RBECT
Revista Brasileira de Ensino de Ciência e Tecnologia

Revista Brasileira de Ensino de Ciência e Tecnologia
ISSN: 1982-873X

https://periodicos.utfpr.edu.br/rbect

Among snakes, lizards, and genomes: the use of DNA sequences to reconstruct phylogenies and track functional traits

Rodrigo de Mello
0000-0001-8367-3769
rdemellobr@gmail.com
Universidade Estadual de Maringá, Maringá, Paraná, Brasil.

Bruno Reis de Almeida Rodrigues
0000-0003-4062-4603
reisbruno.bio@gmail.com
Universidade Católica de Brasília, Brasília, Brasil.

ABSTRACT

Evolution is the unifying principle of the natural sciences, and understanding its mechanisms is essential for students to organize the biological knowledge they acquire. However, the subject is often taught ineffectively. Many students have problems understanding evolutionary concepts, and in turn, interpreting cladograms. Since molecular genetics has become a rich source of evolutionary information and is the main tool used to reconstruct phylogenies in biology, this study used DNA sequences available in public databases and open access bioinformatics tools to reconstruct a cladogram and track ecophysiological traits along the evolutionary history of lizard and snake species from Brazil. Phylogenetic analyses were based on a fragment of 530 base pairs of the mitochondrial cytochrome B (CytB) gene. Next, the shared (or exclusive) ecological traits of the studied species were tracked, focusing on reproductive strategy (viviparity/oviparity) and presence or absence of venom. The results provide theoretical foundations that demonstrate the practical applications of the meaning and importance of interpreting a phylogenetic tree. As such, it paves the way to address gaps in biology teaching, which generally does not sufficiently address macroevolution (e.g., phylogenetic systematics), thus creating barriers to the understanding of evolutionary mechanisms. In this study, we discuss the importance of learning and understanding the processes and mechanisms of evolution that support biodiversity, which requires direct or indirect knowledge about kinship among species. Finally, pedagogical activities are suggested that break down the complex network of interactions between species, environments, and ecological attributes that constitute the vast biodiversity of Brazil.

KEYWORDS: Evolution. Cladograms. Squamata.

INTRODUCTION

When asked to describe a snake, most people would likely say that the animal has an elongated body that is covered with scales, without limbs for locomotion. But is this information enough to characterize a snake? Zoologically, the answer is a categorical no because there are many apodous, or limbless, lizards. In Brazil, one species with these characteristics is the glass snake (*Ophiodes striatus*) which, despite its name and apparent resemblance, is a lizard of the Diploglossidae family. Meanwhile, for some species of snakes, including the anaconda (Eunectes genus), it is possible to see the presence of spurs, which are vestigial limbs. In addition, there are several lizards with serpentine bodies and limited locomotor limbs, including the many species grouped in the Gymnophthalmidae and Scincidae families. Similarly, caecilians and amphisbaenians, respectively known as blind snakes and two-headed snakes in Brazil, share morphological similarities such as a rounded tail that is the same shape as the head, but belong to very different taxonomic groups. While amphisbaenians are reptiles of the Reptilia class (Squamata order), caecilians belong to the Amphibia class (Gymnophiona order). Therefore, these animals are examples of lineages that have undergone convergent evolution in relation to body shape, a process that was influenced by their respective habitats and life ways.

All the lizards, amphisbaenians, and snakes that exist today are scaled reptiles taxonomically designated as Squamata, and their morphological similarities automatically point to a very close evolutionary relationship. As for the origin of these animals, snakes evolved from an ancestor similar to a lizard, not the other way around. We know this because of the large amount of paleontological, morphological, and phylogenetic evidence (PIANKA; VITT, 2003). Unfortunately, the evolutionary context of life histories and the sharing (or exclusivity) of traits are not intuitive to most people, which generates limited perceptions about the origin and diversification of biodiversity. It is a clear indication of how basic education engages superficially (or even erroneously) with relevant content of the curriculum. As noted by Mendes, Rizzo, and Mayrinck (2022), who evaluated the topic of phylogenetic systematics in the textbook collections approved for the 2021 National Textbook Program (PNLD) in Brazil, a large proportion of the textbooks evaluated do not address phylogenetic systematics content in a clear manner, nor do they reasonably consider the historical and philosophical contexts of this science.

Although evolution is the unifying principle of all biology, most students fail to understand concepts related to this topic (BAUM; SMITH; DONOVAN, 2005). In fact, inaccurate perceptions about evolution are common even among students that have graduated with a biology degree (GREGORY; ELLIS, 2009). Among biologists, significant effort is still required to accurately interpret evolutionary relationships (KRELL; CRANSTON, 2004). This suggests that tree thinking does not come naturally to most people. Achieving an intuitive understanding of evolution is not trivial. However, the ideal is that non-specialists and biology students learn to read modern evolutionary trees, just as geography students would read a map. Understanding evolutionary and ecological concepts in various aspects of society is essential; it is key, for example, in any nation that aims to achieve sustainable and environmentally ethical development.

Understanding biodiversity at a phylogenetic level is a key step for any country seeking to delineate and foster conservation actions and efforts to preserve its genetic and historical heritage. Phylogenetic studies produce important information for taxonomy, patterns and dynamics of adaptation, and evolutionary divergence of species. Currently, molecular systematics, based on DNA sequence data, provide a rich source of information to analyze evolutionary relationships; although there are some limitations, it can indicate approximate timeframes and sequences of events that were key factors influencing divergence patterns. While few subjects require knowledge of phylogenetic systematics, most require some knowledge of evolutionary biology, and many scientists and educators agree that it is impossible to fully understand evolution without the ability to accurately interpret phylogenetic trees. As visual representations that highlight the logic of ancestry, they help students to integrate evolutionary concepts and provide an organizational framework to structure knowledge around biological diversity.

Thus, phylogenetic literacy (see DE MELLO, 2021) requires some kind of exposure to tree thinking – which is the ability to conceptualize evolution in visual representations, such as cladograms and phylogenetic trees. Interactive learning strategies have been effective in teaching scientific concepts when compared to traditional teaching methodologies. Therefore, for learning to be effective, it is essential that students engage in practical tasks related to the target content. In this context, reptiles are a group of animals that demonstrate important evolutionary processes due to the rich diversity of forms, behavior, and life histories among species (SITES; REEDER; WIENS, 2011).

The reptile class, which includes thousands of known species from around the world, is divided into four taxonomic orders: Testudines (or Chelonia - turtles and tortoises); Squamata (amphisbaenians, snakes, and lizards); Rhynchocephalia (tuataras, found only in New Zealand); and Crocodilia (gharial, alligators, and crocodiles). Two fundamental characteristics differentiate reptiles from all other animals in nature: ectothermic thermoregulation (i.e., the use of external heat sources to regulate body temperature) and skin covered with scales. According to the latest list of species produced by the Brazilian Society of Herpetology (SBH), Brazil has 856 reptile species, of which 39 are Testudines, six are Crocodilia, and 811 are Squamata (81 amphisbaenians, 295 lizards, and 435 snakes). Including subspecies, the total number of reptiles in the country rises to 889. Thus, the country ranks 3rd in terms of reptile species richness in the world, behind only Australia (1132) and Mexico (980) (GUEDES; ENTIAUSPE-NETO; COSTA, 2023).

Representatives of the Squamata order are currently found on all continents, presenting a broad spectrum of life histories, ecologies, and body shapes that reflect one of the most diverse adaptive radiations among terrestrial vertebrates. As of October 2023, more than 12,000 species have been cataloged (UETZ *et al.*, 2023). The rate of description of new species continues to grow, with a remarkable record of 168 new species described only in 2012 – a rate higher than any year of the last three centuries (PYRON; BURBRINK; WIENS, 2013). In this context, the general objective of this study was to use DNA sequences that are available in a public database and explore the use of biotechnology tools to investigate genetic parameters and the distribution of ecological attributes in the reconstruction of evolutionary history among the sampled species. More specifically, life history traits of lizards and snakes (viviparity, oviparity, and venomousness) were tracked in a phylogenetic tree generated with real data from Brazilian species that have had part of their genome sequenced.

METHODOLOGY

The first step of the methodology was a survey of DNA sequences of Squamata species that occur in Brazil and are included in GenBank (http://www.ncbi.nlm.nih.gov), a free, open access platform produced and maintained by the National Center for Biotechnology Information (NCBI). GenBank is a public database of nucleotide sequences for about 260,000 formally described species. It houses more than 200 million nucleotide sequences from more than 400,000 animals alone.

DNA sequences were collected from the Cytochrome B (CytB) mitochondrial gene. This genomic region was chosen because it is abundantly sequenced for vertebrates. Furthermore, because it is an integral region of mitochondrial DNA (and therefore of maternal origin), it allows us to estimate the matrilineal history of each organism. Because of these characteristics, as well as the fact that it does not experience recombination, mitochondrial DNA is widely used with phylogenetic methods to address explicit evolutionary hypotheses. Additionally, the scientific literature indicates that divergence rates of mitochondrial DNA sequences are exceptionally well suited to explore the genealogy of speciation in animals, as it is fast enough to show population differences across the geographic range of a species, yet slow enough to not become saturated with recurring mutations over a few million years.

Based on the selection of CytB mitochondrial DNA fragment sequences from 27 species, representing 13 families within the Squamata order (Table 1), an alignment was made with a final length of 530 base pairs. Then, a FASTA format (.fas) file was built to conduct the analysis. This file format is commonly used in programs that perform phylogenetic reconstructions from molecular data. The alignment and all analyses, which included the calculation of genetic distance between species, were conducted with the MEGA X software (KUMAR *et al.*, 2018). In research related to the construction of phylogenetic trees, a wide variety of specific programs are used for each stage of the analysis (i.e., alignment, choice of evolutionary models, different statistics for reconstructing phylogenetics, etc.); however, the MEGA X software was chosen due to ease of handling and because it offers a wide spectrum of functions.

Table 1 – Taxa used in the analyses with their respective GenBank access codes. 1 Lizards, 2 Snakes, 3 Amphisbaenians, 4 Amphioxus

Species	Family	GenBank Code
Tropidurus insulanus[1]	Tropiduridae	EF616029.1
Phyllopezus pollicaris[1]	Phyllodactylidae	JQ827196.1
Gonatodes humeralis[1]	Sphaerodactylidae	JQ639603.1
Colobodactylus taunayi[1]	Gymnophthalmidae	JX079919.1
Colombosaura modesta[1]	Gymnophthalmidae	KT254367.1
Heterodactylus imbricatus[1]	Gymnophthalmidae	JX079921.1
Polychrus marmoratus[1]	Polychrotidae	AB266749.1

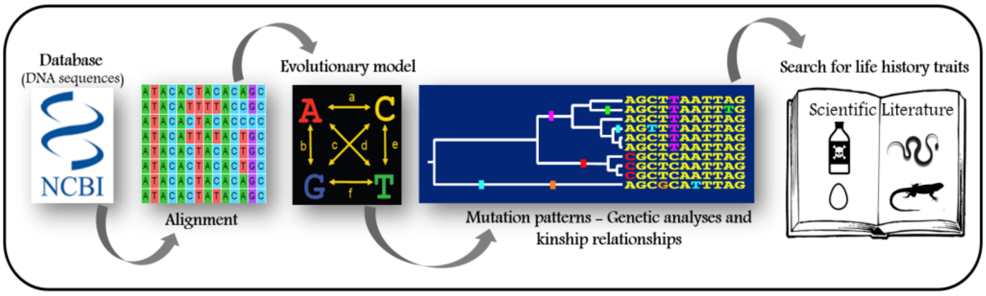
Kentropyx calcarata[1]	Teiidae	JQ639739.1
Tupinambis quadrilineatus[1]	Teiidae	AY217838.1
Mabuya frenata[1]	Scincidae	EU443111.1
Trachylepis atlantica[1]	Scincidae	AF020251.1
Iguana iguana[1]	Iguanidae	DQ239155.1
Crotalus durissus terrificus[2]	Viperidae	AY937301.1
Bothrops alternatus[2]	Viperidae	AF292579.1
Bothrops itapetiningae[2]	Viperidae	AF292582.1
Bothrops moojeni[2]	Viperidae	AF292606.1
Lachesis muta[2]	Viperidae	AF039262.1
Boa constrictor[2]	Boidae	AY575035.1
Epicrates cenchria[2]	Boidae	U69779.1
Eunectes murinus[2]	Boidae	U69808.1
Oxyrhopus guibei[2]	Colubridae	JQ598938.1
Apostolepis dimidiata[2]	Colubridae	JQ598917.1
Sibynomorphus mikanii[2]	Colubridae	JQ598954.1
Leptomicrurus narduccii[2]	Elapidae	EF137412.1
Micrurus mipartitus[2]	Elapidae	EF137414.1
Amphisbaena schmidti[3]	Amphisbaenidae	AY605475.1
Branchiostoma belcheri[4]	Branchiostomatidae	AY700107.1

Source: Prepared by the authors (2023).

Subsequently, an analysis of the composition of nucleotides and phylogenetically informative sites was performed, and the genetic distance was calculated between sampled taxa. The best evolutionary model was also chosen, which identifies the pattern of nucleotide substitutions in the database. Then, the phylogenetic tree was reconstructed using the Maximum Likelihood method with 500 bootstrap replications to determine the retention indexes of each node in the generated tree.

The chosen evolutionary model, which describes the mutational patterns, was the GTR+G+I, which considers different rates of nucleotide substitutions. The species *Branchiostoma belcheri* (amphioxus) was used as an outgroup to root the tree, since it is a basal species in vertebrate phylogeny and adequately polarizes the kinship relationships between the species in the database used herein. Finally, a bibliographic survey was carried out to categorize the species sampled in terms of reproductive strategy (oviparous or ovoviviparous) and snake venomousness to track these traits in the phylogeny. Figure 1 illustrates how mutation patterns in homologous DNA sequences can be used to reconstruct the evolutionary history of species and graphically summarizes the methodology used in this study.

Figure 1 – Schematic diagram of the methodology used in the analysis

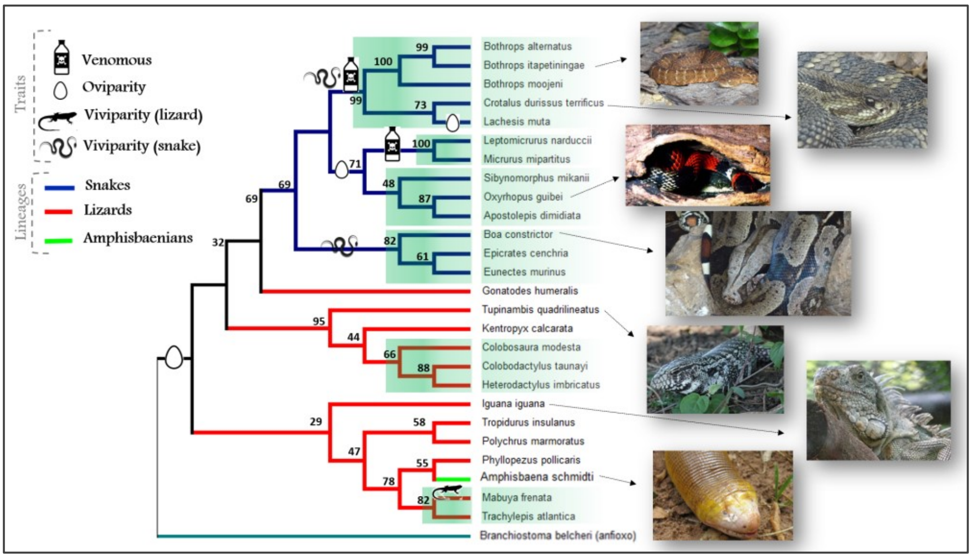


Source: Prepared by the authors (2023).

RESULTS AND DISCUSSION

The kinship generated in the Maximum Likelihood analysis showed the formation of several evolutionary branches. All sampled snakes reflect a monophyletic origin (indicated by green rectangles in Figure 2), while the lizards have several paraphyletic relationships (Figure 2). The average proportion of nucleotide bases in the data used was 28.5% thymine (T), 28.8% cytosine (C), 28.1% adenine (A), and 14.6% guanine (G). Of the 530 analyzed base pairs (pb), 180 were preserved (i.e., without mutation), and of 350 variables, 310 were philologically informative. Despite being based on a 530 bp fragment, with not all nodes being well supported (i.e., high bootstrap values), the cladogram shows that molecular data are a very rich source for phylogenetic systematics, since most kinship relations are consistent with recent studies that used several genes and thousands of pairs of nucleotide sequences. Pyron, Burbrink, and Wiens (2013), for example, used 4,161 species of Squamata based on 12 genes, for a total data matrix with 12,896 bp, while Zheng and Wiens (2016), considering the same 4,161 species, expanded the genome sampling to 52 genes, for a total data matrix of 43,593 bp.

Figure 2 – Phylogenetic tree generated from the reptiles sampled, with bootstrap numbers on each node. Images of some specimens are included to highlight the morphological differences between the species. Source of images: Wikimedia Commons.



Source: Prepared by the authors (2023).

The analysis of genetic diversity parameters highlight that the clusters between more closely related species have shorter genetic distances (e.g., *Bothrops itapetiningae* x *Crotalus durissus* = 0.192), while phylogenetically distant taxonomic groups present higher genetic diversity values (e.g., *Oxyrhopus guibei* x *Tupinambis quadrilineatus* = 0.585), as indicated in bold in Table 2. This pattern reinforces that common ancestry is reflected in the (phylo)genetic profile of the DNA sequences of the sampled taxa, even in a gene fragment of 530 bp, which is extremely small compared to the total genome size of the animals' mitochondria, which is about 16,000 bp.

Table 2 – Genetic distance values calculated for the species illustrated in Figure 2.

Popular name	**Genetic distances between species**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
1. Jararaca	-						
2. Rattlesnake	**0.192**	-					
3. False coral snake	0.285	0.267	-				
4. *Boa constrictor* (*jiboia*)	0.293	0.267	0.302	-			
5. Tupinambis lizard (*teiú*)	0.553	0.517	**0.585**	0.478	-		
6. Iguana	0.439	0.436	0.461	0.391	0.357	-	
7. Amphisbaenian (blind snake)	0.525	0.474	0.497	0.479	0.497	0.307	-

Source: Prepared by the authors (2023).

Regarding phylogenetic reconstruction, in the cladogram, some attributes are shared by Squamata species, emphasizing patterns found in relation to reproductive strategies (oviparity and viviparity), and the presence of venom within snake lineages (Figure 2). The phylogenetic relationships generated provide the same basic information as all evolutionary trees: a historical pattern of ancestry, divergence, and descent. They do this by depicting a series of branches that emerge at points (nodes) which represent common ancestors, who are, in turn, connected to more distant ancestors. These visual representations can provide students with a sense of the historical processes that underpin both sets of relationships and act as a starting point for developing new questions. With this approach, taxa are seen not only as collections of attributes (traits), but also as members of an evolutionary history, which determines how they function within an ecological system.

In the case of scaled reptiles (Squamata), the shared trait of scales to avoid water loss to the environment among lizards, amphisbaenians, and snakes was essential for these vertebrates to definitively colonize the terrestrial environment. In this context, we can amplify the perception that a species that has diversified over geological periods within a natural environment is incapable of responding evolutionarily and ecologically to the rapid changes that human beings have inflicted on the environment. Demonstrating a phylogenetic tree and defining the origin of an attribute in a branch is a very efficient way to show that every descendant of a node must have this attribute. Any biologically important feature can be mapped into a cladogram or phylogenetic tree; in an educational context, this means that the most relevant characteristics can be presented in a unified organizational diagram, as exemplified here with scales, amniotic eggs, and venom.

Tree thinking in this context is important because it embodies a clear understanding of the principle of common ancestry, since it helps students organize their knowledge about biological diversity. As the focus shown here was on reproductive strategies and venomousness, one can graphically explain *where* they appear in the branches of the phylogenetic tree, thus creating a fertile field for evolutionary and ecological discussions within the classroom. This aligns with teaching proposals aimed at scientific literacy (SASSERON; CARVALHO, 2011) and encourages a critical teaching of zoology based on phylogenetic systematics. It also underscores that biology can be framed within a historical-philosophical approach, enabling the teaching of evolution to be a guiding axis of the biological sciences through the fundamental concepts of phylogenetic systematics (SANTOS; KLASSA, 2012).

Oviparity, for example, is found in most lineages (Figure 2), indicating that viviparity has origins in independent lineages as distinct as the families Viperidae (*Lachesis muta*) and Scincidae (*Mabuya frenata*). Awareness of patterns of reproductive strategies is necessary to understand intraspecific (within the same species) and interspecific (between different species) divergences, resulting from the conditions to which a species is subjected, including food availability, thermal variation, water content, concentration of predators in the environment, and suitability of reproductive strategy (SHINE, 2003). Viviparity brings more advantages to species living in regions with cold climates, since lower temperatures are harmful to the development of the embryo inside the egg. Meanwhile, in regions with open phytophysiognomies, such as in Brazilian Cerrado and Caatinga biomes, warmer temperatures can impair the functioning of enzymes that act inside the egg in the process of embryo development.

In relation to tracking venomous species, the tree presented herein helps us to understand some of the complex processes related to the origin and evolution of venom in reptile lineages. In snakes and some lizards, venom is a form of saliva modified throughout their evolutionary history and is a key evolutionary innovation between phylogenetically different lineages; it functions as a defense mechanism, and for predation (killing or subduing prey) and diet-related digestion, as well as a means to assert dominance over members of the same species. The evolution of venomousness may have been responsible for the vast expansion of snakes around the globe (FRY *et al*., 2012).

Snake venom represents an adaptive trait and an example of convergent evolution. It is produced in specialized glands capable of synthesizing and secreting large amounts of biologically active substances, composed mainly of proteins and polypeptides. The various types of venom consist of a complex mixture of organic substances with toxic effects that can act in an isolated or synergistic way, further enhancing local or systemic tissue damage. Diet played a central role in the adaptive radiation of snakes, and venom was an important adaptation in the diversification of these animals (CALVETE *et al*., 2009). As highlighted by these authors, venom represents a fundamental development in the evolution of snakes, as it enabled a transition from a mechanical (constriction) to chemical (venom) means of containing and digesting prey larger than themselves. Therefore, its proteins have multiple functions, which include immobilization, paralysis, killing, and digestion of prey.

All venomous squamates (poisonous snakes and lizards) share a common venomous ancestor and the development of venom in the evolutionary history of snakes is still a matter of debate in academia. It is believed that the origin of venom prompted a rapid diversification of snakes in the Cenozoic period, particularly for species of the Colubridae family and their dispersal across the Americas (WÜSTER *et al*. 2008). Scientists suggest that the reason for this widespread expansion is due to the shift from mechanical to biochemical methods of subduing their prey. In terms of its origin, Fry *et al.* (2012) argued that the most widely accepted hypothesis is that venomousness originated only once around 170 million years ago, and then diversified into the wide range of venom that exist today.

According to this hypothesis, venom originated as a simple set of proteins coupled to a pair of glands. Later, this set of proteins diversified into several lineages, including snakes and lizards of the Anguimorpha and Iguania groups (CASEWELL *et al*., 2013). Several snake lineages subsequently lost the ability to produce venom, usually due to a change in diet (FRY *et al*., 2012). The hypothesis of a unique origin for venom suggests that the mechanism of evolution in most cases occurred due to the duplication of genes followed by natural selection that favored this adaptive trait. Some of the various adaptations that developed through this process include more toxic venom for specific prey in several lineages, proteins that pre-digest the prey, and strategies to track the prey after a bite (e.g., BARLOW *et al*., 2009).

Before the advent of gene sequencing for phylogenetic tree reconstruction, phylogenetics was based on morphology. These traditional relationships suggested that venom originated in multiple lineages among the Squamata approximately 100 million years ago, after which they diversified and evolved independently (FRY *et al*., 2012; CASEWELL *et al*., 2013). Although Pyron and Burbrink (2014) have investigated ecological attributes of lizards and snakes globally, the use of this approach on a more restricted scale is unprecedented with species present in different Brazilian biomes, and it sheds light on how phylogenetics can be used to highlight the evolution of life history traits on a regional scale.

The approach presented in this article aligns with the proposal of Catley (2006), who suggested a paradigm shift in teaching evolution so that students begin to have a more systemic view of evolutionary processes that captures the grandiosity of the theory. In turn, such an approach can incorporate a wide range of evolutionary concepts, from variation in the genome of individuals and natural selection, to mechanisms of speciation, extinction, and formation of higher order groups (cladogenesis).

In the context of teaching evolutionary biology, most of the classes dedicated to this subject are focused on processes related to microevolution, with a significant emphasis on natural selection and modern syntheses of evolutionary theory (e.g., allele frequencies, Hardy-Weinberg Equilibrium). As such, macroevolutionary processes that are visible only at a "phylogenetic scale" become obscure and unintuitive. This also creates a disadvantage for students in that themes related to molecular evolution require a minimum understanding of genetics, which leads many teachers to dedicate few classes to the subject, often at the end of the course after all the prerequisites have been met (TIDON; LEWONTING, 2004). However, the consequence is that students consider evolution as just another subject in the curriculum and are incapable of perceiving it as a unifying theory that provides a framework to organize biological knowledge.

In addition to pedagogical techniques in the classroom, the understanding and acceptance of evolutionary theory depends on several sociocultural factors such as religion, philosophical positioning, family educational level, among others (PENTEADO; KAVALCO; PAZZA, 2012). It has been observed that biology students who learn evolution using tree thinking demonstrate a higher acceptance rate of evolutionary concepts (GIBSON; HOEFNAGELS, 2015). Thus, the development of tree thinking skills is essential and should be considered a priority in the teaching of evolutionary biology (MEISEL, 2010).

In this context, a better understanding of evolutionary biology can contribute to the teaching of other concepts, such as ecology. It is a vital topic for understanding society as a whole, but one that is rarely approached from the perspective that ecological interactions and relationships are products of evolution. However, addressing this issue is not simple and requires an approach that looks to ecosystemic and transdisciplinary thinking. Previous studies have proposed creating connections with educational practices that break away from the reductionism and fragmentation of traditional paradigms, since an ecosystemic and transdisciplinary approach in school environments and pedagogical practices enables education to develop to new levels (e.g., PINHO; QUEIROZ; FREIRE, 2021).

An innovative notion in this transdisciplinarity is that classification is best understood in an ecological context; for example, understanding a set of interrelationships in nature as manifestations of natural selection over long periods of time. As defended and discussed by Santos and Piranha (2018), any subject related to the history of life on Earth requires an interdisciplinary approach that interacts with the phylogenetic system. In the same vein, Chaves, Moraes, and Lira-Da-Silva (2018) pointed out that geological time and the evolutionary process are already considered in the National Common Core Curriculum of Brazilian basic education (BRASIL, 2018). However, rather than presenting adaptation in isolation as the "needs of an organism", adaptation within an evolutionary context is better introduced as characteristics or behaviors that were selected by the environment in the past, favoring the organisms best suited to a particular habitat.

Thus, classification presented in the form of cladogram becomes a powerful pedagogical tool because it is based on observable traits that reflect the history of events that led to evolutionary and ecological patterns among taxa. Visually, it is more didactic and practical to observe the traits investigated here (venomousness and reproductive strategies) along the sampled lineages, highlighting which have multiple or a single common origin. That said, the methodology adopted indicates that the approach of tree thinking is as important for evolutionary biology as the understanding of the mechanisms of natural selection. Thus, phylogenetic reasoning can be powerful in teaching across all fields of biology since tree thinking can be applied at any taxonomic level to discuss and consider the origin and diversification of species.

Brazil is the country most closely associated with the idea of “megadiversity” because it holds the highest levels of biodiversity for many taxonomic groups; the greater the biodiversity of a country, the greater its responsibility to conserve its biological wealth. Therefore, the use of native faunal species as examples in practical approaches to teaching evolution is beneficial, since it highlights the vast diversity of life forms found within the country. As such, when looking at Brazilian lizards and snakes under the prism of evolution, it becomes clear that the country is home to the most majestic faunal populations on the planet. Besides being a powerful tool to provide a deeper understanding in several areas of biological sciences, tree thinking is also extremely useful in raising awareness about the need to conserve Brazilian biodiversity.

FINAL CONSIDERATIONS

Among animal groups, reptiles are used extensively as biological models for evolutionary and speciation studies. They are also considered good models in ecological studies, including those addressing climatic and environmental effects on species distribution. Therefore, reptiles provide a broad spectrum of possible approaches to be investigated within the scope of tree thinking, since phylogenetic trees demonstrate the evolutionary relationships of species or groups of species. Understanding these relationships is crucial to understand evolution. Compared to other methods of classification based on similar traits, evolutionary relationships are unique because they the result of common ancestry.

An important consequence of the evolutionary process is that it produces hierarchically nested relationships between species and species groups. If people understand and interpret this correctly, they will develop an intuitive understanding of evolutionary relationships based on hierarchies. Unfortunately, in biology teaching little time is dedicated to macroevolution, such as phylogenetic systematics, which creates barriers to understanding evolutionary mechanisms. Learning and integrating the processes and mechanisms of evolution that support our biodiversity depends directly or indirectly on one’s knowledge about the relationships between species. Biotechnology and DNA mutation patterns have helped significantly in this field.

The methodology used herein aims to instigate new perspectives for future studies and inform research that seeks to unpack the complex network of interactions between species, environments, and ecological attributes. This type of approach helps science not only shed light on the history of life on Earth, but it can also lead to a greater awareness of the environment and increase conservation efforts to preserve the genetic heritage, historical legacy, and biological wealth that the country holds.

ENTRE COBRAS, LAGARTOS E GENOMAS: O USO DE SEQUÊNCIAS DE DNA NA RECONSTRUÇÃO DE FILOGENIAS PARA RASTREAMENTO DE ATRIBUTOS FUNCIONAIS

RESUMO

A Evolução é o princípio unificador das ciências naturais e sua compreensão é essencial para a organização do conhecimento biológico adquirido por estudantes. Entretanto, este assunto é frequentemente ensinado de forma ineficaz e diversos alunos tem problemas em compreender conceitos evolutivos e, por sua vez, em interpretar cladogramas. Uma vez que a genética molecular se tornou uma rica fonte de informações evolutivas e é a principal ferramenta para a reconstrução de filogenias na Biologia, este trabalho utilizou sequências de DNA disponíveis em bancos de dados públicos e ferramentas de bioinformática de livre acesso para a reconstrução de um cladograma para se rastrear atributos ecofisiológicos na história evolutiva de espécies de lagartos e serpentes da fauna brasileira. As análises filogenéticas empregadas foram baseadas em um fragmento de 530 pares de bases do gene mitocondrial Citocromo B (CytB). Em seguida, os atributos ecológicos compartilhados (ou exclusivos) às espécies investigadas foram rastreados, como estratégia reprodutiva (viviparidade/oviparidade) e presença ou ausência de veneno. Os resultados gerados fornecem alicerces teóricos para enfatizar a aplicação prática do significado e importância de se interpretar uma árvore filogenética, pavimentando discussões para suprir falhas no ensino de Biologia, que em geral não dedica muito tempo a tópicos de macroevolução (e.g., sistemática filogenética), o que gera barreiras para o entendimento dos mecanismos evolutivos. Neste trabalho, discute-se a importância de assimilar e entender os processos e mecanismos de evolução que sustentam a biodiversidade, que depende direta ou indiretamente do conhecimento sobre as relações de parentesco entre as espécies. Por fim, são sugeridas ações pedagógicas para desmembrar a complexa rede de interações entre espécies, ambientes e atributos ecológicos que compõe a imensa biodiversidade brasileira.

PALAVRAS-CHAVE: Evolução. Cladogramas. Squamata.

BIBLIOGRAPHIC REFERENCES

BARLOW, A.; POOK, C. E.; HARRISON, R. A.; WÜSTER, W. Coevolution of diet and prey-specific venom activity supports the role of selection in snake venom evolution. **Proceedings of the Royal Society B: Biological Sciences**, v. 276, n. 1666, p. 2443-2449, 2009. Available at: https://royalsocietypublishing.org/doi/10.1098/rspb.2009.0048. Accessed on: May 12nd, 2021.

BAUM, D. A.; SMITH, S. D.; DONOVAN, S. S. The tree-thinking challenge. **Science**, v. 310, n. 5750, p. 979-980, 2005.

BRASIL. Ministério da Educação. **Base Nacional Comum Curricular**. Brasília, 2018. Available at: http://basenacionalcomum.mec.gov.br/. Accessed on: Jul. 02nd, 2023.

CALVETE, J. J.; FASOLI, E.; SANZ, L.; BOSCHETTI, E.; RIGHETTI, P. G. Exploring the venom proteome of the western diamondback rattlesnake, *Crotalus atrox*, via snake venomics and combinatorial peptide ligand library approaches. **Journal of Proteome Research**, v. 8, n. 6, p. 3055-3067, 2009. Available at: https://pubmed.ncbi.nlm.nih.gov/19371136/. Accessed on: Jun. 08th, 2021.

CASEWELL, N. R.; WÜSTER, W.; VONK, F. J.; HARRISON, R. A.; FRY, B. G. Complex cocktails: the evolutionary novelty of venoms. **Trends in Ecology & Evolution**, v. 28, n. 4, p. 219-229, 2013. Available at: https://pubmed.ncbi.nlm.nih.gov/23219381/. Accessed on: May 15th, 2023.

CATLEY, K. M. Darwin's missing link—a novel paradigm for evolution education. **Science Education**, v. 90, n. 5, p. 767-783, 2006. Available at: https://www.academia.edu/83313820/Darwins_missing_link_a_novel_paradigm_for_evolution_education. Accessed on: Jun. 08th, 2021.

CHAVES, R. S.; MORAES, S. S.; LIRA-DA-SILVA, R. M. Por que Ensinar Tempo Geológico na Educação Básica? **Terra e Didática**, v. 14, n. 3, p. 233-244, 2018. Available at: https://periodicos.sbu.unicamp.br/ojs/index.php/td/article/view/8652309. Accessed on: Jun. 01st, 2021.

DE MELLO, R. Nada em meio ambiente faz sentido exceto à luz da Ecologia e da Evolução. **Revista Brasileira de Meio Ambiente**, v. 10, n. 1, 2021. Available at: https://revistabrasileirademeioambiente.com/index.php/RVBMA/article/view/1008. Accessed on: Apr. 12th, 2021.

FRY, B. G.; SCHEIB, H.; AZEVEDO, I. L. M. J.; SILVA, D. A.; CASEWELL, N. R. Novel transcripts in the maxillary venom glands of advanced snakes. **Toxicon**, v. 59, n. 7-8, p. 696-708, 2012. Available at: https://pubmed.ncbi.nlm.nih.gov/22465490/. Accessed on: May 05th, 2021.

GIBSON, J.; HOEFNAGELS, M. H. Correlations between tree thinking and acceptance of evolution in introductory biology students. **Evolution: Education and Outreach**, v. 8, n. 1, p. 1-17, 2015. Available at: https://evolution-outreach.biomedcentral.com/articles/10.1186/s12052-015-0042-7. Accessed on: Mar. 04th, 2021.

GREGORY, T. R.; ELLIS, C. A. J. Conceptions of evolution among science graduate students. **BioScience**, v. 59, n. 9, p. 792-799, 2009. Available at: https://academic.oup.com/bioscience/article/59/9/792/248601. Accessed on: Feb. 25th, 2021.

GUEDES, T. B.; ENTIAUSPE-NETO, O. M.; COSTA, H. C. Lista de répteis do Brasil: atualização de 2022. **Herpetologia Brasileira**, v. 12 n. 1, p. 56-161, 2023. Available at: https://zenodo.org/record/7829013. Accessed on: Jun. 08th, 2021.

KRELL, F.; CRANSTON, P., S. Which side of the tree is more basal? **Systematic Entomology**, v. 29, n. 3, p. 279-281, 2004. Available at: https://resjournals.onlinelibrary.wiley.com/doi/10.1111/j.0307-6970.2004.00262.x. Accessed on: Jun. 04th, 2021.

KUMAR, S.; STECHER, G.; LI, M.; KNYAZ, C.; TAMURA, K. MEGA X: molecular evolutionary genetics analysis across computing platforms. **Molecular Biology and Evolution**, v. 35, n. 6, p. 1547, 2018. Available at: https://pubmed.ncbi.nlm.nih.gov/29722887/. Accessed on: Jun. 04th, 2021.

MEISEL, R. P. Teaching tree-thinking to undergraduate biology students. **Evolution: Education and Outreach**, v. 3, n. 4, p. 621-628, 2010. Available at: https://pubmed.ncbi.nlm.nih.gov/21572571/. Accessed on: Jun. 02nd, 2021.

MENDES, S. L. D. S. D.; RIZZO, A. E.; MAYRINCK, D. A representação da Sistemática Filogenética nos livros didáticos do novo ensino médio: desafios e perspectivas. **Amazônia: Revista de Educação em Ciências e Matemáticas**, v. 18, n. 40, p. 209-224, 2022. Available at: https://periodicos.ufpa.br/index.php/revistaamazonia/article/view/12742. Accessed on: Jun. 08th, 2021.

PENTEADO, P. R.; KAVALCO, K. F.; PAZZA, R. Influence of sociocultural factors and acceptance of creationism in the comprehension of evolutionary biology in freshman Brazilian students. **Evolution: Education and Outreach**, v. 5, p. 589-

594, 2012. Available at: https://evolution-outreach.biomedcentral.com/articles/10.1007/s12052-012-0456-4. Accessed on: Jun. 08th, 2021.

PIANKA, E. R.; VITT, L. J. **Lizards: windows to the evolution of diversity**. Univ of California Press, 2003.

PINHO, M. J. D.; QUEIROZ, M. C. D. C.; FREIRE, J. C. D. S. Pensamento ecossistêmico e transdisciplinar: trilhando caminhos na perspectiva da Ecologia dos Saberes. **Linhas Críticas**, v. 27, 2021. Available at: https://periodicos.unb.br/index.php/linhascriticas/article/view/34338. Accessed on: Jun. 01st, 2021.

PYRON, R. A.; BURBRINK, F. T. Early origin of viviparity and multiple reversions to oviparity in squamate reptiles. **Ecology Letters**, v. 17, n. 1, p. 13-21, 2014. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1111/ele.12168. Accessed on: Jun. 06th, 2021.

PYRON, R. A.; BURBRINK, F. T.; WIENS, J. J. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. **BMC Evolutionary Biology**, v. 13, n. 1, p. 1-54, 2013. Available at: https://pubmed.ncbi.nlm.nih.gov/23627680/. Accessed on: Jun. 08th, 2021.

SANTOS, C. M. D.; KLASSA, B. Despersonalizando o ensino de evolução: ênfase nos conceitos através da sistemática filogenética. **Educação: Teoria e Prática**, v. 22, n. 40, p. 62-80, 2012. Available at: https://www.periodicos.rc.biblioteca.unesp.br/index.php/educacao/article/view/6271. Accessed on: May 15th, 2021.

SANTOS, C. P. S.; PIRANHA, J. M. Earth history and evolution of life in the high school curriculum of the state of São Paulo. **Terra e Didática**, v. 14, n. 3, p. 296-303, 2018. Available at: https://periodicos.sbu.unicamp.br/ojs/index.php/td/article/view/8653529. Accessed on: May 15th, 2021.

SASSERON, L. H.; CARVALHO, A. M. P. Alfabetização científica: uma revisão bibliográfica. **Investigações em Ensino de Ciências**, v. 16, n. 1, p. 59–77, 2011. Available at: https://ienci.if.ufrgs.br/index.php/ienci/article/view/246. Accessed on: May 15th, 2021.

SHINE, R. Reproductive strategies in snakes. **Proceedings of the Royal Society of London. Series B: Biological Sciences**, v. 270, n. 1519, p. 995-1004, 2003. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1691341/. Accessed on: Mar. 06th, 2021.

SITES, J. R.; J. W.; REEDER, T. W.; WIENS, J. J. Phylogenetic insights on evolutionary novelties in lizards and snakes: sex, birth, bodies, niches, and venom. **Annual Review of Ecology, Evolution, and Systematics**, v. 42, p. 227-244, 2011. Available at: https://www.annualreviews.org/doi/abs/10.1146/annurev-ecolsys-102710-145051. Accessed on: Jun. 05th, 2021.

TIDON, R.; LEWONTIN, R. C. Teaching evolutionary biology. **Genetics and Molecular Biology**, v. 27, p. 124-131, 2004. Available at: https://www.scielo.br/j/gmb/a/8pwzKNbBJXHNNV9vF8rzrdb/. Accessed on: Jun. 06th, 2021.

UETZ, P.; FREED, P.; AGUILAR, R.; REYES, F.; HOSEK, J. (eds.) 2023. **The Reptile Database**. Available at: http://www.reptile-database.org. Accessed on: Nov. 28th, 2023.

WÜSTER, W.; PEPPIN, L.; POOK, C. E.; WALKER, D. E. A nesting of vipers: phylogeny and historical biogeography of the Viperidae (Squamata: Serpentes). **Molecular Phylogenetics and Evolution**, v. 49, n. 2, p. 445-459, 2008. Available at: https://pubmed.ncbi.nlm.nih.gov/18804544/. Accessed on: Mar. 03rd, 2021.

ZHENG, Y.; WIENS, J. J. Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4162 species. **Molecular Phylogenetics and Evolution**, v. 94, p. 537-547, 2016. Available at: https://pubmed.ncbi.nlm.nih.gov/26475614. Accessed on: Jun. 08th, 2021.

Received: Jun. 09th, 2021.
Approved: Dec. 03rd, 2023.
DOI: 10.3895/rbect.v17n1.14708
How to cite: MELLO, R.; RODRIGUES, B. R. A. Among snakes, lizards, and genomes: the use of DNA sequences to reconstruct phylogenies and track functional traits. **Brazilian Journal of Science Teaching and Technology**, Ponta Grossa, v.17, p. 1-17, 2024. Available at: <https://periodicos.utfpr.edu.br/rbect/article/view/14708>. Access on: XXX.
Mailing address: Rodrigo de Mello - rdemellobr@gmail.com