae. By implementing phylogenetic analyses of variance and evolutionary model fitting, we identified that morphological evolution in the group comprised specialization in the use arboreal microhabitat coupled with increases in body slenderness, although slender arboreal species do not necessarily exhibit longer tails. Moreover, species that are fully terrestrial tend to present similar degree of slenderness observed in species that occasionally climb. Evolution of tail length, in contrast, seems uncoupled from the frequency of arboreal use, as all species exploring arboreal microhabitats have similar values of tail length. Overall, our study suggests that the use of special substrates likely imposes different selective regimes over different morphological traits. We propose that even that both traits are related to arboreal life-style in general, tail length apparently reflects arboreality independent of the time spent on arboreal substrate, whereas slenderness evolved in dependency of the frequency of time spent on branches.

Introduction

The question about how organisms interact with complex environmental structures is deeply associated with a complete understanding of the evolutionary history of morphological traits (Williams 1972; Losos 1990, 2011; Revell et al. 2007). Specialization into a particular microhabitat has been often interpreted as a potential selective force that may result on trait optimization that maximizes performance of specific tasks, such as locomotion (Schulte et al. 2004; De Alencar et al. 2017; Pelegrin et al. 2017; Lowie et al. 2019). The conceptual framework that directly correlates the functional morphology to fitness is the main idea supporting interpretations that rescue the idea of convergence in macroevolutionary studies (Arnold 1983; Losos 1994; Wainwright and Price 2016). Conversely, authors have been cautioned to new evidences indicating that shared functions do not guarantee convergence at the morphological level (Thompson et al. 2017). Not rarely, the ability to perform a task in a given environment does not change in despite of substantial variation in morphological traits. Such phenomenon has

been often interpreted as indicative of relaxed selective forces or other constraints acting by trade-off restricting trait's optimization (Wainwright et al. 2005; Maestri et al. 2016; Kulyomina et al. 2019). Still, the morphological association to differential environmental structure along the evolution depend on how the organisms in particular, or lineage specific, use its environment. In this context, despite the presence of global ecomorphological patterns, a closer look at specific lineages will always be necessary to understand the evolution of morphology of its forms in a set region.

The lineage *Serpentes* constitutes a promising model system for ecomorphological studies, given the wide ecological diversity observed in the group (Sheehy et al. 2016) and extensive descriptions of phenotypic associations with microhabitat usage including head shape, trunk elongation and tail length (Lillywhite 2014; Fabre et al. 2016; Sheehy et al. 2016). In some snake families, as *Boidae*, *Pythonidae* and *Viperidae*, slenderer trunks and relatively longer tails are observed in snakes that often use arboreal microhabitats to forage (Martins et al. 2001; Pizzatto et al. 2007; De Alencar et al. 2017); species with these characteristics have been formerly referred to as “semi-arboreal” (Greene, 1997). This correlation has been interpreted as a consequence of physiological implications of climbing, including also an adaptation to deal with gravitational forces on the vertical posture (Lillywhite 1993, 2014; Byrnes et al. 2014). Performance tests suggest that not only slenderer-bodied snakes are able to climb more efficiently on branches, but slenderness is also correlated with a better distribution of body weight, which allows individuals to move over thinner branches and change from branch to branch (Ray 2009, 2012). Gravitation is an important factor during climbing, as snakes likely experience pressure variation in the fluid column between head and tail while in vertical posture (Lillywhite 1993). Changes in relative positioning of specific organs, with the heart located more anteriorly, nearer the head, have been described in arboreal snakes, an adaptation that likely enhances cephalic oxygenation (Seymour 1987; Lillywhite et al. 2012). However, the tail of these climber's snakes also suffered from gravitation effect on the circulatory system, as much as the trunk. Former literature demonstrated that a wide diversity of arboreal snake presents non-compliant tissue compartments correlated with elongated tails, reflecting the capability to avoid blood oedema (Lillywhite 1993, 2015). A convergent pattern has been identified among 15 families exhibiting longer tails in association with specialization in arboreal microhabitats, and elongated tails have been interpreted as a morphological state associated with gravity and blood oedema that independently evolved in snake evolution (Sheehy et al. 2016).

Although several studies discuss convergent patterns of slenderness and tail lengths associated with the general use of arboreal microhabitat (Pizzatto et al. 2007; Sheehy et al. 2016; De Alencar et al. 2017), no study so far focused on morphological evolution within families from

the high colubroidea clade, especially in South American species of Colubridae and Dipsadidae. Colubridae and Dipsadidae comprise high morphological and ecological diversity (Alexander Pyron and Burbrink 2009; Alencar et al. 2013; Bars-clozel et al. 2017), and encompass over 50 genera. In particular, the South American species are widely distributed, with representatives occupying broad latitudinal ranges and others restricted to the rainforest (Cadle 1985; Daza et al. 2009; Museos and Andes 2014). The colubroidea superfamily diverged recently, around 35 Mya, and its species radiated into all major habitats, ranging from open lands to dense forests and occupying all types of microhabitats: fossorial, litter swimmer, terrestrial, semi-arboreal and fully arboreal (Strussmann and Sazima 1993; Martins et al. 2001; Alencar et al. 2013; Guedes et al. 2014; Zaher et al. 2019). The semi-arboreal or arboreal species comprise great variability on the proportion of time spent on tree branches or bushes. Some species are very specialized, and forages, rest and reproduce over trees; others use arboreal microhabitat only to rest. In the present study, we investigate evolutionary patterns of slenderness and tail length in Colubridae and Dipsadidae, testing for relationships with different specialization levels to the arboreal life style.

Materials and Methods

Species sampling and morphological data

Morphological data from 61 species of neotropical Brazilian Colubridae and Dipsadidae snakes were obtained from 969 specimens available at Brazilian scientific collections. Sample size for each species varied between 1 and 20 specimens (average 18 individuals per species), and only adult males were considered. Data from species comprising samples of less than five specimens were included only after exhausting search in all collections did not result in specimens suitable to increase sample size. The total of species represented by less than five individuals corresponded to 9.8% of the total dataset.

We compiled a database using mean values of snout-vent-length (SVL), mid-body circumference (MBC) and tail length for each species. The SVL was assumed as a proxy of body size, consistent with the literature and due to its relationship with several morphological and physiological variables (Lindell 1994; Herrel et al. 2011; Feldman and Meiri 2013). Body slenderness was inferred from MBC. Measurements were obtained using a meter stick, a caliper rule and a string. We measured SVL from the tip of the nose to the posterior end of the cloacal scale following the specimen spine with body contours, and measured MBC at the point corresponding to 50% of the SVL of the individual; tail length was measured from the posterior end of the cloaca to the tail tip.

Ecology classification

In order to understand the evolution of morphological variables in snakes and its relationship with microhabitat use – with special focus on the specialization in arboreality - we established an ecological classification comprising one continuous variable (Arboreality Index) and two types of categorical classification. The Arboreality Index was based on ecological and field notes from current literature, and to each species we assigned a score ranging from 1 to 100. The score for each species corresponded to the number of individuals observed in arboreal microhabitats divided by the number of individuals observed in arboreal plus terrestrial microhabitats (see Supplementary Table S1 for the scores). Complementarily, species were also classified into a two-category based classification that divided species in terrestrial (species never found in arboreal microhabitats) and arboreal (species described at least once in arboreal microhabitats). Finally, we also classified species following the nomenclatural and criterium from Sheehy III and Lilywhite 2016, which grouped part of species generally classified as ‘arboreal’ into a separate and more specialized category. This classification was originally based on the proportion of gravitational stress that the individuals of a particular species experience and is referred to as G-habitats (gravitational habitats). The G-habitat classification divided species into three groups: stenotopically (specialized arboreal species), eurytopically (broadly adapted), and non-scansorial (snake species that never climb). This type of classification also considers species depending on frequency of individuals encountered on each microhabitat. Non-scansorial species retain individuals that never climb. Eurytopically species comprise individuals that are often found on the ground but climb occasionally, less than 50% of the time on arboreal microhabitats. Stenotopically snake species encompass individuals that spend more than 50% of the time on arboreal microhabitats. By using different classification criteria, we refined our interpretation and reduce bias from ecological information. Moreover, we aimed to test evolutionary model fitting, and approach that presumes categorical factors (microhabitat classification) instead of continuous (Arboreality Index) to be assumed as a selective regime.

Statistical Analyses

All statistical analyses and graphical information were implemented in R Core Team environment (2013; R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>). In order to test for interspecific variation, we calculated the mean value of tail length, mid-body circumference and SVL for each species. Prior to analyses, morphological variables were log₁₀ transformed in order

to establish a better data distribution and variance. Allometry of tail length and mid-body circumference with body size (SVL) was tested using phylogenetic regression. After confirming a significant allometric relationship, residuals of tail lengths and MBC regressed by body size were extracted for the subsequent analyses. These variables were then named relative tail length (RTL) and relative mid-body circumference (RMBC).

Phylogenetic hypothesis

Data were analyzed using comparative methods, implemented using a topology based on relationships from the time-calibrated phylogenetic hypothesis proposed by Zaher et al. 2019, which comprised a data matrix of 1278 terminal taxa representing all caenophidian families and a total of 6447 mitochondrial and nuclear sequences (total of 15 genes). The complete phylogeny was trimmed to include only species measured in the present study, maintaining the phylogenetic relationships among species and the divergence time estimates proposed in the original phylogeny by Zaher et al. 2019.

Phylogenetic Comparative Method (PCM) approach

We used Phylogenetic Generalized Least Squares (PGLS; Grafen 1989; Martins and Hansen 1997) to address possible associations between morphology and the Arboreality Index. In PGLS, the original (non-phylogenetically corrected) data are converted by multiplication with the variance - covariance matrix derived from the phylogeny branch length information. PGLS was used to test for correlations between RTL, RMBC and SVL and the Arboreality Index, using *caper* package and considering Brownian-motion as the default for evolutionary models (Pinheiro et al. 2020).

Subsequently, we tested for differences in mean and variances of the morphological variables between each microhabitat categories by performing separate tests for the two types of classification. First, we investigated differences in RTL and RMBC between snakes from arboreal and terrestrial microhabitats. Then we tested for differences in RTL and RMBC between species according to the three G-habitats using analyses of variance (one-way ANOVA) and accounting for phylogeny (“*phylANOVA*” function in *phytools* package; Revell 2012); these were followed by post-hoc “Holm-Sidak” test to verify pairwise differences between groups in the G-habitat classification.

Evolutionary model fitting for tail length and slenderness

Two approaches were used to address morphological evolutionary history in Colubridae and Dipsadidae. Evolutionary model was primarily inferred using only phylogenetic information and morphological data to verify if distribution of RTL and RMBC values in our database fitted better a random distribution or one comprising a tendency (contrasting Brownian Motion with general Ornstein Uhlenbeck; see Supplementary Figure S1). Then, we estimated ancestral states for the continuously valued characters based on the maximum probability of that state having occurred and it precluded all subsequent analyses, using *fastAnc* function (“*phytools*” package; Revell 2012). These were our maximum likelihood estimates (MLE), variances and 95% confidence intervals for each node. Derived from MLE, separated phylogenies containing ancestral reconstructions of RTL, RMBC and SVL and Arboreality and ancestral state reconstructions of microhabitat on the nodes were established using *contMap* function, also provided by *phytools* (Supplementary Figure S2 and Figure S3).

The second approach was used to infer possible selective regimes related to the frequency of arboreal use with the evolution of RTL and RMBC in Colubridae and Dipsadidae snakes. We implemented the model fitting approach proposed by Butler and King 2004 using *OUCH* package. This approach (*OUCH*) rescues the best evolutionary model by first comparing Brownian Motion (BM) with other models comprising selective regimes specified by the researcher, often Ornstein-Uhlenbeck (OU) encompassing one or more adaptive phenotypic optima. We applied this method to ascertain which OU model was the best fit for each morphological variable in our data base: OU1 - single optimum (θ) model, OU2 and OU3 – assuming distinct θ for each microhabitat while having a single selective pull (α) and evolution rate (σ^2). We ran separate tests for RTL and RMBC, where we assumed OU2 model as a hypothesis where RTL and RMBC evolved under only two selective regimes associated with trait change: terrestrial and arboreal. The OU3 model assumed RTL and RMBC evolved under three selective regimes associated with trait change: non-scansorial, eurytopical and stenotopical. To determine which model better fit the data, we compared AICc (AIC corrected for data sample) and chose the model with the lowest value, being AICc differences ≥ 2 considered significant (Anderson 1998).

Results

Mean values for SVL, tail length and MBC accompanied by standard errors, as well as the ecological classifications, Arboreality Index and G-habitat for the 61 snake species included

in this study are provided at Supplementary Table S1. Untransformed values of average SVL for the snakes included in our database ranged from 217 mm² (*Tantilla melanocephala*) to 1534 mm² (*Drymarchon corais*). Untransformed tail lengths ranged from 38.8 mm² (*Atractus reticulatus*) to 790 mm² (*Chironius multiventris*); and Mid-Body-Circumference values ranged from 21 mm² (*Imantodes cenchoa*) to 155 mm² (*Xenodon severus*) (see Figure 1 below). Moreover, the highest RTL value in our database were from *Dendrophidion dendrophis*, and the lowest value, *Apostolepis dimidiata*. Regarding RMBC values, the species *Erythrolamprus poecilogyrus* presented the highest values and *Imantodes cenchoa* was graded with the lowest RMBC.

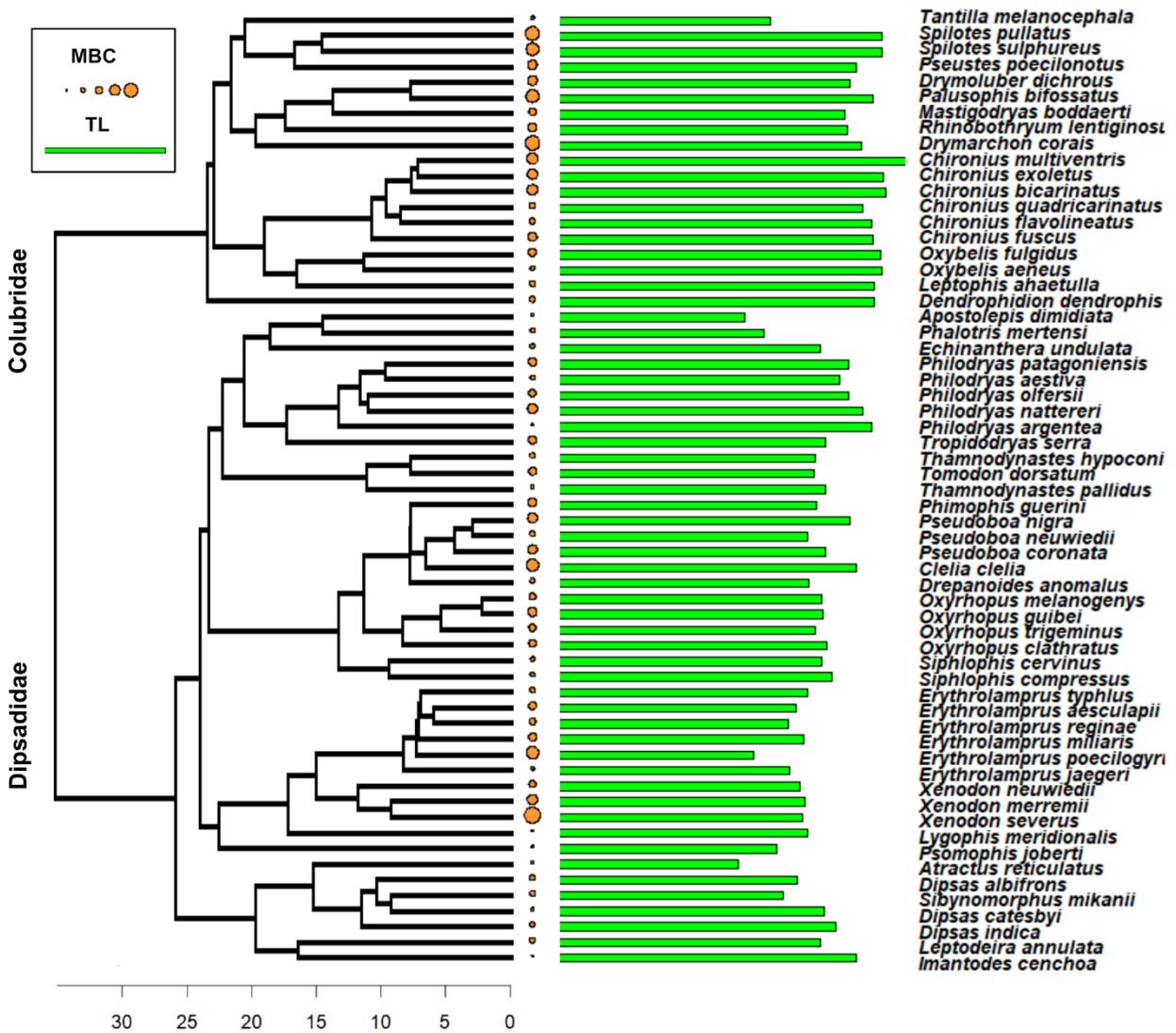


Figure 1 Distribution of tail length (TL) and mid-body circumference (MBC) values of 61 Colubridae and Dipsadidae species included in this study. Yellow-mustard circles represent mid-body circumference of species, and green bars represent the tail length.

Allometric relationships were identified in both log-corrected morphological traits, as illustrated by associations with body size (given by SVL in mm^2 shown in Figure 2). Specifically, longer species (higher values of SVL) exhibited higher values of tail length and MBC. Allometric relationships in these variables were also verified accounting for the effect of the ecological classification (two-categories habitat and G-habitats), and both variables seem correlated with body size at the same magnitude (slopes did not differ; ANOVA: $P < 0.05$, see Supplementary Figure S4).

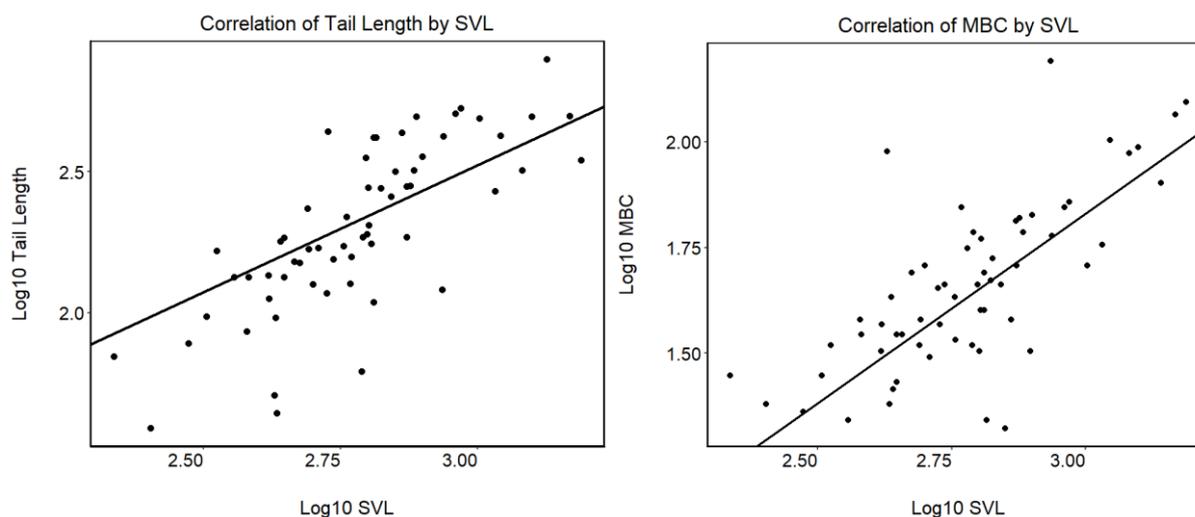


Figure 2 Allometry of tail length and MBC with body size. a) Regression of Log Tail Length by Log10 SVL. b) Regression of Log Mid-Body-Circumference by Log10 SVL. Yellow lines represent the regression slopes and intercept of the residuals corrected by the phylogeny and the black lines, the not corrected ones. Tested with PGLS, both tail length and MBC showed significant correlation with SVL ($P < 0.05$).

Ecomorphological associations with Arboreality Index

Phylogenetic analyses suggested that both RTL and RMBC are highly correlated with the Arboreality Index; as expected, in Colubridae and Dipsadidae snakes the ecological correlations expressed opposite trends in these two morphological traits: while RTL were positively correlated with arboreality (PGLS: $P < 0.05$, Figure 3a), this association was negative in RMBC (PGLS: $P = 0.01$, Figure 3b). Therefore, snakes classified as highly arboreal according to the Arboreality Index tend to exhibit relatively longer tails (RTL) and be comparatively slender (lower values of RMBC). Accordingly, lower values of the Arboreality Index were often associated to species characterized by shorter tails and thicker trunks. Despite the allometric

effect of body size on these morphological variable significant correlations between body size and the Arboreality Index were not detected (PGLS: $P = 0.33$, Figure 3c).

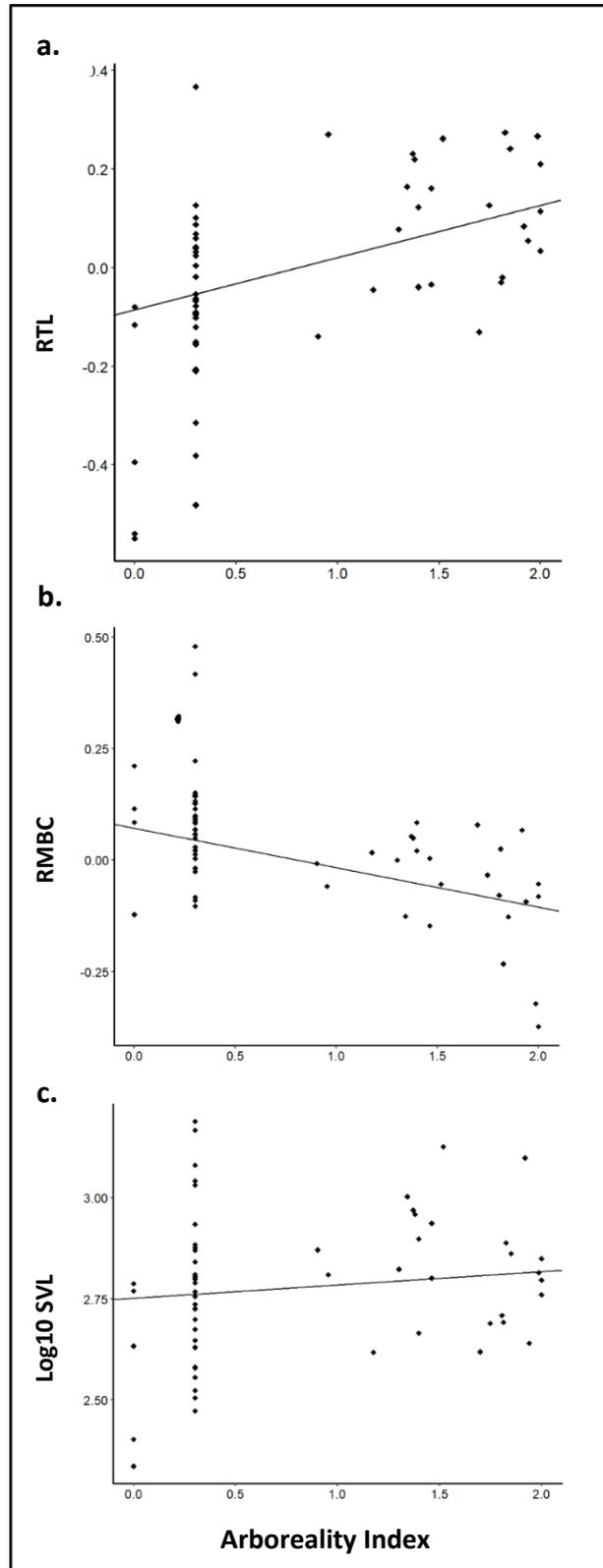


Figure 3 Regressions between average values of morphological variables and the Log10 Arboreal Index, in analyses that considered phylogenetic relationships. a) Relative tail length [$P < 0.05$; $R^2 = 0.16$]. b) Relative mid-body circumference [$P < 0.05$; $R^2 = 0.25$]. c) Snout vent Length [$P > 0.05$; $R^2 = 0.011$].

Ecomorphological associations: Two - categories and G-habitat classification

Analyses performed with microhabitat classification considered as a categorical factor and frequency of arboreal use being grouped revealed various association patterns for RTL and RMBC (Figure 4). For species classified as terrestrial and arboreal, RTL and RMBC differed significantly between categories (one-way ANOVA: RTL and RMBC with $P < 0.01$). Arboreal species on average exhibit longer RTL and lower RMBC values when compared to terrestrial snakes. When microhabitat was established as a G-habitat classification in the analyses, RTL and RMBC differed among the three G-habitats (Figure 4) (one-way ANOVA, *post hoc*: RTL and RMBC with $P < 0.01$). Non-scansorial and eurytopically species differed in mean and variance RTL ($t = 3.55$, $P = 0.03$), a similar contrast identified between stenotopically and non-scansorial species ($t = 3.97$, $P < 0.05$). However, no differences were detected between the two arboreal classifications (eurytopically and stenotopically; $t = 0.53$, $P = 0.60$). Results for RMBC suggested another pattern, where most differences were detected in contrasts between the arboreal classifications (eurytopically and stenotopically; $t = 2.32$, $P = 0.05$) and between ecological extremes (stenotopically *versus* non-scansorial species; $t = 4.90$, $P < 0.05$). In these analyses, we did not detect differences in RMBC between non-scansorial and eurytopically snakes ($t = 2.27$, $P = 0.10$). Results of pairwise comparisons between all categories are detailed at Supplementary Table S2.

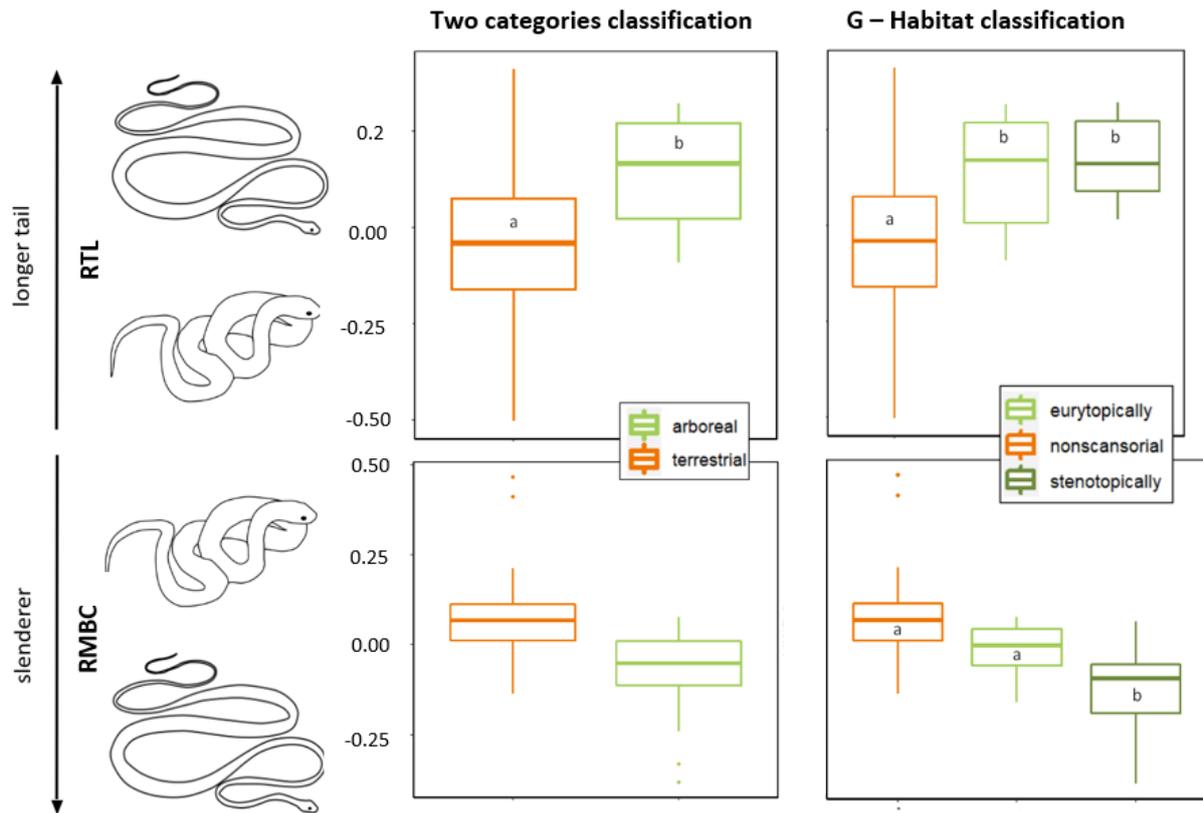


Figure 4 Morphological patterns of snakes occupying different microhabitat, considering the two categorical classification (left) and the G - Habitat classification (right). Middle lines in the boxplots correspond to the median (RTL and RMBC) in each category, boxes and whiskers indicate the quartiles. Different letters represent significant statistical differences between the categories.

Evolutionary Model Fitting of RTL and RMBC under different selective scenarios

Estimates using *simmap* and ancestral state reconstructions for continuous and categorical traits suggest that the use of arboreal microhabitat is a derived condition in the group that evolved more than 20 times within our dataset. Regardless of the type of classification used, ancestral state estimates suggested that ancestral species were probably terrestrial or non-scansorial. In agreement, analyses on morphological data indicated transitions from the ancestral phenotype characterized by intermediate values of body size, tail length and MBC to extremes values of large body size with longer tails and reduced MBC (Supplementary Figure S2)

Evolutionary model fitting compared which selective regimes better explain the evolution of RTL and RMBC in our database and estimated values of phenotypic optima (θ) for each regime; and differences were tested for variance analyses (Supplementary Figure S5).

Comparisons among AICc scores ($\Delta\text{AICc} \leq 2$; Table 1; Burnham and Anderson, 1998) suggested that RTL evolution is equally explained by a regime with two phenotypic optima (OU2; arboreal and terrestrial) and the one comprising three phenotypic optima (OU3; G-Habitat). However, the wAICc scores and predicted values of phenotypic optima indicated for OU3, particularly for the stenotopically regime ($\theta_{\text{steno.}} = 0.98$, Table 2), provided unrealistic values, and therefore discussions of our results do not address this model. Model fitting indicated that change in RMBC probably occurred associated with the three phenotypic optima regime (Table 1), as model with three phenotypic optima had the best AICc score and wAICc. Bootstrap sampling for both variables with a visual density plot of each selective regime showed that RTL probably evolved with two phenotypic optima, and optima values for all three categories were much more clustered than pattern observed in the RMBC distributions (Supplementary Figure S5).

Table 1 Performance of evolutionary models fit for morphological traits. For each model, the likelihood values (*LogLik*), and AIC bias-corrected (AICc) are provided. Scores of Brownian Motion, one phenotypic optima (OU1), two phenotypic optima (OU2) and three phenotypic optima (OU3). Parameter estimates for the two models with best AICc scores; α = strength of selection in OU models; σ = strength of random drift; θ = optimal values for each selective regime.

RTL								
Models	Df	α	σ^2	θ	LogLik	AICc	Δ AICc	w(AICc)
BM	2		0.04		29.78	-55.35	3.6	0.01
OU1	3	0.33	0.05	0.04	28.34	-50.27	8.68	1.19e-4
OU2	4	0.61	0.05	arbo. = 0.63; terr. = -0.03	33.83	-58.95	0	0.64
OU3	5	0.51	0.04	n.s. = -0.03; eury. = 0.55; steno. = 0.98	34.69	-58.29	0.66	0.33
RMBC								
BM	2		0.03		35.70	-67.17	13.93	5.18e-06
OU1	3	2.21	0.08	-0.002	37.79	-69.15	12.08	5.18e-06
OU2	4	3.74	0.11	arbo. = -0.09; terr. = 0.05	43.67	-78.63	2.37	0.08
OU3	5	3.93	0.10	n.s. = 0.06; eury. = -0.03; steno. = -0.17	46.09	-81.10	0	0.91

Best fit scores for Δ AICc and w(AICc) are shown in bold.

Discussion

This study inferred ecomorphological associations and evolution between body shape and the frequency of arboreal use in colubrid snakes. In counterpart to other studies, our data base contemplated only South American species from Colubridae and Dipsadidae. Analyses were performed using comparative phylogenetic regressions and complemented with the evolutionary model fitting approach, being the first study to test evolutionary models to address patterns of tail elongation and slenderness in colubroidea snakes. Model fitting in Colubroidea suggested that slenderness evolution comprised three phenotypic optima: one for fully-terrestrial species (non-scansorial), one for species classified as occasional arboreal (eurytopically), and one for arboreal specialists (stenotopically). Non-scansorial species differed from occasional arboreal species (eurytopically) and arboreal specialists (stenotopically) in body slenderness, a pattern not followed by results for tail length. The evolutionary model that best-fitted tail length evolution comprised only two phenotypic optima, with major differences observed between terrestrial species and arboreal species, with the last comprising all species that uses arboreal microhabitat.

Ecomorphological associations with Arboreal Life-Style

Ecomorphological associations in snakes often describe variation in trunk slenderness and tail lengths associated with differential microhabitat use (Martins et al. 2001; Pizzatto et al. 2007; Alencar et al. 2013; Lillywhite 2014; De Alencar et al. 2017). Studies suggest that these traits not only differ between terrestrial and arboreal species, but also vary depending on the frequency species spend on the arboreal habitat (Martins et al. 2001; De Alencar et al. 2017). These results foster the prediction that species that use the arboreal microhabitat more frequently likely present slenderer bodies and longer tails. Functional interpretations of these patterns often address gravitational force effects influencing evolution of trunk slenderness and tail lengths as a consequence of the upright posture often experienced during locomotion along tree trunks and branches. However, these two traits relate to different physical aspects of arboreality: while slenderness seems directly related to the biomechanics of locomotion, patterns of tail length have been explained by physiological associations with blood circulatory system (Lillywhite and LaFrentz 2000; Astley and Jayne 2007; Byrnes et al. 2014; Lillywhite 2015).

Climbing in snakes is a behavior particularly affected by gravity due to the demands for enhanced grip performance and the characteristics of non-compliant tissues in order to avoid blood oedema. Slenderer trunks allow better grip during climbing, as they facilitate to enfold more loops on a perch (Astley and Jayne 2007, 2009; Jayne and Herrmann 2011). Some very slender arboreal snakes are able to change between tree branches and sustain more than 50% of

body length in the air (“cantilever” behavior) (Lillywhite and LaFrentz 2000; Ray 2009, 2012). Still, non-scansorial animals that never experience vertical locomotion may also undergo different gravitational and biomechanical forces that likely impact slenderness, but in a different way. Behaviors including concertina and a rectilinear type of locomotion, together with substrate characteristics, may impact locomotor performance on horizontal surfaces (Gans 1970; Kelley et al. 1997; Moon and Gans 1998; Moon 2015). Our results for body shape evolution in Brazilian colubrid snakes indicate that slenderer trunks are observed in those species whose utilizes arboreal substrate more frequently when active. Furthermore, our findings for evolutionary model fitting suggest that species that occasionally climb arboreal habitats evolved under one phenotypic optimum distinct from those species more specialized (with less slender bodies). Still, differences in phenotypic optimum of terrestrial species is not significantly different than occasional climbers. These general morphology for occasional climbers (eurytopically species) and fully terrestrial could be related to the fact that they spend as much time on the ground as aboveground, including foraging on the ground (Hartmann and Marques 2005; Bellini et al. 2015), a behavior that probably imposes constraints associated with locomotion on the ground that may express trade-offs for slenderness.

Foraging strategy and prey type likely play important roles in the evolution of slenderness in snakes, due to the known relationship between predator and prey size in snakes (Downes 2002; Pleguezuelos et al. 2007). Arboreal snakes that actively forage over branches depend on climbing performance to successfully capture prey, while species that feed mostly on the ground and only use trees to rest or nest likely experience different selective pressures on locomotor performance affected by slenderness. Functional studies demonstrate that locomotion on perches by snakes of similar size that differ in slenderness relies mostly on behavioral adjustments (Jayne and Herrmann 2011; Jayne et al. 2015). Heavier individuals may change their center of mass (COM) according to perch diameter in order to enhance stabilization and velocity, a behavioral adjustment similar to that described in paddles gecko lizards while moving on perches (Fuller et al. 2011). It is important to mention that thicker snakes often perform better on larger perches (13 to 73mm), while locomotion of slenderer snakes is enhanced in thinner perches (Jayne and Herrmann 2011), so interaction between morphology and specific properties of the structural habitat play a key role in this equation. Other behavioral adjustments comprise shifts from concertina locomotion (periodic static gripping) to undulatory locomotion (periodic contact without gripping), which likely mitigate implications from morphological specialization among non-scansorial and occasional climbers.

Evolutionary patterns of tail length in Colubroidea provide a different story, although arboreality likely represents a similar source of selective pressures. In our dataset, tail length

differs only between non-scansorial species and occasional climbers, a result similar to that described for relative tail lengths in burrowing lizards (Wiens et al. 2006; Shine and Wall 2008). Studies using different ecological classifications reported that fossorial snakes are characterized by relatively short tails, while this trait apparently does not differ between species that move along the surface and those classified as litter-swimmers (Shine and Wall 2008). In Colubroidea, our results indicate that upright locomotion associated with the arboreal lifestyle is probably one of the main selective forces driving tail length evolution in the group. In contrast to results obtained for body slenderness, the “morphological evolutionary jump” in tail length occurred when lineages specialized in the arboreal lifestyle, instead of gradual changes associated with increased frequency in the use of arboreal microhabitats. Similar slenderness patterns between non-scansorial and occasional arboreal species are coupled with similar tail lengths among species that differ in the levels of arboreality, suggesting that a general morphology can be associated with differential use of microhabitat in snakes, especially if behavioral adjustments grant proper performance in arboreal microhabitats. Especially regarding tail lengths, considerable variation is observed among terrestrial species of Colubroidea. Under a macroevolutionary approach, traits comprising considerable variation might reflect weak selective pressures or evolutionary constraints associated with other aspects of life history (Arnold 1992; Wainwright and Price 2016). Our dataset for arboreal species, in contrast, comprised restricted variation, despite mean differences, which might be interpreted as an effect of stabilizing selection maintaining trait averages with restricted variation. The contrasting patterns for tail length and slenderness evolution associated with arboreality observed here suggest that traits might respond differently to specialization of arboreal microhabitats depending on the snake lineage. Morphological convergence associated with similar environments is a central topic in ecomorphology, although the use of similar habitats by phylogenetically-distant species does not necessarily result in morphological similarity (Thompson et al. 2017; Zelditch et al. 2017).

Contrasting patterns identified in Colubroidea, in addition to results described in other arboreal snakes from different families, provide evidence for several factors that might attenuate morphological convergence in specific lineages that differ in geographical distribution, in addition to environmental relationships not addressed here that likely influence evolutionary trajectories despite similarity in microhabitat usage. Other studies had already described ecomorphological associations in snakes, but neither used evolutionary model fitting to test for such associations in New World colubroidea species. Our study corroborates as a proper approach the use of more than one type of ecological classification coupled with integrative analyses combining model fitting with classical phylogenetic statistics to evaluate macroevolutionary patterns in specific lineages.

Supplementary Material

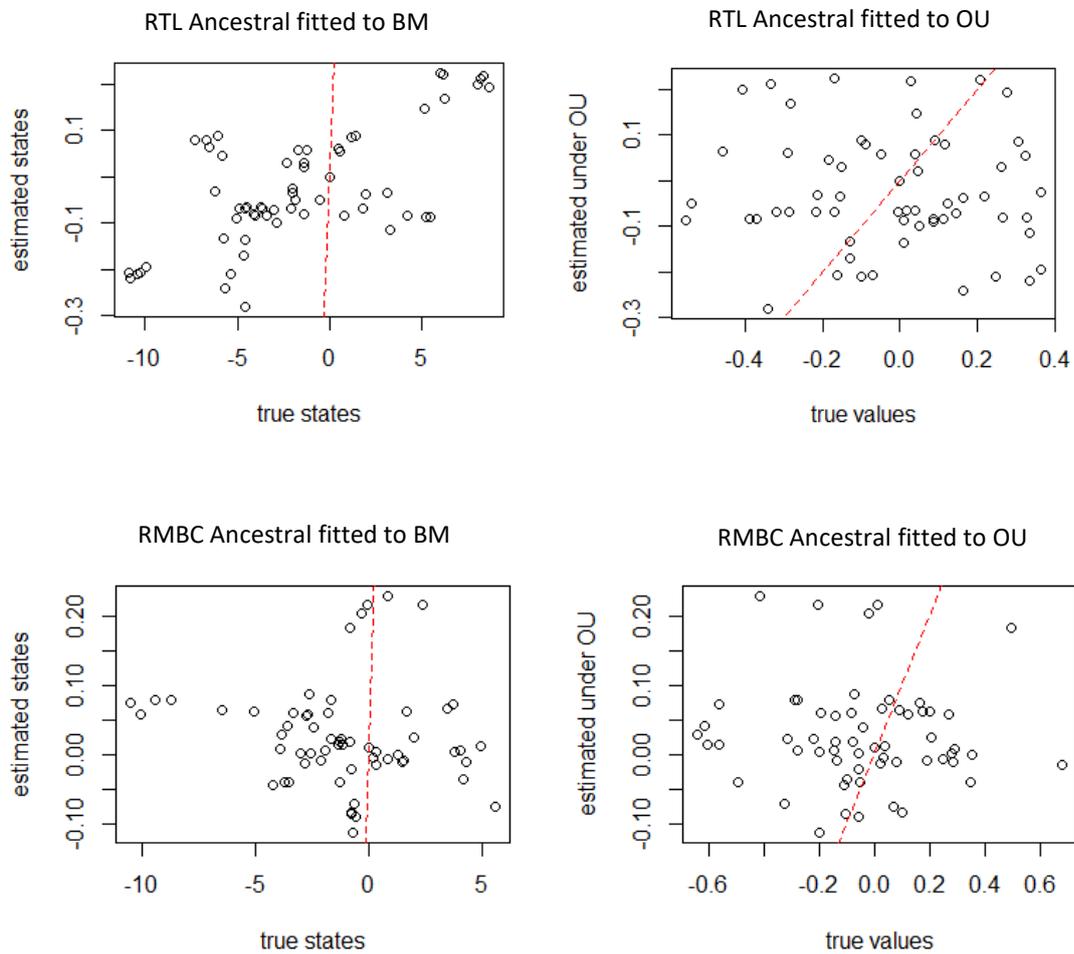
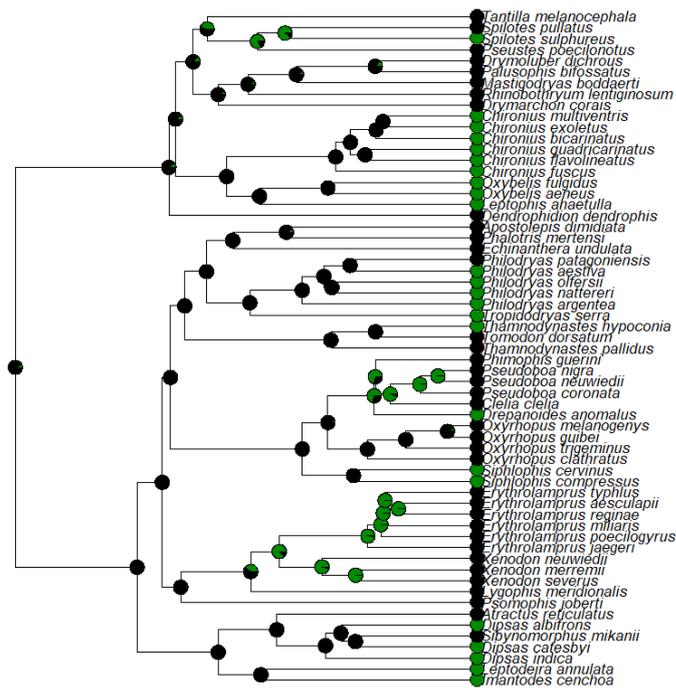


Figure S1. Phylogenetic residual distributions under different evolutionary models. Estimated data of RTL and RMBC fitted against true data distribution under Brownian Motion and Ornstein - Uhlenbeck. Slope proximal to zero express best fitted, consequently the best evolutionary model that fit the data.

a.



b.

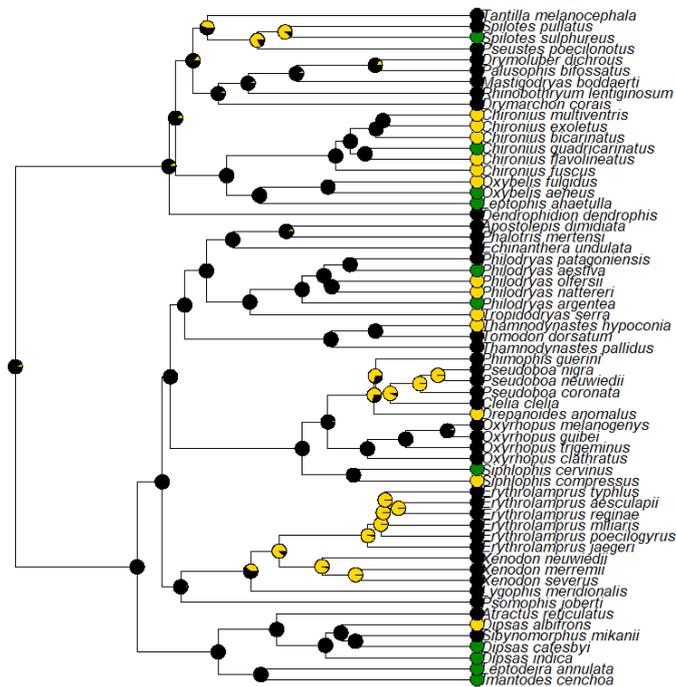


Figure S3. Phylogenetic relationships of 61 species included in this study with ancestral state reconstruction considering both types of ecological classification. Colored circles in the nodes represent the probability of the ancestral state on that particular node corresponding to one of the

ecological classifications. (Left column) arboreal = green, terrestrial = black; (Right column) eurytopically = yellow, stenotopically = green, non-scansorial = black.

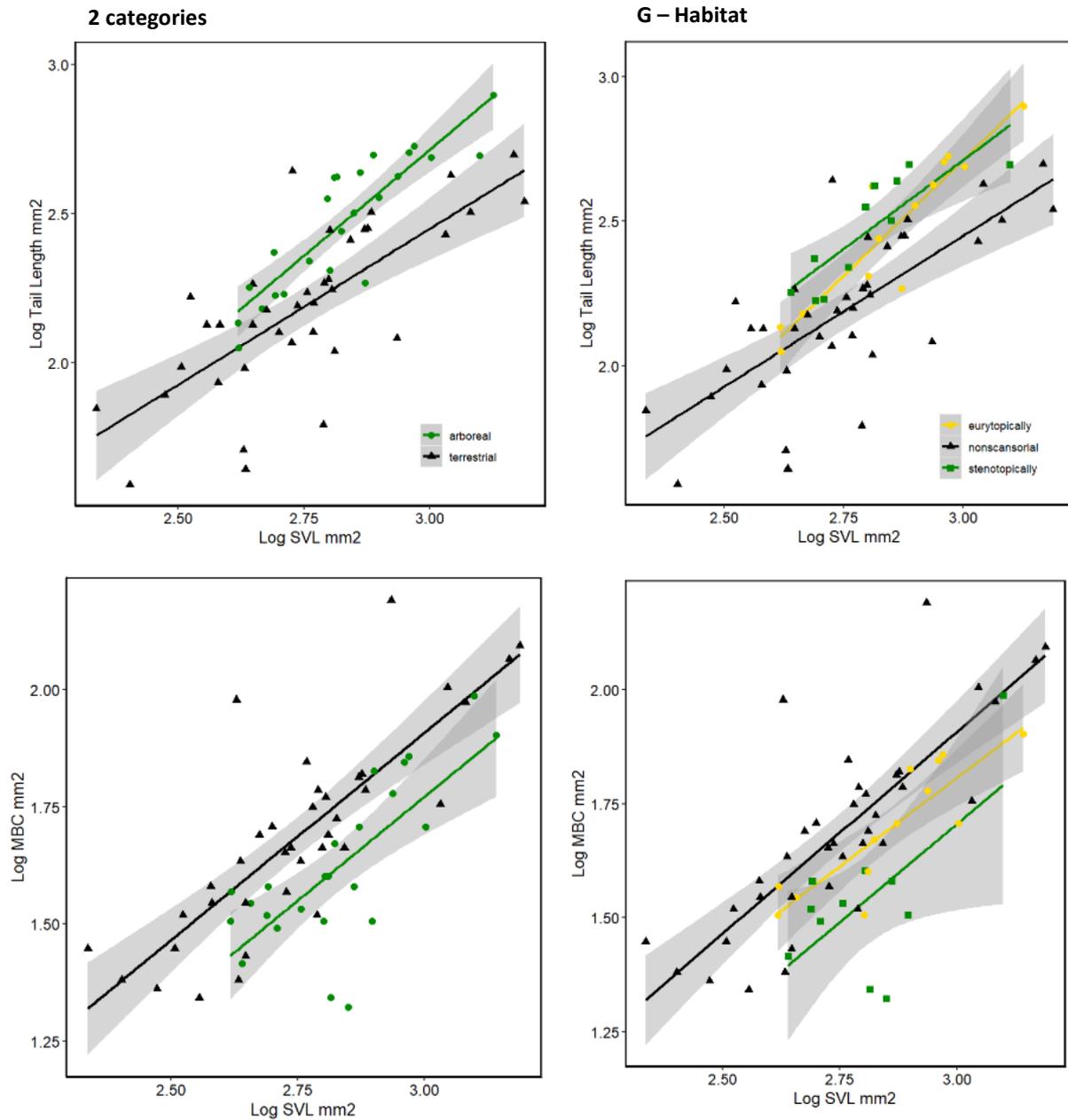


Figure S4. Phylogenetic regressions of morphological variables with body size. Left column classified species as arboreal or terrestrial. Graphs on the right classified species based on G-Habitat categories. Regression slopes did not differ significantly between ecological categories in any analyses (PGLS; $P < 0.05$)

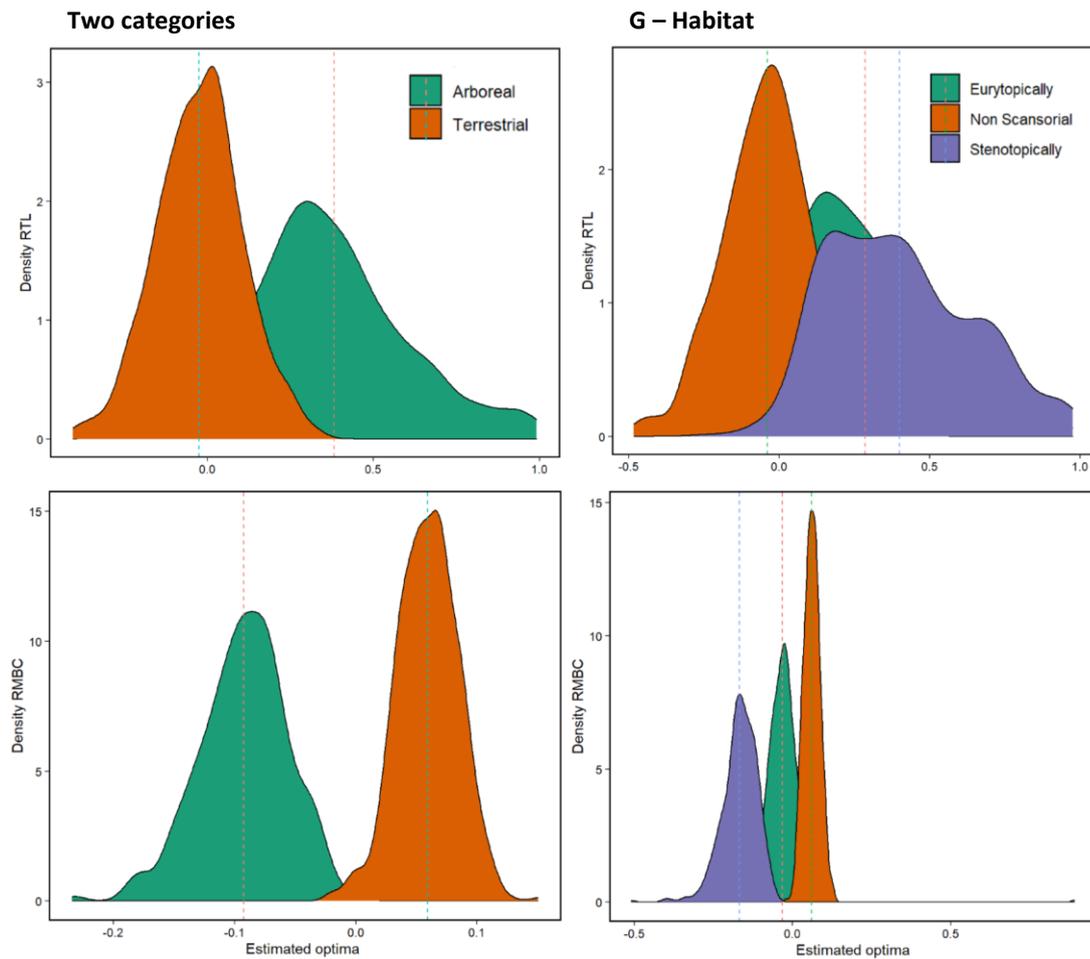


Figure S5. Variance and mean of phenotypic optima (θ) of OU models (2 and 3 optima). Figures at the left provide theta estimated distributions of two-optima regimes model, and in the right correspond to theta estimates for three-optima regimes. Distributions were compiled after bootstraps (nboot = 500 per regime). Dashed lines represent predicted optimal values for each selective regime. Classification using Two Categories; RTL: Arboreal = 0.38 and Terrestrial = -0.02, RMBC: Arboreal = -0.09 and Terrestrial = 0.05. G-habitat Classification RTL: Non-scansorial = -0.03, Eurytopically = 0.28, Stenotopically = 0.40. Classification using Two Categories RMBC: Arboreal = -0.09 and Terrestrial = 0.07. G-habitat Classification: Non-scansorial = 0.06, Eurytopically = -0.03, Stenotopically = -0.16.

Table S1. Ecological and morphological data for 61 snake species included in this study. Species, Tree Tip Order (TTO), Number of individuals (N), Arboreality Index Score (AI), Ecological classifications, Mean values and standard errors (\pm SE) of snout vent-length (SVL), tail length and mid-body circumference. Bibliographical references used for microhabitat identification are represented for each species

Species	T.T.O.	N	A.I.	Two Habitat	G. Habitat	SVL \pm SE	Tail Length \pm SE	MBC \pm SE	Microhabitat References
<i>T. melanocephala</i>	1	20	1	TE	nonscansorial	217.45 \pm 5.98	70.4 \pm 2.33	28.15 \pm 0.54	Bernarde 2004; França and Araújo 2006; Santos-Costa et al. 2015
<i>S. pullatus</i>	2	20	2	TE	nonscansorial	1.470 \pm 40.92	497.55 \pm 12.24	115.6 \pm 4.02	Martins and Oliveira 1998; Bernarde 2004
<i>S. sulphureus</i>	3	20	83	AR	stenotopically	1.255 \pm 55.68	494.6 \pm 21.27	97.35 \pm 5.51	Martins and Oliveira 1998
<i>P. poecilnotus</i>	4	21	2	TE	nonscansorial	762 \pm 22.75	314.95 \pm 9.80	NA	Martins and Oliveira 1998; Santos-Costa et al. 2015
<i>D. dichrous</i>	5	20	2	TE	nonscansorial	741.6 \pm 28.9	280.4 \pm 18.25	65 \pm 3.49	Bernarde 2004
<i>P. bifossatus</i>	6	17	2	TE	nonscansorial	1.110 \pm 34.19	424.94 \pm 18.37	101.18 \pm 4.3	Strussmann and Sazima 1993; França and Araújo 2006
<i>M. boddaerti</i>	7	20	2	TE	nonscansorial	695.15 \pm 22.17	258.35 \pm 8.58	46.4 \pm 1.67	Bernarde 2004
<i>R. lengitinossum</i>	8	9	2	TE	nonscansorial	1.074 \pm 34.96	269.44 \pm 19.35	56.78 \pm 2.45	Strussmann and Sazima 1993; Bernarde 2004
<i>D. corais</i>	9	16	2	TE	nonscansorial	1.543 \pm 44.18	346.69 \pm 7.15	124.25 \pm 3.82	Bernarde 2004
<i>C. multiventris</i>	10	15	33	AR	eurytopically	1.384 \pm 66.18	79 \pm 28.45	80.47 \pm 4.53	Bernarde 2004; Santos-Costa et al. 2015
<i>C. exoletus</i>	11	24	24	AR	eurytopically	913.13 \pm 37.49	507 \pm 19.11	70.21 \pm 3.85	Hartmann et al. 2009a, 2009b
<i>C. bicarinatus</i>	12	20	23,5	AR	eurytopically	932.7 \pm 29.99	531.4 \pm 21.07	71.55 \pm 2.38	Hartmann et al. 2009a, 2009b
<i>C. quadricarinatus</i>	13	18	100	AR	stenotopically	636.94 \pm 12.51	354.19 \pm 8.7	40.33 \pm 1.13	Sawaya et al. 2008
<i>C. flavolineatus</i>	14	15	9	AR	eurytopically	646.47 \pm 10.36	418.47 \pm 9.34	40.13 \pm 1.17	Sawaya et al. 2008
<i>C. fuscus</i>	15	15	29	AR	eurytopically	865.47 \pm 40.29	423.33 \pm 34.48	60.2 \pm 3.4	Martins 1994; Hartmann et al. 2009b
<i>O. fulgidus</i>	16	15	22	AR	eurytopically	1.007 \pm 36.44	488.2 \pm 21.02	50.6 \pm 2.55	Martins and Oliveira 1998
<i>O. aeneus</i>	17	20	67	AR	stenotopically	788.4 \pm 32.56	497.95 \pm 18.67	31.75 \pm 2.05	Martins and Oliveira 1998
<i>L. ahaettula</i>	18	20	71	AR	stenotopically	726.55 \pm 30.3	435.1 \pm 13.88	37.65 \pm 1.8	Martins 1994; Martins and Oliveira 1998
<i>D. dendrophis</i>	19	20	2	TE	nonscansorial	534.15 \pm 9.21	438.84 \pm 21.2	37.05 \pm 1	Bernarde, 2004
<i>A. dimidiata</i>	20	1	1	TE	nonscansorial	430 \pm NA	44 \pm NA	24 \pm NA	Sawaya et al. 2008
<i>P. mertensi</i>	21	20	1	TE	nonscansorial	613.95 \pm 16.97	62.2 \pm 1.67	32.9 \pm 1	Sawaya et al. 2008
<i>E. undulata</i>	22	12	2	TE	nonscansorial	334 \pm 18.54	166.33 \pm 9.75	33.25 \pm 1.55	Hartmann et al. 2009
<i>P. patagoniensis</i>	23	20	2	TE	nonscansorial	671.3 \pm 25.48	278.27 \pm 10.26	53.3 \pm 2.39	Machado Filho 2015
<i>P. aestiva</i>	24	15	56	AR	stenotopically	488.73 \pm 23.87	233.53 \pm 14.06	33.33 \pm 2.11	Goldsmith 1984
<i>P. olfersii</i>	25	20	20	AR	eurytopically	666 \pm 19.77	275.9 \pm 5.65	46.75 \pm 1.55	Bernarde 2004; Hartmann et al. 2009b
<i>P. nattereri</i>	26	20	25	AR	eurytopically	794.6 \pm 28.19	358.05 \pm 12.55	66.5 \pm 2.5	Machado Filho 2015
<i>P. argentea</i>	27	3	97	AR	stenotopically	653.33 \pm 63.66	419 \pm 54.67	22 \pm 1.15	Machado Filho 2015; Santos-Costa et al. 2015
<i>T. serra</i>	28	19	8	AR	eurytopically	742.90 \pm 25.36	184.58 \pm 7.45	50.95 \pm 1.93	Oliveira 2008
<i>T. hypoconia</i>	29	13	25	AR	eurytopically	454 \pm 16.36	151.5 \pm 5.92	35.46 \pm 0.81	Sawaya et al. 2008
<i>T. dorsatum</i>	30	20	2	TE	nonscansorial	472.50 \pm 14.26	149.65 \pm 4.78	48.95 \pm 2.32	Gomes 2016
<i>T. pallidus</i>	31	11	2	TE	nonscansorial	443.64 \pm 13.89	183.91 \pm 6.06	26.91 \pm 0.68	Strussmann and Sazima 1993; Bailey et al. 2005; Vrcibradic et al. 2012
<i>P. guerini</i>	32	9	1	TE	nonscansorial	601.67 \pm 30.4	158.43 \pm 5.93	56.11 \pm 2.76	França and Araújo 2006; Sawaya et al. 2008
<i>P. nigra</i>	33	20	2	TE	nonscansorial	753.4 \pm 28.55	281.6 \pm 8.66	65.55 \pm 2.89	Strussmann and Sazima 1993; Hartmann et al. 2009a; França and Braz 2013
<i>P. neuwiedii</i>	34	3	2	TE	nonscansorial	443.67 \pm 18.28	134 \pm 10.6	35.33 \pm 3.18	Alencar et al. 2013
<i>P. haasi</i>	35	1	2	TE	nonscansorial	617 \pm NA	185 \pm NA	61 \pm NA	Alencar et al. 2013

<i>C. clelia</i>	36	12	2	TE	nonscansorial	1.200 ± 60.38	319.09 ± 20.74	93.83 ± 6.33	Bernarde and Abe 2006; Guimar et al. 2014
<i>D. anomalus</i>	37	20	15	AR	eurytopically	414.6 ± 6.12	136.1 ± 3.82	31.7 ± 0.85	Bernarde 2004
<i>O. melanogenys</i>	38	20	2	TE	nonscansorial	570.3 ± 20.1	171.85 ± 5.5	42.7 ± 2.32	Sérgio and Abe 2006; Guimar et al. 2014
<i>O. guibeii</i>	39	20	2	TE	nonscansorial	639.4 ± 29.05	176.4 ± 4.94	59.45 ± 2.56	Sawaya et al. 2008; Guimar et al. 2014
<i>O. trigeminus</i>	40	1	2	TE	nonscansorial	545 ± NA	155 ± NA	46 ± NA	França et al. 2008
<i>O. clathratus</i>	41	15	2	TE	nonscansorial	629.27 ± 19.1	189.6 ± 6.69	45.67 ± 1.83	Hartmann et al. 2009b; Guimar et al. 2014
<i>S. cervinus</i>	42	7	64	AR	stenotopically	511.71 ± 21.88	170.43 ± 9.66	30.57 ± 1.34	Alencar et al. 2013
<i>S. compressus</i>	43	20	29	AR	eurytopically	632.75 ± 13.76	203.5 ± 5.28	32.45 ± 1.3	Bernarde 2004; Pontes et al. 2009; Alencar et al. 2013
<i>E. typhlus</i>	44	20	2	TE	nonscansorial	381.05 ± 8.41	134.05 ± 4.98	35.2 ± 0.68	Sawaya et al. 2008
<i>E. aesculapii</i>	45	18	2	TE	nonscansorial	645.56 ± 11.57	109.33 ± 2.35	48.83 ± 1.1	Bernarde and Abe 2006; Guimar et al. 2014
<i>E. reginae</i>	46	20	2	TE	nonscansorial	433.95 ± 12.42	96.37 ± 2.48	42.75 ± 1.2	Sawaya et al. 2008
<i>E. miliaris</i>	47	20	2	TE	nonscansorial	500.6 ± 15.08	126.2 ± 3.72	50.55 ± 1.7	Hartmann 2005; da Silva et al. 2010
<i>E. poecilogyrus</i>	48	19	2	TE	nonscansorial	425.68 ± 15.75	95.37 ± 4.57	51.26 ± 1.98	Sawaya et al. 2008; Entiauspene-Neto et al. 2016
<i>E. jaegeri</i>	49	16	2	TE	nonscansorial	321.56 ±	97.2 ± 3.52	27.56 ± 0.9	Sawaya et al. 2008; Entiauspene-Neto et al. 2016
<i>X. neuwiedi</i>	50	20	2	TE	nonscansorial	531.4 ± 11.87	116.65 ± 3.32	45.3 ± 1.51	Pontes et al. 2009
<i>X. merremii</i>	51	20	2	TE	nonscansorial	585.8 ± 11.53	126.85 ± 2.69	69.55 ± 2.93	Sawaya et al. 2008; França and Braz 2013
<i>X. severus</i>	52	7	2	TE	nonscansorial	861.29 ± 70.27	154.71 ± 13.75	121.29 ± 10.9	Sawaya et al. 2008
<i>L. meridionalis</i>	53	4	2	TE	nonscansorial	360.25 ± 22.33	133.75 ± 13.93	21.5 ± 1.55	Sawaya et al. 2008
<i>P. joberti</i>	54	20	2	TE	nonscansorial	297.4 ± 8.06	78.45 ± 1.49	22.7 ± 0.56	Strussmann and Sazima 1993
<i>A. reticulatus</i>	55	18	1	TE	nonscansorial	253.17 ± 6.82	38.89 ± 1.2	24.11 ± 0.59	Passos et al. 2005
<i>D. albifrons</i>	56	2	50	AR	eurytopically	415.5 ± 54.5	111.5 ± 9.5	36.5 ± 10.5	Dias et al. 2018
<i>S. mikanii</i>	57	20	2	TE	nonscansorial	379.4 ± 10.34	85.95 ± 1.59	37.7 ± 0.96	Sawaya et al. 2008
<i>D. catesbyi</i>	58	20	87	AR	stenotopically	437.15 ± 10.94	178.85 ± 6.7	26.4 ± 0.7	Bernarde 2004
<i>D. indica</i>	59	17	100	AR	stenotopically	570.82 ± 18.34	219.27 ± 6.06	34.06 ± 1.66	Martins and Oliveira 1998; Bernarde 2004
<i>L. annulata</i>	60	20	65	AR	stenotopically	492.15 ± 15.44	167.75 ± 6.50	37.85 ± 1.24	Martins 1994; Hartmann et al. 2009b
<i>I. cenchoa</i>	61	20	100	AR	stenotopically	706.9 ± 17.94	317.45 ± 9.4	20.8 ± 1.48	Martins and Oliveira 1998

Table S2. Results of phylogenetic analyses expressed as t , P and F values of Relative Tail Length (RTL) and Relative Mid-Body Circumference (RMBC); comparisons between species grouped in the two-categories classification and among those classified according to the G-habitat. See Fig. 4 for the accompanied visuals.

RTL					
	F		P		one-way ANOVA
Arboreal vs Terrestrial	22.87		0.003		
G Habitat	Stenotopically		Non-scansorial		one-way ANOVA + post hoc "Holm Sidak"
	t	P	t	P	
Eurytopically	- 0.53	0.63	3.55	0.03	
Non-scansorial	- 3.97	0.03	-		
RMBC					
	F		P		one-way ANOVA
Arboreal vs Terrestrial	18.13		0.006		
G Habitat	Stenotopically		Non-scansorial		one-way ANOVA + post hoc "Holm Sidak"
	t	P	t	P	
Eurytopically	2.34	0.04	- 2.22	0.13	
Non-scansorial	4.88	0.01	-		

Table S3. Residuals from the PGLS regression of Tail length and Mid Body Circumference with Body Size.

Species	RTL	RMBC
<i>Tantilla melanocephala</i>	-0.03782	0.19251
<i>Spilotes pullatus</i>	0.07608	0.08163
<i>Spilotes sulphureus</i>	0.13447	0.06413
<i>Pseustes poecilonotus</i>	0.13603	0.05110
<i>Drymoluber dichrous</i>	0.08982	0.09030
<i>Palusophis bifossatus</i>	0.11842	0.12807

<i>Mastigodryas boddaerti</i>	0.07953	-0.03493
<i>Rhinobothryum lentiginosum</i>	-0.07063	-0.10783
<i>Drymarchon corais</i>	-0.09951	0.09214
<i>Chironius multiventris</i>	0.31278	-0.05679
<i>Chironius exoletus</i>	0.26849	0.04355
<i>Chironius bicarinatus</i>	0.27978	0.04754
<i>Chironius quadricarinatus</i>	0.25726	-0.06246
<i>Chironius flavolineatus</i>	0.31730	-0.06780
<i>Chironius fuscus</i>	0.20981	-0.00283
<i>Oxybelis fulgidus</i>	0.21324	-0.13127
<i>Oxybelis aeneus</i>	0.32235	-0.24034
<i>Leptophis ahaetulla</i>	0.28903	-0.13504
<i>Dendrophidion dendrophis</i>	0.41278	-0.02919
<i>Apostolepis dimidiata</i>	-0.50336	-0.13473
<i>Phalotris mertensi</i>	-0.49187	-0.13201
<i>Echinanthera undulata</i>	0.17077	0.09972
<i>Philodryas patagoniensis</i>	0.14862	0.03995
<i>Philodryas aestiva</i>	0.17278	-0.04537
<i>Philodryas olfersii</i>	0.12527	-0.00937
<i>Philodryas nattereri</i>	0.17138	0.07720
<i>Philodryas argentea</i>	0.31418	-0.33154
<i>Tropidodryas serra</i>	-0.09068	-0.01554
<i>Thamnodynastes hypoconia</i>	0.00649	0.00844
<i>Tomodon dorsatum</i>	-0.00750	0.13897
<i>Thamnodynastes pallidus</i>	0.10563	-0.09577
<i>Phimophis guerini</i>	-0.06891	0.10516
<i>Pseudoboa nigra</i>	0.08723	0.09133
<i>Pseudoboa newwiedii</i>	-0.03207	0.01692
<i>Pseudoboa coronata</i>	-0.01897	0.13294
<i>Clelia clelia</i>	-0.04064	0.06564
<i>Drepanoides anomalus</i>	0.00042	0.00371
<i>Oxyrhopus melanogenys</i>	-0.02004	0.01124
<i>Oxyrhopus guibei</i>	-0.05415	0.10513
<i>Oxyrhopus trigeminus</i>	-0.04793	0.05760
<i>Oxyrhopus clathratus</i>	-0.01482	0.00303
<i>Siphlophis cervinus</i>	0.01628	-0.09002
<i>Siphlophis compressus</i>	0.01360	-0.15698
<i>Erythrolamprus typhlus</i>	0.02595	0.07517
<i>Erythrolamprus aesculapii</i>	-0.26644	0.02032
<i>Erythrolamprus reginae</i>	-0.16274	0.11499

<i>Erythrolamprus miliaris</i>	-0.10541	0.13445
<i>Erythrolamprus poecilogyrus</i>	-0.43564	0.46633
<i>Erythrolamprus jaegeri</i>	-0.04603	0.04230
<i>Xenodon neuwiedii</i>	-0.16004	0.05796
<i>Xenodon merremii</i>	-0.16245	0.21233
<i>Xenodon severus</i>	-0.33194	0.41111
<i>Lygophis meridionalis</i>	0.04884	-0.10489
<i>Psomophis joberti</i>	-0.11193	-0.01236
<i>Atractus reticulatus</i>	-0.35108	0.06714
<i>Dipsas albifrons</i>	-0.08482	0.06585
<i>Sibynomorphus mikanii</i>	-0.16360	0.11288
<i>Dipsas catesbyi</i>	0.09980	-0.10612
<i>Dipsas indica</i>	0.08082	-0.09141
<i>Leptodeira annulata</i>	0.02651	0.01356
<i>Imantodes cenchoa</i>	0.16236	-0.38199

Chapter 2

Evolution of Surface Area to Volume ratio (S/V) in South American colubrid snakes does not reflect differences in microhabitat usage or activity period

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Abstract

Implications from the use of different thermal environments are particularly relevant for studies of phenotypic evolution in snakes. Snakes can exhibit specific physiological and behavioral traits that enable the use of environments that differ in thermal characteristics. However, current literature relegates investigations using morphological traits more directly related to heat exchange, such as the Surface Area to Volume ratio (S/V), especially when testing associations with thermal ecology under a macroevolutionary perspective. In this chapter, we evaluated evolutionary associations between S/V and differences in microhabitat use and activity period in South-American Colubroidea snakes. We obtained linear measurements of 61 species and estimated S/V for all species applying geometry calculations commonly used in stereology. Then, we implemented phylogenetic comparative methods to trace evolution of S/V along the phylogeny and to test for changes in S/V associated to the occupation of different microhabitats and shifts in activity periods S/V in Colubroidea. Analyses did not identify differences in S/V among species that use distinct microhabitats or differ in activity periods, despite differences in average body size among ecological categories. Therefore, occupation of different ecological niches during Colubroidea evolution apparently did not involve significant changes in S/V. One possibility is that snakes primarily evolved physiological and behavioral traits that enable to explore a variety of distinct thermal environments. In agreement to previous studies that used different sets of environmental predictors for thermal environment, we interpret that snakes in overall did not evolve specific morphological patterns associated with thermal variables.

Introduction

Interactions with environmental temperatures represent a major factor for phenotypic evolution in most of the existing life in Earth. Morphological, physiological and behavioral traits are often associated with average environmental temperatures and also with patterns of temperature variation (Kearney et al. 2009; Michael J. Angilletta Jr. 2009; Gardner et al. 2011). Thermal heterogeneity in a given environment, such as temperature daily variation or seasonal fluctuations, likely affects evolution of thermal biology in several lineages (Angilletta et al. 2002; Jr et al. 2006; Michael J. Angilletta Jr. 2009). Contingency of environmental temperature oscillation in a determined location, i.e. diel or seasonal, related to how the animal's body temperature respond have originated thermoregulation and thermoconformation strategists (Jr et al. 2006). Yet, not all species occurring in a given thermal environment evolved similar patterns. Animals that actively thermoregulate often use behavioral strategies to deal with temperature

environmental oscillations and maintain body temperatures near the optimal range (Huey and Kingsolver 1989; Navas 1996; Michael J. Angilletta Jr. 2009).

Average air temperature and associated patterns of oscillation determine the thermal environment accessible to a given terrestrial animal, and can be interpreted as a possible source of selective forces. Most studies addressing the Bergman's rule, focused on morphological patterns associated with latitudinal gradients that comprise continuous variation on mean air temperature (Mayr 1955; Zamora-Camacho et al. 2014; Meiri et al. 2015). Evidence supporting the Bergman's rule focused mostly on endotherms, while no consistent pattern has been identified in ectotherms (Olalla-Tárraga et al. 2006; Penniket and Cree 2015; Slavenko et al. 2019). Accordingly, possible effects of different thermal environments derived from the use of distinct microhabitats with local scale heterogeneity remain unclear (Pincebourde et al. 2016). The fossorial habitat for example, is often colder and characterized by more constant temperatures in comparison to the surface (López et al. 1998; Greenville and Dickman 2009). The same is observed in aquatic microhabitats (Strangas et al. 2019). On the counterpart, animals that use arboreal microhabitats are more exposed to the air and wind than terrestrial animals and, depending on the animal size and the diameter of the tree branch, individuals body temperature might fall within different intervals from the operative temperature range (Bakken 1989; Dzialowski 2005; Pincebourde et al. 2016). Furthermore, the time an animal is active may have direct influence on its thermal relationship with the environment as the air temperature commonly decreases during the night (Kronfeld-Schor and Dayan 2003). Diel temperature may vary considerably according to season and location. In South America, for example, temperature variation along a single day may range from 23 to 28°C in latitude near the Ecuador and from 10 to 28°C in southern regions, depending on the season (National Institute of Meteorology, INMET). In Squamata, morphological differences in ectotherms that diverge in activity periods have been addressed more often under intraspecific scales, even though little information is known about relationships between activity patterns and morphological evolution in ectotherms (Zamora-Camacho et al. 2014; Penniket and Cree 2015). Specifically, I am not aware of a study that investigated associations among activity patterns, microhabitat usage and morphological evolution in squamates.

Other traits in Squamata may be also correlated with the use of different thermal environments (Slavenko and Meiri 2015; Bergmann and Morinaga 2019). In some snake lineages, physiological and behavioral parameters might express adaptations to life at different temperatures, which indirectly reflect relaxed selection residing on thermal environments acting on morphological traits. Studies that independently addressed some of these topics in snake species suggest that occupation of environments with low air temperatures occurred in

association with decreased values of optimal temperatures (Shine et al. 2006; Mori and Toda 2018; Paci et al. 2018). Another possible physiological adaptation in the context of thermal heterogeneity grants to some species the ability to remain active at temperatures that are lower than the preferred optimum (Dorcas and Peterson 1998; Putman and Clark 2017; Yamasaki and Mori 2017). Such adaptations have been identified using experimentation with controlled temperatures, where snakes have chosen retreat sites with higher temperatures despite differences with the average body temperature measured in these same snakes in the field (Cox et al. 2017). Such physiological and behavioral adjustments might fit species as thermoconformation strategists. These patterns have been observed only for a few species worldwide, and little information is known about the thermal physiology of South American colubrid. Although current literature supports in some snake species correlations of physiology and behavior with characteristics of the thermal environment, in South American species differences in the thermal environment might have also affected morphological evolution (Pupin et al. 2020). Some morphological characteristics influence heat exchange rates, which is particularly important for animals that thermoregulate. Surface Area to Volume ratio expresses the body proportion exposed to thermal exchange with air for a given volume, and is among one of the most important parameters influencing thermal inertia (Lindsey 1966).

The importance of Surface Area to Volume ratio (S/V) for thermoregulation in ectotherms and its relationship with differences in thermal environments has been addressed by Ray 1960 and Lindsey 1966. Most studies, however, infer S/V using body size (SVL) or body mass (weight) as a proxy for S/V, given the challenges of estimating Surface Area in complex shapes (Ashton and Feldman 2003; Pincheira-donoso et al. 2008; Slavenko and Meiri 2015). Even though, none of these variables provide the same accuracy of precisely measuring both surface area and volume to estimate the S/V ratio. Especially when accounting for associations with specific environmental variables, such as thermic, variation in body size might express associations with other selective pressures, imposed for example by locomotion, or reflect phylogenetic constrains. Differences in S/V might also reflect variation deriving from shape and allometry. The trait S/V has never been directly estimated in Serpentes, although snakes offer an excellent model to obtain these measurements because of their limbless and elongated cylindrical form. Furthermore, the clade Serpentes comprises a remarkable diversity in morphology and ecology, being also very species-rich, which turns the lineage ideal for to ecomorphological studies (Bars-Closel et al. 2018; Harrington et al. 2018). In particular, Colubridae and Dipsadidae are two snake families that comprise highest diversity levels of microhabitat occupation and activity periods. These families aggregate species that are fully terrestrial and also those that are fully arboreal, and comprise species that are only active at night and others only active at daylight

(Martins 1998; Hartmann and Hartmann 2009). South American colubrid snakes also occupy a wide geographic distribution range, with some species distributed from the Amazon to southern Brazil (Guedes et al. 2014; Nogueira et al. 2020). Such wide distribution may be another factor favoring this lineage as a biological system for studies in thermal biology and associated topics that do not focus on particular regional temperatures, as the most species occur under a wide climatic distribution.

Considering the absence of direct estimates for S/V in studies focusing on evolution of thermal biology in snakes, we hypothesize that, by quantifying S/V, ecological associations between S/V ratios, microhabitat usage and activity periods might be detected. Specifically, we evaluate if S/V evolved in association with differences in thermal ecology. To answer these questions, we considered the following hypothesis:

1. Associations with ecological variables likely differ between S/V and body size. This hypothesis fosters discussion regarding the implications of using direct measures of S/V or an indirect proxy in studies evaluating thermal relationships.
2. S/V ratio evolved in association with differential microhabitat usage and activity period. Due to thermal properties of S/V for heat exchange and thermal inertia, we hypothesize that species that are active at the same period of the day might exhibit convergent patterns of S/V, the same being expected for species that are similar in microhabitat use. More specifically, we predict that species that are active at night would present lower values of S/V than diurnal relatives because of high thermal inertia. Regarding differential microhabitat use, we expect that arboreal species would present lower values of S/V in comparison to relative species classified as terrestrial or fossorial.
3. The null hypothesis predicts absence of association between S/V and ecology. The null hypothesis may be corroborated if we do not identify the associations between body size and latitudinal gradients suggested by other studies. In this case, the absence of morphological relationships with parameters related to thermal ecology might indicate that snakes are more prompt to evolve eco-physiological associations with different thermal environments, which ultimately may relax selective pressures derived from thermoregulation acting on morphological evolution.

Materials and Methods

Study Species

We selected 61 species from the colubroidea superfamily in order to compile a database comprising only Neotropical species of Colubridae and Dipsadidae family. Species selection aimed to maximize ecological and phylogenetic diversity (avoiding sampling species from a unique lineage). Specimens were assessed from loans and visits to several Brazilian herpetological collections from different institutions. The sample size for each species varied between 1 and 20 specimens (average of 18 individuals per species), and only adult males were considered. Data from species comprising less than five specimens measured were considered only after exhausted searching in all collections. The total of species represented by less than five individuals totalized less than 10% of the total of species sampled in the final database.

Morphological and Ecological dataset

Snakes are characterized by a generic cylindrical shape, which may be partitioned and approximated to a geometrical form according to the region (i.e., head and tail resemble a cone, trunk is expressed as a cylinder). Therefore, we applied stereology techniques to geometric forms in order to infer total body S/V, given by the sum of S/V estimates for each part of the body (head, trunk and tail).

We first measured snout-to-vent length (SVL) and tail length (TL), and then circumference measures were obtained in equally-spaced points in the head and along the trunk, positioned according to the specimen head and trunk lengths. We obtained two measures of circumference on the head and eleven along trunk length. Head circumferences were obtained at 50% and at 100% (jaw base) of head length, and trunk circumferences were obtained at distances corresponding to 10% of total length (i.e. trunk length was divided in 10 equal parts) from the jaw base to the cloacal scale, with circumference around the cloacal scale being the last circumference measurement.

Each segment (one circumference to the adjacent circumference) was approximated to a geometrical shape depending on the specific segment (cone or cylinder; See Supplementary Figure 1 for the formulas). Specifically, if the proximal circumference had the same value of the distal circumference, the segment was approximated to a cylinder; in contrast, if the distal circumference had a different value of the proximal circumference, the segment was approximated to a cone trunk. Typically, the first segment (tip of the nose to 50% of the head) and the tail segments were approximated to a cone, and most intermediate regions were treated

as cylinders. At the end of all the measurements, we assembled thirteen segments approximated to a given geometrical form, and calculated surface area and volume for each. Subsequently, all individual values for Surface Area and for Volume (i.e. estimates obtained for each segment) were summed in order to estimate the total value for S/V for the specimen (Figure 1) as follows:

$$\sum_{i=1}^{13} (S \text{ and } V \times (\text{Head Length} \times 0.5)) + (S \text{ and } V \times (\text{Trunk Length} \times 0.1)) \\ + S \text{ and } V \times (\text{Tail Length})$$

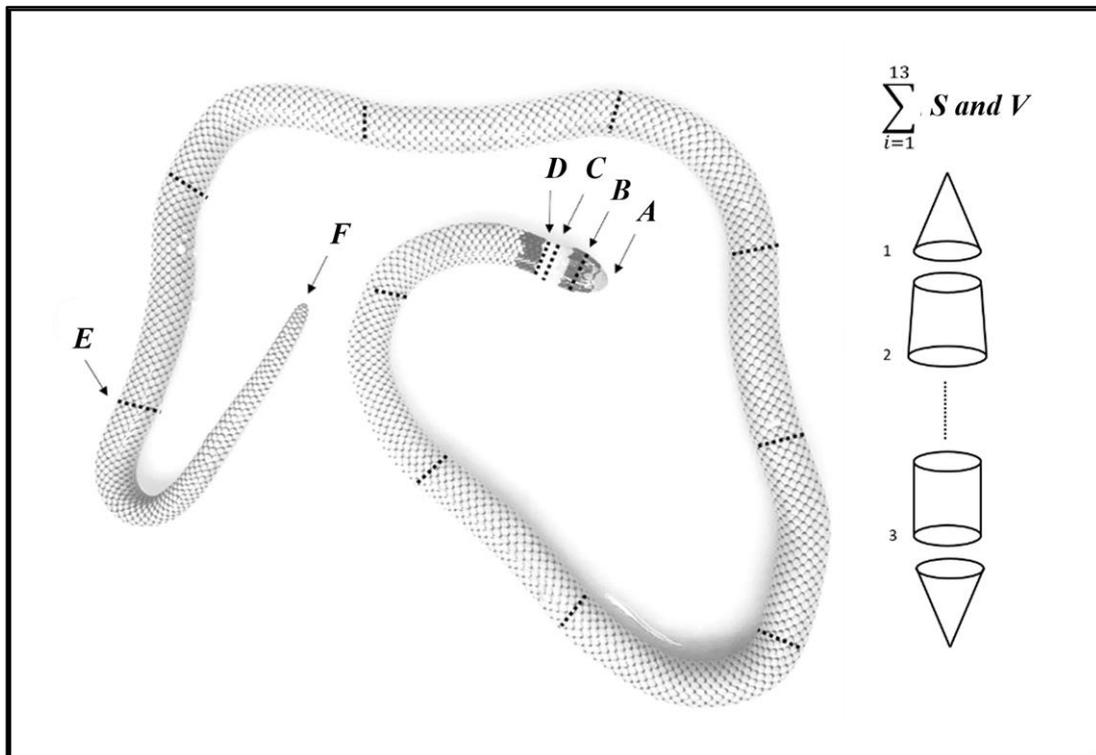


Figure 1 Hypothetical representation of points where circumference measurements were obtained and approximated geometrical form. A. tip of the nose; B. 50% of head length; C. 100% of head length (end of the mandible); D. 1mm after end of the mandible; E. beginning of cloacal scale; F. end of tail. Dash lines over the trunk represent the point of measurement of circumference. Sigma represents the sum of Surface Area and Volume for each segment. 1. represents cone form; 2. represents trunk of cone form; 3 represents cylinder form.

In order to evaluate evolutionary associations between S/V and environmental parameters, we compiled a database for ecological classification; species were classified

according to habitat use (terrestrial and arboreal) and activity period (diurnal and nocturnal). Data for ecological classification were obtained from extensive review of the current literature, mostly based on ecology articles and natural history field notes. Habitat classification was based on previous studies in ecology that defined the species as fossorial, terrestrial or arboreal (we also fit species classified as “semi-arboreal” into the arboreal category). Researchers often define a species as terrestrial when there is no record of individuals observed in arboreal habitats and as arboreal when at least one event of individual observed above the ground was recorded. Species classified as fossorial correspond to those rarely seen above the surface, which usually occupy microhabitats in the underground. In this study, the fossorial category congregates strictly fossorial species (that burrow) and also cryptozoic ones (which move under the litter).

Particularly for the case of *Phimophis guerini*, which is cryptozoic according to some authors, we classified here as a fossorial species. Regarding activity period, snakes are often defined as nocturnal when most observations of active individuals are recorded during the night. For some species, information on activity period recorded the number of active individuals observed at different hours; in these cases, we systematized that the species would be classified as nocturnal when records comprised individuals observed after 7pm (UTC-3). Not rarely, literature sources provide contrasting information regarding habitat usage for some species. In these cases, we adopted the classification that corresponded to 2/3 of the category described by the articles reviewed; the same strategy was adopted for activity period. We also contacted specialists with experience on natural history of Neotropical colubrid snakes to validate the ecological database assembled.

Statistical Analyses

In this chapter we implemented statistical analyses based on comparative methods that incorporate phylogenetic information. Phylogenetic relationships for the colubrid species assembled in our databases were based on the time-calibrated phylogeny proposed by Zaher et al. 2019. The original phylogeny from was trimmed in order to encompass only the species we measured, but maintaining the phylogenetic relationships and divergence estimates proposed in the original article.

Data were first tested for normality using Shapiro Wilk test. Parameters that did not fit the normality premises (SVL) were normalized by log₁₀ transformation. Data for the trait S/V fit the normality and therefore were not transformed. Analyses were performed using mean values of morphological parameters calculated for each species. All analyses were implemented

in RStudio® (version 1.3.1093). We first evaluated the correspondence between S/V and body size (SVL), and tested for correlations using phylogenetic generalized least squares - *pgls* function in *caper* package (version 0.5.2; Orme 2013). For visual purposes, we assembled a phylogenetic scatterplot between S/V by SVL followed by ancestral state reconstruction of both traits. We used the *fancyTree* function implemented by *phytools* package (version 0.7-70; Revell 2020). This function plots a phylogenetic scatterplot matrix comprising *contMap* style trees on the diagonal and phylomorphospace plots in the non-diagonal panels. We also evaluated allometric differences in S/V residing on SVL variation, performing separated tests (microhabitat or activity period) and also combining microhabitat and activity. Correlation slopes of S/V against SVL did not differ among snake species from different microhabitats or between species that differ in activity periods. Tests for allometry considering the ecological categories were implemented using the *gls* function from *nlme* package (version 3.1-149; Pinheiro et al. 2020), with branch lengths controlled with scores from Maximum Likelihood.

Evolutionary associations of S/V were evaluated by first estimating Bloomberg phylogenetic signal of S/V using the *Kcalc* function from *picante* package (version 1.8.2; Kembel et al., 2020). Subsequently, we tested if S/V in the lineage comprises interspecific variation according to differences in microhabitat usage (arboreal and terrestrial) or activity periods (nocturnal and diurnal). In these analyses, we used the *pgls* function from *caper* package to run ANOVA using S/V as the response variable and ecological categories as the predictors. Finally, we also tested for differences in S/V among species using combined ecological classifications (arboreal-diurnal, arboreal-nocturnal, terrestrial-diurnal, terrestrial-nocturnal, fossorial-diurnal and fossorial-nocturnal).

Evolutionary history of microhabitat use and period of activity in Colubroidea was also inferred using *simmap* function. Maximum likelihood estimates (MLE) and 95% confidence intervals for each node on the tree were calculated for a given ecological category. The *phytools* package rescued a tree with branches colored according to microhabitat category and nodes colored based on the probability of activity period.

S/V and Slenderness

Current literature lacks direct measurements of Surface Area and Volume in snakes. A derived prediction from relationships between Surface Area and Volume in animals that differ in body shape would be that in limbless and elongated phenotypes the S/V ratio likely changes according to differences in the degree of elongation and slenderness. Slenderness differs among snake families, and species from Boidae, Pythonidae and Viperidae are often recognized as more

robust snakes while slenderer bodies characterize Elapidae, Colubridae and Dipsadidae. However, patterns of slenderness also vary according to microhabitat usage regardless of the family (Pizzatto et al. 2007; De Alencar et al. 2017). In this context, differences in S/V might either reflect indirect associations with slenderness due to biomechanical relationships or direct associations with the use of different thermal environments. Accordingly, correlations between S/V and slenderness (estimated from phylogenetic residuals of mid-body circumference by SVL) were also tested using phylogenetic GLS from *phytools*. We identified a negative relationship (PGLS; $P < 0.01$, $R^2 = 0.18$) that each increased unit in SVL was coupled with S/V approximately 0.48 unit decrease in S/V, which expresses the trend that relative thicker snakes comprised smaller S/V values (Supplementary S3).

Results

Species included in this study comprised representative species from the Colubridae and Dipsadidae families distributed in South America. The phylogenetic relationships among species and families used in all analyses considered the phylogenetic hypothesis from Zaher et al. 2019. In our dataset, body size (SVL) and Surface Area per Volume ratio (S/V) comprised remarkable variation, ranging from species smaller than 30cm² (217 mm²; *Tantilla melanocephala*) to those larger than one and a half meter (1534 mm²; *Drymarchon corais*). The largest discrepancy in S/V was observed between *T. melanocephala* (= 0.12) and *D. corais* (= 0.78).

Significant allometric relationships between S/V and body size expressed a negative correlation (PGLS; $P < 0.01$). The correlation between traits can be visualized in the scattergram in Figure 2 (regression line provided at Supplementary Figure S2), which connects species based on phylogenetic relationships. Larger snakes tended to comprise lower values of S/V than smaller species (which comprised higher S/V ratios). We also tested for differences among ecological categories in the slope inclinations of relationships between S/V and SVL. When species were classified according to the use of microhabitat or based on combined ecological categories, we did not detect differences in slopes (PGLS/ANOVA; $P < 0.05$) However, when accounting for differences in activity periods, allometric differences were nearly significant (PGLS/ANOVA: $P = 0.07$), and S/V values tended to be greater in nocturnal species. Although we did not detect significant allometric differences when using combined ecological categories, slopes were more elevated in arboreal–nocturnal snakes than in arboreal–diurnal species (see Supplementary Figure S3c) (PGLS/ANOVA: $P = 0.27$).

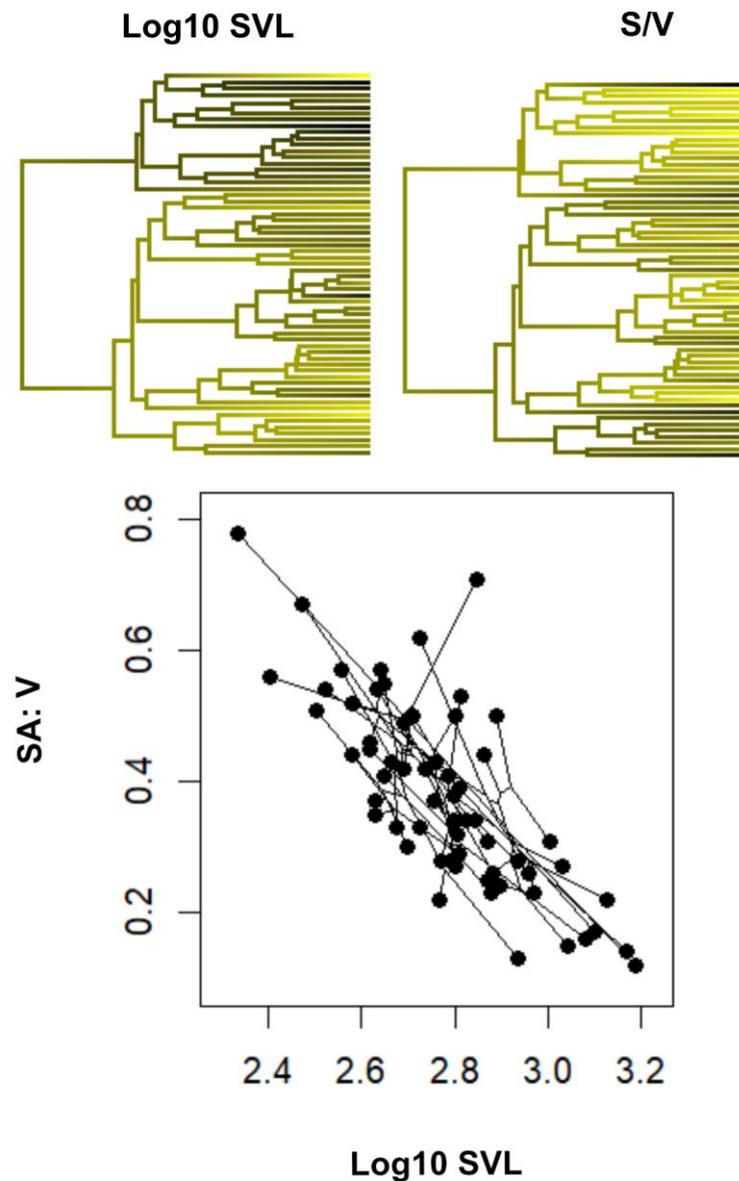


Figure 2 Ancestral State Reconstruction of SVL and S/V in the top-left and top-right, respectively. Yellow-to-black gradients indicate low-to-high values in the trees. Phylogenetic scatterplot of Surface Area to Volume ratio in relation to SVL in the bottom.

Inferences of evolutionary associations between average S/V and ecology were first evaluated based on calculation of phylogenetic signal followed by implementation of phylogenetic ANOVA using *pgls* from the *caper* package. We performed separate analyses considering microhabitat use, activity period and combined ecological categories (Table 1). For comparative purposes, we also report the results obtained using body size as the response variable, with the same predictors used in the analyses for S/V. Phylogenetic signal rescued values near 0.7, which is much higher than 0 (changes fit a star phylogeny) but also do not reach 1 (expressing the phylogenetic relationship among species under Brownian Motion). The

estimated value is large enough to rescue Brownian Motion with phylogeny explaining most S/V patterns, but not entirely. Further, our results indicate lack of association between S/V and ecological parameters, suggesting that there are no differences in average S/V between species that differ in microhabitat use (fossorial, terrestrial and arboreal), activity period (diurnal and nocturnal), or when classified under categories that combine both parameters. Interestingly, however, body size did differ between species according to microhabitat usage, with smaller values observed in fossorial species than in terrestrial and arboreal snakes. Species classified under the combined categories also differed in body size, and fossorial–diurnal species comprised relative lower body size values.

Table 1 Results for Analyses of variance using SVL and S/V, considering the ecological predictors.

Phylogenetic ANOVA		
	Response Variable	
	Body Size (log₁₀ SVL)	S/V
Combined		
<i>R</i> ²	0.100	0.055
<i>F</i>	2.342	1.339
<i>P</i>	0.053 ·	0.149
Microhabitat		
<i>R</i> ²	0.096	0.314
<i>F</i>	4.207	0.941
<i>P</i>	0.01 *	0.396
Activity		
<i>R</i> ²	-0.015	- 0.016
<i>F</i>	0.062	0.012
<i>P</i>	0.803	0.912

Asterisk indicates significant P – value and dot represent values marginally significant.

Ancestral reconstructions of discrete characters suggested the most ancestral state in our dataset probability being terrestrial and diurnal (Figure 3). Scaled loglikelihood at the root corresponded to 0.83 for the terrestrial microhabitat and 0.94 for diurnal activity at this node. According to these estimates, nocturnal activity, fossoriality and arboreality would be derived states in these lineages. Furthermore, nocturnal activity and fossoriality seem to be rare conditions (Figure 3). The reconstructions of S/V and SVL are provided as yellow-to-black gradients at Figure 2, being yellow lower values and black the higher ones.

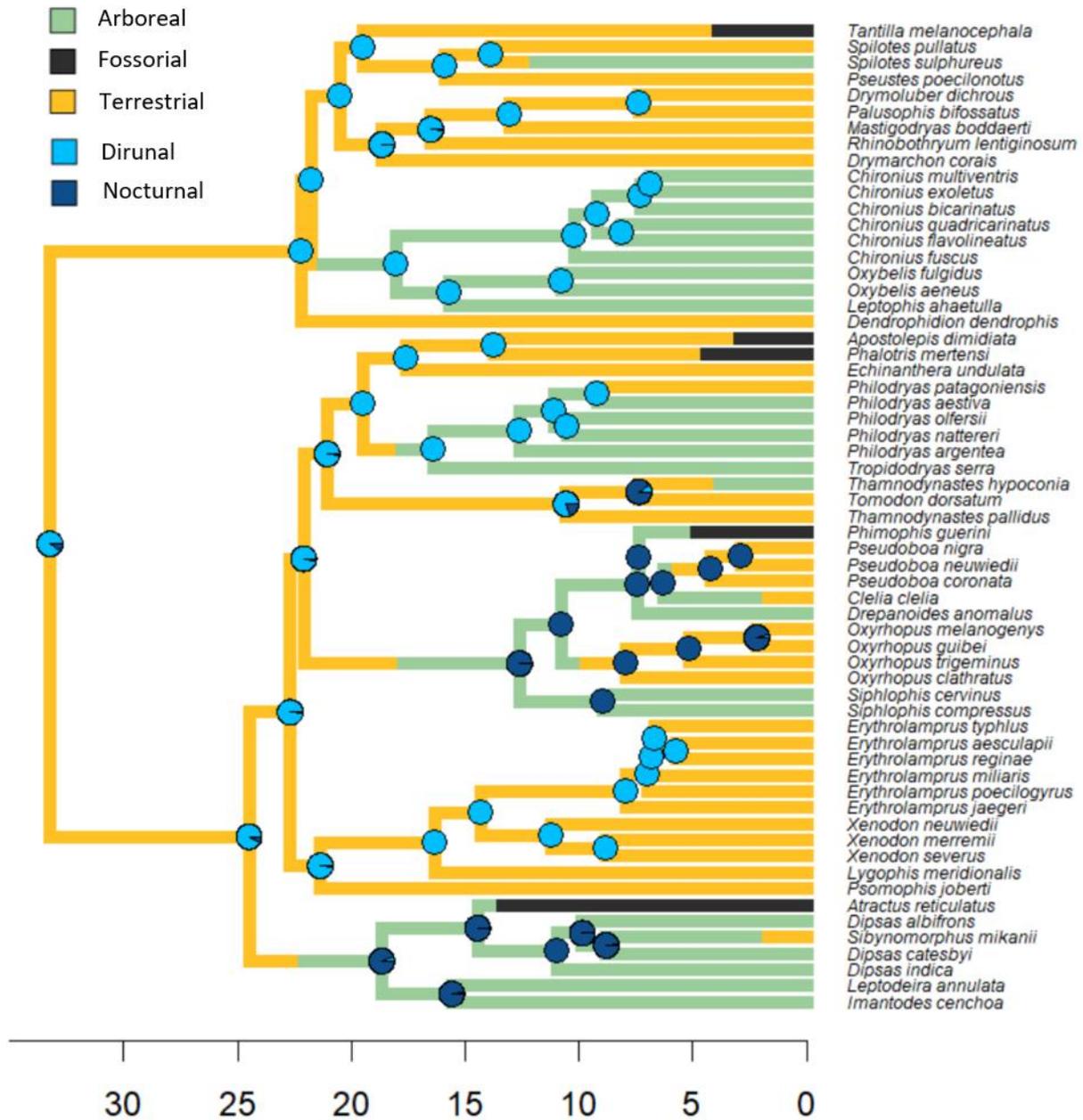


Figure 3 Ancestral state reconstruction of ecological categories. Along with the branch, colors indicate microhabitat categories and colored circles in the nodes correspond to activity period.

Discussion

This study evaluated if snake species that diverge in microhabitat usage and activity periods also differ in S/V ratios. To our knowledge, this is the first study that evaluated the evolution of S/V in a phylogenetic comparative context. The inclusion of microhabitat information in the analysis is particularly relevant for snakes, especially considering eventual

evolutionary trade-offs likely related to limbless locomotion (Shine and Shetty 2001; Gartner 2011; Lillywhite 2014). Analyses performed here did not detect evolutionary associations between average S/V and the type of microhabitat used or the preferred activity period. Despite the lack of correlations between S/V and environmental parameters, allometric relationships of S/V with body size seems to diverge between species that differ in activity periods. Most species expressed negative allometric relationships, with longer snakes expressing lower S/V values. Surprisingly, arboreal-nocturnal species comprised the species with more positive correlations between S/V and SVL in contrast to patterns identified within all other ecological categories (Supplementary Figure S2). We investigated S/V evolution in snake species by directly quantifying Surface Area and Volume, and these values were used in the analyses, which differs from approaches more commonly used in the literature, using body size or body weight as a proxy (Ashton and Feldman 2003; Pincheira-Donoso et al. 2008; Slavenko and Meiri 2015). This approach enabled comparisons of evolutionary associations of S/V and SVL with ecological categories.

Comparisons between S/V and Body length

The Surface Area to Volume ratio is a relative measure that plays a crucial role for dynamics of heat exchange rate in geometric forms (IV and V 2019). Relationships among S/V, heat loss and overall morphology S/V has been classically addressed by pioneer studies on the Bergman's rule (see McNab 1968; Ashton and Feldman 2003; Pincheira-donoso et al. 2008). Most of these studies, however, have inferred S/V based on body size (SVL or mass used as a proxy for S/V). Such approximations might not reflect with the same accuracy the relationship between Surface Area and Volume as estimates of S and V using stereology. For example, two species of similar mass might differ in SVL and therefore comprise different relationships between Surface Area and Volume. This is case for *Spilotes pullatus* and *Palusophis bifossatus*, which are very similar on mass average but differ in body length, with *S. pullatus* being much longer (Feldman and Meiri 2013). Due to the allometric correspondence of body size and S/V in animals, the use of body size or mass in ecological evolutionary studies can produce misleading interpretations, especially because the two variables can have separately evolutionary histories, therefore be under distinct selective pressures. Ferreira-Amado et al. 2019 provided solid evidence for such prediction when analyzed separately patterns of S/V and body size in relation to the same environmental predictors, testing for associations with biogeographic distribution S/V and air humidity in anurans. In the natterjack toad *Epidalea calamita*, different sets of predictors explain the variation of S/V and body size. In colubrid snakes, we identified that body

size is associated with microhabitat and also in combination with activity period, a result that differs from that of S/V, suggesting that these two traits might have evolved differently. Furthermore, analyzes focusing on S/V accounts for selective regimes acting on body size or body mass. Body mass, in particular, might comprise high phylogenetic constraints, even though S/V may change along with differences in shape decoupled from variation in body mass. Moreover, S/V might be directly associated with thermal properties and so be used to predict correlations with thermal environment with less possible bias.

Effects of ecological parameters on S/V evolution

The evolution of S/V might have been directly associated with thermally selective regimes encompassing effects of air temperature during the period the animal is active, but may be also indirectly related to other morphological traits related to other selective pressures. Snake slenderness, for example, has been described as associated to the use of arboreal lifestyle and locomotion over branches, while fossorial species are more often characterized by small and robust bodies that likely evolved in association with burrowing biomechanics (Pizzatto et al. 2007). Our data indicate that S/V is correlated with slenderness, despite the low explanatory capacity for variation in S/V. As discussed in Chapter 1, slenderness in snakes is often correlated with the use of arboreal microhabitats, as exemplified by previous studies with South-American vipers (De Alencar et al. 2017). Despite the trend for S/V increase coupled with slenderness, S/V does not reflect evolutionary associations between slenderness and microhabitat usage, which is often explained by biomechanical constraints (Astley and Jayne 2007; De Alencar et al. 2017). Findings from Chapter 1 suggest that this is an expected pattern, as slenderness only differs between semi-arboreal colubroid snakes and those specialized in arboreal microhabitat, but not between terrestrial and arboreal species.

Conversely, we predicted that differences in the habitat structure used by snakes species might also comprise thermal implications related to different microclimates, so that locomotion on tree branches might expose larger Surface Area of the animal body to air contact and, consequently, to heat exchange (Bakken 1989; Plummer 1993). However, our analyses did not detect any association between S/V and microhabitat. It is possible that the use of particular microhabitats, ultimately with particular microclimates, did not evolved in association with changes in average S/V. Thermal constraints expected to be related to S/V patterns in nocturnal arboreal species might have been relaxed according to seasonal activity. Information available for seasonality of some representatives of the arboreal-nocturnal species included in this study suggest that these are much more active during summer, when air temperature in tropical region

is more constant and elevated (Garreaud et al. 2009). Species such as *T. hypoconia*, *D. albifrons*, *I. cenchoa* are active mainly during summer and spring (September to March; see Hartmann et al. 2010; Bellini et al. 2013; de Sousa et al. 2014). The effect of seasonality in the thermoregulatory strategy has been demonstrated for *Bothrops insularis* and *Boa constrictor*, two species that seem to display a tendency for thermoconformation in hotter seasons (Chiaraviglio et al. 2003; Bovo et al. 2012). In this case, if colubrid nocturnal snakes also present a similar trend, the effect of S/V on heat exchange might have minimum consequences, allowing these species to explore arboreal habitats during the night.

Associations between body size and microhabitat usage suggest that differences in snake length among microhabitats were not coupled with proportional variation in body volume, given that average S/V does not differ between colubrid species that diverged in microhabitat usage. This result agrees with allometric differences in S/V between species ecologically divergent.

Implications of S/V for thermal relationships in ectotherms

In ectotherms, performance of several tasks is affected by body temperature (Huey 1957; Huey and Kingsolver 1989; Angilletta et al. 2002; Michael J. Angilletta Jr. 2009). The capacity of an animal to achieve values near the optimal temperature is important because time spent in thermoregulation may expose individuals and facilitate predation (Dunham et al. 1989; Dzialowski and O'Connor 2001; Gvoždík 2002). In squamates that thermoregulate, S/V plays a special role due to effects on thermal inertia, being potentially targeted by specific selective pressures at different thermal environments (Pincheira-donoso et al. 2008). Variation in body weight (as a proxy to S/V) is related to thermally-constrained environments in some ectotherm lineages (Olalla-Tárraga et al. 2006; Penniket and Cree 2015; Rodrigues et al. 2018; Slavenko et al. 2019). Populational studies also described that larger lizards inhabit colder regions while smaller individuals are often found in a hotter locations (Zamora-Camacho et al. 2014; Penniket and Cree 2015). In contrast, studies focusing on thermal biology in snakes point no correlation between body mass and thermal gradient for some lineages over an interspecific scale (Olalla-Tárraga et al. 2006). Analyses restricted to South American species suggest a slight positive correlation between body mass and average temperature, the opposite trend expected by thermal constraints of Bergman's rule (Slavenko et al. 2019).

The majority of studies available in the current literature are restricted to diurnal species, and associations with nocturnality remained relegated. The largest sampled database used to evaluate associations between activity period and body mass using a phylogenetic comparative approach suggested a trend that differs from our findings (Feldman and Meiri 2014). In

Australian snakes, surface-active nocturnal species are apparently smaller than diurnal, a pattern that may express a weak relationship with environmental temperatures and be mostly explained by phylogeny and biogeography. In that study, the authors also did not identify correlations between body weight and air temperature in diurnal species (Feldman and Meiri 2014). The explanation may reside on the hypothesis that diurnal species experience enhanced thermal quality and increased retreat resources, and therefore may behaviorally thermoregulate regardless of body size (Rosen 1991). The same explanation could be applied to the diurnal species that explores different microclimates hypothesized in this study, where despite differences in temperature and S/V, thermoregulation by finding better thermal quality environments could mitigate S/V associations with microhabitat. Furthermore, studies on functional morphology suggest that nocturnal snakes might actually deal with thermal constraints by physiological adjustments, which might have also evolved in association with thermal relationships. Body temperature in nocturnal snake species often corresponds to the air temperature (Peterson 1987; Anderson et al. 2005; Webb et al. 2015). In Japanese nocturnal snakes, body temperature may be as low as 10° C during activity in the winter (Mori and Toda 2018). The ability to be active at suboptimal body temperatures has been reported in nature, where some species that are specialized to be active in the twilight and during the night experience optimal body temperatures above the air temperature (Peterson 1987; Dorcas and Peterson 1998; Llewelyn et al. 2005; Cruz et al. 2008). Functional studies suggest that nocturnal snake species have better performance in the low temperatures at night (Llewelyn et al. 2006).

Eco-physiological and behavioral evolutionary responses suggest that, at least for some snakes, thermoconformation with displaced body optimal temperature might be a condition that evolved more frequently than morphological patterns associated to the use of distinct thermal environments (Mori and Toda 2018). In other squamates such as *Liolaemus* lizards, preferential temperature comprises very low phylogenetic signal, despite significant correlations between body temperatures, biogeographic distribution and environmental air temperature. In these lizard species, body size and rates of heat exchange are not associated to thermal environment (Labra et al. 2009). Another possible explanation for the lack of association between S/V and thermal parameters of the environment might rely on the fact that some squamates do not necessarily fit precisely into thermoconformation or thermoregulatory categories. Some animals, including lizards and snakes, perform all regular activities without reaching the optimum value for body temperatures (Huey and Bennett 1987; DeNardo et al. 2002; Sperry et al. 2013; Huey et al. 2014). In these cases, even if performance is affected by thermoregulation inefficiency, the fitness apparently is not too affected, suggesting that selection on traits related to thermoregulation might be weak.

Evolution of S/V in South American Colubridae and Dipsadidae snakes apparently did not relate to differences in microhabitat usage (arboreal, terrestrial or fossorial) or activity period (diurnal or nocturnal). Our findings suggest that species sampled are similar in average S/V despite ecological divergence, so that other traits might have evolved in association to differences in thermal environments imposed by divergence in microhabitat and activity period. Accordingly, colonization of novel thermal environments might have comprised changes in physiology and behavior, rather than morphology. Furthermore, we propose that the evaluation of direct estimates of S/V may produce results in macroevolutionary analyses that differ from those resulting from the use of S/V proxies such as body mass or body length.

Supplementary Material

Calculating Surface Area and Volume using Geometry

Inference of Surface Area for each segment was calculated using the following formulas, based on the specific geometrical shape. For trunk of cone we used: $\pi \times g \times (R + r)$, for cone we used: $\pi \times r \times g$ and for cylinder we used $2 \times \pi \times R \times h$. Inference of body Volume for each segment was calculated using the following formulas, based on the specific geometrical shape. For trunk of cone we used: $\pi \times h / 3 \times (R^2 + R \times r + r^2)$, for cone we used: $\pi \times r^2 \times h / 3$ and for cylinder we used $\pi \times r^2 \times h$. As $\pi = 3,14$; g = generatrix; h : height (distance between circumference measures); R = ray of the smaller circumference; r = ray of the larger circumference. Generatrix values varied based on the circumference values and height: $\sqrt{(R - r)^2 + h^2}$). Representation of the geometric shapes and respective properties as follows:

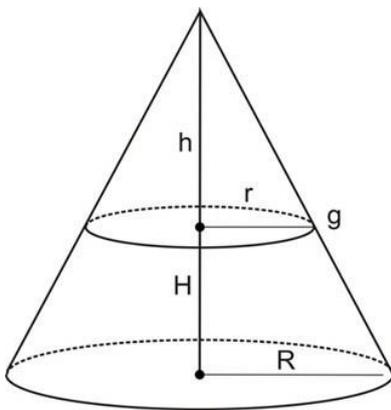


Figure S1. All species correlation of Surface Area per Volume by Body Size. $R^2: 0.632$; $P = 1.21e-14$.

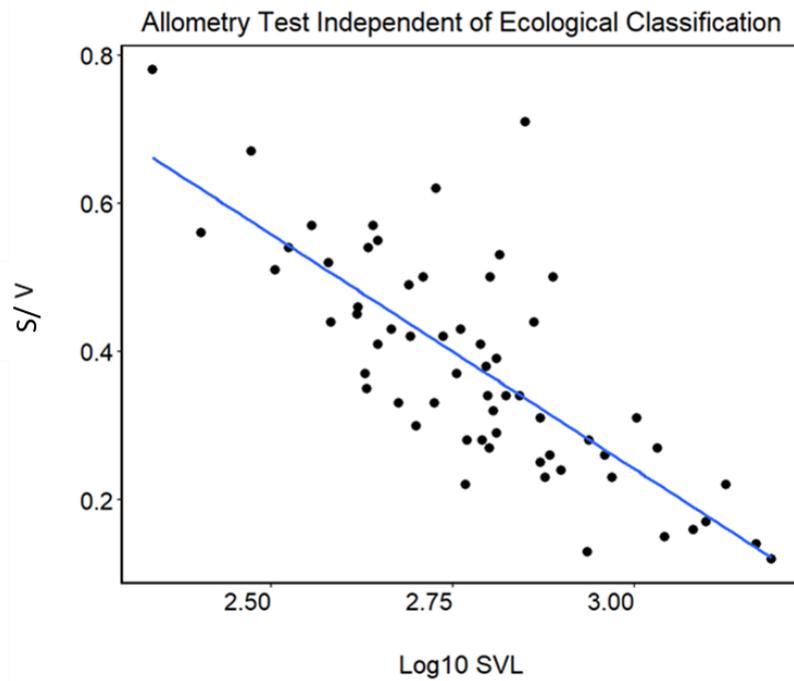
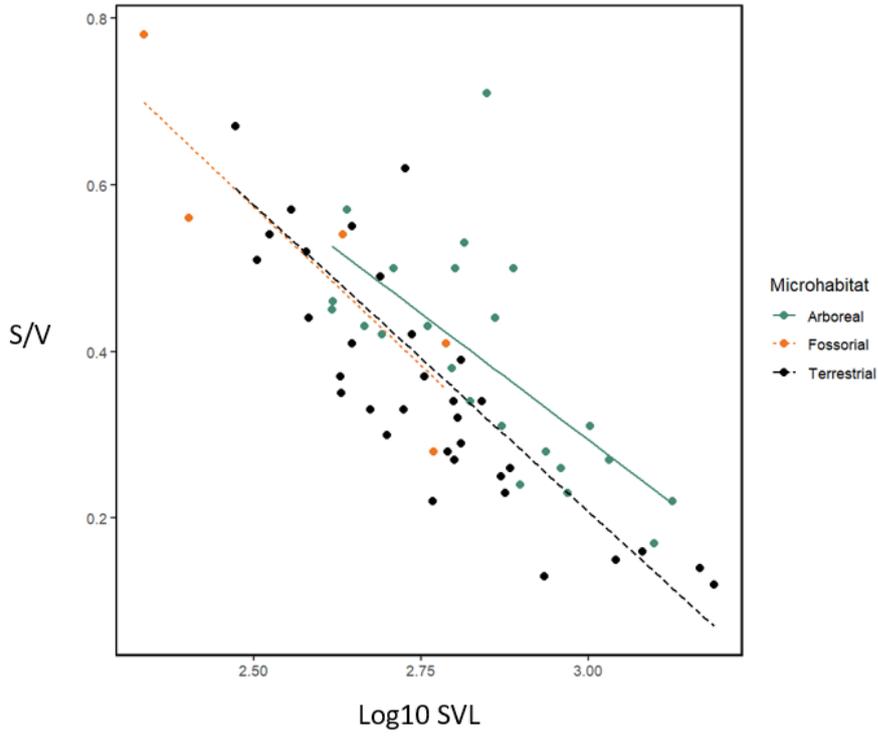
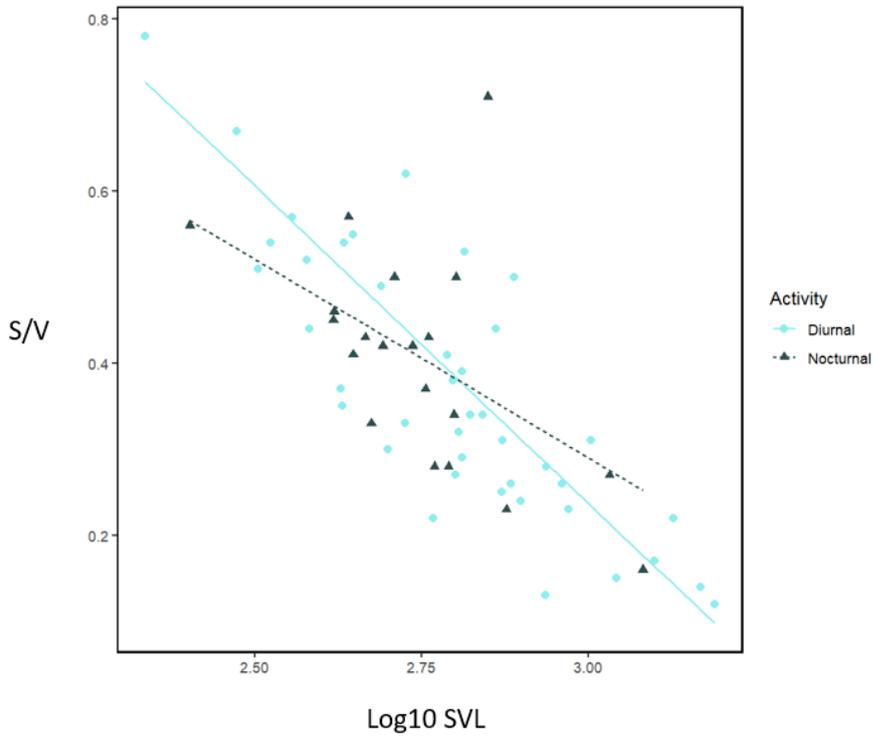


Figure S2. Allometric relationships in S/V among ecological categories. a. considering only microhabitat: Terrestrial, Arboreal and Fossorial; b. considering only activity period: Diurnal and Nocturnal; c. considering combined microhabitat and activity period (Terrestrial/ Diurnal (TD), Terrestrial/ Nocturnal (TN), Fossorial/ Diurnal (FD), Fossorial/ Nocturnal (FN), Arboreal/ Diurnal (AD) and Arboreal/ Nocturnal (AN)).

a.



b.



c.

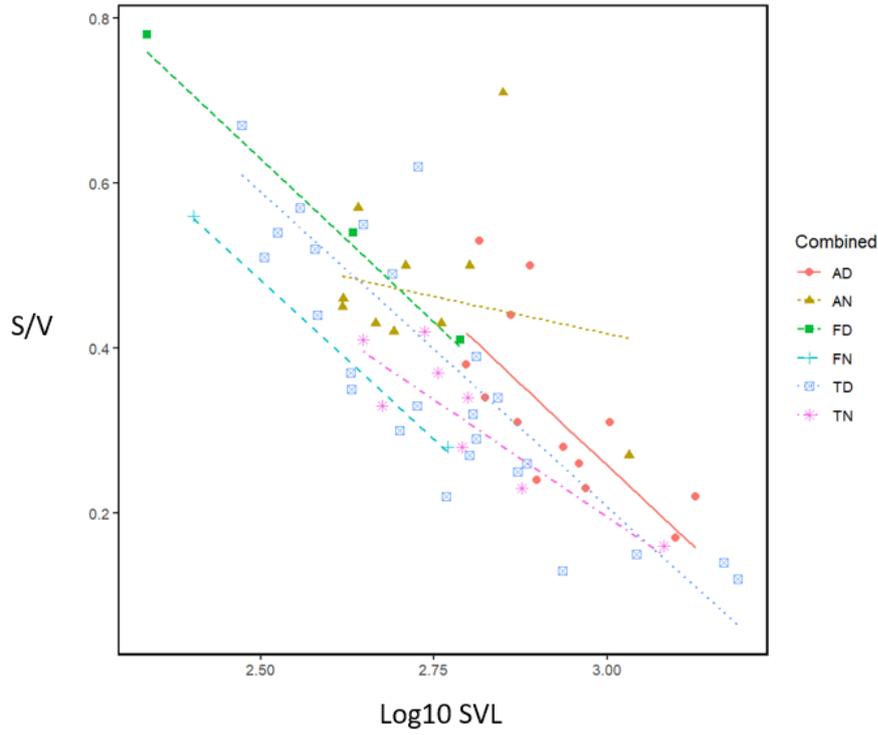


Figure S3. Phylogenetic linear regression between Surface Area per Volume and slenderness (Mid-Body Circumference), $P < 0.05$.

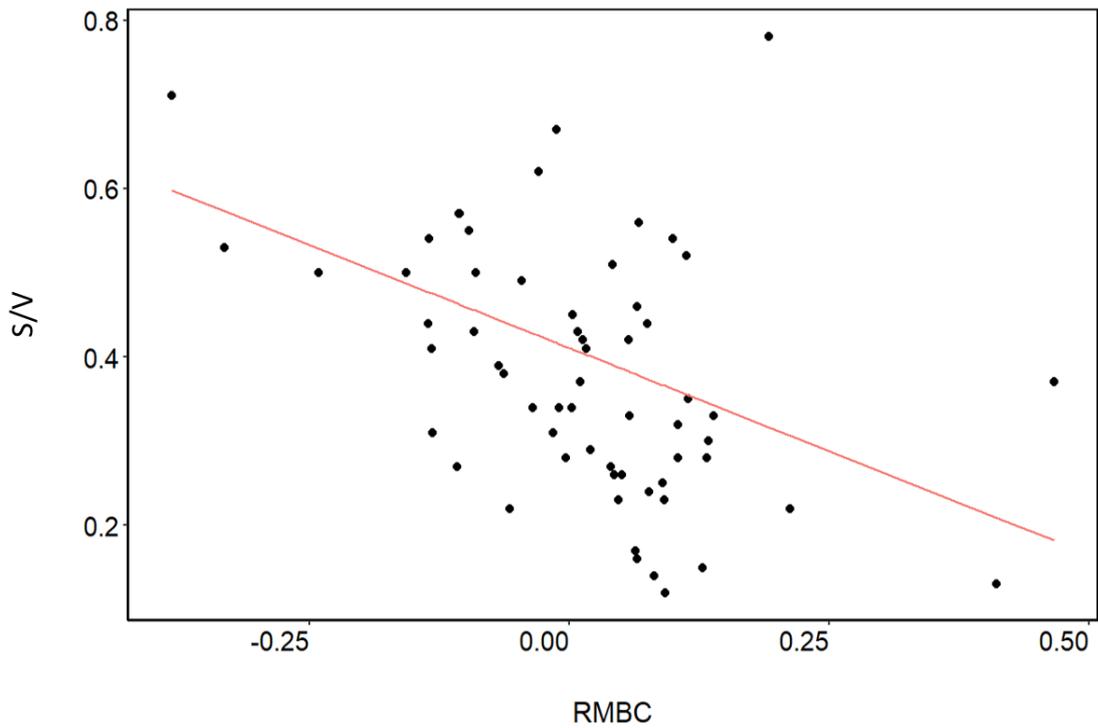


Table S1. Homogeneity test among slopes of S/V by SVL among ecological categories. P-values in bold indicate significant relationships.

S/V ~	Chi- squared	<i>P - value</i>
SVL x Microhabitat	0.804	0.66
SVL x Activity	03.17	0.07
SVL x Combined	6.48	0.27

Table S2. Ecological and morphological information of 61 snake species included in this study. Species; Tree Tip Order (TTO); Sample size (Number of individuals; N); Ecological classification; mean values and standard errors (\pm SE) of snout vent-length (SVL), Surface Area per Volume (S/V); bibliographical references used for microhabitat identification are provided for each species.

Species	Tree tip order	N	Microhabitat	Activity	SVL \pm	SE	Surface Area per Volume	Microhabitat References
<i>T. melanocephala</i>	1	20	Diurnal	Fossorial	217.45 \pm	5.98	0.78	Bernarde, 2004; dos Santos-Costa, 2015; França and Araújo, 2006
<i>S. pullatus</i>	2	20	Diurnal	Terrestrial	1.470 \pm	40.92	0.14	Martins and Oliveira, 1998; Bernarde, 2004
<i>S. sulphureus</i>	3	20	Diurnal	Arboreal	1.255 \pm	55.68	0.17	Martins and Oliveira, 1998
<i>P. poecilonotus</i>	4	21	Diurnal	Terrestrial	762 \pm	22.75	0.26	dos Santos-Costa, 2015; Martins and Oliveira, 1998
<i>D. dichrous</i>	5	20	Diurnal	Terrestrial	741.6 \pm	28.9	0.25	Bernarde, 2004
<i>P. bifossatus</i>	6	17	Diurnal	Terrestrial	1.110 \pm	34.19	0.15	Sazima, 1993; França et al., 2008
<i>M. boddaerti</i>	7	20	Diurnal	Terrestrial	695.15 \pm	22.17	0.34	Bernarde, 2004
<i>R. lengitinossum</i>	8	9	Nocturnal	Arboreal	1.074 \pm	34.96	0.27	Bernarde, 2004; Strussman and Sazima, 1993
<i>D. corais</i>	9	16	Diurnal	Terrestrial	1.543 \pm	44.18	0.12	Bernarde, 2005
<i>C. multiventris</i>	10	15	Diurnal	Arboreal	1.384 \pm	66.18	0.22	Bernarde, 2004
<i>C. exoletus</i>	11	24	Diurnal	Arboreal	913.13 \pm	37.49	0.26	Hartmman et al., 2009
<i>C. bicarinatus</i>	12	20	Diurnal	Arboreal	932.7 \pm	29.99	0.23	Hartmman et al., 2009
<i>C. quadricarinatus</i>	13	18	Diurnal	Arboreal	636.94 \pm	12.51	0.38	Sawaya et al., 2008
<i>C. flavolineatus</i>	14	15	Diurnal	Terrestrial	646.47 \pm	10.36	0.39	Sawaya et al., 2008
<i>C. fuscus</i>	15	15	Diurnal	Arboreal	865.47 \pm	40.29	0.28	Martins, 1994
<i>O. fulgidus</i>	16	15	Diurnal	Arboreal	1.007 \pm	36.44	0.31	Martins and Oliveira, 1998
<i>O. aeneus</i>	17	20	Diurnal	Arboreal	788.4 \pm	32.56	0.5	Martins and Oliveira, 1998
<i>L. ahaettula</i>	18	20	Diurnal	Arboreal	726.55 \pm	30.3	0.44	Martins, 1994; Martins and Oliveira, 1998
<i>D. dendrophis</i>	19	20	Diurnal	Terrestrial	534.15 \pm	9.21	0.62	Bernarde, 2004
<i>A. dimidiata</i>	20	1	Diurnal	Fossorial	430 \pm	NA	0.54	Sawaya et al., 2008
<i>P. mertensi</i>	21	20	Diurnal	Fossorial	613.95 \pm	16.97	0.41	Sawaya et al., 2008
<i>E. undulata</i>	22	12	Diurnal	Terrestrial	334 \pm	18.54	0.54	Hartmman et al., 2009
<i>P. patagoniensis</i>	23	20	Diurnal	Terrestrial	671.3 \pm	25.48	0.27	Machado Filho, 2015
<i>P. aestiva</i>	24	15	Diurnal	Terrestrial	488.73 \pm	23.87	0.49	Goldsmith, 1984
<i>P. olfersii</i>	25	20	Diurnal	Arboreal	666 \pm	19.77	0.34	Hartmman et al., 2009; Bernarde, 2004
<i>P. nattereri</i>	26	20	Diurnal	Arboreal	794.6 \pm	28.19	0.24	Machado Filho, 2015
<i>P. argentea</i>	27	3	Diurnal	Arboreal	653.33 \pm	63.66	0.53	dos Santos-Costa, 2015; Machado Filho, 2015
<i>T. serra</i>	28	19	Diurnal	Arboreal	742.90 \pm	25.36	0.31	Martins and Oliveira, 1998
<i>T. hypoconia</i>	29	13	Nocturnal	Arboreal	454 \pm	16.36	0.43	Sawaya et al., 2008
<i>T. dorsatum</i>	30	20	Nocturnal	Terrestrial	472.50 \pm	14.26	0.33	Gomes, 2016

<i>T. pallidus</i>	31	11	Diurnal	Terrestrial	443.64 ±	13.89	0.55	Strussman and Sazima, 1993; Vrcibradic et al., 2014; Bailey et al., 2005
<i>P. guerini</i>	32	9	Nocturnal	Fossorial	601.67±	30.4	0.28	Sawaya et al., 2008; França and Braz, 2013
<i>P. nigra</i>	33	20	Nocturnal	Terrestrial	753.4 ±	28.55	0.23	Strussman and Sazima, 1993; França and Braz, 2013; Hartmman et al., 2009
<i>P. neuwiedii</i>	34	3	Nocturnal	Terrestrial	443.67 ±	18.28	0.41	Pontes et al., 2009
<i>P. haasi</i>	35	1	Nocturnal	Terrestrial	617 ±	NA	0.28	Alencar et al., 2013
<i>C. clelia</i>	36	12	Nocturnal	Terrestrial	1.200 ±	60.38	0.16	Alencar et al., 2013; Bernarde and Abe, 2006
<i>D. anomalus</i>	37	20	Nocturnal	Arboreal	414.6 ±	6.12	0.45	Bernarde, 2004
<i>O. melanogenys</i>	38	20	Nocturnal	Terrestrial	570.3 ±	20.1	0.37	Alencar et al., 2013; Bernarde and Abe, 2006
<i>O. guibei</i>	39	20	Diurnal	Terrestrial	639.4 ±	29.05	0.32	Alencar et al., 2013; Sawaya et al., 2008
<i>O. trigeminus</i>	40	1	Nocturnal	Terrestrial	545 ±	NA	0.42	França et al., 2006
<i>O. clathratus</i>	41	15	Nocturnal	Terrestrial	629.27 ±	19.1	0.34	Alencar et al., 2013; Hartmman et al., 2009
<i>S. cervinus</i>	42	7	Nocturnal	Arboreal	511.71 ±	21.88	0.5	Alencar et al., 2013
<i>S. compressus</i>	43	20	Nocturnal	Arboreal	632.75 ±	13.76	0.5	Bernarde, 2004
<i>E. typhlus</i>	44	20	Diurnal	Terrestrial	381.05 ±	8.41	0.44	Martins et al., 2008
<i>E. aesculapii</i>	45	18	Diurnal	Terrestrial	645.56 ±	11.57	0.29	Alencar et al., 2013; Bernarde and Abe, 2006
<i>E. reginae</i>	46	20	Diurnal	Terrestrial	433.95 ±	12.42	0.35	Martins et al., 2008
<i>E. miliaris</i>	47	20	Diurnal	Terrestrial	500.6 ±	15.08	0.3	Silvia et al., 2010; Hartmman, 2005
<i>E. poecilogyrus</i>	48	19	Diurnal	Terrestrial	425.68 ±	15.75	0.37	Entiauspe-Neto et al., 2016; Marques and Martins, 2008
<i>E. jaegeri</i>	49	16	Diurnal	Terrestrial	321.56 ±	8.75	0.51	Sawaya et al., 2008; Entiauspe-Neto et al., 2016
<i>X. neuwiedi</i>	50	20	Diurnal	Terrestrial	531.4 ±	11.87	0.33	Pontes et al., 2009
<i>X. merremii</i>	51	20	Diurnal	Terrestrial	585.8 ±	11.53	0.22	Sawaya et al., 2008; França and Braz, 2013
<i>X. severus</i>	52	7	Diurnal	Terrestrial	861.29 ±	70.27	0.13	Sawaya et al., 2008
<i>L. meridionalis</i>	53	4	Diurnal	Terrestrial	360.25 ±	22.33	0.57	Sawaya et al., 2008
<i>P. joberti</i>	54	20	Diurnal	Terrestrial	297.4 ±	8.06	0.67	Rodrigues and Prudente, 2011; Strussman and Sazima, 1993
<i>A. reticulatus</i>	55	18	Nocturnal	Fossorial	253.17 ±	6.82	0.56	Passos et al., 2005
<i>D. albifrons</i>	56	2	Nocturnal	Arboreal	415.5 ±	54.5	0.46	Dias, 2018
<i>S. mikanii</i>	57	20	Diurnal	Terrestrial	379.4 ±	10.34	0.52	Sawaya et al., 2008
<i>D. catesbyi</i>	58	20	Nocturnal	Arboreal	437.15 ±	10.94	0.57	Bernarde, 2004
<i>D. indica</i>	59	17	Nocturnal	Arboreal	570.82 ±	18.34	0.43	Bernarde, 2004; Martins e Oliveira, 1998

<i>L. annulata</i>	60	20	Nocturnal	Arboreal	492.15 ±	15.44	0.42	Martins, 1994; Hartmman et al., 2009
<i>I. cenchoa</i>	61	20	Nocturnal	Arboreal	706.9 ±	17.94	0.71	Martins, 1994; Martins and Oliveira, 1998

General Conclusion

Although all snakes have a cylindrical shape, elongated trunk and absence of limbs, the general body shape may vary considerably. Under an evolutionary perspective, the diversity of shapes and patterns can be related to the parameters of environmental structure where animals live. Within the lineage of the South American colubroidea sampled, a high degree of ecological diversity was demonstrated from the current literature, congregating species that are from specialists to the arboreal way of life until strictly terrestrial. Additionally, it has been shown that some species are active only during the night, an apparently derived condition of the lineage. Thus, the intention of the present study was to test whether the diversity of ecological could reflect evolutionary associations with the morphology of these snakes.

The first chapter of the present study indicated that even the snakes which explore arboreal microhabitat gradually, that is, some species spend more time over trees than others, the patterns found in the size of the tail and the degree of slenderness do not vary concomitantly in relation to the degree of arboreality. While longer tails can be seen in animals that use the arboreal microhabitat regardless of how frequent it is used, the increase in slenderness stands out only among the species that use the tree microhabitat more frequently compared to those that use less. However, no difference was found in the degree of relative tail length between terrestrial species and those that use arboreal micro-habitat less frequently. The use of arboreal micro-habitat in turn appears to be a state derived from way of life, with the ancestor of the lineage probably being terrestrial. These findings are in agreement with our current understanding about how the function morphology of different parts of the body relates to the use of arboreal microhabitat.

In relation to changes in body shape, which in turn are reflected in the Surface Area by Volume ratio, it can be observed that these do not present any type of relationship with environmental parameters here studied, such as the use of different microhabitats and activity periods. Thus, this work suggests that throughout the evolution of the snake species sampled, the variation between microclimates derived from different microhabitats does not explain the diversity of the Surface Area to Volume ratio. It is possible that the existing variation of this morphological variable is, in fact, explained only by different degrees of slenderness. As it has been discussed in Chapter 1, environmental pressures related to the biomechanics of locomotion affect the present patterns of slenderness, which consequently can have effects on the S/V ratios. Thus, the thermal relationships of these animals with the environment in which they live are probably mediated mainly by behavioral and physiological mechanisms.

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Snake's species included in this study



Tantilla melanocephala



Spilotes pullatus



Spilotes sulphureus



Pseustes poecilonotus



Drymoluber dichrous



Palusophis bifossatus



Mastigodryas boddaerti



Rhinnobothryum lentiginosum



Drymarchon corais



Chironius multiventris



Chironius bicarinatus



Chironius exoletus



Chironius quadricarinatus



Chironius flavolineatus



Chironius fuscus



Oxybelis fulgidus



Oxybelis aeneus



Dendrophidion dendrophis



Apostolepis dimidiata



Phalotris mertensi



Echisanthera undulata



Philodryas patagoniensis



Philodryas aestiva



Philodryas olfersii



Philodryas nattereri



Philodryas argentea



Tropidodryas serra



Thamnodynastes hypoconia



Tomodon dorsatum



Thamnodynastes pallidus



Phimophis guerini



Pseudoboa nigra



Pseudoboa neuwiedii



Pseudoboa coronata



Clelia clelia



Drepanoides anomalus



Oxyrhopus melanogenys



Oxyrhopus guibei



Oxyrhopus trigeminus



Oxyrhopus clathratus



Shiphlophis cervinus



Shiphlophis compressus



Erythrolamprus typhlus



Erythrolamprus aesculapii



Erythrolamprus reginae



Erythrolamprus miliaris



Erythrolamprus poecilogyrus



Erythrolamprus jaegeri



Xenodon neuwiedii



Xenodon merremii



Xenodon severus



Lygophis meridionalis



Psomophis joberti



Atractus reticulatus



Dipsas albifrons



Sibynomorphus mikanii



Dipsas catesbyi



Dipsas indica



Leptodeira annulata



Imantodes cenchoa

Image's references

Tantilla melanocephala

Sawaya, R. J., Marques, O. A. V., & Martins, M. (2008). Composição e história natural das serpentes de Cerrado de Itirapina, São Paulo, sudeste do Brasil. *Biota Neotropica*, 8(2), 0-0.

Spilotes pullatus

Carlos Cândido - <http://ardobrasil.blogspot.com/2010/10/spilotes-pullatus-linnaeus-1758.html>

Spilotes suphureus

Sawaya, R. J., Marques, O. A. V., & Martins, M. (2008). Composição e história natural das serpentes de Cerrado de Itirapina, São Paulo, sudeste do Brasil. *Biota Neotropica*, 8(2), 0-0.

Pseustes poecilonotus

Magnus Manske - https://en.wikipedia.org/wiki/Phrynonax_poecilonotus

Drymoluber dichrous

<http://reptile-database.reptarium.cz/species?genus=Drymoluber&species=dichrous>

Palusophis bifossatus

José Reynaldo da Fonseca - <https://pt.wikipedia.org/wiki/Jararacu%C3%A7u-do-brejo>

Mastigodryas boddaerti

Claudia Koch - <http://reptile-database.reptarium.cz/species?genus=Mastigodryas&species=boddaerti>

Rhinobothryum lentiginosum

<http://reptile-database.reptarium.cz/species?genus=Rhinobothryum&species=lentiginosum>

Drymarchon corais

Bernard Dupont - [https://commons.wikimedia.org/wiki/File:Indigo_Snake_\(Drymarchon_corais\)_ \(7774161874\).jpg](https://commons.wikimedia.org/wiki/File:Indigo_Snake_(Drymarchon_corais)_ (7774161874).jpg)

Chironius multiventris

<http://reptile-database.reptarium.cz/species?genus=Chironius&species=multiventris>

Chironius exoletus

Jacob Loyacano - <https://www.flickr.com/photos/fins72/24726939105/>

Chironius bicarinatus

Miguel Rangel Jr - https://commons.wikimedia.org/wiki/File:Chironius_bicarinatus_Instituto_Butant%C3%A3.jpg

Chironius quadricarinatus

Ivan Sazima - <http://serpentesbrasileiras2.blogspot.com/2011/04/chironius-quadricarinatus-boie-1827.html>

Chironius flavolineatus

Sawaya, R. J., Marques, O. A. V., & Martins, M. (2008). Composição e história natural das serpentes de Cerrado de Itirapina, São Paulo, sudeste do Brasil. *Biota Neotropica*, 8(2), 0-0.

Chironius fuscus

<http://reptile-database.reptarium.cz/species?genus=Chironius&species=fuscus>

Oxybelis fulgidus

JD Wilson - https://www.discoverlife.org/mp/20p?see=I_JDW655&res=640

Oxybelis aeneus

https://www.discoverlife.org/mp/20p?see=I_JDW649&res=640

Leptophis ahaetulla

https://www.discoverlife.org/mp/20p?see=I_JDW509&res=640

Dendrophidion dendrophis

<https://alchetron.com/Dendrophidion#dendrophidion-829e3545-1972-4727-8292-a72ac114b32-resize-750.jpg>

Apostolepis dimidiata

Sawaya, R. J., Marques, O. A. V., & Martins, M. (2008). Composição e história natural das serpentes de Cerrado de Itirapina, São Paulo, sudeste do Brasil. *Biota Neotropica*, 8(2), 0-0.

Phalotris mertensi

<http://reptile-database.reptarium.cz/species?genus=Phalotris&species=mertensi>

Echinanthera undulata

<http://reptile-database.reptarium.cz/species?genus=Echinanthera&species=undulata>

Philodryas patagoniensis

<http://reptile-database.reptarium.cz/species?genus=Philodryas&species=patagoniensis>

Philodryas aestiva

<http://reptile-database.reptarium.cz/species?genus=Philodryas&species=aestiva>

Philodryas olfersii

<http://reptile-database.reptarium.cz/species?genus=Philodryas&species=olfersii>

Philodryas nattereri

Teles, D. A., Oliveira, M. C., Lima, V. F., Pinto, C. L. M., Silva, É. G., Silva, C. F., & Almeida, W. O. (2019). New record of *Physaloptera* sp. (Nematoda: Physalopteridae) parasitizing *Philodryas nattereri* (Ophidia: Dipsadidae) in Brazil. *Herpetology Notes*, 12, 1031-1034.

Philodryas argentea

https://inpn.mnhn.fr/espece/cd_nom/721412?lg=en

Tropidodryas serra

<http://reptile-database.reptarium.cz/species?genus=Tropidodryas&species=serra>

Thamnodynastes hypoconia

<http://reptile-database.reptarium.cz/species?genus=Thamnodynastes&species=hypoconia>

Tomodon dorsatus

<http://reptile-database.reptarium.cz/species?genus=Tomodon&species=dorsatus>

Thamnodynastes pallidus

Mejía, R. Á. El libro “Serpientes Venenosas: Lecciones aprendidas desde Colombia”, cuyos autores principales son Carlos Alberto Cañas Dávila, médico Internista de la Fundación Valle del Lili, y Fernando Castro Herrera, biólogo de la Universidad del Valle–Cali-, nos ofrece un novedoso y actualizado texto relacionado con los aspectos biológicos, toxicológicos y médicos de las serpientes ponzoñosas.

Phimophis guerini

<http://reptile-database.reptarium.cz/species?genus=Phimophis&species=guerini>

Pseudoboa nigra

<http://reptile-database.reptarium.cz/species?genus=Pseudoboa&species=nigra>

Pseudoboa neuwiedii

<http://reptile-database.reptarium.cz/species?genus=Pseudoboa&species=neuwiedii>

Pseudoboa coronata

<http://reptile-database.reptarium.cz/species?genus=Pseudoboa&species=coronata>

Clelia clelia

<http://reptile-database.reptarium.cz/species?genus=Clelia&species=clelia>

Drepanoides anomalus

<http://thoruto2000.blogspot.com/2012/12/drepanoides-anomalus.html>

Oxyrhopus melanogenys

<http://reptile-database.reptarium.cz/species?genus=Oxyrhopus&species=melanogenys>

Oxyrhopus guibei

<http://reptile-database.reptarium.cz/species?genus=Oxyrhopus&species=guibei>

Oxyrhopus trigeminus

<http://reptile-database.reptarium.cz/species?genus=Oxyrhopus&species=trigeminus>

Oxyrhopus clathratus

<http://reptile-database.reptarium.cz/species?genus=Oxyrhopus&species=clathratus>

Siphlophis cervinus

https://pt.m.wikipedia.org/wiki/Ficheiro:Siphlophus_cervinus.jpg

Siphlophis compressus

<https://srelherp.uga.edu/jd/jdweb/Herps/species/Forsnake/Sipcom.htm>

Erythrolamprus typhlus

<http://reptile-database.reptarium.cz/species?genus=Erythrolamprus&species=typhlus>

Erythrolamprus aesculapii

<http://reptile-database.reptarium.cz/species?genus=Erythrolamprus&species=aesculapii>

Erythrolamprus reginae

<http://reptile-database.reptarium.cz/species?genus=Erythrolamprus&species=reginae>

Erythrolamprus miliaris

<http://reptile-database.reptarium.cz/species?genus=Erythrolamprus&species=miliaris>

Erythrolamprus poecilogyrus

<http://reptile-database.reptarium.cz/species?genus=Erythrolamprus&species=poecilogyrus>

Erythrolamprus jaegeri

Sawaya, R. J., Marques, O. A. V., & Martins, M. (2008). Composição e história natural das serpentes de Cerrado de Itirapina, São Paulo, sudeste do Brasil. *Biota Neotropica*, 8(2), 0-0.

Xenodon neuwiedii

<http://reptile-database.reptarium.cz/species?genus=Xenodon&species=neuwiedii>

Xenodon merremii

https://commons.wikimedia.org/wiki/File:Xenodon_merremii_in_Len%C3%A7%C3%B3is_Maranhenses_National_Park_-_ZooKeys-246-051-g007-G.jpeg

Xenodon severus

<http://reptile-database.reptarium.cz/species?genus=Xenodon&species=severus>

Lygophis meridionalis

Sawaya, R. J., Marques, O. A. V., & Martins, M. (2008). Composição e história natural das serpentes de Cerrado de Itirapina, São Paulo, sudeste do Brasil. *Biota Neotropica*, 8(2), 0-0.

Psomophis joberti

<http://reptile-database.reptarium.cz/species?genus=Psomophis&species=joberti>

Atractus reticulatus

<http://reptile-database.reptarium.cz/species?genus=Atractus&species=reticulatus>

Dipsas albifrons

<http://reptile-database.reptarium.cz/species?genus=Dipsas&species=albifrons>

Dipsas mikanii

Sawaya, R. J., Marques, O. A. V., & Martins, M. (2008). Composição e história natural das serpentes de Cerrado de Itirapina, São Paulo, sudeste do Brasil. *Biota Neotropica*, 8(2), 0-0.

Dipsas catesbyi

<http://reptile-database.reptarium.cz/species?genus=Dipsas&species=catesbyi>

Dipsas indica

<https://www.flickr.com/photos/fins72/24593683112>

Leptodeira annulata

[https://commons.wikimedia.org/wiki/File:Banded_Cat-eyed_Snake_\(Leptodeira_annulata\)__\(26785836919\).jpg](https://commons.wikimedia.org/wiki/File:Banded_Cat-eyed_Snake_(Leptodeira_annulata)__(26785836919).jpg)

Imantodes cenchoa

<http://reptile-database.reptarium.cz/species?genus=Imantodes&species=cenchoa>

